UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

CARACTERIZACIÓN DE LA CALIDAD DE PRODUCTOS CÁRNICOS CRUDO-CURADOS MEDIANTE ULTRASONIDOS DE SEÑAL

TESIS DOCTORAL

Presentada por:
Edith Corona Jiménez

Dirigida por:
Dr. D. José Javier Benedito Fort
Dr. D. José Vicente García Pérez

Valencia, 2012

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

D. JOSÉ JAVIER BENEDITO FORT, CATEDRÁTICO DE UNIVERSIDAD DEL DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS, Y D. JOSÉ VICENTE GARCÍA PÉREZ, PROFESOR TITULAR DE UNIVERSIDAD DEL DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS DE LA UNIVERSIDAD POLITÉCNICA DE VALENCIA

CERTIFICAN: que la memoria titulada "CARACTERIZACIÓN DE LA CALIDAD DE PRODUCTOS CÁRNICOS CRUDO-CURADOS MEDIANTE ULTRASONIDOS DE SEÑAL", que para aspirar al grado de Doctor en Ciencia y Tecnología de Alimentos presenta Dña. Edith Corona Jiménez, realizada bajo nuestra dirección en el Departamento de Tecnología de Alimentos de la Universidad Politécnica de Valencia, cumple las condiciones adecuadas para su aceptación como Tesis Doctoral, por lo que

AUTORIZAN: A la interesada a su presentación en el Departamento de Tecnología de Alimentos de la Universidad Politécnica de Valencia.

Y para que conste a los efectos oportunos presentamos la referida memoria, firmado el presente certificado en Valencia 21 de diciembre del 2012.

Fdo. Dr. D José Javier Benedito Ford

Fdo. Dr. D. José Vicente García Pérez

A Dios, por todas sus bondades.

A mis padres, mis hermanas y mi hermano, porque son lo único que realmente tengo en mi vida, lo que más quiero. Gracias por estar siempre a mi lado y por su infinito cariño.
***Los amo**.*

"El querer alcanzar un sueño implica ser la forma más grande y poderosa de exigirse a uno mismo. Sólo la constancia, la perseverancia, el trabajo duro y el apoyo incondicional de todos los que te quieren son el mejor motivo para seguir. Las reglas fundamentales son nunca pasar por encima de los demás y no importar el caer, sino el volver a levantarse para recorrer ese largo camino al sueño y cuando por fin éste se logra, la PLENA FELICIDAD se alcanza y uno se da cuenta de que tan fuerte puede llegar a ser uno mismo y que la felicidad es un estado hermoso y maravillosamente inexplicable."

Edith Corona Jiménez

Valencia, España 2012

AGRADECIMIENTOS

Deseo manifestar mi agradecimiento a todas aquellas personas e instituciones que colaboraron de alguna forma en el desarrollo del presente trabajo:

A la Universidad Politécnica de Valencia y al Departamento de Tecnología de Alimentos, porque la presente Tesis Doctoral se desarrolló dentro de sus instalaciones.

A todos los integrantes del grupo de Análisis y Simulación de Procesos Agroalimentarios (ASPA). A su director el Doctor Antonio Mulet Pons.

A los directores de este trabajo, el Doctor José Javier Benedito Fort y el Doctor José Vicente García Pérez. Porque el elevado compromiso que tienen con su propio trabajo ha resultado ser mi mayor reto, pero también mi mayor logro. Porque todo lo que me han enseñado y que he aprendido de ustedes no sólo ha sido en el ámbito laboral, sino también en el aspecto personal. Gracias.

Al Ministerio de Economía y Competitividad del Gobierno de España, por aportar los recursos necesarios para llevar a cabo esta investigación (FPI 2008-2012-BES-2008-004294; Proyecto AGL 2007-65923-C02-02; Proyecto RTA 2010-00029-C04-02; Estancias breves para el Investigador en Formación. FPI-2011).

A todo el personal de Tecnología de los Alimentos, Departamento de Producción Animal y Ciencia de los Alimentos de la Facultad de Veterinaria de la Universidad de Extremadura (España) por su disposición y colaboración.

Al Doctor Tomás Gómez Álvarez-Arenas del grupo de investigación UMEDIA (Ultrasound for Medical and Industrial Applications), CSIC; en Madrid (España) por su disposición y colaboración.

I would like to express my gratitude to the Food Physics Group, of the School of Food Science and Nutrition at the University of Leeds (United Kingdom); especially to Professor Malcolm J. W. Povey, head of the research group, and to my supervisor Doctor Nicholas J. Watson. Thank you for the technical assistance, the scientific explanations, continuous support and for conversations; thank you as well for the good moments and coffee/tea time with British biscuits.

A la Vicedirectora de la Escuela Oficial de Idiomas de Valencia, Catedrática Liv Ingrid Ostbye Roggen y al Profesor Juan Miguel Muñoz Olmos por toda su ayuda, afecto y cuidados, porque el estar lejos de casa resulta muchas veces difícil, pero personas como ustedes hacen que uno se sienta protegido y acompañado, por hacer que su casa sea mi segundo hogar. Gracias.

A todos los que de alguna manera han contribuido y me han acompañado durante cada uno de los días de este largo y hermoso camino, haciéndolo fácil de recorrer. Aquellos con los que comencé esta aventura, los que aún están y los que ya se han ido, pero que aún siguen en la distancia desde cualquier parte del mundo. Resulta difícil colocar nombres, pero quiero agradecer a todos y cada uno de ustedes por compartir conmigo las alegrías y tristezas, las sonrisas y lágrimas, los problemas y sus soluciones, los obstáculos y facilidades, las adversidades y fortunas, los desacuerdos y conciliaciones, la algarabía y el sosiego, la extravagancia y naturalidad, las locuras y cordura, por enseñarme y aprender, por lo cotidiano y lo inhabitual, sus idiomas y tradiciones, su respeto y afecto, su apoyo y consejos, por sostenerme e impulsarme cuando más lo necesité, por su consuelo y ayuda desinteresada, por caminar junto a mí, por las reuniones y sus buenos momentos, los viajes y sus distancias recorridas, el día a día y sus sorpresas, pero sobretodo por su amistad y su entrañable cariño, porque en resumen, lo mejor que me pudieron dar durante todo este tiempo es el regalo de tener su compañía y amistad, y con ella, mantener mi sonrisa y alegría. Los llevo a todos y a cada uno de ustedes en mis recuerdos por el resto de mis días. Muchas gracias por estar ahí, los quiero.

Esta tesis está dedicada **a mis padres Yolanda Jiménez y Teodoro Corona**, por ser el mejor ejemplo de templanza, perseverancia, esfuerzo y trabajo, porque gracias a sus esfuerzos nunca me ha hecho falta nada, por el gran amor y cuidados que tienen por mí y por mis hermanos, por el simple hecho de ser mis padres, mi mayor motivo. **A mis hermanos Ana, Hugo y Lygia**, por ser mi ejemplo a seguir, porque admiro su inteligencia, empeño y sencillez, por su cariño y complicidad, por su apoyo en todo momento y por ser mi mayor alegría y lo más lindo que tengo en mi vida. A todos ustedes gracias, porque sin ustedes, nada de esto sería posible. **Gracias**.

Finalmente quiero agradecer **a Dios,** porque tu grandeza está en cada uno de los días que me has concedido vivir, porque por más difíciles que éstos puedan parecer, no dejan de ser un regalo para mí, por todas tus bondades y la fuerza que siempre me das. **Gracias.**

ÍNDICE

ÍNDICE DE FIGURAS

Chapter 3 (Apartado 3).

Chapter 4 (Apartado 4).

Chapter 5 (Apartado 5).

ÍNDICE DE TABLAS

Chapter 3 (Apartado 3).

ABSTRACT / RESUMEN / RESUM

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 on 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 views.

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 and 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 drying 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. 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 to 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 % w.b.), 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 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 non-contact 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.

RESUMEN

Los productos cárnicos crudo-curados provenientes del cerdo Ibérico son muy apreciados por el consumidor como consecuencia de su elevada calidad tanto organoléptica como nutricional. El aumento de la demanda de estos productos en el mercado empuja a las industrias productoras a una mejora continua, buscando nuevas formulaciones y presentaciones, así como, optimizando los procesos de elaboración e implementando nuevas técnicas de conservación y distribución. La calidad final de los productos cárnicos crudo-curados puede variar tanto por la materia prima utilizada, como por los diferentes procesos a los que son sometidos. En este sentido, la **grasa** es uno de los principales componentes que influye en la calidad y que a su vez, depende de **la raza y la alimentación del animal**. Así pues, el contenido de grasa, así como el estado físico (líquido y/o sólido) en el que se encuentre, afectarán a las propiedades sensoriales y en particular a la textura de los productos cárnicos curados. Por lo tanto, resulta de interés evaluar el **contenido, estado y tipología** de la grasa para estimar la calidad de los productos cárnicos crudo-curados.

En el momento actual, el consumo de jamón ibérico está desplazándose de la compra de piezas enteras para su loncheo manual, a productos loncheados mecánicamente y envasados al vacío. Este formato de producto hace necesario el uso adicional de tecnologías convencionales de conservación, como el **almacenamiento refrigerado**, o de nuevas tecnologías como las **altas presiones hidrostáticas** (APH). Estas técnicas de conservación permiten prolongar la vida útil del producto pero pueden afectar a la calidad del mismo, provocando cambios en propiedades tan importantes como su textura. En este sentido, también es de gran interés la caracterización de los cambios sufridos por los productos cárnicos curados sometidos a estos métodos de conservación (frío y APH).

Hasta el momento, se han utilizado diversas técnicas instrumentales y sensoriales para la evaluación de diferentes parámetros de calidad de la grasa de los productos cárnicos crudo-curados, tales como su textura, el tipo de grasa, la cantidad de la misma y el estado en el que se encuentra a diferentes temperaturas.

No obstante, la integridad de las muestras sometidas a estas técnicas convencionales se ve afectada de forma considerable, además de que su implementación en las líneas de producción es compleja y costosa. De esta manera, la búsqueda de técnicas **no destructivas**, fáciles de implementar y de bajo coste, que permitan la caracterización en línea de los productos cárnicos curdo-curados resulta interesante desde el punto de vista tanto científico como tecnológico.

Los **ultrasonidos** de baja intensidad o señal son una técnica de evaluación no destructiva, rápida, sencilla y económica, por lo que pueden ser utilizados como una herramienta fiable para la caracterización de diversos alimentos y ser integrados en los sistemas de producción con objeto de su optimización. La medida de la velocidad de los ultrasonidos ha permitido estimar de manera no destructiva la composición y los cambios sufridos durante el proceso de elaboración de un amplio número de alimentos, así como de las materias primas empleadas.

En base a lo anteriormente expuesto, el objetivo general de este trabajo fue evaluar la utilidad de los ultrasonidos de señal para caracterizar de manera no destructiva los productos cárnicos crudo-curados provenientes del cerdo Ibérico en función de la tipología de la grasa, su contenido graso y sus propiedades texturales. Asimismo se pretende evaluar el uso de esta técnica para la caracterización no destructiva de los cambios sufridos durante el almacenamiento refrigerado de productos cárnicos crudo-curados y tras su tratamiento por altas presiones hidrostáticas.

Para la consecución de este objetivo general, se han llevado a cabo cinco estudios. En el primero, el objetivo fue evaluar la viabilidad del uso de los ultrasonidos para caracterizar el proceso de cristalización de manteca de cerdo Ibérico durante su almacenamiento a bajas temperaturas, así como, desarrollar un modelo matemático que permitiera estimar el contenido de grasa sólida durante su cristalización. Para alcanzar estos objetivos, se realizaron medidas de la velocidad de los ultrasonidos en muestras de manteca Ibérica durante su almacenamiento (11 días) a diferentes temperaturas (0, 3, 5, 7, 10 y 20 ºC). Asimismo, se determinó el comportamiento térmico de las muestras mediante

Calorimetría Diferencial de Barrido (DSC) y se realizaron ensayos de punción para determinar las características texturales de la muestra, principalmente su dureza. Los resultados experimentales mostraron dos incrementos pronunciados en la velocidad de los ultrasonidos durante el almacenamiento isotermo. Este comportamiento se relacionó con la cristalización de los triglicéridos en función de su grado de saturación, que fue observada en los resultados obtenidos mediante DSC. En las medidas texturales llevadas a cabo durante el almacenamiento isotermo se observó el mismo comportamiento, encontrándose dos incrementos pronunciados de la dureza de las muestras. Por otro lado, el proceso de cristalización de la grasa en dos etapas fue descrito adecuadamente mediante un modelo matemático basado en la ecuación de Avrami, estableciéndose una relación entre la velocidad de los ultrasonidos y el tiempo de almacenamiento isotermo (% var >99.9). Además, se desarrolló un modelo matemático para estimar la evolución del porcentaje de grasa sólida durante el almacenamiento isotermo, considerando un proceso de cristalización en dos etapas. Por lo tanto, las medidas de velocidad de los ultrasonidos permitieron caracterizar el patrón de cristalización de la manteca de cerdo Ibérica sometida a un proceso de almacenamiento isotermo a bajas temperaturas, lo que dio paso al siguiente estudio.

El segundo estudio se llevó a cabo en muestras de grasa subcutánea (tejido adiposo) de cerdos Ibéricos alimentados en dos diferentes sistemas (montanera y cebo). El objetivo de este apartado fue evaluar el uso de los ultrasonidos para caracterizar los cambios texturales del tejido adiposo que tienen lugar durante el almacenamiento a bajas temperaturas, así como discriminar entre los dos tipos de grasa. Para ello, se realizaron medidas de la velocidad de los ultrasonidos en las grasas durante su almacenamiento isotermo durante 20 días, ensayándose diferentes temperaturas (0, 2, 5, 7 y 10 ºC). Los cambios en la dureza de las grasas durante el almacenamiento se determinaron mediante ensayos de punción. La composición del perfil de ácidos grasos y el comportamiento térmico de los dos tipos de grasa fueron obtenidos mediante análisis por cromatografía de gases y medidas de DSC, respectivamente. Así, en ambas grasas, se observaron dos incrementos pronunciados de su dureza durante el almacenamiento, al igual que en el estudio previo con la manteca de cerdo. Estos cambios en la textura fueron

atribuidos a la cristalización de triglicéridos con diferente grado de insaturación, hecho que se correspondió con los dos picos de cristalización de grasa encontrados en los análisis de DSC. Por otro lado, la grasa de cebo presentó valores medios de la dureza superiores ($p<0.05$) a los de la grasa de montanera durante todo el periodo de almacenamiento, lo que permitió discriminar entre los dos tipos de grasa. Las diferencias en la dureza se relacionaron con el mayor contenido ($p<0.05$) de ácidos grasos saturados de las muestras de cebo comparado con las de montanera. De manera similar a la dureza, las medidas ultrasónicas permitieron observar dos aumentos pronunciados en la velocidad durante el tiempo de almacenamiento, así como discriminar entre los dos tipos de grasa. En este sentido, la velocidad de los ultrasonidos tras la cristalización de los triglicéridos más saturados fue 2.8 % superior para cebo que para montanera, siendo este porcentaje del 5.2 % tras la cristalización de la totalidad de triglicéridos. Finalmente, tanto los cambios texturales, como los de la velocidad de los ultrasonidos observados durante el proceso de cristalización de las dos grasas, fueron descritos adecuadamente mediante un modelo matemático basado en la ecuación de Avrami. Así pues, los resultados obtenidos pusieron de manifiesto que las medidas de ultrasonidos pueden ser utilizadas para caracterizar de manera no destructiva la cristalización de la grasa subcutánea de cerdo Ibérico y los cambios en sus propiedades texturales ocurridos durante su almacenamiento a bajas temperaturas, así como para discriminar entre los dos tipos de grasa.

De los resultados obtenidos en los dos primeros trabajos, se confirmó la viabilidad del uso de los ultrasonidos para la monitorización de los procesos de cristalización de grasa de cerdo ibérico, que dan lugar a cambios texturales en la misma, durante el almacenamiento refrigerado, así como para discriminar entre grasas de diferente origen (montanera y cebo). Otro de los objetivos de la Tesis era estudiar el uso de los ultrasonidos para estimar el contenido graso y el estado de la grasa en productos cárnicos curados, donde la grasa es únicamente un componente del sistema, así como para discriminar estos productos en función del tipo de grasa con el que han sido elaborados. A diferencia de la materia prima ensayada en los dos primeros trabajos, estos productos están formados por tejido magro y grasa y han sido sometidos a un proceso de curado. En un primer caso,

se trabajó con sistemas modelo a base de carne magra y grasa (picadas y embutidas), lo que permite disponer de una mayor homogeneidad composicional en las muestras que la encontrada en productos como el jamón curado. De esta manera, el tercer estudio se realizó en salchichas curadas elaboradas con diferente contenido y tipo de grasa. El objetivo del estudio fue el de evaluar la viabilidad del uso de los ultrasonidos para determinar el contenido de grasa y su estado, así como para discriminar entre los distintos tipos de grasa utilizados en su formulación. Se elaboraron diferentes lotes de salchichas con distintos tipos de grasa (montanera, cebo, manteca y aceite de girasol) y niveles de contenido graso (desde 3 a 17 %). Se realizaron medidas de la velocidad de los ultrasonidos a diferentes temperaturas (2, 6, 10, 15, 20 ,25 y 30 ºC), también se determinó el perfil de ácidos grasos por cromatografía de gases y el comportamiento fundente de las muestras mediante medidas de DSC y finalmente, se determinó la composición química de las salchichas. Los resultados mostraron que la composición del perfil de ácidos grasos (grado de saturación) afectó al comportamiento fundente de las muestras (temperaturas y entalpías de fusión), lo que permitió distinguir entre los diferentes lotes de salchichas. Por otra parte, para todos los lotes de salchichas con alto contenido de grasa, se observó un descenso en la velocidad media de los ultrasonidos con el aumento de la temperatura en un rango de 2 a 30 ºC. El descenso de la velocidad fue relacionada con los resultados obtenidos en los análisis de DSC que mostraron que para ese rango de temperaturas se presentó una fusión pronunciada de las grasas. Por lo tanto, a mayor temperatura, mayor porcentaje de grasa fundida y menor velocidad de los ultrasonidos. Así, se encontraron relaciones lineales significativas ($p<0.05$) entre el porcentaje de grasa fundida en las muestras y la velocidad de los ultrasonidos, que fueron diferentes para las salchichas elaboradas con distintos tipos de grasa. Por lo tanto, los ultrasonidos fueran capaces no solo de estimar el porcentaje de grasa fundida, sino también de identificar los distintos tipos de grasa utilizados en la formulación de las salchichas. Por otro lado, la dependencia de la velocidad de los ultrasonidos con la temperatura permitió la estimación de la composición, y especialmente del contenido en grasa (% var 96.1), a través de la medida de la velocidad de los ultrasonidos en las salchichas a 2 y 25 ºC y empleando un modelo semi-empírico. Estos resultados revelan la posibilidad de utilizar los ultrasonidos como

herramienta no destructiva para distinguir el tipo de grasa contenida en productos cárnicos crudo-curados formulados mediante mezclas de carne magra y grasa, así como determinar el contenido y estado de la grasa, lo que contribuiría a una mejor clasificación de estos productos.

Tras haber observado que los ultrasonidos resultan ser una técnica adecuada para caracterizar de manera no destructiva la grasa de cerdo Ibérico y los productos cárnicos crudo-curados formulados (sistemas modelo de composición homogénea) elaborados a partir de ésta, en el siguiente trabajo se estudió la aplicación de la técnica en jamón Ibérico curado, donde existe una mayor heterogeneidad composicional. Así pues, en el cuarto estudio, se evaluó la posibilidad de la aplicación de los ultrasonidos no solo para caracterizar la composición y textura del jamón Ibérico, sino también para caracterizar los posibles cambios que puede sufrir el mismo como consecuencia de la influencia que tienen los procesos de conservación y almacenamiento. En este sentido, se determinó la viabilidad del uso de ultrasonidos para estimar el efecto que tanto el tratamiento por altas presiones, como el almacenamiento a bajas temperaturas, puede ejercer sobre las propiedades texturales del jamón Ibérico. A tal fin, se llevaron a cabo medidas de la velocidad de los ultrasonidos en dos diferentes lotes de jamón Ibérico loncheado, apilado y envasado al vacío. En el primero, se estudió el efecto de las altas presiones (600 MPa/6 min) y las medidas de ultrasonidos se realizaron considerando un rango de temperaturas de 2 a 25 ºC (2, 6, 10, 15, 20 y 25 ºC) sobre muestras tratadas y no tratadas, de dos zonas diferentes del jamón (punta y babilla). En el segundo, se estudió el efecto del almacenamiento en refrigeración (6 ºC) y las medidas de ultrasonidos se realizaron únicamente a esta temperatura al inicio y al final del periodo de almacenamiento (120 días). En ambos estudios, las medidas ultrasónicas se realizaron sobre la superficie del paquete y se complementaron con análisis de textura instrumental y composición química. Los resultados mostraron que el contenido graso del jamón Ibérico influyó de manera significativa ($p<0.05$) en la dureza de las muestras ($R>0.80$). Por otra parte, se observó que existía una relación lineal ($p<0.05$) entre la temperatura y la velocidad de los ultrasonidos ($R>0.96$), disminuyendo ésta con el aumento de la temperatura, siendo aquellas zonas cuyo contenido graso era más elevado, en las que mayor caída de la

velocidad se encontró (-2.5 m/s $ºC^{-1}$ para muestras con % grasa >36.34 %). Este hecho se relacionó con la disminución del ratio grasa sólida/líquida en las muestras, aumentando el contenido de grasa líquida, disminuyendo la dureza y por tanto la velocidad. Así, se encontró una relación lineal significativa ($p<0.05$) entre la dureza de las muestras y la velocidad de propagación los ultrasonidos ($R>0.84$), observándose que a mayor dureza de las muestras, mayor velocidad de propagación de los ultrasonidos. Por otro lado, el tratamiento por altas presiones implicó cambios estructurales en el jamón, en concreto, se produjo tras el tratamiento un incremento de la dureza del 18 % para las muestras de punta (% grasa >36.34 %) y un 12 % para las de babilla (% grasa <21.07 %), diferencia que puede estar relacionada con el mayor contenido de grasa en las muestras de punta. Estos cambios en la dureza dieron lugar a un incremento en la velocidad de los ultrasonidos, observándose un aumentó de 13 y 9 m/s en muestras de punta y babilla, respectivamente. De esta forma, los ultrasonidos han permitido caracterizar de manera no destructiva los cambios texturales que tuvieron lugar después del tratamiento por altas presiones en jamón Ibérico loncheado y envasado al vacío, así como discriminar entre paquetes de dos zonas diferentes del jamón. Por último, el almacenamiento en refrigeración provocó un aumento significativo ($p<0.05$) en la dureza de las muestras (promedio de 1.10 N) y en la velocidad de los ultrasonidos (promedio de 70 m/s). Este aumento se relacionó con la cristalización de la grasa y por lo tanto, con el aumento del ratio grasa sólida/líquida. Los resultados muestran que las medidas no destructivas de ultrasonidos permitieron evaluar los cambios texturales ocurridos por el almacenamiento a bajas temperaturas del jamón Ibérico, loncheado y envasado al vacío. Por lo tanto, los ultrasonidos resultan ser una herramienta útil para evaluar y controlar de manera no destructiva, el contenido de grasa y la textura del jamón Ibérico envasado al vacío, así como los cambios ocurridos después de su tratamiento por altas presiones o durante su almacenamiento a temperaturas de refrigeración.

Se ha demostrado que las técnicas de ultrasonidos por contacto directo realizadas en este trabajo contribuyen a la evaluación no destructiva de productos cárnicos crudo-curados envasados al vacío. Sin embargo, para la realización de las medidas es necesario el uso de un material de acople sobre el film plástico del

paquete, lo que lleva aparejado la necesidad de su colocación y posterior retirada. En este sentido, el uso de técnicas ultrasónicas sin contacto permitiría un ahorro de tiempo y de recursos en la realización de medidas acústicas en este tipo de productos cárnicos. Por otro lado, en este trabajo, las medidas de ultrasonidos se han llevado a cabo con un transductor de 1 MHz con el objeto de medir propiedades macroscópicas, como la composición media o la textura, de un área de medida relativamente amplia. El uso de frecuencias mayores abriría las puertas a una mayor focalización de las medidas, lo que permitiría analizar aspectos como la estructura de los tejidos cárnicos. En este contexto, se realizó un quinto trabajo con el objetivo de implementar otro tipo de técnicas acústicas sin contacto y evaluar su aplicación para la caracterización de productos cárnicos crudo-curados. Por un lado, se realizaron medidas de la velocidad de los ultrasonidos sin contacto y por contacto en paquetes de jamón loncheado y envasado al vacío con diferentes espesores. Mediante las medidas sin contacto fue posible realizar de forma simultánea la medida de la velocidad y del espesor de la muestra sin necesidad de un calibre. En referencia a los resultados obtenidos a partir de las medidas sin contacto, la velocidad media en los paquetes fue de 1846 m/s, mientras que para las medidas por contacto fue de 1842 m/s. Las desviaciones entre las medidas fueron en gran medida relacionadas con la heterogeneidad de la estructura y composición de la muestra. No obstante, se encontró una adecuada correlación ($p<0.05$) entre las dos técnicas, por lo que tanto el espesor de los paquetes como la velocidad de los ultrasonidos pueden ser estimados satisfactoriamente con medidas sin contacto. Así pues, se demostró la viabilidad del uso de los ultrasonidos sin contacto para la caracterización del jamón curado, haciendo las medidas más sencillas y rápidas. Por otro lado, se llevaron a cabo medidas de microscopía de barrido acústico en muestras de jamón curado (1 mm de espesor) y en chorizo (5 mm de espesor) a 6 ºC. Se obtuvieron imágenes que mostraron que existen diferencias en la intensidad de la reflexión de la señal de los ultrasonidos sobre la muestra, encontrándose dos regiones de la imagen diferentes. La región con mayor intensidad de reflexión de la señal correspondió al tejido magro, mientras que una menor intensidad fue obtenida para el tejido graso. Las medidas presentaron una resolución espacial sobre la superficie de la muestra (ejes x e y) de 1 mm y de 300 μm sobre la profundidad de la misma (eje z). Estas medidas presentan la ventaja de poder

hacer un análisis de la estructura de muestras opacas a la luz, como es el caso de los productos cárnicos, pero que pueden ser atravesados para su análisis por ondas ultrasónicas. Así pues, la microscopía acústica permitió la caracterización de los distintos tejidos existentes en los productos cárnicos curados, lo que resulta útil para predecir su contenido en grasa y su distribución a nivel microscópico, favoreciendo la clasificación de los productos. La implementación de estas dos técnicas acústicas permitiría la caracterización no destructiva de productos cárnicos curados, mejorando y optimizando los procesos de producción y el control de calidad de los mismos.

Finalmente, se puede concluir que las medidas ultrasónicas han permitido caracterizar y determinar de manera no destructiva la composición y textura de productos cárnicos crudo-curados, así como discriminar entre diferentes lotes en función del tipo y el contenido de grasa. Además, resultaron ser una herramienta fiable, rápida y económica para monitorizar los cambios texturales ocurridos tras la aplicación de tratamientos de conservación por altas presiones y durante procesos de almacenamiento refrigerado, lo que puede permitir la mejora y optimización de los procesos de elaboración y distribución de los productos cárnicos crudo-curados. El uso de técnicas sin contacto permitiría reducir los tiempos de medida, al eliminar la necesidad del medio de acople y no ser necesaria una medida independiente del espesor de la muestra.

RESUM

Els productes carnis curats provinents del porc Ibèric són molt apreciats pel consumidor com a conseqüència de la seua elevada qualitat tant organolèptica com a nutricional. L'augment de la demanda d'aquests productes en el mercat espenta a les indústries productores a una millora contínua, cercant noves formulacions i presentacions, així com, optimitzant els processos d'elaboració i implementant noves tècniques de conservació i distribució. La qualitat final dels productes carnis curats pot variar tant per la matèria primera utilitzada, com pels diferents processos als quals són sotmesos. En aquest sentit, el **greix** és un dels principals components que influeix en la qualitat i que al seu torn, depèn de **la raça i l'alimentació** de l'animal. Així doncs, el contingut de greix, així com l'estat físic (líquid i/o sòlid) en el qual es trobe, afectaran a les propietats sensorials i en particular a la textura dels productes carnis curats. Per tant, resulta d'interès avaluar el **contingut, estat** i **tipologia del greix** per a estimar la qualitat dels productes carnis cru-curats.

En el moment actual, el consum de pernil ibèric està desplaçant-se de la compra de peces senceres per al seu tallat manual, a productes tallats mecànicament i envasats al buit. Aquest format de producte fa necessari l'ús addicional de tecnologies convencionals de conservació, com **l'emmagatzematge refrigerat**, o de noves tecnologies com les **altes pressions hidrostàtiques** (APH). Aquestes tècniques de conservació permeten perllongar la vida útil del producte però poden afectar a la qualitat del mateix, provocant canvis en propietats tan importants com la seua textura. En aquest sentit, també és de gran interès la caracterització dels canvis patits pels productes carnis curats sotmesos a aquests mètodes de conservació (fred i APH).

Fins al moment, s'han utilitzat diverses tècniques instrumentals i sensorials per a l'avaluació de diferents paràmetres de qualitat del greix dels productes carnis cru-curats, tals com la seua textura, el tipus de greix, la quantitat de la mateixa i l'estat en el qual es troba a diferents temperatures. No obstant açò, la integritat de les mostres sotmeses a aquestes tècniques convencionals es veu afectada de forma considerable, a més de que la seua implementació en les línies

de producció és complexa i costosa. D'aquesta manera, la cerca de tècniques **no destructives**, fàcils d'implementar i de baix cost, que permeten la caracterització en línia dels productes carnis curats resulta interessant des del punt de vista tant científic com a tecnològic.

Els **ultrasons** de baixa intensitat o senyal són una tècnica d'avaluació no destructiva, ràpida, senzilla i econòmica, per la qual cosa poden ser utilitzats com una eina fiable per a la caracterització de diversos aliments i ser integrats en els sistemes de producció a fi de la seua optimització. La mesura de la velocitat dels ultrasons ha permès estimar de manera no destructiva la composició i els canvis patits durant el procés d'elaboració d'un ampli nombre d'aliments, així com de les matèries primeres empleades.

Sobre la base de l'anteriorment exposat, l'objectiu general d'aquest treball va ser avaluar la utilitat dels ultrasons de senyal per a caracteritzar de manera no destructiva els productes carnis cru-curats provinents del porc Ibèric en funció de la tipologia del greix, el seu contingut gras i les seues propietats texturals. Així mateix es pretén avaluar l'ús d'aquesta tècnica per a la caracterització no destructiva dels canvis patits durant l'emmagatzematge refrigerat de productes carnis cru-curats i després del seu tractament per altes pressions hidrostàtiques.

Per a la consecució d'aquest objectiu general, s'han dut a terme cinc estudis. En el primer, l'objectiu va ser avaluar la viabilitat de l'ús dels ultrasons per a caracteritzar el procés de cristal·lització de llard de porc Ibèric durant el seu emmagatzematge a baixes temperatures, així com, desenvolupar un model matemàtic que permetera estimar el contingut de greix sòlid durant la seua cristal·lització. Per a aconseguir aquests objectius, es van realitzar mesures de la velocitat dels ultrasons en mostres de llard Ibèric durant el seu emmagatzematge (11 dies) a diferents temperatures (0, 3, 5, 7, 10 i 20 ºC). Així mateix, es va determinar el comportament tèrmic de les mostres mitjançant Calorimetria Diferencial de Barrido (DSC) i es van realitzar assajos de punció per a determinar les característiques texturals de la mostra, principalment la seua duresa. Els resultats experimentals van mostrar dos increments pronunciats en la velocitat dels ultrasons durant l'emmagatzematge isoterm. Aquest comportament es va relacionar amb la cristal·lització dels triglicèrids en funció del seu grau de

saturació, que va ser observada en els resultats obtinguts mitjançant DSC. En les mesures texturals dutes a terme durant l'emmagatzematge isoterm es va observar el mateix comportament, trobant-se dos increments pronunciats de la duresa de les mostres. D'altra banda, el procés de cristal·lització del greix en dos etapes va ser descrit adequadament mitjançant un model matemàtic basat en l'equació de Avrami, establint-se una relació entre la velocitat dels ultrasons i el temps d'emmagatzematge isoterm (% var >99.9). A més, es va desenvolupar un model matemàtic per a estimar l'evolució del percentatge de greix sòlid durant l'emmagatzematge isoterm, considerant un procés de cristal·lització en dues etapes. Per tant, les mesures de velocitat dels ultrasons van permetre caracteritzar el patró de cristal·lització del llard de porc Ibèric sotmès a un procés d'emmagatzematge isoterm a baixes temperatures, la qual cosa va donar pas al següent estudi.

El segon estudi es va dur a terme en mostres de greix subcutani (teixit adipós) de porcs Ibèrics alimentats en dos diferents sistemes (montanera i cebo). L'objectiu d'aquest apartat va ser avaluar l'ús dels ultrasons per a caracteritzar els canvis texturals del teixit adipós que tenen lloc durant l'emmagatzematge a baixes temperatures, així com discriminar entre els dos tipus de greix. Per a açò, es van realitzar mesures de la velocitat dels ultrasons en els greixos durant el seu emmagatzematge isoterm durant 20 dies, assajant-se diferents temperatures (0, 2, 5, 7 i 10 ºC). Els canvis en la duresa dels greixos durant l'emmagatzematge es van determinar mitjançant assajos de punció. La composició del perfil d'àcids grassos i el comportament tèrmic dels dos tipus de greix van ser obtinguts mitjançant anàlisi per cromatografia de gasos i mesures de DSC, respectivament. Així, en ambdues grasses, es van observar dos increments pronunciats de la seua duresa durant l'emmagatzematge, igual que en l'estudi previ amb el llard de porc. Aquests canvis en la textura van ser atribuïts a la cristal·lització de triglicèrids amb diferent grau d'insaturació, fet que es va correspondre amb els dos pics de cristal·lització de greix trobats en les anàlisis de DSC. D'altra banda, el greix de cebo va presentar valors mitjans de la duresa superiors ($p<0.05$) als del greix de montanera durant tot el període d'emmagatzematge, la qual cosa va permetre discriminar entre els dos tipus de greix. Les diferències en la duresa es van relacionar amb el major contingut ($p<0.05$) d'àcids grassos saturats de les mostres

de cebo comparat amb les de montanera. De manera similar a la duresa, les mesures ultrasòniques van permetre observar dos augments pronunciats en la velocitat durant el temps d'emmagatzematge, així com discriminar entre els dos tipus de greix. En aquest sentit, la velocitat dels ultrasons després de la cristal·lització dels triglicèrids més saturats va ser 2.8 % superior per a cebo que per a montanera, sent aquest percentatge del 5.2 % després de la cristal·lització de la totalitat de triglicèrids. Finalment, tant els canvis texturals, com els de la velocitat dels ultrasons observats durant el procés de cristal·lització dels dos greixos, van ser descrits adequadament mitjançant un model matemàtic basat en l'equació de Avrami. Així doncs, els resultats obtinguts van posar de manifest que les mesures d'ultrasons poden ser utilitzades per a caracteritzar de manera no destructiva la cristal·lització del greix subcutani de porc Ibèric i els canvis en les seues propietats texturals ocorreguts durant el seu emmagatzematge a baixes temperatures, així com per a discriminar entre els dos tipus de greix.

Dels resultats obtinguts en els dos primers treballs, es va confirmar la viabilitat de l'ús dels ultrasons per al monitoratge dels processos de cristal·lització de greix de porc ibèric, que donen lloc a canvis texturals en la mateixa, durant l'emmagatzematge refrigerat, així com per a discriminar entre greixos de diferent origen (montanera i cebo). Un altre dels objectius de la Tesi era estudiar l'ús dels ultrasons per a estimar el contingut gras i l'estat del greix en productes carnis curats, on el greix és únicament un component del sistema, així com per a discriminar aquests productes en funció del tipus de greix amb el qual han sigut elaborats. A diferència de la matèria primera assajada en els dos primers treballs, aquests productes estan formats per teixit magre i grassa i han sigut sotmesos a un procés de curat. En un primer cas, es va treballar amb sistemes model composats de carn magra i grassa (picades i embotides), la qual cosa permet disposar d'una major homogeneïtat composicional en les mostres que la trobada en productes com el pernil curat. D'aquesta manera, el tercer estudi es va realitzar en salsitxes curades elaborades amb diferent contingut i tipus de greix. L'objectiu de l'estudi va ser el d'avaluar la viabilitat de l'ús dels ultrasons per a determinar el contingut de greix i el seu estat, així com per a discriminar entre els diferents tipus de greix utilitzats en la seua formulació. Es van elaborar diferents lots de salsitxes cru-curades preparades amb diferents tipus

de greix (montanera, cebo, llard i oli de gira-sol) i nivells de contingut gras (3-17 %). Es van realitzar mesures de la velocitat dels ultrasons a diferents temperatures (2, 6, 10, 15, 20,25 i 30 ºC), també es va determinar el perfil d'àcids grassos per cromatografia de gasos i el comportament fundent de les mostres mitjançant mesures de DSC i finalment, es va determinar la composició química de les salsitxes. Els resultats van mostrar que la composició del perfil d'àcids grassos (grau de saturació) va afectar al comportament fundent de les mostres (temperatures i entalpies de fusió), la qual cosa va permetre distingir entre els diferents lots de salsitxes. D'altra banda, per a tots els lots de salsitxes amb alt contingut de greix, es va observar un descens en la velocitat mitjana dels ultrasons amb l'augment de la temperatura en un rang de 2 a 25 ºC. El descens de la velocitat va ser relacionada amb els resultats obtinguts en les anàlisis de DSC que van mostrar que per a aqueix rang de temperatures es va presentar una fusió pronunciada dels greixos. Per tant a major temperatura, major percentatge de greix fos i menor velocitat dels ultrasons. Així, es van trobar relacions lineals significatives ($p<0.05$) entre el percentatge de greix fos en les mostres i la velocitat dels ultrasons, que van ser diferents per a les salsitxes elaborades amb diferents tipus de greix ($R>0.97$). Per tant, els ultrasons foren capaços no solament d'estimar el percentatge de greix fos, sinó també d'identificar els diferents tipus de greix utilitzats en la formulació de les salsitxes. D'altra banda, la dependència de la velocitat dels ultrasons amb la temperatura va permetre l'estimació de la composició, i especialment del contingut en greix (% var 96.1), a través de la mesura de la velocitat dels ultrasons en les salsitxes a 2 ºC i 25 ºC i emprant un model semi-empíric. Aquests resultats revelen la possibilitat d'utilitzar els ultrasons com a eina no destructiva per a distingir el tipus de greix contingut en productes carnis cru-curats formulats mitjançant mescles de carn magra i grassa, així com determinar el contingut i estat del greix, la qual cosa contribuiria a una millor classificació d'aquests productes.

Després d'haver observat que els ultrasons resulten ser una tècnica adequada per a caracteritzar de manera no destructiva el greix de porc Ibèric i els productes carnis cru-curats formulats (sistemes model de composició homogènia) elaborats a partir d'aquesta, en el següent treball se estudià l'aplicació de la tècnica en pernil Ibèric curat, on existeix una major heterogeneïtat

composicional. Així doncs, en el quart estudi, es va avaluar la possibilitat de l'aplicació dels ultrasons no solament per a caracteritzar la composició i textura del pernil Ibèric, sinó també per a caracteritzar els possibles canvis que pot patir el mateix com a conseqüència de la influència que tenen els processos de conservació i emmagatzematge. En aquest sentit, es va determinar la viabilitat de l'ús d'ultrasons per a estimar l'efecte que tant el tractament per altes pressions, com l'emmagatzematge a baixes temperatures, pot exercir sobre les propietats texturals del pernil Ibèric. A tal fi, es van dur a terme mesures de la velocitat dels ultrasons en dos diferents lots de pernil Ibèric tallat, apilat i envasat a buit. En el primer, es va estudiar l'efecte de les altes pressions (600 MPa/6 min) i les mesures d'ultrasons es van realitzar considerant un rang de temperatures de 2 a 25 ºC (2, 6, 10, 15, 20 i 25 ºC) sobre mostres tractades i no tractades, de dues zones diferents del pernil (punta i babilla). En el segon, es va estudiar l'efecte de l'emmagatzematge refrigerat (6ºC) i les mesures d'ultrasons es van realitzar únicament a aquesta temperatura a l'inici i al final del període d'emmagatzematge (120 dies). En tots dos estudis, les mesures ultrasòniques es van realitzar sobre la superfície del paquet i es van complementar amb anàlisi de textura instrumental i composició química. Els resultats van mostrar que el contingut gras del pernil Ibèric va influir de manera significativa ($p<0.05$) en la duresa de les mostres ($R>0.80$). D'altra banda, es va observar que existia una relació lineal ($p<0.05$) entre la temperatura i la velocitat dels ultrasons ($R>0.96$), disminuint aquesta amb l'augment de la temperatura, sent aquelles zones el contingut gras de les quals era més elevat en les quals major caiguda de la velocitat es va trobar (-2.5 m/s $ºC^{-1}$ per a mostres amb % greix >36.34 %). Aquest fet es va relacionar amb la disminució del ràtio grassa sòlida/líquida en les mostres, augmentant el contingut de greix líquid, disminuint la duresa i per tant la velocitat. Així, es va trobar una relació lineal significativa ($p<0.05$) entre la duresa de les mostres i la velocitat de propagació dels ultrasons ($R>0.84$), observant-se que a major duresa de les mostres, major velocitat de propagació dels ultrasons. D'altra banda, el tractament per altes pressions va implicar canvis estructurals en el pernil, en concret, es va produir tras el tractament un increment de la duresa del 18 % per a les mostres de punta (% greix >36.34 %) i un 12 % per a les de babilla (% greix <21.07 %), diferencia que pot estar relacionada amb el major contingut de greix en les mostres de punta. Aquests canvis en la duresa van donar lloc a un

increment en la velocitat dels ultrasons, observant-se un augment de 13 m/s i 9 m/s en mostres de punta i babilla, respectivament. D'aquesta forma, els ultrasons han permès caracteritzar de manera no destructiva els canvis texturals que van tenir lloc després del tractament per altes pressions en pernil Ibèric tallat i envasat a vuit, així com discriminar entre paquets de dues zones diferents del pernil. Finalment, l'emmagatzematge en refrigeració va provocar un augment significatiu ($p<0.05$) en la duresa de les mostres (mitjana de 1.10 N) i en la velocitat dels ultrasons (mitjana de 70 m/s). Aquest augment es va relacionar amb la cristal·lització del greix i per tant amb l'augment del ràtio grassa sòlida/líquida. Els resultats mostren que les mesures no destructives d'ultrasons van permetre avaluar els canvis texturals ocorreguts per l'emmagatzematge a baixes temperatures del pernil Ibèric, tallat i envasat a buit. Per tant, els ultrasons resulten ser una eina útil per a avaluar i controlar de manera no destructiva, el contingut de greix i la textura del pernil Ibèric envasament a buit així com els canvis ocorreguts després del seu tractament per altes pressions o durant el seu emmagatzematge a temperatures de refrigeració.

S'ha demostrat que les tècniques d'ultrasons per contacte directe realitzades en aquest treball permeten l'avaluació no destructiva de productes carnis cru-curats envasats a buit. No obstant açò, per a la realització de les mesures és necessari l'ús d'un material d'acoble sobre el film plàstic del paquet, la qual cosa porta aparellat la necessitat de la seua col·locació i posterior retirada. En aquest sentit, l'ús de tècniques ultrasòniques sense contacte permetria un estalvi de temps i de recursos en la realització de mesures acústiques en aquest tipus de productes carnis. D'altra banda, en aquest treball, les mesures d'ultrasons s'han dut a terme amb un transductor d'1 MHz amb l'objecte de mesurar propietats macroscòpiques, com la composició mitjana o la textura, d'un àrea de mesura relativament àmplia. L'ús de majors freqüències, obriria les portes a una major focalització de les mesures, la qual cosa permetria analitzar aspectes com l'estructura dels teixits carnis. En aquest context, es va realitzar un cinquè treball amb l'objectiu d'implementar un altre tipus de tècniques acústiques sense contacte i avaluar la seua aplicació per a la caracterització de productes carnis cru-curats. D'una banda, es van realitzar mesures de la velocitat dels ultrasons sense contacte i per contacte en paquets de pernil tallat i envasat a buit amb

diferents espessors. Mitjançant les mesures sense contacte va ser possible realitzar de forma simultània la mesura de la velocitat i de l'espessor de la mostra sense necessitat d'un calibre. En referència als resultats obtinguts a partir de les mesures sense contacte, la velocitat mitjana en els paquets va ser de 1846 m/s, mentre que per a les mesures per contacte va ser de 1842 m/s. Les desviacions entre les mesures van ser relacionades en gran mesura amb l'heterogeneïtat de l'estructura i composició de la mostra. No obstant açò, es va trobar una adequada correlació ($p<0.05$) entre les dues tècniques, per la qual cosa tant l'espessor dels paquets com la velocitat dels ultrasons poden ser estimats satisfactòriament amb mesures sense contacte. Així doncs, es va demostrar la viabilitat de l'ús dels ultrasons sense contacte per a la caracterització del pernil curat, fent les mesures més senzilles i ràpides. D'altra banda, es van dur a terme mesures de microscòpia per barrido acústic en mostres de pernil curat (1 mm d'espessor) i en xoriço (5 mm d'espessor) a 6 ºC. Es van obtenir imatges que van mostrar que existeixen diferències en la intensitat de la reflexió del senyal dels ultrasons sobre la mostra, trobant-se dues regions de la imatge diferents. La regió amb major intensitat de reflexió del senyal va correspondre al teixit magre, mentre que una menor intensitat va ser obtinguda per al teixit gras. Les mesures van presentar una resolució espacial sobre la superfície de la mostra (eixos x i y) de 1 mm i de 300 μm sobre la profunditat de la mateixa (eix z). Aquestes mesures presenten l'avantatge de poder fer una anàlisi de l'estructura de mostres opaques a la llum, com és el cas dels productes carnis, però que poden ser travessats per a la seua anàlisi per ones ultrasòniques. Així doncs, la microscòpia acústica va permetre la caracterització dels diferents teixits existents en els productes carnis curats, la qual cosa resulta útil per a predir el seu contingut en greix i la seua distribució a nivell microscòpic, afavorint la classificació dels productes. La implementació d'aquestes dues tècniques acústiques permetria la caracterització no destructiva de productes carnis curats, millorant i optimitzant els processos de producció i el control de qualitat dels mateixos.

Finalment, es pot concloure que les mesures dels ultrasons han permés caracteritzar i determinar de manera no destructiva la composició i textura de productes carnis cru-curats, així com discriminar entre diferents lots en funció del tipus i el contingut de greix. A més, van resultar ser una ferramenta fiable,

ràpida i econòmica per a monitoritzar els canvis texturals ocorreguts després de l'aplicació de tractaments de conservació per altes pressions i durant processos d'emmagatzemament refrigerat, la qual cosa pot permetre la millora i optimització dels processos d'elaboració i distribució dels productes carnis crucurats. L'ús de tècniques sense contacte permetria reduir els temps de mesura, a l'eliminar la necessitat del mig d'acoble i no ser necessària una mesura independent de la grossària de la mostra.

1. INTRODUCCIÓN

1.1. Justificación e interés del trabajo

Los productos crudo-curados elaborados a base de carne de cerdo Ibérico tienen una gran tradición dentro del territorio Español, siendo muy apreciados por los consumidores como consecuencia de su elevada calidad (Petrón et al., 2004; Ventanas et al., 2007b). La creciente demanda de este tipo de productos ha llevado a esta industria a ser uno de los segmentos del sector cárnico español más dinámico, tratando de buscar nuevas formulaciones, estrategias de producción, formatos y presentaciones, así como mejoras en los canales de distribución y en los métodos de conservación. Con estas medidas se pretende en todo momento obtener productos con características organolépticas muy específicas que satisfagan el gusto del consumidor, además de ampliar nuevos mercados, introduciendo estos productos en otros países.

Los productos Ibéricos crudo-curados, son alimentos elaborados a partir de músculos enteros (como el jamón Ibérico) (Toldrá y Aristoy, 2010), o mezclas de tejido magro y grasa (embutidos como salchichas, el fuet, el salchichón o el chorizo, entre otros) (Toldrá, 2002) provenientes de cerdos Ibéricos. Las características finales de los productos Ibéricos crudo-curados varían enormemente dependiendo no solo de la materia prima utilizada, sino también de los diferentes procesos a los que son sometidos. En este sentido, los embutidos Ibéricos, son elaborados a partir de carne y grasa triturada, mezclada con sal, otras especias y aditivos, posteriormente embutidos en tripa natural o artificial y sometidos a un proceso de fermentación, secado y maduración (Arnau et al., 2007; Fernández-López et al., 2008; González-Fernández et al., 2006; Mendoza et al., 2001). Ambos tipos de productos (embutidos crudo-curados y el jamón Ibérico) cuentan con un alto contenido en grasa, la cual influye enormemente en las características sensoriales y texturales y por lo tanto, en su calidad final. Entre otros factores, la calidad de la **grasa** depende del tipo de **raza y alimentación del animal**, que afectan a los productos denominados y clasificados como "de Ibérico" (BOE 2007). Así, existe un elevado interés en la búsqueda de tecnologías que permitan clasificar los productos cárnicos crudo-curados en función de su **tipología y contenido de grasa**.

Las necesidades de almacenamiento, distribución y venta hacen que se apliquen técnicas de conservación para aumentar la vida útil de los productos cárnicos crudo-curados, esto es especialmente interesante en aquellos formatos loncheados. Además de técnicas convencionales de conservación, como la refrigeración, se está buscando la aplicación de nuevas tecnologías, como las altas presiones hidrostáticas. Estas técnicas de conservación pueden influir en el **estado físico** (sólido/líquido) de la grasa y por tanto, en propiedades de calidad como la textura y la liberación de aromas. Además, las nuevas tendencias de los mercados y las preferencias de los consumidores por adquirir productos más saludables y bajos en grasa, hacen que se tienda a la reducción del contenido graso, viéndose modificadas algunas de las propiedades sensoriales, entre las que destaca la textura (Fernández-Ginés et al., 2005; Jiménez-Colmenero et al., 2001; Muguerza et al., 2003; Ortiz-Somovilla et al., 2005).

Actualmente, el control de la calidad de estos productos se lleva a cabo solo en algunas etapas del proceso, mediante evaluaciones sensoriales llevadas a término por expertos y que en algunos casos pueden resultar imprecisas y subjetivas. Así, la industria cárnica busca implementar nuevas tecnologías innovadoras que sean capaces de evaluar los productos cárnicos crudo-curados con diferentes tipos de grasa en función de la raza y alimentación del animal, así como estimar el **contenido de grasa**, su **estado físico** y sus **propiedades texturales**, además de monitorizar los cambios sufridos durante y después de la aplicación de los métodos de conservación. Para este fin, es necesaria la obtención de información objetiva y la cuantificación precisa de parámetros que permitan optimizar y estandarizar los procesos. En este sentido, se dispone de diferentes técnicas que han sido aplicadas al estudio de la caracterización de los productos cárnicos crudo-curados Ibéricos. Ejemplos de éstas técnicas son los análisis sensoriales (Ruiz et al., 1998; Ruiz et al., 2002; Ventanas et al., 2007a; Ventanas et al., 2007b) los fisicoquímicos (García-Esteban et al., 2004; Garcia-Rey et al., 2005; Gimeno et al., 2000; González-Fernández et al., 2006; Guerrero et al., 1999), la Calorimetría Diferencial de Barrido (Benedito et al., 2001) y la cromatografía de gases (Niñoles et al., 2007; Ventanas et al., 2007a). No obstante, las citadas técnicas suponen, en mayor o menor grado, la destrucción o alteración de la muestra sometida a ensayo. Como alternativa, se han

desarrollado otras técnicas que permiten realizar la caracterización y monitorización de los cambios que tienen lugar en el proceso de elaboración de los productos cárnicos de manera no destructiva, como la Resonancia Magnética Nuclear (RMN) (Straadt et al., 2012), la impedancia bioeléctrica (Daza et al., 2006), la difracción por Rayos-X o la tomografía axial computarizada (Santos-Garcés et al., 2010; Santos-Garcés et al., 2012). Sin embargo, su elevado coste, complejidad y dificultad de implementación en las líneas de producción limitan su aplicación industrial, por lo que el interés por la búsqueda de técnicas no destructivas, rápidas y fáciles de incorporar a las líneas de elaboración se mantiene en el sector cárnico.

1.2. El cerdo Ibérico y los productos cárnicos crudo-curados

1.2.1 El Cerdo Ibérico

Existe una gran diversidad de razas ganaderas autóctonas en España, siendo el cerdo Ibérico el que más desarrollo y adaptación presenta en la dehesa. Esta raza está principalmente ubicada en el sudoeste de la Península Ibérica, abarcando las Comunidades Autónomas de Andalucía, Castilla la Mancha, Castilla y León y Extremadura (Figura 1).

Figura 1. Principales comunidades autónomas productoras de cerdo Ibérico en España.

Actualmente existe una norma de calidad para la protección de los animales y productos crudo-curados provenientes de raza Ibérica publicada en el 2001 (B O E, 2001), siendo revisada y adaptada a las nuevas condiciones y necesidades de mercado en el 2007 (B O E, 2007) como consecuencia de la evolución de la oferta y la demanda. Por lo tanto, mediante el Real Decreto 1469/2007 (B O E, 2007) se fija la vigente *Norma de calidad para la carne, el jamón, la paleta y la caña de lomo Ibéricos*, por la cual se definen y establecen las características de calidad que deben cumplir los productos etiquetados como

Ibéricos, y de este modo evitar fraudes. Dicha norma también pretende contribuir a evitar la pérdida de la raza Ibérica estableciendo los requisitos necesarios para su regulación y mantenimiento. Asimismo, pretende preservar los recursos del ecosistema natural denominado *dehesa arbolada* regulando su aprovechamiento, para poder llevar a cabo un adecuado régimen de alimentación de los animales basado en el aprovechamiento de los recursos de bellota y pastizal propios de la dehesa. Por otra parte, quedan estandarizadas las condiciones de alimentación de los animales, que se clasifican en cuatro tipos en función de su alimentación (de bellota o montanera, de recebo, de cebo de campo y de cebo). Finalmente, la norma busca estandarizar los productos crudo-curados, estableciendo el proceso de elaboración, así como regulando el etiquetado.

Por otra parte, las categorías definidas en la norma en función de la raza son: "Ibérico puro" o "Ibérico". Se denomina "Ibérico puro" cuando los progenitores del cerdo, madre y el padre, son reproductores Ibéricos puros, mientras que el producto "Ibérico" se obtiene a partir de cerdos procedentes del cruce de madre "Ibérica" y macho "Duroc puro" o "Duroc". Para considerar un animal como "Ibérico" debe presentar un mínimo de 50 % de sangre Ibérica, por lo que se exige que sea la madre la que aporte la raza Ibérica pura. La importancia de la raza Ibérica radica en las características que aporta a la carne, entre las que destaca la facilidad de acumular grasa, lo que se traduce en una mayor cantidad de grasa infiltrada en los músculos. Esta grasa infiltrada influye notablemente en las características finales de los productos crudo-curados Ibéricos.

Como ya se ha indicado, con respecto a las categorías de clasificación en función de la alimentación, las establecidas por la norma son: de *Bellota o terminado en montanera*, de *Recebo o terminado en Recebo*, de *Cebo de campo* y de *Cebo*. A continuación se detallan las características más importantes de cada una de estas categorías.

- ✓ Bellota o terminado en montanera

Este sistema de alimentación consiste en el aprovechamiento exclusivo de bellotas, hierba y demás recursos naturales de la dehesa. La bellota

principalmente aporta a la dieta del animal un 60 % de ácido oleico (C18:1 n9) del total de ácidos grasos, mientras que la hierba proporciona proteína y ácido linolénico (C18:3 n3). El rápido engorde del animal durante la montanera es favorecido por el elevado aporte energético de la bellota, proporcionando mayor cantidad de grasa y una mejor distribución (Ventanas, 2006). La norma indica que el peso medio de entrada en montanera estará comprendido entre 92 y 115 kg, siendo la reposición de montanera como mínimo de 46 kg durante una estancia mínima de 60 días. La edad y peso mínimos al sacrificio serán de 14 meses y 117 kg, respectivamente.

- ✓ Recebo o terminado en Recebo

Se obtiene a partir de animales que después de reponer un mínimo de peso en montanera su cebo es completado mediante el uso de piensos constituidos por cereales y leguminosas. El peso medio de entrada de los animales debe de ser entre 92 y 115 kg reponiendo en montanera como mínimo 29 kg durante una estancia de 60 días y siendo sacrificados con un mínimo de 14 meses de edad y un peso de 117 kg.

- ✓ Cebo de Campo

Esta clasificación se consigue a partir de animales alimentados por piensos constituidos por cereales y leguminosas, complementando su alimentación mediante una estancia de 60 días en campo, recibiendo también una dieta a base de pienso. El peso de entrada en la fase de cebo de campo será de aproximadamente entre 92 y 115 kg, y siendo sacrificados con un mínimo de 12 meses y peso de 117 kg.

- ✓ Cebo

Esta alimentación se basa durante toda la vida del animal en piensos constituidos a partir de cereales y leguminosas, siendo la edad mínima al sacrificio de 10 meses y de 117 kg de peso medio.

En general, los piensos compuestos utilizados durante el periodo de Cebo de campo y de Cebo, son enriquecidos con subproductos y semillas con un

elevado contenido de ácido oleico, con el fin de favorecer la acumulación de grasa y aproximarse a las características proporcionadas por un sistema de engorde de Montanera o Recebo. Sin embargo, los productos obtenidos a partir de animales alimentados mediante los últimos sistemas mencionados, generan alimentos crudo-curados de mayor calidad comparados con los Cebo de campo y Cebo. No obstante, a pesar de la elevada calidad de los productos derivados de animales alimentados en la dehesa y el aumento de la demanda de esos productos, existen factores limitativos resultantes de la producción de la bellota y de la misma superficie de la dehesa, lo que hace que aumente la elaboración de productos provenientes de animales de raza Ibérica alimentados bajo el sistema de Cebo. En este sentido, los datos publicados por el Registro Informativo de Organismos Independientes de Control del Ibérico (RIBER) (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2012) muestran que los animales Ibéricos de Cebo, principalmente, y montanera son los más producidos (Figura 2). Es necesario indicar que desde el año 2009 se está observando un descenso en el número de cabezas de animales ibéricos alimentados en montanera.

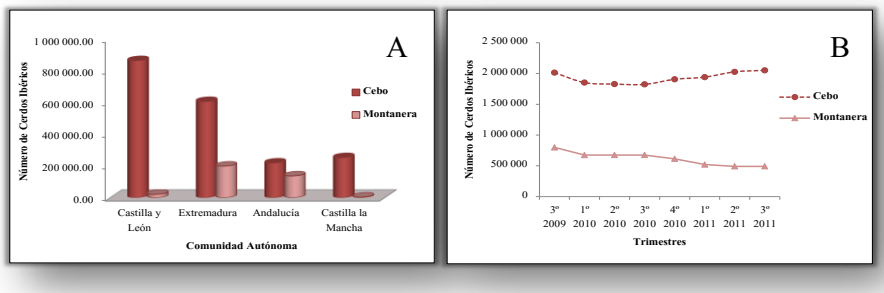


Figura 2. A) Censo de Cerdos Ibéricos alimentados en el sistema de Cebo y Montanera por Comunidad Autónoma en 2011. B) Evolución del censo de cerdos Ibéricos alimentados en el sistema de Cebo y Montanera por trimestre durante el periodo 2009 - 2011. Fuente: RIBER-MAGRAMA, 2012.

1.2.2 Importancia de la carne del Cerdo Ibérico

La producción de cerdos Ibéricos se encuentra principalmente destinada a la obtención de materia prima para la elaboración de productos crudo-curados de alta calidad. Para lograr este propósito, la industria cárnica requiere de carne de calidad, la cual depende principalmente de su composición y estructura.

El músculo estriado voluntario o esquelético denominado comúnmente como *carne* se encuentra acompañado por grasa y está estructurado por fibras individuales ordenadas paralelamente (Figura 3), rodeadas de una envoltura de tejido conectivo llamado *Endomisio.* Este conjunto de fibras forman fascículos rodeados por tejido conectivo llamado en este caso *Perimisio*. De igual modo, los fascículos forman una estructura superior rodeado por una membrana de tejido conectivo denominada *Epimisio* y así, constituyendo el músculo completo. Por lo tanto, el tejido conectivo mantiene las fibras musculares unidas, permitiendo que la fuerza de contracción generada por las fibras actúe sobre el músculo entero. La fuerza del tejido conectivo se encuentra dada por su principal componente, el colágeno, y por la presencia de elastina. El colágeno se conforma de fibras que forman estructuras muy fuertes, mientras que las fibras de elastina son elásticas (Purslow, 2005).

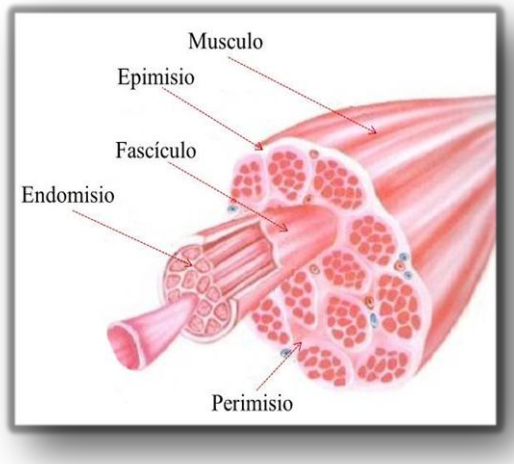


Figura 3. Estructura del musculo. Fuente: Fennema, 2000.

Por otra parte, la grasa que acompaña a la carne, es un componente que se encuentra depositado y distribuido en el músculo de diferentes maneras. Así, la grasa puede ser subcutánea, (depositada bajo la piel del animal), intermuscular (depositada entre los músculos) o intramuscular (infiltrada en el músculo) (Timón et al., 2001).

En el caso del cerdo Ibérico, concretamente la grasa intramuscular, es uno de los principales factores que influyen en la calidad de la carne, ya que la **cantidad**, **composición**, así como el **estado físico** en el que se encuentre determina en gran medida muchas de las características sensoriales y texturales de los productos crudo-curados. Desde el punto de vista tecnológico, el contenido

graso condiciona la penetración de la sal, especias y aditivos, asimismo, influye en el proceso de desecación del producto y en el desarrollo de aromas y sabores característicos del mismo (García et al., 1991). Por otra parte, la consistencia de la grasa tiene un importante efecto en las características texturales de los productos crudo-curados, ya que determina su facilidad de manipulación, apariencia, además de su dureza. En éste sentido, una fluidificación elevada de la grasa por temperaturas a las cuales se ve favorecida la presencia de ácidos grasos en estado líquido (Niñoles et al., 2010), aportará una jugosidad, brillo y suavidad característicos de los productos Ibéricos crudo-curados (Ruiz y Lopez Bote, 2002). Por otra parte, la cristalización de la grasa, favorecida por las bajas temperaturas, incrementa el contenido de la fracción sólida, confiriéndole dureza a la muestra (McClements et al., 1992), así como su grado de palatabilidad. En este sentido, el punto de fusión y de cristalización de la grasa, está determinado por el grado de instauración de los triglicéridos (Himawan et al., 2006), siendo el ácido Oleico, uno de los ácidos grasos mayoritarios en el perfil de ácidos grasos de la grasa de cerdo Ibérico (Fernández et al., 2007), contribuyendo a la consistencia característica de este tipo de productos (Ventanas et al., 2007b). Así, en función de la temperatura, el **estado físico** (sólido/líquido) de la grasa, determinado por su composición, afectará a las propiedades texturales de los productos crudo-curados.

La grasa proveniente de animales de raza Ibérica cuenta con propiedades específicas que son determinantes en la producción de alimentos crudo-curados de gran calidad (Ventanas et al., 2007b). Como ya se mencionó en el apartado 1.2.1, estas propiedades son consecuencia de factores como la conservación del cruce entre animales autóctonos de zonas de la península Ibérica (agrupación Ibérica racial), así como su alimentación mediante la explotación en extensivo, por medio del aprovechamiento óptimo de los recursos naturales o mediante el aporte de piensos controlados.

1.2.3 Productos cárnicos crudo-curados y su calidad

Los productos cárnicos crudo-curados Ibéricos son aquellos que parten de carne fresca de cerdo Ibérico y no son sometidos a tratamientos térmicos, sino que son elaborados mediante un proceso de secado y madurado en cámaras de temperatura y humedad controladas. Los productos cárnicos crudo-curados pueden ser aquellos presentados en forma de embutidos (como las salchichas fermentadas crudo-curadas, chorizos, salchichones, fuet, longaniza, etc…) o aquellos que son formados por piezas enteras (como el lomo embuchado, el jamón o la paleta que son las extremidades traseras y delanteras del cerdo, respectivamente).

Dentro del amplio abanico de productos cárnicos Ibéricos en forma de embutidos, son los crudo-curados los que presentan mayor número de variantes. Los más importantes y conocidos son las salchichas fermentadas crudo-curadas, los chorizos, el salchichón, la chistorra, el salami, el fuet y las longanizas. Estos productos embutidos parten de carne fresca picada, mezclada con grasa, sal, diferentes especias, aditivos y otros ingredientes que se embuten dentro de tripas naturales o artificiales con diferentes diámetros. Posteriormente, son fermentados hasta un pH deseado para estabilizar el producto, obteniendo así, los aromas y sabores deseados al final de su proceso de fermentación. Finalmente, son secados y madurados lo que le confiere las características texturales y sensoriales finales.

En el caso de la elaboración del jamón Ibérico crudo-curado se deben considerar tres factores importantes que determinan su calidad, que son la raza de los animales, su alimentación y las etapas de elaboración para la obtención del alimento. Así, las piezas obtenidas de animales de raza Ibérica, cebados mediante cualquiera de los diferentes sistemas de alimentación de los animales descritos anteriormente (apartado 1.2.1.), son sometidas a un proceso de *Salazonado*, en la que se incorpora sal común y sales nitrificantes (nitratos y nitritos) para favorecer la deshidratación y conservación de las piezas. Posteriormente mediante el *Lavado* de las piezas se elimina la sal adherida a su superficie y se pasa a la fase de *Asentamiento o Post-salado*, donde las piezas son colocadas en cámaras de temperatura y humedad relativa controlada (entre 3 y 6 ºC; H. R. de 80 y 90 %) para favorecer que la sal se reparta homogéneamente en toda la pieza cárnica y se

elimine de manera paulatina el agua superficial, estabilizándose el producto enzimática y microbiológicamente. La siguiente etapa es la de *Secado-maduración*, donde las piezas son tradicionalmente colocadas en secaderos naturales provistos con ventanales que permiten el control de la ventilación, obteniendo así las condiciones adecuadas de humedad relativa y temperatura de una manera natural. No obstante y teniendo en cuenta la dependencia de los secaderos naturales de las condiciones ambientales que no son compatibles con los requerimientos industriales, actualmente, también se utilizan secaderos automatizados que permiten una elaboración similar a la tradicional a menor coste y permiten unas condiciones ambientales constantes que facilitan la producción y reducen el tiempo de proceso. En esta etapa, el rango de temperaturas utilizadas oscila entre 15 y 30 ºC y la humedad entre 60 y 80 %. Durante este periodo, se lleva a cabo una gradual deshidratación, denominada sudado, y que también favorece la difusión de la grasa que se introduce entre las fibras musculares, lo que repercute favorablemente en la retención de aromas, Finalmente las piezas son sometidas al proceso de *Envejecimiento* en bodegas a temperaturas de entre 8 a 22 ºC, según la zona geográfica y la época del año, y humedad relativa entre 60 y el 80 %. El periodo en bodega oscila entre los 18 meses y los 2.5-3 años (Ventanas, 2006), en donde prosiguen los procesos bioquímicos con intervención de la flora microbiana confiriéndole un aroma y sabor característico a este producto.

La calidad de estos productos depende en gran medida de la materia prima utilizada, siendo la carne y la grasa los factores más importantes, que influyen en las características finales de calidad del producto.

1.2.4 Conservación de productos cárnicos crudo-curados

Con el fin de mantener y prolongar la vida útil de los diferentes productos cárnicos crudo-curados, se han desarrollado diferentes técnicas para su conservación, ya sea por medio de la adición de sales y aditivos (conservantes, antioxidantes, etc.), modificando parámetros para tener una deshidratación óptima, o por medio de la aplicación de tecnologías de conservación tradicionales (atmosferas protectoras, refrigeración o congelación) o de nuevas tecnologías (pulsos eléctricos, campos magnéticos, irradiación, pulsos de luz o

las altas presiones). Estas últimas son cada vez más utilizadas ya que con su aplicación se obtienen alimentos con una mayor vida útil, minimizándose el efecto sobre las características nutricionales y organolépticas, mejorando la distribución y la venta al por menor (sin refrigeración) .

Dentro de los sistemas de conservación tradicionales destaca el uso de la sal común. Una de las principales características de la sal y por la cual se utiliza en la elaboración de productos crudo-curados es su acción de inhibición del crecimiento microbiano (Andrés et al., 2005b; Costa-Corredor et al., 2010). No obstante, se ha constatado que las concentraciones de sal utilizadas en productos comerciales no son lo suficientemente elevadas para inhibir el crecimiento de las formas bacterianas esporuladas (Betts et al., 2007). Así, el efecto de la sal ha de complementarse con otras técnicas que pueden potenciar su efecto, como la adición de otras sustancias con acción conservadora como las sales nitrificantes (nitritos y nitratos) (Sebranek y Bacus, 2007). Los nitritos y nitratos son aditivos alimentarios con actividad conservante empleados en los diferentes productos crudo-curados. El uso de estos conservantes se fundamenta principalmente en su capacidad de impedir el crecimiento de microorganismos patógenos, como el *Clostridium botulinum*, bacteria responsable de la producción de la toxina botulínica, la cual tiene graves efectos sobre la salud humana. Otra de las propiedades de los nitritos y nitratos es que aportan propiedades antioxidantes, además de que desarrollan y estabilizan el aroma y el color rojo característico de los productos crudo-curados debido a la formación de la nitrosomioglobina. Sin embargo, estos conservantes son promotores de la formación de nitrosaminas y metahemoglobina, que son compuestos potencialmente cancerígenos y afectan el transporte del oxígeno, por lo que los valores admitidos en los alimentos son muy bajos, estando la ingesta diaria admitida de las sales nitrificantes regulada (FAO/OMS (JEFCA), 1990; Diario Oficial de la Unión Europea, 2006). Actualmente, la Union Europea busca la manera de promover la eliminación de estos conservantes, y reducir de esta manera posibles riesgos tóxicos para el consumidor. No obstante, la disminución del uso de conservantes en los productos crudo-curados puede suponer, al mismo tiempo, un riesgo microbiológico, por lo que es necesario complementar la acción de los aditivos conservantes con otras técnicas de conservación.

Otra de las formas de conservación de productos cárnicos crudo-curados tiene lugar a través de su envasado y mantenimiento a bajas temperaturas (refrigeración). En este sentido, el mercado de los productos crudo-curados ha tenido cambios en la presentación de los mismos, desarrollando nuevos formatos que faciliten su distribución, almacenamiento y consumo y en muchos casos requieren de un almacenamiento en refrigeración (Quintavalla y Vicini, 2002). Así, actualmente se está produciendo un aumento constante de los formatos loncheados, en tiras o tacos, a la par que un descenso de las presentaciones tradicionales, en forma de piezas enteras. Los nuevos desarrollos en el envasado de estas presentaciones pretenden solventar algunas deficiencias, como evitar la adhesión entre lonchas y minimizar las reacciones de oxidación y por lo tanto cambios de color y sabor. Asimismo, se están desarrollando nuevos materiales (de permeabilidad controlada) y tecnologías de envasado en atmosfera protectora como son el envasado al vacío (Cilla et al., 2006b) y el envasado en atmósfera modificada (García-Esteban et al., 2004; Parra et al., 2010). El primero de ellos previene la contaminación del producto, ya que extrayendo el aire que rodea el producto se obtiene una atmósfera libre de oxígeno que retarda el crecimiento de bacterias y hongos y la oxidación del producto, prolongando su vida útil. El envasado en atmosfera modificada consiste en envasar el alimento extrayendo por completo el aire y rodeándolo de una atmosfera cuya composición es diferente a la del aire. Por medio de la optimización de la composición de la mezcla de gases, principalmente el dióxido de carbono, oxígeno y nitrógeno, se persigue la inhibición del crecimiento de algunos microorganismos, incluyendo bacterias contaminantes. Aún a pesar de que el envasado prolonga la vida útil del alimento, es necesario que sea complementado por otros métodos de conservación como el almacenamiento a bajas temperaturas. Así, el almacenamiento a bajas temperaturas de los productos cárnicos crudo-curados retarda la actividad enzimática y ralentiza el desarrollo de los microorganismos, evitando la degradación de los mismos (Cilla et al., 2006a; Cilla et al., 2006b). Sin embargo, durante el periodo de almacenamiento a bajas temperaturas existe una disminución de la calidad de los diferentes productos crudo-curados, como consecuencia de cambios que afectan tanto a la grasa como al agua. Entre otros se dan procesos de migración de agua y/o cristalización de agua y grasa, lo que repercute tanto en el tejido magro, como en el tejido adiposo (Leygonie et al.,

2012; Sato, 2001), alterando las propiedades sensoriales y en particular la textura de los productos cárnicos crudo-curados.

Es necesario indicar que en la industria alimentaria se suelen combinar varias tecnologías de conservación, que si bien por sí solas resultan insuficientes para detener la acción de los microorganismos presentes, en conjunto, proporcionan una estabilidad microbiana que garantiza la seguridad del alimento. En este sentido, el uso de nuevas tecnologías de conservación como la irradiación (Cava et al., 2005), los pulsos eléctricos (Jaeger et al., 2009) y las altas presiones hidrostáticas (Hugas et al., 2002; Mañas y Pagán, 2005; Patterson, 2005), resultan de gran interés al ser efectivas en la eliminación o reducción del contenido de microorganismos, tanto patógenos como causantes del deterioro del alimento, cuando son aplicadas y combinadas con otros métodos de conservación tradicionales. El uso combinado de diferentes tecnologías es especialmente interesante en los productos cárnicos crudo-curados loncheados, donde durante el proceso de loncheado puede producirse una contaminación del producto, quedando además una gran superficie del mismo expuesta a agentes externos.

En la industria cárnica, las altas presiones hidrostáticas han sido aplicadas en productos cárnicos comerciales como el jamón cocido (Garriga et al., 2004), pollo rebozado (Jiménez-Colmenero et al., 1998), y lomo de ternera marinado (Garriga et al., 2004), entre otros. En los productos cárnicos crudo-curados, la aplicación del tratamiento por "altas presiones" es de especial interés, ya que se trata de una tecnología no térmica que conserva el alimento por medio de la reducción de microorganismos ligados al deterioro de la carne. Así, las altas presiones han sido aplicadas en productos crudo-curados como el fuet y chorizo (Marcos et al., 2007), el salchichón (Rubio et al., 2007) y jamón crudo-curado (Serra et al., 2007b; Zhifei et al., 2012). No obstante, a pesar de ser una técnica viable para la conservación de alimentos, se ha demostrado que los productos crudo-curados sometidos a altas presiones sufren alteraciones que repercuten en sus propiedades sensoriales, viendose modificados aspectos como el color, el sabor y la textura (Andrés et al., 2005a; Fuentes et al., 2010; Serra et al., 2007a; Serra et al., 2007b). Por lo tanto, para la aplicación de altas presiones, además de la seguridad microbiológica, se ha de considerar el efecto de la presurización

sobre las propiedades sensoriales de los productos cárnicos crudo-curados. Este hecho hace que la búsqueda de las condiciones óptimas de aplicación debe perseguir tanto mejorar la conservación del alimento como evitar o minimizar la degradación de sus propiedades sensoriales y funcionales.

1.3. Caracterización de la calidad de productos cárnicos crudo-curados

Diversas han sido las metodologías y técnicas que se han utilizado para la caracterización y el análisis de los productos cárnicos crudo-curados con el fin de evaluar y determinar su calidad. No obstante, muchos de los análisis que se llevan a cabo son técnicas subjetivas, destructivas o muy costosas, que previenen su utilización como técnicas de control en las líneas de producción. En el caso de los *análisis sensoriales*, su principal limitación viene derivada de las particularidades de las opiniones y gustos propios de la persona que lleva a cabo la evaluación. Estos análisis se llevan a cabo mediante un grupo de catadores que evaluan el alimento a través de diferentes ensayos, siguiendo unas pautas predeterminadas en condiciones normalizadas obteniendo así una medida de la calidad. Por lo tanto, no resulta una técnica adecuada para ser utilizada en una línea de producción.

En el caso de las técnicas de *análisis fisicoquímico* de alimentos, las limitaciones vienen dadas fundamentealmete porque son **destructivas** lo que imposibilita su uso en línea para toda la producción. Así, estas técnicas únicamente son empleadas en algunas etapas o al final del proceso en una muestra concreta de todo el lote. En el análisis de la composición química del producto se determinan las fracciones de sus componentes mayoritarios, agua, grasa, proteína y cenizas, siendo el contenido de grasa uno de los más relevantes en los productos cárnicos crudo-curados. En el método oficial de extracción de grasa mediante *Soxhlet* (AOAC, 1997; procedimiento 991.36), los lípidos apolares (triglicéridos y colesterol esterificado) son extraidos, a excepción de los fosfolípidos por lo que la cuantificación de lípidos totales no es posible (Ruiz y Petrón, 2001). Así, este método se utilizado habitualmente en productos cárnicos como diversos musculos de cerdo (Koch et al., 2011a), jamon curado (Corino et al., 2003) y lomo curado (Ruiz-Ramírez et al., 2005). En vistas a caracterizar la

composición de la fracción grasa, otro parámetro de importancia es la *composicición de ácidos grasos* mediante la obtención de ésteres metílicos y su cuantificación por cromatografía gaseosa (Ansorena y Astiasar, 2004). Esta técnica ha permitido detectar diferencias en la composición de la grasa del musculo de cerdos de raza Ibérica y cruzados con Duroc (Carrapiso et al., 2003), así como de animales con diferentes sistemas de alimentación en carne fresca (Cava et al., 1997) y en jamón curado (Petrón et al., 2004). También ha sido utilizada para determinar los efectos del empaquetado y almacenamiento sobre la composición de ácidos grasos de salchichas fermentadas crudo-curadas (Ansorena y Astiasar, 2004).

En el caso de la fracción grasa, resulta muy interesante caracterizar su comportamiento cuando es sometida a cambios de temperatura. En este sentido, se utiliza la *Calorimetría Diferencial de Barrido (DSC)* que permite evaluar el comportamiento térmico (transición vítrea, cristalización, fusión, etc) de los alimentos ya sea en un rango de temperaturas, o a temperatura constante (procesos isotérmicos). Así, Bell et al. (2007), utilizaron esta tecnica para observar cambios durante la cristalización de manteca vegetal. También, se ha evaluado el comportamiento fundente de grasa tanto subcutanea (Niñoles et al., 2007; Niñoles et al., 2010), como intramuscular (Niñoles et al., 2011) de cerdo Ibérico, así como en mezclas cárnicas (Benedito et al., 2001) y jamon crudo-curado (Niñoles et al., 2008).

Por otra parte, existen técnicas como la *determinación de la textura* mediante métodos instrumentales donde a partir de ensayos de punción o compresión (análisis de perfil de textura-TPA) se pueden evaluar las propiedades texturales de los productos crudo-curados debidas a cambios de composición o al procesado. Así, se han realizado análisis de compresión en muestras de salchichas fermentadas crudo-curadas (Herranz et al., 2005; Ruiz-Capillas et al., 2012), chorizo (Casquete et al., 2012; Gimeno et al., 2000; Gonzalez-Fernandez et al., 2006) y jamón crudo-curado (Benedini et al., 2012; Costa-Corredor et al., 2009). Por otra parte, los ensayos de punción han sido utilizados en muestras de grasa de cerdo Ibérico (Santacatalina et al., 2011) y jamón crudo-curado (Niñoles et al., 2008), entre otros.

En general, la industria cárnica persigue remplazar las ya mencionadas tècnicas analíticas tradicionales **destructivas,** por técnicas mas innovadoras que no afecten la integridad del producto, permitiendo su utilización en las líneas de producción y así, optimizar los procesos y desarrollar productos seguros de más calidad. Las nuevas tecnologías no destructivas empleadas hasta el momento se basan en el uso de equipos de alto nivel tecnológico, capaces de determinar de forma rápida una o varias características de los productos cárnicos crudo-curados. Un ejemplo de éstas técnicas aplicadas es la Resonancia Magnética Nuclear (RMN), que se ha utilizado para evaluar y caracterizar la calidad de productos del cerdo y de la carne fresca proveniente de cerdos de diferentes cruces de razas no comerciales y comerciales (Straadt et al., 2012). Asimismo, las Imágenes de RMN han sido empleadas para clasificar jamones frescos y crudo-curados provenientes de cerdos Ibéricos alimentados con dos sistemas de alimentación distintos, relacionándose las imágenes con las características texturales y composicionales de los jamones (Pérez-Palacios et al., 2010; Pérez-Palacios et al., 2011). Por otra parte, esta técnica ha sido utilizada para la estimación de la composición del tejido magro y graso de la canal de cerdos (Jia et al., 2010). Otra técnica utilizada es la Tomografía Axial Computarizada (TAC), que ha sido empleada para predecir la cantidad y distribución del contenido de sal y agua en jamon curado durante el proceso de secado y al final del proceso de elaboración (Fulladosa et al., 2010; Håseth et al., 2012; Santos-Garcés et al., 2010; Santos-Garcés et al., 2012). La Impedancia Bioeléctrica ha sido utilizada para predecir la composición de cerdos Ibéricos vivos como paso previo a la selección de la materia prima (Daza et al., 2006). La Espectroscopía Infraroja se ha usado para clasificar jamones crudo-curados en función de sus propiedades texturales y de color (Garcia-Rey et al., 2005; Ortiz et al., 2006). No obstante para algunas de estas técnicas, no resulta fácil su implementación en las lineas de producción, ya que además de ser costosas, no son fáclmente adaptables, lo que restringe su aplicación e implica un gran reto para la investigación y desarrollo en la industria cárnica. No es así el caso de los ultrasonidos de señal, donde es posible el uso de equipos portátiles, sencillos, no invasivos, de bajo coste, precisos y de fácil incorporación en las lineas de producción. Es por ello que resulta de interés su aplicación y desarrollo en la industria de los alimentos y en particular en el sector cárnico. Así, los

ultrasonidos ya han sido aplicados para caracterizar las propiedades fundentes de la grasa de jamones Ibéricos curados (Niñoles et al., 2010), así como para músculos provenientes de cerdos de diferente raza y régimen de alimentación (Niñoles et al., 2011)

1.4. Ultrasonidos

1.4.1 Generalidades

Los ultrasonidos son ondas elásticas con frecuencias mayores a las audibles por el ser humano (>20 kHz), las cuales necesitan un medio para propagarse (Mulet et al., 1999). Las ondas acústicas, como cualquier otra onda viene definida por su frecuencia (f), velocidad (v) e intensidad o amplitud (A), siendo clasificadas como *longitudinales, transversales* y *de Rayleigh*. Las ondas *longitudinales* son aquellas en las que las partículas del medio se mueven en la dirección del desplazamiento de la propagación de la onda, mientras que en las *transversales*, el movimiento es perpendicular al de la onda. Las ondas *de Rayleigh* son aquellas en las que su desplazamiento se produce por la superficie del medio.

Las ondas acústicas se dividen en cinco grupos: infrasonido (<20 Hz), espectro audible (20 Hz a 18 kHz), ultrasonidos de alta intensidad (18-100 kHz), ultrasonidos de baja intensidad (100 kHz – 1 MHz) y los ultrasonidos médicos o de diagnóstico (>1 MHz) (Mason, 1993). Por otra parte, las aplicaciones de los ultrasonidos se dividen en dos grupos, por un lado estan los *ultrasonidos de potencia* o *alta intensidad,* con frecuencias entre 20 kHz y 100 kHz e intensidades por encima de 1 Wcm^{-2}, que se caracterizan por provocar cambios que afectan a los procesos o productos. Por otra parte, los ultrasonidos de *baja potencia* o *de señal* presentan frecuencias desde 100 kHz hasta 1 MHz y menores intensidades (<1 Wcm^{-2}) (en algunas aplicaciones se emplean mayores frecuencias) y son usados para monitorizar los procesos o productos ya que no producen cambios en ellos.

Dentro del ámbito alimentario, por un lado, los *ultrasonidos de potencia* han sido utilizados para mejorar los fenómenos de transporte (Cárcel et al., 2012; Ozuna et al., 2011), así como para la mejora de la inactivación de

microorganismos (Ortuño et al., 2012; Chandrapala et al., 2012). Por otro lado, *los ultrasonidos de señal* se han empleado para estimar la composición, textura y en el control de diversos alimentos (Awad et al., 2012). En el apartado 1.4.3. se hace una revisión detellada de las aplicaciones de ésta técnica en el ámbito alimentario.

1.4.2 Ultrasonidos de baja potencia o de señal

La información que los *ultrasonidos de baja potencia* proporcionan cuando se propagan a través de un medio permite estimar algunas de las propiedades fisicas del alimento analizado. Las medidas ultrasónicas se pueden realizar utilizando los modos *pulso-eco* o *trasmisión-recepción* (Awad et al., 2012; Mulet et al., 1999).

En el *modo pulso-eco*, se pueden utilizar dos transductores (emisor y receptor), ambos colocados en la misma cara del material o bien un solo transductor, el cuál actúa como emisor y receptor. Sin embargo en este último caso, la señal recibida de las reflexiones producidas en el alimento puede ser enmascarada por las vibraciones del transductor. Con objeto de evitarlo, se hace necesario el uso de una línea de retardo para separar la señal recibida de la emitida. En el caso del *modo transmisión-recepción*, se colocan dos transductores, cada uno en una de las caras opuestas del material a estudiar, uno de los transductores emite la señal ultrasónica mientras que el otro transductor recibe la energía transmitida a través del material. Así, a partír de esos dos modos de medición, se pueden obtener medidas de la *velocidad*, el *coeficiente de atenuación* y el *espectro de frecuencias*.

La ***velocidad de los ultrasonidos*** *(v)* es la distancia recorrida por la onda ultrasónica por unidad de tiempo, siendo constante para un material a determinadas condiciones y depende solo de sus propiedades físicas (modulo de elasticidad y densidad). Las ecuaciones que permiten estimar la velocidad de los ultrasonidos depende de si el medio por el que se transmiten es sólido o líquido.

- ✓ *Velocidad en sólidos*.

La velocidad de las ondas longitudinales en un medio sólido v_s está relacionada con las constantes elásticas para un medio homogéneo, isotrópico y elástico (ecuación 1).

$$v_s = \sqrt{\frac{K+\frac{4G}{3}}{\rho}} \qquad \text{(ecuación 1)}$$

donde *K* es el módulo de compresibilidad, *G* es el módulo de elasticidad transversal y ρ es la densidad del material. Cuando la longitud de onda es mayor en comparación con el diámetro de la muestra, la velocidad se calcula mediante la ecuación 2.

$$v_s = \sqrt{\frac{E}{\rho}} \qquad \text{(ecuación 2)}$$

donde *E* representa el módulo de Young.

Las ondas transversales, se atenúan más que las longitudinales, por lo que se aplican en alimentos sólidos de pequeño espesor. Para este tipo de ondas, la velocidad se calcula a partir de la ecuación 3:

$$v_s = \sqrt{\frac{G}{\rho}} \qquad \text{(ecuación 3)}$$

✓ *Velocidad en líquidos*

Las ondas longitudinales son las únicas que pueden propagarse a traves de medios líquidos o gases. Así, asumiendo que existen condiciones adiabáticas durante la propagación de la onda, la velocidad en gases o líquidos v_l viene determinada por la ecuación 4:

$$v_l = \sqrt{\frac{1}{\rho\beta}} \qquad \text{(ecuación 4)}$$

donde β es el coeficiente de compresibilidad adiabática, que es igual a la inversa de la temperatura (K) en gases ideales.

Las medidas de velocidad pueden llevarse a cabo mediante el *modo pulso-eco* o *trasmisión-recepción* y la misma se calcula a partir de las medidas independientes de la distancia recorrida y del tiempo transcurrido entre la salida de la onda desde el emisor y su llegada al receptor. Así, el tiempo utilizado para el cálculo de la velocidad se conoce como *tiempo de vuelo* (t_v), que es el tiempo transcurrido desde que el equipo generador envía la señal a través del transductor emisor hasta que el transductor receptor detecta la llegada de la señal.

Las ondas al propagarse por un medio pierden energía, la cual es absorbida por el mismo medio; a esta pérdida de energía se le conoce como ***atenuación***, la cual es característica del material y proporciona información acerca de sus propiedades físicas. La atenuación ha sido relacionada con parámetros físicos en líquidos (Pierre et al., 2012) y en sólidos (Koch et al., 2011a; Koch et al., 2011b). Las medidas de atenuación se pueden realizar mediante el cálculo experimental del coeficiente de atenuación (α) utilizando la ecuación 5.

$$\alpha = \frac{1}{d} \ln\left(\frac{A_1}{A_2}\right) \qquad \text{(ecuación 5)}$$

donde A_1 y A_2 son las amplitudes de la señal antes y despues de atravesar la longitud de la muestra (d), en el caso de realizar la medida en modo trasmisión-recepción. Cuando las medidas se lleva a cabo en el modo pulso-eco, A_1 y A_2 representan la amplitud de la onda del primer y segundo ecos.

Por otra parte, cuando los transductores son excitados mediante un pulso eléctrico emiten una onda con una composición en frecuencias característica, que se propaga a través del material en estudio. A partir de la señal temporal y aplicando la transformación de Fourier (FFT, Oppenhein, & Schafer, 1989), se puede obtener la magnitud energética para cada frecuencia (***espectro de frecuencias***). Así, la transformada de Fourier relaciona la señal temporal con el dominio de frecuencia, descomponiendo o separando la misma en una suma de ondas de diferentes frecuencias. El estudio del espectro de las señales al atravesar un medio aporta información sobre como éste modifica cada una de las frecuencias de una onda sonora, siendo la distribución del espectro característica de cada material (Mulet et al., 1999).

1.4.3 Métodos y aplicaciones en tecnología de alimentos

Los ultrasonidos de señal pueden cosniderarse una técnica muy interesante para la industria de alimentos. Por medio de la adquisición de medidas ultrasónicas en tiempo real, las propiedades de los alimentos pueden ser evaluadas de manera no destructiva, permitiendo el ajuste de las formulaciones y de los parámetros de proceso. Esto contribuye a la optimización de los mismos siendo posible la mejora y mantenimiento de la calidad del producto final.

La fiabilidad y el potencial del uso de los ultrasonidos como una herramienta analítica para la caracterización de diversos alimentos ha sido demostrada en una amplia gama de productos. En este sentido, los ultrasonidos han sido empleados para evaluar la calidad de las frutas y vegetales (Mizrach, 2008). Así, la madurez del aguacate y la harenosidad de la manzana se evaluaron mediante medidas de atenuación (Mizrach et al., 1999a; Bechar et al., 2005; Mizrach et al., 2003). Los ultrasonidos han permitido determinar la firmeza, el contenido de azucar y el estado de hidratación de frutas como el mango (Mizrach et al., 1997; Mizrach et al., 1999b), la ciruela (Mizrach, 2004), el melón (Mizrach et al., 1991; Mizrach et al., 1994) y la naranja (Jiménez et al., 2012). En vegetales, la firmeza, textura, contenido en sólidos solubles y acidéz de tomates, zanahorias y patatas han sido caracterizados mediante medidas de atenuación y velocidad de los ultrasonidos (Mizrach, 2007; Mizrach, 2008; Nielsen et al., 1998). También, se han utilizado en procesos de fermentación durante la fabricación de pan (Ross et al., 2004; Skaf et al., 2009) y de bebidas alcoholicas (Resa et al., 2009), así como en la determinacion de la composición del queso curado y fresco a diferentes temperaturas (Benedito et al., 2002; Telis-Romero et al., 2011) y en la estimación del contenido de grasa sólida, los cambios en la cristalización y el estado físico y polifórmico de grasas animales y vegetales (Awad, 2004; McClements & Povey, 1992). En el sector cárnico, los ultrasonidos han sido utilizados para caracterizar y estimar la composición y propiedades texturales de canales (Koch et al., 2011b) y de musculos (Koch et al., 2011a) de animales de diferentes razas y sistemas de alimentación (Niñoles et al., 2011). En productos elaborados a base de carne, se ha determinado la composición de mezclas de carne (Benedito et al., 2001), y se ha evaluado las propiedades

texturales (Lllul et al., 2002a; Llull et al., 2002b) y la composición (Simal et al., 2003) de productos cárnicos fermentados.

En los trabajos comentados anteriormente, la realización de las medidas de ultrasonidos se ha llevado a cabo mediante medidas *por contacto* en las que los transductores se encuentran en contacto directo con el alimento, siendo necesario en la mayoría de las ocasiones la aplicación de un medio de acople (gel, aceite, agua, etc.) que permita la transmisión de los ultrasonidos hasta la muestra, eliminando el aire entre el transductor y la muestra, y evitando así la reflexión de la señal en la superficie del tranductor. A pesar de que los transductores usados en esas medidas son adecuados en las lineas de producción, en algunos casos, el uso de los medios de acople dificulta su aplicación, especialmente en aquellas etapas del proceso donde se debe evitar la contaminación del alimento y cuando las medidas y el manejo del producto deben realizarse de forma muy rápida. Por tal motivo, se han desarrollado las técnicas de ultrasonidos *sin contacto* (*air-coupled*) que cada vez son mas aplicados para la caracterización, inspección y evaluación de la calidad de diferentes materiales. En esta técnica, los transductores no estan en contacto con el alimento, lo que resulta de gran interes para la industria, siendo su principal limitación la menor cantidad de energía introducida en el material, lo que supone un problema importante en materiales altamente atenuantes. Sin embrago, su naturaleza mínimamente invasiva permite que esta técnica pueda ser aplicada en una amplia gama de productos dentro de las líneas de producción. Así, las técnicas ultrasónicas sin contacto ha sido utilizada para medir la velocidad de los ultrasonidos y el coeficiente de atenuación en diferentes materiales sólidos como la fibra de carbono, polímeros reforzados (Schindel y Hutchins, 1995), materiales sólidos permeables y porosos (Nagy, 1993; Gómez Álvarez-Arenas et al., 1995) y películas de polipropileno (Gómez Álvarez-Arenas et al., 2010). Su aplicación en el área de los alimentos ha servido para inspeccionar y detectar cuerpos extraños contenidos en productos alimenticios comerciales como el queso, chocolate, pastas y productos enlatados (Pallav et al., 2009). Gan et al. (2002) detectaron y evaluaron la presencia y las variaciones en la consistencia de sólidos de almidón dentro de recipientes para microondas. Asimismo, se han caracterizado los cambios fisicoquímicos en bebidas dentro de recipientes de vidrio o botellas de polímeros (Meyer et al.,

2006). De manera similar, se caracterizó y estudió la estabilidad con el tiempo en la distribución de partículas en emulsiones, dispersiones y sistemas coloidales (Nelson et al., 2001). También se han empleado los ultrasonidos sin contacto para detectar cambios fisicoquímicos en productos lácteos (Gan et al., 2006; Pallav et al., 2009; Saggin y Coupland, 2001). Hasta el momento, no se ha evaluado la aplicación de ésta técnica en el sector cárnico por lo que es de gran interés el desarrollo de esta tecnología, fundamentalmente para productos cárnicos loncheados y envasados a vacío como el jamón curado.

Los ultrasonidos son una tecnología que también ofrece una vía para adquirir imágenes superficiales e internas de diversos materiales. En este sentido, es posible analizar las propiedades elásticas de los materiales mediante imágenes generadas a partir de ondas acústicas que atraviesan los materiales, incluso aquellos que son opacos (Wickramasinghe, 1984). En este sentido, *la microscopía acústica* ofrece la capacidad de generar imágenes de modo no destructivo de los materiales. Las forma más común de obtener imágenes por medio de un microscopio de barrido acústico es en modo pulso-eco y la utilización de un único transductor (Parker et al., 2010). La aplicación de esta técnica ha sido empleada en el estudio de diversos materiales, tales como circuitos integrados (Parker et al., 2010) o películas multicapa (Caneret al., 2003). Actualmente, su desarrollo y aplicación en la industria de los alimentos es escasa pero de gran interés, habiendo sido utilizada en el estudio de paquetes flexibles de alimentos (Ozguler et al., 1998) y en tejidos biológicos como la piel de cebolla (Parker et al., 2010). No obstante, la microscopía acústica respresenta un desafío en la investigación conducente a la caracterización de alimentos en particular en los productos cárnicos crudo-curados.

Finalmente, destacar que los diferentes métodos de análisis de alimentos mediante ultrasonidos resultan de gran interés en la industria alimentaria, como muestra el continuo incremento del número de aplicaciones reflejado en la bibliografía (Awad et al., 2012; Chandrapala et al., 2012), especialmente en el sector de los productos cárnicos.

2. OBJETIVOS

El objetivo principal de esta Tesis Doctoral consistió en evaluar la viabilidad del uso de los ultrasonidos de señal para caracterizar de manera no destructiva los productos cárnicos crudo-curados provenientes del cerdo Ibérico, en función de la tipología de la grasa, su contenido graso y sus propiedades texturales. Asimismo, se planteó el uso de esta técnica para la caracterización no destructiva de los cambios sufridos por los productos cárnicos crudo-curados tanto durante su almacenamiento refrigerado, como tras su tratamiento por altas presiones hidrostáticas. Para alcanzar este objetivo general se establecieron los siguientes objetivos parciales:

- Estudiar el uso de los ultrasonidos de señal para caracterizar el proceso de cristalización de manteca de cerdo Ibérico durante su almacenamiento a bajas temperaturas, así como, desarrollar un modelo matemático que permita monitorizar el proceso de cristalización y estimar el contenido de grasa sólida.

- Evaluar el uso de los ultrasonidos de señal para determinar los cambios texturales del tejido adiposo que tienen lugar durante su almacenamiento a bajas temperaturas, así como discriminar entre diferentes tipos de grasa subcutánea en función de la alimentación de los cerdos de los que provienen. Desarrollar modelos matemáticos que permitan describir los cambios texturales y de velocidad de los ultrasonidos durante la cristalización de la grasa.

- Estimar el contenido graso y el estado de la grasa en productos cárnicos formulados crudo-curados y envasados al vacío mediante la aplicación no destructiva de ultrasonidos de señal. Clasificar mediante ultrasonidos de forma no destructiva estos productos, en función del tipo de grasa con el que han sido elaborados.

- Evaluar la viabilidad de la aplicación de los ultrasonidos de señal para caracterizar no destructivamente el contenido y distribución de grasa así como la textura de jamón Ibérico loncheado y envasado al vacío. Estimar mediante ultrasonidos los posibles cambios texturales que

puede sufrir el jamón como consecuencia de los procesos de conservación a bajas temperaturas y del tratamiento por altas presiones.

- Determinar si las técnicas de ultrasonidos sin contacto pueden ser empleadas para la caracterización del jamón curado loncheado y envasado al vacío, reemplazando a las técnicas ultrasónicas convencionales por contacto.

- Estudiar la aplicación de la microscopía acústica para la caracterización microestructural de productos cárnicos crudo-curados.

3. METODOLOGÍA

3.1 Plan de Trabajo

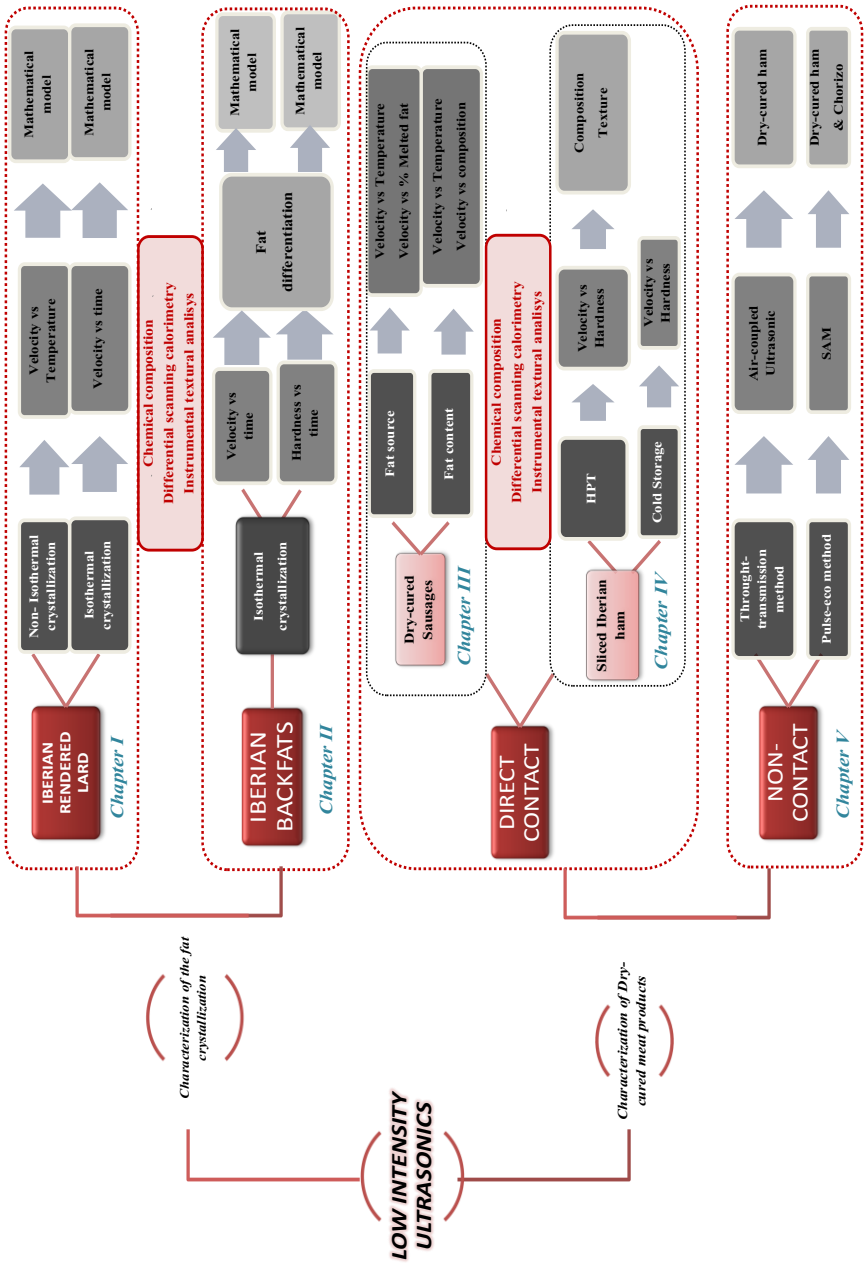


Figura 1. Plan de Trabajo (Working Plan)

En base a los objetivos planteados, se elaboró un plan de trabajo (Figura 1) que dio lugar a los cinco capítulos que forman la sección de Resultados, en formato de artículo científico, de esta Tesis Doctoral. La metodología específica de cada uno de los trabajos se detalla en los distintos capítulos, de manera que en esta sección únicamente se describirán con mayor profundidad las técnicas ultrasónicas utilizadas.

Los cinco capítulos se dividen en dos grandes apartados (Figura 1), el primero alude a los trabajos realizados para caracterizar la cristalización de grasas ibéricas mediante ultrasonidos. Así, en el *Apartado 1* (artículo publicado en Food Research International), se monitorizó mediante el uso de los ultrasonidos el proceso de cristalización de manteca de cerdo Ibérico durante su almacenamiento. Se desarrolló un modelo matemático basado en el modelo de Avrami con el objetivo de describir los cambios en la velocidad de los ultrasonidos que tienen lugar durante el almacenamiento isotermo. Asimismo, se desarrolló un modelo predictivo para estimar el contenido de grasa sólida durante la cristalización isoterma de la manteca de cerdo Ibérico, a partir de la velocidad de propagación de los ultrasonidos. En el *Apartado 2* (artículo enviado a Food Research International), se realizaron medidas de velocidad de los ultrasonidos y textura en dos diferentes tipos de grasas subcutáneas de cerdo Ibérico (montanera y cebo) durante su almacenamiento a bajas temperaturas. Se aplicó el modelo predictivo desarrollado en el *Apartado 1* para describir los cambios de la velocidad y se extendió la aplicación del modelo para caracterizar los cambios en las propiedades texturales de las grasas que ocurren durante el almacenamiento a bajas temperaturas.

El segundo apartado de esta tesis doctoral (Figura 1) engloba los trabajos realizados para la caracterización de productos cárnicos crudo-curados mediante ultrasonidos. Los tres capítulos se dividen en dos grandes sub-apartados en relación a las técnicas ultrasónicas aplicadas: contacto directo o sin contacto. Así, en el *Apartado 3* (artículo enviado a Food Science and Technology International) se utilizaron técnicas ultrasónicas por contacto directo para estimar el contenido graso y el estado de la grasa en productos cárnicos crudo-curados formulados, así como para discriminar el tipo de grasa con la que han sido elaborados. Para ello

se realizaron medidas de la velocidad de los ultrasonidos y de composición de muestras de salchichas crudo-curadas elaboradas con diferente contenido y tipo de grasa (manteca Ibérica, grasa subcutánea Ibérica de cerdos de cebo y montanera y aceite de girasol). Se desarrolló un modelo semi-empírico para estimar el contenido graso a partir de la velocidad de los ultrasonidos a 2 y 25 ºC. En el *Apartado 4* (artículo enviado a Meat Science) se realizaron medidas de la velocidad ultrasónica para determinar el contenido y distribución de la grasa en jamón ibérico loncheado y envasado al vacío, así como su textura. Asimismo, se estudió mediante medidas de la velocidad de los ultrasonidos el efecto que tienen el tratamiento de las altas presiones y el almacenamiento a bajas temperaturas sobre las propiedades texturales de este producto.

Finalmente, en el *Apartado 5* (artículo enviado a Sensors) se utilizaron técnicas acústicas sin contacto directo para caracterizar diversos productos cárnicos crudo-curados con el objetivo de evaluar esta tecnología y así reemplazar las técnicas convencionales de ultrasonidos por contacto. Así, se realizaron medidas de la velocidad de ultrasonidos sin contacto en muestras de jamón loncheado y envasado al vacío. Por otro lado, se realizaron medidas de microscopía acústica para caracterizar los tejidos que conforman los productos cárnicos crudo-curados.

Como ya se ha comentado, en esta sección se procede únicamente a describir las técnicas de ultrasonidos empleados en los diferentes trabajos llevados a cabo en la presente Tesis doctoral.

3.2 Ultrasonidos de señal

En esta Tesis Doctoral se utilizaron ultrasonidos de señal para la caracterización de diferentes tipos de productos procedentes del cerdo Ibérico realizándose medidas de los ultrasonidos tanto en medios líquidos como en medios sólidos utilizando diferentes tipos de técnicas ultrasónicas.

3.2.1 Medidas en medios líquidos

El sistema de ultrasonidos para la medida de las propiedades acústicas de medios líquidos (Figura 2) constaba de los siguientes elementos:

- Cilindro de aluminio portamuestras.
- Baño de temperatura controlada (± 0.1 ºC, Frigiterm, P-selecta, Abrera, España) para el atemperamiento de las muestras.
- Dos transductores piezoeléctricos (emisor y receptor) (1MHz, 0.5'' diámetro de cristal, A303S, Panametrics, Waltham, MA, USA).
- Termopar tipo K.
- Controlador de procesos (E5CK, Omron, Madrid, España), conectado a través de la interfase RS232 a un ordenador portable con una tarjeta de adquisición de datos (PCI 5112, National Instruments, Madrid, España).
- Generador-receptor de ultrasonidos (5058PR, Panametrics, Waltham, MA, USA).

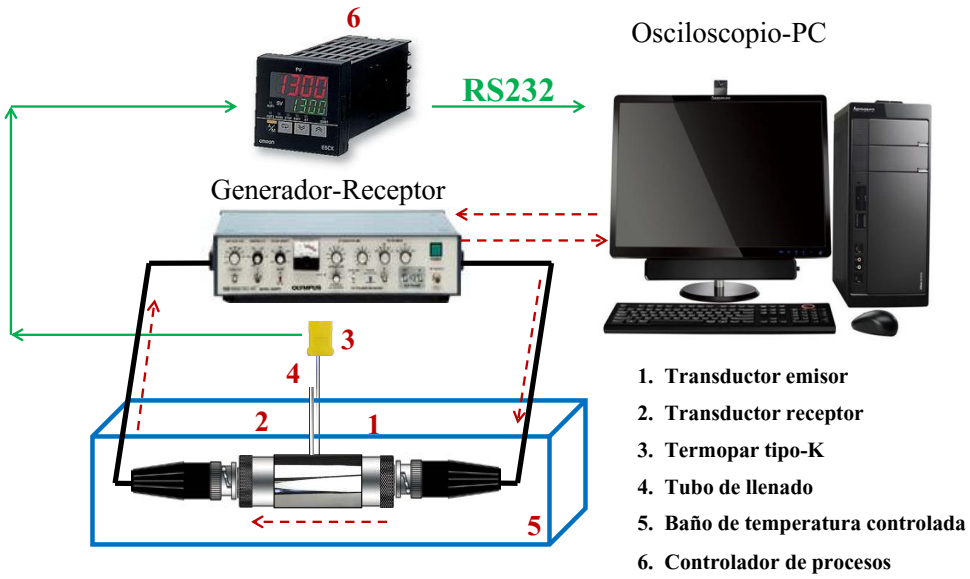


Figura 2. Sistema de medida de las propiedades ultrasónicas en líquidos.

El cilindro de aluminio mecanizado (17.8 mm de diámetro, 25 mm de longitud) tenía insertados y fijados dos transductores focalizados (emisor y receptor) en cada uno de sus lados (Figura 2). El procedimiento de medida comprendía los siguientes pasos. Las muestras líquidas se introducían en el interior del cilindro por uno de los dos tubos de aluminio (tubo de llenado) que existían en la parte central-superior del mismo. El segundo tubo servía para introducir el termopar tipo K, que medía la temperatura de la muestra y que estaba conectado al controlador. El cilindro portamuestras a su vez, era colocado dentro del baño de temperatura controlada. El generador-receptor producía una corriente eléctrica que llegaba al transductor emisor y se transformaba en una onda ultrasónica mediante la vibración del cristal piezoeléctrico del transductor. La onda atravesaba la muestra introducida en el cilindro y llegaba al transductor receptor. Una vez la vibración había sido convertida en señal eléctrica mediante el proceso inverso al producido en el transductor emisor, ésta era recogida mediante el generador-receptor donde se filtraba y amplificaba (filtro de la señal

de 0.3 MHz) para posteriormente ser digitalizada por la tarjeta de adquisición de datos insertada en el PC (amplitud del pulso de excitación del transductor de 1000 V, escala del eje de amplitudes del osciloscopio de 2.0 V/división; una ganancia de 30 dB, adquisiciones a 25 Mmuestras/s y un total de 25000 puntos adquiridos). La señal en formato digital era adquirida, mediante un software específico (Visual Basic, 6.0 Microsoft), que permitió determinar el tiempo que tarda la onda ultrasónica en atravesar la muestra. Así, conociendo la distancia entre los transductores, la velocidad se calcula a partir del tiempo de vuelo y el tiempo que transcurre desde que la corriente eléctrica es generada hasta que se recibe (tiempo de retardo) (1.7 μs), calculado mediante la calibración con agua destilada.

3.2.2 Medidas en medios sólidos

En la presente Tesis Doctoral se realizaron medidas sobre muestras de sistemas modelo de salchichas crudo-curadas envasadas al vacío, así como en jamón Ibérico curado loncheado y envasado al vacío mediante la técnica de *ultrasonidos por contacto*. Por otro lado, se llevaron a cabo medidas ultrasónicas en muestras de jamón loncheado y envasado al vacío, así como en muestras de chorizo y jamón curado mediante técnicas de *ultrasonidos sin contacto*. A continuación, las dos diferentes técnicas se presentan en detalle.

3.2.2.1 Ultrasonidos por contacto

Para realizar las medidas de los ultrasonidos por contacto fue necesario el uso de un dispositivo de medida del espesor de la muestra y de un sistema de medida de ultrasonidos que se detallan a continuación.

- ✓ *Dispositivo de medida del espesor de la muestra.*

Para la correcta determinación de la velocidad de los ultrasonidos en una muestra sólida es necesaria una medida exacta de su espesor. En este sentido, la medida de los espesores de las muestras analizadas mediante ultrasonidos se realizó a través de un dispositivo de medida (Figura 3) diseñado específicamente para esta aplicación y que permite soportar condiciones de trabajo adversas, como medidas en el interior de cámaras de refrigeración.

El sistema permite una amplitud de medida máxima de 27±0.01 cm, que los transductores se encuentren posicionados paralelamente y que la presión ejercida sobre la muestra sea constante. El dispositivo constaba de una plataforma sobre la cual se colocaban las muestras. En la plataforma se fijaba el transductor receptor, mientras que el transductor emisor se sitúa en un brazo deslizante que se encuentra unido a un carro posicionador formado por un tornillo sinfín y un motor paso a paso (motor unipolar paso a paso de 4 fases, RS 440-458, Taiwan). En el carro se encontraba acoplada una célula de carga de aluminio de 40 kg de fuerza máxima (1040-41, Tedea Huntleigh, Canoga Park, USA) y el transductor emisor, que se encontraba adecuadamente alineado y enfrentado con el transductor receptor. Un controlador de presión unido a la célula de carga permitía fijar la presión que los transductores realizan sobre la muestra (45 N). El dispositivo de medida era controlado por un PLC, programado para detener el motor paso a paso cuando la señal del controlador de presión indicaba que se había alcanzado la fuerza de consigna.

Por otra parte, la lectura del carro posicionador, que proporciona el espesor de la muestra, se realizaba mediante un "encoder" relativo (BHK 06.24K500-B6-5, Baumer Electric, Barcelona, España), conectado a un lector de posición que a su vez iba conectado al PC a través de una interfaz RS232. El PLC, el controlador de presión y el lector de posición se situaron en el interior de una carcasa en la que se ubicaban los botones para accionar el sistema (encendido y apagado, subida y bajada del carro).

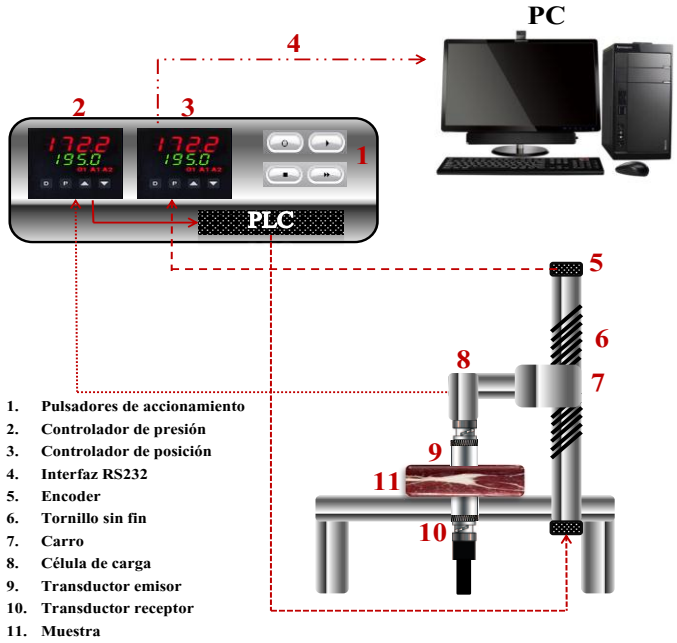


Figura 3. Dispositivo de medida del espesor de la muestra.

✓ *Sistema de medida de ultrasonidos*

Las medidas ultrasónicas se realizaron con transductores piezoeléctricos de banda estrecha (Figura 4). En los *Apartado 3* y *5*, se realizaron medidas en muestras de salchichas crudo-curadas y jamón crudo-curado loncheado y envasado al vacío, respectivamente. Así, con el fin de caracterizar la mayor área posible del alimento, fueron usados dos transductores de 1 MHz y 0.75'' de diámetro de cristal (A314S-SU, Panametrics, Waltham, MA, USA), (Figura 5a). En *Apartado 4*, se utilizaron transductores de cristal de 1 MHz y 0.5'' de diámetro (A303S-SU, Panametrics, Waltham, MA, USA) (Figura 5b). Además en las medidas ultrasónicas, se utilizaron un generador-receptor (5058PR, Panametrics, Waltham, MA, USA) y un osciloscopio-PC digital (TDS5034, Tektronix, Bearverton, Oregon, USA).

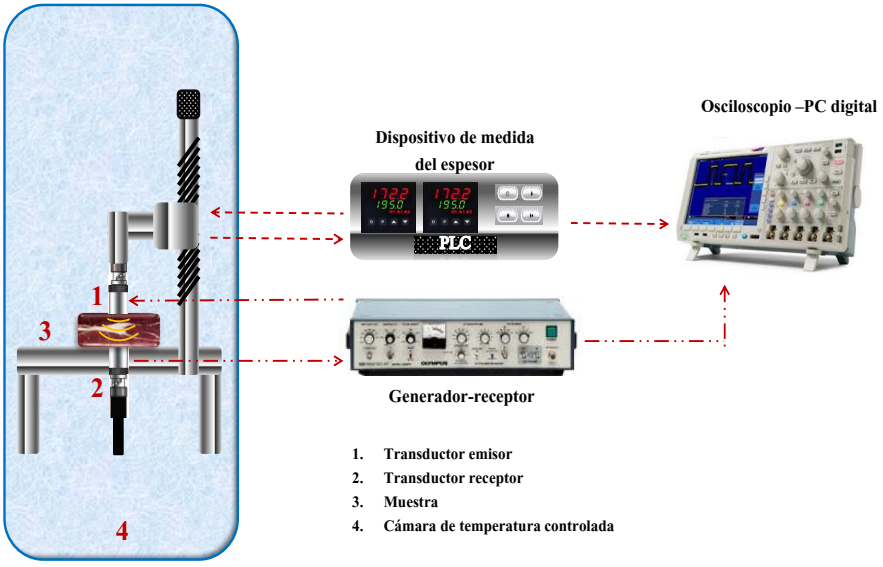


Figura 4. Sistema de medida de ultrasonidos en sólidos.

Figura 5. Transductores piezoeléctricos de 1 MHz de a) 0.75” y b) 0.5”.

La generación y recepción de las señales ultrasónicas se realizó de igual forma que la descrita en el apartado *3.2.1*. Posteriormente, la señal eléctrica filtrada, y amplificada en el receptor era enviada al osciloscopio que la digitalizaba (promedio de 5 señales) y la enviaba a un PC (incluido en el osciloscopio) a través de una interfaz GPIB. En el PC la señal era digitalizada y procesada para determinar el tiempo de vuelo. En este caso, la medida del tiempo

de retardo calculado (6.5 μs) en el sistema ultrasónico se realizó utilizando cilindros de metacrilato de 40 cm de diámetro y diferentes alturas. El cálculo de la velocidad de los ultrasonidos se realizó a partir del tiempo que tarda la onda acústica en atravesar la muestra y el espesor de la muestra con ayuda de un software desarrollado específicamente para esta aplicación en Visual Basic. Tanto las muestras, como el sistema de medida de los ultrasonidos y el de medida del espesor, se colocaron dentro de una cámara de temperatura controlada dado la sensibilidad de la velocidad ultrasónica a la temperatura de la muestra. Para evitar la presencia de aire entre el transductor y la superficie de la muestra, se utilizó como medio de acople aceite de oliva (Figura 6).

Las medidas de la velocidad llevadas a cabo tanto en las muestras de salchichas crudo-curadas, como en los paquetes de jamón Ibérico crudo-curado, se realizaron utilizando una amplitud del pulso de excitación del transductor de 100 V y la escala del eje de amplitudes del osciloscopio de 200 mV/división. Se operó con una ganancia de 30 dB, realizándose adquisiciones a 250 Mmuestras/s, con un total de 25000 puntos adquiridos. Finalmente, se aplicó un filtro de la señal para eliminar las bajas frecuencias (<0.3 MHz).

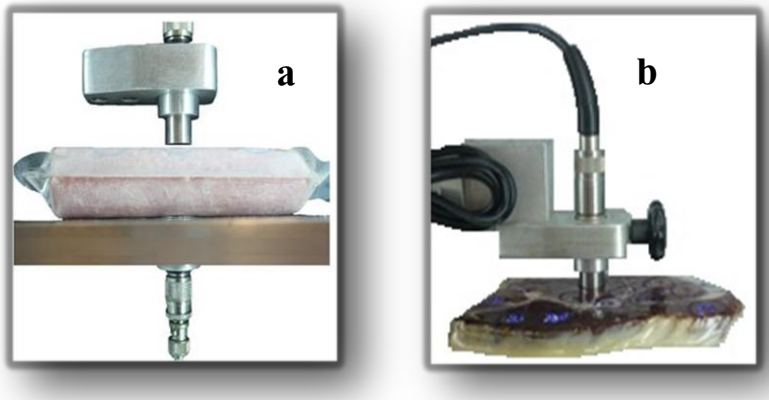


Figura 6. Medida de los ultrasonidos en productos cárnicos crudo-curados. a) Sistemas modelo de salchichas crudo-curadas, b) jamón loncheado y envasado al vacío

3.2.2.2 *Ultrasonidos sin contacto (air-coupled)*

El sistema para realizar las medidas sin contacto (Figura 7) (por aire) constaba de dos transductores (0.75 MHz, 0.79" diámetro de cristal), un generador-receptor (P/R4077, Panametrics, Waltham, MA, USA) y un osciloscopio digital (TDS5034, Tektronix, Bearverton, Oregon, USA). Los transductores fueron diseñados y construidos en el Centro de acústica aplicada y evaluación no destructiva (CAEND) en Madrid, España; por el grupo del Doctor Tomas Gómez Álvarez-Arenas. En el *Apartado 5* del capítulo de Resultados de la presente Tesis Doctoral, se detalla la construcción de los transductores, así como también el procedimiento a seguir para la determinación de las medidas de la velocidad y el espesor (Figura 8).

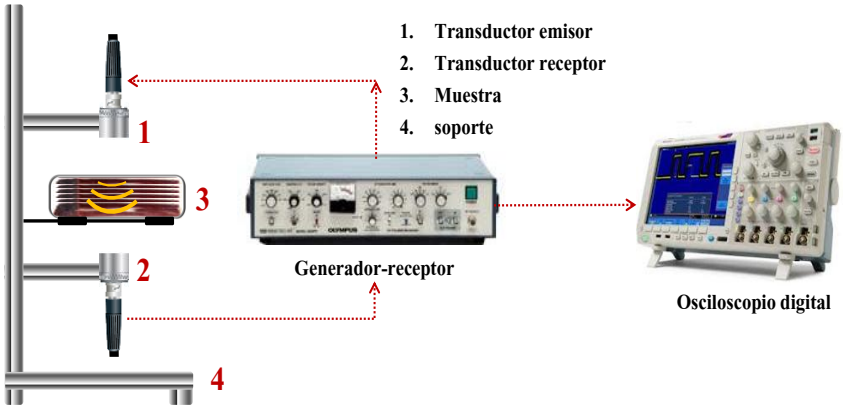


Figura 7. Sistema de medida de ultrasonidos sin contacto.

Figura 8. Medida de los ultrasonidos sin contacto en productos cárnicos crudo-curados

3.2.2.3 *Ultrasonidos sin contacto (Microscopía Acústica)*

Se realizaron medidas de ultrasonidos sin contacto para caracterizar microscópicamente muestras de jamón crudo-curado y chorizo. El microscopio acústico de barrido (Figura 9) consistía en una plataforma de barrido acústica (Figura 10), un generador-receptor (320, UTEX, Ontario, Canadá) y un osciloscopio digital (Xi-64, Lecroy Waverunner, Berkshire, Reino Unido). El equipo se encuentra localizado en las instalaciones de la Universidad de Leeds (Reino Unido).

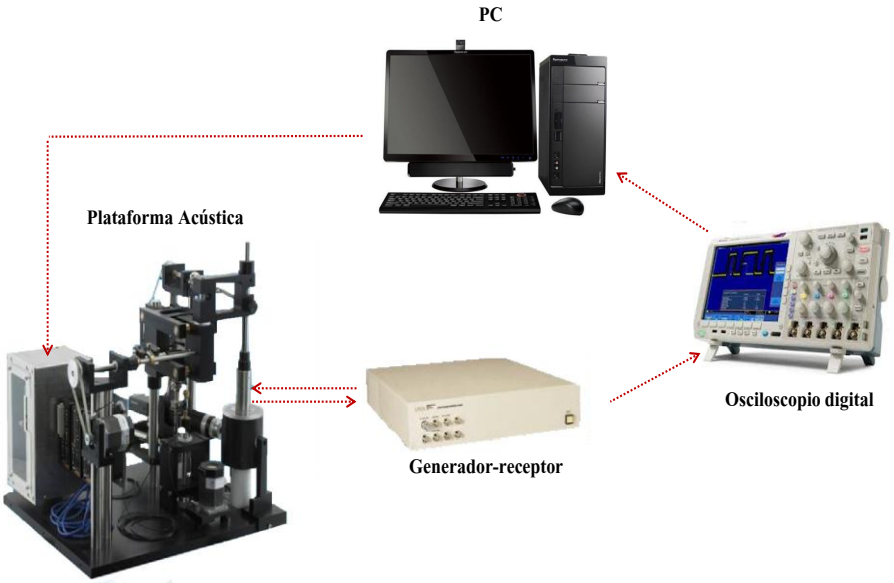
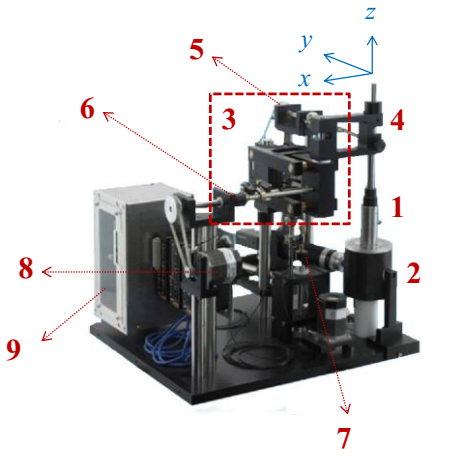


Figura 9. Sistema de microscopía acústica mediante ultrasonidos.

La plataforma acústica (Figura 10) consistía en un sistema de posicionamiento con un brazo vertical donde en su extremo inferior se encontraba conectado un único transductor (10 MHz, 0.5'' diameter, V311, Panametrics, Waltham, MA, USA). Por lo tanto, las medidas se realizaron mediante el modo pulso-eco, utilizando un transductor de inmersión focalizado, con una distancia focal de 6 mm y una resolución de 300 μm.



1. **Brazo vertical con transductor emisor - receptor**
2. **Unidad portamuestras termostatada**
3. **Sistema de posicionamiento**
4. **Brazo vertical (3D)**
5. **Control del eje *y***
6. **Control del eje *x***
7. **Control del eje *z***
8. **Motor paso a paso**
9. **Controlador del motor paso a paso**

Figura 10. Plataforma acústica

El sistema de posicionamiento combina una alta precisión espacial con un amplio rango de movimiento. El mismo se encuentra conformado de brazos y articulaciones rotacionales basados en el movimiento circular en las tres dimensiones (*xyz*). Este sistema cuenta con un brazo vertical móvil en el que se conecta el transductor y permite centrar el transductor en la posición deseada de la muestra. De esta manera, la plataforma acústica permite realizar el posicionamiento automático del transductor en las tres dimensiones (*xyz*). Para realizar las medidas, es necesario el uso de un medio de acople líquido (agua Milli-Q) que permita que la señal ultrasónica pueda llegar hasta la muestra adecuadamente. La plataforma acústica cuenta con una unidad portamuestras termostada (±0.01 ºC) donde se deposita la muestra y el medio de acople, (Figura 11).

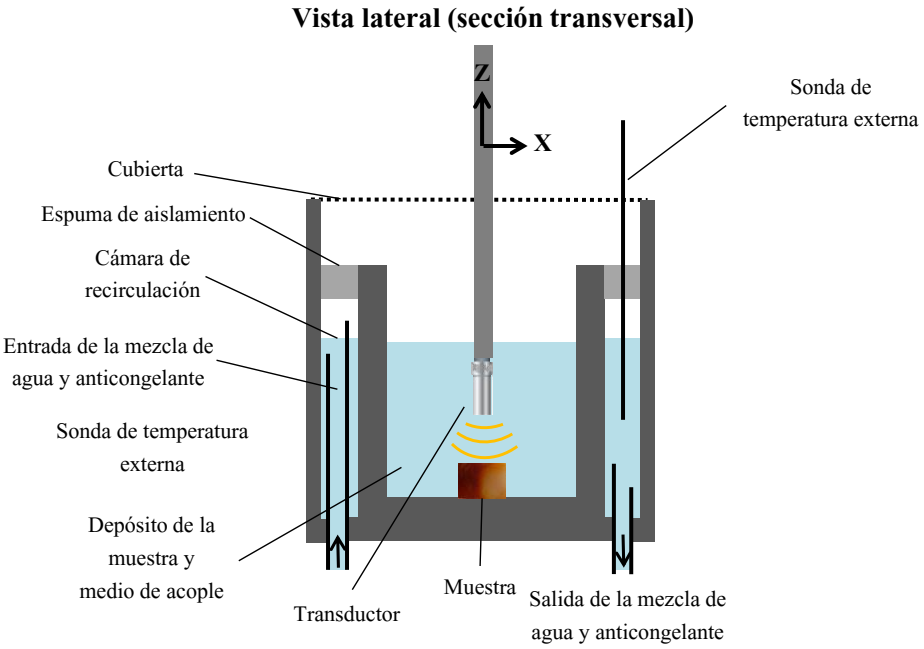


Figura 11. Unidad portamuestras termostatada utilizada para las medidas de microscopía acústica.

La unidad portamuestras (Figura 11) dispone de una camisa externa donde se recircula una mezcla de agua y anticongelante para mantener y controlar la temperatura de la muestra y el medio de acople. El líquido refrigerante se impulsa mediante una bomba de un baño de temperatura controlada (Haake B5/DC50, Basingstoke, UK) que permite regular la temperatura de la camisa y todo el baño de la muestra, en un rango de 0 a 80 ºC. El microscopio de barrido acústico puede operar en tres modos de digitalización de imagen: A-scan, B-scan y C-scan. El A-scan permite obtener una imagen a partir de la medición del tiempo de vuelo realizado en una posición única. El B-scan consiste en la realización de varias medidas del tiempo de vuelo realizadas lateralmente a través de la muestra (recorriendo el eje *x* o *y*). Por último el modo C-scan consiste en llevar a cabo mediciones en un área (recorriendo los ejes *x* e *y*).

El sistema es completamente automático, así, desde el software de control se le indica a la plataforma acústica la posición de partida en la que

debe colocarse el transductor sobre la muestra y la dirección en la cual debe realizar el barrido de la misma, así como el modo de digitalización de la imagen. Una vez posicionado el transductor sobre cada punto de medida, el ordenador envía una señal al generador-receptor, el cual proporciona un impulso de onda cuadrada de 50 ns de duración y una amplitud de 300 V al transductor emisor. Así, este transductor emite la onda de ultrasonidos que viaja a través del medio de acople atravesando la muestra, se refleja sobre la base del baño que contiene la muestra y es recibida por el transductor que en este caso actúa como receptor. El transductor puede recibir reflexiones de la señal sobre diferentes interfaces, las cuales al llegar al transductor son convertidas en impulsos eléctricos. La señal resultante de las diferentes reflexiones es filtrada y amplificada en el receptor y enviada al osciloscopio que la digitaliza y exporta a un PC donde era almacenada para su posterior análisis. Para la obtención de las imágenes se desarrolló específicamente un software utilizando Matlab® R2011. Las imágenes eran construidas a partir de la intensidad de la reflexión de la señal de los ultrasonidos representada en una escala de color. Así, el color blanco correspondió a la amplitud 0 de la señal y el color negro a la máxima amplitud de reflexión de la misma.

4. RESULTS

CHAPTER 1

(*Apartado 1*)

ULTRASONIC MONITORING OF LARD CRYSTALLIZATION DURING STORAGE

J.V. Santacatalina, J.V. García-Pérez, E. Corona, J. Benedito

Food Research International

(2011), 44, 146-155

Ultrasonic monitoring of lard crystallization during storage

J.V. Santacatalina, J.V. García-Pérez, E. Corona, J. Benedito

Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento Tecnología de Alimentos, Universidad Politécnica de Valencia, Camí de Vera s/n, E46022, Valencia, Spain.

Abstract

This work addresses the use of ultrasonics as a non-destructive technique with which to monitor lard crystallization during cooling and storage. The ultrasonic velocity was measured during both the isothermal crystallization of lard (at 0, 3, 5, 7, 10 and 20 ºC) and during the non-isothermal crystallization. In addition, lard crystallization was also studied through Differential Scanning Calorimetry (DSC) and instrumental texture analysis (penetration tests). The measurement of the ultrasonic velocity allowed the bulk crystallization to be detected. The evolution of the ultrasonic velocity and the textural measurements during isothermal crystallization showed two steep increases, which may be explained by the two fractions of triglycerides found in DSC thermograms. At 7, 10 and 20 ºC the second fraction did not crystallize within the first 11 days of storage. A two-step crystallization model based on the Avrami model was used to properly describe (%var >99.9 and RMSE<1.99 m s^{-1}) the relationship between the ultrasonic velocity and the isothermal crystallization time. In addition, a model was developed to estimate the percentage of solid fat content during isothermal crystallization. Therefore, it may be pointed out that ultrasonic techniques could be useful to monitor the crystallization pattern of complex fats during long periods of storage.

Key words: *Ultrasound; Crystallization; Storage; Fat; Modeling*

1. Introduction

Rendered lard is the fat obtained by rendering the fatty tissue of the pig. It is mainly used for cooking or as a raw material for manufacturing other products, such as bakery products or pastries. Lard may be considered as a mixture of several fatty acids. The composition of lard largely varies according to the breed, the feeding, the pig's activity and the weather conditions (Graciani, 2006), but it mainly consists of a few long chain major fatty acids: C14 1.5 %, C16 27.3 %, C16:1 2.3 %, C18 16.6 %, C18:1 36.6 % and C18:2 9.4 % (Ling-Zhi, Hong, Yuan, & Xuebing, 2009).

An understanding of the fat crystallization process plays a critical role in determining the overall product quality (Foubert, Vanrolleghem, Vanhoutte, & Dewettinck, 2002). Moreover, the kinetics of fat crystallization is important for controlling operations in the food industry in order to achieve the desired product characteristics (Metin & Hartel, 1998). Several properties of fat-containing products, such as appearance, texture, mouthfeel and stability, are related to the characteristics of the crystal network formed by its constituent lipid species (Narine & Marangoni, 2002). The macroscopic properties of this fat crystal network are influenced by the chemical composition, the solid fat content (SFC) and the polymorphism, size, shape and distribution of the crystals (deMan, 1999). Fat crystallization is also influenced by the cooling rate. In this regard, fast lard crystallization leads to higher SFC levels and a large number of heterogeneously distributed, small crystals with a less stable conformation, producing a harder fat (Campos, Narine, & Marangoni, 2002).

On-line measurement of food properties can lead to an improvement in the product quality and profitability of manufacturing processes (Martini, Bertoli, Herrera, Neeson, & Marangoni, 2005a). These measurements provide information about the product characteristics (temperature, composition, structure, etc.) that can be useful for monitoring and controlling food quality (Benedito, Cárcel, Rosselló, & Mulet, 2001). The SFC measures the percentage of crystallized fat at a given temperature and is a good indicator of the functional characteristics of the fat at that temperature. Thus, measuring the SFC has been widely used in the quality control of fats and fat-containing products (Foubert,

Dewettinck, Janssen, & Vanrolleghem, 2006). Several techniques have been used to study the SFC of foods, such as differential scanning calorimetry (DSC) (Metin & Hartel, 1998; Toro-Vázquez, Briceno-Montelongo, Dibildox-Alvarado, Charo-Alonso, & Reyes-Hernández, 2000), pulsed Nuclear Magnetic Resonance (pNMR) (Wright, Narine, & Marangoni, 2001; Singh, McClements & Marangoni, 2004), NMR-MOUSE (NMR mobile universal surface explorer) (Martini, Herrera, & Marangoni, 2005c), polarized light microscopy (Saggin & Coupland, 2002; Singh et al., 2004), dilatometry (Timms, 2005), transmittance/turbidity measurements (Marangoni, 1998), light scattering techniques (Wright, Hartel, Narine, & Marangoni, 2000) and X-ray diffraction (Kloek, Walstra, & Van Vliet, 2000; Kalnin, Lesieur, Artzner, Keller, & Ollivon, 2005).

Low intensity ultrasound has been widely used to determine the physicochemical properties of many foods (Coupland, 2004). This technique can be used to measure food properties such as the composition, structure and physical state. In general, ultrasonic velocimetry techniques are capable of rapid, accurate and non-destructive measurements, and are particularly suitable for on-line measurements. Ultrasonic velocity measurements have been used to estimate the solid fat content of oils and fats such as anhydrous milk fat and cocoa butter (Miles, Fursey, & Jones, 1985; Singh et al., 2004) and used to determine the chemical structure of liquid triglycerides (Javanaud & Rahalkar, 1988; McClements & Povey, 1988a). Moreover, ultrasonic techniques have been used to take on-line measurements of the SFC in several fats (Martini et al., 2005a, c; Martini, Bertoli, Herrera, Neeson, & Marangoni, 2005b). Martini et al. (2005a) used ultrasonic velocity measurements to carry out on-line monitoring of the crystallization process of edible fats. These authors observed that the velocity increased when the temperature of the sample decreased until reaching the crystallization temperature. Thereafter, velocity remained constant until crystallization began, which produced a new velocity increase.

Due to the importance of the fat crystallization to the overall product quality, it is also relevant to use mathematical models to predict the fat crystallization pattern (Foubert, Dewettinck, & Vanrolleghem, 2003). Several of

these models have been used to describe the isothermal crystallization kinetics of fats. Berg & Brimberg (1983) confirmed that empirical equations used for aggregation and flocculation could also be used to describe fat crystallization. These authors used the experimental results of palm oil and hardened soy oil obtained from the literature. Foubert et al. (2002) developed a new model to describe the crystallization of cocoa butter and milk fat, available in both an algebraic and a differential equation form. However, the Avrami and Gompertz empirical models are by far the most frequently used in the literature to describe the isothermal crystallization of fats and oils (Wright et al., 2000; Vanhoutte, Dewetinck, Foubert, Vanlerberghe, & Huyghebaert, 2002).

The aim of this work was to evaluate the feasibility of using ultrasonics to monitor lard crystallization during storage and to develop a mathematical model to estimate the SFC during the crystallization of lard.

2. Materials and methods

2.1 Raw material

The experiments were carried out using Iberian rendered lard (El Pozo Alimentacion S.A., Alhama de Murcia, Spain) purchased in a local market. The lard was packaged in plastic tubs of 400 g. The ingredients declared by the manufacturer on the label were Iberian lard and antioxidants E-321 and E-320.

2.2 Differential scanning calorimetry (DSC)

The thermal behavior of the samples was analyzed by DSC (DSC5200CU, Seiko Instruments, Inc, Torrance, CA, USA). An empty aluminum crucible was used as the reference material and liquid nitrogen was used as the cooling fluid. To perform the analysis, from 18 to 20 mg of dry sample were introduced in the aluminum crucible. The lard was dried in an oven at 105 ºC for 24 hours to avoid the influence of water. The crucible was hermetically sealed and weighed on a balance (± 0.01 mg, ER-182A, AND, Tokyo, Japan).

During DSC experiments, the temperature was raised from 25 to 70 ºC at a heating rate of 5 ºC min^{-1}. Then, the sample was tempered at 70 ºC for 1 min to destroy any crystal memory and, afterwards, it was cooled from 70 to -50 ºC

using 5 cooling rates: 0.2, 0.5, 1, 5 and 10 ºC min^{-1}. Thereafter, temperature was held at -50 ºC for 30 min. Finally, the sample was heated from -50 to 55 ºC at 5 ºC min^{-1}. Following this procedure, a DSC curve was obtained for the cooling of each sample at the 5 different cooling rates tested. The peak maximum temperature (T_{max}, ºC), onset temperature (T_o, ºC) and latent heat (ΔH, J/g) were computed from the thermograms (Cebula & Smith, 1992).

2.3. Ultrasonic set-up

The experimental set-up used in the ultrasonic measurements is depicted in Figure 1. The sample was introduced in the aluminum cylindrical, ultrasonic cell (diameter 17.8 mm, length 25 mm). Two (emitter and receiver) narrow band piezoelectric transducers (1MHz, 0.5'' crystal diameter, A303S, Panametrics, Waltham, MA, USA) were inserted in each side of the cell. The temperature was measured by using a K-type thermocouple (Figure 1) placed in the center of the cell. The cell was placed in a temperature-controlled bath (± 0.1 ºC, Frigiterm, P-selecta, Abrera, Spain). The ultrasonic pulser-receiver instrument (5058PR, Panametrics, Waltham, MA, USA) excited the emitter through an electrical spike pulse, which turned into an ultrasonic wave due to the vibration of the transducer piezoelectric crystal. The signal that reached the receiver-transducer was filtered and amplified by the pulser-receiver and thereafter captured and digitized by a data acquisition card (PCI 5112, National Instruments, Austin, USA). Specific software was programmed in Visual Basic and used to determine the ultrasonic velocity from the digitized signal, by considering the time of flight and the distance between transducers. Time of flight is defined as the time that the ultrasonic wave needs to go through the sample, while the distance between the transducers was calculated using distilled water.

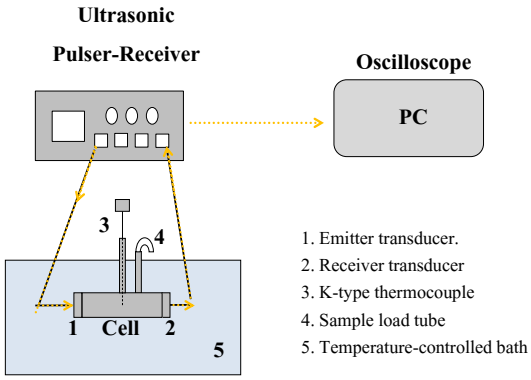


Figure 1. Diagram of the ultrasonic equipment for monitoring lard crystallization.

In order to take ultrasonic measurements, a sample of lard, previously stored at 2 ºC, was heated at 80 ºC for 15 min to ensure the complete destruction of the fat crystals. After heating, the sample was manually stirred until a temperature of 50 ± 0.1 ºC was reached, then, it was placed into the ultrasonic cell (Figure 1), which had been tempered in a water bath at 40 ºC for 10 min. Once the sample reached a homogeneous temperature of 40 ºC, it was introduced into the temperature-controlled bath (Figure 1) where six different storage temperatures were tested: 0, 3, 5, 7, 10 and 20 ºC. The ultrasonic velocity measurements were taken every 10 minutes during storage times ranging between 62 and 263 hours. In that way, a non-isothermal experiment was conducted from the initial sample temperature up to the bath temperature. Once the temperature was reached, the isothermal storage experiment began.

In the non-isothermal period, the average cooling rate was calculated as the ratio of the difference between the initial sample temperature (40 ºC) and the storage temperature and the time that samples needed to reach the storage temperature. Thereby, cooling rates from 1.4 to 3.6 ºC min^{-1} were tested, which allowed the evaluation of the influence of the cooling rate on the ultrasonic velocity, until the temperature of the bath was reached. In this case, the velocity was measured, at least, for each degree of temperature variation.

2.4. Instrumental texture analysis

Instrumental texture analysis was performed in order to study the possible texture changes that could take place during isothermal crystallization and that could be linked to velocity changes. For that purpose, the lard was previously melted at 80 °C for 30 min in a vacuum oven and then it was placed in Petri dishes (60 ml) and manually stirred until reaching 50 °C. The lard samples in Petri dishes were stored in a temperature-controlled chamber at 0±0.5 ºC. The textural properties of the lard samples were analyzed by penetration tests using a universal texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, England) placed in the same temperature-controlled chamber, which allowed the temperature of the samples to be kept constant during the experiments. Penetration tests were performed every hour for 105 hours using a conical probe with an angle of 40º. For each measurement, a different Petri dish was used, in which 5 different penetrations were carried out and averaged. The penetration distance was of 5 mm and the test speed of 1 mm s^{-1}. The maximum force values (MF, N) were computed from the force versus distance records.

2.5. Modeling of ultrasonic velocity

The empirical Avrami and Gompertz models are among the most popular mathematical expressions to describe the isothermal crystallization of oils and fats. The Avrami model (Avrami, 1940; equation 1) is the equation most frequently used to describe the isothermal crystallization kinetics of fats (Toro-Vazquez et al., 2000; Wright et al., 2000).

$$f(t) = a' \cdot \left(1 - \exp\left(-k \cdot t^{n}\right)\right) \tag{1}$$

where f is the amount (%) of solid fat at time t (s), a' (%) is the value for f when t approaches infinity, k is the crystallization rate constant (s^{-n}) dependent on the crystallization temperature and n is the Avrami exponent (dimensionless).

Due to the fact that ultrasonic velocity has been linked to the solid fat content (SFC) for different types of fats (Singh et al., 2004, Martini et al., 2005a), the Avrami model was used to describe the ultrasonic velocity changes

during the isothermal crystallization of lard. Thus, the ultrasonic velocity as a function of crystallization time is depicted in equation 2.

$$V(t) = a \cdot \left(1 - \exp\left(-k \cdot t^{n}\right)\right) \tag{2}$$

where V is the ultrasonic velocity (m s^{-1}) at time t (s) and a the ultrasonic velocity at infinite time.

The Gompertz model has been frequently reported to describe bacterial growth. Based on the similarities found between bacterial growth and fat crystallization, Kloek et al. (2000), Vanhoutte (2002) and Vanhoutte et al. (2002) fitted crystallization curves to a re-parameterized Gompertz equation (equation 3), as reported by Zwietering, Jongenburger, Rombouts, & Van't Riet (1990).

$$f(t) = a' \cdot \exp\left\{-\exp\left[\frac{\mu \cdot e}{a'}(\lambda - t) + 1\right]\right\} \tag{3}$$

where e is a constant value: 2.718281, μ is the maximum increase rate in crystallization (m s^{-2}) and λ is the time (s) needed to start the exponential increase of velocity.

As for the Avrami model, the Gompertz equation was modified to estimate the ultrasonic velocity during the isothermal crystallization of lard (equation 4).

$$V(t) = a \cdot \exp\left\{-\exp\left[\frac{\mu \cdot e}{a}(\lambda - t) + 1\right]\right\} \tag{4}$$

2.6. Modeling of solid fat content

From the ultrasonic velocity values, the percentage of solid fat as a function of crystallization time can be estimated. To this end, a model based on the equation used by Singh et al. (2004) was developed (equation 5).

$$\frac{1}{V^2} = \frac{\theta_L}{V_L^2} + \frac{\theta_S}{V_S^2} \tag{5}$$

where θ_L and θ_S are the liquid and solid fractions of the lard sample ($\theta_L + \theta_S = 1$) for the same temperature at which the ultrasonic velocity was measured and V_L and V_S are the ultrasonic velocities when the sample is fully liquid or solid at that temperature, respectively. Measuring the ultrasonic velocity and using equation 5, the percentage of solid fat content (SFC= $100 \cdot \theta_S = (1 - \theta_L) \cdot 100$) may be calculated when V_L and V_S are known.

2.7. Statistical analysis

The fit of the Avrami and Gompertz models to the experimental data was performed using the Solver optimization tool of Microsoft Excel spreadsheet 2003, which applies the optimization method of Generalized Reduced Gradient. The model parameters (Avrami: *n, k, a*; Gompertz: *a*, μ, λ) were identified by minimizing the sum of the squared differences between the experimental and estimated ultrasonic velocities.

The goodness of the fit of each model to the experimental data was evaluated by the percentage of explained variance (% var, equation 6) (Berthouex & Brown, 1994; Simal, Benedito, Clemente, Femenia, & Rosselló, 2003) and the root mean square error (RMSE, equation 7) between the experimental and the estimated data (Váquiro, Bon, & Díez, 2008).

$$\%\,\mathrm{var} = \left(1 - \frac{S_{YX}^2}{S_Y^2}\right) \cdot 100 \tag{6}$$

$$RMSE = \sqrt{\frac{\sum (V_{\exp} - V_{calc})^2}{N}} \tag{7}$$

The percentage of explained variance represents the relative variance explained by the model (S_{YX}, equation 8) regarding the total variance (S_Y, equation 9).

$$S_{YX} = \frac{\sum (V_{calc} - V_{\exp})^2}{N - 2} \tag{8}$$

$$S_Y = \frac{\sum (V_{\exp} - \overline{V}_{\exp})^2}{N - 1} \quad (9)$$

V_{calc} and V_{exp} being the calculated and experimental ultrasonic velocities, respectively, $\overline{V}_{\exp}$, the average of the experimental ultrasonic velocity data and *N*, the total number of data.

3. Results and discussion

3.1. Monitoring non-isothermal lard crystallization by DSC

Differential scanning calorimetry analyses were performed in order to characterize the thermal behavior of the lard. Five cooling rates (10, 5, 1, 0.5 and 0.2 ºC min^{-1}) were used to study their influence on the fat crystallization process. The five DSC curves obtained for the tested cooling rates are shown in figure 2. From the DSC curve at the lowest cooling rate (0.2 ºC min^{-1}), two main peaks were found. These peaks were related to the crystallization of different fractions of lard triglycerides (Campos et al., 2002). Thus, peak C could be related to the crystallization of a fraction containing more unsaturated triglycerides, while peak A-B may be linked to the crystallization of more saturated triglycerides.

When the cooling rate increased, several phenomena were observed: the splitting of peak A-B into two (A and B), peak displacement to lower temperatures and peak broadening. The cause of these phenomena is related to the short crystallization time at high cooling rates (Cebula & Smith, 1991). Due to these fast rates, the time to crystallize at a given temperature is short, leading to supercooling (peak displacement). In addition, the short crystallization time also meant that triglycerides with similar crystallization temperatures did not crystallize simultaneously, causing the observed peak split and broadening.

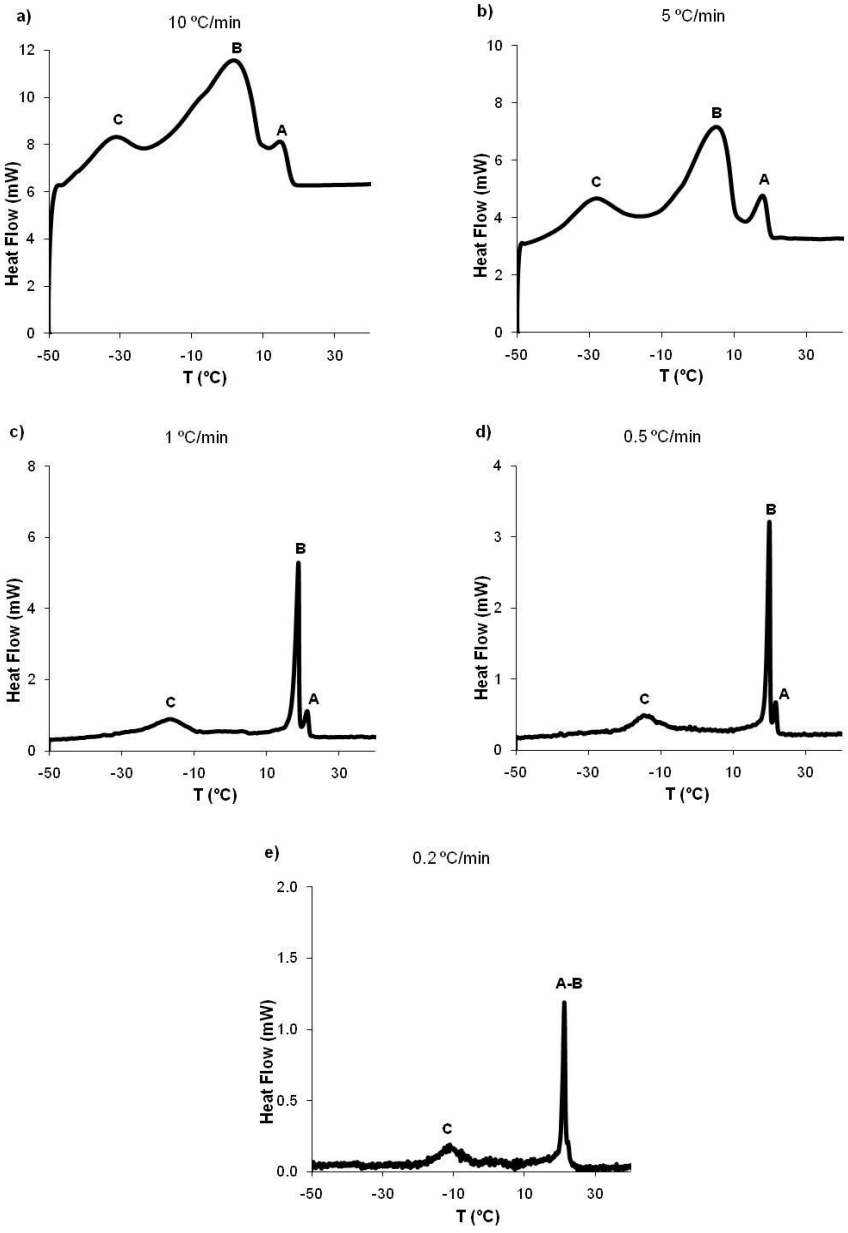


Figure 2. DSC curves of Iberian lard at different cooling rates: a) 10 ºC/min; b) 5 ºC/min; c) 1 ºC/min; d) 0.5 ºC/min; e) 0.2 ºC/min.

For all the thermograms, peak onset temperature (T_o), maximum temperature (T_{max}) and latent heat (ΔH) of the crystallization peaks were computed (Table 1). The latent heat of peaks A and B were jointly determined due to the impossibility of separately calculating each one of them, since both peaks were very close or merged depending on the cooling rate. The peak onset temperature and maximum temperature of all the peaks decreased when the cooling rate increased as a consequence of the already mentioned supercooling effect. In particular, T_o of peak A decreased from 23.2 to 18.3 ºC when the rate increased from 0.2 to 10 ºC min^{-1} and T_{max} of peak C fell from -11.1 to -31.1 ºC at cooling rates of 0.2 and 10 ºC min^{-1}, respectively (table 1).

Table 1. Results of the DSC analyses of lard at different cooling rates.

Cooling rate (ºC/min)	Peak	ΔH (J/g)	T_{max} (ºC)	T_o (ºC)
10	Peak A	36.1±3.1[a]	14.7±0.6[a]	18.3±0.3[a]
	Peak B		1.8±0.4[a]	9.6±0.6[a]
	Peak C	9.0±2.3[a]	-31.1±1.1[a]	-21.0±0.8[a]
5	Peak A	37.2±2.9[a]	17.8±1.1[b]	20.0±0.9[b]
	Peak B		5.1±1.3[b]	11.3±1.0[b]
	Peak C	14.7±2.6[b]	-28.1±0.8[b]	-13.5±0.3[b]
1	Peak A	26.3±1.1[b]	21.3±1.2[c]	22.0±1.0[c]
	Peak B		18.9±0.2[c]	19.4±0.4[c]
	Peak C	13.3±0.9[b]	-16.7±0.1[c]	-9.5±0.1[c]
0.5	Peak A	27.3±1.2[b]	21.9±0.8[c]	22.5±0.7[d]
	Peak B		20.1±0.6[d]	20.6±0.5[d]
	Peak C	11.3±1.0[b]	-14.7±0.3[d]	-7.6±0.4[d]
0.2	Peak A-B	28.2±0.9[b]	22.4±0.7*	23.2±0.5*
	Peak C	12.5±0.8[b]	-11.1±0.2[e]	-5.4±0.2[e]

Average ± Standard deviation.

a, b, c, d, e: different letters in the same column and for the same peak indicate a significant difference ($p<0.05$)

*Not considered in the ANOVA since peaks A and B are merged.

According to Campos et al. (2002), the cooling rate not only displaces the crystallization temperatures, but also affects the fat crystals' morphology and stability. Using X-ray diffraction, these authors found two polymorphic forms of the crystals in lard samples. The polymorphs observed were the *β'* phase, when

lard was quickly crystallized, and both the $\beta' + \beta$ phases for slow crystallization rates. The polymorphic form of a fat is related to its chemical composition and crystallization conditions. At high cooling rates of the sample, the crystal formation is less stable because the molecules are forced to crystallize rapidly producing broader peaks and lower temperatures than when using low cooling rates. Similar results were reported by Kalnin et al. (2005) in DSC experiments of lard. These authors studied polymorphism by X-ray diffraction and used different cooling rates of between 10 and 0.15 ºC min^{-1} observing different crystalline forms depending on the cooling rate and the final temperature. Less stable forms (α) were formed at high rates, which were transformed into more stable forms (β and β') during storage. Thereby, at least 3 peaks (depending on the cooling rate) were identified, but in every case, the higher the cooling rate, the lower the onset temperature. Therefore, the results obtained in this work agree with that found in previous literature.

3.2. Monitoring non-isothermal lard crystallization by ultrasonic measurements

Ultrasonic velocity measurements were taken during the cooling stage. As already mentioned in section 2.3, the average cooling rate was calculated as the ratio of the difference between the initial sample temperature (40 ºC) and the storage temperature and the time that samples needed to reach the storage temperature. In this way, cooling rates between 1.4 and 3.6 ºC min^{-1} were tested.

Figure 3 shows the variation of the ultrasonic velocity with the temperature at different average cooling rates. A similar shape of the curve was found in every case: at high temperatures, a first linear part in which the ultrasonic velocity is inversely related to the temperature, which corresponds to the temperatures where the lard remains completely liquid. Then, an unstable area where the onset of crystallization led to a higher velocity increase in line with the decrease of temperature, followed by a sudden increase of the temperature and velocity, due to the bulk crystallization (Figure 3). The temperature increase is explained by the latent heat released during crystallization. Finally, another area of an almost linear increase of the ultrasonic velocity in line with the drop in temperature is observed, while the crystallization process continues. Thus, the crystals that began to form during the temperature decrease (non-isothermal

crystallization) should include the more saturated fraction of triglycerides observed in the DSC curves (peak A-B, figure 2) since the onset crystallization temperature in the thermograms (23.6 ºC at 1 ºC min^{-1}) is similar to the temperature of the bulk crystallization observed in figure 3 (23.5 ºC at 1.4 ºC min^{-1}).

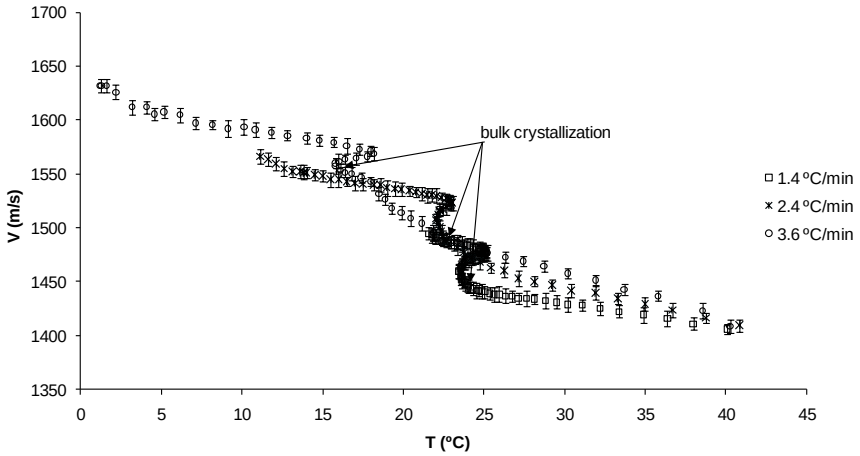


Figure 3. Influence of the cooling rate on the ultrasonic velocity variation during the cooling of lard.

In general terms, the experimental results showed that the ultrasonic velocity increased as the solid fat content increased (Saggin & Coupland, 2002; Singh et al., 2004) and the sample temperature decreased. These results coincide with previous studies carried out on other fats such as cheese fat (Mulet, Benedito, Bon, & Sanjuán, 1999), olive oil (Benedito, Mulet, Velasco, & Dobarganes, 2002) or Iberian pig backfat (Niñoles, Clemente, Ventanas, & Benedito, 2007). The results also showed that the cooling rate affected the ultrasonic velocity measurements, since for the same temperature, the higher the cooling rate, the higher the ultrasonic velocity. This is because the heat transfer between the high viscosity liquid lard and the cylinder walls, the temperature of which is similar to that of the bath due to the high conduction of the aluminum and the great turbulence in the bath, occurs mainly by conduction, since the sample was not agitated within the cylinder. Thus, lard began to solidify during crystallization close to the walls and ended in the center. It should be taken into

consideration that the higher the cooling rate, the higher the temperature gradient from the walls to the center of the sample where the temperature is measured (Figure 1). As a consequence, for the same temperature at the center of the sample, lower temperatures from the walls to the center should be found when using high rather than low cooling rates leading to a lower average temperature. Therefore, this lower average temperature, even one that can lead to crystallization starting close to the wall, produces a higher ultrasonic velocity since the wave crosses the whole sample in the cylinder and is affected by the average physical properties determined by the average sample temperature.

The cooling rate also affected the onset temperature of the bulk crystallization. It was reduced as the cooling rate was increased (approximately, 23.5 ºC for cooling at 1.4 °C min^{-1}, 22.5 ºC at 2.4 ºC $min^{-1}$and 15.8 ºC at 3.6 ºC min^{-1}, Figure 3). Thus, if the cooling rate increases, a supercooling of the sample occurs, crystallizing at lower temperatures. Therefore, the onset of crystallization is related to the cooling rate and conditions. This fact was also found in the DSC experiments (Figure 2), where a supercooling was observed at high cooling rates, leading to a peak displacement to lower temperatures. Thereby, ultrasonic measurements could be used to study fat crystallization as an alternative technique to DSC. Singh et al. (2004) concluded that the differences between the ultrasonic velocities in fat applying different cooling rates depended on the polymorphism and microstructure of the crystals. According to these authors, ultrasonic velocity is a reliable indicator of the onset of crystallization in fat.

3.3. Monitoring isothermal lard crystallization by ultrasonic measurements

Ultrasonic velocity measurements were taken in isothermal crystallization experiments in order to study the lard behavior during cold storage. Lard samples were analyzed at different crystallization temperatures: 0, 3, 5, 7, 10 and 20 ºC and velocity curves were represented versus crystallization time (Figure 4). For the same crystallization time, the lower the storage temperature, the higher the ultrasonic velocity. Two steep increases were observed in the velocity curves obtained at 0, 3 and 5 ºC, which suggests that, at these temperatures, crystallization occurred in two steps during storage. However, a single step was observed at 7, 10 and 20 ºC. The first increase appeared during the first hour and

it may be linked to the crystal formation of the most saturated fraction found by DSC (peak A-B, figure 2). This fraction began to crystallize with the cooling of the sample, as mentioned in section 3.2, and ended in the initial period of the isothermal crystallization. Once the most saturated fraction was fully crystallized, the velocity remained constant for several hours. The length of this constant velocity period was dependent on the storage temperature; the higher the temperature, the longer the constant period. Thereby, the start of the second velocity increase was delayed, 13 hours for 0 ºC, 32 hours for 3 ºC and 84 hours for 5 ºC, and it was linked to the crystallization of more unsaturated triglycerides of lard. This second increase also ended in a constant velocity period. As already mentioned, the experiments at 7, 10 and 20 ºC only presented the first increase of the ultrasonic velocity and the first constant velocity period lasted until the end of the storage (Figure 4). This behavior suggests that the most unsaturated triglyceride fraction did not crystallize in 263 storage hours at 7, 10 and 20 ºC. The crystallization of the different fraction of triglycerides should be linked to a change in the physical properties of the samples, in particular, their texture.

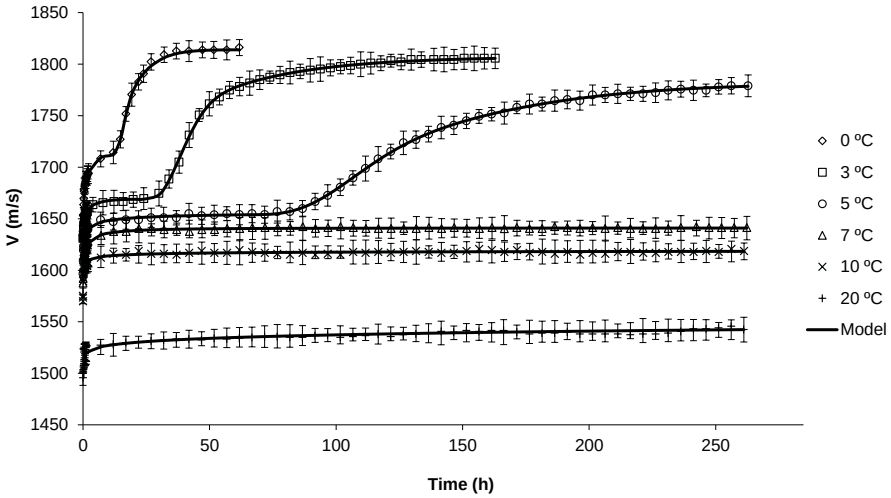


Figure 4. Changes and modeling of the ultrasonic velocity during the isothermal crystallization of lard at different storage temperatures. Note: To improve figure art quality only one measurement each five hours is plotted.

The results of the instrumental texture experiments during lard storage at 0 ºC show a similar pattern to that found for velocity. Thereby, two steep increases of the maximum penetration force values were also observed during the storage (Figure 5). The first increase was observed during the first hour and was related, as was velocity, with the crystallization of the more saturated fraction of lard triglycerides. Then, the maximum force remained constant until the twelfth hour of storage, when the second increase began. As already mentioned, this increase was linked to the crystallization of the more unsaturated fraction of triglycerides. The maximum penetration force is linked to the hardness of the lard, therefore, the longer the storage time, the harder the lard samples, which suggests a more crystallized lard (Campos et al., 2002). According to the experimental results and literature, it may be concluded that lard crystallization during storage could be monitored through the ultrasonic velocity, since this technique showed a good agreement with both thermal and textural analyses. Therefore, ultrasonic velocity can be used both to follow the changes in the thermal behavior of fat and to estimate the textural changes that occur during lard crystallization.

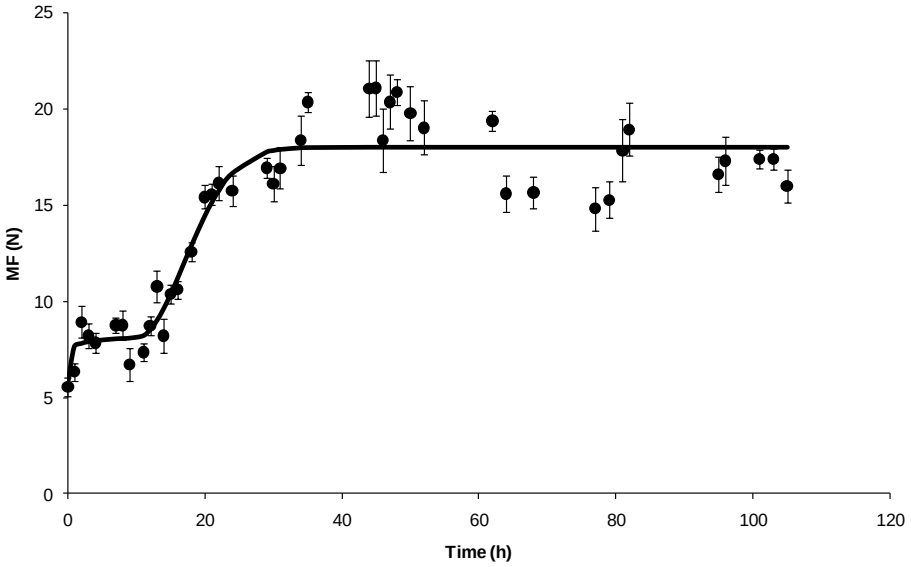


Figure 5. Change of the maximum penetration force (MF) with storage time of lard at 0 ºC. Continuous line shows a polynomial fit of experimental data. Note: Each point is the average of 5 replicates.

3.4. Modeling ultrasonic velocity

According to the experimental results, a two-step procedure was applied in the modeling of the ultrasonic velocity during the isothermal crystallization of lard. The first step consisted of testing the ability of both proposed models (Avrami and Gompertz) to describe only the first velocity increase (Figure 4) related to the crystallization of the most saturated triglycerides. This step was conducted in order to choose the most appropriate model for describing the changes in the ultrasonic velocity which take place during crystallization. Thereby, the second step was devoted to the modeling of the entire crystallization curve, including the two ultrasonic velocity increases, by using the previously chosen model.

In the first step, the experimental data of the ultrasonic velocity in lard samples during the first 12 hours of isothermal crystallization were used, which includes the beginning of the isothermal crystallization and the first constant velocity period. The parameters of the Avrami and Gompertz models (equations 2 and 4) were estimated for each crystallization temperature by fitting the respective model to the experimental data (Table 2), as described in section 2.6. The model parameter which was most seriously affected by the crystallization temperature was the *a* parameter (Table 2), which is the constant ultrasonic velocity attained when the crystallization stage finalized. The parameter *a* decreased when the temperature rose, since the higher the temperature used, the lower the velocity obtained in the constant period (Figure 4). This fact was observed for both models (Table 2). The rest of the variables did not show a clear influence of the crystallization temperature.

Table 2. Results for the modeling of the ultrasonic velocity during the isothermal crystallization of lard at short times: percentage of explained variance (%var), root mean square error (RMSE) and model parameters.

T (ºC)	Avrami Model					Gompertz Model				
	a (m/s)	*k* (s^{-n})	*n*	%var	RMSE (m/s)	*a* (m/s)	μ (m/s^2)	λ (min)	%var	RMSE (m/s)
0	1714	0.013	0.53	99.99	1.92	1706	0.02	0.00001	98.45	6.3
3	1669	0.019	0.5	99.99	0.86	1663	0.02	0.00001	97.49	4.52
5	1648	0.027	0.49	99.93	1.24	1645	0.022	0.00001	96.44	3.32
7	1637	0.022	0.48	99.9	1.5	1634	0.016	0.00001	97.47	3.36
10	1621	0.095	0.29	99.68	1.45	1612	0.041	0.00001	94.78	2.91
20	1528	0.0001	1.19	99.93	1.16	1528	0.009	0.00001	99.95	1.09

Table 2 also shows the values for the percentage of explained variance (% var) and root mean square error (RMSE) for each model at the different temperatures tested. It may be observed that the Avrami model fitted the experimental data better than the Gompertz model, providing a higher percentage of explained variance and a lower root mean square error. The goodness of the fit for both models is also illustrated in Figure 6, where the experimental and calculated ultrasonic velocities for experiments at 3 ºC are depicted together for comparison. This figure shows how the Avrami model provides a better agreement between the experimental and calculated data than the Gompertz model. Therefore, it may be concluded that the Avrami model is suitable for describing the ultrasonic velocity changes that take place during the isothermal storage of lard. For this reason, it will be used to model the complete crystallization process of lard.

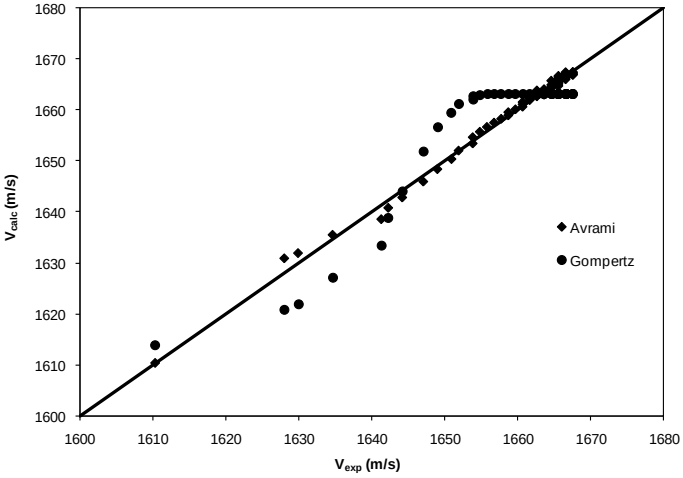


Figure 6. Fit of Avrami and Gompertz models to the experimental data during the isothermal crystallization at 3ºC.

As for temperatures of 7, 10 and 20 ºC, a single velocity increase is observed (Figure 4). The tested Avrami model (Table 2) is adequate for describing the velocity changes for the storage period of 263 hours (Figure 4). However, for lower temperatures (0, 3 and 5 ºC), a new model must be developed to take the two velocity increases into account. For that purpose, both velocity increases were separately modeled using the Avrami equation and the models were joined by applying fuzzy logic, according to the results of Váquiro et al. (2008) for modeling the drying kinetics of mango. Therefore, equation 10 was used to calculate the ultrasonic velocity.

$$V = A_1 \cdot Avr_1 + A_2 \cdot Avr_2 \tag{10}$$

where *Avr1* and *Avr2* are the Avrami equations for the first and second velocity increases, respectively, and A_1 and A_2 are the exponential membership functions for each of the fuzzy sets. A_1 and A_2 can be calculated using expressions 11 and 12.

$$A_1 = \left\{ \begin{array}{l} \exp\left[-\left(\frac{t - \alpha}{\beta} \right)^2 \right] \text{ if } t > \alpha \\ 1 \quad \text{if } t \leq \alpha \end{array} \right\} \tag{11}$$

$$A_2 = 1 - A_1 \tag{12}$$

where α is the time the second velocity increase starts and β a parameter that controls the curvature index at the junction of the two fuzzy sets (represented by the two Avrami equations).

By following this procedure, an equation with eight parameters was obtained: six for the two Avrami models (a_1, k_1, n_1, a_2, k_2 *and* n_2), α and β (table 3). The same optimization procedure as that illustrated in section 2.6 was used to calculate the parameters. The high values of the percentage of explained variance (higher than 99.9%) and the low value of root mean square error (lower than 1.99 m s^{-1}, table 3) suggest that the two-step crystallization model developed was appropriate for modeling the ultrasonic velocity during isothermal lard crystallization. Figure 4 shows the fits of the two-step crystallization models to the experimental data for storage temperatures of 0, 3 and 5 ºC.

Table 3. Results for the modeling of the ultrasonic velocity during the complete isothermal crystallization of lard at 0, 3 and 5 ºC: percentage of explained variance (%var), root mean square error (RMSE) and model parameters

T (ºC)	a_1 (m/s)	k_1 (s^{-n})	n_1	a_2 (m/s)	k_2 (s^{-n})	n_2	α (h)	β (s)	% var	RMSE (m/s)
0	1716	0.015	0.5	1814	$1.46 \cdot 10^{-7}$	1.45	11.82	15700	99.99	1.99
3	1669	0.006	0.59	1807	$1.55 \cdot 10^{-5}$	0.95	26.51	52223	99.99	1.38
5	1656	0.13	0.26	1781	$3.59 \cdot 10^{-10}$	1.68	69.57	127696	99.99	1.19

The results showed in this work agree with those reported in literature, since crystallization has been well described by also using two steps in other foodstuffs. Thus, Foubert et al. (2003) developed a two-step crystallization model by adding two Foubert models to adequately describe the isothermal crystallization of milk fat and cocoa butter monitored by X-ray diffraction.

According to these authors, since fat is a complex mixture of triglycerides, crystallization in a two-step process may be due to the crystallization of different fractions of triglycerides or the formation of different polymorphic forms of crystals. These processes lead to curves which rise to an intermediate plateau and then increase again to a second plateau, the same like the experimental results obtained in this work. Vanhoutte (2002) combined two Gompertz equations to fit the two-step crystallization of milk fat. Marangoni & McGauley (2003) found crystallization curves in two steps for the isothermal crystallization of cocoa butter below 20 ºC and used the Avrami model to fit the experimental data. Herrera, de Leon Gatti, & Hartel (1999) also used the Avrami model to describe the isothermal crystallization of milk fat below 25 ºC.

3.5. Modeling solid fat content

The ultrasonic velocity of a fatty material increases as its solid fat content increases, hence, literature widely agrees that the ultrasonic velocity measurements can be used to determine the solid fat content of bulk fats and emulsions (Miles et al., 1985; McClements & Povey, 1987, 1988, 1992; McClements, Povey, & Dickinson, 1993). The physicochemical properties and sensory attributes of most products containing fat are determined by the fraction of fat that is solid at a given temperature. Therefore, it is very important to measure the variation in the solid fat content (SFC) of a food. In the food industry, solid fat content values measured at different temperatures can be used to predict important attributes such as mouth-feel and hardness (Singh et al., 2004). The SFC may change during the manufacturing and storage of various products, such as chocolate, butter, margarine and shortenings. Therefore, it is important to be able to measure this parameter to control the ingredients and manufacturing conditions used for optimum product quality (Saggin & Coupland, 2002). Thus, the estimation of the solid fat content during crystallization by means of non-destructive ultrasonic velocities may be considered as a matter of relevant research.

Singh et al. (2004) found a linear correlation between the ultrasonic velocity and the solid fat content for concentrated samples (SFC> 20%) of milk fat and cocoa butter. Martini et al. (2005a) also determined the solid fat content

by using on-line ultrasonic measurements. In this work, the ultrasonic velocity measurements at different temperatures were also used to estimate the percentage of solid fat in lard during the isothermal crystallization. The procedure followed is illustrated in this section in detail.

Equation 5 could be used to estimate the percentage of solid fat as a function of crystallization time using ultrasonic velocity data. If it is considered that there are two fractions of triglycerides with different crystallization patterns, equation 5 should be modified, turning into equation 13.

$$\frac{1}{V^2} = \frac{1 - SFC}{V_L^2} + \frac{\theta_{S1}}{V_{S1}^2} + \frac{\theta_{S2}}{V_{S2}^2} \qquad (13)$$

Subscripts 1 and 2 refer to the more and less saturated triglyceride fractions, respectively.

In order to use equation 13 to estimate the SFC from the ultrasonic velocity, the following parameters should be calculated: V_L, V_{SI}, V_{S2} and the maximum values of θ_{S1} and θ_{S2}.

In order to calculate V_L, additional experiments were carried out. The ultrasonic velocity was measured at each degree from 55 to 45 ºC and it should be mentioned that, in order to avoid any influence of the cooling rate, the ultrasonic measurement was taken holding the sample at a constant temperature for at least 10 min. From these data, the linear equation that relates the ultrasonic velocity and the sample temperature for liquid lard was obtained (equation 14). According to the literature, it may be assumed that the relationship is valid for the whole temperature range tested (Singh et al., 2004).

$$V_L = -3.81 \times T + 1532.5 \qquad R^2=0.99 \qquad (14)$$

The maximum values of θ_{S1} and θ_{S2} were determined by comparing the latent heat of crystallization for both triglyceride fractions and were calculated as the percentage of the total area of the two main peaks found in DSC (Table 1, Figure 2). Average values of θ_{S1} = 0.7 and θ_{S2} = 0.3 were obtained for the different cooling rates.

On the one hand, V_{S1} was obtained by solving equation 13 when the first fraction was completely solid and the second fraction had not started to crystallize, that is when V is equal to the constant velocity after the first increase (velocity of the first plateau, a_1). In this case: $\theta_{S1} = 0.7$ and $\theta_{S2} = 0$. On the other hand, V_{S2} was obtained by solving equation 13 when the whole sample was solid (SFC= $\theta_{S1} + \theta_{S2} = 1$), that is when both triglyceride fractions were crystallized. At this moment, V is equal to the constant velocity after the second increase (velocity of the second plateau, a_2).

By introducing the values of the parameters into equation 13 and solving it for the solid fat content (SFC), an equation is obtained to estimate the SFC during isothermal crystallization at each temperature studied. The model developed to estimate the SFC was split into two equations (expressions 15 and 16), depending on whether the ultrasonic velocity was lower or higher than a1. In the first case, when the ultrasonic velocity was lower than a1, θ_{S1} was between 0 and 0.7 and $\theta_{S2} = 0$ (equation 15), in the second case $\theta_{S1} = 0.7$ and θ_{S2} was between 0 and 0.3 (equation 16).

$$SFC = \frac{\left(V_{S1}^2 - \frac{V_{S1}^2 \times V_L^2}{V^2} \right)}{V_{S1}^2 - V_L^2} \qquad \text{if } V < a_1 \qquad (15)$$

$$SFC = \frac{\left[\left(\frac{1}{V^2} - \frac{0.7}{V_{S1}^2} \right) \times V_L^2 \times V_{S2}^2 \right] + 0.7 \times V_L^2 - V_{S2}^2}{V_L^2 - V_{S2}^2} \qquad \text{if } V \geq a_1 \qquad (16)$$

Following the aforementioned procedure, the solid fat content estimated from the ultrasonic velocities using a two-step crystallization pattern is shown in Figure 7. As should be expected, the SFC decreased when the storage temperature rose for the same experimental time (Figure 7). Obviously, in order to reach 100% of SFC, the higher the temperature, the longer the storage time.

For isothermal crystallization experiments performed at 7, 10 and 20 ºC, the same model as that described above was used but the second triglyceride fraction did not crystallize (only 70% of SFC was achieved, figure 7). Therefore, the ultrasonic non-destructive measurements can be useful to estimate the changes in the solid fat content that take place during food processing and storage. As SFC greatly influences the physicochemical properties and sensory attributes such as the textural properties of food products, ultrasonic velocity can be useful to optimize processing and storage conditions in order to improve product quality.

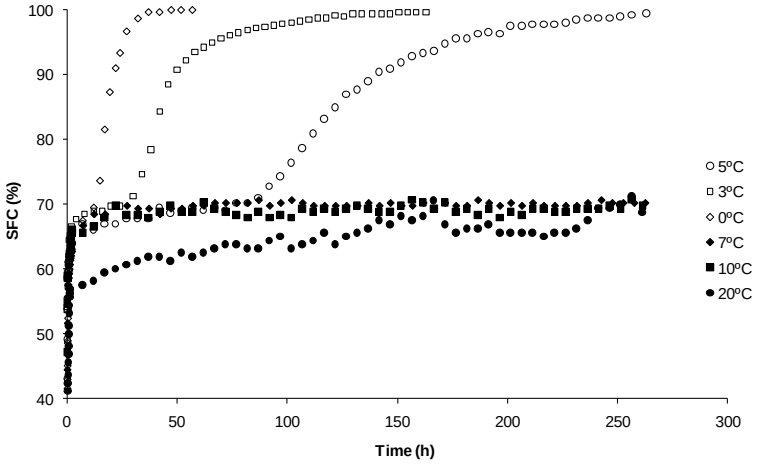


Figure 7. Estimation of the solid fat content during the isothermal crystallization of lard at different storage temperatures from ultrasonic measurements.

4. Conclusions

Ultrasonic velocity was used to monitor the lard crystallization during cooling and storage. During non-isothermal crystallization, the cooling rate affected the crystallization pattern, and obviously the ultrasonic velocity. The ultrasonic velocity measurements during the isothermal crystallization showed two steep increases due to the crystallization of the two fractions of triglycerides (more saturated and unsaturated) found by DSC. These results were confirmed by instrumental texture experiments, where two steep increases of the maximum penetration force were also observed during storage, due to the crystallization of

the two fractions of triglycerides. The crystallization pattern was well described using a two-step crystallization model based on the Avrami equation, which establishes a relationship between the ultrasonic velocity and the isothermal crystallization time. From the ultrasonic velocity, a model was also developed to estimate the percentage of solid fat content during isothermal crystallization. Both models, with minor modifications, may be useful to estimate the crystallization pattern of complex fat products such as chocolate, cream, margarine or butter. Therefore, ultrasonics may be considered as a suitable technique for monitoring the fat crystallization during cooling and long storage periods and, as a consequence, it may be used to estimate the textural and organoleptic properties of fat-containing products.

References

Avrami, M. (1940). Kinetics of phase change II. Transformation-time relations for random distribution of nuclei. *Journal Chemical Physics*, 8, 212-224.

Benedito, J., Cárcel, J.A., Rosselló, C., & Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Journal of Meat Science*, 57, 365-370.

Benedito, J., Mulet, A., Velasco, J., & Dobarganes, M.C. (2002). Ultrasonic assesment of oil quality during frying. *Journal of Agricultural and Food Chemistry*, 50, 4531-4536.

Berg, T.G.O. & Brimberg, U.I. (1983). Über die kinetik der Fettkristallisation. *Fette Seifen Anstrichmittel*, 85, 142-149.

Berthouex, P.M. & Brown, L.C. (1994). Statistics for environmental engineers. *New York: Lewis Publishers Inc*.

Campos, R., Narine, S.S., & Marangoni, A.G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Research International*, 35, 971-981.

Cebula, D.J. & Smith, K.W. (1991). Differential scanning calorimetry of confectionery fats. Pure triglycerides: Effects of cooling and heating rate variation. *Journal of the American Oil Chemists' Society*, 68, 591-595.

Cebula, D.J. & Smith, K.W. (1992). Differential scanning calorimetry of confectionery fats. Part II: Effects of blends and minor components. *Journal of the American Oil Chemists' Society*, 69, 992-998.

Coupland, J. N. (2004). Low intensity ultrasound. *Food Research International*, 37, 537-543.

deMan, J.M. (1999). Relathionship among chemical, physical and textural properties of fats. In N. Widlak (Ed.), *Physical properties of fats, oils and emulsifiers* (pp 79-95). *Champaign: AOCS Press.*

Foubert, I., Vanrolleghem, P.A., Vanhoutte, B., & Dewettinck, K. (2002). Dynamic mathematical model of the crystallization kinetics of fats. *Food Research International*, 35, 945-956.

Foubert, I., Dewettinck, K., & Vanrolleghem, P.A. (2003). Modelling of the crystallization kinetics of fats. *Trends in Food & Science Technology*, 14, 79-92.

Foubert, I., Dewettinck, K., Janssen, G., & Vanrolleghem, P.A. (2006). Modelling two-step isothermal fat crystallization. *Journal of Food Engineering*, 75, 551-559.

Graciani, E. (2006). Los aceites y grasas: composición y propiedades. *Sevilla: Mundiprensa.*

Herrera, M. L., de Leon Gatti, M., & Hartel, R. W. (1999). A kinetic analysis of crystallization of a milk fat model system. *Food Research International*, 32, 289-298.

Javanaud, C. & Rahalkar, R.R. (1988). Velocity of sound in vegetable Oils. *Fat Science Technology*, 90, 73-75.

Kalnin, D., Lesieur, P., Artzner, F., Keller, G., & Ollivon, M. (2005). Systematic investigation of lard polymorphism using combined DSC and time-

resolved synchrotron X-ray diffraction. *European Journal of Lipid Science and Technology*, 107, 594-606.

Kloek, W., Walstra, P., & Van Vliet, T. (2000). Crystallization kinetics of fully hydrogenated palm oil in sunflower oil mixtures. *Journal of the American Oil Chemists' Society,* 77, 389-398.

Ling-Zhi, C., Hong, Z., Yuan, X., & Xuebing, X (2009). Physical characterization of lard partial acylglycerols and their effects on melting and crystallization properties of blends with rapeseed oil. *Journal of Agricultural and Food Chemistry*, 57, 5020-5027.

Marangoni, A.G. (1998). On the use and misuse of the Avrami equation in characterization of the kinetics of fat crystallization. *Journal of the American Oil Chemists' Society*, 75, 1465-1467.

Marangoni, A.G. & McGauley, S.E. (2003). Relationship between crystallization behaviour and structure in cocoa butter. *Crystal Growth and Design*, 3, 95-108.

Martini, S., Bertoli, C., Herrera, M.L., Neeson, I., & Marangoni, A.G. (2005a). In-situ monitoring of solid fat content by means of p-NMR and ultrasonics. *Journal of the American Oil Chemists' Society*, 82, 305-312.

Martini, S., Bertoli, C., Herrera, M.L., Neeson, I., & Marangoni, A.G. (2005b). Attenuation of ultrasonics waves: Influence of microstructure and solid fat content. *Journal of the American Oil Chemists' Society*, 82, 319-328.

Martini, S., Herrera, M.L., & Marangoni, A.G. (2005c). New technologies to determine solid fat content on-line. *Journal of the American Oil Chemists' Society*, 82, 313-317.

McClements, D.J. & Povey, M.J.W. (1987). Solid fat content determination using ultrasonic velocity measurements. *Ultrasonics*, 25, 340.

McClements, D.J. & Povey, M.J.W. (1988). Comparison of pulsed NMR and ultrasonic velocity techniques for determining solid fat contents. *Journal of Science of Food and Technology*, 23, 159-170.

McClements, D.J. & Povey, M.J.W. (1992). Ultrasonic analysis of edible fats and oils. *Ultrasonics,* 30, 383-388.

McClements, D.J., Povey, M.J.W., & Dickinson, E. (1993). Absorption and velocity dispersion due to crystallization and melting of emulsion droplets. *Journal of the American Oil Chemists' Society*, 31, 433-437.

Metin, S. & Hartel, R.W. (1998). Thermal analisis of isothermal crystallization kinetics in blends of cocoa butter with milk fat or milk fat fractions. *Journal of the American Oil Chemists' Society*, 75, 1617-1624.

Miles, C.A., Fursey, G.A.J., & Jones, R.C.D. (1985). Ultrasonic estimation of solid/liquid ratios in fats, oils and adipose tissue. *Journal of Science of Food and Agriculture*, 36, 215-228.

Mulet, A., Benedito, J., Bon, J., & Sanjuán, N. (1999). Low intensity ultrasonics in food technology. *Food Science and Technology International*, 5, 285-297.

Narine, S.S. & Marangoni, A.G. (2002). Structure and mechanical properties of fat crystal networks. Advances in Food and Nutrition *Research International*, 44, 33-45.

Niñoles, L., Clemente, G., Ventanas, S., & Benedito, J. (2007). Quality assesment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Saggin, R. & Coupland, J.N. (2002). Measurement of solid fat content by ultrasonic reflectance in model systems and chocolate. *Food Research International*, 35, 999-1005.

Simal, S., Benedito, J., Clemente, G., Femenia, A., & Rosselló, C. (2003). *Ultrasonic determination of the composition of a meat-based product. Journal of Food Engineering*, 58, 253-257.

Singh, A.P., McClements, D.J., & Marangoni, A.G. (2004). Solid fat content determination by ultrasonic velocimetry. *Food Research International*, 37, 545-555.

Timms, R.E. (2005). SFI or SFC? Why the difference? *Inform*, 16, 74-75.

Toro-Vázquez, J.F., Briceno-Montelongo, M., Dibildox-Alvarado, E., Charo-Alonso, M., & Reyes-Hernández, J. (2000). Cristallization kinetics of palm stearin in blends with sesame seed oil. *Journal of the American Oil Chemists' Society*, 77, 297-310.

Vanhoutte, B. (2002). Milk fat crystallization: fractionation and texturisation. Ph.D Thesis. Ghent University.

Vanhoutte, B., Dewetinck, K., Foubert, I., Vanlerberghe, B., & Huyghebaert, A. (2002). The effect of phospholipids and water on the isothermal crystallization of milk fat. *European Journal of Lipid Science and Technology*, 104, 490-495.

Váquiro, H.A., Bon, J., & Díez, J.L. (2008). Fuzzy logic application to drying kinetics modelling. *The International Federation of Automatic Control* (17th World Congress, Seoul, July 2008).

Wright, A.J., Hartel, R.W., Narine, S.S., & Marangoni, A.G. (2000). The effect of minor components on milk fat crystallization. *Journal of the American Oil Chemists' Society,* 77, 463-475.

Wright, A.J., Narine, S.S., & Marangoni, A.G. (2001). Comparison of experimental techniques used in lipid crystallization studies. In N. Widlak, R.Hartel, & S. Narine (Eds.), Crystallization and solidification properties of lipids (pp 120-131). *Champaign: AOCS Press*.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., & Van't Riet, K. (1990). Modeling of the bacterial growth curve. Applied Environmental Microbiology, 56,1875-1881.

CHAPTER 2

(*Apartado 2*)

ULTRASONIC CHARACTERIZATION OF IBERIAN PORK FAT CRYSTALLIZATION DURING COLD STORAGE

E. Corona, J.V. García-Pérez, J.V. Santacatalina, S. Ventanas, J. Benedito

Food Research International

(Submitted)

Ultrasonic characterization of Iberian pork fat crystallization during cold storage

E. Corona[a], J.V. García-Pérez[a], J.V. Santacatalina[a], S. Ventanas[b], J. Benedito[a]

a. Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camí de Vera s/n, E46022, Valencia, Spain.

b. Tecnología de los Alimentos, Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Avd.de la Universidad s/n, 10071 Cáceres, Spain.

Abstract

In this work, the feasibility of using ultrasonic techniques, namely the measurement of ultrasonic velocity at 2, 5, 7 and 10 ºC, with which to characterize the crystallization pattern of two Iberian backfats (Montanera and Cebo) during cold storage was evaluated. Moreover, the fatty acid profile, thermal behavior and textural properties of fat were also determined.

Both fats became harder during cold storage, showing a two-step pattern related with the separate crystallization of the different existing triacylglycerols. Due to a greater content of saturated triacylglycerols, Cebo fat was harder than Montanera. It was observed that the harder the fat, the higher the ultrasonic velocity. Thus, ultrasonic measurements were useful both to monitor the textural changes taking place during cold storage and to differentiate between Cebo and Montanera fats. Therefore, considering the relevance of both fat type and state, ultrasound emerges as an accurate technology for monitoring the overall quality of dry-cured products during cold storage.

Key words: *Ultrasound; Fat; Crystallization; Texture; Modeling*

1. Introduction

In the southwestern Iberian Peninsula, the Iberian pig is an autochthonous breed traditionally reared and fed under an extensive production system, "Montanera", which is a diet based on acorns and grass. Alternatively, Iberian pigs are also reared in a more intensive system, "Cebo", which is based on concentrate feeds (Timón, Martín, Petrón, Jurado, & Garcia, 2001a). The meat of the Iberian pigs is used to obtain different dry-cured products of high sensory quality and wide consumer acceptance (Ventanas, Ventanas, Ruiz, & Estévez, 2005).

The influence of the rearing system led to modifications in the fatty acid profile of the raw material, thus affecting the sensory characteristics of the derived Iberian meat products (Carrapiso, Bonilla, & García, 2003; Cava, Ventanas, Ruiz, Andrés, & Antequera, 2000; Niñoles, Clemente, Ventanas, & Benedito, 2007). In this regard, tissues from Iberian pigs reared on the Montanera system contain higher levels of unsaturated fatty acids, particularly of oleic acid, than those derived from pigs fed on Cebo system with concentrate feeds (Petrón, Muriel, Timón, Martín, & Antequera, 2004; Timón, Ventanas, Carrapiso, Jurado, & García, 2001b). Positive significant correlations have been found between the level of unsaturated fatty acids and particular sensory characteristics such as, the brightness, oiliness, aroma and juiciness of Iberian dry-cured hams (Carrapiso et al., 2003; Cava et al., 2000; Ruiz, Ventanas, Cava, Andrés, & García, 2000).Therefore, dry-cured Iberian products derived from Montanera Iberian pigs show a much higher overall sensory quality than those produced from Cebo Iberian pigs. The Iberian dry-cured products obtained from Montanera–reared pigs are highly appreciated by Spanish consumers due to their distinctive and characteristic sensory traits and the market prices are much higher compared to those for products from Cebo Iberian pigs. However, although there is a law (BOE, 2007) to regulate the Iberian meat product market, in some cases Iberian meat products derived from Cebo pigs are labeled as "Montanera". As a result, it is important to find technologies that can adequately identify and classify the different dry-cured Iberian products in function of the rearing system used in order to prevent possible fraud and maintain quality standards.

In order to extend the shelf-life of dry-cured meat products, mainly when they are sliced and vacuum-packaged, preservation technologies, such as freezing and refrigeration, are used during distribution and retail sale (Cilla, Martínez, Beltrán, & Roncáles, 2006a; Cilla, Martínez, Beltrán, & Roncalés, 2006b). Nevertheless, different studies have reported that storage time and fat content exerted a notable effect on the textural properties of dry cured products due to the crystallization of the fat and the increase in the solid fat content at low temperatures (Campos, Narine, & Marangoni, 2002; Cilla et al., 2006b; García-Esteban, Ansorena, & Astiasarán, 2004; Papadima, & Bloukas, 1999; Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2007a, Rubio et al., 2007b; Santacatalina, García-Pérez, Corona, & Benedito, 2010). Therefore, monitoring the changes that take place during the cold storage of fat is relevant since the texture of dry-cured products is directly related to these variations. Moreover, previous studies carried out in Iberian dry-cured products (Ruiz et al., 2000; Ventanas, Ventanas & Ruiz, 2007; Ventanas, Estévez, Andrés & Ruiz, 2008) and dry-cured meat model systems (Fuentes, Ventanas, Estévez, Carrapiso & Ventanas, 2010) have reported the effect of fat content and composition on the release of volatile compounds and, thus, on the perception of the related sensory attributes, particularly of odour and flavour perception.

Different techniques have been used to monitor changes during fat crystallization, such as instrumental textural analysis (Shi, Liang & Hartel, 2005), differential scanning calorimetry (DSC) (Bell, Gordon, Jirasubkunakorn, & Smith, 2007; Danthine & Deroanne, 2004), X-ray diffraction (Kalnin, Lesieur, Artzner, Keller, & Ollivon, 2005; Marangoni, & Narine, 2002), pulsed Nuclear Magnetic Resonance (pNMR) (Singh, McClements, & Marangoni, 2004; Wright, Narine, & Marangoni, 2001) and polarized light microscopy (PLM) (Campos, et al., 2002; Singh et al., 2004). However, the search for non-destructive techniques, like ultrasound, that could be used to monitor textural changes provoked by the crystallization of complex fats during cold storage is of great interest to the industry. Among other advantages of ultrasound, it is rapid, low cost and easy to be automated on-line (Mulet, Benedito, Bon, & Sanjuan, 1999). Low intensity ultrasound has been used to assess the textural properties of many foods, such as Mahon Cheese (Benedito, Carcel, González, & Mulet, 2002;

Benedito, Carcel, Clemente, & Mulet, 2000a, Benedito, Carcel, González, & Sanjuan, 2000b) and meat-based products (Sobrassada) (Llull, Simal, Femenia, Benedito, & Rosselló, 2002; Llull, Simal, Benedito, & Rosselló, 2002). Ultrasonic measurements were also useful to characterize and classify subcutaneous dry cured fat from dry cured Iberian hams with different genetics and raised on different feeding systems (Niñoles, Sanjuan, Ventanas, & Benedito, 2008). Furthermore, the increase in the solid fat content was monitored during lard crystallization using ultrasonic measurements (Santacatalina et al., 2010). Thereby, the main objective of this work was to test the feasibility of low intensity ultrasound for both the characterization of the textural changes in Iberian Montanera and Cebo fat during cold storage and the discrimination between both fat types.

2. Materials and methods

2.1 Raw material

Experiments were carried out using two Iberian subcutaneous fats: Montanera and Cebo. In both fats, a homogeneous sample of 5000 g was obtained from the backfat of ten Iberian pigs, approximately 0.5 kg of fat per animal. For each fat type, the fat was separated from the skin prior to the experiments, milled and homogenized by melting at 70 ºC for 30 min and finally stored at 2 ºC.

2.2 Fatty acid composition

Fatty acid methyl esters (FAMEs) of both fats were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992). Lipids were previously extracted by using the Folch et al. (1957) procedure. FAMEs were analysed by a gas chromatograph (HP-5890A, Hewlett Packard, USA) equipped with a flame ionisation detector (FID). Separation was carried out on a polyethylene glycol–TPA modified fused silica semicapillary column (30 m long, 0.53 mm id, 1 μm film thickness) maintained at 225°C. Injector and detector temperatures were 230°C. Carrier gas was nitrogen at a flow rate of 1.8 mL/min. Individual FAMEs peaks were identified by comparing their retention times with those of standards (Sigma, St.

Louis). Results are expressed as percentage of the total fatty acids analyzed. Eight repetitions per fat were performed.

2.3 Differential scanning calorimetry (DSC)

The thermal behavior of the samples was monitored using differential scanning calorimetry (DSC) (DSC5200CU, Seiko Instruments, Inc., USA). Each fat type was analyzed at least in triplicate. Approximately 18 to 20 mg of dry fat were introduced into a standard aluminum DSC crucible and then hermetically sealed and weighed on a balance (± 0.01 mg, ER-182A, AND, Japan). The sample was previously dried in an oven at 105 ºC until constant weight (approximately 24 hours) to avoid the influence of water. An empty and hermetically sealed aluminum DSC crucible was used as the reference material and liquid nitrogen was used as the cooling fluid. The sample was heated from 25 ºC to 70 ºC at 5 ºC/min. Next, the sample was tempered at 70 ºC for 1 min to destroy any crystal memory. Afterwards, the sample was cooled from 70 to -50 ºC using a wide range of cooling rates (0.2, 0.5, 1, 5 and 10 ºC/min) in order to characterize the crystallization pattern of both Montanera and Cebo fats. Following this procedure, DSC curves were obtained for the cooling of both fats at the five different cooling rates tested. The peak maximum temperature (T_{max}, ºC), onset temperature (T_o, ºC) and latent heat (ΔH, J/g) were computed from the thermograms (Cebula & Smith, 1992).

2.4. Instrumental texture analysis

The textural changes of the fat samples during cold storage were analyzed using a universal texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, England) linked to a computer for data acquisition and processing. In order to perform textural measurements, the fat was previously melted at 70 °C for 30 min in a vacuum oven (EV50, Raypa Vacuterm, Terrasa, Spain) and then placed in Petri dishes (volume of fat 60 mL, layer thickness 10 mm) and manually stirred until reaching 50 °C. Petri dishes and the textural analyzer were placed in the same temperature-controlled chamber (±0.1 ºC) (AEC330R, Infrico, Barcelona, Spain); which allowed the temperature of the samples to be kept constant during the instrumental test. Three storage temperatures were tested: 0, 2 and 5 ºC. From the first hour of storage, one Petri dish was measured every

hour for approximately 5 days, using a conical probe (angle 40º, penetration distance 5 mm and crosshead speed 1 mm/s). For each time, five penetrations were carried out on the sample placed in each Petri dish. Finally, the maximum force (MF, N) was computed from the force versus distance records and averaged for the 5 measurements.

2.5. Ultrasonic set-up and measurements

Figure 1 shows the experimental set-up used in the ultrasonic measurements. The experimental set-up consisted of an aluminum cylindrical ultrasonic cell (diameter 17.8 mm, length 25 mm) where the sample was introduced. A narrow band piezoelectric transducer (1MHz, 0.5'' crystal diameter, A303S, Panametrics, Walthman, MA, USA) was inserted in each side of the cell, the path length being 25 mm. A thermocouple K-type was used to measure the sample temperature; it was placed in the center of the cell, which was kept in a temperature-controlled bath (± 0.1 ºC) (Frigiterm-30, P-selecta, Barcelona, Spain). The ultrasonic pulser-receiver instrument (5058PR, Panametrics, Walthman, MA, USA) supplied the electrical spike pulse to the emitter-transducer generating the ultrasonic wave, which propagated through the sample and was received by the receiver-transducer. Then, the signal was filtered (0.3 MHz LP5) and amplified (40 dB) by the pulser-receiver and thereafter, captured and digitized (100 MS/s) by a data acquisition card (PCI 5112, National Instruments, USA) installed in a PC. Specific software was programmed in Visual Basic for the determination of the ultrasonic velocity, taking the time of flight and the distance between transducers into account. Time of flight is defined as the time the ultrasonic wave needs to go through the sample, while the distance between the transducers was calculated using distilled water.

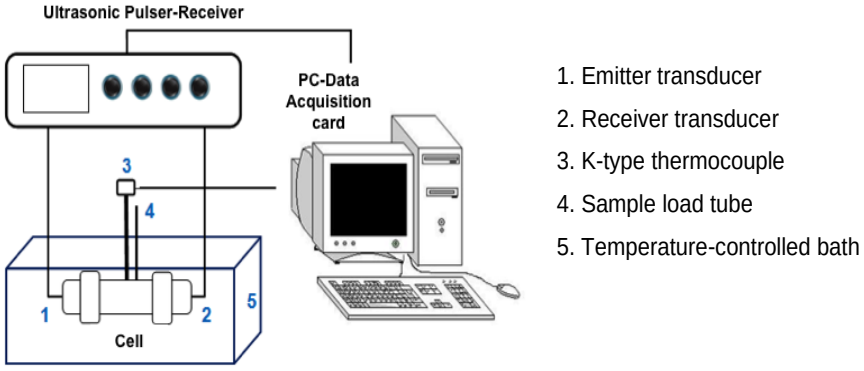


Figure 1. Ultrasonic system for monitoring isothermal crystallization

The experiments were carried out on samples of fat that had previously been stored at 2 ºC, then heated at 70 ºC for 30 min in a vacuum oven (EV50, Raypa Vacuterm, Terrassa, Spain) to ensure the complete destruction of the fat crystals. Thereafter, the sample was manually stirred until a temperature of 50 ± 0.1 ºC was reached and placed into the ultrasonic cell (Figure 1). Once the sample was introduced into the ultrasonic cell, it was placed in a bath at 45 ºC for 10 min to assure tempering. Then the ultrasonic cell was introduced into the temperature-controlled bath set to the experimental temperature (0, 2, 5, 7 and 10 ºC). Once the sample reached the bath temperature, the isothermal storage experiment began and the ultrasonic velocity was measured every 10 min during storage times ranging for between 100 and 480 hours (depending on the storage temperature). Three replicates were carried out for each temperature and type of fat.

As the ultrasonic set-up allowed long periods of cold storage to be monitored in an automated and non-destructive way, a wide range of storage temperatures were considered. Therefore, not only were conventional storage temperatures (0, 2 and 5 ºC) considered but tests were also performed at temperatures on the limits of cold storage (7 and 10 ºC).

2.6. Modeling of ultrasonic velocity and statistical analysis

A modified Avrami equation (Eq. 1), previously used in the literature by Santacatalina et al. (2010), was used to describe the ultrasonic velocity changes during isothermal crystallization of Montanera and Cebo fats.

$$V(t) = a \cdot \left(1 - \exp\left(-k \cdot t^{n}\right)\right) \tag{1}$$

where V is the ultrasonic velocity (m/s) at time t (s), a the ultrasonic velocity at infinite time (m/s), k is the crystallization rate (s^{-n}) dependent on the crystallization temperature and n the Avrami exponent (dimensionless).

For two-stage crystallization, which is common in complex fats, Santacatalina et al. (2010) proposed the following expression based on Eq. 1.

$$V = A_1 \cdot Avr_1 + A_2 \cdot Avr_2 \tag{2}$$

where Avr_1 and Avr_2 are the modified Avrami equations (Eq. 1) for the first and second crystallization stages, respectively, and A_1 and A_2 can be calculated using equations 3 and 4.

$$A_1 = \left\{ \begin{array}{l} \exp\left[-\left(\dfrac{t - \alpha}{\beta} \right)^2 \right] \text{ if } t > \alpha \\ 1 \quad \text{if } t \le \alpha \end{array} \right\} \tag{3}$$

$$A_2 = 1 - A_1 \tag{4}$$

where α is the time when the second crystallization stage starts and β a parameter that controls the curvature index at the junction of the two fuzzy sets (represented by the two Avrami equations).

The precision with which the model fits the experimental data was evaluated by the percentage of explained variance (% VAR) and the root mean square error (RMSE, m/s) (Váquiro, Bon, & Díez, 2008).

The analysis of variance (ANOVA) was carried out in order to determine the significant influence of the rearing system (Montanera and Cebo) on the fatty acid profile, thermal properties, ultrasonic velocity and texture. The ANOVAs were performed using the Statgraphics Centurion XVI statistics software and Fisher's least significant difference (LSD) intervals were computed at a 95 % confidence level.

3. Results and discussion

3.1 Fatty acid profile

The fatty acid profile in Montanera and Cebo fats are shown in Table 1, where the content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) can be compared for both fats. The results show that there was a significant difference ($p<0.05$) between the SFA content of both fats, being higher for Cebo (45.13 %) than for Montanera (41.83 %). SFA content is affected by the rearing system, which involves lower SFA percentages in Montanera fat from pigs fed on a more extensive, natural diet based on acorns and grass than Cebo animals (Carrapiso et al., 2003; Niñoles et al., 2007). The main SFA in both fats were palmitic (C16) and stearic acids (C18), and the differences in SFA content were mainly due to the differences in stearic acid. These results agree with those previously found by Petrón et al. (2004), who reported higher proportions of palmitic and stearic acids in fat from Iberian pigs fed on concentrate feed.

Table 1. Fatty acid composition (%) of Iberian Montanera and Cebo backfats.

	CEBO	MONTANERA
C12	0.06±0.04[a]	0.070±0.014[b]
C14	1.51±0.09[a]	1.67±0.08[a]
C16	30.1±0.8[a]	29.1±0.9[a]
C16:1	2.03±0.04[a]	1.98±0.04[a]
C17	0.35±0.02[a]	0.31±0.02[a]
C17:1	0.301±0.009[a]	0.260±0.006[b]
C18	12.7±0.5[a]	10.46±0.3[b]
C18:1 (n-9)	42.3±0.5[a]	45.07±1.08[b]
C18:1 (n-7)	3.37±0.11[a]	2.58±0.13[b]
C18:2	4.2±0.3[a]	6.3±0.3[b]
C18:3 (n-3)	0.24±0.12[a]	0.41±0.09[a]
C20	0.4±0.2[a]	0.246±0.112[a]
C20:1	1.20±0.09[a]	0.7±0.03[b]
C20:2	0.44±0.13[a]	0.32±0.04[a]
C20:3 (n-6)	0.17±0.09[a]	0.12±0.02[a]
C20:4	0.40±0.12[a]	0.20±0.09[a]
C20:3 (n-3)	0.31±0.11[a]	0.15±0.05[a]
C24:0	0[a]	0.021±0.002[a]
SFA	**45.13±0.14[a]**	**41.83±0.16[b]**
MUFA	**49.15±0.09[a]**	**50.62±0.25[b]**
PUFA	**5.72±0.14[a]**	**7.55±0.08[b]**
MUFA+PUFA	**54.87±0.07[a]**	**58.17±0.19[b]**

Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA) and Polyunsaturated Fatty Acid (PUFA).

Average values ± Standard deviation are shown from eight replicates. Different letters (a and b) in the same row denote a significant difference ($p<0.05$).

On the other hand, in the case of the unsaturated fatty acid content (MUFA+PUFA), Montanera showed a significantly ($p<0.05$) higher content than Cebo (58.17 % versus 54.87 %) (Table 1). For both MUFA and PUFA fractions, as expected, Montanera presented a significantly ($p<0.05$) higher content than

Cebo, which is mainly due to the difference in the oleic (C18:1; 45.07 % for Montanera and 42.3 % for Cebo) and linoleic acid contents (C18:2; 6.3 % for Montanera and 4.2 % for Cebo). The high content of oleic acid in both fats is explained by the feeding system. As previously mentioned, Montanera animals are mainly fed on acorns and grass, which are rich sources on this fatty acid (Niñoles et al., 2007). In the case of Cebo animals, the concentrate feeds used also contain a high content of oleic acid, aiming to simulate the supply provided by acorns.

The fatty acid composition of Montanera and Cebo fats found in this work was very similar to others previously reported (Carrapiso et al., 2003; Ventanas, Tejeda, & Estévez, 2008). The fatty acid profile has a great influence on the sensory and textural properties of meat products (Ruiz-Carrascal et al., 2000). Although triacylglycerols (TGs) have not been identified in this work, it could be assumed that the level of unsaturation in TGs is directly linked to the fatty acid composition. Consequently, the crystallization pattern of Iberian Montanera and Cebo fats is also, to a certain extent, related with the fatty acid composition. This issue is addressed in the following section.

3.2. Thermal behavior characterization of Iberian pork fats.

The thermal behavior of Montanera and Cebo subcutaneous fat was analysed by recording DSC curves at different cooling rates of 0.2, 0.5, 1, 5 and 10 ºC/min. Different cooling rates were used with the aim of identifying the crystallization pattern of both fats and finding the rate that provides the best differentiation between animals fed using Montanera from those fed with concentrate feed.

Similar DSC curves were found for both fats, showing two main peaks (A and B) for every cooling rate (Figure 2). This fact has already been observed and linked to the crystallization of different TGs in fats with a complex chemical composition (Campos et al., 2002). Peak A may be related with the crystallization of the most highly saturated TGs, while peak B may be linked to the crystallization of the most unsaturated TGs. In this regard, Díaz, García-Regueiro, Casillas & De Pedro (1996) and Petrón et al. (2004) found that the main TGs for Montanera and Cebo fats were OOL, POL, OOO, POO and PSO.

Petron et al. (2004) also observed a fatty acid composition of both fats similar to that found in the present work. In particular they found that Montanera fat had 3 % more oleic acid (major fatty acid) than Cebo (in the present work, 2.8 %). Despite the small difference between the fatty acid compositions of both fat types, significant differences in the TGs composition were found due to the effect of the diet (Petron et al., 2004). Whereas Montanera fat contained more OOO, OLL and OOL, the content of PPL, PPO, PSO, PPS and SSO was higher in Cebo fat (Díaz et al., 1996; Petron et al., 2004).

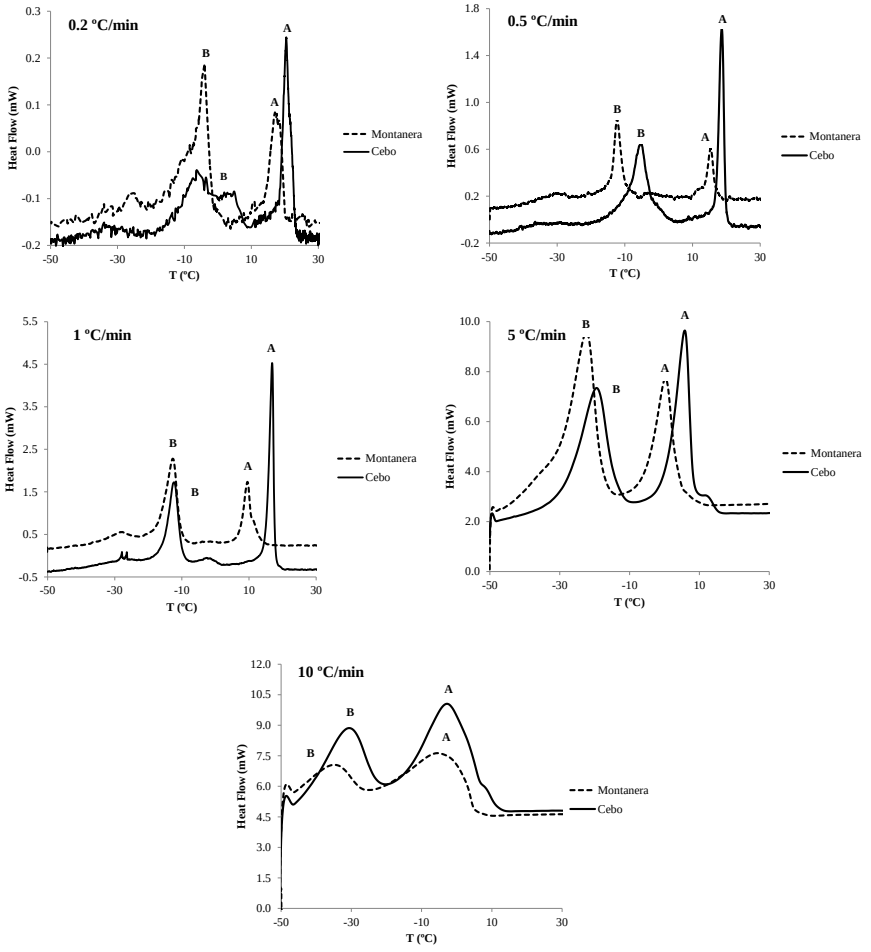


Figure 2. DSC thermograms of Montanera and Cebo fats at different cooling rates: 0.2, 0.5, 1, 5 and 10 ºC/min.

On the other hand, Figure 2 also shows how the cooling rate affected the thermograms. Both peaks (A and B) were broadened and displaced to lower temperatures when the cooling rate increased. These results are explained by a kinetic limitation due to the short crystallization time at high cooling rates (Cebula & Smith, 1991). In the case of peak displacement, for the fastest rates, only a very short time is needed to crystallize at a given temperature, forcing the TGs to crystallize at lower temperatures, a phenomenon known as supercooling. The short crystallization time also involves peak broadening, which appears because TGs with similar crystallization temperatures are not able to crystallize simultaneously due to a time limitation.

In order to quantify the influence of the cooling rate, the peak onset temperature (T_o), the maximum crystallization temperature (T_{max}) and the latent heat (ΔH) were determined for peaks A and B (Table 2). For each fat, when the cooling rate increased (from 0.2 to 10 ºC/min), the T_o and the T_{max} fell significantly ($p<0.05$) in both A and B peaks. In the case of Montanera, the T_o of peak A decreased from 20.5 (at 0.2 ºC/min) to 5.2 ºC (at 10 ºC/min) and for peak B, from 1.6 (at 0.2 ºC/min) to -26.0 ºC (at 10 ºC/min). The T_{max} for Montanera fell from 18.1 to 5.0 ºC for peak A and in peak B from -5.8 to -34.5 ºC when the rate increased from 0.2 to 10 ºC/min. The effect of the cooling rate has previously been studied in lard (Kalnin et al., 2005) using the combined DSC and X-ray diffraction techniques. Different crystalline forms appeared in function of the cooling rate: the forms being less stable (α) at high rates, but changing into more stable forms (β and β') during the storage of lard. Campos et al. (2002) linked the cooling rate to the stability of the polymorphic forms present in the lard using the X-ray diffraction technique. The abovementioned studies also found that the higher the cooling rate, the lower the T_o and T_{max}, which is coherent with the results obtained in this work.

Table 2. Thermal properties of Montanera and Cebo backfats obtained from DSC using different cooling rates: peak maximum temperature (T_{max}), onset temperature (T_o) and latent heat (ΔH).

PEAK A						
Cooling rate	T_{max} (ºC)		T_o (ºC)		ΔH (J/g)	
(ºC/min)	Cebo	Montanera	Cebo	Montanera	Cebo	Montanera
0.2	21.2±1.1^{a}	18.1±1.3^{b}	23.9±0.3^{a}	20.5±0.6^{b}	24.1±1.1^{a}	18.3±1.3^{c}
0.5	19.7±1.6^{a}	14±2^{c}	21.4±1.1^{b}	18.2±1.2^{c}	25.2±0.7^{ab}	17.5±1.0^{c}
1	18.5±0.6^{b}	9.4±0.5^{d}	20.1±0.8^{b}	15.2±1.8^{d}	24.1±0.6^{a}	16.2±0.9^{d}
5	6.4±1.1^{de}	7.6±0.5^{d}	16.7±0.6^{c}	10.5±1.0^{e}	25.2±1.1^{ab}	18.3±0.6^{c}
10	-1.7±0.5^{f}	5.0±0.7^{e}	14.7±1.1^{d}	5.2±0.7^{f}	23.3±1.3^{a}	13.5±0.5^{e}
PEAK B						
Cooling rate	T_{max} (ºC)		T_o (ºC)		ΔH (J/g)	
(ºC/min)	Cebo	Montanera	Cebo	Montanera	Cebo	Montanera
0.2	-5.9±0.6^{a}	-5.8±1.5^{a}	9.2±0.6^{a}	1.6±0.6^{b}	30.5±0.7^{a}	42.2±0.7^{b}
0.5	-5.0±0.7^{a}	-10.6±1.1^{b}	4.1±1.3^{c}	-7.0±0.8^{d}	31.5±1.3^{a}	42.0±0.8^{b}
1	-10.1±0.4^{b}	-14±1.6^{c}	-6.0±1.3^{d}	-8.8±0.4^{de}	28.2±0.3^{c}	41.8±1.1^{b}
5	-19±1.2^{d}	-29.1±0.8^{e}	-10.4±1.3^{e}	-20.3±0.6^{f}	29.8±0.9^{ca}	37.8±1.1^{d}
10	-31.1±0.8^{e}	-34.5±1.4^{f}	-23.3±1.8^{g}	-26.01±0.8^{h}	12.2±1.2^{f}	25.2±0.8^{e}

Average values ± Standard deviation are shown from three replicates.

(a, b, c, d, e, f, g, h) show homogeneous groups established from LSD intervals ($p<0.05$) for each parameter (T_{max}, T_o and ΔH) and peak (A and B).

For both A and B peaks, it could be stated that Montanera presented lower T_o and T_{max} than Cebo. This fact may be observed in the thermograms of the displacement of the peaks for both fats and could be linked to the higher level of unsaturation of TGs in Montanera. In the case of peak A, Cebo showed a significantly ($p<0.05$) higher T_o than Montanera, the faster the cooling rate (ΔT_o=3.4 ºC at 0.2 ºC/min and 9.7 ºC at 10 ºC/min), the greater the difference (ΔT_o). For the same peak, the T_{max} was significantly higher ($p<0.05$) for Cebo than Montanera at the lowest cooling rates (from 0.2 to 1 ºC/min), but at 5 ºC/min, the difference disappeared and at 10 ºC, Montanera presented a significantly ($p<0.05$) higher T_{max} than Cebo. In the case of peak B, T_o and T_{max} values were significantly ($p<0.05$) higher for Cebo than Montanera (average ΔT_o=6.6 ºC and ΔT_{max}=5.7 ºC), with the exception of the T_{max} at 0.2 ºC/min.

Thermograms of Montanera and Cebo fats also differed in the area (latent heat of crystallization) of peaks A and B (Table 2). For peak A, the latent heat was significantly ($p<0.05$) higher in Cebo than Montanera, which could be expected given the higher level of saturation in Cebo TGs. Peak B was observed to behave in the opposite way, thus, the higher level of unsaturation in Montanera than in Cebo involved a significantly ($p<0.05$) higher latent heat for all cooling rates. Himawan, Starov & Stapley (2006) reported that the crystallization pattern is very sensitive, even to small differences in fatty acid composition, which explained the significant ($p<0.05$) differences observed between Iberian Montanera and Cebo fats. It should be pointed out that, in the present study, significant ($p<0.05$) differences in the fatty acid composition were found between Montanera and Cebo fats. However, through the use of specific fat sources found in the animals' concentrate feed, it might be possible to steer the Cebo fats in such a way as to mimic the composition of Montanera fat (Ventanas et al., 2008), which would hinder the differentiation between both rearing systems using DSC.

3.3 Characterization of the textural measurements during cold storage.

The textural properties of Montanera and Cebo fats were monitored during cold storage at different temperatures (0, 2 and 5 ºC). The maximum penetration force (MF) (Figure 3) was measured due to its direct relationship with fat hardness. For both fats, the penetration force increased during storage for each of the experimental temperatures tested. For experiments carried out at 0 and 2 ºC, the curves increased steeply twice during storage. The first increase took place during the first hours of storage; this fact could be linked to the crystallization of the most highly saturated TGs found by DSC (peak A). Once the most highly saturated TGs were fully crystallized, the maximum penetration force remained constant for a few hours, implying that cold storage progressed without changes in the solid/liquid ratio. How long the constant penetration force period lasted was dependent on storage temperature, showing that the higher the storage temperature, the longer the constant period. Thus, for Cebo fat, the constant period was extended to 35 hours at 2 ºC, but only to 15 hours at 0 ºC. Campos, et al. (2002) observed that, when the fat system is rapidly chilled, the nucleation rate is high and the crystallization takes place in a short period of time. Finally, the second steep increase in the penetration force occurred due to the

crystallization of the most unsaturated TGs, which was related to peak B found in DSC, as previously explained in section 3.2. The second increase also ended in a constant penetration force period. It should be commented on that, in the case of samples stored at 5 ºC, the most unsaturated TGs did not crystallize during the 120 hours of storage.

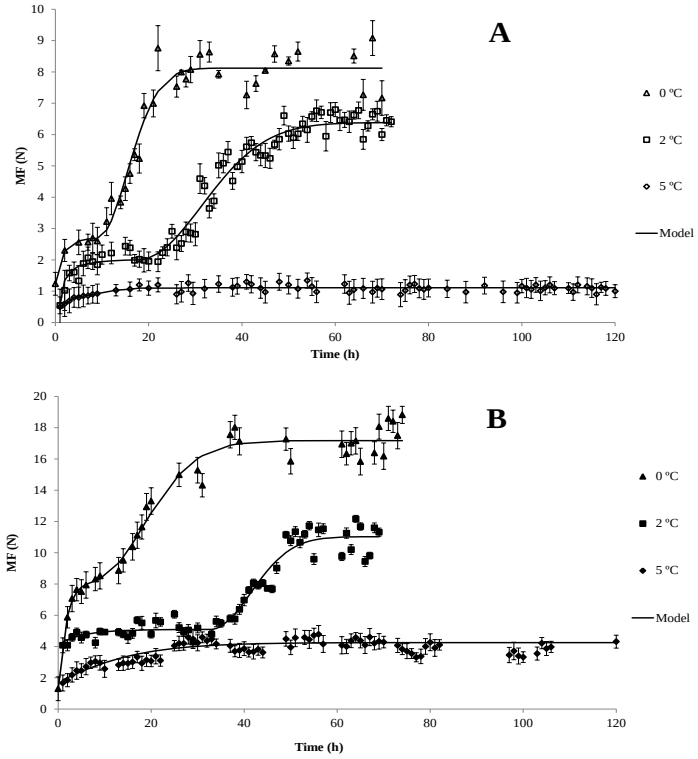


Figure 3. Changes and modeling of the maximum penetration force (MF) at different temperatures of Montanera (A) and Cebo (B) fats during cold storage. Average values ± Standard deviation are plotted from five replicates.

The degree of hardness of the fat also varied in function of the cold storage temperature. Thus, the lower the storage temperature, the higher the penetration force. At 0 ºC, Cebo fat showed a maximum penetration force close to 17 N whereas at 2 ºC, it was of only 10 N (Figure 3B). At 5 ºC, the maximum penetration force was even lower (4 N) than at 2 ºC, due to the fact that only the most highly saturated TGs crystallized and therefore, the solid/liquid ratio was

lower than at 2 and 0 ºC. Montanera fat was observed to behave similarly. Low storage temperatures cause a rapid crystallization, leading to the formation of small fat crystals, which display a more homogeneous mass spatial distribution involving a denser and harder structure (Campos et al., 2002).

When comparing the different penetration forces of Montanera and Cebo fats during storage at the same temperature (Figure 3), the results showed that Cebo was harder than Montanera fat. Thus, the constant penetration force values after the first increase were almost three times higher for Cebo than Montanera fat (8 versus 2.5 N at 0 ºC). This fact may be related with the higher content of saturated TGs in Cebo fat (Petrón et al., 2004), which increases the solid/liquid ratio, resulting in a harder texture. When the most unsaturated TGs are fully crystallized, which coincides with the end of the second increase in the penetration force, Cebo fat still reached a higher maximum penetration force than Montanera (16 versus 8 N at 0 ºC). This fact may be explained by considering the characteristic crystallization pattern of saturated and unsaturated TGs. Saturated fatty acids can easily align themselves to form a compact mass because they are relatively linear molecules (Himawan et al., 2006). The unsaturated fatty acids have kinks in their aliphatic chains, causing a zig-zag bending, which has implications for crystal packaging. This fact makes more unsaturated TGs have a less dense structure, with lower melting temperatures than saturated TGs with the same chain length (Himawan et al., 2006). Therefore, the higher level of saturation in Cebo fat implies a more compact fat crystal packaging, resulting in a harder texture during crystallization than in the case of Montanera fat. In addition to the fat composition, the differences in the textural parameters could also be attributed to differences in the structure of the samples. In this regard, after only a short period of time, the use of the Montanera system leads to a significant increase in the fat content of the animal. As a result, the connective tissue is expected to be less structured and consistent than that of intensively reared animals (Niñoles et al., 2008).

The crystallization of the TGs in Iberian pig fat caused changes in their textural properties during cold storage. These changes largely varied depending

on whether it was Montanera or Cebo fat, since the different rearing systems used lead to varying levels of unsaturation/saturation in fatty acid profiles.

3.4 Monitoring of ultrasonic velocity during cold storage.

Ultrasonic monitoring was performed during the isothermal crystallization at different storage temperatures (0, 2, 5, 7 and 10 ºC) for both Montanera and Cebo fats. The ultrasonic velocity measurements started when the temperature of the sample reached the storage temperature, thus, the crystallization always followed an isothermal pattern.

Figure 4 shows the two-step sigmoid curves of ultrasonic velocity versus crystallization time. A similar curve shape had already been observed for the change of textural parameters during storage (Figure 3). Thus, ultrasonic velocity increased with time, showing a pattern with two steep increases (Figure 4). As already reported in section 3.3, the crystallization of the TGs shown in Figure 2 brings about the increase in the fat solid/liquid ratio, which results in a harder sample with a higher elastic modulus where ultrasound waves travel faster than in samples with a higher liquid content. In the experiments at 10 ºC, only the first increase in the ultrasonic velocity was observed, which then remained constant until the end of the storage (480 h). At 10 ºC, the most unsaturated TGs did not crystallize and, consequently, only the first increase in the ultrasonic velocity was observed. This behavior also occurred during the textural measurements at the highest cold storage temperature (Figure 3).

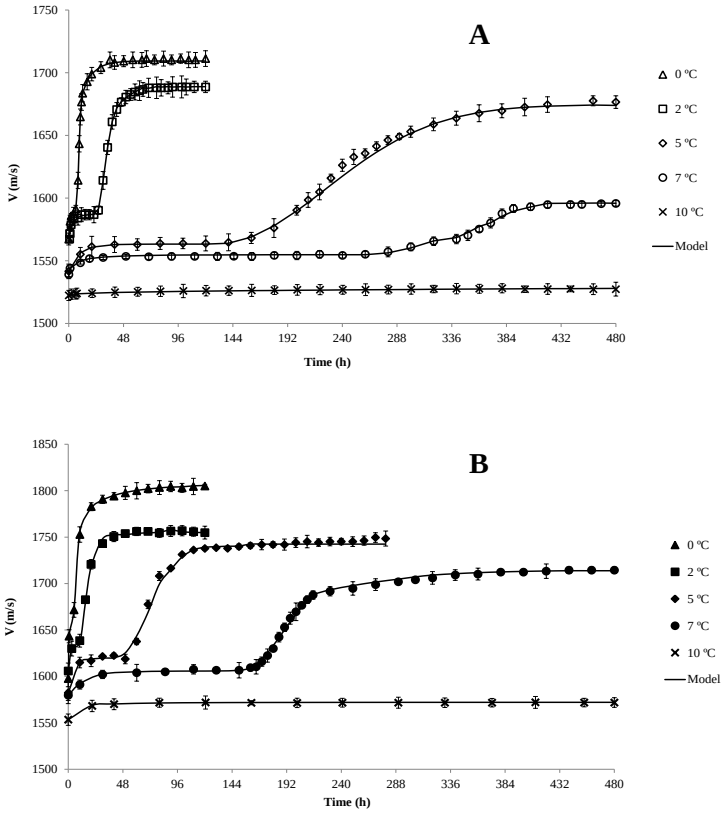


Figure 4. Changes and modeling of the ultrasonic velocity during cold storage at different temperatures of Montanera (A) and Cebo (B) fats. Average values ± Standard deviation are plotted from three replicates.

On the other hand, ultrasonic measurements showed that the higher the storage temperature, the lower the velocity. In the case of Montanera fat, the ultrasonic velocity increased from 1525 to 1705 m/s (Figure 4A) when storage temperature was reduced from 10 to 0 ºC, while the ultrasonic velocity in Cebo changed from 1570 to 1800 m/s for the same range. As previously mentioned, high crystallization temperatures lead to larger fat crystals and a softer crystalline structure than low temperatures. In addition, it should be remarked that the ultrasonic velocity in liquid and solid fats also falls as the temperature rises (Benedito, Cárcel, Roselló & Mulet, 2001)

Finally, in a similar way to textural measurement, the ultrasonic velocity allowed the two types of fats to be differentiated. When comparing the curves of

Iberian Montanera and Cebo fats (Figure 4), the highest values of ultrasonic velocity at the same storage temperature were always found in Cebo. Thus, at 0 ºC, the maximum ultrasonic velocity was 90 m/s higher for Cebo than Montanera (1800 versus 1710 m/s). As explained in section 3.3, the higher level of saturation in Cebo TGs implies a harder crystalline structure in which ultrasound is propagated faster than in Montanera.

Therefore, ultrasound permitted an optimum monitoring of the change in the textural properties brought about by crystallization during cold storage. In particular, the last stage in the manufacturing of dry-cured Iberian ham involves a storage temperature of around 18 ºC. At this temperature, the most unsaturated TGs are liquid and they will become partly or totally solid during the cold storage of distribution and retail sale. Furthermore, the crystallization pattern of Iberian fat will depend on the TGs composition, which is characteristic for each breed and rearing system (Carrapiso et al., 2003; Petrón et al., 2004; Niñoles et al., 2007).

Thus, due to the great influence that the state of the fat has on the quality of the meat products (texture and aroma release) which contain Iberian fat, ultrasound could also be used as an alternative to DSC or textural methods to identify the overall quality of Iberian products and to monitor the changes that take place during cold storage. This application is highly relevant as ultrasound could be measured on the surface of dry-cured vacuum packaged Iberian ham and used as a non-destructive technology which is easily automated on-line.

3.5 Modeling of the change in the ultrasonic velocity and textural properties during cold storage.

Modeling the ultrasonic velocity change during the isothermal crystallization of Iberian fat is relevant for predicting and quantifying the crystallization process during the cold storage of Iberian products. A mathematical model developed by Santacatalina et al. (2010), based on the Avrami model, was applied to describe the evolution of the ultrasonic velocity during crystallization of both Montanera and Cebo fats (Figure 4).

Table 3 shows the results obtained for the modeling of the ultrasonic velocity. The Avrami model fitted the experimental data of ultrasonic velocity well. In Figure 4, it may be observed that the model followed the same trend as the experimental data, the % VAR and RMSE values being higher than 99.5 % and lower than 3.5 m/s for Cebo fat and higher than 99.7 % and lower than 2.3 m/s for Montanera fat, respectively. Both statistical parameters indicated that the two-step crystallization model developed by Santacatalina et al. (2010) was appropriate for modeling the change which took place in the ultrasonic velocity during the cold storage of the two types of fat used in this work.

Table 3. Results for the modeling of the ultrasonic velocity during cold storage of Montanera and Cebo fats at different temperatures (0, 2, 5 and 7 ºC). Model parameters, percentage of explained variance (% VAR) and root mean square error (RMSE).

MONTANERA										
T (ºC)	a_1 (m/s)	K_1 (s^{-n})	n_1	a_2 (m/s)	K_2 (s^{-n})	n_2	α (h)	β (s)	VAR (%)	RMSE (m/s)
0	1647	6.90E-03	0.4	1708	3.70E-06	1.2	7.4	7451	99.8	1.8
2	1587	7.90E-04	0.8	1689	2.20E-08	1.5	23.3	33760	99.9	0.8
5	1564	1.50E-04	0.9	1673	3.00E-11	1.9	134	441687	99.7	2.3
7	1555	3.90E-03	0.5	1602	7.50E-09	1.9	257.5	435664	99.8	1.6
10	1527	2.00E-01	0.1	-	-	-	-	-	81.5	0.6
CEBO										
T (ºC)	a_1 (m/s)	K_1 (s^{-n})	n_1	a_2 (m/s)	K_2 (s^{-n})	n_2	α (h)	β (s)	VAR (%)	RMSE (m/s)
0	1666	3.00E-03	0.7	1808	1.80E-02	0.4	3.9	14043	99.9	0.9
2	1636	5.00E-03	0.6	1753	4.70E-06	2.2	8.4	16300	99.6	2.8
5	1621	3.00E-03	0.6	1742	1.90E-03	2	42.1	141693	99.5	3.5
7	1606	5.80E-02	0.3	1714	7.00E-03	3	167.3	1556138	99.7	2.2
10	1572	1.30E-02	0.5	-	-	-	-	-	77.4	0.9

Subscripts 1 and 2 denote the first and second steep increases in the ultrasonic velocity versus time curve, respectively.

The crystallization temperature affected the a_1 and a_2 parameters. These parameters are the values of the plateau in the first and second increases in the ultrasonic velocity when the crystallization of both saturated and unsaturated TGs is completed. Thus, for both parameters, the lower the temperature, the higher the velocity plateau. As already mentioned, the faster the crystallization (low temperatures), the harder the sample and, as a consequence, the higher the velocity. The relationship between a_1 and a_2 and the crystallization temperature was well described by significant ($p<0.05$) linear regressions. For the 0 to 10 ºC temperature range, a linear relationship (Cebo Eqs. 5 and 6 and Montanera Eqs. 7 and 8) was found between the parameters a_1 and a_2 and the crystallization temperature:

$$a_1 = -8.7(T) + 1661 \qquad R^2=0.97 \qquad (5)$$

$$a_2 = -11.8(T) + 1795 \qquad R^2=0.88 \qquad (6)$$

$$a_1 = -10.7(T) + 1628 \qquad R^2=0.89 \qquad (7)$$

$$a_2 = -13.6(T) + 1716 \qquad R^2=0.84 \qquad (8)$$

where T is the crystallization temperature (ºC).

These equations could be used to estimate the maximum velocity and, therefore, the maximum texture reached by a sample stored in a temperature range of 0 to 10 ºC. As an example, if Cebo fat was crystallized at 6 ºC, the a_1 and a_2 values would be 1609 and 1724 m/s, respectively. On the other hand, when comparing Montanera and Cebo fats (Table 3), the highest values of the a_1 and a_2 parameters for each temperature were obtained for Cebo. As already mentioned, the higher content of saturated fatty acids present in Cebo fat leads to a more compact TGs packaging, involving higher values of the elastic modulus, which, in turn, leads to a faster propagation of the ultrasonic wave. Therefore, the parameters a_1 and a_2 allow the different crystallization pattern of Montanera and Cebo fats to be identified and quantified.

The temperature was also found to influence the α parameter of the modified Avrami equation. This parameter represents the time (in hours) when the second crystallization stage starts and is characteristic of the onset time of crystallization of the most unsaturated TGs. In this case, exponential equations were identified in order to quantify the influence of the crystallization temperature on the α parameter (Cebo Eq. 9 and Montanera Eq. 10):

$$\alpha = 3.35\, exp(0.537\, T) \qquad R^2=0.99 \qquad (9)$$

$$\alpha = 8.03\, exp(0.519\, T) \qquad R^2=0.99 \qquad (10)$$

Using Eq. 9, therefore, it could be predicted that, at 6 ºC, the crystallization of the most unsaturated TGs in Cebo would begin at 84 hours. At high temperatures, the exponential relationship indicates that the crystallization of the most unsaturated TGs would only occur at very long storage times. Thus, although Eqs. 9 and 10 are only valid in the range of 0 to 7 ºC, the α parameter at 10 ºC for Montanera should be 1441 hours, which could explain why the crystallization of the most unsaturated TGs was not observed for 5 storage days (480 hours) at this temperature (Fig. 4). However, it could also be possible that the most unsaturated TGs cannot crystallize at 10 ºC. The main influence of the origin of the fat on the relationship between α and temperature was found in the pre-exponential factor, which was 3.35 in the case of Cebo and 8.03 for Montanera (Eqs. 9 and 10), respectively. This means that the crystallization of the most unsaturated TGs in Montanera is delayed if compared to that in Cebo, which can also be seen in Fig. 4.

As reported in section 3.4, similar behavior between the ultrasonic velocity (Fig. 4) and the textural changes (Fig. 3) was observed during cold storage. For this reason, the Avrami equation was also applied to describe the changes in the maximum penetration force, related with the hardness of the samples (Figure 3) during cold storage. In this regard, the changes in the penetration force as a function of crystallization time were modeled using Eq. 11.

$$FM(t) = a' \cdot \left(1 - \exp\left(-k' \cdot t^{n'}\right)\right) \quad (11)$$

where *FM* is the maximum penetration force (N) at time *t* (s), *a'* the maximum force at infinite time, *k'* is the crystallization rate constant (s^{-n}) dependent on the crystallization temperature and *n'* is the Avrami exponent (dimensionless).

Eq. 11 was modified to include the two step crystallization process (Eq. 12):

$$FM = A_1' \cdot Avr_1' + A_2' \cdot Avr_2' \quad (12)$$

where Avr_1' and Avr_2' are the Avrami equations (Eq. 11) for the first and second maximum force increases, respectively, and A_1' and A_2' are calculated in a similar way to the ultrasonic velocity using equations 3 and 4.

Figure 3 shows the fit of the Avrami model for both Cebo and Montanera fats; in spite of the great variability of the experimental data, the model adequately described the behavior of the textural changes during cold storage. The results showed the influence of the crystallization temperature on the a'_1 and a'_2 parameters (Table 4); therefore, the lower the temperature used, the higher the maximum force and the harder the sample. When comparing the two types of fat at the same temperature (0 ºC, Table 4), the highest values of the a'_1 and a'_2 parameters were obtained for Cebo (9.0 and 17.0 N) and the lowest for Montanera (2.7 and 8.1 N). As already explained, this fact arises from the differing fatty acid profile that leads to a different TGs crystallization. The differences in the connective tissue (Niñoles et al., 2008) could also lead to the variations in the texture of the two types of fat. Therefore, the modified Avrami model was adequate for the description, quantification and prediction of the ultrasonic and textural changes that took place during cold storage as a result of the Iberian fat crystallization. In addition, the model could be used to differentiate between both Iberian fats from different rearing systems.

Table 4. Results for the modeling of the maximum penetration force during cold storage of Montanera and Cebo fats at different temperatures (0, 2 and 5 ºC). Model parameters, percentage of explained variance (% VAR) and root mean square error (RMSE).

MONTANERA										
T (ºC)	a'_1 (N)	K'_1 (s^{-n})	n'_1	a'_2 (N)	K'_2 (s^{-n})	n'_2	α (h)	β (s)	VAR (%)	RMSE (N)
0	2.7	7.60E-04	0.8	8.1	8.1	8.2	9	33249	95.3	0.09
2	2.1	1.50E-06	1.3	6.5	8	12.2	17.8	69111	95.4	0.05
5	2.1	3.50E-07	1.2	-	-	-	-	-	66.9	0.02
CEBO										
T (ºC)	a'_1 (N)	K'_1 (s^{-n})	n'_1	a'_2 (N)	K'_2 (s^{-n})	n'_2	α (h)	β (s)	VAR (%)	RMSE (N)
0	9	1.70E-04	1	17	3.60E-08	1.6	1	65423	97	0.13
2	5.2	1.00E-04	0.9	11	8.05	7.8	32.8	43926	94.3	0.09
5	5.2	3.30E-02	1.2	-	-	-	-	-	58.5	0.04

Subscripts 1 and 2 denote the first and second steep increases in the maximum penetration force versus time curve, respectively.

4. Conclusions

In this work, textural changes were observed in two types of Iberian backfats (Montanera and Cebo) during cold storage. Thus, fat hardness, instrumentally characterized as maximum penetration force, showed two steep increases due to the crystallization of the most saturated and unsaturated TGs. During crystallization, Cebo fat was always harder than Montanera, which was explained by taking their different fatty acid composition into account. Ultrasonic velocity permitted an optimum monitoring of fat crystallization. Thus, this technology successfully identified the TGs crystallization and characterized the different crystallization pattern of Montanera and Cebo fats. Finally, a two step model based on the Avrami equation was used to describe the textural and ultrasonic changes in both Montanera and Cebo fats during the crystallization process.

Therefore, the results obtained in this work indicate that the ultrasonic technique could be a reliable method with which to characterize the

crystallization of fats from Iberian pigs and their textural properties during cold storage. Further work should be carried out in the future to establish the feasibility of using non-destructive ultrasonic techniques in the behavior characterization of dry-cured Iberian products during cold storage, in order to identify the progress of crystallization and to differentiate between Montanera and Cebo products.

References

Bell, A., Gordon, M. H., Jirasubkunakorn, W. & Smith, K. W. (2007). Effects of composition on fat rheology and crystallisation. *Food Chemistry*, 101, 799-805.

Benedito, J., Carcel, J. A., Clemente, J. & Mulet, A. (2000a). Cheese maturity assessment using ultrasonics. *Journal of Dairy Science*, 83, 248-254.

Benedito, J., Carcel, J. A., González, R. & SanJuan, N. (2000b). Prediction of Instrumental and Sensory textural characteristics of Mahon cheese from ultrasonic measurements. *Journal of Texture Studies*, 31, 631-643.

Benedito, J., Carcel, J. A., Roselló, C. & Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 57, 365-370.

Benedito, J., Carcel, J. A., González, R. & Mulet, A. (2002). Application of low intensity ultrasonics to cheese manufacturing process. *Ultrasonics.* 40, 19-23.

BOE (2007). Real Decreto 1469/2001 por el que se aprueba la norma de calidad para el jamón Ibérico, paleta ibérica y caña de lomo ibérico elaborados en Espana. *Boletín Oficial del Estado de de 3 de Noviembre de 2007*.

Campos, R., Narine, S.S., & Marangoni, A.G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Research International*, 35, 971-981.

Carrapiso, A. I., Bonilla, F. & García, C. (2003). Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. *Meat Science*, 65, 623-629.

Cava, R., Ruiz, J., López-Bote, C., Martín, L., García, C., Ventanas, J. & Antequera, T. (1997). Influence of finishing diet on fatty acid profiles of intramuscular lipids, triglycerides and phospholipids in muscle of Iberian pig. *Meat Science*, 45, 263-270.

Cava, R., Ventanas, J., Ruiz, J., Andrés, A. & Antequera, T. (2000). Sensory characteristics of Iberian ham: Influence of rearing system and muscle location. *Food Science and Technology International*, 6 (3), 235-242.

Cebula, D.J. & Smith, K.W. (1991). Differential scanning calorimetry of confectionery fats. Pure trygliceride: Effects of cooling and heating rate variation. *Journal of the American Oil Chemists' Society*, 68, 591-595.

Cebula, D.J. & Smith, K.W. (1992). Differential scanning calorimetry of confectionery fats. Part II: Effects of blends and minor components. *Journal of the American Oil Chemists' Society*, 69, 992-998.

Cilla, I., Martínez, L., Beltrán, J. A. & Roncalés, P., (2006a). Effect of low-temperature preservation on the quality of vacuum-packaged dry-cured ham: Refrigerated boneless ham and frozen ham cuts. *Meat Science*, 73, 12-21.

Cilla, I., Martínez, L., Beltrán, J. A. & Roncalés, P., (2006b). Dry-cured ham quality and acceptability as affected by the preservation system used for retail sale. *Meat Science*, 73, 581-589.

Danthine, S. & Deroanne, C. (2004). Influence of SFC, microstructure and polymorphism on texture (hardness) of binary of fats involved in the preparation of industrial shortenings. *Food Research International*, 37, 941-948.

Diaz, I., Garcia-Regueiro, J.A., Casillas, M. & De Pedro, E. (1996). Triglyceride composition of fresh ham fat from iberian pigs produced with different systems of animal nutrition. *Food Chemistry*, 55, 383-387.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226, 497-509.

Fuentes, V., Ventanas, J., Estévez, M., Carrapiso, A. I. & Ventanas, S. (2010). Application of Time-intensity technique to study the effect of fat composition on texture and flavour perception in a dry-cured model product. *Oral presentation in the Fourth European Conference on Sensory and Consumer Research (Vitoria, Spain).*

García-Esteban, M., Ansorena, D. & Astiasarán, I. (2004). Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on color, texture and microbiological quality. *Meat Science*, 67, 57-63.

Himawan, C., Starov, V. M. & Stapley, A. G. F. (2006). Thermodynamic and kinetic aspects of fat crystallization. *Advances in Colloid and Interface Science*, 122, 3-33.

Kalnin, D., Lesieur, P., Artzner, F., Keller, G. & Ollivon, M. (2005). Systematic investigation of lard polymorphism using combined DSC and time-resolved synchrotron X-ray diffraction. *European Journal of Lipid Science and Technology*, 107, 594-606.

Llull, P., Simal, S., Femenia, A., Benedito, J. & Rosselló, C. (2002). The use of ultrasound velocity measurement to evaluate the textural properties of sobrassada from Mallorca. *Journal of Food Engineering*, 52, 323-330.

Llull, P., Simal, S., Benedito, J. & Rosselló, C. (2002). Evaluation of textural properties of meat-based product (sobrassada) using ultrasonic techniques. *Journal of Food Engineering*. 53, 279-285.

Marangoni, A. G. & Narine, S. S. (2002). Identifying key structural indicators of mechanical strength in networks of fat crystals. *Food Research International*. 35, 957-969.

Mulet, A., Benedito, J., Bon, J. & Sanjuán, N. (1999). Low intensity ultrasonics in food technology. *Food Science and Technology International*, 5, 285-297.

Niñoles, L., Clemente, G., Ventanas, S. & Benedito, J. (2007). Quality assesment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Niñoles, L., Sanjuan, N., Ventanas, S. & Benedito, J. (2008). Ultrasonic and sensory characterization of dry-cured ham fat from Iberian pigs with different genetics and feeding backgrounds. *Meat Science*, 80, 896-902.

Papadima, S. N. & Bloukas, J. G. (1999). Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science*, 51, 103-113.

Petrón, M. J., Muriel, E., Timón, M. L., Martín, L. & Antequera, T. (2004). Fatty acids and triacylglycerols profiles from different types of Iberian dry-cured hams. *Meat Science*, 68, 71-77.

Rubio, B., Martínez, B., García-Cachán, M.D., Rovira, J. & Jaime, I. (2007a). The effects of high pressure treatment and of storage periods on the quality of vacuum-packed "salchichón" made of raw material enriched in monounsaturated and polyunsaturated fatty acids. *Innovative Food Science & Emerging Technologies*, 8, 180-187.

Rubio, B., Martínez, B., Sánchez, M. J., García-Cachán, M. D., Rovira, J. & Jaime, I. (2007b). Study of the shelf life of a dry fermented sausage "salchichón" made from raw material enriched in monounsaturates and polyunsaturated fatty acids and stored under modified atmospheres. *Meat Science*. 76, 128-137.

Ruiz-Carrascal, J., Ventanas, J., Cava, R., Andrés, A. I. & García, C. (2000). Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. *Food Research International*, 33, 91-95.

Sandler, S. R., & Karo, W. (1992). Source book of advances organic laboratory preparations. *San Diego: Academic Press*.

Santacatalina, J. V., Garcia-Perez, J. V., Corona, E. & Benedito, J. (2010). Ultrasonic monitoring of lard crystallization during storage. *Food Research International*, 44, 146-155.

Shi, Y., Liang, B. & Hartel, R. W. (2005). Crystal morphology, microstructure, and textural properties of model lipid systems. *Journal of the American Oil Chemists Society*, 82, 399-408.

Singh, A.P., McClements, D.J. & Marangoni, A.G. (2004). Solid fat content determination by ultrasonic velocimetry. *Food Research International*, 37, 545-555.

Timón, M. L., Martín, L., Petrón, M. J., Jurado, A. & Garcia, C. (2001a). Composition of subcutaneous fat from dry-cured Iberian hams as influenced by pig feeding. *Journal of the Science of food and Agriculture*, 82, 186-191.

Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A. & García, C. (2001b). Subcutaneous and intermuscular fat characterization of dry-cured Iberian hams. *Meat Science*, 58, 85-91.

Váquiro, H. A., Bon, J. & Díez, J. L. (2008). Fuzzy logic application to drying kinetics modelling. *The International Federation of Automatic Control (17th World Congress, Seoul, July 2008)*.

Ventanas, S., Estévez, M., Andrés, A. I. & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME–DED. *Meat Science*, 79, 172–180.

Ventanas, S., Tejeda, F. J., & Estévez, M. (2008). Chemical composition and oxidative status of tissues from Iberian pigs as affected by diets: extensive feeding v.oleic acid- and tocopherol-enriched mixed diet. *Animal*, 2: 621-630.

Ventanas, S., Ventanas, J., & Ruiz, J. (2007). Sensory characteristics of Iberian dry-cured loin: influence of crossbreeding and rearing system. *Meat Science*, 75, 211–219.

Ventanas, S., Ventanas, J., Ruiz, J., & Estévez, M. (2005). Iberian pigs for the development of high-quality cured products. *Recent Research Developments of Agricultural & Food Chemistry*, 6: 27-54.

Wright, A.J., Narine, S.S. & Marangoni, A.G. (2001). Comparison of experimental techniques used in lipid crystallization studies. In N. Widlak, R.Hartel, & S. Narine (Eds.), Crystallization and solidification properties of lipids (pp 120-131). *Champaign: AOCS Press*.

CHAPTER 3

(*Apartado 3*)

ULTRASONIC CHARACTERIZATION OF THE FAT SOURCE AND COMPOSITION OF FORMULATED DRY-CURED MEAT PRODUCTS

E. Corona, J.V. García-Pérez, S. Ventanas, J. Benedito

Food Science and Technology International

(Submitted)

Ultrasonic characterization of the fat source and composition of formulated dry-cured meat products

E. Corona[a], J.V. García-Pérez[a], S. Ventanas[b], J. Benedito[a]

a. Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camí de Vera s/n, E46022, Valencia, Spain.

b. Tecnología de los Alimentos, Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Avd.de la Universidad s/n, 10071 Cáceres, Spain.

Abstract

The aim of this work was to test the feasibility of using ultrasonic measurements to estimate the fat content (FC) and identify the fat sources used in formulated dry-cured meat products. For that purpose, dry-cured sausages were prepared using different fat sources (montanera and cebo backfats, Iberian lard and sunflower oil) and contents (FC from 3 to 17 % w.b.) and characterized by measuring the ultrasonic velocity (at 2, 6, 10, 15, 20 and 25 ºC), fatty acid profile, thermal behavior and composition. The fatty acid composition affected the sausages melting behavior, which also involved different ultrasonic velocities depending on the fat source used for the sausage formulation. Significant ($p<0.05$) linear relationships were established between the ultrasonic velocity and the percentage of melted fat, with which the sausage batches were differentiated according to the fat source used. The ultrasonic velocity dependence on temperature allowed the determination of the fat content (explained variance 96.1 %) by measuring the ultrasonic velocity in the dry-cured sausages at 2 ºC and 25 ºC and using a semi-empirical equation. Therefore, the ultrasonic measurements could be considered a reliable tool for the characterization and differentiation of formulated dry-cured meat products with different fat sources and contents.

Key words: *Ultrasonics, modeling, meat products, non-destructive testing*

1. Introduction

Dry-cured pork meat products are highly appreciated by consumers in Spain and Mediterranean countries. They can be classified in two main groups depending on whether the product is formulated, such as different kinds of sausages ("chorizo", "salchichon", sobrassada, etc.), or constitutes a specific portion of the animal, this being the case of Iberian and Serrano ham and also pork loin. Both groups also greatly differ in the fat content, actually being much higher in those formulated. An increase in the intake of fatty dry-cured meat products is not recommended from a human diet point of view, at least, for some population groups and highly fatty products, which is more noticeable for the most saturated fat sources. Thus, the meat industry strives to develop new products with better nutritional properties (Muguerza, Gimeno, Ansorena & Astiasarán, 2004), considering the new trend of markets and consumer preferences for low fat products. In this regard, new formulations have been tested, mostly affecting the fat content (García, Dominguez, Galvez, Casas & Selgas, 2002; Mendoza, García, Casas & Selgas, 2001). Changes in the fat content affect not only the composition but also the textural properties and flavor (Muguerza, Fista, Ansorena, Astiasaran & Bloukas, 2002; Papadima & Bloukas, 1999). Likewise, the fat source appears to have an important effect on these properties (Murgueza et al., 2002; Muguerza, Ansorena, Bloukas & Astisarán, 2003; Murgueza et al., 2004; Niñoles, Mulet, Ventanas & Benedito., 2010; Valencia, Ansorena & Astiasarán, 2006; Ventanas, Ventanas, Jurado, & Estévez, 2006).

Common techniques for analyzing the fatty composition of dry-cured meat products include chemical analysis (Mendoza et al., 2001; Papadima et al., 1999), gas-chromatography (Murqueza et al., 2003) and Differential Scanning Calorimetry (DSC) (Benedito, Carcel, Rossello, & Mulet, 2001). Moreover, these techniques, together with the sensory and instrumental texture analysis (Ventanas, Ruiz, García, & Ventanas, 2007), have been applied for the identification of the fat source of dry-cured meat products. Thus, Rubio et al. (2007) evaluated the addition of meat with sunflower and soya oils in salchichon samples through textural profile analysis and sensory evaluation. While Muguerza, Gimeno, Ansorena, Bloukas & Astiasarán (2001) used textural

analysis for characterizing the partial replacement of pork backfat with a pre-emulsion of olive oil in chorizo samples. However, these technologies require sample destruction for analysis and in addition, are high time consuming. Thereby, new technologies with greater non-destructive character are being tested for evaluating the fat content, identifying the fat source and quantifying their influence on sensory and textural parameters, such as near infrared spectroscopy (Fenández-Cabanás, Polvillo, Rosríguez-Acuña, Botella & Horcada, 2011; Ortiz-Somovilla, España-España, Gaitán-Jurado, Pérez-Aparicio & De Pedro-Sanz, 2007), magnetic resonance imaging (MRI) (Pérez-Palacios, Antequera, Durán, Caro & Rodríguez, 2010), computed tomography (Garcia-Gil et al., 2012) and ultrasound (Llull, Simal, Benedito & Roselló, 2002), among others.

Ultrasound could be considered an accurate, affordable, rapid and non-destructive analytical technique with potentially easy implementation in on-line applications. The reliability of ultrasound as analytical technique has been shown through the characterization of several food properties. Thus, the spoilage of edible oil during frying (Benedito, Mulet, Velasco, & Dobarganes, 2002), the solid content of semi-crystalline fats (Coupland, 2004) and the cheese composition (Telis-Romero, Váquiro, Bon, & Benedito, 2011) have been characterized using ultrasonic measurements. In the meat food sector, ultrasonic measurements have been used to determine the composition of raw meat mixtures (Benedito et al., 2001), to classify pig backfat from animals of different breeds and feeding regimes (Niñoles, Clemente, Ventanas, & Benedito, 2007; Niñoles, Sanjuan, Ventanas & Benedito, 2008) or to characterize the composition and texture of different muscles from Iberian pigs (Niñoles, Mulet, Ventanas & Benedito, 2011). In previous works, ultrasound has been used to monitor the crystallization process of lard (Santacatalina, Garcia-Perez, Corona, & Benedito, 2011), as well as to differentiate the crystallization pattern of montanera and cebo Iberian backfats. However, its ability to classify dry-cured meat products, where fat is only a component of a complex matrix that has been subjected to drying, according to its fat content and source has not been addressed. As a first approach, it seems reasonable to address this objective in formulated dry-cured meat products, where the fat is uniformly distributed, to evaluate in the following

stages this issue in whole pieces, such as Iberian ham or loin with greater heterogeneity in fat distribution. Therefore, the main aim of this work was to evaluate the feasibility of using ultrasonic measurements to estimate the fat source and content of formulated vacuum packaged dry-cured meat products.

2. Materials and methods

2.1 Raw material and sample preparation

The formulated meat products (sausages) were manufactured using fresh pork lean tissue (Large White x Landrace breed) and different fat sources: two types of Iberian subcutaneous backfats (montanera and cebo), commercial sunflower oil (Consum. S. Coop, Valencia, Spain) and Iberian rendered lard (El Pozo Alimentación S.A., Alhama de Murcia, Spain), to obtain a wide range of fat saturation/unsaturation levels. Every fat and the fresh pork meat were obtained from local markets.

Two sets of experiments were performed. On the one hand, in order to assess the influence of the fat source (saturation/unsaturation) on the ultrasonic measurements (FAT SOURCE, FS set), four batches of sausages were prepared with the same fat content using the different fat sources (montanera, cebo, lard and sunflower oil). The samples were manufactured with a fat content of 12 % (wet basis, w.b.), which includes a 3±0.5 % of the native fat from the pork lean tissue and 9 % of added fat. On the other hand, in order to study the effect of fat content on the ultrasonic velocity (COMPOS set), three batches of sausages were prepared with different fat sources (montanera, cebo and lard) and three levels of fat content (6, 10 and 15 % w.b.), which also included the 3 % of the native fat from pork lean tissue. In addition, a control sample was also elaborated using only the pork lean meat. Since the objective of this study was to determine the feasibility of using ultrasound for fat source and content assessment in a homogeneous sample and thereafter, apply the technique to products like ham, the range of fat contents was chosen according to the fat content naturally found in Iberian dry-cured ham. Thereby, 13 different batches of sausages were carried out (4 in FS and 9 in COMPOS set, respectively).

The sausages for both experiment sets were separately prepared following the same procedure. The mixture of pork lean and fat was minced to a particle size of about 8 mm and afterwards mixed in a vacuum mixer (UMC 5 15, Stephan, Germany) with the following ingredients: 2.10 % salt, 0.015 % nitrites, 0.05 % ascorbic acid and 1 % w.b. Glucono D-Lactone. The sausage mixture was stuffed (BB12, Mainca, Spain) into artificial collagen casings (40 mm Ø) in pieces of 166±11 g. For each batch of both FS and COMP sets, at least four sausages were prepared. The sausages were placed in a drying chamber (CME, Vizuete, Spain) at temperatures ranging from 22±1 ºC to 10±1 ºC, and relative humidity from 95 % to 75 % until the end of the ripening process (15 days). Once the ripening was finalized, the sausages were taken from the casings and vacuum packaged. During this drying period, samples lost approximately 15±7 % of the initial weight. The final composition of the sausages was affected by the dehydration suffered during the ripening stage. In Table 1, the average fat, moisture and protein+others contents in the dry-cured sausages are reported. Finally, the ultrasonic measurements were performed for all the batches.

Table 1. Composition of the different dry-cured sausages batches.

SET	FAT SOURCE	Fat (% w.b.)	Moisture (% w.b.)	Protein+others* (% w.b.)
FS	Lard	15.6±0.7	47.3±0.7	37.2
	Cebo	15.8±1.3	48±0.5	36.2
	Montanera	14.6±1.2	50.8±0.3	34.6
	Sunflower oil	12.5±0.5	47.4±0.5	40.1
COMPOS	Lard	7.6±0.6	56.5±1.1	35.9
	Lard	13.5±0.5	54.2±1.1	32.3
	Lard	15.6±0.5	48.9±0.6	35.5
	Cebo	7.3±0.3	53.2±0.5	39.5
	Cebo	11.1±0.6	51.9±0.8	37.1
	Cebo	16.6±1.3	48.2±0.5	35.2
	Montanera	7.0±0.3	53.8±0.9	39.3
	Montanera	12.1±0.4	50.9±0.6	37.1
	Montanera	19.6±0.3	46.3±0.6	34.1
Pork lean		3.1±0.4	58.3±0.6	38.6

Average values ± Standard deviation from 12 replicates.

***calculated by difference**

2.2 Chemical composition

Fatty acid methyl esters (FAMEs) of both fats were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992). Lipids were previously extracted by using the Folch et al. (1957) procedure. FAMEs were analysed by a gas chromatograph (HP-5890A, Hewlett Packard, USA) equipped with a flame ionisation detector (FID). Separation was carried out on a polyethylene glycol–TPA modified fused silica semicapillary column (30 m long, 0.53 mm id, 1 μm film thickness) maintained at 225 °C. Injector and detector temperatures were 230°C. Carrier gas was nitrogen at a flow rate of 1.8 mL/min. Individual FAMEs peaks were identified by comparing their retention times with those of standards (Sigma, St. Louis). Results are expressed as percentage of the total fatty acids analyzed. Eight repetitions per sausage batch were performed.

The fat (FC) and the water contents (WC) analysis of the dry-cured sausages were carried out in triplicate according to the AOAC procedures 991.36 and 950.46, respectively (AOAC, 1997).

2.3 Thermal behavior

The thermal behavior of every sausages batch was monitored using differential scanning calorimetry (DSC) (DSC5200CU, Seiko Instruments, Inc., USA). Approximately 18 to 20 mg of dry sausage sample were introduced into a standard aluminum DSC crucible and then hermetically sealed and weighed on a balance (± 0.01 mg, ER-182A, AND, Japan). The sample was previously dried in an oven at 105 ºC until constant weight (approximately 24 hours) to avoid the influence of water. An empty and hermetically sealed aluminum DSC crucible was used as the reference material and liquid nitrogen was used as the cooling fluid. The sample was heated from 25 ºC to 70 ºC at 5 ºC/min. Next, the sample was tempered at 70 ºC for 1 min to destroy any crystal memory. Afterwards, the sample was cooled from 70 to -50 ºC using a 5 ºC/min cooling rate. Thereafter, temperature was held at -50 ºC for 30 min. Finally, the sample was heated from -50 to 55 ºC at 5 ºC min^{-1}. Following this procedure, DSC curves were obtained for the melting of the different fats at the cooling rate tested. The peak maximum temperature (T_{max}, ºC), onset temperature (T_o, ºC) and latent heat (ΔH, J/g) were

computed from the thermograms (Cebula & Smith., 1992). The percentage of melted fat (% MF) at every temperature was also obtained. This percentage was computed at temperature intervals of 1ºC as the ratio between the accumulated latent heat for each temperature, and the total latent heat for the complete heating cycle.

2.4 Ultrasonic velocity measurements

The experimental set-up for ultrasonic velocity measurements (Niñoles et al., 2010) consisted of a couple of narrow-band ultrasonic transducers (5 MHz, 0.75" diameter, V308, Panametrics, Waltham, MA, USA), a pulser-receiver (5058PR, Panametrics, Waltham, MA, USA) and a digital storage oscilloscope (TDS5034, Tektronix, Bearverton, Oregon, USA). A custom digital height gage was designed and built, and linked to the computer by a RS232 interface to measure the sample thickness. The ultrasonic velocity was computed from the time of flight (averaged for 5 signals) and the sample thickness and determined for all the samples at 2, 6, 10, 15, 20, 25 and 30 ºC in a temperature-controlled chamber (±0.5 ºC) (AEC330R, Infrico, Spain).

2.5 Experimental procedure

In every vacuum packaged dry-cured sausage, three zones of measurement were uniformly distributed and marked on their surface. Each zone was coincident with the surface of the transducer (2.5 cm^2) used in the ultrasonic measurements. The ultrasonic velocity was measured in triplicate in each zone, thus nine measurements were carried out per sausage, being the total number of measurements of 504 for each temperature.

After the ultrasonic measurements, the fatty acid composition and the DSC analysis were carried out for each dry-cured sausage batch. Moreover, the water (WC) and fat (FC) contents were determined in each zone of measurement.

2.6 Statistical analysis

The influence of the fat source on the fatty acid profile, thermal properties and ultrasonic velocity were statistically evaluated by the analysis of variance (ANOVA). Moreover, linear relationships were established between the

ultrasonic velocity and water and fat content using the "Regression Model Selection" tool. In both cases, the Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA) software was used, being Fisher's Least Significant Difference (LSD) intervals computed at a 95 % confidence level.

3. Results and discussion

3.1 Fatty acid profile

The fatty acid profile for the dry-cured sausages of FS set (sunflower oil, montanera, cebo and lard fat sources) was analyzed (Table 2), being assessed the saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) contents. These samples were elaborated with an initial fat content of 12 % w.b., which increased up to 14.6±1.5 % w.b. after the ripening process (Table 1).

On the one hand, the results show that sunflower oil presented a statistically significant ($p<0.05$) different content in SFA, MUFA, PUFA and total unsaturated fatty acid (MUFA+PUFA) content than the other batches. Low SFA (23.9 %) content and high MUFA+PUFA (76.1 %) content were found for sausages prepared with sunflower oil, being the most abundant fatty acid the linoleic acid (C18:2, 46.4 %). This fact involves a lower melting temperature and therefore, confers a more liquid state for these samples (Himawan, Starov & Stapley, 2006).

On the other hand, when montanera, cebo and lard sausages are compared, it was observed that montanera sausages presented a significantly ($p<0.05$) higher MUFA+PUFA (61.6 %) and lower SFA contents (38.4 %). Likewise, for these three batches, the main fatty acid was the oleic acid, C18:1 (n-9), being its content significantly ($p<0.05$) higher in montanera (42.2 %) than cebo (37.2 %) and lard (34.7 %) samples. These results could be explained considering the influence of the feeding system of the animals. In this regard, Iberian pigs fed in montanera system tend to accumulate high levels of unsaturated fatty acids mostly due to the high oleic acid content of acorns (Niñoles et al., 2008; Ruiz-Carrascal, Ventanas, Cava, Andrés & García, 2000). Several works (Niñoles et

al., 2007; Petrón, Muriel, Timón, Martín & Antequera, 2004; Ruiz-Carrascal et al., 2000) have reported the influence of the fatty acid profile on the sensory properties and therefore, on the overall quality of dry cured products. In this regard, higher levels of unsaturation lead to an increase in oiliness, brightness and aroma release.

Table 2. Fatty acid composition (%) of dry-cured sausages elaborated with different fat sources (FS set: Sunflower oil, Montanera, Cebo and Lard). Average fat content 14.6 ± 1.5 % w.b.

	SUNFLOWER OIL	MONTANERA	CEBO	LARD
C12	0.051±0.002^{a}	0.093±0.005^{a}	0.090±0.004^{a}	0.11±0.08^{a}
C14	0.71±0.04^{a}	1.6±0.1^{b}	2.1±0.1^{c}	2.1±0.1^{c}
C16	15.2±0.7^{a}	26.1±0.5^{b}	31.1±0.4^{c}	31.0±1.0^{c}
C16:1	1.0±0.1^{a}	2.4±0.1^{b}	2.8±0.1^{c}	2.9±0.1^{d}
C17	0.15±0.02^{a}	0.34±0.01^{c}	0.32±0.04^{b}	0.36±0.01^{d}
C17:1	0.11±0.01^{a}	0.31±0.01^{c}	0.26±0.01^{b}	0.32±0.04^{c}
C18	7.4±2.4^{a}	9.8±0.1^{ab}	12.2±0.2^{bc}	13.7±2.0^{c}
C18:1 (n-9)	24.7±4.8^{a}	42.2±0.8^{c}	37.2±0.6^{b}	34.7±0.7^{b}
C18:1 (n-7)	1.9±0.2^{a}	3.2±0.1^{b}	3.5±0.1^{c}	3.3±0.1^{bc}
C18:2	46.4±2.5^{c}	10.4±0.1^{b}	6.9±0.2^{a}	8.8±0.3^{ab}
C18:3 (n-3)	0.31±0.04^{a}	0.66±0.14^{b}	0.54±0.03^{b}	0.53±0.10^{b}
C20	0.35±0.09^{a}	0.44±0.15^{a}	0.51±0.02^{a}	0.35±0.20^{a}
C20:1	0.55±0.01^{a}	0.81±0.05^{b}	0.57±0.01^{a}	0.50±0.03^{a}
C20:2	0.33±0.20^{a}	0.48±0.13^{a}	0.45±0.02^{a}	0.36±0.10^{a}
C20:3 (n-6)	0.16±0.10^{a}	0.40±0.18^{a}	0.37±.09^{a}	0.22±0.15^{a}
C20:4	0.42±0.13^{a}	0.50±0.03^{ab}	0.78±0.11^{b}	0.52±0.28^{ab}
C20:3 (n-3)	0.10±0.08^{a}	0.28±0.14^{a}	0.34±0.15^{a}	0.18±0.15^{a}
C20:5	0.21±0.03^{b}	0.023±0.003^{a}	0.011±.005^{a}	0.022±0.009^{a}
C24:0	0.032±0.001^{a}	0.061±0.014^{b}	0.031±0.008^{a}	0.033±0.009^{a}
C22:6	-	0.005±0.011^{a}	-	-
C24:1	0.003±0.006^{a}	-	-	-
SFA	**23.9±2.9^{a}**	**38.4±0.6^{b}**	**46.3±0.5^{c}**	**47.6±1.1^{c}**
MUFA	**28.2±4.8^{a}**	**48.9±0.7^{c}**	**44.3±0.6^{bc}**	**41.7±0.8^{b}**
PUFA	**47.9±1.9^{c}**	**12.7±0.3^{b}**	**9.4±0.6^{a}**	**10.7±0.8^{ab}**
MUFA+PUFA	**76.1±2.9^{c}**	**61.6±0.6^{b}**	**53.6±0.5^{a}**	**52.4±1.1^{a}**

Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA) and Polyunsaturated Fatty Acid (PUFA).

Average values ± Standard deviation from 8 replicates. Different letters (a, b, c and d) in the same row denote a significant difference ($p<0.05$).

3.2. Thermal behavior characterization of Iberian pork fats.

It is well known that the melting behavior of the fat is one of the most important functional properties in many dry-cured meat products, since the liquid/solid ratio of the fat greatly contributes to the sensory perception of consumers (Niñoles et al., 2010). In this regard, the thermal properties of the dry-cured sausages of FS set elaborated with sunflower oil, montanera, cebo and lard fat sources were analyzed by DSC (Figure 1, Table 3), in which the melting behavior of triacylglycerols is observed. Although triacylglycerols were not identified in this work, it could be assumed than the level of unsaturation on triacylglycerols is directly linked to the fatty acid composition. According to this fact, sunflower oil sausages should have a higher level of unsaturation than the other batches due to its significantly higher content of MUFA+PUFA.

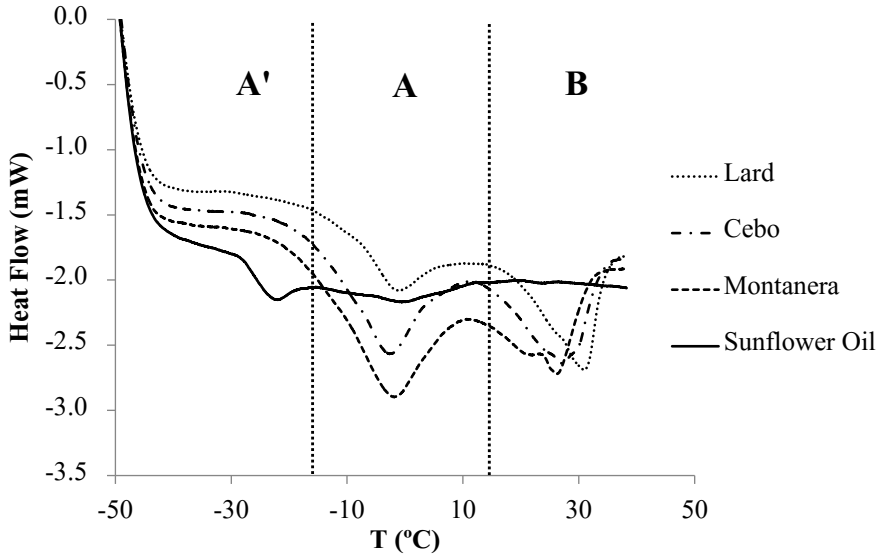


Figure 1. DSC thermograms of dry-cured sausages elaborated with different fat sources (FS set, sunflower oil, montanera, cebo and lard). Average fat content 14.6 ± 1.5 % w.b.

Two main endothermal peaks were identified in the DSC melting curves (Figure 1) in each batch. The peaks A' and A for sunflower oil sausages and the peaks A and B for montanera, cebo and lard ones. The enthalpy of the endothermal peaks A' and A should correspond to the melting of the most

unsaturated triacylglycerols, while the peak B should be linked to the most saturated ones (Niñoles et al., 2007; Niñoles et al., 2010).

Table 3 shows the thermal properties calculated from the DSC curves. For sunflower oil sausages, the peak A' showed higher melting latent heat (0.81 J/g) than peak A due to a higher content of unsaturated triacylglycerols (O'Brien, 2009; Ortuño Sánchez, 2006), and a lower peak temperature (-22.2 ºC versus -0.55 of peak A). The peak A could be linked to the melting of the unsaturated triacylglycerols of the native fat of pork lean.

Table 3. Thermal properties, peak maximum temperature (Tmax), onset temperature (To) and latent heat (ΔH), of dry-cured sausages elaborated with different fat sources (FS set: Sunflower oil, Montanera, Cebo and Lard). Average fat content 14.6 ± 1.5 % w.b.

		SUNFLOWER OIL	MONTANERA	CEBO	LARD
ΔH (J/g)	Peak A'	0.81±0.03	-	-	-
	Peak A	0.10±0.05^{a}	6.6±0.5^{c}	4.2±0.1^{b}	3.0±0.3^{b}
	Peak B	-	5.7±0.1^{a}	5.7±0.4^{a}	5.8±0.4^{a}
Tmax (ºC)	Peak A'	-22.2±0.1	-	-	-
	Peak A	-0.55±0.41^{a}	-3.3±0.6^{a}	-2.0±0.3^{b}	-1.6±0.5^{bc}
	Peak B	-	26.7±0.5^{a}	26.9±0.6^{a}	30.2±0.7^{b}
To (ºC)	Peak A'	-28.1±0.1	-	-	-
	Peak A	-13.8±0.1^{c}	-18.2±0.4^{a}	-16.0±0.1^{b}	-10.8±0.7^{d}
	Peak B	-	13.7±0.5^{a}	15.0±0.9^{b}	18.1±0.1^{c}

Average values ± Standard deviation are shown. Different letters (a, b, c and d) in the same row denote a significant difference ($p<0.05$) for each parameter (Tmax, To and ΔH) and peak (A', A and B).

On the other hand, for the other samples and peak A, montanera sausages presented a significantly ($p<0.05$) higher latent melting heat (6.6 J/g) and lower peak temperature (-3.3 ºC) than cebo and lard sausages due to its higher content of unsaturated triacylglycerols. On the contrary, the lowest latent heat (3.08 J/g)

and the highest peak temperature (-1.6 ºC) for peak A were found in lard, which is linked to its low content of unsaturated triacylglycerols.

In the peak B, which would correspond with the melting of the most saturated triacylglycerols, no significant ($p<0.05$) differences for the latent heat were observed between the samples of montanera, cebo and lard. However, lard samples presented a significant ($p<0.05$) higher maximum peak temperature (30.2 ºC) and onset temperature (18.1 ºC) than montanera and cebo ones, which involves that triacylglycerols should present in lard a higher level of saturation.

From the experimental results, it could be pointed out that the melting behavior of the sausages, provided by DSC technique, contributes to its discrimination regarding the fat source used during the elaboration. Nevertheless, this technique is high time consuming and require the sample destruction, which limits its use as a quality control technique in food processing and hinders its on-line implementation.

3.3. Ultrasonic velocity.

3.3.1. Influence of temperature

Figure 2 shows the influence of temperature on the ultrasonic velocity (Figure 2A) and the percentage of melted fat (% MF) (Figure 2B) for the dry-cured sausages of FS set elaborated with sunflower oil, montanera, cebo and lard fat sources and a final fat content of 14.6±1.5 %. For every sausage batch, the curves showed that the higher the temperature in the range 2 to 30ºC, the lower the ultrasonic velocity. This change could be linked with the increase of the percentage of melted fat (%MF) (Figure 2B), which was calculated from the DSC thermograms (Figure 1). Indeed, the melting peaks are noticeable in the thermograms in this temperature range except for sunflower oil. The greater change in the % MF and decrease in the ultrasonic velocity (Figure 2A and B) was found in the temperature ranges from 2-10 ºC and from 20-30 ºC. This higher fat melting involves the larger amount of released fat, which is related to the sensory properties of dry-cured Iberian products. Fat melting results in an increase of the liquid/solid fat ratio (McClements, 1997) and, therefore, in a decrease of the ultrasonic velocity of the sausages. This velocity decrease is also

ascribed to the superimposed effect of the negative temperature coefficient of the ultrasonic velocity in fatty matrixes (Povey, 1997). A similar trend of the ultrasonic velocity with the temperature was observed in dry-cured subcutaneous fat (Niñoles et al., 2010) and meat mixtures (Benedito et al., 2001).

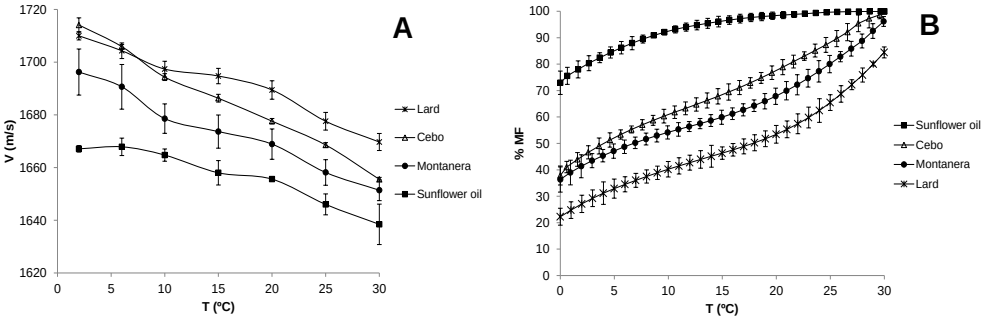


Figure 2. Influence of temperature on the average ultrasonic velocity (A) and the percentage of melted fat (% MF) (B) of dry-cured sausages elaborated with different fat sources (FS set, sunflower oil, montanera, cebo and lard). Average fat content 14.6 ± 1.5 % w.b.

From 2 to 30 ºC, only a 27 % fat was melted for sunflower oil sausages, which corresponds with the lowest ultrasonic velocity decrease (28 m/s, from 1667 to 1638 m/s). This fact could be mainly linked with the melting of only the most saturated triacylglycerols of the native fat of the meat due to that sunflower oil is completely melted in this temperature range. For cebo, montanera and lard batches, the average decrease of velocity was 48 m/s for a change of the % MF of 60 %. It should be remarked that in lard sausages only the 85 % of the fat was melted at 30 ºC, suggesting that at this temperature the most saturated triacylglycerols are still in the solid state. Moreover, the ultrasonic velocity, at this temperature, was the highest for lard sausages (1669 m/s).

3.3.2. Characterization of the fat source and melted fat

The ultrasonic velocity measurements showed the feasibility to discriminate the four different dry-cured sausage batches of FS set (Figure 2A). The results showed that the highest velocity for all temperatures was obtained for lard sausages (except at 2 ºC), while the lowest values were recorded for sunflower oil ones. An intermediate ultrasonic velocity was found for cebo and

montanera samples. This fact suggests that the different melting behavior of triacylglycerols (Niñoles et al., 2010), given by the different fatty acid composition (Himawan et al., 2006), affect the sausage structure at a given temperature and the ultrasonic velocity is prone to these changes. For every temperature, the highest % MF was obtained for sunflower oil samples, which corresponds with the lowest ultrasonic velocity (Figure 2A). On the contrary, the lowest % MF and the highest velocity were obtained for lard samples. However, when cebo and montanera batches are compared, a contradictory result was found, due to cebo presented higher % MF and higher ultrasonic velocity for the same temperature than montanera. Therefore, in these products a direct relationship between % MF and ultrasonic velocity was not found, since the higher liquid/solid fat ratio in cebo does not involve a lower ultrasonic velocity than in montanera. In order to understand this result, it should be considered that montanera feeding system leads to a sharp increase in the backfat content of the animals in a short period of time, being the connective and fatty tissue less structured and consistent than in cebo reared animals (Niñoles et al., 2008). Thus, montanera backfat becomes softer than cebo ones, which explains the lower ultrasonic velocity values reported for montanera sausages. This effect of the structure tends to disappear at high temperatures (30 ºC) due to the fat melting process, as observed in Figure 2A where montanera and cebo sausages presented a non-significant ($p<0.05$) different ultrasonic velocity. A similar behavior was observed in samples of dry-cured ham fat (Niñoles et al., 2008), being montanera fat softer than cebo one at the same temperature, which was also confirmed from the measurement of ultrasonic velocities.

Significant ($p<0.05$) relationships were found between the average ultrasonic velocity and the % MF measured in the range from 2 to 30 ºC (Figure 3) with correlation coefficients higher than 0.97 for montanera, cebo and lard sausages. While for the sunflower oil batch, a lower correlation coefficient (0.76) was obtained, that could be given by the narrow range of % MF and velocity for this batch compared to the remaining batches. Thereby, through the use of equations shown in Figure 3 for montanera, cebo and lard sausages, it could be predicted the % MF in a non-destructive way. Therefore, the ultrasonic measurements could be a reliable technique for estimating the percentage of

melted fat in vacuum packaged dry-cured sausages, which could be used for assessing the textural properties of the samples at different temperatures, as well as for differentiating samples with different fat sources. In addition, the direct measurement of the ultrasonic velocity on the package does not perturb the product allowing the on-line measurement and minimizing the risk of contamination, which is a great advantage for the food industry.

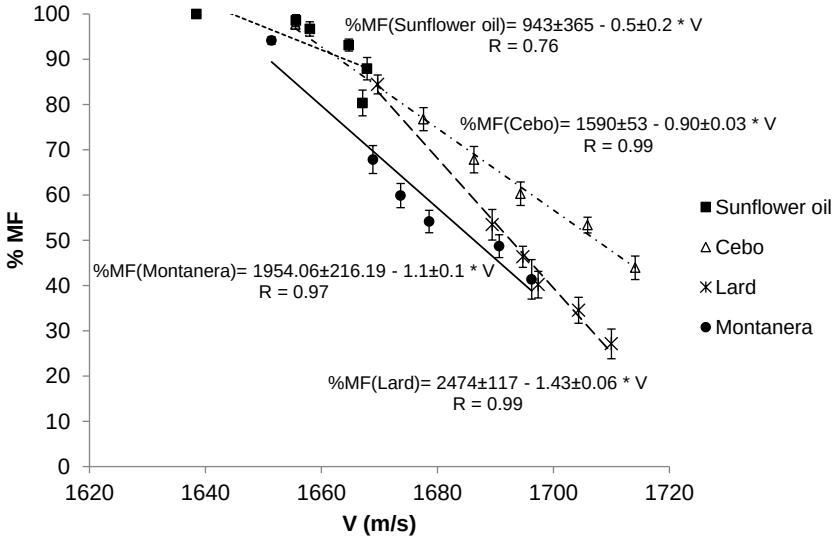


Figure 3. Relationship between the ultrasonic velocity (V) and the percentage of melted fat (% MF) of dry-cured sausages elaborated with different fat sources (FS set, sunflower oil, montanera, cebo and lard). Average fat content 14.6 ± 1.5 % w.b.

3.3.3. Characterization of the fat and water content

In this section, the use of low intensity ultrasound to assess the fat and water content on vacuum packaged dry-cured sausages was addressed. For that purpose, as explained in section 2.1, dry-cured sausages of COMPOS set were elaborated with different fat sources (montanera, cebo and lard) and 3 fat contents 7.3±0.5, 12.2±1.1 and 17.2±1.9 % w.b. The ultrasonic measurements were performed at different temperatures from 2 to 25 ºC.

For samples with low fat content (7 %), a significant ($p<0.05$) increase in ultrasonic velocity was observed for cebo and lard sausages (Figure 4). This

result is linked to the increase of the ultrasonic velocity in water with the increase of temperature (Povey & McClements., 1988). Thus, the decrease of velocity with the increase of temperature, observed in Figure 2, due to fat melting is counteracted by the opposite behavior of the ultrasonic velocity in water (Povey, 1997). Notwithstanding, this fact becomes more evident as moisture increases and fat diminishes. Thus, in the control sample without added fat (3 % fat, and high moisture content, 58.3 %), the ultrasonic velocity increased from 1669 m/s at 2 ºC to 1698 m/s at 30 ºC. A similar finding was observed by Benedito et al, (2001) in lean tissue samples of raw meat mixtures, who reported that in low fat lean tissues, the main influence of temperature on velocity was given by the water content of samples. For montanera sausages containing 7 % of fat, the velocity kept almost constant in this temperature range. For sausages with higher fat contents, 12 % and 17 %, there is a decrease in velocity with the increase of temperature, such as observed in batches of FS set, being the higher the fat content, the higher the velocity drop. Thus, montanera batch showed a decrease of ultrasonic velocity of 7 m/s for 12 % of fat content, while for 17 % of fat content the velocity decrease was of 26 m/s.

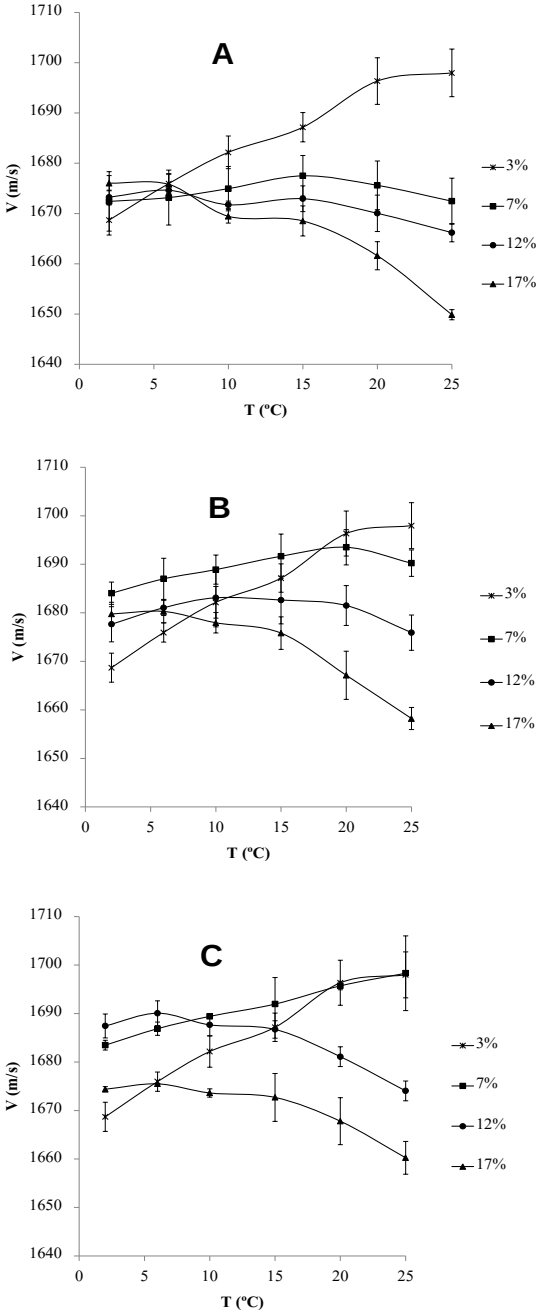


Figure 4. Variation of the average ultrasonic velocity with temperature in dry-cured sausages elaborated with different fat sources (FS set, montanera A, cebo B and lard C) and contents.

From Figure 4, it is observed that the ultrasonic measurements also could be used to differentiate the fat content for all batches. Although, the ability of the ultrasonic measurements for fat content differentiation was temperature dependent. For low temperatures, it is difficult to separate the sausages according to its fat content due to the similar ultrasonic velocity in the solid fat and the non-fatty fraction (mainly protein and water). As temperature increases, the fat melts and the ultrasonic velocity in samples with different fat content starts to be differentiated due to the ultrasonic properties of liquid fat are very different to those of non-fatty fraction. In this regard, the higher the content of liquid fat, the lower the ultrasonic velocity (McClements, 1997). Thus, for temperatures higher than 15 ºC, the sausages with different fat content may be correctly differentiated, being found the highest differences for the measurements carried out at 25 ºC.

Figure 5 shows the influence of both the water (WC) and the fat (FC) content on the ultrasonic velocity measured at 20 ºC (a common consumption temperature) for dry-cured sausages elaborated with cebo backfat. A significant ($p<0.05$) linear relationship was found (Figure 5) between the fat and water content and the ultrasonic velocity ($R=0.98$ and $R=0.97$, respectively). The curves for montanera and lard sausages showed a similar behavior ($R>0.95$ for FC and $R>0.96$ for WC). Thus, it seems that there exists a strong relationship between the global composition of the dry-cured sausages and the ultrasonic velocity. In this regard, Benedito et al. (2001) proposed a semi-empirical equation to estimate the composition of raw meat mixtures (fatty and lean tissues) considering the temperature dependence on the ultrasonic velocity and assuming samples behave as a three constituents system (fat, water and protein+others). The model also assumes that the temperature dependence of the ultrasonic velocity is different for the three constituents. Thereby, from the measurement of the ultrasonic velocity at 2 and 25 ºC, and using the semi-empirical model proposed by Benedito et al. (2001), the assessment of the composition of the dry-cured sausages was performed. In Figure 6, the comparison of actual fat contents on the dry-cured sausages of COMPOS set and those computed from the model is depicted. It is observed that the simulation results largely agree with the experimental data of the fat content, being reached

an explained variance of 96.1 %. A slightly lower explained variance was obtained for the water content (87.4 %). Moreover, the model also provided an accurate assessment of the fat content of sausages of FS set, thus the average figures estimated for the different batches differed an average of 3.1 % of the experimental values (Table 1). Therefore, the ultrasonic measurements could be useful for the characterization of the composition of dry-cured sausages, which is directly related to sensory properties, and so to the perception of consumers.

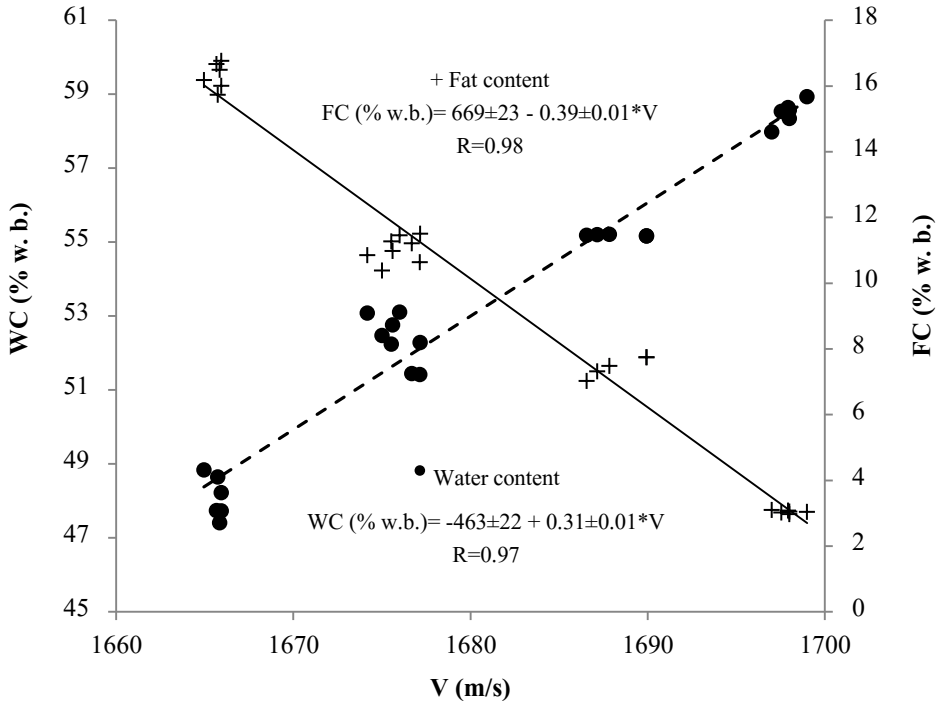


Figure 5. Influence of the water (WC) and fat (FC) content on the ultrasonic velocity measured at 20 ºC for dry-cured sausages elaborated with Cebo fat (COMPOS set).

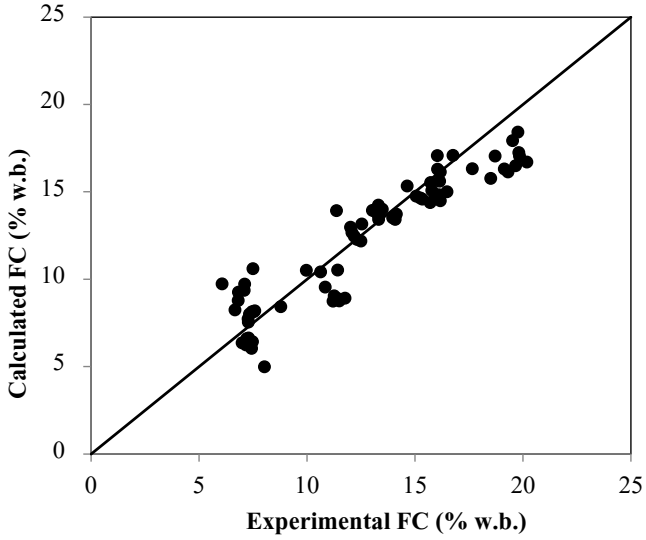


Figure 6. Experimental and calculated fat content (FC) of dry-cured sausages of COMPOS set using a mathematical model based on the measurement of the ultrasonic velocity at 2 and 25 ºC.

4. Conclusions

This work highlights the reliability of the ultrasonic techniques, and namely the measurement of the ultrasonic velocity at different temperatures, to not only identify the fat source used in the formulation of dry-cured sausages but also predict its composition. In this regard, the decrease of the ultrasonic velocity with the temperature increase, as a consequence of the fat melting, was significantly ($p<0.05$) different depending on the fat used in the formulation, which allow differentiating the different sausage batches. A semi-empirical model, which considers the temperature dependence on the ultrasonic velocity, allowed for accurate composition evaluation, mainly the fat content of the dry-cured sausages. Therefore, the non-destructive and non-invasive ultrasonic measurements can be used to classify formulated dry-cured meat products according to its fat content or source, which are relevant quality traits greatly linked to the sensory properties and preferences of consumers. Further work must validate the reliability of this technology with other dry-cured meat products with high economical added value, like Iberian hams where also exists more heterogeneity in the fat distribution.

References

Association of Official Analytical Chemist. *Official Methods of Analysis; AOAC, International,* 16 edn. Washington, DC, 1997.

Benedito, J., Carcel, J. A., Rossello, C. & Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 57, 365-370.

Cava, R., Ruiz, J., López-Bote, C., Martin, L., García, C., Ventanas, J. & Antequera, T. (1997). Influence of finishing diet on fatty acid profiles of intramuscular lipids, triglycerides and phospholipids in muscles of the Iberian pig. *Meat Science*, 2, 263-270.

Cebula, D.J. & Smith, K.W. (1992). Differential scanning calorimetry of confectionery fats. Part II: Effects of blends and minor components. *Journal of the American Oil Chemists' Society*, 69, 992-998.

Fernández-Cabanás, V. M., Polvillo, O., Rodríguez-Acuña, R., Botella, B. & Horcada, A. (2011). Rapid determination of the fatty acid profile in pork dry-cured sausages by NIR spectroscopy. *Food Chemistry*, 124, 373-378.

Funami, T., Yotsuzuka, F., Yada, H., & Nakao, Y. (1998). Thermoirreversible Characteristics of Curdlan Gels in a Model Reduced Fat Pork Sausage. *Journal of Food Science*, 63, 575–579.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226, 497-509.

García, M. L., Dominguez, R., Galvez, M. D., Casas, C. & Selgas, M. D. (2002). Utilization of cereal and fruit fibres in low fat dry fermented sausages. *Meat Science*, 60, 227-236.

Garcia-Gil, N., Santos-Garcés, E., Muñoz, I., Fulladosa, E., Arnau, J. & Gou, P. (2012). Salting, drying and sensory quality of dry-cured hams subjected to different pre-salting treatments: Skin trimming and pressing. *Meat Science*, 90, 389-392.

Himawan, C., Starov, V. M. & Stapley, A. G. F. (2006). Thermodynamic and kinetic aspects of fat crystallization. *Advances in colloid and interface science*, 122, 3-33.

Llull, P., Simal S., Benedito, J. & Roselló, C. (2002b). Evaluation of textural properties of meat-based product (sobrassada) using ultrasonic techniques. *Journal of Food Engineering*, 53, 279-285.

McClements, D. J. & Povey, M. J. W. (1988). Investigation of phase transitions in glycerine/paraffin oil mixtures using ultrasonic velocity measurements. *Journal of the American Oil Chemists´Scociety*., 65. 1791-1795.

McClements, D. J. (1997). Ultrasonic characterization of food and drinks:Principles, methods and applications. *Critical Reviews in Food Science and Nutrition*, 37, 1-46.

Mendoza, E., García, M. L., Casas, C. & Selgas, M. D. (2001). Inulin as fat substitute in low fat, dry fermented sausages. *Meat Science*, 57, 387-393.

Muguerza, E., Ansorena, D., Bloukas, J. G. & Astisarán, I. (2003). Effect of fat level and partial replacement of pork backfat with olive oil on the lipid oxidation and volatil compouns of greek dry fermented sausages. *Journal of Food Science*, 68, 1531-1536.

Muguerza, E., Fista, G., Ansorena, D., Astiasaran, I. & Bloukas, J. G. (2002). Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, 61, 397-404.

Muguerza, E., Gimeno, G., Ansorena, A. & Astiasarán, I. (2004). New formulatuions for healthier dry fermented sausages: a review. *Food Science & Technology*, 15, 452-457.

Muguerza, E., Gimeno, O., Ansorena D., Bloukas, J. G. & Astiasarán, I. (2001). Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of Chorizo de Pamplona - a traditional Spanish fermented sausage. *Meat Science*, 59, 251-258.

Niñoles, L., Clemente, G., Ventanas, S. & Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Niñoles, L., Mulet, A., Ventanas, S. & Benedito, J. (2010). Ultrasonic assessment of the melting behaviour in fat from Iberian dry-cured hams. *Meat Science*, 85, 26-32.

Niñoles, L., Mulet, A., Ventanas, S. & Benedito, J. (2011). Ultrasonic characterisation of B. femoris from Iberian pigs of different genetics and feeding systems. *Meat Science*, 89, 174-180.

Niñoles, L., Sanjuan, N., Ventanas, S. & Benedito, J. (2008). Ultrasonic and sensory characterization of dry-cured ham fat from Iberian pigs with different genetics and feeding backgrounds. *Meat Science*, 80, 896-902.

O'Brien, R. D. (2009). Fats and Oils: Formulating and Processing for Applications. Third edition. *United States of America: CRC Press, Taylor & Francis Group*.

Ortiz-Somovilla, V., España-España, F., Gaitán-Jurado, A. J., Pérez-Aparicio, J. & De Pedro-Sanz, E. J. (2007). Proximate analysis of homogenized and minced mass of pork sausages by NIRS. *Food Chemistry*, 101, 1031, 1040.

Ortuño Sánchez, M. F. (2006). Manual práctico de aceites esenciales, aromas y perfumes. *Spain: AIYANA ediciones*.

Papadima, S. N. & Bloukas, J. G. (1999). Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science*, 51, 103-113.

Pérez-Palacios, T., Antequera, T., Durán, M. L., Caro, A. & Rodríguez, P. G. (2010). MRI-based analysis, lipid composition and sensory traits for studying Iberian dry-cured hams from pigs fed with different diets. *Food Research International*, 43, 248-254.

Petrón, M. J., Muriel, E., Timón, M. L., Martín, L. & Antequera, T. (2004). Fatty acids and tryacylglycerols profiles from different types of Iberian dry-cured hams. *Meat Science*, 68, 71-77.

Povey, M. J. W. & McClements, D. J. (1988). Ultrasonics in food engineering. Part I: Introduction and experimental methods. *Journal of Food Engineering*, 8, 217-245.

Povey, M. J. W. (1997). Ultrasonic techniques for fluids characterization. *San Diego, California, USA: Academic Press.*

Rubio, B., Martínez, B., Sánchez, M. J., García-Cachán, M. D., Rovira, J. & Jaime, I. (2007). Study of the shelf life of a dry fermented sausage "salchichón" made from raw material enriched in monounsaturated and polyunsaturated fatty acids and stored under modified atmospheres. *Meat Science*, 76, 128-137.

Ruiz-Carrascal, J., Ventanas, J., Cava, R., Andrés, A. I. & García, C. (2000). Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. *Food Research International*, 33, 91-95.

Sandler, S. R., & Karo, W. (1992). Source book of advances organic laboratory preparations. *San Diego: Academic Press*.

Santacatalina, J. V., Garcia-Perez, J. V. Corona, E. & Benedito, J. (2011). Ultrasonic monitoring of lard crystallization during storage. *Food Reseatch International*, 44, 146-155.

Valencia, I., Ansorena, D. & Astiasarán, I. (2006). Stability of linseed and antioxidants containing dry fermented sausages: a study of the lipid fraction during different storage conditions. *Meat Science*, 73, 269-277.

Ventanas, S., Ruiz, J., García, C. & Ventanas, J. (2007). Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science*, 77, 324-330.

CHAPTER 4

(*Apartado 4*)

ULTRASONIC ASSESSMENT OF TEXTURAL CHANGES IN VACUUM PACKAGED SLICED IBERIAN HAM INDUCED BY HIGH PRESSURE TREATMENT OR COLD STORAGE

E. Corona, J.V. García-Pérez, A. Mulet, J. Benedito

Meat Science
(Submitted)

Ultrasonic assessment of textural changes in vacuum packaged sliced Iberian ham induced by high pressure treatment or cold storage

E. Corona, J.V. García-Pérez, A. Mulet, J. Benedito

Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camí de Vera s/n, E46022, Valencia, Spain.

Abstract

Ultrasonic measurements were used to characterize the effect of High Pressure Treatment (HPT) (600 MPa/6 min) or cold storage (6 ºC/120 days) on the textural properties of vacuum packaged dry-cured ham. The ultrasonic velocity, textural properties and fat content were determined in the ham packages. The ultrasonic velocity was related to the ham hardness, which depends on the sample composition. HPT induced molecular alterations which resulted in an average increase in the hardness of lean tissue of 0.2 N and one of 0.3 N in that of fatty tissue. These textural changes give rise to a velocity increase (8 m/s for lean and 17 m/s for fatty tissue). The cold storage of the Iberian ham also led to an increase in hardness (average 1.10 N) and ultrasonic velocity (average 70 m/s). Therefore, the non-destructive ultrasonic technique could be a reliable method with which to assess the textural changes induced by HPT or cold storage on packaged dry-cured ham.

Key words: *Ultrasound; Non-destructive; Texture; High pressure treatment; Cold storage.*

1. Introduction

Nowadays, many meat products are commercialized already packaged, which represents an added difficulty when carrying out quality control on individual packages. This is particularly true in the case of Iberian ham. Dry-cured Iberian ham is a traditional Spanish dry-cured meat product of high sensory quality, which is in great consumer demand and is of economic importance. Although traditionally the whole Iberian ham has been commercialized with the hoof, both the difficulties involved in handling a whole ham and a change in consumer preferences have led to new retail presentations being adopted. Thus, sliced Iberian ham is also commercialized vacuum-packed or in modified atmosphere packages, which are commonly preserved by cold storage, in order to extend its shelf life and reduce spoilage (Cilla, Martínez, Beltrán, & Roncalés, 2006a; Cilla, Martínez, Beltrán, & Roncalés, 2006b; Parra et al., 2010). In addition to cold storage, new preservation technologies, such as High Pressure Treatment (HPT), are also being explored. HPT is considered to be a very promising and efficient non-thermal technology for the preservation of packaged sliced Iberian ham, since it reduces the microbial load and therefore, prolongs the shelf life (Andrés, Moller, Adamsen, & Skibsted, 2004; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010).

Both cold storage and HPT can affect ham characteristics (Colmenero, 2002; Parra et al., 2010) and, as such, molecular alterations have been observed in Iberian ham that has been subjected to these preservation techniques, inducing textural changes (Chapleau & Lamballerie-Anton, 2003). Thus, for quality assurance purposes it is important to assess the changes that can be induced by HPT or cold storage. To this end different techniques have been used to characterize the changes brought about in meat products during cold storage or HPT, such as Differential Scanning Calorimetry (DSC) (Zhu, LeBail, Chapleau, Ramaswamy, & de Lamballerie-Anton, 2004), chemical analysis such as the determination of lipid and protein oxidation (Cava, Ladero, González, Carrasco, & Ramírez, 2009), physicochemical and volatile compound analysis (Campus, Flores, Martínez, & Toldrá, 2008; Serra, Sárraga, Grèbol, Guàrdia, Guerrero et al., 2007), microbiological analysis (Garriga, Aymerich, Monfort, & Hugas, 2004) or sensory and instrumental texture analysis (Mor-Mur & Yuste, 2003;

Serra, Grèbol, Guàrdia, Guerrero, Gou et al., 2007). Using the aforementioned techniques implies the destruction of the sample, which prevents its use in automated on-line applications for industrial purposes. In this regard, the search for non-destructive technologies, such as ultrasound, nuclear magnetic resonance (NMR), computed tomography, near-infrared (NIR) or fluorescence spectroscopy, among others, which could evaluate the quality of cold stored or HPT samples, is of great interest for the food industry.

Ultrasound parameters, like velocity or attenuation, have been related to the textural properties of many foods, such as pork meat (Koch et al., 2011a; Koch, et al., 2011b; Niñoles, Mulet, Ventanas, & Benedito, 2011), meat mixtures (Benedito, Carcel, Rossello, & Mulet, 2001), fat from Iberian dry-cured hams (Niñoles, Mulet, Ventanas, & Benedito, 2010) and Iberian lard (Santacatalina, Garcia-Perez, Corona, & Benedito, 2011). Ultrasonic techniques are rapid, non-destructive and suitable for on-line measurements. The packaging material may represent an obstacle in the measurements, as it could mask the changes taking place in the product. Therefore, the aim of this work was to evaluate the feasibility of using low intensity ultrasound to assess the effect of high pressure treatment and cold storage on the textural properties of vacuum packaged, sliced dry-cured Iberian ham.

2. Materials and methods

2.1. Raw material preparation, high pressure treatment and cold storage

In order to explore the ability of using ultrasound to assess changes in vacuum packaged sliced ham, two sets of experiments were designed; the aim of the first was to determine the influence of HPT on the textural properties and the second to assess the effect of cold storage. The hams were obtained from Iberian pigs (reared in free-range), fattened on a mixture of grass and commercial concentrate feeds.

2.1.1. HPT set

From dry-cured Iberian hams (20 hams weighing an average of 7 kg each one) two different cuts (Babilla and Punta; Figure 1A) were considered due to

their different characteristics. Both ham cuts were separately sliced (1 mm thickness; mechanical slicing) and vacuum-packaged (20±2 slices; 90±9 g per package). A total of 90 packages were prepared per cut, and split into two batches. One batch was subjected to HPT (CENTA Institute, Monells, Girona, Spain) at 600 MPa and 12 ºC for 6 min (Wave 6500/120, NC Hyperbaric, Burgos, Spain), while the other batch of Punta and Babilla packages remained as control, both being characterized for comparison from compositional, textural and ultrasonic measurements.

2.1.2. Cold storage set.

In order to estimate the influence of cold storage on the textural properties of dry-cured Iberian ham, whole Iberian hams (5 hams/ 7 kg) were boned, mechanically sliced (1 mm thickness) and vacuum packaged (90 packages of 90±2 g each one). The packages were separated into two batches (45 packages per batch); the first one, considered as control, was characterized in a similar way to the control batch for the HPT set. The second batch was stored at 6±1 ºC for 120 days, which is an average storage time of vacuum packaged sliced dry-cured Iberian ham during retail sale.

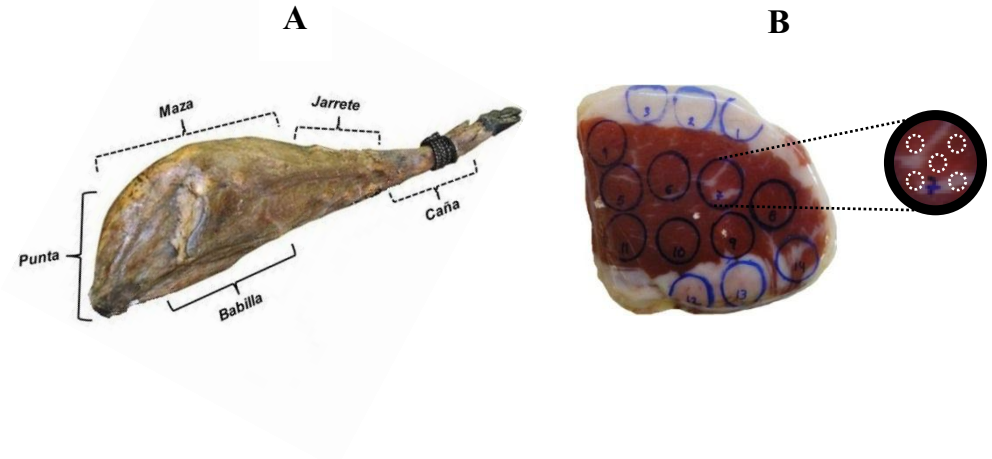


Figure 1. (A) Characteristic parts of Iberian ham and (B) Location of zones for the ultrasonic measurements on the surface of vacuum packaged, sliced Iberian ham. In the detail, the white dotted circles represent the pattern of the 5 penetrations carried out in the textural analysis in each zone.

In order to carry out the ultrasonic, textural and analytical characterization, in every sample from both sets of experiments, several zones of measurement were marked on the surface of each package, as shown in Figure 1B. The zones of measurement (from 10 to 14) were uniformly distributed over the surface of each package, including points in lean, marbled and fatty tissues. The surface of each zone tested coincided with the surface of the transducer (1.6 cm^2) used in the ultrasonic measurements. Thus, the total number of zones of measurement per analysis was 2,520 for the HPT set and 1,260 for the cold storage set.

In the HPT set, the ultrasonic and textural measurements were carried out on the control samples and after HPT processing, whereas, in the cold storage set, the control batch was measured at the beginning of storage and the stored batch after 120 days.

2.2. Composition analysis and tissue classification

For the HPT set, the moisture and fat (FC) contents were determined in triplicate for each one of the zones of measurement located in the ham packages, following the AOAC procedures (AOAC, 1997).

The estimation of fat content from images (FCI) may be considered a useful alternative for vacuum packaged sliced dry-cured ham. Thus, in the cold storage set, image analysis was used to classify each zone of measurement into three categories according to the fat content: fatty (FCI>70%), lean (FCI<23%) and marbled (23<FCI<70%). For that purpose, images of the packages were taken and each one of the zones of measurement was analysed using the Image J software (Research Service Branch, National Institute of Mental Health, US, available as freeware from http://rsbweb.nih.gov/ij/). The RGB images were converted to the binary system, where the lean tissue was characterized as the color black and the fat as white. Finally, the area of both colors was measured in pixels and the relative percentage calculated. Thus, depending on the percentage of white, the samples were classified as fatty (white>70%), lean (white<23%) and marbled (23<white<70%).

2.3. Ultrasonic velocity measurements

The experimental set-up consisted of a pair of narrow-band ultrasonic transducers (1 MHz, 0.5" diameter, A303S-SU, Panametrics, Waltham, MA, USA), a pulser-receiver (5058PR, Panametrics, Waltham, MA, USA) and a digital storage oscilloscope (TDS5034, Tektronix, Bearverton, Oregon, USA). A custom digital height gage was designed and built, and linked to the computer by a RS232 interface to measure the sample thickness. The sliced-ham packages were placed between the transducers. The ultrasonic velocity in the zones of measurement was computed in triplicate from the time of flight and the thickness provided by the height gage. The ultrasonic measurements for the different batches were taken at 6 ºC in a temperature-controlled chamber (±0.5 ºC) (AEC330R, Infrico, Spain), as this is the usual refrigeration storage temperature.

Temperature influences food texture and so, in order to study the effect of temperature on the ultrasonic velocity, the measurements were carried out in both the control and treated batches of the HPT set at 2, 6, 10, 15, 20 and 25 ºC (temperature range from refrigeration to consumption).

2.4. Instrumental textural analysis

The textural properties of the samples were analyzed using a texture analyzer machine (TA-XT2i, Stable Micro Systems, Surrey, England) linked to a computer for data acquisition and processing. Texture analysis was carried out at storage temperature (6±0.5 ºC) in a cooling chamber (AEC330R, Infrico, Spain). Due to the destructive nature of the test, only one temperature was considered for comparison purposes. The textural analysis consisted of puncture tests carried out by using a flat-ended cylindrical stainless steel probe (2 mm diameter), with a penetration distance of 5 mm and a crosshead speed of 1 mm/s. For each zone of measurement located on the ham packages, five penetrations were carried out following the pattern shown in Figure 1B. Finally, hardness was determined as the average of the maximum force (MF, N) computed from the force versus distance records for the five punctures carried out in each zone.

2.5. Statistical analysis

The influence of the following factors; fat content, ham cut (Punta and Babilla), HPT or cold storage on ham hardness and ultrasonic velocity was analysed by using analysis of variance (Multifactorial ANOVA). Moreover, aiming to quantify the influence of those factors, simple and generalized regression models were established. The statistical analysis was performed by using Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA), determining the Least Significant Differences (LSD) intervals determined considering a confidence level of 95 %.

3. Results and discussion

3.1. Influence of the composition on the textural properties.

The fat content (FC) is one of the most important factors affecting the quality and sensory characteristics, like texture, of raw and dry-cured meat products (Wood et al., 2008). Therefore, the influence of FC on the hardness of sliced Iberian ham was addressed by measuring the maximum penetration force (MF).

Figure 2 shows the significant ($p<0.01$) linear relationship between the average MF and the FC for all the zones of measurement located in the vacuum packages of Punta (Figure 2A) and Babilla (Figure 2B) control batches ($R=0.84$ and $R=0.78$ for Punta and Babilla batches, respectively) of the HPT set. As expected for both Punta and Babilla batches, the higher the fat content, the lower the penetration force. As observed in Figure 2 (A and B), there exists a high experimental variability, which is especially important at low fat contents ($FC<23\%$) where several points with $MF>3.5$ N are found. This variability could be linked to the effect that the moisture content has on the zones of measurement with a low fat content. At these low fat points of measurement, the lower the water content, the harder the ham is, which could be linked to the formation of crust in the lean tissue of the ham caused by drying (Serra, Ruiz-Ramírez, Arnau, & Gou, 2005). Therefore, once the influence of the water content (WC) was included in the linear relationship, a small increase was observed in the correlation coefficients ($R=0.86$ and $R=0.80$ for Punta and Babilla, respectively).

The small improvement found when both water and fat content are jointly considered in the model would indicate that the experimental dispersion observed should be mostly linked to sample variability. In this regard, the FC is an average measurement of the amount of fat contained in the zone of measurements, which showed an average standard deviation of 0.2 (wt %) between replicates. This small deviation indicates that the sample variability observed in Figures 2 A and B should be mainly linked to the textural measurements. It should be pointed out that the textural measurements were the average of five punctures of 3.14 mm^2. The highly heterogeneous nature of the sample (distribution of fat, muscle and connective tissue) involved a large standard deviation in the hardness measurement; this was 0.55 N for Punta and 0.44 N for Babilla in zones with a high fat content (>60%) and 0.31 for Punta and 0.25 for Babilla in zones with a low fat content (<20%). This fact would explain the variability found in Figures 2 A and B.

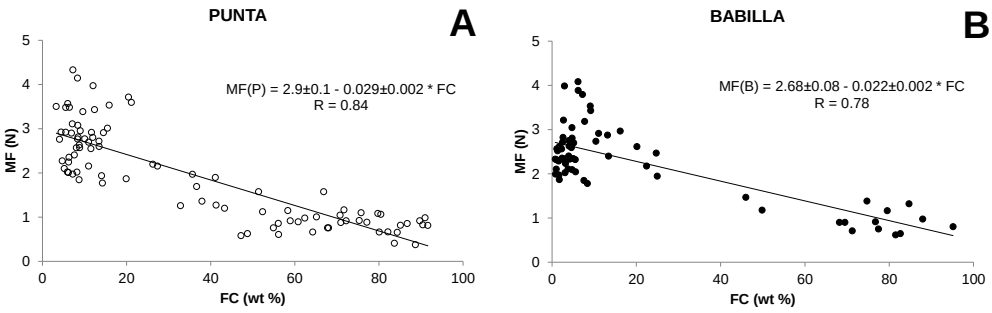


Figure 2. Influence of fat content on texture (MF) in vacuum packaged, sliced Iberian ham for untreated Babilla and Punta batches of the HPT set (T=6 ºC).

On the other hand, when the FC of different ham cuts is compared, a significant ($p<0.05$) difference may be observed. Thus, the Babilla cut showed an average FC of 21.07 %, while the FC of Punta was 36.34 %, which is linked to the anatomical characteristics of both cuts (Molinero et al., 2008). These differences lead to a significantly ($p<0.05$) lower average MF in Punta (2.16 N) than in Babilla (2.65 N). In a similar way, Ruiz-Carrascal, Ventanas, Cava, Andrés, and García (2000) observed that the FC was negatively related to the

hardness of dry cured ham samples, as a result of the lower resistance to shearing and the ease of fracture compared with other muscle components. Also, working on samples of Iberian dry-cured loins, Ventanas, Ventanas, and Ruiz (2007), found that hardness was negatively correlated with the FC and, therefore, with the marbling of the pieces.

3.2. Influence of temperature on the ultrasonic velocity measurements

The average ultrasonic velocity measured at the different experimental temperatures in every one of the control packages of Punta and Babilla batches from the HPT set are plotted in Figure 3. As can be observed, under the experimental conditions tested (0 – 20 ºC), temperature exerts a strong influence on the ultrasonic velocity. Thereby, the ultrasonic velocity decreased as the temperature was raised. This phenomenon is linked to both the reduction of the solid/liquid ratio as a consequence of the fat melting, and to the negative temperature coefficient of the ultrasonic velocity of solid and liquid fats (McClements & Povey, 1988). Similar behavior was observed by other authors when analyzing samples of pork fat (Niñoles, Clemente, Ventanas, & Benedito, 2007), meat-based products (Sobrasada de Mallorca) (Llull, Simal, Benedito, & Roselló, 2002b) and lard samples (Santacatalina et al., 2011).

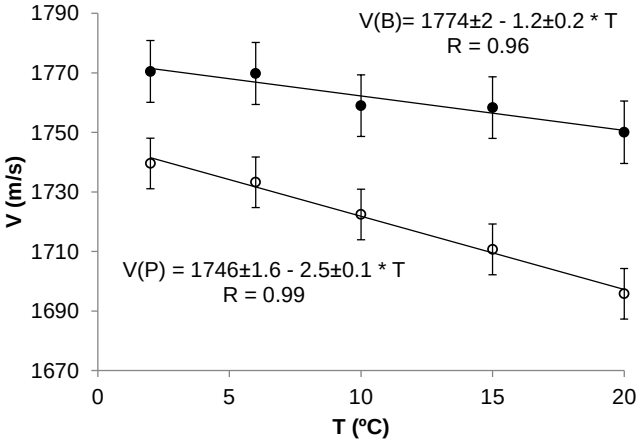


Figure 3. Temperature dependence of the ultrasonic velocity in vacuum packaged, sliced Iberian ham for Babilla (B, ●) and Punta (P, ○) control samples of HPT set. Average±LSD ($p<0.05$).

A significant ($p<0.05$) linear relationship between the velocity and temperature was found both for Punta and Babilla batches, with the linear regression coefficients (R) being higher than 0.96 (Figure 3). There was a significantly ($p<0.05$) steeper temperature-related decrease in ultrasonic velocity for Punta (-2.5 m/s per ºC) than for Babilla (-1.2 m/s per ºC). The HPT processed samples were observed to behave in a similar way (-2.4 m/s per ºC and R=0.94 for Punta and -1.1 m/s per ºC and R=0.97 for Babilla) The differences observed between Punta and Babilla could be ascribed to the higher fat content of the Punta cut (72 % higher), which means that, when the temperature increases, the higher liquid fat content leads to a softer sample wherein the velocity is lower. In a previous study performed on dry-cured subcutaneous fat from Iberian pigs of different breeds and reared on differing feeding systems, Niñoles et al. (2010) also observed a reduction in ultrasonic velocity linked with temperature over a similar temperature range.

Figure 3 also shows that a significant ($p<0.05$) difference exists at every temperature between the ultrasonic velocity of Punta and Babilla batches. Thereby, the ultrasonic velocity can be used to differentiate both cuts, since Babilla always showed significantly ($p<0.05$) higher values than Punta (an average of 1760 m/s and 1720 m/s for Babilla and Punta, respectively). As already mentioned, this is linked to the influence of the FC (21.07 % and 36.34 % for Babilla and Punta, respectively) on the ultrasonic wave propagation.

From these results, it can also be concluded that ultrasonic velocity measurements are highly dependent on temperature and, therefore, this variable must be accurately controlled during ultrasonic measurements in dry-cured ham. Moreover, the ultrasonic velocity and its temperature dependence are linked to the fat content and, accordingly, this acoustic parameter can be used to differentiate batches of vacuum packaged, sliced Iberian ham which have differing fat contents.

3.3. Relationship between texture and ultrasonic velocity measurements

Previous works have reported that there exists a close relationship between the ultrasonic velocity and the square root of the maximum penetration force

(MF) (Benedito et al., 2001; Llul, Simal, Femenia, Benedito, & Roselló, 2002a) since the ultrasonic velocity is linked to the square root of the elastic modulus of the material (Povey & McClements, 1988) considering an homogeneous, isotropic and elastic medium.

It was found that experimental results show (Figure 4C and D) the existence of a significant ($p<0.01$) relationship between the ultrasonic velocity and the square root of the MF ($R=0.87$ for Babilla and $R=0.84$ for Punta batches). The large dispersion observed could be linked, as previously pointed out, to the variability in the textural measurements due to the highly heterogeneous nature of the tissue distribution of the samples. The effect packaging had on the variability of ultrasonic velocity measurements was calculated to be around 5 m/s, negligible compared to the velocity variation (300 m/s) brought about by the change in the textural properties of the samples. Therefore, the non-destructive ultrasonic measurements taken out directly on the surface of packaged, dry-cured sliced ham could be considered a reliable technology.

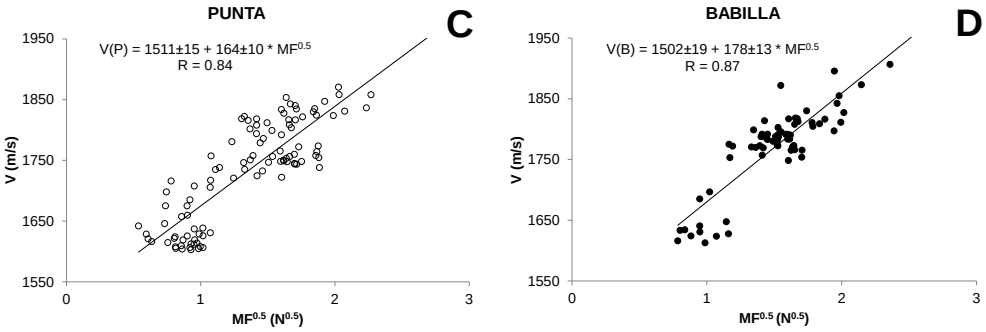


Figure 4. Relationship between the ultrasonic velocity (V) and the square root of the maximum penetration force ($MF^{0.5}$) in vacuum packaged, dry-cured Iberian ham from Babilla and Punta control batches of the HPT set (T=6 ºC).

Therefore, the harder the meat, which is affected by its composition (moisture and fat content), the faster the ultrasonic wave propagation. In a similar way, Benedito, Carcel, Clemente & Mulet (2000), showed that a decrease in the water content of cheese caused an increase in the deformability modulus, and, therefore, an increase in velocity. Moreover Niñoles, Sanjuan, Ventanas &

Benedito (2008) and Niñoles et al. (2011) also reported that the ultrasonic velocity was related with the textural parameters, which were dependent on the fatty acid composition of dry-cured ham fat and *Biceps femoris* muscle from Iberian pigs with different feeding regimes and rearing systems. From the results of the present work, it can be concluded that ultrasonic techniques could be considered a reliable tool with which to characterize the texture of vacuum packaged, dry-cured Iberian ham in a non-destructive way, thus characterizing important parameters that influence the overall quality of the sliced ham.

3.4. Influence of composition on ultrasonic velocity measurements

Figure 5 shows the relationship between the ultrasonic velocity (at 6 ºC) and the fat content of sliced Iberian ham for every zone of measurement in the control packages of Punta (Figure 5E) and Babilla (Figure 5F) batches of the HPT set. As can be observed, the higher the fat content, the lower the ultrasonic velocity for both Babilla and Punta batches. A significant ($p<0.01$) linear regression was established between the velocity and the fat content for each batch (Punta R= 0.89, Babilla R=0.88). Linear regressions (Figure 5E and F) showed that an increase of 1 % in the fat content corresponded to a velocity decrease of 2.1 m/s and 2.5 m/s in Babilla and Punta, respectively.

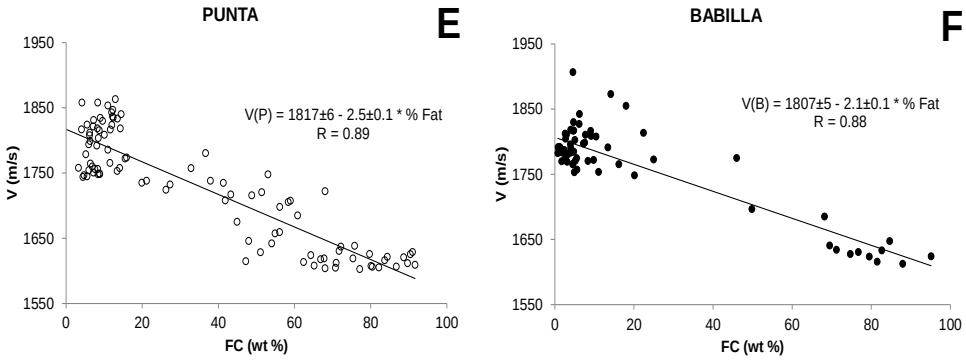


Figure 5. Relationship between the ultrasonic velocity (T=6 ºC) and the fat content in vacuum packaged, sliced Iberian ham from Babilla and Punta control batches of the HPT set.

In order to check whether the water content could account for the variability observed, this parameter was also considered in the correlation. A small increase in the correlation coefficient was observed (Punta R= 0.93, Babilla R= 0.91), thus indicating that variability would be mostly linked to sample heterogenity.

3.5. Influence of High Pressure Treatment (HPT) on the textural and ultrasonic velocity measurements.

As already mentioned, HP treatment is considered to be an effective, non-thermal preservation technology (Patterson, 2005). Nowadays, this emerging technology is applied to meat and dry-cured meat products, enabling the reduction of microorganisms linked to spoilage and extending the shelf life of these products. Nevertheless, this treatment induces changes, modifying the sensory characteristics and, in particular, the texture of meat products, which could influence their consumer acceptance (Cheftel & Culioli, 1997; Fuentes et al., 2010; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998; Marcos, Aymerich, Guardia, & Garriga, 2007; Ueno, Ikeuchi, & Suzuki, 1999; Zhifei et al., 2012). For this reason, it is important to assess on-line the changes induced by HPT processing for a better quality control. Thus, the ability of ultrasound measurements to detect these changes in vacuum packaged HPT samples will be addressed.

In order to identify whether the observed trends were statistically significant, Generalized Linear Models (GLM) were used to obtain correlations between the variables. For that purpose, the influence of a categorical factor was evaluated at 6 ºC. This was the HPT at two levels: Treated (HPT) and Untreated samples (Control), plus a numerical one (FC) for the hardness, characterized as the square root of MF. The FC was included in the analysis since it exerts a strong influence on hardness, as observed in section 3.1. The analysis of variance showed that both factors were statistically significant ($p<0.05$). HPT ham was harder than the control one (Figures 6A and B). This fact was observed for both Punta (Figure 6A) and Babilla (Figure 6B) cuts, thus, in Punta samples, hardness increased from 1.37±0.01 to 1.61±0.02 $N^{0.5}$ (18 %), while in Babilla ones, it increased from 1.55±0.02 to 1.73±0.04 $N^{0.5}$ (12 %). Hence, it seems that HPT

affects the ham structure, creating molecular alterations and leading to an increase in hardness. In the literature, the most important changes produced by HPT in dry-cured meat products have been related with lipid and myofibrillar protein (myosin and actin) interactions (Andrés, Adamsen, Moller, Ruiz, & Skibsted, 2006; Andrés et al., 2004; Campus et al., 2008; Norton & Sun, 2008). These changes include protein and lipid oxidation and changes in volatile compounds, color, hardness and juiciness (Fuentes et al., 2010). In the specific case of proteins, the pressure can lead to the unfolding/denaturation of the polypeptide chain (Hendrickx et al., 1998). The depolymerization of myofibrillar proteins and their solubilisation (Campus et al., 2008; Cheftel et al., 1997), play an important role in both developing and catalyzing protein and lipid oxidation (Andrés et al., 2004; Campus, 2010; Estévez, Morcuende, & Ventanas, 2008). Both protein and lipid oxidation processes generate the formation of secondary products (carbonyl derivatives and aldehydes from protein and lipid oxidation, respectively) which could interact, reducing the protein functionality (Ventanas, Estevez, Tejeda, & Ruiz, 2006), affecting the juiciness and tenderness (Chelh, Gatellier, & Santé-Lhoutellier, 2006) and, as consequence, bringing about an increase in hardness (Fuentes et al., 2010), all of which coincides with the results obtained in the present work. Similar results were reported by Fuentes et al. (2010), where pressurized Iberian ham samples (600 MPa/ 6 min) were harder, less juicy, less doughy and more difficult to chew than non-pressurized ones. Moreover, working on pressurized hams at 600 MPa for 6 min, Serra, Grèbol, Guàrdia, Guerrero, Gou et al. (2007), observed a decrease in crumbliness and an increase in fibrousness, which led to harder samples.

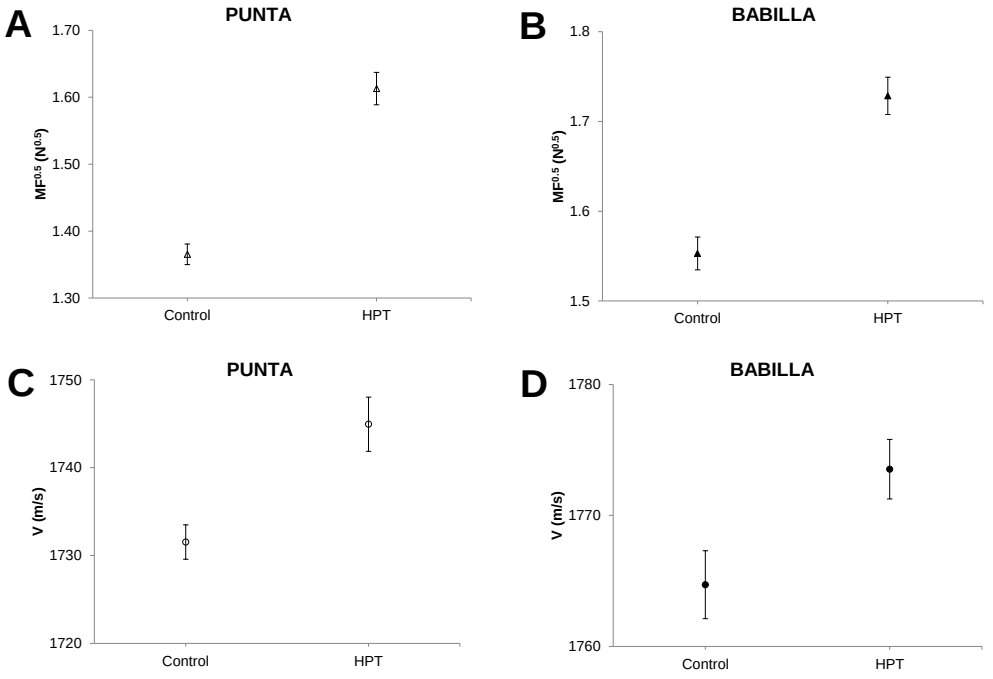


Figure 6. Effect of high pressure treatment (HPT) on the square root of the maximum penetration force ($MF^{0.5}$) (A, B) and ultrasonic velocity (V) (C, D) in vacuum packaged, sliced Iberian ham from Babilla and Punta batches. Average and LSD Intervals at a confidence level of 95% are plotted (T=6 ºC).

On the other hand, a greater increase in hardness was observed for Punta (18 %) samples than Babilla (12 %). Thus, the FC influenced the increase in hardness, which could be linked to the release of fat after HPT and to the changes in the transition temperature of lipids (Simonin, Duranton, & de Lamballerie, 2012). In this regard, Carballo, Fernandez, Carrascosa, Solas, and Jiménez-Colmenero (1997), found that HPT promoted the rupture of adipocytes, leading to a release of fat. On the other hand, complex systems of triacylglycerols show that pressure produces changes in the transition temperatures (Huppertz, Kelly, & Fox, 2002), which are displaced to higher values (Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002). In this regard, the crystallization and melting temperatures of lipids rise and the liquid lipids crystallize under pressure at room temperature, leading to the formation of the more stable, denser crystals (Cheftel

et al., 1997). Therefore, samples with a high fat content would have more fat released from adipocytes, which could be more easily crystallized under pressure. This fact could be linked to the textural changes observed in dry-cured ham samples with a high FC caused by the HPT used in the present work.

The GLM allows the influence of HPT on the hardness of sliced Iberian ham to be quantified (Equations 1 and 2). The influence of HPT was significantly ($p<0.05$) greater in Punta than in Babilla cuts. Thus, for the same FC, HPT brought about an increase in the hardness of Babilla slices of 0.176 $N^{0.5}$ while, in the case of Punta samples, the increase was of 0.248 $N^{0.5}$ (an increase 40% higher).

$$MF^{0.5}(\text{Babilla}) = 1.81 \pm 0.02 - 0.088 \pm 0.014 * \text{HPT} - 0.010 \pm 0.001 * \text{FC} \quad (1)$$

$$\text{MF}^{0.5}(\text{Punta}) = 1.93 \pm 0.02 - 0.124 \pm 0.014 * \text{HPT} - 0.0122 \pm 0.004 * \text{FC} \quad (2)$$

where HPT refers to the high pressure treatment; thus, HPT= 1 for control samples and -1 for HPT ones.

Similarly, a GLM was proposed to quantify the influence of a categorical factor, the HPT at two levels: Treated (HPT) and Untreated samples (Control), and a numerical one (FC) on the ultrasonic velocity (Equations 3 and 4). The GLM indicated that HPT samples exhibited significantly ($p<0.05$) higher ultrasonic velocity than control ones (Figure 6C and D). The difference was of 9 m/s (0.5 %) for Babilla and 13 m/s (0.75 %) for Punta, which agrees with the greater increase in hardness for Punta (18 %) than Babilla (12 %).

$$\text{V (Babilla)} = 1801.52 \pm 2.14 - 4.4 \pm 1.7 * \text{HPT} - 1.83 \pm 0.07 * (\text{FC}) \quad (3)$$

$$\text{V (Punta)} = 1821.9 \pm 2.8 - 6.7 \pm 1.8 * \text{HPT} - 2.31 \pm 0.06 * (\text{FC}) \quad (4)$$

where HPT= 1 for control samples and -1 for HPT treated samples for both equations.

The HPT processing of sliced Iberian ham induced modifications in molecular properties, causing an impact on textural properties, which can be adequately assessed by the use of a non-destructive technique, such as low intensity ultrasound. It should be highlighted that ultrasonic measurements can be directly carried out on the plastic surface of ham packages without affecting the product. By measuring the ultrasonic velocity before and after the HPT, a rapid and non-destructive assessment of the textural changes induced by HPT could be obtained which would allow samples to be classified according to their characteristics.

3.6. Influence of cold storage on the textural and ultrasonic velocity measurements.

It has been reported that changes might appear during low temperature storage, affecting the overall quality of the dry-cured ham (Cilla et al., 2006a). In this regard, the cold storage set experiments were conducted to assess the influence of cold storage on the textural changes of dry-cured Iberian ham and to evaluate the viability of using ultrasonic measurements to assess these textural changes in packaged ham non-destructively. As the stored, packaged ham would be aimed at consumers, visual appearance is an important factor, which explains why image analysis was considered for the evaluation of the fat content (FCI). For this purpose, and in order to take the influence of fat content into account, zones of measurement were marked on the surface of the sliced Iberian ham packages. As already explained, image techniques were employed to measure their fat content and classify them into three groups (fatty, marbled and lean tissue), and they were characterized by ultrasonic measurements and textural analysis at the beginning (1 day) and the end (120 days) of storage.

Figure 7A shows that the level of ham hardness of the three groups under study was significantly ($p<0.05$) different. Thus, lean tissue (FCI<23 %) showed higher values of hardness (2.02 $N^{0.5}$ for 1 day and 3.09 $N^{0.5}$ for 120 days of storage) than marbled zones (23<FCI<70%), with the fatty zones (FCI>70) being the softest (1.15 $N^{0.5}$ for 1 day and 2.3 $N^{0.5}$ for 120 days of storage). Similarly, significantly ($p<0.05$) higher values of velocity (Figure 7B) were obtained for the lean tissue (1832 m/s at the beginning and 1894 m/s at the end of storage), while

the lowest velocity was found for the fatty zones (1743 and 1849 m/s for 1 day and 120 days of storage, respectively). These results coincide with those obtained in HPT set assays (section 3.4): the higher the fat content, the softer the ham and the lower the ultrasonic velocity.

Storage at 6 ºC exerted an effect on the hardness of the ham (Figure 7A), thus, a significant ($p<0.05$) increase in the $MF^{0.5}$ was observed at the end of storage for the three different levels of fat content. For the lean zones, the increase in hardness was 1.07 $N^{0.5}$, while for the marbled and fatty zones an increase of 1.09 and 1.15 $N^{0.5}$ were obtained, respectively. Therefore, the effect of storage was more marked on the fatty zone (100 % MF increase) than on the marbled (74 % MF increase) and lean tissues (53 % MF increase).

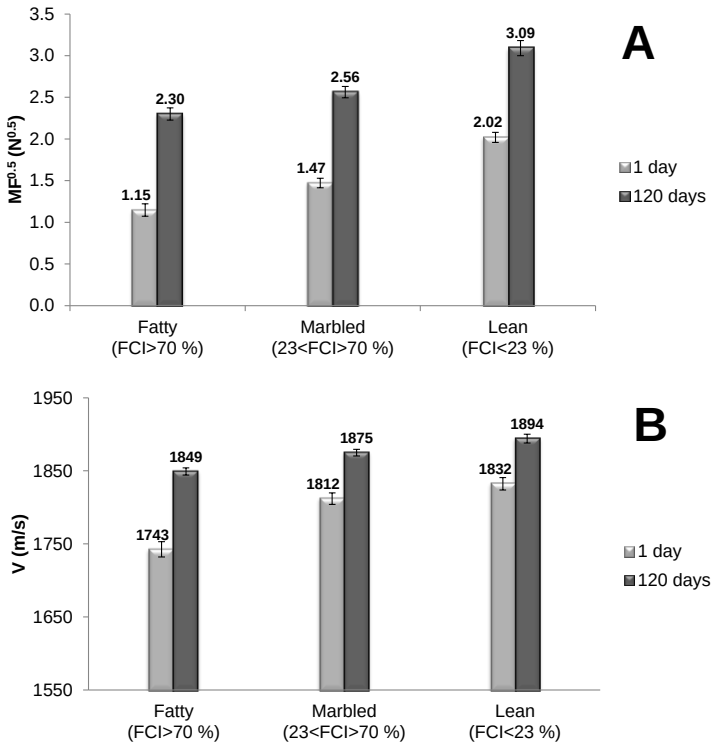


Figure 7. Effect of cold storage (6 ºC - 120 days) on the square root of the maximum penetration force ($MF^{0.5}$) and the ultrasonic velocity (V) in the different types of tissue of vacuum packaged, sliced Iberian ham (T=6 ºC). Average and LSD intervals at a confidence level of 95%.

The increase in hardness was also well monitored by the ultrasonic techniques, since a significant increase ($p<0.05$) in the ultrasonic velocity was found after 120 days of cold storage. The increase in velocity was of 62 m/s for the lean and 63 m/s for the marbled zones, while, in the fatty zones, the velocity after storage was 106 m/s higher. These results agree with the significantly greater increase in hardness found in the fatty tissue. The increase in hardness and velocity could be explained by the fat crystallization and the increase in the fat solid/liquid ratio. At the beginning of storage, the fat can be partially liquid; thereafter, there is an increase in the fat solid/liquid ratio which determines the increase in velocity. Other authors observed that Iberian lard (Santacatalina et al., 2011) and milk and cocoa butter behaved similarly during storage (Singh, McClements, & Marangoni, 2003). Accordingly, due to their higher fat content, fatty zones underwent more intense changes than marbled and lean tissues.

The results obtained in this section indicate that the non-destructive measurement of ultrasonic velocity could be used to assess the textural changes undergone by sliced Iberian ham during cold storage.

4. Conclusions

The increase in hardness of vacuum packaged, sliced, dry-cured Iberian ham during cold storage, as well as that provoked by high pressure treatment, was adequately assessed by ultrasonic measurements. Therefore, the non-destructive ultrasound techniques could be considered a reliable technique with which to evaluate the textural changes undergone by this product during retail sale and storage. Ultrasonic measurements could also be considered for performing quality control of packaged samples after HPT processing. These results could be extended to other meat packaged products. For a particular industrial application, the effect of temperature should be considered.

References

Andrés, A. I., Adamsen, C. E., Moller, J. K. S., Ruiz, J., & Skibsted, L. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research Technology*, 222, 486–491.

Andrés, A. I., Moller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2004). High pressure treatment of dry-cured Iberian ham. Effects on radical formation, lipid oxidation and color. *European Food Research and Technology*, 219, 205-210.

AOAC. (1997). Official methods of analysis of AOAC International 16 ed. (*Association of Official Analytical Chemist International* AOAC, Gaithersburg, USA).

Benedito, J., Carcel, J., Clemente, G., & Mulet, A. (2000). Cheese maturity assessment using ultrasonics. *Journal of Dairy Science*, 83, 248-254.

Benedito, J., Carcel, J. A., Rossello, C., & Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 57, 365-370.

Campus, M. (2010). High pressure processing meat, meat products and seafood. *Food Engineering Reviews*, 2, 256-273.

Campus, M., Flores, M., Martínez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, 80, 1174-1181.

Carballo, J., Fernández, P., Carrascosa, A. V., Solas, M. T., & Jiménez-Colmenero, F. (1997). Characteristics of low and high fat beef patties: effect of high hydrostatic pressure. *Journal of Food Protection*, 60, 48-53.

Cava, R., Ladero, L., González, S., Carrasco, A., & Ramírez, M. R. (2009). Effect of pressure and holding time on colour, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage. *Innovative Food Science and Emerging Technologies*, 10, 76-81.

Chapleau, N. J., & Lamballerie-Anton, M. I. (2003). Changes in myofibrillar proteins interactions and rheological properties induced by high-pressure processing. *European Food Research and Technology*, 216, 470-476.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: a review. *Meat Science*, 46, 211-236.

Chelh, I., Gatellier, P., & Sante´-Lhoutellier, V. (2006). Characterization of fluorescent Schiff bases formed during oxidation of pig myofibrils. *Meat Science*, 76, 210-215.

Cilla, I., Martínez, L., Beltrán, J. A., & Roncalés, P. (2006a). Effect of low temperature preservation on the quality of vacuum-packaged dry-cured ham: Refrigerated boneless ham and frozen ham cuts. *Meat Science*, 73, 12-21.

Cilla, I., Martínez, L., Beltrán, J. A., & Roncalés, P. (2006b). Dry-cured ham quality and acceptability as affected by the preservation system used for retail sale. *Meat Science*, 73, 581-589.

Colmenero, F., (2002). Muscle protein gelation by combined use of high pressure/temperature. *Food Science & technology*, 13, 22-30.

Estévez, M., Morcuende, D., & Ventanas, S. (2008). Determination of oxidation. In L. M. L. Mollet, & F. Toldrá (Eds.). Handbook of processed meat and poultry analysis, (pp. 141-162). *Boca Raton, FL, USA: CRC Press*.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science*, 85, 506-514.

Garriga, L., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science & Emerging Technologies*, 5, 451-457.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., & Weemaes, C. (1998). Effects of high pressure on enzymes related to food quality. *Trends in Food Science & Technology,* 9, 197-203.

Huppertz, T., Kelly, A. L., & Fox, P. F. (2002). Effects of high pressure on constituents and properties of milk. *International Dairy Journal*, 12, 561-572.

Koch, T., Lakshmanan, S., Brand S., Wicke M., Raum K., & Mörlein, D. (2011a). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle. *Meat Science*, 88, 51-58.

Koch, T., Lakshmanan, S., Brand S., Wicke M., Raum K., & Mörlein. (2011b). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: II. Skin and backfat. *Meat Science*, 88, 67-74.

Llul, P., Simal, S., Femenia, A., Benedito, J., & Roselló, C. (2002a). The use of ultrasound velocity measurement to evaluate the textural properties of sobrasada from Mallorca. *Journal of Food Engineering*, 52, 323-330.

Llull, P., Simal S., Benedito, J., & Roselló, C. (2002b). Evaluation of textural properties of meat-based product (sobrassada) using ultrasonic techniques. *Journal of Food Engineering*, 53, 279-285.

Marcos, B., Aymerich, T., Guardia, M. D., & Garriga, M. (2007). Assessment of high hydrostatic pressure and stated culture on the quality properties of low-acid fermented sausages. *Meat Science*, 76, 46-53.

McClements, D. J., & Povey, M. J. W. (1988). Investigation of phase transitions in glycerine/paraffin oil mixtures using ultrasonic velocity measurements. *Journal of the American Oil Chemists 'Society*, 65. 1791-1795.

Molinero, C., Martínez, B., Rubio, B., González-Fernández, C., García-Cachán, M. D., & Jaime, I. (2008). The Anatomical origin of raw meat affects the sensory and chemical characteristics of dry-cured beef product: Cecina de León. *Food Science and Technology*, 14, 225-232.

Mor-Mur, M., & Yuste, J. (2003). High pressure processing applied to cooked sausage manufacture: physical properties and sensory analysis. *Meat Science,* 65, 1187-1191.

Niñoles, L., Clemente, G., Ventanas, S., & Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Niñoles, L., Mulet, A., Ventanas, S., & Benedito, J. (2010). Ultrasonic assessment of the melting behaviour in fat from Iberian dry-cured hams. *Meat Science*, 85, 26-32.

Niñoles, L., Mulet, A., Ventanas, S., & Benedito, J. (2011). Ultrasonic characterization of B. femoris from Iberian pigs of different genetics and feeding systems. *Meat Science*, 89, 174-180.

Niñoles, L., Sanjuan, N., Ventanas, S., & Benedito, J. (2008). Ultrasonic and sensory characterization of dry-cured ham fat from Iberian pigs with different genetics and feeding backgrounds. *Meat Science*, 80, 896-902.

Norton, T., & Sun, D. W. (2008). Recent advances in the use of High Pressure as an effective processing technique in the food industry. *Food Bioprocess Technology*, 1, 2-34.

Parra, V., Viguera, J., Sánchez, J., Peinado, J., Espárrago, F., Gutiérrez, J. I., & Andrés, A. I. (2010). Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. *Meat Science*, 84, 760-768.

Patterson, M. F., (2005). Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, 98, 1400-1409.

Povey, M. J. M., & McClements, D. J., (1988). Ultrasonics in food engineering. Part I: Introduction and experimental methods. *Journal of Food Engineering*, 8, 217-245.

Ruiz-Carrascal, J., Ventanas, J., Cava, R., Andrés, A. I., & García, C. (2000). Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. *Food Research International*, 33, 91-95.

Santacatalina, J. V., Garcia-Perez, J. V. Corona, E., & Benedito, J. (2011). Ultrasonic monitoring of lard crystallization during storage. *Food Research International*, 44, 146-155.

Serra, X., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M., Sárraga, C., Monfort, J. M., & Arnau, J. (2007). High pressure applied to frozen ham at different process stages. 2. Effect on the sensory attributes and on the color characteristics of dry-cured ham. *Meat Science*, 75, 21-28.

Serra, X., Ruiz-Ramírez, J., Arnau, J., & Gou, P. (2005). Texture parameters of dry-cured ham *m. biceps femoris* samples dried at different levels as a function of water activity and water content. *Meat Science*, 69, 249-254.

Serra, X., Sárraga, C., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M, Monfort, J. M., & Arnau, J. (2007). High pressure applied to frozen ham at different process stages. 1. Effect on the final physicochemical parameters and on the antioxidant and proteolytic enzyme activities of dry-cured ham. *Meat Science*, 75, 12-20.

Simonin, H., Duranton, F., & de Lamballerie, M. (2012). New Insights into the High-Pressure Processing of Meat and Meat Products. *Comprehensive Reviews in Food Science and Food Safety*, 11, 285-306.

Singh, A. P., McClements, D. J., & Marangoni, A. G. (2003). Solid fat content determination by ultrasonic velocimetry. *Food Research International* , 37, 545-555.

Trujillo, A. J., Capellas, M., Saldo, J., Gervilla, R., & Guamis, B. (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science and Emerging Technologies*, 3, 295-307.

Ueno, Y., Ikeuchi, Y., & Suzuki, A. (1999). Effects of high pressure treatments on intramuscular connective tissue. *Meat Science*, 52, 143-150.

Ventanas, S., Estevez, M., Tejeda, J. F., & Ruiz, J. (2006). Protein and lipid oxidation in *Longissimus dorsi* and dry-cured loin from Iberian pigs as affected by crossbreeding and diet. *Meat Science*, 72, 647-655.

Ventanas, S., Ventanas, J., & Ruiz, J. (2007). Sensory characteristics of Iberian dry-cured loins: Influence of crossbreeding and rearing system. *Meat Science*, 75, 211-219.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343-358.

Zhifei, H., Yechuan, H., Hongjun, L., Gang, Q., Ting, W., & Jiayi, Y. (2012). Effect of high-pressure treatment on the fatty acid composition of intramuscular lipid in pork. *Meat Science*, 90, 170-175.

Zhu, S. M., LeBail, A., Chapleau, N., Ramaswamy, H. S., & de Lamballerie-Anton, M. (2004). Pressure shift freezing of pork muscle: Effect on color, drip loss, texture and protein stability. *Biotechnology Progress*, 203, 939-945.

CHAPTER 5

(*Apartado 5*)

NEW TRENDS IN ULTRASONIC SENSING IN DRY-CURED MEAT PRODUCTS

E. Corona, J.V. García-Pérez, T.E. Gómez Álvarez-Arenas, N. Watson, M. J. W. Povey, J. Benedito

Sensors
(Submitted)

New trends in ultrasonic measurements in dry-cured meat products

E. Corona[1], J.V. García-Pérez[1], T.E. Gómez Álvarez-Arenas[2], N. Watson[3], M. J. W. Povey[3], J. Benedito[1]

[1] Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camí de Vera s/n, E46022, Valencia, Spain

[2]UMEDIA (Ultrasound for Medical and Industrial Applications) research group, CSIC, Serrano 144, 28006 Madrid, Spain

[3] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, United Kingdom.

Abstract

In this work, the feasibility of using non-contact ultrasonic techniques (air-coupled and scanning acoustic microscopy, SAM) for characterizing different dry-cured meat products was assessed. Air-coupled ultrasonic measurements were performed on vacuum packaged sliced dry-cured ham, and compared with contact measurements. The average ultrasonic velocity in dry-cured ham was 1846±49 m/s and 1842±42 m/s for air-coupled and contact measurements, respectively. The deviation (1 % relative error) between both techniques was related with the influence of the heterogeneous structure and composition of dry-cured ham and the transducer focusing. The SAM was used to characterize dry-cured ham and chorizo samples. B-scan images for dry-cured ham and chorizo showed two main different reflections from the sample, which were linked to reflections in the lean and fatty tissues. Therefore, the results show that contact ultrasonic measurements could be replaced by the air-coupled technique, reducing the measuring time and the material handling. On the other hand, SAM technique allows the microscopic characterization of dry-cured meat products.

Key words: *Ultrasound; Air-coupled; Scanning acoustic microscopy; Dry-cured meat products*

1. Introduction

Dry-cured meat products, such as, salchichón, chorizo, dry-cured loin or dry-cured ham, among others, are products with high quality and consumer acceptance in Spain and other Mediterranean countries. The raw materials and the conditions of the curing process are important factors that have a great influence on the final quality of these products. Fat content, its distribution, state and fatty acid composition are several of the most important factors related to the raw materials. The dry-cured meat industry is interested in obtaining reliable information about the quality of their products. In this regard, several techniques have been developed to objectively measure the meat quality traits. However, some of these techniques are invasive and the structure of food is affected (Damez & Clerjon, 2008), therefore the search for non-destructive techniques is of great interest.

Non-destructive techniques have been developed for assessing the quality of raw materials and dry-cured meat products. Among these techniques it could be cited the nuclear magnetic resonance (NMR) (Straadt, Aaslyngb, & Bertram, 2012), X-ray technologies (Santos-Garcés et al., 2010; Håseth, SØrheim, HØy, & Egelandsdal, 2012), near infrared spectroscopy (NIRS) (García-Rey, García-Olmo, De Pedro, Quiles-Zafra, & Luque de Castro, 2005) and ultrasound (Benedito, Carcel, Rossello, & Mulet, 2001b; Niñoles, Mulet, Ventanas, & Benedito, 2010; Santacatalina, Garcia-Perez, Corona, & Benedito, 2011). The implementation of these techniques in the meat industry could lead to a better control of raw materials and intermediate and final products, thus improving the elaboration processes and obtaining a higher quality of meat and dry-cured meat products.

Low intensity ultrasound can provide a rapid, accurate, on-line, inexpensive and non-destructive food and process characterization (Benedito, Carcel, Gonzalez, & Mulet, 2002; Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). Several studies have shown the potential of ultrasound as an analytical tool to characterize various properties of different foods, such as cheese (Benedito, Carcel, Gisbert, & Mulet, 2001a; Telis-Romero, Váquiro, Bon, & Benedito, 2011), fruits (Mizrach, 2000; Kim, Lee, Kim, & Cho, 2009),

vegetables (Mizrach, 2007; Schössler, Thomas, & Knorr, 2012). In the meat sector, ultrasonic contact measurements had been used to determine the composition of raw meat mixtures (Benedito et al., 2001b), or for the evaluation of the textural properties (Llull, Simal, Benedito, & Roselló, 2002) and the composition of fermented meat products (Simal, Benedito, Clemente, Femenia, & Rosseló, 2003). Moreover, this technique was used to characterize and classify pig backfat and to find differences in composition and texture of muscles from animals of different breeds and feeding regimes (Mörlein, Rosner, Brand, Jenderka, & Wicke, 2005; Niñoles, Clemente, Ventanas, & Benedito, 2007; Niñoles, Mulet, Ventanas, & Benedito, 2011). In a similar way, ultrasonic measurements have been used to characterize porcine *Longissimus* muscle (Koch et al., 2011a) and backfat samples of pork carcasses (Koch et al., 2011b), by means of in-vitro measurements of ultrasonic velocity. Therefore, the contact ultrasonic technique has been proved to be useful for the characterization of several meat products aiming to improve their quality and safety. However, sometimes the use of contact ultrasonic measurements involves the need of a couplant to improve the matching between the sample and the transducer, for example in the packaged products. Thus, for those applications where the use of couplants can affect the product quality or where the time of measurement restricts the application and removal of the couplant, air-coupled ultrasonic measurements can be a reliable alternative.

Air-coupled ultrasonic technique is a promising non-destructive technique, due to its minimally invasive nature and broad application range, including its on-line application. However, the most important challenge lies in the several orders of magnitude acoustic impedance mismatch between air and materials of the transducer and the sample. The impedance mismatch causes high interfacial reflection and low acoustic transmission efficiency. This, along with the attenuation of ultrasound in the air, has hampered developing a practical non-contact ultrasound such as inspection method. Therefore, the use of efficient ultrasound transducers must be significantly improved. Even so, this technique has been used for the successfully inspection and detection of foreign bodies in commercially-available food products, such as cheese, chocolate, dough-based products and canned food products (Pallav, Hutchins, & Gan, 2009). Meyer,

Hindle, Sandoz, Gan, & Hutchins (2006) used air-coupled ultrasonic measurements to detect physicochemical changes of liquid beverages, as well as to examine the time dependent processes in milk-based drinks. Gan, Hutchins, & Billson (2002) evaluated the possibility to detect variations in consistency of starchy solids distributed into microwaveable containers, as well as to detect the liquid level within polymer drinks bottles using air-coupled tomographic images.

On the other hand, sometimes it is necessary to obtain microscopic information of the food products, in contrast with the macroscopic information provided by most of the aforementioned ultrasonic applications. In those cases acoustic microscopy can be considered. Acoustic waves offer versatile modality with which to test a wide range of media. The internal elastic properties of material can be analyzed by acoustic waves that can access deep into materials, including many that are optically opaque. Thus, acoustic boundaries generate reflections. The time of flight (TOF) of these reflections allows mapping out the surface and sub-surface structure of the sample. The amplitude and phase of the returning signal conveys mechanical information, which can reveal elastic moduli, compressibility, stress, adhesion properties, thermophysical properties and phonon transport (Parker, Nelson, & Povey, 2010). An important feature of acoustic waves is their scalability. Therefore, the scanning acoustic microscope (SAM) is a precision device which uses tightly focused ultrasound to map the acoustic contrast (scale) within a sample. SAM has been used to accurately produce an image of the internal structure of opaque materials using ultrasonic waves (Wickramasinghe, 1984). SAM generates details in a non-destructive way of the surface and sub-surface structure of materials and can be operated in transmission or reflection mode, with reflection-mode being the most common due to the use of a single transducer (Parker et al., 2010). SAM has been used to study different items, such as integrated circuits (Parker et al., 2010), multilayer films (Caner, Hernandez, Pascall, & Riemer, 2003), flexible food packages (Ozguler, Morris, & O´Brien, 1998) and biological tissues such as onion skin (Parker et al., 2010). However, to our knowledge, no attempt has been done to apply this technology to meat products.

Therefore, the aim of this work was to assess the feasibility of using new acoustic techniques such as air-coupled ultrasound and scanning acoustic microscopy for the characterization of different dry-cured meat products.

2. Materials and methods

The experiments were conducted using two different non-contact ultrasonic techniques on dry-cured meat products. On one hand, air-coupled ultrasonic measurements (Figure 1) were carried out on samples of vacuum packaged sliced dry-cured ham. These were compared with measurements performed on the same samples using conventional contact ultrasonic measurements, in order to determine if similar information is obtained and consequently if contact measurements could be replaced by air-coupled ones.

On the other hand, the acoustic imaging was performed using scanning acoustic microscopy (SAM) (Figure 2) in dry-cured ham and chorizo samples with the aim of characterizing the fat/lean structure and its distribution.

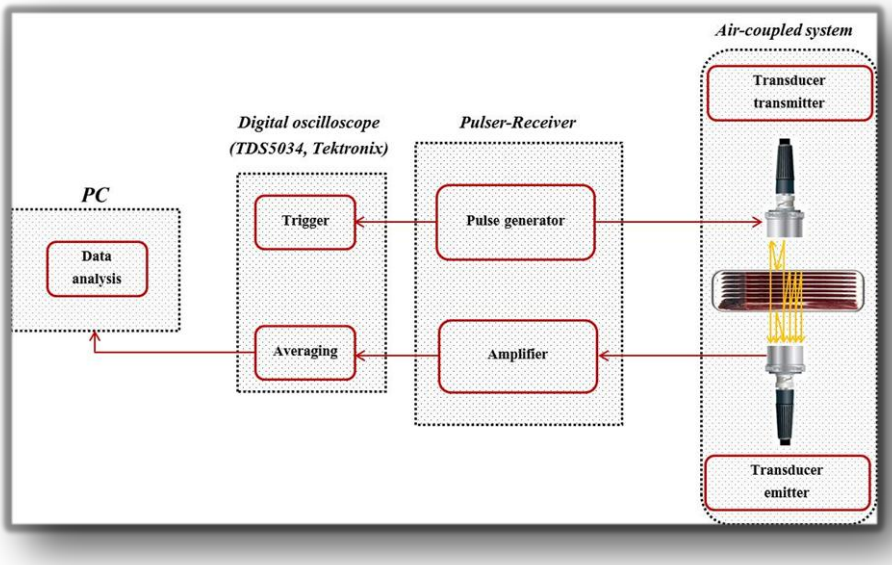


Figure 1. Experimental set up for air-coupled ultrasonic measurements

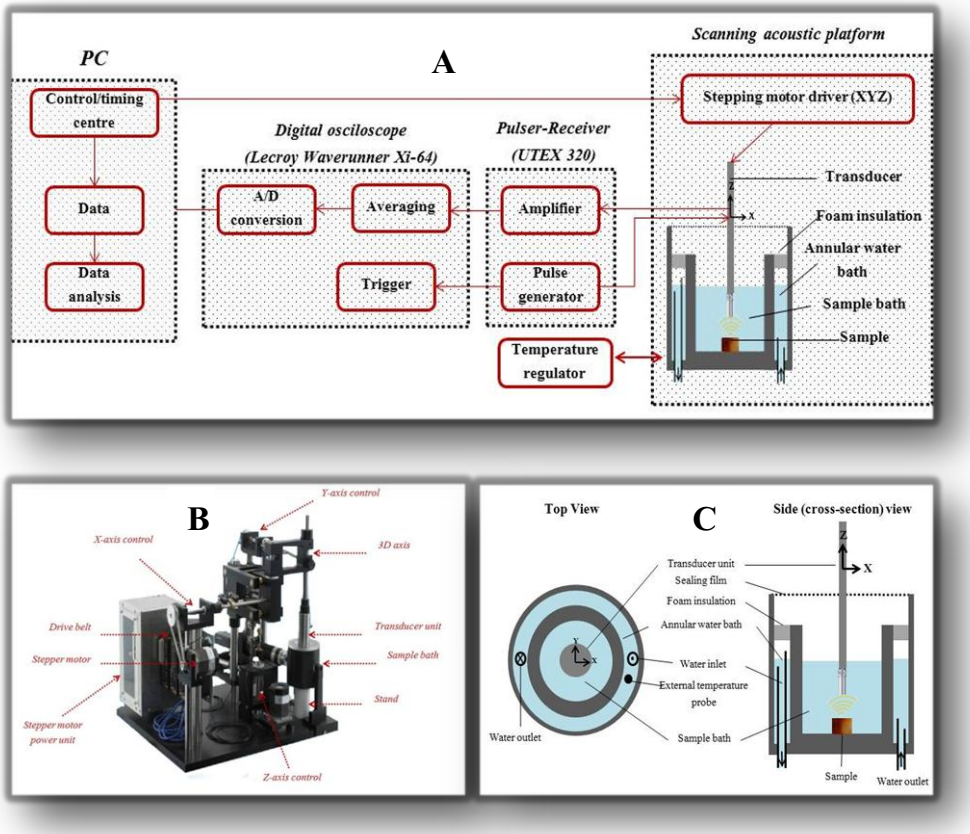


Figure 2. Experimental set up for scanning acoustic microscopy measurements. (A) Schematic of the SAM system, (B) Scanning acoustic platform and (C) schematic views of the sample unit from the top and side (cross-section).

2.1 Air-coupled measurements

A) Vacuum packaged dry-cured ham

Sliced dry-cured ham was purchased in a local market and vacuum packaged using three different thicknesses (Thin: 5.2-7.4 mm, Medium: 7.8-9.8 mm and Thick: 8.4-10.6 mm; at least 3 packages per thickness), which are usual thicknesses found in the retail distribution of this product. Zones of measurement were marked (from 4 to 10 zones per package) and uniformly distributed on the surface of each package including zones in lean, marbled and fatty tissues. Each

point was coincident with the surface of the transducers (2.5 cm^2) used in both types of ultrasonic measurements (contact and air-coupled). Packages consisted of a LDPE bag (thickness 90 μm, density 950 Kg/m^3). Ultrasound velocity in the LDPE bag was measured at 10 MHz using a conventional through transmission and gel-coupling system, obtaining a value of 1970 m/s. As the bag is very thin and the acoustic properties of the LDPE are close to those of the packaged ham, the influence of the package on the ultrasonic measurements was considered negligible.

B) Ultrasonic contact measurements

The experimental set-up consisted of two narrow-band ultrasonic transducers (1 MHz, 0.75" diameter, A314S-SU, Panametrics, Walthman, MA, USA), a pulser-receiver (5058PR, Panametrics, Walthman, MA, USA) and a digital storage oscilloscope (TDS5034, Tektronix, Bearverton, Oregon, USA). A custom digital height gauge was designed and built, 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 zones of measurement was computed in triplicate from the time of flight obtained from the signal (average of five signal acquisitions), and the thickness provided by the height gauge. The ultrasonic velocity was measured at 6 ºC in a temperature-controlled chamber (±0.1 ºC) (AEC330R, Infrico, Spain).

C) Ultrasonic air-coupled measurements

The experimental set-up (Figure 1) consists of a couple of specially designed and built air-coupled transducers (Gómez Álvarez-Arenas 2004), with centre frequency of 0.75 MHz (constructed in UMEDIA (CSIC), Madrid), with two-way insertion loss of -30 dB and 75% bandwidth. Active element is a 1-3 piezocomposite disk, 20 mm diameter, made of piezoelectric fibers embedded in an epoxy matrix (65 % vol. concentration of ceramic). Matching to the air is achieved by attaching a stack of three quarter-wavelength matching layers. Finally, a backing of epoxy resin loaded with tungsten particles is added. The two transducers used were positioned in opposition. The transmitter was driven by a negative square semi cycle tuned to the centre frequency of the transducers

with amplitude of 200 V (P/R 4077, Panametrics, Waltham, MA, USA). This transducer transmits an ultrasonic signal that travels across the air-gap between transmitter and receiver and is eventually detected by the receiver. This transducer converts the ultrasonic wave into an electrical signal. This signal is then amplified and filtered by the receiver and digitized in a digital oscilloscope (TDS5034, Tektronix, Bearverton, Oregon, USA).

The measuring procedure is as follows. First the signal received without any sample is acquired and FFT is calculated. Both FFT amplitude $A_0(\omega)$ and signal amplitude in the time domain $S_0(t)$ are stored as references. Then the sample is put in between the transducers at normal incidence. Figure 3 shows some of the measured signals.

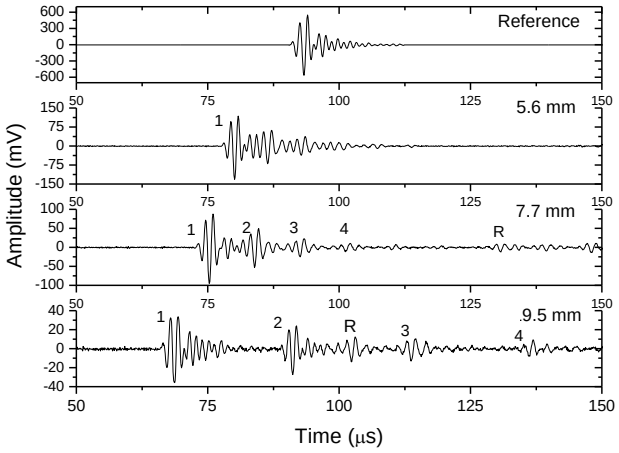


Figure 3. Transmitted signal through different samples. 1: through transmitted pulse, 2, 3 or 4 reverberations within the sample. R: reverberation within the airgap between sample and transducers.

Then, amplitude of the FFT of the through transmitted signal and all reverberations within the sample $A(\omega)$ are calculated. Both $A(\omega)$ and the signal amplitude in the time domain S(t) are stored. The insertion loss (IL) is then calculated.

$$IL = 20/log(A(\omega)/A_0(\omega)) \quad (1)$$

Some of the measurements are shown in Figure 4. The pattern of thickness resonances is clearly observed in all cases.

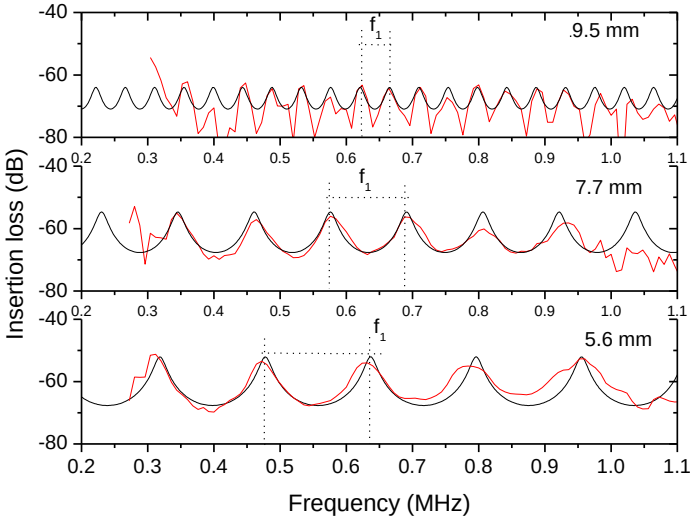


Figure 4. Insertion loss versus frequency for three of the studied packages of different thickness.

To calculate the time of flight of the ultrasound in the sample, first the cross-correlation of $S_0(t)$ and $S(t)$ is computed, then the Hilbert transform was calculated to compute the envelope and from the location of the maximum, the time of flight is obtained.

Ultrasound velocity in the sample (v), time of flight (t) and thickness (h) are related by:

$$v = \frac{h}{h/v_f - t} \tag{2}$$

where v_f is the ultrasound velocity in the air. In addition, thickness resonances (f_n) of a plane and isotropic plate, at normal incidence, are given by:

$$f_n = {}^{nv}/_{(2h)},\ n=1, 2, 3\ldots. \tag{3}$$

If *t* and f_1 (first-order thickness resonance) are measured, then, from Eq. 2 and 3 it is possible to work out *v* and *h*. The possibility of obtaining the thickness

of the sample directly from the ultrasonic measurements is very interesting (Gómez Álvarez-Arenas 2010, Rupitsch et al. 2012) as it optimizes the process and reduces the manipulation of the package. The ultrasonic velocity was measured at 6 ºC in a temperature-controlled chamber (±0.1 ºC) (AEC330R, Infrico, Spain).

2.2 Scanning Acoustic Microscopy measurements (SAM)

A) Raw material

Samples of dry-cured ham and chorizo were used. The sliced dry-cured ham (1 mm of thickness) samples were cut (AS3914, Severin, Bristol, UK) with 10 mm of length and 5 mm of width. The chorizo samples were cut considering lean and fatty tissue into parallelepiped (10x5x5 mm), taking care to maintain smooth surfaces. Any deviations would cause large angle reflections and reduced received signals. The acoustic microscopy measurements were carried out at 6±0.01 ºC.

B) SAM measurements

The scanning acoustic microscope (Figure 2A) consisted of a scanning acoustic platform (Figure 2B), a pulse-receiver (320, UTEX, Ontario, Canada) and a digital oscilloscope (Xi-64, Lecroy Waverunner, Berkshire, UK). The pulse-receiver generates a square wave pulse of 50 ns duration and 300 V amplitude to excite the transducer and receive the returning signal. The digital oscilloscope converts the signal and exported it to a PC for storage and analysis.

The scanning acoustic platform consisted of a positional system and a point focused transducer (10 MHz, 0.5'' diameter, V311, Panametrics, Waltham, MA, USA). This transducer has a 6 mm focal distance and a resolution of approximately 300 microns. The positioning system combines high spatial precision with a large and configurable range of motion. The positioning system is constructed of arms and rotational joints and based on arcular motion in the xyz directions. The transducer is connected to the bottom of a moving vertical arm which is used to focus the transducer on the sample. The acoustic platform can perform automated positioning of the transducer in three dimensions (*xyz*).

The transducer is immersed in the coupling fluid (Millipore water), which allows that frequency signals can travel properly. In addition, the platform includes a sample unit with a bath (Figure 2C) which incorporates an outer fluid jacked for temperature control. A water-antifreeze mixture is circulated through this jacket by an external temperature bath/circulator (Haake B5/DC50, Basingstoke, UK), which enables to regulate the temperature of the sample bath over the range 0-80 ºC. The velocity of sound varies with temperature at up 3 m/s per ºC in water. This can introduce considerable thermal aberration in the image and so it is essential for temperature stabilization. However, the temperature variations can be reduced by operating at temperatures with minimal gradient. Therefore, it is important to have a uniform temperature between the transducer and the sample to prevent the thermal image aberrations. The variation of the temperature in the bath sample is approximately 0.01 ºC. Thus, a 0.003 % of variation in velocity sound is obtained if is taking into account the temperature dependence on the velocity of sound. The temperature variation grows at higher ambient temperature. However, the corresponding variation in velocity of sound is approximately 0.01 %, which is negligible for most purposes.

The SAM can be operated in A-scan, B-scan and C-scan mode. The A-scan is a time-of-flight measurement performed at a single lateral position, B-scan consist of a multiple time-of-flight measurements performed laterally across the sample (*x* or *y*) and a C-scan consist of multiple measurements across an area (*x* and *y*). C-scan mode often requires many measurements increasing scan time; therefore, the faster measurements are obtained by A-scan and B-scan. For this work, measurements of B-scan were performed on the samples.

3. Results and discussion

A) Air-coupled

Figure 5 shows the results for the air-coupled and contact ultrasonic measurements of sample thickness and ultrasonic velocity. A significant ($p<0.05$) linear relationship was obtained between air-coupled and contact measurements, for both, thickness and ultrasonic velocity when all zones of measurement located in the vacuum packages were considered. The correlation coefficients were R=0.98 for thickness and R=0.88 for ultrasonic velocity. The thickness

measurements were slightly greater for the contact measurements (average 8.38±1.6 mm) than for the air-coupled ones (average 8.13±1.71mm), which is observed in Figure 5 and could be due to the use of couplants in the former technique.

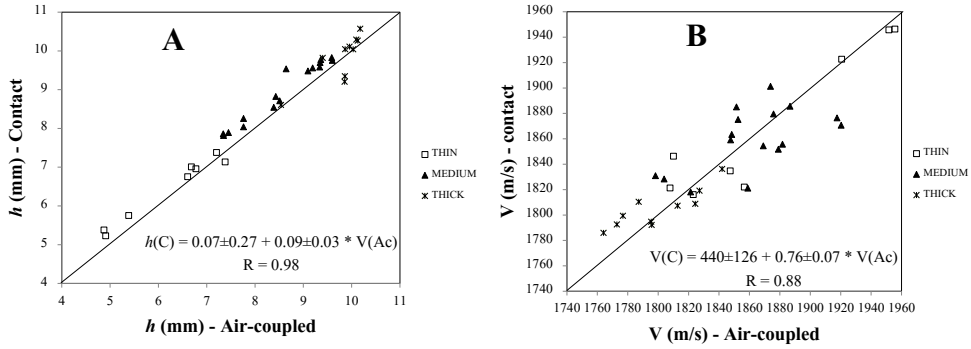


Figure 5. Comparison between air-coupled and contact ultrasonic measurements of thickness and velocity in vacuum packaged dry-cured ham.

However, this small difference in the thickness measurement does not affect the measurement of the ultrasonic velocity, being 1846±49 m/s and 1842±42 m/s for air-coupled and contact ultrasonic measurements, respectively. From Figure 5 it is also observed that the thickness of the sample does not affect the ultrasonic velocity measurements since similar deviations are found for the thin, medium and thick packages of dry-cured ham. Saggin & Coupland, (2001) reported that the air-coupled ultrasonic velocity measurements in food items (measured at 23±2 ºC) was significantly ($p<0.05$) lower than velocity measured by contact technique. This fact was linked with the possible slight compression of foods between the two transducers used in the contact technique. In particular, for luncheon meat, the average ultrasonic velocity measured by the contact method was 1668 m/s, while for air-coupled one it was 1562 m/s, more than 100 m/s of difference. In the present work, the difference in the average value of the ultrasonic velocity between both techniques was only of 4 m/s and the relative error in the estimation of contact ultrasonic velocity from air-coupled measurements was 1%. In a similar way, Gan, Pallav, & Hutchins (2006),

compared between both contact and air-coupled ultrasonic measurements for characterizing palm oil samples. Those authors observed a slight variation in their results between the contact and non-contact techniques as a consequence of the heating of the ultrasonic transducers in the contact technique, which affected the measurements. However, it was concluded that in spite of these differences, there was a good correlation between both acoustic techniques when applied to monitoring the crystallization of palm oil samples. On the other hand, the variability in the relationship between air-coupled and contact ultrasonic velocity measurements found in the present work (Figure 5) could be attributed to the heterogeneous structure and composition of the ham sample. In this regard, as the focusing of the transducer for contact and air-coupled measurements is different and also the alignment of the transducer in the air-coupled technique was not as accurate as for the contact one, the ultrasonic velocity could be measured in slightly separated areas for each system, giving rise to differences in the measurements of the time of flight.

The results obtained in this section indicate that both contact and air-coupled ultrasonic measurements can be carried out directly on the plastic surface of vacuum packaged sliced dry-cured ham. Likewise, it was demonstrated that both ultrasonic techniques are well correlated ,which indicates that contact measurements could be replaced or complemented by the air-coupled technique for a better characterization of dry-cured vacuum packaged ham. The advantage of using the air-coupled technique relies on the reduction of the measuring time since it is not necessary to place and remove the coupling medium on the sample surface. Moreover, it is not necessary to perform independent measurements of the sample thickness and therefore, transducers have not to be moved. Thus, the implementation of an innovative air-coupled ultrasonic system could lead to a better analysis of the total food area, obtaining an accurate measurement of the properties of products with high composition heterogeneity like dry-cured Iberian ham. The possible implementation of this air-coupled technique as a cost-effective and reliable method to characterize and inspect dry-cured products is very attractive for the industry. This technique could lead to the process optimization, reducing the manipulation of the package and controlling on-line the quality of meat and dry-cured meat products.

B) Scanning Acoustic Microscopy

Figure 6 shows the dry-cured ham (A) and chorizo (B) samples and the B-scans obtained for each one (C and D, respectively). The color scale of B-scans shows the magnitude of the reflected signal, thus, the color white corresponded to the zero signal and the color black to the maximum signal reflection.

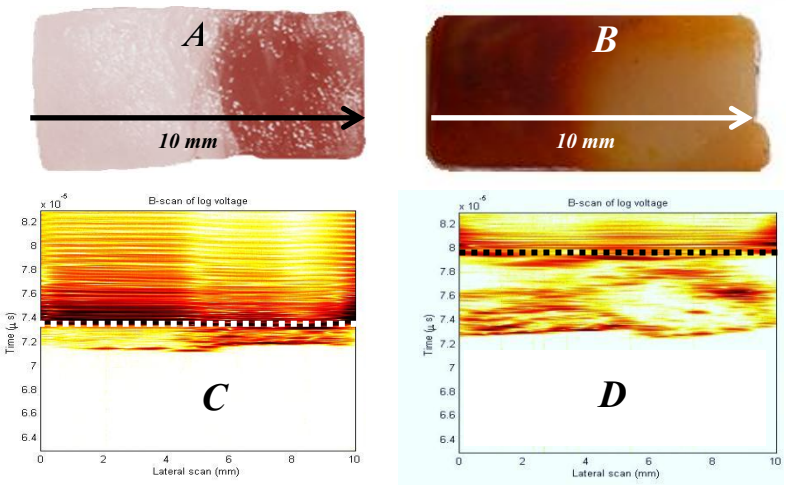


Figure 6 Samples of (A) dry-cured ham and (B) Chorizo. B-scans of (C) dry-cured ham and (D) chorizo samples.

For both, dry-cured ham and chorizo, the B-scans showed that the x-axis correspond to the scan position (10 mm) and the y-axis the time-of-flight. Thus, the first reflection received (~7.2 μs) corresponds to the top surface of the samples. The region between the first received signal and the dotted lines corresponded to the thickness of samples; therefore, the thinner area corresponds to dry-cured ham (1 mm) and the thicker area to chorizo thickness (5 mm). The dotted lines indicate the position of the glass slide where samples were placed and the signals above this line, corresponded to the reflections and echoes emanating from the slide itself.

For dry-cured ham, two different regions of sample were analyzed; the fat and lean tissue (Figure 6A). The B-scan image (Figure 6C) clearly identifies

these two different regions. The light region is the fat tissue, while the dark region corresponded with the lean tissue. Differences between both tissues were clearly visible. A large acoustic impedance difference through the fluid-sample interface, results in a large amplitude reflected signal. Different reflections are obtained from the subsurface of sample, which are caused by back-scattering from the different tissues, therefore, the differences of composition between lean and fatty tissue, such as the cell walls, the adipocytes, proteins, among others, may contribute to the different reflections and signal intensities obtained in the analysis.

In the chorizo sample (Figure 6B), the B-scan image showed different levels of back-scattering, this corresponds with the heterogeneous structure of sample. The fat distribution in sample was observed. The weakest reflection (light/white regions) was linked with the presence of the fatty tissue, therefore, three fat globules were observed within the sample (Figure 6D). Parker et al., (2010) reported a high contrast and resolution imaging of biological tissues with acoustic microscope measurements, such as onion skin, showing that the stronger reflection of signal was obtained for cell walls than the cell centre. This fact was linked with the larger impedance in cell wall than the rest of the cell. Those authors reported that the acoustic microscopy is a powerful method to obtain images of microstructure of different materials. In this work the possibility of using scanning acoustic microscopy in dry-cured meat products was demonstrated. This would allow product classification according to intramuscular fat content and distribution at microscopic level in a fast, inexpensive and non-destructive way in food processes.

4. Conclusions

Novel non-contact ultrasonic techniques (air-coupled and scanning acoustic microscopy, SAM) were used to characterize dry-cured meat products. Air-coupled measurements were compared in the meat samples with the contact ultrasonic technique. The comparison reveals a close agreement between both sets of measurements. The variability in the relationship between contact and air-coupled ultrasonic measurements was largely attributed to the heterogeneous structure and composition of ham samples and the transducer focusing. However,

air-coupled ultrasonic technique can be adequately applied to vacuum packaged dry-cured meat products reducing the measuring time. This time reduction is due to the lack of couplants and to the simultaneous measurement of ultrasonic velocity and sample thickness without the use of additional thickness gauges.

The feasibility of using SAM to characterize the structure of dry-cured meat products was demonstrated. Differences between lean and fatty tissues are clearly visible at microscopic scale. For the B-scan images, the good contrast of the tissue structure was observed, exhibiting the stronger reflection in lean tissue and the most weak for fatty one. This could allow the characterization of dry-cured meat products with heterogeneous structure and composition. Therefore, SAM could be a reliable technique for prediction of fat content and their distribution at microscopic scale in dry-cured meat products, which can be used for classification purposes.

Therefore, implementation of air-coupled and SAM techniques would allow characterize dry-cured meat products in on-line food processes, without affecting their structure, improving and optimizing the production processes and monitoring the quality of meat and dry-cured meat products.

References

Benedito, J., Carcel, J. A., Gisbert, M. & Mulet, A. (2001a). Quality control of cheese maturation and defects using ultrasonics. *Journal of Food Science*, 66, 100-104.

Benedito, J., Carcel, J. A., Gonzalez, R. & Mulet, A. (2002). Application of low intensity ultrasonics to cheese manufacturing processes. *Ultrasonics*, 40, 19-23.

Benedito, J., Carcel, J. A., Rossello C. & Mulet, A. (2001b). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 365-370.

Caner, C., Hernandez, R. J., Pascall, M. & Riemer, J. (2003). The use of mechanical analyses, scanning electron microscopy and ultrasonic imaging to study the effects of high-pressure processing on multilayer films. *Journal of the Science of Food and Agriculture*, 83, 1095-1103.

Chandrapala, J., Oliver, C., Kentish, S. & Ashokkumar, M. (2012). Ultrasonics in food processing-Food quality assurance and food safety. *Trends in Food Science and Technology*, 26, 88-98.

Damez, J. L. & Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. *Meat Science*, 80, 132-149.

Gan, T. H., Hutchins, D. A. & Billson, D. R. (2002). Preliminary studies of a novel air-coupled ultrasonic inspection system for food containers. *Journal of Food Engineering*, 53, 315-323.

Gan, T. H., Pallav, P. & Hutchins, D. A. (2006). Non-contact ultrasonic quality measurements of food products. *Journal of Food Engineering*, 77, 239-247.

García-Rey, R. M., García-Olmo, J., De Pedro, E., Quiles-Zafra, R. & Luque de Castro, M. D. (2005). Prediction of texture and colour of dry-cured ham by visible and near infrared spectroscopy using a fiber optic probe. *Meat Science*, 70, 357-363.

Gómez Álvarez-Arenas, T. E. (2004). Acoustic impedance matching of piezoelectric pransducers to the air. *Trans. on Ultrasound., Ferroelect. & Frec. Contrl*. 51, 624-633.

Gómez Alvarez-Arenas, T. E. (2010). Simultaneous determination of the ultrasound velocity and the thickness of solid plates from the analysis of thickness resonances using air-coupled ultrasound. *Ultrasonics*, 50, 104-109

Håseth, T., SØrheim, O., HØy, M. & Egelandsdal, B. (2012). Use of computed tomography to study raw ham properties and predict salt content and distribution during dry-cured ham production. *Meat Science*, 90, 858-864.

Kim, K. B., Lee, S., Kim, M. S, & Cho, B. K. (2009). Determination of apple firmness by nondestructive ultrasonic measurement. *Postharvest Biological Technology*, 52, 44-48.

Koch, T., Lakshmanan, S., Brand, S., Wicke, M., Raum, K. & Mörlein, D. (2011a). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle. *Meat Science*, 88, 51-58.

Koch, T., Lakshmanan, S., Brand, S., Wicke, M., Raum, K. & Mörlein, D. (2011b). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: II. Skin and backfat. *Meat Science*, 88, 67-74.

Llull, P., Simal S., Benedito, J. & Roselló, C. (2002). Evaluation of textural properties of meat-based product (sobrassada) using ultrasonic techniques. *Journal of Food Engineering*, 53, 279-285.

Meyer, S., Hindle, S. A., Sandoz, J. P., Gan, T. H. & Hutchins, D. A. (2006). Non-contact evaluation of milk-based products using air-coupled ultrasound. *Measurement Science and Technology*, 17, 1838-1846.

Mizrach, A. (2000). Determination of avocado and mango fruit properties by ultrasonic technique. *Ultrasonics*, 38, 717-722.

Mizrach, A. (2007). Nondestructive ultrasonic monitoring of tomato quality during shelf-life storage. *Postharvest Biological Technology*, 46, 271-274.

Mörlein, D., Rosner, F., Brand, S., Jenderka, K. -V. & Wicke, M. (2005). Non-destructive estimation of the intramuscular fat content of the longissimus muscle of pigs by means of spectral analysis of ultrasound echo signals. *Meat Science*, 69, 187-199.

Niñoles, L., Clemente, G., Ventanas, S. & Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Niñoles, L., Mulet, A., Ventanas, S. & Benedito, J. (2010). Ultrasonic assessment of the melting behaviour in fat from Iberian dry-cured hams. *Meat Science*, 85, 26-32.

Niñoles, L., Mulet, A., Ventanas, S. & Benedito, J. (2011). Ultrasonic characterization of B. femoris from Iberian pigs of different genetics and feeding systems. *Meat Science*, 89, 174-180.

Ozguler, A., Morris, S. A. & O´Brien, W. D. (1998). Ultrasonic imaging of micro-leaks and seal contamination in flexible food packages by the pulse-echo technique. *Journal of Food Science*, 63, 673-678.

Pallav, P., Hutchins, D. A. & Gan, T. H. (2009). Air-coupled ultrasonic evaluation of food materials. *Ultrasonics*, 49, 244-253.

Parker, N. G., Nelson, P. V. & Povey, M. J. W. (2010). A versatile scanning acoustic platform. *Measurement Science and Technology*, 21, 045901.

Rupitsch, S., Glaser, D., & Lerch, R. (2012). Simultaneous determination of speed of sound and sample thickness utilizing coded excitation. 2012 I*EEE International Ultrasonic Symposium, P2E-1 (in press)*.

Saggin, R. & Coupland, J. N. (2001). Non-contact ultrasonic measurements in food materials. *Food Research International*, 34, 865-870.

Santacatalina, J. V., Garcia-Perez, J. V., Corona, E. & Benedito, J. (2011). Ultrasonic monitoring of lard crystallization during storage. *Food Research International*, 44, 146-155.

Santos-Garcés, E., Gou, P., Garcia-Gil, N., Arnau, J. & Fulladosa, E. (2010). Non-destructive analysis of aw, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering*, 101, 187-192.

Schössler, K., Thomas, T. & Knorr, D. (2012). Modification of cell structure and mass transfer in potato tissue by contact ultrasound. *Food Research International*, 49, 425-431.

Simal, S., Benedito, J., Clemente, G., Femenia, A. & Rosseló, C. (2003). Ultrasonic determination of the composition of a meat-based product. *Journal of Food Engineering*, 58, 253-257.

Straadt, I. K., Aaslyngb, M. D. & Bertram, H. C. (2012). Assessment of meat quality by NMR - an investigation of pork products originating from different breeds. *Magnetic Resonance in Chemistry*, 49, 71-78.

Telis-Romero, J., Váquiro, H. A., Bon, J. & Benedito, J. (2011). Ultrasonic assessment of fresh cheese composition. *Journal of Food Engineering*, 103, 137-146.

Wickramasinghe, H.K. (1984). Acoustic microscopy: present and future. Physical Science, Measurement and Instrumentation, Management and Education - Reviews, *IEEE Proceedings* A, 282-291.

5. DISCUSIONES GENERALES

Tal y como se ha puesto de manifiesto en el apartado de introducción, existe la necesidad de poder evaluar y caracterizar de manera no destructiva los productos cárnicos crudo-curados provenientes de cerdos Ibéricos en función del tipo y contenido de grasa, así como estimar los cambios texturales ocurridos durante los procesos de conservación y almacenamiento. En base a estos antecedentes, la presente Tesis Doctoral se ha llevado a cabo con el fin de evaluar la viabilidad del uso de los ultrasonidos de señal para cubrir estas necesidades y poder caracterizar los productos cárnicos crudo-curados de manera no destructiva, rápida y con un bajo coste. Cabe resaltar que los ultrasonidos son una técnica portable y de fácil adaptación, lo que puede facilitar su implementación en las líneas de producción.

Durante los procesos de almacenamiento de los productos cárnicos crudo-curados, tienen lugar una serie de cambios en sus propiedades texturales, que van a determinar la calidad final del producto. En este sentido, la grasa y el estado en el que se encuentra (sólido/líquido) son factores determinantes en las propiedades texturales de este tipo de productos, por lo que es importante monitorizar y caracterizar aquellos cambios producidos en la grasa durante su almacenamiento. Así pues, en el presente trabajo y considerando los antecedentes descritos al inicio del mismo, se planteo como primer objetivo el evaluar la viabilidad del uso de los ultrasonidos para la caracterización del proceso de cristalización de la manteca de cerdo Ibérico durante su almacenamiento (*Apartado I*). Para alcanzar este objetivo se estudió la cristalización no isoterma de la grasa mediante análisis de calorimetría diferencial de barrido (DSC), observándose dos picos, que fueron relacionados con la cristalización de triglicéridos con diferente grado de insaturación (Campos et al., 2002). Por otro lado, se observó que la velocidad ultrasónica durante la cristalización no isoterma (etapa de atemperamiento) aumentó con el incremento del contenido de grasa sólida (Singh et al., 2004), como consecuencia de la disminución de la temperatura. Asimismo, la velocidad de enfriamiento influyó en la cristalización y por lo tanto en la velocidad de los ultrasonidos. Por otra parte, las medidas de ultrasonidos en las muestras de manteca durante su almacenamiento isotermo (11 días) a diferentes temperaturas (0, 3, 5, 7, 10 y 20 ºC) mostraron dos aumentos pronunciados en la velocidad de los ultrasonidos, lo que fue relacionado con la cristalización de triglicéridos con

diferente grado de insaturación, hecho que fue previamente observado en el análisis de DSC. De forma similar a la velocidad de los ultrasonidos, se observaron dos incrementos en la dureza de las muestras con el tiempo de almacenamiento. Por otra parte, el proceso de cristalización de la grasa en dos etapas se describió mediante un modelo matemático basado en la ecuación de Avrami, que permitió establecer una relación entre la velocidad de los ultrasonidos y el tiempo de almacenamiento (% var >99.9 y RMSE <1.99 ms^{-1}). Considerando que el ratio grasa sólida/líquida determina las propiedades texturales de los productos cárnicos crudo-curados (Singh et al., 2004), resulta importante estimar su evolución durante el almacenamiento isotermo de estos productos, mediante técnicas no destructivas como son los ultrasonidos. Para ello, en el presente trabajo se desarrolló un modelo matemático que permitió estimar de manera adecuada la evolución del porcentaje de grasa sólida, considerando los dos aumentos de la velocidad de los ultrasonidos durante la cristalización. En general, tanto el modelo que describe el proceso de cristalización de la grasa, como el que estima el porcentaje de grasa sólida, resultaron útiles para caracterizar los cambios en la grasa durante el proceso de cristalización, por lo que las medidas de la velocidad de los ultrasonidos permitieron monitorizar de manera no destructiva la cristalización de la manteca de cerdo Ibérica durante su almacenamiento isotermo. No obstante, la calidad de la grasa de cerdo depende de la raza y alimentación del animal (Ventanas et al., 2006; Niñoles et al., 2007), que influyen en el perfil de ácidos grasos y por lo tanto en su nivel de insaturación, factor que a su vez puede afectar al comportamiento de la grasa durante su almacenamiento. En este sentido, con el objetivo de evaluar el uso de los ultrasonidos para caracterizar los cambios en la textura de la grasa durante su almacenamiento en refrigeración, así como discriminar entre el tipo de grasa en función de su origen, se analizaron muestras de grasa subcutánea (tejido adiposo) de cerdos Ibéricos alimentados en montanera y cebo (*Apartado II*). Los resultados mostraron que la composición en ácidos grasos influyó en las diferentes propiedades térmicas de las grasas Ibéricas durante su cristalización, pudiéndose discriminar entre ellas (montanera y cebo) a través de las medidas de DSC. Así, en este trabajo, el perfil de ácidos grasos, mostró que la grasa de montanera tiene un mayor ($p<0.05$) contenido en ácidos grasos insaturados (58.2 %) y menor en saturados (41.8 %), comparado con la

grasa de cebo (54.9 % y 45.1 %, respectivamente). Los resultados del perfil de ácidos grasos presentados en este trabajo, fueron similares a los mostrados por otros autores (Petrón et al., 2004; Ventanas et al., 2008). Al igual que Himawan et al (2006), en el presente trabajo se ha observado que la cristalización de la grasa es sensible a pequeñas diferencias en la composición de ácidos grasos. Por otro lado, los cambios en la dureza de las dos grasas durante su cristalización isoterma mostró un comportamiento similar al observado en la manteca de cerdo Ibérico (*Apartado I*), encontrándose dos incrementos pronunciados, relacionados con la cristalización de triglicéridos con diferente grado de insaturación. Como ya se ha mencionado, el patrón de cristalización de las grasas está estrechamente relacionado con la composición en ácidos grasos, lo que resultó en diferencias de la dureza entre los dos tipos de grasas. Así, los valores medios de la dureza de la grasa de cebo (10.7 N) fueron mayores ($p<0.05$) a los de la grasa de montanera (4.4 N) durante todo el periodo de almacenamiento isotermo, lo que permitió discriminar entre las mismas. Por otra parte, las medidas de los ultrasonidos mostraron un comportamiento similar a la dureza, observándose dos aumentos pronunciados de la velocidad durante el almacenamiento. Además, los dos tipos de grasas Ibéricas pudieron ser diferenciados mediante las medidas de la velocidad ya que la velocidad de los ultrasonidos en la grasa de cebo siempre fue más alta que en montanera, en concreto un 2.8 % superior (44 m/s) tras la cristalización de los triglicéridos más saturados y 5.2 % (86 m/s) tras la cristalización de los más insaturados. Al igual que en la manteca de cerdo Ibérico (*Apartado I*), los cambios de la velocidad de los ultrasonidos durante el almacenamiento isotermo de las grasas de montanera y cebo fueron descritos adecuadamente (% var >99.5 y RMSE <3.5 ms^{-1}) mediante la aplicación de un modelo desarrollado a partir de la ecuación de Avrami (*Apartado II*). El mismo modelo fue adaptado y empleado para describir los cambios en la dureza de los dos tipos de grasas Ibéricas durante su cristalización isoterma (% var >94.3 y RMSE <0.13 N). Por lo tanto, a partir de las medidas de la velocidad de los ultrasonidos se pudo caracterizar la cristalización de la grasa subcutánea de cerdo Ibérico, así como los cambios texturales que tienen lugar durante su almacenamiento a bajas temperaturas, además el uso de los ultrasonidos permitió discriminar entre los dos tipos de grasas analizadas (montanera y cebo).

Los resultados obtenidos en esta Tesis Doctoral ponen de manifiesto que los ultrasonidos resultan una herramienta fiable para evaluar y monitorizar los cambios texturales que tienen lugar en los procesos de cristalización de la grasa, durante su almacenamiento a bajas temperaturas. Además los ultrasonidos son una técnica útil para discriminar entre diferentes tipos de grasa en función de su origen y por tanto, en función de su composición. No obstante, el estudio bibliográfico pone de manifiesto la necesidad de caracterizar los productos cárnicos crudo-curados, donde la grasa es uno de los principales componentes del sistema, pero no el único. En este sentido, es de interés el poder determinar el contenido graso y el estado en el que se encuentra la grasa (sólida/líquida) en los productos cárnicos crudo-curados, lo que va a determinar en gran medida su calidad. Asimismo, resultaría útil el poder discriminar estos productos en función del tipo de grasa con el que han sido elaborados. Estas necesidades llevaron a establecer en esta Tesis Doctoral, el objetivo de estudiar el uso de una técnica no destructiva, como son los ultrasonidos de señal, para conseguir una adecuada caracterización de los productos cárnicos crudo-curados. Para conseguir este objetivo de forma racional, se caracterizaron en primer lugar (*Apartado III*) sistemas modelo consistentes en salchichas crudo-curadas, cuya homogeneidad composicional resulta ser mayor que la de otros productos como el jamón curado. Así, a partir del análisis de salchichas crudo-curadas elaboradas con diferente contenido y tipo de grasa (manteca Ibérica, grasa subcutánea Ibérica de cerdos de cebo y montanera y aceite de girasol), se observó que la propiedades térmicas correspondientes a la fusión de las grasas, estaban relacionadas con la composición de ácidos grasos (grado de insaturación), permitiendo los análisis de DSC discriminar entre los diferentes tipos de lotes de salchichas. Por otro lado, los resultados experimentales mostraron que en muestras con un elevado contenido graso, la velocidad de los ultrasonidos disminuyó cuando la temperatura aumentó en el rango de 2 a 30 ºC. Esta disminución de la velocidad fue consecuencia de que para ese rango de temperaturas, los análisis de DSC mostraron una importante fusión de las grasas, por lo que al aumentar la temperatura, existe un mayor porcentaje de grasa fundida y por lo tanto, una menor velocidad de los ultrasonidos. Así, la fusión de la grasa resulta en un incremento del ratio liquido/sólido (McClements, 1997) lo que afecta a los ultrasonidos, ya que la velocidad de estos es menor en medios líquidos que en

sólidos. Se establecieron relaciones lineales ($p<0.05$)) entre el porcentaje de grasa fundida y la velocidad de los ultrasonidos en las muestras, que fueron diferentes para los lotes elaborados con distintos tipos de grasa. Esto permitió no solo estimar el porcentaje de grasa fundida, sino también el poder identificar y discriminar entre las diferentes grasas utilizadas para la elaboración de las salchichas. Por otra parte, considerando el efecto de la temperatura sobre la velocidad de los ultrasonidos, se desarrolló un modelo semi-empírico que permitió estimar el contenido graso (% var 96.1) de los sistemas modelo a partir de la velocidad de los ultrasonidos a 2 y 25 ºC. Por lo tanto, los resultados encontrados muestran que los ultrasonidos son una técnica fiable para distinguir el tipo de grasa y determinar el contenido y estado de la misma de manera no destructiva y rápida en productos cárnicos crudo-curados, lo que permitiría su implementación en la clasificación de estos productos.

Como ya se ha comentado con anterioridad, la caracterización de muestras con mayor heterogeneidad composicional, como el jamón curado, resulta ser de un gran interés para la industria, teniendo en cuenta el alto valor añadido de este producto. A pesar de que ya se han empleado diversas técnicas no destructivas para caracterizar este alimento, resulta difícil su implementación al tratarse de técnicas complejas y de difícil adaptación, lo que limita su uso en las líneas de producción. De esta forma y considerando que los ultrasonidos son una técnica de fácil adaptación y bajo coste, que ha demostrado ser útil para caracterizar adecuadamente y de manera no destructiva la grasa de cerdo Ibérico y productos cárnicos crudo-curados formulados, se evaluó su aplicación (*Apartado IV*) para determinar la composición y textura del jamón Ibérico, así como para caracterizar los posibles cambios que pueden ocurrir en el mismo como resultado de los procesos de conservación por altas presiones (600 MPa/6 min) y el almacenamiento a bajas temperaturas. Los resultados experimentales mostraron que la dureza de las muestras se relacionó linealmente con el contenido en grasa ($R>0.80$), disminuyendo la dureza con el aumento del contenido en grasa, un hecho ya observado con anterioridad por otros autores (Ruiz-Carrascal et al., 2000; Ventanas et al., 2007a). De los resultados obtenidos en el estudio de muestras de jamón Ibérico de dos zonas del jamón (punta y babilla), se observó que la disminución de la velocidad de los ultrasonidos con el incremento de la

temperatura (2 a 25 ºC) fue mayor (-2.5 m/s $ºC^{-1}$) en aquellas zonas con un elevado contenido graso (punta; % grasa>36.34 %). Este comportamiento, que ya había sido observado anteriormente en los resultados de las salchichas crudo-curadas con un elevado contenido graso (*Apartado III*), se debe a una disminución del ratio grasa sólida/líquida, dando lugar a una disminución de la dureza y a su vez de la velocidad de los ultrasonidos. Así, la velocidad de los ultrasonidos en el jamón Ibérico (*Apartado IV*) se relacionó linealmente ($p<0.05$; $R>0.84$) con la dureza de las muestras.

Dado el potencial que tiene el tratamiento con altas presiones como técnica de conservación de productos cárnicos crudo-curados, se realizaron medidas de la velocidad de los ultrasonidos a diferentes temperaturas (2, 6, 10, 15, 20 y 25 ºC) en paquetes de jamón Ibérico curado loncheado y envasado al vacío, tratados y no tratados con altas presiones. Los resultados mostraron que el tratamiento por altas presiones induce cambios en la textura del jamón, aumentando la dureza un 18 % para muestras con un elevado contenido graso (zona punta; %grasa >36.34 %), mientras que para un menor contenido graso (zona babilla; %grasa <21.07 %) el aumento fue del 12 %. Las diferencias encontradas pueden estar relacionadas con el mayor contenido de grasa en las muestras de punta, que puede ser fácilmente liberada y cristalizada bajo presión (Carballo et al., 1997; Cheftel et al., 1997; Trujillo et al., 2002; Simonin et al., 2012). Los cambios en la dureza resultaron en un incremento de la velocidad de los ultrasonidos, por lo que la velocidad en las muestras de punta aumentó una media de 13 m/s, mientras que en babilla el aumento fue inferior, de 9 m/s. Así, además de poder caracterizar los cambios texturales del jamón Ibérico producidos por el tratamiento por altas presiones, se ha podido discriminar entre paquetes de diferentes zonas del jamón, con diferente contenido graso, de manera no destructiva y rápida. Por otra parte, el almacenamiento a baja temperatura (6 ºC) durante un tiempo prolongado (120 días) dio lugar a un aumento de la dureza (1.10 N) y por lo tanto de la velocidad de los ultrasonidos (70 m/s), como consecuencia del incremento del ratio de grasa sólida/líquida, debido a la cristalización de la misma. Este fenómeno de cristalización de la grasa ya fue encontrado en el *Apartado II*, donde también se observaron cambios importantes en la textura del tejido adiposo, debido a la paulatina cristalización de los

diferentes. Como conclusión, se puede afirmar que mediante las medidas no destructivas de ultrasonidos se consiguió evaluar los cambios en la textura del jamón Ibérico que tienen lugar durante el almacenamiento a baja temperatura y después del tratamiento por altas presiones.

No obstante, a pesar de que en la presente Tesis Doctoral se ha puesto de manifiesto la viabilidad del uso de las técnicas ultrasónicas por contacto para caracterizar productos cárnicos crudo-curados, resulta interesante la investigación e implementación de nuevas técnicas acústicas que permitan reducir tanto los recursos (medios de acople), como el tiempo de medida, así como evaluar estos productos a nivel microestructural, lo que permitiría analizar aspectos como la micro-estructura de los tejidos cárnicos. Por ello, considerando los dos últimos objetivos planteados en esta Tesis Doctoral, se implementó otro tipo de técnicas acústicas sin contacto y de microscopía acústica para evaluar su aplicación en la caracterización de productos cárnicos crudo-curados (*Apartado V*). Las medidas acústicas convencionales por contacto en paquetes de jamón loncheado y envasado al vacío fueron similares a las medidas realizadas utilizando una técnica acústica sin contacto. Se obtuvo una adecuada correlación ($p<0.05$) entre ambas técnicas para la determinación del espesor y la velocidad de los ultrasonidos en las muestras (error relativo de 3.91 % y 1.26 %, respectivamente), lo que abre las puertas al empleo de los ultrasonidos sin contacto para la caracterización de productos cárnicos crudo-curados. Cabe resaltar que para las medidas sin contacto, tanto la velocidad como el espesor fueron determinados simultáneamente sin necesidad de un calibre, lo que permite que las medidas sean más rápidas y sencillas.

En cuanto a las técnicas de microscopía acústica de barrido, las imágenes obtenidas de muestras de jamón curado y chorizo mostraron que la mayor intensidad de la reflexión de la señal se dio en el tejido magro, siendo de menor intensidad aquella correspondiente al tejido graso. Así pues, ha sido posible realizar una evaluación microestructural mediante la propagación de las ondas ultrasónicas en muestras opacas a la luz, como lo son los productos cárnicos crudo-curados, caracterizando así, los distintos tejidos encontrados en los mismos. Considerando lo anteriormente expuesto, la aplicación de esta técnica

favorecería la clasificación de los productos cárnico crudo-curados en función de su contenido graso y distribución a nivel microscópico. Cabría concluir que estas dos novedosas técnicas acústicas resultan ser una herramienta útil para la caracterización de los productos cárnicos crudo-curados, permitiendo optimizar los procesos de producción y mejorando el control de la calidad de los mismos.

En general, considerando los resultados obtenidos en cada uno de los apartados que comprenden la presente Tesis Doctoral, se puede concluir, que los ultrasonidos permiten caracterizar y determinar de manera no destructiva la composición y textura de los productos cárnicos crudo-curados. Asimismo, tienen la facultad de discriminar entre diferentes contenidos y tipos de grasa. Igualmente, resultan ser una técnica fiable para evaluar y monitorizar de manera rápida y económica, los cambios texturales que tienen lugar tras la aplicación de tratamientos de conservación por altas presiones y durante los procesos de almacenamiento refrigerado, lo que permite que se pueda conseguir una optimización de los procesos de elaboración y distribución de los productos cárnicos crudo-curados. Además, la aplicación de técnicas sin contacto reducirían los tiempos de medida al descartar el uso de medios de acople y medir de manera simultánea el espesor de la muestra y la velocidad de los ultrasonidos en la misma. Por último, destacar que los ultrasonidos son una técnica de medida rápida y de fácil adaptación, lo que facilita su implementación en las líneas de producción.

6. CONCLUSIONS

From the results obtained in the present work, the main extracted conclusions have been grouped in two sections according to the type of product analyzed, fat or dry-cured products.

✓ ***Characterization of fat crystallization by ultrasound***

- Differential Scanning Calorimetry (DSC) showed two main peaks during non-isothermal crystallization of Iberian fats, namely lard and cebo and montanera backfats, due to the crystallization of triacylglycerols (TGs) with different level of saturation, which is directly linked to the fatty acid composition.
- The different cooling rates affected the crystallization pattern, modifying the enthalpy and crystallization temperatures of the two peaks observed.
- Textural analysis showed that the isothermal crystallization of the Iberian fats took place in two-steps. Thus, the two steep increases of the hardness observed during the cold storage were linked to the crystallization of TGs with different level of saturation, as found in the DSC analysis. The crystallization pattern of fats was affected by the temperature set in the cold storage.
- The ultrasonic velocity showed a similar pattern than texture during the isothermal crystallization of Iberian fats, thus, the velocity showed two increases during cold storage, due to the crystallization of TGs with different level of saturation.
- The percentage of solid fat content was estimated using a model, which considers ultrasonic velocity measurements during the isothermal storage and takes into account the two-step crystallization pattern.
- The ultrasonic and instrumental texture measurements allowed characterizing and differentiating the isothermal crystallization pattern of Iberian backfats from pigs with different rearing systems (Cebo and Montanera). Thereby, during the cold storage, Cebo fat was harder than Montanera, this fact involved that ultrasonic wave propagated faster in Cebo than Montanera fat.

- A two-step crystallization model based on the Avrami equation was used to properly describe the relationship between the ultrasonic velocity and the isothermal crystallization time. Likewise, the model was also satisfactorily used to describe the hardness increase during the cold storage.
- The Iberian fat crystallization during cold storage was well monitored through the measurement of the ultrasonic velocity, since this technique showed a good agreement with both thermal and textural analyses. Therefore, the ultrasonic velocity can be used both to estimate the textural changes that occur during fat crystallization and to distinguish between different fat sources (cebo and montanera) according to its crystallization pattern.

✓ ***Characterization of dry-cured meat products by ultrasound***

- The fatty acid composition of dry-cured sausages affected the fat melting behaviour, which allowed distinguishing between the different fat sources used (Iberian lard, sunflower oil and montantera and cebo backfats).
- An increase of temperature from 2 to 25 ºC caused a decrease of the ultrasonic velocity in dry-cured sausages elaborated with high fat content (>14%). The decrease of the ultrasonic velocity was linked to the fat melting observed in the DSC curves.
- The ultrasonic velocity measurements allowed estimating the percentage of melted fat at different temperatures and identifying the different fat sources used in the formulation of sausages. The sausage batches were better differentiated from the ultrasonic velocity measurements at temperatures close to the consumption (20 ºC).
- By using a semi-empirical model based on the measurement of the ultrasonic velocity at 4 and 25ºC, the fat content of dry-cured sausages was assessed The model provided a close agreement between experimental and calculated fat contents.

- The ultrasonic velocity adequately assessed the fat content in sliced dry-cured vacuum packaged Iberian ham, which is in a certain way a product with larger fat distribution heterogeneity than dry-cured sausages.
- The fat content had an important influence on the hardness of Iberian ham. Thereby, the ultrasonic velocity was also well related to the textural properties of the ham.
- The ultrasonic velocity in dry-cured ham decreased with the increase of temperature due to the fat melting. Thus, the higher the fat content of the sample, the higher the velocity drop.
- The high pressure treatment (HPT) involved an increase of the Iberian ham hardness, which was larger in the fatty than the lean tissue. This increase of hardness involved an increase in the ultrasonic velocity. Therefore, the ultrasonic measurements could be considered to be used for quality control purposes of packaged samples after HPT processing.
- The ultrasonic measurement allowed discriminating between two different zones of the Iberian ham (Punta and Babilla) subjected to HPT.
- The cold storage of Iberian ham involved a hardness increase, due to the fat crystallization, which was also confirmed by the increase of the ultrasonic velocity.
- Therefore, the ultrasound's ability to non-destructively monitor textural changes occurred in sliced vacuum packaged Iberian ham after the application of preservation treatments, such as HPT and cold storage, has been proved.
- The implementation of non-contact ultrasonic techniques, such as the air-coupled ultrasonic measurements could be used to characterize dry-cured ham replacing the traditional ultrasonic contact techniques. The use of this non-contact technique could reduce the measuring time by removing the need of couplants. In addition, this new technique also provides a simultaneous measurement of velocity and thickness.

- The scanning acoustic microscopy allowed characterizing the different tissues contained in dry-cured meat products, which is useful to predict not only the fat content but also the fat distribution at microscopic level, giving rise to a better product classification.

7. REFERENCIAS

Andrés, A. I., Adamsen, C. E., Moller, J. K. S., Ruiz, J. & Skibsted, L. (2005). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research Technology*, 222, 486-491.

Andrés, A. I., Ventanas, S., Ventanas, J. & Cava, R. (2005). Physicochemical changes throughout the ripening of dry cured hams with different salt content and processing conditions. *European Food Research and Technology*, 221, 30–35.

Ansorena, D. & Astiasar, I. (2004). Effect of storage and packaging on fatty acid composition and oxidation in dry fermented sausages made with added olive oil and antioxidants. *Meat Science*, 67, 237-244.

Arnau, J., Serra, X., Comaposada, J., Gou, P. & Garriga, M. (2007). Technologies to shorten the drying period of dry-cured meat products. *Meat Science*, 77, 81-89.

Awad, T. S. (2004). Ultrasonic studies of the crystallization behavior of two palm fats O/W emulsions and its modification. *Food Research International*, 37, 579-586.

Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D. & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International*, 48, 410-427.

Bechar, A., Mizrach, A., Barriero, P. & Landahl, S. (2005). Determination of mealiness in apples using ultrasonic measurements. *Biosystems Engineering*, 91, 329-334.

Benedini, R., Parolari, G., Toscani, T. & Virgili, R. (2012). Sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content. *Meat Science*, 90, 431-437.

Benedito, J., Carcel, J. A., Gonzalez, R. & Mulet, A. (2002). Application of low intensity ultrasonics to cheese manufacturing processes. *Ultrasonics*, 40, 19-23.

Benedito, J., Carcel, J. A., Rossello C. & Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 365-370.

Betts, G., Everis, L. & Betts, R. (2007). Microbial issues in reducing salt in food products. En D. &. Kilcast, Reducing salt in foods (págs. 174-200). *Boca Raton, Finland: CRC Press LLC.*

Boletín Oficial del Estado; Real Decreto 1083/2001, de 5 de Octubre, por el que se aprueba la norma de calidad para jamón Ibérico, paleta Iberica y caña de lomo Iberico elaborados en España (5 de Octubre de 2001). *B. O. E. 37830-37833.*

Boletín Oficial del Estado; Real Decreto 1469/2007, de 2 de Noviembre, por el que se aprueba la norma de calidad para jamón Ibérico, paleta Iberica y caña de lomo Iberico elaborados en España. (2 de Noviembre de 2007). *B. O. E. 45087-45104.*

Campos, R., Narine, S. S. & Marangoni, A. G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Research International*, 35, 971-981.

Caner, C., Hernandez, R. J., Pascall, M. & Riemer, J. (2003). The use of mechanical analyses, scanning electron microscopy and ultrasonic imaging to study the effects of high-pressure processing on multilayer films. *Journal of the Science of Food and Agriculture*, 83, 1095-1103

Carballo, J., Fernández, P., Carrascosa, A. V., Solas, M. T., & Jiménez-Colmenero, F. (1997). Characteristics of low and high fat beef patties: effect of high hydrostatic pressure. *Journal of Food Protection*, 60, 48-53.

Carrapiso, A. I., Bonilla, F. & García, C. (2003). Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. *Meat Science*, 65, 623-629.

Casquete, R., Benito, M. J., Martín, A., Ruiz-Moyano, S., Aranda, E. & Córdoba, M. G. (2012). Use of autochthonous pediococcus acidilactici and

staphylococcus vitulus starter cultures in the production of "Chorizo" in 2 Different Traditional Industries. *Journal of Food Science*, 77, M70-M79.

Cava, R., Ruiz, J., López-Bote, C., Martin, L., García, C., Ventanas, J. & Antequera, t. (1997). Influence of finishing diet on fatty acid profiles of intramuscular lipids, triglycerides and phospholipids in muscles of the Iberian pig. *Meat Science*, 2, 263-270.

Cava, R., Tárrega, R., Ramirez M. R., Mingoarranz, F.J. & Carrasco, A. (2005). Effect of irradiation on colour and lipid oxidation of dry-cured hams from free-range reared and intensively reared pigs. *Innovative Food Science and Emerging Technologies*, 6, 135-141.

Chandrapala, J., Oliver, C., Kentish, S. & Ashokkumar, M. (2012). Ultrasonics in food processing-Food quality assurance and food safety. *Food Science & Technology*, 26, 88-98.

Cheftel, J. C. & Culioli, J. (1997). Effects of high pressure on meat: a review. *Meat Science*, 46, 211-236.

Cilla, I., Martínez, L., Beltrán, J. A. & Roncalés, P. (2006). Dry-cured ham quality and acceptability as affected by the preservation system used for retail sale. *Meat Science*, 73, 581-589.

Cilla, I., Martínez, L., Beltrán, J. A. & Roncalés, P. (2006). Effect of low-temperature preservation on the quality of vacuum-packaged dry-cured ham: Refrigerated boneless ham and frozen ham cuts. *Meat Science*, 73, 12-21.

Corino, C., Magni, S., Pastorelli, G., Rossi, R. & Mourot, J. (2003). Effect of conjugated linoleic acid on meat quality, lipid metabolism, and sensory characteristics of dry-cured hams from heavy pigs. *Journal of Animal Science*, 81, 2219-2229.

Costa-Corredor, A., Muñoz, I., Arnau, J. & Gou, P. (2010). Ion uptakes and diffusivities in pork meat brine-salted with NaCl and K-lactate. *LWT - Food Science and Technology*, 43, 1226-1233.

Costa-Corredor, A., Serra, X., Arnau, J. & Gou, P. (2009). Reduction of NaCl content in restructured dry-cured hams: Post-resting temperature and drying level effects on physicochemical and sensory parameters. *Meat Science*, 93, 390-397.

Daza, A., Mateos, A., Ovejero, I. & López Bote, C. J. (2006). Prediction of body composition of Iberian pigs by means biolectrical impedance. *Meat Science*, 72, 43-46.

Diario Oficial de la Unión Europea. (2006). Directiva 2006/52/CE del parlamento europeo y del consejo de 5 de julio de 2006.

FAO/OMS (JEFCA). Joint FAO/OMS Expert Comittee on Food Additives (JECFA). Comité Científico de la Alimnetación Humana de la CEE. Informe de la 15a reunion del comite del codex sobre productos carnicos elaborados. *Copenhague, 8–12 de octubre de 1990*.

Fennema, O. (2000). Química de los Alimentos. 2a. Edición. *Zaragoza, España: ACRIBIA, S. A.*

Fernández, M., Ordóñez, J. A., Cambero, I., Santos, C., Pin, C. & de la Hoz, L. (2007). Fatty acid compositions of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry*, 101, 107-112.

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., & Pérez-Alvarez, J. A. (2005). Meat products as functional foods: a review. *Journal of Food Science*, 70, 37-43.

Fernández-López, J., Sendra, E., Sayas-Barberá , E., Navarro, C. & Pérez-Alvarez, J. A. (2008). Physico-chemical and microbiological profiles of ‘‘salchichón” (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science*, 80, 410-417.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M. & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science*, 85, 506-514.

Fulladosa, E., Santos-Garcés, E., Picouet, P. & Gou, P. (2010). Prediction of salt and water content indry-cured hams by computed tomography. *Journal of Food Engineering*, 96, 80-85.

Gan, T. H., Pallav, P. & Hutchins, D. A. (2006). Non-contact ultrasonic quality measurements of food products. *Journal of Food Engineering*, 77, 239-247.

Gan., T. H., Hutchins, D. A. & Billson, D. R. (2002). Preliminary studies of a novel air-coupled ultrasonic inspection system for food containers. *Journal of Food Engineering* , 53, 315-323.

García, C., Berdagué, J. J., Antequera, T., López-Bote, C., C'ordoba, J. J. & Ventanas, J. (1991). Volatile components of dry cured Iberian ham. *Food Chemistry*, 23-32.

García-Esteban, A., Ansorena, D. & Astiasarán, I. (2004). Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on colour, texture and microbiological quality. *Meat Science*, 67, 57-63.

García-Íñiguez de Ciriano, M., Berasategi, I., Navarro-Blasco, Í., Astisarán, I. & Ansorena, D. (2012). Reduction of sodium and increment of calcium and ω3 polyunsaturated fatty acids in dry fermented sausages: effects on the mineral content, lipid profile and sensory quality. *Journal of the Science of Food and Agriculture*, doi: 10.1002/jsfa.5811.

Garcia-Rey., R. M., Garcia-Olmo, J., De Pedro, E., Quiles-Zafra, R. & De Castro, M. D. L. (2005). Prediction of texture and colour of dry-cured ham by visible and near infrared spectroscopy using a fibre optic probe. *Meat Science*, 70, 375-363.

Garriga, M., Grébol, N., Aymerich, M. T., Monfort, J. M. & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, 5, 451-457.

Gimeno, O., Ansorena, D., Astiasarán, I. & Bello, J. (2000). Characterization of chorizo de Pamplona: instrumental measurements of colour and texture. *Food Chemistry*, 69, 195-200.

Gómez Álvarez-Arenas, T. E., Calás, H., Ealo Cuello, J., Ramos Fernández, A. & Muñoz, M. (2010). Noncontact ultrasonic spectroscopy applied to the study of polypropylene ferroelectrets. *Journal of Applied Physics*, 108, 074110.

Gómez Álvarez-Arenas, T. E., Elvira, L. & Riera, E. (1995). Generation of the slow wave to characterize air-filled porous fabrics. *Journal of Applied Physics*, 78, 2843-2845.

Gómez Álvarez-Arenas, T., Corona, E. & Benedito, J. (2009). Non-contact ultrasonic assessment of the properties of vacuum-packaged dry-cured ham. Proceedings IEEE International Ultrasonics Symposium, 2541-2544.

Gómez, J., Sanjuán, N., Peña, R., Escrivá, L. & Clemente, G. (2011). Difusión de nitratos en carne de cerdo. Vl Congreso Nacional de Ciencia y Tecnología de los Alimentos. *Valencia, España: Universidad Politécnica de Valencia*.

Gonzalez-Fernandez, C., Santos, E. M., Rovira, J. & Jaime, I. (2006). The effect of sugar concentration and starter culture on instrumental and sensory textural properties of chorizo-Spanish dry-cured sausage. *Meat Science*, 74, 467-475.

Guerrero, L., Gou, P., Alonso, P. & Arnau, J. (1999). Study of the Physicochemical and Sensorial Characteristics of Dry-Cured Hams in Three Pig Genetic Types. *Journal of the Science of Food and Agriculture*, 70, 526-530.

Håseth, T., SØrheim, O., HØy, M. & Egelandsdal, B. (2012). Use of computed tomography to study raw ham properties and predict salt content and distribution during dry-cured ham production. *Meat Science*, 90, 858-864.

Herranz, B., de la Hoz, L., Hierro, E., Fernández, M. & Ordóñez, J. A. (2005). Improvement of the sensory properties of dry-fermented sausages by the addition of free amino acids. *Food Chemistry*, 91, 673-682.

Himawan, C., Starov, V. M. & Stapley, A. G. F. (2006). Thermodynamic and kinetic aspects of fat crystallization. *Advances in Colloid and Interface Science*, 122, 3-33.

Hugas, M., Garriga, M. & Monfort, J. M. (2002). New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, 62, 359-371.

Jaeger, H., Balasa, A. & Knorr, D. (2009). Food Industry Applications for Pulsed Electric Fields. *Electrotechnologies for Extraction from Food Plants and Biomaterials*, 181-216.

Jia, J., Schinckel, A. P., Forrest, J. C., Chen, W. & Wagner, J. R. (2010). Prediction of lean and fat composition in swine carcasses from ham area measurements with image analysis. *Meat Science*, 85, 240-244.

Jiménez, N., Picó, R., Camarena, F., Redondo, J. & Roig, B. (2012). Ultrasonic evaluation of the hydration degree of the orange peel. *Postharvest Biology and Technology*, 67, 130-137.

Jiménez-Colmenero, F., Carballo, J.& Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. *Meat Science*, 59, 5–13.

Jiménez-Colmenero, F., Fernandez, P., Carballo, J. & Fernández-Martín, F. (1998). High-pressure-cooked low-fat pork and chicken batters as affected by salt levels and cooking temperature. *Journal of Food Science*, 63, 656-659.

Koch, T., Lakshmanan, S., Brand, S., Wicke, M., Raum, K. & Mörlein, D. (2011a). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle. *Meat Science*, 88, 51-58.

Koch, T., Lakshmanan, S., Brand, S., Wicke, M., Raum, K. & Mörlein, D. (2011b). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: II. Skin and backfat. *Meat Science*, 88, 67-74.

Leygonie, C., Britz, T. J. & Hoffman, C. L. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, 91, 93-98.

Lllul, P., Simal, S., Femenia, A., Benedito, J. & Roselló, C. (2002a). The use of ultrasound velocity measurement to evaluate the textural properties of sobrasada from Mallorca. *Journal of Food Engineering*., 52, 323-330.

Llull, P., Simal S., Benedito, J. & Roselló, C. (2002b). Evaluation of textural properties of meat-based product (sobrassada) using ultrasonic techniques. *Journal of Food Engineering*, 53, 279-285.

Lorenzo, J. M. & Franco, D. (2012). Fat effect on physico-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage Lipolysis, proteolysis and sensory properties. *Meat Science*, 92, 704-714.

Mañas, P. & Pagán, R. (2005). A Review: Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology*, 98, 1387-1399.

Marcos, B., Aymerich, T., Guardia, M. D. & Garriga, M. (2007). Assessment of high hydrostatic pressure and starter culture on the quality properties of low-acid fermented sausages. *Meat Science*, 76, 46-53.

Mason, T. J. (1993). Sonochemistry: A technology for tomorrow. *Chemistry and Industry*, 2, 47-50.

McClements, D. J. & Povey, M. J. W. (1992). Ultrasonic analysis of edible fats and oils. *Ultrasonics*, 30, 383-388.

Mendoza, E., García, M. L., Casa, C. & Selgas, M. D. (2001). Inulin as fat substitute in low fat, dry fermented sausages. *Meat Science*, 57, 387-393.

Meyer, S., Hindle, S. A., Sandoz, J. P. Gan, T. H. & Hutchins, D. A. (2006). Non-contact evaluation of milk-based products using air-coupled ultrasound. *Measurement Science and Technology*, 17, 1838-1846.

Ministerio de Agricultura, Alimentación y Medio Ambiente. (2012). Recuperado el 03 de Septiembre de 2012, de http://www.magrama.gob.es/es/alimentacion/temas/calidad-agroalimentaria/calidad-comercial/mesa-del-iberico/riber-publico/censos-animales-productos-comercializados/

Mizrach, A. (2004). Assessing plum fruit quality attributes with an ultrasonic method. *Food Research International*, 37, 627-631.

Mizrach, A. (2008). Ultrasonic technology for quality evaluation of fresh fruit and vegetables in pre- and postharvest processes. *Postharvest Biology and Technology*, 48, 315-330.

Mizrach, A., Bechar, A., Grinshpon, Y., Hofman, A., Egozi, H. & Rosenfeld, L. (2003). Ultrasonic classification of mealiness in apples. *Transactions of the ASAE*, 46, 397-400.

Mizrach, A., Flitsanov, U. & Fuchs, Y. (1997). An ultrasonic nondestructive method for measuring maturity of mango fruit. *Transactions of the ASAE*, 40, 1107-1111.

Mizrach, A., Flitsanov, U., El-Batsri, R. & Degani, C. (1999a). Determination of avocado maturity by ultrasonic attenuation measurements. *Scientia Horticulturae*, 80, 173-180.

Mizrach, A., Flitsanov, U., Schmilovitch, Z. & Fuchs, Y. (1999b). Determination of mango physiological indices by mechanical wave analysis. *Postharvest Biology and Technology*, 16, 179-186.

Mizrach, A., Galili, N., Rosenhouse, G. & Teitel, D. C. (1991). Acoustical, mechanical, and quality parameters of winter-grown melon tissue. *Transactions of the ASAE*, 34, 2135-2138.

Mizrach, A., Galili, N., Teitel, D. C. & Rosenhouse, G. (1994). Ultrasonic evaluation of some ripening parameters of autumn and winter-grown galia melons. *Scientia Horticulturae*, 56, 291-297.

Muguerza, E., Ansorena, D., Bloukas, J. G. & Astiasarán, I. (2003). Effect of fat level and partial replacement of pork backfat with olive oil on the lipid oxidation and volatile compounds of greek dry fermented sausages. *Journal of Food Science*, 68, 1531-1536.

Mulet, A., Benedito, J., Bon, J. & Sanjuan, N. (1999). Review: Low intensity ultrasonics in food technology. *Food Science and Technology International*, 5, 285-297.

Nagy, P. B. (1993). Slow wave propagation in air-filled permeable solids. The *Journal of the Acoustical Society of America*, 93, 3224-3234.

Nelson, P. V., Povey, M. J. W. & Wang, Y. (2001). An ultrasound velocity and attenuation scanner for viewing the temporal evolution of a dispersed phase in fluids. *Review of Scientific Instruments*, 72, 4234-41.

Nielsen, M., Martens, H. J. & Kaack, K. (1998). Low frequency ultrasonics for texture measurements in carrots (Daucus carota L.) in relation to water loss and storage. *Postharvest Biological*, 14, 297-308.

Niñoles, L., Clemente, G., Ventanas, S. & Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Niñoles, L., Mulet, A., Ventanas, S. & Benedito, J. (2010). Ultrasonic assessment of the melting behaviour in fat from Iberian dry-cured hams. *Meat Science*, 85, 26-32.

Niñoles, L., Mulet, A., Ventanas, S. & Benedito, J. (2011). Ultrasonic characterisation of B. femoris from Iberian pigs of different genetics and feeding systems. *Meat Science*, 89, 174-180.

Niñoles, L., Sanjuan, N., Ventanas, S. & Benedito, J. (2008). Ultrasonic and sensory characterization of dry-cured ham fat from Iberian pigs with different genetics and feeding backgrounds. *Meat Science*, 80, 896-902.

Oppenhein, A. V. & Schafer, R. W. (1989). Discrete-time Signal Processing. *New Jersey, USA. Prentice Hall International, Inc*.

Ortiz, M. C., Sarabia, L., García-Ray, R. & Castro, M. D. L. (2006). Sensitivity specificity of PLS-class modeling for five sensory characteristics of dry-cured ham using visible and near infrared spectroscopy. *Analytica Chimica Acta*, 558, 125-131.

Ortiz-Somovilla, V., España-España, F., De Pedro-Sanz, E. J. & Gaitán-Jurado, A. J. (2005). Meat mixture detection in Iberian pork sausages. *Meat Science*, 71, 490-497.

Ortuño, C., Martínez-Pastor, M. T., Mulet, A. & Benedito, J. (2012). An ultrasound-enhanced system for microbial inactivation using supercritical carbon dioxide. *Innovative Food Science and Emerging Technologies*, 15, 31-37.

Ortuño, C., Perez-Munuera, I., Puig, A., Riera, E. & García-Pérez, J. V. (2010). Influence of power ultrasound application on mass transport and microestructure or orange peel during hot air drying. *Physics Procedia*, 153-159.

Ozguler, A., Morris, S. A. & O´Brien, W. D. (1998). Ultrasonic imaging of micro-leaks and seal contamination in flexible food packages by the pulse-echo technique. *Journal of Food Science*, 63, 673-678.

Ozuna, C., Cárcel, J. A., García-Pérez., J. V. & Mulet, A. . (2011). Improvement of water transport mechanisms during potato drying by applying ultrasound. *Journal of the Science of Food and Agriculture*, 91, 2511-2517.

Pallav, P., Hutchins, D. A. & Gan, T. H. (2009). Air-coupled ultrasonic evaluation of food materials. *Ultrasonics*, 49, 244-253.

Parker, N. G., Nelson, P. V. & Povey, M. J. W. (2010). A versatil scanning acoustic platform. *Mesaurement Science and Technology*, 21, 045901.

Parra, V., Viguera, J., Sánchez, J., Peinado, J., Espárrago, F., Gutierrez, J. I. & Andrés, A. I. (2010). Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. *Meat Science,* 84, 760-768.

Parra, V., Viguera, J., Sánchez, J., Peinado, J., Espárrago, F., Gutierrez, J. I. & Andrés, A. I. (2012). Effect of exposure to light on physico-chemical quality attributes of sliced dry-cured Iberian ham under different packaging systems. *Meat Science*, 90, 236-243.

Patterson, M. F. (2005). A Review: Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, 98, 1400-1409.

Pérez-Palacios, T., Antequera, T., Durán, M. L., Caro, A., Rodríguez, P. G. & Ruiz, J. (2010). MRI-based analysis, lipid composition and sensory traits for studying Iberian dry-cured hams from pigs fed with different diets. *Food Research International*, 43, 248-254.

Pérez-Palacios, T., Antequera, T., Durán, M. L., Caro, A., Rodríquez, P. G. & Palacios, R. (2011). MRI-based analysis of feeding background effect on fresh Iberian ham. *Food Chemestry*, 126, 1366-1372.

Petrón, M. J., Muriel, E., Timón, M. L., Martín, L. & Antequera, T. (2004). Fatty acids and triacylglycerols profiles from different types of Iberian dry-cured hams. *Meat Science*, 68, 71-77.

Pierre, J., Elias, F. & Leroy, V. (2012). A technique for measuring velocity and attenuation of ultrasound in liquid foams. *Ultrasonics*, 10.1016/j.ultras.2012.10.011.

Povey., M. J. W. & McClements, D. J. (1988). Ultrasonics in food engineering. Part I: Introduction and experimental methods. *Journal of Food Engineering*, 8, 217-245.

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, 70, 435-447.

Quintavalla, S. & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science*, 62, 373-380.

Resa, P., Elvira, L., de Espinosa, F. M., González, R. & Barcenilla, J. (2009). On-line ultrasonic velocity monitoring of alcoholic fermentation kinetics. *Bioprocess and Biosystems Engineering*, 32, 321-331.

Registro informativo de organismos independientes de control del ibérico (RIBER) (2012). Ministerio de Agricultura, Alimentación y Medio Ambiente. http://www.magrama.gob.es/es/alimentacion/temas/calidad-agroalimentaria/calidad-comercial/mesa-del-iberico/riber-publico/default.aspx Visitado el 10 Octubre 2012.

Ross, K. A., Pyrak-Nolte, L. J. & Campanella, O. H. (2004). The use of ultrasound and shear oscillatory test to characterize the effect of mixing time on the rehological properties of dough. *Food Research International*, 37, 567-577.

Rubio, B., Martínez, B., García-Cachán, M. D., Rovira, J. & Jaime, I. (2007). The effects of high pressure treatment and of storage periods on the quality of vacuum-packed "salchichón" made of raw material enriched in monounsaturated and polyunsaturated fatty acids. *Innovative Food Science & Emerging Technologies*, 8, 180-187.

Ruiz, J. & Lopez Bote, C. (2002). Improvement of dry-cured hams quality by lipid modification through dietary means. Research Advances in the Quality of Meat and Meat Products, Research Singpost, *Trivandrum, Kerala India,* 2555-271.

Ruiz, J. & Petrón, M. J. (2001). Métodos para la clasificación de la materia prima. En: Tecnología del jamón Ibérico: De los sistemas tradicionales a la explotación racional del sabor y el aroma. *Madrid, España: Editorial Mundi Prensa*.

Ruiz, J., García, C., Muriel, E., Andrés, A. I. & Ventanas, J. (2002). Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science*, 61, 347-354.

Ruiz, J., Ventanas, J., Cava, R., Timón, M. L. & García, C. (1998). Sensory characteristics of Iberian ham: influence of processing time and slice location. *Food Research International* , 31, 53-58.

Ruiz-Capillas, C., Triki, M., Herrero, A. M., Rodriguez-Salas, L. & Jiménez-Colmenero, F. (2012). Konjac gel as pork backfat replacer in dry fermented sausages: Processing and quality characteristics. *Meat Science*, 92, 144-150.

Ruiz-Carrascal, J., Ventanas, J., Cava, R., Andrés, A. I. & García, C. (2000). Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. *Food Research International*, 33, 91-95.

Ruiz-Ramírez, J., Serra, X., Arnau, J. & Gou, P. (2005). Profiles of water content, water activity and texture in crusted dry-cured loin and in non-crusted dry-cured loin. *Meat Science*, 69, 519-525.

Saggin, R. & Coupland, J. N. (2001). Non-contact ultrasonic measurements in food materials. *Food Research International*, 34, 865-870.

Santacatalina, J. V., Garcia-Perez, J. V., Corona, E. & Benedito, J. (2011). Ultrasonic monitoring of lard crystallization during storage. *Food Research International*, 44, 146-155.

Santos-Garcés, E., Gou, P., Garcia-Gil, N., Arnau, J. & Fulladosa, E. (2010). Non-destructive analysis of aw, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering*, 101, 187-192.

Santos-Garcés, E., Muñoz, I., Gou, P., Sala, X. & Fulladosa, E. (2012). Tools for studying dry-cured ham processing by using computed tomography. *Journal of Agricultural and Food Chemistry*, 60, 241-249.

Sato, K. (2001). Crystallization behaviour of fats and lipids-a review. *Chemical Engineering Science*, 56, 2255-2265.

Schindel, D. W. & Hutchins, D. A. . (1995). Through-thickness characterization of solids by wideband air-coupled ultrasound. *Ultrasonics*, 33, 11-17.

Sebranek, J. & Bacus, J. N. (2007). Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Science*, 77, 136-147.

Serra, X., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M., Sárraga, C., Monfort, J. M. & Arnau, J. (2007b). High pressure applied to frozen ham at different process atages. 2. Effect on the sensory attributes and on the colour characteristics of dry-cured ham. *Meat Science*, 75, 21-28.

Serra, X., Sárraga, C., Grébol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M., Monfort, J. M. & Arnau, J. (2007a). High pressure applied to frozen ham at different process satges. 1. Effect on the final physicochemical parameters and on the antioxidant and proteolytic enzyme activities of dry-cured ham. *Meat Science*, 75, 12-20.

Simal, S., Benedito, J., Clemente, G., Femenia, A. & Rosselló, C. (2003). Ultrasonic determination of the composition of a meat-based product. *Journal of Food Engineering*, 58, 253-257.

Simonin, H., Duranton, F., & de Lamballerie, M. (2012). New Insights into the High-Pressure Processing of Meat and Meat Products. *Comprehensive Reviews in Food Science and Food Safety*, 11, 285-306.

Singh, A. P., McClements, D. J. & Marangoni, A. G. (2004). Solid fat content determination by ultrasonic velocimetry. *Food Research International*, 37, 545-555.

Straadt, I. K., Aaslyngb, M. D. & Bertram, H. C. (2012). Assessment of meat quality by NMR - an investigation of pork products originating from different breeds. *Magnetic Resonance in Chemistry*, 49, 71-78.

Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A. & García, C. (2001). Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams. *Meat Science*, 58, 85-91.

Toldrá , F. (2002). Dry-cured meat products. Ed. F. Toldrá. *Trumbull, CT: Food & Nutrition Press.*

Toldrá, F. & Aristoy, M. C. (2010). Dry-Cured Ham, in Handbook of Meat Processing. Ed. F. Toldrá. *Oxford, UK: Wiley-Blackwell*.

Trujillo, A. J., Capellas, M., Saldo, J., Gervilla, R., & Guamis, B. (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science and Emerging Technologies*, 3, 295-307.

Valencia, I., Ansorena, D. & Astiasarán, I. (2006). Stability of linseed and antioxidants containing dry fermented sausages: a study of the lipid fraction during different storage conditions. *Meat Science*, 73, 269-277.

Ventanas, J. (2006). El jamón Ibérico. De la dehesa al paladar. *Madrid: Ediciones Mundi-Prensa.*

Ventanas, S., Ruiz, J., García, C. & Ventanas, J. (2007b). Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science*, 77, 324-330.

Ventanas, S., Ventanas, J., Tovar, T., García, C. & Estévez, M. (2007a). Extensive feeding versus oleic acid and tocopherol enriched mixed diets for the production of Iberian dry-cured hams: Effect on chemical composition, oxidative status and sensory traits. *Meat Science*, 77, 246-256.

Ventanas, S., Tejeda, J. F. & Estévez. (2008). Chemical composition and oxidative status of tissues from Iberian pigs as affected by diets: extensive feeding v. oleic acid- and tocopherol-enriched mixed diets. *Animal*, 2, 621-630.

Wickramasinghe, H.K. (1984). Acoustic microscopy: present and future. Physical Science, Measurement and Instrumentation, Management and Education - Reviews, *IEEE Proceedings* A, 282-291.

Zhifei, H., Yechuan, H., Hongjun, L., Gang, Q., Ting, W. & Jiayi, Y. (2012). Effect of high-pressure treatment on the fatty acid composition of intramuscular lipid in pork. *Meat Science*, 90, 170-175.