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CARACTERIZACIÓN MEDIANTE ULTRASONIDOS DE SEÑAL DE LOS CAMBIOS COMPOSICIONALES DEL JAMÓN CURADO DURANTE SU PROCESADO

TESIS DOCTORAL

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DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

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Que la memoria titulada "*CARACTERIZACIÓN MEDIANTE ULTRASONIDOS DE SEÑAL DE LOS CAMBIOS COMPOSICIONALES DEL JAMÓN CURADO DURANTE SU PROCESADO*", presentada por Dña. Marta de Prados Pedraza para aspirar al grado de Doctora en Ciencia, Tecnología y Gestión Alimentaria y realizada bajo nuestra dirección en el Departamento de Tecnología de Alimentos de la Universitat Politècnica de València, cumple las condiciones adecuadas para su aceptación como Tesis Doctoral, por lo que

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RESUMEN

El jamón curado presenta una elevada variabilidad en su calidad final debido a la heterogeneidad de la materia prima y a los múltiples factores que afectan al procesado. El objetivo general de este trabajo fue evaluar el uso de los ultrasonidos de señal para estimar diferentes parámetros composicionales del jamón curado durante su elaboración, en particular su contenido en sal y grasa. Para ello, se llevaron a cabo experiencias de caracterización ultrasónica en tres de las fases del procesado del jamón curado: producto fresco, salado y curado.

En la primera fase del procesado, se midió la velocidad de los ultrasonidos en jamones frescos a 2ºC. Se observó que un incremento del contenido en grasa conllevó un aumento de la velocidad ultrasónica, siguiendo una relación lineal. Mediante un modelo basado en la velocidad se predijo el contenido en grasa de los jamones frescos con un error (RMSE) del 2.97% y se clasificó correctamente el 89% de los jamones en tres categorías según su contenido en grasa (<14, 14-26 y >26% b.h.). Los resultados obtenidos se compararon con el uso de rayos-X para la estimación del contenido en grasa de manera no destructiva. Los modelos predictivos basados en la técnica de rayos-X proporcionaron un error en la estimación de grasa ligeramente superior (RMSE=4.65%) y un porcentaje de jamones correctamente clasificados menor (65%). Asimismo, la combinación de ultrasonidos y rayos-X, no mejoró la precisión del modelo predictivo del contenido en grasa en jamones frescos obtenido mediante las medidas ultrasónicas.

En la fase de salado, se midió la velocidad ultrasónica, en modo transmisión-recepción, en muestras modelo formuladas a partir de *Biceps femoris* (BF) con contenido en agua y sal predefinidos. Se observó un efecto significativo ($p<0.05$) de ambos componentes (sal y agua) sobre la velocidad ultrasónica, que se cuantificó mediante relaciones lineales. Así, se puso de manifiesto que el contenido en sal tuvo un mayor efecto (13m/s por cada 1% b.h. de ganancia de sal) sobre la velocidad ultrasónica, que el contenido en agua (5m/s por cada

1% b.h. de pérdida de agua). Asimismo, se midió la velocidad ultrasónica, en modo transmisión-recepción, en *Longissimus dorsi* (LD) y BF, así como en jamones, antes y después del salado (hasta 16 días). En todas las muestras, la velocidad ultrasónica aumentó durante el salado, debido a la pérdida de agua y a la ganancia de sal, alcanzando variaciones de velocidad de 61.5 y 49.3m/s en LD y BF salados 168h y de 46.8m/s en jamones salados 16 días. La pendiente de la relación entre la ganancia de sal y la variación de velocidad fue similar en LD y BF salados en salmuera (13.3m/s % b.h.), en los jamones salados en seco (12.7m/s % b.h.) e incluso similar a la mostrada en la bibliografía para una disolución salina (13.7m/s % b.h.). El hecho de que las pendientes fuesen similares indicó que una misma ganancia de sal da lugar a una misma variación de velocidad, independientemente del tipo de salado (formulación, salado en salmuera o en seco) y de la estructura del producto (disolución o carne). Mediante la relación entre la ganancia de sal y la variación de velocidad durante el salado (R^2>0.76) fue posible determinar el contenido en sal con un error de predicción (RMSE) del 0.48% en LD y BF y del 0.44% en jamones. La precisión alcanzada en estas predicciones pone de manifiesto que la técnica de ultrasonidos es adecuada como método de clasificación de las piezas saladas en diferentes niveles de sal, pero su uso como método analítico no sería recomendable.

Dado que las medidas de velocidad antes y después del salado permitirían clasificar las piezas en diferentes niveles de sal, pero no reducir el número de piezas saladas por defecto o en exceso; se abordó la monitorización del salado en seco de LD y BF mediante la medida on-line de la velocidad ultrasónica. Así, la velocidad aumentó de manera progresiva durante el salado de LD y BF, lo que demostró la capacidad de los ultrasonidos en modo transmisión-recepción para monitorizar este proceso. La pendiente de la relación entre la ganancia de sal y la variación de velocidad durante la monitorización del salado de LD y BF (13.9m/s % b.h.) fue similar a las obtenidas previamente cuando la velocidad se media antes y después del salado en LD y BF y en jamones y a la encontrada en

la bibliografía (13.6±0.8m/s % b.h.). Se desarrolló un modelo predictivo, basado en la relación entre la ganancia de sal y la variación de velocidad, que proporcionó un error (RMSE=0.43%) similar en la predicción del contenido en sal al obtenido cuando las medidas se habían realizado antes y después del salado (RMSE=0.48% en LD y BF, RMSE=0.44% en jamones).

Por otro lado, también se consideró el uso del tiempo de vuelo, medido en modo pulso-eco, para monitorizar el proceso de salado de jamones enteros. En este caso, las señales obtenidas presentaron niveles de energía muy bajos, por lo que el método empleado hasta el momento para el cálculo del tiempo de vuelo (método del umbral de energía) conllevó errores importantes en su determinación. Por ello, se estudiaron otros métodos de análisis de señal (método de la correlación cruzada y el método del espectro de frecuencias) para la estimación del tiempo de vuelo y se concluyó que la energía de la señal es determinante a la hora de seleccionar el método más adecuado para su determinación. En este sentido, el método de la correlación cruzada, entre señales capturadas cada hora, resultó el más apropiado para calcular la variación de tiempo de vuelo durante el proceso de salado de jamones. Determinada la metodología de análisis de señal más adecuada, se realizaron medidas del tiempo de vuelo en LD y jamones durante su salado (hasta 30 días). La variación de tiempo de vuelo disminuyó progresivamente durante el salado, debido a la pérdida de agua y ganancia de sal, pero también a la reducción del espesor y a la desnaturalización proteica y compactación de las fibras (aumento de dureza) que sufre la muestra. Se alcanzó una variación del tiempo de vuelo total de -7.0μs en LD salados 72h y -17.6μs en jamones salados 30 días. Aunque los modelos predictivos del contenido en sal basados en el tiempo de vuelo tuvieron un error de predicción (RMSE=0.73% en LD y RMSE=0.57% en jamones) superior a los modelos basados en la medida de la velocidad (RMSE=0.45±0.03% b.h.), éstos permitieron clasificar correctamente el 85% de los LD y el 90% de los jamones, en tres niveles de contenido en sal (<2.5, 2.5-4.0 y >4.0% b.h. en LD y <2.0, 2.0-3.0 y >3.0% b.h. en jamones). En

LD, se logró una ligera mejora en el porcentaje de muestras correctamente clasificadas (95%) con la inclusión en el modelo predictivo del peso de la muestra y el tiempo de salado. Los resultados de este estudio demostraron el potencial de los ultrasonidos para predecir on-line la ganancia de sal y monitorizar el proceso de salado, lo que podría ser de utilidad para determinar el momento preciso en el que las muestras han alcanzado el nivel de sal deseado. Además, el uso del modo pulso-eco, respecto al modo transmisión recepción, facilitaría la implementación a nivel industrial de la tecnología ultrasónica para la monitorización de la etapa de salado en pila, ya que no necesita de la medida del espesor de la muestra y requiere un único transductor.

En el producto acabado, la velocidad ultrasónica se midió en modo transmisión-recepción en porciones de jamón curado a 2 y 15ºC. El contenido en grasa y sal influyó significativamente ($p<0.05$) en la medida de velocidad. Se desarrolló un modelo predictivo del contenido de sal basado en la medida de velocidad a 15ºC, que proporcionó un error (RMSE) del 0.69% en su estimación. Mediante la tecnología basada en rayos-X, se desarrolló un modelo predictivo (RMSE=0.43%) que permitió una mejor caracterización del contenido en sal que la obtenida mediante las medidas de ultrasonidos. No se observó una mejora de la predicción combinando ambas tecnologías. Por otro lado, mediante un modelo semi-empírico y la medida de la velocidad a 2 y 15ºC, se estimó el contenido en grasa (RMSE=6.70%), agua y proteínas+otros. Este modelo clasificó correctamente el 77% de las porciones en tres categorías en función del contenido en grasa (<25, 25-40 y >40% b.h.), lo que demostró la capacidad de esta tecnología no destructiva para determinar el contenido en grasa, así como, la composición global en el jamón curado. El modelo basado únicamente en las variables de rayos-X, mostró un error de predicción de grasa, en las porciones curadas, ligeramente superior (RMSE=7.00%). La combinación de ambas tecnologías redujo el error de predicción (RMSE) del contenido en grasa hasta el 5.60%. Sin embargo, el uso combinado de ambos

sistemas supondría una elevada inversión inicial, lo que dificultaría su implementación en la industria.

En base a los resultados obtenidos en la presente Tesis Doctoral, se puede concluir que los ultrasonidos de señal han demostrado ser una técnica viable para clasificar de forma no destructiva los jamones frescos en diferentes niveles según su contenido en grasa. Por otro lado, también han demostrado ser una técnica no destructiva que permite predecir el contenido en sal y clasificar, en base a este parámetro, la carne de cerdo sometida a un proceso de salado. Mediante ultrasonidos, también es posible monitorizar el proceso de salado, con el objetivo de predecir la ganancia de sal y finalizar el proceso cuando se alcance el contenido en sal deseado. Por último, la tecnología ultrasónica puede emplearse para clasificar porciones de jamón curado en diferentes niveles de grasa, así como, para determinar su composición global. Por lo tanto, los ultrasonidos de señal son una herramienta no destructiva, rápida, sencilla y económica, capaz de evaluar el contenido en sal y grasa en el jamón curado durante su procesado y cuyo uso permitiría aumentar la homogeneidad y calidad del producto acabado.

RESUM

La qualitat final del pernil curat presenta una elevada variabilitat a causa de l'heterogeneïtat de la matèria primera i dels diversos factors que afecten el processament del pernil. L'objectiu general d'aquest treball és avaluar l'ús dels ultrasons de senyal per a estimar diversos paràmetres composicionals del pernil curat durant el procés d'elaboració; en particular, el contingut de sal i de greix. Per a fer-ho, es van dur a terme experiments de caracterització ultrasònica en tres fases del processament del pernil curat: producte fresc, salat i curat.

En la primera fase del processament es va mesurar la velocitat dels ultrasons en el pernil fresc a 2ºC. Les dades permeteren observar que un increment del contingut de greix comportava un augment de la velocitat ultrasònica seguint una relació lineal. Mitjançant un model basat en la velocitat es va predir el contingut de greix dels pernils frescos amb un error (RMSE) del 2,97% i es va classificar correctament el 89% dels pernils en tres categories segons el contingut de greix (<14, 14-26 i >26% b. h.). Els resultats obtinguts es van comparar amb l'ús dels raigs X per a l'estimació del contingut de greix de manera no destructiva. Els models predictius basats en la tècnica dels raigs X proporcionaren un error en l'estimació de greix lleugerament superior (RMSE=4,65%) i un percentatge de pernils correctament classificats més baix (65%). Així mateix, la combinació d'ultrasons i raigs X no millorà la precisió del model predictiu del contingut de greix en el pernil fresc obtingut mitjançant les dades ultrasòniques.

En la fase de salament es va mesurar la velocitat ultrasònica en mode transmissió-recepció, en mostres model formulades a partir de *biceps femoris* (BF) amb continguts d'aigua i sal preestablerts. Es va observar un efecte significatiu ($p<0,05$) dels dos components (aigua i sal) sobre la velocitat ultrasònica, que es va quantificar mitjançant relacions lineals. Així, es va posar de manifest que el contingut de sal tenia més efecte (13 m/s per cada 1% b.h.

de guany de sal) sobre la velocitat ultrasònica, que el contingut d'aigua (5 m/s per cada 1% b. h. de pèrdua d'aigua). Així mateix, es va mesurar la velocitat ultrasònica en mode transmissió-recepció en *longissimus dorsi* (LD) i BF, com també en pernils, abans i després de salar-los (fins a 16 dies). En totes les mostres, la velocitat ultrasònica augmentà durant el salament a causa de la pèrdua d'aigua i el guany de sal, i s'assoliren variacions de velocitat de 61,5 i 49,3 m/s en LD i BF salats 168 h, i de 46,8 m/s en pernils salats 16 dies. El pendent de la relació entre el guany de sal i la variació de velocitat fou similar en LD i BF salats en salmorra (13,3 m/s b. h.), en els pernils salats en sec (12,7 m/s % b. h.) i, fins i tot, similar al que es pot trobar en la bibliografia per a una dissolució salina (13,7 m/s % b. h.). El fet que els pendents siguen similars indica que el mateix guany de sal dóna lloc a la mateixa variació de velocitat, independentment del tipus de salament (formulació, salat en salmorra o en sec) i de l'estructura del producte (dissolució o carn). Mitjançant la relació entre el guany de sal i la variació de velocitat durant el salament (R^2<0,76), fou possible determinar el contingut de sal amb un error de predicció (RMSE) del 0,48% en LD i BF; i del 0,44% en pernils. La precisió assolida en aquestes prediccions posa de manifest que la tècnica dels ultrasons és adequada com a mètode de classificació de les peces salades en diversos nivells de sal; però l'ús com a mètode analític no seria recomanable.

Atès que els mesuraments de velocitat abans i després del salament permetrien classificar les peces en diversos nivells de sal, però no reduir el nombre de peces salades per defecte o en excés, es va abordar el monitoratge del salament en sec de LD i BF mitjançant el mesurament en línia de la velocitat ultrasònica. Així, la velocitat augmentà de manera progressiva durant el salament de LD i BF, cosa que demostrà la capacitat dels ultrasons en mode transmissió-recepció per a monitorar aquest procés. El pendent de la relació entre el guany de sal i la variació de velocitat durant el monitoratge del salament de LD i BF (13,9 m/s % b. h.) fou similar als obtinguts abans, quan la velocitat es va mesurar abans i després del salament en LD i BF i en pernils, i

similar també a la que es pot trobar en la bibliografia (13,6 ± 0,8 m/s % b. h.). Es va bastir un model predictiu, basat en la relació entre el guany de sal i la variació de velocitat, que proporcionà un error similar (RMSE = 0,43%) en la predicció del contingut de sal a l'obtingut quan els mesuraments s'havien fet abans i després del salament (RMSE = 0,48% en LD i BF, RMSE = 0,44% en pernils).

D'altra banda, també es va considerar l'ús del temps de vol, mesurat en mode impuls-eco, per a monitorar el procés de salament de pernils sencers. En aquest cas, els senyals obtinguts presentaren nivells d'energia molt baixos. Així, el mètode emprat fins ara per a calcular el temps de vol (mètode del llindar d'energia) comportà errors importants en la determinació d'aquest valor. I és per això que es van estudiar altres mètodes d'anàlisi de senyal (mètode de la correlació encreuada i mètode de l'espectre de freqüències) per a l'estimació del temps de vol, i es va concloure que l'energia del senyal és determinant a l'hora de seleccionar el mètode de determinació més adequat. En aquest sentit, el mètode de la correlació encreuada, entre els senyals capturats cada hora, resultà el mètode més apropiat per a calcular la variació del temps de vol durant el procés de salament de pernils. Una vegada determinada la metodologia d'anàlisi més adequada, es van mesurar temps de vol en LD i en pernils durant el procés de salament en sec (fins a 30 dies). La variació del temps de vol va disminuir progressivament durant el salament, a causa de la pèrdua d'aigua i del guany de sal, però també per la reducció de l'espessor i per la desnaturalització proteica i compactació de les fibres (augment de duresa) que experimentà la mostra. Es va aconseguir una variació del temps de vol total de -7,0 μs en LD salat 72 h; i -17,6 μs en pernils salats 30 dies. Encara que els models predictius del contingut de sal basats en el temps de vol tingueren un error de predicció (RMSE = 0,73% en LD i RMSE = 0,57% en pernils) superior als models basats en el mesurament de velocitat (RMSE = 0,45 ± 0,03% b. h.). Tot i així, aquests models permeteren classificar correctament el 85% dels LD i el 90% dels pernils en tres nivells de sal (<2,5, 2,5-4,0 i >4,0% b.

h. en LD; i <2,0, 2,0-3,0 i >3,0% b. h. en pernils). En LD es va aconseguir una lleugera millora en el percentatge de mostres correctament classificades (95%) amb la inclusió en el model predictiu del pes de la mostra i del temps de salament. Els resultats d'aquest estudi demostraren el potencial dels ultrasons per a predir en línia el guany de sal i monitorar el procés de salament, potencial que podria ser de gran utilitat per a determinar en quin instant la mostra assoleix el nivell de sal desitjat. A més a més, l'ús del mode impuls-eco, respecte al mode transmissió-recepció, facilitaria la implementació industrial de la tecnologia ultrasònica per al monitoratge de l'etapa de salament en pila, ja que no caldria mesurar l'espessor de la mostra i només caldria un transductor.

En el producte acabat, la velocitat ultrasònica es mesurà en mode transmissió-recepció en porcions de pernil curat a 2 i 15ºC. El contingut de greix i sal va influir de manera significativa ($p<0,05$) en el valor de la velocitat. Es va desenvolupar un model predictiu del contingut de sal basat en el mesurament de velocitat a 15ºC que va proporcionar un error (RMSE) del 0,69% en l'estimació. Mitjançant la tecnologia basada en raigs X es va desenvolupar un model predictiu (RMSE = 0,43%) que va permetre una millor caracterització del contingut de sal que l'obtinguda mitjançant els ultrasons. No es va observar cap millora de la predicció combinant les dues tecnologies. D'altra banda, mitjançant un model semiempíric i el mesurament de la velocitat a 2 i 15ºC, es va estimar el contingut de greix (RMSE = 6,70%), d'aigua, de proteïnes i d'altres elements. Aquest model classificà correctament el 77% de les porcions en tres categories segons el contingut de greix (<25, 25-40 i >40% b. h.), cosa que demostrà la capacitat d'aquesta tecnologia no destructiva per a determinar el contingut de greix i, també, per a determinar la composició global del pernil curat. El model basat únicament en les variables de raigs X mostrà un error de predicció de greix, en les porcions curades, lleugerament superior (RMSE = 7,00%). La combinació de les dues tecnologies reduí l'error de predicció (RMSE) del contingut de greix fins al 5,60%. No obstant això, l'aplicació en la indústria

de l'ús combinat dels dos sistemes és dificultada per l'elevada inversió inicial que caldria.

Segons els resultats obtinguts en aquesta tesi doctoral, es pot concloure que els ultrasons de senyal han demostrat ser una tècnica viable per a classificar de forma no destructiva els pernils frescos en diferents nivells de greix. D'altra banda, també han demostrat ser una tècnica no destructiva que permet predir el contingut de sal i classificar la carn de porc sotmesa a un procés de salament. Mitjançant ultrasons també és possible monitorar el procés de salament, amb l'objectiu de predir el guany de sal i finalitzar el procés quan s'haja assolit el contingut de sal desitjat. Per últim, la tecnologia ultrasònica pot usar-se per a classificar porcions de pernil curat en diversos nivells de greix, com també per a determinar la composició global d'aquestes porcions. Per tant, l'ús d'ultrasons de senyal, que són una eina no destructiva, ràpida, senzilla i econòmica capaç d'avaluar el contingut de sal i greix en el pernil curat durant el processament, permetria augmentar l'homogeneïtat i la qualitat del producte acabat.

ABSTRACT

The final quality of the dry-cured ham has a great variability due to the heterogeneity of the raw meat and the multiple operational factors. The main objective of this Thesis was to evaluate the feasibility of using low-intensity ultrasound to estimate different compositional parameters of dry-cured ham during its elaboration, in particular the salt and fat contents. For that purpose, ultrasonic characterization experiments were carried out in the three main stages of dry cured ham processing (raw, salted and dry-cured product).

Firstly, the ultrasonic velocity was measured in fresh hams at 2ºC. An increase of the fat content involved an increase in the ultrasonic velocity, which followed a linear relationship. Using a model based on the ultrasonic velocity, the fat content in the fresh hams was predicted with an error (RMSE) of 2.97% and the 89% of the fresh hams were correctly classified into three levels of fattiness (<14, 14-26 and >26% w.b.). The results obtained were compared with the fat content estimation by using X-ray technology. The predictive models based on the X-ray technology provided an error in the fat content estimation slightly higher (RMSE=4.65%) and a percentage of fresh hams correctly classified lower (65%) than the ones obtained by using ultrasound. Likewise, the combination of both, ultrasound and X-ray technologies, did not improve the accuracy of the fat content prediction in the fresh hams obtained using only ultrasound.

In the salting stage, the ultrasonic velocity was measured, by the through-transmission mode, in formulated model samples from minced *Biceps femoris* (BF) with pre-set water and salt contents. A significant ($p<0.05$) effect of both components (salt and water) on the ultrasonic velocity was observed. Thus, the salt content had a more marked effect on the ultrasonic velocity (13m/s per 1% w.b. of salt gain) than the water content (5m/s per 1% w.b. of water loss). Furthermore, the ultrasonic velocity was measured, by the through-transmission mode, in *Longissimus dorsi* (LD) and BF, as well as, in hams,

before and after salting (up to 16 days). In all the cases, the ultrasonic velocity increased during salting, due to the water loss and the salt gain, obtaining ultrasonic velocity variations of 61.5 and 49.3m/s in LD and BF salted for 168h and of 46.8m/s in hams salted for 16 days. The slope of the relationship between the salt gain and the ultrasonic velocity variation was similar in LD and BF salted in brine (13.3m/s % w.b.) and in hams dry salted (12.7m/s % w.b.), and even, similar to the one reported in the bibliography for an aqueous solution (13.7m/s % w.b.). The fact that the slopes were similar indicate that a given salt gain causes a similar ultrasonic velocity variation, regardless of the salting type (formulation, brining and dry salting) and of the sample structure (water solution, minced meat or muscle). Using the relationship between the salt gain and the velocity variation during salting (R^2>0.76), the salt gain was computed with an error of 0.48% in LD and BF and of 0.44% in hams. These results confirm that the ultrasonic technique is a suitable classification method of salted pieces into different levels of salt, but its use as an analytic method is not recommendable.

Since the measurement of the ultrasonic velocity before and after salting could classify the pieces into different levels of salt content, but not reduce the number of over-salted or insufficiently salted pieces; the monitoring of the dry salting of LD and BF muscles was addressed by measuring the ultrasonic velocity using the through-transmission mode. Thus, the ultrasonic velocity progressively increased during LD and BF dry salting, which showed the ability of this technique to monitor the salting process. The slope of the relationship between the salt gain and the ultrasonic velocity variation obtained during the monitoring of the LD and BF salting process (13.9m/s % w.b.) was similar to the ones obtained previously in formulated samples, LD and BF and hams (avg. 13.6±0.8m/s % w.b.). The predictive model based on the relationship between the salt gain and the velocity variation provided an error in the salt content prediction (RMSE=0.43%) similar to the ones obtained when the velocity was

measured before and after salting (RMSE=0.48% in LD and BF, RMSE=0.44% in hams).

On the other hand, the use of the time of flight, measured by the pulse-echo mode, was also considered to monitor the ham dry-salting process. In this case, the ultrasonic signals presented a low energy level, so the general method used to calculate the time of flight (energy threshold method) provided significant errors in its determination. Thus, other methods of signal analysis were studied (the cross-correlation method and the phase spectrum method) to calculate the time of flight. This study revealed that the signal energy determines the most appropriated method for computing the time of flight. In this context, the cross-correlation method between signals captured in a time interval of 1 hour resulted the most appropriated method to calculate the time of flight variation during the ham salting process. Thereby, the measurement of the time of flight was carried out in LD and in hams during their dry salting (up to 30 days). The time of flight decreased progressively during dry salting due to the salt gain and the water loss, but also to the thickness reduction, and the protein denaturalization and compaction of the myofibrillar structure (increase in hardness). The total time of flight variation achieved was -7.0μs in LD salted 72h and -17.6μs in hams salted 30 days. Predictive models of the salt gain, based on the time of flight, had errors (RMSE=0.73% in LD and RMSE=0.57% in hams) higher than those based on the ultrasonic velocity (avg. RMSE=0.45±0.03%). However, the models based on the time of flight classified correctly the 85% of LD and 90% of hams into three groups of salt content (<2.5, 2.5-4.0 and >4.0% w.b. in LD and <2.0, 2.0-3.0 and >3.0% w.b. in hams). A slight improvement in the percentage of correctly classified (95%) LD muscles was found when the model incorporated the sample weight and the salting time. The results obtained confirm that the use of the ultrasonic pulse-echo technique is of great potential in the prediction of the salt gain, particularly for classification purposes. In addition, it could be useful for non-destructive monitoring of the LD and hams dry salting, and thus, determining

the optimal salting time in each piece. Furthermore, the use of the pulse-echo mode, compared to the use of the through-transmission mode, facilitates the industrial implementation of the ultrasonic technique due to a single transducer is used and the sample thickness measurement is not required.

In the finished product, the ultrasonic velocity was measured, by the through-transmission mode, in dry-cured ham portions at 2 and 15ºC. The salt and fat contents affected significantly ($p<0.05$) the velocity measurement. A predictive model for the salt content based on the ultrasonic velocity measured at 15ºC was developed and provided an error (RMSE) of 0.69%. Using the X-ray technology, a predictive model was also developed (RMSE=0.43%) and provided a better salt content prediction than the one obtained with the ultrasonic measurements. No improvement in the prediction was observed by combining both technologies. On the other hand, using a semi-empirical model and the velocity measurements at 2 and 15ºC, the fat (RMSE=6.70%), water and protein+others contents were estimated. This model classified correctly the 77% of the portions into three fat content categories (<25, 25-40 and >40% w.b.), which showed the ability of this non-destructive technology for determining the fat content, as well as, the global composition in the dry-cured ham. The model based on the X-ray variables showed an error of the prediction for the fat content slightly higher (RMSE=7.00%) than the ultrasonic model. The combination of both technologies reduced the prediction error (RMSE) up to 5.60%. However, it should be taken into account that, the use of both technologies would result in a high initial investment which would hinder its industrial implementation.

According to the results obtained in this Thesis, it could be concluded that the low-intensity ultrasound is a non-destructive, fast, simple and low-cost tool that could classify the fresh hams into different levels of fat content. On the other hand, this non-destructive technique could predict the salt content and classify, according to this parameter, the salted pork meat. In addition, ultrasound could monitor the salting process and determine the ending time of

this stage when the targeted salt content was achieved. Finally, the ultrasonic technology could classify the dry-cured ham portions into different levels of fat content, as well as, to determine their overall composition. Therefore, the use of low-intensity ultrasound could evaluate the salt and fat contents in the dry-cured ham during its processing, and therefore, contribute to raise the homogeneity and quality in the final product.

1. INTRODUCCIÓN

1. INTRODUCCIÓN

1.1 PRODUCTOS CÁRNICOS CURADOS

La gran variedad de productos cárnicos procedentes del cerdo, junto con su alto valor nutritivo y sensorial, explica la importancia de estos productos en la dieta mediterránea. La clasificación de los productos elaborados a partir del cerdo se puede realizar según si el producto es fresco o procesado. Los productos procesados pueden clasificarse a su vez según si son tratados con calor o no. Los **productos curados** entran dentro de la categoría de productos cárnicos de cerdo procesados no tratados con calor (MAGRAMA, 2015). Estos productos se pueden dividir en dos grupos: los productos con integridad anatómica, que se presentan en forma de piezas curadas enteras (jamón y paleta curada y lomo embuchado) y los productos sin integridad anatómica, que se presentan en forma de embutidos curados (chorizo, salchichón, sobrasada, fuet, chistorra, salami, etc.). A pesar de la gran variedad de materias primas y modos de elaboración, el objetivo principal en la producción de los productos curados se centra en la estabilización de la materia prima, es decir, obtener un producto estable a nivel microbiológico a temperatura ambiente, a partir de un producto perecedero como es la carne fresca (Ventanas, 2001; Ruiz, 2005). Este primer objetivo se consigue gracias al efecto bacteriostático de la sal común y las sales curantes (nitratos y nitritos), así como al descenso del contenido en agua en la materia fresca. Asimismo, se persigue desarrollar las características sensoriales propias de los productos curados, es decir, la formación de compuestos con sabor y aroma que conferirán al producto sus características finales de calidad. Para ello, tienen lugar transformaciones químicas y bioquímicas de los componentes de la carne, principalmente lípidos y proteínas (Toldrá et al., 1998; Gilles, 2009).

1.1.1 JAMÓN CURADO

Dentro de los productos curados, el jamón es un producto tradicional español que destaca por su importancia económica. De hecho, España es el principal

productor mundial de jamón y paleta curada con una producción de 254.000 toneladas en 2013 (Jimenez-Colmenero et al., 2010; ANICE, 2015). En España, el **proceso de elaboración** del jamón curado sigue un sistema tradicional que ha sido utilizado durante muchos años y que comprende las etapas mostradas en la Figura 1.1. En primer lugar, los jamones frescos pasan por un proceso de *acondicionamiento* (sangrado, perfilado, etc.). A continuación, las piezas son sometidas a un proceso de *selección* que consiste en la clasificación de las mismas según su aspecto externo, peso, pH, y raza y edad del animal (Ventanas, 2001). Posteriormente, son sometidas a una fase de *salado* en la que se incorpora sal común (NaCl) y sales nitrificantes (nitratos y nitritos). Entre los procedimientos de salado, se puede distinguir entre el salado en seco y el salado en húmedo. El sistema tradicionalmente utilizado es el salado en seco en pila, que consiste en cubrir la superficie de las piezas con sal humectada y hacer capas alternas de sal/jamones (Zumalacarregui, 1997; Ventanas, 2003). En este tipo de salado, es imprescindible controlar la temperatura (T^a<5ºC) y la humedad relativa (75-95%) de la cámara para facilitar la solubilización de la sal alrededor de la superficie de la pieza, y favorecer así, su difusión al interior, limitando también el crecimiento microbiano. Las piezas se mantienen en estas condiciones por un tiempo que depende de su peso (0.65-1 días/kg producto). El salado en húmedo puede realizarse por inmersión de los jamones en salmuera o mediante su inyección en el producto. En el salado húmedo, es fundamental la concentración de la salmuera, ya que la corteza de la pieza puede quedar quemada con salmueras saturadas (Armenteros, 2010). Independientemente del tipo de salado empleado, en esta fase se producen fenómenos osmóticos de interés que contribuyen a estabilizar el producto. Por un lado, tiene lugar el transporte de agua desde el producto hasta la solución concentrada o sal humectada que lo rodea. Por otro lado, se produce la difusión de solutos que migran desde la salmuera/sal humectada que rodea la pieza hacia su interior. Ambos fenómenos contribuyen a la disminución de la actividad de agua del producto, mejorando su estabilidad. Tras el salado, las piezas se *lava*n para eliminar el exceso de sal y pasan a un periodo de

asentamiento o *post-salado* durante más de 40 días a temperaturas entre 3-4ºC y humedad relativa del 75-80%. En esta etapa, la pieza continúa perdiendo agua y la sal se difunde homogéneamente por su interior. Posteriormente, comienza la etapa de *secado-maduración* con el fin de potenciar la difusión de la sal y la deshidratación, así como, el desarrollo del sabor, olor y aroma. Las piezas permanecen en los secaderos durante 110-121 días incrementado la temperatura de 5 hasta 34ºC y reduciendo la humedad relativa del 80 al 60%. Finalmente, y según las tendencias actuales, se concluye con una fase de *envejecimiento* de mínimo 210 días (contando desde que comienza la etapa de salado) con temperatura de 12-20ºC y humedad relativa de 50-80%. En esta última fase, prosiguen las reacciones bioquímicas que permiten mejorar las características de sabor, olor y aroma del producto final (Bello, 2008).

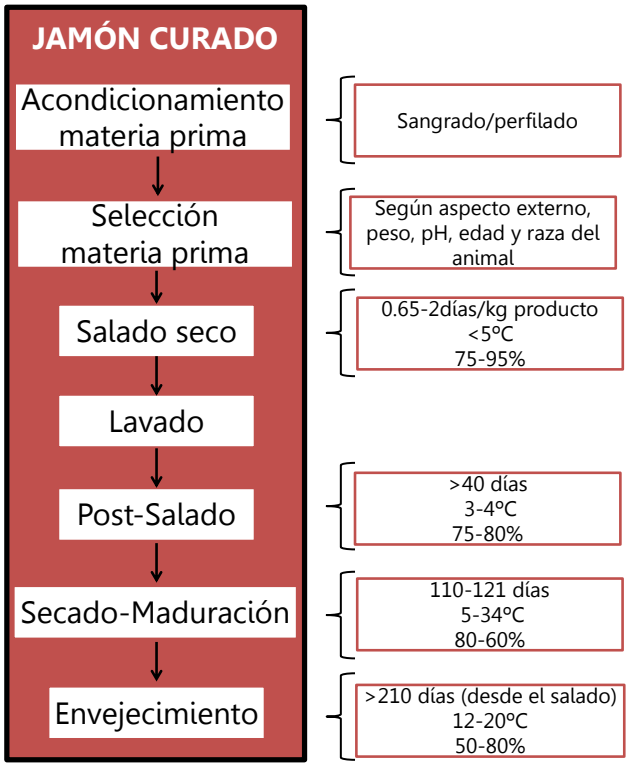

Figura 1.1 Diagrama del proceso de elaboración del jamón curado.

1.1.2 INFLUENCIA DE LA SAL Y LA GRASA EN LOS FACTORES DE CALIDAD DEL JAMÓN CURADO

El jamón curado es un producto muy apreciado por los consumidores como consecuencia de su elevada calidad. Esa calidad viene definida por parámetros como el sabor, aroma, color, composición y textura del producto (Ventanas, 2001). La sal y la grasa son dos componentes que destacan por determinar la calidad del jamón. Ambos componentes desempeñan funciones importantes en este producto, estando presentes en concentraciones significativas en el mismo.

El jamón curado destaca por ser **fuente** de sal y grasa en la dieta (BEDCA, 2015). Así, en jamones curados de diferente origen (Serranos, Ibéricos, Parma, San Danielle, etc.) el contenido en sal varía entre 3.7 y 10.3% b.h. (Ventanas 2001) y la grasa total entre 17.2 y 29.5% b.h. (Jiménez-Colmenero et al., 2010; Fulladosa et al., 2013). Dado que una ingesta excesiva de sal y grasa en la dieta puede dar lugar a problemas para la salud, como hipertensión o enfermedades cardiovasculares (Matthews et al., 2005; Fernández et al., 2007; He et al., 2012), las tendencias de consumo actuales se orientan hacia la reducción de la ingesta alimentaria de estos dos compuestos. En este contexto, la organización mundial de la salud (OMS) recomienda una ingesta de sal de <2g/día y de grasa que represente entre el 15 y el 30% de la ingesta calórica diaria total (OMS, 2015). Además, el Reglamento (CE) nº 1924/2006 aprobó que en el etiquetado de un producto podría aparecer la denominación "bajo en sal" o "bajo en grasa" cuando se reduzca, respecto al producto estándar del mercado, el contenido en sal un 25% y en grasa un 30%. Teniendo en cuenta lo anteriormente comentado, se han realizado numerosos esfuerzos para la reducción del contenido en sal y grasa en el jamón (Jiménez-Colmenero, 2000; Ruusunen et al., 2005; Aaslyng et al., 2014), así como, para la sustitución de la sal común (NaCl) por otro tipo de sales (KCl, $CaCl_2$, $MaCl_2$, etc.) (Costa-Corredor et al., 2009). Sin embargo, la reducción o sustitución del NaCl puede producir

la aparición de defectos sensoriales y dar lugar a riesgos microbiológicos (Costa-Corredor et al., 2009; Santos-Garcés, 2012).

La sal y la grasa son dos componentes que desempeñan un **papel** relevante en la elaboración del jamón curado. Por una parte, la incorporación de sal (NaCl) en el jamón durante la etapa de salado tiene una función conservante, ya que permite estabilizar microbiológicamente el producto gracias al descenso de la actividad de agua (Matthews et al., 2005; Costa, 2010). El NaCl también contribuye a modificar la textura del producto gracias a su efecto sobre el aumento de la solubilidad de las proteínas e inhibición enzimática (Bello, 2008). Asimismo, este componente modifica aspectos organolépticos, como el sabor, proporcionando el gusto a salado característico de los productos cárnicos curados (Costa, 2010).

Por otra parte, la grasa del jamón curado, que se presenta como grasa subcutánea (depositada bajo la piel del animal), intermuscular (entre los músculos) e intramuscular (infiltrada en los músculos), influye en la penetración de NaCl y otras sales curantes en la etapa de salado (Cierach et al., 2011) y afecta a la pérdida de agua durante el proceso de salado, secado/maduración y envejecimiento (Garcia-Gil et al., 2012; Čandek-Potokar et al., 2012). De la misma manera, la grasa afecta a las características texturales, ya que su composición y estructura determinan su estado (ratio grasa sólida/líquida) y por lo tanto, la apariencia y dureza del producto (Corona, 2013). Asimismo, la grasa es un componente fundamental en el desarrollo de aromas y sabores (Larrea, 2003), a través de procesos de degradación que comienzan con la lipólisis y continúan con la oxidación. En la lipólisis, diferentes procesos enzimáticos actúan sobre los lípidos dando lugar a la liberación de ácidos grasos, y en la oxidación, dichos ácidos grasos sufren una serie de reacciones oxidativas que darán lugar a compuestos con sabor y aroma (Gilles, 2009). Por tanto, la sal y la grasa son dos componentes de gran importancia, tanto a nivel tecnológico en la elaboración del jamón curado, como a nivel sensorial en la calidad final del mismo.

No obstante, uno de los principales problemas en la elaboración del jamón curado es la elevada **variabilidad** en el contenido en sal y grasa en el producto final, tanto entre lotes como entre jamones de un mismo lote. En el caso del contenido en grasa, esta variabilidad es consecuencia de las características de la materia prima utilizada, es decir, de la edad, sexo, raza y sistema de alimentación y crianza del animal (Zumalacarregui, 1997; Ventanas, 2001; Niñoles, 2007) (Figura 1.2). Pese a que los jamones frescos se pueden clasificar previamente a la entrada del proceso según esos criterios (edad, raza y sistema de alimentación y crianza del animal), las piezas no presentan el mismo contenido en grasa dentro de un lote de jamones. Esto conlleva que los fenómenos de transferencia de materia durante el salado (ganancia de sal y pérdida de agua) y en las fases de secado-maduración y envejecimiento (pérdida de agua) difieran en cada pieza. Por ello, disponer de técnicas de caracterización que permitan obtener lotes de jamones frescos con contenido en grasa homogéneo es interesante para la industria cárnica, ya que permitiría un comportamiento más uniforme de las piezas durante las distintas etapas y, por tanto, optimizaría el proceso de elaboración. Asimismo, obtener lotes de jamones frescos con contenido en grasa uniforme resulta relevante para la fabricación de jamón cocido, debido a que el contenido en grasa afecta a la adherencia entre partes magras de la pieza entera y a la aceptabilidad por parte del consumidor.

En el caso de la sal, la variabilidad es consecuencia del comportamiento no uniforme de los jamones durante el proceso de salado. Esto se debe a la heterogeneidad de la materia prima utilizada, en cuanto a peso, conformación, composición y estructura del jamón fresco, así como a los pretratamientos que se pueden llevar a cabo sobre las piezas (congelación-descongelación, grado de perfilado de la corteza, etc.) (Gou et al., 2004; Ramírez et al., 2007; Castro-Giráldez et al., 2010; Čandek-Potokar et al., 2012; Reig et al., 2013) (Figura 1.2). Por otro lado, la variabilidad en el contenido final en sal puede ser también debida al propio proceso de salado, en cuanto a condiciones de procesado no

homogéneas (temperatura y humedad relativa), uso de un mismo tiempo de salado para todos los jamones (independientemente de sus características), tamaño de los cristales de sal, grado de humedad de la sal, formación de salmuera entre la superficie del jamón y la sal, posición del jamón en las pilas de sal, etc. (Barat et al., 2004; Van Nguyen et al., 2010; Albarracín et al., 2011) (Figura 1.2). Todo ello conlleva que los fenómenos de transferencia de materia durante el salado (ganancia de sal y pérdida de agua) difieran en cada jamón, lo que a su vez afecta al comportamiento individual de cada pieza en las siguientes etapas del proceso, dando lugar a características nutricionales y organolépticas muy variables en el lote final. Hasta el momento, los esfuerzos han ido encaminados a predecir el contenido en sal en las piezas tras el salado, con el propósito de clasificarlas en diferentes lotes de contenido en sal y asignar a cada lote las condiciones más adecuadas en las etapas posteriores (Manzocco et al., 2013; Fulladosa et al., 2015). Sin embargo, la determinación del contenido en sal en el producto tras el salado no permite eliminar la existencia de jamones defectuosos (con alto o bajo contenido en sal), ya que no es posible modificar la ganancia de sal que ha alcanzado el producto. Por ello, el poder monitorizar la ganancia de sal en el jamón durante su salado, con el propósito de finalizar esta etapa cuando se haya alcanzado el contenido en sal establecido para cada pieza, sería de gran interés para la industria cárnica.

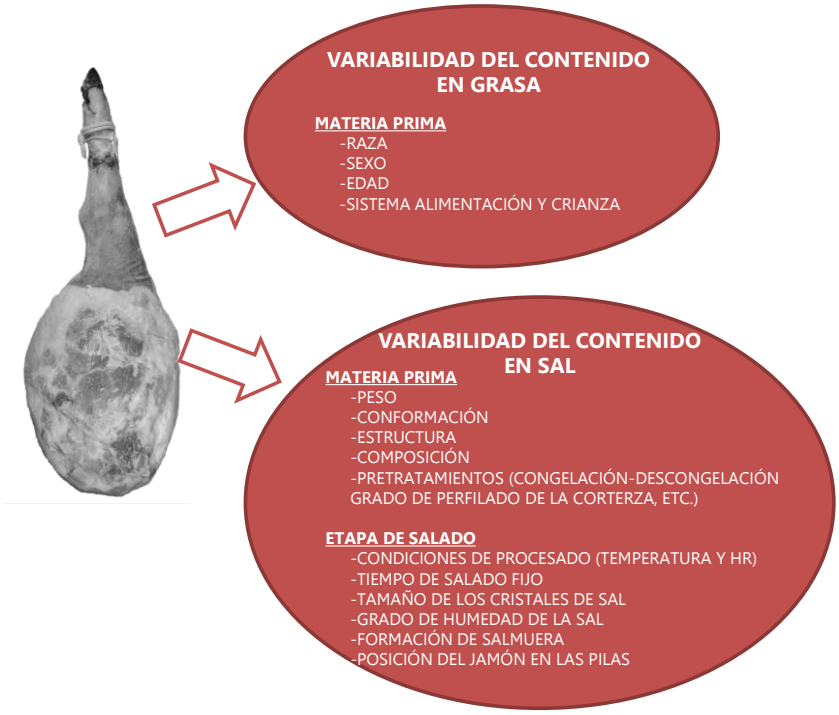

Figura 1.2 Factores que influyen sobre la variabilidad del contenido en sal y grasa en el jamón curado.

Por tanto, teniendo en cuenta la elevada variabilidad del contenido en grasa y sal en el jamón curado, y que además, estos dos componentes (sal y grasa) están presentes en elevadas concentraciones y desempeñan funciones relevantes en el mismo, el objetivo de las empresas productoras es caracterizar el jamón curado en cuanto a su contenido en sal y grasa, para conseguir, de esta manera, productos con calidad final homogénea que satisfagan los requerimientos de los consumidores.

1.2 MÉTODOS DE CARACTERIZACIÓN DE LOS PRODUCTOS CÁRNICOS: DETERMINACIÓN DEL CONTENIDO EN SAL Y GRASA

Para determinar el contenido en sal y grasa en productos cárnicos existen diferentes técnicas destructivas de análisis. Sin embargo, actualmente también

han aparecido nuevas técnicas no destructivas que intentan sustituir a las anteriores. A continuación se describen cada una de ellas.

1.2.1 TÉCNICAS DESTRUCTIVAS DE ANÁLISIS

Tradicionalmente se han empleado métodos sensoriales, químicos y físico-químicos para determinar el contenido en sal y grasa en productos cárnicos. Los **métodos sensoriales** están basados en la evaluación del producto por parte de un panel de catadores debidamente seleccionados y entrenados. Dentro de los **métodos químicos** y **físico-químicos,** destacan los métodos oficiales de análisis. En este sentido, los métodos para determinar el contenido en sal en carne son: método Volhard (ISO 1841-1-1996), método potenciométrico (ISO, 1841-2-1996; NMKL 178, 2004) y método volumétrico (AOAC 935.47-1987). En el caso de la grasa, se emplea el método gravimétrico para la determinación de grasa total (ISO 1443-1973; NMKL 131, 1989) y de grasa libre (ISO 1444-1996) y los métodos de extracción con solvente (AOAC 985.15-1991; AOAC 991.36-1996) para la determinación de grasa cruda en productos cárnicos. Asimismo, la cromatografía de gases se utiliza para determinar la composición en ácidos grasos de la grasa y la calorimetría diferencial de barrido (DSC) para estudiar las transiciones de fase (cristalización, fusión, transición vítrea, etc.).

No obstante, estas técnicas de análisis son destructivas, lentas, laboriosas y presentan un alto coste. Todo ello implica que su utilización como técnicas de control en las líneas de producción no sea viable. Por ello, la industria cárnica está interesada en remplazar estos métodos de análisis por técnicas que sean rápidas, fiables, precisas, que no afecten a la integridad del producto (no destructivas ni invasivas). De esta manera, sería posible su utilización en las líneas de producción y en el análisis de todas las piezas de un lote. Todo ello, permitiría obtener lotes con un contenido en grasa y sal homogéneo, permitiendo optimizar los procesos productivos, y por tanto, mejorando la calidad final del producto.

1.2.2 NUEVAS TÉCNICAS NO DESTRUCTIVAS

Las nuevas tecnologías no destructivas empleadas para la caracterización de productos cárnicos siguen, en la mayoría de los casos, en fase de investigación, y 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 alimentos. Como ejemplo de estas técnicas cabría citar: la Resonancia Magnética Nuclear (RMN), la Resonancia Magnética de Imagen (RMI), la Espectroscopia de Infrarrojo Cercano (NIR), los Rayos X y los Ultrasonidos de Señal (US). Estas tecnologías están basadas en las relaciones obtenidas entre los parámetros propios de la técnica y las características físico-químicas del alimento. En el presente apartado se detallan las aplicaciones más recientes de estas tecnologías en productos cárnicos. Dado que en la presente Tesis Doctoral se utilizaron los US como técnica de caracterización de los productos cárnicos, esta tecnología se describirá con mayor profundidad en los apartados 1.3 y 1.4.

<u>RESONANCIA MAGNÉTICA NUCLEAR (RMN) Y RESONANCIA MAGNÉTICA DE IMAGEN (RMI)</u>

La Resonancia Magnética Nuclear (RMN) se basa en el hecho de que la mayoría de los núcleos atómicos absorben energía en el rango de radiofrecuencias del espectro electromagnético (Damez et al., 2013). Esta técnica puede utilizarse para estudiar núcleos atómicos con un número impar de protones o neutrones, como ocurre en los átomos ^{1}H, ^{13}C, ^{19}F y ^{31}P. El más utilizado es el ^{1}H debido a que está presente en la mayoría de compuestos. La técnica se basa en que los ^{1}H en diferentes moléculas exhiben diferentes frecuencias de absorción. Esto da lugar a que cada molécula tiene un patrón característico en el espectro de RMN lo que proporciona información sobre el tipo de molécula (Vázquez, 2015). La Resonancia Magnética de Imagen (RMI) es una aplicación más compleja de RMN y proporciona una imagen de la estructura interna del alimento. Esta tecnología se basa en que cuando el núcleo ^{1}H se somete a un

campo magnético intenso se excita y cuando cesa el pulso de radiación sobre el núcleo éste retorna a su estado inicial emitiendo ondas de radiofrecuencia. La intensidad de esas ondas emitidas disminuye con el tiempo a medida que los núcleos vuelven a su posición inicial y se relajan. El proceso de relajación dura un tiempo que es característico para cada tejido. Convirtiendo el valor de tiempo de relajación en tonalidades de grises, se obtiene la imagen de los diferentes tejidos.

Estas técnicas han permitido la caracterización de la carne, en cuanto a su contenido en grasa, agua y sal. Así, Davenel et al. (2012) estimaron el contenido en grasa en carne de cerdo; y Maria et al. (2010) y Siciliano et al. (2013) determinaron la composición de ácidos grasos en carne de vacuno y de cerdo, respectivamente. La RMN y RMI también se han utilizado para describir la difusión y distribución de agua y sal en carne de cerdo durante su salado en salmuera (Vestergaard et al., 2005b). Otra aplicación importante de esta tecnología se basa en diferenciar productos cárnicos de animales con distinto régimen de alimentación. Así, Pérez-Palacios et al. (2010 y 2011) diferenciaron jamones frescos y curados provenientes de cerdos Ibéricos con distintos sistemas de alimentación (montanera y montanera pero cebados con concentrado alto oleico). Hasta el momento, la aplicación de la RMN y RMI en la industria alimentaria, está limitada por el alto coste del equipo, la necesidad de importantes medidas de seguridad, la dificultad de adaptación de la tecnología a las líneas de producción y la exigencia de operarios con elevada cualificación. Asimismo, en el caso de la RMN se requieren tamaños de muestra pequeños.

ESPECTROSCOPÍA DE INFRARROJOS CERCANO (NIR)

Esta técnica se basa en la absorción de energía por parte de un alimento como consecuencia de la vibración de sus moléculas cuando interaccionan con radiación electromagnética con una longitud de onda entre 780 y 2500nm. El valor de absorbancia de una muestra que contenga una sustancia con

capacidad de absorber radiación NIR, es directamente proporcional a la concentración de la sustancia que absorbe esta radiación. De esta forma, los valores de absorbancia obtenidos en una muestra se pueden relacionar con la concentración del compuesto existente, siempre que éste absorba radiación NIR. La NIR ha sido útil en la predicción del contenido en grasa y su composición en ácidos grasos en carne de vacuno (Wold et al., 2011), de cordero (Pullanagari et al., 2015) y de cerdo (Barlocco et al., 2006; Prevolnik et al., 2011; Gou et al., 2013). La NIR también se ha utilizado para estimar el contenido en agua y sal en salchichas de cerdo fermentadas (Collell et al., 2010), en *Biceps Femoris* de jamones curados (Prevolnik et al., 2011) y en lonchas de jamón curado envasadas a vacío (Gou et al., 2013). Los mismos componentes se han determinado durante el proceso de secado de jamón (Collell et al., 2011) y de salchichas (Collell et al., 2012). Otros parámetros tecnológicos (color, pH, capacidad de retención de agua, actividad de agua, etc.) y atributos sensoriales (textura, olor, sabor, jugosidad, terneza, etc.) en una amplia variedad de productos cárnicos, también han sido predichos con esta tecnología (Prieto et al., 2009). Sin embargo, la tecnología NIR tiene una serie de inconvenientes como son: la alta inversión inicial para la adquisición del equipo y su compleja calibración, así como, su limitada capacidad de penetración, lo que permite solamente un análisis superficial de la muestra analizada.

RAYOS X

Las aplicaciones de Rayos X se basan en la diferente atenuación de los Rayos X (radiación electromagnética con una longitud de onda de 0.01-10nm) que producen tejidos de diferentes densidades, permitiendo de esta manera diferenciar estructuras biológicas. Varias tecnologías de Rayos X se basan en este principio: radiografía de Rayos X, inspectores de Rayos X, absorciometría de Rayos X de doble energía (DEXA o DXA), tomografía computerizada (CT) y tomografía microcomputerizada. Inicialmente estas tecnologías fueron empleadas para la diagnosis médica, sin embargo, su aplicación se ha

extendido en los últimos años en la tecnología alimentaria. Así, las tecnologías de Rayos X se han utilizado para determinar la composición (grasa y magro) de animales vivos, canales y piezas de carne fresca en vacuno (Brienne et al., 2001; Frisullo et al., 2010), cordero (Hunter et al., 2011) y cerdo (Brienne et al., 2001, Marcoux et al., 2003; Vester-Christensen et al., 2009; Picouet et al., 2010). En este sentido, se han desarrollado equipos de Rayos X para medir online la relación grasa/magro en recortes de carne de vacuno y cerdo (Damez et al., 2013). Asimismo, los Rayos X han demostrado ser una herramienta útil para estimar el contenido en agua y sal en el músculo *Longissimus dorsi* de cerdo (Vestergaard et al., 2004) y en jamones curados en las etapas iniciales de su procesado (Fulladosa et al., 2010) y en la etapa de secado (Santos-Garcés et al., 2010). Numerosos estudios de Rayos-X han profundizado en el estudio del proceso de salado en productos cárnicos. Así, la difusión de la sal en el músculo *Semimembranosus* de cerdo (Picouet et al., 2013) y su distribución en jamones curados durante su salado (Vestergaard et al., 2005a; Håseth et al., 2012) también ha sido investigada con Rayos X. Además, recientemente, Fulladosa et al. (2015) evaluaron la capacidad de esta tecnología para monitorizar la ganancia de sal durante el salado en jamones curados. Como las tecnologías anteriormente mencionadas, los Rayos X presentan algunas desventajas como son la necesidad de la calibración del equipo para cada componente (grasa, sal,…) y matriz cárnica a analizar, una inversión inicial elevada y las medidas de seguridad necesarias para evitar riesgos para la salud de los operarios.

OTRAS TECNOLOGÍAS

También se han utilizado otras tecnologías no invasivas, tales como imagen hiperespectral para predecir el contenido en humedad de la carne de cerdo durante su salado (Liu et al., 2014), imagen por fluorescencia para estudiar la distribución de la grasa en carne de pollo o la imagen térmica para monitorizar el grado de cocción deseado del pollo (Chen et al., 2013).

Todas las técnicas no destructivas anteriormente comentadas (RMN, RMI, NIR, Rayos-X, etc.) se han aplicado a nivel de laboratorio. En algunos casos, como la tecnología de Rayos-X, su aplicación a nivel industrial ya existe, sin embargo, se centra en sistemas online de análisis de canales en mataderos. Por ello, en base a las demandas del sector, se debería trabajar en el desarrollo de equipos que permitan la caracterización de productos cárnicos y la monitorización online de sus procesos a nivel industrial. Además, estas técnicas deben de ser eficientes, simples de utilizar, fáciles de adaptar a las líneas de producción y tener un bajo consumo energético.

1.3 ULTRASONIDOS

El uso de ultrasonidos como técnica de caracterización presenta una serie de ventajas respecto al empleo de otras nuevas tecnologías no destructivas, como son: la facilidad de implementación en entornos industriales, el bajo consumo de energía, el bajo coste del equipamiento, la nula peligrosidad y no requerir operarios con alto grado de formación. Las aplicaciones de ultrasonidos se han extendido en el campo de la medicina (ecografías), así como, en la industria química, cosmética, textil y petroquímica (Turantaş et al., 2015). En el campo de la industria alimentaria, la aplicación de esta tecnología ha llegado con posterioridad, sin embargo, ha suscitado un gran interés por sus múltiples aplicaciones en la mejora de la calidad y seguridad de los alimentos (Awad et al., 2012).

1.3.1 GENERALIDADES

Los ultrasonidos son ondas elásticas con frecuencias que exceden el límite audible por el ser humano (≈20kHz). Al igual que cualquier onda elástica, necesitan un medio sólido, líquido o gaseoso para propagarse. El medio por el cual viajan ha de poseer masa y elasticidad, lo que implica que estas ondas no pueden viajar a través del vacío (Pascual et al., 1997). La fuente de producción de los ultrasonidos suele ser un cuerpo vibrante. El movimiento de dicho cuerpo genera una vibración que se comunica a las moléculas del medio, que a

su vez transmiten el movimiento a las moléculas adyacentes, y retornan a su posición original (Ortuño, 2014). Las ondas acústicas están definidas por su frecuencia (Hz), velocidad (m s^{-1}), longitud de onda (m), presión (N m^{-2}), intensidad (W m^{-2}) y potencia (W) acústica, densidad de energía (J m^{-3}) y atenuación (neper m^{-1}).

Las ondas acústicas pueden clasificarse en base a diferentes criterios:

En función de la frecuencia, las ondas acústicas se pueden dividir en cinco grupos: infrasonidos (frecuencia <20Hz), espectro audible (20Hz-18kHz), ultrasonidos de alta intensidad (20-100kHz), de baja intensidad (100kHz-1MHz) y de diagnóstico (>1MHz). Otra clasificación viene dada por la dirección de desplazamiento de las partículas del medio respecto a la dirección de propagación de la onda. Así, se pueden distinguir tres tipos, ondas longitudinales, transversales y de superficie o de Rayleigh. Las ondas longitudinales son aquellas en las que las partículas se mueven en la dirección de desplazamiento de la onda. En cambio, en las ondas transversales las partículas se mueven perpendicularmente al movimiento de la onda. Y, por último, las ondas de superficie, son ondas transversales que se propagan solamente en la superficie de cuerpos elásticos (Mulet et al., 1999).

En el caso de los ultrasonidos, estos también se pueden clasificar en función de su aplicación. Por un lado, los **ultrasonidos de potencia o alta intensidad**, con frecuencias entre 20kHz y 100kHz e intensidades por encima de 1W cm^{-2}, se caracterizan por su baja frecuencia y son empleados para mejorar etapas del procesado, fundamentalmente mejoran procesos de transferencia de materia. Así, se han utilizado para mejorar procesos de extracción de compuestos naturales (Ahmad-Qasem et al., 2013) o para facilitar los fenómenos de transporte en el secado de productos cárnicos (Ozuna et al., 2014) o vegetales (Santacatalina et al., 2014). Actualmente, se ha extendido su uso como método de inactivación de microorganismos (Ortuño et al., 2014) y para la inhibición de

la actividad enzimática (Povey et al., 1998). Asimismo, se han utilizado para mejorar la estabilidad de emulsiones (Ramisetty et al., 2015).

Por otro lado, los **ultrasonidos de señal o baja intensidad,** que presentan frecuencias desde 100kHz hasta 1MHz y menores intensidades (<1W cm^{-2}), se han empleado para caracterizar diversos parámetros físico-químicos de los medios donde se propagan, sin provocar cambios en los mismos (Mulet et al., 1999; Scanlon, 2004). En el apartado 1.3.5 y 1.4 se hace una revisión detallada de la aplicación de los ultrasonidos de señal en la industria alimentaria.

1.3.2 ULTRASONIDOS DE SEÑAL O DE BAJA INTENSIDAD

Los ultrasonidos de señal (US) se caracterizan por ser una técnica de análisis que permite obtener información del medio donde se propagan, fundamentalmente a partir de cuatro parámetros: la velocidad, la atenuación, la impedancia acústica y el espectro de frecuencias (McClements, 1995; Niñoles, 2007; Awad et al., 2012). Estos parámetros están relacionados con las propiedades físico-químicas del medio, y por tanto, pueden proporcionar información de la composición, la estructura y el estado físico del mismo.

La **velocidad** (V) a la que viaja una onda ultrasónica a través de un material es constante para un medio homogéneo y depende únicamente de sus propiedades físicas, en particular del módulo de deformación o módulo de Young (E) y de la densidad (ρ) (Ec. 1.1) (Mulet et al., 1999). Dado que el módulo de Young y la densidad de un material dependen de su estructura, composición y estado físico, las medidas de velocidad ultrasónica pueden proporcionar información sobre esas propiedades (McClements, 1995).

$$V = \sqrt{\frac{E}{\rho}} \qquad \text{(Ec. 1.1)}$$

La velocidad ultrasónica (V) de un material se puede determinar a partir del producto entre la longitud de onda (λ) y la frecuencia (f) (Ec. 1.2) o a partir del

cociente entre la distancia recorrida (e) y el tiempo que necesita la onda en recorrerla (Tv) (Ec. 1.3) (Toozandehjani et al., 2015).

$$V = \lambda f \qquad \text{(Ec. 1.2)}$$

$$V = \frac{e}{Tv} \qquad \text{(Ec. 1.3)}$$

El coeficiente de **atenuación** (α) se define como la pérdida de energía (amplitud de la onda) que sufre una onda, por unidad de distancia recorrida en el medio que atraviesa. Esta pérdida de energía puede ser debida a la conversión de la onda en calor (absorción) o a la dispersión o difracción de la misma (Toozandehjani et al., 2015). La atenuación de las ondas ultrasónicas es característica del material y proporciona información sobre sus propiedades físicas (Mulet et al., 1999). Su estimación puede llevarse a cabo a partir de la ecuación 1.4.

$$\alpha = \frac{1}{e} \ln\left(\frac{A_1}{A_2}\right) \qquad \text{(Ec. 1.4)}$$

Donde A_1 y A_2 son las amplitudes de la señal antes y después de atravesar el espesor de la muestra, respectivamente y e es la distancia recorrida.

La **impedancia acústica** (z) es un parámetro característico de cada material y determina la fracción de la onda ultrasónica que es reflejada por la superficie del material sobre el que incide. La impedancia acústica de un determinado medio depende de sus propiedades físicas, siendo el producto de su densidad por la velocidad de propagación del sonido en el mismo (Kuttruff, 1991). Cuando una onda ultrasónica, que viaja por un medio, incide sobre un segundo medio, la proporción de energía reflejada depende de las impedancias acústicas de los dos materiales, como se expresa en la Ec. 1.5. La reflexión será pequeña cuando las impedancias acústicas de los dos materiales sean similares y será mayor cuando sean diferentes (Pascual et al., 1997; Awad et al., 2012).

$$R = \frac{A_T}{A_t} = \frac{z1 - z2}{z1 + z2} \qquad \text{(Ec. 1.5)}$$

Donde, R es el cociente entre la amplitud de la onda reflejada (A_T) y la incidente (A_t). Y z_1 y z_2 son las impedancias acústicas de los materiales 1 y 2.

Cuando los transductores de ultrasonidos son excitados mediante un pulso eléctrico, emiten una onda con una composición en frecuencias específica para cada transductor. La distribución de energía para cada frecuencia es lo que se denomina el **espectro de frecuencias** y se obtiene a partir de la señal temporal, aplicando la transformada de Fourier. Cuando una onda de ultrasonidos atraviesa un material, éste modifica de forma característica la amplitud de cada frecuencia del espectro, por tanto, el estudio del espectro de frecuencias de la onda antes y después de atravesar la muestra, aporta información sobre sus propiedades (Corona, 2013).

1.3.3 VENTAJAS E INCONVENIENTES DE LOS ULTRASONIDOS DE SEÑAL

Los ultrasonidos de señal presentan múltiples ventajas. Así, los US son una técnica rápida, versátil, precisa, no invasiva y no destructiva. Comparado con otras técnicas no destructivas, la adquisición de un equipo de US es una inversión inicial relativamente baja. Asimismo, los costes de operación son reducidos debido a que la preparación de la muestra es rápida y sencilla y se realiza con poca manipulación. Además, su aplicación tampoco genera ningún residuo. Asimismo, permite estimar parámetros químicos (composición, acidez, etc.) y físicos (textura, densidad, viscosidad, temperatura, etc.) en diferentes medios, incluso en los ópticamente opacos. Otra ventaja de los US, es que su portabilidad y fácil adaptación a las líneas de producción permite que esta tecnología sea útil para la monitorización online de procesos industriales. Al contrario que otras técnicas de análisis no destructivo que sólo analizan la superficie (p.e. NIR), los US permiten estudiar el interior de los productos evaluados.

Sin embargo, los US también presentan una serie de inconvenientes. Las medidas son dependientes de la temperatura, por lo que es necesario su control. Además, la presencia de gas entre la muestra y el transductor puede atenuar la señal ultrasónica, evitando su propagación a través de la misma. Esto implica, en muchos casos, el uso de un material de acople (agua, aceite, etc.) entre el transductor y la muestra para eliminar el aire. De forma similar, la presencia de gas en materiales porosos (como las frutas) atenúa la señal, dificultando el empleo de esta tecnología en estos productos. Asimismo, las medidas proporcionan información de una pequeña área, por lo que se requieren múltiples medidas para cubrir toda la superficie de la muestra. Otro inconveniente, es que la técnica ultrasónica es poco sensible a componentes minoritarios tales como vitaminas, minerales, etc.

1.3.4 MODOS DE MEDIDA ULTRASÓNICOS

Las medidas ultrasónicas pueden llevarse a cabo mediante dos modos: el modo transmisión-recepción y el modo pulso-eco (McClements, 1995; Povey et al., 1998; Mulet et al., 1999; Mohammadi et al., 2014).

MODO TRANSMISIÓN-RECEPCIÓN

En este modo, los transductores (emisor y receptor) son colocados sobre las caras opuestas del material analizado (Figura 1.3). El transductor emisor genera la onda ultrasónica que atraviesa la muestra y es detectada por el transductor receptor (Povey et al., 1998). En principio, las medidas ultrasónicas en modo transmisión-recepción son simples de realizar. Sin embargo, hay una serie de factores que deben ser considerados, como son un buen acople entre transductor y muestra y una buena alineación entre los dos transductores, de forma que las caras de ambos transductores se mantengan paralelas durante la medida.

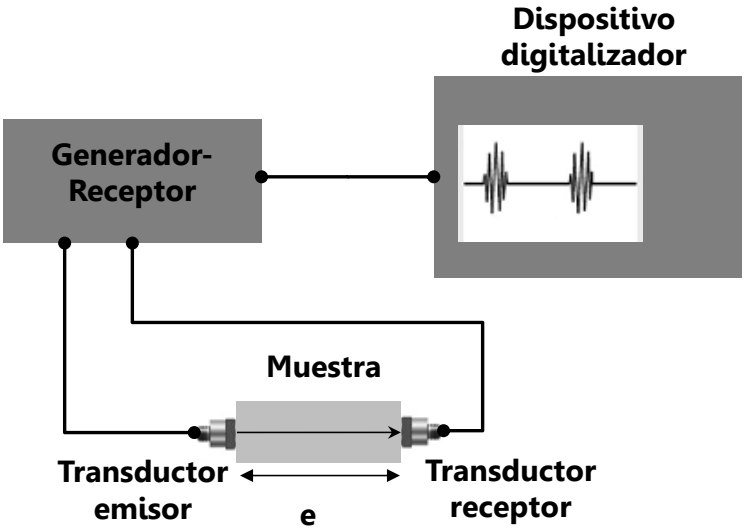

Figura 1.3 Diagrama de medida ultrasónica en modo transmisión-recepción. Donde: e indica el espesor de la muestra.

MODO PULSO-ECO

El modo pulso-eco se caracteriza porque los transductores se localizan en la misma cara de la muestra. En este caso, se puede utilizar dos transductores, uno que funcione como emisor y el otro como receptor; o puede utilizarse un único transductor que actuará tanto de emisor como de receptor de la señal (Awad et al., 2012). En este último caso, el transductor genera la onda ultrasónica que viaja a través de la muestra, se refleja en la interfase muestra-medio y retorna atravesando de nuevo la muestra al mismo transductor (Figura 1.4). En este caso, la vibración del transductor durante la emisión puede enmascarar la señal recibida de las reflexiones producidas en el interior del alimento (p.e. defectos internos) e incluso la reflexión muestra-medio. Con el objeto de evitar este problema, se puede utilizar una línea de retardo para separar la señal recibida de la emitida (Benedito, 1998). En este modo, la onda atraviesa en dos ocasiones el espesor (e) de la muestra, lo que da lugar a una mayor atenuación de la misma.

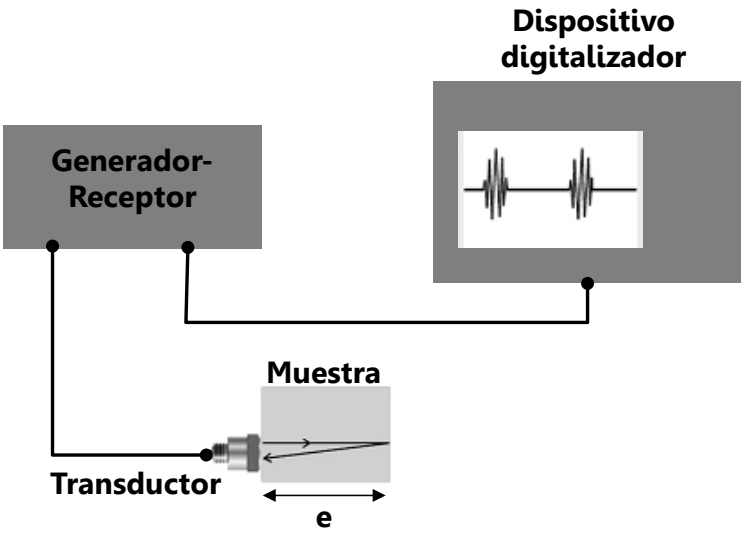

Figura 1.4 Diagrama de medida ultrasónica en modo pulso-eco. Donde: e indica el espesor de la muestra.

1.3.5 APLICACIONES DE LOS ULTRASONIDOS DE SEÑAL EN TECNOLOGÍA DE ALIMENTOS

El uso de los ultrasonidos de señal es relativamente reciente en la industria alimentaria. Sin embargo, cada vez son más numerosas en este sector sus aplicaciones, tanto para evaluar las propiedades de un producto, como para supervisar las variables de un proceso. En la mayoría de los casos, las aplicaciones se centran en la determinación de la composición, estructura y estado físico del alimento o en la detección de defectos internos en el mismo. Asimismo, existen aplicaciones basadas en la monitorización online de las propiedades de los alimentos durante diferentes etapas del procesado. A continuación, se describen las aplicaciones más recientes de los US para diferentes grupos de alimentos:

ACEITES Y GRASAS VEGETALES

Los aceites y grasas son productos alimentarios importantes, ya que el tipo y la proporción en que se utilicen para la fabricación de alimentos (margarina, helados, chocolate, etc.) influyen en sus propiedades reológicas (densidad, viscosidad, temperatura de fusión, etc.) y organolépticas (sabor, color, etc.) (Martini et al., 2006). Por tanto, la caracterización de las propiedades físico-químicas de grasas y aceites vegetales es esencial para asegurar la calidad de sus productos derivados. Por ello, características como el contenido, la densidad, la viscosidad, las transiciones de fase y el polimorfismo de las grasas, han sido evaluadas con US (Awad et al., 2012). Así, Sankarappa et al. (2005) estudiaron la relación entre la densidad y la medida de la velocidad ultrasónica en diferentes tipos de aceites comestibles (coco, girasol, cártamo, ricino y cacahuete). Izbaim et al. (2009) determinaron que las medidas de velocidad pueden ser un indicador de la calidad del aceite de soja durante la fritura. Para ello, compararon los valores de los indicadores convencionales de la calidad del aceite de soja (porcentaje de ácidos grasos libres y de compuestos polares) con las medidas de velocidad. De forma similar, Benedito et al. (2002b) relacionaron las medidas ultrasónicas (velocidad y atenuación) y las propiedades físico-químicas (viscosidad y compuestos polares) del aceite de oliva virgen calentado a 200ºC durante diferentes tiempos, para determinar mediante US la calidad del mismo durante la fritura. Otra de las aplicaciones más relevante de los US, es la monitorización de la cristalización de la grasa (Awad, 2004; Gan et al., 2006; Povey et al., 2009). En este sentido, Awad (2004) midió la velocidad de los ultrasonidos durante la cristalización de emulsiones de aceite de palma comestible (con y sin aditivo emulsionante hidrofóbico) para monitorizar las transiciones de fase, nucleación y crecimiento de los cristales. De forma similar, Povey et al. (2009) utilizaron la velocidad ultrasónica para medir el ratio de nucleación y las cinéticas de cristalización en emulsiones de mantequilla de cacahuete durante su cristalización.

PRODUCTOS LÁCTEOS

Los productos lácteos suelen ser sistemas complejos, que además sufren complejos cambios durante su procesado. En este contexto, los US se han empleado principalmente en la monitorización y control de calidad de los productos lácteos (leche, queso, yogur, etc.), relacionando los parámetros ultrasónicos con las variaciones de sus propiedades físico-químicas durante el procesado y almacenamiento (Mohammadi et al., 2014). Así, Koc et al. (2008) utilizaron los US para monitorizar el proceso de cuajado de la leche entera, que posteriormente se utilizaba para la fabricación de quesos; y Gan et al. (2006) monitorizaron el proceso de coagulación de productos a base de leche (bebida láctea con sabor a fresa y a plátano) mediante medidas ultrasónicas sin contacto, concluyendo que dicha técnica podía sustituir a las técnicas ultrasónicas por contacto. Los ultrasonidos de señal también se han utilizado para estimar la composición de helados (Bahram-Parvar, 2015) y de quesos frescos y curados (Benedito et al., 2002a; Telis-Romero et al., 2011). Por último, algunas de las aplicaciones se han enfocado a la detección de defectos en queso (agujeros, grietas etc.) (Benedito et al., 2001b y 2002a; Leemans et al., 2009) y de cuerpos extraños (plásticos, cristal, madera, piedras o metal) en leche y yogur (Chandrapala et al., 2012).

PRODUCTOS PANARIOS

Los US también han sido empleados para evaluar características de calidad de los productos panarios. Así, Alava et al. (2007) demostraron que los parámetros ultrasónicos (velocidad y atenuación) permiten diferenciar tipos de harina. Por su parte, Gómez et al. (2008) y Elfawakhry et al. (2013) mostraron el potencial de los US para caracterizar las propiedades físicas (densidad, reológicas, etc.) de masas de bizcocho y masas panarias, respectivamente. Además, Elmehdi et al. (2003) emplearon los US para evaluar la estructura de la miga del pan. La caracterización online del proceso de fermentación de masas panarias también se ha abordado mediante el uso de técnicas ultrasónicas (Skaf et al., 2009). Por

último, los US también han sido utilizados como sensores para detectar el nivel crujiente de galletas (Chen et al., 2005).

PRODUCTOS VEGETALES

Hay un elevado número de aplicaciones para evaluar la calidad de productos vegetales mediante ultrasonidos. Así, la mayor parte de los estudios se han centrado en buscar relaciones entre los parámetros ultrasónicos (velocidad, atenuación e impedancia acústica) y la firmeza/textura/madurez de frutas como el aguacate, mango, tomate, ciruela, manzana, pera y naranja (Mizrach et al., 1996; Mizrach, 2000; Mizrach, 2004; Camarena et al., 2006; Mizrach, 2007; Kim et al., 2009; Morrison et al., 2014). Los US también se han utilizado para evaluar cambios bioquímicos en zumos. Así, los parámetros ultrasónicos se han utilizado para determinar el contenido en diferentes azúcares (fructosa, glucosa, sacarosa…) en zumo de mango (Valente et al., 2013) y el contenido de azúcar y la viscosidad en zumo de naranja reconstituido (Kuo et al., 2008). Por último, Cheng et al. (1994) utilizaron el espectro de frecuencias para detectar agujeros en patatas. Sin embargo, hay que tener en cuenta que la mayoría de productos vegetales tienen grandes coeficientes de atenuación debido a la presencia de aire en los espacios intercelulares. Esto da lugar a una baja capacidad de penetración de las ondas ultrasónicas lo que resulta en un importante problema para analizar la señal (Chandrapala, 2015).

PRODUCTOS PESQUEROS

Actualmente, los estudios de la aplicación de US en productos pesqueros son escasos, centrándose en la determinación composicional en diferentes tipos de pescado (salmón, caballa, bacalao, etc.) (Ghaedian et al., 1998, Suvanich et al., 1998, Sigfusson et al., 2001). Asimismo, el potencial de los US para monitorizar el contenido en hielo en porciones de merluza durante su congelación, también ha sido evaluado por Aparicio et al. (2008).

OTROS PRODUCTOS

Los US han sido utilizados para detectar miel adulterada (Singh et al., 1995, Chandrapala, 2015) así como, para evaluar la calidad de huevos de gallina en diferentes condiciones de almacenamiento (Aboonajmi et al., 2010) o determinar el contenido en alcohol y azúcar en bebidas alcohólicas (Krause et al., 2011). En bebidas alcohólicas también se ha monitorizado su proceso de fermentación mediante medidas ultrasónicas (Resa et al., 2009). Por último, las medidas ultrasónicas se han aplicado para monitorizar el proceso de maduración del tofu (Ting et al., 2009).

1.4 APLICACIÓN DE ULTRASONIDOS DE SEÑAL EN PRODUCTOS CÁRNICOS

Las aplicaciones de los US en el sector cárnico son múltiples y variadas. Dado que la composición y estructura son los principales factores que determinan la calidad de la carne, la mayoría de los estudios se han centrado en estimar estos dos factores mediante la medida de los parámetros ultrasónicos. No obstante, también se han desarrollado otras aplicaciones ultrasónicas para monitorizar los procesos que sufren los productos cárnicos o sus componentes, principalmente la grasa.

ANIMALES VIVOS, CANALES Y MÚSCULOS

Las aplicaciones de los US en la industria cárnica comenzaron a desarrollarse en las granjas y mataderos. Así, los ultrasonidos se han empleado para predecir el porcentaje de grasa intramuscular y tejido magro, el espesor de la grasa dorsal y de los músculos, entre otros aspectos de calidad, en animales vivos, canales y músculos de cerdo (Newcom et al., 2002; Môrlein et al., 2005; Koch et al., 2011a; Koch et al., 2011b; Lakshmanan et al., 2012; Ayuso et al., 2013), cordero (Ribeiro et al., 2008; Thériault et al., 2009; Emenheiser et al., 2010), vaca (Miles et al., 1987; Whittaker et al., 1992; Faulkner et al., 1990; Peña et al., 2014; Emenheiser et al., 2014, Scholz et al., 2015), cabrito (Delfa et al., 1999) y pollo

(Kleczek et al., 2009). Concretamente, la aplicación ultrasónica para predecir grasa y magro en canales es la más extendida en las últimas décadas. Otra aplicación diferente, se basó en la estimación del peso de la pechuga de pollo a partir de comparar las medidas ultrasónicas con la forma y tamaño de la pechuga del animal vivo (Larivière et al., 2009). De forma similar, Ayuso et al. (2013) realizaron medidas ultrasónicas en cerdos ibéricos vivos para evaluar el peso del jamón y la paleta. Como muestra el alto número de referencias bibliográficas, el desarrollo de la tecnología ultrasónica para la caracterización de parámetros de calidad en canales es extenso y ha evolucionado notablemente con el tiempo, existiendo en la actualidad equipos ultrasónicos comerciales capaces de clasificar automáticamente las canales para establecer así su valor de mercado (Llull, 2001).

CARNE Y GRASA

El avance de la tecnología ultrasónica en el sector cárnico se ha extendido a la caracterización de productos cárnicos frescos. En este sentido, los US se han empleado para caracterizar la composición de productos cárnicos de pollo (Chanamai et al., 1999). En este estudio, la velocidad ultrasónica se relacionó con el contenido en grasa y con el contenido en sólidos no grasos de muestras de pollo con composición conocida. De forma similar, Miles et al. (1977) obtuvieron una buena correlación entre la velocidad ultrasónica y el contenido en grasa en músculos, tejido adiposo y mezclas cárnicas (grasa y magro) de carne de vacuno, tanto fresca como congelada. Por otra parte, Dwyer et al. (2001) y Swatland (2001) emplearon los US para detectar cambios de textura en carne de vacuno en animales viejos y sometidos a estrés, respectivamente. Ambos autores concluyeron que las medidas ultrasónicas pueden servir como indicadores de calidad de la carne de vacuno. A pesar de que, como se ha comentado anteriormente, las técnicas de ultrasonidos se han empleado en la caracterización de productos cárnicos de pollo y vacuno; donde se han desarrollado más aplicaciones es para la caracterización de productos cárnicos de cerdo. Así, Benedito et al. (2001a) estimaron la composición (grasa, agua y

proteínas+otros) en mezclas cárnicas frescas de cerdo (tejido magro y graso) con medidas de velocidad ultrasónica a dos temperaturas diferentes. De igual forma, se ha determinado el contenido en grasa y agua a través de la medida de la velocidad ultrasónica en *Biceps femoris* fresco de cerdo ibérico (Niñoles et al., 2011).

Otra aplicación relevante de los US en carne de cerdo, consiste en utilizar la medida de velocidad a diferentes temperaturas para caracterizar y clasificar grasa subcutánea fresca y músculos *Biceps Femoris* frescos, según la raza (ibérico puro y cruces) y el sistema de alimentación (bellota o montanera y cebo con concentrados alto y bajo oleico) de los cerdos ibéricos de los que proceden (Niñoles et al., 2007; Niñoles et al., 2011). En estos estudios también se emplearon otras técnicas destructivas (análisis de ácidos grasos, Calorimetría Diferencial de Barrido (DSC) y ensayos de punción) para caracterizar y clasificar estos productos. En general, la tecnología ultrasónica mostró mayor capacidad que las técnicas destructivas empleadas para diferenciar de manera rápida los diferentes productos cárnicos. Por ello, los US demostraron ser una alternativa a los métodos convencionales para caracterizar y clasificar productos ibéricos en función de la raza y alimentación. Los US también permiten evaluar el comportamiento de cristalización de la grasa, lo que resulta de gran interés por la influencia que el estado de la misma tiene sobre la calidad de los productos cárnicos (aroma y textura), en especial en los productos ibéricos. En este sentido, Corona et al. (2014a) analizaron los patrones de cristalización de grasa ibérica fresca. De estos estudios se desprende que los US también se presentan como una alternativa a los métodos tradicionales (DSC y textura) para caracterizar las propiedades de cristalización de grasas ibéricas de manera fiable y no destructiva.

PRODUCTOS CRUDO-CURADOS

En los productos cárnicos de cerdo, el desarrollo de la tecnología US ha permitido abordar el estudio de sus productos curados. En este sentido, Simal

et al. (2003) y Llull et al. (2002) estimaron la composición (grasa, agua y proteínas+otros) y la textura de la sobrasada mediante medidas de velocidad a diferentes temperaturas. De forma similar, se ha determinado el contenido en grasa y agua a través de la medida de la velocidad ultrasónica en productos formulados crudo-curados (Corona et al., 2014b) y en lonchas de jamón curado ibérico (Corona et al., 2013a). En el último estudio, también se evaluó la viabilidad de esta tecnología no destructiva para estimar los cambios de textura que sufren las lonchas de jamón curado ibérico envasadas a vacío, como consecuencia de los procesos de conservación a baja temperatura y del tratamiento de altas presiones (Corona et al., 2013a). Al igual que en el caso de productos frescos de cerdo, mediante las medidas de US se caracterizó y clasificó grasa subcutánea curada y productos formulados crudo-curados procedente de carnes de cerdo ibérico, según el origen genético (ibérico puro y cruces) y sistema de alimentación (bellota o montanera y cebo con concentrados alto y bajo oleico) de los animales (Niñoles et al., 2008; Corona et al., 2014b). Igual que en el caso de los productos frescos de cerdo, se concluyó, que los US se presentan como una alternativa rápida, no destructiva y fiable para clasificar los diferentes productos curados en función del origen genético y de la alimentación de los animales. Por otra parte, los US también permiten evaluar el comportamiento de fusión de la grasa ibérica curada (Niñoles et al., 2010). Recientemente, Nowak et al. (2015) compararon propiedades mecánicas estimadas con medidas de US y determinadas con medidas convencionales (test compresión) en salchichas curadas, concluyendo que no existían diferencias significativas entre los dos métodos (US y método convencional) utilizados.

En los trabajos comentados anteriormente, las medidas ultrasónicas fueron llevadas a cabo por contacto. Es decir, el transductor y el alimento se encuentran en contacto directo, siendo necesario en la mayoría de los casos un material de acople (agua, grasa, etc.) que elimine el aire entre transductor-muestra, y que permita, por tanto, la transmisión de la señal ultrasónica a

través de la muestra. Una alternativa frente a las medidas por contacto es la técnica de ultrasonidos sin contacto (aire-acoplado), donde los transductores no tocan la muestra. En este campo, Corona et al. (2013b) comparó el uso de ultrasonidos con y sin contacto para caracterizar lonchas de jamón curado envasadas a vacío, obteniendo resultados similares con ambas técnicas. Hasta el momento, no existen más aplicaciones de esta técnica en el sector cárnico. Sin embargo, su aplicación tiene gran interés para reemplazar las técnicas ultrasónicas por contacto, ya que se minimiza la posibilidad de contaminación cruzada y se aumenta la velocidad de análisis.

MONITORIZACIÓN DE PROCESOS

Una de las aplicaciones ultrasónicas más prometedoras en la industria cárnica es la monitorización online de propiedades físico-químicas de alimentos cárnicos durante su procesado. Así, Sigfusson et al. (2004) monitorizaron el proceso de congelación de carne congelada de pollo y vacuno mediante US, estimando el porcentaje de carne congelada. En productos cárnicos de cerdo, Santacatalina et al. (2011) y Corona et al. (2014a) usaron los US para monitorizar el proceso de cristalización de la manteca de cerdo ibérico y grasa fresca ibérica durante su almacenamiento a baja temperatura. Como se desprende del análisis bibliográfico realizado, las aplicaciones para la monitorización de procesos cárnicos mediante ultrasonidos es aun escasa, por lo que resulta de gran interés el desarrollo de la tecnología en este campo, fundamentalmente en la monitorización de etapas complejas del procesado de productos cárnicos curados, como es el caso del salado y curado del jamón.

1.5 JUSTIFICACIÓN E INTERÉS DEL TRABAJO

Tal y como se ha comentado, el jamón curado es un producto cárnico muy apreciado por los consumidores. Sin embargo, los parámetros de calidad de los jamones de un mismo lote presentan una elevada variabilidad debido a la heterogeneidad de la materia prima y a los múltiples factores que afectan al procesado. Entre los parámetros de calidad que difieren en el producto

acabado destacan el contenido en grasa y sal. Estos dos componentes desempeñan funciones importantes en el jamón, y además, están presentes en elevadas concentraciones en el mismo, lo que tiene implicaciones para la salud de los consumidores, especialmente en la población hipertensa o con enfermedades cardiovasculares. Así, las empresas productoras de jamón están interesadas tanto en caracterizar de forma no destructiva el contenido en sal y grasa del jamón, con el objetivo de obtener productos con calidad homogénea, como en reducir el contenido en estos componentes para satisfacer los requerimientos actuales de la población, pero sin afectar a la calidad del producto final.

Las metodologías tradicionalmente empleadas para determinar el contenido en sal y grasa en los productos cárnicos son destructivas, lentas, laboriosas y presentan un alto coste en la adquisición de reactivos. Asimismo, se han desarrollado nuevas técnicas no-destructivas (Rayos-X, RMI, RMN, etc.) para la caracterización de alimentos, pero su uso presenta una serie de inconvenientes como son: el alto coste del equipo, elevadas medidas de seguridad, necesidad de operarios con elevada cualificación, etc. Todo ello implica que, actualmente, su utilización no sea viable como técnicas de control en las líneas de producción.

Una alternativa a estas técnicas no destructivas, son los ultrasonidos de señal que se caracterizan por ser una técnica rápida, versátil, fiable y económica, y cuya portabilidad y adaptación a las líneas de producción la habilita para monitorizar procesos alimentarios. Como se ha mostrado en la revisión bibliográfica, los ultrasonidos de señal presentan un gran interés para la industria alimentaria como consecuencia de su potencial para determinar de manera no destructiva la composición y textura de una amplia variedad de alimentos (productos lácteos, vegetales, panarios, etc.). Su uso como técnica de análisis no destructiva está basado en la relación existente entre los parámetros ultrasónicos (velocidad, atenuación y espectro de frecuencias) y las propiedades físico-químicas del alimento. En productos cárnicos, se puede

encontrar un número importe de estudios de investigación que emplean los ultrasonidos como técnica de análisis no destructivo, lo que se explica por la importancia económica de la carne y sus productos derivados. Además en los últimos años se ha profundizado en el desarrollo de la aplicación ultrasónica en productos cárnicos curados, lo cual ha permitido avanzar en la caracterización composicional y estructural, así como en la clasificación de estos productos. Sin embargo, no existe en la bibliografía trabajos que aborden la caracterización del jamón curado en cuanto a contenido en sal y grasa durante su procesado mediante ultrasonidos de señal. Así, teniendo en cuenta lo anteriormente comentado, parece interesante utilizar esta tecnología no destructiva para caracterizar el jamón fresco en cuanto a contenido en grasa. Esto permitiría obtener lotes de jamones frescos con contenido en grasa homogéneo, lo que daría lugar a un comportamiento similar de las piezas durante el proceso de elaboración. Asimismo, la caracterización del contenido en sal del jamón tras el salado, permitiría clasificar las piezas saladas en diferentes niveles de sal, posibilitando un comportamiento más homogéneo de las piezas en las siguientes etapas de elaboración, y por tanto, mejorando la calidad del producto final. En este sentido, aún más interesante sería caracterizar el jamón durante su salado, es decir, monitorizar on-line la ganancia de sal en el producto, con el objetivo de alcanzar un contenido de sal previamente establecido en cada pieza. Finalmente, la caracterización del contenido en grasa y sal del producto final, permitiría proporcionar información al consumidor sobre la composición del jamón curado que va a adquirir.

Por tanto, la aplicación de los ultrasonidos de señal para caracterizar el jamón curado durante diferentes etapas de su elaboración, permitiría optimizar el proceso, y por tanto, obtener jamones curados con parámetros de calidad más homogéneos. Asimismo, permitiría reducir el contenido en sal en este producto y proporcionar información al consumidor de su composición final.

2. OBJETIVOS

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El desarrollo de nuevas tecnologías fiables, rápidas y no destructivas, que permitan la caracterización del jamón curado durante su procesado, tiene un gran interés para la industria cárnica. La aplicación de estas tecnologías permitiría obtener lotes de productos curados con características más homogéneas. En este contexto, el objetivo principal de esta Tesis doctoral consistió en evaluar la viabilidad del uso de los ultrasonidos de señal para estimar diferentes parámetros composicionales del jamón curado, fundamentalmente su contenido en sal y grasa, durante diferentes etapas de su procesado. Para alcanzar este objetivo general se establecieron los siguientes objetivos parciales:

-Predecir el contenido graso en jamones frescos, y así caracterizar la materia prima del proceso, a partir de la velocidad ultrasónica. Determinar la viabilidad de la tecnología ultrasónica para clasificar los jamones en diferentes categorías en función de su contenido en grasa.

-Determinar el efecto del contenido en sal y agua en muestras cárnicas formuladas (*Biceps femoris*) con composición predefinida, sobre la medida de la velocidad ultrasónica.

-Analizar la capacidad de los ultrasonidos de señal, en modo transmisión-recepción, para estimar el contenido en sal en carne de cerdo (músculos *Biceps femoris* y *Longissimus dorsi*) salada en salmuera.

-Evaluar la viabilidad del uso de los ultrasonidos de señal, en modo transmisión-recepción, para monitorizar online el proceso de salado en seco de *Biceps femoris* y *Longissimus dorsi*. Analizar la aplicabilidad de modelos matemáticos, basados en la velocidad ultrasónica, para predecir el contenido en sal en músculos y jamones enteros.

-Determinar si los ultrasonidos, en modo pulso-eco, pueden ser empleados para monitorizar online el salado en seco de *Longissimus dorsi* y jamones a partir del tiempo de vuelo.

-Desarrollar una metodología de análisis de la señal ultrasónica, obtenida en modo pulso-eco, que sea adecuada para calcular el tiempo de vuelo durante el salado en seco de jamones.

-Clasificar *Longissimus dorsi* y jamones según la ganancia de sal, a partir de un modelo basado en el tiempo de vuelo obtenido en las medidas ultrasónicas en modo pulso-eco.

-Abordar el uso de los ultrasonidos de señal, para estimar el contenido en grasa y sal en porciones de jamón curado y así, clasificarlas según su nivel de estos componentes.

3. METODOLOGÍA

3. METODOLOGÍA

3.1 PLAN DE TRABAJO

En base a los objetivos planteados en la presente Tesis Doctoral, se desarrolló el plan de trabajo general que se muestra en la Figura 3.1. El plan experimental se estructuró en tres bloques que coinciden con los capítulos en los que ha sido dividida la sección de resultados de esta Tesis Doctoral. Los tres capítulos principales se corresponden con los ensayos de caracterización ultrasónica llevados a cabo en tres de las fases de procesado del jamón curado: producto fresco (capítulo 4.1), salado (capítulo 4.2) y curado (capítulo 4.3).

En el procesado del jamón curado, el contenido graso afecta tanto a la ganancia de sal durante la etapa de salado como a la pérdida de agua durante la etapa de secado. La correcta clasificación de los jamones frescos en lotes con un contenido en grasa homogéneo, permitiría reducir la variabilidad durante el resto del procesado, y por lo tanto, la heterogeneidad del producto final. En este contexto, el **capítulo 4.1** se centra en la aplicación de ultrasonidos de señal (US) para caracterizar el contenido en grasa en jamones frescos (Figura 3.1). Para ello, se realizaron medidas de velocidad ultrasónica (V) en modo transmisión-recepción (MTR) en jamones frescos provenientes de cerdos de diferentes razas (*Large White*, *Duroc-Large White* e *Ibérico-Duroc*) a 2ºC y se determinó el contenido en grasa en todas las piezas de jamón. En el mismo capítulo, se desarrolló un modelo simple basado en la V para clasificar los jamones en diferentes niveles de contenido graso (artículo publicado en *Meat Science*).

La ganancia de sal durante el salado de piezas de jamón de un mismo lote es variable. Las variaciones del contenido de sal tras el salado pueden dar lugar a un exceso o deficit en el contenido en sal en los jamones curados. Un exceso de sal resulta en productos que pueden ser perjudiciales para la salud de la población hipertensa o con enfermedades cardiacas. Una insuficiente cantidad de sal puede causar defectos sensoriales, así como problemas microbiológicos

en los jamones. Así, el **capítulo 4.2** de esta Tesis Doctoral engloba la caracterización del contenido en sal, mediante la medición ultrasónica antes y después del salado, de muestras de carne de cerdo con diferente grado de complejidad estructural (desde cilindros de carne triturada hasta jamones), así como la monitorización online del salado mediante ultrasonidos (Figura 3.1). Este capítulo se divide en dos partes, atendiendo al proceso de salado ensayado: salado en salmuera (apartado 4.2.1) y salado en seco (apartados 4.2.2, 4.2.3 y 4.2.4). Así, en el **apartado 4.2.1** se utilizaron medidas ultrasónicas en MTR para determinar el contenido en sal en cilindros de carne salados en salmuera (Figura 3.1). Para ello, se realizaron medidas de composición y V en cilindros de carne de músculos *Longissimus dorsi* (LD) y *Biceps femoris* (BF). Previamente, se prepararon muestras modelo con concentración de agua y sal conocidas a partir de BF triturado, con el objetivo de separar y cuantificar el efecto individual de estos parámetros sobre la V. En el caso de los cilindros de LD y BF, se salaron en salmuera a diferentes tiempos (24, 48, 96 y 168h) a 2ºC, y se midió la V antes y después del salado para estudiar la influencia conjunta de la ganancia de sal y la pérdida de agua en la medida ultrasónica. Además, se desarrolló un modelo matemático para estimar el contenido en sal en las muestras cárnicas de cerdo a partir de la variación de velocidad (ΔV), calculada como la diferencia de V antes y después del proceso de salado (artículo publicado en *Journal of Food Engineering*).

En los **apartados 4.2.2, 4.2.3 y 4.2.4** se estudió la aplicación de ultrasonidos para la monitorización del proceso de salado en seco y para la predicción del contenido en sal en músculos LD y BF y jamones salados. Para ello, se utilizaron dos modos ultrasónicos diferentes (Figura 3.1), con el MTR (apartado 4.2.2) se midió online la V cada 5 minutos en una zona de los músculos LD y BF durante su salado a diferentes tiempos (6, 12, 24, 36 y 48h) y 2ºC, para monitorizar el proceso de salado en seco. Asimismo, el MTR se utilizó para medir la V antes y después del salado de jamones *Large White* a diferentes tiempos (2, 4, 7, 11 y 16 días). En todos los productos frescos y salados se determinó

experimentalmente el contenido en sal y agua. Además, se obtuvieron modelos simples que relacionaron el parámetro ultrasónico ΔV con el contenido en sal, con el propósito de predecir la ganancia de sal en los músculos LD y BF y jamones durante su salado en seco (artículo publicado en *Food Control*).

Por otro lado, para facilitar la aplicación industrial de la tecnología ultrasónica, se empleó el modo pulso-eco (MPE) (apartados 4.2.3 y 4.2.4) para monitorizar online el proceso de salado en seco de músculos LD y jamones y para predecir la ganancia de sal en estos productos. Para ello, se midió el tiempo de vuelo (Tv) cada 5 minutos en jamón entero *Large White* (durante 4, 7, 11 y 16 días) mientras las muestras eran saladas (apartado 4.2.3). Se utilizaron tres métodos diferentes para calcular el Tv a partir de la señal (método del umbral de energía, método de la correlación cruzada y método del espectro de fase), seleccionándose el que permitía un cálculo más preciso del Tv (correlación cruzada) (apartado 4.2.3). Asimismo, se midió el Tv cada hora en 4 zonas del músculo LD (durante 6, 12, 24, 48 y 72h) y del jamón entero *Large White* (durante 4, 7, 10, 11, 12, 14, 15, 16, 20 y 30 días), mientras las muestras eran saladas (apartado 4.2.4). En este apartado se presentaron modelos basados en parámetros ultrasónicos (variación del tiempo de vuelo (ΔTv) y tiempo de vuelo inicial (Tv_0)) y operaciones (peso de la muestra y tiempo de salado) para clasificar según diferentes niveles de contenido en sal los músculos LD y los jamones tras el salado (apartado 4.2.4) (artículo enviado *Meat Science*).

Finalmente, la industria cárnica tiene interés por la caracterización no destructiva del contenido en grasa y sal de los jamones curados antes de su envasado. Esto permitiría definir en el etiquetado la cantidad aproximada de estos componentes (grasa y sal), y por tanto, proporcionar a los consumidores información nutricional del producto final. Por ello, el último capítulo (**capítulo 4.3**) abarca la caracterización composicional del jamón curado mediante medidas de US (Figura 3.1). En este trabajo se midió la V con el MTR en porciones de jamón curado de distintas razas (*Large White*, *Duroc* e *Ibérico*) a dos temperaturas diferentes (2 y 15ºC). El contenido en sal, agua y grasa

también fue determinado en todas las porciones. Además, se desarrollaron dos modelos matemáticos empíricos, uno que relacionó la V medida a 15ºC (V_{15}) con el contenido en sal y el otro que relacionó la variación de velocidad entre 2 y 15ºC ($\Delta V=V_2-V_{15}$) con el contenido en grasa. Asimismo, se aplicó un modelo semi-empírico para estimar el contenido en grasa, agua y proteínas+otros a partir de la datos de V a 2 y 15ºC. Estos modelos se utilizaron para clasificar los jamones curados según su contenido en grasa y sal (artículo publicado en el *Journal of Food Engineering*).

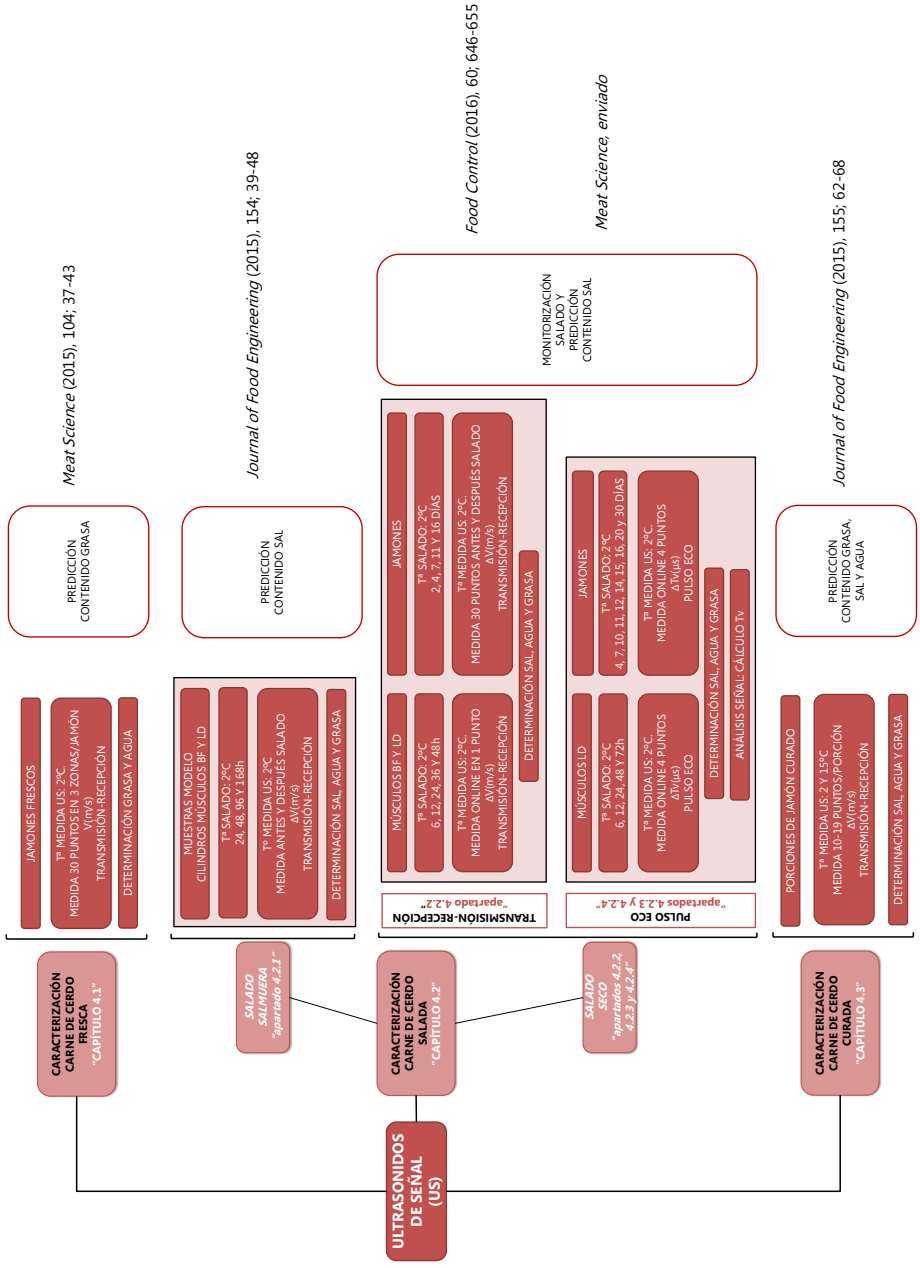


Figura 3.1 Plan de trabajo de la Tesis Doctoral. Dónde: US son los ultrasonidos de señal, Tª la temperatura (ºC), V la velocidad ultrasónica (m/s), ΔV la variación de velocidad ultrasónica (m/s) y ΔTv la variación de tiempo de vuelo (μs).

3.2 MATERIA PRIMA

Para la caracterización mediante ultrasonidos de señal de la composición del jamón curado durante su procesado, se analizaron tres tipos de materia prima: fresca (capítulo 4.1), salada (capítulo 4.2) y curada (capítulo 4.3) (Figura 3.1). A continuación, se indica de forma general el tipo y número de muestras empleado en cada capítulo, así como, la preparación de aquellas muestras en las que fue necesaria su manipulación. En cada uno de los capítulos de resultados, en su sección de materiales y métodos, se describirá con más detalle las características y preparación de las muestras empleadas.

En el trabajo del **capítulo 4.1** se utilizaron 80 jamones frescos de diferentes razas: 31 *Large White* (LW), 9 *Duroc-Large White* y 40 *Ibérico-Duroc*.

En las experiencias llevadas a cabo en el **apartado 4.2.1** se emplearon dos tipos de muestras diferentes. Por una parte, se obtuvieron muestras cilíndricas (3.7cm de diámetro y 6±1cm de longitud) de 12 LD y 16 músculos BF. Por otra parte, se formularon muestras modelo con contenido en agua y sal conocido a partir de 2 músculos BF. En el trabajo del **apartado 4.2.2**, se emplearon 15 músculos BF (1.2±0.1kg y 19.1±2.4cm de largo) y LD (1.0±0.1kg y 20.0±0.2cm de largo), manteniendo el ancho y el espesor original del músculo. Asimismo, en este trabajo se utilizaron 30 jamones LW. De forma similar, en las experiencias del **apartados 4.2.3** se utilizaron 4 jamones LW y en las del **apartado 4.2.4** se emplearon 20 músculos LD (1.0±0.1kg y 20.0±0.5cm de largo) y 10 jamones LW.

Finalmente, en los trabajos llevados a cabo en el **capítulo 4.3**, se emplearon 114 porciones de jamón curado de LW-*Landrace* y 162 de *Ibérico*, obtenidas a razón de 6 porciones/jamón.

3.3 MEDIDAS DE ULTRASONIDOS DE SEÑAL

En el presente apartado se describen los elementos de los equipos de ultrasonidos y los modos de medida empleados para la caracterización

composicional de carne de cerdo fresca, salada y curada, mediante ultrasonidos de señal.

3.3.1 ELEMENTOS DE LOS SISTEMAS DE MEDIDA DE ULTRASONIDOS DE SEÑAL

Los elementos que constituyeron los sistemas de ultrasonidos para la medida de las propiedades acústicas en muestras cárnicas fueron: transductores, generador-receptor, sistema digitalizador de la señal, dispositivo de medida del espesor y multiplexador. A continuación, se detallan las características de cada uno de los elementos empleados.

TRANSDUCTORES

Las medidas ultrasónicas se realizaron con varios tipos de transductores piezoeléctricos de banda estrecha:

- **T1.** Transductor de 1MHz y 0.75" de diámetro de cristal (modelo A314S-SU, Panametrics, Waltham, USA).
- **T2.** Transductor de 1 MHz y 0.5" de diámetro de cristal (modelo A303S, Panametrics, Waltham, USA).
- **T3.** Transductor de 1 MHz y 0.5" de diámetro de cristal (modelo A103S-RM, Panametrics, Waltham, USA).

GENERADOR-RECEPTOR

Se emplearon dos tipos de generador-receptor:

- **GR1.** (modelo 5058PR, Panametrics, Waltham, USA).
- **GR2.** (modelo 5077PR, Panametrics, Waltham, USA).

DISPOSITIVO DE DIGITALIZACIÓN DE LA SEÑAL

Se utilizaron dos sistemas digitalizadores:

- **O-PC.** Un osciloscopio-PC (modelo TDS5034, Tektronix, Inc. Bearverton, USA).
- **T-PC.** Una tarjeta digitalizadora (PCI-5112, National Instruments, Austin, USA) insertada en un ordenador portable.

DISPOSITIVO DE MEDIDA DEL ESPESOR

Se emplearon dos sistemas de medida para determinar el espesor de las muestras cárnicas:

- **ME1.** Calibre de altura digital comercial (192-633 Serie, Mitutoyo, Japan).
- **ME2.** Calibre de altura digital diseñado y construido específicamente por el Grupo ASPA de la UPV.

El dispositivo de medida de espesor diseñado por el Grupo ASPA (**ME2**) garantizaba que los transductores estuvieran paralelos y que la presión ejercida sobre la muestra fuese constante. Además, estaba diseñado para una lectura máxima de 27cm con una precisión de ±0.01mm. El sistema constaba de una plataforma para disponer la muestra, donde se fijó el transductor receptor (Figura 3.2). El otro transductor, el transductor emisor, que estaba perfectamente alineado y enfrentado con el transductor receptor, se colocó en un brazo deslizante perpendicular a la plataforma. El brazo tenía movimiento de subida y bajada gracias a que se encontraba unido a un carro posicionador accionado por un tornillo sin fin y un motor paso a paso (motor unipolar paso a paso de 4 fases, RS 440-458, Taiwan). Al carro estaba acoplada una célula de carga de aluminio de 40kg de fuerza máxima (1040-41, Tedea Huntleigh, Canoga Park, USA). Un controlador de presión (K3NV-LC1C, Omron, Japan) unido a la célula de carga permitía fijar la presión que los transductores ejercían sobre la muestra. El sistema de medida de espesor estaba unido a un PLC (EC-20HR, Hitachi, Japan) que se encargaba de detener el motor paso a paso cuando el controlador de presión alcanzaba el valor de consigna. Por otro lado, el espesor de la muestra se determinaba a partir de la lectura de posición

del carro deslizante mediante un encoder (Baumer electric, BHK 06.24K500-B6-5, Suiza) conectado a un lector de posición (K3NC-PB1A, Omron, Japan). La lectura de la posición del carro o espesor de la muestra, se enviaba a un PC a través de una interfaz RS232 (Figura 3.2). El lector de posición, el controlador de presión y el PLC se encontraban en el interior de una carcasa con botones frontales para poder accionar el sistema, en cuanto a subida y bajada del carro deslizante, encendido y apagado y valor de presión de consigna ejercida por los transductores sobre la muestra.

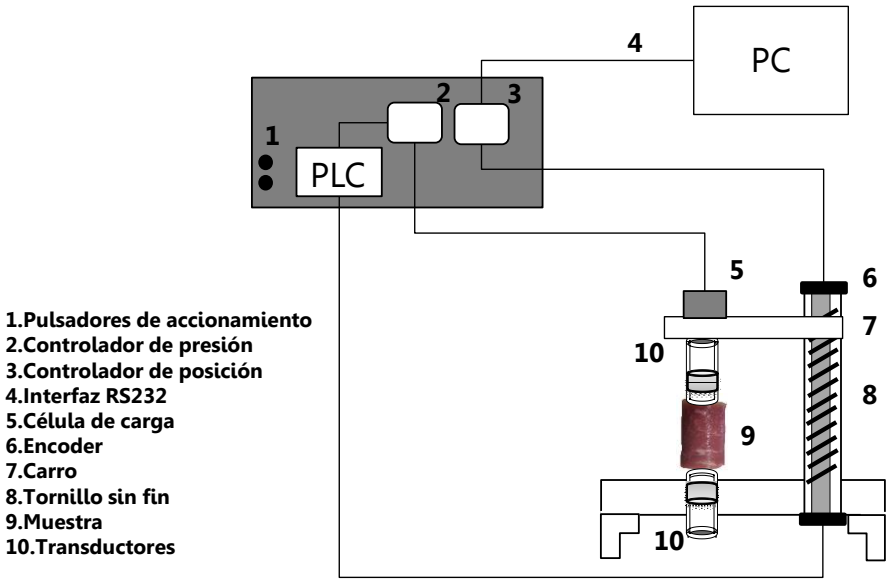


Figura 3.2 Dispositivo de medida de espesor de la muestra (**ME2**) desarrollado por el Grupo ASPA.

SISTEMA MULTIPLEXADOR DE GENERACIÓN Y RECEPCIÓN

Una de las limitaciones asociadas a las medidas ultrasónicas es la reducida área de estudio de la muestra, lo que viene definido por la superficie del transductor empleado. En este sentido, se desarrolló un sistema de generación/recepción que permita multiplexar la señal a varios transductores,

pudiendo así ampliar el área de estudio y realizar medidas simultáneas en varios puntos de las muestras cárnicas.

- **MPX.** Equipo multiplexador de generación-recepción diseñado y construido por el Grupo ASPA de la UPV.

Este dispositivo (**MPX**) estaba constituido por una interfaz de 4 salidas optoacopladas a relé (CEBEC T1 V3, Electronic circuits, Spain) y una tarjeta de entradas/salidas (E/S) digitales (NI 6501, National Instruments, Austin, USA) (Figura 3.3). El sistema se accionaba de la siguiente manera: el PC enviaba una instrucción a la tarjeta de E/S digitales a través de una conexión USB. La tarjeta de E/S se encargaba de accionar de manera secuencial cada uno de los relés para permitir el paso de la señal del generador por cada uno de los cuatro transductores (Figura 3.3). Para ello, la tarjeta E/S utilizaba una codificación de 0 y 1, el 0 significaba que el puerto del relé estaba abierto impidiendo el paso de la señal y el 1 significaba que el puerto estaba cerrado permitiendo el paso de la señal. Dado que la tensión de la señal eléctrica que le llegaba de la tarjeta de E/S digitales no era suficiente para accionar los relés, se incorporó un convertidor de señal de tensión de 5 a 24V. Una vez la señal llegaba al transductor, ésta traspasaba la muestra y retornaba al mismo transductor, que enviaba la señal al generador-receptor y éste posteriormente al equipo de digitalización. Para evitar interferencias y ruidos eléctricos que pudieran interferir en la medida ultrasónica, todos los componentes de la interfaz estaban apantallados y el sistema de multiplexado estaba encerrado dentro una jaula de Faraday. Asimismo, esta jaula de Faraday junto con todos los componentes del sistema de multiplexado se encontraban en el interior de una carcasa de plástico con conectores para poder ensamblar el cableado de los transductores, el generador-receptor y el PC.

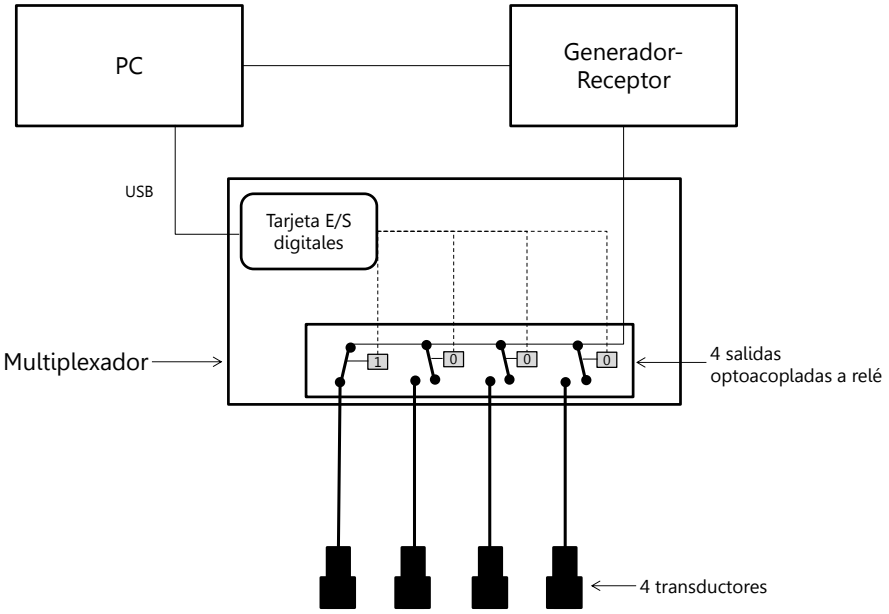


Figura 3.3 Equipo multiplexador de generación-recepción de la señal ultrasónica para 4 transductores desarrollado por el Grupo ASPA.

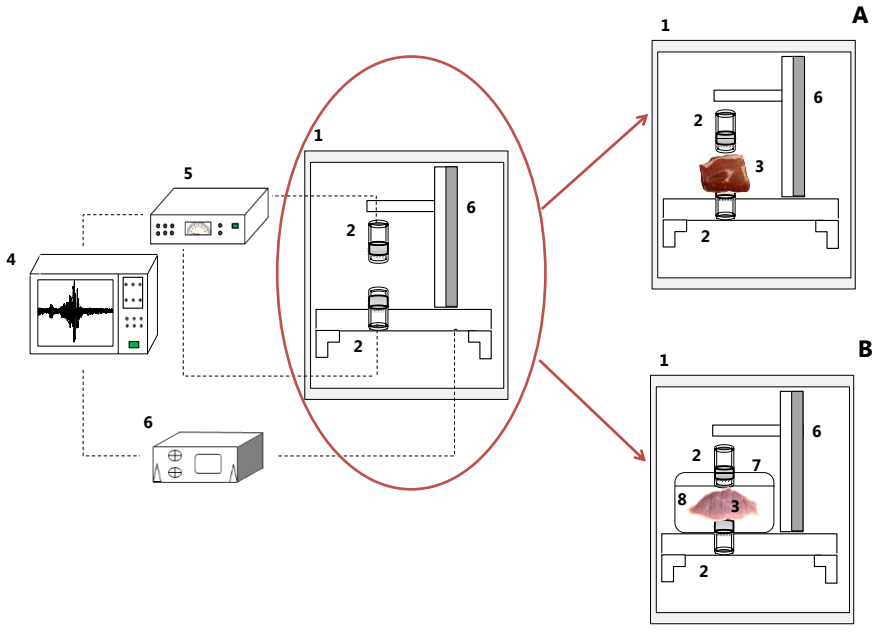
3.3.2 SISTEMAS Y PROCEDIMIENTOS DE MEDIDA ULTRASÓNICOS EMPLEADOS EN LOS DIFERENTES CAPÍTULOS

MEDIDAS EN MODO TRANSMISIÓN-RECEPCIÓN (MTR)

Para llevar a cabo las medidas en modo transmisión-recepción (MTR) fue necesario el uso de un dispositivo de medida de espesor y de un sistema de medida de ultrasonidos constituido por: un par de transductores (emisor y receptor), un generador-receptor y un dispositivo digitalizador de la señal (Figura 3.4).

El procedimiento de medida comprendía los siguientes pasos: La muestra se colocaba entre la pareja de transductores, los cuales se ajustaban al espesor de la misma con el dispositivo de medida del espesor. El generador-receptor emitía una señal eléctrica de excitación con una amplitud y duración específicas que llegaba al transductor emisor. Al mismo tiempo, el generador

enviaba otra señal al sistema digitalizador (señal del trigger o disparo) para indicar el momento exacto en que la señal de excitación había sido enviada a la muestra. El transductor emisor convertía la señal eléctrica de excitación en una onda ultrasónica mediante la vibración del cristal piezoeléctrico. La onda ultrasónica atravesaba la muestra y llegaba al transductor receptor, donde se convertía en señal eléctrica mediante el proceso inverso al producido en la cerámica piezoeléctrica del receptor. Esta señal se recibía en el generador-receptor donde se filtraba y amplificaba, para posteriormente ser enviada al sistema digitalizador que estaba insertado en el PC (Figura 3.4). La señal en formato digital se adquiría mediante una aplicación informática específica de Visual Basic (2010, Microsoft Corporation, USA) que calculaba los parámetros ultrasónicos a partir de la señal y el espesor de la muestra, y además almacenaba los datos.



1. Cámara de refrigeración 2. Transductores 3. Muestra 4. Sistema digitalizador de la señal-PC 5. Generador-Receptor 6. Dispositivo de medida del espesor 7. Contenedor 8. Sal

Figura 3.4 Montaje experimental para la medida ultrasónica con el modo transmisión-recepción (MTR) en los trabajos de predicción del contenido en grasa en jamones

frescos, predicción del contenido en sal en cilindros de LD y BF y en jamones, y predicción de la composición en porciones de jamón curado (**A**) y en el trabajo de la monitorización del salado de músculos LD-BF (**B**).

Las medidas ultrasónicas en MTR se utilizaron para predecir el contenido en grasa en jamones frescos (**capítulo 4.1**). Para ello, se llevaron a cabo 30 medidas en tres zonas del jamón (20 en la maza, 5 en la babilla y punta) a 2ºC (Figura 3.4A). Los elementos del equipo de ultrasonidos utilizados fueron: el dispositivo de medida de espesor (**ME1**), un par de transductores (emisor y receptor) **T1**, el generador-receptor **GR1** y el dispositivo digitalizador de la señal **O-PC**. Los parámetros de configuración del generador-receptor utilizados fueron: frecuencia de repetición 100Hz, factor Damping 100Ω, altura del pulso de 200V, atenuación de 0dB, Vernier de 0dB, ganancia de 40dB, fase normal, modo transmisión-recepción y filtro de 0.3MHz (HP) y OUT (LP). Los parámetros de digitalización utilizados fueron: en la escala del eje vertical se utilizó un rango de ±0.8V y en el eje horizontal una velocidad de digitalización o adquisición (Va) de 125 Mpuntos/s y un total de 25000 ptos adquiridos. Además, se estableció que se adquirieran un 10% de los puntos (pre-trigger=2500 puntos) antes del disparo o trigger y el 90% restante (22500 puntos) después. Dada la sensibilidad de las medidas ultrasónicas a la temperatura, los jamones se mantuvieron antes de la medida 24 h a 2ºC y las medidas se realizaron dentro de una cámara de refrigeración con temperatura controlada (Figura 3.4A).

El MTR también se utilizó para predecir el contenido en sal en muestras cilíndricas de carne (**apartado 4.2.1**). Para ello, las medidas se realizaron en muestras modelo colocadas en el interior de un cilindro metálico y en cilindros de carne antes y después de su salado en salmuera a 2ºC. Asimismo, el MTR también se empleó para la predicción de la composición (agua, sal y grasa) de porciones de jamón curado (**capítulo 4.3**). Para ello, se tomaron medidas en 10-19 puntos de la superficie de las porciones a dos temperaturas (2 y 15ºC). En estos dos casos (apartado 4.2.1 y capítulo 4.3), se emplearon los mismos

elementos que en el capítulo 4.1 (**T1**, **GR1** y **O-PC**), excepto el sistema de medida de espesor que fue el **ME2** (Figura 3.4A). Pese a que el ME1 permitía una mayor velocidad de medida, se decidió utilizar el ME2 porque permitía controlar la presión ejercida sobre la muestra con más exactitud, asimismo, estaba adaptado para trabajar en el interior de cámaras de refrigeración de pequeño tamaño donde se producen fenómenos de condensación. En las medidas en cilindros de carne y muestras modelo (apartado 4.2.1) el único parámetro de digitalización diferente a los empleados en el capítulo 4.1 fue la velocidad de adquisición cuyo valor fue de 250Mmuestras/s. Asimismo, en las medidas en porciones de jamón curado (capítulo 4.3), el único parámetro de configuración del generador-receptor que cambio respecto a los empleados en el capítulo 4.1 fue la altura del pulso de excitación que fue de 400V, para alcanzar un mayor poder de penetración en las muestras curadas, donde la atenuación a los ultrasonidos es más elevada debido a su mayor espesor.

Finalmente, el MTR se empleó en las experiencias del **apartado 4.2.2** para monitorizar el salado en seco en los músculos LD y BF y determinar su ganancia de sal. Para ello, se realizaron medidas online cada 5 minutos en un punto de las piezas de LD y BF durante su salado a 2ºC (Figura 3.4B). Asimismo, el MTR también se empleó para predecir el contenido en sal en jamones. En este caso, se realizaron 30 medidas en tres zonas del jamón (20 en la maza, 5 en la babilla y punta) antes y después de su salado también a 2ºC (Figura 3.4A). Los elementos del equipo utilizados en este caso fueron: la pareja de transductores **T1** en jamones y **T2** en músculos LD y BF, siendo el resto de elementos iguales que los utilizados en el apartado 4.2.1 y capítulo 4.3 (**GR1**, **O-PC**, **ME2**). El único parámetro de digitalización que se modificó fue la velocidad de adquisición cuyo valor fue de 250Mmuestras/s, el resto de los parámetros fueron los mismos que en el capítulo 4.1.

El parámetro ultrasónico estudiado en el MTR fue la **velocidad de los ultrasonidos (V),** que se obtuvo a partir del cociente entre el espacio recorrido por la onda (dado por el espesor de la muestra medido con el dispositivo de

medida del espesor) y el tiempo que tarda la onda en recorrerlo. El tiempo que necesita la onda para atravesar la muestra se calcula a partir de la diferencia entre el tiempo de vuelo (Tv) y el tiempo de retardo (Tr). El Tr hace referencia al retraso ocasionado por los elementos que forman el sistema de medida (cables, transductores, etc.) y se calcula determinando el tiempo de vuelo en una serie de cilindros de metacrilato de 40cm de diámetro y diferentes alturas (1 a 6cm). Representando gráficamente el tiempo de vuelo frente a la altura de los cilindros, el valor del Tr viene dado por la ordenada en el origen de la relación lineal obtenida. El Tv se define como el intervalo de tiempo transcurrido desde que el generador envía la señal de excitación hasta que el receptor detecta la llegada de la onda, y se calcula a partir de la señal digitalizada. Existen diferentes métodos para calcular el Tv a partir de la señal digitalizada. En la presente Tesis doctoral se empleó el método del umbral de energía para calcular el Tv en las señales ultrasónicas obtenidas en MTR. En el procedimiento de cálculo del Tv mediante el método del umbral de energía (Figuras 3.5A y B), se establece un umbral de energía superior (fijado en 0.1 V) y se busca el primer punto de la señal digitalizada (punto TA, Figura 3.5B) que supere ese umbral. Una vez localizado, se retrocede hasta encontrar el primer punto que quede por debajo de un umbral inferior fijado en 0.03 V, siendo ese punto el de llegada de la señal (TB) (Figura 3.5B). A partir del valor de ese punto se calcula el Tv según la ecuación 3.1.

$$Tv = \frac{TB - 2500}{Va} \qquad \text{(Ec. 3.1)}$$

Donde: TB es el punto de llegada de la señal, 2500 es el número de puntos antes del trigger y Va es la velocidad de digitalización o adquisición (puntos/s).

En la aplicación desarrollada específicamente para el cálculo del Tv (Visual Basic 2010, Microsoft Corporation, USA) se estableció un salto de 3250 puntos para descartar el pre-trigger y el ruido correspondiente a la excitación del

transductor. A partir de esos 3250 puntos se procedió a la búsqueda del punto TB y se calculó el Tv con la Ec. 3.1, tal y como se ha explicado anteriormente.

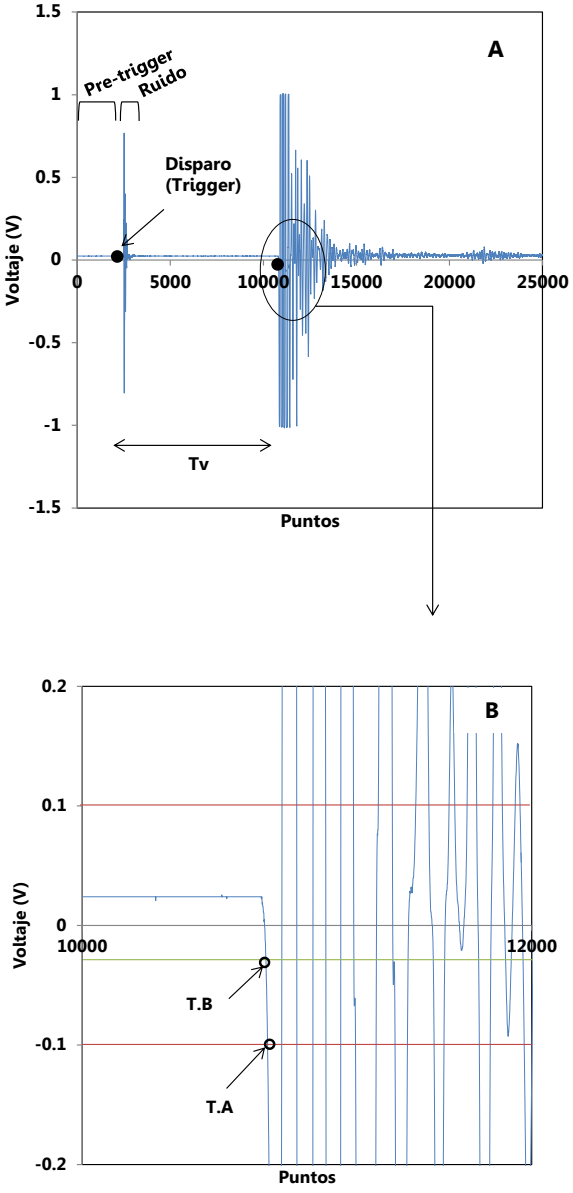
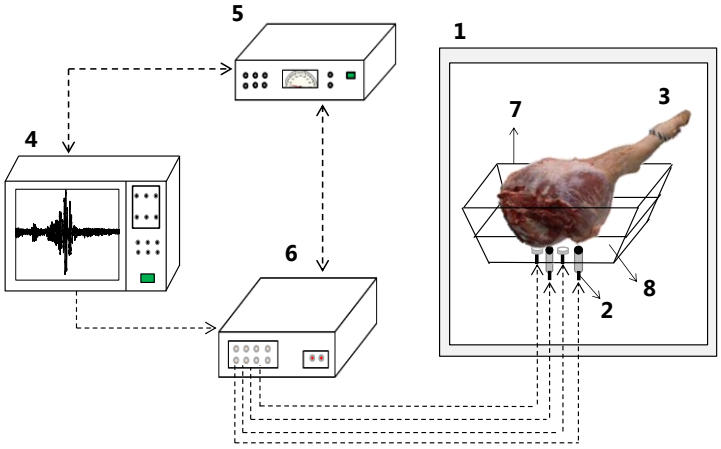


Figura 3.5 Procedimiento de cálculo del tiempo de vuelo (Tv) con el método del umbral de energía en señales ultrasónicas en MTR.

MEDIDAS EN MODO PULSO-ECO (MPE)

El sistema para realizar las medidas en modo pulso-eco (MPE) constaba de una base con cuatro transductores, un multiplexador, un generador-receptor y un dispositivo digitalizador de la señal (Figura 3.6).

El procedimiento para realizar la medida comprendía los siguientes pasos: el ordenador enviaba una instrucción al multiplexador para alimentar, con la señal de excitación del generador-receptor, cada uno de los cuatro transductores de forma secuencial (Figura 3.6). Al mismo tiempo, para la excitación de cada transductor, el generador mandaba otra señal al sistema digitalizador (señal del trigger o disparo) para indicar el momento exacto de excitación de los transductores. Los transductores, que trabajaban en MPE, convertían la señal eléctrica de excitación en una onda ultrasónica que se propagaba a través de la muestra y que al entrar en contacto con la interfase muestra/sal, se reflejaba, atravesaba de nuevo la muestra y volvía al transductor, donde era convertida de nuevo en una señal eléctrica. Ésta retornaba al multiplexador, que la mandaba al generador-receptor donde era filtrada y amplificada. Por último, la señal era enviada a una tarjeta digitalizadora integrada en un PC, que se encargaba de su digitalización. La señal en formato digital se adquiría mediante una aplicación informática específica de Visual Basic (2010, Microsoft Corporation, USA), que almacenaba los datos.



1. Cámara de refrigeración 2. Transductores 3. Muestra 4. Sistema digitalizador de la señal 5. Generador-Receptor 6. Multiplexador 7. Contenedor 8. Sal

Figura 3.6 Montaje experimental para la medida ultrasónica con el modo pulso-eco (MPE) en las experiencias de monitorización del salado de músculos LD y jamones.

Las medidas ultrasónicas en MPE se llevaron a cabo para caracterizar el contenido en sal y monitorizar el salado en seco de músculos LD y jamones (**apartados 4.2.3 y 4.2.4**). Para ello, se realizaron medidas cada 5 minutos en 4 puntos del jamón (apartado 4.2.3) y cada hora en 4 puntos del músculo LD y del jamón (apartado 4.2.4) durante su salado en seco a 2ºC. En este caso, los elementos del equipo de ultrasonidos utilizados fueron una base con 4 transductores (dos de tipo **T2** y otros dos de tipo **T3**), el generador-receptor (**GR1** para el músculo LD y **GR2** para el jamón), el dispositivo digitalizador de la señal (**T-PC**) y el multiplexador (**MPX**) (Figura 3.6). Los parámetros de digitalización utilizados fueron: en la escala del eje vertical se utilizó un rango de ±2V y en el eje horizontal una velocidad de digitalización o adquisición (Va) de 100 Mpuntos/s y un total de 25000 ptos adquiridos. Los puntos adquiridos antes y después del disparo fueron los mismos que en MTR.

El parámetro ultrasónico estudiado en el MPE fue el **tiempo de vuelo (Tv)**. Para calcularlo se utilizaron dos métodos diferentes: el método del umbral de energía y el método de la correlación cruzada.

El procedimiento del cálculo del Tv mediante el método del umbral de energía fue el mismo que el explicado anteriormente para el MTR, con la diferencia de que en el caso de señales obtenidas en MPE, la señal ultrasónica atraviesa dos veces la muestra antes de ser detectada por el receptor, por lo que el valor de Tv de la Ec. 3.1 se ha de dividir por 2. Asimismo, en la aplicación informática LABVIEW™ 2015 (National Instruments, Austin, TX, USA) se establecieron unos parámetros de búsqueda diferentes para descartar el ruido. Como ejemplo, en la Figura 3.7 se muestra la señal ultrasónica obtenida en MPE en un jamón durante su salado. Como se observa, se han de descartar los primeros 2500 puntos correspondientes al pre-trigger, posteriormente se han de descartar 12500 puntos que se corresponden con el ruido (Figura 3.7). Este ruido engloba el primer tren de ondas después del trigger, correspondiente a la vibración del transductor, el segundo tren de ondas corresponde a la reflexión entre la grasa subcutánea y el magro de jamón, y el resto de ruido debido a reflexiones de la onda antes de que ésta se refleje en la cara opuesta del jamón y regrese al transductor emisor (Figura 3.7).

Además, al final de cada tiempo de salado se obtuvo la variación del tiempo de vuelo (ΔTv) como la diferencia entre el Tv inicial y final.

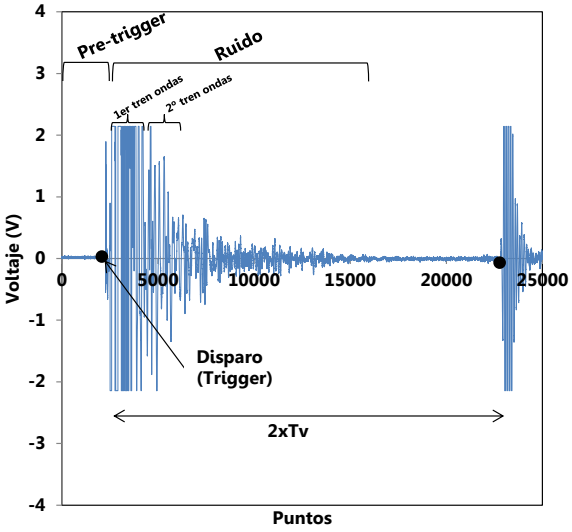


Figura 3.7 Procedimiento de cálculo del tiempo de vuelo (Tv) con el método del umbral de energía en señales ultrasónicas en MPE.

La otra metodología utilizada para el cálculo del Tv fue la de la correlación cruzada, que se utiliza en el campo del tratamiento de señales para identificar similitudes entre señales moduladas en el tiempo. Este método utilizaba dos señales, una de ellas como referencia y realizaba el producto escalar entre las dos, desplazando la segunda señal respecto a la primera, por lo que si ambas tenían el mismo número de puntos (p.e. 10000) el vector de la correlación cruzada tendría el doble de esos puntos (20000). La técnica permitía calcular el desplazamiento en el espacio temporal de una señal respecto a la de referencia, por lo que el parámetro realmente calculado fue la ΔTv. Para el cálculo de la ΔTv con el método de la correlación cruzada, se utilizó una aplicación informática desarrollada en el lenguaje de programación de LABVIEW™ 2015 (National Instruments, Austin, USA). En la Figura 3.8, se muestra la correlación cruzada de una misma señal (real proveniente de una experiencia de salado de jamón), utilizada como referencia y como señal a desplazar. La señal original contenía 25000 puntos, y al igual que en el método del umbral se eliminaron 2500 puntos para descartar la zona del pre-trigger y

12500 puntos para descartar la zona del ruido, por lo que las señales empleadas constaban de 10000 puntos. El gráfico de la correlación cruzada (Figura 3.8, derecha) muestra un valor máximo (PM) en la posición 10000 que coincide con el momento de la superposición exacta de las dos señales.

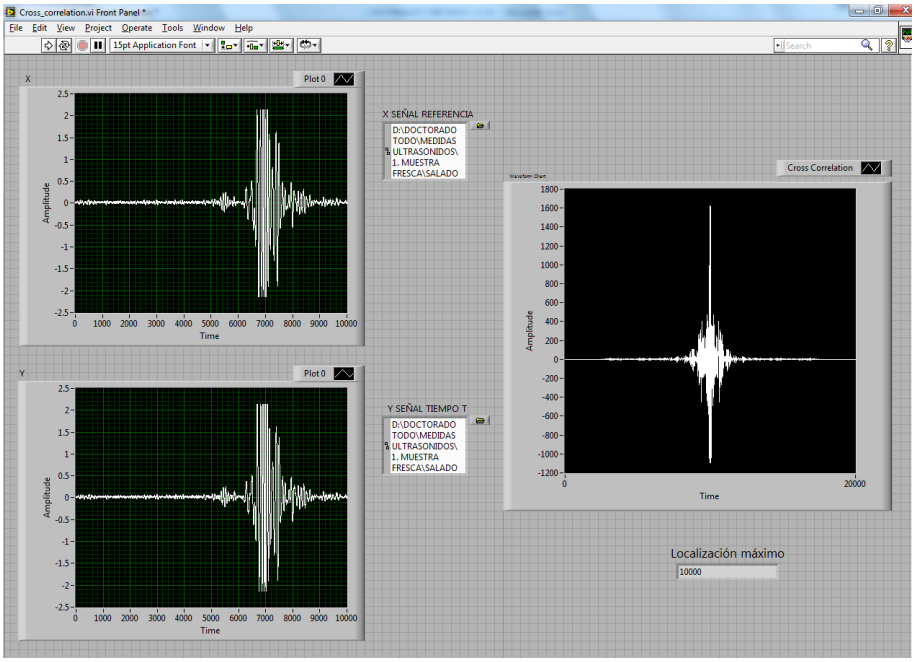


Figura 3.8 Resultados de la correlación cruzada para una misma señal ultrasónica obtenida con el MPE.

En el caso de aplicar el método a dos señales desplazadas en el tiempo, si la localización del máximo (PM) es en un punto inferior a 10000 quiere decir que el Tv en la señal que se desplaza es menor que en la referencia. Por el contrario, si PM se sitúa en un punto mayor de 10000 indicaría que el Tv en la señal de referencia es menor. Así, mediante la Ec. 3.2 se puede calcular la variación del Tv entre dos señales.

$$\Delta Tv = \frac{PM - LS}{2 \times Va} \qquad \text{(Ec. 3.2)}$$

Donde: PM es el punto del vector de la correlación cruzada donde se encuentra el máximo; LS es la longitud de la señal (tras eliminar pre-trigger y ruido, 10000 en el caso de la Figura 3.8) y Va es la velocidad de digitalización o adquisición (puntos/s).

3.4 MEDIDAS DE RAYOS-X

La presente Tesis Doctoral se ha desarrollado bajo el marco de los proyectos de investigación "Optimización y control de la calidad tecnológica, nutricional y organoléptica de jamones serranos e ibéricos (RTA 2010-00029-C04-01/02)" y "Caracterización y detección objetiva de defectos de textura en jamón curado mediante tecnologías no destructivas. Desarrollo y evaluación de medidas correctoras (RTA 2013-00030-C03-02)". Los objetivos de estos proyectos abarcaron la evaluación de metodologías no destructivas tanto para optimizar los procesos de elaboración de jamón serrano e ibérico como para caracterizar la composición y la presencia de defectos de calidad (como la pastosidad). Dichos proyectos se llevaron a cabo de forma coordinada entre varios grupos de investigación (IRTA, UNEX, INIA, CTC y UPV). Entre ellos cabe destacar el grupo de "Investigación y Tecnologías Agroalimentarias (IRTA)", con el que se trabajó de forma conjunta en dos trabajos reflejados en la sección de resultados de la presente Tesis Doctoral. Así, el primer trabajo conjunto pretendía analizar la viabilidad del uso de rayos-X y ultrasonidos de señal para predecir, tanto de forma individual como conjunta, el contenido en grasa de jamones frescos, así como, determinar la fiabilidad de las dos tecnologías para clasificar los jamones en categorías preestablecidas según su contenido en grasa (**capítulo 4.1** de resultados). En el segundo trabajo, se evaluó la capacidad de las dos tecnologías tanto para predecir el contenido en grasa y sal en porciones de jamones curados (**capítulo 4.3** de resultados) como para clasificar dichas porciones en categorías preestablecidas según los valores de grasa y sal predichos. El equipamiento de rayos-X y la metodología utilizada en cada uno de los trabajos, se describe en los capítulos correspondientes de la sección de resultados de la presente Tesis Doctoral.

3.5 MÉTODOS DE ANÁLISIS QUÍMICO

El contenido en agua, grasa y sal se determinó en las diferentes muestras cárnicas estudiadas mediante ultrasonidos en la presente Tesis Doctoral. A continuación, se describe de forma general el procedimiento llevado a cabo para su determinación.

DETERMINACIÓN DEL CONTENIDO EN AGUA

El contenido en agua de las muestras cárnicas se determinó secando 3g de muestra en una estufa a 102ºC hasta alcanzar peso contante, siguiendo el método 950.46 (AOAC, 1997).

DETERMINACIÓN DEL CONTENIDO EN SAL

Para determinar el contenido en sal se homogeneizaron las muestras (1g para muestras frescas y 0.5g para muestras saladas) en 100mL de agua destilada mediante un ULTRATURRAX (T25, IKA Labortechnik, Germany) a 9500rpm durante 5 min. El extracto obtenido se filtró y se analizó una muestra de 500µL mediante un equipo analizador de cloruros (926 Mark 2 Chloride Analyzer, Sherwood, Reino Unido).

DETERMINACIÓN DEL CONTENIDO EN GRASA

Para la determinación de grasa se utilizó el método AOAC 991.36 (AOAC, 1997), que consiste en la extracción del contenido graso total de la muestra cárnica mediante el método de Shoxlet con un disolvente orgánico (éter de petróleo).

Los resultados finales se expresaron como porcentaje de agua, sal y grasa en la muestra en base húmeda (% b.h.). Todas las determinaciones se realizaron por triplicado.

4. RESULTS AND DISCUSSION

CHAPTER 4.1

FRESH PORK MEAT CHARACTERIZATION

Meat Science. 2015, 104, 37-43.

Non-destructive determination of fat content in green hams using ultrasound and X-rays

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Abstract

This work addresses the use of ultrasound (US) and medical dual X-rays absorptiometry methods to predict the fat content in green pork hams. Ultrasonic velocity (υ) and X-ray absorption were measured in 78 green hams. An increase in the fat content involved an increase in υ and a decrease in the X-ray attenuation measured at 2ºC. Models developed to predict the fat content from the ultrasonic velocity or X-ray parameters provided errors of 2.97% and 4.65%, respectively. The combination of both US and X-ray technologies did not improve prediction accuracy. These models allowed green hams to be classified into three levels of fatness, with 88.5% and 65.4% of the hams correctly classified when using models based on ultrasonic and X-ray parameters, respectively. Therefore, US and X-rays emerge as useful quality control technologies with which to estimate the fat content in green pork hams.

Keywords: *Non-destructive analysis, Green ham, Meat products, Ultrasound, X-rays.*

1. INTRODUCTION

The total fat content of green (raw) hams is a key issue, since it affects the processing of both cooked and dry-cured hams. In cooked hams, intramuscular fat can affect the binding strength and consumer acceptability. In dry-cured hams, the fat content has a great influence on the salt uptake during the salting process (Cierach, & Modzelewska-Kapitula, 2011) and on the weight losses during drying (Čandek-Potokar, & Škrlep, 2012; Garcia-Gil et al., 2012). The development of online non-invasive technologies as a means of predicting the fat content in green hams is of special interest for the meat industry, since they would make it possible to classify the product into different fat categories which would allow the elaboration processes to be optimized. These techniques need to be robust and cost-effective for being used in the industry.

New techniques are being tested with which to carry out the non-destructive determination of the composition of the meat products. For live animals and carcass inspection, reliable ultrasonic devices are available for the measurement of lean and fat content (Miles, Fisher, Fursey, & Page, 1987; Miles, Fursey, Page, & Fisher, 1990), as well as the depth of subcutaneous fat, in particular sites of the animal. Miles and Fursey (1977) related the ultrasonic velocity to the fat content of meat muscles, comminuted tissue, meat mixtures and dehydrated muscles. In this regard, Koch et al. (2011a) estimated the intramuscular fat content of porcine *Longissimus dorsi* muscle by using ultrasound velocity and attenuation. Corona, García-Pérez, Ventanas, and Benedito (2014) and Benedito, Carcel, Rosello, and Mulet (2001) have also used ultrasound to determine the composition of a formulated dry-cured meat product (sausage) and raw meat mixtures, respectively. Most of the aforementioned ultrasonic studies rely on the measurement of the ultrasonic velocity, because it is the simplest and most reliable ultrasonic measurement. However, each ultrasonic measurement provides information on a reduced area of the sample which implies that, if large samples are to be analyzed, multiple measurements are required. Moreover, the results are largely

dependent on the temperature and anisotropy meat tissue (Miles & Fursey 1977). In this regard, other non-destructive techniques, such as X-rays, do not require a precise temperature control.

There are several X-ray technologies that, based on the differential X-ray attenuation produced by the different tissue density, permit meat composition to be determined. X-ray computed tomography has been used to predict the lean/fat content in animal carcasses (Vester-Christensen et al., 2009) and bone-in green hams (Picouet, Muñoz, Fulladosa, Daumas, & Gou, 2014) and to determine the intramuscular fat content of meat (Font-i-Furnols, Brun, Tous, & Gispert, 2013). Brienne, Denoyelle, Baussart, and Daudin (2001) used Medical Dual Energy X-Ray Absorptiometry (DEXA) to predict the fat content in pork meat/fat mixtures and beef muscles. Although a low correlation was observed between the percentage of fat obtained through chemical analyses and the percentage estimated from the Beer-Lambert equation, they proposed different corrections and obtained an improvement. However, corrections are specific for each sample format and DEXA equipment. Mercier et al. (2006) used the ratio between the coefficients of attenuation of the two X-ray energy levels obtained with a medical DEXA to predict the fat content in legs of lamb carcasses. The predictions underestimated the fat content, probably because dissected fat was used instead of chemically analyzed fat for predictive model development. López-Campos, Larsen, Prieto, Juárez, and Aalhus (2013) reported that DEXA technology may also be useful for the objective estimation of the intramuscular fat content in beef. Nevertheless, the medical devices used in the aforementioned studies are not suitable for working in industrial environments at the required speed. In this sense, other authors demonstrated that non-medical X-ray instruments also allow the online determination of the salt uptake in whole bone-in hams during the salting procedure (Fulladosa, Muñoz, Serra, Arnau, & Gou, 2014) and the accurate estimation of the fat content of boned and packaged meat trimmings (Hansen et al., 2003).

Nevertheless, more research is needed before using ultrasound and DEXA technologies to determine the composition of products in which the fat is not uniformly distributed or that contain bones. Fat content determination in whole pieces, such as green bone-in hams, is still a challenge because among others the presence of bones and the existence of different muscles with a high degree of heterogeneity in terms of their fat content and distribution. Besides, combining the information obtained from acoustic and electromagnetic waves as a means of achieving more accurate predictions is worth investigating. Thus, the aim of the present study was to analyze the ability of ultrasound and DEXA techniques to predict both separately and jointly the fat content of green hams and to determine the feasibility of using them for industrial classification purposes.

2. MATERIAL AND METHODS

2.1 SAMPLES

Thirty nine green hams from 'White' pigs (crosses containing Duroc (CDU) or Large White (CLW)), average weight 11.1±0.8 kg, and 39 green hams from 'Iberian' pigs (crosses containing at least 50% Iberian breed (CIB)), average weight 10.6±1.2 kg, were purchased in 2 different slaughterhouses. The hams were taken to the pilot plant in refrigerated storage and kept at 2±2 ºC for less than 2 days before the non-destructive measurements were conducted. The different genetic source of the hams allowed for a wide range of fat contents.

2.2 ULTRASONIC MEASUREMENTS

A specific device was designed and assembled for ultrasonic measurements; it mainly consisted of a couple of narrow-band ultrasonic transducers (1 MHz, 0.75'' crystal diameter, A314S-SU model, Panametrics, Waltham, MA, USA), a pulser-receiver (Model 5058PR, Panametrics, Waltham, MA, USA) and a digital oscilloscope (Tektronix, TDS5034, Digital phosphor oscilloscope. Tektronix inc. Bearverton, OR, USA). A digital height (192-633 Serie, Mitutoyo, Japan) gage

was linked to the computer by a RS 232 interface in order to measure the sample thickness (±0.01 mm) (Figure 1A).

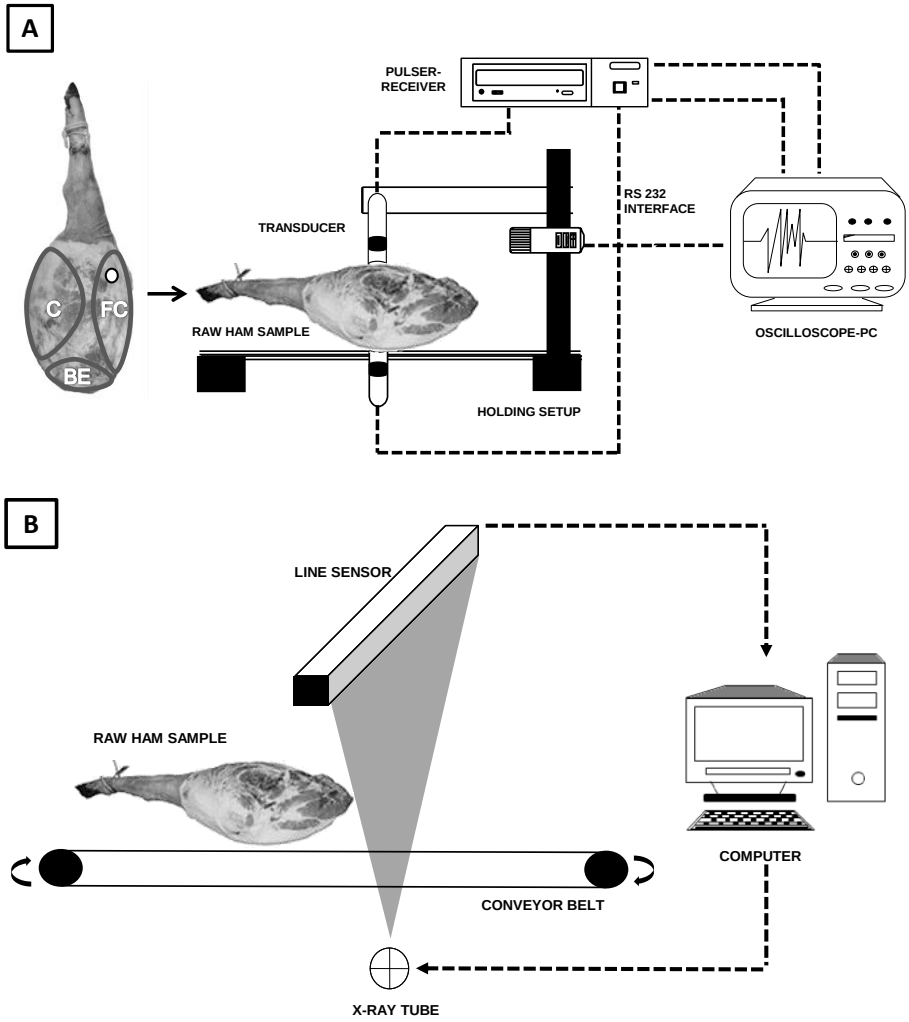

Figure 1. The experimental set-up used in the ultrasonic **(A)** and X-ray **(B)** measurements and location of ultrasonic measurements zones. C. Cushion, FC. Fore cushion and BE. Butt end.

The ultrasonic velocity was computed from the time of flight (an average of 3 signal acquisitions) and the sample thickness. In order to assess the ultrasonic velocity, the system delay was taken into account, which was determined from the pulse transit time measured across a set of methacrylate cylinders of different thicknesses. The delay time was then obtained from the intercept on the y-axis of the time versus thickness graph.

The ultrasonic measurements were taken in three zones of the ham (FC, BE and C), as shown in Figure 1A. The number of experimental measurements carried out in each zone depended on the hams' surface and weight. On average, 20 measurements were carried out in the cushion (C) and 5 in the fore cushion (FC) and butt end (BE). Measurements were carried out in triplicate. The hams were kept at 2±2 ºC for 24 h before the ultrasonic velocity was measured in place. The ultrasonic velocity in the ham was calculated as the average of the 30 ultrasonic velocities measured in all the ham zones. The average ultrasonic velocity was correlated to the fat content of the green hams.

2.3 X-RAY ABSORPTIOMETRY MEASUREMENTS

A commercially available X-ray inspector model X20V G90 (Multiscan technologies, S.L, Cocentaina, Spain) was used to scan the samples. X-rays were emitted from below the samples and the transmitted X-rays were measured in the upper part of the equipment while a conveyor belt moves the sample through at 0.33 m s^{-1} (Figure 1B). The device uses low-energy X-rays to obtain images (matrixes of values, 4000 x 1280 pixels) of the scanned object in the horizontal plane. Samples were scanned at three different voltages and intensities, specifically 90 kV and 4 mA, 70 kV and 8 mA and 50 kV and 15 mA, in exactly the same position and location in order to combine the information obtained from the three matrixes of values. Matrixes of attenuation values were imported and analyzed using a specific Matlab code (MATLAB, Ver. 7.7.0, The Mathworks Inc., Natick, MA, USA).

The global X-ray attenuation value (A) for each sample and used energy was obtained by the following equation:

$$A = -Ln\frac{\sum I_{(i;j)}}{\sum I0_{(i;j)}} \quad \text{(Eq. 1)}$$

where I is the of the radiation transmitted through each pixel of the matrix (i;j); I_0 is the energy of the incident radiation to each pixel of the matrix (i;j); i ranges from 1 to 4000 and j ranges from 1 to 1280. Therefore, attenuation values for measurements carried out at 50, 70 and 90 kV were obtained (A_{50}, A_{70} and A_{90}).

According to the Beer-Lambert law, X-Ray attenuation is proportional to the thickness and composition of the sample (n components):

$$A = L \cdot \sum_{i=1}^{n} \varepsilon_i \cdot c_i = \frac{L}{V} \cdot \sum_{i=1}^{n} \varepsilon_i \cdot M_i \quad \text{(Eq. 2)}$$

where L is the sample thickness (m), V is the sample volume (m^3), ε_i is the absorptivity coefficient of component i ($m^2\ kg^{-1}$), which is dependent on the X-ray energy, and c_i and M_i are the concentration (kg m^{-3}) and the mass (kg) of absorbing component i, respectively.

Eq (2) can be converted into Eq (3) by dividing by the ham weight (M_t):

$$\frac{A \cdot V}{L \cdot M_t} = \sum_{i=1}^{n} \varepsilon_i \cdot X_i \quad \text{(Eq. 3)}$$

where X_i is the mass fraction of component i.

Since hams do not have a uniform thickness, an average thickness was estimated as the ratio between V and the sample surface in the scan (S). Then, a new parameter (A_T) can be calculated from Eq. (3):

$$A_T = \frac{A \cdot S}{M_t} = \sum_{i=1}^{n} \varepsilon_i \cdot X_i \qquad \text{(Eq. 4)}$$

The correlation between A_T, obtained at different voltages (A_{T50}, A_{T70} and A_{T90}), with the fat content was analyzed.

2.4 DISSECTION AND CHEMICAL ANALYSIS

After the ultrasound and X-ray measurements, the lean and fat tissues for each ham were dissected, weighed and minced together. Afterwards, the fat and moisture contents of the mixture were determined. The moisture was analyzed by drying at 103±2 ºC until reaching constant weight (ISO 1442, 1997). The total fat content was estimated by near infrared spectroscopy using a FoodScanTM Lab (Foss Analytical, Dinamarca) according to AOAC (2007). All analyses were performed in triplicate. The fat (X_f) and moisture (X_w) contents of the whole hams were calculated by referring the mixture composition to the ham weight.

2.5 DEVELOPMENT OF PREDICTIVE MODELS AND STATISTICAL ANALYSIS

The green hams used in this study were split into two sets. The first set (Model Calibration, MC) included 52 hams and was used to develop predictive models using ultrasonic and X-ray absorptiometry parameters. The rest of the hams (26) were used for model validation (MV set). In order to cover a wide range of fat content in each set of hams (Table 1), they were sorted according to the experimental fat content and for each group of 3 hams, 2 hams were systematically included in the MC set and 1 in the MV set. In addition, the hams of the MV set were divided into 3 groups according to their fat content (low<14%, medium 14-26% and high>26% fat content level).

Predictive models were established to find single and multiple regression models between the fat content and the ultrasonic and X-ray variables. For that purpose, the XLSTAT 2009 statistical package (Microsoft Office, Redmond, WA, USA) was used. Regarding the ultrasonic measurements, only the ultrasonic

velocity (υ) was used because other variables, such as attenuation and the frequency spectrum analysis, did not provide relevant information. For X-ray measurements, A_T values obtained at different energies were used. The combination of US and X-ray parameters was also investigated. In this case, the independent variables of the model were selected by the Stepwise method, the levels of significance to enter and keep the dependent variables in the model being p=0.05 and p=0.1, respectively. The reliability of the predictive models was given by the coefficient of determination (R^2) and the Root Mean Square Error of Calibration (RMSEC). For the validation data set, the Root Mean Square Error of Validation (RMSEV) was also calculated.

3. RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION

The chemical composition of the green hams used in this study is shown in Table 1. The fat and moisture content ranged between 6.5 and 41.0% w.b. and 39.9 and 70.2% w.b., respectively. These ranges of fat and moisture contents cover the fat and moisture contents of the majority of hams usually found on the market (Serra, & Fulladosa, 2011; Blasco et al., 1994).

Table 1. Average, minimum and maximum values of moisture (X_w % w.b.) and fat (X_f % w.b.) contents for validation and calibration ham sets.

	n	X_w (% w.b.)			X_f (% w.b.)		
		MEAN	**MIN**	**MAX**	**MEAN**	**MIN**	**MAX**
CALIBRATION (MC)	52	57.0	40.9	70.2	21.7	6.5	39.8
VALIDATION (MV)	26	56.4	39.9	68.3	22.4	8.6	41.0

3.2 INFLUENCE OF FAT ON ULTRASONIC VELOCITY

Figure 2 (A and B) shows the relationship between the ultrasonic velocity (υ) and the fat (X_f) and moisture contents (X_w) in the 78 green hams analyzed. It

should be highlighted that the υ reported in each point of Figure 2 is the average ultrasonic velocity of a whole ham (30 measurements distributed in the three zones, Figure 1A), as explained in section 2.2. There is great experimental variation in the ultrasonic response to differences in moisture and fat content, which is especially noticeable for fat contents between 20 and 28% w.b. (Figure 2). This general variability could be linked to the highly heterogeneous nature of the ham, which is a piece made up of subcutaneous fat and different muscles, also containing a heterogeneous distribution of intramuscular fat and connective tissue. In addition, the breed of the pig and feeding system could significantly modify the υ in the fatty fraction and affect the protein content in the lean tissue, which would also determine the υ in the muscles (Niñoles, Mulet, Ventanas, & Benedito, 2011; Niñoles, Sanjuan, Ventanas, & Benedito, 2008).

As can be observed in Figure 2A, an increase in the fat content involved an increase in the υ measured at 2 ºC. Thus, on average, an increase in the fat content of 5% corresponded to an increase of 8.4 m s^{-1} in the υ. This result is explained by considering that, at low temperatures, the ultrasonic velocity in the fatty tissue is higher than in lean tissue. This fact has been previously reported by Benedito et al. (2001), who found an ultrasonic velocity of 1610.0-1620.0 m s^{-1} in fatty tissues and 1530.0-1555.0 m s^{-1} in lean tissues at 4 ºC. Similarly, Miles and Fursey (1977) reported ultrasonic velocities at 4 ºC in intact beef muscles around 1530 m $s^{-1}$and significant higher (1650 m s^{-1}) for beef adipose tissue. The ultrasonic velocity in fatty tissue is so high at this temperature because it depends on the solid/liquid ratio which affects its textural properties; consequently, as the state of the fat at low temperatures is mainly solid, in which ultrasound propagates faster, the υ reaches its highest values. In contrast, the ultrasonic velocity in lean tissue is lower because the main component in raw meat is water and the ultrasonic velocity in water at 2 ºC is 1412.8 m s^{-1} (Kinsler, Frey, Coppens, & Sanders, 1982). The ultrasonic velocity in the whole ham is lower (1531.1-1586.9 m s^{-1}, Figure 2A) than in the

fatty tissue because it is greatly influenced by the water content of the lean tissue.

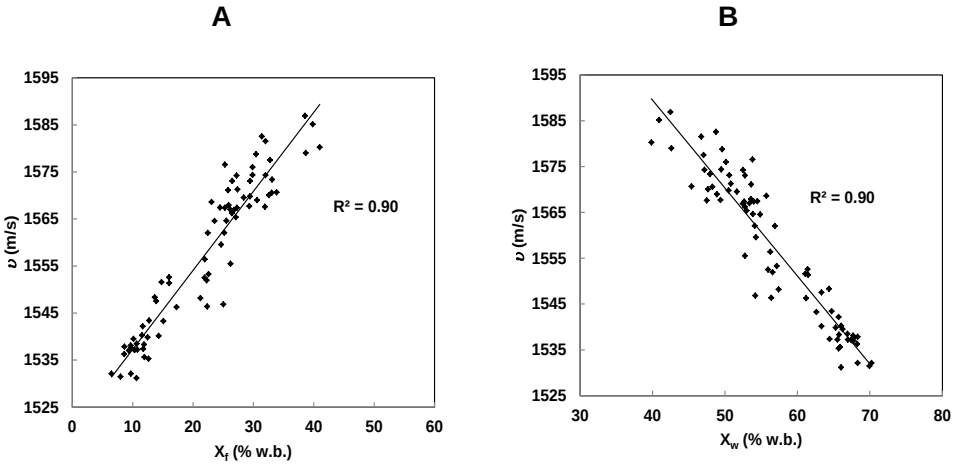

Figure 2. Relationship between the ultrasonic velocity and the fat (X_f) **(A)** and moisture contents (X_w) **(B)** for raw hams.

It should be emphasized that the influence of the fat content on the υ in ham is highly temperature dependent. In this regard, the υ in pure fat decreases with the rise in temperature (McClements, & Povey, 1992). This fact has also been observed in different meat products, where velocity was measured at between 2 and 38 ºC (Corona et al., 2014; Koch et al., 2011b; Niñoles et al., 2008; Chanamai, & Mc.Clements, 1999), the reduction in velocity being mainly ascribed to the fat melting as the temperature rises. The temperature used (2 ºC) is appropriate for fat content assessment, since there is a remarkable difference between the υ in the fatty and lean tissues. As the temperature increases, the ultrasonic velocity in fat falls and that of lean tissue goes up, leading to similar υ values for both tissues, which hinders the fat content estimation.

The moisture content was found to have the opposite effect on υ to that reported in the case of fat (Figure 2B). Thus, in average terms, an increase in the moisture content of the green ham of 5% corresponded to a decrease of

9.6 m s^{-1} in υ. As previously mentioned, the υ in water is lower than the velocity in the other components of ham (fat and protein+others) (Benedito et al., 2001); therefore, as the water content increases, the υ in the ham decreases. The influence of the moisture content on υ has also been reported in the curing process of *Biceps femoris* and *Longissimus dorsi* muscles and sobrassada (a dry-cured minced meat product), where the υ increased due to the dehydration (Niñoles, 2007; Llull, Simal, Benedito, & Roselló, 2002). Likewise, Koch et al. (2011a) indicated that the water loss in thawed *Longissimus dorsi* muscle entailed an increase in υ.

The water and fat contents of hams have the opposite effect on the υ and, at the same time, they show a high negative correlation in fresh hams (non-dried hams). Therefore, it is expected that, although both fat and water affect υ, there will be a relationship between the υ and each component. For a low correlation between fat and water contents, the influence of both water and fat contents on the υ should be assessed.

3.3 INFLUENCE OF FAT ON X-RAY ABSORPTIOMETRY PARAMETERS

Figure 3A shows the relationship between X-ray attenuation values (A) obtained at different energies and the measured fat content of the hams. There was an increase in A values when the X-ray energy decreased. This fact is linked to the greater absorption phenomena which exist at low energies than at high ones (Kalender, 2005). Whatever the energy considered, an increase in the percentage of fat content involved a decrease in A. Non-significant differences in the slope of the A *vs* X_f were detected ($p>0.05$), due to the large experimental variation of attenuation not explained by the fat content. It has been described that X-ray attenuation at low energies is dependent on both fat content and the product thickness (Hansen et al., 2003), which is not constant in hams. The variation in ham weight could also increase the variation in A values.

A_T is proportional to the attenuation (A) and to the ratio between sample surface in the scan (S) and the ham weight (M_t) (Eq. 4). This ratio is related to the composition but also to the shape of the ham. As shown in Figure 3A, although a drop in the fat content produces an increase in A, it simultaneously increases the density and consequently, for a constant sample surface in the scan, it decreases the ratio S/M_t. Therefore, a decrease in the fat content has an opposite effect on the two factors of Eq. (4), and the resulting effect on A_T is unknown. In the present study, A_T was found to be positively correlated to the fat content at the three different voltages and intensities studied, specifically 90 kV and 4 mA (R^2=0.57), 70 kV and 8 mA (R^2=0.53) and 50 kV and 15 mA (R^2=0.34) (see Figure 3B).

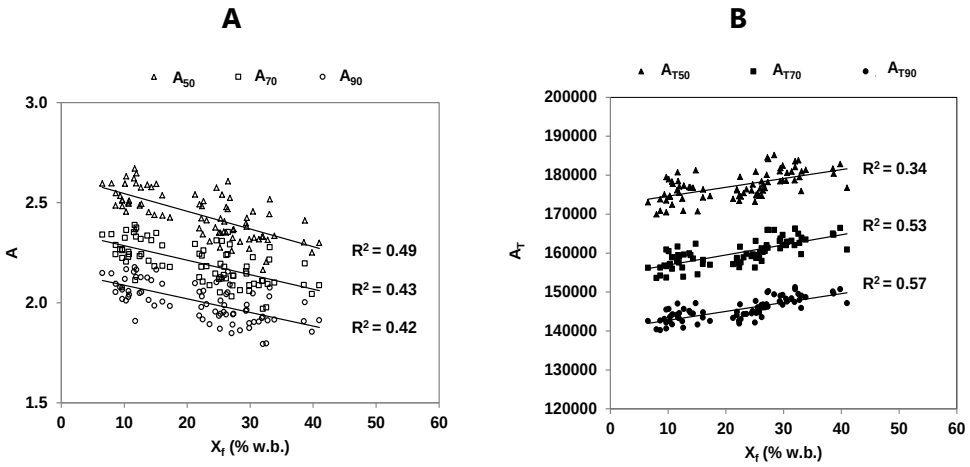

Figure 3. Relationship between X-ray parameters, A **(A)** and A_T **(B)**, obtained at different X-ray energies (50, 70 and 90 kV) and the fat content (X_f) for raw hams.

3.4 PREDICTIVE MODELS

A linear model was established for MC set between the fat content and the ultrasonic velocity (Eq. 5); the RMSEC being 2.90% (Table 2) and R^2=0.89 (Eq. 5). This could be considered a robust model because very different samples were used in the study.

$$X_f(\%\,w.b.) = 0.54 \cdot \upsilon - 821.99 \qquad \text{(Eq. 5)}$$

where X_f is the fat content and υ is the ultrasonic velocity. The slope of Eq. (5) indicates that an increase of 1 m s^{-1} in the ultrasonic velocity led to an increase of 0.54% in the fat content.

Miles and Fursey (1977), using the reciprocal of the squared ultrasonic velocity ($1/\upsilon^2$) at 0 °C, reported less satisfactory predictive models of fatness for comminuted beef muscles (R^2 < 0.54). These authors assessed the fat composition in meat muscles and mixtures of lean and fatty tissues. However, in the present work the fat content assessment is conducted on a much complex medium (whole bone-in ham) which includes different types of muscles, connective tissue and subcutaneous fat, which highlights the relevance of the results for implementing quality control systems in the meat industry. Miles and Fursey (1977) reported that the best temperature for conducting the ultrasonic measurements was 37 °C, however in the present work the temperature chosen was 2 °C since it is the most commonly one used for refrigeration of green hams prior to classification and processing. When analysing fresh *Biceps femoris* at 0 ºC, Niñoles et al. (2011) found that an increase of 1 m s^{-1} in the ultrasonic velocity implied an intramuscular fat content increase of 0.34%. The different coefficient value found by Niñoles et al. (2011) (0.34 compared to 0.54) could be due to the great experimental variability (R^2=0.59) found by these authors, which greatly increases the standard error of the estimated coefficient. However, Park, Whittaker, Miller, and Hale (1994) suggested that the increase of 1 m s^{-1} in the ultrasonic velocity measured at 22 ºC led to a reduction of 0.21% in the fat content of *Longissimus dorsi* muscle, which may be explained by considering the fact that the fat melts at high temperatures.

A multiple regression analysis was performed to study the relationship between the fat and moisture contents and the ultrasonic velocity. The analyses detected a severe collinearity between both variables (the fat and moisture contents), due to the VIF being higher than 5 ($VIF_{f\text{-}w}$=8.2), caused by the inherent relationship between the fat and moisture contents in the green hams.

Therefore, including the moisture content in the model does not lead to a better explanation of the experimental variability observed in the ultrasonic velocity.

The fat content was also predicted by means of X-ray parameters (A_T) at three different energies. The predictive model (Eq. 6) showed a RMSEC of 4.20% and a R^2 of 0.80.

$$X_f\,(\%\,w.b.) = -279.643 - 0.00473 \cdot A_{T50} + 0.00806 \cdot A_{T70} - 0.00103 \cdot A_{T90} \quad \text{(Eq. 6)}$$

Predictive errors were high in comparison to what occurs using technologies in which the thickness of the sample is not critical, such as the ham grading system based on electromagnetic induction measurements (Serra, & Fulladosa, 2011), or in technologies in which thickness determination is inherent to the measurement, such as in the case of US. There are only slight X-ray attenuation differences between fatty and lean tissues and a more accurate thickness correction (including a laser volume sensor) could help to obtain better models.

The use of hams from different animal breeds increases the robustness of the models, but may also have an adverse effect on the predictive errors. Figure 4 shows the typical geometry of scanned ham surfaces from different crossbreeds. CLW hams exhibited a different shape from CDU and CIB hams. In Eq. (4), an average thickness was used instead of the real thickness. The error of this approximation may depend on the dimensional conformation of the hams. Therefore, new models were developed by discarding the CLW hams and, thus, considering only the hams with a similar geometry. From this approach, the errors (RMSEC=2.23%) were smaller than the ones obtained using all the hams (RMSEC=4.20%), pointing to the importance of the homogeneous conformation of the hams. In contrast, since the geometry is not important in US technology, the error is similar (3.02% *vs* 2.90%) when using υ.

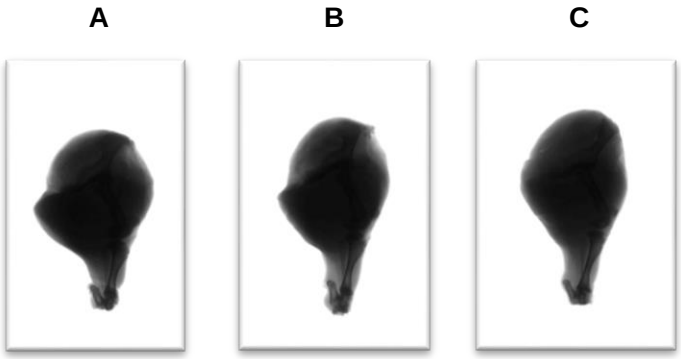

Figure 4. Scanned surface of hams from crosses containing Large white and Landrace (A), Duroc (B) or Iberian (C) breeds.

When using all the hams, the stepwise regression analysis including both the US and X-ray parameters, showed that the parameter which provided the most relevant information for fat content prediction (Table 2) was the υ. The addition of X-ray parameters to the model did not decrease the prediction error. In contrast, when discarding CLW hams, the most relevant information is provided by A_{T50} and A_{T70} and υ is not included in the model.

Table 2. Parameters of raw hams fat predictive models using X-Ray and ultrasound measurements.

Crossbreeds used	Technology	MODEL VARIABLES	RMSEC(%)	R^2	RMSEV(%)
CLW, CDU, CIB	**US**	υ	2.90	0.89	2.97
CLW, CDU, CIB	**X-Rays**	A_{T50} ,A_{T70}, A_{T90}	4.20	0.80	4.65
CDU, CIB	**US**	υ	3.02	0.59	3.29
CDU, CIB	**X-Rays**	A_{T50} ,A_{T70}, A_{T90}	2.23	0.79	3.27

3.5 VALIDATION AND CLASSIFICATION TESTS

Figure 5 depicts the relationship between the fat contents measured and predicted using ultrasound (A, Eq. 5, R^2=0.90) and X-ray (B, Eq. 6, R^2=0.67) models (Table 2). RMSEV were 2.97% and 4.65% for ultrasound and X-rays,

respectively, both providing a reliable, non-destructive measurement of the fat content of green hams over a wide range of fat content (from 6.5 to 41.0% w.b.). The number of validation errors decreases when CLW hams are excluded from the model for X-rays (RMSEV=3.27%). Miles et al. (1987) reported standard deviations of the residuals of around 1.85 for the ultrasonic estimation of the fat content in specific sites of the beef carcass. In other studies, the ultrasonic velocity has been used to estimate the fat content of green meat mixtures and fish (Benedito et al., 2001; Ghaedian, Coupland, Decker, & McClements, 1998) obtaining a better correlation than in the present study, (R^2=0.99, in both cases). In all likelihood, this fact could be explained by considering that highly homogeneous samples were tested in the former studies. The green hams used in the present study, however, are heterogeneous; this is due to several factors, the fat distribution within the samples, the connective tissue characteristics, the different moisture and fat profiles and the existing bones and skin, among others.

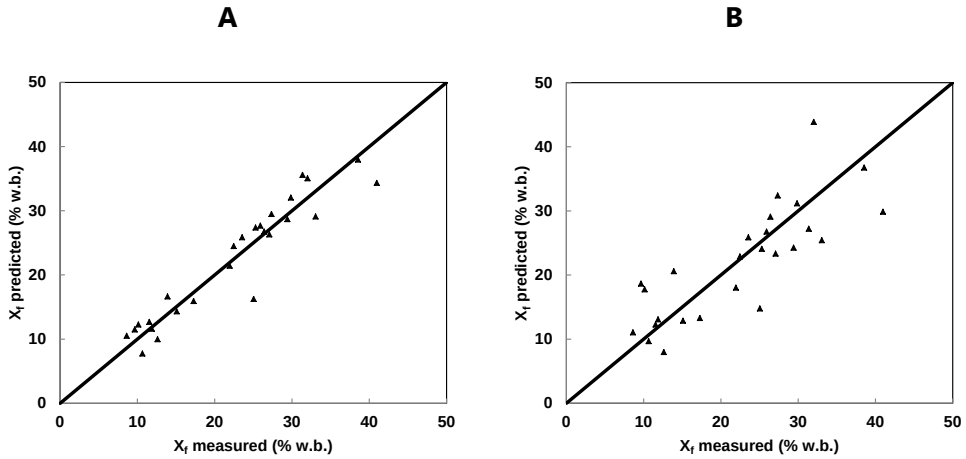

Figure 5. Validation of the predictive model for fat content (X_f) estimation of raw hams based on ultrasonic (A) and X-ray absorptiometry (B) measurements.

In order to evaluate the feasibility of using the ultrasonic and X-ray models to classify the hams into different categories according to their fat content, the MV set hams (Table 1) were classified into three groups: low (<14%), medium

(between 14 and 26%) and high (>26%) fat content levels (Table 3). Once the estimated fat content was calculated from Eq. (5) and (6) and compared with the measured one, the classification performance was assessed. In average terms, whereas the ultrasonic model classified 88.5% correctly, the X-ray model only classified 65.4% of the MV ham set (Table 3). The ultrasonic model was able to correctly classify 87.5 and 100.0% of the ham pieces, in the low and high fat content groups, respectively. However, for a medium fat content, the percentage of correctly classified hams was reduced to 75.0% (Table 3). In contrast, the X-ray model provided similar percentages for every category.

Table 3. Classification of raw hams according to the fat content (X_f) (low X_f<14%, medium 14≤X_f≤26% and high X_f>26%) by using the predictive model based on ultrasonic and X-Ray measurements.

FAT LEVEL	X_f (% w.b.)	% CLASSIFICATION	
		US	X-Rays
LOW	**<14**	87.5	70.0
MEDIUM	**14-26**	75.0	62.5
HIGH	**>26**	100.0	75.0
TOTAL		88.5	65.4

Thus, US could better classify hams into different groups of fatness, which would be highly relevant for industrial quality control purposes. It would be necessary to develop a prototype which permits a rapid measurement before implementing this technology industrially as a means of easily and rapidly sorting and processing the raw material according to the fat content. X-rays could also be useful, especially if a specific calibration is developed for each kind of raw material in order to overcome the variability produced by the different conformation of the hams. In this case, the device is already suitable for industrial conditions and works at production speed.

As previously explained, it is not worth combining X-ray and US sensors together in an instrument because it does not offer a significant improvement and it would increase the cost of the device.

4. CONCLUSIONS

Ultrasound velocity and X-ray attenuation are influenced by the composition of the hams, allowing predictive models to be developed for the fat content with errors of 2.97% and 4.65% for US and X-ray, respectively, when all the hams are used, when all the hams are used. When discarding hams with a different geometry (CLW hams), the X-ray predictive error improved, decreasing to 3.27%. Nevertheless, in no case did the combination of parameters obtained from both technologies improve the prediction accuracy. These predictive models permitted a satisfactory classification of the hams into three fat levels (<14, 14-26 and >26% fat content), demonstrating the feasibility of these non-destructive techniques for ham classification purposes. Research should be conducted in order to include accurate sample geometry corrections in the X-ray technique and to develop fast ultrasonic devices to be used online.

ACKNOWLEDGEMENTS

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REFERENCES

Association of Official Analytical Chemists (2007). Official method 2007.04, Determination of Fat, Moisture, and Protein in Meat and Meat Products. Official Methods of Analysis of AOAC International (18th ed.), Association of Official Analytical Chemists International, Gaithersburg, MD.

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

Blasco, A., Gou, P., Gispert, M., Estany, J., Soler, J., Diestre, A., & Tibau, J. (1994). Comparison of five types of pig crosses. I. Growth and carcass traits. *Livestock Production Science*, 40(2), 171-178.

Brienne, J. P., Denoyelle, C., Baussart, H., & Daudin, J. D. (2001). Assessment of meat fat content using dual energy X-Ray absorption. *Meat Science*, 57(3), 235-244.

Čandek-Potokar, M., & Škrlep, M. (2012). Factors in pig production that impact the quality of dry-cured ham: A review. *Animal*, 6(2), 327-338.

Chanamai, R., & Mc.Clements, D. J. (1999). Ultrasonic determination of chicken composition. *Journal Agricultural Food Chemistry,* 47, 4686-4692.

Cierach, M., & Modzelewska-Kapitula, M. (2011). Effects of ph values and fat content on sodium chloride diffusion rate in pork. *Journal of Food Processing and Preservation,* 35, 129-142.

Corona, E., García-Pérez, J. V., Ventanas, S., & Benedito, J. (2014). Ultrasonic characterization of the fat source and composition of formulated dry-cured meat products. *Food Science and Technology International*, 20(4), 275-285.

Font-i-Furnols, M., Brun, A., Tous, N., & Gispert, M. (2013). Use of linear regression and partial least square regression to predict intramuscular fat of pig loin computed tomography images. *Chemometrics and Intelligent Laboratory Systems*, 122, 58-64.

Fulladosa, E., Muñoz, I., Serra, X., Arnau, J., & Gou, P. (2014). X-Ray absorptiometry for non-destructive monitoring of the salt uptake in

bone-in raw hams during salting. *Food Control*. DOI: 10.1016/j.foodcont.2014.06.023.

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(2), 386-392.

Ghaedian, R., Coupland, J. N., Decker, E. A., & McClements, D. J. (1998). Ultrasonics determination of fish composition. *Journal of Food Engineering,* 35, 323-337.

Hansen, P. W., Tholl, I., Christensen, C., Jehg, H. C., Borg, J., Nielsen, O., Østergaard, B., Nygaard, J., & Andersen, O. (2003). Batch accuracy of on-line fat determination. *Meat Science*, 64, 141-147.

ISO 1442 (1997). Meat and meat products. Determination of moisture content (Reference method). International organization for standardization, Geneva.

Kalender, W. A. (2005). *Computed tomography: Fundamentals, system technology, image quality, applications.* (2nd ed.) Erlangen, Germany: Publicis. Corperate Publishing (p. 304).

Kinsler, L. E., Frey, A. R., Coppens, A. B., & Sanders, J. V. (1982). *Fundamentals of Acoustics.* (3 rd ed.) New York, U.S.A.: John Wiley and Sons (Chapter 4).

Koch, T., Brand, S., Lakshmanan, S., Raum, K., Wicke, M., & 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). The use of ultrasound velocity measurement to evaluate the textural properties of sobrassada from Mallorca. *Journal of Food Engineering*, 52, 323-330.

López-Campos, O., Larsen, I., Prieto, N., Juárez, M., & Aalhus, L. (2013). Preliminary evaluation of dual energy x-Ray absorptiometry to assess intramuscular fat from the *Longissimus Thoracis* muscle of beef. *E-Book of proceedings of the 59th International Congress of Meat Science and Technology.* (S7B-17), Izmir, Turkey.

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

Mercier, J., Pomar, C., Marcoux, M., Goulet, F., Thériault, M., & Castonguay, F. W. (2006). The use of dual-energy X-Ray absorptiometry to estimate the dissected composition of lamb carcasses. *Meat Science*, 73(2), 249-257.

Miles, C.A., Fisher, A.V., Fursey, G.A.J., & Page, S.J. (1987). Estimating beef carcass compo-sition using the speed of ultrasound. Meat Science, 21(3), 175–188.

Miles, C.A., & Fursey, G.A.J. (1977). Measurement of the fat content of meat using ultra-sonic waves. Food Chemistry, 2(2), 107–118.

Miles, C.A., Fursey, G.A.J., Page, S.J., & Fisher, A.V. (1990). Progress towards using the speed of ultrasound for beef leanness classification. Meat Science, 28(2), 119–130.

Niñoles, L. (2007) *Contribución a la caracterización de la calidad de productos de cerdo mediante ultrasonidos de señal.* (PhD thesis) Spain: Universitat Politècnica de València.

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(3), 896-902.

Park, B., Whittaker, A. D., Miller, R. K., & Hale, D. S. (1994). Predicting fat in beef Longissumus muscle from speed of sound. *Journal of Animal Science*, 72, 109-116.

Picouet, P. A., Muñoz, I., Fulladosa, E., Daumas, G., & Gou, P. (2014). Partial scanning using computed tomography for fat weight prediction in green hams: Scanning protocols and modelling. *Journal of Food Engineering* DOI: 10.1016/j.jfoodeng.2014.06.012

Serra, X., & Fulladosa, E. Online total fat prediction in green hams. (2011). Newsletter Q-PorkChains, 8, 8. Retrieved from http://www.q-porkchains.org/news/~/media/Qpork/docs/pdf/newsletter/QPorkChains_Newsletter_8_march2011.ashx

Vester-Christensen, M., Erbou, S. G. H., Hansen, M. F., Olsen, E. V., Christensen, L. B., Hviid, M., Ersbøll, B. K., & Larsen, R. (2009). Virtual dissection of pig carcasses. *Meat Science*, 81(4), 699-704.

CHAPTER 4.2

SALTED PORK MEAT CHARACTERIZATION

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Non-destructive salt content prediction in brined pork meat using ultrasound technology

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Abstract

This works aims to show the feasibility of using low-intensity ultrasound (US) to predict the salt content in brined Biceps femoris (BF) and Longissimus dorsi (LD) pork muscles. For this purpose, meat samples were salted in brine solution (20% w/w at 2 ºC) for different times (24, 48, 96 and 168 h) and the US velocity measured before and after salting. In addition, model samples with preset water and salt contents were formulated and the US velocity was measured. In the model samples, the salt content (X_S) had a more marked effect on the US velocity (13.0 m/s per ΔX_S = 1% wet basis) than the water content (5.0 m/s per ΔX_W = 1% wet basis). The salt gain and the water loss during brining caused the US velocity to increase (61.5 and 49.3 m/s for 168 h in LD and BF, respectively). Significant linear relationships between the US velocity and both factors (X_S and X_W) were established (R^2>0.771). A predictive model of the salt content based on the US velocity was proposed; this was successfully validated (average estimation error 0.48%), which shows the feasibility of using US for industrial quality control purposes.

Keywords: *Non-destructive technology; Ultrasound; Salt content prediction; Pork meat*

1. INTRODUCTION

In the last few decades, the application of ultrasound in the food industry has been the focus of increased attention. In particular, as a non-destructive technology, the use of low-intensity ultrasound has been the subject of many contributions aiming to characterize the structural and compositional properties of food commodities (Povey and Harden, 1981; Contreras et al., 1992; Ghaedian et al., 1998; Mizrach, 2000; Aparicio et al., 2008; Corona et al., 2014). In the meat industry, low intensity ultrasound (US) has already been used to characterize animal carcasses. Thus, Morlein et al. (2005) have classified pig carcasses based on the intramuscular fat content of Longissimus dorsi by means of the spectral analysis of US echo signals (at ≈ 3.5 MHz). Faulkner et al. (1990) and Ribeiro et al. (2008) used ultrasonic measurements to estimate the fat cover and carcass composition in cows and in growing lambs, respectively. Llull et al. (2002) evaluated the textural properties of a pork meat-based product (sobrassada) from ultrasonic velocity measurements. Corona et al. (2014) tested the feasibility of using ultrasonic velocity measurements to estimate the fat content and identify the fat source used in formulated dry-cured sausages. Similarly, the fat, water and protein content were assessed in raw meat mixtures by using ultrasonic velocity (Benedito et al., 2001). In addition, the time of flight of ultrasound waves has also been used to assess the percentage of frozen meat in blocks of chicken and beef during freezing (Sigfusson et al., 2004). However, the ability of ultrasound to determine the salt content in meat products, such as salted hams or loin, has not been reported in the literature.

Salting is among the most relevant stages in the processing of dry-cured meat products, since salt inhibits foodborne pathogens and spoilage bacteria growth (Liu et al., 2013) and contributes to necessary flavor and textural modifications of the raw meat (Larrea et al., 2006). However, the salting process of whole pieces, such as ham or loin, is complex since the biological and chemical characteristics of meat are greatly heterogeneous (Vestergaard et al., 2007).

Consequently, the use of a fixed salting time/product mass ratio is rarely optimal. In addition, the conventional analytical techniques used for the salt content measurement in meat products are not suitable for quality control purposes, due to the fact that they are destructive, laborious and time-consuming. Thus, great effort has been made by the meat industry to search for non-destructive techniques which allow reliable real time measurements of the salt content. Some of the most relevant non-destructive technologies previously applied to characterize the salt content in meat-based products are microwave dielectric spectroscopy (Castro-Giráldez et al., 2010), computer tomography (CT) (Vestergaard et al., 2004), near infrared spectroscopy (NIR) (Collell et al., 2011; Prevolnika et al., 2011) and hyperspectral imaging (Liu et al., 2013). These techniques have great potential for the rapid and non-destructive prediction of salt content. However, some of these techniques have limitations which include the high cost, the limited capacity of the equipment to be modified for on-line measurements and the reduced penetration capacity, for which reason they are barely used in the meat industry. In contrast, ultrasound is especially suitable for use as an industrial process quality control tool (Scanlon, 2004), because of its affordable portable equipment and easy adaptability to the process lines. Moreover, ultrasound can also be used to measure the internal composition of thick opaque samples. Therefore, the objective of this study was to test the feasibility of using low-intensity ultrasound to predict the salt content in brined pork meat (*Biceps femoris* and *Longissimus dorsi* muscles).

2. MATERIALS AND METHODS

2.1 RAW MATERIAL

Biceps femoris (BF) and *Longissimus dorsi* (LD) muscles were obtained from *Large White* breed pigs from a local market. In both muscles, the subcutaneous fat was removed before processing. The pieces selected had a pH of 5.65±0.05, which was measured at three different points along the muscle avoiding fatty

areas. Two different types of samples were prepared and analyzed by ultrasound. On the one hand, cylindrical samples (0.037 m in diameter (Ø) and 0.060±0.010 m in length (L)) were taken from 16 BF and 12 LD muscles by using a cylindrical cutter. The cut of the muscle was carried out perpendicularly to the direction of the fibers. On the other hand, model samples were formulated with specific moisture and salt contents, for which purpose 2 BF muscles were ground.

2.2 SALTING EXPERIMENTS IN BRINE SOLUTION

BF and LD cylindrical samples were placed into metallic cylinders (0.04 m in diameter and 0.07 m in length) in order to avoid the radial mass transfer during salting (Figure 1A and B). The base's cylindrical perimeter of the sample in contact with the brine was sealed with contact adhesive (Loctite Superglue-3, Loctite, Henkel) to avoid brine penetration along the walls, while the upper base was covered with a plastic film and paraffin to avoid sample dehydration. Salting experiments were carried out in a thermostatic bath with 15 L of brine 20% NaCl (w/w), which was held at 2ºC, a common temperature for meat salting, and vigorously stirred (Figure 1A). In each experiment, 12 samples were partially immersed in the brine, supported by a hollow polystyrene sheet. During salting, three samples were taken out after 24, 48, 96 and 168 h and analyzed. The salting experiments were replicated four times for both BF and LD muscles, using 3 LD and 4 BF muscles in each salting experiment. Additionally, equilibrium salting experiments were carried out keeping 4 BF and 4 LD cylindrical samples in the brine until constant weight was achieved.

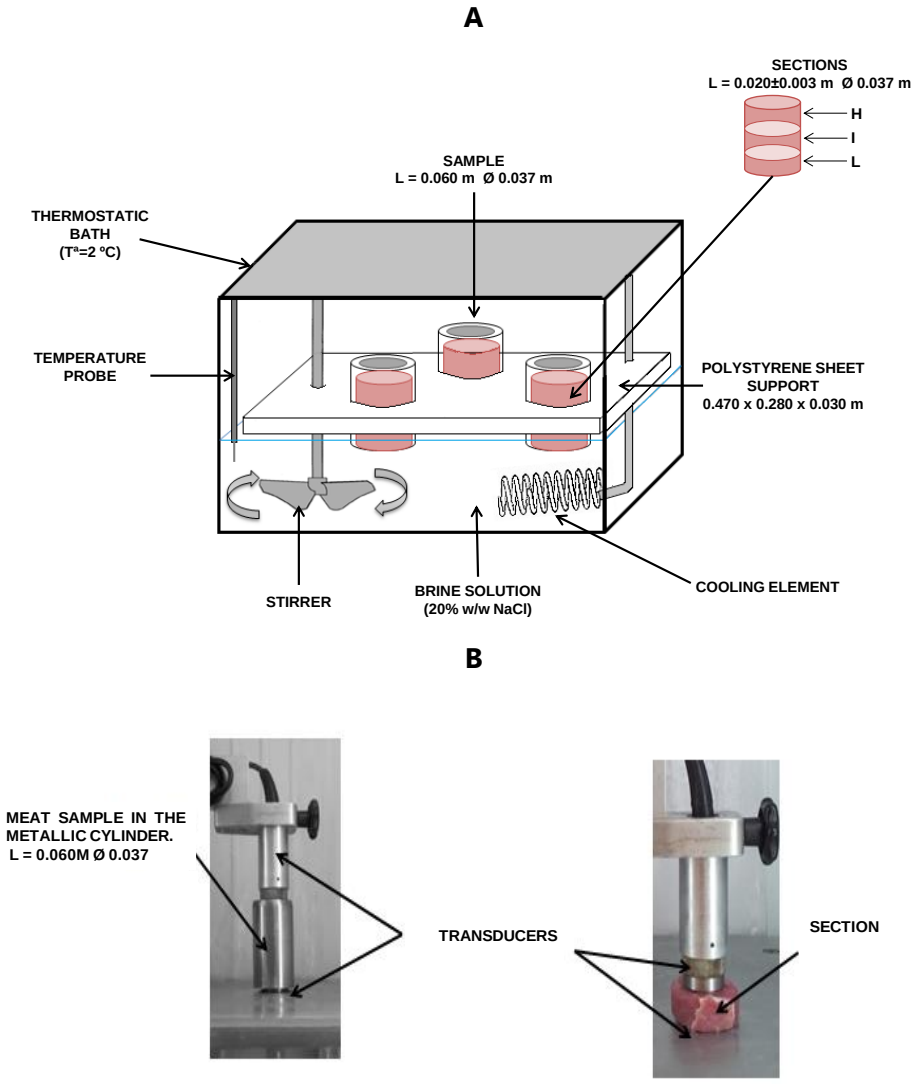

Figure 1. (A) Scheme of experimental device used for meat salting brine solution including a detail of a meat sample section (H, I and L'). (B) Ultrasonic measuring procedure in the meat sample inside the metallic cylinder (left) and in the meat sample section (right).

2.3 MODEL SAMPLES WITH PRESET WATER AND SALT CONTENT

Model samples with preset water and salt contents were formulated from ground BF (Table 1). During salting, there exists an inherent relationship

between water and salt content, thereby, the experiments with model samples aim to separate the specific effect of both variables on the ultrasonic properties.

Table 1. Model samples with preset salt and water content formulated from ground *Biceps Femoris* by:

SET 1			
	BATCHES ΔX_{W0} (% w.b.)		
	-3	**0 (Raw meat)**	**3**
Salt content (X_S % w.b.)	*0*	*0*	*0*
	1	*1*	*1*
	2	*2*	*2*
	3	*3*	*3*
	4	*4*	*4*

SET 2			
	BATCHES ΔX_{S0} (% w.b.)		
	0 (Raw meat)	**2**	**4**
Water content (X_W % w.b.)	*71*	*69*	*68*
	72	*70*	*69*
	73	*71*	*70*
	74	*72*	*71*
	75	*73*	*72*
	76	*74*	*73*
	77	*75*	*74*

Set 1. Modifying the salt content in batches with different initial water content (X_{W0}).
Set 2: Modifying the water content in batches with different initial salt content (X_{S0}).

Two sets of model samples were prepared to cover the typical water and salt content range found during the salting of pork products, such as ham or loin

(Table 1). In Set 1, three batches with a different initial water content (X_{W0}) were prepared by adding (ΔX_{W0} = +3% w.b.) or removing (ΔX_{W0} = -3% w.b.) water from the raw meat (ΔX_{W0} = 0). Afterwards, five different kinds of samples were formulated for each batch by adding salt (X_S from 0 to 4% w.b.) to the ground BF (Table 1 Set 1). In order to increase the water content, distilled water was added to the ground BF, while for dehydration, the ground BF was homogenously placed in a plate and dried in an air-forced oven (40 ºC). Similarly, in Set 2, three batches were prepared by adding salt (ΔX_{S0} = 2 and 4% w.b.) to the raw meat (ΔX_{S0} = 0). In this case, 7 different samples were formulated with water contents from 68 to 77% w.b. for each batch (Table 1). For each set, metallic cylinders (0.033 m in diameter and 0.025 m in length) were stuffed with the formulated model samples and vacuum treated (VAC-10, Edesa, Spain) in order to remove the air incorporated during grinding and blending.

2.4 ULTRASOUND MEASUREMENTS

The experimental set-up used for the ultrasonic measurements consisted of a pair of narrow-band ultrasonic transducers (1 MHz and 0.75'' crystal diameter, A314S-SU model, Panametrics, MA, U.S.A.), a pulser-receiver (5058PR model, Panametrics, MA, U.S.A.) and a digital storage oscilloscope (TDS5034 model, Tektronix, WA, U.S.A.). A custom designed digital height gage, linked to the computer by a RS232 interface, was used to measure the sample thickness with a precision of ±0.01 mm.

Ultrasonic measurements were carried by through-transmission mode placing the meat sample between the two ultrasonic transducers (Figure 1B). The ultrasonic velocity (V) was computed as the ratio between the sample thickness (measured by the digital height gage) and the time of flight (time elapsed from the departure of the wave from the emitter to the arrival at the receiver). Time of flight was computed averaging 3 signal acquisitions, and the In order to compute V, the system delay (0.789μs) was taken into account, which was

determined from the pulse transit time measured across a set of methacrylate cylinders of different thicknesses (from 1 to 6cm).

V was measured in the model samples and in the raw and the salted cylindrical samples (after 24, 48, 96 and 168 h) (Figure 1B). After the V measurement, each brined sample was sliced into 3 sections (0.020±0.003 m in thickness), as shown in Figure 1A and B. The L' section refers to the one in contact with the brine solution, the I section is the intermediate zone of the sample and the H section is the upper one furthest from the brine solution. V was also measured in each section (H, I and L') (Figure 1B).

The ultrasonic measurement was carried out in triplicate at 2 ºC inside a temperature controlled chamber (Arco SP 350, Comersa, Spain). Prior to measuring V, the samples were tempered for 24 h as a consequence of the influence of the temperature. In the brined samples, the variation of ultrasonic velocity (ΔV) was assessed as the difference between the V after (t = 24, 48, 96 and 168 h) and before salting (0 h) ($\Delta V = V_t - V_{0h}$).

2.5 CHEMICAL COMPOSITION

The water, salt and fat contents were determined in the raw muscles. In addition, the salt and water content were also analyzed in each section of the brined samples.

The water content was determined by oven drying to constant weight at 102 ºC following the standard AOAC method 950.46 (AOAC, 1997). The salt content was analyzed after sample homogenization in 100 mL of distilled water at 9500 rpm in an ULTRATURRAX (T25, IKA Labortechnik, Germany) for 5 minutes. Supernatant was filtered through membrane filters (45 µm) and a 500 µl aliquot sample was taken and titrated in Chloride Analyzer equipment (Chloride Meter 926 L, Ciba Corning, U.K.) (Cárcel et al., 2007). The fat content was determined by using Shoxlet equipment and following the AOAC 991.36 (AOAC 1997). All analyses were performed in triplicate.

The salt (X_S), water (X_W) and fat (X_F) contents were expressed as percentages (%) in wet basis (w.b.). The salt gain (ΔX_S) and the water loss (ΔX_W) were also calculated for each salting time in the salting experiments.

2.6 MASS TRANSPORT MODELLING

Mass transport was addressed in order to gain insight into the rate of water loss and salt gain during meat salting, which would allow the existing relationship between both variables to be quantified. The salt and water transport during the salting experiments was modeled by considering a governing diffusion mechanism. In terms of mass transfer, cylindrical samples behaved as infinite-slab geometry bodies due to the fact that radial transfer was prevented by the metallic wall. Equation (Eq. 1) shows the governing diffusion equation for the salt taking into account the homogeneity and isotropicity of the solid and considering the effective diffusivity and the volume to be constant.

$$\frac{\partial X'_S(x,t)}{\partial t} = D_S\left(\frac{\partial^2 X'_S(x,t)}{\partial x^2}\right) \qquad \text{(Eq. 1)}$$

where X'_S is the local salt content (% w.b.), D_S is the effective diffusivity of the salt (m^2/s), x is the transport direction (m) and t is the time (s).

In order to solve the differential equation (Eq. 1), the initial and boundary conditions were assumed (Barat et al., 2011). The initial salt concentration in the sample was considered to be homogenous, the external resistance to mass transport was taken to be negligible due to the high turbulence created in the brine solution and the solid presented a symmetrical diffusion profile. Thereby, the solution of equation (Eq. 1) is presented in equation (Eq. 2) in terms of the local salt content at a specific time (Crank, 1975).

$$X'_S = X_{Se} + (X_{S0} - X_{Se}) \cdot \sum_{n=0}^{\infty} \frac{-1^n}{2n+1} \exp\left(\frac{-D_S(2n+1)^2\pi^2 t}{4L^2}\right) \cdot \cos\frac{(2n+1)\pi x}{2L} \qquad \text{(Eq. 2)}$$

By integrating equation (Eq. 2) for the solid volume (0<x<L), a relationship between the average salt content (X_S) in the sample and the time was obtained (Eq. 3).

$$X_S = X_{Se} + (X_{S0} - X_{Se}) \cdot \sum_{n=0}^{\infty} \frac{(8)}{\pi^2 (2n+1)^2} \exp\left(\frac{-D_S (2n+1)^2 \pi^2 t}{4L^2} \right) \quad \text{(Eq. 3)}$$

where X_{S0} and X_{Se} are the initial sal content and the salt content at the equilibrium, respectively. Analogous expressions to equations (Eq. 2) and (Eq. 3) were used to model the water transport.

Equation (Eq. 3) was fitted to the experimental salt content in order to identify the characteristic effective diffusivity (D_S) of the salt. D_S was estimated using the optimization tool, SOLVER, available in the spreadsheet Excel ™ (Microsoft, WA, USA). The optimization procedure consists of minimizing the sum of the squared differences between experimental and calculated equation (Eq. 3) salt content. Following the same procedure, the effective diffusivity of the water (D_W) during salting was also estimated.

2.7 REGRESSION MODELS

Multiple and simple regression models were developed between the dependent variable (ΔV) and the compositional factors (ΔX_S and ΔX_W), considering the LD and BF samples both separately and jointly. For that purpose, the BF and LD samples were split into two sets. The first set (model calibration, MC) was used to develop the models, including 36 samples (n_{MC}) when the BF and LD muscles were studied separately and 72 when the BF-LD muscles were studied jointly. The rest of the samples (n_{MV} = 12 or 24) were used for the model validation (MV). The regression analysis was performed by using Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

The accuracy of each model was estimated by computing the squared of the linear regression coefficient (R^2) and the Root Mean Square Error of Prediction (RMSE) value (Eq. 4).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_p - y_i)^2}{n}} \quad \text{(Eq. 4)}$$

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

3. RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION

The chemical composition of the raw BF and LD muscles used in this work is shown in Table 2. Non-significant ($p>0.05$) differences were observed between their pH and salt, fat and water contents of both muscles. However, it should be remarked that a marked variability was found in their water and fat content. The fat content ranged from 0.06 to 5.81% w.b. in LD and from 0.18 to 3.79% w.b. in BF, while the water content varied from 73.1 to 75.5% w.b. in LD and from 74.1 to 76.9% w.b. in BF (Table 2).

Table 2. Average values and standard deviations of salt (X_S), water (X_W), fat (X_F) content (% w.b.), pH and initial ultrasonic velocity (V_{0h}) of LD and BF raw muscles.

SAMPLE	X_S (% w.b.)	X_W (% w.b.)	X_F (% w.b.)	pH	V_{0h} (m/s)
LD	0.19±0.03	74.5±0.8	1.79±1.35	5.60±0.17	1557.0±6.3
BF	0.17±0.05	75.6±0.7	0.98±0.74	5.54±0.15	1533.9±4.0

3.2 INFLUENCE OF THE SALT AND WATER CONTENT ON THE ULTRASONIC VELOCITY FOR MODEL SAMPLES

Figure 2 shows the influence of the salt (X_S) and water (X_W) content on the ultrasonic velocity (V) in formulated (ground BF) Set 1 (Figure 2A) and Set 2

(Figure 2B) model samples. These experiments aim to split the effect of X_S and X_W on the V, as already mentioned.

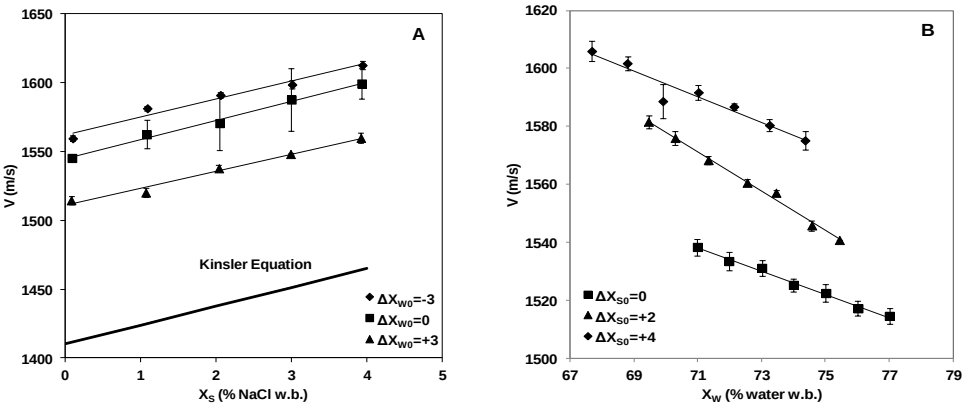

Figure 2. Influence of the salt (X_S) and water (X_W) content on the ultrasonic velocity (V) for formulated model samples. (A). Set 1 and Kinsler Equation (Eq. 5). (B). Set 2.

Figure 2A illustrates the influence of X_S on V by comparing the behavior of three batches of model samples with different initial water content. The initial V_{0h} was different in each batch, the average values being 1559.5, 1545.2 and 1514.5 m/s for model samples of ΔX_{W0} -3, 0 (raw meat) and +3% w.b., respectively. This experimental variability is mainly ascribed to the fact that V is dependent on the water content, which is different for each batch analyzed. Ultrasound travels faster in the solids (protein and fat), with high elastic modulus (Benedito et al., 2000), than in liquids (water), which explains the fact that V decreases as the moisture content rises. Otherwise, the increase of the salt content caused a rise of V due to the net gain of soluble solid which increased the bulk modulus of the saline solution contained in the meat. Thus, for the different batches of Set 1, significant ($p<0.05$) linear relationships were found between V and X_S with coefficients of determination (R^2) between 0.967 and 0.991 (Figure 2 and Table 3). The slope of the linear relationships (Table 3) represents the variation in V for a given change of X_S, e.g. a slope value of 12.9 indicates that a change in salt of ±1% causes a ΔV of ±12.9 m/s. As observed in Figure 2A and Table 3, similar slopes were obtained for every batch. Figure 2A

and Eq. 5 also shows the experimental Kinsler Equation (Eq. 5) at 2 ºC, which represents the influence of X_S on V for an aqueous solution at this temperature (Kinsler et al., 1982). It is worth mentioning that the slope of the linear relationship for the meat model samples was similar to that of the Kinsler Equation (Eq. 5). Therefore, it could be assumed that a given salt gain causes a similar ultrasonic velocity increase, regardless of the sample structure. No previous studies have reported how the salt content modifies the ultrasonic velocity in meat products.

$$V = 13.7Xs + 1410 \quad \text{(Eq. 5)}$$

Table 3. Linear regression models between ultrasonic velocity (V) and the salt (X_S) and water (X_W) in model samples.

		Equation	R^2
Batches Set 1	**ΔX_{W0} = -3% w.b.**	$V = 12.9 \pm 0.5 X_S + 1562 \pm 2$	0.967
	ΔX_{W0} = 0% w.b. (raw meat)	$V = 13.9 \pm 2.3 X_S + 1545 \pm 6$	0.991
	ΔX_{W0} = +3% w.b.	$V = 12.3 \pm 0.4 X_S + 1511 \pm 1$	0.982
Batches Set 2	**ΔX_{S0} = 0% w.b. (raw meat)**	$V = -4.0 \pm 0.2 X_W + 1822 \pm 15$	0.992
	ΔX_{S0} = 2% w.b.	$V = -6.8 \pm 0.1 X_W + 2052 \pm 22$	0.994
	ΔX_{S0} = 4% w.b.	$V = -4.4 \pm 0.2 X_W + 1902 \pm 13$	0.931

As regards the specific effect of the X_W on V (Figure 2B and Table 3), it was found to produce the opposite effect to X_S. For the three batches of Set 2, the higher the X_W (from 67.7 to 77.0% w.b.), the lower the V. In the case of the raw meat (ΔX_{S0} = 0), V varied from 1538.4 to 1514.6 m/s. Significant linear relationships ($p<0.05$) were found between V and X_W and R^2 ranged from 0.931-0.994. Similar slopes were found for batches of raw meat and ΔX_{S0} = +4% w.b, whereas a more pronounced drop in the V was found in the batch with the intermediate salt content (ΔX_{S0} = +2% w.b.), leading to a significantly ($p<0.05$) larger slope. This fact could be linked to the complex interaction of the salt with the meat protein matrix, since intermediate salt contents could

induce protein solubilization (Nguyen et al., 2010; Du et al., 2010), causing severe structural modifications that affect the V. The influence of X_W on V has already been reported in literature. Thus, Niñoles et al. (2011) proposed a similar linear relationship ($V = 1820.2 - 3.6X_W$, $R^2 = 0.790$ at 0 ºC) for the raw BF of Iberian pigs (X_W from 66.0 to 73.8% w.b.) to those found in Table 3. V has also been measured in a minced cured meat product (X_W from 18.9 to 39.8% w.b.) by Llull et al. (2002) who observed a reduction in V as the water content rised. Pascual et al. (1997) reported that V in beef meat (1572 m/s) was lower than in pork (1589 m/s) and lamb (1588 m/s) meat because of the higher X_W.

In Table 3, it may be observed that the average slope of the V-X_S relationship (13.0±0.8 m/s % w.b.) was much higher than that found for the V-X_W (-5.0±1.5 m/s % w.b.). This fact indicates that a change of 1% w.b. in salt would produce a change of 13.0 m/s, while a change of 1% w.b. in water would only result in a change of 5.0 m/s. Thereby, considering the data reported by Albarracín (2009) for typical compositional changes during ham salting, a salt gain of 4% would lead to an ultrasonic velocity increase of 52.1 m/s, while a decrease of 7% in the water content would increase V by 34.5 m/s. Therefore, from the results obtained for model samples, a rise of 86.6 m/s in V could be expected during ham salting for a salt gain of 4% and a water loss of 7%. In order to confirm this result, the salting of BF and LD pork muscles was studied, as well as how it experimentally affects the ultrasonic velocity. First of all, the salt and water transport during salting was thoroughly addressed in order to analyze the flow rates and quantify the existing relationship between both variables.

3.3 KINETICS OF WATER LOSS AND SALT GAIN DURING SALTING

Figure 3 shows the evolution of the X_S and X_W during salting of the BF and LD in the calibration sample set. As shown in Figure 3, the BF and LD samples gained salt and lost water during the salting process. A great experimental dispersion was observed, which was more marked for the water than for the

salt content for both muscles. On average terms, the experimental dispersion was also larger in LD than in BF (Figure 3).

The rate of salt gain and water loss reduced as salting progressed (Figure 3), which is a common fact in diffusion-governing transport. This fact demonstrates that both mechanisms are coupled and the rate is controlled by the effective diffusivities of both components. At the end of salting (168 h), more salt was gained and more water lost in LD than in BF muscle. Thus, the X_S increased from 0.19 to 3.47% in BF and from 0.20 to 4.21% in LD. However, in the case of the X_W, it decreased from 75.4 to 72.3% w.b. in BF and from 74.5 to 69.7% w.b. in LD. This could be linked to the different structure and initial composition of the BF and LD (Table 1).

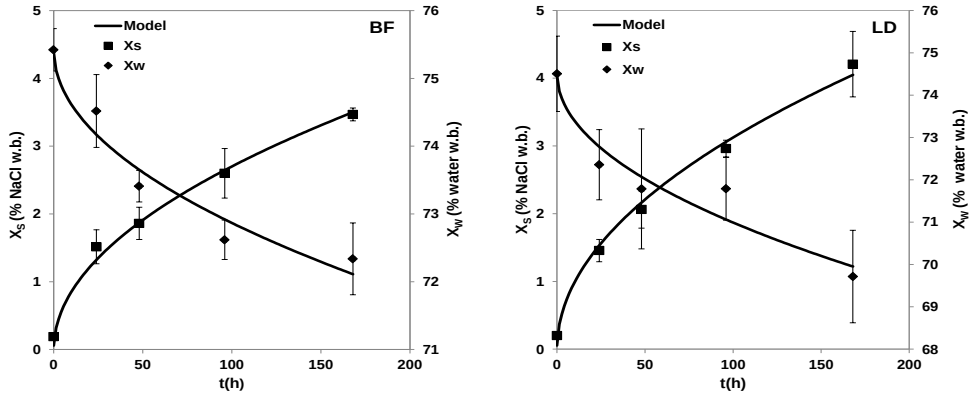

Figure 3. Experimental kinetics of water loss (X_W) and salt gain (X_S) in BF and LD samples of the calibration set during brining at 2 ºC and diffusion model.

BF and LD muscles achieved the equilibrium after 288 h with X_S of 14.4 and 13.2% w.b. and X_W of 65.2 and 65.0% w.b., respectively. The X_S and X_W figures obtained at equilibrium were consistent with the previously published results (Graiver et al., 2006; Håseth et al., 2009; Aliño et al., 2010).

Experimental kinetics of water loss and salt gain during salting were modeled by using a diffusion model (Eq. 3). The model provided a satisfactory fit of the experimental data (X_S and X_W) in both LD and BF muscles, as shown in Figure 3.

The fit was more accurate for X_S than X_W in both muscles due to its lower experimental dispersion. The explained variances for the water loss kinetics were of 0.969 and 0.939 for BF and LD, respectively, whereas higher explained variances were found for the salt gain kinetics (BF 0.996 and LD 0.993) due to its lower experimental dispersion.

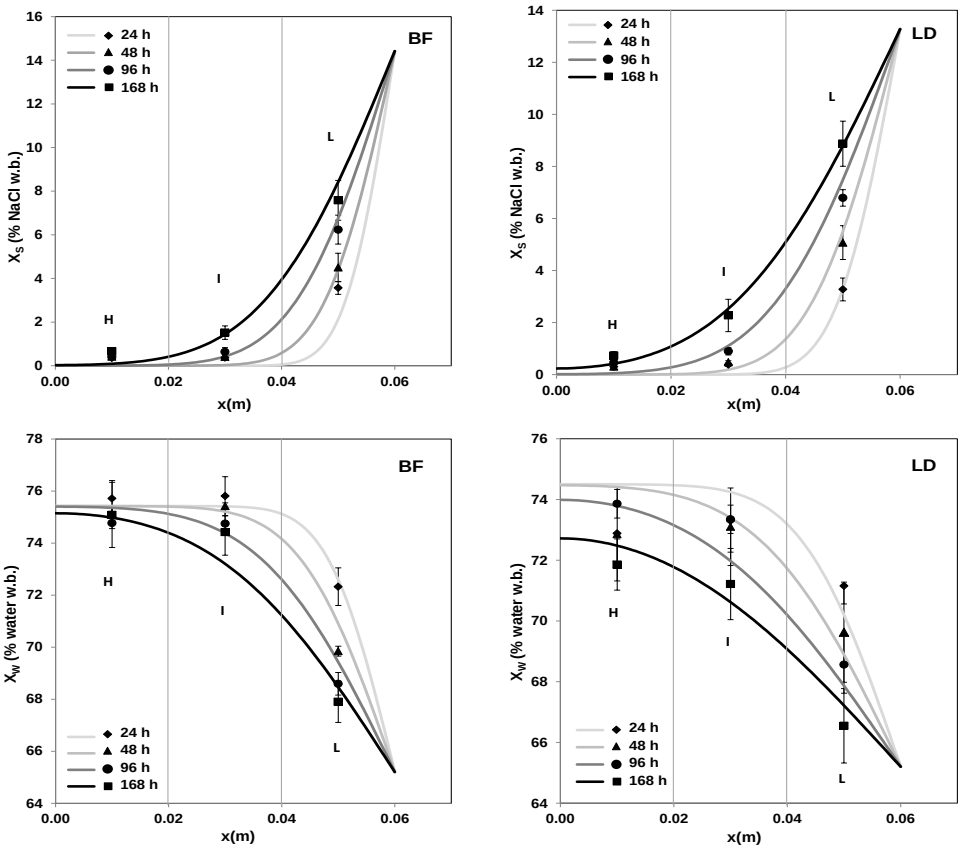

Figure 4. Simulated diffusion profile (continuous line) of salt (X_S) and water content (X_W) in BF and LD muscles and experimental salt and water content (dots) in the sections (H, I and L') of the samples for the calibration set during brining at 2 ºC (24, 48, 96 and 168 h).

The effective diffusivity identified by fitting the model to the experimental data (Eq. 3) was of 4.88 x 10^{-10} m^2/s for the water and 2.75 x 10^{-10} m^2/s for the salt transport in BF and 1.07 x 10^{-9} m^2/s for the water and 4.35 x 10^{-10} m^2/s for the

salt in LD. The values obtained for the effective diffusivities were consistent with others already reported in the literature. Siró et al. (2008) and Costa-Corredor et al. (2010) proposed a diffusion coefficient for the salt transport of 4.00 x 10^{-10} m^2/s and 3.18 x 10^{-10} m^2/s in LD and BF samples brined (20% w/w, brine concentration) at 5 and 2 ºC, respectively. Barat et al. (2011) proposed a diffusion coefficient of 6.17 x 10^{-10} m^2/s for the salt transport and 1.18 x 10^{-9} m^2/s for the water transport in brined pork meat (15% w/w, brine concentration) at 4 ºC.

The models were validated from the validation set, and the prediction of X_S was found to be more accurate (R^2 = 0.968) than in the case of X_W (R^2 = 0.938). Moreover, the diffusion profile for the salt and water contents in BF and LD was simulated from the identified effective diffusivities and compared to the experimental X_S and X_W of the sections (H, I and L') into which the samples were divided (Figure 4). In general terms, the calculated diffusion profiles for the salt content were close to the experimental ones of the meat sections, which reinforces the hypothesis that diffusion may be considered to be the predominant mass transport mechanism. From the effective diffusivities identified and using simplified diffusion models (only one term of the summatory of equation (Eq. 3)), particular numerical relationships for BF and LD samples could be established between X_S and X_W.

3.4 EVOLUTION OF THE ULTRASONIC VELOCITY VARIATION DURING SALTING

Initial ultrasonic velocity showed a marked experimental variability (V_{0h}; 1557.0±6.3 m/s for the LD and 1533.9±3.98 m/s for the BF), which was not ascribed to pH, fat or water content of the fresh muscles (Table 2), since significant relationships were not found ($p>0.05$) between these parameters and the ultrasonic velocity. As a consequence of this variability, it was considered convenient to assess the ultrasonic velocity variation (ΔV) between the final (after salting) and the initial ultrasonic velocity of the raw sample.

Thereby, the dispersion of the ΔV was lower than that of V and this parameter could be used as an indicator of the progress of the salting process.

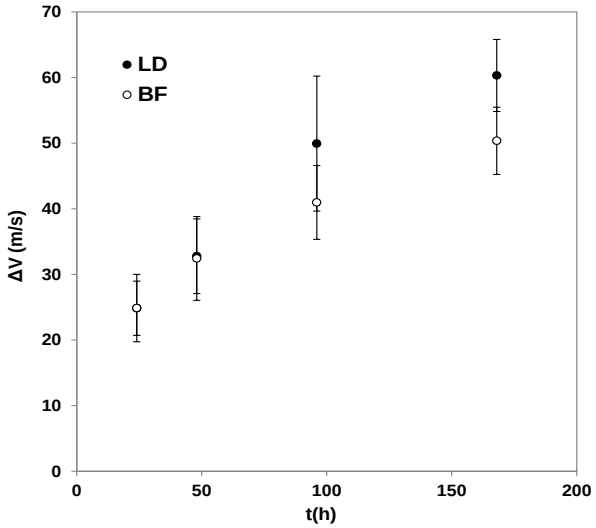

Figure 5. Ultrasonic velocity variation (ΔV) in BF and LD samples during brining at 2 ºC.

The evolution of the ΔV in the BF and LD samples during salting is shown in Figure 5. As is observed, ΔV increased during salting due to the net increase in solids ascribed to the salt gain and water loss. This fact has also been observed in fish products, where the V increased along with the solid-non-fat content (Ghaedian et al., 1998), but there have not been studies for meat products. In the range of 0 to 168 h of salting, the ΔV increased more in LD than in BF, 61.5 versus 49.3 m/s (Figure 5). This result is consistent with the final X_S reached in both muscles; since the LD muscle achieved a higher X_S than the BF (4.21% w.b. in LD and 3.47% w.b. in BF). As shown in Figure 5, despite using the ΔV, there was a large dispersion in both muscles for each specific salting time, especially after 96 and 168 h. As previously remarked, this could be linked to the experimental variability provoked by the heterogeneity of the raw meat samples, which led to a marked variability in the final salt content (Figure 3), especially after 96 and 168 h.

From the results found for formulated model samples (section 3.2), equation (Eq. 6) was obtained to assess ΔV as a function of the experimental ΔX_S and ΔX_W. Experimental and calculated ΔV are plotted in Figure 6.

$$\Delta V_{CAL} = 13.0 \cdot \Delta X_{S\,EXP} - 5.0 \cdot \Delta X_{W\,EXP} \quad \text{(Eq. 6)}$$

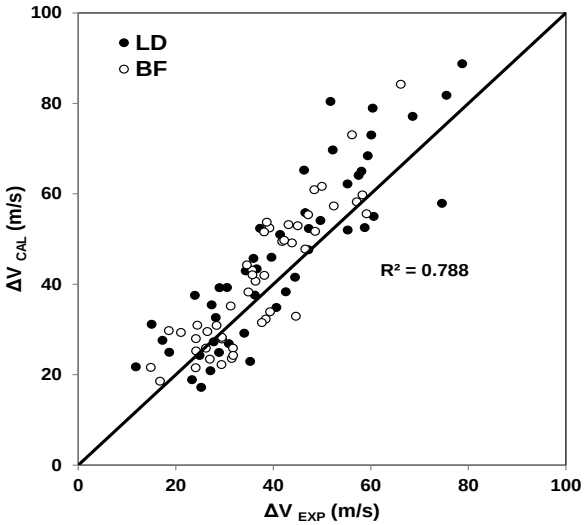

Figure 6. Calculated (ΔV_{CAL} using Eq. 6) and experimental (ΔV_{EXP}) ultrasonic velocity variation in BF and LD samples.

Figure 6 displays an acceptable correlation between the experimental and calculated ΔV in BF and LD samples (R^2 = 0.788). Consequently, it strengthens the hypothesis that ΔV is mainly caused by the salt gain and water loss during salting, as well as revealing that the influence of both variables should be non-dependent on the matrix structure. Major deviations were found at high ΔV (>50 m/s), where calculated values were higher than the experimental ones (Eq. 6). This could be associated to several factors, among others, the non-homogeneous distribution of salt in LD and BF samples, as observed in diffusion profiles (Figure 4), compared to the homogeneous distribution found in the model samples. In addition, the interaction effect of ΔX_W and ΔX_S on ΔV was not included in equation (Eq. 6), and this is probably more remarkable at high ΔX_W and ΔX_S.

The results reported in this section confirm that the changes in ultrasonic velocity during salting could be predicted from the salt gain and water loss. However, it is necessary to go further to assess the salt gain and water loss during brining from the change in the ultrasonic velocity. In this sense, it should be considered that salt gain is the main aim of salting rather than water loss, since the water content is going to be reduced in further processing steps of dry-cured meat products, such as drying and maturing and ageing. As a consequence, ultrasonic measurements should be mainly addressed to provide a good estimation of the salt content. For that purpose, a thorough analysis of how the ultrasonic velocity varies as a function of ΔX_W and ΔX_S is required, all of which is addressed in the following section.

3.5 SALT CONTENT PREDICTION FROM ULTRASONIC VELOCITY

The correlation between the ΔV and the salt gain (ΔX_S) and water loss (ΔX_W) of the BF and LD samples and their corresponding sections (H, I and L') are plotted in Figures 7 and 8, respectively. The goodness of the linear fit was better in the sections (Figure 8) than in the whole samples (Figure 7) (e.g. R^2 0.893 against 0.823 for Xs in BF), which could be explained by the wider experimental range of X_S and X_W covered by the sections. Due to the fact that the transport of salt and water is coupled, there is multicollinearity between the ΔX_S and ΔX_W factors that affects V. Thus, the relationship found between ΔV and ΔX_W was, to some extent, disturbed by ΔX_S and vice versa. This is the reason why the slopes of the linear relationships plotted in Figures 7 and 8 were higher than the ones shown in Table 3 for model samples, which was especially noticeable for X_W. Thus, in order to better quantify the effect of the water loss and salt gain on the ultrasonic velocity during salting, multiple linear regression models were established between the response variable (ΔV) and the compositional factors (ΔX_S and ΔX_W), considering LD and BF samples not only separately but also jointly.

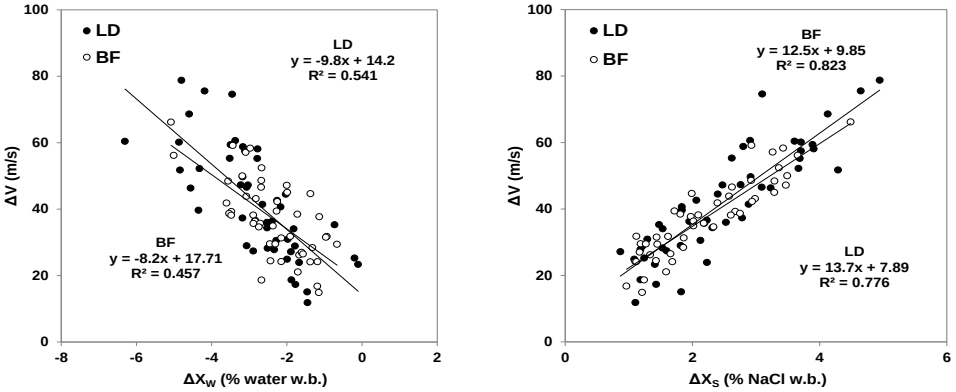

Figure 7. Relationships between the ultrasonic velocity variation (ΔV) and the salt gain (ΔX_S) and water loss (ΔX_W) in BF and LD samples.

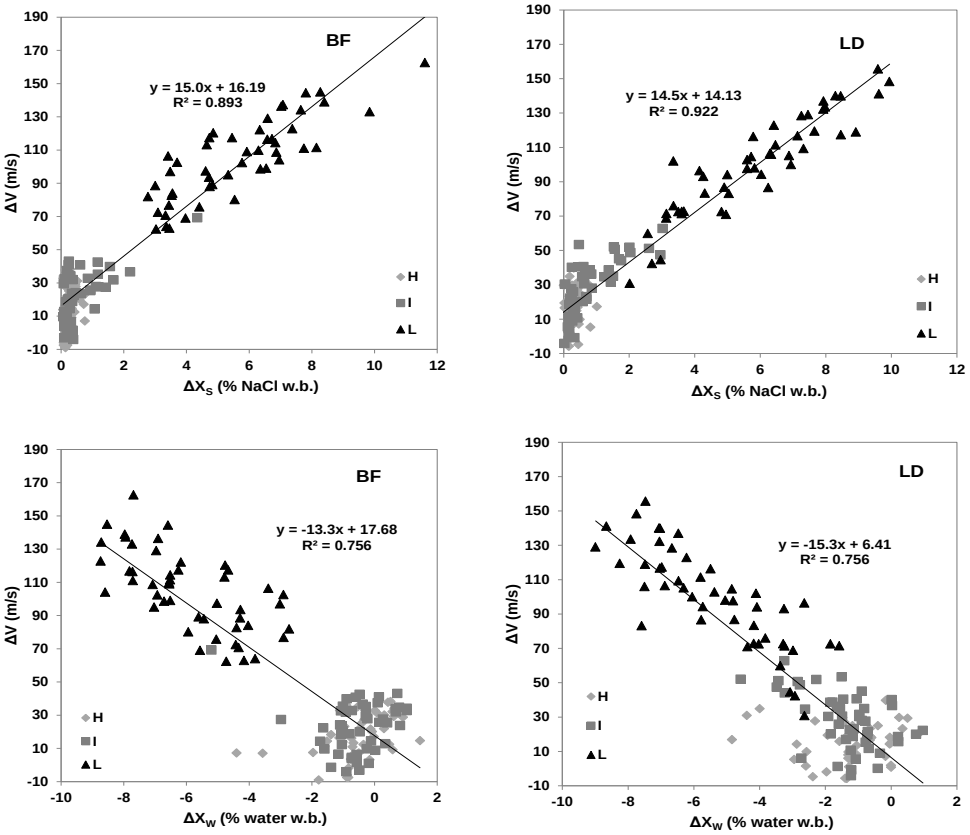

Figure 8. Relationships between the ultrasonic velocity variation (ΔV) and the salt gain (ΔX_S) and water loss (ΔX_W) in the sections (H, I and L') of brined BF and LD samples.

The performance of the regression models is displayed in Table 4 for both calibration (MC) and validation (MV) sample sets and compared with the simple linear regression models (Figure 7) only considering ΔX_S as factor. In overall terms, the accuracy of the models was similar for LD and BF samples. In the case of simple regression models, R_{MV}^2 and R_{MC}^2 figures were close to 0.8, except for the validation set of BF where R_{MV}^2 reached 0.865. The joint analysis of LD and BF samples did not reduce the accuracy of the models, which is relevant for industrial applications as the ultrasonic variation is not considered to be dependent on the meat source.

Table 4. Linear regression models between ultrasonic velocity variation (ΔV) and the salt gain (ΔX_S) and water loss (ΔX_W) in brined BF and LD samples.

SAMPLES	MODELS	n_{MC} [a]	$RMSE_{MC}$ (%) [b]	R^2_{MC} [c]	n_{MV} [a]	$RMSE_{MV}$ (%) [b]	R^2_{MV} [c]
LD	$\Delta V=8.09+13.06\Delta X_S-0.87\Delta X_W$	36	7.66	0.795	12	7.74	0.771
	$\Delta V=8.94+13.81\Delta X_S$	36	7.68	0.794	12	8.27	0.775
BF	$\Delta V=10.23+12.13\Delta X_S-0.03\Delta X_W$	36	4.84	0.800	12	5.21	0.865
	$\Delta V=10.25+12.15\Delta X_S$	36	4.84	0.800	12	5.21	0.865
LD-BF	$\Delta V=7.75+12.35\Delta X_S-1.29\Delta X_W$	72	6.51	0.800	24	6.21	0.814
	$\Delta V=8.67+13.43\Delta X_S$	72	6.58	0.796	24	6.45	0.804

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

The inclusion of the ΔX_W factor did not imply a better prediction of ΔV. Thus, statistical parameters for LD were similar when considering only ΔX_S (R^2_{MV} = 0.775 and $RMSE_{MV}$ = 8.27%) or ΔX_S and ΔX_W (R^2_{MV} = 0.771 and $RMSE_{MV}$ = 7.74%), which was also found for BF and BF-LD samples. Consequently, there was a non-significant ($p>0.05$) influence of ΔX_W on ΔV. This differed from the behavior observed in the model samples, which could be linked to several factors. Firstly, the influence of ΔX_W on the calculated ΔV (Eq. 6) was much

milder than that of ΔX_S (Table 3), e.g. in equation (Eq. 6), the coefficient for ΔX_S (13.0 m/s) doubled the one for ΔX_W (-5.0 m/s) (Table 3). Secondly, the greater variability of the water content during salting also contributes to a reduction in the significance of the ΔX_W factor over the ΔV. Finally, the existing relationship between ΔX_W and ΔX_S (see section 3.3.) implies that the effect of ΔX_W on the ΔV is included in the X_S coefficient and y-intercept value of the simple linear relationships shown in Table 4, both of them being, to some extent, overestimated. Therefore, the relationship found for LD-BF samples can be simplified considering only one factor (ΔX_S) and a simple model could easily be derived for predicting ΔX_S as a function of ΔV (Eq. 7).

$$\Delta Xs = \frac{\Delta V - 8.67}{13.43} \qquad \text{(Eq. 7)}$$

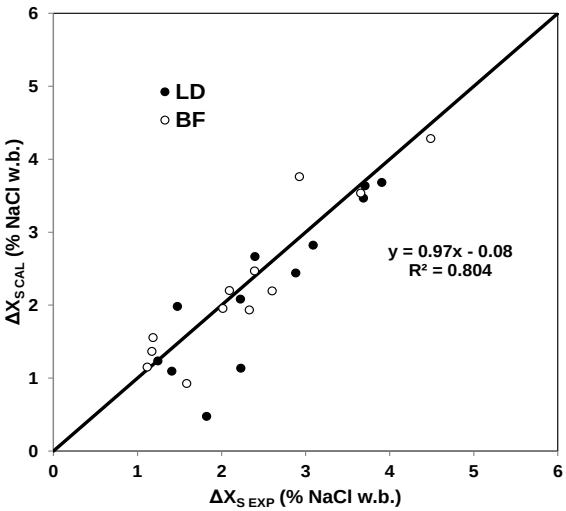

Figure 9. Calculated ($\Delta X_{S\ CAL}$ using Eq. 7) and experimental ($\Delta X_{S\ EXP}$) salt gain in the BF and LD samples for the validation set.

In Figure 9, the salt content ($\Delta X_{S\ CAL}$) calculated by using equation (Eq. 7) is plotted against the experimental one ($\Delta X_{S\ EXP}$) for the BF and LD samples of the validation set. In overall terms, a good correlation was found (R^2 = 0.804) and a random distribution between experimental and calculated values appeared

(Figure 9). ΔX_S was predicted with an error of under 0.25% w.b. in 50% of the samples from the validation set and with an error of under 0.42% w.b. in 75%,whereas the average error of the salt estimation was 0.48%. It should be remarked that outliers have not been removed from the analysis (Figure 9). Similarly, an error of 0.30% in non-destructive salt prediction for dry-cured hams is reported by Fulladosa et al. (2010) using computed tomography. Therefore, and considering the errors of the estimation, ultrasound inspection should not be presented as an analytical tool for estimating the salt content in pork meat. Otherwise, it has a great potential for non-invasive and non-destructive industrial applications for online quality control purposes.

4. CONCLUSIONS

The feasibility of using low-intensity ultrasound to assess the salt gain in brined pork meat has been demonstrated. The ultrasonic velocity in LD and BF muscles increased during salting. The influence of the water loss on the ultrasonic velocity during brining was simplified due to that the transport of water and salt are coupled. Thereby, a simple predictive model of the ultrasonic velocity change during salting was developed and validated to estimate the salt gain (average error 0.48%). Further work is required to customize the ultrasonic inspection for whole pieces, such as ham or loin, to be used in industry for online quality control purposes, which would greatly contribute to improve the homogeneity in the salt content in dry-cured meat products.

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REFERENCES

Albarracín, W. (2009). *Salado y descongelado simultáneo en salmuera para la obtención de jamón curado de cerdo de raza Ibérica.* (PhD thesis) Spain: Universitat Politècnica de València.

Aliño, M., Grau, R., Fernández-Sánchez, A., Arnold, A., & Barat, J.M. (2010). Influence of brine concentration on swelling pressure of pork meat throughout salting. *Meat Science*, 86, 600-606.

Aparicio, C., Otero, L., Guignon, B., Molina-García, A.D., & Sanz, P.D. (2008). Ice content and temperature determination from ultrasonic measurements in partially frozen foods. *Journal of Food Engineering*, 88, 272-279.

Association of Official Analytical Chemists (AOAC) (1997). Official methods of analysis. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Barat, J.M., Baigts, D., Aliño, M., Fernández, F.J., & Pérez-García, V.M. (2011). Kinetics studies during NaCl and KCl pork meat salting. *Journal of Food Engineering*, 160, 102-110.

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.

Cárcel, J.A., Benedito, J., Bon, J., & Mulet, A. (2007). High intensity ultrasound effects on meat salting. *Meat Science*, 76, 611-619.

Castro-Giráldez, M., Fito, P.J., & Fito, P. (2010). Application of microwaves dielectric spectroscopy for controlling pork meat (*Longissimus dorsi*) salting process. *Journal of Food Engineering*, 97, 484-490.

Collell, C., Gou, P., Arnau, J., & Comaposada, J. (2011). Non-destructive estimation of moisture, water activity and NaCl at ham surface during resting and drying using NIR spectroscopy. *Food Chemistry*, 129, 601-607.

Contreras, N.I., Fairley, P., McClements, D.J., & Povey, M.J.W. (1992). Analysis of the sugar content of fruits juices and drinks using ultrasonic velocity measurements. *International Journal of Food Science and Technology*, 27, 515-529.

Corona, E., García-Pérez, J.V., Ventanas, S., & Benedito, J. (2014). Ultrasonic characterization of the fat source and composition of formulated dry-cured meat products. *Food Science and Technology International*, 20, 275-285.

Costa-Corredor, A., Muñoz, I., Arnau, J., & Gou, P. (2010). Ion uptakes and diffusivities in pork meat brine-salted with NaCl and K-lactate. *Food Science and Technology*, 43, 1226-1233.

Crank, J. (1975). The mathematics of diffusion. Clarendon Press. Oxford Londres, Reino Unido.

Du, L., Zhou, G-H., Xu, X-L., & Li, C-B. (2010). Study on kinetics of mass transfer in water-boiled salted duck during wet-curing. *Journal of Food Engineering*, 100, 578-584.

Faulkner, D.B., Parrett, D.F., Mckeith, F.K., & Berger, L.L. (1990). Prediction of fat cover and carcass composition from live and carcass measurements. *Journal of Animal Science*, 68, 604-610.

Fulladosa, E., Santos-Garcés, E., Picouet, P., & Gou, P. (2010). Prediction of salt and water content in dry-cured hams by computed tomography. *Journal of Food Engineering*, 96, 80-85.

Ghaedian, R., Coupland, J.N., Decker, E.A., & McClements, D.J. (1998). Ultrasonic determination of fish composition. *Journal of Food Engineering*, 35, 323-337.

Graiver, N., Pinotti, A., Califano, A., & Zaritzky, N. (2006). Diffusion of sodium chloride in pork tissue. *Journal of Food Engineering*, 77, 910-918.

Håseth, T.T., HØy, M., Egelandsdal, B., & SØrheim, O. (2009). Nondestructive analysis of salt, water and protein in dried salted cod using computed tomography. *Food Engineering and Physical Properties*, 74, 147-153.

Kinsler, L.E., Frey, A.R., Coppens, A.B., & Sanders, J.V. (1982). Fundamentals of acoustics. (3 rd ed.) New York, U.S.A.: John Wiley and Sons (Chapter 4).

Larrea, V., Hernando, I., Quiles, A., Lluch, A.M., & Perez-Munuera, I. (2006). Changes in proteins during Teruel dry-cured ham processing. *Meat Science*, 74, 586-593.

Liu, D., Qu, J., Sun, D-W., Pu, H., & Zeng, X-A. (2013). Non-destructive prediction of salt content and water activity of porcine meat slices by hyperspectral imaging in a salting process. *Innovative Food Science and Emerging Technologies*, 20, 316-323.

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

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

Morlein, 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.

Nguyen, M.V., Arason, A., Thorarinsddottir, K.A., Thorkelsson, G., & Gudmundsdóttir, A. (2010). Influence of salt concentration on the salting kinetics of cod loin (*Gadus morhua*) during brine salting. *Journal of Food Engineering*, 100, 225-231.

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.

Pascual, A., Gisbert, M., Tomas, D., & López, N. (1997). Aplicación de los ultrasonidos en tecnología de la carne y los productos cárnicos (I). *Alimentacion. Equipos y Tecnología*, 6, 75-80.

Povey, M.J.W., & Harden, C.A. (1981). An application of the ultrasonic pulse echo technique to the measurement of the crispness of biscuits. *Journal of Food Technology*, 16, 167-175.

Prevolnika, M., Škrlep, M., Janeš, L., Velikonja-Bolta, Š., Škorjanc, D., & Čandek-Potokar, M. (2011). Accuracy of near infrared spectroscopy for prediction of chemical composition, salt content and free amino acids in dry-cured ham. *Meat Science*, 88, 299-304.

Ribeiro, F.R.B., Tedeschi, L.O., Stouffer, J.R., & Carstens, G.E. (2008). Technical note: A novel technique to assess internal body fat of cattle by using real-time ultrasound. *Journal of Animal Science*, 86, 763-767.

Scanlon, M.G. (2004). Low-intensity ultrasound for food research and the food industry. *Food Research International*, 37, 535-536.

Sigfusson, H., Ziegler, G.R., & Coupland, J.N. (2004). Ultrasonic monitoring of food freezing. *Journal of Food Engineering*, 62, 263-269.

Siró, I., Vén, C., Balla, C., Jónás, G., Zeke, I., & Friedrich, L. (2008). Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. *Journal of Food Science*, 91, 363-362.

Vestergaard, C., Andersen, B.L., & Adler-Nissen, J. (2007). Sodium diffusion in cured pork determined by 22Na radiology. *Meat Science*, 76, 258-265.

Vestergaard, C., Risum, J., & Adler-Nissen, J. (2004). Quantification of salt concentration in cured pork by computed tomography. *Meat Science*, 68, 107-113.

Food Control. 2016, 60, 646-655.

Ultrasonic characterization and online monitoring of pork meat dry salting process

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Abstract

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

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

1. INTRODUCTION

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

In the meat industry, dry salting is the most commonly-used salting process for the whole anatomical piece meat products and consists of covering the meat with coarse salt (Barat, Grau, Pagan-Moreno, & Fito, 2004). Usually, several salt/product layers are superimposed (Ventanas, 2001) and a particular salting time, temperature and relative humidity conditions are established for an entire batch (Jurado, Carrapiso, García, & Timón, 2002; Bello, 2008). Consequently, the salt content of meat pieces in the same batch varies greatly, not only due to the salting process itself but also to the heterogeneity in the weight, shape, composition and structure of the fresh meat (Gou, Composada, & Arnau, 2004; Ramírez & Cava, 2007; Castro-Giráldez, Fito, & Fito, 2010; Čandek-Potokar & Škrlep, 2012; Reig, Aristoy, & Toldrá, 2013). The variability linked to the dry salting process arises from the non-homogeneous ambient conditions of the salting chamber, the different position in the salting layers, the formation of brine between the sample surface and the dry salt and the size of the salt crystals, among other factors (Barat, Grau, Pagan-Moreno, & Fito, 2004; Van Nguyen, Arason, Thorarinsdottir, Thorkelsson, & Gudmundsdottir, 2010; Albarracín, Sánchez, Grau, & Barat, 2011). As a consequence of the variable salt absorption in meat pieces from the same batch, the behavior of each salted piece is different in the subsequent stages of the product manufacturing process, which gives rise to heterogeneous sensory and nutritional

characteristics of the final batch (Garcia-Gil, Muñoz, Santos-Garcés, Arnau, & Gou, 2014). In addition, due to the above-mentioned variability in the salting process, meat products are commonly over-salted to ensure the product's safety, which increases the energy consumption, lengthens the process time and has a great impact on the product quality (Garcia-Gil et al., 2012). Thus, the online monitoring of the salt content of meat products during salting could be a useful tool in the meat industry with which to describe the salt evolution and to determine the optimal salting time, according to the salt content targeted for each particular piece.

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

the salt gain in meat products with great structural complexity, such as whole hams, or to perform the online evaluation of the salt gain evolution in meat muscles during dry salting.

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

2. MATERIALS AND METHODS

2.1 MEAT SAMPLING

Fifteen fresh *Longissimus dorsi* (LD) and *Biceps femoris* (BF) pork muscles from *Large White* breed pigs were obtained from a local market. Muscles were selected with a pH ranging between 6.4 and 5.5. In both muscles, the subcutaneous fat and external connective tissue were removed. Samples of 20±2cm in length (L) and 1.0±0.1kg were obtained from each muscle, keeping the original width (Z) and thickness (T) of the muscle (Figure 1). Meat muscles were used for the online monitoring of dry salting and the salt gain estimation using ultrasound.

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

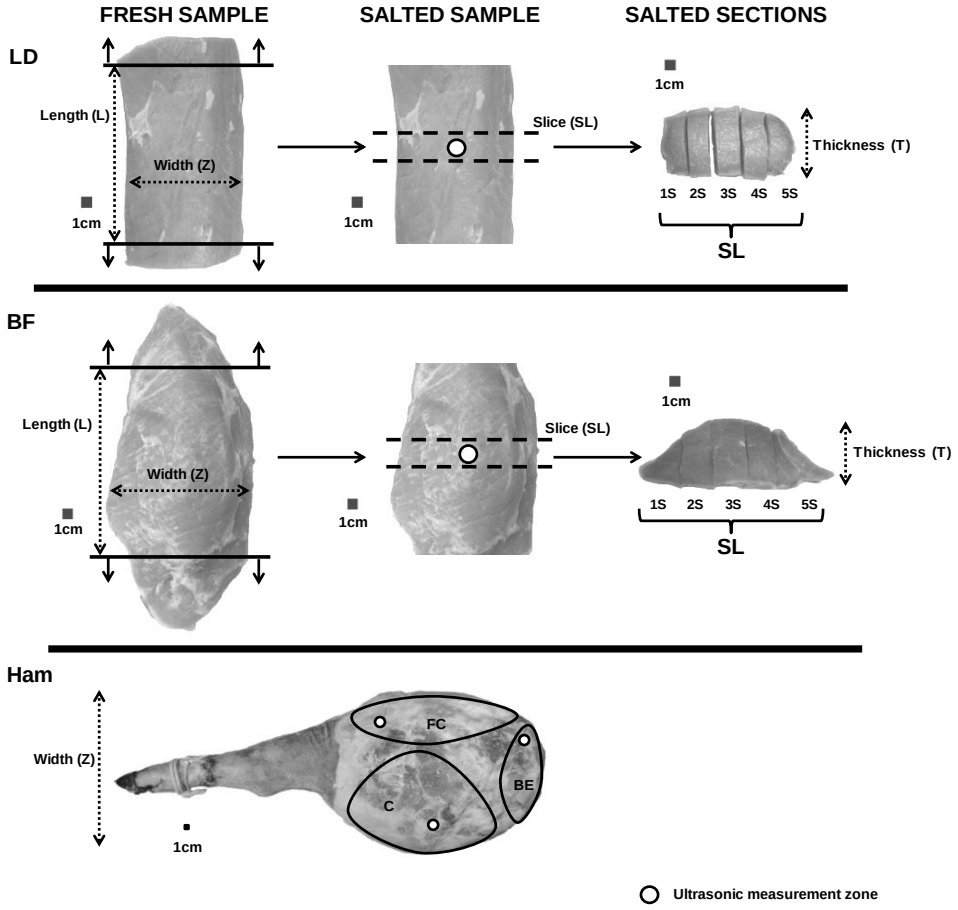

Figure 1. Sample preparation. Fresh sample, salted sample and sections (1S, 2S, 3S, 4S and 5S) of Longissimus dorsi (LD) and Biceps femoris (BF) muscles. Ultrasonic measurement zones in muscles and hams. C. Cushion, FC. Fore Cushion and BE. Butt End.

2.2 DRY SALTING EXPERIMENTS

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

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

2.3 ULTRASOUND MEASUREMENTS

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

Figure 2 shows the experimental set-up used for the measurement of the ultrasonic velocity during the dry salting experiments on LD and BF muscles. For the purposes of carrying out the ultrasonic measurements while the LD and BF meat was being salted, the sample was placed on 2kg of salt inside a plastic container (30x25x15cm) (Figure 2) and the transducers were coupled to the sample's thickness. Next, three temperature sensors were introduced; one was placed in the sample, one in the salt and the third one close to the transducer and the rest of the salt was added until the sample was covered. In this case, the transducers used had a small contact surface (A303S model, 1.77cm^2) so as to maximize the contact area between the meat sample and the salt. The ultrasonic velocity (V) was measured by the through-transmission mode at time intervals of 5min. Due to the fact that the meat sample shrinks during salting, the position of the upper transducer was manually adjusted both initially and

during the process with a force of 1N to maintain the contact between the sample and the transducers.

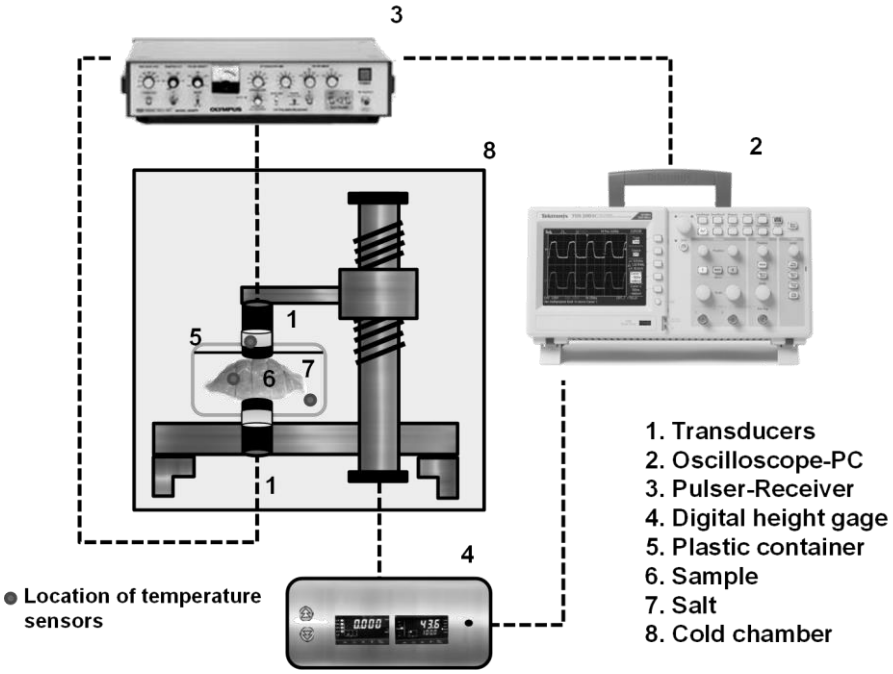

Figure 2. Experimental set-up for online ultrasonic measurements in *Longissimus dorsi* (LD) and Biceps femoris (BF) muscles during dry salting.

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

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

2.4 DETERMINATION OF FAT, WATER AND SALT CONTENTS

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

The fat, salt and water contents were determined in the fresh muscles and hams. To this end, a representative piece of each muscle was taken after obtaining the fresh muscle samples. In the case of the hams, 5 additional hams from the same batch were used for the purposes of measuring the initial average fat, salt and water contents of the fresh samples. In addition, the salt and water contents were also analyzed in each section of the salted LD and BF samples and in the mixture of lean and fatty tissues of salted hams. Thus, the fat content was determined by using the Shoxlet extraction method following AOAC 991.36 (AOAC, 1997). The water content was determined by oven drying

to constant weight at 102ºC following the standard AOAC method 950.46 (AOAC, 1997). The salt content was analyzed after sample homogenization (1g for fresh samples and 0.5g for salted samples) in 100mL of distilled water at 9500rpm in an ULTRATURRAX (T25, IKA Labortechnik, Germany) for 5min. Supernatant was filtered through membrane filters (45µm) and a 500µl aliquot sample was taken and titrated in a Chloride Analyzer equipment (Chloride Meter 926L, Ciba Corning, U.K.) (Cárcel, Benedito, Bon, & Mulet, 2007). All the analyses were performed in triplicate.

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

2.5 STATISTICAL ANALYSIS AND REGRESSION MODELS

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

Simple regression models were developed between the dependent variable (ΔV) and the salt gain both for the muscles (considering the LD and BF samples jointly) and the hams. In order to evaluate the capacity of the models for salt content determination, both the BF and LD samples and the hams were split into two sets. The first set (model calibration, MC), comprising 20 samples (n_{MC}), was used to develop the models. The rest of the samples (n_{MV} = 10) were used

for the model validation (MV). The regression analysis was performed by using Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

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

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

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

3. RESULTS AND DISCUSSION

3.1. FRESH MEAT CHARACTERIZATION

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

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

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

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

As previously mentioned, the salt absorption in the samples depends on their shape and dimensions, among other things. These factors are characteristic for each fresh ham, BF and LD sample. In the case of hams, the thickness and width of the pieces varied greatly (T = 10.7-12.8cm and Z = 28.7-34.3cm). In that of muscles, the BF samples were not only more irregular than the LD ones (Figure 1) but also thicker and wider (T = 4.4-6.4cm and Z = 14.9-18.7cm) than the LD samples (T = 3.6-5.3cm and Z = 10.7-12.0cm) (Table 1).

3.2. SALTING KINETICS IN DRY-SALTED MUSCLES AND HAMS

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

hams (Table 1). In fact, significant ($p<0.05$) relationships were found between the muscle dimensions (T and Z) and the compositional changes (ΔX_S and ΔX_W). Thus, the greater thickness and width of BF could explain its smaller ΔX_S and ΔX_W compared to LD (Table 2).

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

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

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

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

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

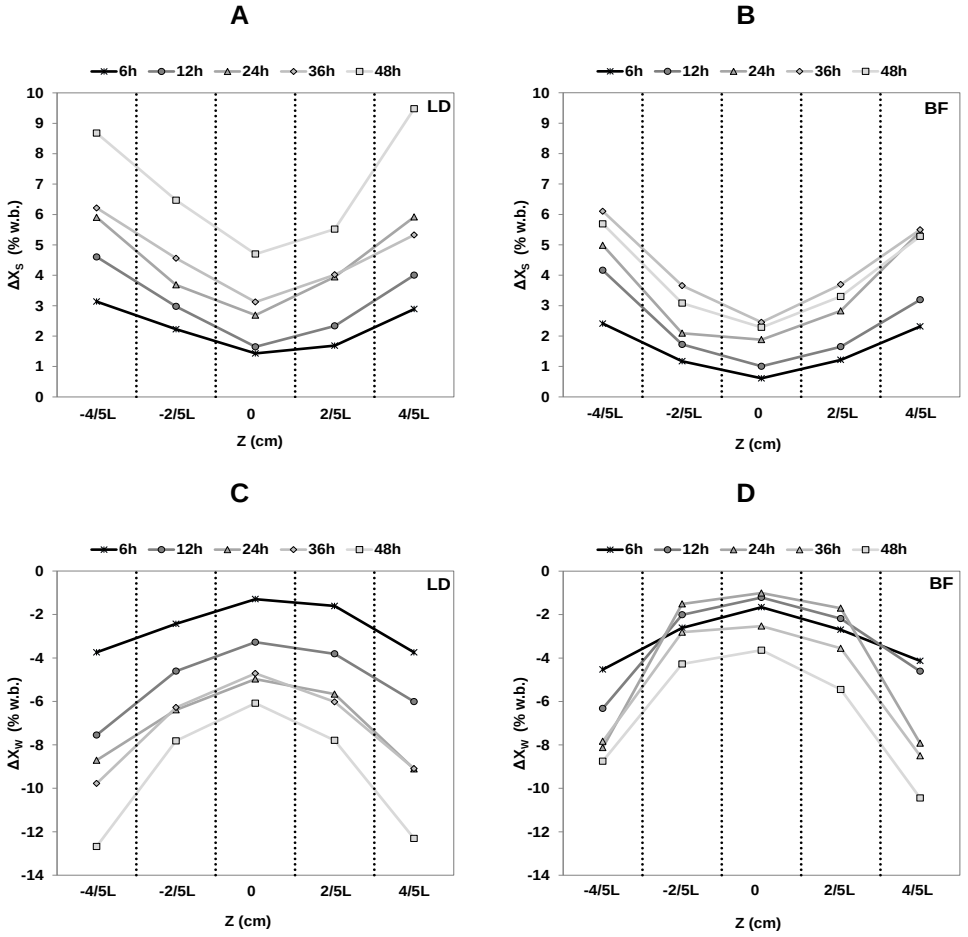

Figure 3. Profiles of salt gain (ΔX_S) and water loss (ΔX_W) in the slice (SL) of *Longissimus dorsi* (LD) (**A** and **C**) and *Biceps femoris* (BF) (**B** and **D**) muscles during dry salting (6, 12, 24, 36 and 48h) at 2ºC.

In Figure 3, the profile of the salt gain and water loss in the sections (from 1S to 5S) of the slice (SL) of the LD and BF samples is plotted at different salting times (6, 12, 24, 36 and 48h). In general terms, the ΔX_S and ΔX_W profiles exhibited a reasonably good symmetry in both muscles (Figure 3). As expected, the most marked compositional changes (water and salt) took place in the end sections (1S and 5S), the ΔX_S and ΔX_W in these sections being significantly ($p<0.05$) higher than those in the central (3S) and intermediate (2S and 4S) ones (Figure 3). As an example, the ΔX_S was 5.8±1.1% w.b. and the ΔX_W was -9.4±1.7% w.b. in the end sections (avg. 1S and 5S) of LD after 36h of dry salting, while the ΔX_S was 4.3±0.3 and 3.1±0.1% w.b. and the ΔX_W was -6.2±1.1 and -4.7±1.1% w.b. in the intermediate (avg. 2S and 4S) and central (3S) sections, respectively, for the same muscle and salting time. The differences between the sections grew as salting progressed, bending the initially flat profiles (Figure 3). The salt and water profiles also illustrate the fact that the compositional changes in LD were bigger than in BF. Figure 4 shows the relationship between the composition of SL and that of the zone of ultrasonic measurement (3S) for the salt gain (A) and the water loss (B). As can be observed, the ΔX_S and ΔX_W in 3S were lower than those found in the whole SL slice. Those differences could be explained by considering that, although the thickness of 3S was similar to that of the intermediate sections (2S and 4S), it was thicker than the end sections (1S and 5S) (Figure 1). Moreover, the contact area between the sample and the salt was larger in the external sections (1S and 5S), which also helps to increase the differences between the SL and 3S salt contents. Additionally, the transducers' surface (A303S model, 1.77cm^2) was in contact with the 3S section, which may hinder the mass transfer (salt and water) due to the fact that it reduces the contact area between the sample and the salt (Figure 1). Despite the experimental variability, significant ($p<0.05$) relationships were observed between the composition of 3S and SL in both muscles studied, showing high correlation coefficients (avg. R^2 = 0.898) (Figure 4). These results demonstrate that employing ultrasound to assess the compositional changes occurring in a particular zone could be used to evaluate

the changes in the whole meat piece, which would be of great interest for industrial purposes. Relationships like those shown in Figure 4 are dependent on the shape and dimensions of the meat piece; accordingly, if these factors are sufficiently different from those considered in the present study, new relationships should be developed. Alternatively, a larger number of transducers could be used to assess an average composition of the whole meat piece, as was done in the present study with an irregularly-shaped product, such as ham.

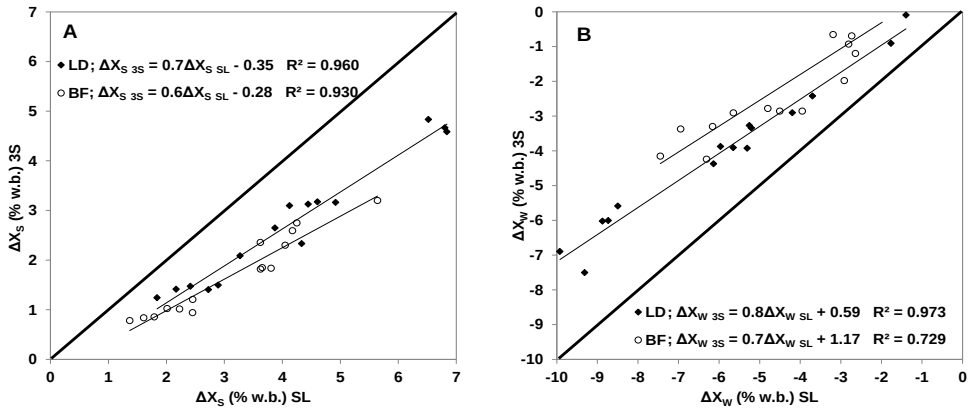

Figure 4. Relationship between the salt gain (ΔX_S) (**A**) and the water loss (ΔX_W) (**B**) in the slice (SL) and ultrasonic measurement section (3S) of *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles during dry salting at 2ºC.

3.3. ULTRASONIC MONITORING OF THE DRY SALTING OF MUSCLES

Figure 5 shows the change in the ultrasonic velocity (V) of the LD samples during dry salting at 2ºC for 12 and 24h. The same behavior (data not shown) was observed after the LD samples were submitted to the dry salting process for 6, 36 and 48h; in the case of the BF samples, this behavior was observed after every salting time (6, 12, 24, 36 and 48h). The initial V in the fresh muscle (V_0) varied markedly (1558.3±22.7m/s for the LD and 1525.8±10.5m/s for the BF). In previous studies, V has been linked to the meat composition and in particular to its water and fat content (Simal, Benedito, Clemente, Femenia, &

Rosselló, 2003; Corona, García-Pérez, Ventanas, & Benedito, 2014). However, in the present study, the V_0 was not significantly ($p>0.05$) related either to the water or the fat contents, probably because the analytical determinations in the fresh sample were not carried out where the V_0 was measured. In addition to composition, the great variability found for V_0 could be explained by the differences in the amount of connective tissue and its distribution among the muscles of different animals, which could lead to differences in their textural properties.

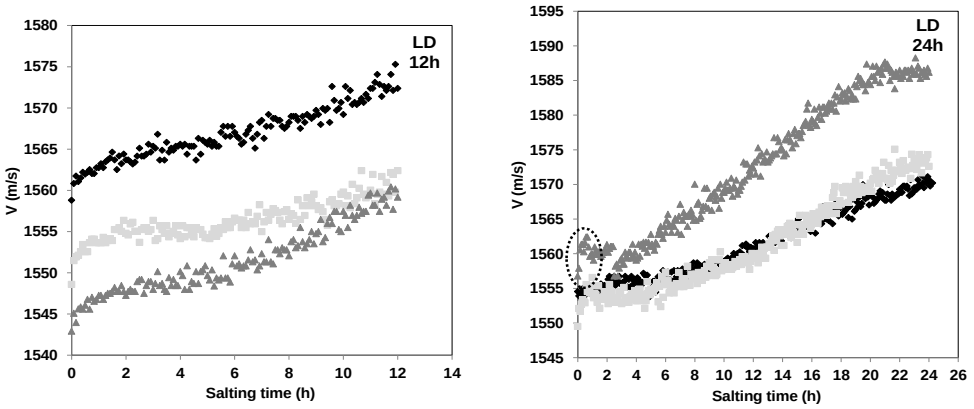

Figure 5. Ultrasonic velocity (V) evolution in the ultrasonic measurement zone (3S) of the *Longissimus dorsi* (LD) muscle during dry salting (12 and 24h) at 2ºC. Dotted circle indicates an initial sharp increase in the ultrasonic velocity (V) during ultrasonic monitoring. Each series correspond to a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

Figure 5 illustrates how the V increased gradually during dry salting. This behavior could be explained by the fact that ultrasound travels faster in solids, with a high elastic modulus (Benedito, Cárcel, Clemente, & Mulet, 2000), than in liquids (water). Thus, the rise in V is caused by the increase in the solid content, due to the salt gain and water loss that takes place during salting. The same behavior was observed by De Prados, García-Pérez, and Benedito (2015b), who reported an increase in the V in brine-salted cylindrical LD and BF samples. Similarly, Kinsler, Frey, Coppens, and Sanders (1982) observed that the greater the salt concentration in an aqueous solution, the higher the V. De Prados,

García-Pérez, and Benedito (2015b) measured the V in small cylindrical samples before and after salting; however, in the present study, the V was measured during dry salting in LD and BF muscle pieces (≈1kg) at intervals of 5min, which proves the feasibility of the online ultrasonic monitoring.

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

Despite the progressive increase in ultrasonic velocity during dry salting, some unexpected behavior was observed in the first hours of the process. As an example, Figure 5 illustrates this behavior for a 24h dry salting trial of LD. A sharp increase in V was found in the first 5h of dry salting (Figure 5), which was observed in almost all the trials. This behavior is considered to be unexpected because it does not match the normal kinetics for salt and water diffusion in meat. Due to the marked temperature effect on the V (Povey & Scanlon, 1983; Mulet, Benedito, Bon, & Sanjuan, 1999), it is thought that this steep temperature increase could be associated with a possible temperature rise caused by the sample positioning in the ultrasonic experimental set-up. However, a non-significant ($p>0.05$) relationship was found between the measured temperature and the rise in V. Thus, two tests were conducted in order to explain this steep initial increase in V. First, a methacrylate cylinder (6cm in length and 4cm in diameter) was covered with coarse salt (NaCl moisturized at 10% w/w) for 24h and V was measured every 5min. The results showed a constant V (Figure 6A), which was expected due to fact that the methacrylate cylinder is not affected by salt absorption. In the second test, the V was measured every 5min in the same methacrylate cylinder, but without salt for the first 6h. Afterwards, 1mL of water was added on both flat surfaces of the cylinder, between the sample and the transducers, and subsequently covered

with salt. During the first 6h the V was almost constant, followed by an increase for approximately 2h when water and salt were added (Figure 6B). This behavior could be explained by considering the formation of a salt solution between the transducers and the samples' surfaces. Therefore, the sharp increase in V during the first 5h of dry salting (Figure 5) could be linked to the formation of a salt solution between the transducer and the meat due to the initial extraction of water from the most external meat layers.

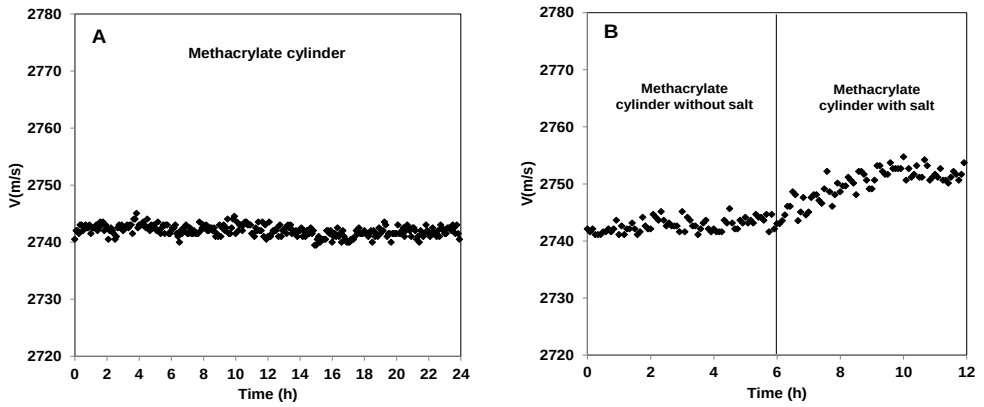

Figure 6. Ultrasonic velocity (V) evolution: (**A**) in a methacrylate cylinder (6cm in length and 4cm in diameter) covered with salt for 24h at 2ºC. (**B**) in a methacrylate cylinder; 6h without salt and 6h with salt and 1mL of water on the transducer's surfaces at 2ºC.

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

In order to obtain a good estimator of the dry salting progress based on the ultrasonic parameters and bearing in mind the great variability of V_0, the total velocity variation (ΔV) during salting after 6, 12, 24, 36 and 48h in LD-BF muscles and 2, 4, 7, 11 and 16 d in hams was considered (Table 2). The increase in ΔV during dry salting was related to the increase in the solid content of the sample (muscles and hams), as previously mentioned. In muscles, the ΔV value of the LD samples was higher than that of BF, the average ΔV of LD being 59.5m/s and that of BF 30.6m/s after 48h of dry salting. These results are

consistent with the greater compositional changes (salt and water) which occur in LD (ΔX_S = 6.7% w.b. and ΔX_W = -9.2% w.b., Table 2) compared to BF. In hams, the ΔV was 46.8m/s after 16 d of dry salting. Different values of ΔV were obtained in hams and LD-BF muscles for a similar salt gain and water loss (Table 2). As an example, the ΔV was 21.9m/s in hams for a ΔX_S of 1.1% w.b. and a ΔX_W = -5.1% w.b. after 2 d of salting. In contrast, the ΔV was 4.5m/s in BF for a ΔX_S of 1.6% w.b. and a ΔX_W = -3.2% w.b. after 6h of salting. De Prados, García-Pérez, and Benedito (2015b) also found higher values of ΔV for a similar salt gain and water loss in LD and BF samples that had been brine-salted for different times (24, 48, 96 and 168h). These authors found that cylindrical LD and BF samples reached a ΔV of 32.6m/s for ΔX_S = 1.9% w.b. and ΔX_W = -2.3% w.b., after 24h of brine salting. This difference could be linked to the type of ultrasonic measurement. In the present study, the ultrasonic velocity was measured online in LD and BF muscles at time intervals of 5min; consequently, transducers and muscles were in continuous contact during salting. This fact prevented the salt from being in direct contact with the sample surface where the ultrasonic velocity was being measured. On the contrary, in the hams used in the present study and in De Prados, García-Pérez, and Benedito (2015b), the V was measured before and after salting; therefore, the meat surface where transducers were located for velocity measurements had been in contact with salt, which can lead to protein denaturation, salt intake and water loss, giving rise to a fast surface textural increase, and, therefore, to a higher initial ΔV.

Despite the fact that ΔV reduced the wide dispersion of the V, there was still a great variability for each specific time (Table 2). As previously mentioned, this could be linked to the experimental variability provoked by the heterogeneity of the fresh meat samples and the salting process itself, which resulted in salted samples with different water and salt contents after the same salting time. The relationship between the salt gain (ΔX_S) and the ultrasonic velocity variation (ΔV) in both muscles (LD-BF) is shown in Figure 7. Since both muscles (LD and BF) exhibited the same behavior in the ΔV *vs* ΔX_S plot, a single

relationship was considered for both muscles. Figure 7 also shows the ΔV *vs* ΔX_S relationship for whole hams, whose slope is quite similar to the one found for muscles. A significant correlation ($p<0.05$) was found between ΔV and ΔX_S in LD-BF and in hams as well as between ΔV and ΔX_W in LD-BF. An increase in ΔX_S produced a rise in ΔV of hams and both muscles (LD-BF) (Figure 7), whilst the opposite trend was found in the case of an increase in ΔX_W ($\Delta V = -6.4\Delta X_W+2.9$ for LD-BF and $\Delta V = -1.9\Delta X_W+22.6$ for hams). However, the goodness of the fit was much more satisfactory for ΔX_S ($R^2 = 0.903$ in both muscles and $R^2 = 0.758$ in hams) than for ΔX_W ($R^2 = 0.611$ in both muscles and $R^2 = 0.200$ in hams). The poorer fit for ΔX_S and ΔX_W in hams could be attributed to the higher degree of variability linked to a greater structural (connective tissue, fat profile, bones and different types of muscles) complexity. Moreover, in hams, the salt and water content determinations were carried out considering the whole muscular tissue of each piece, while the V was measured at 30 points and the initial salt content and moisture are average values for 5 hams from the same batch. In contrast, the ΔV in muscles was related to the compositional changes in 3S (ultrasonic measurement zone).

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

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

surface in contact with the transducers is negligible, and therefore, the relationship between ΔV and ΔX_S is similar for both samples (muscles and hams).

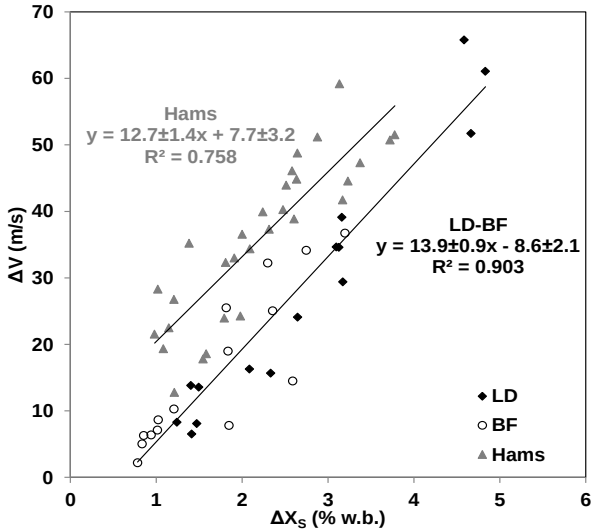

Figure 7. Relationship between the salt gain (ΔX_S) and the ultrasonic velocity variation (ΔV) in the ultrasonic measurement zone (3S) of the *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and hams.

The slope of the ΔV vs ΔX_S relationship was similar to that reported by Kinsler, Frey, Coppens, and Sanders (1982), who found that the ΔV increased by 13.7m/s per 1% increase in ΔX_S in a saline solution at 2ºC. De Prados, García-Pérez, and Benedito (2015b) also found slopes of 12.5m $s^{-1}\%^{-1}$ for BF and 13.7m $s^{-1}\%^{-1}$ for LD for the ΔV *vs* ΔX_S relationship and of -9.8m $s^{-1}\%^{-1}$ (LD) and -8.2m $s^{-1}\%^{-1}$ (BF) for the ΔV *vs* ΔX_W relationship, in brine-salted cylindrical samples at 2ºC. The fact that the slopes of the ΔV *vs* ΔX_S relationship found in this study (for whole hams and two different muscles) and those of Kinsler, Frey, Coppens, and Sanders (1982) and De Prados, García-Pérez, and Benedito (2015b) were similar, indicates that the same increase in the ΔX_S (1%) implies a similar change in the ΔV (≈13-14m/s), regardless of the type of salting process and the structure of the product. Therefore, according to these results, the ultrasonic parameter (ΔV) could be used to monitor the salt gain during dry salting in

meat products of great structural complexity, such as hams, or even in products which are different in nature, such as cheese or fish.

3.5. SALT CONTENT PREDICTION IN MUSCLES AND HAMS

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

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

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

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

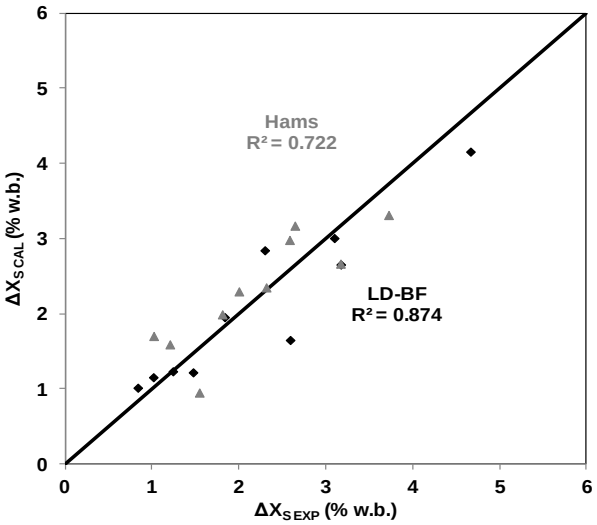

Figure 8. Calculated ($\Delta X_{S\ CAL}$) and experimental ($\Delta X_{S\ EXP}$) salt gain in *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and hams for the validation set.

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

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

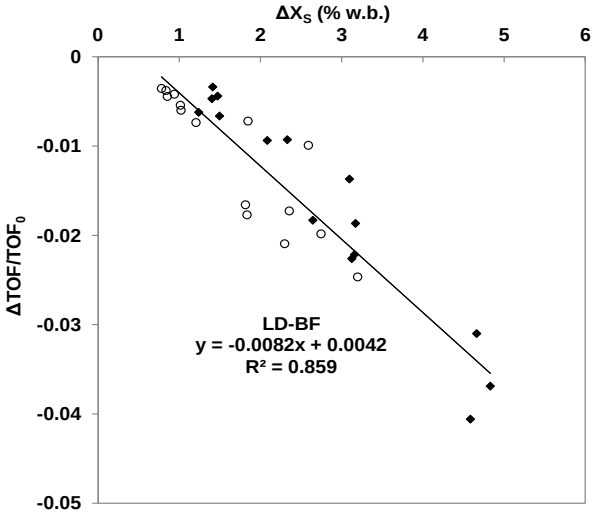

Figure 9. Relationship between the salt gain (ΔX_S) and the relative increase in the time of flight ($\Delta TOF/TOF_0$) in the ultrasonic measurement zone (3S) of the *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles.

Despite the correlation coefficients between the compositional changes and $\Delta TOF/TOF_0$ (R^2 0.859 for ΔX_S and 0.526 for ΔX_W; $RMSE_{\Delta XS}$ 0.46%) being slightly poorer than in the case of the ΔV (R^2 0.903 for ΔX_S and 0.611 for ΔX_W; $RMSE_{\Delta XS}$ 0.36%), the TOF could be considered a good ultrasonic parameter with which to characterize the salting process for online quality control purposes. The main advantage of considering the time of flight is that it is not necessary to measure the sample thickness by means of some electronic gage, something quite difficult to implement in an industrial environment where the pile dry salting is conducted. Moreover, in this study, the time of flight parameter was calculated by means of the through-transmission mode; however, by changing the ultrasonic arrangement, it could be obtained by means of the pulse-echo mode, which uses a single transducer that acts as emitter and receiver. This would reduce the cost of the industrial devices and would also minimize the impact of the measurements on the salt and water transfer. However, further work is required to test the feasibility of the pulse-echo mode on the

continuous monitoring of the salting process in meat products, such as muscles, but mostly in hams, which have a greater anatomical complexity.

4. CONCLUSIONS

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

ACKNOWLEDGEMENTS

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REFERENCES

Albarracín, W., Sánchez, I. C., Grau, R., & Barat, J. M. (2011). Salt in food processing; usage and reduction: a review. International Journal of Food Science and Technology, 46, 1329-1336.

Association of Official Analytical Chemist (AOAC). Official Methods of Analysis. (1997). (16th ed.). Washington: AOAC, International.

Barat, J. M., Grau, R., Pagan-Moreno, M. J., & Fito, P. (2004). Replacement of pile salting by simultaneous brine thawing-salting in Spanish cured ham manufacturing. Meat Science, 66, 603-608.

Barbin, D. F., ElMasry, G., Sun, D-W., & Allen, P. (2013). Non-destructive determination of chemical composition in intact and minced pork using near-infrared hyperspectral imaging. Food Chemistry, 138, 1162-1171.

Bello, J. (2008). Jamón curado: Aspectos científicos y tecnológicos: Perspectiva desde la Unión Europea. (1st ed.). Spain: Ed. Díaz de Santos, (Chapter 3).

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

Binkerd, E. F., & Kolari, O. E. (1975). The history and use of nitrate and nitrite in the curing of meat. Food and Cosmetics Toxicology, 13, 655-661.

Čandek-Potokar, M., & Škrlep, M. (2012). Factors in pig production that impact the quality of dry-cured ham: a review. Animal, 6, 327-338.

Cárcel, J. A., Benedito, J., Bon, J., & Mulet, A. (2007). High intensity ultrasound effects on meat salting. Meat Science, 76, 611-619.

Castro-Giráldez, M., Fito, P. J., & Fito, P. (2010). Non-equilibrium thermodynamic approach to analyze the pork meat (Longissimus dorsi) salting process. Journal of Food Engineering, 99, 24-30.

Corona, E., García-Pérez, J. V., Ventanas, S., & Benedito, J. (2014). Ultrasonic characterization of the fat source and composition of formulated dry-cured meat products. Food Science and Technology International, 20, 275-285.

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

De Prados, M., Fulladosa, E., Gou, P., Muñoz, I., García-Pérez, J. V., & Benedito, J. (2015a). Non-destructive determination of fat content in green hams using ultrasound and X-rays. Meat Science, 104, 37-43.

De Prados, M., García-Pérez, J. V., & Benedito, J. (2015b). Non-destructive salt content prediction in brined pork meat using ultrasound technology. Journal of Food Engineering, 154, 39-48.

Fulladosa, E., De Prados, M., García-Pérez, J. V., Benedito, J., Muñoz, I., Arnau, J., et al. (2015a). X-ray absorptiometry and ultrasound technologies for non-destructive compositional analysis of dry-cured ham. Journal of Food Engineering, 155, 62-68.

Fulladosa, E., Muñoz, I., Serra, X., Arnau, J., & Gou, P. (2015b). X-ray absorptiometry for non-destructive monitoring of the salt uptake in bone-in raw hams during salting. Food Control, 47, 37-42.

Garcia-Gil, N., Muñoz, I., Santos-Garcés, E., Arnau, J., & Gou, P. (2014). Salt uptake and water loss in hams with different water contents at the lean surface and at different salting temperatures. Meat Science, 96, 65-72.

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, 386-392.

Gou, P., Composada, J., & Arnau, J. (2004). Moisture diffusivity in the lean tissue of dry-cured ham at different process times. Meat Science, 67, 203-209.

Grau, R., Albarracín, W., Toldrá, F., Antequera, T., & Barat, J. M. (2008). Study of salting and post-salting stages of fresh and thawed Iberian hams. Meat Science, 79, 677-682.

Hæggström, E., & Luukkala, M. (2001). Ultrasound detection and identification of foreign bodies in food products. Food Control, 12, 37-45.

Jurado, A., Carrapiso, A. I., García, M., & Timón, M. L. (2002). Elaboración del jamón ibérico en cámaras de atmósfera controlada. Mundo Ganadero, 148, 60-62.

Kinsler, L. E., Frey, A. R., Coppens, A. B., & Sanders, J. V. (1982). Fundamentals of acoustics. (3 rd ed.). New York: John Wiley and Sons, (Chapter 4).

Mulet, A., Benedito, J., Bon, J., & Sanjuan, 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 assessment of Iberian pigs through back fat 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.

Povey, M. J. W., & Scanlon, M. G. (1983). Ultrasonic absorption in a polystyrene latex. Journal of Colloid and Interfacial Science, 93, 565-567.

Ramírez, R., & Cava, R. (2007). Carcass composition and meat quality of three different Iberian Duroc genotype pigs. Meat Science, 75, 388-396.

Reig, M., Aristoy, M-C., & Toldrá, F. (2013). Variability in the contents of pork meat nutrients and how it may affect food composition databases. Food Chemistry, 140, 478-482.

Røra, A. M., Furuhaug, R., Fjæra, S. O., & Skjervold, P. O. (2004). Salt diffusion in pre-rigor filleted atlantic salmon. Aquaculture, 232, 201-211.

Schivazappa, C., Degni, M., Nanni Costa, L., Russo, V., Buttazzoni, L., & Virgili, R. (2002). Analysis of raw material for the prediction of proteolysis of Parma ham. Meat Science, 60, 77-83.

Schöck, T., & Becker, T. (2010). Sensor array for the combined analysis of water–sugar–ethanol mixtures in yeast fermentations by ultrasound. Food Control, 21, 362-369.

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.

Van Nguyen, M., Arason, S., Thorarinsdottir, K. A., Thorkelsson, G., & Gudmundsdottir, A. (2010). Influence of salt concentration on the salting kinetics of cod loin (Gardu Morhua) during brine salting. Journal of Food Engineering, 100, 225-231.

Ventanas, J. (2001). Tecnología del jamón ibérico. De los sistemas tradicionales a la explotación racional del sabor y aroma. (1st ed.). Madrid: Ed. Mundi-Prensa, (Chapter 2).

Whittaker, A. D., Park, B., Thane, B. R., Miller, R. K., & Savell, J. W. (1992). Principles of ultrasound and measurement of intramuscular fat. Journal of Animal Science, 70, 942-952.

Ultrasonic online monitoring of the ham salting process. Methods for signal analysis: Time of flight calculation

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Abstract

In the dry-cured ham industry, an accurate control of the dry salting process results especially complex because of the high heterogeneity of the meat pieces and the effect of the different operational variables. The main objective of this study was to evaluate the feasibility of using ultrasound for the online monitoring of ham dry salting. For that purpose, hams were dry salted at different times (4, 7, 11 and 16 days) at 2ºC. Ultrasonic measurements were carried out automatically in the cushion zone during the salting process by the pulse-echo mode. Several signal analysis methods were considered to calculate the time of flight (TOF), studying which one allowed a better salting monitoring. The energy threshold, phase spectrum and cross correlation (between consecutive signals) methods were affected by the signal energy and could only be applied for specific ranges of signal amplitude/energy. By contrast, the cross-correlation method between non-consecutive signals (separated 1 h) was suitable for any signal energy, as was able to adequately monitor the dry salting process of hams. Moreover, the TOF parameter calculated by the cross-correlation method for signal analysis, was accurately related to the salt gain (R^2=0.88) after ham salting. This technique does not require the measurement of the sample thickness and can be carried out using an array of transducers located under the hams. Consequently, ultrasonic pulse echo measurements, could be considered as a simple, reliable and effective technique for industrial monitoring of ham dry salting.

Keywords: *Online salting monitoring, Ham, Time of flight, Energy threshold method, Cross-correlation method, Phase spectrum method*

1. INTRODUCTION

The online monitoring of food processes allows controlling the physico-chemical changes that take place in food matrices during manufacturing, in order to achieve the expected organoleptic and safety attributes. Nowadays, several new non-destructive techniques (X-Rays, microwaves, ultrasound, etc.) have been developed or adapted to measure a wide range of quality parameters during food processing.

In dry cured ham manufacturing, the dry salting stage consists in placing the hams, subcutaneous fat side down, piled and surrounded by coarse salt at 2-4ºC and relative humidity of 90-95% (Toldrà, 2010). The monitoring of the dry salting process is of great interest for the meat industry, since the salt gain in a batch of hams is largely variable as a consequence of the high heterogeneity of the meat pieces and the effect of the different operational variables (De Prados et al., 2015). The variable salt gain causes a non-uniform behaviour of hams during the next process stages, and consequently, final dry-cured hams of the same batch have heterogeneous sensory properties, affecting their quality.

Low-intensity ultrasound is one of the most promising non-destructive technologies for food process monitoring and optimization since it is accurate, fast, easy to implement on-line and relatively inexpensive. In this regard, the ultrasonic measurements have been used to monitor a wide range of food processes. For example, ultrasounds have been used to monitor the rennet of whole milk during cheese manufacturing (Koc et al., 2008), the alcoholic fermentation in synthetic broths (glucose, fructose and sucrose) and in natural media (must and wort) (Resa et al., 2009), the temperature and the ice content of fish (hake) during freezing (Aparicio et al., 2008), the ripening of tofu (Ting et al., 2009) or the crystallization of palm oil in O/W emulsions (Awad, 2004). Recently, De Prados et al. (2016) used the ultrasonic through-transmission technique to monitor the dry salting process of pork meat (*Biceps femoris* and *Longissimus dorsi* muscles). In most of these studies, velocity, calculated from

the time of flight (TOF) and the sample thickness, is the ultrasonic parameter obtained from the ultrasonic signal. Overall, the energy threshold method is the most often used to determine the TOF from the ultrasonic signals. However, when the signal is strongly attenuated in the sample, this method can lead to miscalculation of the TOF and other approaches for signal analysis, such as the cross-correlation and phase spectrum methods, must be considered (Leemans et al., 2009; Pallav et al., 2009; Sarabia et al., 2013).

In the work of De Prados et al. (2016), the through-transmission method was used for monitoring of meat muscles dry-salting. This set-up allows obtaining good signal amplitude, since the ultrasonic wave has to cross the sample only once. However, this configuration could bring about important problems when being implemented on-line for monitoring dry-salting of hams, since transducers should be located in both faces of piled hams and they should be perfectly aligned during the whole salting process. Another alternative might be the use of only one transducer located at the bottom of hams and working in the pulse-echo mode. However, using this simple and easy to implement on-line arrangement, the ultrasonic signal should cross twice a complex and with large thickness medium made of skin, bones and different muscles with a high degree of heterogeneity, which could hinder the measurement of the TOF.

Therefore, the aim of this study was to investigate the feasibility of using an ultrasound pulse-echo technique for the online monitoring of the dry salting process of hams and to determine the best signal analysis method to calculate the changes in the TOF during salting.

2. MATERIALS AND METHODS

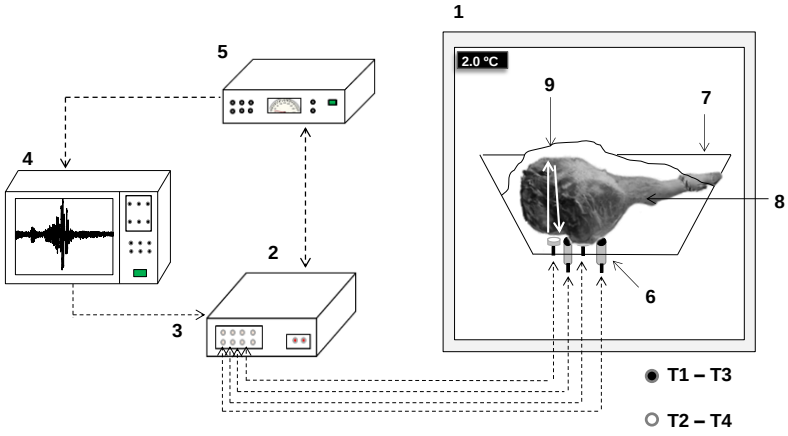
2.1 SAMPLES AND DRY SALTING PROCESS

Hams from the *Large White* breed were obtained from a local market. All the hams were dry salted by covering the piece with 15kg of coarse salt (NaCl moisturized at 10% w/w) at 2±1ºC in a cold chamber (AEC330r, Infrico, Spain).

Fresh hams and coarse salt were previously stored for 24h at 2ºC for the purposes of tempering. One ham was used for each salting time considered (4, 7, 11 and 16 days).

2.2 ULTRASOUND EXPERIMENTAL SET-UP

Ultrasonic measurements were carried out during the dry salting experiments of hams. The ultrasonic experimental set-up used (Figure 1) consisted of four narrow-band piezoelectric transducers of 1MHz and 0.5" crystal diameter, two of them type A (T1 and T3, A303S model, Panametrics, Waltham, MA, USA) and the other two type B (T2 and T4, A103S-RM model, Panametrics, Waltham, MA, USA); a pulser-receiver instrument (5077PR, Panametrics, Waltham, MA, USA), a digital input/output USB device (NI 6501, National Instruments, Austin, TX, USA) that a controlled the multiplexation and a high-speed digitizer (PXI/PCI-5112, National Instruments, Austin, TX, USA) installed in a PC.



1. Cold Chamber 2. Multiplexer 3. USB 4. Data Adquisition Card-PC 5. Pulser-Receiver
6. Tranducers 7. Container 8. Ham 9. Salt

Figure 1. Experimental set-up for online ultrasonic measurements in hams during dry salting.

For the purposes of carrying out the ultrasonic measurements while the ham was salted, the sample was placed in direct contact with the four transducers

(T1, T2, T3 and T4, Figure 1) and on a layer of 5kg of salt inside a plastic container (120x35x20cm). Afterwards, two temperature sensors were placed both in the salt and on the surface of the sample; and the remaining 10 kg of salt were added until the sample was entirely covered. The ultrasonic measurements were carried out using the pulse-echo mode at intervals of 5min in the cushion zone of the ham. By means of the multiplexation device, the signal for each transducer was digitized (25kpoints at 100Msamples/s, 10% pre-trigger points) and stored for subsequent signal analysis.

2.3 METHODS FOR SIGNAL ANALYSIS

Different signal analysis methods (energy threshold, cross-correlation and phase spectrum) were studied to determine which was the most appropriated to calculate the TOF for monitoring the salting process of hams.

The energy threshold method (ETM) determined the TOF in the signal. For that, the signal must surpass the noise caused by the own transducer's vibration after emission and the reflections of the ultrasonic wave on interfaces close to the transducer surface (zone A Figure 2); and then, the TOF is calculated in the arrival of the wavefront from the reflection on the interface sample/salt (zone B, Figure 2) when the amplitude of the signal exceeded the established threshold (0.1V) (Sarabia et al., 2013).

Additionally, the cross-correlation method (CCM) (Leemans et al., 2009) was considered. This method calculates the time of flight variation (ΔTOF) between two signals. For that purpose, the CCM calculates an array of the scalar products between the two signals in the signal's zone corresponding to the sample (non-noise) (zone B, Figure 2), displacing the first signal with respect to the second one (Leemans et al., 2009). The maximum value of the array determines when the two signals are overlapped and the position of the maximum in the array allowed calculating the ΔTOF. Several approaches were considered to calculate the ΔTOF in a measuring point for each salting time. First, the ΔTOF was calculated using the CCM between the initial signal or

reference signal (0h, RS) and the signal at that salting time (CCM-RS). Secondly, the CCM was performed for each pair of consecutive signals (separated 5min) and then the ΔTOF for each salting time was calculated by the addition of the ΔTOF between consecutive signals from the beginning of salting until the salting time considered (CCM-CS). Finally the same procedure was followed but performing the CCM between signals separated 1h (CCM-NCS).

Finally, the phase spectrum method (PSM) was used to calculate the ΔTOF between consecutive signals separated each 5min. For that purpose, the Fast Fourier Transform (FFT) transforms the signal data from the time domain to the frequency domain. The FFT of the signal is a complex number, whose module and phase give place to the module spectrum and phase spectrum, respectively. In the phase spectrum the frequency range where the signal presents the highest energy is established. In this range, the average of phase differences between the signals under analysis is calculated. So, the phase differences and the accumulated phase differences between the signals were calculated, and then, the between two signals to calculate the ΔTOF between both.

For each method, a specific software programmed in LABVIEWTM 2015 (National Instruments, Austin, TX, USA) was used.

2.4 CHEMICAL ANALYSIS

The salt and water content were determined in the salted ham. For that, four cylindrical salted samples (204±21g), which included the ultrasonic measurement zones, were taken by using a cylindrical cutter (5cm in diameter). Each cylindrical salted sample was grinded and homogenized before analytical determinations. The water content was determined by oven drying to constant weight at 102ºC following the standard AOAC method 950.46 (AOAC, 1997). The salt content was analyzed after sample homogenization (1g for fresh samples and 0.5g for salted samples) in 100mL of distilled water using an ULTRATURRAX (T25, IKA Labortechnik, Germany) at 9500rpm for 5min.

Supernatant was filtered through membrane filters (45µm) and a 500µl aliquot sample was taken and titrated in a Chloride Analyzer equipment (Chloride Meter 926L, Ciba Corning, U.K.) (Cárcel et al., 2007). All analyses were performed in triplicate. As the ham's integrity cannot be altered before salting, the initial average values of salt and water content were obtained from 6 hams of the same breed purchased to the same supplier. The final salt gain (ΔX_S) and the water loss (ΔX_W) were also calculated for each salting time.

3. RESULTS AND DISCUSSION

3.1 ULTRASONIC ONLINE MONITORING OF HAM DRY SALTING

As an example of the obtained ultrasonic signals, Figure 2 shows the first and last signal of one of the type A transducers in the 11 days salting trial (T1) The energy received in the zone A represents the own transducer's vibration after emission and also reflections of the ultrasonic wave on interfaces close to the transducer surface (for example, from the interface subcutaneous fat/lean meat). On the other hand, zone B includes the reflection of the wave on the interface meat/salt, and thus, shows the arrival of the wavefront when it has crossed twice the whole sample thickness. As can be observed in Figure 2 the TOF decreased from the first to the last day of salting in the 11 days salting trial. This same behaviour, a decrease of the TOF with the salting time, was observed in all the experiments carried out (4, 7, 11 and 16 days). In the example of Figure 2, the 11 days ultrasonic signal was displaced 10.5µs (calculated by the ETM) to the left compared to the 0 day signal, which illustrates the TOF decrease. This behaviour can be explained by the fact that ultrasound travels faster in solids with high elastic modulus, than in liquids (water) (Benedito et al., 2000). Thus, the water loss and the salt gain that takes place during salting increase the solid content in the ham, resulting into an increase of the ultrasonic velocity, and therefore, a decrease of the TOF. In this context, De Prados et al. (2015 and 2016) observed an ultrasonic velocity increase in *Biceps femoris* and *Longissimus dorsi* muscles brined salted and dry

salted, respectively. Moreover, the TOF decrease could be also associated to the sample's contraction (thickness reduction) during salting. The TOF decrease observed during salting could be used to monitor the progress of the salting process and to determine the salt content evolution. For that purpose, an accurate calculation of the TOF is required.

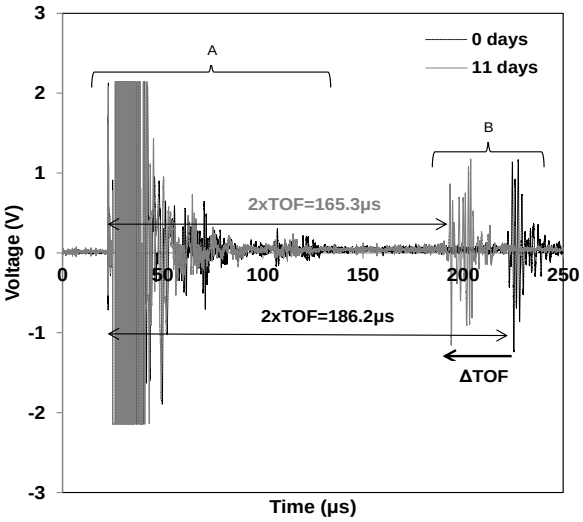


Figure 2. Changes in the ultrasonic signal during dry salting of ham (sample salted for 11 days at 2ºC) in the transducer type A (T1). Zone A represents the transducer's vibration after emission and the reflections close to the transducer's surface and zone B the reflection on the interface meat/salt (when the wave has crossed twice the sample).

3.2 TIME OF FLIGHT CALCULATION BY USING THE ENERGY THRESHOLD METHOD

Figure 3 depicts the TOF evolution of the ultrasonic signals corresponding to the four measuring points (T1, T2, T3 and T4) in the ham dry salted for 16 days at 2ºC. A similar behaviour was observed for the different measuring points in the remaining salting times assayed (data not shown). Figure 3 shows a downward trend of the TOF during salting time, which, as previously mentioned, is related to the increase of the meat solid content. In addition, the initial TOF (TOF_0) and the TOF evolution were different for each transducer (T1,

T2, T3 and T4). This fact could be linked to the compositional and structural differences in the ham, which lead to variations in the salt and water contents among measurement points during salting. Moreover, the different TOF_0 value could also be due to the thickness differences among measurement points in the ham.

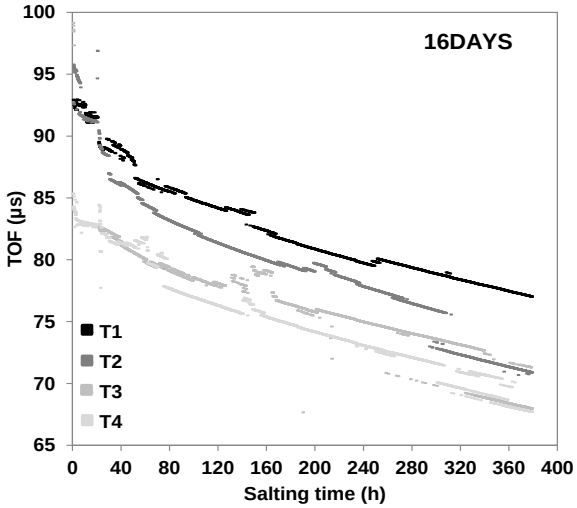


Figure 3. Time of flight (TOF) evolution in the ham dry salted for 16 days at 2ºC. TOF calculated with the ETM. T1, T2, T3 and T4 make reference to the four measuring points/transducers.

Taking into account the ultrasonic signal displacement shown in Figure 2 and the nature of the salt and water diffusion during salting, a progressive decrease of the TOF during salting could be expected. However, several abrupt changes in the TOF evolution (Figure 3) were observed in all the salting experiments. These abrupt changes might not be related to compositional variations due to they occurred randomly and appeared upward and downward (Figure 3). In order to find the origin of this problem, several ultrasonic signals during salting were analysed. As an example, the ultrasonic signals (non-noise) (zone B, Figure 2) in T4 at 0, 127, 254 and 380h during the salting were represented in Figure 4.

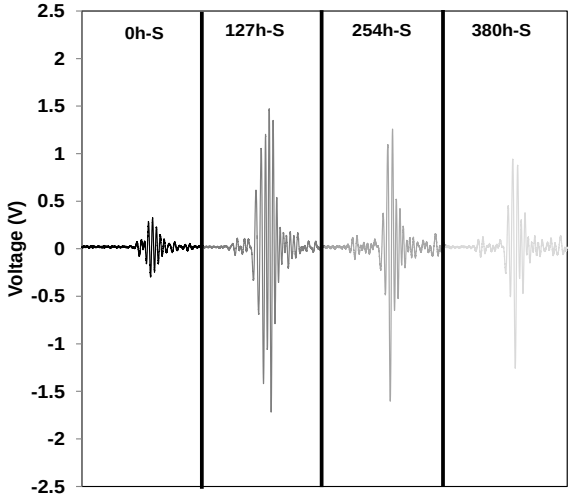


Figure 4. Change of the amplitude in the ultrasonic signals of T4 in the ham dry salted for 16 days at 2ºC (0, 127, 254 and 380h).

As can been observed in Figure 4, the amplitude in the ultrasonic signals fluctuated during salting. The random fluctuations in the signal amplitude make that the energy threshold method detects the wavefront arrival in a particular position and when the signal amplitude decreases or increases with time, the new peak crossing the threshold can randomly be displaced backward or forward independently of the salt gained by the samples as observed in Figure 3. The salt gain, water loss, sample's contraction and the chemical and structural changes in the protein matrix during salting might be the reason for the amplitude fluctuation in the ultrasonic signal. Different energy thresholds (0.05-1.2V) were considered in order to study its influence on the TOF calculation. However, the abrupt changes in the TOF evolution appeared in all cases.

When an ultrasonic signal presents a high energy level, despite of the amplitude fluctuations, the peak corresponding to the arrival wavefront will always overcome the established energy threshold (Sarabia et al., 2013), and thus, the ETM will always locate the arrival wavefront from the same peak,

avoiding fluctuations in the TOF calculation. However, the ultrasonic waves found in this study were attenuated after crossing twice the ham, and therefore, fluctuations in the signal energy resulted in miscalculations of the TOF evolution by the ETM. Therefore, the ETM, which was used by De Prados et al. (2016) for monitoring the salting process in LD and BF muscles, cannot estimate accurately the TOF in the attenuated ultrasonic signals obtained during the ham salting process.

3.3 TIME OF FLIGHT CALCULATION BY USING THE CROSS-CORRELATION METHOD

An alternative to the ETM to analyze the ultrasonic signals and to calculate the TOF is the cross-correlation method (CCM). Leemans et al. (2009) used this method to calculate the TOF and detect foreign bodies in cheese. Similarly, Pallav et al. (2009) analyzed ultrasonic signals with the cross-correlation method to determine the TOF and detect foreign bodies and additives in food. This method compares two ultrasonic signals and calculates the time of flight variation (ΔTOF) between them. In the present section, the ΔTOF was calculated between the initial ultrasonic signal or reference signal (0h-RS) and the remaining signals measured during the dry salting of hams (CCM-RS).

Figure 5A shows the ΔTOF decrease during salting at 2ºC. Although the cross-correlation method is not conditioned by the amplitude fluctuations of the arrival wavefront, the abrupt changes in the ΔTOF evolution also appears in this case. The abrupt changes observed in Figure 5A could be explained by the shape change in the ultrasonic signal during salting compared with the reference signal. As an example, Figure 6 shows the change in the shape of the signals from the RS to the signals obtained at 48, 100 and 168h in the ham dry salted for 7 days at 2ºC (transducer type A, T1). In Figure 6 is only represented the ultrasonic signal zone corresponding to the sample (non-noise) (zone B, Figure 2). As can be appreciated, the energy distribution of the time domain ultrasonic signal changes during the dry salting process. As previous

mentioned, these changes could be linked to the compositional changes (salt gain and water loss), the thickness reduction and the protein denaturing suffered by the ham during salting.

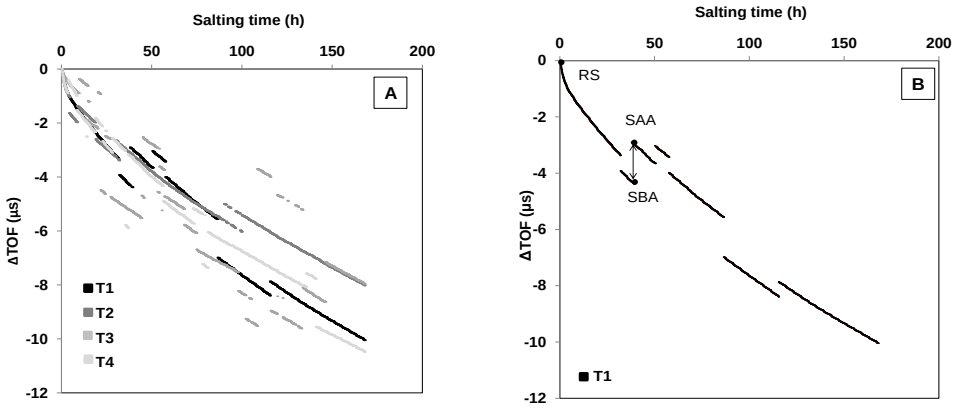


Figure 5. Time of flight variation (ΔTOF) evolution in the ham dry salted for 7 days at 2ºC. ΔTOF calculated using the CCM-RS. T1, T2, T3 and T4 make reference to the four measuring points/ transducers used **(A)**. Time of flight variation (ΔTOF) evolution in T1 of the ham dry salted for 7 days at 2ºC. ΔTOF calculated using the CCM-RS. The arrow shows an abrupt change in the ΔTOF evolution. SBA makes reference to the signal before the abrupt change and SAA after the abrupt change **(B)**.

As an example, the abrupt change observed in Figure 5B was analyzed. Figure 7A shows the overlap of RS and SBA signals obtained by the cross-correlation method (where the maximum of the cross-correlation array is found) while Figure 7B shows the overlap of RS and SAA signals). The maximum value obtained in the cross-correlation between RS-SBA (91.23μs) was quite different to the one obtained in the correlation between RS-SAA (94.04μs) which gives rise to the abrupt change observed in Figure 5B.

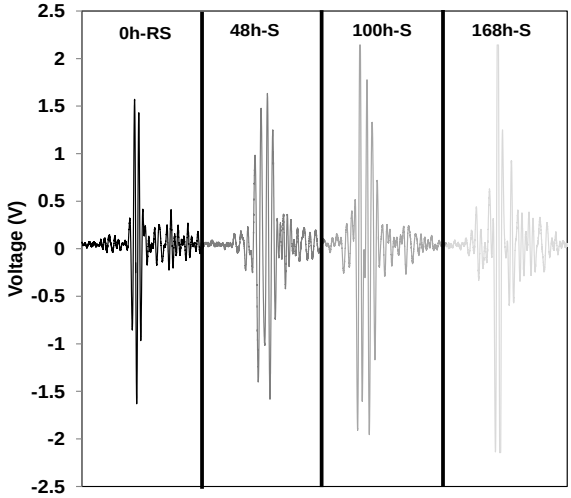


Figure 6. Change of the shape in the ultrasonic signals of T1 in the ham dry salted for 7 days at 2ºC (0, 48, 100 and 168h).

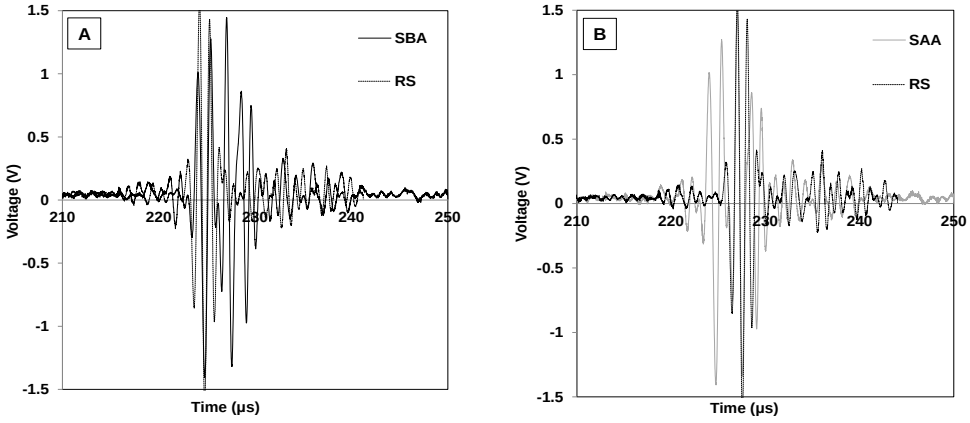


Figure 7. Overlapping between RS-SBA (**A**) and RS-SAA (**B**) ultrasonic signals for the maximum value of the cross-correlation array (transducer T1 in the ham dry salted for 7 days at 2ºC).

According to the results obtained in this section, the compositional and structural changes that take place in the meat during the salting process

modify the shape of the ultrasonic signal which results into miscalculation of the ΔTOF when CCM-RS is applied.

3.3.1 ΔTOF CALCULATION USING THE CROSS-CORRELATION METHOD BETWEEN CONSECUTIVE SIGNALS

From the analysis of the ultrasonic signals, it was observed that the changes of the shape in the ultrasonic signal did not happen abruptly between consecutive signals (5 min) but progressively during salting. Thus, in order to solve the problems found in the calculation of the ΔTOF with the CCM-RS discussed in the previous section, the cross-correlation method was applied between consecutive ultrasonic signals (separated each other 5min) (CCM-CS). Therefore, the ΔTOF between each pair of signals was calculated and the relationship between the ΔTOF and the salting time was represented by using the accumulated sum.

Figure 8 shows the ΔTOF evolution, calculated with the CCM-CS, in hams dry salted for 4, 7, 11 and 16 days. As can be observed, the ΔTOF decreased during salting and no abrupt change was found. In the 4, 7 and 11 days salting trials, the final ΔTOF value in T1, T2, T3 and T4 measuring points differed less than 7% between each other. The small difference between transducers might be explained by the heterogeneous salting process which, as mentioned in section 3.2, gives rise to different salt content, thickness reduction and protein denaturation in the T1-T4 measuring points, and therefore, to differences in the final ΔTOF. Additionally, the final average ΔTOF value in the 4, 7 and 11 days salting experiments, calculated with the CCM-CS, was not significantly ($p>0.05$) different from the values obtained with the CCM-RS (Table 1).

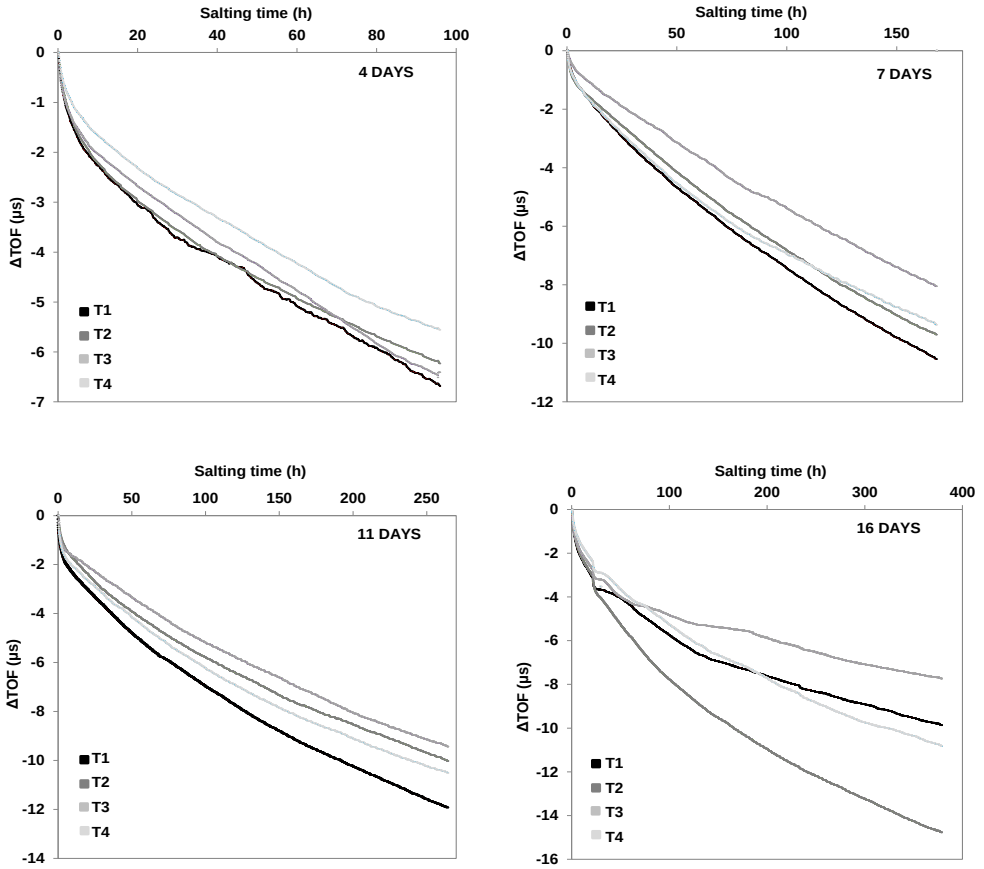


Figure 8. Time of flight variation (ΔTOF) evolution in hams dry salted for 4, 7, 11 and 16 days at 2ºC. ΔTOF calculated with the CCM-CS. T1, T2, T3 and T4 make reference to the four measuring points/ transducers used.

However, in the ΔTOF of T1, T3 and T4 transducers during the 16 days salting trial an anomalous decrease trend was observed (Figure 8). As a consequence, the final ΔTOF value using the CCM-CS was -9.8,-7.7 and -10.8μs for T1, T3 and T4, respectively, much lower than those calculated using the CCM-RS (-11.3, -11.9 and -12.7μs for the T1, T3 and T4, respectively). Only T2 calculated with the CCM-CS (-14.8μs) had a similar decrease to that observed with the CCM-RS (-14.9μs) and a similar trend to that observed in the rest of salting times (Figure 8). In order to explain these differences, the energy content of the signal was

calculated by integrating the time domain signal corresponding to the reflection in the meat/salt interface (zone B, Figure 2). In this context, a lower ultrasonic signal energy was observed in the T1, T3 and T4 of the 16 days salting experiment (3.7±0.3Vμs) compared to the rest of transducers and salting experiments (11.3±3.6Vμs). Therefore, for the monitoring of ham dry salting, the CCM-CS was suitable for ultrasonic signals with energy levels over 4Vμs. However, for signals with lower energy levels, this methodology was not able to detect the small TOF changes between consecutive signals (separated each other 5min) and therefore was not appropriated for the TOF calculation. Thus, in order to analyze ultrasonic signals with low energy levels, the development of complementary methodologies is necessary.

Table 1. Final average time of flight variation (ΔTOF) values calculated using the different signal analysis methods (CCM-RS, CCM-CS, CCM-NCS and PSM).

	CCM-RS(μs)	**CCM-CS(μs)**	**CCM-NCS(μs)**	**PSM(μs)**
4 days	-6.2 ± 1.9^{a1}	-6.2 ± 0.5^{a4}	-5.5 ± 0.5^{ab6}	-3.8 ± 1.2^{b10}
7 days	-9.1 ± 1.3^{c2}	-9.3 ± 1.0^{c5}	-8.8 ± 0.8^{c7}	-5.4 ± 1.2^{d11}
11 days	-9.5 ± 1.4^{e2}	-10.4 ± 1.1^{e5}	-10.3 ± 1.3^{e8}	-9.5 ± 0.5^{e12}
16 days	-12.7 ± 1.6^{f3}	-10.8 ± 2.9^{g5}	-14.4 ± 0.8^{f9}	-13.6 ± 0.4^{f13}

Means values and standard deviations between T1, T2, T3 and T4. Different letters in the same row indicate significant ($p<0.05$) differences between the methods and different numbers in the same column significant ($p<0.05$) differences between salting times. CCM-RS makes reference to the cross-correlation method between the initial ultrasonic signal or reference signal (0h-RS) and the remaining signals. CCM-CS the cross-correlation method between consecutive signals (each 5min) y CCM-NCS between non-consecutive signals (each 1h). PMS is the spectrum phase method between signals separated 5min.

3.4 ΔTOF CALCULATION FOR LOW ENERGY ULTRASONIC SIGNALS

Two alternative methodologies to calculate the TOF were studied: the cross-correlation method between non-consecutive ultrasonic signals (separated 1h) (CCM-NCS) and the phase spectrum method (PSM).

3.4.1 CALCULATION OF TOF USING THE CROSS-CORRELATION METHOD BETWEEN NON-CONSECUTIVE ULTRASONIC SIGNALS

As can be observed in Figure 9, the ΔTOF evolution calculated using CCM-NCS for T1, T2, T3 and T4 transducers had a similar behaviour than the ΔTOF calculated using CCM-CS for T2. Moreover, the final ΔTOF value in T1 was -13.8μs, -15.7 μs in T2, -14.2μs in T3 and -14.0μs in T4 using the CCM-NCS, similar values that the one obtained in T2 (-14.8μs) using the CCM-CS. Therefore, the CCM-NCS solved the miscalculation of the ΔTOF caused by the low energy level of the ultrasonic signals found in the 16 days salting experiment (Figure 7). In addition, this method showed a very similar decrease of the ΔTOF with salting time for the rest of transducers and salting times (data not shown), to the one found for the CCM-CS (Figure 8). Consequently, non-significant ($p>0.05$) differences were observed in the final average ΔTOF value between the CCM-CS and the CCM-NCS (Table 1).

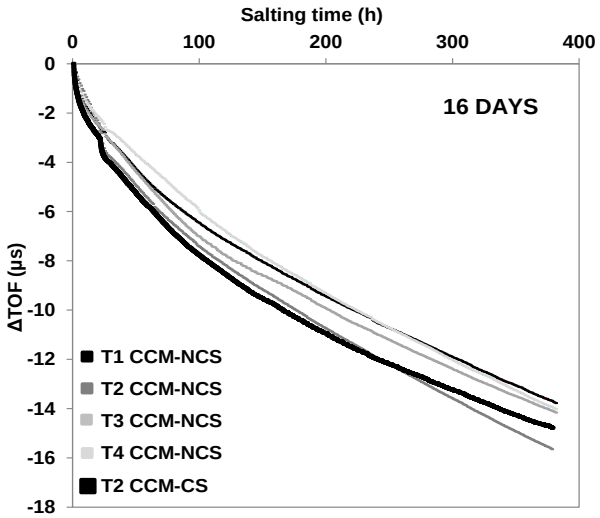


Figure 9. Time of flight variation (ΔTOF) evolution in the ham dry salted for 16 days at 2ºC. ΔTOF calculated with the CCM-NCS for the T1, T2 T3 and T4 and with the CCM-CS for the T2. T1, T2, T3 and T4 make reference to the four measuring points/transducers.

3.4.2 TOF CALCULATION USING THE PHASE SPECTRUM METHOD

When calculating the ΔTOF by the phase spectrum method (PSM), a similar trend in all the transducers (T1, T2, T3 and T4) during the 16 days salting experiment was observed (Figure 10). In particular, after the 16 days salting the ΔTOF was -13.4μs in T1, -14.2 μs in T2, -13.2μs in T3 and -13.7μs in T4. In addition, a similar final average ΔTOF was observed in the 16 days salting experiment with the PSM (-13.6±0.4μs) and the CCM-NCS (-14.4±0.8μs) (Table 1). In the 11 days salting experiment, the ΔTOF evolution was similar in all the transducers and non-significant ($p>0.05$) differences were observed in the final average ΔTOF values obtained with the PSM, the CMM-CS and the CCM-NCS (Table 1). However, the final ΔTOF values were lower than expected in T2 and T3 in the 4 days salting trial and in all the transducers in the 7 days salting experiment (Figure 10). Additionally, overall significant ($p<0.05$) differences in the 4 and 7 days salting experiment were found in the final average ΔTOF values when it was calculated using the PSM and the cross correlation methods (CMM-CS and CCM-NCS) (Table 1). From the analysis of the signal obtained from T2 and T3 in the 4 days salting trial and in all the transducers in the 7 days salting experiment, it could be observed that the signals were partially saturated (voltage >2V, data not shown), and therefore, the calculation of the frequency distribution through the FFT is conditioned by this fact, invalidating the use of the PSM for the calculation of the ΔTOF.

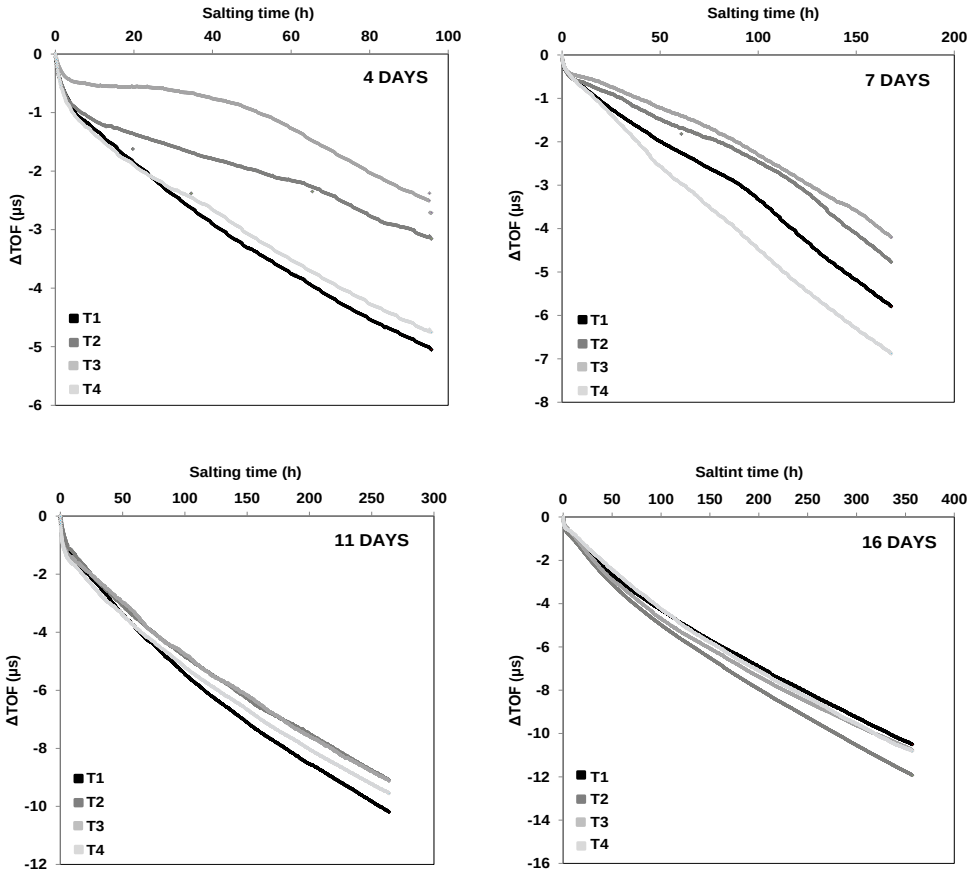


Figure 10. Time of flight variation (ΔTOF) evolution in hams dry salted for 4, 7, 11 and 16 days at 2ºC. ΔTOF calculated with the PSM. T1, T2, T3 and T4 make reference to the four measuring points/transducers used.

Therefore, after reviewing the results of the different signal analysis methods, it can be concluded that the energy of the time domain signal determines the most appropriated method to calculate the TOF (Figure 11). Two limits (upper and lower) were defined, in order to establish the best signal analysis method to be used. An upper limit of 2V was established for the peak-peak amplitude (saturated signals). In addition, a lower limit was also defined (4Vµs) for the integral of the time domain signal. According to these limits, the CCM-NCS (each 1h) and the PSM could be used to calculate the TOF for ultrasonic signals

with low energy level (Integral<4Vμs) (Figure 11). For ultrasonic signals with moderate energy level (Integral>4Vμs; peak-peak amplitude< 2V), the CCM-CS (each 5min), the CCM-NCS (each 1h) and the PSM were suitable methods to calculate the ΔTOF. For saturated ultrasonic signals (peak-peak amplitude>2V), the PSM could not be applied, but the CCM-CS and CCM-NCS provided trustworthy results. As can be observed, the CCM-NCS provided reliable results for any energy level, so it can be considered the most appropriated method to calculate the ΔTOF during the ham salting process.

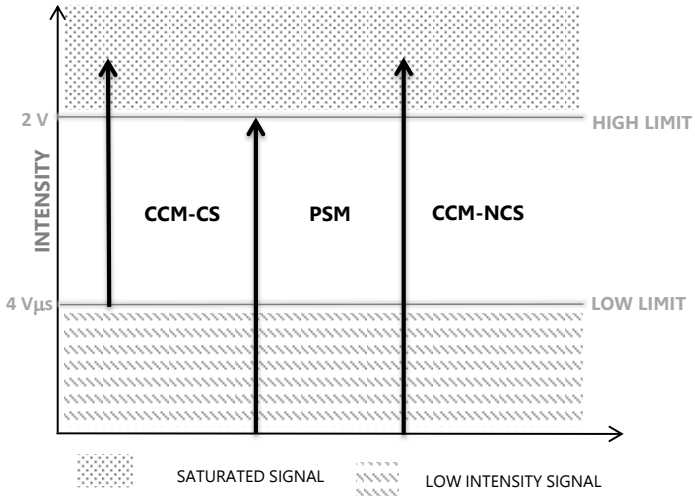


Figure 11. Field of application for each signal analysis method to calculate the ΔTOF during the ham salting process depending on the signal energy level. CCM-RS makes reference to the cross-correlation method between the initial ultrasonic signal or reference signal (0h-RS) and the remaining signals. CCM-CS the cross-correlation method between consecutive signals (each 5min) y CCM-NCS between non-consecutive signals (each 1h). PMS is the spectrum phase method between signals separated 5min.

3.5 PREDICTION OF THE SALT GAIN THROUGH THE ΔTOF

As mentioned in section 3.1, the TOF decrease found during ham salting was influenced by the compositional changes (salt gain and water loss) as well as

by the sample's contraction and structural changes that take place during salting. When the final ΔTOF value was related with the salt gain (ΔX_S), a great variability was found (data not shown). In order to take into account the initial sample thickness, which can affect the relationship between the change in the time of flight and the salt gain, the TOF_0 was considered for the estimation of the salt gain. Therefore, the relationship between the salt gain and the $\Delta TOF \cdot TOF_0$ was studied (Figure 12). Since in previous sections, the CCM-NCS has shown to be the only one that can be applied regardless of the energy content of the ultrasonic signals, it was the method chosen to calculate the ΔTOF in all the experiments. A significant ($p<0.05$) relationship between the ΔX_S and the $\Delta TOF \cdot TOF_0$ was found, showing a high correlation coefficient ($R^2=0.88$, Figure 12). Consequently, ultrasonic pulse echo measurements, using the cross-correlation method between ultrasonic signals taken each hour, could be considered a reliable and effective technique for calculating the ΔTOF and predicting the salt gain during ham dry salting.

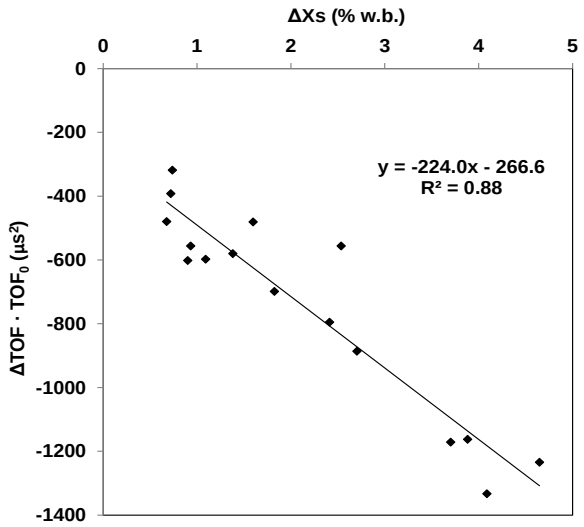


Figure 12. Relationship between the ultrasonic parameter ($\Delta TOF \cdot TOF_0$) and the salt gain (ΔXs).

Several studies have shown the relationship between the ultrasonic velocity and the solid content in a food staff. In this regard, Valente et al. (2013) showed that the ultrasonic velocity increased with the increase in the solid content during mango ripening. De Prados et al. (2015 and 2016) reported that the ultrasonic velocity rose in pork meat (*Biceps femoris y Longissimus dorsi*) during salting. The ultrasonic velocity measurements require the sample's thickness measurement by means of some electronic gage, something quite difficult to implement in the industrial environment where the pile ham dry salting is conducted. By contrast, the pulse-echo TOF measurement presents the advantage of not requiring the measurement of the sample thickness and the location of the ultrasonic transducers only in contact with one of the ham surfaces, making easier the ultrasonic industrial application.

4. CONCLUSIONS

The time of flight decreased progressively during the dry salting process of hams. The signal energy determined the best method to calculate the time of flight variation. The energy threshold method was not able to provide reliable results in any range of energy. The phase spectrum was suitable for ultrasonic signals with low and moderate energy levels, but not for saturated ones. The cross-correlation method between consecutive ultrasonic signals (separated 5min) could be used when the energy levels were moderate or high. The cross-correlation method between non-consecutive signals (separated 1h) provided reliable results for any energy level, so it is the most appropriated method to calculate the ΔTOF and monitoring the ham salting process. By using this method, the time of flight parameter ($\Delta TOF \cdot TOF_0$) was significantly ($p<0.05$) correlated to the salt gain in the hams. Therefore, the ultrasound pulse-eco technique could be a feasible non-destructive tool to monitor the dry salting process of hams, and thus, to be used for the quality control in the ham industry.

ACKNOWLEDGEMENTS

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REFERENCES

Aparicio, C., Otero, L., Guignon, B., Molina-García, A.D., & Sanz, P.D. (2008). Ice content and temperature determination from ultrasonic measurements in partially frozen foods. *Journal of Food Engineering*, 88, 272-279.

Association of Official Analytical Chemist (AOAC). Official Methods of Analysis. (1997). 16th Edn. Washington, DC: AOAC, International.

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

Cárcel, J. A., Benedito, J., Bon, J., & Mulet, A. (2007). High intensity ultrasound effects on meat salting. *Meat Science*, 76(4), 611-619.

De Prados, M., García-Pérez, J. V., & Benedito, J. (2015). Non-destructive salt content prediction in brined pork meat using ultrasound technology. *Journal of Food Engineering,* 154, 39-48

De Prados, M., García-Pérez, J. V., & Benedito, J. (2016). Ultrasonic characterization and online monitoring of pork meat dry salting process. *Food Control*, 60, 646-655

Koc, A. B., & Ozer, B. (2008). Nondestructive monitoring of renetted whole milk during cheese manufacturing. *Food Research International*, 41, 745-750.

Leemans, V. & Destain, M. F. (2009). Ultrasonic internal defect detection in cheese. *Journal of Food Engineering*, 90, 333-340.

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

Resa, P., Elvira, L., de Espinosa, F. M., Gonzalez, R., & Barcenilla, J. (2009). On-line ultrasonic velocity monitoring of alcoholic fermentation kinetics. *Bioprocessing Biosystem Engineering*, 32, 321-331.

Sarabia E. G., Llata, J. R., Robla, S. Torre-Ferrero C., & Oria, J. P. (2013). Accurate estimation of airborne ultrasonic time-of-flight for overlapping echoes. *Sensors*, 13, 15465-15488.

Ting, C. -H., Kuo, F. -J., Lien, C. -C., & Sheng, C. -T. (2009). Use of ultrasound for characterising the gelation process in heat induced CaSO4•2H2O tofu curd. *Journal of Food Engineering*, 93(1), 101-107.

Toldrà, F. (2010). Handbook of Meat Processing. Blackwell Publishing. Iowa, USA. Chapter 20. 351-362

Valente, M., Prades, A., & Laux, D. (2013). Potential use of physical measurements including ultrasound for a better mango fruit quality characterization. *Journal of Food Engineering*, 116, 57-64.

Meat Science, submitted.

Assessment of time of flight by ultrasonic pulse-echo mode as a tool to monitor pork loin and ham dry salting

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Abstract

This work explores the feasibility of using the ultrasonic pulse-echo technique for both the continuous monitoring of the dry salting process and also for the prediction of salt gain in loins and hams. For this purpose, online ultrasound measurements were taken during dry salting of loins (*Longissimus dorsi*) and hams at different times (up to 30 days). From the time-domain ultrasonic wave, the time of flight (TOF) was estimated as well as its variation between two signals (ΔTOF). The progressive decrease in TOF during dry salting was due to the water loss and the reduction in thickness, but was also a consequence of the salt gain in the sample. Predictive models based on the ultrasonic parameters (ΔTOF and initial time of flight, TOF_0) correctly classified 85% of the loins and 90% of the hams into three groups of salt content (low/medium/high). A slight improvement in the percentage of correctly classified loins (95%) was found when the model incorporated the sample weight and the salting time. The results obtained confirm that use of the ultrasonic pulse-echo technique is of great potential in the non-destructive monitoring of the dry salting in pork loins and hams, as well as in the prediction of the salt gain for classification purposes.

Keywords: *Pork meat, Curing, Ultrasound, Non-destructive technology, Process control*

1. INTRODUCTION

Dry salting is the traditional technique used in meat products with anatomical integrity, such as hams or loins, in order to obtain dry-cured products. In the dry-cured loin and ham industries, an accurate control of the dry salting process is especially complex because of the high degree of heterogeneity in the meat pieces (pH, weight, fat content and moisture, size of the piece…), the effect of the pre-treatments on the product (skin-trimming of the fresh ham or the freezing/thawing processes) and the influence of the different process variables (temperature, relative humidity, position in the salt pile, size of the coarse salt…) (Costa-Corredor, Muñoz, Arnau, & Gou, 2010; García-Gil, Muñoz, Santos-Garcés, Arnau, & Gou, 2014; Fulladosa, Muñoz, Serra, Arnau, & Gou, 2015). All of these factors lead to there being a highly variable salt content in the batches of dry cured hams and loins. This salt content variability is of great concern to the meat industry as an excessive amount of salt produces a too salty taste (Ruusunen & Puolanne, 2005), but an insufficient amount may cause sensory defects, such as pastiness and softness (Albarracín, Sánchez, Grau, & Barat, 2011) or microbiological problems (Desmond, 2006). Thus, the meat industry demands non-destructive quality control techniques that allow the salt content to be determined after the salting stage for quality control purposes.

Nowadays, the feasibility of using several non-destructive technologies (X-Ray, NMR, ultrasound...) to determine the salt content in meat products has been tested. In this regard, Fulladosa, Muñoz, Serra, Arnau, and Gou (2015) predicted the salt content in bone-in hams after salting by using an X-Ray inspector. Similarly, Manzocco et al. (2013) proposed predictive models with which to estimate the salt content in ham muscles by using magnetic resonance imaging (MRI). In this study, image analysis was carried out in different stages of dry-cured ham processing (before salting, after salting and at different times during resting, maturing and ageing). Recently, ultrasound has also been applied to predict the salt content in brine salted pork meat (De Prados, García-Pérez, & Benedito, 2015) and in dry-salted hams (De Prados, García-Pérez, & Benedito,

2016), by taking ultrasonic measurements before and after salting. An accurate prediction of the salt content after the salting may allow the products to be classified according to the different levels of salt, which can be used for the purposes of optimizing the subsequent processing stages. However, the measurement after salting does not permit the correction of the variability in the batch salt content by reducing the number of over-salted or insufficiently salted pieces. Thereby, the use of non-destructive quality control techniques to monitor the salt gain during the dry salting process is gaining importance in the meat industry as a means of meeting the target salt content in each piece. In this context, ultrasound has been used by De Prados, García-Pérez, and Benedito (2016) to monitor the dry salting process of *Longissimus dorsi* and *Biceps Femoris* pork muscles. In this study, the ultrasonic velocity was measured online during salting by the ultrasonic through-transmission mode. That mode is characterized by the use of two transducers in direct contact with two opposite and parallel sides of the sample, which makes its implementation in a ham or loin salting pile very complicated. Otherwise, the ultrasonic equipment could be greatly simplified by using the pulse-echo mode, where a single transducer can be placed on one side of the sample, simultaneously acting as emitter and receiver (Mulet, Benedito, Bon, & Sanjuan, 1999; Awad, Moharram, Shaltout, Asker, & Youssef, 2012). The use of one transducer located in the ham's base during dry salting would facilitate the ultrasonic implementation, reducing the cost and the impact of the ultrasonic measurement on the mass transfers (salt and water).

The pulse-echo mode is most commonly used to detect internal defects in metallic materials, but it has also been applied in food characterization. Thus, the pulse-echo mode has been used to determine the ripeness of avocados (Gaete-Garretón, Vargas, León, & Pettorino, 2005), to detect anomalies in the internal structure of Mahon cheese (Benedito, Cárcel, Gisbert, & Mulet, 2001) or bone fragments in deboned chicken breasts (Correia, Mittal, & Basir, 2008) and to measure the sugar content and viscosity of reconstituted orange juice (Kuo,

Sheng, & Ting, 2008). Another application of the pulse-echo mode consisted in the monitoring of the cooling and freezing processes in several food products (gelatin, chicken, salmon, beef and yoghurt) (Sigfusson, Ziegler, & Coupland, 2001 and 2004). However, no studies have been found so far in the literature regarding either the online ultrasonic monitoring of the salting process in meat products or the salt gain assessment by means of the pulse-echo mode. Moreover, the ultrasonic monitoring of salting in structural and compositional complex meat pieces, such as whole hams, has not been addressed elsewhere.

The objective of this paper was to investigate the feasibility of applying ultrasound in the pulse-echo mode for both the online monitoring of dry salting in loins and hams and the prediction of the final salt gain.

2. MATERIALS AND METHODS

2.1 FRESH MEAT SAMPLING

Ten fresh hams and twenty loins (*Longissimus dorsi*) from *Large White* breed pigs were obtained in a local market. Loin and ham pieces were selected with a pH range of between 5.3 and 5.8. In loins, the subcutaneous fat and external connective tissue were removed and samples of 20.0±0.5cm in length (l) and with an average weight of 1.0±0.1kg (wg) were obtained, keeping the original width (z) and thickness (e) of the muscle.

2.2 DRY SALTING PROCESS

Dry salting experiments in loins and hams were carried out at 2±1ºC by covering the sample with 6kg or 15kg of coarse salt, respectively (NaCl moisturized at 10% w/w) (Figure 1). The fresh samples and coarse salt were previously stored for 24h at 2ºC for tempering purposes. The thickness (e) was measured before and after dry salting in the loins and hams and the thickness reduction (Δe) was calculated. In the case of loins, four replicates were carried out for each salting time (6, 12, 24, 48 and 72h), while in that of hams, one was used for each salting time (4, 7, 10, 11, 12, 14, 15, 16, 20 and 30 days).

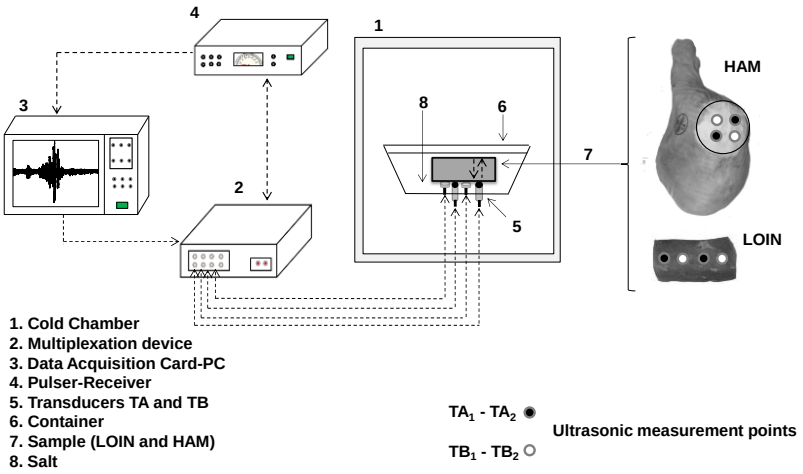

Figure 1. Experimental set-up for online ultrasonic measurements in loins and hams during dry salting.

2.3 ULTRASOUND EXPERIMENTAL SET-UP

Figure 1 shows the experimental set-up used for the ultrasonic measurements during the dry salting of loins and hams. The experimental set-up consisted of four narrow-band piezoelectric transducers of 1MHz and 0.5" crystal diameter, two of them type TA (TA_1 and TA_2) (A303S model, Panametrics, Waltham, MA, USA) and the other two type TB (TB_1 and TB_2) (A103S-RM model, Panametrics, Waltham, MA, USA). The pulse generation and reception (5058PR, Panametrics, Waltham, MA, USA for loins and 5077PR, Panametrics, Waltham, MA, USA for hams) was multiplexed to the transducers using a digital input/output device (NI 6501, National Instruments, Austin, TX, USA) and a high-speed digitizer (PXI/PCI-5112, National Instruments, Austin, TX, USA) installed in a PC (Figure 1). The multiplexation unit allowed the signal from the pulser to reach the first transducer every 1h. The ultrasonic signal generated in this transducer crossed the sample, was reflected in the meat/salt interface and returned to the same transducer, sent to the receiver through the multiplexation device and digitized by the oscilloscope (Figure 1). This operation was sequentially repeated in the rest of the transducers by the action of the multiplexation unit.

The sample was placed on a layer of salt in direct contact with the four transducers (TA_1, TA_2, TB_1 and TB_2, which corresponds to the four ultrasonic measurement points) inside a plastic container (30x25x15cm for loins and 120x35x20cm for hams) (Figure 1). Afterwards, two type-K thermocouples were located in the salt and the sample, respectively; and the rest of the salt was added (total salt amount of 2kg for loins and 5kg for hams) until the sample was entirely covered. In the case of hams, a part of the skin in the cushion zone ($177\pm14cm^2$) was removed, which coincides with the region where the transducers were placed (Figure 1) and 1mL of water was added on the transducers' surfaces in order to guarantee the acoustic matching, thus, improving the signal intensity. The ultrasonic measurements were taken by the pulse-echo mode at intervals of 1h in the central part of the loins and in the cushion zone of the hams (Figure 1).

The time of flight represents the time which elapses between the pulser sending the signal to the transducer acting as emitter, until the signal crosses the sample twice and is detected in the transducer acting as receiver. The time of flight variation (ΔTOF) between two ultrasonic signals was the ultrasonic parameter considered for the online monitoring of the loins and hams. For that purpose, the cross correlation method (Leemans & Destain, 2009) was employed to calculate the ΔTOF between ultrasonic signals one hour apart by using a specific software developed in LABVIEW™ 2015 (National Instruments, Austin, TX, USA) and the final ΔTOF for the different salting times was computed. The initial time of flight (TOF_0), which corresponds to the raw meat, was calculated through the energy threshold method (Avanesians & Momayez, 2015), using the same software.

2.4 CHEMICAL ANALYSIS

The fat, salt and water contents were determined in the fresh muscles and hams. To this end, a piece (200±50g) was taken from each loin after obtaining the fresh muscle sample for the salting process. In the case of the hams, as the

sample integrity cannot be altered before salting, the average values of the fat, salt and water contents were obtained from 30 hams of the same breed purchased from the same supplier.

Once the salting finalized, the excess salt was removed from the surface of the loins and hams and four cylindrical salted samples (64±13g for loins and 172±39g for hams) corresponding to the ultrasonic measurement points (Figure 1), were taken by using a cylindrical cutter (5cm in diameter). Each cylindrical salted sample was ground and homogenized before the analysis. The analyses of the fat and water contents were carried out according to AOAC procedures 991.36 and 950.46, respectively (AOAC, 1997). The salt content was analyzed following the process described by De Prados, García-Pérez, and Benedito (2015 and 2016). All the analyses were performed in triplicate.

The salt (X_S), water (X_W) and fat (X_F) contents of the fresh samples and the salted loin and ham cylinders were expressed as percentages (%) in wet basis (w.b.). The final salt gain (ΔX_S) and the water loss (ΔX_W) were also calculated as an average of the four cylindrical samples from the loins and hams at each salting time.

2.5 STATISTICAL ANALYSIS AND DEVELOPMENT OF PREDICTIVE MODELS

The influence of the salting time on the ΔX_S, ΔX_W, Δe and ΔTOF in loins and hams was evaluated by means of an analysis of variance. Similarly, the analysis of variance was carried out in order to determine the significant influence of the type of transducer (TA and TB) on the final ΔTOF value during salting. Additionally, a multiple regression model was used to evaluate the influence of the salting time, ΔX_S, ΔX_W and Δe on the ΔTOF. In every case, the Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA) software was used and a significance level of 95% was fixed.

The ultrasonic (ΔTOF and TOF_0) and sample parameters (weight, wg) and salting time (t) were used as independent variables so as to predict the salt

gain (ΔX_S) in loins and hams. For that purpose, both the loins and hams were split into two sets; a model set (M) and a validation set (V). The model set (M) included 15 loins and 7 hams, chosen randomly. Their ultrasonic measurement points (n_{ump}=60 for loins and 28 for hams) were used to develop multiple regression models with the Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA). The optimal number of independent variables and the interactions in the model were obtained using the Marquardt method. The p-value used to keep the independent variables in the model was 0.05. The validation set included 5 loins (n_{ump}=20) and 3 hams (n_{ump}=12). The overall classification capacity was tested using the optimal model for salt gain prediction. For this purpose, loins and hams and their corresponding ultrasonic measurement points from sets M and V were classified into three different categories. Loins with a salt content of <2.5% w.b. were considered to have a low level of salt, those with a salt content of >4.0% w.b. a high level of salt and the remaining ones to have a moderate level of salt. For hams, three categories were also considered (low <2.0% w.b., medium 2.0-3.0% w.b. and high >3.0% w.b. salt content level). The levels of salt in loins were higher than in hams due to the fact that the loins are not as thick as hams, so actually, they tend to be saltier.

3. RESULTS AND DISCUSSION

3.1 FRESH SAMPLE CHARACTERIZATION

As shown in Table 1, a similar initial salt content (X_S) was observed in loins and hams. However, significant differences ($p<0.05$) were found between the fat (X_F) and water (X_W) contents in fresh loins and hams, the fat content being greater in hams than in loins. The ranges of X_F, X_S, and X_W found in the present study (Table 1) coincide with the ones reported for *Large White* breed loins (*Longissimus dorsi*) and hams in the literature (Cierach & Modzelewska-Kapituła, 2011; Moreiras, Carbajal, Cabrera, & Cuadrado, 2013). Additionally, a high degree of variability was found in the X_F (0.3-6.1% w.b. for loins and 7.5-

26.4% w.b. for hams) and X_W (67.9-75.6% w.b. for loin and 56.3-70.2% w.b. for ham) compared to the that observed in the X_S (0.10-0.26% w.b. for loins and 0.16-0.33% for hams), which is especially evident in the case of hams (Table 1). This compositional variability, especially the fat content and its distribution, should be considered when designing the salting process due to the fact that it may affect the mass transfer (salt gain and water lost) during salting (Cierach & Modzelewska-Kapituła, 2011; De Prados, García-Pérez, & Benedito, 2015).

Table 1. Fat (X_F), water (X_W) and salt content (X_S), thickness (e), width (z), length (l) and weight (wg) of the fresh loins and hams*.

	LOINS	**HAMS**
X_F (% w.b.)	2.4 ± 1.9^{a}	$14.0\pm3.7^{b*}$
X_W (% w.b.)	72.7 ± 2.1^{c}	$65.5\pm2.9^{d*}$
X_S (% w.b.)	0.18 ± 0.05^{e}	$0.23\pm0.05^{e*}$
e (cm)	5.0 ± 0.8^{f}	15.7 ± 0.6^{g}
z (cm)	11.0 ± 0.8^{j}	27.8 ± 2.8^{i}
l (cm)	20.0 ± 2.0^{j}	49.4 ± 6.0^{k}
wg (kg)	1.0 ± 0.1^{l}	10.3 ± 0.9^{m}

3.2 COMPOSITIONAL CHANGES DURING DRY-SALTING

Figure 2 illustrates the kinetics of salt gain and water loss in loins and hams during dry salting. The ΔX_S values in the present work were slightly lower than the ones reported by other studies. For example, De Prados, García-Pérez, and Benedito (2016) reported a salt gain of between 2.1 and 6.7% w.b. in loins salted from 6 to 48h. When studying hams that had been dry salted for between 2 and 16 days, Fulladosa, Muñoz, Serra, Arnau, and Gou (2015) and Håseth, Sørheim, Høy, and Egelandsdal (2012) found a salt content ranging from 0.8 to 4.8% w.b. and from 1.2 to 4.5% w.b., respectively. This lower salt gain could be explained by considering that other authors reported the salt content as an average of the whole piece. However, in the present study, the

salt gain shown represents the average value between the four cylindrical samples corresponding to the four ultrasonic measurement points of the cushion zone of the ham (Figure 1), which is its thickest part and where the salt diffusion takes longer (Toldrá & Wai-Kit, 2008; Håseth, Sørheim, Høy, & Egelandsdal, 2012). On the other hand, the ΔX_S and ΔX_W in loins and hams showed a marked experimental variability, especially the ΔX_W (Figure 2). As previously mentioned, salting is a complex process affected by the different process variables (temperature, size of coarse salt, quantity of salt, position in the salt pile, salting time...) and the high degree of compositional heterogeneity of the meat pieces. In this study, the process variables were accurately controlled and the pieces (loins and hams) were individually salted (not pile salted). Thus, this great variability in the ΔX_S and ΔX_W could be mainly ascribed to the highly heterogeneous nature of the fresh meat pieces in terms of the content and distribution of the water, fat and connective tissue, among other things. This fact is more relevant in hams due to the presence of both the skin and bone and also to that of different muscles with a greater degree of heterogeneity than in single muscles.

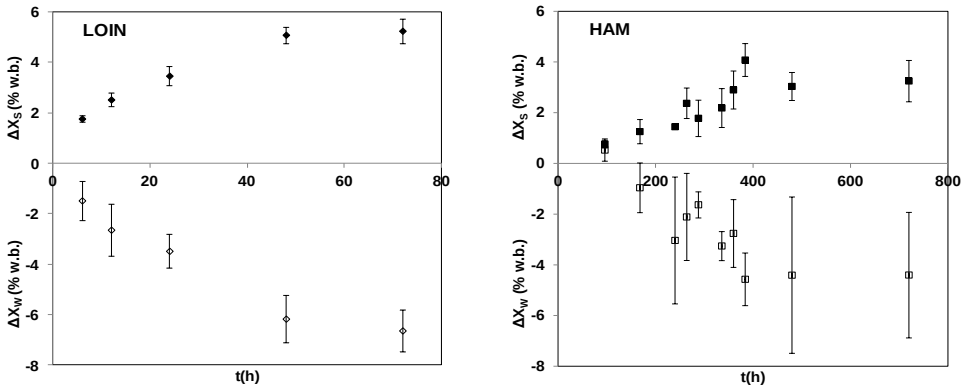

Figure 2. Experimental kinetics of salt gain (ΔX_S) and water loss (ΔX_W) in loins and hams during dry salting at 2ºC.

3.3 ULTRASONIC MONITORING OF THE DRY SALTING PROCESS

A gradual decrease of the TOF during salting was found. As an example, Figure 3 shows the displacement in the time domain of the ultrasonic signal from the position at time 0 to the one captured after 30 days of salting. As observed, the ultrasonic signal after 30 days is displaced to the left (Figure 3), which illustrates a shortening of the TOF. This could be explained by the fact that ultrasound travels faster in solids, with a high elastic modulus (Benedito, Cárcel, Clemente, & Mulet, 2000), than in liquids (water). Thus, the increase in the solid content during salting, as a result of the salt gain and water loss, leads to an increase in the sample's ultrasonic velocity, and thereby, a decrease in the TOF. When analyzing different kinds of samples (water, fish, juice and meat), several studies have reported an increase in the ultrasonic velocity in line with an increase in the solid content (Kinsler, Frey, Coppens, & Sanders, 1982; McClements, 1995; Ghaedian, Coupland, Decker, & McClements, 1998; Kuo, Sheng, & Ting, 2008; De Prados, García-Pérez, & Benedito, 2015 and 2016).

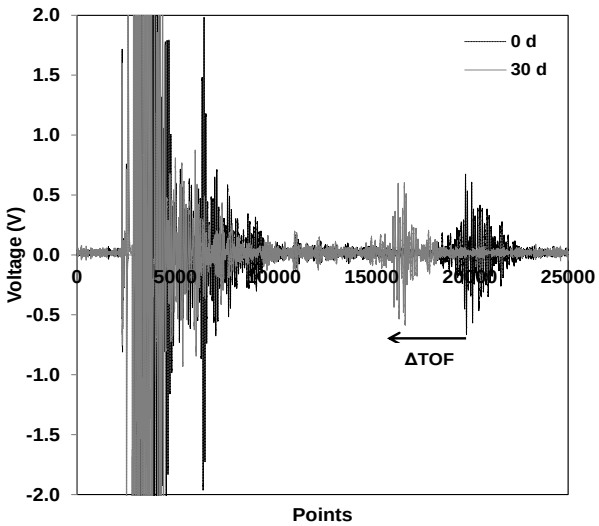

Figure 3. Variation of the time of flight (ΔTOF) between the first and last ultrasonic signals captured in a ham dry salted for 30 days at 2ºC.

Figure 4 illustrates the evolution of the ΔTOF in loins and hams during different salting experiments (24, 72h for loins and 11, 20 days for hams). The same behavior (data not shown) was observed in the remaining experiments (6, 12 and 48h dry salting of loins and 4, 7, 10, 12, 14, 15, 16 and 30 days' dry salting of hams). As can be appreciated, the ΔTOF decreased gradually due to the above mentioned increase in the solid content of the sample. This result is consistent with the increase in the salt content and the decrease in the water content in loins and hams during dry salting, as observed in Figure 2. The ΔTOF evolution was different for each ultrasonic measurement point corresponding to the different transducers (TA_1, TA_2, TB_1 and TB_2) (Figure 4). This fact could be linked to the heterogeneity of the compositional changes (ΔX_S and ΔX_W) at each ultrasonic measurement point during salting, which was especially evident in the ham salting experiments (Figure 2). Non-significant ($p>0.05$) differences were observed in the ΔTOF evolution between type TA and TB transducers (Figure 4). Therefore, both types of transducers could be considered equivalent for the purposes of monitoring the salting process.

An unexpected, fast decrease in the ΔTOF was observed during the first few hours of dry salting (Figure 4) for both loins and hams, which was not coherent with the kinetics for salt and water diffusion (Figure 2). Similar behavior was observed in the evolution of the ultrasonic velocity in *Longissimus dorsi* and *Biceps femoris* during dry salting (De Prados, García-Pérez, & Benedito, 2016). De Prados, García-Pérez, and Benedito (2016) associated this behavior with the formation of a salt solution between the transducers and the meat, due to the initial extraction of water from the external meat layers, which could partly explain the important decrease in the ΔTOF during the first 3h, as may be observed in Figure 4. Additionally, the fast initial ΔTOF could also be associated with the textural changes that take place on the meat surface due to the effect of the salt in contact with the sample. Thus, a test was conducted in order to prove this hypothesis. Two loins (1kg) were salted for 1 and 3h, respectively and the hardness, characterized as the maximum penetration force (N), was

evaluated in each loin before and after salting. Penetration test was conducted with a 6mm flat cylinder probe, a crosshead speed of 1mm/s and strain of 20% (penetration distance of 10mm). The results showed that, after 1 and 3h, the salted loins were significantly ($p<0.05$) harder (14.7±1.8N at 1h and 21.4±3.4N at 3h) than the fresh ones (8.6±4.0N). Ruiz-Ramírez, Arnau, Serra, and Gou (2005) related the increase in hardness with the increase in the salt content in the dry cured pork muscles (Biceps femoris and Semimembranosus). This increase in hardness was linked to the compaction of the myofibrillar structure due to the salt content and an inhibitory effect of salt on the calpains activity (Ruiz-Ramírez, Arnau, Serra, & Gou, 2005; Lorenzo, Fonseca, Gómez, & Domínguez, 2015). Thus, the salt gain and water loss that takes place in the external meat layers during the first few hours gives rise to a great increase in the salt concentration in these layers, leading to a rapid surface textural increase which would explain the fast decrease in the ΔTOF.

The TOF and velocity measurements have been used to monitor other food processes. Thus, Sigfusson, Ziegler, and Coupland (2001 and 2004) monitored the cooling and freezing process in different food products by measuring the TOF by means of the pulse-echo mode. Recently, De Prados, García-Pérez, and Benedito (2016) monitored the meat dry salting process online. In that work, the ultrasonic velocity was measured online in *Longissimus dorsi* and *Biceps femoris* by using the through-transmission mode, where the sample's thickness had to be measured and two transducers were in direct contact with two opposite and parallel sides of the sample (De Prados, García-Pérez, & Benedito, 2016). This fact complicates the implementation of ultrasonic technology in the meat industry where pile salting is used. On the contrary, in the present study, the ultrasonic measurements were taken in loins and hams by using the pulse-echo mode, which is characterized by the use of a single transducer and the sample's thickness does not have to be measured. The use of a single transducer on the loin or ham's base during pile salting would simplify the

industrial implementation, reduce the cost of the device and minimize the impact of the ultrasonic measurement on the mass transfers (salt and water).

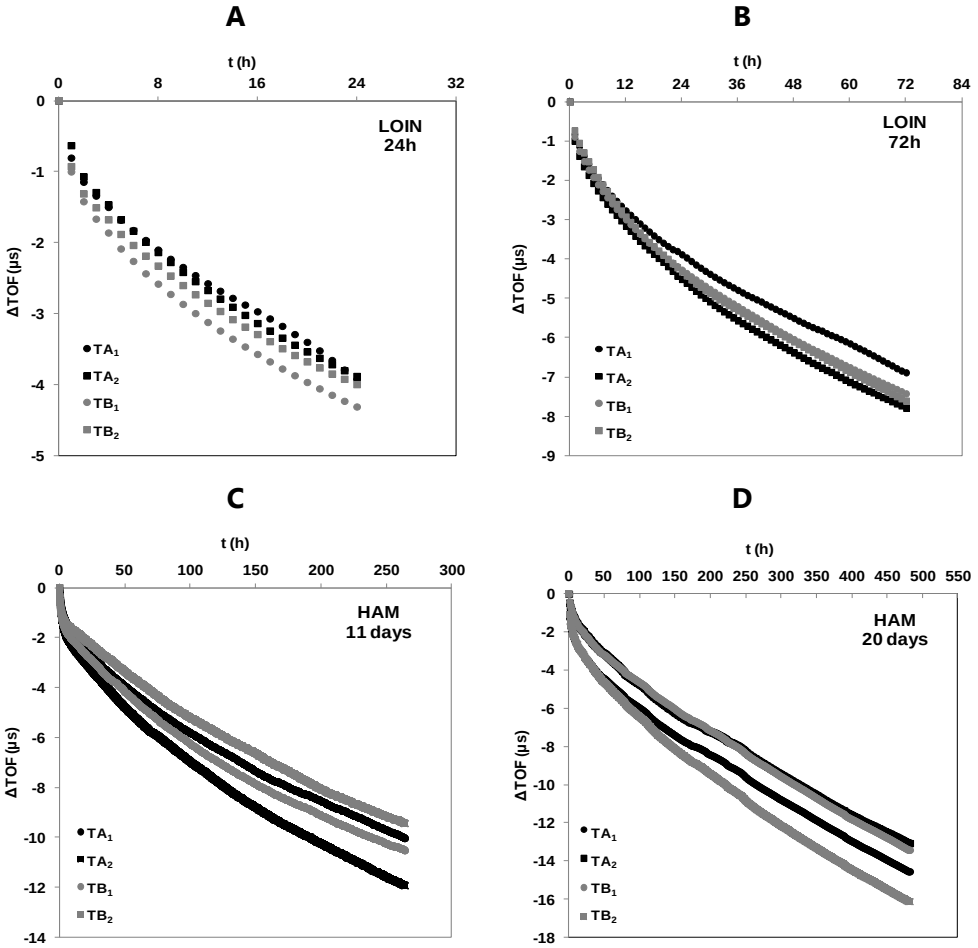

Figure 4. Time of flight variation (ΔTOF) in loins (**A** and **B**) and hams (**C** and **D**) during dry salting (24-72h for loins and 11-20 days for hams) at 2ºC. Each series corresponds to a different ultrasonic measurement point (TA_1, TA_2, TB_1 and TB_2).

Therefore, the results reported in this section confirm that the ΔTOF measured by using the pulse-echo mode could be a useful ultrasonic parameter for the purposes of performing the online monitoring of the salting process in individual loins (average thickness 5.0cm), as well as in more complex and thicker meat pieces, such as hams (average thickness 15.7cm). Additionally, the

ultrasonic pulse-echo technique represents a significant improvement for the industrial application of the system compared to the ultrasonic through-transmission technique due to the fact that it avoids the need to measure the sample thickness and the number of transducers required is reduced.

3.4 INFLUENCE OF THE DRY SALTING ON THE TIME OF FLIGHT

Table 2 shows the thickness reduction (Δe), salt gain (ΔX_S), water loss (ΔX_W) and time of flight variation (ΔTOF) in loins and hams during dry salting at 2ºC. As appreciated in Table 2, the longer the salting time, the higher the ΔTOF. Thus, the ΔTOF increased in loins from -2.7μs (6h) to -7.0μs (72h) and in hams from -5.5μs (4 days) to -17.6μs (30 days) (Table 2). As previously mentioned, and as observed in Table 2, the ΔTOF during salting was related to the ΔX_S and ΔX_W in the samples (loins and hams). Overall, the higher the ΔX_S and ΔX_W, the higher the ΔTOF. In addition, water loss during salting leads to meat shrinkage (García-Gil, Muñoz, Santos-Garcés, Arnau, & Gou, 2014), and thus, to thickness reduction (Δe) (Table 2), which could also contribute to shortening the time of flight. Despite the fact that the ΔX_W and Δe factors affect ΔTOF, the multiple regression model used showed that both factors were statistically non-significant ($p>0.05$) on the ΔTOF prediction, which can be attributed to the fact that the magnitude of the individual effect of these factors can be masked by their highly variable nature in the salting process. Thus, according to the statistical analysis, the ΔTOF is mainly related to the salt gain in the sample during salting.

Previous results have shown a similar relationship between the ΔX_S and the change of the ultrasonic velocity regardless of the sample nature (formulated samples from ground pork meat, pork muscles or water solution) (De Prados et al., 2015). Consequently, as velocity of ultrasound in a sample is the ratio between the sample thickness (e) and the TOF, the ΔX_S must be related not only to the ΔTOF, but also to the initial sample thickness and the thickness change (Δe). As far as in the present work the effect of Δe on ΔX_S has been

found to be negligible, the models for predicting ΔX_S should consider both ΔTOF and the initial e. As an example of the need of considering the initial sample thickness, it can be observed that the ΔTOF was -3.3μs in loins (e_{avg}= 5.0cm) for a ΔX_S of 2.5% w.b. after 12h of salting, while the ΔTOF was -11.2μs in hams (e_{avg}=15.7cm) for a similar ΔX_S (2.2% w.b.) after 14 days of salting. However, when the ΔTOF/e was computed for these two cases, a similar value was found for loins (-0.7μs/cm) and hams (-0.8μs/cm). Since measuring the thickness before salting could be complex on an industrial scale, the initial time of flight (TOF_0), related to the sample thickness and velocity of sound in the raw meat, could be used.

Table 2. Salt gain (ΔX_S), water loss (ΔX_W), thickness reduction (Δe) and time of flight variation (ΔTOF) in loins and hams during dry salting at 2ºC.

SAMPLE	TIME	Δe (cm)	ΔX_S (% w.b.)	ΔX_W (% w.b.)	ΔTOF (μs)
LOIN	**6h**	-0.23±0.58^{a}	1.8±0.2^{a}	-1.5±1.5^{a}	-2.7±0.8^{a}
	12h	-0.47±0.99^{a}	2.5±0.5^{b}	-2.6±1.9^{b}	-3.3±0.6^{b}
	24h	-0.24±0.45^{a}	3.5±0.7^{c}	-3.5±1.2^{b}	-4.6±1.0^{c}
	48h	-1.15±0.59^{a}	5.1±0.6^{d}	-6.2±1.8^{c}	-6.0±0.7^{d}
	72h	-0.45±0.48^{a}	5.3±0.9^{d}	-6.6±1.6^{c}	-7.0±0.9^{e}
HAM	**4 days**	-1.71±0.49^{abc}	0.8±0.1^{a}	0.5±0.3^{a}	-5.5±0.5^{a}
	7 days	-1.36±0.27^{bc}	1.3±0.3^{ab}	-1.0±0.6^{b}	-8.8±0.8^{bc}
	10 days	-1.28±1.88^{bc}	1.4±0.1^{b}	-3.0±1.5^{de}	-8.0±1.0^{b}
	11 days	-1.17±0.63^{bc}	2.4±0.4^{de}	-2.1±1.1^{bcd}	-10.3±1.3^{cd}
	12 days	-0.40±0.50^{c}	1.8±0.5^{bc}	-1.6±0.3^{bc}	-11.0±1.5^{d}
	14 days	-0.93±0.49^{bc}	2.2±0.5^{cd}	-3.3±0.4^{de}	-11.2±1.0^{d}
	15 days	-2.88±1.31^{a}	2.9±0.5^{ef}	-2.8±0.8^{cd}	-13.1±1.5^{e}
	16 days	-2.18±0.89^{ab}	4.1±0.4^{g}	-4.6±0.7^{f}	-14.2±0.4^{e}
	20 days	-5.15±1.24^{d}	3.0±0.3^{f}	-4.4±1.9^{ef}	-14.3±1.4^{e}
	30 days	-4.75±1.27^{d}	3.2±0.5^{f}	-4.4±1.6^{ef}	-17.6±0.5^{f}

3.5 PREDICTIVE MODELS FOR SALT GAIN AND CLASSIFICATION OF LOINS AND HAMS

Since the ΔTOF is related to the salt content and the TOF_0 is related to the initial thickness, both ultrasonic parameters (ΔTOF and TOF_0) were used as factors with which to develop predictive models for the salt gain. The ultrasonic measurement points of the M set were used to develop the predictive models as mentioned in section 2.5. Eq. 1 and Eq. 2 show the best models obtained for the salt gain estimation in loins and hams by combining the two ultrasonic variables (ΔTOF and TOF_0) from the pulse-echo measurements. The R^2 and RMSE were 0.787 and 0.73% for loins (Eq. 1) and 0.774 and 0.57% for hams (Eq. 2). Additionally, other predictive models were established, including two variables which can be easily measured at industrial level: one regarding the sample (the initial weight, wg) and the other one related to the salting process (the salting time, t) (Eq. 3 for loin and Eq. 4 for ham). The inclusion of t and wg provided additional information that improved the predictive models, obtaining a reduced model error (RMSE=0.45% for loins using Eq. 3 and 0.43% for hams using Eq. 4) and an increase in the determination coefficient (R^2= 0.923 for loins and 0.891 for hams). Similar results were obtained by Fulladosa, Muñoz, Serra, Arnau, and Gou (2015) and Håseth et al. (2008) using X-Rays. In these studies, the salt prediction was more accurate than in the present analysis, the RMSE being 0.30% for hams of different breeds (Fulladosa, Muñoz, Serra, Arnau, & Gou, 2015) and 0.20-0.40% for ground pork *Semimembranosus* muscles (Håseth et al., 2008).

LOIN $$\Delta Xs = 3.524 - 0.0039 \cdot TOF_0^2 - 0.0255 \cdot TOF_0 \cdot \Delta TOF \quad \text{(Eq. 1)}$$

HAM $$\Delta Xs = 0.253 - 0.0003 \cdot TOF_0^2 - 0.0125 \cdot \Delta TOF_0 - 0.0066 \cdot TOF_0 \cdot \Delta TOF \quad \text{(Eq. 2)}$$

LOIN
$$\Delta Xs = 1.568 + 0.143 \cdot t - 0.0010 \cdot t^2 - 0.0414 \cdot \Delta TOF^2 + 0.0431 \cdot t \cdot wg - 0.0034 \cdot t \cdot TOF_0 - 0.0107 \cdot t \cdot \Delta TOF \quad \text{(Eq. 3)}$$

HAM
$$\Delta Xs = -39.051 + 0.140 \cdot t - 0.00003 \cdot t^2 + 3.550 \cdot wg - 0.00004 \cdot TOF_0^2 - 0.0120 \cdot t \cdot wg + 0.0001 \cdot t \cdot TOF_0 + 0.00004 \cdot t \cdot \Delta TOF \quad \text{(Eq. 4)}$$

where ΔX_S is the salt gain (% w.b.), t the salting time (h), wg the initial sample weight (kg), TOF_0 the initial time of flight (μs) and ΔTOF the time of flight variation (μs).

The usefulness of ultrasound as a reliable method of classifying loins and hams according to the different levels of salt gain was tested by using the best predictive models (Eq. 1, Eq. 2, Eq. 3 and Eq. 4). For that purpose, the validation (V) and model (M) sets of the whole loins and hams and each ultrasonic measurement point were classified into three different categories of salt gain (ΔX_S), as mentioned in section 2.5. Similar percentages of correctly classified samples (CC) at the ultrasonic measurement points (79% in loins and 75% in hams) and in the whole pieces (85% in loins and 90% in hams) were computed (Table 3) by using only the ultrasonic parameters (Eq. 1 and 2). On the other hand, the classification improved by using Eqs. 3 and 4, especially in the case of loins (Table 3). In this regard, the percentage of CC ultrasonic measurement points increased from 79% to 86% for loins and from 75% to 78% for hams. In the case of whole loins, the percentage of CC samples increased from 85% to 95% whereas no improvement was found for whole hams.

Table 3. Classification of loins and hams according to different levels of salt gain (ΔX_S) by using the best predictive models (Eqs.1 and 3 for loins and Eqs.2 and 4 for hams).

			Ultrasonic measurement points				Whole loins				
			CC for different levels of ΔX_S (% w.b.)				CC for different levels of ΔX_S (% w.b.)				
		n_{UMP}	<2.5	2.5-4.0	>4.0	TOTAL	n_S	<2.5	2.5-4.0	>4.0	TOTAL
LOINS	Eq.1 M	60	11/19 (58%)	15/17 (88%)	23/24 (96%)	63/80 (**79%**)	15	3/4 (75%)	4/5 (80%)	6/6 (100%)	17/20 (**85%**)
	Eq.1 V	20	4/5 (80%)	4/6 (67%)	6/9 (67%)		5	1/1 (100%)	1/1 (100%)	2/3 (67%)	
	Eq.3 M	60	16/19 (84%)	14/17 (82%)	24/24 (100%)	69/80 (**86%**)	15	4/4 (100%)	5/5 (100%)	6/6 (100%)	19/20 (**95%**)
	Eq.3 V	20	5/5 (100%)	4/6 (67%)	6/9 (67%)		5	1/1 (100%)	1/1 (100%)	2/3 (67%)	

			Ultrasonic measurement points				Whole hams				
			CC for different levels of ΔX_S (% w.b.)				CC for different levels of ΔX_S (% w.b.)				
		n_{UMP}	<2.0	2.0-3.0	>3.0	TOTAL	n_S	<2.0	2.0-3.0	>3.0	TOTAL
HAMS	Eq.2 M	28	9/12 (75%)	6/9 (67%)	6/7 (86%)	30/40 (**75%**)	7	2/3 (67%)	2/2 (100%)	2/2 (100%)	9/10 (**90%**)
	Eq.2 V	12	4/6 (67%)	3/4 (75%)	2/2 (100%)		3	1/1 (100%)	1/1 (100%)	1/1 (100%)	
	Eq.4 M	28	10/12 (83%)	5/9 (56%)	7/7 (100%)	31/40 (**78%**)	7	2/3 (67%)	2/2 (100%)	2/2 (100%)	9/10 (**90%**)
	Eq.4 V	12	5/6 (83%)	2/4 (50%)	2/2 (100%)		3	1/1 (100%)	1/1 (100%)	1/1 (100%)	

M and V refer to the model and validation set, respectively.
n_{UMP} and n_S are the number of ultrasonic measurement points (UMP) and samples (S) in each set.
CC represents the correctly classified samples and is expressed as the percentaje of correctly classified n_{UMP} or n_S (in parenthesis/brackets) and as the ratio between the

4. CONCLUSIONS

The gradual shortening of the time of flight during the dry salting of loins and hams was mainly related to the salt gain. 85% of the loins and 90% of the hams were correctly classified by using predictive models based on the ultrasonic parameters. A slight improvement in the percentage of correctly classified samples was achieved in loins (95%) with the inclusion of the sample's weight and salting time in the predictive model. Therefore, the ultrasound pulse-echo mode could be a useful technique for continuous dry salting monitoring, as well as for the salt gain prediction for classification purposes. In addition, the pulse-echo technique is characterized by the use of a single transducer on one side of the sample, which facilitates the industrial implementation of this non-destructive technique compared to the through-transmission mode.

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REFERENCES

Albarracín, W., Sánchez, I. C., Grau, R., & Barat, J. M. (2011). Salt in food processing; usage and reduction: a review. International Journal of Food Science and Technology, 46, 1329-1336.

Association of Official Analytical Chemist (AOAC). Official Methods of Analysis. (1997). 16th Edn. Washington, DC: AOAC, International.

Avanesians, P., & Momayez, M. (2015). Wave separation: Application for arrival time detection in ultrasonic signals. Ultrasonics, 55, 15-25.

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.

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

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

Cierach, M., & Modzelewska-Kapituła, M. (2011). Effects of ph values and fat content on sodium chloride diffusion rate in pork. Journal of Food Processing and Preservation, 35, 129-142.

Correia, L. R., Mittal, G. S., & Basir, O. A. (2008). Ultrasonic detection of bone fragment in mechanically deboned chicken breasts. Innovative Food Science & Emerging Technologies, 9 (1), 109-115.

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, 8, 1226-1233.

De Prados, M., García-Pérez, J. V., & Benedito, J. (2015). Non-destructive salt content prediction in brined pork meat using ultrasound technology. Journal of Food Engineering, 154, 39-48.

De Prados, M., García-Pérez, J. V., & Benedito, J. (2016). Ultrasonic characterization and online monitoring of pork meat dry salting process. Food Control, 60, 646-655.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. Meat Science, 74, 188-196.

Fulladosa, E., Muñoz, I., Serra, X., Arnau, J., & Gou, P. (2015). X-ray absorptiometry for non-destructive monitoring of the salt uptake in bone-in raw hams during salting. Food Control, 47, 37e42.

Gaete-Garretón, L., Vargas, Y., León, C., & Pettorino, A. (2005). A novel non-invasive ultrasonic method to assess avocado ripering. Food Engineering and Physical Properties, 70, 187-191.

García-Gil, N., Muñoz, I., Santos-Garcés, E., Arnau, J., & Gou, P. (2014). Salt uptake and water loss in hams with different water contents at the lean surface and at different salting temperatures. Meat Science, 96, 65-72.

Ghaedian, R., Coupland, J. N., Decker, E. A., & McClements, D.J. (1998). Ultrasonic determination of fish composition. Journal of Food Engineering, 35, 323-337.

Håseth, T. T., Høy, M., Kongsro, J., Kohler, A., Sørheim, O., & Egelandsdal, B. (2008). Determination of sodium chloride in pork meat by computed tomography at different voltages. Journal of Food Science, 73(7), E333-E339.

Håseth, T. 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.

Kinsler, L. E., Frey, A. R., Coppens, A. B., & Sanders, J. V. (1982). Fundamentals of acoustics. John Wiley and Sons (eds.), (Chapter 5). New York, USA.

Kuo, F. J., Sheng, C. T., & Ting, C. H. (2008). Evaluation of ultrasonic propagation to measure sugar content and viscosity of reconstituted orange juice. Journal of Food Engineering, 86(1), 84-90.

Leemans, V., & Destain, M.-F. (2009). Ultrasonic internal defect detection in cheese. Journal of Food Engineering, 90, 333-340.

Lorenzo, J. M., Fonseca, S., Gómez, M., & Domínguez, R. (2015). Influence of the salting time on physico-chemical parameters, lipolysis and proteolysis of dry-cured foal "cecina". LWT - Food Science and Technology, 6, 332-338.

Manzocco, L., Anese, M., Marzona, S., Innocente, N., Lagazio, C., & Nicoli, M. C. (2013). Monitoring dry-curing of S. Daniele ham by magnetic resonance imaging. Food Chemistry, 141, 2246-2252.

McClements, J. (1995). Advance in the application of ultrasound in food analysis and processing. Trends in Food Science & Technology, 6, 293-299.

Moreiras, O., Carbajal, A., Cabrera, L., & Cuadrado, C. (2013). Tablas de composición de alimentos. Pirámide (Eds), Madrid, Spain.

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

Ruiz-Ramírez, J., Arnau, J., Serra, X., & Gou P. (2005). Relationship between water content, NaCl content, pH and texture parameters in dry-cured muscles. Meat Science, 70, 579-587.

Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. Meat Science, 70, 531-541.

Sigfusson, H., Ziegler, G. R., & Coupland, J. N. (2001). Ultrasonic monitoring of unsteady state chilling of food products. Transactions of the ASAE, 44(5), 1235-1240.

Sigfusson, H., Ziegler, G. R., & Coupland, J. N. (2004). Ultrasonic monitoring of food freezing. Journal of Food Engineering, 62(3), 263-269.

Toldra, F., & Wai-Kit, N. (2008). Dry-Cured Meat Products. Wiley-Blackwell (Eds) Manufacturing of Dry-Cured Hams (Chapter 3). USA.

CHAPTER 4.3

DRY-CURED PORK MEAT CHARACTERIZATION

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X-ray absorptiometry and ultrasound technologies for non-destructive compositional analysis of dry-cured ham

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Abstract

The characterization of dry-cured ham according to salt and fat contents is of great interest to industry and consumers. In this study, the feasibility of using non-destructive technologies such as X-rays and ultrasound (US) for this purpose was evaluated in dry-cured ham portions. Predictive models for fat and salt contents were based on the measurement of X-ray attenuation at different incident energies and the US velocity when the ham was at 2 and 15 ºC. A semi-empirical model based on the US measurements was also developed. Salt content was better predicted by X-ray technology (RMSEV=0.43%) than US (RMSEV=0.69%) and their combination had little impact on the accuracy of the prediction. US predicted fat content slightly better (RMSEV=6.70%) than X-rays (RMSEV=7.00%), and their combination increased the accuracy of the prediction (RMSEV=5.60%). Using the best models, 81% of samples were correctly classified into three salt content categories with X-rays whereas 71% of samples were correctly classified into three fat content categories by combining X-rays and US.

Keywords: *Non-destructive, Modelling, Dry-cured ham, Salt, Fat, X-rays, Ultrasound, Composition.*

1. INTRODUCTION

The characterization of food products according to their composition is of interest to industry in order to provide consumers with nutritionally defined products. Well-characterized products allow consumers to choose foodstuffs according to their needs and/or preferences. Besides, the selection of products with a lower salt or fat content than the conventional ones may have a special relevance. In this regard, nutritional claims such as "reduced salt" (25% less salt in comparison to standard salt content on the market) or "reduced fat content" (30% less fat in comparison to standard fat content on the market) may have competitive advantages. However, information, such as 'reduced salt content' or 'reduced fat content', should be confirmed in order to ensure compliance with European regulations (Regulation 1924/2006). In the case of entire pieces of meat, such as dry-cured ham, this characterization is of special complexity since the variation of fat and salt contents within batch, batch-to-batch or even within one ham is high. Variations in the global salt content of the product at the end of the process is due to both the raw ham characteristics and the salting process conditions (Garcia-Gil et al., 2011; Guerrero et al., 2004; Picouet et al., 2013).

Recently, several non-destructive technologies based on near infrared spectroscopy (Gou et el., 2013), dielectric time domain reflectometry (Fulladosa et al., 2013; Rubio-Celorio et al., 2014) and hyperspectral imaging (Liu et al., 2014) have arisen as useful techniques to determine salt and water contents and water activity (a_w) in dry-cured ham. However, these technologies only analyze the composition of the outermost layers of a product (because of their limited penetration capacity), which is not representative of the whole piece. Mincing of the sample would be necessary to obtain representative homogeneous surfaces which allow the measurement of the average composition of a whole meat piece. In contrast, resonance magnetic imaging (Fantazzini et al., 2009) or computed tomography (Fulladosa et al, 2010; Håseth et al., 2012; Santos-Garcés et al., 2010, 2014) are able to

non-invasively predict the composition of any point of a piece, but nowadays they are not currently applicable on-line on an industrial scale due to their high security requirements and cost. Other technologies based on X-ray absorptiometry and ultrasound (US) can be applied on-line and could be useful to predict the composition of entire pieces of ham. X-ray absorptiometry has previously been used to determine carcass and raw meat composition (Brienne et al., 2001, Hansen et al., 2003). US has also previously been used to determine the composition of meat-based products (Simal et al., 2003) and characterize the texture and sensory traits of subcutaneous fat from dry-cured hams (Niñoles et al., 2008). Nevertheless, no studies using these technologies for salt or fat content determination in dry-cured ham were found in the literature.

The aim of the present study was to evaluate the prediction accuracy of salt and fat contents in dry-cured hams by means of X-ray and ultrasound technologies and to study the viability of a classification system of dry-cured hams according to a predicted composition.

2. MATERIALS AND METHODS

2.1 SAMPLES

Forty-six hams from different breeds and origins were selected/elaborated in order to obtain a wide range of salt and fat contents. Nineteen green hams from pigs consisting of crosses of Large White and Landrace breeds (W, White hams), characterised by a lower fat content than IB hams, were purchased at different commercial slaughterhouses. The W hams were salted for 0.6, 0.7, 0.8, 1.1, 1.2, 1.3, 1.4 and 1.5 days/kg of green ham in order to obtain a wide variation of salt contents and followed a traditional drying process (Gou et al. (2012). When the drying process had finished, 27 commercial dry-cured hams from pigs consisting of at least 50% Iberian breed (IB, Iberian hams) characterised by a high fat content were selected at the end of the salting process according to their salt and fat contents, using computed tomography

equipment (Santos-Garcés et al., 2012). All the hams, W and IB, were boned, trimmed of skin and external yellow subcutaneous fat and lean according to the common practices in industry, formatted in blocks of constant thickness (76.3±3.5 mm) and divided into six portions (n=276) for further X-ray analysis. After X-ray measurements, the ham portions were individually vacuum packed in plastic bags before US measurements were carried out.

2.2 X-RAY ABSORPTIOMETRY (X-RAY)

A X-ray inspector model X20V G90 (Multiscan technologies, S.L, Cocentaina, Spain) was used to scan the dry-cured ham portions (Figure 1A). X-rays were emitted from below the samples and the transmitted X-rays were measured at the upper part of the device while a conveyor belt moved the sample through it. The system used low-energy X-rays to obtain images (matrices of attenuation values, 4000 x 1280 pixels) of the scanned object at a horizontal plane with a constant speed (20 m/min). Three different voltages and intensities, specifically 90 kV and 4 mA, 70 kV and 8 mA and 50 kV and 15 mA, were used to scan the ham portions each one in exactly the same position.

Matrices of attenuation values were imported from the X-ray inspector device. The global X-ray attenuation value (A) of the sample was obtained by the following equation:

$$A = -\sum \mathrm{Ln}\left(\frac{I_{(i;j)}}{I_{0(i;j)}}\right) \qquad \text{(Eq. 1)}$$

where I is the intensity of the transmitted radiation through each pixel ($i;j$); I_o is the energy of the incident radiation to each pixel ($i;j$); i ranges from 1 to 4000 and j ranges from 1 to 1280. The global X-ray attenuation values for each incident energy (50, 70 and 90 kV) were referred to sample weight (A_{90}/weight, A_{70}/weight and A_{50}/weight).

2.3 ULTRASOUND (US)

Figure 1B shows the experimental set-up used for the ultrasonic measurements. The experimental set-up consisted of a couple of narrow-band ultrasonic transducers (1 MHz, 0.75'' crystal diameter, A314S-SU model, Panametrics, Waltham, MA, USA), a pulser-receiver (Panametrics, Model 5058PR, Waltham, USA) and a digital storage oscilloscope (Tektronix, TDS5034, Digital phosphor oscilloscope. Tektronix inc. Bearverton, Oregon. USA). The sample was placed between the transducers (Figure 1B). A custom designed digital height gage, linked to the computer by a RS232 interface, was used to measure the thickness of the packaged ham, with an accuracy of ±0.01 mm. The ultrasonic velocity in the sample was computed from the time of flight obtained from the signal and the thickness read from the RS232 interface. The time of flight for a sample was computed by averaging the value obtained from five signal acquisitions. To compute the ultrasonic velocity, the system delay was taken into account. In order to determine this delay, the pulse transit time was measured across a set of calibration cylinders of different thicknesses. The delay time was then obtained from the intercept on the y-axis of the time versus thickness graph.

In order to carry out the ultrasonic measurements, several zones of measurement (circles) were marked on the surface of each package, as shown in Figure 1B. The zones of measurement (from 10 to 19, depending on the package surface) were uniformly distributed over the surface of each portion. The surface of each zone tested coincided with the surface of the transducer used in the ultrasonic measurements. The measurement of the ultrasonic velocity was carried out in triplicate for each zone of measurement at two different temperatures (2 and 15 ºC).

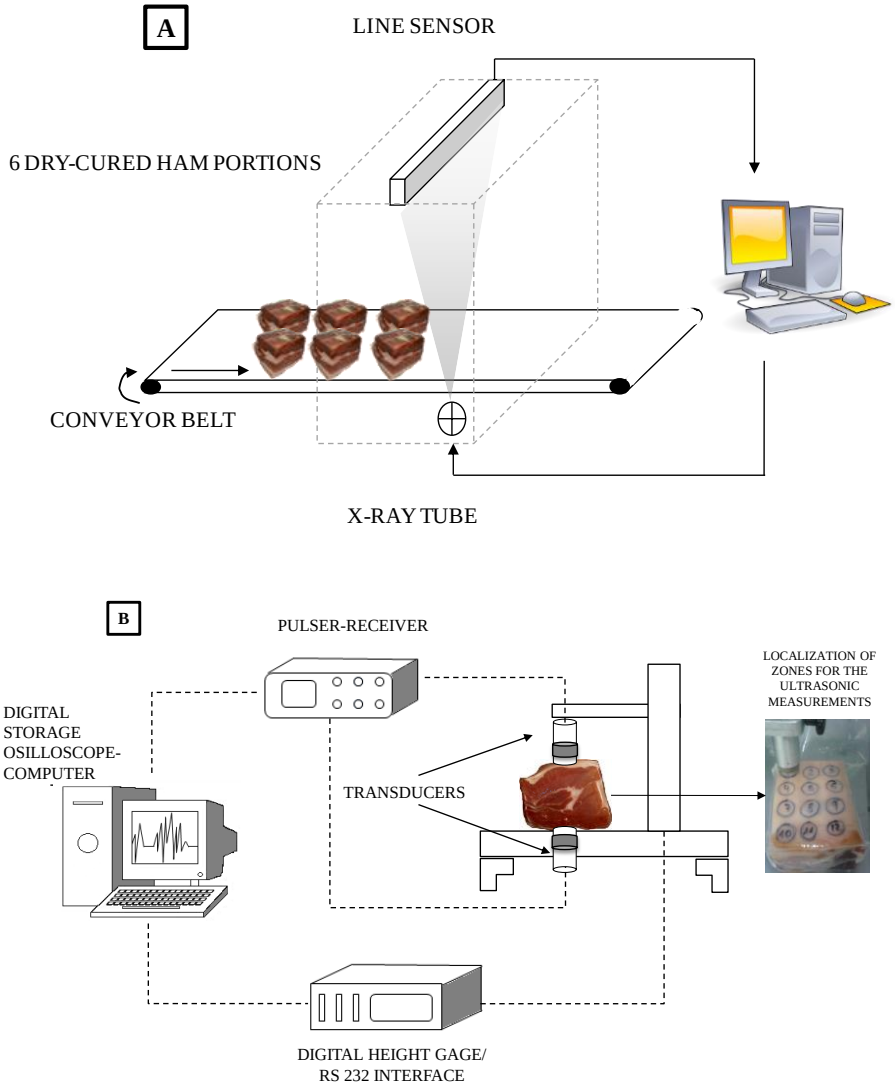

Figure 1. Experimental set-up used for the X-ray (A) and ultrasonic (B) measurements.

2.4 CHEMICAL ANALYSIS

Following the US measurements, ham portions were unpacked, ground and homogenized for chemical analysis. Water content was analysed by drying at 103±2 ºC until reaching a constant weight (AOAC, 1990). Chloride content was determined according to ISO 1841-2 using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland) and expressed as salt content. The

total fat content was estimated by near infrared spectroscopy using a FoodScanTM Lab (Foss Analytical, Dinamarca) (AOAC, 2006). All the analyses were performed in triplicate.

2.5 CALIBRATION AND VALIDATION DATA SETS

For each component (fat and salt content), the samples (n=276) were divided into two sets (calibration and validation data sets) in order to develop and validate the predictive compositional models. To define both sets, the samples were sorted according to their fat or salt content. Subsequently, they were distributed alternatively, two dry-cured ham portions for the calibration set (184 samples) and one dry-cured ham portion for the validation set (92 samples). In this way, both sets of samples covered a similar range for each component (Table 1). The validation set was also used to test the overall classification capacity using the predictive models chosen for salt and fat prediction. For this purpose, samples were classified into three different categories depending on their salt content. Hams with a salt content of ≤ 4% were considered to have a low level of salt, hams with a salt content of > 5.5% a high level of salt and those remaining to have a moderate level of salt. Similarly, samples with a predicted fat content of ≤ 25% were considered to have a low level of fat, whereas samples with a fat content of > 40% were considered to have a high level of fat and the rest to have a moderate level of fat.

Table 1. Sample composition for calibration and validation sets.

	Set	n	Salt content range (%)	Water content range (%)	Fat content range (%)
For salt predictive model	**Calibration**	184	2.08-7.35	21.67-51.81	6.82-57.22
	Validation	92	2.34-7.16	18.63-50.68	7.61-59.05
For fat predictive model	**Calibration**	184	2.27-7.18	18.63-51.81	6.82-59.05
	Validation	92	2.08-7.35	22.59-50.68	7.61-55.73

2.6 PREDICTIVE MODELS AND STATISTICAL ANALYSIS

A_{90}/weight, A_{70}/weight and A_{50}/weight and ultrasonic velocity (v) at 2 (v_{T1}) and 15 ºC (v_{T2}) were used as independent variables to develop linear predictive models. REG procedure from XLSTAT package (Addinsoft, Paris, France) was used to estimate model parameters for each technology (X-rays and US). The independent variables included in the models were identified using Stepwise method. Levels of significance to enter and keep the independent variables in the model were p=0.05 and p=0.1, respectively.

Additionally, a semi-empirical model to estimate the composition of dry-cured ham as a function of the ultrasonic velocity was used (Benedito et al., 2001). This model considers that ultrasonic velocity in a medium is dependent on the particular velocity within the different components of that medium. In this case it is assumed that samples behave as a three component system (fat, water and proteins+others) and the temperature dependence of the ultrasonic velocity in the different components is considered (Eqs. 2-4).

$$\frac{100}{v_{T1}^2} = \frac{x_f}{v_{f\,T1}^2} + \frac{x_w}{v_{w\,T1}^2} + \frac{x_{p+o}}{v_{p+o\,T1}^2} \qquad \text{(Eq. 2)}$$

$$\frac{100}{v_{T2}^2} = \frac{x_f}{v_{f\,T2}^2} + \frac{x_w}{v_{w\,T2}^2} + \frac{x_{p+o}}{v_{p+o\,T2}^2} \qquad \text{(Eq. 3)}$$

$$x_f + x_w + x_{p+o} = 100 \qquad \text{(Eq. 4)}$$

where v is the ultrasonic velocity in the dry cured ham, v_f, v_w and v_{p+o} are the ultrasonic velocities in the fat, water and protein+other components, while subscripts T_1 and T_2 refer to the ham temperature (2 and 15 ºC, respectively). x_f, x_w and x_{p+o} are the composition of fat, water and protein+other components, respectively. Eqs. from 5 to 9 show the temperature dependence of the ultrasonic velocity on water, fat and protein+other dry components of IB and W hams. Firstly, Eqs. 5 and 6 were

experimentally determined in this work for subcutaneous fat taken from dry-cured W and IB hams following the experimental procedure reported by Niñoles et al., (2008). Secondly, Eq. 7 (Kinsler et al., 1982) was obtained from literature. Thirdly, the A, B, C, D coefficients of Eqs. 8 and 9 were identified by fitting the model to the calibration set. Thereby, in the validation set, measuring v at T_1 and T_2 and calculating v_f, v_w and v_{p+o} at T_1 and T_2 from Eqs 5-9, the composition of the dry-cured ham could be estimated from Eqs. 2-4.

$$v_{fIB}=1666.7-5.47T \quad \text{(Eq. 5)}$$

$$v_{fLW}=1646.7-4.84T \quad \text{(Eq. 6)}$$

$$v_{w(IB-LW)}=1403+5T-0.06T^2+0.0003T^3 \quad \text{(Eq. 7)}$$

$$v_{p+oIB}=A-BT \quad \text{(Eq. 8)}$$

$$v_{p+o\ LW}=C-DT \quad \text{(Eq. 9)}$$

The feasibility of X-ray and US models to predict fat and salt contents was evaluated by determining the coefficient of determination (R^2) and the Root Mean Square Error (RMSE) of calibration (RMSEC) and validation (RMSEV) and the residual predictive deviation (RPD) statistic. RPD is the ratio between the standard deviation of the reference values and the error of validation.

Overall correctness of sample classification in the three groups of salt or fat contents (low, moderate and high) was evaluated through the percentage of correctly classified samples from the validation set.

3. RESULTS AND DISCUSSION

3.1 SALT PREDICTIVE MODELS

X-ray attenuation values obtained at three different energies (50, 70 and 90 kV) were correlated to the salt content (Figure 2A). The attenuation increased with the salt content due to the high density of Na^+ and Cl^- ions. This increase was

more pronounced at 50 kV than at high-energy radiations because of the fact that low-energy radiations are more sensitive to density variations caused by inorganic substances such as Na^+ and Cl^- ions. This was reported by Fulladosa et al (2014) who, when using the same technology, reported that the variation of the attenuation of raw bone-in hams during salting was higher at low X-ray energies (50 kV) than at high energies (70 and 90 kV). The experimental variation of X-ray attenuation between samples with the same salt content could be caused mainly by their variation in water content (different levels of dryness) which produces variations of samples density. In previous studies dealing with samples having variable thicknesses, other authors have highlighted the importance of this parameter (Brienne et al., 2001; Hansen et al., 2003). However, the thickness effect could be considered negligible in the formatted ham portions.

The salt content affected the US measurements at 15 ºC (Figure 2B), but not at 2 ºC. An increase of salt content gave rise to higher ultrasonic velocities. This fact was reported by Kinsler et al., (1982), who found that an increase of salt content in an aqueous solution of sea water causes an increase of the ultrasonic velocity due to a rise in the solid content of the medium when measured at any temperature. Therefore, the higher the solid/liquid ratio, the higher the ultrasonic velocity. The same effect was observed in the salting process of pork muscles (García-Pérez et al., 2012 and De Prados et al., 2014) and hams (De Prados et al., 2012) due not only to the salt gain but also to the water loss. Figure 2B shows that experimental data presented a large dispersion. This fact could be ascribed to the complex sample analyzed in this study, which differs not only in salt content but also in water and fat content and the type of fat and protein matrix (W and IB hams). Moreover, meat curing can affect the state of proteins and lipids due to the complex reactions taking place during process, as a result of these changes, the tissue mechanical properties could be modified. All these factors affect ultrasonic velocity (Niñoles et al., 2008; Corona et al., 2013; Llull et al., 2002) and are partially responsible for the experimental dispersion.

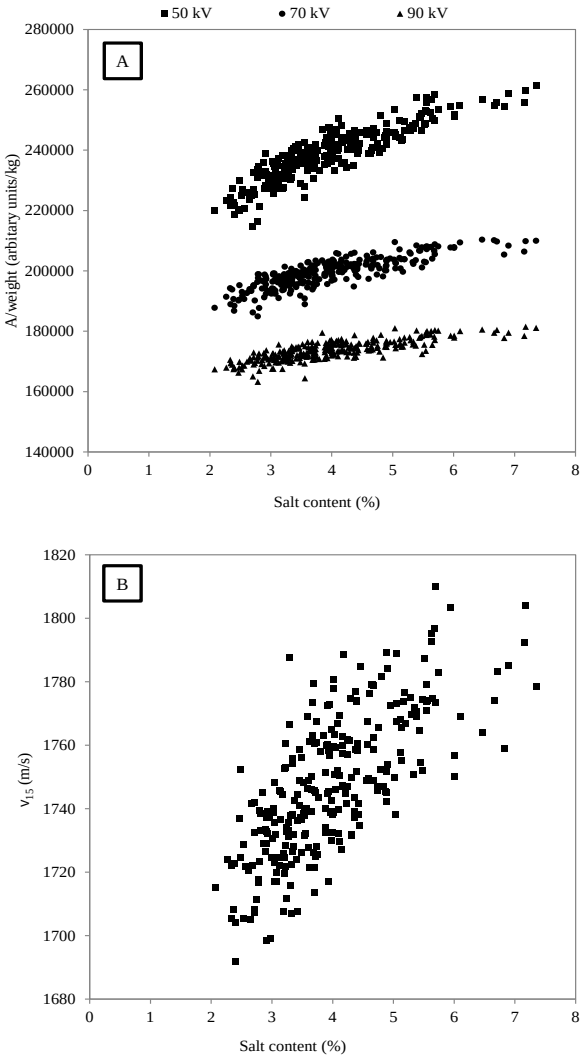

Figure 2. Influence of salt content of dry-cured ham portions on X-ray attenuation (A) and ultrasonic velocity at 15 ºC (B).

The parameters of the models developed to predict the salt content in dry-cured ham portions using X-rays and US are presented in Table 2. X-ray technology can predict salt content (RMSEV=0.43%) using only the information obtained at 50 kV. The portions were taken from formatted dry-cured hams and therefore no important variation in portion thicknesses. The thickness effect on the attenuation of X-rays was then considered negligible. Moreover,

the residual errors of salt prediction (measured – predicted) presented a random distribution and were not significantly ($p>0.05$) related to fat or water content (results not shown). This demonstrates the robustness of this salt content predictive model to fat or water content variations.

Table 2. Statistical parameters of salt predictive models using X-ray and US technologies.

	X-ray	**US**	**X-ray + US**
Calibration			
n	184	184	184
R^2	0.781	0.528	0.804
RMSEC (%)	0.48	0.71	0.46
Model variables	A_{50}/weight	v_{15}	A_{50}/weight, v_{15}
Validation			
n	92	92	92
R^2	0.814	0.549	0.824
RMSEV (%)	0.43	0.69	0.42
RPD	2.3	1.5	2.4

Simple regression models using the US velocity at 15 ºC as variable showed higher prediction errors in comparison to X-ray based models (Table 2). The slope of the linear relationship (15.85 m s^{-1} $\%^{-1}$) shows that increasing the salt content by 1%, the velocity increases 15.85 m/s (Figure 2B). Kinsler et al. (1982) reported a similar slope (16.5 m s^{-1} $\%^{-1}$) for the linear relationship between ultrasonic velocity (at 15 ºC) and salt content for a water/salt solution, which indicates that the influence of salt on ultrasonic velocity could be non-dependent on the matrix where ultrasound is propagated. Nevertheless, although the increase of salt content affected the ultrasonic velocity, it was not the only factor. The large variability of dry-cured ham composition (fat and

water content and type of fat and protein matrix) diminishes the importance of the salt as a factor which affects the ultrasonic velocity. As for X-rays, errors of salt prediction were randomly distributed and were not significantly ($p>0.05$) related to fat or water content (results not shown). However, the use of an US based model for the salt prediction did not provide reliable results (Table 2). Moreover, the addition of US variables into the predictive model of X-ray barely improved the model (Table 2). RPD value was 2.3 for X-ray absorptiometry, 1.6 for ultrasound technology and 2.4 when combining both technologies. Conzen (2006) considered a model good for quality control purposes with an RPD > 5, but models with lower RPD values could still be used for screening or classification purposes. In this case, models will not permit an exact estimate of the composition of the product but they could be useful for classifying products into different salt level groups, a relevant usefulness for industrial applications.

3.2 FAT PREDICTIVE MODELS

Global X-ray attenuation values at the three different energies were correlated to fat content, and the most important variation in attenuation values was found mainly at 50 kV (Figure 3A), as in the case of salt. The correlation was lower than in the case of the salt content.

In the case of US, a different effect of the fat content on the ultrasonic velocity at 2 and 15 ºC was found. At 2 ºC, ultrasonic velocity was not related ($p>0.05$) to the fat content, which points to the fact that ultrasound velocity in the fatty matrix is similar to that in the protein matrix (for both W and IB). When temperature rises to 15 ºC, the ultrasonic velocity shows a significant ($p<0.05$) decrease with the increase of the fat content ($R^2=0.393$). Consequently, the ultrasonic velocity in the fatty tissue has to be lower than in the protein matrix. This difference in velocity between the fatty and protein matrixes as temperature increases from 2 to 15ºC could be linked to the melting of the fat triglycerides (Corona et al., 2014a,b), which causes a decrease of the fat

solid/liquid ratio and produces a drop of the ultrasonic velocity (Benedito et al., 2001). In addition, the velocity in the protein matrix would increase as temperature rises as a consequence of the increase of velocity in water for higher temperatures (Eq. 7). Therefore, in order to take advantage of the different effect of temperature on the fatty and protein matrixes, it was considered convenient to assess the variation of the velocity between 2 and 15 ºC ($\Delta v = v_2 - v_{15}$) for each zone of measurement and average them for each ham portion. In this way, samples with a higher fat content would suffer a larger velocity drop between 2 and 15ºC, due to fat melting, than those with a lower fat content. These results are shown in Figure 3B where it can be observed that as expected, the higher the fat content, the higher the ultrasonic velocity variation (Δv). In spite of the fact that there is an important part of Δv variability that can be explained by fat content (R^2=0.678), there is still a large experimental dispersion (Figure 3B). These results point to the fact that not only the fat but also other components, such as proteins, salt and water affect the ultrasonic velocity. As previously mentioned, during meat curing, fat and protein matrix can suffer important modifications. However, textural changes that take place in dry-cured ham curing are mostly linked to the water loss, thus being the major responsible of ultrasonic velocity modifications.

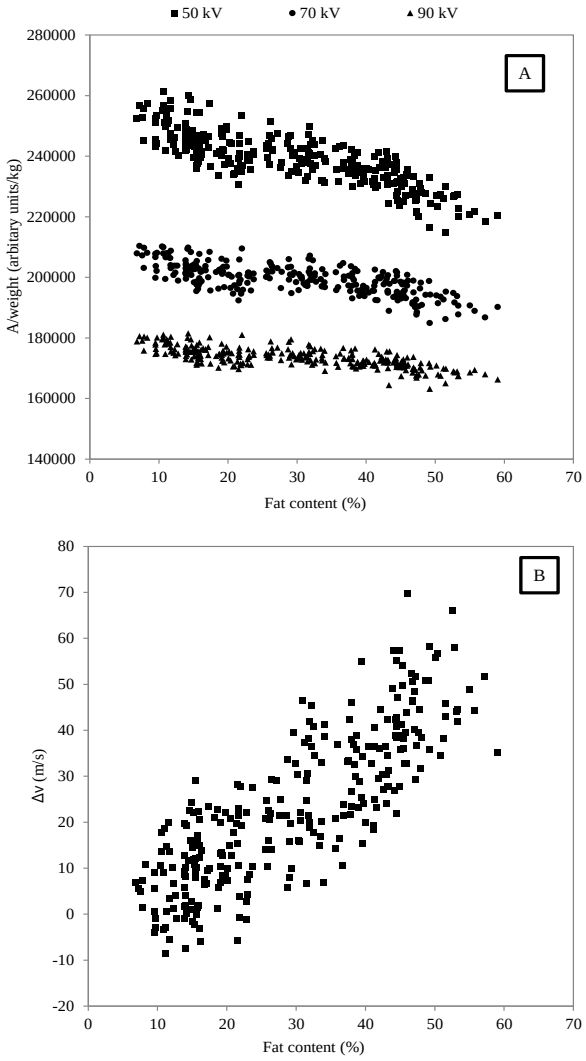

Figure 3. Influence of fat content of dry-cured ham portions on the X-ray attenuation (A) and on the increment of ultrasound velocity between 2 and 15 ºC (B).

The parameters of the predictive model using X-rays are presented in Table 3. By using only attenuation values at 50 kV the predictive error was 8.02%. The addition of the information obtained at 70 kV to the model, improved the prediction (RMSEV=7.00%).

The use of only Δv or the combination with v_{15} provided a similar prediction error (RMSEV=6.88%) to the model of X-rays (Table 3). The effect of temperature on US velocity depends on the sample composition (Niñoles et al., 2008). Therefore, the US velocity in such a complex medium as dry-cured ham is weighed from the particular velocity of each component. Thereby, a semi-empirical model was developed (Eqs. from 2 to 9) where a 3 component system (water, fat and protein+others) were considered (see section 2.6). The coefficients (A=4239.7, B=64.06, C=3373.9 and D=39.20) from Eqs. 8 and 9 were obtained. This compositional model provided a similar estimation of the fat content (RMSEV=6.70%) in comparison to the individual models for US and X-rays. The main advantage of this semi-empirical model is that it not only estimates the fat content but also provides a good estimation of the water (Table 4) and protein+others (results not shown), which allows the characterization of the global composition of the dry-cured ham portions (Ghaedian et al., 1997, Benedito et al., 2001, Corona et al., 2013). However, the use of such a model requires the ultrasonic velocity measurement at two temperatures, which represents a drawback for industrial application.

Table 3. Statistical parameters of fat predictive models using X-ray and US technologies.

	X-ray	**US**	**X-ray + US**
Calibration			
n	184	184	184
R^2	0.672	0.739	0.844
RMSEC (%)	7.87	7.01	5.42
Model variables	A_{50}/weight, A_{70}/weight	Δv, v_{15}	Δv, A_{50}/weight
Validation			
n	92	92	92
R^2	0.732	0.738	0.825
RMSEV (%)	7.00	6.88	5.60
RPD	1.9	2.0	2.4

The estimation of fat content was improved by combining the results of X-rays and US (Table 3). A regression model using the ultrasonic velocity variation with temperature (Δv) and X-ray attenuation at 50 kV (A_{50}/weight) as factors, provided a significantly higher RPD value (2.4) and a lower RMSEV (5.60%). However, it should be taken into account that the use of the combination of these technologies would result in a high investment and application cost to industry. It must be pointed out that, using these technologies, fat distribution within the sample (intermuscular, intramuscular and subcutaneous fat content) cannot be distinguished or evaluated separately.

Table 4. Statistical parameters of US semi-empirical compositional model.

	Fat content	**Water content**
Calibration		
n	184	184
R^2	0.805	0.732
RMSEC (%)	6.56	4.88
Model variables	v_2 , v_{15}, T	v_2 , v_{15}, T
Validation		
n	92	92
R^2	0.804	0.718
RMSEV (%)	6.70	4.85
RPD	2.01	1.51

3.3 USEFULNESS OF X-RAY AND US TO CLASSIFY SAMPLES ACCORDING TO THEIR COMPOSITION

The usefulness of X-ray and US technologies as reliable methods to classify dry-cured ham samples into different levels of saltiness and fatness was evaluated by using the best predictive models. Figure 4, where experimental

and predicted data are plotted for the validation set, illustrates the accuracy of the proposed models for the prediction of salt and fat content.

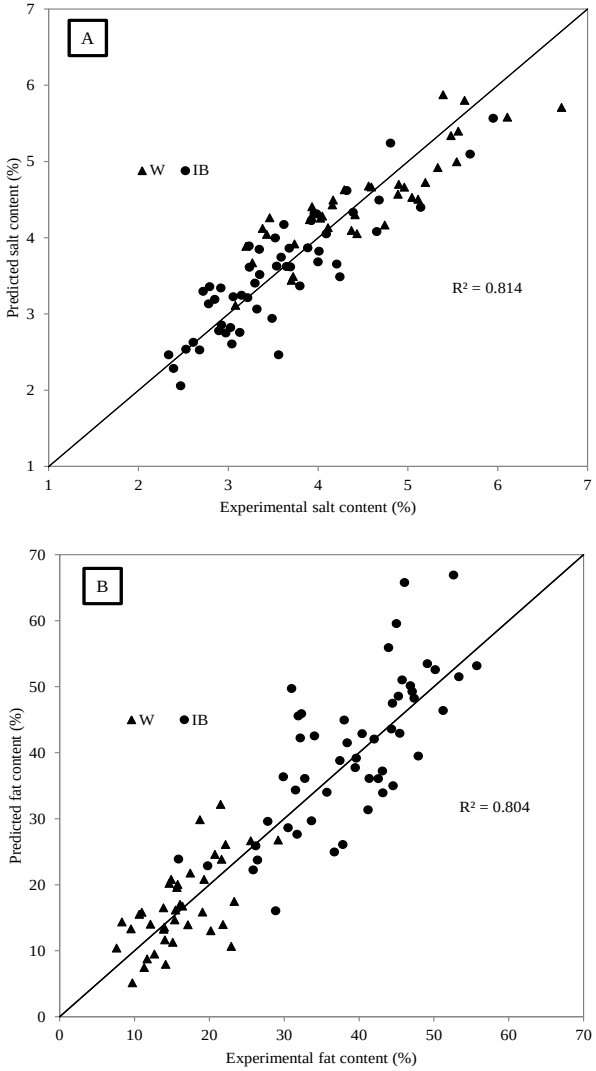

Figure 4. Predicted versus experimental salt and fat contents of dry-cured ham portions from the validation data set using X-ray model for salt content prediction (A) and US semi-empirical model for fat content prediction (B).

The salt content of portions from the validation set was predicted using the model developed using X-rays, which uses only one variable for the prediction

(A_{50}/weight) (Table 2). Furthermore, the overall correctness of the classification in the three pre-defined categories (low, moderate and high) was 81%, which demonstrates the practical relevance of using X-ray technology for non-destructive salt content analysis. Discrepancies were found between low and moderate salt levels and between moderate and high salt levels but not between low and high salt levels. Only 4 of 46 portions (error of 8.7%) were classified as having a low salt level when in fact they had a moderate salt level. Using this technology, errors in nutritional claims such as "reduced salt content" could be further reduced if a more restrictive threshold value was used to define the low level salt groups.

The fat content was predicted using a combination of X-ray and US parameters (Table 3). Using this model, the overall correct classification of portions according to fat content level was 71%. The practical implementation of dual technology equipment based on both US and X-ray technologies could be complicated because the cost of the equipment would increase significantly and individual calibrations for both equipments would be required. Therefore, it seems more reasonable to try the individual use of one technology instead of a combination of the two. Using the semi-empirical model obtained from US measurements, the correctness of the classification increased to 77%. At low fat contents, the semi-empirical model was able to correctly classify 92% of the pieces, however for moderate and high fat contents, the percentage of well classified hams was reduced to 58 and 73%, respectively. Only 4 of 26 portions (error of 15.3%) were classified as having a low fat level when in fact they had a moderate fat level. Thus, using this technology, nutritional claims such as "reduced fat content" could be ensured if a restrictive threshold value was used to define the low level fat groups.

As already mentioned, one of the disadvantages of US characterization is the need to measure the velocity at two different temperatures. This fact makes the management of the product difficult and lengthtens the time of product analysis. However, measuring the portions (blocks or thick slices) at the end of

the curing stage at room temperature (15-20 ºC) and after a certain storage period in refrigeration (2-4 ºC) could be considered. This strategy would allow the partial characterization of the global composition (x_f, x_w and x_{p+o}) of the dry-cured ham portions.

It must be stated that there are other well established and commercially available methods that allow evaluation of physical and chemical composition of muscle foods (Damez and Clerjon, 2013). However, most of them are destructive, or do not penetrate enough in the product, or are medical systems non-adapted for food control at the industry. Development of better adapted equipment for the food industry is on the focus of device manufactures.

4. CONCLUSION

X-rays and US technologies can predict salt contents of dry-cured ham portions with an error of 0.43% and 0.69%, and fat content with an error of 7.00% and 6.70%, respectively. In the case of fat, the combination of both technologies decreases fat prediction error to 5.60%. The identification of samples that could be labelled with nutritional claims such as "reduced salt" or "reduced fat" is feasible using X-rays and US. Therefore, the individual or combined use of these technologies present a high interest for industrial application and quality control purposes.

ACKNOWLEDGEMENTS

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REFERENCES

AOAC. (1990). Official Method 950.46, Moisture in meat, B. Air drying. In K. Helrich (Ed.) (15th ed.). Official methods of analysis of the association of

official analytical chemists (Vol. II, pp. 931). Arlington: Association of Official Analytical Chemists, Inc.

AOAC. (2006). Official method 991.36, fat (crude) in meat and meat products. Retrieved from: http://www.aoac.org/oma_revision/toc.htm

Benedito, J., Carcel, J. A., Rossello, C. & Mulet, A. (2001). Composition Assessment of Raw Meat Mixtures using Ultrasonics. Meat Science. 57:365-370

Brienne, J. P., Denoyelle, C., Baussart, H. & Daudin, J. D. (2001). Assessment of Meat Fat Content using Dual Energy X-ray Absorption. Meat Science. 57:235-244

Conzen, J. P. (2006). Multivariate Calibration, a Practical Guide for Developing Methods in the Quantitative Analytical Chemistry. Ettlingen, Germany: Bruker Optik GmbH

Corona, E., García-Pérez, J.V., Santacatalina, J.V., Ventanas, S. & Benedito, J. (2014a). Ultrasonic Characterization of Pork Fat Crystallization during Cold Storage. Journal of Food Science. In press.

Corona, E., García-Pérez, J.V., Ventanas, S. & Benedito, J. (2014b). Ultrasonic Characterization of the Fat Source and Composition of Formulated Dry-cured Meat Products. Food Science and Technology International. Food Science Technology International. 20 (4), 275–285.

Damez, J.L., Clerjon, S., 2013. Quantifying and predicting meat and meat products quality attributes using electromagnetic waves: An overview. Meat Science 95, 879–896.

De Prados, M. García-Pérez, J.V., Fulladosa, E. & Benedito, J. (2014). Monitorización del Salado de Pila de *Longissimur dorsi* mediante Ultrasonidos de Señal. Congreso Iberiamericano de Ingeniería de Alimentos. (9CIBIA) Valencia, Spain

De Prados, M., Pérez-Muelas, N., Rossello, C. García-Pérez, J.V., Ventanas, S. & Benedito, J. (2012). Use of Low Intensity Ultrasound to Monitoring Dry-cured Ham Salting. Institute of Food Technology 12. (IFT12) Annual Meeting and Food Expo. Las Vegas, NV USA.

Fantazzini, P., Gombia, M., Schembri, P., Simoncini, N. & Virgili, R. (2009). Use of Magnetic Resonance Imaging for Monitoring Parma Dry-cured Ham Processing. Meat Science. 82(2):219-227

Fulladosa, E., Duran-Montgé, P., Serra, X., Picouet, P., Schimmer, O. & Gou, P. (2013). Estimation of Dry-cured Ham Composition using Dielectric Time Domain Reflectometry. Meat Science. 93(4):873-879

Fulladosa, E., Muñoz, I., Serra, X., Arnau, J. & Gou, P. (2014). The Use of X-ray Absorptiometry to Predict the Salt Uptake in Bone-in Hams during Salting. Submitted to Food Research International

Fulladosa, E., Santos-Garcés, E., Picouet, P. & Gou, P. (2010). Prediction of Salt and Water Content in Dry-cured Hams by Computed Tomography. Journal of Food Engineering. 96(1):80-85

Garcia-Gil, N., Santos-Garcés, E., Muñoz, I., Fulladosa, E., Arnau, J. & Gou, P. (2011). Salting, Drying and Sensory Quality of Dry-cured Hams Subjected to Different Pre-salting Treatments: Skin Trimming and Pressing. Meat Science. 90(2):386-392

García-Pérez, J., De Prados, M., Pérez-Muelas, N., Cárcel, J. & Benedito, J. (2012). Ultrasonic Characterization of Pork Meat Salting. International Symposium on Ultrasound in the Control of Industrial Processes (UCIP 2012)

Ghaedian, R., Decker, E. A., & McClements, D. J. (1997). The Use of Ultrasound to Determine Cod Fillet Composition. Journal of Food Science. 62:500-504

Gou, P., Santos-Garcés, E., Hoy, M., Wold, J. P., Liland, K. H. & Fulladosa, E. (2013). Feasibility of NIR Interactance Hyperspectral Imaging for On-line Measurement of Crude Composition in Vacuum Packed Dry-cured Ham Slices. Meat Science. 95(2):250-255

Gou, P., Arnau, J., Garcia-Gil, N., Fulladosa, E., Serra, X. (2012). Dry-cured ham. In: Handbook of meat and meat processing. Second edition. p. 673-688

Guerrero, L., Gobantes, I., Oliver, M. A., Arnau, J., Guàrdia, M. D., Elvira, J., Riu, P., Grèbol, N. & Monfort, J. M. (2004). Green Hams Electrical Impedance Spectroscopy (EIS) Measures and Pastiness Prediction of Dry-cured Hams. Meat Science. 66:289-294

Hansen, P. W., Tholl, I., Christensen, C., Jehg, H. C., Borg, J., Nielsen, O., Ã˜stergaard, B., Nygaard, J. & Andersen, O. (2003). Batch Accuracy of On-line Fat Determination. Meat Science. 64(2):141-147

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(3):858-864

ISO 1841-2 (1996). Meat and Meat Products. Determination of Chloride Content -Part 2: Potentiometric Method (Reference method). Geneva: International Organization for Standardization.

Kinsler, L.E., Frey, A.R., Coppens, A.B. & Sanders, J.V. (1982). Fundamentals of Acoustics. John Wiley and Sons. U.S.A.

Liu, D., Sun, D.W., Qu, J., Zeng, X.A., Pu, H. & Ma, J. (2014). Feasibility of Using Hyperspectral Imaging to Predict Moisture Content of Porcine Meat during Salting. Food Chemistry. 152:197-204

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

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(3):896-902

Picouet, P. A., Gou, P., Fulladosa, E., Santos-Garcés, E. & Arnau, J. (2013). Estimation of NaCl Diffusivity by Computed Tomography in the *Semimembranosus* Muscle during Salting of Fresh and Frozen/thawed Hams. LWT. Food Science and Technology. 51(1):275-280

Regulation (EC) No 1924/2006 of the European parliament and of the council of 20 December 2006 on nutrition and health claims made on foods. p.9-25

Rubio-Celorio, M., Garcia-Gil, N., Gou, P., Arnau, J. & Fulladosa, E. (2014). Effect of Temperature, High Pressure and Freezing/thawing of Dry-cured Ham Slices on Dielectric Time Domain Reflectometry Response. Submitted to Meat Science

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(2):187-192

Santos-Garcés, E., Muñoz, I., Gou, P., Garcia-Gil, N., Fulladosa, E. (2014). Including Estimated Intramuscular Fat Content from Computed Tomography Images Improves Prediction Accuracy of Dry-cured Ham Composition. Meat Science. 96:943–947

Santos-Garcés, E., Muñoz, I., Gou, P., Sala, X. & Fulladosa, E. (2012). Tools for Studying Dry-cured Ham Processing by using Computed Tomography. Journal Agriculture Food Chemistry. 60(1):241-249

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

5. DISCUSIÓN GENERAL

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El jamón curado es un producto cárnico muy apreciado por los consumidores. Sin embargo, debido a la heterogeneidad de la materia prima y a los múltiples factores que afectan a su procesado, la composición de los jamones de un mismo lote presenta una elevada variabilidad. La aplicación de una tecnología no destructiva para caracterizar el jamón durante las diferentes etapas de su elaboración permitiría mejorar el proceso y así, obtener jamones curados con parámetros finales de calidad más homogéneos. La estimación mediante ultrasonidos de señal del contenido en grasa del jamón a la entrada del proceso y de su contenido en sal en la etapa de salado, permitiría diseñar mejor las etapas posteriores de su procesado y así, reducir la variabilidad composicional en los lotes de jamones de manera no destructiva, rápida, sencilla y a un bajo coste. Asimismo, la determinación del contenido en sal y grasa en el jamón curado mediante el empleo de esta tecnología ultrasónica, proporcionaría información nutricional que podría recogerse en el etiquetado del producto final, siendo de gran utilidad para el consumidor desde un punto de vista nutricional.

Caracterización del producto fresco mediante ultrasonidos de señal

La bibliografía previa pone de manifiesto que las medidas ultrasónicas han servido para estimar el contenido en grasa en diferentes productos cárnicos de cerdo, tales como canales (Lakshmanan et al., 2012), músculos, como *Biceps femoris* (BF) (Niñoles et al., 2011), y productos formulados (Corona et al., 2014b). De forma similar, el potencial de los ultrasonidos de señal para predecir el contenido en grasa en jamones frescos quedó demostrado en el capítulo 4.1 de la presente Tesis Doctoral. Así, los resultados del capítulo 4.1 mostraron que el contenido en grasa afectó de manera significativa ($p<0.05$) a la medida de la velocidad de los ultrasonidos (V) en jamones frescos. Así, un aumento del 5% del contenido en grasa en los jamones frescos conllevó un aumento de 8.4m/s de la V medida a 2ºC. Este hecho se explica porque los

ultrasonidos viajan a mayor velocidad en medios sólidos que en líquidos. Así pues, dado que a bajas temperaturas (2ºC), la mayor parte de la grasa está en estado sólido, la V en el tejido graso es mayor que en tejido magro (Benedito et al., 2001a). En este sentido, a mayor contenido en grasa, mayor fracción de sólidos en la muestra y mayor valor de la V en la carne (Mc Clements et al., 1992). Sin embargo, a altas temperaturas (25ºC), debido a la fusión de los triglicéridos, la V en el tejido graso es menor que en el tejido magro, lo que hace que la V descienda con el aumento del contenido en grasa (Benedito et al., 2001a). Asimismo, a temperaturas intermedias (12-16ºC), dado que la V es similar en el tejido graso y magro no existe una relación entre el contenido en grasa y la medida de la V (Benedito et al., 2001a). Por tanto, para poder estimar el contenido en grasa en carne fresca a partir de la V es necesario trabajar bien a temperaturas por debajo de 5ºC o a temperaturas por encima de 25ºC. En este sentido, en el capítulo 4.1 de la presente Tesis Doctoral, la V se midió a 2ºC ya que es una temperatura empleada habitualmente en la conservación de la carne fresca, lo que permitiría su uso en la industria de elaboración de jamón curado en la etapa de recepción y clasificación de la materia prima. El rango de temperaturas por encima de 25ºC, podría ser únicamente interesante para la estimación del contenido en grasa de los jamones en el matadero, tras el sacrificio de los animales. A partir de la relación lineal ($p<0.05$) entre la medida de V a 2ºC y el contenido en grasa de los jamones frescos, se estableció un modelo predictivo (R^2=0.89 y RMSE=2.90%) que permitió clasificar los jamones frescos en tres categorías según su contenido de grasa (<14, 14-26 y >26% b.h.) con un porcentaje global de piezas correctamente clasificadas del 89%. La presente Tesis Doctoral se ha desarrollado en el marco de dos proyectos de investigación (RTA 2010-00029-C04-01/02; RTA 2013-00030-C03-02), que han sido llevados a cabo de forma coordinada entre varios grupos de investigación. En el primer proyecto, se trabajó conjuntamente con un grupo de investigación del IRTA (Monells, Girona), que evaluó la viabilidad de la tecnología de rayos-X para predecir el contenido en grasa en los mismos jamones empleados en la presente Tesis Doctoral. Sin embargo, el error de predicción (RMSE=4.20%) y el

porcentaje global de piezas correctamente clasificadas (65%) obtenidos con los parámetros medidos con rayos-X fueron peores a los obtenidos con el modelo basado en los parámetros ultrasónicos. Además, tampoco se observó ninguna mejora en la predicción del contenido en grasa utilizando la combinación de ambas tecnologías. En bibliografía se encontraron aplicaciones de los ultrasonidos para predecir el contenido en grasa en carne fresca. Así, Park et al. (1994) estimaron el contenido en grasa en músculos frescos de *Longissimus dorsi* (LD) de vacuno (R^2=0.81 y RMSE=1.40%) midiendo la velocidad de los ultrasonidos a 23ºC. De forma similar, Benedito et al. (2001a) determinaron el contenido en grasa en mezclas frescas cárnicas de cerdo midiendo la velocidad a dos temperaturas (4 y 25ºC). Sin embargo, la medida de la V a dos temperaturas dificulta la implementación de la tecnología ultrasónica a nivel industrial. Por tanto, en este primer estudio, los ultrasonidos de señal demostraron ser una técnica fiable para clasificar jamones frescos en grupos con contenido en grasa homogéneo, lo que permitiría fijar condiciones específicas de procesado en función del nivel graso de los jamones, y por lo tanto, un comportamiento más homogéneo de cada grupo durante las siguientes fases de procesado.

Caracterización del producto salado mediante ultrasonidos de señal

Los ultrasonidos de señal resultaron ser una técnica no destructiva adecuada para caracterizar productos cárnicos durante y tras el salado, trabajando tanto en modo transmisión-recepción (MTR) (apartados 4.2.1 y 4.2.2) como en modo pulso-eco (MPE) (apartados 4.2.3 y 4.2.4). El contenido en agua y sal tuvieron un efecto significativo ($p<0.05$) sobre la V, medida en MTR, en muestras modelo de BF formuladas con contenido en sal y agua predefinido (apartado 4.2.1). Por un lado, el aumento del contenido en agua en las muestras modelo dio lugar a un descenso de la V, lo que ya había sido observado en otros productos cárnicos, tales como sobrasada (Llull et al., 2002) o músculos BF de cerdo (Niñoles et al., 2011). Por otro lado, el aumento del contenido en sal conllevó un aumento de la V. En la misma línea, se ha puesto de manifiesto

que la velocidad de los ultrasonidos aumentó en zumos (Kuo et al., 2008) y soluciones acuosas (Krause et al., 2011), conforme aumentó el contenido en azúcar; y en pescado cuando se incrementó el contenido en sólidos no grasos (Ghaedian et al., 1998). Conocida la influencia individual de la sal y el agua sobre la V en carne, se llevaron a cabo medidas de V en MTR antes y después del salado, en cilindros de LD y BF, sus correspondientes secciones saladas en salmuera (apartado 4.2.1) y en jamones salados en seco (apartado 4.2.2). En todos los casos, los resultados mostraron un aumento de la V durante el salado. En este sentido, dado que los ultrasonidos viajan más rápido en sólidos, con elevados módulos elásticos (Benedito et al., 2000; Chandrapala, 2015) que en líquidos (agua), el aumento de la V es atribuible al incremento del contenido en sólidos que tiene lugar en la carne durante el salado, debido a la ganancia de sal (ΔX_S) y la pérdida de agua (ΔX_W). La alta variabilidad experimental en las medidas de velocidad inicial en los cilindros de LD y BF y en los jamones se asoció a la heterogeneidad en la composición y estructura de la carne (Reig et al., 2013) y conllevó la necesidad de utilizar la variación de velocidad (ΔV) como un indicador más adecuado del progreso de la etapa de salado, que la simple medida de la V. Así, se encontraron relaciones lineales significativas ($p<0.05$) entre la ΔX_S y la ΔV medida antes y después del salado en los cilindros de LD y BF salados en salmuera y en sus secciones (apartado 4.2.1), así como, en los jamones salados en seco (apartado 4.2.2) (Figura 5.1). De forma similar, se encontraron relaciones lineales significativas ($p<0.05$) entre la ΔX_W y la ΔV. Un incremento de la ganancia de sal produjo un aumento de la variación de velocidad (Figura 5.1). Por otro lado, la pérdida agua durante el salado resultó también en un aumento de la variación de velocidad (resultados no mostrados). La pendiente de la relación ΔX_S *vs* ΔV fue similar en los cilindros y secciones de LD y BF y en los jamones (13.5±1.0m/s % b.h.) e incluso similar a la mostrada por Kinsler et al. (1982), quienes encontraron que la V aumentó en 13.7m/s con un incremento del 1% de la concentración de sal en una disolución salina a 2ºC (Figura 5.1). El hecho de que las pendientes fuesen similares indicó que un mismo ΔX_S da lugar a una misma ΔV,

independientemente del tipo de salado (en salmuera o en seco) y de la estructura del producto (disolución o carne).

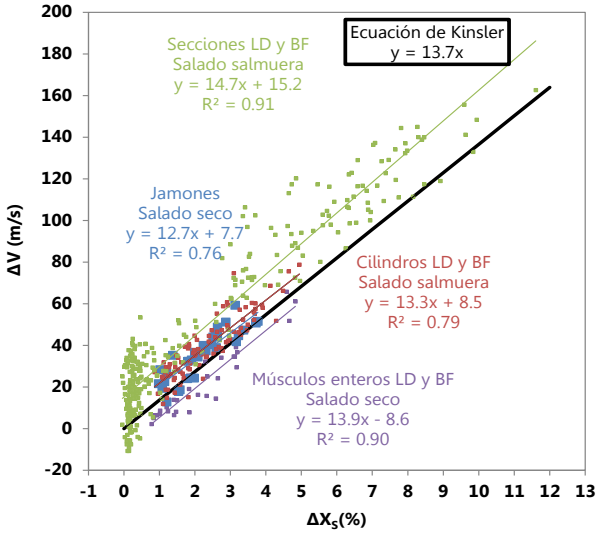

Figura 5.1 Relación entre la ganancia de sal (ΔX_S) y la variación de velocidad ultrasónica (ΔV) medida antes y después del salado para cilindros y secciones de LD y BF (apartado 4.2.1) y jamones (apartado 4.2.2) y durante la monitorización del salado en seco de las piezas de LD y BF (apartado 4.2.2).

Dado que el contenido en agua continúa descendiendo en las siguientes etapas del procesado del jamón (secado-maduración y envejecimiento) (Bello, 2008), estimar la ganancia de sal tras el salado resulta de mayor interés que determinar la pérdida de agua en esa etapa del procesado. En este sentido, se desarrollaron modelos predictivos basados en la ΔV para estimar la ganancia de sal, que proporcionaron un error de estimación (RMSE) de 0.48% en los cilindros de LD y BF (apartado 4.2.1) y de 0.44% en los jamones (apartado 4.2.2). Teniendo en cuenta los errores de estimación de la ganancia de sal, los ultrasonidos de señal no se presentan como una herramienta analítica para determinar el contenido en sal en los productos cárnicos, aunque pueden ser utilizados como una herramienta de clasificación para el control de calidad en la etapa de salado. En esta misma línea, los ultrasonidos se han utilizado para

predecir el contenido en sólidos en otros productos. Así, Valente et al. (2013) estimaron el contenido en sacarosa del mango utilizando un modelo predictivo basado en la medida de velocidad ultrasónica y en el contenido en sólidos solubles del fruto (R^2=0.81, RMSE=12.3g/L≈1.13%). De forma similar, Krause et al. (2011) desarrollaron modelos basados en medidas de velocidad ultrasónica y temperatura para determinar el contenido en sacarosa (R^2=0.95, RMSE=0.40%) y el contenido alcohólico (R^2=0.99, RMSE=0.18%) en soluciones acuosas. Sin embargo, hasta el momento, no se ha encontrado ningún estudio relacionado con la predicción del contenido en sal en productos cárnicos mediante ultrasonidos.

Las medidas de V antes y después del salado permitirían estimar el contenido en sal y clasificar las piezas en lotes homogéneos que podrían procesarse de manera diferente, pero no permitirían corregir la variabilidad del contenido en sal, reduciendo el número de piezas saladas por defecto o en exceso. En este sentido, sería de gran interés para la industria, el desarrollo de técnicas que permitiesen monitorizar cómo la pieza se va salando y aumenta su contenido en sal, con el objetivo de finalizar el proceso de salado cuando se alcance la ganancia de sal deseada. Así, se monitorizó online el salado en seco de las piezas de LD y BF con medidas de velocidad ultrasónica en MTR (apartado 4.2.2). Los resultados mostraron un aumento progresivo de la V durante el salado, lo que demostró la capacidad de los ultrasonidos para monitorizar este proceso. Como ocurrió en los cilindros y secciones de LD y BF (apartado 4.2.1) y en los jamones (apartado 4.2.2), la ΔV total obtenida durante la monitorización del salado de piezas de LD y BF se vio influenciada de forma significativa ($p<0.05$) por la ΔX_W y ΔX_S. Además, como se observa en la Figura 5.1, la pendiente de la relación ΔV *vs* ΔX_S en las piezas de LD y BF (13.9m/s % b.h.) fue similar a las encontradas en cilindros y secciones de LD y BF, jamones y a la obtenida por Kinsler et al. (1982) para una disolución salina (13.6±0.8m/s % b.h.). Aunque las pendientes fueron similares, se observó un valor diferente de la ordenada en el origen en la experiencia de monitorización del salado de

las piezas de LD y BF (valor de ordenada negativo) y en las experiencias donde se midió la V antes y después del salado en cilindros y secciones de LD y BF y jamones (valor de ordenada positivo) (Figura 5.1). Este hecho se asoció al efecto de la sal sobre la textura en la superficie de la carne donde se realiza la medida de la V. Ruiz-Ramírez et al. (2005) relacionaron los cambios de textura de la carne durante el salado (aumento de dureza) con la compactación de la estructura miofibrilar debido al contenido en sal y al efecto inhibitorio de la sal sobre la actividad de las calpaínas. Así, en los cilindros y secciones de LD y BF y jamones, la V se midió antes y después del salado, por lo que la superficie de la carne, donde se colocaban los transductores, estuvo en contacto con la sal, produciéndose un aumento de la dureza superficial, y por lo tanto, de la ΔV. En cambio, en la monitorización del proceso de salado de las piezas de LD y BF, los transductores estuvieron siempre en contacto con la muestra. De esta manera, la penetración de sal en los puntos de medida se vería dificultada, resultando en un menor aumento de la dureza superficial, y por tanto, de la ΔV. A partir de la ΔV total durante la monitorización del salado en seco de las piezas de LD y BF se estableció un modelo predictivo de la ganancia de sal con un error de estimación (RMSE=0.43%) similar a los obtenidos en cilindros de LD y BF (RMSE=0.48%) (apartado 4.2.1) y jamones (RMSE=0.44%) (apartado 4.2.2).

Además de la V (apartados 4.2.1 y 4.2.2), otro parámetro ultrasónico utilizado para monitorizar el proceso de salado fue el tiempo de vuelo (Tv), medido en MPE (apartados 4.2.3 y 4.2.4). La medida del Tv presenta la ventaja, respecto de la V, que no necesita de la medida del espesor de la muestra, lo que simplifica la medida ultrasónica. En las experiencias de jamones frescos (capítulo 4.1) y de cilindros de LD y BF (apartado 4.2.1), piezas de LD y BF y jamones salados (apartado 4.2.2), para determinar la V se calculó el Tv con el método del umbral de energía. Este método calcula el Tv en el frente de llegada de la señal que atraviesa la muestra cuando la misma sobrepasa un umbral de energía establecido (0.1V para el presente estudio). En señales con niveles altos de energía, pese a que existan fluctuaciones de la amplitud de la señal, el método

del umbral permite calcular correctamente el Tv, ya que el pico correspondiente al frente de llegada de la señal siempre supera el umbral de energía establecido. Sin embargo, las señales ultrasónicas obtenidas con el MPE durante el salado de jamones (apartado 4.2.3) presentaron niveles bajos de energía como consecuencia de la atenuación tras cruzar la señal dos veces la muestra y de las deficiencias en el acople transductor/jamón. Así, el método de umbral de energía conllevó errores significativos en el cálculo del Tv. El estudio de otros métodos de análisis de señal para calcular el Tv (método de la correlación cruzada y del espectro de fase obtenido a partir de la Transformada Rápida de Fourier) (apartado 4.2.3) mostró que el nivel de energía de la señal es determinante a la hora de seleccionar el método más conveniente para calcular el Tv. Así, el método del espectro de fase fue útil para calcular la variación de tiempo de vuelo (ΔTv) entre dos señales cuando éstas no estaban saturadas (amplitud señal pico-pico<2V). Por otro lado, el método de la correlación cruzada resultó adecuado para la calcular la ΔTv entre señales consecutivas (intervalo 5min) cuando la integral de la señal temporal era superior a 4Vμs. Finalmente, el método de la correlación cruzada entre señales separadas cada hora fue apropiado para calcular la ΔTv para cualquier nivel de energía. Así pues, mediante esta última metodología de cálculo, se determinó la ΔTv durante el salado de piezas de LD y jamones (apartado 4.2.4). En ambos casos, la ΔTv disminuyó progresivamente durante el salado, lo que corroboró la capacidad de los ultrasonidos para monitorizar el proceso de salado. Este resultado es consistente con el aumento de ΔV observado en los apartados 4.2.1 y 4.2.2. La pérdida de agua y la ganancia de sal durante el salado aumentan el contenido en sólidos en la muestra, lo que da lugar a que los ultrasonidos viajen más rápido (aumenta la V) (Benedito et al., 2000), y por lo tanto, disminuya el tiempo necesario para que la onda atraviese la muestra. Como en el caso de la ΔV medida en el salado de cilindros de LD y BF (apartado 4.2.1), piezas de LD y BF y jamones (apartado 4.2.2), se encontraron relaciones lineales significativas ($p<0.05$) entre la ΔTv *vs* ΔX_W y ΔTv *vs* ΔX_S. Asimismo, el descenso del tiempo de vuelo también podría estar influenciado

por la reducción del espesor (García-Gil et al., 2014) y el grado de desnaturalización proteica y compactación de las fibras (Ruiz-Ramírez et al., 2005; Soyer et al., 2011) (aumento de dureza) que sufre la muestra durante el salado.

Por otra parte, conforme la pieza tiene mayor espesor, mayor cantidad de sal tiene que penetrar en la misma para conseguir un mismo contenido en sal, y por lo tanto, mayor es la ΔTv. En este sentido, en el apartado 4.2.4 se observó que para una misma ganancia de sal en piezas de LD (ΔX_S=2.5% b.h.) y en jamones (ΔX_S=2.2% b.h.), la ΔTv fue notablemente mayor en jamones (-11.2µs) que en piezas de LD (-3.3µs), lo que se explica por la diferencia de espesor entre los jamones (15.7±0.6cm de espesor) y las piezas de LD (5.0±0.8cm de espesor). Así pues, dado que el espesor inicial de la muestra afecta a la relación ΔTv *vs* ΔX_S, para el desarrollo de los modelos predictivos de la ganancia de sal, se incorporó el tiempo de vuelo inicial (Tv_0), que está relacionado con el espesor inicial de la muestra, como factor corrector. Los modelos con las variables de tiempo de vuelo (Tv_0 y ΔTv) mostraron un error de predicción de sal (RMSE) en piezas de LD (0.73%) y jamones (0.57%) superior a los modelos obtenidos con la variable velocidad (ΔV) en cilindros de LD y BF (0.48%), piezas de LD y BF (0.43%) y jamones (0.44%). El modelo predictivo del contenido de sal basado únicamente en los parámetros ultrasónicos Tv_0 y ΔTv clasificó correctamente el 85% de las piezas de LD, en tres categorías con diferente contenido en sal (<2.5, 2.5-4.0 y >4.0% b.h.). De la misma manera, empleando un modelo basado en el Tv_0 y la ΔTv, se clasificaron correctamente el 90% de los jamones en tres categorías (<2.0, 2.0-3.0 y >3.0% b.h.). La inclusión de otras variables del proceso (tiempo de salado y peso de la muestra) en el modelo predictivo mejoraron ligeramente la clasificación de las piezas de LD (95%), manteniéndose el mismo porcentaje de jamones correctamente clasificados (90%).

Los resultados de este trabajo han puesto de manifiesto que la tecnología ultrasónica es fiable para la clasificación de jamones tras el salado, lo que

permitiría diseñar de manera óptima las siguientes etapas de su procesado. Además, la medida del ΔTv en MPE se presenta como un método de inspección o monitorización online de la ganancia de sal durante el salado, que podría emplearse para el control de calidad del proceso, como ya se vio en el caso de la medida de la ΔV en MTR para piezas de LD y BF. Sin embargo, en la industria, donde predomina el salado en pila, la implementación de la tecnología ultrasónica en MPE presenta importantes ventajas respecto al MTR. Así, el MTR requiere el uso de dos transductores perfectamente alineados y enfrentados, localizados en cada una de las caras del jamón, lo que supone importantes problemas para monitorizar online el salado en pila de jamones. En cambio, el MPE se caracteriza por usar un único transductor que actúa tanto de emisor como de receptor (Mulet et al., 1999). El uso de un único transductor localizado en la base del jamón mientras éste se sala en pila, simplificaría la implementación de esta tecnología en el entorno industrial, reduciría el coste del montaje, así como, minimizaría el impacto de la medida sobre la transferencia de sal y agua al reducirse el número de transductores que se apoyan sobre la superficie de la muestra.

Caracterización del producto curado mediante ultrasonidos de señal

Una de las necesidades más importantes de la industria cárnica es la determinación de la composición de los productos cárnicos curados de manera no destructiva. En este sentido, el Reglamento (CE) nº 1924/2006 exige la declaración en el etiquetado de las cantidades de nutrientes (sal, grasa, azúcar, etc.) contenidos en los alimentos. En el caso de productos curados no formulados como el jamón, resulta complicado proporcionar la información nutricional en el etiquetado debido a su elevada variabilidad. En consecuencia, la última parte de la presente Tesis, se centró en evaluar la viabilidad del uso de los ultrasonidos de señal para predecir el contenido en sal y grasa en porciones de jamón curado (capítulo 4.3). Al igual que ocurrió en los estudios anteriores referidos a cilindros de LD y BF (apartados 4.2.1), piezas de LD y BF y jamones (apartado 4.2.2), a medida que aumentó el contenido en sal en las

muestras curadas, se incrementó la V en la muestra medida a 15ºC. Sin embargo, el modelo que se desarrolló para predecir el contenido en sal usando la V a 15ºC, mostró errores de predicción elevados (RMSE=0.69%). Este hecho se explica considerando que la elevada variabilidad composicional del jamón curado (contenido en grasa y agua, tipo de matriz proteica y grasa, etc.) afecta notablemente a la V (Niñoles et al., 2008), lo que hace que disminuya la importancia relativa del contenido en sal sobre la velocidad ultrasónica. En este último estudio, también se evaluó la capacidad de la tecnología de rayos-X para predecir el contenido en sal y grasa en las mismas porciones de jamón curado. Los modelos de rayos-X obtuvieron un error de predicción del contenido de sal menor (RMSE=0.43%) que los modelos ultrasónicos. La combinación de ambas tecnologías no mejoró la precisión de la predicción de sal en las muestras curadas.

Por otra parte, a diferencia de lo que ocurrió en jamones frescos, donde la velocidad ultrasónica medida a 2ºC aumentó con el contenido en grasa (capítulo 4.1), en las porciones de jamón curado, la velocidad medida a 2ºC no se relacionó con el contenido en grasa. Esto se podría asociar a que la velocidad de los ultrasonidos en el tejido magro curado es mayor que en el tejido magro fresco, como consecuencia de las modificaciones sufridas durante el proceso de curado (cambios de textura, pérdida de agua, etc.). Así, la velocidad en el tejido graso y magro curado se igualarían a 2ºC, haciendo que la diferencia de V entre ambos tejidos, necesaria para la estimación del contenido graso de la carne, desaparezca. Por otro lado, se encontró una disminución de la V medida a 15ºC, con el aumento en el contenido graso. En este caso, al igual que sucede en la carne fresca, la velocidad en el tejido graso es menor que en tejido magro curado, debido a la fusión de los triglicéridos (Corona et al., 2014a), lo que hace que a mayor contenido graso, menor sea la V en la muestra. En el capítulo 4.3, para la realización de las medidas ultrasónicas, se seleccionaron dos temperaturas comunes en la elaboración del jamón curado. Así, la medida a 15ºC se podría realizar durante la etapa de

secado-maduración y la de 2ºC durante la etapa de refrigeración. Considerando el efecto de la temperatura sobre la medida de V, se desarrolló un modelo semi-empírico que permitió estimar el contenido en grasa (RMSE=6.70%, varianza explicada 93.6%), agua y proteínas+otros de las porciones de jamón curado a partir de la medida de velocidad a 2 y 15ºC. Utilizando un modelo semi-empírico similar, se ha determinado el contenido en grasa en salchichas curadas (varianza explicada 96.1%) realizando medidas de V a 2 y 25ºC (Corona et al., 2014b) y en sobrasada (varianza explicada 97.6%) realizando medidas de V a 4 y 25ºC (Simal et al., 2003). Mediante el uso del modelo semi-empírico fue posible clasificar correctamente el 77% de las porciones analizadas en tres niveles de grasa (<25, 25-40 y >40% b.h.). En cambio, el modelo basado en las variables de rayos-X mostró errores de predicción de grasa en las porciones curadas ligeramente superiores (RMSE=7.00%) a los obtenidos con el modelo basado en variables ultrasónicas, por lo que no se empleó para clasificar las porciones. La combinación de ambas tecnologías redujo el error de predicción (RMSE) de grasa en las porciones curadas hasta el 5.60%. Sin embargo, el uso de ambas tecnologías supondría una elevada inversión inicial, lo dificulta su aplicación en la industria. Estos resultados revelan la posibilidad de utilizar los ultrasonidos como herramienta no destructiva para clasificar el jamón curado según diferentes niveles de grasa. Además, la caracterización de la composición media (agua, grasa y proteínas+otros) de las porciones de jamón curado mediante la medida de la V en el producto curado, al final de la etapa de secado-maduración (15-20ºC) y tras un periodo en refrigeración (2-4ºC), permitiría proporcionar información nutricional del producto final al consumidor.

6. CONCLUSIONES

6. CONCLUSIONES

Las principales conclusiones alcanzadas en la presente Tesis Doctoral se han agrupado en tres apartados según el tipo de producto cárnico caracterizado mediante ultrasonidos: fresco, salado y curado.

Caracterización del producto fresco mediante ultrasonidos de señal

-La velocidad de los ultrasonidos medida a 2ºC en jamones frescos estuvo influenciada por el contenido en grasa del mismo. Un aumento del contenido en grasa conllevó un aumento de la velocidad.

-Se desarrolló un modelo basado en la medida de la velocidad ultrasónica a 2ºC que predijo el contenido en grasa con un error del 2.97%. Dicho modelo permitió clasificar correctamente el 89% de los jamones en función de su contenido en grasa (<14, 14-26 y >26% b.h).

-Mediante un modelo basado en la tecnología de rayos-X fue posible predecir el contenido en grasa en los jamones frescos con un error del 4.65% y se clasificó correctamente el 65% de los mismos.

-La combinación de ambas tecnologías (ultrasonidos y rayos-X) no mejoró la precisión del modelo predictivo del contenido en grasa basado en las medidas ultrasónicas.

-A nivel industrial, la medida ultrasónica a 2ºC en el jamón fresco permitiría su clasificación en grupos con contenido en grasa homogéneo, lo que repercutiría de manera positiva en la homogeneidad y la calidad del producto final.

Caracterización del producto salado mediante ultrasonidos de señal

-El contenido en agua y sal de las muestras modelo, formuladas con *Biceps femoris* (BF), tuvo un efecto significativo ($p<0.05$) sobre la velocidad ultrasónica.

-Se observó un aumento de la velocidad ultrasónica tras el salado, tanto en *Longissimus dorsi* (LD) y *Biceps femoris* (BF) como en jamones, lo que se asoció a la pérdida de agua y ganancia de sal.

-De igual forma, la velocidad de los ultrasonidos aumentó progresivamente durante el salado de LD y BF, lo que demostró la capacidad de la medida de velocidad en modo trasmisión-recepción para monitorizar el proceso de salado.

-Debido a la variabilidad composicional y estructural de la carne fresca, la variación de velocidad durante el salado fue el parámetro ultrasónico más apropiado para describir los cambios composicionales que sufre el producto.

-La variación de velocidad se correlacionó de manera significativa ($p<0.05$) con la ganancia de sal y la pérdida de agua en LD y BF, así como, en jamones. La ganancia de sal y la pérdida agua, que tienen lugar durante el salado, provocaron un aumento de la variación de velocidad. Además, se identificó que la ganancia de sal tuvo una mayor influencia sobre la variación de velocidad que la pérdida de agua.

-Una misma ganancia de sal (p.e. 1% b.h.) da lugar a una misma variación de velocidad (13-14m/s), independientemente del tipo de salado (en salmuera o en seco) y de la estructura del producto (disolución o carne).

-Se desarrollaron modelos basados en la variación de velocidad durante el salado, los cuales permitieron estimar la ganancia de sal en LD y BF salados en salmuera (RMSE=0.48%) y en LD y BF (RMSE=0.43%) y jamones (RMSE=0.44%) salados en seco. La precisión de la estimación, aunque no permite el uso de esta técnica como método analítico de determinación de sal, sí permitiría la aplicación del sistema ultrasónico para tareas de control de calidad en la industria cárnica.

-La variación del tiempo de vuelo disminuyó progresivamente durante el salado tanto en LD como en jamones, lo que demostró la viabilidad de la

medida de este parámetro, en modo pulso-eco, para monitorizar el proceso de salado.

-La energía de la señal ultrasónica determinó el método más adecuado para calcular la variación del tiempo de vuelo durante el salado de jamones. Así, el método de la correlación cruzada entre señales no consecutivas (separadas cada hora), resultó el más conveniente para calcular el tiempo de vuelo y monitorizar el proceso de salado de jamones.

-La medida del tiempo de vuelo en modo pulso-eco, comparado con la medida de la velocidad en modo trasmisión-recepción, facilita la implementación de la tecnología ultrasónica para la monitorización del proceso de salado. Así, el uso de un único transductor, localizado en la base del jamón mientras éste se sala en la pila, simplificaría la implementación de esta tecnología en el entorno industrial, reduciría el coste del montaje y minimizaría el impacto de la medida sobre la transferencia de sal y agua.

-El uso de modelos de predicción basados en el tiempo de vuelo permitió clasificar correctamente el 85% de LD y el 90% de los jamones en tres categorías según su contenido en sal (<2.5, 2.5-4.0 y >4.0% b.h. en LD y <2.0, 2.0-3.0 y >3.0% b.h. en jamones). El porcentaje de LD correctamente clasificados ascendió hasta el 95% cuando se incluyó el peso de la muestra y el tiempo de salado en el modelo predictivo.

-La aplicación de la tecnología ultrasónica en la etapa de salado de productos cárnicos como jamones y lomos, podría llevarse a cabo con dos enfoques diferentes. El primero consistiría en realizar medidas ultrasónicas en modo transmisión-recepción antes y después del salado. Esto permitiría clasificar las piezas en lotes con contenido en sal homogéneo y obtener un comportamiento más uniforme de las piezas en las siguientes etapas del procesado. En el segundo caso, se podrían realizar medidas ultrasónicas online en modo pulso-eco durante el salado. De esta manera, se monitorizaría la etapa de salado, con el objetivo de determinar la ganancia de sal en cada

momento, y poder así, finalizar el proceso, una vez alcanzado el contenido en sal deseado.

Caracterización del producto curado mediante ultrasonidos de señal

-En porciones de jamón curado, la medida de la velocidad ultrasónica a 15ºC aumentó con el aumento del contenido en sal.

-Se desarrolló un modelo predictivo del contenido en sal basado en la medida de velocidad a 15ºC, que no proporcionó resultados satisfactorios para la clasificación del jamón curado en función del contenido en sal (RMSE=0.69%). Este hecho puede ser debido a que el resto de factores composicionales y estructurales del jamón curado afectan significativamente a la medida de la velocidad ultrasónica, haciendo que disminuya la importancia relativa del efecto de la sal sobre la velocidad.

-El contenido en sal en las porciones de jamón curado se predijo con un error del 0.43% empleando un modelo basado en diferentes parámetros obtenidos mediante un análisis con rayos-X. Sin embargo, la combinación de ultrasonidos y rayos-X no mejoró la precisión de la predicción del contenido en sal.

-La velocidad ultrasónica en las porciones de jamón curado disminuyó con el aumento de la temperatura (de 2 a 15ºC), debido a la fusión de las grasas. A mayor contenido en grasa, mayor resultó la disminución de la velocidad al aumentar la temperatura.

-Utilizando un modelo semi-empírico, basado en las medidas de velocidad a 2 y 15ºC, se determinó el contenido en grasa con un error del 6.70%. Este modelo también proporcionó resultados fiables para la determinación del contenido en agua y proteínas+otros. Mediante el empleo de dicho modelo semi-empírico, fue posible clasificar correctamente el 77% de las porciones de jamón curado en tres niveles según el contenido en grasa (<25, 25-40 y >40% b.h.).

-Los parámetros obtenidos mediante rayos-X permitieron predecir el contenido en grasa en las porciones de jamón curado con un error del 7.00%. El uso combinado de ultrasonidos y rayos-X mejoró la predicción del contenido en grasa del jamón curado (5.60%), sin embargo, esta combinación no parece ser una opción económicamente viable para la industria cárnica.

-El uso de la tecnología ultrasónica, se podría llevar a cabo a nivel industrial realizando medidas ultrasónicas en el jamón al final de la etapa de secado-maduración (15-20ºC) y tras un periodo en refrigeración (2-4ºC), lo que permitiría clasificar el jamón curado según su contenido en grasa, así como, estimar su contenido aproximado en agua y proteínas+otros. Todo ello, posibilitaría definir en el etiquetado la cantidad de estos componentes, y por tanto, proporcionar a los consumidores información nutricional del producto final.

Conclusión general

La presente Tesis doctoral mostró la viabilidad del uso de los ultrasonidos de señal para clasificar los jamones frescos en función de su contenido en grasa, lo que permitiría gestionar lotes homogéneos de materia prima que posteriormente tendrían un comportamiento más uniforme en el proceso de elaboración. Por otra parte, se demostró la capacidad de esta técnica no destructiva para determinar el contenido en sal en las piezas cárnicas como método de control de calidad. Concretamente, realizando medidas ultrasónicas antes y después del salado, se podrían clasificar las piezas cárnicas saladas en lotes con contenido en sal similar, lo que permitiría diseñar las etapas posteriores con las condiciones más adecuadas para cada lote. Asimismo, se mostró la utilidad de las medidas ultrasónicas en continuo para monitorizar el proceso de salado de carne, con el objetivo de predecir la ganancia de sal durante esta etapa y finalizarla cuando se haya alcanzado el contenido en sal deseado. Por último, los ultrasonidos de señal pueden ser considerados una tecnología viable para clasificar porciones de jamón curado

en función de su contenido en grasa, así como, para estimar su composición global, lo que proporcionaría información nutricional del producto final al consumidor.

7. RECOMENDACIONES

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A partir de los resultados obtenidos en esta Tesis Doctoral, se puede sugerir continuar la investigación profundizando en los siguientes temas:

Transferencia de materia

- ✓ Cuantificar el efecto sobre la trasferencia de materia durante el salado de la presencia de uno o varios transductores en la superficie de la carne.
- ✓ Evaluar la aplicación de la tecnología de ultrasonidos de señal para la monitorización del salado de jamones de diferentes razas (ej. ibéricos) y de productos de diferente naturaleza (bacalao, mojama, etc.).

Nuevos modelos predictivos

- ✓ Establecer relaciones entre los cambios composicionales durante el salado y parámetros ultrasónicos distintos a la velocidad ultrasónica y al tiempo de vuelo, tales como la atenuación y diferentes parámetros del espectro de frecuencias.

Mejora del equipo ultrasónico y la aplicación industrial

- ✓ Mejorar el diseño de los transductores empleando materiales que maximicen su resistencia a la acción de la sal, y por lo tanto, su durabilidad. Asimismo, intentar reducir el tamaño de los transductores sin afectar a la capacidad de penetración de las ondas. Por otro lado, diseñar un sistema de array de transductores dispuestos en una malla, de modo que colocándola bajo la pieza cárnica y con sólo una medida ultrasónica, se obtenga información de toda la pieza.
- ✓ Evaluar la viabilidad del uso de los ultrasonidos de señal sin contacto para caracterizar el jamón fresco, que presenta una menor atenuación. La aplicación de los ultrasonidos de señal sin contacto en las líneas de producción presenta ventajas respecto a las técnicas ultrasónicas por

contacto, ya que permitiría realizar medidas ultrasónicas en los alimentos de forma muy rápida y evitar la contaminación superficial del alimento.

- ✓ Diseñar un sistema ultrasónico automatizado acoplado en las líneas de producción para determinar el contenido en grasa en jamones frescos y curados.
- ✓ Estudiar la viabilidad técnica y económica de la monitorización del proceso de salado en pila de jamones mediante ultrasonidos de señal. Desarrollar un modelo de gestión de proceso e incorporarlo a un software, de tal forma que, conocidos los datos obtenidos de la monitorización del salado de cada pieza, se decida qué condiciones de proceso son las más adecuadas en las siguientes etapas de procesado.

Otras aplicaciones de ultrasonidos de señal en productos cárnicos

- ✓ Evaluar el uso de los ultrasonidos de señal para detectar los cambios estructurales que ocurren en la matriz proteica de la carne durante el salado, post-salado y secado-maduración con el objetivo de detectar problemas de pastosidad o textura blanda en el jamón.
- ✓ Analizar la capacidad de la tecnología ultrasónica para diferenciar grasa subcutánea e intramuscular en el jamón.

8. CONTRIBUCIÓN CIENTÍFICA

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Artículos científicos

De Prados, M., Fulladosa, E., Gou, P., Muñoz, I., García-Pérez, J.V. & Benedito, J. (2015). Non-destructive determination of fat content in green hams using ultrasound and X-rays. *Meat Science*, 104, 37-43.

Fulladosa, E., De Prados, M., García-Pérez, J.V., Benedito, J., Muñoz., I., Arnau, J. & Gou, P. (2015). X-ray absorptiometry and ultrasound technologies for non-destructive compositional analysis of dry-cured ham. *Journal of Food Engineering*, 155, 62-68.

De Prados, M., García-Pérez, J.V. & Benedito, J. (2015). Non-destructive salt content prediction in brined pork meat using ultrasound technology. *Journal of Food Engineering*, 154, 39-48.

De Prados, M., García-Pérez, J.V. & Benedito, J. (2016). Ultrasonic characterization and online monitoring of pork meat salting process. *Food Control*, 60, 646-655.

Martínez, G., De Prados, M., García-Pérez, J.V. & Benedito, J. (2016). Ultrasonic online monitoring of the ham salting process. Methods for signal analysis: Time of flight calculation.

De Prados, M., García-Pérez, J.V. & Benedito, J. (2016). Assessment of time of flight by ultrasonic pulse-echo mode as a tool to monitor pork loin and ham dry salting. *Meat Science*, submitted.

Contribuciones a congresos

García-Pérez, J.V., De Prados, M., Perez-Muelas, N., Carcel, J. A. & Benedito, J. Ultrasonic characterization of pork meat salting. International Symposium on Ultrasound in the Control of Industrial Processes. 18-20 April 2012. Madrid (Spain).

De Prados, M., Perez-Muelas, N., Rossello, C., García-Pérez, J. V. & Benedito, J. Use of low intensity ultrasound to monitoring dry-cured ham salting. Institute of Food Technologists (IFT) Annual Meeting and Food Expo. 25-29 June 2012. Las Vegas (U.S.A.).

Benedito, J., García-Pérez, J.V. & De Prados, M. Use of low intensity ultrasonics to control the salt and fat content during ham processing. Congreso Mundial del Jamón. 29-31 May 2013. Ourique (Portugal).

De Prados, M., García-Pérez, J.V., Benedito, J., Fulladosa, E., Gou, P. & Muñoz, I. Assesment of fat content on raw pork ham using non-destructive ultrasonic and x-ray techniques. Congreso Mundial del Jamón. 29-31 May 2013. Ourique (Portugal).

Fulladosa, E., De Prados, M., García-Pérez, J.V., Benedito, J. Muñoz, I., Arnau, J. & Gou, P. Determination of dry-cured ham composition using X-ray absorptiometry and ultrasound technologies. 59th International Congress of Meat Science and Technology (ICOMST). 18-23 August 2013. Izmir (Turkey).

De Prados, M., García-Pérez, J.V., Fulladosa, E., Mulet, A. & Benedito, J. Monitorización del salado en pila de *Longissimus dorsi* mediante ultrasonidos de señal. Congreso Iberoamericano de Ingeniería de Alimentos 9 (CIBIA9). 13-16 January 2014. Valencia (Spain).

De Prados, M., García-Pérez, J.V., Benedito, J. & Mulet, A. Use of low intensity ultrasound to monitor the dry salting process of pork biceps femoris muscle.

Institute of Food Technologists (IFT). Annual Meeting and Food Expo. 20-24 June 2014. New Orleans (U.S.A.).

De Prados, M., Martinez-Escriva, G., Perez-Muelas, N., Benedito, J. & García-Pérez, J.V. Caracterización y monitorización no destructiva del salado de lomo y jamón de cerdo mediante ultrasonidos de señal. VII Congreso Español de Ingeniería de Alimentos (CYTA/CESIA). 7-10 April 2015. Badajoz (Spain).

García-Pérez, J.V., de Prados, M., Martinez-Escriva, G., Gonzalez, R., Mulet, A. & Benedito, J. Exploring the use of low-intensity ultrasonics as a tool for assessing the salt content in pork meat products. International Congress on Ultrasonics (ICU). 11-14 May 2015. Mezt (France).

Martinez-Escriva, G., De Prados, M., Mulet, A., Fernandez-Salguero, J., García-Pérez, J.V. & Benedito, J. Ultrasound on-line monitoring of ham dry salting. 12th International Congress on Engineering and Food (ICEF12). 14-18 June 2015. Québec (Canada).

De Prados, M., Martinez-Escriva, G., Diaz, K., Benedito, J. & García-Pérez, J.V. Monitorización a tiempo real del salado de jamón y lomo de cerdo. III Congreso internacional de calidad y seguridad alimentaria (ACOFESAL). 10-12 June 2015. Valencia (Spain).

9. REFERENCIAS

9. REFERENCIAS

Aaslyng, M.D., Vestergaard, C., Koch, A.G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, 96, 47-55

Aboonajmi, M., Akram, A., Nishizu, T., Kondo, N., Setarehdan, S.K., Rajabipour, A. (2010). An ultrasound based technique for the determination of poultry egg quality. *Research Agriculture Engineering*, 56(1), 26-32

Ahmad-Qasem, M.H., Cánovas, J., Barrajón-Catalán, E., Micol, V., Cárcel, J.A., García-Pérez, J.V. (2013). Kinetic and compositional study of phenolic extraction from olive leaves (*var. Serrana*) by using power ultrasound. *Innovative Food Science and Emerging Technologies*, 17, 120-129

Alava, J.M., Sahi, S.S., Garcıa-Alvarez, J., Turo, A., Chavez, J.A., Garcıa, M.J. (2007). Use of ultrasound for the determination of flour quality. *Ultrasonics*, 46, 270-276

Albarracín, W., Sánchez, I.C., Grau, R., Barat, J.M. (2011). Salt in food processing; usage and reduction: a review. *International Journal of Food Science and Technology,* 46, 1329-1336

Aparicio, C., Otero, L., Guignon, B., Molina-García, A.D., Sanz, P.D. (2008). Ice content and temperature determination from ultrasonic measurements in partially frozen foods. *Journal Food Engineering*, 88, 272-279

Armenteros, M. (2010). Reducción de sodio en lomo y jamón curados. Efecto sobre la proteolisis y las características sensoriales. Tesis Doctoral. Universitat Politècnica de València

Association of Official Analytical Chemist (AOAC). Official Methods of Analysis. (1997). (16th ed.). Washington: AOAC, International

Awad, T.S. (2004). Ultrasonic studies of the crystallization behavior of two palm fats O/W emulsions and its modification. *Food Research International,* 37(6), 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: Review. *Food Research International*, 48, 410-427

Ayuso, D., González, A., Hernández, F., Corral, J.M., Izquierdo, M. (2013). Prediction of carcass composition, ham and foreleg weights, and lean meat yields of Iberian pigs using ultrasound measurements in live animals. *Journal Animal Science*, 91 (4), 1884-1892

Bahram-Parvar, M. (2015). A review of Modern instrumental techniques for measurements of ice cream characteristics. *Food Chemistry,* 188, 625-631

Barat, J.M., Grau, R., Pagan-Moreno, M.J., Fito, P. (2004). Replacement of pile salting by simultaneous brine thawing-salting in Spanish cured ham manufacturing. *Meat Science*, 66, 603-608

Barlocco, N., Vadell, A., Ballesteros, F., Galietta, G., Cozzolino, D. (2006). Predicting intramuscular fat, moisture and Warner–Bratzler shear force in pork muscle using near infrared reflectance spectroscopy. *Animal Science*, 82, 111-116

Bello, J. (2008). El jamón curado. Aspectos científicos y tecnológicos: perspectiva desde la Unión Europea. Ed: Díaz de Santos. Madrid. España

Benedito, J. (1998). Contribución a la caracterización de quesos mediante el uso de ultrasonidos de señal. Tesis Doctoral. Universitat Politècnica de València

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., Gisbert, M., Mulet, A. (2001b). Quality control of cheese maturation and defects using ultrasonics. *Journal of Food Science*, 66(1), 100-104

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

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

Benedito, J., Mulet, A., Velasco, J., Dobarganes, M.C. (2002b). Ultrasonic assessment of oil quality during frying. *Journal Agriculture Food Chemistry*, 50, 4531-4536

Brienne, J.P., Denoyelle, C., Baussart, H., Daudin, J.D. (2001). Assessment of meat fat content using dual energy X-ray absorption. *Meat Science*, 57, 235-244

Camarena, F., Martinez-Mora, J.A. (2006). Potential of ultrasound to evaluate turgidity and hydration of the orange peel. *Journal Food Engineering*, 75, 503-507

Čandek-Potokar, M., Škrlep, M. (2012). Factors in pig production that impact the quality of dry-cured ham: A review. *Animal*, 6(2), 327-338

Castro-Giráldez, M., Fito, P.J., Fito, P. (2010). Non-equilibrium thermodynamic approach to analyze the pork meat (*Longissimus dorsi*) salting process. *Journal of Food Engineering*, 99, 24-30

Chanamai, R., Mc.Clements, D.J. (1999). Ultrasonic determination of chicken composition. *Journal Agricultural Food Chemistry*, 47, 4686-4692

Chandrapala, J. (2015). Low intensity ultrasound applications on food systems. *International Food Research Journal*, 22 (3), 888-895

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

Chen, J.S., Karlsson, C., Povey, M. (2005). Acoustic envelope detector for crispness assessment of biscuits. *Journal of Texture Studies*, 36(2), 139-156

Chen, Q., Zhang, C., Zhao, J., Ouyang, Q. (2013). Recent advances in emerging imaging techniques for non-destructive detection of food quality and safety. *Trends in Analytical Chemistry*, 52, 261-274

Cheng, Y., Haugh, C.G. (1994). Detecting hollow heart in potatoes using ultrasound. *Transaction of the ASAE*, 37, 217-222

Cierach, M., Modzelewska-Kapitula, M. (2011). Effects of ph values and fat content on sodium chloride diffusion rate in pork. *Journal of Food Processing and Preservation*, 35, 129-142

Collell, C., Gou, P., Picouet, P., Arnau, J., Comaposada, J. (2010). Feasibility of near-infrared spectroscopy to predict aw and moisture and NaCl contents of fermented pork sausages. *Meat Science*, 85, 325-330

Collell, C., Gou, P., Picouet, P., Arnau, J., Comaposada, J. (2011). Non-destructive estimation of moisture, water activity and NaCl at ham surface during resting and drying using NIR spectroscopy. *Food Chemistry*, 129, 601-607

Collell, C., Gou, P., Picouet, P., Arnau, J., Muñoz, I., Comaposada, J. (2012). NIR technology for on-line determination of superficial aw and moisture content during the drying process of fermented sausages. *Food Chemistry*, 135, 1750-1755

Corona, E. (2013). Caracterización de la calidad de productos cárnicos crudo-curados mediante ultrasonidos de señal. Tesis Doctoral. Universitat Politècnica de València

Corona, E., García-Pérez, J.V., Gómez Alvarez-Arenas, T.E., Watson, N., Povey, M.J.W., Benedito, J. (2013b). Advances in the ultrasound characterization of dry-cured meat products. *Journal of Food Engineering*, 119, 464-470

Corona, E., García-Pérez, J.V., Mulet, A., Benedito, J. (2013a). Ultrasonic assessment of textural changes in vacuum packaged sliced Iberian ham induced by high pressure treatment or cold storage. *Meat Science*, 95, 389-395

Corona, E., García-Pérez, J.V., Santacatalina, J.V., Ventanas, S., Benedito, J. (2014a). Ultrasonic characterization of pork fat crystallization during cold storage. *Journal of Food Science*, 79(5), E828-E838

Corona, E., García-Pérez, J.V., Ventanas, S., Benedito, J. (2014b). Ultrasonic characterization of the fat source and composition of formulated dry-cured meat products. *Food Science and Technology International*, 20, 275-285

Costa, A. (2010). Modificacions en la dinàmica de processat i en les característiques finals de productes carnis amb contingut reduït de sodi. Tesis Doctoral. Universitat de Girona

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*, 83, 390-397

Damez, J-L., Clerjon, S. (2013). Quantifying and predicting meat and meat products quality attributes using electromagnetic waves: An overview. *Meat Science*, 95, 879-896

Davenel, A., Bazin, C., Quellec, S., Challois, S., Gispert, M., Mercat, M.J., Muller, N. (2012). High throughput determination of intramuscular fat content by magnetic resonance imaging. *Journees de la Recherche Porcine en France*, 44, 53-54

Delfa, R., Teixeira, A., Gonzalez, C., Torrano, L., Valderrábano, J. (1999). Use of ultrasounds in blanca celtiberica live kids as predictors of carcasses composition. *Archivos de Zootecnia*, 48, 123-134

Dwyer, C., Mullen, A.M., Allen, P., Buckin, V., (2001). Anisotropy of ultrasonic velocity as a method of tracking postmortem ageing in beef. Proceedings 47th ICoMST, Poland, 4 (P4), pp. 250

Elfawakhry, H., Hussein, M.A., Becker, T. (2013). Investigations on the evaluation of rheological properties of cereal based viscoelastic fluids using ultrasound. *Journal of Food Engineering*, 116, 404-412

Elmehdi, H.M., Page, J.H., Scanlon, M.G. (2003). Using ultrasound to investigate the cellular structure of bread crumb. *Journal of Cereal Science*, 38, 33-42

Emenheiser, J.C., Greiner, S.P., Lewis, R.M., Notter, D.R. (2010). Validation of live animal ultrasonic measurements of body composition in market lambs. *Journal Animal Science*, 88, 2932-2939

Emenheiser, J.C., Tait Jr., R.G., Shackelford, S.D., Kuehn, L.A., Wheeler, T.L., Notter, D.R., Lewis, R.M. (2014). Use of ultrasound scanning and body condition score to evaluate composition traits in mature beef cows. *Journal Animal Science*, 92, 3868-3877

Faulkner, D.B., Parrett, D.F., Mckeith, F.K., Berger, L.L. (1990). Prediction of fat cover and carcass composition from live and carcass measurements. *Journal of Animal Science*, 68(3), 604-610

Fernández, M., Ordoñez, 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

Frisullo, P., Marino, R., Laverse, J., Albenzio, M., Del Nobile, M.A. (2010). Assessment of intramuscular fat level and distribution in beef muscles using X-ray microcomputed tomography. *Meat Science*, 85(2), 250-255

Fulladosa, E., Duran-Montgé, P., Serra, X., Picouet, P., Schimmer, O., Gou, P. (2013). Estimation of dry-cured ham composition using dielectric time domain reflectometry. *Meat Science*, 51, 873-879

Fulladosa, E., Muñoz, I., Serra, X., Arnau, J., Gou, P. (2015). X-ray absorptiometry for non-destructive monitoring of the salt uptake in bone-in hams during salting. *Food Control*, 47, 37-42

Fulladosa, E., Santos-Garcés, E., Picouet, P., Gou, P. (2010). Prediction of salt and water content in dry-cured hams by computed tomography. *Journal Food Engineering*, 96(1), 80-85

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

García-Gil, N., Muñoz, I., Santos-Garcés, E., Arnau, J., Gou, P. (2014). Salt uptake and water loss in hams with different water contents at the lean surface and at different salting temperatures. *Meat Science*, 96, 65-72

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(2), 386-392

Ghaedian, R., Coupland, J.N., Decker, E.A., McClements, D.J. (1998). Ultrasonic determination of fish composition. *Journal Food Engineering*, 35, 323-337

Gilles, G. (2009). Dry cured ham quality as related to lipid quality of raw material and lipid changes during processing: a review. *Grasas y Aceites*, 60(3), 297-307

Gómez, M., Oliete, B., García-Álvarez, J., Ronda, F., Salazar, J. (2008). Characterization of cake batters by ultrasound measurements. *Journal of Food Engineering,* 89, 408-413

Gou, P., Composada, J., Arnau, J. (2004). Moisture diffusivity in the lean tissue of dry-cured ham at different process times. *Meat Science*, 67, 203-209

Gou, P., Santos-Garcés, E., Hoy, M., Wold, J.P., Liland, K.H., Fulladosa, E. (2013). Feasibility of NIR interactance hyperspectral imaging for on-line measurement of crude composition in vacuum packed dry-cured ham slices. *Meat Science*, 95(2), 250-255

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(3), 858-864

He, F.J., Campbell, N.R.C., MacGregor, G.A. (2012). Reducing salt intake to prevent hypertension and cardiovascular disease. *Revista Panam Salud Pública*, 32(4), 293-300

Hunter, T.E., Suster, D., Dunshea, F.R., Cummins, L.J., Egan, A.R., Leury, B.J. (2011). Dual energy absorptiometry (DXA) can be used to predict live animal and whole carcass composition of sheep. *Small Ruminant Research*, 100(2), 143-152

Izbaim, D., Taifi, N., Malanine, M., Faiz, B., Moudden, A., Maze, G. (2009). Use of ultrasonics for the quality assessment of frying oil. 3rd International Conference on Integrity, Reliability and Failure, Porto/Portugal, 20-24 July

Jiménez-Colmenero, F. (2000). Relevant factors in strategies for fat reduction in meat products. *Trends in Food Science & Technology*, 11, 56-66

Jiménez-Colmenero, F., Ventanas, J., Toldrà, F. (2010). Nutritional composition of dry-cured hams and its role in a healthy diet. *Meat Science*, 84(4), 585-593

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

Kinsler, L.E., Frey, A.R., Coppens, A.B., Sanders, J.V. (1982). Fundamentals of acoustics. (3rd ed.). (Chapter 4). Ed. John Wiley and Sons. New York. U.S.A.

Kleczek, K., Wawro, K., Wilkiewicz-Wawro, E., Makowski, W., Konstantynowicz, K. (2009). Relationships between breast muscle thickness measured by ultrasonography and meatiness and fatness in broiler chickens. *Archives Animal Breeding*, 52, 538-545

Koc, A.B., Ozer, B. (2008). Nondestructive monitoring of renetted whole milk during cheese manufacturing. *Food Research International*, 41, 745-750

Koch, T., Brand, S., Lakshmanan, S., Raum, K., Wicke, M., 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

Krause, D., Schöck, T., Hussein, M.A., Becker, T. (2011). Ultrasonic characterization of aqueous solutions with varying sugar and ethanol

content using multivariate regression methods. *Journal of Chemometrics*, 25, 216-223

Kuo, F.J., Sheng, C.T., Ting, C.H. (2008). Evaluation of ultrasonic propagation to measure sugar content and viscosity of reconstituted orange juice. *Journal of Food Engineering*, 86 (1), 84-90

Kuttruff, H. (1991). Ultrasonics. Fundamentals and applications. Ed. Elsevier Applied Science. London. United Kingdom

Lakshmanan, S., Koch, T., Brand, S., Männickae, N., Wicke, M., Mörlein, D., Raum, K. (2012). Prediction of the intramuscular fat content in loin muscle of pig carcasses by quantitative time-resolved ultrasound. *Meat Science*, 90, 216-225

Larivière, J.M., Michaux, C., Verleyen, V., Hanzen C., Leroy, P. (2009). Non-Invasive methods to predict breast muscle weight in slow-growing chickens. *International Journal of Poultry Science*, 8(7), 689-691

Larrea, V. (2003). Caracterización química y microestructural del jamón curado denominación de Origen Teruel. Tesis Doctoral. Universitat Politècnica de València

Leemans, V., Destain, M.F. (2009). Ultrasonic internal defect detection in cheese. *Journal of Food Engineering*, 90(3), 333-340

Liu, D., Sun, D.W., Qu, J., Zeng, X.A., Pu, H., Ma, J. (2014). Feasibility of using hyperspectral imaging to predict moisture content of porcine meat during salting. *Food Chemistry*, 152, 197-204

Llull, C.P. (2001). Ús dels ultrasounds de señal per a l'avaluació de les característiques texturales y de composición de productes cárnics cru curats: aplicación a la sobrasada de Mallorca. Tesis Doctoral. Universitat de les Illes Balears

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

Manzocco, L., Anese, M., Marzona, S., Innocente, N., Lagazio, C., Nicoli, M.C. (2013). Monitoring dry-curing of S. Daniele ham by magnetic resonance imaging. *Food Chemistry*, 141, 2246-2252

Marcoux, M., Bernier, J.F., Pomar, C., (2003). Estimation of Canadian and European lean yields and composition of pig carcasses by dual-energy X-ray absorptiometry. *Meat Science*, 63(3), 359-365

Maria, R.M., Colnago, L.A., Forato, L.A., Bouchard, D. (2010). Fast and simple nuclear magnetic resonance method to measure conjugated linoleic acid in beef. *Journal of Agricultural and Food Chemistry*, 58(11), 6562-6564

Martini, S., Awad, T.S., Marangoni, A.G. (2006). Structure and properties of fat crystal networks. Chapter Modifying food lipids for use in foods (pp. 349-380). Ed. In F. Gunstone. Cambridge. U.K.

Matthews, K., Strong, M. (2005). Salt, its role in meat products and the industry's action plan to reduce it. *British Nutrition Foundation Nutrition Bulletin*, 30, 55-61

McClements, D.J. (1995). Advances in the applications of ultrasound in food analysis and processing. *Trends in Food Science & Technology*, 6, 293-299

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

Miles, C.A., Fisher, A.V., Fursey, G.A.J., Page, S.J. (1987). Estimating beef carcass composition using the speed of ultrasound. *Meat Science*, 21(3), 175-188

Miles, C.A., Fursey, G.A.J. (1977). Measurement of the fat content of meat using ultrasonic waves. *Food Chemistry*, 2(2), 107-118

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

Mizrach, A. (2004). Assessing plum fruit quality attributes with an ultrasonic method. *Food Research International*, 37, 627-631

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

Mizrach, A., Galili, N., Gan-mor, S., Flitsanov, U., Prigozin, I. (1996). Models of ultrasonic parameters to assess avocado properties and shelf life. *Engineering Research*, 65(4), 261-267

Mohammadi, V., Ghasemi-Varnamkhasti, M., Ebrahimi, R., Abbasvali, M. (2014). Ultrasonic techniques for the milk production industry. *Measurement*, 58, 93-102

Môrlein, 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

Morrison, D.S., Abeyratne, U.R. (2014). Ultrasonic technique for non-destructive quality evaluation of oranges. *Journal of Food Engineering*, 141, 107-112

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

Newcom, D.W., Baas, T.J., Lampe, J.F. (2002). Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *Journal Animal Science*, 80, 3046-3052

Niñoles, L. (2007) Contribución a la caracterización de la calidad de productos de cerdo mediante ultrasonidos de señal. Tesis Doctoral. Universitat Politècnica de València

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 system. *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

Nowak, K.W., Markowski, M., Daszkiewicz, T. (2015). Ultrasonic determination of mechanical properties of meat products. *Journal of Food Engineering*, 147, 49-55

Ortuño, C. (2014). Aplicación de ultrasonidos de potencia para la mejora de procesos de inactivación con fluidos supercríticos. Tesis Doctoral. Universitat Politècnica de València

Ortuño, C., Quiles, A., Benedito, J. (2014). Inactivation kinetics and cell morphology of *E. coli* and *S. cerevisiae* treated with ultrasound-assisted supercritical CO_2. *Food Research International*, 62, 955-964

Ozuna, C., Cárcel, J.A., Walde, P.M., García-Pérez, J.V. (2014). Low-temperature drying of salted cod (*Gadus morhua*) assisted by high power ultrasound: Kinetics and physical properties. *Innovative Food Science & Emerging Technologies*, 23, 146-155

Park, B., Whittaker, A.D., Miller, R.K., Hale, D.S. (1994). Predicting fat in beef *Longissimus* muscle from speed of sound. *Journal of Animal Science*, 72, 109-116

Pascual, A., Gisbert, M., Tomas, D., López, N. (1997). Aplicación de los ultrasonidos en tecnología de la carne y los productos cárnicos (I). Alimentación. *Equipos y Tecnología*, 75-80

Peña, F., Molina, A., Juárez, M., Requena, F., Avilés, C., Santos, R., Domenech, V., Horcada, A. (2014). Use of serial ultrasound measures in the study of growth- and breed-related changes of ultrasonic measurements and relationship with carcass measurements in lean cattle breeds. *Meat Science*, 96, 247-255

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 Chemistry*, 126, 1366-1372

Picouet, P., Teran, F., Gispert, M., Font, I., Furnols, M. (2010). Lean content prediction in pig carcasses, loin and ham by computerized tomography (CT) using a density model. *Meat Science*, 86(3), 616-622

Picouet, P.A., Gou, P., Fulladosa, E., Santos-Garcés, E., Arnau, J. (2013). Estimation of NaCl diffusivity by computed tomography in the

Semimembranosus muscle during salting of fresh and frozen/thawed hams. *LWT Food Science Technology*, 51(1), 275-280

Povey, M.J.W., Awad, T.S., Huo, R., Ding, Y.L. (2009). Quasi-isothermal crystallization kinetics, non-classical nucleation and surfactant-dependent crystallisation of emulsions. *European Journal of Lipid Science and Technology*, 111(3), 236-242

Povey, M.J.W., Mason, T.J. (1998). Ultrasound in food processing. Chapter 3, 5 and 6. Ed. Blackie Academic & Professional of Thomson Science. London, U.K.

Prevolnik, M., Škrlep, M., Janeš, L., Velikonja-Bolta, Š., Škorjanc, D., Čandek-Potokar, M. (2011). Accuracy of near infrared spectroscopy for prediction of chemical composition, salt content and free amino acids in dry-cured ham. *Meat Science*, 88, 299-304

Prieto, N., Roehe, R., Lavín, P., Batten, G., Andrés, S. (2009). Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. *Meat Science*, 83, 175-186

Pullanagari, R.R., Yule, I.J., Agnew, M. (2015). On-line prediction of lamb fatty acid composition by visible near infrared spectroscopy. *Meat Science*, 100, 156-163

Ramírez, R., Cava, R. (2007). Carcass composition and meat quality of three different Iberian *Duroc* genotype pigs. *Meat Science*, 75, 388-396

Ramisetty, K.A., Pandit, A.B., Gogate, P.R. (2015). Ultrasound assisted preparation of emulsion of coconut oil in water: Understanding the effect of operating parameters and comparison of reactor designs. *Chemical Engineering and Processing: Process Intensification,* 88, 70-77

Reglamento (CE) No 1924/2006 of the European parliament and of the council of 20 December 2006 on nutrition and health claims made on foods. pp.9-25

Reig, M., Aristoy, M-C., Toldrá, F. (2013). Variability in the contents of pork meat nutrients and how it may affect food composition databases. *Food Chemistry*, 140, 478-482

Resa, P., Elvira, L., de Espinosa, F.M., Gonzalez, R., Barcenilla, J. (2009). On-line ultrasonic velocity monitoring of alcoholic fermentation kinetics. *Bioprocessing Biosystem Engineering*, 32, 321-331

Ribeiro, F.R.B., Tedeschi, L.O., Stouffer, J.R., Carstens, G.E. (2008). Technical note: A novel technique to assess internal body fat of cattle by using real-time ultrasound. *Journal of Animal Science*, 86(3), 763-767

Ruiz, J.L. (2005). Textura de músculos de cerdo y de jamón curado con distintos niveles de NaCl, pH y contenido de agua. Tesis Doctoral. Universidad Autónoma de Barcelona

Ruiz-Ramírez, J., Arnau, J., Serra, X., Gou, P. (2005). Relationship between water content, NaCl content, pH and texture parameters in dry-cured muscles. *Meat Science*, 70, 579-587

Ruusunen, M., Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science*, 70, 531-541

Sankarappa, T., Kumar, M.P., Ahmad, A. (2005). Ultrasound velocity and density studies in some refined and unrefined edible oils. *Physics and Chemistry of Liquids*, 43(6), 507-514

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

Santacatalina, J.V., Rodríguez, O., Simal, S., Cárcel, J.A., Mulet, A., García-Pérez, J.V. (2014). Ultrasonically enhanced low-temperature drying of apple: Influence on drying kinetics and antioxidant potential. *Journal of Food Engineering*, 138, 35-44

Santos-Garcés, E. (2012). Applications of computer tomography in dry-cured meat products. Tesis Doctoral. Universitat de Girona

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 Food Engineering*, 101(2), 187-192

Scanlon, M.G. (2004). Low-intensity ultrasound for food research and the food industry. *Food Research International*, 37, 535-536

Scholz, A.M., Bünger, L., Kongsro, J., Baulain, U., Mitchell, A.D. (2015). Non-invasive methods for the determination of body and carcass composition in livestock: dual-energy X-ray absorptiometry, computed tomography, magnetic resonance imaging and ultrasound: invited review. *Animal*, 9(7), 1250-1264

Siciliano, C., Belsito, E., De Marco, R., Di Gioia, M.L., Leggio, A., Liguori, A. (2013). Quantitative determination of fatty acid chain composition in pork meat products by high resolution 1H NMR spectroscopy. *Food Chemistry*, 136(2), 546-554

Sigfusson, H., Decker, E.A., McClements, D.J. (2001). Ultrasonic characterization of Atlantic mackerel (*Scomber scombrus*). *Food Research International*, 34(1), 15-23

Sigfusson, H., Ziegler, G.R., Coupland, J.N. (2004). Ultrasonic monitoring of food freezing. *Journal Food Engineering*, 62, 263-269

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, V.R., Dwivedi, S. (1995). Ultrasonic detection of adulteration in fluid foods. Proceedings RC IEEEEMBS &14th BMESI (pp. 1.73–1.74)

Skaf, A., Nassar, G., Lefebvre, F., Nongaillard, B. (2009). A new acoustic technique to monitor bread dough during the fermentation phase. *Journal of Food Engineering*, 93, 365-378

Soyer, A., Ugˇuz, S., Dalmis¸, Ü. (2011). Proteolytic changes during processing in turkish dry-cured meat product (*pastirma*) with different salt levels. *Journal of Food Quality*, 34, 212-219

Suvanich, V., Ghaedian, R., Chamai, R., Decker, E.A., McClements, D.J. (1998). Prediction of approximate fish composition from ultrasonic properties: catfish, cod, flounder, mackerel and salmon. *Journal of Food Science*, 63(6), 966-968

Swatland, H.J. (2001). Elastic deformation in probe measurements on beef carcasses. *Journal Muscle Foods*, 12, 97-105

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

Thériault, M., Pomar, C., Castonguay, F.W. (2009). Accuracy of real-time ultrasound measurements of total tissue, fat, and muscle depths at different measuring sites in lamb. *Journal Animal Science*, 87, 1801-1813

Ting, C.H., Kuo, F.J., Lien, C.C., Sheng, C.T. (2009). Use of ultrasound for characterising the gelation process in heat induced $CaSO_4{\cdot}2H_2O$ tofu curd. *Journal of Food Engineering*, 93(1), 101-107

Toldrá, F., Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Review in Food Science*, 38, 331-352

Toozandehjani, M., Matori, K.A., Ostovan, F., Mustapha, F., Zahari, N.I., Oskoueian, A. (2015). On the correlation between microstructural evolution and ultrasonic properties: a review. *Journal Material Science*, 50, 2643-2665

Turantaş, F., Kılıç, G.B., Kılıç, B. (2015). Ultrasound in the meat industry: General applications and decontamination efficiency. *International Journal of Food Microbiology*, 198, 59-69

Valente, M., Prades, A., Laux, D. (2013). Potential use of physical measurements including ultrasound for a better mango fruit quality characterization. *Journal of Food Engineering*, 116, 57-64

Van Nguyen, M., Arason, S., Thorarinsdottir, K.A., Thorkelsson, G., Gudmundsdottir, A. (2010). Influence of salt concentration on the salting kinetics of cod loin (*Gardu Morhua*) during brine salting. *Journal of Food Engineering*, 100, 225-231

Vázquez, R. (2015). Estudio metabólomico de biomarcadores nutricionales en estudios de intervención mediante Resonancia Magnética Nuclear. Intervención con alimentos, complementos y patrones alimentarios. Tesis Doctoral. Universidad de Barcelona

Ventanas, J. (2001). Tecnología del jamón ibérico. De los sistemas tradicionales a la explotación racional del sabor y aroma. Ed. Mundi-prensa. Madrid. España

Ventanas, J. (2003). Avances en la ciencia, tecnología y comercialización del jamón. Ed. Conjamón (II Congreso mundial del jamón) Capítulo 3. Cáceres. España

Vester-Christensen, M., Erbou, S.G.H., Hansen, M.F., Olsen, E.V., Christensen, L.B., Hviid, M. (2009). Virtual dissection of pig carcasses. *Meat Science*, 81(4), 699-704

Vestergaard, C., Erbou, S.G., Thauland, T., Adler-Nissen, J., Berg, P. (2005a). Salt distribution in dry-cured ham measured by computed tomography and image analysis. *Meat Science*, 69(1), 9-15

Vestergaard, C., Risum, J., Adler-Nissen, J. (2005b). 23Na-MRI quantification of sodium and water mobility in pork during brine curing. *Meat Science*, 69(4), 663-672

Vestergaard, C., Risum, J., Adler-Nissen, J., (2004). Quantification of salt concentration in cured pork by computed tomography. *Meat Science*, 68, 107-113

Whittaker, A.D., Park, B., Thane, B.R., Miller, R.K., Savell, J.W. (1992). Principles of ultrasound and measurement of intramuscular fat. *Journal of Animal Science*, 70, 942-952

Wold, J.P., O'Farrell, M., Høy, M., Tschudi, J. (2011). On-line determination and control of fat content in batches of beef trimmings by NIR imaging spectroscopy. *Meat Science*, 89(3), 317-324

Zumalacarregui, J.M. (1997). Tecnología del jamón curado. *Ciencia y Tecnología de los alimentos*, 5, 134-138

Páginas web

www.iso.org International Organitation for Standarization (Consulta 13.05.15)

www.aoac.org AOAC international (Consulta 13.05.15)

www.nmkl.org Nordic Comité of Food Analysis (Consulta 13.05.15)

http://www.magrama.gob.es/ Ministerio de agricultura, alimentación y medio ambiente (consulta 18.05.15)

www.who.int Organización Mundial de la Salud

www.bedca.net Base de datos española de composición de alimentos

www.anice.es Asociación Nacional de Industrias de la Carne de España