Universitat de Girona

TECHNOLOGIES TO DETERMINE QUALITY PARAMETERS AND THE EFFECT OF HIGH PRESSURE PROCESSING ON DRY-CURED HAM

Marc RUBIO CELORIO

Dipòsit legal: Gi. 1892-2015
http://hdl.handle.net/10803/319951

DOCTORAL THESIS - TECHNOLOGIES TO DETERMINE QUALITY PARAMETERS AND THE EFFECT OF HIGH PRESSURE PROCESSING ON DRY-CURED HAM - Marc Rubio Celorio

Universitat de Girona

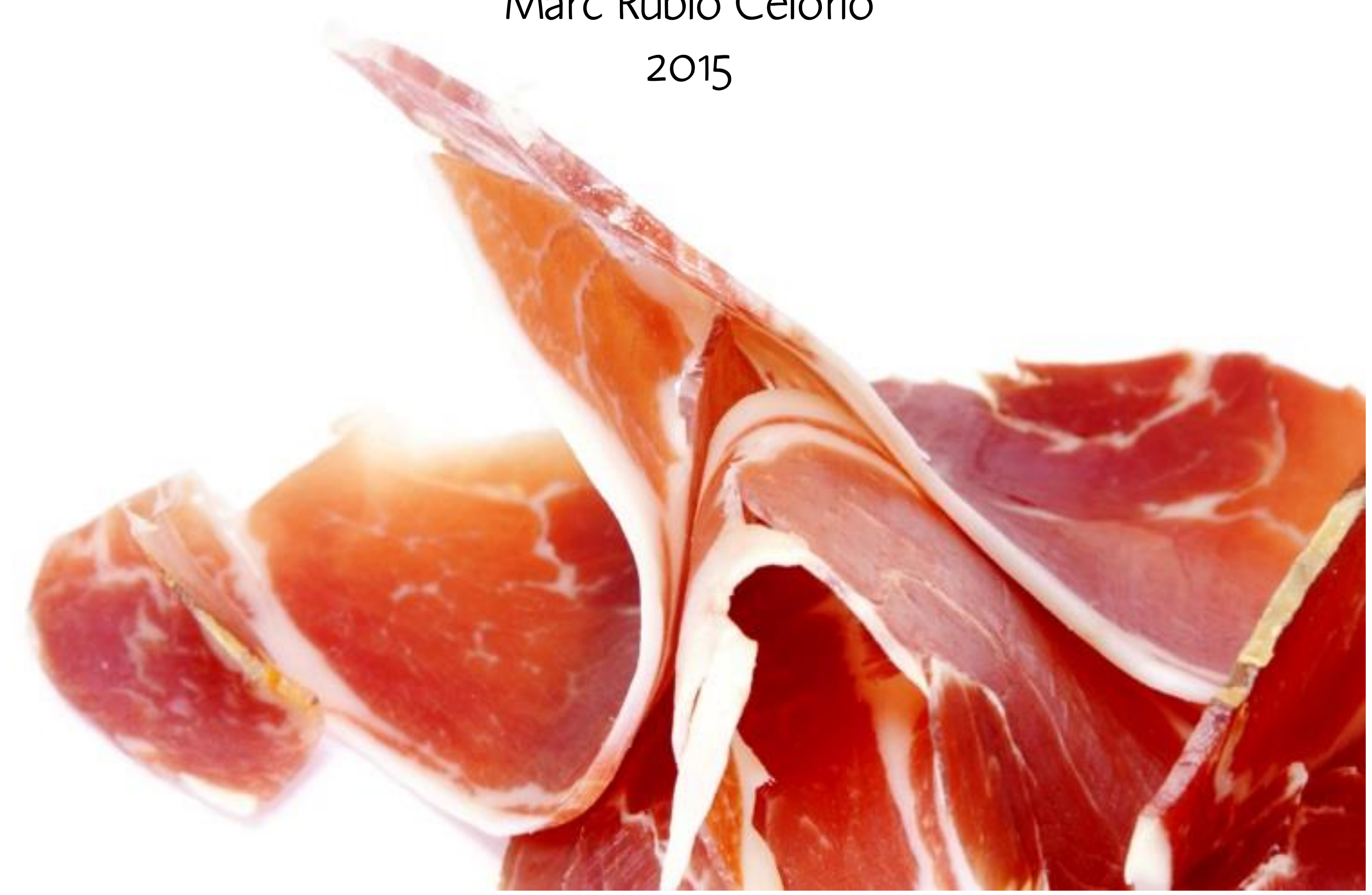
IRTA
RESEARCH & TECHNOLOGY
FOOD & AGRICULTURE

Doctoral Thesis

TECHNOLOGIES TO DETERMINE QUALITY PARAMETERS AND THE EFFECT OF HIGH PRESSURE PROCESSING ON DRY-CURED HAM

Marc Rubio Celorio

2015



Doctoral Thesis

TECHNOLOGIES TO DETERMINE QUALITY PARAMETERS AND THE EFFECT OF HIGH PRESSURE PROCESSING ON DRY-CURED HAM

Marc Rubio Celorio

2015

PhD degree in Technology

ELENA FULLADOSA	**NÚRIA GARCIA-GIL**	**ELENA SAGUER**
Supervisor	**Supervisor**	**Tutor**

Thesis presented to compete for the Philosophiae Doctor degree at the University of Girona

Dr. **ELENA FULLADOSA I TOMÀS**, researcher affiliated to "Institut de Recerca I Tecnologies Agroalimentàries (IRTA)" Monells (Girona, Spain) and Dr. **NÚRIA GARCIA-GIL**

CERTIFY:

That this work, entitled "*Technologies to determine quality parameters and the effect of High Pressure Processing on dry-cured ham***"**, presented by **MARC RUBIO CELORIO** to obtain the title of doctor, has been carried out under the direction of Dr. **ELENA FULLADOSA I TOMÀS** and Dr. **NÚRIA GARCIA-GIL**, and meets the requirements to qualify for the International Mention.

ELENA FULLADOSA **NÚRIA GARCIA-GIL**

Supervisor **Supervisor**

This work was carried out at the Food Technology Programme at the research centre IRTA-Monells within the framework of project RTA2010-00029-CO4-01 of the “Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria” (INIA) of Spain and the COST Action FA1102.

El dolç no seria tan dolç

si no existís l'amarg.

Acknowledgements

First of all, I want to express my deepest gratitude to my supervisors Dr. Elena Fulladosa and Dr. Núria Garcia-Gil, my "scientific mums", who accompanied me on this journey and taught me lots of things beyond this thesis.

I would also like to thank Dr. Jacint Arnau, as a head of the Food Technology Programme, for giving me the chance of working with this wonderful team of people.

All the researchers of the Food Technology Programme are also acknowledged. Especially Dr. Pere Gou, for his invaluable help with the statistical analysis, Dr. Israel Muñoz for his amazing brain using Mat Lab, Dr. Maria Dolors Guàrdia and Dr. Anna Claret for their amazing sensorial skills and Dr. Pierre Picouet for his physical speeches.

The author would also like to thank Dr. Elena Saguer (Universitat de Girona) for her tuition in my Final Degree Project, my Final Master Project and now in my thesis.

A warm thanks to all the support technicians, now my friends, who have helped me somehow at some point during my four years at IRTA: Cristina Canals, Jordi Garcia, Grau Matas, Dani Pascual, Marta Baret, Raquel Cama, Gustavo Rodríguez, Quim Arbonés and Bernardo Guerra, and to all the PhD students who have shared this tricky road with me: Eva Santos, Carles Collell, Joan Montasell, Héctor Mora, Wondessen Bekele and Oxana Lazo.

Acknowledgments are also extended to Dr. Marta Castro and Dr. Pedro Fito (Universidad Politécnica de Valencia) and to Dr. Hanne Bertram (Aarhus University) for allowing me carry out a research stay at their respective centres and being such marvellous professional co-authors in the papers presented in this thesis.

A mis padres,

Candela y Juan,

por su infatigable apoyo

y su incondicional amor.

Published works

This thesis is based on the following papers:

Paper I

Muñoz, I., **Rubio-Celorio, M.**, Garcia-Gil, N., Guàrdia, M.D., & Fulladosa, E., (2015). Computer image analysis as a tool for classifying marbling: a case study in dry-cured ham. *Journal of Food Engineering,* Submitted (JFOODENG-D-15-00248).

Quality index of the journal according to JCR Science Edition 2013:

Impact factor: 2.576

Position in Food Science & Technology Category: 23/123 (1st quartile)

Paper II

Rubio-Celorio, M., Garcia-Gil, N., Gou, P., Arnau, J., & Fulladosa, E. (2015). Effect of temperature, high pressure and freezing/thawing of dry-cured ham slices on dielectric time domain reflectometry response. *Meat Science*, 100, 91-95.

Quality index of the journal according to JCR Science Edition 2012:

Impact factor: 2.754

Position in Food Science & Technology Category: 17/124 (1st quartile)

Paper III

Rubio-Celorio, M., Fulladosa, E., Garcia-Gil, N., & Bertram, H.C. (2015). Multiple spectroscopic approach to elucidate water distribution and water-protein interactions in dry-cured ham after high pressure processing. *Food of Journal Engineering*, Submitted (JFOODENG-D-15-00439).

Quality index of the journal according to JCR Science Edition 2013:

Impact factor: 2.576

Position in Food Science & Technology Category: 23/123 (1st quartile)

Paper IV

Rubio-Celorio, M., Garcia-Gil, N., Castro-Giraldez, M., Fulladosa, E., & Fito, P.J. (2015). Changes on slice conformation and microstructure in dry-cured ham subjected to different high pressure levels. In progress (to be submitted).

Table of contents

Abbreviations

CIA	Computer Image Analysis
CT	Computed Tomography
DXA	Dual energy X-ray Absorptiometry
HPP	High Pressure Processing
MI	Multispectral Imaging
NIRS	Near Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
SEM	Scanning Electron Microscopy
TDR	Time Domain Reflectometry
TEM	Transmission Electron Microscopy

List of figures and tables

Abstract

The research for new technologies able to determine quality parameters as well as study the effect of High Pressure Processing (HPP) on dry-cured ham is of scientific interest because the information obtained is useful for understanding the changes to which the product is subjected. Besides, this information is valuable for the dry-cured ham industry, as it could provide products which are better characterized for consumers and of higher quality.

The main objective of this thesis was to study the potential of different technologies to determine quality parameters of dry-cured ham and elucidate the effect of HPP on the product. Several studies were carried out to achieve this aim, the results of which were submitted to different journals included in the Science Citation Index (Papers). In Paper I, a reference marbling scale was elaborated and Computer Image Analysis (CIA) was used to develop a system to objectively classify dry-cured ham slices into marbling categories. In Paper II, the effect of temperature, HPP and freezing/thawing treatment on the prediction of salt and water contents in dry-cured ham was studied by means of Time Domain Reflectometry (TDR). In Paper III, the effect of HPP on dry-cured ham was elucidated by means of Nuclear Magnetic Resonance (NMR), TDR, and Multispectral Imaging (MI). In Paper IV, conformational changes and microstructural modifications as studied by Cryo-SEM were evaluated.

Results showed that CIA allows the classification of samples according to their marbling with an accuracy of 89%, and can therefore be regarded as an objective and reliable sorter of dry-cured ham slices based on this attribute (Paper I). On the other hand, a decrease of the TDR signal intensity in pressurized dry-cured ham was observed, which produced changes in salt but not in water content predictions using the available predictive models (Paper II). A reallocation of the water populations from water closely associated with the macromolecules to water entrapped within the myofibrillar matrix and a new arrangement of the proteins in dry-cured ham treated by HPP were demonstrated as studied by NMR and MI (Paper III). A macroscopic compression and a compaction of the muscle fibres in dry-cured ham were observed after HPP, which agreed with the previous results (Paper IV). The changes due to the application of HPP produce structural modification in dry-cured ham, resulting in sensory changes which can negatively affect the quality of the product, but which could also be used to improve some of its attributes.

The technologies tested in this thesis appear to be promising as tools to determine quality parameters in dry-cured ham. Some of them are currently available for industry and others could be implemented on-line in the future.

Resum

La investigació en noves tecnologies que permetin determinar paràmetres de qualitat, així com estudiar l'efecte de las altes pressions hidrostàtiques (HPP) en pernil curat és d'interès científic perquè la informació obtinguda pot ser útil per entendre els canvis als quals està sotmès el producte. A més, aquesta informació pot tenir un valor important per a la indústria del pernil curat, permetent-li oferir als consumidors productes de més qualitat i millor caracteritzats.

L'objectiu principal d'aquesta tesi va ser estudiar el potencial de diferents tecnologies per determinar paràmetres de qualitat del pernil curat i per estudiar l'efecte de les altes pressions en aquest producte. Es varen dur a terme varis estudis per assolir aquest objectiu, els resultats dels quals varen ser enviats a diferents revistes incloses en el *Science Citation Index* (Papers) per a la seva publicació. En el Paper I, es va elaborar una escala de referència de vetejat i es va utilitzar l'anàlisi d'imatge (*Computer Image Analysis*, CIA) per desenvolupar un sistema automàtic per classificar objectivament llesques de pernil curat en diferents categories de vetejat. En el Paper II, es va avaluar l'efecte de la influència de la temperatura, les altes pressions i la congelació/descongelació en la predicció del contingut de sal i aigua en pernil curat mitjançant espectometria de microones, concretament amb el sistema *Time Domain Reflectometry* (TDR). En el Paper III, es va estudiar l'efecte de les altes pressions en pernil curat per mitjà de Ressonància Magnètica Nuclear (RMN), TDR i Imatge Multiespectal (MI). En el Paper IV, es varen avaluar els canvis conformacionals i les modificacions microestructurals mitjançant *Cryo-SEM*.

Els resultats varen mostrar que la tecnologia CIA permet classificar mostres segons el seu vetejat amb una precisió del 89%, pel que podria ser considerada com una eina objectiva i fiable per classificar llesques de pernil curat en funció d'aquest atribut (Paper I). Per altra banda, es va observar un descens en la intensitat de les mesures TDR després de sotmetre a altes pressions el pernil curat, la qual cosa va produir canvis en la predicció del contingut de sal però no del contingut d'aigua utilitzant els models predictius disponibles en el mercat (Paper II). També es va demostrar mitjançant NMR i MI que les altes pressions provoquen una redistribució de les poblacions d'aigua, en la que part de l'aigua passa d'estar fortament lligada a estar més lliure i retinguda a la matriu miofibril·lar, així com una nova conformació de les proteïnes (Paper III). Així mateix, les altes pressions varen causar una compressió macroscòpica i una compactació de les fibres musculars en pernil curat, la qual cosa concorda amb els resultats anteriors (Paper IV). Aquest canvis deguts a l'aplicació d'altes pressions produeixen modificacions estructurals en el pernil curat, donant lloc a canvis en les propietats sensorials que poden afectar negativament la qualitat del producte, però que també poden ser utilitzats per millorar alguns dels seus atributs.

Les tecnologies estudiades en aquesta tesi són prometedores com a eines per a determinar paràmetres de qualitat en pernil curat. Algunes d'elles ja estan actualment disponibles per a la indústria i d'altres podrien arribar a implentar-se en línia en un futur.

Resumen

La investigación en nuevas tecnologías que permitan determinar parámetros de calidad, así como estudiar el efecto de las altas presiones hidrostáticas (HPP) en jamón curado es de interés científico porque la información obtenida puede ser útil para entender los cambios a los que está sometido el producto. Además, esta información puede tener un valor importante para la industria del jamón curado, permitiéndole ofrecer a los consumidores productos de mayor calidad y mejor caracterizados.

El objetivo principal de esta tesis es estudiar el potencial de diferentes tecnologías para determinar parámetros de calidad del jamón curado y para estudiar el efecto de las altas presiones en este producto. Se llevaron a cabo varios estudios para alcanzar este objetivo, los resultados de los cuales se enviaron a distintas revistas incluidas en el *Science Citation Index* (Papers) para su publicación. En el Paper I, se elaboró una escala de referencia de veteado y se utilizó el análisis de imagen (*Computer Image Analysis*, CIA) para desarrollar un sistema para clasificar objetivamente lonchas de jamón curado en distintas categorías de veteado. En el Paper II, se evaluó el efecto de la influencia de la temperatura, las altas presiones y la congelación/descongelación en la predicción del contenido de sal y agua en jamón curado mediante espectrometría de microondas, concretamente con el sistema *Time Domain Reflectometry* (TDR). En el Paper III, se estudió el efecto de las altas presiones en jamón curado mediante Resonancia Magnética Nuclear (RMN), TDR e Imagen Multiespectral (MI). En el Paper IV, se evaluaron los cambios conformacionales y las modificaciones microestructurales mediante *Cryo-SEM*.

Los resultados mostraron que la tecnología CIA permite clasificar muestras según su veteado con una precisión del 89%, pudiéndose considerar como una herramienta objetiva y fiable para clasificar lonchas de jamón curado en función de este atributo (Paper I). Por otro lado, se observó un descenso de la intensidad de las mediciones TDR después de someter a altas presiones el jamón curado, lo cual produjo cambios en la predicción del contenido de sal pero no del contenido de agua utilizando los modelos predictivos disponibles en el mercado (Paper II). También se demostró mediante RMN y MI que las altas presiones provocan una redistribución de las poblaciones de agua, en la que parte del agua pasa de estar fuertemente ligada a estar más libre y retenida en la matriz miofibrilar, así como una nueva conformación de las proteínas (Paper III). Asimismo, las altas presiones causaron una compresión macroscópica y una compactación de las fibras musculares en jamón curado, lo cual concuerda con los resultados anteriores (Paper IV). Estos cambios debidos a la aplicación de altas presiones producen modificaciones estructurales en el jamón curado, dando lugar a cambios en las propiedades sensoriales que pueden afectar negativamente a la calidad del producto, pero que también pueden ser utilizados para mejorar algunos de sus atributos.

Las tecnologías estudiadas en esta tesis son prometedoras como herramientas para determinar parámetros de calidad en jamón curado. Algunas de ellas ya están actualmente disponibles para la industria y otras podrían llegar a implementarse en línea en un futuro.

Keywords

Dry-cured ham; emerging technologies; electromagnetic field; high pressure processing; nuclear magnetic resonance; time domain reflectometry; multispectral imaging; salt content; water content; microstructure.

1. INTRODUCTION

Dry-cured ham is considered an important meat product in the Mediterranean region, appreciated by consumers because of their flavour and texture characteristics (Møller, Adamsen, & Skibsted, 2003; Morales, Guerrero, Aguiar, Guàrdia, & Gou, 2013), being one of the products with greatest relevance and tradition in Spain (Jimenez-Colmenero, Ventanas, & Toldrà, 2010).

During the last decades, dry-cured ham production has become mainly industrial, although it is still based on the traditional elaboration process. Green hams are rubbed with a mixture of sodium chloride and curing salts and then covered with salt (usually for 1 day/kg of green product) to achieve the target salt uptake. After this process, hams are hung in a cold room at 3 – 4ºC for a resting period at a relative humidity of 75 - 80% until the salt content has been homogenized throughout the whole ham in order to avoid microbiological hazards. Finally, during the drying process, the temperature is progressively increased (from 5 to 30°C) while the relative humidity is decreased (60-75%) in order to decrease the water content in hams. When the weight loss in hams reaches approximately 33% and the characteristic texture and aroma of dry-cured ham are obtained the process is finished (Arnau, Hugas, & Monfort, 1987).

1.1 Justification of the work

The composition of dry-cured hams present on the market is variable because it depends on the features of the animals (breed, age, gender, feed, etc.), the characteristics of the raw material (weight, pH, etc.) and the technological process followed by the industry (salting time, temperature, humidity, etc.) (Arnau, Guerrero, Gou, & Monfort, 2001) resulting in dry-cured hams with very variable salt, water and fat contents. For consumers, salty taste and intramuscular fat are two of the most important attributes when purchasing dry-cured ham (Morales, Guerrero, Claret, Guàrdia, & Gou, 2008). Currently, there is a general tendency to reduce the NaCl content in dry-cured hams in accordance with World Health Organization recommendations and the increase of consumer demands for dry-cured ham with a lower salt content (Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2006). Besides, due to the EU Regulation 1169/2011 demands mandatory nutrition information on processed foods from 13 December 2016, it will be crucial to individually characterize the final product. Categorize it according to consumer preferences it is also a milestone for the dry-cured ham industry. On the other hand, intramuscular fat content, and to greater extent its spatial distribution (marbling), is one of the features that most influence the acceptability and palatability of dry-cured ham (Font-i-Furnols, Tous, Esteve-Garcia, & Gispert, 2012).

To satisfy the current consumer demands and to succeed in today's highly competitive market, the industry has been forced to increase the use of technologies which permit the monitoring of the elaboration process and perform an on-line quality control as well as a product characterization. There are many tools available to determine quality parameters in meat products but they are often destructive, time-consuming or difficult to implement on-line. Up to now, destructive analytical methods have been used to measure the variation of the physicochemical properties of a product. In the food industry, the idea of replacing these traditional systems for more optimized innovative ones is growing. Therefore, the implementation of non-destructive technologies for monitoring the process and for providing objective information on specific parameters is a challenge facing industry. Using these technologies results are usually obtained within a shorter period of time in comparison to the conventional analytical techniques (Valous, Mendoza, & Sun, 2010). Besides, some of these technologies are non-destructive, allowing the analysis of meat products without altering their properties, which is useful for monitoring and controlling the product during the industrial process. The increased use of non-destructive evaluation methods leads to a better understanding of the materials and processes involved, resulting in meat products which are safer and of better quality (Reh, 2008). The sophistication of non-destructive methods has rapidly evolved with modern technologies (Mix, 2005). For the particular case of dry-cured ham, these technologies are of interest due to the elevated cost of each piece.

Nowadays, consumers demand highly safe products which are, at the same time, reduced in salt content. To match these demands, alternative non-thermal preservation technologies have been investigated (Aymerich, Picouet, & Monfort, 2008). High Pressure Processing (HPP) is currently being used in industry to eliminate pathogenic microorganisms, to extend product shelf-life and to improve the safety of commercial processed meat products (Bajovic, Bolumar,

& Heinz, 2012). Nevertheless, HPP can affect the sensory characteristics and the quality of the products (Sun & Holley, 2010). In dry-cured ham, high pressure affects colour and colour stability (Andrés, Møller, Adamsen, & Skibsted, 2004), texture (Serra et al., 2006), and produces a significant increase of saltiness perception (Fulladosa, Serra, Gou, & Arnau, 2009). Due to this fact, the assessment of all these effects on the product is important for the industry.

Thus, the research for new technologies able to determine quality parameters as well as study the effect of HPP on dry-cured ham is of scientific interest because the obtained information could be useful for the understanding of the structural changes to which the product is subjected. Besides, this information could be valuable to the dry-cured ham industry, as it would provide products of higher quality and better characterized for consumers.

1.2 Technologies for measuring dry-cured meat parameters

Technologies based on the use of electromagnetic radiation at different wavelengths (Figure 1) have been demonstrated to be useful for the prediction of food composition in industry (see reviews Scotter, 1997; Damez & Clerjon, 2008; Valous, Mendoza, & Sun, 2010; Damez & Clerjon, 2013). The widespread use of electromagnetic waves for meat quality assessment is due to their practicality and their ability to explore the material. Depending on the frequency or the wavelength used, electromagnetic waves are more or less likely to be reflected in the meat or be transmitted and absorbed, offering the possibility to objectively quantify quality factors through physical measurements (Damez & Clerjon, 2008). These methods of assessment can either measure meat component properties directly or calculate them indirectly (Monin, 1998) by using correlations between one or several biophysical measurements and meat component properties (Brunton, Lyng, Zhang, & Jacquier, 2006; Swatland, 1997). Non-destructive analyses are based on the resolution of equipment sensors combined with the mathematical capabilities of current computer software. Nevertheless, these methods must be previously calibrated for each type of component and product. These calibrations can allow a subsequent fast, non-destructive analysis of the studied product as well as the simultaneous determination of multiple components, such as water and salt contents or fat content and its distribution (marbling).

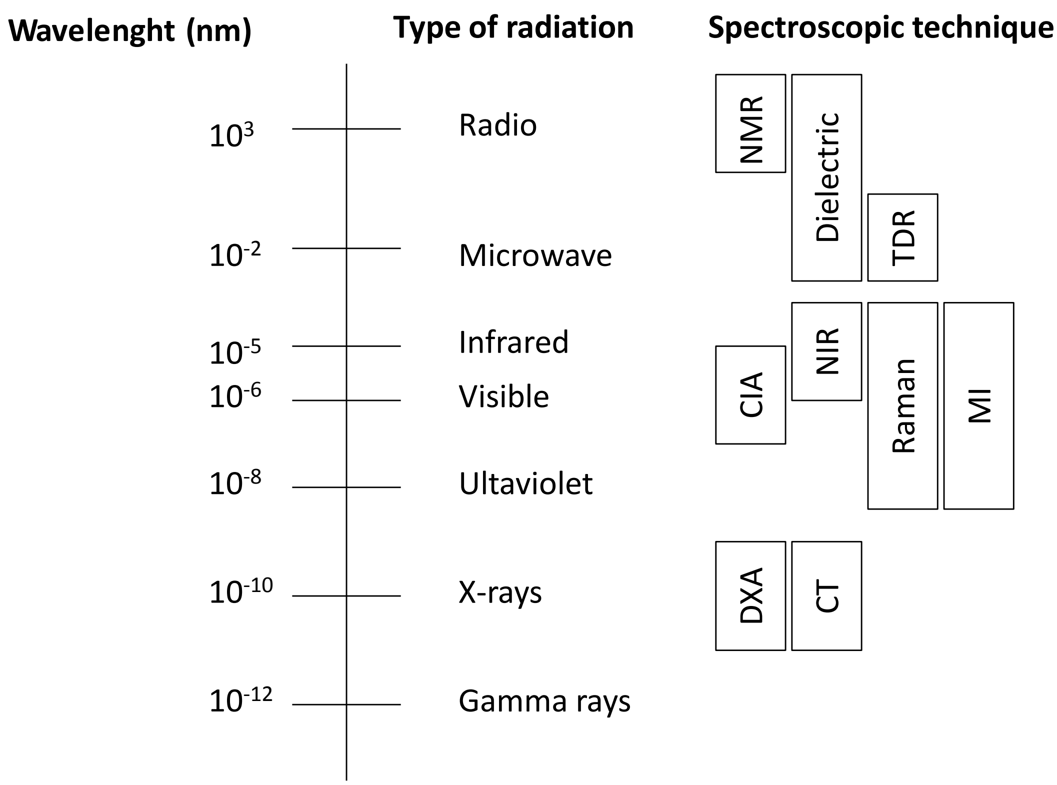


Figure 1. Electromagnetic spectrum and related technologies

From less to more energy of the electromagnetic spectrum (Figure 1), there are several technologies which can be applied in the meat field:

Nuclear Magnetic Resonance (NMR) aligns the hydrogen nuclei in a magnetic field and subsequently perturbs them from their equilibrium state by a radiofrequency pulse, to which they will subsequently return by a process described as relaxation. In **Low-field NMR relaxometry** technique, this relaxation decay provides direct information about the compartmentalization and mobility of water in heterogeneous systems such as meat (Bertram & Andersen, 2004). Low field NMR relaxometry has been used in meat to determine fat and water contents (Sørland, Larsen, Lundby, Rudi, & Guiheneuf, 2004), to study the mechanisms determining the development of sensory attributes during pork cooking (Bertram, Aaslyng, & Andersen, 2005), to evaluate water migration and water-binding within the meat pork matrix upon salting (Bertram, Meyer, Wu, Zhou, & Andersen, 2008; McDonnell et al., 2013), to evaluate water properties during cooking of pork (Bertram, Engelsen, Busk, Karlsson, & Andersen, 2004), to predict water holding capacity in pork (Bertram, Dønstrup, Karlsson, & Andersen, 2002), to study changes in distribution and mobility of water as a result of HPP of sausages (Møller et al., 2011) and for monitoring Parma dry-cured ham processing (Fantazzini, Gombia, Schembri, Simoncini, & Virgili, 2009).

Dielectric spectrometry determines the dielectric properties of biological tissues and can be used to describe physicochemical aspects, components interactions and structural changes in foodstuffs (Castro-Giráldez, Fito, Toldrá, & Fito, 2009; Içier & Baysal, 2004). Nowadays dielectric spectroscopy is a technique already used to analyse different properties of meat and meat products, for example to evaluate meat structure (Clerjon & Damez, 2007), to evaluate meat freshness (Damez, Clerjon, Abouelkaram, & Lepetit, 2008), to detect added water in pork (Kent, Knöchel, Daschner, & Berger, 2001; Kent, Peymann, Gabriel, & Knight, 2002), to determine fat composition of minced meat (Borgaard, Christensen, & Jespersen, 2003; Kent, Lees, & Roger, 1993), to relate dielectric properties of meat and meat products with meat structural proteins denaturation (Bircan & Barringer, 2002; Brunton, Lyng, Zhang, & Jacquier, 2006), to control pork salting process (Castro-Giráldez, Fito, & Fito, 2010) and to identify pork quality (Castro-Giráldez, Aristoy, Toldrá, & Fito, 2010). On the other hand, there are modalities such as **Time Domain Reflectometry (TDR)** which shows the response obtained from the interaction of an electromagnetic pulse containing a wide range of frequencies in the range of microwaves simultaneously with the sample (Miura, Yagihara, & Mashimo, 2003). TDR has also been used to develop models for fast estimation of water and salt contents in dry-cured ham (Fulladosa, Duran-Montgé, Serra, Picouet, Schimmer, & Gou, 2013).

Near Infrared (NIR) spectroscopy has its basis in the different vibration modes of molecules which are caused by their interaction with electromagnetic radiation absorbed at wavelengths between 750 and 2500 nm. NIR spectroscopy has been proved to be a useful technique to predict water and salt contents and a_w in minced fermented sausages (Collell, Gou, Picouet, Arnau, & Comaposada, 2010) and for the classification of dry- cured hams as a function of their texture and colour (García-Rey et al., 2005).

Computer Image Analysis (CIA), which includes the capturing, processing and analysis of images, allows a rapid, objective assessment of the visual characteristics of the product, as well as quality features that cannot be visually differentiated by human inspection, i.e., structural and textural characteristics (Mendoza et al., 2009). CIA has been used to estimate marbling scores in beef ribeyes (McDonald and Chen, 1991), to evaluate marbling by enhancing the colour contrast of meat samples (Faucitano et al., 2005), to recognize fat streaks in dry-cured ham (Cernadas et al., 2002) and to estimate fat content of dry-cured ham slices (Widiyanto et al., 2013). Moreover, there are techniques such as **Multispectral Imaging** that consists of capturing images at different frequencies across the electromagnetic spectrum. This technology has been proved to be useful for studying changes in meat colour during storage (Christiansen, Carstensen, Møller, & Nielsen, 2012) and for evaluating changes in the colour of minced cured restructured ham subjected to HPP (Bak, Lindahl, Karlsson, & Orlien, 2012).

Raman spectroscopy is a vibrational spectroscopic technique that relies on the inelastic scattering of monochromatic light, usually from a laser in the visible, IR, or near-UV spectra (Damez & Clerjon, 2008). Raman spectroscopy has great potential for biochemical tissue analysis at both the macroscopic and microscopic scale. One of the great advantages of this technique is its ability to provide information on the concentration, structure and interaction of biochemical molecules in their microenvironments within intact cells and tissues (i.e., in situ), non-destructively, and without homogenization, extraction, or the use of dyes, labels, or other contrast enhancing agents (Damez & Clerjon, 2008). This technology has been used for investigating the influence of ageing and cooking on the porcine *Longissimus dorsi* muscles (Beattie, Bell, Borggaard, & Moss, 2008), for in-situ characterization of meat ageing (Schmidt et al., 2009) and for predicting quality traits of pork at slaughtering process (Scheier, Scheeder, & Schimdt, 2015).

X-rays technologies are based on the different X-ray attenuations produced by the different tissues (Seeram, 2009), which allow the distinction between biological structures (Skejervold *et* *al.,* 1981). **Dual energy X-ray absorptiometry (DXA)** involves the acquisition of 2D images combining two different X-ray energy levels which are directed in a one angled direction. DXA has been used to determine fat content in different types of boned fresh meat (Brienne *et al.,* 2001), for meat tenderness evaluation (Kroger et al., 2006), to determine fat content in green hams (Prados et al., 2015), and for monitoring of the salt uptake in ham salting (Fulladosa, Muñoz, Serra, Arnau, & Gou, 2015).

Computed Tomography (CT) is able to carry out a 3D representation of an object by stacking several tomograms of the scanned object. Vestergaard et al. (2005) correlated computed tomograms of dry-cured hams at various stages of the production process with the final salt content of the product and Santos-Garcés et al. (2010) predicted salt and water contents and a_w in dry-cured ham.

Each of the above mentioned technologies varies in its fundamental basis, has advantages and drawbacks, and can be more or less suitable for different industrial applications depending on the product features and industrial requirements (Table 1). Their different possibilities make them interesting from a scientific point of view as they permit the study of products by different approaches.

Aside from these spectroscopic techniques, electron microscopy has also been widely used to study meat and meat product structure (Damez & Clerjon, 2008). This family of techniques consists of the use of electron bundles to illuminate a specimen and create an enlarged image which provides an image observation with great resolution and magnification. **Scanning Electron Microscopy (SEM)** results in images with a great depth-of-field yielding a characteristic 3D display that provides a greater insight into the surface structure of a biological sample. SEM is a high-performance tool for investigating process-related changes in meat ultrastructure. In **Transmission Electron Microscopy (TEM)**, electrons are passed through the sample. Resolution is higher than in SEM and the sample can be stained with heavy metals to improve image quality. Microstructural changes showing the modification of the actin–myosin complex in dry-cured ham subjected to HPP have been studied using SEM (Garcia-Gil et al., 2014) and TEM (Picouet et al., 2012). One remarkable technique is **Cryo-Scanning Electronic Microscopy (Cryo-**

SEM), in which observation is carried out after a cryofixation which avoids introducing artifacts during processing due to the instantaneous sample fixation at low temperature. Cryo-SEM has been used to study the effect of cooking on the colour and texture of beef steaks (Garcia-Segovia, Andres-Bello, and Martinez-Monzo, 2007), to study the effect of freezing on the ultrastructure of pig muscle cells (Ngapo, Babare, Reynolds, & Mawson, 1999) and to monitor microstructural changes during dry-cured ham processing (Larrea, Perez-Munuera, Hernando, Quiles, Llorca, & Lluch, 2007).

Table 1. Summary of the characteristic features of each technology

Technology	Advantages	Drawbacks
Nuclear Magnetic Resonance	Accuracy	Destructive method Difficult to implement on-line Elevated costs
Dielectric spectrometry	Short measuring times Limited sample preparation	Contacting method Superficial measurement
Near Infrared spectroscopy	Non-contacting, rapid Low cost of equipment maintenance and chemical-free Short measuring times with limited sample preparation	Superficial measurement Complex data analysis required
Computer Image Analysis	Quick and objective generation of precise descriptive data Consistent, efficient and cost-effective Reduces human involvement Robust and competitively priced sensing technique	Object identification is considerably more difficult in unstructured scenes Artificial lighting needed for obscure or dark conditions
Raman spectroscopy	Non-contacting, rapid, small sample portion	Complex data analysis required
Computed Tomography	Information on 3D-structure of food products and composition	Difficult to implement on-line Elevated cost Training to interpret the output Long acquisition times

1.3 High Pressure Processing (HHP)

High Pressure Processing (HPP) is a non-thermal preservation technology that currently uses an isostatic pressure between of 100 and 600 MPa at room temperature. The pressure chamber is loaded and closed, degassed and the pressure is transmitted by pumps through a liquid, generally water. The technology is based on the principle of Le Châtelier and the isostatic rule, so pressure is transmitted in a uniform and instantaneous manner and the product, or its constituents, suffer volume changes under pressure (Hugas, Garriga, & Monfort, 2002)**.** HPP is currently being used in industry to eliminate pathogenic microorganisms, to extend product shelf-life and to improve the safety of commercial processed meat products (Aymerich, Picouet, & Monfort, 2008; Bajovic, Bolumar, & Heinz, 2012). Pressure levels applied for the pasteurization of meats and meat products range from 400 to 600 MPa with short processing times of 3–7 min at room temperature (Cheftel & Culioli, 1997). HPP is becoming a common non-thermal treatment in industry to extend shelf-life in dry-cured ham and to fulfil the requirements of a non-presence of *Listeria monocytogenes* on some exigent markets such as in the case of the United States. Nevertheless, HPP can influence meat protein conformation and induce protein denaturation, aggregation or gelation, which can affect the appearance and quality of the products. The means whereby HHP treatment exerts effects on meat protein structure are through the rupture of non-covalent interactions within protein molecules, and a subsequent re-formation of intra- and inter- molecular non covalent bonds within or among protein molecules (Cheftel & Culioli, 1997; Sun & Holley, 2010). Pressurization also causes a loosening of the meat protein matrix and alterations in the water distribution in meat (Bertram, Wu, Straadt, Aagaard, & Aaslyng, 2006). In dry-cured ham, pressures higher than 600 MPa modify the non-covalent interactions between muscle proteins and sodium ions and between muscle proteins and water molecules, thus altering the distribution of both sodium and water (Picouet et al 2012).

2. OBJECTIVES

The main objective of this thesis was to study the potential of different technologies to determine quality parameters of dry-cured ham and elucidate the effect of High Pressure Processing used on dry-cured ham.

The following specific objectives were carried out within the framework of the main objective:

I. To develop a sensory marbling grading scale and a system to objectively classify dry-cured ham slices into categories of marbling using Computer Image Analysis.

II. To study the effect of common treatments used in the industry for the prediction of salt and water contents in dry-cured ham slices using Time Domain Reflectometry.

III. To elucidate the effects of High Pressure Processing and of raw material on the water distribution in dry-cured ham by means of Nuclear Magnetic Resonance relaxometry, Time Domain Reflectometry and Multispectral Imaging.

IV. To elucidate the effect of High Pressure Processing on dry-cured ham in terms of conformational changes and microstructural modifications as studied by Cryo-SEM.

3. METHODOLOGY

3.1 Working plan

To achieve the objectives raised in this thesis, four studies were performed (Figure 2). The studies mentioned have been published or submitted to the international scientific journals included in the Science Citation Index.

In **Paper I,** high quality images of dry-cured ham slice were acquired with a photographic system. A sensory marbling grading scale using these images was developed by a panel of experts who did not only take into account the amount of visual fat content, but also the fat flecks distribution. This scale was used for the design of an automatic classification system for dry-cured ham slices according to a marbling score from 1 to 7 based on segmenting intramuscular fat by means of Computer Image Analysis in combination with pattern recognition techniques.

In **Paper II**, the effect of temperature, High Pressure Processing (HPP) and freezing/thawing of dry-cured ham slices on the prediction of salt and water contents in dry-cured ham slices using Time Domain Reflectometry (TDR) was studied. To evaluate the effect of temperature, TDR measurements were taken at different temperatures between 5 and 26 ºC at intervals of 2 ºC. To evaluate the effect of HPP and freezing/thawing TDR measurements were taken before and after the treatments.

In **Paper III**, a multiple spectroscopic approach including Nuclear Magnetic Resonance relaxometry, TDR, and Multispectral Imaging, combined with physico-chemical analyses was used to elucidate the impact of different HPP levels (200, 400 and 600 MPa) and of raw material (low and high pH_{24SM}) on the distribution and physico-chemical state of the intrinsic water populations and water-protein interactions in dry-cured ham.

In **Paper IV**, mass, thickness, volume and surface area of dry-cured ham slices were determined before and after applying different HPP levels (200, 400 and 600 MPa) in order to study conformational changes in slices. Cryo-SEM micrographies were obtained to evaluate microstructural changes

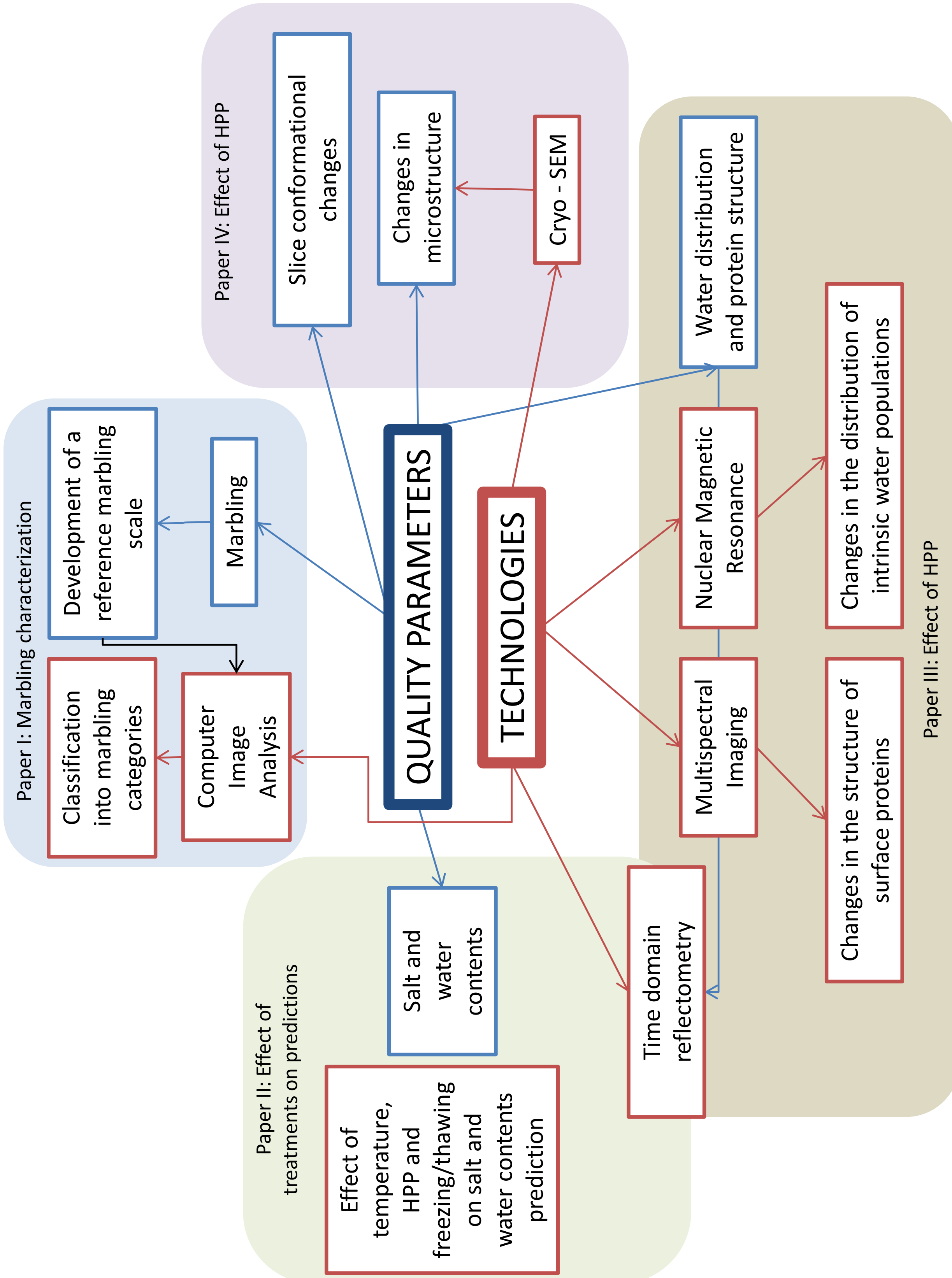


Figure 2. Working plan

3.2 Specifications of the used technologies

3.2.1 High Pressure Processing (HPP)

Dry-cured ham slices were vacuum packaged in plastic bags of multilayer polyamide/polyethylene (water permeability of 2.6 g/m^2/d at 23°C and 85% RH, Sacoliva® S.L., Spain) and pressurized at 200, 400 and 600 MPa for 5 min with water at 10 °C as pressure-transmitting medium (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain) (Figure 2). Pressurization rate was 220 MPa/min and the time for decompression was ≤10s.

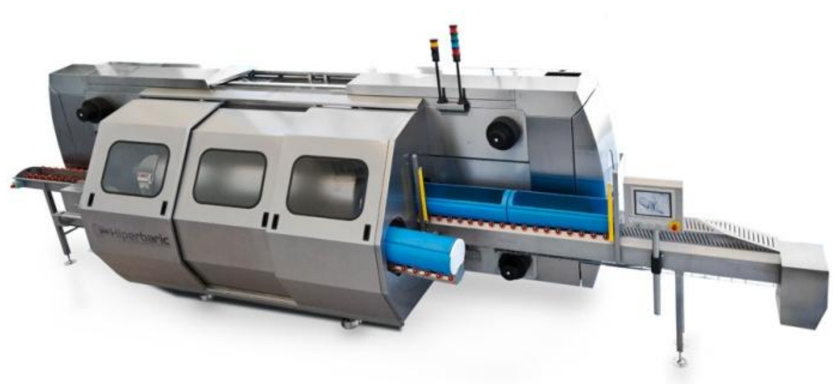


Figure 3. High Pressure Processing equipment

3.2.2 Computer Image Analysis

High quality images of the dry-cured ham slices with different levels of marbling were acquired with a photographic system that includes a calibrated digital camera Canon EOS 50D with a picture resolution of 15.1 megapixels. A total of 48 features (geometrical and textural) were extracted from the images. Using all this data, several classifiers were built using two machine learning techniques, Support Vector Machines (SVM) and Neural Networks (NN).

3.2.3 Time Domain Reflectometry (TDR)

Time domain reflectometry (TDR) measurements were carried out before and after treatments with the device RFQ Scan 3.0 (Sequid GmbH, Bremen, Germany) (Figure 3). Salt and water contents were predicted using the commercial modules based on principal components regression models developed by Sequid GmbH (Bremen, Germany) which were developed from the results obtained by Fulladosa et al. (2013). TDR curves were retrieved using a Matlab (Mathworks, 2007) script made in-house, in order to visually compare the responses.

Figure 4. Sequid RFQ Scan 3.0 device

3.2.4 Nuclear Magnetic Resonance (NMR) relaxometry

NMR relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) (Figure 7) operating at 23.2 MHz and equipped with an 18 mm variable temperature probe, applying a CPMG sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The obtained relaxation data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler, Reeds, and Dawson (1981) and carried out in MatLab (The Mathworks Inc., Natick, MA, USA) using in-house scripts.

Figure 5. Maran Benchtop Pulsed NMR Analyzer

3.2.5 Multispectral imaging

Images were acquired using a VideometerLab vision system (Videometer A/S, Denmark) (Figure 8) which acquires multi-spectral images at 18 different wavelengths ranging from UV (405 nm) to short wave NIR (970 nm). The acquisition system records surface reflections with a standard monochrome charge coupled device chip. The software VideometerLab 3 (version 2.12) was used to obtain the spectra and the L*a*b* values.

Figure 6. VideometerLab vision system

3.2.6 Cryo-Scanning Electronic Microscopy (Cryo-SEM)

Samples (5 x 3 x 2 mm) were immersed in slush N_2 (-210ºC) and then quickly transferred to the Cryostage at 1 kPa where sample fracture took place. The sublimation (etching) was carried out at -95ºC. The final point was determined by direct observation in the microscope at 5 kV. Then the samples were coated with gold in vacuum (0.2 kPa) for 3 min, with an ionization current of 2 mA. The observation in the scanning electron microscope was carried out in a CryoACryostage CT-1500C unit (Oxford Instruments, Witney, UK), coupled to a Jeol JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan) at 15 kV, a working distance of 15 mm and a temperature ≤ -130ºC (Figure 4).

Figure 7. Jeol JSM-5410 scanning electron microscope

4. RESULTS

Paper I

Muñoz, I., **Rubio-Celorio, M.**, Garcia-Gil, N., Guàrdia, M.D., & Fulladosa, E., (2015). Computer image analysis as a tool for classifying marbling: a case study in dry-cured ham. *Journal of Food Engineering,* Submitted (JFOODENG-D-15-00248).

Computer image analysis as a tool for classifying marbling: a case study in dry-cured ham

Israel Muñoz*, Marc Rubio-Celorio, Núria Garcia-Gil, Maria Dolors Guàrdia, Elena Fulladosa

IRTA. XaRTA. Food Technology. Finca Camps i Armet, E-17121 Monells (Girona), Spain

*Author for correspondence: israel.munoz@irta.cat

Abstract

Marbling in sliced dry-cured ham affects consumer acceptability and the sensory quality of the product. This study presents an automated marbling grading system of dry-cured ham slices which allows the characterization and classification of the product. Firstly, a sensory marbling grading scale was developed by a panel of experts who did not only take into account the amount of visual fat content, but also the distribution of the fat flecks. This scale was used for the design of an automatic classification system of dry-cured ham based on segmenting intramuscular fat. 643 regions of interest (ROI) of the slice were categorized by a panel of experts using the marbling grading scale and later segmented by the computer system. From the segmented ROI, 48 features (geometrical and textural) were extracted. Using all the data several classifiers were built using two machine learning techniques, Support Vector Machines (SVM) and Neural Networks (NN). Different feature selection algorithms were tested to select the optimal subset of features. Results show that with a reduced number of features, 89% of the samples could be correctly classified. Performance was better for SVM algorithms than for NN.

Key words: marbling, dry-cured ham, image analysis, pattern recognition, neural networks, support vector machines, non-destructive classification.

1. Introduction

Marbling is one of the characteristics which influences the acceptability of meat and meat products (Antequera et al., 1992; Ventanas et al., 2007; Morales et al., 2008). Intramuscular fat (IMF) can be defined as the fat located throughout skeletal muscle, whereas marbling can be defined as the amount and spatial distribution of the visible fat, which appears as fine flecks in the muscle giving it an appearance similar to marble (Cernadas et al., 2002). IMF is moderately related to the amount of marbling or visual fat (Faucitano et al., 2004). Differences in eating quality can be attributed to the fat distribution in meat rather than to the total intramuscular fat content (Albrecht et al., 1996). It is not only the amount but also the spatial distribution of intramuscular fat streaks –the marbling- which have an effect on the sensory attributes of dry-cured ham and are used to characterize and classify the product (Cernadas et al., 2002). Marbling score determination has traditionally been performed by panels of trained judges, using descriptive analysis methods. The evaluation of marbling in the pork industry relies mainly on subjective comparison with raw pork meat marbling standards or pictures such as those from the National Pork panellist employee (NPPC, 1999). The National Pork Producers Council marbling standards show pictures of pork samples containing low to high intramuscular fat content with standardized numerical marbling scores from 1.0 (devoid) to 10.0 (abundant). However, the visual assessment leads to inconsistencies in pork quality from different companies, increases labour costs, has low repeatability, and is easily influenced by environment.

Dry-cured ham is especially appreciated for its sensory properties. However, there is only a five score reference scale available (http://www.irta.cat/ca-ES/RIT/Noticies/Documents/Jornada_pernil_sensojam_guiametodologica_def.pdf) which permits a rough evaluation and classification of the product. Therefore, it would be of interest to have a more specific reference scale to evaluate marbling since this development could be used as the basis for the development of fast, non-destructive methods which would allow an online characterization of marbling on an industrial scale.

Computer image analysis (CIA) has emerged as a reliable alternative for the marbling assessment of different food products. CIA, which includes capturing, processing and analyzing of images, allows the rapid and objective assessment of visual characteristics of the product, as well as quality features that cannot be visually differentiated by human inspection, i.e., structural and textural characteristics (Mendoza et al., 2009) through the extraction of suitable features (Brosnan and Sun, 2004). McDonald and Chen (1991) used video image processing to estimate marbling scores in beef ribeyes. More recently, a segmentation based approach was reported by Jackman et al. (2009) which used K-means clustering to segment images of beef *Longissimus dorsi* muscle into background, lean muscle, and intra muscular fat area. One of the most usual techniques for marbling detection is line detection algorithms. Faucitano et al. (2005) evaluated marbling by enhancing the colour contrast of pork meat samples using chemical pre-treatments and line detection algorithms. They found significant correlations between the percentage of areas detected as marbling with respect to the muscle area and the number of areas/cm^2. They also found that neither large nor small detected flecks are representative of marbling content. Liu et al. (2012) also used a line detection algorithm for determining a marbling score of pork loins. In dry cured ham, Cernadas et al. (2002) used a multi-scale line detection framework for the recognition of fat streaks showing promising results. Widiyanto et al. (2013) were able to obtain good estimations of the fat content of slices of dry-cured ham using fuzzy c-means and bias field estimation.

Pattern recognition has been widely used in combination with CIA for classification tasks. Pattern recognition consists of extracting a set of features (geometric, texture, etc) from the segmented image and assigning a category out of a given set of categories to the data obtained. A model is built using this data in combination with one of the existing techniques of pattern recognition. New images are then classified applying the model to the features extracted from the segmentation of these images. In quality assessment of foodstuffs, several pattern recognition techniques have been used for quality evaluation. For example, Cano Marchal et al. (2013) used support vector machines and artificial neural networks for the evaluation of olive oil impurities in virgin oil using CIA. However, no studies have been found in the literature available which applies CIA for the classification of dry-cured ham slices based on marbling. Marbling assessment in dry-cured ham is of great interest to industry as it conditions the quality of the product and its commercial value. Therefore, it is important to establish a basis for the development of industrial systems to assess marbling in this product.

The main objective of this research was to define a precise marbling reference scale for sliced dry-cured ham and to develop a system to objectively classify slices into the categories of this reference scale using CIA in combination with pattern recognition techniques.

2. Materials and methods

2.1 Sampling

180 commercial dry-cured hams obtained from different ham producers which were crosses from different pig breeds (Large White, Landrace, Duroc and Iberian) were taken in order to obtain a batch of hams with a wide range of marbling. A 2 cm thick slice containing *Semimembranous* (SM), *Semitendinosus* (ST) and *Biceps femoris* (BF) muscles was obtained at 10 cm from the aitch bone in the distal direction (at the widest part of the ham) and packed into plastic bags of polyamide/polyethylene (oxygen permeability of 50 $cm^3/m^2/24h$ at 23 °C and water permeability of 2.6 $g/m^2/24h$ at 23 °C and 85% RH, Sacoliva® S.L., Spain).

2.2 Chemical analysis

Total fat content was measured in some samples by Soxtec extraction (Soxtec HT 6- 1043 and Service Unit 1046) according to ISO 1443 (1973); the analytical standard deviation was 0.32%.

2.3 Image acquisition

High quality images were acquired with a photographic system that included a calibrated digital camera Canon EOS 50D with a picture resolution of 15.1 megapixels and an objective Canon EF-S 18-200 mm f/3,5-5,6 IS. The camera was mounted onto a photographic bench in the middle of a closet (1.06 m x 1.06 m x 2.50 m) with black and a circle of 8 equidistant halogen lights Solux Q50MR16 CG/47/36º 12volts/50watt/4700 K (Eiko Ltd., Shawnee, Kansas, E.U.A.) to obtain a correct lighting environment for image capture which ensured consistent colour and lighting. White balance was carried out with a white card (Lastolite*)* in order to electronically adjust the colour reproduction without showing colour dominants. The camera was connected to a PC into which the images with RAW format were uploaded. Dry-cured ham slices were positioned 30 cm below the camera lens. An image of the entire slice surface was taken against a uniform black background. Both sides of the dry-cured ham slices were photographed (n=360) and all the images were taken during the same session. Capture One PRO 5.0 software (Phase One A/S Inc., Frederiksberg, Denmark) was used to carry out the white balance of the RAW images and digitalize them to 667 x 1000 pixels resulting in a .tif file with 16 bits color and 4 MB. This was considered to be high enough in quality for computer image analysis. With this setting one pixel of the image corresponded to 0.3968 mm^2. With the aim of obtaining the maximum fidelity to the original samples the work screen was calibrated for the sensory analysis of marbling (NEC Multisync LCD 2690 $WUXI^2$).

2.4 Sensory analysis

Sensory analysis (marbling evaluation) of the samples was carried out by six trained panellists (ISO 8586-2: 2012) and consisted at a visual assessment of the marbling of the selected Regions of interest (ROIs). These ROI were evaluated through the 362 acquired images and corresponded to SM, BF and ST muscles. Marbling was scored by consensus by means of scoring scale from 1 (minimum marbling) to 10 (maximum marbling) at intervals of 0.5. When scoring marbling, the panellists did not only focus on the total amount of marbling, but also on the distribution of the fat streaks through the muscle and the union with the subcutaneous fat. BF and SM were selected because they are considered to be the most representative muscles of dry-cured ham (Arnau et al., 1995; Boadas et al., 2001). Only for marbling reference scale development, ST muscle was also used because the BF and SM muscle did not reach such a high values of marbling. A total of 643 ROIS were evaluated. Marbling evaluation was performed in triplicate by the expert panellists showing a standard deviation of 0.5 point score. From all the analyzed samples a 9 point marbling scale (from 1 to 9) was developed.

2.5 Image analysis and segmentation

Regions of interest (ROIs) corresponding to the BF and SM muscles were manually selected from the images (Figure 1). Some samples were discarded from the study due to defects on the surface (such as cuts and phosphate crystals) which made them unsuitable for the CIA. Although ST muscles were used to develop the reference marbling scale, they were not used for classification purposes because of the different marbling characteristics of this muscle in comparison to BF and SM. For this reason, ST muscles should be classified using a different approach from the one used for BF and SM muscles which is presented in this work. Only marbling scores from BF and SM muscles ranging from 0.5 to 6 were used. Very few samples with marbling scores above 6 in BF and SM muscles were found. The number of samples ranged from a minimum of 8 for a marbling score of 6 to a maximum of 136 for a marbling score of 2.5.

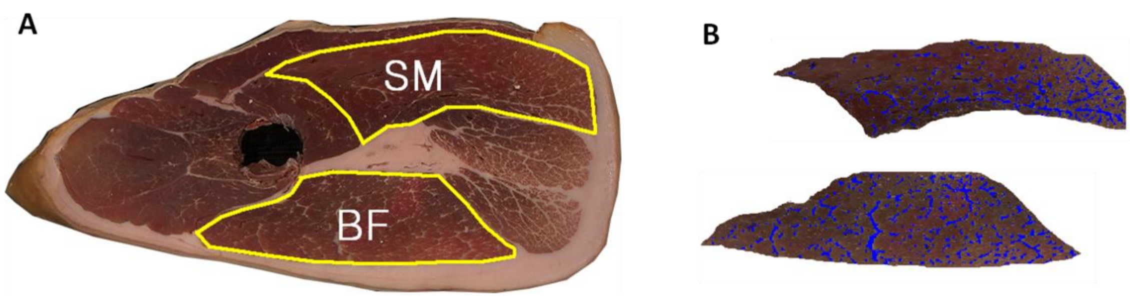


Figure 1. Selection of the two regions of interest of the dry-cured ham slice: *Biceps femoris* (BF) and *Semimebranosus* (SM) muscles (A) and segmentation of the muscles with its identified fat streaks (B).

IMF was segmented for each ROI using the following procedure. Firstly, colour components RGB (Red/Green/Blue) were extracted from the images. After testing, the G component was selected for image processing, as it was the one which produced better results for the procedure. This will be detailed below.

After selecting the G component, a median filter using a 3x3 neighbourhood was applied for removing noise from the image. The most suitable segmentation technique was found to be the Discrete Fourier transform (DFT) (Rangayyan, 2004). The G channel was transformed using DFT and a Gaussian high pass filter with a cut-off frequency of 250 and applied to the resulting image. After filtering, the images were transformed back using the inverse discrete Fourier transform and pixels were labelled as IMF if their value was above a predetermined threshold. This procedure was partly based on the work by Santos et al. (2014). Next, two morphological operations were applied. Firstly, a bridge operations was used to connect unconnected pixels and a dilate operation was applied 20 times to the resulting segmented image. At each dilate operations pixels were labelled as IMF if the value of the blue channel (B) was above a predetermined threshold. Finally, IMF regions with less than 2 pixels were discarded.

2.6 Feature extraction

Segmented IMF has to be transformed into useful information in order to classify it. In this work, 48 features were extracted from each segmented ROI. These features were classified into two groups, geometric and textural features, which are described in Table 1 and Table 2, respectively.

Table 1. Description of the 30 geometrical selected features.

Code	Description
1	Proportion of IMF flecks in relation to the total ROI area (%)
2	Number of IMF flecks
3	Number of IMF flecks/cm^2
4	Average surface of IMF flecks (cm^2)
5	Average length of IMF flecks (cm)
6	Average width of IMF flecks (cm)
7	Average length of IMF flecks for the three largest fleck areas (cm)
8	Average width of IMF flecks for the three largest fleck areas (cm)
9	Sum of the length of IMF flecks (cm)
10	Sum of the width of IMF flecks (cm)
11	Sum of the length of the three largest IMF flecks (cm)
12	Sum of the width of the three largest IMF flecks (cm)
13	Proportion of the length of IMF flecks for the three largest fleck areas in relation to the total length of all IMF flecks (%)
14	Proportion of the width of IMF flecks for the three largest fleck areas in relation to the total width of all IMF flecks (%)
15	Proportion of the area of three largest IMF flecks in relation to the total IMF flecks (%)
16	The average length of the skeleton of IMF flecks (cm)
17	The average length of the skeleton for the three largest fleck areas (cm)
18	Proportion of the length of the skeletons of IMF flecks for the three largest fleck areas in relation to respect to the total length of all IMF flecks (%)
19	Sum of the length of the skeletons of IMF flecks for the three largest fleck areas (cm)
20	Proportion of IMF flecks area with size equal or less than 20 pixels in relation to the total area (%)
21	Proportion of IMF flecks area with size equal or less than 20 pixels in relation to the total area of IMF flecks (%)

22	Proportion of the number of IMF flecks area with size equal or less than 20 pixels in relation to the total number of IMF flecks (%)
23	The average largest distance from a point inside the IMF fleck to the border (cm)
24	The average largest distance from a point inside the IMF fleck to the border for the three largest fleck areas (cm)
25	Proportion of the largest distance for the three largest fleck areas in relation to the sum of the largest distances for all IMF flecks (%)
26	Sum of the largest distance from a point inside the IMF fleck to the border for the three largest fleck areas (cm)
27	The average number of intersection points of the skeleton
28	The average number of intersection points of the skeleton for three largest IMF flecks
29	Proportion of the intersection points of the skeleton for three largest IMF flecks in relation to the total number of intersection points (%)
30	Sum of the total number of intersection points for the three largest IMF flecks areas

Table 2. Description of the 18 textural (Haralick, 1979) selected features.

Code	Feature name
31	Contrast
32	Correlation
33	Energy
34	Homogeneity
35	Autocorrelation
36	Cluster prominence
37	Cluster shade
38	Entropy
39	Maximum probability
40	Sum of squares: Variance
41	Sum average
42	Sum of variance
43	Sum of entropy
44	Difference entropy
45	Information measure of correlation 1
46	Information measure of correlation 2
47	Inverse difference normalized
48	Inverse difference moment normalized

30 geometrical features (Table 1) were calculated from the binary image obtained after segmentation of IMF for each ROI. Some of these geometrical features have already been used in previous works on marbling characteristics (Faucitano et al., 2005; Albrecht et al., 2006; Yang et al., 2006; Peña et al., 2013). The length and width of IMF flecks were computed using the

length of the major and minor axis of the ellipse which had the same normalized central moments as the region.

18 Haralic textural features (Haralick, 1979) were extracted from the IMF segmented ROI in order to obtain information regarding the pixel distribution in the image. Textural features have been widely used in previous works for evaluation of quality in different foodstuffs (ElMasry et al., 2007; Huang et al., 2013). To calculate the features a co-occurrence matrix C must be calculated (Eq.1). As IMF segmented ROI were binary images, the grey-levels were 0 and 1, so the resulting matrix was 2x2. Only horizontal offset and a distance of 1 pixel were considered for the computation of co-occurrence matrix.

$$C(i,j)_{\Delta x,\Delta y} = \sum_{n=1}^{N}\sum_{m=1}^{M}\begin{cases}1 \text{ if } f(m,n)=i \text{ and } f(m+\Delta x, n+\Delta y)=j\\ 0 \text{ elsewhere}\end{cases} \quad (1)$$

Feature normalization was performed on the data set to standardize the range of values of all the features. This can speed up the learning process of classifiers which are based on gradient descend algorithms and in other algorithms prevents attributes with large ranges from influencing the outcome of the algorithm. In this work z-score normalization was applied (Eq.2).

$$x' = \frac{x-\mu_i}{\sigma_i} \quad (2)$$

μ_i and σ_i are the average value and the standard deviation for the *ith* feature, respectively. x' is the normalized value of x.

2.7 Feature Selection

In order to reduce the size of the feature vector several techniques have previously been tested: F-score (Chen and Lin, 2005), Recursive Feature Elimination (SVM-RFE) (Guyon et al., 2002), Sequential Forward Selection (SFS), Sequential Back Selection (SBS) and Plus-L Minus-R Selection (RLS) (John et al., 1994). SVM-RFE procedure was only applied to SVM using linear kernels. No standard procedure exists for non-linear kernels.

2.8 Design of the classifier

Two different machine learning techniques were used for building the classifier. Both, Support Vector Machines (SVMs) and Artificial Neural Networks (ANNs) were selected for this work as they have been successfully applied to wide range of problems in previous studies (Cano Marchal et al., 2013; Donis-González et al., 2013).

2.8.1 Support vector machines

SVM is a supervised learning technique which is widely used for classification and regression problems (Vapnik, 1995). SVM is a binary classification method originally used to separate the positive (+1) and negative classes (-1) by calculating the hyperplane (also known as linear kernel) which maximizes the distance to the closest samples of both classes. By introducing some changes, using what is called the "kernel trick", the problem can be extended to non-linear separation surfaces for the two classes, such as polynomial or radial basis functions.

For some problems, it may not be possible to calculate the hyperplane (or non-linear separation surface) which completely separates completely '+1' and '-1' samples. For this reason parameter *C* is used to account for the misclassified samples. C controls the relative importance of maximizing the margin and minimizing the error, ensuring that most examples are correctly classified. For *C=0* no importance is given to misclassified examples, whereas as C increases, the importance given to misclassified samples increases. This is an important parameter as it may affect the results.

The binary classification problem can easily be extended to multiclassification by combining several binary classifiers (Hsu and Lin, 2002). There are different strategies for implementing the multiclassification problem, one against one, one against all and a directed acyclic graph (DAGSVM).

2.8.2 Artificial Neural Networks (ANN)

ANN is also a supervised learning technique which has been applied to a wide range of problems, such as classification, prediction, pattern recognition, function approximation, etc.

(Duda et al., 2000). These networks are composed of simple elements called neurons, which are interconnected. Each neuron acts as a processing unit which produces a numerical output depending on the numerical inputs from other neurons and an activation function which processes these inputs. The parameters of network which must be determined are the weights of the connections between neurons and the bias of the activation function of each neuron.

Among the different network typologies available, feed-forward networks were chosen for this classification task. Three layered networks were used in this paper, an input layer, a hidden layer and an output layer. The number of neurons in the input layer is equal to the number of attributes of the feature vectors used for training. The number of neurons at the output is equal to the number of classes which are trained.

One of the advantages of ANN over SVM is that ANN architecture allows the modelling of complex relationships between inputs which are not possible in SVMs.

2.8.3 Algorithm parameter adjustment

ANN with a hidden layer and one vs. one SVM and one vs. one DAGSVM were selected for the classification of the slices. One vs. all SVM was not included in the analysis because some previous tests showed that performance was much lower than for the one vs. one SVM (results not shown).

For the ANN classifier the default parameters included in the function were used for training. Training was limited to 500 cycles. The number of nodes of the hidden layer was selected by trial and error. The performance of the ANN was evaluated for 1 feature and 48 features for different numbers of neurons were selected in the hidden layer. The range of neurons which produced the best result for both cases was similar within a range of the number of neurons (4-8 neurons). For this reason, 6 neurons in the hidden layer were chosen. A conjugate gradient algorithm was used for the training of the neural network, the weights of the connections and the bias of the activation functions.

For the SVM classifier different kernel functions and values of C were tested for the whole data set. Linear and radial basis function kernels showed the best results for SVM, other kernels, such as polynomial, presented much poorer results. For DAGSVM only the linear kernel showed good results while for other kernels results were much worse. For SVM and DAGSVM using a linear kernel, the best results were obtained for values of C between 0.6 and 1.5, for the two one vs. one classifiers. For this reason a value of 1 was selected for C. For the radial kernel, the selected value of (parameter of the radial kernel) was the default value in LibSVM (1/number of features) (Chang and Lin, 2011) and the value of C selected was 5. In LibSVM, it is possible to apply different values of C for each class in order to balance the different number of samples for each class. Classes with a small number of samples should usually have higher values of C in order to make up for their small number. Several optimization techniques were used to find the optimal set of C values for a selection of features which presented good results. However, results did not show any significant improvement and the same C value was used for all the classes.

For both learning techniques, L=4 and R=3 were selected after some testing for the feature selection algorithm RLS.

2.8.4 Algorithm implementation

Matlab 2008b and its image processing toolbox (The MathWorks, Inc., United States) were used for selecting and segmenting the images of the dry-cured hams. It was also applied to calculate the geometric features after segmentation of the images. Two Matlab toolboxes freely available from Matlabcentral were used for calculating the skeleton intersection points and the textural features. SVM multiclass algorithms were implemented using the Matlab version of LIBSVM (Version 3.18) (Chang and Lin, 2011) running on Windows XP (a software widely used in the scientific community to work with support vector machines).

Artificial Neural Networks were implemented using Netlab a freely downloadable (http://www.aston.ac.uk/eas/research/groups/ncrg/resources/netlab/) toolbox for Matlab which implements a series of Neural Network algorithms for pattern recognition (Nabney, 2004).

2.9 Validation

Performance of the classifier was evaluated as the ratio of correctly classified samples in relation to the total number of samples. A sample was considered to be correctly classified if the category in which was classified was within a distance of 0.5 to the correct category. The reason for this tolerance is that in this study, standard deviation of sensory analysis may also have differed in 0.5 scores as previously described.

K-fold cross-validation was used to validate the classifier. 10-fold stratified cross-validation, this means that 90% of the samples were used for training and 10% for validation. Stratified cross-validation guaranteed that the class distribution was similar in all training and test subsets.

3. Results and discussion

3.1 Development of a marbling dry-cured ham reference scoring scale

The ranges of marbling scores using BF muscle (1-7), SM muscle (0.5-6) and ST muscle (4-9) were representative of the whole scoring scale and permitted the development of a precise marbling reference scoring scale (Figure 2). For elaborating the reference scoring scale of marbling, BF muscle was used for scores from 1 to 7 whereas for scores representing marbling 8 and 9, ST muscle was used because the BF muscle did not reach such a high value of marbling in any sample.

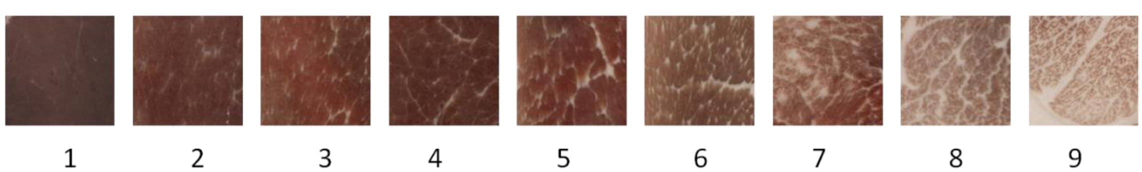


Figure 2. Reference scale of marbling

BF and SM muscle are the muscles considered to be the most representative of dry-cured ham (Arnau et al., 1995; Boadas et al., 2000), therefore they were considered the most useful for classifying the whole slice in terms of marbling. In this study, it was also found that the marbling values between BF and SM muscle were highly correlated (r = 0.84). Besides, marbling scores were highly correlated to the analytical fat content of the ROI. Figure 3 shows that the sensorial score increased with a higher fat content following an exponential tendency (R^2 = 0.80) in the selected ROIs. Santos-Garcés et al. (2014) also found an exponential relationship between the percentage of fat area from computer tomography images and the analytical fat content in dry-cured ham. In contrast, Faucitano et al. (2004) reported that marbling scores and analytical fat content were linearly correlated (*r* = 0.86) in raw pork meat. It must be remarked that ROIs from Iberian dry-cured hams did not follow this tendency, showing higher values of fat in all the marbling scores. The reason for this could be the higher proportion of intramuscular fat that cannot be visually differentiated by human inspection or CIA in this breed. Further studies will be needed to elucidate the effect of the pig breed in the correlation between sensorial score and fat content. Different pig breeds could also lead to different kinds of marbling when described in terms of Haralick and texture attributes.

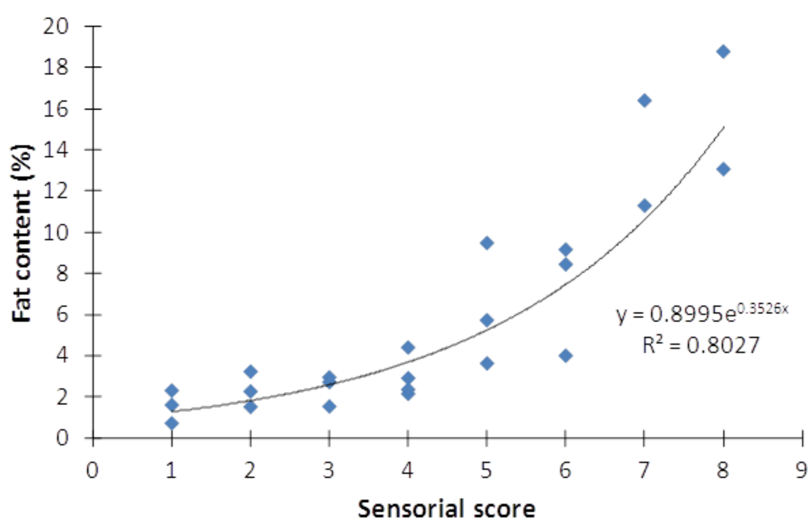


Figure 3. Correlation between sensorial score and analytical fat content.

3.2 Classification techniques performance and marbling features

Results showed a very good performance for the three classification techniques with accuracies ranging from 86% to 89% approximately (Table 3). From all the analysed classifiers and feature selection techniques the best overall classifiers were the SVM (SVM and DAGSVM) with linear and radial kernels using the RLS selection technique obtaining a performance of 88.96%. All other classification and feature selection techniques performed slightly lower as shown in Table 3. When comparing NN performance to SVM techniques performance, results showed that the performance was slightly higher for both SVM (SVM and DAGSVM) techniques. With respect to the feature selection techniques, the best results were observed for RLS and SFS techniques in almost all cases, whereas F-score showed the worst performance for SVM (SVM and DAGSVM) and SBS for the NN classifier. The reason for the bad performance of F-scores was probably due to the independent assessment of features which did not take into account interactions among features, unlike other feature selection methods.

Table 3. Best performances obtained by different combinations of classification and feature selection techniques.

Classifier	Classifier characteristics	Feature selection techniques	Number of features	Best achieved performance (%)
SVM	Linear Kernel	F-Score	26	86.47
SVM	Linear Kernel	SVM-RFE	6	87.40
SVM	Linear Kernel	SFS	12	88.18
SVM	Linear Kernel	SBS	5	87.71
SVM	Linear Kernel	RLS	18	88.96
SVM	Radial Kernel	F-Score	26	86.62
SVM	Radial Kernel	SFS	11	88.49
SVM	Radial Kernel	SBS	28	87.56
SVM	Radial Kernel	RLS	7	88.96
DAGSVM	Linear Kernel	F-Score	25	86.94
DAGSVM	Linear Kernel	SVM-RFE	3	87.09
DAGSVM	Linear Kernel	SFS	6	88.65
DAGSVM	Linear Kernel	SBS	6	87.40
DAGSVM	Linear Kernel	RLS	6	88.65
NN	6 hidden layer	F-Score	13	87.71
NN	6 hidden layer	SFS	3	88.02
NN	6 hidden layer	SBS	4	87.09
NN	6 hidden layer	RLS	5	87.25

When using only one feature for classification, NN showed the best results with a performance of 86.94% (Table 4). This result can be explained by the fact that the NN can better approximate the separation surface between different marbling scores because of the larger number of parameters that can be adjusted in a NN.

Table 4. Performances using only one feature

Classifier	Classifier characteristics	Feature selection	Feature code	Performance (%)
SVM	Linear Kernel	SFS	37	84.91
SVM	Radial Kernel	SFS	42	85.85
DAGSVM	Linear Kernel	SFS	35	83.83
NN	6 hidden layer	SFS	38	86.94

In contrast to previous works (Liu et al., 2012; Huang et al., 2013) in which the proportion of the segmented intramuscular fat area was regarded as the best feature to measure quantitative marbling in meat products, in this work, the features which produced the best performance belong to the Haralick textural features: autocorrelation (35), cluster shade (37), entropy (38) and sum of variance (42). Although, it is difficult to have a clear physical interpretation of these parameters, they are all related to distribution of fat in the slices. However, it is not easy to discern why some features were preferred to others. Autocorrelation can be thought as a measure of the regularity of the texture of an image as well as the fineness/coarseness of the texture (Tuceryan and Jain, 1998). Cluster shade is a measure of the skewness of a image, whereas entropy measures spatial disorder or complexity of an image, the entropy being higher when the image is not texturally uniform. Figure 4 shows how an increase of autocorrelation, cluster shade and entropy produce an increase in the marbling score. Sum of variance has a difficult physical interpretation, although can be observed an increase of this parameter when the marbling score decreases (Figure 4). These four features gave better results than the IMF feature area because marbling (spatial distribution of visible fat) is not only related to the amount of IMF, but also to pixel distribution. In this study, correlations of these attributes with respect to the proportion of the segmented intramuscular fat area were high ($r > 0.9$). This means that these features also gave an indirect measure of the segmented intramuscular fat area. Detection of quality defects such as steatosis could be feasible using these features.

When all attributes were used (Table 5) performance was, in most cases, lower than the performance with one attribute (Table 4). This was especially noticeable for the NN classifier, which suffered from dimensionality and overfitting. This fall in the performance of NN did not improve by adding or removing neurons from the hidden layer.

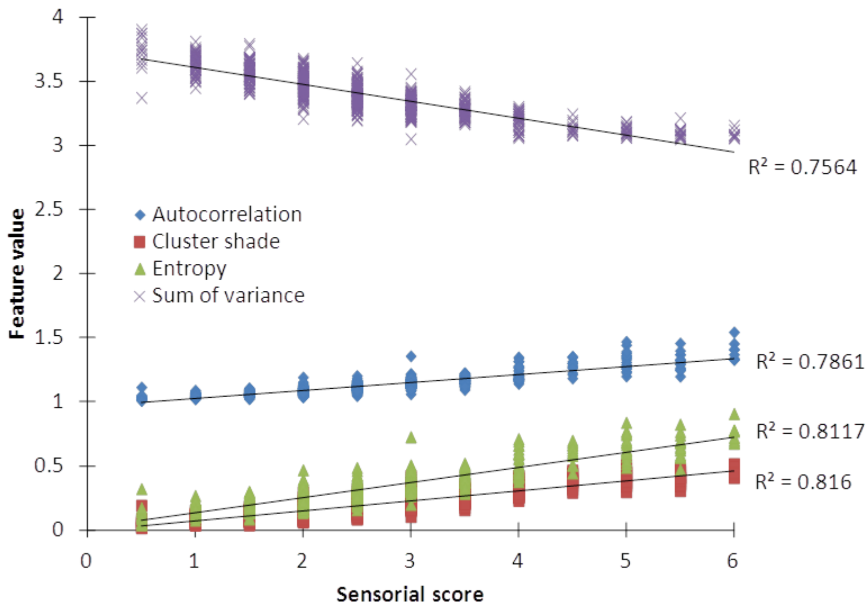


Figure 4. Correlation between sensorial score and autocorrelation, cluster shade, entropy and sum of variance.

Table 5. Performance obtained for each classifier using all the features.

Classifier	Classifier features	Performance (%)
SVM	Linear Kernel	84.60
SVM	Radial Kernel	84.29
DAGSVM	Linear Kernel	85.38
NN	6 hidden layer	80.09

Table 6 shows in detail the best performing combination of features for the best performing combinations (performance above 88%) presented in Table 3. It can be observed that there were 18 features which were not used in any of the presented feature selection algorithms. Among the most used features, which were used on at least three occasions, there was the proportion of the segmented intramuscular fat area (1) with respect to overall area, which as has been already mentioned is widely considered as the most representative feature for evaluating quantitatively marbling and highly correlated to the mentioned texture features (35, 37, 38 and 42). However, it was not the feature which was the most important in the results presented. The most important feature was the number of IMF fleck areas (2) which appeared in 5 cases. This feature alone performed very badly, around 65% (average of the different classification techniques, results not shown), when it was used alone for training. However, in combination with other features it contributed to improve the performance of the classifier. The reason for the contribution of this feature can probably be explained by the fact that the marbling scoring scale is not only defined using the IMF content, but also by the distribution of IMF flecks which can somehow be related to the number of IMF fleck areas. It can be observed in the images that the larger the number of the marbling flecks, the more regularly the fat is distributed. The average width of IMF flecks (6) was also an important feature as it was used on 4 occasions. This feature was not among the best predictors of marbling grading, around 75% when used alone (results not shown), but contained information which could contribute to the evaluation of the distribution of IMF flecks and the marbling score. This could be explained by the fact that IMF streaks are usually lengthened. However, when IMF is concentrated in few but large regions, the average width of these regions increases.

Table 6. Feature subsets for the classifiers with an accuracy above 88% .

Classifier	Classifier features	Feature selection	Feature codes
SVM	Linear Kernel	SFS	1,4,5,6,15,31,14,21,29,35,37,39
SVM	Linear Kernel	RLS	1,6,15,33,20,24,29,35,36,37,38,39,40, 41,43,44,47,30
SVM	Radial Kernel	SFS	1,2,6,8,31,34,37,40,42,47,10
SVM	Radial Kernel	RLS	2,6,32,41,45,10,30
DAGSVM	Linear Kernel	SFS	2,32,20,24,35,11
DAGSVM	Linear Kernel	RLS	2,32,20,24,35,11
NN	6 hidden layer	SFS	2,38,47

Among the textural features, there were three which appeared in at least three cases: autocorrelation (35), cluster shade (37), inverse difference normalized (47). Inverse difference normalized is a measure of the uniformity of the image. These three texture parameters were all good predictors the marbling grading with performance above 80% in all the three cases (results not shown). Other significant features, used in the 3 cases, was the proportion of IMF flecks with a size equal or less than 20 pixels with respect to the total area (20), which can interpreted as a measure of the distribution of IMF in small patches which is important for the evaluation of marbling, and the average largest distance from a point inside the IMF fleck to the border for the three largest fleck areas (24), which can also give an indirect idea on the size of the flecks.

Among the features which were not selected, there was the number of IMF flecks/cm^2 (3) and the sum of the length of IMF flecks (42) which were among the features which Faucitano et al. (2005) found to be good predictors of marbling in pork.

Overall, the best performance of the classifiers was obtained by combining several features. The increase in performance by adding features was higher for SVM techniques than for NN where performance barely increased (from 86.94 to 88.02%). The peak performance was obtained for a small subset of features for the NN technique whereas for the SVM technique it was as high as 28. After the peak in performance it started to decrease.

Results showed that both marbling characterization and grading is feasible using non-expensive technologies which would be easy to implement in industry. For industrial applications, the convenience of analysing the entire slice surface or only the most characteristic muscle needs to be evaluated for each case. If only the analysis of a part of the slice surface is of interest, pattern recognition systems able to automatically segment a given muscle in the dry-cured ham slice could be useful (Widiyanto et al., 2013).

Computer image analysis could also be implemented to characterize other kinds of images such as tomograms obtained from computed tomography (Peña et al., 2013; Font i Furnols et al., 2013) or nuclear magnetic resonance imaging (Monziols et al., 2005). These technologies allow the non-destructive inspection and characterization of the internal parts of a product and computed image analysis would allow the characterization and grading of features, such as marbling, or detection of defects. However, to implement this characterization/grading online, the image resolution of industrial equipment that is currently under development needs to be evaluated first.

4. Conclusions

A marbling reference scoring scale in dry-cured ham was developed allowing a standardization of the evaluation of this attribute. An automatic system for an accurate, objective and reliable classification of dry-cured ham slices based on this marbling scale was achieved using computer image analysis. The best results were obtained with the Support Vector Machines classifier combining several features showing an accuracy performance of 89%. This system is accurate enough to develop a future automatic grading system in terms of marbling for industrial processing lines.

5. Acknowledgements

This work was supported by projects RTA 2010-00029-CO4-01 and RTA 2013-00030-CO3-01 from INIA (Spain). Acknowledgements are extended to INIA for financing the doctorate studies of Marc Rubio at Girona University. The authors would also like to acknowledge the contribution of Albert Rosell in the sensory analysis.

6. References

AOAC, 1990. Official Methods of Analysis. 15th ed. Washington, USA: Association of Official Analytical Chemists.

Albrecht, E., Wegner, J., & Ender, K. (1996). A new technique for objective evaluation of marbling in beef. *Fleischwirtschaft*, 76, 1145-1148.

Albrecht, E., Teuscher, F., Ender, K., & Wegner, J. (2006). Growth and breed-realted changes of marbling characteristics in cattle. *Journal of Animal Science*, 84(5), 1067-1075.

Antequera, T., López-Bote, C.J., Córdoba, J.J., García, C., Asensio, M.A., Ventanas, J., & Díaz, Y., (1992). Lipid oxidative changes in the processing of Iberian pig hams. *Food Chemistry*, 45 (105), 105–110.

Arnau, J., Guerrero, L., Casademont, G., & Gou, P. (1995). Physical and chemical changes in different zones of normal and PSE dry-cured ham during processing. Food Chemistry, 52, 63–69.

Boadas, C., Gou, P., Valero, A., & Arnau, J. (2001). Changes in different zones of dry-cured ham during drying. Moisture and sodium chloride content. Fleischwirtschaft, 81, 91–93.

Brosnan, T., & Sun, D.W. (2004). Improving quality inspection of food products by computer vision - a review. *Journal of Food Engineering*, 61(1), 3-16.

Cano Marchal, P., Martínez Gila, D., Gámez García, J., & Gómez Ortega, J. (2013). Expert system based on computer vision to estimate the content of impurities in olive oil samples. *Journal of Food Engineering*, 119, 220-228.

Cernadas, E., Dur, M. L., & Antequera, T. (2002). Recognizing marbling in dry-cured Iberian ham by multiscale analysis. *Pattern Recognition Letters*, 23, 1311–1321.

Chang, C.-C., & Lin, C.-J., (2011). LIBSVM: a library for support vector machines. *ACM Transactions on Intelligent Systems Technology*, 2, 1–27.

Chen, Y.W., & Lin, C.J., (2005). Combining SVMs with various feature selection strategies. Available from http://www.csie.ntu.edu.tw/~cjlin/ papers/features.pdf.

Donis-González, I.R, Guyer, D.E., Leiva-Valenzuela, G.A., & Burns, J., (2013). Assessment of chestnut (Castanea spp.) slice quality using color images. *Journal of Food Engineering*, 115, 407-414.

Duda, R.O., Hart, P.E. & Stork, D.G., (2000). *Pattern Classification* (2nd ed.). Wiley-Interscience.

ElMasry, G., Wang, N., ElSayed, A., Ngadi, M., (2007). Hyperspectral imaging for nondestructive determination of some quality attributes for strawberry. *Journal of Food Engineering,* 81(1), 98-107.

Faucitano, L., Huff, P., Teuscher, F., Garipey, C. & Wergner, J., (2005). Application of computer image analysis to measure pork marbling characteristics. *Meat Science*, 69, 537-543.

Faucitano, L., Rivest, J., Daigle, J. P., Lévesque, J., & Gariepy, C. (2004). Distribution of intramuscular fat content and marbling within the longissimus muscle of pigs. *Canadian Journal of Animal Science*, 3, 57–62.

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.

Guyon, I., Weston, J., & Barnhill, S., (2002). Gene selection for cancer classification using support vector machines. *Machine Learning*, 46, 389–422.

Haralick, R.M., (1979). Statistical and structural approaches to texture. In *Proceedings of the IEEE*, 67, 786–804.

Hsu, C., & Lin, C., (2002). A comparison of methods for multiclass support vector machines. *IEEE Transactions on Neural Networks*, 13 (2), 415–425.

Huang, H., Liu, L., Ngadi, M.O., & Gariépy, C., (2013). Prediction of pork marbling scores using pattern analysis techniques. *Food Control*, 31, 224-229.

ISO 1443 (1973). *Determination of Total Fat Content*. International Organization for

Standardization, Geneva.

ISO 8586-2 (2012). *Sensory analysis*. General guidance for the selection, training and monitoring of assessors -Part 2: Expert sensory assessors. International Organization for

Standardization, Geneva.

Jackman, P., Sun, D-W., & Allen, P. (2009). Automatic segmentation of beef longissimus dorsi muscle and marbling by an adaptable algorithm. *Meat Science*, 83(2), 187-194.

John, G.H., Kohavi, R. & Pfleger, K. (1994). Irrelevant features and the subset selection problem. In *Machine Learning: Proceedings of the Eleventh International Conference*, 121-129.

Liu, L., Ngadi, M.O., Prasher, S.O., & Gariépy, C., (2012). Objective determination of pork marbling scores using the wide line detector. *Journal of Food Engineering*, 110, 497-504.

Mendoza, F., Valous, NA., Sun, DW., & Allen. P. (2009). Characterization of fat-connective tissue size distribution in pre-sliced pork hams using multifractal analysis. *Meat Science*, 4, 713-22

McDonald, T. P., & Chen, Y. R. (1991). Visual characterization of marbling in beef ribeyes and its relationship to taste parameters. *Transactions of the ASAE*, 34(6), 2499-2504.

Monziols, M., Collewet, M., Mariette, F., Kouba, M., & Davenel, A. (2005). Muscle and fat quantification in MRI gradient echo images using a partial volume detection method. Application to the characterization of pig belly tissue. *Magnetic Resonance Imaging*, 23 (6), 745-755

Morales, R., Guerrero, L., Aguiar, a P.S., Guàrdia, M.D., & Gou, P., (2013). Factors affecting dry-cured ham consumer acceptability. *Meat science*, 95(3), 652–657.

Nabney, I.T, (2004). *Netlab Algorithms for Pattern Recongnition*. Springer Verlag.

NPPC (1999). Marbling standards. Des Moines, USA: National Pork Producers Council.

Peña, F., Molina, A., Avilés, C., Juárez, M., & Horcada, A., (2013). Marbling in the longissimus thoracis muscle from lean cattle breeds. Computer image analysis of fresh versus stained meat samples. *Meat Science*, 95, 512-519.

Rangayyan, M. R., (2004). *Biomedical image analysis*. CRC Press.

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.

Tuceryan, M., & Jain, A.K., (1998). In C.H. Chen, L.F. Pau, P.S.P. Wang (Eds.). *Handbook of Pattern Recognition and Computer Vision* (2nd edition; pp 207-248). World Scientific Publishing Co.

Ventanas, S., Ruiz, J., García, C., & Ventanas, J. (2007). Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science*, 77(3), 324-30.

Vapnik, V.N., (1995). *The Nature of Statistical Learning Theory* (2th ed.). Springer-Verlag, New York.

Widiyanto, S., Cufí, X., Rubio, M., Muñoz, I., Fulladosa, E., & Martí, R. (2013). Automatic intra muscular fat analysis on dry-cured ham slices. In *Proceedings of ibPRIA*, 873-880.

Yang, X.J., Albrecht, E., Ender, K., Zhao, R.Q., & Wegner, J., (2006). Computer image analysis of intramuscular adipocytes and marbling in the Longissimus muscle of cattle. *Journal of Animal Science*, 84(1), 3251-3258.

Paper II

Rubio-Celorio, M., Garcia-Gil, N., Gou, P., Arnau, J., & Fulladosa, E. (2015). Effect of temperature, high pressure and freezing/thawing of dry-cured ham slices on dielectric time domain reflectometry response. *Meat science*, 100, 91-95.

Meat Science 100 (2015) 91–96

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of temperature, high pressure and freezing/thawing of dry-cured ham slices on dielectric time domain reflectometry response

Marc Rubio-Celorio, Núria Garcia-Gil, Pere Gou, Jacint Arnau, Elena Fulladosa *

IRTA, XaRTA, Food Technology, Finca Camps i Armet, E-17121 Monells, Girona, Spain

ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form 2 October 2014
Accepted 5 October 2014
Available online 12 October 2014

Keywords:
Time domain reflectometry
Non-destructive
Dielectric properties
Temperature
High pressure
Freezing/thawing

ABSTRACT

Dielectric Time Domain Reflectometry (TDR) is a useful technique for the characterization and classification of dry-cured ham according to its composition. However, changes in the behavior of dielectric properties may occur depending on environmental factors and processing. The effect of temperature, high pressure (HP) and freezing/thawing of dry-cured ham slices on the obtained TDR curves and on the predictions of salt and water contents when using previously developed predictive models, was evaluated in three independent experiments. The results showed that at temperatures below 20 °C there is an increase of the predicted salt content error, being more important in samples with higher water content. HP treatment caused a decrease of the reflected signal intensity due to the major mobility of available ions promoting an increase of the predicted salt content. Freezing/thawing treatment caused an increase of the reflected signal intensity due to the microstructural damages and the loss of water and ions, promoting a decrease of the predicted salt content.

1. Introduction

There are many tools available to determine salt and water contents in food products but they are often destructive, time-consuming or difficult to implement on-line. Technologies based on the use of electromagnetic radiation at different wavelengths have been demonstrated to be useful for the prediction of food composition in industry (Damez & Clerjon, 2008; Scotter, 1997; Valous, Mendoza, & Sun, 2010). These technologies allow the analysis of food without altering its properties and the results are usually obtained within a shorter period of time in comparison to conventional analytical techniques. Each technology varies in its fundamental basis, advantages and drawbacks, and can be more or less suitable for different industrial applications depending on the product features and industrial requirements. While X-rays can penetrate thick products giving a different response depending on the product density (Brienne, Denoyelle, Baussart, & Daudin, 2001; Fulladosa, Muñoz, Serra, Arnau, & Gou, 2015; Fulladosa, Santos-Garcés, Picouet, & Gou, 2010; Hansen et al., 2003; Santos-Garcés, Gou, Garcia-Gil, Arnau, & Fulladosa, 2010), Near Infrared spectroscopy (Collell, Gou, Arnau, & Comaposada, 2011; García-Rey, García-Olmo, De Pedro, Quiles-Zafra, & Luque de Castro, 2005), Raman Spectroscopy or others (Berhe, Engelsen, Hviid, & Lametsch, 2014; Schmidt, Scheier, & Hopkins, 2013) measure the surface of the product and give direct or indirect composition information. Dielectric microwave spectrometry allows the determination of the dielectric properties of a sample. When a microwave frequency radiation interacts with the sample, a change in the rotation of the polar molecules is produced and dielectric parameters, which can be related to composition and quality parameters, can be calculated. Water is the main polar molecule in food and therefore, the number of water molecules, as well as their relationship with their environment, determines the dielectric properties of the food. Microwave spectrometry has been be used to determine the presence of added water in different pork products (Kent, Peymann, Gabriel, & Knight, 2002); to control pork salting process (Castro-Giráldez, Fito, & Fito, 2010) and to identify pork quality (Castro-Giráldez, Botella, Toldrá, & Fito, 2010). Besides, the dielectric Time Domain Reflectometry (TDR) measurement system shows the response of the interaction of an electromagnetic pulse containing a wide range of frequencies in the range of microwaves simultaneously (up to 5×10^9 Hz) with the sample (Miura, Yagihara, & Mashimo, 2003). Using this system, in contrast to conventional microwave dielectric measurements, dielectric parameters cannot be specifically determined but the obtained response is related to the dielectric properties of the sample. In TDR, the response is represented by a curve, which integrates the information of: 1) the shape of the reflected signal obtained for each frequency and 2) the turnaround time for each frequency. According to Miura, Yagihara, & Mashimo (2003), TDR appears to be the most suitable method for the investigation of the dielectric properties of food because of its fast response. TDR has been used to predict the storage time of different species of fish (Kent et al., 2004) and to predict water and salt contents in dry-cured ham (Fulladosa, Duran-Montgé, Serra,

* Corresponding author. Tel.: +34 972 630 052; fax: +34 972 630 980.
E-mail address: elena.fulladosa@irta.cat (E. Fulladosa).

http://dx.doi.org/10.1016/j.meatsci.2014.10.005
0309-1740/

Picouet, Schimmer, & Gou, 2013). Nevertheless, the effect of environmental factors and some treatments commonly used in the meat industry on the predictions have not previously been studied.

The main objective of this research was to study the effect of sample temperature and the application of high pressure (HP) and freezing/thawing treatments on the obtained TDR curves and on the predictions of salt and water contents in dry-cured ham slices when using previously developed predictive models.

2. Materials and methods

2.1. Sampling

The cushion part of forty-three dry-cured hams proceeding from animals of crosses of Large White and Landrace breeds containing *Semimembranosus* (SM), *Semitendinosus* (ST) and *Biceps femoris* (BF) muscles was excised and vacuum packed in plastic bags of polyamide/polyethylene (oxygen permeability of 50 $cm^3/m^2/24$ h at 23ªC and water permeability of 2.6 $g/m^2/24$ h at 23 °C and 85% RH, Sacoliva® S.L., Spain). From this part, one 1.25 cm thick slice was obtained from each ham for each of the different experiments described below.

Two different regions of interest (ROIs) were defined on the slices. The ROIs were on the SM muscle (ROI 1) and on the BF muscle (ROI 2) (Fig. 1). These two muscles are known to have very different compositions in terms of salt and water contents (Arnau, Guerrero, Casademont, & Gou, 1995), so they were very suitable for the approach of this work. On the other hand, ST muscle was discarded due to the high content of intramuscular fat that presented this muscle which could interfere in a proper measurement.

2.2. Equipment

The device RFQ Scan 3.0 (Sequid GmbH, Bremen, Germany) was used to obtain time domain reflectometry (TDR) curves from dry-cured ham samples. This TDR equipment generates a step signal which is then applied to the material under test via an open-ended coaxial line sensor, which must be in close contact with the sample. The TDR applies a step signal with a 100 ps rise time and a repetition frequency of 20 MHz, corresponding to a frequency range from 5×10^7 Hz to approximately 5×10^9 Hz. The device measures the time domain waveform with a time-base resolution of 10 ps (Schimmer, Osen, Schönfeld, & Hemmy, 2009) and a TDR curve is acquired.

2.3. Salt and water content predictions from TDR curves

TDR curves were retrieved using a MATLAB R2007B (The MathWorks, Inc., United States) script made in-house, in order to visually compare the average response before and after treatments. Although the exact relationship between permittivity and TDR curves is rather complex, the intensity of the normalized signal (y-axis) of the TDR curve represents the overall reflected energy of the sample. Thus, when increasing the signal intensity, it can be assumed that there is an increase of the permittivity (ε_r) due to either the increase of the dielectric constant (ε'') or to the decrease of the loss factor (ε'') (see Eq. (1)) (Risman, 1991).

$$\varepsilon_r = \varepsilon' - j \cdot \varepsilon''. \quad (1)$$

Salt and water contents were predicted using the commercial modules based on principal component regression models developed by Sequid GmbH (Bremen, Germany) based on the results obtained by Fulladosa, Duran-Montgé, Serra, Picouet, Schimmer, & Gou (2013). The predictive error of the commercial modules is 0.29% for salt content and 2.50% for water content.

2.4. Experimental protocols

2.4.1. Experiment 1: temperature effect

TDR measurements were taken at three different points on the two ROIs of a slice (Fig. 1) from six dry-cured hams at different temperatures between 5 and 26 °C at intervals of 2 °C. To do so, the slices were kept in sealed plastic bags of polyamide/polyethylene (oxygen permeability of 50 $cm^3/m^2/24$ h at 23ªC and water permeability of 2.6 $g/m^2/24$ h at 23 °C and 85% RH, Sacoliva® S.L., Spain) at different set temperatures using a heating oven (Model EC-360, Radiber S.A., Barcelona, Spain) and the sample temperature was monitored before the measurement. The ROIs were then dissected and individually minced to chemically determine salt and water contents.

2.4.2. Experiment 2: effect of HP treatment

TDR measurements were taken at three different points on the two ROIs of a slice (Fig. 1) from ten dry-cured hams from the same batch at 20 °C. Thereafter, samples were vacuum packaged in the plastic bags previously described in Section 2.4.1 and pressurized at 600 MPa for 5 min with water at 10 °C as pressure-transmitting medium (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain). The pressurization rate was 220 MPa/min and the time for decompression was $\leq$10 s. The samples were then kept at 20 °C for 2 h, unpacked, and TDR measurements were again taken on the same ROIs.

2.4.3. Experiment 3: effect of freezing/thawing treatment

TDR measurements were taken at three different points on the two ROIs of a slice (Fig. 1) from twenty-seven dry-cured hams from different breeds at 20 °C. Thereafter, the samples were vacuum packed in the plastic bags previously described in Section 2.4.1, frozen at −18 °C for three days and then thawed at room temperature for 4 h. The samples were then kept at 20 °C for 2 h, unpacked, and TDR measurements were again taken on the same ROIs.

2.5. Chemical analysis

NaCl content was determined according to ISO 1841–2 (1996) using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland); the analytical standard deviation was 0.05%. The water

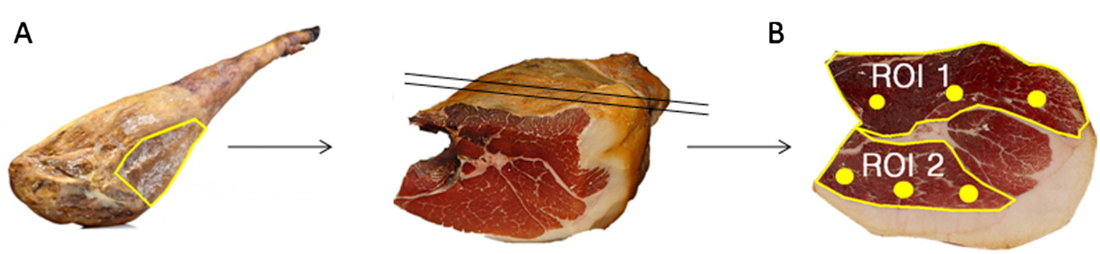


Fig. 1. Dissection of the cushion from the entire ham (A). Slice from the cushion with points of measurement on the different regions of interest (ROIs). ROIs were on the SM muscle (ROI 1) and on the BF muscle (ROI 2) (B).

content was analyzed by drying at 103 °C ± 2 °C until reaching a constant weight (AOAC, 1990); the analytical standard deviation was 0.25%. All analyses were performed in duplicate.

2.6. Statistical analysis

The average of the three TDR measurements on each ROI was used in the data analysis.

Measured salt contents (Experiment 1) were compared to predicted values by using the Root Mean Square Error of Validation (RMSEV). The RMSEV value is given by the Eq. (2):

$$\mathrm{RMSEV} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)}{n}} \quad (2)$$

where, n is the number of samples, $\hat{y}_i$ is the predicted value of the response, and y_i is the value of the response measured by a reference method.

The effect of temperature on the salt content prediction error (Experiment 1) was assessed by simple regression.

3. Results and discussion

3.1. Temperature effect

The alignment of water molecules when electromagnetic radiation in microwave frequencies is applied has been described to be temperature dependent in different food products (Sosa-Morales, Valerio-Junco, López-Malo, & García, 2010). The temperature dependence of dielectric properties is complex, and dielectric constants may increase or decrease with temperature depending on the food product (Venkatesh & Raghavan, 2004). In the case of dry-cured ham (Experiment 1), which is a product that contains a high amount of NaCl, although the temperature produced TDR curve intensity variations, a clear tendency as a function of temperature was not observed (data not shown). Nevertheless, the temperature affected the salt content prediction (Fig. 2A), but not the water content prediction (Fig. 2B). The salt content was underestimated at temperatures below 20 °C, with the exception of two samples (non-solid markers in Fig. 2A), which belonged to the driest ham (water content of BF and SM muscles of 40.20% and 39.83%, respectively). Moreover, at temperatures below 20 °C there was a clear increase of the predicted salt content error when the temperature was decreased. In contrast, no salt content error increase was observed above 20 °C. Therefore, the salt content prediction error was not affected by temperature in the range of temperatures between 20 and 26 °C probably because the predictive models of Fulladosa, Duran-Montgé, Serra, Picouet, Schimmer, & Gou (2013) were developed in this range of temperatures.

Fig. 3 shows the average predicted salt content at temperatures between 20 and 26 °C versus the measured salt contents. The error of prediction was 0.42%. However, by excluding the two points belonging to the driest ham (non-solid markers in Fig. 3), the error of prediction was 0.30%, similar to that found by Rubio, Fulladosa, Garcia-Gil, Gou, and Arnau (2013). As observed by Fulladosa, Duran-Montgé, Serra, Picouet, Schimmer, & Gou (2013), the predictive model slightly underestimated the salt content at higher salt contents, which was also shown by Rubio, Fulladosa, Garcia-Gil, Gou, & Arnau (2013).

The dependence of predicted salt content on temperature (at temperature below 20 °C) was fitted to linear regression models. The slope of the linear regression between salt prediction error and temperature varied from sample to sample. Fig. 4 shows the relationship between these slopes and the measured water content for each sample. In this figure it can be observed that the temperature effect on salt prediction was more important in samples with higher water content.

3.2. Effect of HP and freezing/thawing treatments

In non-treated samples, different reflected intensity signals between muscles were found (Fig. 5) due to the different composition. BF muscle showed higher predicted water and salt contents (5.85 ± 1.18% salt content; 55.00 ± 5.72% water content) than SM muscle (4.96 ± 1.02% salt content; 49.61 ± 5.68% water content). Fulladosa, Duran-Montgé,

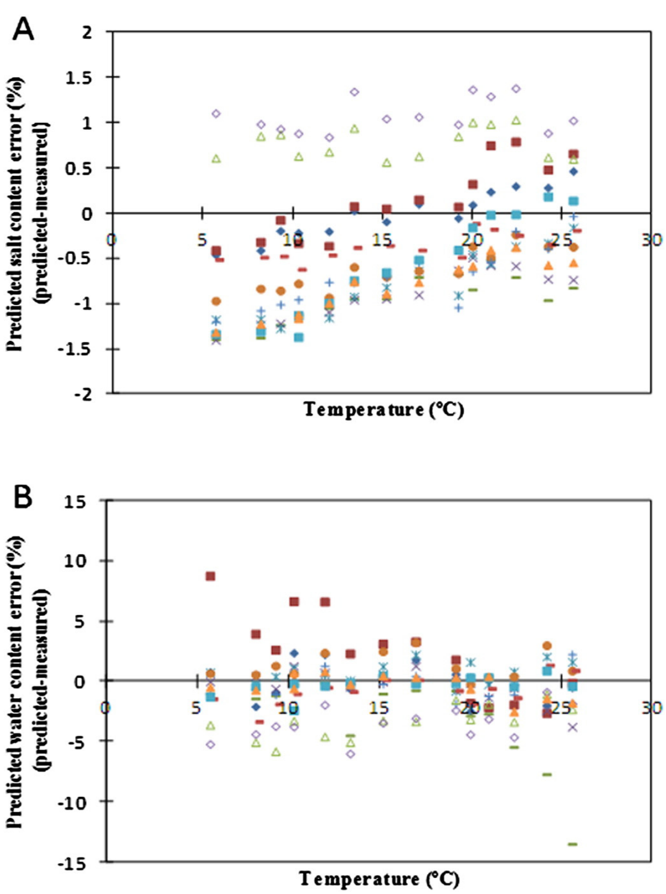


Fig. 2. Salt (A) and water (B) content prediction error (predicted-measured) using commercial modules (Sequid GmbH, Bremen, Germany) as a function of sample temperature. Each series represents a different sample. The non-solid markers belong to the driest ham.

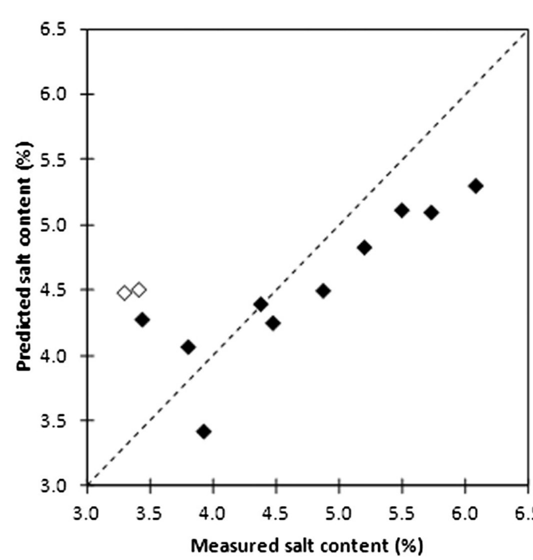


Fig. 3. Average predicted salt content at temperatures between 20 and 26 °C versus the measured salt content. The non-solid markers belong to the driest ham. The dashed-line represents the perfect 1:1 relationship between x and y.

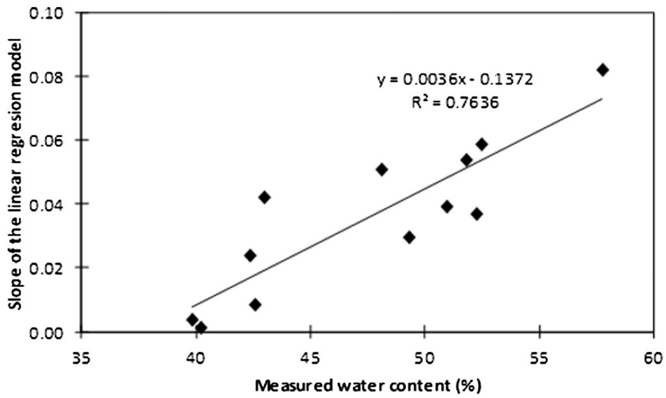


Fig. 4. Slope of the linear regression model between salt prediction error and temperature (at a temperature below 20 °C) versus water content for each sample.

Serra, Picouet, Schimmer, & Gou (2013) found that an increment of either salt content or water content in dry-cured ham samples produced a decrease of the reflected signal intensity of the TDR curve.

Important changes between the acquired TDR curves before and after HP (Experiment 2) (Fig. 5A) and freezing/thawing (Experiment 3) (Fig. 5B) treatments in both SM and BF muscles were found. Changes of the reflected signal intensity after the treatments could have been due to the fact that HP and freezing/thawing treatments produced damages to the microstructure of the meat which in turn could have produced modifications on the mobility of water and ions (Bertram, Wu, Straadt, Aagaard, & Aaslyng, 2006; Cheftel & Culioli, 1997; Leygonie, Britz, & Hoffman, 2012; Ngapo, Babare, Reynolds, & Mawson, 1999). However, the effect for each treatment was different, i.e. a decrease of the reflected signal intensity after the HP treatment and an increase after the freezing/thawing treatment.

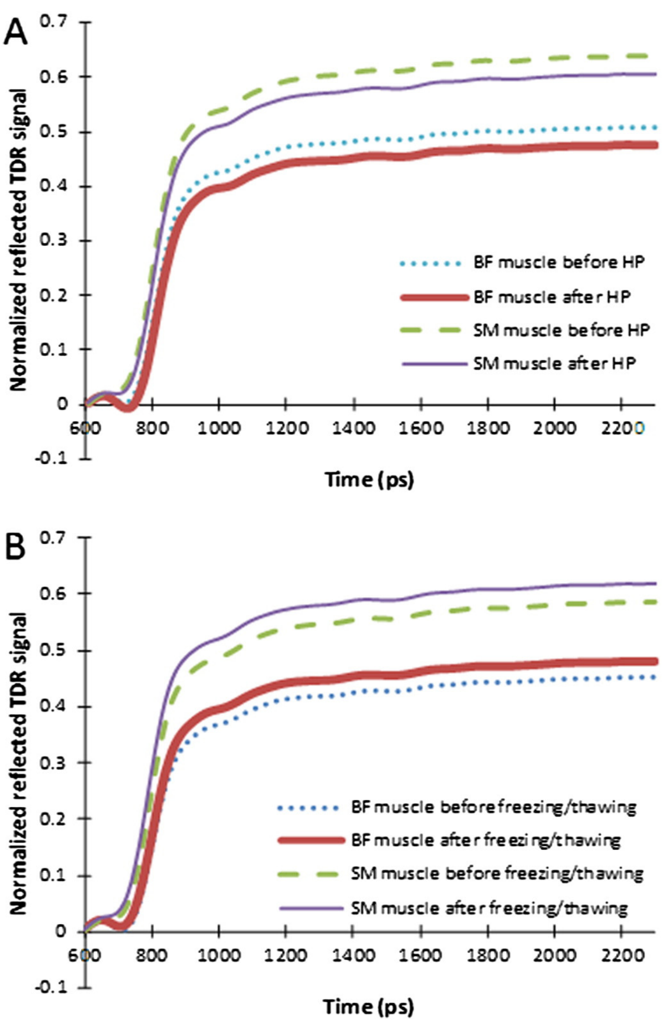


Fig. 5. Average reflected TDR-signals of dry-cured ham samples before and after HP (A) and freezing/thawing (B) treatments.

In the case of HP treatment, Picouet et al. (2012) observed modifications of myofibril ultrastructure and denaturation of muscle proteins in dry-cured ham subjected to 600 MPa, facilitating the liberation of a part of sodium. Fulladosa, Serra, Gou, and Arnau (2009) found a lower water holding capacity after pressurization of dry-cured ham, which facilitates the mobility of ions in water. According to Fulladosa, Duran-Montgé, Serra, Picouet, Schimmer, & Gou (2013) the increased presence of available ions causes a decrease of the reflected signal intensity of TDR curves. Therefore, the aforementioned modifications may have been the reason for the TDR signal decrease. Furthermore, differences in the dielectric properties of pork meat due to spatial disposition e.g. the fiber direction with respect to the measurement have also been reported (Castro-Giráldez, Aristoy, Toldrá, & Fito, 2010). Garcia-Gil, Santos-

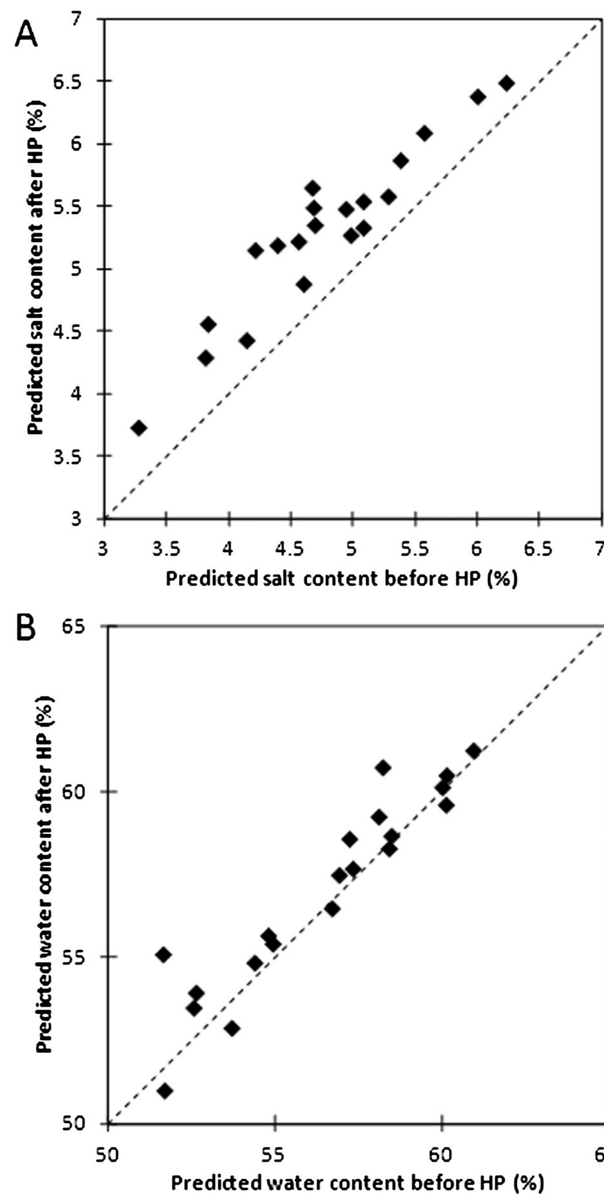


Fig. 6. Predicted salt (A) and water (B) contents before HP treatment versus after HP treatment. The dashed-line represents the perfect 1:1 relationship between x and y.

Garcés, Fulladosa, Laverse, Del Noile, & Gou (2014) observed a compaction of the muscle fibers during HP treatments which could therefore also have contributed to the decrease of the reflected signal intensity (Fig. 5A).

Regarding the salt and water content predictions, Fig. 6A shows a clear increase of the predicted salt content due to HP treatment (from 4.77 ± 0.73% to 5.30 ± 0.68%), while the predicted water content was barely modified (from 56.44 ± 2.97% to 57.08 ± 2.92%) (Fig. 6B).

In the case of the freezing/thawing treatment, the increase of the reflected energy (Fig. 5B) has previously been described in raw fish after one or multiple freezing/thawing processes (Schimmer, Oberheitmann, Baumann, & Knöchel, 2007). The increase of TDR curves in this case was attributed to the loss of water and ions during the breakage of structures by ice crystals. In dry-cured ham, no studies dealing with the effect of freezing/thawing treatments on the microstructure were found in literature. Nevertheless, because dry-cured ham is a much drier product in comparison to raw meat or fish, similar but less important microstructural damages would be expected. During freezing/thawing processes in dry-cured ham a formation of ice crystals as well as salt and phosphate crystals takes place (Arnau, Guerrero, & Gou, 1998), which could contribute to microstructural damages.

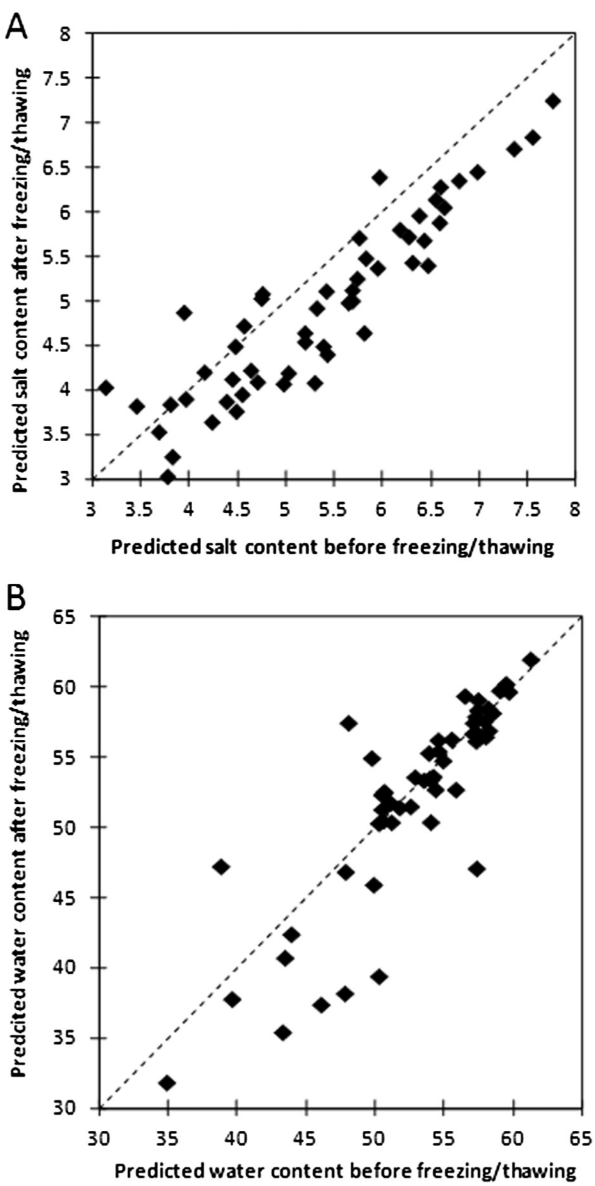


Fig. 7. Predicted salt (A) and water (B) contents before freezing/thawing treatment versus after freezing/thawing treatment. The dashed-line represents the perfect 1:1 relationship between x and y.

With regard to the salt and water content predictions, Fig. 7A shows a clear decrease of the predicted salt content due to freezing/thawing treatment (from 5.40 ± 1.17% to 5.00 ± 1.06%), while the predicted water content was barely modified (from 52.31 ± 6.23% to 52.02 ± 7.04%) (Fig. 7B) as occurred in the case of HP treatment. The prediction of water content was more affected by freezing/thawing treatment in samples with lower water content predictions (Fig. 7B), probably due to fact that the hams with the lowest water content were also the ones with the highest fat content, which could have interfered with the TDR measurements.

Further studies using conventional dielectric spectroscopy, able to determine the dielectric constant and the loss factor, would be necessary to elucidate the effect of HP and freezing/thawing treatment on dry-cured ham.

4. Conclusions

Temperature had a significant effect in salt content predictions. Furthermore, when predicting salt content, the effect of temperature is more pronounced in samples with high water content. HP and freezing/thawing treatment affect the obtained TDR curve in an opposite way. These changes in the TDR curves produce changes in salt but not in water content predictions. For these reasons, when implementing this technology in industry, predictive models would have to be constructed at the operational temperature and specific models to predict salt content would have to be developed for dry-cured hams subjected to HP and freezing/thawing treatments.

Conflict of Interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgments

This work has been supported by project RTA2010-00029-CO4-01 of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) of Spain. Acknowledgments are extended to INIA for financing the doctorate studies of Marc Rubio at Girona University. The authors would also like to acknowledge the contribution of Cristina Canals, Raquel Cama and Jordi Garcia in the sample analyses.

References

AOAC (1990). Official method 950.46, moisture in meat, B. Air drying. In K. Helrich (Ed.), *Official methods of analysis of the association of official analytical chemists, Vol. II*. (pp. 931). Arlington: Association of Official Analytical Chemists Inc.

Arnau, J., Guerrero, L., Casademont, G., & Gou, P. (1995). Physical and chemical changes in different zones of normal and PSE dry cured ham during processing. *Food Chemistry*, *52*, 63–69.

Arnau, J., Guerrero, L., & Gou, P. (1998). The precipitation of phosphates in meat products. *Fleischwirtschaft International*, *3*, 46–47.

Berhe, D.T., Engelsen, S.B., Hviid, M.S., & Lametsch, R. (2014). Raman spectroscopic study of effect of the cooking temperature and time on meat proteins. *Food Research International*, *66*, 123–131.

Bertram, H.C., Wu, Z., Straadt, I.K., Aagaard, M., & Aaslyng, M.D. (2006). Effects of pressurization on structure, water distribution, and sensory attributes of cured ham: Can pressurization reduce the crucial sodium content? *Journal of Agriculture and Food Chemistry*, *54*, 9912–9917.

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.

Castro-Giráldez, M., Aristoy, M.C., Toldrá, F., & Fito, P. (2010a). Microwave dielectric spectroscopy for the determination of pork meat quality. *Food Research International*, *43*(10), 2369–2377.

Castro-Giráldez, M., Fito, P.J., & Fito, P. (2010b). Application of microwave dielectric spectroscopy for controlling pork meat (*Longissimus dorsi*) salting process. *Journal of Food Engineering*, *97*(4), 484–490.

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

Collell, C., Gou, P., Arnau, J., & Comaposada, J. (2011). Non-destructive prediction of moisture, water activity and NaCl at ham surface during resting and drying using NIR spectroscopy. *Food Chemistry*, *129*(2), 601–607.

Damez, J.L., & Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. *Meat Science*, *80*(1), 132–149.

Fulladosa, E., Duran-Montgé, P., Serra, X., Picouet, P., Schimmer, O., & Gou, P. (2013). Prediction 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. (2015). X-ray absorptiometry for non-destructive monitoring of the salt uptake in bone-in raw 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 of Food Engineering*, *96*(1), 80–85.

Fulladosa, E., Serra, X., Gou, P., & Arnau, J. (2009). Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, *82*(2), 213–218.

Garcia-Gil, N., Santos-Garcés, E., Fulladosa, E., Laverse, J., Del Nobile, M.A., & Gou, P. (2014). High pressure induces changes in texture and microstructure of muscles in dry-cured hams. *Innovative Food Science and Emerging Technologies*, *22*, 63–69.

García-Rey, R.M., García-Olmo, J., De Pedro, E., Quiles-Zafra, R., & Luque de Castro, M.D. (2005). Prediction of texture and colour of dry-cured ham by visible and near infrared spectroscopy using a fiber optic probe. *Meat Science*, *70*(2), 357–363.

Hansen, P.W., Tholl, I., Christensen, C., Jehg, H. -C., Borg, J., Nielsen, O., Ostergaard, B., et al. (2003). Batch accuracy of on-line fat determination. *Meat Science*, *64*(2), 141–147.

ISO 1841–2 (1996). *Meat and meat products. Determination of chloride content — Part 2: Potentiometric method (reference method)*. Geneva: International Organization for Standardization.

Kent, M., Knöchel, R., Daschner, F., Schimmer, O., Oehlenschläger, J., Floberg, U.B.P., Tejada, M., et al. (2004). Time domain reflectometry as a tool for the prediction of quality in foods. *International Agrophysics*, *18*, 225–229.

Kent, M., Peymann, A., Gabriel, C., & Knight, A. (2002). Determination of added water in pork products using microwave dielectric spectroscopy. *Food Control*, *13*(3), 143–149.

Leygonie, C., Britz, T.J., & Hoffman, L.C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, *91*(2), 93–98. Matlab R2007B (The MathWorks, Inc., United States).

Miura, N., Yagihara, S., & Mashimo, S. (2003). Microwave dielectric properties of solid and liquid foods investigated by time-domain reflectometry. *Journal of Food Science*, *68*(4), 1396–1403.

Ngapo, T.M., Babare, I.H., Reynolds, J., & Mawson, R.F. (1999). Freezing rate and frozen storage effects on the ultrastructure of samples of pork. *Meat Science*, *53*, 159–168.

Picouet, P.A., Sala, X., Garcia-Gil, N., Nolis, P., Colleo, M., Parella, T., & Arnau, J. (2012). High pressure processing of dry-cured ham: Ultrastructural and molecular changes affecting sodium and water dynamics. *Innovative Food Science & Emerging Technologies*, *16*, 335–340.

Risman, P. (1991). Terminology and notation of microwave-power and electromagnetic energy. *The Journal of Microwave Power and Electromagnetic Energy*, *26*, 243–248.

Rubio, M., Fulladosa, E., Garcia-Gil, N., Gou, P., & Arnau, J. (2013). Estimación del contenido de sal en lonchas, porciones y piezas enteras deshuesadas de jamón curado. *Proceedings, 7th world congress of dry-cured ham*978-989-98363-0-3.

Santos-Garcés, E., Gou, P., Garcia-Gil, N., Arnau, J., & Fulladosa, E. (2010). Non-destructive analysis of a_w, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering*, *101*(2), 187–192.

Schimmer, O., Oberheitmann, B., Baumann, F., & Knöchel, R. (2007). Instantaneous distinction between double and single frozen fish using a new handheld time domain reflectometer. *Proceedings of the 7th International Conference on Electromagnetic Wave Interaction with Water and Moist Substances, Hamamatsu, Japan, 2007*.

Schimmer, O., Osen, R., Schönfeld, K., & Hemmy, B. (2009). Detection of added water in seafood using a dielectric time domain reflectometer. *Proceedings, 8th International Conference on Electromagnetic Wave Interaction With Water and Moist Substances, ISEMA 2009, Espoo, Finland* (pp. 350–357).

Schmidt, H., Scheier, R., & Hopkins, D.L. (2013). Preliminary investigation on the relationship of Raman spectra of sheep meat with shear force and cooking loss. *Meat Science*, *93*(1), 138–143.

Scotter, N.G. (1997). Non-destructive spectroscopic techniques for the measurement of food quality. *Trends in Food Science & Technology*, *81*, 285–292.

Sosa-Morales, M.E., Valerio-Junco, L., López-Malo, A., & García, H.S. (2010). Dielectric properties of foods: Reported data in the 21st Century and their potential applications. *LWT - Food Science and Technology*, *43*(8), 1169–1179.

Valous, N.A., Mendoza, F., & Sun, D. -W. (2010). Emerging non-contact imaging, spectroscopic and colorimetric technologies for quality evaluation and control of hams: A review. *Trends in Food Science & Technology*, *21*(1), 26–43.

Venkatesh, M.S., & Raghavan, G.S.V. (2004). An overview of microwave processing and dielectric properties of agri-food materials. *Biosystems Engineering*, *88*, 1–18.

Paper III

Rubio-Celorio, M., Fulladosa, E., Garcia-Gil, N., & Bertram, H.C. (2015). Multiple spectroscopic approach to elucidate water distribution and water-protein interactions in dry-cured ham after high pressure processing. *Food of Journal Engineering,* Submitted (JFOODENG-D-15-00439).

Multiple spectroscopic approach to elucidate water distribution and water-protein interactions in dry-cured ham after high pressure processing

Marc Rubio-Celorio[1], Elena Fulladosa[1*], Núria Garcia-Gil[1], Hanne Christine Bertram[2]*

[1]*IRTA. XaRTA. Food Technology. Finca Camps i Armet, E-17121 Monells (Girona), Spain*

[2]Aarhus University, Dept. Food Science, Research Centre Aarslev, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

*Authors for correspondence: elena.fulladosa@irta.cat, hannec.bertram@agrsci.dk

Abstract

High Pressure Processing (HPP) produces modifications of water-protein interactions and sensory changes. The effect of three high pressure levels (200, 400 and 600 MPa) on the water distribution of dry-cured ham with different raw meat pH_{24SM} was studied using nuclear magnetic resonance relaxometry (NMR), time domain reflectometry (TDR) and multispectral imaging (MI). Firstly, the effect of different pressure levels was studied. Secondly, the effect of both high pressure and pH_{24SM} was evaluated. The results obtained with the different technologies showed a reallocation of the water populations and a new arrangement of the proteins. The effect of HPP increased when pressure level was increased, showing an inflection point between 200 and 400 MPa. Besides, it was found that pH_{24SM} plays an essential role in the curing process resulting in dry-cured ham with different properties at the end of the process.

Key words: meat, water loss, low-field NMR relaxation, time domain reflectometry, multispectral imaging

1. Introduction

High Pressure Processing (HPP) is currently being used in industry to eliminate pathogenic microorganisms (especially *Listeria moncytogenes*), to extend product shelf-life and to improve the safety of commercial processed meat products (Aymerich, Picouet, & Monfort, 2008; Bajovic, Bolumar, & Heinz, 2012). Pressure levels applied for the pasteurization of meats and meat products range from 400 to 600 MPa with short processing times of 3–7 min at room temperature (Cheftel & Culioli, 1997). Nevertheless, HPP can influence meat protein conformation and induce protein denaturation, aggregation or gelation, which can affect the appearance and the quality of the products. The means whereby HHP treatment exerts effects on meat protein structure are through the rupture of non-covalent interactions within protein molecules, and a subsequent re-formation of intra- and inter- molecular bonds within or among protein molecules (Cheftel & Culioli, 1997; Sun & Holley, 2010). Pressurization also causes a loosening of the meat protein matrix, alterations in the water distribution in meat (Bertram, Wu, Straadt, Aagaard, & Aaslyng, 2006) and can also modify non-covalent interactions between muscle protein and sodium ions or water molecules in cured products (Picouet et al., 2012).

Different spectroscopic technologies may provide information regarding the effects produced on dry-cured ham by HPP. Proton nuclear magnetic resonance (NMR) relaxometry provides direct information about the compartmentalization and mobility of water in meat. NMR relaxometry has been widely applied in meat science. For a review see Bertram & Andersen (2004). NMR relaxometry has been used in meat to determine fat and water contents (Sørland, Larsen, Lundby, Rudi, & Guiheneuf, 2004), to study the mechanisms determining the development in sensory attributes during pork cooking (Bertram, Aaslyng, & Andersen, 2005), to evaluate water migration and water-binding within the meat pork matrix upon salting (Bertram, Meyer, Wu, Zhou, & Andersen, 2008; McDonnell et al., 2013), to evaluate water properties during cooking of pork (Bertram, Engelsen, Busk, Karlsson, & Andersen, 2004), to predict water holding capacity in pork (Bertram, Dønstrup, Karlsson, & Andersen, 2002) and to study changes in distribution and mobility of water as a result of high pressure processing of sausages (Møller et al., 2011). Other technologies such as microwave spectrometry and time domain reflectometry (TDR), by means of the determination of the dielectric properties of biological tissues, can also provide information about the linking state of water and salt contents. Microwave dielectric spectra has been used to determine the presence of added water in different pork products (Kent, Peymann, Gabriel, & Knight, 2002), to control pork salting process (Castro-Giráldez, Fito, & Fito, 2010), to identify pork quality (Castro-Giráldez, Aristoy, Toldrá, & Fito, 2010) and to study the microstructural changes in dry-cured ham subjected to HPP (Rubio-Celorio, Castro-Giraldez, Garcia-Gil, Fulladosa, & Fito, 2015). Dielectic TDR has also been used to develop models for fast estimation of water and salt contents in dry-cured ham (Fulladosa, Duran-Montgé, Serra, Picouet, Schimmer, & Gou, 2013) and factors affecting the TDR response have been studied (Rubio-Celorio, Garcia-Gil, Gou, Arnau, & Fulladosa, 2014). Structural changes caused by HPP may also be elucidated using multispectral imaging since light reflection at different wavelenghts depends not only on the composition but also on the structure of the protein matrix. This technology has been proved to be useful for studying changes in meat colour during storage (Christiansen, Carstensen, Møller, Nielsen, 2012) and for evaluating changes in the colour of minced cured restructured ham subjected to HPP (Bak, Lindahl, Karlsson, & Orlien, 2012).

Since dry-cured ham is a high value product that needs to be pressurized before commercialization in some countries, several studies elucidating the impact of pressurization on different parameters have been conducted during the last decade. The effect of HPP on colour (Andrés, Møller, Adamsen, & Skibsted, 2004; Cava, Ladero, González, Carrasco, & Ramírez, 2009; Fuentes, Utrera, Estévez, Ventanas, & Ventanas, 2014), on volatile compounds (Rivas-Cañedo, Fernández-García, & Nuñez, 2009), on protein and lipid oxidation (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010), on saltiness perception (Fulladosa, Serra, Gou, & Arnau, 2009; Serra et al., 2007) and on texture and microstructure (Garcia-Gil et al., 2014) have been studied. Nevertheless, no information is available regarding the interaction between raw meat pH and the effect of different high pressure levels and no information related to the effects of HPP on dry-cured ham originating from different raw meat pH has been found. pH measured in the *Semimembranosus* muscle at 24 hours post-mortem (pH_{24SM}) is considered to have a great impact on the quality and technological parameters of both fresh meat and processed meat products as pH influences the three-dimensional structure of proteins (Anfinsen, 1973) and thus the allocation and mobility of water (Bertram, Whittaker, Andersen, & Karlsson, 2003). This results in changes in the structural conditions of muscles and influences the texture of dry-cured ham at the end of the process (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006).

The main objective of this work was to elucidate the impact of HPP processing (200, 400 and 600 MPa) and of raw material (low and high pH_{24SM}) on the distribution and physico-chemical state of the intrinsic water populations and water-protein interactions in dry-cured ham. For this purpose a multiple spectroscopic approach including NMR relaxometry, dielectic TDR, and multispectral imaging, combined with physico-chemical analyses was applied.

2. Materials and methods

2.1 Sampling and HPP treatments

The experimental work was divided into two experiments. In experiment 1, in order to study the effect of different pressure levels, 10 dry-cured hams originating from raw hams with a pH_{24SM} between 5.63 and 5.76 were selected from a commercial dry-cured ham producer. In experiment 2, in order to study the combined effect of both high pressure and pH_{24SM}, 20 additional dry-cured hams were selected at the end of the process and grouped into two classes according to their pH_{24SM}: 10 hams originating from hams with a low pH_{24SM} (5.42-5.54) and 10 hams originating from hams with high pH_{24SM} (6.09-6.40). In both experiments the hams came from animals which were crosses of Large White and Landrace breeds and dry-cured hams were elaborated using a traditional procedure (Arnau, Guerrero, Gou, & Monfort, 2001) until reaching a similar weight loss (~30%).

In all cases, *Biceps femoris* (BF) muscle was excised (Figure 1) and vacuum packaged in plastic bags of multilayer polyamide/polyethylene (oxygen permeability of 50 cc/m^2/24h at 23ºC and water permeability of 2.6 g/m^2/24h at 23 °C and 85% RH, Sacoliva® S.L., Spain) for one month in order to reach a homogenous composition of salt and water contents throughout the muscle. Sampling was performed as specified in Figure 1. In brief, four 40 mm thick slices *a* were obtained from the central part of the dissected muscle for NMR, TDR and MI analyses. Between them, 3 mm thick slices *b* were taken for water loss analyses. The two edges *c* of the muscle were minced together and used for physicochemical analyses. All the samples were individually vacuum packed.

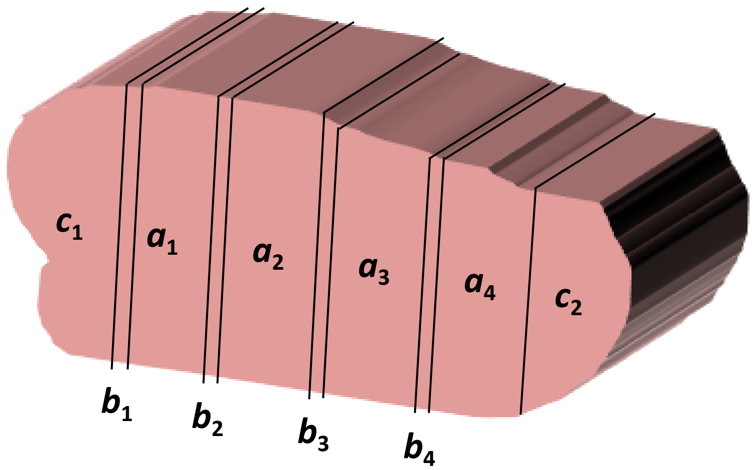


Figure 1. Sampling procedure of *Biceps Femoris* in dry-cured hams.

For each ham, one slice *a* and one slice *b* were kept as a control (non-pressurized) and the other slices were pressurized at 200, 400 or 600 MPa for 5 min with water at 10 °C as pressure-transmitting medium (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain). Pressurization rate was 220 MPa/min and the time for decompression was ≤ 10 s. In order to minimize the muscular position effect, the different pressure treatments were alternated in the different positions. All the measurements described below were performed 7 days after pressurization.

2.2 Physicochemical analyses

NaCl content was determined according to ISO 1841-2 (1996) using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland); the analytical standard deviation was 0.05%. Water content was analyzed by drying at 103 ºC ± 2 ºC until reaching constant weight (AOAC, 1990); the analytical standard deviation was 0.25%. The final pH of the dry-cured ham was directly measured on the *Biceps femoris* muscle with a pHmeter (Crison GLP 21, Crison

Instruments, S.A., Alella, Spain). Water loss determinations were carried out following the procedure used by Picouet et al., (2012). In brief, squares of 10×40 mm^2 from 3 mm thick slices were weighed and then centrifuged at 17,400 g for 1 h at 4 °C (Beckman J2-MC, Beckman Instruments Inc., Palo Alto, California). Supernatants were decanted and the ham pieces were weighed again. Supernatant volume was expressed as the sample weight loss percentage. All analyses were performed in duplicate.

2.3 NMR relaxation measurements

Proton NMR T2 relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) operating at 23.2 MHz and equipped with an 18 mm variable temperature probe, applying a CPMG sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). Samples (approx. 40 mm long and 10 mm in diameter) were analyzed in triplicate at 25 °C. The obtained T2 relaxation data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler, Reeds, and Dawson (1981) and carried out in MatLab (The Mathworks Inc., Natick, MA, USA) using in-house scripts. Distributed exponential fitting results were presented in a plot of relaxation amplitude versus relaxation time, over a predefined range of characteristic relaxation times. In this study a fitting of 256 logarithmically distributed relaxation times from 0.5 ms to 3000 ms was established. Areas and mean relaxation times of the T2 populations observed were calculated.

2.4 Time domain reflectometry (TDR) measurements

The device RFQ Scan 3.0 (Sequid GmbH, Bremen, Germany) was used to obtain TDR curves from the dry-cured ham samples. This TDR equipment generates a step signal which is then applied to the material under test via an open-ended coaxial line sensor, which must be in close contact with the sample. The TDR applies a step signal with a 100 ps rise time and a repetition frequency of 20 MHz, corresponding to a frequency range from $5x10^7$ Hz to approximately $5x10^9$ Hz. The device measures the time domain waveform with a time-base resolution of 10 ps (Schimmer, Osen, Schönfeld, & Hemmy, 2009) and a TDR curve is acquired.

2.5 Multispectral imaging (MI) measurements

Images were acquired using a VideometerLab vision system (Videometer A/S, Denmark) which acquires multi-spectral images at 18 different wavelengths ranging from UV (405 nm) to short wave NIR (970 nm). The acquisition system records surface reflections with a standard monochrome charge coupled device chip. The software VideometerLab 3 (version 2.12) was used to obtain the spectra and the L*a*b* values.

2.6 Statistical analysis

An analysis of variance (ANOVA) was performed with XLSTAT-Pro (Win) 7.5.3 (Addinsoft SARL, Paris, France) for each batch. For experiment 1, the model included pressure treatment as a fixed effect and ham as a random effect. For experiment 2, the model included pressure treatment, pH group and their interaction as fixed effects and ham as a random effect. Differences between means were tested with Tukey test.

3. Results and discussion

3.1 Experiment 1: Effect of high pressure levels

Physicochemical analyses of salt (4.27 ± 0.33%) and water contents (52.72 ± 1.16%) showed that the samples used in the study were similar and comparable, forming a homogeneous group of study which have both parameters in the range of the dry-cured hams commonly found on the market.

Table 1. Mean ± standard deviation of water losses (%) of samples subjected to different high pressure levels.

Control	200 MPa	400 MPa	600 MPa
4.74 ± 0.68^{c}	5.45 ± 0.94^{b,c}	6.33 ± 1.07^{a,b}	7.30 ± 0.59^{a}

^{a,b,c} Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

Different high pressure levels produced important changes on water loss, showing a steady linear increase when the pressure level was increased (Table 1). This can be related to crucial changes in the meat protein structure, such as modification of myofibril ultrastructure and protein denaturation, which becomes more severe with an increase in pressure level (Garcia-Gil et al. 2014; Picouet et al. 2012). It has previously been described that pressure levels above 200 MPa affect the water binding properties of sausages (Colmenero, Carballo, Fernández, Barreto, & Solas, 1997). Furthermore, Fulladosa, Serra, Gou, & Arnau (2009) also found higher water losses after pressurization of dry-cured ham at 600 MPa. Picouet et al. (2012) found significant differences in the water losses between dry-cured hams pressurized at 300 and 600 MPa, but not between non-pressurized hams and 300 MPa. In the present work, significant differences were only found between pressure levels with a difference of more than 200 MPa. Picouet et al. (2012) also reported an increase of 0.82% in the water loss for each 100 MPa in the range from 0 to 900 MPa, which is higher than the increase found in the present work (+0.43%). This difference could be due to the different chemical composition of the hams and to the range of high pressure levels to which the samples were subjected.

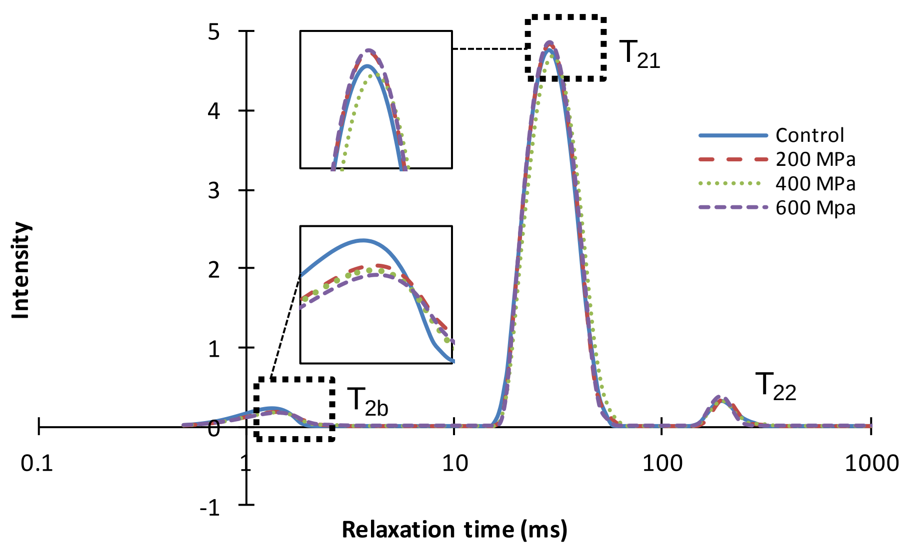


Figure 2. Distributed proton T2 relaxation times of dry-cured ham samples subjected to different high pressure treatments using NMR.

Figure 2 shows the distributed T2 relaxation times of the dry-cured hams samples subjected to different pressures. Data obtained on the dry-cured ham revealed the presence of three populations of water: water closely associated with macromolecules (T_{2B}), myofibrillar water (T_{21}) and extra-myofibrillar water (T_{22}). The obtained populations were very similar to those previously reported by Bertram, Karlsson, Rasmussen, Dontrup, & Petersen (2001) and Bertram, Donstrup, Karlsson, & Anderssen (2002) in fresh meat. Besides, the three populations displayed relaxation times which were also very similar: 0-4 ms (T_{2B}), 15-65 ms (T_{21}) and 140-350 ms (T_{22}). Differences in T_{2B} and T_{21} water populations were observed between the dry-cured ham samples subjected to different pressure levels (Figure 2). Statistical analysis also showed significant differences in the T_{2B} and T_{21} areas between the HPP treated samples and the control samples (non-pressurized samples) (Table 2).

Table 2. Mean ± standard deviation of the area under the curve of the water populations obtained with NMR of samples subjected to different pressure levels.

	Control	**200 MPa**	**400 MPa**	**600 MPa**
T_{2B} area	4.59 ± 0.35^{a}	4.02 ± 0.94^{b}	3.79 ± 0.50^{b}	3.65 ± 0.55^{b}
T_{21} area	92.06 ± 0.29^{b}	92.74 ± 0.50^{a}	93.02 ± 0.68^{a}	93.15 ± 0.63^{a}
T_{22} area	3.32 ± 0.52	3.15 ± 0.41	3.16 ± 0.52	3.17 ± 0.40

^{a,b,c} Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

Differences in the T_{2B} water population between the different pressure levels are likely to have been a direct result of a modification in the water-protein interactions caused by protein conformational modifications due to high pressure and the subsequent re-formation of intra- and inter- molecular bonds within or among protein molecules (Cheftel & Culioli, 1997; Sun & Holley, 2010). Changes in intra- and inter- molecular bonds within or among protein molecules induce changes in the accessibility of protons that can exchange with water protons. This phenomenon can affect the relaxation rate of the water closely associated with the macromolecules. Thus, the interactions between the protons in the proteins and the water protons in the approximate hydration shell surrounding the proteins are expressed in the characteristics of the T_{2B} population.

Changes in the T_{21} water population due to pressure probably reflect an alteration in the functionality of the myofibrillar proteins (Pearce, Rosenvold, Andersen, & Hopkins, 2011), resulting in a less binding strength that produces an increase in the amount of water entrapped within the myofibrillar matrix, which is in agreement with the increase in water loss with increasing pressures. In fresh meat, T_{22} area is positively correlated with potential drip loss (Bertram, Karlsson, Rasmussen, Dontrup, & Petersen, 2001; Bertram, Donstrup, Karlsson, & Anderssen, 2002), but the present study reveals that a considerably lower water content in dry-cured ham imposes a different relation between the T_{22} area and water loss in this type of meat product.

It must be remarked that the T_{2B} area tended to decrease while the T_{21} area tended to increase with increased pressure, being inversely proportional. This could be explained by the fact that part of the bound water tightly associated with macromolecules was reallocated into the myofibrilar protein network retaining the majority of the water. These results are in agreement with Rubio-Celorio, Garcia-Gil, Castro-Giraldez, Fulladosa, & Fito (2015) who reported that HHP produces elastic and shear forces that cause transformations in the microstructure, particularly a reduction of interstitial space between both bundles of muscle fibres and muscles fibres and an interruption of the internal ultrastructure of myofibrils. This compacting effect can be related to the capability to retain water in the pressurized muscle proteins found in this study. Furthermore, the higher the pressure applied the higher the water losses due to the change of bound water into bulk water.

In the case of TDR, although its response is known to be dependent on the water binding (Miura, Yagihara, & Mashimo, 2003), no differences between the control and the HPP-treated samples were detected in the present study (data not shown). Despite the fact that HPP was found to cause a decrease of the reflected signal intensity in dry-cured ham in another study (Rubio-Celorio, Garcia-Gil, Gou, Arnau, & Fulladosa, 2014), in the present study no tendency could be established. This could be due to the different origin, composition and processing conditions of the hams between the present study and the study by Rubio-Celorio, Garcia-Gil, Gou, Arnau, & Fulladosa (2014). Since many factors influence TDR measurements, this technology cannot be considered sensitive to small variations. However, conventional microwave dielectric measurements might provide specifically dielectric parameters, which could be related to the microstructural changes caused by HHP. Further experimental work is needed in this area to better understand the dielectric and microstructural changes on dry-cured ham subjected to different high pressure levels.

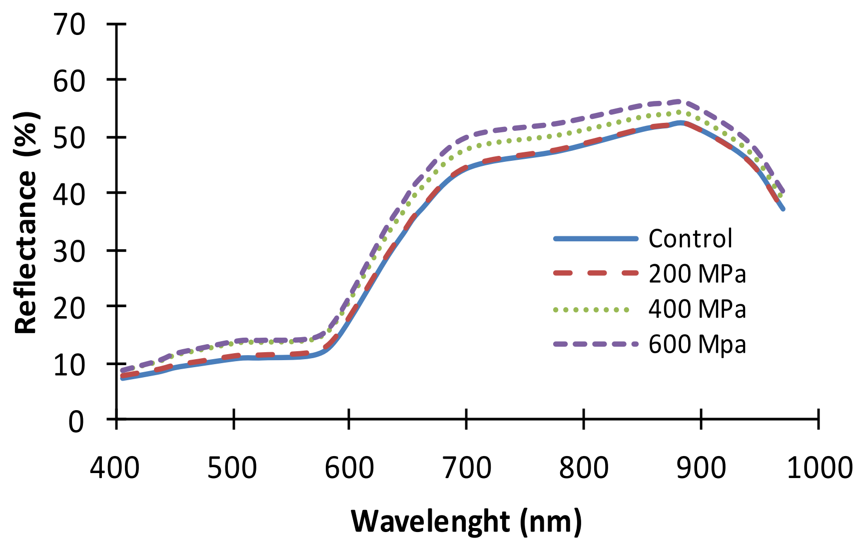


Figure 3.Average reflectance curve of the samples subjected to different high pressure treatments using MI.

Figure 3 shows the spectra between 405 and 970 nm obtained with multispectral imaging of samples subjected to different high pressure levels. It was clear that the reflectance increased at all the analysed wavelengths with increasing pressure level, with a distinct separation between 200 and 400 MPa. Statistical analysis showed that the area under the reflectance spectra increased when the pressure level was increased showing a point of inflection at pressures between 200 and 400 MPa (Table 3), which is in accordance with previous studies (Bak, Lindahl, Karlsson, & Orlien, 2012). This increase of reflectance of light at different wavelengths was attributed to changes in the surface protein conformation which it turn increases the light scattering (Folkestad, et al. 2008). Bajovic, Bolumar, & Heinz (2012) reported that meat muscular proteins, including myofibrillar proteins, were unfolded up to a pressure of 300 MPa. Sun & Holley, 2010, also described an increase of denaturation, gel formation and agglomeration of proteins above this pressure level in meat. In the case of dry-cured ham, Picouet et al. (2012) identified a point of inflection of the protein denaturation at 340 MPa in dry-cured ham determined by a sigmoid equation. The present study is in agreement with this prediction, showing changes between 200 and 400 MPa.

Table 3. Mean ± standard deviation of the reflectance area under the curve between 405 and 970 nm and L*a*b* values of samples subjected to different pressure levels.

	Control	**200 MPa**	**400 MPa**	**600 MPa**
Reflectance area	512.36±19.19[b]	514.42±12.19[b]	555.99± 21.79[a]	577.84± 28.82[a]
L*	41.71± 1.85[b]	42.95± 0.94[b]	46.71± 1.65[a]	47.80± 2.38[a]
a*	20.25± 1.04[a]	19.44± 1.27[a,b]	18.43± 1.07[b]	18.03± 1.59[b]
b*	14.20± 0.82	14.39 ±1.28	14.32± 1.03	14.51± 0.99

[a,b,c] Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

Reflectance and L* value are two parameters which are strongly correlated. In the present study, L* value followed an identical behaviour to the reflectance, with a marked increase with increasing pressure and an inflection point between 200 and 400 MPa (Table 4). Mor-Mur & Yuste (2003) related the increase in L* value of pressurized cooked sausages to a new arrangement of surface proteins promoted by HHP due to the coagulation or denaturation of proteins, which would increase the reflected/absorbed light ratio, also resulting in the observed increase in reflectance (Table 3). Furthermore, a critical pressure for L* value has been previously reported in beef (Marcos, Kerry, & Mullen, 2010) and in pork (Tintchev et al., 2010). This brightening effect has previously been explained by molecular changes mainly introduced by denaturation of myoglobin and/or modification or disruption of the porphyrine ring in the myoglobin molecule. Hughes, Oiseth, Purslow, & Warner (2014) reported that the consequential modifications in structure, which could generate a lighter beef meat colour as a result of HPP, evolve from changes in myofibrillar packing, alterations in the refraction of the sarcoplasm and muscle fiber diameter reduction.

It was also found that the more pressure applied, the lower the a* value, while the b* value remained unaltered (Table 3). These findings also agree with previous studies which reported an increase in lightness (L*) and a decrease in redness (a*) on cured meat products (Ferrini, Comaposada, Arnau, & Gou, 2012) and on dry-cured hams (Andrés, Møller, Adamsen, & Skibsted, 2004; Fulladosa, Serra, Gou, & Arnau, 2009) as a result of pressurization.

3.2 Experiment 2: effect of pH_{24SM} and high pressure levels

Salt and water contents in hams from low and high pH groups differed from the batch of hams used in Experiment 1 (Table 4), thus no comparison with the previous experiment can be done due to the effect that chemical composition can produce on the analyzed parameters. Slight differences in salt and water contents between hams of different pH are unavoidable because of the salt content uptake and drying process is different in raw hams with different pH_{24SM} (Arnau, Guerrero, Casademont & Gou, 1995). Nevertheless, differences were not significant for either salt or water contents between the low and high pH groups. The final pH measurements of the dry-cured hams at the end of the process demonstrated that the difference of pH between the two groups was reduced in comparison to their pH_{24SM}. However, both pH_{24SM} and ultimate pH were significantly different between the low and high pH groups. The low pH group increased in pH throughout the curing process, as was expected according to the literature found (Arnau, Guerrero, Casademont & Gou, 1995), and the high pH group maintained a similar pH as in the case of previous studies (Gou, Morales, Serra, Guàrdia, & Arnau, 2008) (Table 4). Moreover, high pH hams were more visually affected than low pH hams after HPP. pH_{24SM} can affect the dynamics of the curing process resulting in dry-cured ham with different properties at the end of process. In dry-cured ham it has been reported that high pH_{24SM} increases softness (Arnau, Guerrero & Sàrraga, 1997) which would agree with the present study (Table 4).

Table 4. Visual and physicochemical characterization of low and high pH ham groups.

	Salt content (%)	Water content (%)	pH_{24SM}	Ultimate pH	Appearance after HPP
Low pH	3.88 ± 0.34	57.24 ± 1.04	5.50 ± 0.03^{y}	5.89 ± 0.06^{y}	Normal
High pH	4.25 ± 0.36	58.60 ± 1.8	6.22 ± 0.10^{x}	6.17 ± 0.08^{x}	Pale color, cracked surface and pastiness texture

^{x,y} Different letters indicate significant differences ($P \leq 0.05$) between low and high pH groups within each analysed parameter.

Table 5 shows the water losses of the samples with different pH_{24SM} subjected to different pressure treatments. In both pH groups, samples treated with 600 MPa were significantly different from the rest of the pressure treatments. Nevertheless, in contrast to experiment 1, no clear linear tendency was found for the water losses as a function of pressure. Figure 4 shows slight visual differences in the T_{2B} and T_{21} water populations as a function of pressure but the statistical analysis of the areas under the curve found these variations not significantly different in both pH groups ($P > 0.05$) (Table 6). The features that condition the water loss and the water population distribution of a product are numerous and diverse and may be especially complex and heterogeneous for dry-cured ham. Moreover, no effect of pH on the water losses of control and HP treated samples was observed ($P > 0.05$) (Table 5). To the contrary, significant differences in the areas under the curve between low and high pH groups for the two water populations (T_{2B} and T_{21}) were found ($P \leq 0.05$) (Table 6). Thus, a pH effect was not evident on water loss determined gravimetrically but on water distribution determined by NMR relaxation. This finding implies that NMR relaxation is more sensitive to a pH-induced effect on water binding properties than gravimetrical methods, which is consistent with previous findings on effects of creatine supplementation (Young et al., 2007) and freezer storage (Bertram, Andersen, & Andersen, 2007) on water binding properties of pork. Further investigations should be conducted in order to clarify this point.

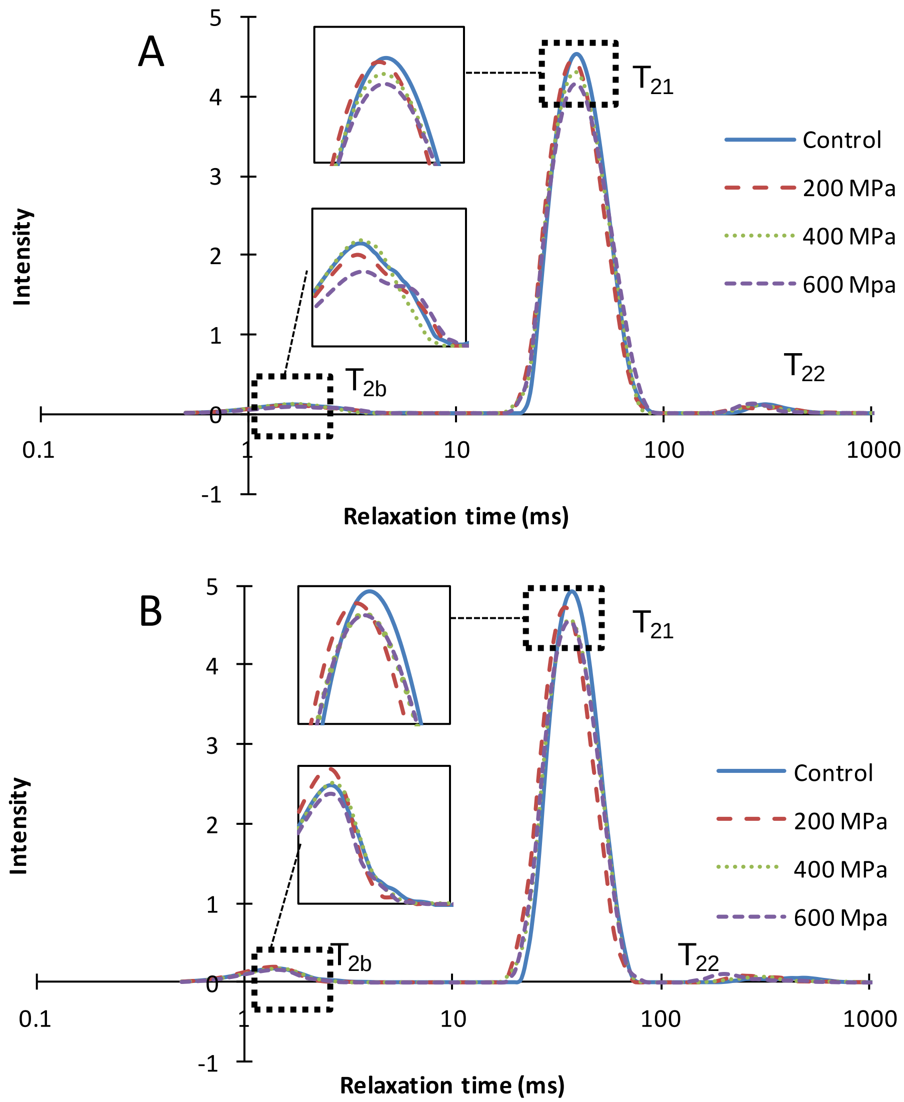


Figure 4. Distributed proton T2 relaxation times of dry-cured ham samples subjected to different high pressure treatments for low pH group (**A**) and high pH group (**B**) using NMR.

Table 5. Mean ± standard deviation for water losses (%) of samples from the low and high pH groups subjected to different pressure levels

	Control	200 MPa	400 MPa	600 MPa
Low pH	3.12 ± 0.65[c]	4.45 ± 1.1[b]	3.62 ± 0.85[b,c]	7.42 ± 0.92[a]
High pH	3.53 ± 1.00[b]	4.01 ± 0.80[b]	3.49 ± 0.62[b]	8.86 ± 2.37[a]

[a,b,c] Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

[x,y] Different letters indicate significant differences ($P \leq 0.05$) between low and high pH groups within each analysed parameter.

Table 6. Mean ± standard deviation of the area under the curve of the populations obtained with NMR relaxometry of of samples from the low and high pH groups subjected to different pressure levels.

		Control	200 MPa	400 MPa	600 MPa
T_{2B} area	**Low pH**	3.42±0.78[y]	3.18±0.75[y]	3.31±0.58[y]	2.81±0.75[y]
	High pH	4.35±0.78[x]	4.54±0.43[x]	4.20±0.46[x]	3.79±0.56[x]
T_{21} area	**Low pH**	94.97±1.16	95.13±0.89[x]	95.11±0.63[x]	95.47±0.68[x]
	High pH	94.25±0.78	93.71±0.43[y]	94.30±0.46[y]	94.17±0.56[y]
T_{22} area	**Low pH**	1.60±0.28	1.69±0.53	1.57±0.41	1.73±0.54
	High pH	1.38±0.37	1.73±0.49	1.48±0.47	2.02±1.30

[a,b,c] Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

[x,y] Different letters indicate significant differences ($P \leq 0.05$) between low and high pH groups within each analysed parameter.

Figure 5 shows, as in the case of experiment 1, that the reflectance values obtained with multispectral imaging increase with increasing pressure level, with a clear separation between 200 and 400 MPa. For both pH groups, significant differences in reflectance area, L* and a* values were again evidenced between 200 and 400 MPa (Table 7). Nevertheless, the increase or decrease of the reflectance and color parameters as a function of pressure level was similar for both low and pH group (~20-25%). Interestingly, significant differences between the two groups of pH were observed regarding reflectance and colour parameters ($P \leq 0.05$) (Table 7). This fact agrees with Morales, Serra, Guerrero & Gou (2007) who reported that dry-cured hams with high pH_{24SM} presented a softer texture. In the present study can be observed that the high pH hams displayed a higher reflectance which can be related to a higher light scattering due to a higher superficial protein denaturation and thus to a softer texture.

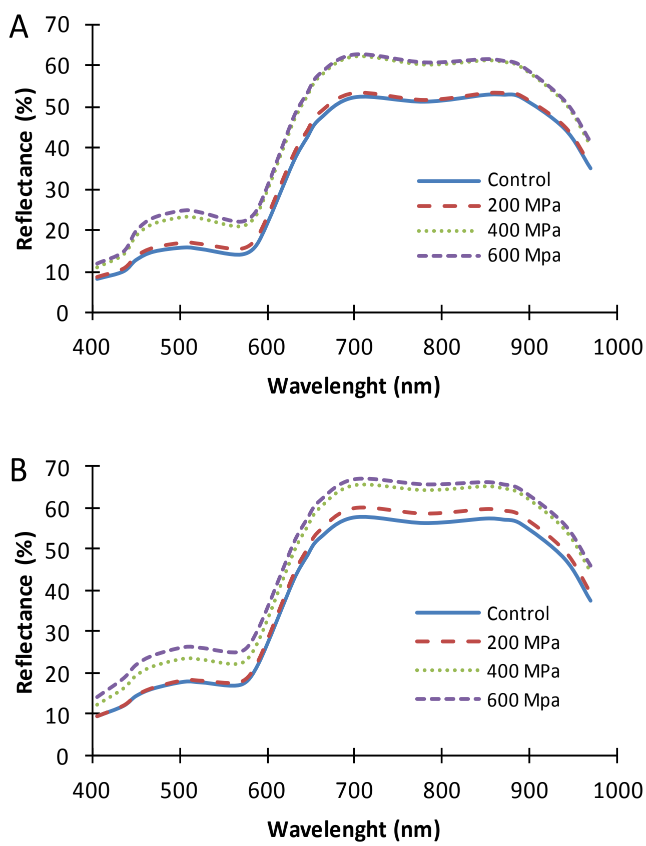


Figure 5. Average reflectance of the samples subjected to different high pressure treatments for low pH group (**A**) and high pH group (**B**) using MI.

Table 7. Mean ± standard deviation of the reflectance area under the curve between 405 and 970 nm and L*a*b* values of samples from the low and high pH groups subjected to different pressure levels using MI.

		Control	200 MPa	400 MPa	600 MPa
Reflectance area	**Low pH**	576.32± 28.88^{b,y}	592.50± 22.66^{b,y}	711.68± 65.14^{a}	725.00± 58.88^{a,y}
	High pH	642.32± 28.88^{b,x}	664.26±42.68^{b,x}	753.34± 48.22^{a}	790.58± 58.58^{a,x}
L*	**Low pH**	48.87±3.24^{b,y}	49.83±2.12^{b,y}	56.61±3.64^{a}	57.67±3.52^{a,y}
	High pH	51.57±2.01^{c,x}	52.85±2.64^{c,x}	57.45±2.96^{b}	61.03±3.02^{a,x}
a*	**Low pH**	18.37±2.57^{a}	17.74±1.74^{a,y}	14.80±1.75^{b,y}	14.05±2.26^{b,y}
	High pH	20.07±1.09^{a}	19.89±1.51^{a,x}	17.73±1.16^{b,x}	15.76±1.40^{c,y}
b*	**Low pH**	12.92±0.45^{y}	13.13±0.91^{y}	12.68±1.22	12.39±1.22
	High pH	14.37±0.52^{b,x}	15.72±0.81^{a,x}	13.38±0.70^{c}	12.48±1.09^{d}

^{a,b,c} Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

^{x,y} Different letters indicate significant differences ($P \leq 0.05$) between low and high pH groups within each analysed parameter.

3.3 General considerations

Generally, when studying dry-cured hams produced from a raw material with a wide range of pH_{24SM}, a more diverse response to HPP in terms of effect on water loss and intrinsic water distribution is found, thus highlighting the importance of the characteristics of dry-cured ham when analyzing these parameters. Further studies would be of interest for a better understanding of the effect of pH_{24SM} on the water binding properties of dry-cured ham subjected to HPP.

The increase in water loss, the reallocation of the water population and the rearrangement of proteins after HPP could partially explain the increase of saltiness and hardness observed in pressurized dry-cured ham reported in previous studies (Fulladosa, Serra, Gou, & Arnau, 2009; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010). Changes in water populations evidenced in the present NMR results and the increase of water loss could probably explain the increase of saltiness since this could make Na+ ions more available to the gustative papillae. The increase of hardness could be ascribed to a new conformation of the proteins resulting in changes in the matrix texture as demonstrated by the present multispectral results.

4. Conclusions

HPP has an effect on water losses, water distribution, reflectance and colour on the dry-cured hams with medium pH. Water loss and NMR results showed a reallocation of the water populations. Reflectance and L* value increased when pressure level was increased, with an inflection point between 200 and 400 MPa, demonstrating an increase of the light scattering. Besides, pH_{24SM} can affect the dynamics of the curing process resulting in different properties of the dry-cured ham at the end of process. Differences in water population distribution were found in samples with different pH. However, samples are not differently affected when submitted to HPP.

5. Acknowledgements

This work was supported by projects RTA2010-00029-CO4-01 and RTA 2013-00030-CO3-01 from INIA (Spain). HCB wishes to thank the Danish research council FTP for its financial support to the NMR activities throughout the project #274-09-107. Acknowledgements are extended to INIA for financing the doctorate studies of Marc Rubio-Celorio at Girona University. The authors would also like to acknowledge the contribution of Nina Eggers, Jens Madsen and Rita Albrechtsen from Aarhus University in sample analyses.

6. References

Andrés, AI, Møller, JKS, Adamsen, CE & Skibsted, L (2004). High pressure treatment of dry-cured lberian ham: effect on radical formation, lipid oxidation and colour. *European Food Research Technology*, 219, 205-210.

Anfinsen, C.B. 1973. Principles that govern the folding of protein chains. *Science,* 181, 223-230.

AOAC (1990).Official method 950.46, moisture in meat, B. Air drying. In K. Helrich (Ed.), *Official methods of analysis of the association of official analytical chemists* (Vol. II, p. 931). Arlington: Association of Official Analytical Chemists Inc.

Arnau, J., Guerrero, L., Casademont, G., & Gou, P. (1995). Physical and chemical changes in different zones of normal and PSE dry-cured ham during processing. *Food Chemistry*, 52, 63–69.

Arnau J., Guerrero L. and Sarraga C. (1997). Effects of temperature during the last month of ageing and of salting time on dry-cured ham aged for six-months. *Journal of the Science of Food and Agriculture*, 74,193-198.

Arnau, J., Guerrero, L., Gou, P., Monfort, JM. (2001). Tecnología, microbiología y principales problemas tecnológicos del jamón curado. En: *Enciclopedia de la carne y de los productos cárnicos*. Edit. Martín & Macías.

Aymerich, T., Picouet, P. A, & Monfort, J. M. (2008). Decontamination technologies for meat products. *Meat science*, 78(1-2), 114–129.

Bajovic, B., Bolumar, T., & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science*, 92(3), 280–289.

Bak, K. H., Lindahl, G., Karlsson, A. H., & Orlien, V. (2012). Effect of high pressure, temperature, and storage on the color of porcine longissimus dorsi. *Meat science*, 92(4), 374–381.

Bertram, H.C., Karlsson A.H., Rasmussen, M., Dønstrup, S., Petersen, O.D. & Andersen, H.J. (2001). Origin of multi-exponential T2 Relaxation in Muscle Myowater. *Journal of Agricultural and Food Chemistry*, 49, 3092-3100

Bertram, H. C., Dønstrup, S., Karlsson, A. H., & Andersen, H. J. (2002). Continuous distribution analysis of T(2) relaxation in meat-an approach in the determination of water-holding capacity. *Meat science*, 60(3), 279–285.

Bertram, H.C., Whittaker, A.K., Andersen, H.J. & Karlsson, A.H. (2003). pH-dependence of the progression in NMR T2 relaxation times in post mortem muscle. *Journal of Agricultural and Food Chemistry*, 51, 4072-4078.

Bertram, H. C., & Andersen, H. J. (2004). Applications of NMR in meat science. *Annual Reports on NMR Spectroscopy*, 53, 157-202.

Bertram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H., & Andersen, H. J. (2004). Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN(-) gene. *Meat science*, 66(2), 437–446.

Bertram, H. C., Aaslyng, M. D., & Andersen, H. J. (2005). Elucidation of the relationship between cooking temperature, water distribution and sensory attributes of pork - a combined NMR and sensory study. *Meat science*, 70(1), 75–81.

Bertram, H. C., Wu, Z., Straadt, I. K., Aagaard, M., & Aaslyng, M. D. (2006). Effects of pressurization on structure, water distribution, and sensory attributes of cured ham: can pressurization reduce the crucial sodium content? *Journal of agricultural and food chemistry*, 54(26), 9912–7.

Bertram, H.C., Andersen, R.H. & Andersen, H.J. (2007). Development in myofibrillar water distribution of two pork qualities during 10-month freezer storage. *Meat Science*, 75, 128-133.

Bertram, H.C., Meyer, R.L., Wu, Z., Zhou, X. & Andersen, H.J. (2008). Water distribution and microstructure in enhanced pork. *Journal of Agricultural and Food Chemistry*. 56, 7201-7207.

Butler, J. P., Reeds, J. A., & Dawson, S. V. (1981). Estimating solutions of 1st kind integral-equations with nonnegative constraints and optimal smoothing. *SIAM Journal of Numeric Analysis*, 18, 381–397.

Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *American Journal of Physics*, 94, 630–638.

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(4), 484–490.

Castro-Giráldez, M., Aristoy, M.C., Toldrá, F., & Fito, P. (2010). Microwave dielectric spectroscopy for the determination of pork meat quality. *Food Research International*, 43(10), 2369–2377.

Cava, R., Ladero, L., González, S., Carrasco, A., & Ramírez, M. R. (2009). Effect of pressure and holding time on colour, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage. *Innovative Food Science & Emerging Technologies*, 10(1), 76–81.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, 46(3), 211–236.

Christiansen, A.N., Carstensen, J.M., Møller, F., Nielsen, A.A. (2012). Monitoring the change in colour of meat: a comparison between traditional and kernel based orthogonal transformations. *Jounal of Spectral Imaging*, 3 (a1), 1-10.

Colmenero, F.J., Carballo, J., Fernández, P., Barreto, G., & Solas, M.T. (1997). High-pressure-induced changes in the characteristics of low-fat and high-fat sausages. Journal of the Science of Food and Agriculture, 45 (1), 61-66.

Ferrini, G., Comaposada, J., Arnau, J., & Gou, P. (2012). Colour modification in a cured meat model dried by Quick-Dry-Slice process® and high pressure processed as a function of NaCl, KCl, K-lactate and water contents. *Innovative Food Science & Emerging Technologies*, 13, 69–74.

Folkestad, A., Wold, J. P., Rørvik, K.-A., Tschudi, J., Haugholt, K. H., Kolstad, K., & Mørkøre, T. (2008). Rapid and non-invasive measurements of fat and pigment concentrations in live and slaughtered Atlantic salmon (Salmo salar L.). *Aquaculture*, 280(1-4), 129–135.

Fuentes, V., Utrera, M., Estévez, M., Ventanas, J., & Ventanas, S. (2014). Impact of high pressure treatment and intramuscular fat content on colour changes and protein and lipid oxidation in sliced and vacuum-packaged Iberian dry-cured ham. *Meat science*, 97(4), 468–474.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat science*, 85(3), 506–14.

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., Serra, X., Gou, P., & Arnau, J. (2009). Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, 82(2), 213–218.

Garcia-Gil, N., Santos-Garcés, E., Fulladosa, E., Laverse, J., Del Nobile, M. A., & Gou, P. (2014). High pressure induces changes in texture and microstructure of muscles in dry-cured hams. *Innovative Food Science & Emerging Technologies*, 22, 63–69.

Gou, P., Morales, R., Serra, X., Guàrdia, M.D. & Arnau, J. (2008). Effect of a 10-day ageing at 30 ºC on the texture of dry-cured hams processed at temperatures up to 18 ºC in relation to raw meat pH and salting time. *Meat Science*, 80(4): 1333-1339.

Hughes, J., Oiseth, S., Purslow, P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, 98(3), 520–532.

ISO 1841-2 (1996). *Meat and meat products*. Determination of chloride content -Part 2: Potentiometric method (Reference method). Geneva: International Organization for Standardization.

Kent, M., Peymann, A, Gabriel, C., & Knight, A. (2002). Determination of added water in pork products using microwave dielectric spectroscopy. *Food Control*, 13(3), 143–149.

Marcos, B., Kerry, J. P., & Mullen, A.M. (2010). High pressure induced changes on sarcoplasmic protein fraction and quality indicators. Meat Science, 85(1), 115–120.

McDonnell, C. K., Allen, P., Duggan, E., Arimi, J. M., Casey, E., Duane, G., & Lyng, J. G. (2013). The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: a low field NMR study. *Meat science*, 95(1), 51–58.

Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear times. Reviews of Scientific Instruments, 29, 688–691.

Miura, N., Yagihara, S., & Mashimo, S. (2003). Microwave Dielectric Properties of Solid and Liquid Foods Investigated by Time-domain Reflectometry. *Journal of Food Science*, 68(4), 1396–1403.

Mor-Mur, M., & Yuste, J. (2003). High pressure processing applied to cooked sausage manufacture: physical properties and sensory analysis. *Meat science*, 65(3), 1187–91.

Møller, S. M., Grossi, A., Christensen, M., Orlien, V., Søltoft-Jensen, J., Straadt, I. K., Thybo, A. K., et al. (2011). Water properties and structure of pork sausages as affected by high-pressure processing and addition of carrot fibre. *Meat science*, 87(4), 387–93.

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes--a review. *Meat science*, 89(2), 111–24.

Picouet, P., Sala, X., Garcia-Gil, N., Nolis, P., Colleo, M., Parella, T., & Arnau, J. (2012). High pressure processing of dry-cured ham: Ultrastructural and molecular changes affecting sodium and water dynamics. *Innovative Food Science & Emerging Technologies*, 16, 335–340.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009). Volatile compounds in dry-cured Serrano ham subjected to high pressure processing. Effect of the packaging material. *Meat science*, 82(2), 162–169.

Rubio-Celorio, M., Garcia-Gil, N., Gou, P., Arnau, J., & Fulladosa, E. (2015). Effect of temperature, high pressure and freezing/thawing of dry-cured ham slices on dielectric time domain reflectometry response. *Meat science*, 100, 91-95.

Rubio-Celorio, M., Castro-Giraldez, M., Garcia-Gil, N., Fulladosa, E., & Fito, P.J. (2015). Study of high pressure processing effect on dry-cured ham by dielectric spectroscopy. *Food Research International.* In press.

Rubio-Celorio, M., Garcia-Gil, N., Castro-Giraldez, M., Fulladosa, E., & Fito, P.J. (2015). Microstructural changes on sliced dry-cured ham subjected to high pressure processing. *LWT - Food Science and Technology*. In press.

Ruiz-Ramírez, J., Arnau, J., Serra, X., & Gou, P. (2006). Effect of pH24, NaCl content and proteolysis index on the relationship between water content and texture parameters in biceps femoris and semimembranosus muscles in dry-cured ham. Meat Science, 72, 185–194.

Schimmer, O., Osen, R., Schönfeld, K., & Hemmy, B. (2009). Detection of added water in seafood using a dielectric time domain reflectometer. *Proceedings, 8th international conference on electromagnetic wave interaction with water and moist substances,* ISEMA 2009, Espoo, Finland (pp. 350–357).

Serra, X., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M. (2007). High pressure applied to frozen ham at different process stages. 2. Effect on the sensory attributes and on the colour characteristics of dry-cured ham. *Meat Science*, 75(1), 21–28.

Sun, X. D., & Holley, R. a. (2010). High hydrostatic pressure effects on the texture of meat and meat products. *Journal of food science*, 75(1), 17–23.

Sørland, G. H., Larsen, P. M., Lundby, F., Rudi, A.-P., & Guiheneuf, T. (2004). Determination of total fat and moisture content in meat using low field NMR. *Meat science*, 66(3), 543–550.

Tintchev, F., Wackerbarth, H., Kuhlmann, U., Toepfl, S., Knorr, D., Hildebrandt, P., et al. (2010). Molecular effects of high-pressure processing on food studied by resonance Raman. *Annals of the New York Academy of Sciences*, 1189, 34–42.

Young, J.F., Bertram, H.C., Theil, P.K., Petersen, A.-G.D. Petersen, Poulsen, K.A., Rasmussen, M., Malmendal, A., Nielsen, N.C., Vestergaard, M. & Oksbjerg, N. (2007). In vitro and in vivo studies of creatine monohydrate supplementation to Duroc and Landrace pigs. *Meat Science*, 76, 342-351.

Paper IV

Rubio-Celorio, M., Garcia-Gil, N., Castro-Giraldez, M., Fulladosa, E., & Fito, P.J. (2015). Changes on slice conformation and microstructure in dry-cured ham subjected to different high pressure levels. In progress (to be submitted).

Changes on slice conformation and microstructure in dry-cured ham subjected to different high pressure levels

Marc Rubio-Celorio[1], Núria Garcia-Gil[1], Marta Castro-Giraldez[2], Elena Fulladosa[1]*, Pedro J. Fito[2]*

[1] *IRTA. XaRTA. Food Technology. Finca Camps i Armet, E-17121 Monells (Girona), Spain*

[2] *Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain*

Abstract

Safety of dry-cured ham can be improved using High Pressure Processing (HPP) but it is linked to strucutal modifications which result in changes of the quality parameters of the product. The aim of this work was to analyze the effect of different high pressure levels (200, 400 and 600 MPa) on dry-cured ham in terms of slice conformation and microstructure. In this work, mass, thickness, volume and surface of dry-cured ham slices were determined in order to study conformational changes and Cryo-SEM micrographies were obtained to evaluate microstructural changes. The results showed that HPP causes a macroscopic compression of the product evidenced in a slice thickness decrease occurred at 400 MPa and in a slice surface decrease occurred at 600 MPa. Pressurized samples showed a compaction of the muscle fibresand a denaturation of the myofibrillar proteins which spreads radially out when pressure level increases.

Keywords: High Pressure Processing, dry-cured ham, microstructure, mechanical forces.

5. GENERAL DISCUSSION

The results have been individually discussed in each of the papers enclosed in the previous section and, in this part, a general and integrative overview of the published results is presented.

This thesis was aimed at studying the potential of different technologies to determine quality parameters of dry-cured ham and elucidate the effect of High Pressure Processing (HPP) on the product. In Papers I and II, calibrations to evaluate marbling and salt and water contents were developed, and the effect of some factors on these predictions was studied. In Papers III and IV, the effect of High Pressure Processing on water populations distribution and protein structure in dry-cured ham was assessed.

Fat content and marbling have been identified as important attributes for dry-cured ham characterization since they are important for both producers and consumers (Morales, Guerrero, Claret, Guàrdia, & Gou, 2008; Font-i-Furnols, Tous, Esteve-Garcia, & Gispert, 2012). In Paper I, a sensory marbling grading scale was achieved and further used to characterize and classify dry-cured ham slices according to this feature. For the time being, there was only a five score reference scale available (Claret, Guerrero, Guàrdia, Garcia-Gil, & Arnau, 2009), but this has been extended to a nine score scale in the present paper. This more extesive scale was also used for the design of an automatic classification system of dry-cured ham based on segmenting intramuscular fat by means of Computer Image Analysis (CIA). The overall correctness of the classification was 89% with a precision of 0.5 score, which demonstrates the practical relevance of using CIA as an objective and reliable sorter of dry-cured ham slices based on their marbling. Thus, CIA would be accurate enough to develop an automatic, rapid, inexpensive method of characterization and classification of the product according to its marbling level for industrial processing lines. This development could have an important impact on the industry and on the market because it would allow producers to classify commercial dry-cured ham slices in terms of marbling thus enabling the consumers to choose the product according to their preferences. The implementation in industry would not be difficult because of the low cost of the necessary equipment and the maintenance required.

Other parameters of interest in dry-cured ham are salt and water contents due to their influence on safety and sensory quality (Benedini, Parolari, Toscani, & Virgili, 2012). Besides, the amount of salt in the product is the main parameter determining saltiness and also affects the occurrence of texture defects, which are more prone to occur in reduced salt content dry-cured hams (Arnau, Hugas, & Monfort, 1987; Arnau, Guerrero, Gou, & Monfort, 2001;). In a previous study (Fulladosa et al. 2013), predictive models to determine salt and water contents in dry-cured ham using Time Domain Reflectometry (TDR) were developed. The device RFQ Scan 3.0 (Sequid GmbH, Bremen, Germany) with the commercial modules based on the results obtained by Fulladosa et al. (2013) is operational with predictive errors of 0.29% for salt content and 2.50% for water content (Annex 1). An industrial validation of this technology showed that this technology was useful for categorizing different retail formats of dry-cured ham, such as slices, portions and entire boned hams, according to their salt content (Annex 2). The results showed that the prediction error of salt content for white hams was 0.37, 0.43 and 0.62%, for slices, portions and entire hams, respectively. This technology is a fast alternative to the traditional physicochemical analyses and could be useful to verify the fulfilment of the "*ETG Jamón Serrano*" product specifications, classify the final product according to its salt content and select dry-cured hams with reduced salt content, which could be labelled as such. Nevertheless, whereas in the case of CIA it has been seen that its implementation would be relatively easy and direct, in the case of TDR, although it is also a non-destructive technology, some adaptations need to be made for an on-line implementation. Besides, the effect of sample temperature, HPP and freezing/thawing treatments on the developed TDR predictive models was not studied. The results presented in Paper II demonstrate that temperature had a significant effect in salt content predictions, especially in samples with high water content. A decrease of the reflected signal intensity after applying HPP was observed, whereas an increase after the freezing/thawing treatment was observed. These changes in the TDR curves produced changes in salt water content predictions but not in water content predictions when using the modules implemented in the device. As demonstrated in Paper II, it should be mentioned that some factors and acquisition conditions must be taken into account when using this technology because they can modify the obtained response and might increase the prediction error.

Paper II was the first step in studying the effect of HPP on dry-cured ham slices using Time Domain Reflectometry (TDR). A further step was taken in Paper III to more accurately assess the effect of HPP and pH on dry-cured ham. On this occasion three levels of pressure (200, 400 and 600 MPa) and two raw materials (low and high pH_{24SM}) were used. Although in Paper II a decrease in the intensity of the TDR was observed, in Paper III no tendency could be established according to the pressure levels. This was attributed to the fact that the batch of dry-cured hams used in Paper III was more variable, in terms of composition and processing conditions, than the batch used in Paper II, which could have interfered with the results. Although there was probably an effect of HPP in the TDR response, it may be not significant within the range of the error of the equipment. Besides, Nuclear Magnetic Resonance (NMR) relaxometry and Multispectral Imaging (MI) measurements were also performed in Paper III in order to elucidate the effect of high pressure on the water distribution and on the conformation of the proteins of dry-cured ham with different raw meat pH_{24SM}. NMR results showed how part of the bound water tightly associated with macromolecules was reallocated into the myofibrilar protein network retaining the majority of water after HPP. Moreover, MI showed an increase of reflectance when pressure level was increased, with an inflection point between 200 and 400 MPa, demonstrating an increase of the light scattering, which was attributed to changes in the surface protein conformation. These results are consistent with the literature, since several authors have previously reported an inflection point of the protein denaturation between 200 and 400 MPa (Picouet et al., 2012; Bajovic, Bolumar, & Heinz, 2012; Bak, Lindahl, Karlsson, & Orlien, 2012). These findings also agree with those in Paper IV, in which both macroscopic and microscopic compression of the product was demonstrated. HPP produced changes on the volume, surface area and thickness of dry-cured ham slices to a different extents depending on the pressure applied, which partly explains the modifications in the water populations and protein structure. Furthermore, the observation of the micrographs presented in Paper IV showed a the compaction of the muscle fibres and the denaturation of the myofibrillar proteins, which also coincides with the increase in water loss with increasing pressures found in Paper III. Moreover, in Paper III it was found that pH_{24SM}, which plays an important role in the elaboration process resulting in dry-cured ham with different properties at the end of the process, affected the water populations, surface proteins and colour. However, samples were not differently affected by their pH when submitted to HPP. Further studies are needed for a better understanding of the effect of pH_{24SM} on the water binding properties of dry-cured ham subjected to HPP.

Due to the consolidation of HHP in the industry, it would be of interest to investigate the effects produced by this treatment on dry-cured ham. In this context, some of the technologies tested in the present thesis, such as NMR, MI and Cryo-SEM, appear to be useful tools to study the changes in the microstructure of dry-cured ham due to HPP. With a deeper knowledge of the structural mechanisms involved in HPP, it would be possible to prevent some of the drawbacks of using HPP treatment and even use it for seeking beneficial effects on an industrial scale. For instance, the use of HPP would be beneficial for developing products with a reduced salt content, because it increases safety and tends to increase the saltiness perception. Besides, it has also been reported that HPP increases hardness in pressurized dry-cured which could partly solve the soft textural defects related to the decrease of salt content (Fulladosa, Serra, Gou, & Arnau, 2009; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2006).

All the technologies tested in the present thesis appear to be promising as tools to determine quality parameters or study the effect of HPP or other processing procedures in dry-cured ham. Some of them could even be implemented in the future for on-line use in the industry. Emergent technologies draw the attention of researchers in the food field, as the increase of books, reviews, congresses and training schools dedicated to this subject in the last decade demonstrate. These technologies are versatile and their possibilities are numerous. For instance, preliminary studies have shown TDR to be promising technology for pastiness detection, a common texture defect found in dry-cured ham (Annex 3). The whole potential of these technologies has yet to be exploited and further efforts need to be made to discover more of their applications.

6. CONCLUSIONS

According to the objectives raised in this thesis and to the results presented in the papers that form it, it can be concluded that:

I. A 9 score marbling scale in dry-cured ham was achieved allowing a standardization of the evaluation of this attribute.

II. An automatic system for an accurate classification of dry-cured ham slices based on this marbling scale was achieved using Computer Image Analysis, which is accurate enough to develop a future automatic grading system in terms of marbling for industrial processing lines.

III. When using TDR the salt content was underestimated at temperatures below 20 ºC, this effect being more pronounced in samples with high water content.

IV. A decrease of the TDR signal intensity after applying HPP and an increase after the freezing/thawing treatment were observed, which produced changes in salt but not in water content predictions.

V. NMR demonstrated a reallocation of the water populations from water closely associated with the macromolecules to water entrapped within the myofibrillar matrix in dry-cured ham at any pressure level, which is in agreement with the increase in water loss found after HPP.

VI. Reflectance and L* value increased in dry-cured ham when pressure level was increased, with an inflection point between 200 and 400 MPa, demonstrating an increase of the light scattering which was attributed to changes in the protein structure.

VII. Differences in water population distribution were found in dry-cured hams with different pH_{24SM}, the T_{2B} population being larger in high pH dry-cured hams, but were not differently affected when submitted to HPP.

VIII. HPP causes a macroscopic compression of dry-cured ham evidenced in a slice thickness decrease occurred at 400 MPa and in a slice surface decrease occurred at 600 MPa, which agrees with the compaction of the muscle fibresand the denaturation of the myofibrillar proteins observed at microscopic level.

7. REFERENCES

Andrés, AI, Møller, JKS, Adamsen, CE & Skibsted, L. (2004). High pressure treatment of dry-cured Iberian ham: effect on radical formation, lipid oxidation and colour. *European Food Research Technology*, 219, 205-210.

Arnau, J., Guerrero, L., Gou, P., Monfort, J.M. (2001). Tecnología, microbiología y principales problemas tecnológicos del jamón curado. En: *Enciclopedia de la carne y de los productos cárnicos*. Ed. Martín & Macías

Arnau, J., Hugas, M., & Monfort, J.M. (1987). *Jamón curado: Aspectos técnicos* (1st edition). Barcelona: Institut de Recerca i Tecnologia Agroalimentaries. *ISBN: 84-404-1575-3*.

Aymerich, T., Picouet, P. A., & Monfort, J. M. (2008). Decontamination technologies for meat products. *Meat Science*, 78(1-2), 114–129.

Bajovic, B., Bolumar, T., & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science*, 92(3), 280–289.

Bak, K. H., Lindahl, G., Karlsson, A. H., & Orlien, V. (2012). Effect of high pressure, temperature, and storage on the color of porcine longissimus dorsi. *Meat Science*, 92(4), 374–381.

Beattie, R., Bell, S. E. J., Borggaard, C., & Moss, B. W. (2008). Preliminary investigations on the effects of ageing and cooking on the Raman spectra of porcine longissimus dorsi. *Meat Science*, 80, 1205-1211.

Benedini, R., Parolari, G., Toscani, T., & Virgili, R. (2012). Sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content. *Meat Science*, *90*(2), 431–437.

Bertram, H. C., & Andersen, H. J. (2004). Applications of NMR in meat science. *Annual Reports on NMR Spectroscopy*, 53, 157-202.

Bertram, H. C., Aaslyng, M. D., & Andersen, H. J. (2005). Elucidation of the relationship between cooking temperature, water distribution and sensory attributes of pork - a combined NMR and sensory study. *Meat Science*, 70(1), 75–81.

Bertram, H. C., Dønstrup, S., Karlsson, A. H., & Andersen, H. J. (2002). Continuous distribution analysis of T(2) relaxation in meat-an approach in the determination of water-holding capacity. *Meat Science*, 60(3), 279–285.

Bertram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H., & Andersen, H. J. (2004). Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN(-) gene. *Meat Science*, 66(2), 437–446.

Bertram, H. C., Wu, Z., Straadt, I. K., Aagaard, M., & Aaslyng, M. D. (2006). Effects of pressurization on structure, water distribution, and sensory attributes of cured ham: can pressurization reduce the crucial sodium content? *Journal of Agricultural and Food Chemistry,* 54(26), 9912–7.

Bertram, H.C., Meyer, R.L., Wu, Z., Zhou, X. & Andersen, H.J. (2008). Water distribution and microstructure in enhanced pork. *Journal of Agricultural and Food Chemistry*. 56, 7201-7207.

Bircan, C., & Barringer, S. A. (2002). Determination of protein denaturation of muscle foods using dielectric properties. *Journal of Food Science*, 67, 202–205

Borgaard, C., Christensen, L. B., & Jespersen, B.L. (2003). Reflection mode microwave spectroscopy for on-line measurement of fat in trimmings. *49th ICoMST, 31 August– 5 September, Campinas, Brazil.*

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.

Brunton, N. P., Lyng, J. G., Zhang, L., & Jacquier, J. C. (2006). The use of dielectric properties and other physical analyses for assessing protein denaturation in beef biceps femoris muscle during cooking from 5 to 85 degrees C. *Meat Science*, 72(2), 236–244.

Butler, J. P., Reeds, J. A., & Dawson, S. V. (1981). Estimating solutions of 1st kind integral-equations with nonnegative constraints and optimal smoothing. *SIAM Journal of Numeric Analysis*, 18, 381–397.

Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *American Journal of Physics*, 94, 630–638.

Castro-Giráldez, M., Aristoy, M.C., Toldrá, F., & Fito, P. (2010). Microwave dielectric spectroscopy for the determination of pork meat quality. *Food Research International*, 43(10), 2369–2377.

Castro-Giráldez, M., Fito, P.J., & Fito, P. (2010). Application of microwave dielectric spectroscopy for controlling pork meat (Longissimus dorsi) salting process. *Journal of Food Engineering*, 97(4), 484–490.

Cernadas, E., Dur, M. L., & Antequera, T. (2002). Recognizing marbling in dry-cured Iberian ham by multiscale analysis. *Pattern Recognition Letters*, 23, 1311–1321.

Claret, A., Guerrero, L., Guàrdia, M.D., Garcia-Gil, N., Arnau, J. (2009). Desarrollo de escalas de referencia para determinados atributos sensoriales del jamón curado de cerdo blanco. *V Congreso Mundial del Jamón*, 6-8 May, Aracena, Spain.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, 46(3), 211–236.

Chen, P., & Sun, Z. (1991). A review of non-destructive methods for quality evaluation and sorting of agricultural products. *Journal of Agricultural Engineering Research*, 49, 85-98.

Christiansen, A.N., Carstensen, J.M., Møller, F., Nielsen, A.A. (2012). Monitoring the change in colour of meat: a comparison between traditional and kernel based orthogonal transformations. *Jounal of Spectral Imaging*, 3 (1), 1-10.

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(2), 325–30.

Damez, J.L., Clerjon, S., Abouelkaram, S., & Lepetit, J. (2007). Dielectric behavior of beef meat in the 1–1500 kHz range: Simulation with the Fricke/Cole–Cole model. *Meat Science*, 77(4), 512–519.

Damez, J.L., Clerjon, S., Abouelkaram, S., & Lepetit, J. (2008). Beef meat electrical impedance spectroscopy and anisotropy sensing for non-invasive early assessment of meat ageing. *Journal of Food Engineering*, 85(1), 116–122.

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.

Damez, J.L., & Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. *Meat Science*, 80(1), 132–149.

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

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), 219–27.

Faucitano, L., Huff, P., Teuscher, F., Garipey, C. & Wergner, J., (2005). Application of computer image analysis to measure pork marbling characteristics. *Meat Science*, 69, 537-543.

Font-i-Furnols, M., Tous, N., Esteve-Garcia, E., & Gispert, M. (2012). Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. *Meat Science*, 91(4), 448–53.

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., Serra, X., Gou, P., & Arnau, J. (2009). Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, 82(2), 213–218.

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, 37–42.

Garcia-Gil, N., Santos-Garcés, E., Fulladosa, E., Laverse, J., Del Nobile, M. A., & Gou, P. (2014). High pressure induces changes in texture and microstructure of muscles in dry-cured hams. *Innovative Food Science & Emerging Technologies*, 22, 63–69.

García-Rey, R. M., García-Olmo, J., De Pedro, E., Quiles-Zafra, R., & Luque de Castro, M. D. (2005). Prediction of texture and colour of dry-cured ham by visible and near infrared spectroscopy using a fiber optic probe. *Meat Science*, 70(2), 357–63.

Garcia-Segovia, P., Andres-Bello, A., & Martinez-Monzo, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (M. pectoralis). *Journal of Food Engineering*, 80(3), 813–821.

Guàrdia, M. D., Guerrero, L., Gelabert, J., Gou, P., & Arnau, J. (2006). Consumer attitude towards sodium reduction in meat products and acceptability of fermented sausages with reduced sodium content. *Meat Science*, 73(3), 484–90.

Hugas, M., Garriga, M., & Monfort, J. M. (2002). New mild technologies in meat processing: high pressure as a model technology. *Meat Science 62*, 359–371.

Içier, F. & Baysal, T. (2004). Dielectrical Properties of Food Materials-1: Factors Affecting and Industrial Uses. *Critical Reviews in Food Science and Nutrition,* 44, 465-471.

Jimenez-Colmenero, F., Ventanas, J., & Toldra, F. (2010). Nutritional composition of dry-cured hams and its role in a healthy diet. *Meat Science*, 84 (4), 585-593.

Kent, M., Knöchel, R., Daschner, F., Berger, U-K. (2001). Composition of foods including added water using microwave dielectric spectra. *Food Control,* 12, 467-482

Kent, M., Lees, A., Roger, A. (1993). Estimation of the fat content of minced meat using a portable microwave fat meter. *Food Control, 4, 222-225.*

Kröger, C., Bartle, C.M., West, J.G., Purchas, R.W., & Devine, C.E. (2006). Meat tenderness evaluation using dual energy X-ray absorptiometry (DEXA). *Computers and Electronics in Agriculture*, 54 (2), 93-100.

Larrea, V., Perez-Munuera, I., Hernando, I., Quiles, A., Llorca, E., & Lluch, M. A. (2007). Microstructural changes in Teruel dry-cured ham during processing. *Meat Science*, 76(3), 574–582.

McDonald, T. P., & Chen, Y. R. (1991). Visual characterization of marbling in beef ribeyes and its relationship to taste parameters. *Transactions of the ASAE*, 34(6), 2499-2504.

McDonnell, C. K., Allen, P., Duggan, E., Arimi, J. M., Casey, E., Duane, G., & Lyng, J. G. (2013). The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: a low field NMR study. *Meat Science*, 95(1), 51–58.

Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear times. *Reviews of Scientific Instruments*, 29, 688–691.

Mendoza, F., Valous, N. A., Allen, P., Kenny, T. A., Ward, P., & Sun, D.W. (2009). Analysis and classification of commercial ham slice images using directional fractal dimension features. *Meat Science*, 81(2), 313-320.

Miura, N., Yagihara, S., & Mashimo, S. (2003). Microwave Dielectric Properties of Solid and Liquid Foods Investigated by Time-domain Reflectometry. *Journal of Food Science*, 68(4), 1396–1403.

Mix, P. E. (2005). Introduction to non-destructive testing - A training guide, (2nd ed.). New Jersey: Wiley Interscience. (pp. 1-14).

Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2003). Spectral characterisation of red pigment in Italian-type dry-cured ham e increasing lipophilicity during processing and maturation. *European Food Research and Technology*, 216, 290-296.

Møller, S. M., Grossi, A., Christensen, M., Orlien, V., Søltoft-Jensen, J., Straadt, I. K., Thybo, A. K., (2011). Water properties and structure of pork sausages as affected by high-pressure processing and addition of carrot fibre. *Meat Science*, 87(4), 387–93.

Monin, G. (1998). Recent methods for predicting quality of whole meat. *Meat Science*, 49(1), 231–243.

Morales, R., Guerrero, L., Aguiar, a P. S., Guàrdia, M. D., & Gou, P. (2013). Factors affecting dry-cured ham consumer acceptability. *Meat Science*, 95(3), 652–657.

Morales, R., Guerrero, L., Claret, A., Guàrdia, M. D., & Gou, P. (2008). Beliefs and attitudes of butchers and consumers towards dry-cured ham. *Meat Science*, 80(4), 1005–1012.

Ngapo, T. M., Babare, I. H., Reynolds, J., & Mawson, R. F. (1999). Freezing rate and frozen storage effects on the ultrastructure of samples of pork. *Meat Science*, 53(3), 159–168.

Picouet, P., Sala, X., Garcia-Gil, N., Nolis, P., Colleo, M., Parella, T., & Arnau, J. (2012). High pressure processing of dry-cured ham: Ultrastructural and molecular changes affecting sodium and water dynamics. *Innovative Food Science & Emerging Technologies*, 16, 335–340.

Reh, C. (2008). An overview of sensor technology in practice: the user's view. In J. Irudayaraj, & C. Reh (Eds.), *Nondestructive testing of food quality*, 1-31. Ames: Wiley-Blackwell.

Santos-Garcés, E., Gou, P., Garcia-Gil, N., Arnau, J., & Fulladosa, E. (2010a). 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.

Scheier, R., Scheeder, M., & Schmidt, H. (2015). Prediction of pork quality at the slaughter line using a portable Raman device. *Meat Science*, *103*, 96–103.

Schmidt, H., Blum, J., Sowoidnich, K., Sumpf, B., Schwagele, F., & Kronfeldt, H.D. (2009). In-situ characterization of meat aging with diode-laser Raman spectroscopy. *Proceedings of SPIE: The International Society for Optical Engineering*, 7315, 731509.

Scotter, N.G. (1997). Non-destructive spectroscopic techniques for the measurement of food quality. *Trends in Food Science & Technology*, 81, 285–292.

Serra, X., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M. (2007). High pressure applied to frozen ham at different process stages. 2. Effect on the sensory attributes and on the colour characteristics of dry-cured ham. *Meat Science*, 75(1), 21–28.

Sørland, G. H., Larsen, P. M., Lundby, F., Rudi, A.-P., & Guiheneuf, T. (2004). Determination of total fat and moisture content in meat using low field NMR. *Meat science*, 66(3), 543–550.

Sun, X. D., & Holley, R.A. (2010). High hydrostatic pressure effects on the texture of meat and meat products. *Journal of Food Science*, 75(1), 17–23.

Swatland, H. J. (1997). Observations on rheological, electrical, and optical changes during rigor development in pork and beef. *Journal of Animal Science*, 75(4), 975–985.

Valous, N.A., Mendoza, F., & Sun, D.W. (2010). Emerging non-contact imaging, spectroscopic and colorimetric technologies for quality evaluation and control of hams: A review. *Trends in Food Science & Technology*, 21(1), 26–43.

Vestergaard, C., Erbou, S.G., Thauland, T., Adler-Nissen, J., Berg, P., (2005). Salt distribution in dry-cured ham measured by computed tomography and image analysis. *Meat Scienc*e, 69 (1), 9–15.

Widiyanto, S., Cufí, X., Rubio, M., Muñoz, I., Fulladosa, E., & Martí, R. (2013). Automatic intra muscular fat analysis on dry-cured ham slices. In *Proceedings of ibPRIA*, 873-880.

ANNEX 1. DISSEMINATION PAPER

Rubio, M., Fulladosa, E., Duran, P., Garcia-Gil, N. (2011). Determinación no destructiva de los contenidos de agua y sal en jamón curado mediante el equipo Sequid RFQ-Scan. *Eurocarne,* 202, 82-87

Rubio, M., Fulladosa, E., Duran, P., Garcia-Gil, N. (2011). Determinación no destructiva de los contenidos de agua y sal en jamón curado mediante el equipo Sequid RFQ-Scan. *Eurocarne*, 202, 82-87

http://www.eurocarne.com/articulos-ficha?codigo=15835&ano=2011

Introducción

Los productores de jamón curado están interesados en disponer de herramientas rápidas y no destructivas que les permitan predecir la composición y calidad de sus productos, de forma que se pueda realizar un control en línea y clasificar el producto final obtenido según sus características nutricionales, como por ejemplo el contenido de sal. Esta información podría ser añadida en el etiquetado del producto, lo que permitiría al consumidor elegir de acuerdo con sus preferencias.

La espectrometría de microondas permite determinar las propiedades dieléctricas de los alimentos, pudiéndose correlacionar con parámetros de calidad y composición de los alimentos. Varios investigadores han utilizado la espectrometría de microondas en el campo de la alimentación. Lleó et al. (2007) determinaron el contenido de azúcares y la firmeza de me locotones. Asimismo, Castro-Giráldez et al. (2010) la utilizaron para medir el grado de maduración en manzanas, el cual está altamente relacionado con su contenido en azúcares. Kent et al. (2000) determinaron parámetros de calidad y frescura en distintas muestras de pescado. En este estudio se demostró la capacidad de esta tecnología para determinar si el producto era fresco o había sido congelado. Durante la congelación se producen cambios en la distribución del agua debido a los daños producidos en los tejidos durante este proceso. Kent et al. (2000) también utilizaron la técnica para detectar si se había añadido agua de manera fraudulenta en muestras de gambas y distintas especies de pescado. Para esta detección se basaron en el hecho de que si se añade agua al alimento, las sales iónicas se diluyen o difunden al exterior, lo que hace variar las propiedades dieléctricas del producto en cuestión. (...)

ANNEX 2. CONGRESS COMMUNICATION

Rubio, M., Fulladosa, E., Garcia-Gil, N., Gou, P. & Arnau, J. Estimación del contenido de sal en lonchas, porciones y piezas enteras deshuesadas de jamón curado. VII Congreso Mundial del Jamón. Ourique, Portugal. 2013

ESTIMATING SALT CONTENT IN SLICES, PORTIONS AND ENTIRE BONED PIECES OF DRY-CURED HAM Panel IV

Marc Rubio, Elena Fulladosa, Núria Garcia-Gil, Pere Gou and Jacint Arnau

IRTA. XaRTA. Food Technology. Finca Camps i Armet, E- 17121 Monells, Spain

This work evaluates the ability of Sequid RFQ-Scan device, based on microwave spectrometry, to estimate the salt content in different retail formats of dry-cured ham from Iberian pigs (IB), consisting of at least 50% Iberian breed, and white pigs (W), consisting of *Large White* x *Landrace* crosses. 51 slices (30 IB + 21 W), 306 portions (180 IB + 126 W) and 51 entire boned hams (30 IB + 21 W) were used. Salt content was estimated on the lean part of slices and portions, and on the *Biceps femoris* muscle in entire boned hams using the salt content prediction module available in the device. The samples were minced for physicochemical determination of salt content. The prediction error of salt content for the Iberian hams was 0.88, 1.29 and 1.63%, for the slices, portions and entire hams, respectively. Whereas for the white hams was 0.37, 0.43 and 0.62%, respectively. The prediction accuracy of salt content in slices of the white hams was higher than in the Iberian hams, which can be attributed to a higher content of intramuscular fat of Iberian hams. Furthermore, the accuracy for portions and entire boned hams was lower than in slices, due to the measuring points in the total sample were less representative. It can be concluded that Sequid RFQ-Scan device is useful to categorize different retail formats of dry-cured ham according to salt content.

ESTIMACIÓN DEL CONTENIDO DE SAL EN LONCHAS, PORCIONES Y PIEZAS ENTERAS DESHUESADAS DE JAMÓN CURADO

Marc Rubio, Elena Fulladosa, Núria Garcia-Gil, Pere Gou y Jacint Arnau

IRTA. XaRTA. Tecnología alimentaria. Finca Camps i Armet, E-17121 Monells, España

INTRODUCCIÓN

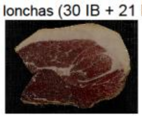
El desarrollo de métodos no destructivos y rápidos para determinar en línea la composición del jamón es de interés para la industria. La espectrometría de microondas se revela como una herramienta capaz de determinar las propiedades dieléctricas de los alimentos, las cuales se pueden correlacionar con parámetros de calidad y composición del jamón curado. En este estudio se evaluó la capacidad del equipo Sequid RFQ-Scan, basado en espectrometría de microondas, para estimar el contenido de sal global en lonchas, porciones y jamones enteros deshuesados.

MATERIALES Y MÉTODOS

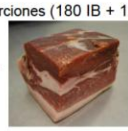
Cerdos ibéricos (IB)
(mínimo 50% raza ibérica)

Cerdos blancos (B)
(Large White x Landrace)

51 lonchas (30 IB + 21 B)



306 porciones (180 IB + 126 B)



51 enteros deshuesados (30 IB + 21 B)

Se estimó con el equipo RFQ-Scan el contenido de sal utilizando el módulo de predicción disponible en el equipo desarrollado con los datos de Fulladosa et al. (2013).

El contenido de sal fue determinado mediante valoración potenciométrica (ISO 1841-2, 1996).

DETERMINACIÓN DE LOS AJUSTES Y ERRORES DE PREDICCIÓN

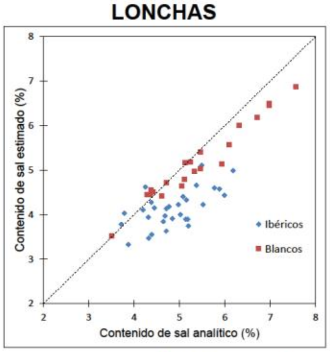
RESULTADOS Y DISCUSIÓN

Tabla 1. Errores de las predicciones del contenido de sal

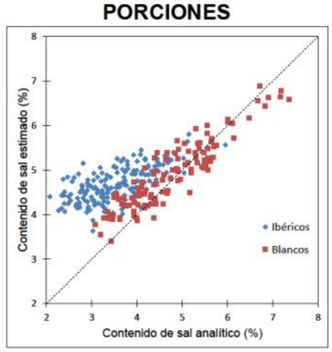
Errores de predicción (%)		
	Jamones ibéricos	Jamones blancos
Lonchas	0,88	0,37
Porciones	1,29	0,43
Enteros deshuesados	1,63	0,62

- La precisión en la estimación del contenido global de sal en las lonchas de jamones blancos fue mayor que en jamones ibéricos, lo cual se atribuye a un mayor contenido de grasa intramuscular de los jamones ibéricos (Tabla 1 y Figura 1).
- La precisión en porciones y enteros fue menor que en lonchas debido a una menor representatividad de los puntos de medida respecto al total de la muestra (Tabla 1 y Figura 1).

LONCHAS



PORCIONES



ENTEROS

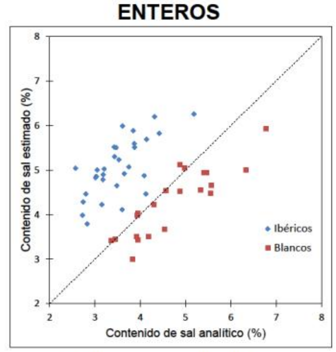


Figura 1. Relación entre el contenido de sal analítico y predicho en lonchas, porciones y jamones enteros deshuesados. ------ bisectriz.

CONCLUSIÓN

Se puede concluir que el equipo Sequid RFQ-Scan, basado en espectrometría de microondas, es útil para categorizar diferentes formatos comerciales de jamón curado de acuerdo con su contenido de sal.

REFERENCIAS

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.

AGRADECIMIENTOS

Este trabajo ha sido financiado por el proyecto RTA2010-00029-CO4-01 del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) de España. Marc Rubio agradece al INIA su beca FPI para poder asistir al VII Congreso Mundial del Jamón.

ANNEX 3. CONGRESS COMMUNICATION

Rubio, M., Fulladosa, E., Claret, A., Guàrdia, M. D., & Garcia-Gil, N. Detection of pastiness in dry-cured ham using dielectric time domain reflectometry. 59th International Congress of Meat Science and Technology - ICoMST 2013. Izmir, Turkey. 2013

DETECTION OF PASTINESS IN DRY-CURED HAM USING DIELECTRIC TIME DOMAIN REFLECTOMETRY

M. Rubio, E. Fulladosa, A. Claret, M.D. Guàrdia, and N. Garcia-Gil

IRTA. XaRTA. Food Technology. Finca Camps i Armet, E-17121 Monells, Spain

INTRODUCTION

Pastiness is one of the major texture defects in dry-cured ham. It is commonly evaluated by sensory analysis and instrumental methods, but these methods are laborious and time-consuming. The development of screening methods which could be related to sensory texture attributes would be a great asset to the meat industry. Time Domain Reflectometry (TDR) has shown to be a rapid and effective tool for meat quality assessment. The **aim** of this work was to assess the feasibility of TDR to classify dry-cured ham slices according to their pastiness level.

MATERIALS AND METHODS

Sampling

25 commercial packages of 12 slices 1.5 mm thick of dry-cured ham showing **absence of pastiness**

5 packages

10 slices from each of 5 packages were used to develop a pastiness reference scale

20 packages

10 slices from each of 20 packages were used for TDR measurements

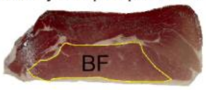
Protocol

Addition of 250 µl of protease (Delvolase®) (50% v/v) on each slice face

Action time of protease (T=20±2ºC): 0.5, 1, 4, 8 and 24 h

Sensory analysis

The pastiness of the slices was assessed on *Biceps femoris (BF)* muscle by 2 expert panellists



Spectroscopic analysis

The Sequid-RFQ Scan 3.0 device was used to obtain TDR curves on *Biceps femoris (BF)*

Statistical analysis

Discriminant analysis

RESULTS AND DISCUSSION

Sensory analysis

Action time of protease	Average intensity	Standard deviation	Levels of pastiness
Control (0h)	0.09	0.17	Absence of pastiness
0.5h	2.70	0.54	Moderate pastiness
1h	3.71	0.86	
4h	5.61	0.70	Intense pastiness
8h	8.00	0.53	
24h	9.30	0.29	Discarded

Table 1. Pastiness intensity increased as increased the action time of the protease. However, the increase was not lineal. Three levels of pastiness were established: absence, moderate and intense.

Spectroscopic analysis

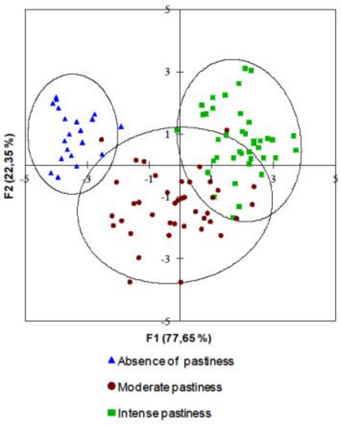


Figure 1. Three different clusters corresponding to the levels different groups of pastiness intensity can be clearly observed when performing discriminant analysis.

	Level of pastiness	Real: Absence	Moderate	Intense	Total
Predicted	Absence	20	1	0	21
	Moderate	0	36	3	39
	Intense	0	3	37	40
	Total	20	40	40	100
	% of correct classification	100	90	92.5	**93**

Table 2. Overall correctness of classification of dry-cured ham slices according to the three levels of pastiness established using the TDR device was **93%**.

CONCLUSION

TDR is useful for the classification of dry-cured ham slices according to the three levels of sensory pastiness established.

ACKNOWLEDGEMENTS

This work has been partially funded by the project RTA2010-00029-CO4-01 of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) of Spain. Marc Rubio thanks INIA the travel grant to attend to the 59th ICOMST.