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Tesis Doctoral

DEVELOPMENT AND EVALUATION OF TOOLS TO IMPROVE THE TEXTURE AND QUALITY OF DRY-CURED HAM

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UNIVERSIDAD DE ZARAGOZA
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Development and evaluation of tools to improve the texture and quality of dry-cured ham

Ph. D Thesis by Elena Coll Brasas
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Hi ha una força motriu més ponderosa que el vapor,
l'electricitat i l'energia atòmica: la voluntat

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Table of contents

Abbreviations

HPP: High Pressure Processing

TPA: Texture Profile Analysis

SR: Stress Relaxation

F_0: Initial Force

Y_2: Force Decay at 2 seconds

Y_{90}: Force Decay at 90 seconds

BF: *Biceps femoris* muscle

SM: *Semimembranosus* muscle

RH: Relative Humidity

NPG: Non-Pastiness Group

MPG: Medium Pastiness Group

HPG: High Pastiness Group

NPN: Non-Protein Nitrogen

TN: Total Nitrogen

PI: Proteolysis Index

ZnPP: Zinc-protoporphyrin

RMSE: Root Mean Square Error

SS: Standard Salting

SR: Salt Reduced

NNSR: Non-Nitrite Salt Reduced

ww: wet weight basis

dm: dry matter basis

a_w: water activity

RoI: Region of Interest

HPP7: HPP at 7 °C

HPP20: HPP at 20 °C

HPP35: HPP at 35 °C

ITAS: Instrumental Texture Analysis at the Surface.

PuS: power ultrasound heating system

Traditional Speciality Guaranteed (TSG)

Protected Designation of Origin (PDO)

Protected Geographical Indication (PGI)

Summary (in English)

Dry-cured ham is considered a typical product of the Mediterranean area made of pig's hind leg which is dry-salted and has a long maturation period. However, there are other typical dry-cured products such as the dry-cured lamb ham, Fenalår, from Norway, which is dry-salted and smoked followed by a short maturation period. The texture of dry-cured products is an important quality criterion for the consumers. However, texture development and also texture defects of dry cured products from both pork and lamb are a complex issue since many factors are involved. For this reason, there is an increasing interest of the industry in finding solutions to reduce the incidence of products with soft and/or defective textures.

The main objective of this PhD Thesis dissertation was to develop and evaluate tools to improve the manufacturing process and the quality of two examples of dry-cured products; dry-cured ham and Fenalår. For this reason, different studies were conducted and herein presented as different papers. First one addresses the main factors affecting texture development and texture prediction modelling (presented in paper I). The second paper presents the results of predictive microbiology used to study food safety in relation to manufacturing process with reduced salt and nitrites addition (in paper II). Next study investigated a method to non-destructively evaluate the end of process based on the measurements taken at the surface of the ham (in paper III). The results of an attempt to develop a method to evaluate pastiness with a rheometer to avoid the tedious sensory analysis are presented in paper IV. Finally, the use of high pressure treatments as a corrective action for products with defective textures is presented in paper V.

In order to study texture development in more detail, mathematical models were developed (paper I), that relate processing conditions and raw material characteristics to the texture of the product. These models not only allow the effect of these factors to be evaluated but could also enable further optimisation of the process in the industry. The results showed that salt content, pH, raw material characteristics and processing conditions (drying temperature and final weight loss) are important factors affecting texture development, as shown in paper I, while affecting product safety. In paper II, reduced salt content not only resulted in softer textures but also in an increase of microbiological hazards especially when no nitrite was used as evaluated by predictive microbiology models. Furthermore, texture defects in both dry-cured products represent an important challenge for the industry.

Despite the efforts to improve the manufacturing process, some products may still be defective and the need for new tools and/or technologies with potential for industrial application is emerging. In this Thesis, a methodology to evaluate whether the product had the appropriate texture to be sent to the market or not (paper III) and an analysis

to instrumentally evaluate pastiness defect in sliced dry-cured ham (paper IV) were studied. Results presented in paper III show that ITAS method allowed to non-destructively assess processing end point; this methodology consist of simulating the tactile assessment of ham surface as currently made by the experts. The maximum classification accuracy was 82.1% obtained when combining measurements with subcutaneous fat thickness. The implementation of this technology in industry would not only improve the quality of the product but also economic, energy and space savings, as it would enable better adjustment of required drying time for each ham.

Results of the study in paper IV represent a first attempt for the development of a method to evaluate pastiness using a rheometer to avoid the tedious sensory analysis. An increase in viscosity of water extracts with an increase in pastiness intensity, show the ability to instrumentally detect this defect using the rheometer. However, there are factors other than viscosity that can affect/alter the results and this method allows discriminating only samples with high pastiness intensity from those with medium or non-pastiness defect.

Corrective actions for dry-cured ham slices using HPP treatments at different temperatures were also analysed (paper V). The obtained results show that while temperatures of 7 °C and 20 °C are useful to correct ham slices with medium pastiness, higher temperature (35 °C) is needed in the case of high pastiness. Therefore, knowing textural properties of the product is key information to define optimal HP processing conditions when aiming to improve texture without deteriorating colour and aroma.

The methodologies developed and evaluated in the present work can be used to improve the manufacturing process and quality of dry-cured ham. They represent a first step in development of new tools for industrial use.

Resumen (in Spanish)

El jamón curado es considerado un producto típico de la región mediterránea elaborado con las patas traseras del cerdo, las cuales se salan y curan durante un período largo. Sin embargo, existen otros productos curados como el jamón curado de cordero, el Fenalår, típico de Noruega, que se sala, se ahúma y se cura durante un período más corto de tiempo. La textura de los productos curados es un criterio de calidad importante para los consumidores. Sin embargo, el desarrollo de la textura, así como también de los defectos de textura de los productos curados tanto de cerdo como de cordero son un tema complejo puesto que hay muchos factores involucrados. Por este motivo, hay un interés creciente de la industria alimentaria en encontrar soluciones para reducir la incidencia de productos con texturas blandas y/o defectuosas.

El principal objetivo de esta Tesis es desarrollar y evaluar herramientas para mejorar el proceso de elaboración y la calidad de dos productos curados: el jamón curado y el Fenalår. Para ello, se han llevado a cabo diferentes estudios los cuales se presentan en esta Tesis en diferentes artículos científicos. El artículo I aborda los principales factores que afectan al desarrollo de la textura y a la modelización de la textura. El segundo artículo muestra los resultados de la microbiología predictiva utilizada para estudiar la seguridad alimentaria en relación con el proceso de elaboración y la reducción del contenido de sal, así como también la omisión de nitrificantes (artículo II). En el artículo III se investigó un método para evaluar de manera no destructiva el punto final del proceso en función de las medidas obtenidas en la superficie del jamón. En el artículo IV, se presentan los resultados obtenidos de un primer intento para el desarrollo de un método para evaluar la pastosidad con el reómetro y evitar así los tediosos análisis sensoriales. Finalmente, el uso de un tratamiento de altas presiones como acción correctora para productos con texturas defectuosas se presenta en el artículo V.

Con la finalidad de estudiar de forma más detallada el desarrollo de la textura, se desarrolló un modelo matemático (artículo I), que relaciona las condiciones de procesado y las características de la materia prima con la textura del producto. Este modelo no sólo permite evaluar el efecto de estos factores, sino que también podría permitir una optimización del proceso de elaboración a nivel industrial. Los resultados mostraron que el contenido de sal, el pH, las características de la materia prima y las condiciones de procesado (temperatura de secado y merma final) son factores importantes que afectan al desarrollo de la textura, tal y como se presenta en el artículo I, y a la vez comprometen la seguridad del producto. En el artículo II, la reducción del contenido de sal no sólo dio como resultado texturas más blandas, sino que también produjo un incremento de los riesgos microbiológicos, especialmente cuando se omitieron los nitrificantes, tal y como muestran los resultados obtenidos mediante los

modelos de microbiología predictiva. Además, los defectos de textura en ambos productos curados representan un importante desafío para la industria.

A pesar de los esfuerzos realizados para mejorar el proceso de elaboración, es posible que algunos productos sigan siendo defectuosos, evidenciando así la necesidad de nuevas herramientas y/o tecnologías con potencial para su aplicación a nivel industrial. En esta Tesis, se estudió una metodología para evaluar si el producto tenía o no la textura adecuada para su comercialización (artículo III), así como también un análisis para evaluar instrumentalmente el defecto de pastosidad en jamón curado loncheado (papel IV). Los resultados presentados en el artículo III muestran que el método ITAS permitió evaluar de manera no destructiva el punto final del proceso; esta metodología consiste en simular la valoración táctil en la superficie del jamón realizada actualmente por los expertos. La máxima precisión de clasificación fue del 82,1%, obtenida al combinar las mediciones junto con el espesor de la grasa subcutánea. La implantación de esta tecnología a nivel industrial no sólo mejoraría la calidad del producto, sino que también supondría un ahorro económico, energético y de espacio, ya que permitiría un mejor ajuste del tiempo de secado necesario para cada jamón.

En el artículo IV se presentan los resultados obtenidos en un primer estudio para el desarrollo de un método para evaluar la pastosidad utilizando un reómetro. Los resultados obtenidos mostraron un aumento de la viscosidad de los extractos a medida que aumentaba la intensidad del defecto de pastosidad, mostrando así la posibilidad de detectar instrumentalmente este defecto utilizando el reómetro. Sin embargo, hay otros factores a parte de la viscosidad que pueden afectar/alterar los resultados y el reómetro sólo permite discriminar las muestras con una pastosidad intensa de aquellas que tienen un defecto medio o que no son pastosas.

También se han llevado a cabo acciones correctoras en lonchas de jamón curado utilizando tratamientos mediante altas presiones a diferentes temperaturas (artículo V). Los resultados obtenidos muestran que, si bien las temperaturas de 7 °C y 20 °C son útiles para corregir el defecto en las lonchas con pastosidad media, se necesita una temperatura superior (35 °C) para las de pastosidad intensa. Por lo tanto, conocer las características iniciales de textura del producto es clave para definir las condiciones óptimas para su procesado mediante altas presiones cuando se busca mejorar la textura sin deteriorar el color ni el aroma.

Las metodologías desarrolladas y evaluadas en esta Tesis pueden utilizarse para mejorar el proceso de elaboración y la calidad del jamón curado. Además, representan un primer paso en el desarrollo de nuevas herramientas para uso industrial.

Resum (in Catalan)

El pernil curat és considerat un producte típic de la regió mediterrània, el qual s'elabora a partir de les potes del darrera del porc, es sala i es cura durant un llarg període de maduració. No obstant això, hi ha altres productes curats com és el cas del pernil curat de xai, el Fenalår, típic de Noruega, que es sala, es fuma i es cura durant un període de temps més curt. La textura dels productes curats és un criteri de qualitat important pels consumidors. Tanmateix, el desenvolupament de la textura així com també dels defectes de textura dels productes curats tant de porc com de xai és un tema complex ja que hi intervenen molts factors. Per aquest motiu, hi ha un interès creixent de la indústria alimentària per trobar solucions per tal de reduir la incidència de productes amb textures toves i/o defectuoses.

El principal objectiu d'aquesta Tesis és desenvolupar i avaluar eines per a millorar el procés d'elaboració i la qualitat de dos productes curats: el pernil curat i el Fenalår. Per fer-ho, s'han dut a terme diferents estudis els quals es presenten en aquesta Tesi en diferents articles científics. El primer tracta dels principals factors que afecten al desenvolupament de la textura i la modelització d'aquesta (presentat en l'article I). L'article II, estudia la seguretat alimentària en relació amb el procés d'elaboració i la reducció del contingut de sal així com també la omissió dels nitrificants mitjançant la microbiologia predictiva. En l'article III, s'ha investigat un mètode per avaluar de manera no destructiva el final del procés en funció de les mesures obtingudes a la superfície del pernil. En l'article IV s'ha fet un primer intent per al desenvolupament d'un mètode per avaluar la pastositat utilitzant el reòmetre per tal d'evitar els tediosos anàlisis sensorials. Finalment, l'article V presenta l´ús d'un tractament d'altes pressions com a acció correctora per a productes amb textures defectuoses.

Per tal d'estudiar més detalladament el desenvolupament de la textura, es va desenvolupar un model matemàtic (article I), que relaciona les condicions de processat i les característiques de la matèria primera amb la textura. Aquest model no només permet avaluar l'efecte d'aquests factors, sinó que també podria permetre una major optimització del procés a la indústria. Els resultats van mostrar que el contingut de sal, el pH, les característiques de la matèria primera i les condicions de processat (temperatura d'assecat i minva final) són factors importants que afecten al desenvolupament de la textura, tal i com es presenta en l'article I, i a la vegada també comprometen la seguretat del producte. En l'article II, la reducció del contingut de sal no només ha resultat en l'aparició de Fenalårs amb textures més toves, sinó que també en un increment dels riscos microbiològic, especialment quan es s'han omès els nitrificants, tal i com mostren els resultats obtinguts mitjançant els models de microbiologia predictiva. A més, els defectes de textura en els productes curats representen un important repte per a la indústria.

Tot i això, malgrat els esforços realitzats per a millorar el procés d'elaboració, és possible que alguns productes segueixin resultant defectuosos, evidenciant així la necessitat de noves eines i/o tecnologies amb potencial d'aplicació industrial. En aquesta Tesi, es va estudiar una metodologia per avaluar si el producte tenia o no la textura adequada per a la seva comercialització (article III), així com també un anàlisi per avaluar instrumentalment el defecte de pastositat en pernil curat llescat (paper IV). Els resultats presentats en l'article III mostren que el mètode ITAS va permetre avaluar de manera no destructiva el punt final del procés; aquesta metodologia simula la valoració tàctil que realitzen actualment els experts en la superfície del pernil. La màxima precisió de classificació va ser del 82,1%, obtinguda al combinar les mesures dels paràmetres texturals amb el gruix del greix subcutani. La implementació d'aquesta tecnologia a nivell industrial no només milloraria la qualitat del producte, sinó que també suposaria un estalvi econòmic, energètic i d'espai, ja que permetria un millor ajust del temps d'assecat necessari per a cada pernil.

L'article IV presenta els resultats obtinguts en un primer intent per al desenvolupament d'un mètode per tal d'avaluar la pastositat utilitzant un reòmetre i evitar així els tediosos anàlisis sensorials. Els resultats obtinguts van mostrar un augment de la viscositat dels extractes a mesura que augmentava la intensitat del defecte de pastositat, mostrant la possibilitat de detectar instrumentalment aquest defecte utilitzant el reòmetre. No obstant això, hi ha altres factors a part de la viscositat que poden afectar/alterar els resultats i el reòmetre només permet discriminar les mostres amb una pastositat intensa d'aquelles que tenen un defecte mitjà o que no són pastoses.

En l'article V, s'han analitzat les accions correctores en pernil curat llescat utilitzant tractaments d'altes pressions a diferents temperatures. Els resultats obtinguts mostren que, si bé les temperatures de 7 °C i 20 °C són útils per corregir les llesques amb pastositats mitges, es necessita una temperatura superior (35 °C) en el cas de les llesques amb pastositat intensa. Per tant, conèixer les característiques inicials de textura del producte és clau per a definir les condicions òptimes de processat per altes pressions quan es busca millorar la textura sense deteriorar ni el color ni l'aroma.

Les metodologies desenvolupades i avaluades en aquesta Tesi poden utilitzar-se per a millorar el procés d'elaboració i la qualitat del pernil curat. A més, representen un primer pas en el desenvolupament de noves eines per a l'ús industrial.

Keywords

Dry-cured ham, pork, lamb, Fenalår, manufacturing process, texture, modelisation, raw material characteristics, rheology, predictive models, sensory characteristics, salt and nitrite reduction.

List of publications

The following list contains the publications resulting from this Doctoral Thesis, referred in Roman numerals:

Paper I

Coll-Brasas, E., Gou, P., Arnau, J., Olmos, A. & Fulladosa, E. (2021). Processing parameters involved in the development of texture and tyrosine precipitates in dry-cured ham: Modelisation of texture development. *Meat Science*, *172*. 108362. https://doi.org/10.1016/j.meatsci.2020.108362. (JCR, impact: 3.126; 19/129 1st quartile, category Food Science and Technology)

Author's contribution: the experimental study (characterizing the hams and analyzing the results) and writing the paper with the contributions from the other authors.

Paper II

Coll-Brasas, E., Possas, A., Berg, P., Grabež, V., Egelandsdal, B., Bover-Cid, S. & Fulladosa, E. (2021). Physicochemical characterization of boned Fenalår and safety implications of its elaboration procedures. *Food Control, 119*. 107460. https://doi.org/10.1016/j.foodcont.2020.107460. (JCR, impact: 3.011; 36/135 1st quartile, category Food Science and Technology)

Author's contribution: the experimental study (controlling and characterizing the Fenalårs at each step of the manufacturing process, doing the physicochemical characterization of the final product and analyzing the results) and writing the paper with the contributions from the other authors.

Paper III

Fulladosa, E., Guerrero, L., Illiana, A., Olmos, A., **Coll-Brasas, E.**, Gou, P. & Arnau, J. (2021). Instrumental texture analysis on the Surface of dry-cured ham to define the end of the process. *Meat Science, 172*. 108334. https://doi.org/10.1016/j.meatsci.2020.108334. (JCR, impact: 3.126; 19/129 1st quartile, category Food Science and Technology)

Author's contribution: the experimental study (characterizing the hams performing the texture analysis) and contribute on writing the paper.

Paper IV

Coll-Brasas, E., Laguna, L., Tárrega, A., Arnau, J., Gou, P. & Fulladosa, E. (2021). Evaluation of dry-cured ham pastiness using a rheometer. Submitted to LWT – Food Science and Technology. (JCR, impact: 3.714; 23/135 1st quartile, category Food Science and Technology)

Author's contribution: the experimental study (selecting and preparing the hams for the rheological analyses, performing the HPP treatments and also analyzing the results) and writing the paper with the contributions from the other authors.

Paper V

Coll-Brasas, E., Arnau, J., Gou, P., Lorenzo Rodríguez, J. M., García-Pérez, J. V. & Fulladosa, E. (2019). Effect of high pressure processing temperature on dry-cured hams with different textural characteristics. *Meat Science, 152*, 127-133. https://doi.org/10.1016/j.meatsci.2019.02.014. (JCR, impact: 3.126; 19/129 1st quartile, category Food Science and Technology)

Author's contribution: the experimental study (characterizing the hams before and after the HPP treatments as well as analyzing the results) and writing the paper with the contributions from the other authors.

The following list contains a publication in a Dissemination Journal resulting from this Doctoral Thesis:

Included in Annex I

Coll-Brasas, E., Arnau, J., Lorenzo, J. M., Purriños, L., García-Pérez, J.V., Benedito, J. & Fulladosa, E. Cómo evaluar no destructivamente defectos de textura en jamón curado. (2016). *Eurocarne* núm. 246, pg. 92-96.

Author's contribution: writing the paper with the contributions from the other authors.

The following list contains the communications in different Congresses:

In 2019

Coll-Brasas, E., Olmos, A., Arnau, J., Gou, P. & Fulladosa, E. (2019). Modelisation of dry-cured ham texture to define optimal processing conditions. 65th International Congress of Meat Science and Technology (ICoMST). August 4th – 9th. Potsdam, Germany.

Coll-Brasas, E., Laguna, L., Tárrega, A., Arnau, J. & Fulladosa, E. (2019). Feasibility of dry-cured ham pastiness evaluation using a rheometer. 65th International Congress of Meat Science and Technology (ICoMST). August 4th – 9th. Potsdam, Germany.

Coll-Brasas, E., Possas, A., Berg, P., Egelandsdal, B., Livden, T., Bover-Cid, S. & Fulladosa, E. (2019). Potential microbial risks associated with innovative Fenalår elaboration procedures. 65th International Congress of Meat Science and Technology (ICoMST). August 4th – 9th. Potsdam, Germany.

Coll-Brasas, E., Kåsin, K., Grabež, V., Fulladosa, E, Livden, T., Berg, P., Thauland Håseth, T. & Egelandsdal, B. (2019). Comparison of colour and texture between entire and boned dry-cured leg of lamb. 65th International Congress of Meat Science and Technology (ICoMST). August 4th – 9th. Potsdam, Germany.

In 2017

Coll-Brasas, E., Arnau, J., Gou, P., Lorenzo, J. M., García, J.V., Benedito, J. & Fulladosa, E. (2017). Effect of high pressure at different temperatures on texture of dry-cured ham with different textural characteristics. 63th International Congress of Meat Science and Technology (ICoMST). August 13th – 18th. Cork, Ireland.

Coll-Brasas, E., Arnau, J., Gou, P., Lorenzo, J. M., García, J.V., Benedito, J. & Fulladosa, E. (2017). Effect of temperature during HP processing on colour and colour stability of dry-cured ham. 63th International Congress of Meat Science and Technology (ICoMST). August 13th – 18th. Cork, Ireland.

1 Introduction

Contents

1.1 Dry-cured ham manufacturing processes and main factors affecting its quality

Nowadays, the consumption of dry-cured products is widespread throughout the world and its flavour and texture characteristics are highly appreciated by consumers. However, the production of dry-cured products was originally characteristic for the European countries. For instance, dry-cured pork ham is considered a typical product of the Mediterranean area, Spain and Italy are the main producers, although it is also manufactured outside Southern Europe, China, Middle East region and in the Balkan area. The Mediterranean types of dry-cured ham (e.g., Spanish Traditional Speciality Guaranteed (TSG) "Jamón Serrano", Protected Designation of Origin (PDO) "Jamón de Teruel", PDO "Jamón del Valle de Los Pedroches", PDO "Jamón de Guijuelo", PDO "Dehesa de Extremadura", "Jamón de bellota 100% ibérico PDO Jabugo", Protected Geographical Indication (PGI) "Jamón de Serón" or PGI "Jamón de Trevélez"; Italian "Prosciutto crudo", PDO "Prosciutto di Parma" or PDO "Culatello di Zibello"; French PGI "Jambon de Bayonne" or Croatian PGI "Istarski pršut" among others) are characterized by a dry-salting process and a long maturation period (from 7 to over 24 months). However, the Northern Europe and Balkan types of dry-cured ham (e.g., "Westphalia ham" from pork in Germany, PGI "Njeguški pršut" from pork in Montenegro, PGI "Kraški pršut" from pork in Slovenia, "Zlatiborska pršuta" from pork in Serbia or PGI bone in "Fenalår" from lamb in Norway among others) are characterized by a dry-salting process followed by a smoking and a short maturation period from 2 to 8 months. The smoking process, characteristic of hams from the cold countries, not only provides the typical external colour and flavour, but also an antioxidant activity and prevents mould growth (Lenges, 1986).

Although there are important differences in the elaboration process and on the sensory characteristics of dry-cured hams between countries, all the processes have two steps in common: the salting and the drying steps. Raw material characteristics (genetics, breeds, weight or trimming), the treatments used (such as different salting treatments, smoking, etc.), and the duration of the drying phase are important factors contributing to the quality and characteristics of the final product (Gou *et al.*, 2012).

The characteristics of the raw material are mainly due genetics, rearing (e.g., animal feeding, age or season, breed, among others) factors, peri-slaughter treatments and the ham handling (Alonso *et al.*, 2009; Gou *et al.*, 2012). For Spanish TSG "Jamón Serrano" production, the rear legs of pork (with weights ranging from 9 to 14 kg) are used, whereas rear legs of lamb (with weights ranging from 1.5 to 4 kg) for Fenalår production are used. In the case of bone in production (Figure 1 a) the bones are kept in during salting, whereas for restructured ham production (Figure 1 b), the hams are boned and salted using a tumbler.

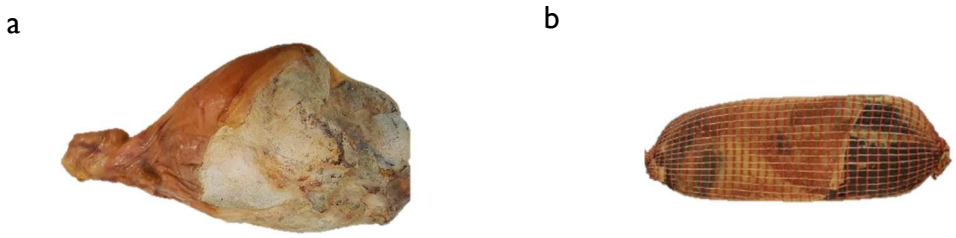

Figure 1. a: Detail of a bone-in dry-cured pork ham and b: Detail of a restructured dry-cured Norwegian lamb ham (b).

In hams with a thicker external fat layer, the dehydration process is slower than in the ones with thinner fat layer which suffer a higher water loss in the external muscular layers. This is due to by different water dynamics, as water molecules move along the inner ham muscles, requiring more time if the ham is of a bigger size (Pinna *et al.*, 2020). This fact is not so crucial for both bone in and restructured Fenalår production, since most of the fat layer is removed. However, a small amount of fat is kept as adipose tissue to give the characteristic flavour. Contrary to bone-in dry-cured pork ham production, there is no specific rule regarding the fat thickness for Fenalår production.

While in Northern European countries consumers prefer lean hams, many consumers in the Mediterranean area accept a certain amount of infiltrated fat in this product (Gou *et al.*, 2012). Hams from pork that contain almost no subcutaneous fat should be avoided, as they end up having a saltier taste, a higher water loss and generally a low quality, and are less appreciated by consumers (Cilla, Altarriba, *et al.*, 2006; Cilla, Martínez, Guerrero, *et al.*, 2006; Resano, Sanjuán, *et al.*, 2010a).

The pH in the *Semimembranosus* muscle at >24h *post mortem* (pH_{SM24h}) is another important parameter that affects the quality of the ham. However, the pH values can vary considerably between the muscles of the same ham (Arnau *et al.*, 1995; Gou *et al.*, 2012). For microbiological safety reasons, the majority of authors recommend avoiding green hams that have a $pH_{SM24h} > 6.2$ (Leistner, 1986) for both pork and lamb. Hams with a pH_{SM24h} higher than 6.2 also leads to an increase in the incidence of appearance problems (Dark, Firm and Dry; DFD hams), such as a shiny aspect of the lean meat and phosphate precipitates (Arnau *et al.*, 1998), and texture problems, such as a soft texture in the internal part of the ham (Morales *et al.*, 2007; Pinna *et al.*, 2020). Hams with a $pH_{SM24h} < 5.6$ for pork (Petrova *et al.*, 2015; Ruiz-Ramírez *et al.*, 2006) and a $pH_{SM24h} < 5.8$ for lamb (Christie, 2007) are also not recommended as they are more prone to develop pastiness defects (Tabilo *et al.*, 1999), especially when producing with low salt content (Ruiz-Ramírez *et al.*, 2006).

The salt is essential for the elaboration of dry-cured hams. Dry-cured ham industry seeks to obtain hams with sufficient salt concentration not only to ensure the product safety during the process (since the salt contributes to the reduction in the water activity) but also to provide the characteristic colour and taste typical of the cured products, and to reduce the incidence of soft and/or defective textures. However, salt absorption can be different between hams and/or between the batches since many factors can influence. The salt content in dry cured hams (expressed on dry matter basis) can range between 8 to 15 %, being the concentration higher in the case of Fenalår production in comparison to dry-cured pork ham. This variability represents an important problem for the sector, since a high salt content represents health risk (an excessive sodium intake can produce adverse cardiovascular effects related to hypertension (Mozaffarian *et al.*, 2014)) and flavour problems, whereas a low salt content produces textural and appearance problems in addition to compromising the safety of the product. Moreover, the reduction of salt content in dry-cured ham batches in which the salt content is heterogeneous, leads to a high percentage of hams with defective textures and safety concern. However, nowadays, the consumption of meat products with lower salt content is becoming more important in agreement with NAOS strategy (Strategy for Nutrition, Physical Activity and the Prevention of Obesity) which recommends to reduce the salt content of the products in a heart-healthy diet (AESAN, 2005). This strategy not only increases the microbiological risk, but also involves important technological changes in the industry, as the high incidence of defective hams, cause important problems during slicing due to a high adhesiveness between slices (Gou *et al.*, 2008). Therefore, there is a strong interest not only of the industry but also society to have healthy products with nutritional claims such as "salt reduced" (25% less than the reference product in agreement with the EU regulation (1924/2006)), as long as the product has the same quality as the traditional product. Non-destructive *in-line* systems are currently being introduced in the industry that validate such nutritional claims (Giró-Candanedo *et al.*, 2020).

However, a system to individually add the appropriate amount of salt according to the characteristics of each ham, or either a methodology to obtain hams with a certain amount of salt is not available and not only would improve the process but also the quality of the final product.

I.2 Development of texture in dry-cured ham

The texture of dry-cured ham is an important quality criterion not only for the consumers but also for the industry (Cilla *et al.*, 2005; Resano, Sanjuán, *et al.*, 2010b; Schivazappa & Virgili, 2020). The development of texture in dry-cured products is the consequence of various factors such as raw material (genetics, pH, enzymatic activity,

among others) and processing conditions. Moreover, dry cured ham anatomy is complex, and it has a big heterogeneity which can vary depending on the selected sampling area (Guerrero, Guàrdia & Arnau, 1998) due to the anatomical differences and the processing conditions (such as duration, relative humidity and/or the temperature of the different stages of the process (Arnau, Guerrero & Gou, 1997; Guerrero, Gou & Arnau, 1999; Pinna *et al.*, 2020; Ruiz-Ramírez *et al.*, 2005)). Throughout the elaboration process of dry-cured ham, different biochemical reactions occur resulting in the modification of the sensory properties of the ham, leading to the flavour and texture development and also its textural characteristics (Hansen-Moller, Hinrichsen, & Jacobsen, 1997; Petrova *et al.*, 2015; Ruiz et al., 1999). In particular, during curing, the main biochemical mechanism is the proteolysis activity, which contributes to the progressive texture development by the breakdown of the muscle proteins (Monin *et al.*, 1997; Petrova *et al.*, 2015; Sentandreu, Coulis & Ouali, 2002), to the taste through the generation of small peptides and free amino acids and to the aroma by further degradation of some free amino acids (Toldrá & Flores, 1998). However, although these changes contribute to improve the quality of dry-cured ham, when the extent of proteolysis is exceeded, the structure is severely damaged and unpleasant textures appear (Arnau *et al.*, 1998; Pinna *et al.*, 2020; Ruiz-Ramírez *et al.*, 2006; Toldrá, 2006) which negatively affects consumers acceptability (Cilla *et al.*, 2006). Therefore, meat with a high proteolytic potential is less suitable for the elaboration of dry-cured hams, especially for a salt reduced elaboration process (Petrova *et al.*, 2015; Virgili *et al.*, 1995). The selection of raw material could be improved by selecting breeds or husbandry practices less prone to develop high proteolysis activity.

The main texture defects are excessive softness (Pinna *et al.*, 2020) and pastiness (Morales *et al.*, 2007), which can produce important problems at industry during slicing (Gou *et al.*, 2008) and reduce the quality of the product (Morales *et al.*, 2008). Pastiness defect is as a mouth sensation described as the feeling, like a flour-water paste, during the mastication process (Guerrero *et al.*, 1999) and should be well-differentiated from softness that is as a palatable texture in the mouth (Resano *et al.*, 2010a). The pastiness defect is mainly associated with an excessive proteolytic activity (Cilla *et al.*, 2005) which produce irreversible damages in the structure (see Figure 2), whereas softness is due to the use of high temperatures during ageing (Arnau *et al.*, 1997).

In dry-cured ham production, pastiness defect has a considerable incidence for the industry, estimated to up 12 % (Tapiador & García-Garrido, 2003) with the *Biceps femoris* is the muscle the more prone to develop this defect.

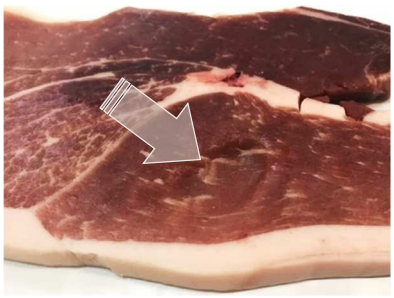

Figure 2. Detail of pastiness defect in the *Biceps femoris* muscle of dry-cured pork ham.

To date, different destructive instrumental techniques have been used to characterize the texture of dry-cured ham at the end of process. Instrumental texture analyses such as Texture Profile Analysis (TPA) and/or Stress Relaxation (SR) test have been proved to be good to evaluate the texture of dry-cured ham (Morales *et al.*, 2007) due to their reproducibility and possible standardization (Brady, 1985). The TPA test, which simulates the mastication process by applying compression-decompression cycles (Bonilla *et al.*, 2002; Guerrero *et al.*, 1999; Ruiz-Ramirez *et al.*, 2005), has been used to evaluate different texture parameters on dry-cured ham such as hardness, cohesiveness, elasticity and/or adhesiveness, among others. For instance, Ruiz-Ramírez *et al.*, (2005) used different texture parameters such as hardness, cohesiveness and/or elasticity to correlate them with the humidity of the product. On the other hand, the SR test, which applies a compression force using a cylindrical probe that keeps the sample deformed during a fixed period of time, evaluates the viscoelastic nature of the sample (Morales, Guerrero, *et al.*, 2007). Morales, Serra, Guerrero, *et al.*, (2007) used SR test to evaluate soft textures depending on the meat quality characteristics and the processing conditions. However, instrumental texture analyses are only able to detect and quantify certain physical parameters that have to be later interpreted in terms of sensory perception (Ruiz-Ramírez, Gou & Arnau, 2003). Sensory analysis allows to evaluate the intensity of the defect (Guàrdia *et al.*, 2010), as well as product acceptability and other sensory parameters which are difficult to quantify with instrumental techniques. In some studies, pastiness has also been related to the proteolysis index (PI), assessed with a chemical analysis and expressed as a percentage of the ratio between non-protein nitrogen (NPN) and the total nitrogen (TN) (Careri *et al.*, 1993; Schivazappa *et al.*, 2002). López-Pedrouso *et al.*, (2018) also proved the proteolysis index to be a reliable indicator of the extent of protein hydrolysis and several studies also demonstrate proteolysis index as good estimate of pastiness defect (Careri *et al.*, 1993; García-Garrido *et al.*, 1999; Morales *et al.*, 2008; Ruiz-Ramírez *et al.*, 2006). Rheological measurements could be useful to evaluate pastiness. However, quantifying the sensory mouth-feel feelings

with an instrumental technique is not easy (Laguna *et al.*, 2017), and effect of different experimental conditions needs to be evaluated.

These textural problems can be addressed at industrial level by optimising the elaboration process. However, despite the importance and the economic impact of the problem, this aspect has not been deeply studied yet. A system to characterise the product using *in-line* non-destructive technologies is neither available on the market. Some authors used non-destructive technologies such as Magnetic Induction (MI) (Schivazappa *et al.*, 2017), Computed Tomography (CT) (Harkouss *et al.*, 2018) or ultrasounds (Contreras, Benedito & Garcia-Perez, 2021) to predict the product characteristics in the whole ham. In sliced dry-cured ham Time Domain Reflectometry (TDR) to classify hams according to their pastiness defect (Rubio-Celorio *et al.*, 2013) or Near Infrared Imaging (NIRs) to predict sensory attributes (Pérez-Santaescolástica, *et al.*, 2019, Fraeye, *et al.*, 2019) has been used. However, to predict pastiness defect in whole hams during the elaboration process is still not possible.

I.3 Colour formation in dry-cured ham

The colour is another important quality attribute for dry-cured ham, because the consumers base their purchase decision mainly on the appearance of the product, which emphasizes the importance of maintaining an attractive colour of the product. Traditionally, dry-cured hams from both pork and lamb are generally cured using salt and nitrite agents, which plays an important role in the color development and as a preservative (antioxidant properties) (Sebranek & Bacus, 2007; Skibsted, 2011), exerting an anticlostridial effect in cured meat.

However, Food and Agricultural Organization (FAO) and World Health Organisation (WHO) and also consumer awareness regarding the use of additives, drive the producer to reduce the use of nitrites, since an excessive amount could be detrimental to human health. In order to reduce and/or eliminate nitrites in cured products is necessary to first ensure the adequate amount of salt for the safety of the product as well as to reformulate and modify the manufacturing processes to obtain "clean label" products ("Natural" and or "without additives").

For instance, in the Italian PDO Parma ham, the omission of nitrifying agents is mandatory (Parolari, 1996; Toldrá, 2002), whereas in Spain this is becoming popular. However, the elaboration processes and the raw materials used in the Italian Parma ham industry differ from the ones used for Spanish or Norwegian dry-cured ham production. Thus, it is important that the elimination of the curing agents does not affect the typical colour characteristics of the traditional product.

In this sense, the Figure 3 shows the differences of colour of a dry-cured ham section when produced using nitrites (a) or without nitrites (b), obtaining a pale and brownish colour of the ham when nitrites are omitted.

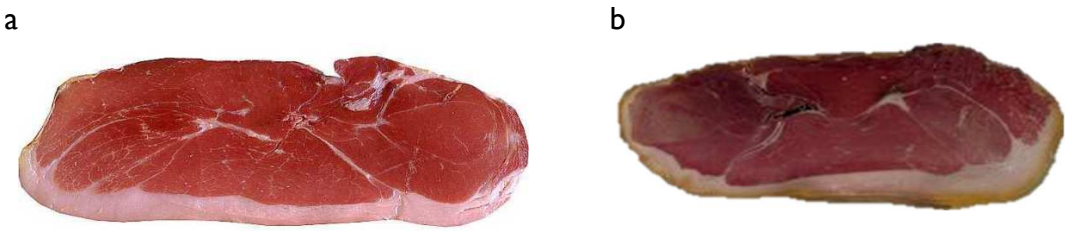

Figure 3. Colour difference of dry-cured ham cured with (a) and without nitrites (b).

When curing using nitrites, the nitrite is reduced to nitric oxide (NO), which is combined with the myoglobin pigment forming nitrosylmyoglobin (being the principal chromophore), responsible for the stable reddish colour in cured meat products (Arnau *et al.*, 2013; Cassens *et al.*, 1979; Chensha *et al.*, 2016; Colioni, 2010) (see Figure 4). However, when nitrite is omitted nitrosylmyoglobin is not formed, instead Zn-protoporphyrin (ZnPP) is the responsible of the red colour (Wakamatsu *et al.*, 2004) (see Figure 4).

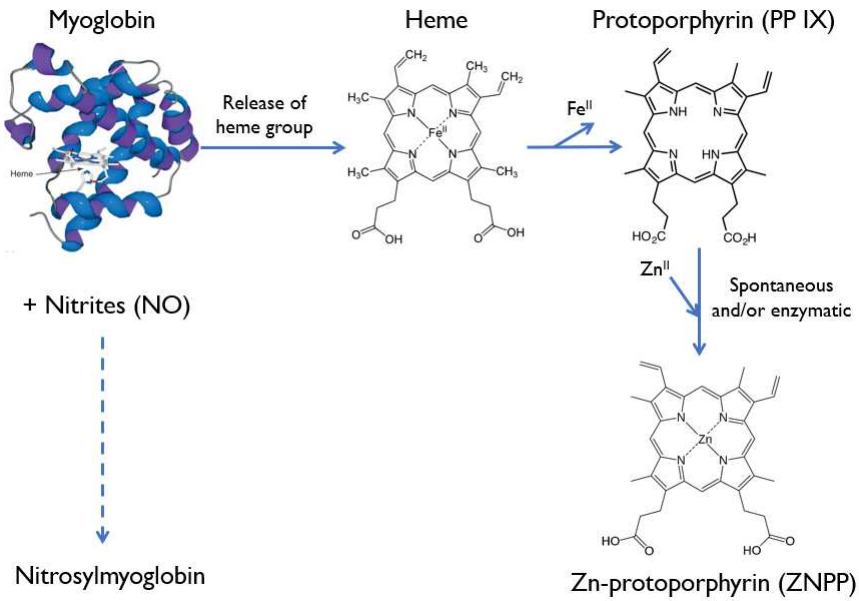

Figure 4. Schematic representation of both Nitrosylmyoglobin and Zinc protoporphyrin formation. Obtained and modified from Becker *et al.*, (2012) and Wilson & Reeder (2006).

In brief, the heme group of myoglobin is the main responsible pigment of both meat and cured products. The heme group, consisting on a tetrapyrrole ring structure, also known

as protoporphyrin IX, has an iron atom inside. However, under certain conditions, this coordinated heme iron can be released and the protoporphyrin formed can incorporate a zinc atom in the heme moiety forming the ZnPP (Labbé & Dewanji, 2004; Mwangi *et al.*, 2014). The ZnPP is the main stable pigment in non-nitrified hams (Wakamatsu, Nishimura & Hattori, 2004). The ZnPP formation is mainly believed to be of enzymatic origin (Wakamatsu, Nishimura, *et al.*, 2004), with the endogenous enzyme ferrochelatase (also known as Znchelatase) playing a crucial role in its formation (Benedini, Raja & Parolari, 2008; Ghadiri Khozroughi *et al.*, 2018).

Unlike nitrosylmyoglobin, ZnPP requires longer curing processes to achieve the reddish colour, as time is an important factor for ZnPP formation (De Maere *et al.*, 2017; De Maere *et al.*, 2016) and has a lower redness intensity. In line with the "clean label" tend, a better knowledge of the factors involved in ZnPP formation could help to develop strategies to obtain a final product with a better and homogenous reddish colour. To date, however, the colour formation of Fenalårs under different salting treatments or when no nitrite is added has not been studied yet, nor has its impact on product safety.

I.4 Use of mathematical models on dry-cured ham elaboration process

According to McMeekin *et al.*, (2008), a model can be defined as "the description of a system, theory, or phenomenon that accounts for its known or inferred properties and may be used for further study of its characteristics". However, in science, a model is a simplified description of relationships between observations of the system (responses) and the factors that are believed to cause the observed responses. This description can be expressed quantitatively in one or more mathematical relationships or equations, which can be used to predict the response of the system to changes in the variables (McMeekin *et al.*, 2008). In this sense, mathematical models are very useful to predict the final characteristics of the product, the quality parameters or the safety evaluations for dry-cured ham and allow to avoid the expensive, lengthy and usually destructive experiments (Chabbouh *et al.*, 2012). However, a compromise between the simplicity of the model and a good description of the experimental results should be ensured, by analysing the particular model to be used according to the objective of the study to be carried out (Gómez *et al.*, 2019).

In this sense, food safety authorities have applied predictive microbiology models as effective tools to assess the microbiological safety consequences of changes in food processing and preservation (Messens *et al.*, 2018), including salt reduction in Spanish cured meat products (Zurera-Cosano *et al.*, 2011). In this case, predictive models are mathematical tools to estimate microbial behaviour in foods, i.e. growth, transfer, survival or inactivation, as affected by a range of intrinsic and environmental factors, such

as pH, a_W, nitrite and temperature, without the need for time-consuming challenge tests (Perez-Rodriguez & Valero, 2013).

Other mathematical models have also been used to predict the time course of salt content, water content, PI, a_w and total weight loss during the initial stages (salting and post-salting) of dry-cured ham production with good prediction results (Harkouss *et al.*, 2018). In another study, mathematical models combined with computational textures obtained by Magnetic Resonance Imaging (MRI) were applied to non-destructively evaluate salt diffusion in Iberian hams at the end of the post-salting stage (Caballero *et al.*, 2016). Mathematical models have also been proposed to compensate for temperature fluctuations when using NIRs to determine sodium content in sliced vacuum-packed dry-cured ham (Campos *et al.*, 2018). However, a model for predicting texture development depending as a function of raw material characteristics and processing conditions has not yet been developed.

I.5 High pressure processing (HPP)

Various technologies are used in the food industry to improve the quality of the final product, and High Pressure Processing (HPP) is one of the most important. In short, HPP is a non-thermal technology of food preservation used for a wide range of products to ensure their microbiological safety and also to extend the shelf-life of the product while maintaining its quality. The applied pressure ranges from 200 to 800 MPa for the shortest possible time (Lamballerie-Anton, Taylor & Culioni, 2002), with the most common treatment for dry-cured ham being at 600 MPa for 6 minutes.

One of the main advantages of this technology is that the pressure is transferred immediately and isostatically to the product, regardless of size, shape, and food composition resulting in highly homogenous products (Deliza *et al.*, 2005).

Figure 5. Dry-cured ham samples ready to be submitted to an HPP treatment.

During the process, the pressure chamber is loaded, closed and degassed, and the pressure is transmitted by the pumps through a liquid, generally water. When the defined

pressure is reached, the valves are closed, and the pressure remains constant during a defined period of time. During the treatment, HPP propagates evenly and instantaneously and the product, or its components undergo a change in volume under pressure. Precisely, high pressure accelerates reactions involving a volume change at the molecular level and they are key to understanding the biological effects on macromolecules and microorganisms (Hugas, Garriga & Monfort, 2002). HPP treatments also affect ultrastructure, by inducing protein denaturation, aggregation or gelation (Cheftel & Culioli, 1997) which affects product quality characteristics such as texture and colour. These changes in structure result from the rupture of non-covalent interactions within the protein molecules and a subsequent re-formation of intra- and inter- molecular non-covalent bonds within or between the protein molecules (Carlez, Veciana-Nogues & Cheftel, 1995; Cheftel & Culioli, 1997). The degree of denaturation of myofibrillar protein varies according to pressure, processing time, temperature, and pH (Garcia-Gil *et al.*, 2014; Huppertz, Fox & Kelly, 2004). Moreover, when pressure is applied together with heat, the modifications of ultrastructure are more severe/intense than when pressure or temperature are applied alone (Cheftel & Culioli, 1997), which leads to significant colour changes (decrease in redness intensity and increase in lightness due to protein denaturation or modification (Clariana *et al.*, 2012; Fuentes *et al.*, 2010)). Previous studies had already demonstrated that HPP treatments can also lead to lipid oxidation in dry-cured ham samples (Fuentes *et al.*, 2010) affecting the volatile profile (Rivas-Cañedo *et al.*, 2021) and altering the sensory characteristics of the final product (Clariana *et al.*, 2011; Fuentes *et al.*, 2010; Serra *et al.*, 2007). Several authors had also described that, after HPP treatment, hams experience an increase in hardness and fibrousness, while pastiness decreases (Fulladosa *et al.*, 2009; Lorido *et al.*, 2015). In other studies, moderate thermal treatments (about 30 °C) were applied on dry-cured ham samples to improve the texture and reduce the occurrence of soft textures (Gou *et al.*, 2008; Morales, Arnau, *et al.*, 2008; Morales, Serra *et al.*, 2007). However, the influence of temperature applied together with pressure has not yet been studied before. Further studies are also needed to minimize the effects on aroma and colour characteristics.

2 Objectives

The main objective of this PhD Thesis dissertation was to develop and evaluate tools to improve the processing and quality of dry-cured ham.

In order to achieve the main objective, several specific goals were planned:

1) (i) To study the effect of raw material pH and processing conditions (salting time, drying temperature and target weight loss) on texture development, white film intensity and tyrosine crystals incidence in dry-cured ham; (ii) to develop texture prediction models with the aim of optimizing dry-cured ham elaboration production process.

II) (i) To characterize colour, texture and physicochemical properties of restructured Fenalårs using Standard Salting, Salt Reduced and a Non-Nitrite Salt Reduced treatments; (ii) to assess the microbiological safety implications of different salting treatments in production process.

III) (i) To define the optimal measurement conditions (probe size and anatomical location) of a non-destructive Instrumental Texture Analysis on the Surface of the dry-cured ham (ITAS), to evaluate whether or not the product has the appropriate texture to be sent to the market; (ii) to evaluate the usefulness of including information on weight loss and subcutaneous fat thickness to improve the prediction of product texture; (iii) to evaluate the use of ITAS to define the internal textural characteristics of ham.

IV) (i) To evaluate the rheological behaviour of water extracts obtained by mimicking *in vitro* mastication of dry-cured ham samples with different pastiness intensities when analysed at different temperatures (25 and 37 °C); (ii) to evaluate the changes in sensory pastiness and flow behaviour of water extracts obtained from samples with modified texture after being subjected to HPP at different temperatures (7 °C, 20 °C and 35 °C); (iii) to perform a characterisation of commercial dry-cured ham samples in terms of sensory pastiness and viscosity of the water extracts.

V) To study the effect of HPP at different temperatures (7 °C, 20 °C and 35 °C) on instrumental and sensory texture, colour and colour stability of samples with different textural characteristics (samples with no, medium or high pastiness defect).

Material and methods | 3

Contents

In order to achieve the main objective described in the previous section, several studies were conducted using different methods and techniques. Each of these studies included in this PhD Thesis dissertation as a separate paper, represents a step towards achieving the goal.

Briefly, Paper I, evaluated the effect of raw material pH (pH_{SM24h}) and processing conditions (salting time, drying temperature and target weight loss) on texture. All the factors were later used to develop a mathematical model to predict the optimal texture in dry-cured pork ham which could be used to optimize manufacturing procedures in the industry. For this purpose, different temperatures (10, 15 and 20 °C) and weight losses (33, 36 and 40%) were evaluated.

In the Paper II, the effect of different salting treatments (Standard Salting (SS), Salt Reduced (SR) and a Non-Nitrite Salt Reduced (NNSR) treatments) on restructured Fenalår as well as the safety implications of the process were evaluated using predictive microbiology. The effects on colour, texture and physicochemical properties of the final product were also studied. For this purpose, the Fenalårs were characterised during the process by measuring pH and a_w. Subsequently, the behaviour of health-threatening microorganisms such as *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus* and *Clostridium botulinum* non-proteolytic and proteolytic was assessed and compared by applying the predictive models available in ComBase Predictor (www.combase.cc).

Although the mathematical models from papers I and II can help to optimise the quality and safety of dry-cured ham, the industry also need instruments and methodologies to analyse product characteristics at the end of the process. In Paper III, a new texture analysis on the surface of the whole ham was developed to non-destructively predict the internal texture of the ham and whether it has reached the end of the process. Therefore, three different anatomical measurement locations and three different probe sizes (large, medium and small) were used to simulate tactile texture assessment performed by an expert in the industry.

In the Paper IV, a first step for the development of a new method for the instrumental assessment of pastiness defect in the laboratory was performed. For this reason, the rheological behaviour of water extracts which were obtained *in vitro* mimicking the mastication of dry-cured ham samples with different pastiness intensities (no, medium and high pastiness) were studied at different temperatures 25 and 37 °C. Rheological changes due to different high hydrostatic pressure processing at different temperatures (7 °C (HPP7), 20 °C (HPP20) and 35 °C (HPP35)) were also evaluated.

Despite efforts to optimize the manufacturing procedures in order to achieve the maximum quality, part of the hams may still present pastiness defect. In the Paper V, a corrective action to improve defective dry-cured ham textures was evaluated. For this purpose, HPP treatments were applied at different temperatures (7 °C, 20 °C and 35

°C) on sliced dry-cured ham with different pastiness intensities in order to correct the texture without producing significant changes on the colour, sensory and the physicochemical characteristics, which were evaluated accordingly.

All the abovementioned Papers have been published or submitted to international scientific journals listed in the Journal Citation Index (JCI) and the material and methods have been described in detail in the relevant sections. However, some general aspects related to the methodologies and procedures used in this Thesis that are not described in the papers are explained below.

3.1 Manufacturing processes of dry-cured ham from pork and lamb (Fenalår)

In this Thesis, bone in dry-cured pork hams and restructured dry-cured Norwegian lamb hams (Fenalår) were produced, controlled and analysed. Dry-cured pork ham is considered a typical product of the Mediterranean area, whereas Fenalår is a traditional dry-cured Norwegian lamb ham. Although there are important differences between products in terms of sensory characteristics and on the manufacturing process (see Table 1), both products have some steps in common: preparation of the raw material, salting, a cold phase and a drying process. In both, a cold phase at 2 – 4 °C is needed to ensure product safety and during drying the temperature is increased progressively until reaching the target ham weight loss. During this process, the characteristic aroma and taste of the ham are developed.

During the production of bone-in dry-cured ham, all the process is performed with the bone inside. Cutting of the skin in different shapes and a rubbing is performed but no netting is necessary. The hams are pile dry-salted followed by a long drying process (from 9 to 12 months) because of the size of the ham.

In contrast, in restructured Fenalår production, the hams are first boned, salted, shaped using nets and thereafter dried. It is important to mention that, for restructured Fenalår production, two boned legs are needed in order to produce a restructured Fenalår. In this case, a salting using tumblers and/or vacuum bags is performed instead of the traditional pile salting procedure. A smoking step is usually applied closer to the end of the curing process. In this case, the whole process is shorter (from 3 to 4 months) in comparisons to bone-in dry-cured ham.

Table 1: Comparisons between bone in dry-cured pork ham and restructured dry-cured lamb ham elaboration processes.

Bone in dry-cured pork ham		**Restructured dry-cured lamb ham, Fenalår**	
Cutting	Skin trimming maintaining a minimum thickness of subcutaneous fat.	Cutting	Boning, connective tissue removed and part of the subcutaneous fat.
Rubbing	To eliminate the blood in veins and arteries and to facilitate a good distribution of the curing salts.	Rubbing (manual)	To increase the muscle binding using fine salt.
		Netting	Double rubber
Salting	Hams are salted using a mixture of sodium chloride and curing salts (in some cases) and pile salted at 0-4 °C.	Salting	Hams are salted using a mixture of nitrite-salt and fine salt in vacuum bags/tumblers.
Cold phase	Hams are stored at 3-4 °C with a RH of 75-80% for 4-6 weeks.	Cold phase	Hams are stored at 2-4 °C until equalization for 4-6 weeks.
Drying	The temperature is progressively increased from around 10-12 °C to approximately 28-34 °C with a RH of 60-80% for 3-4 months until reaching a weight loss of 33-40% depending on the dry-cured ham type: Bodega hams from 7 to 9 months; Reserva from 9 to 12 months or Gran Reserva from 15 to 18 months.	Drying	Hams are first dried at 18 °C with a RH of 60% for 48 h and then are moved to 13 °C and a RH of 60-74% for about 2 months until reaching a weight loss of 34-36%.
		Smoking	Closer to the end of the drying step, hams are smoked with friction-smoke from beech wood.
		Pressing	After smoking to increase muscle binding and at the end of process to shape the ham.
Whole ham		Whole ham	
Ham section		Ham section	

3.2 Analytical methods and techniques

In order to achieve the specific goals set out in each paper of the Thesis, different techniques and analyses were used in both pork and lamb products (Figure 6).

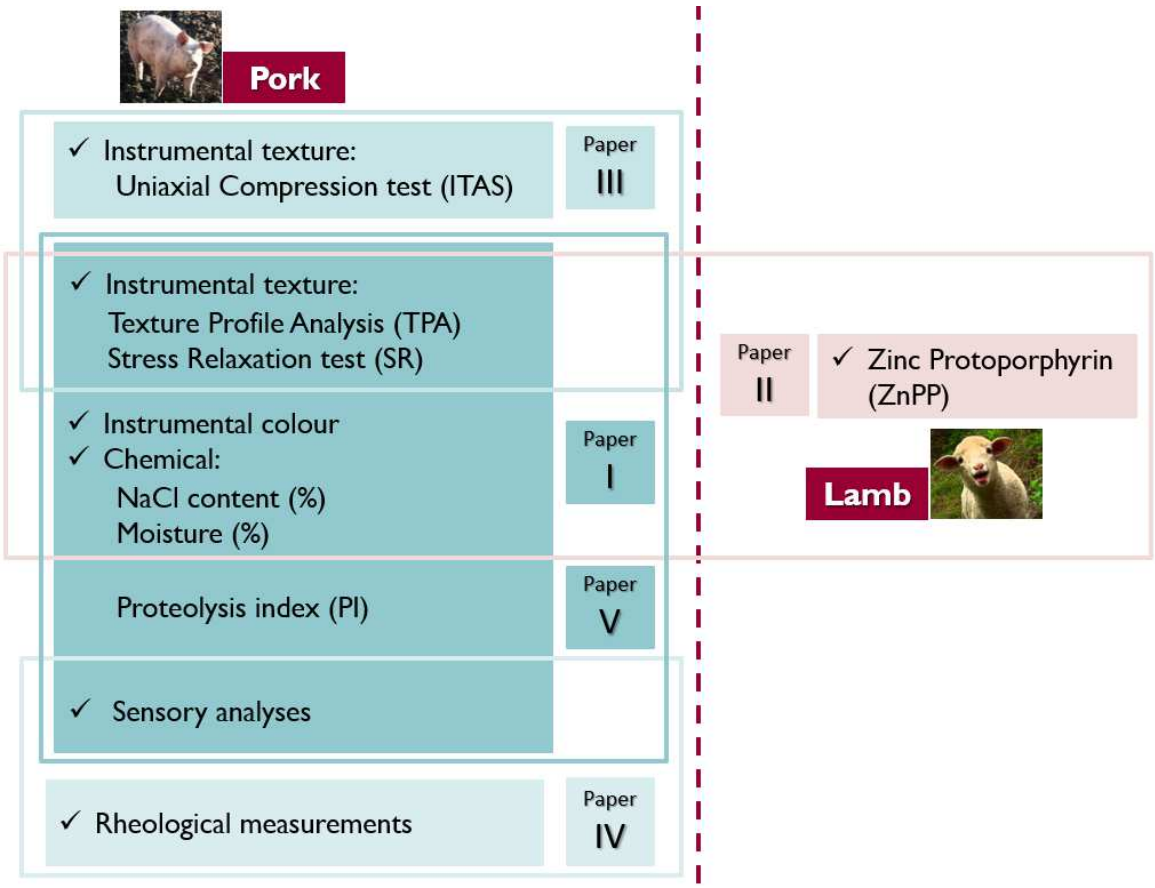

Figure 6. Schematic representation of the used analyses and techniques on each of the papers of this Thesis.

3.2.1 Instrumental analysis

Colour was determined using a colorimeter Minolta Spectrophotometer CM 700d (Konica Minolta Optics, Inc. Japan) (Figure 7) in the CIE–LAB space (Commission Internationale de l'Eclairage, 1976) lightness (L*), redness (a*) and yellowness (b*)) with an illuminant D65 with 2°.

Figure 7. Colorimeter used to measure the colour in the CIE-LAB space.

Texture was measured using different tests:

Stress Relaxation test (SR): was performed using a Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, England) equipped with 30 kg load cell and a 60 mm diameter compression plate. Samples were compressed to 25% of their original weight/height, perpendicular to the muscle fibre bundle direction, at a crosshead speed of 1 mm/s and at a temperature of 4 °C ± 2 °C. Parameters: initial force (F_0, kg) and force decay at 2 s (Y_2) and 90 s (Y_{90}). The Y(t) was calculated according to Morales, Guerrero, *et al.*, (2007) as follows:

$$Y_{(t)} = \frac{F_0 - F_{(t)}}{F_0}$$

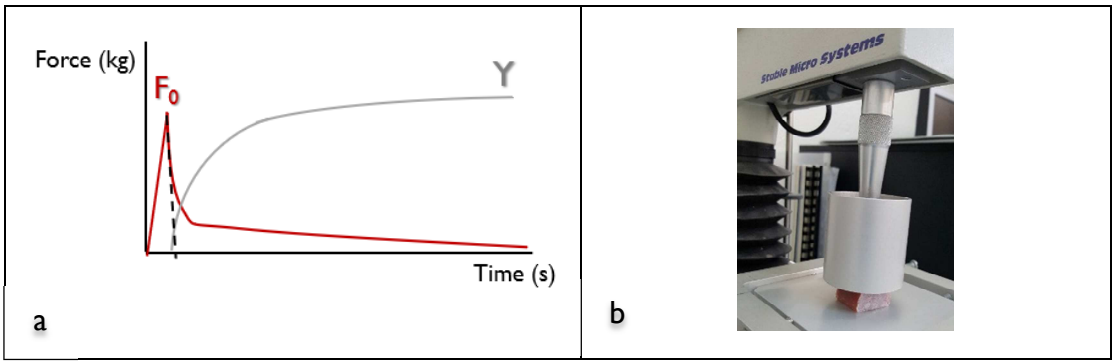

Figure 8. Force versus time curve from a Stress Relaxation test (a). Sample compressed during a Stress Relaxation test (b).

Texture Profile Analysis (TPA): was performed according to Morales, Guerrero, *et al.*, (2007) using a Texture Analyser TA HP plus (Stable Micro Systems Ltd., Surrey, England) provided with 250 kg load cell and a 75 mm diameter compression plate. Samples were compressed twice to 75% of their original height (time = 0 seconds between the two compression cycles), perpendicular to the muscle fibre bundle direction, at a crosshead speed of 1 mm/s and at a temperature of 4 °C ± 2 °C. Force versus time was recorded and the following parameters: hardness (kg), cohesiveness (dimensionless) and springiness (dimensionless).

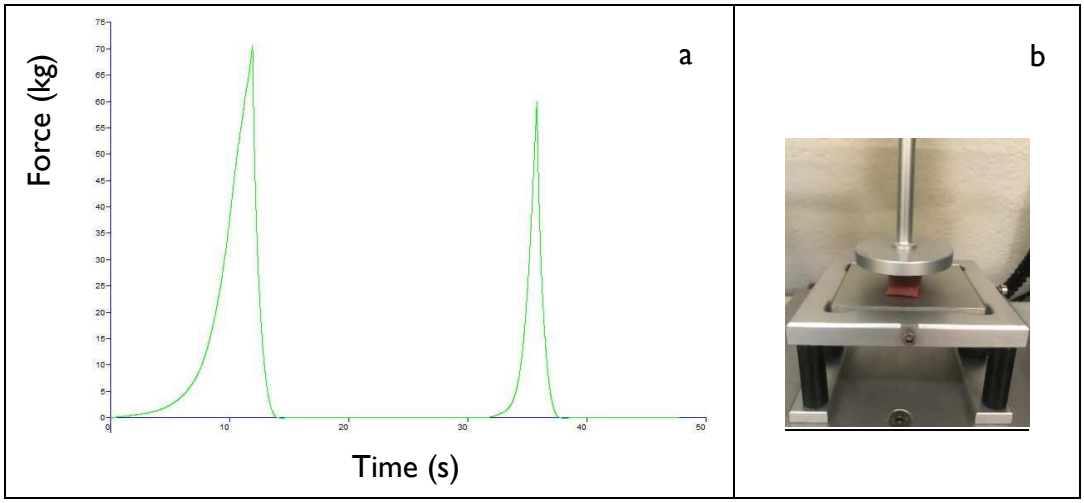

Figure 9. Force versus time curve from a Texture Profile Analyses (TPA) test (a). Sample compressed during a TPA test (b).

Instrumental texture analysis at the surface (ITAS) of dry-cured ham: was performed using a Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, England) provided with 30 kg load cell and different probes (Figure 10) and compression was performed at a crosshead speed of 40 mm/s. Maximum compression force (F_{max}, N); area under the curve until maximum compression force (A_1, N·mm) representing the total work performed to compress the ham; area under the curve after the maximum compression force (A_2, N·mm) representing sample recovery to its initial state after being compressed (related to some extent with the elasticity of the product), and the slope of the curve between the 20% and 80% of the maximum compression force (Slope, Ng/mm) representing the Young's modulus or modulus of elasticity.

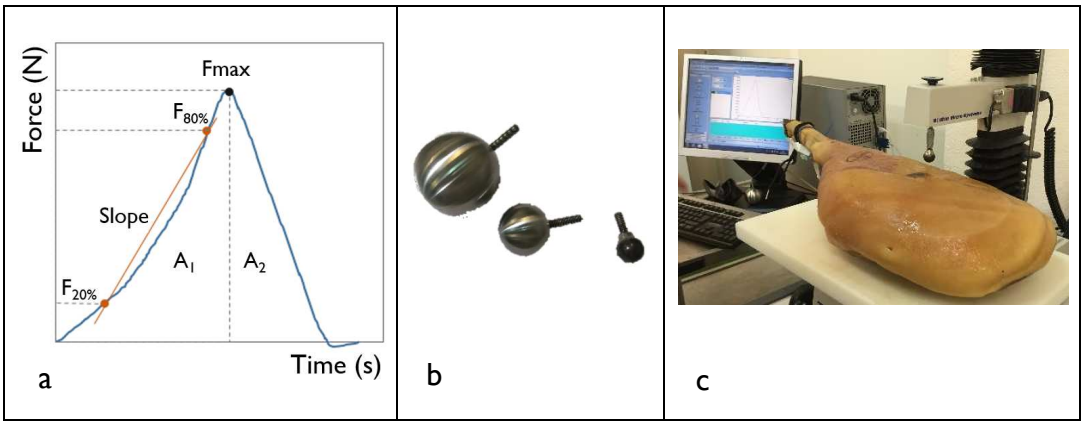

Figure 10. Example of acquired force/time curve and textural parameters determined (a). Large, medium and small probes used for instrumental texture evaluation on the surface of the ham (b). Instrumental texture analysis on the ham surface (c).

Rheology: flow curves of liquid extracts were obtained using a RheoStress controlled stress rheometer monitored by Rheowin Pro Software v. 3.1 (Haake, Karslruhe, Germany) with a parallel-plates sensor system (60mm) with a gap between plates of 0.5mm. Sample was placed in a pre-heated plate at 37 °C. The temperature was controlled by using a Phoenix P1Circulator device (Thermo Haake, Karslruhe, Germany); to avoid evaporation, a temperature cover was used. Flow curves were obtained from stepped shear stress ramp (steady state approximation: 20 s per point). Ranges of shear stresses, in logarithmic distribution, were used to obtain shear rates between 0.05 and 100 s^{-1}. Data from the flow curves were fitted to the Ostwald de Waele fit ($\sigma = K\dot{\gamma}^n$), where *k* (Pa·s) is the consistency index and *n* is the flow index.

Figure 11. Rheometer used to perform the analysis with the parallel-plates of 60 mm of diameter.

3.2.2 Physicochemical analysis

Salt content (NaCl): determined according to ISO 1841-2 (1996) by using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland).

Moisture: drying at 103 ± 2 °C until a constant weight was according to AOAC (1990).

Proteolysis index (PI): Non-protein nitrogen content (NPN) was determined by precipitation of proteins with trichloroacetic acid (Gáspár, 1984) followed by determination of the total nitrogen (TN) in the extract with the Kjeldahl method ISO 937 (1978). Proteolysis index (PI) was calculated as a percentage of the ratio between NPN and TN.

Zn-protoporphyrin content (ZnPP): quantitatively extracted in subdued light conditions with ethyl acetate/acetic acid/dimethyl sulfoxide solvent mixture (10:2:1, v/v/v) as described by Bou, *et al.*, (2018).

3.2.3 High Pressure Processing (HPP)

Samples were submitted to 600 MPa during 6 min in NC Hyperbaric WAVE 6000/120 equipment (NC Hyperbaric, Burgos, Spain) using an initial temperature of 7 °C (HPP7), 20 °C (HPP20) and 35 °C (HPP35).

Figure 12. High Pressure Processing machine used for testing.

3.3 Mathematical algorithms

An algorithm is a finite sequence of well-defined computer-implementable instructions, typically to solve a class of problems or to perform a computation. Algorithms are used as specifications for performing calculations, data processing and automated reasoning (Math Vault, 2020; Merriam-Webster (n.d.)). In the present PhD Thesis, mathematical algorithms were developed or used.

In paper I, a predictive model was developed to predict the texture of the dry-cured ham (Y_{90}) at the end of processing as a function of the processing characteristics (salting time, weight loss and drying temperatures) was developed.

In the case of paper V, predictive microbiology models were used in order to evaluate the safety of the restructured Fenalår according to treatment by using intrinsic and environmental factors, such as pH, a_w, nitrite and temperature.

3.4 Working plan

Another way to gather all this concepts together is presented in Figure 13.

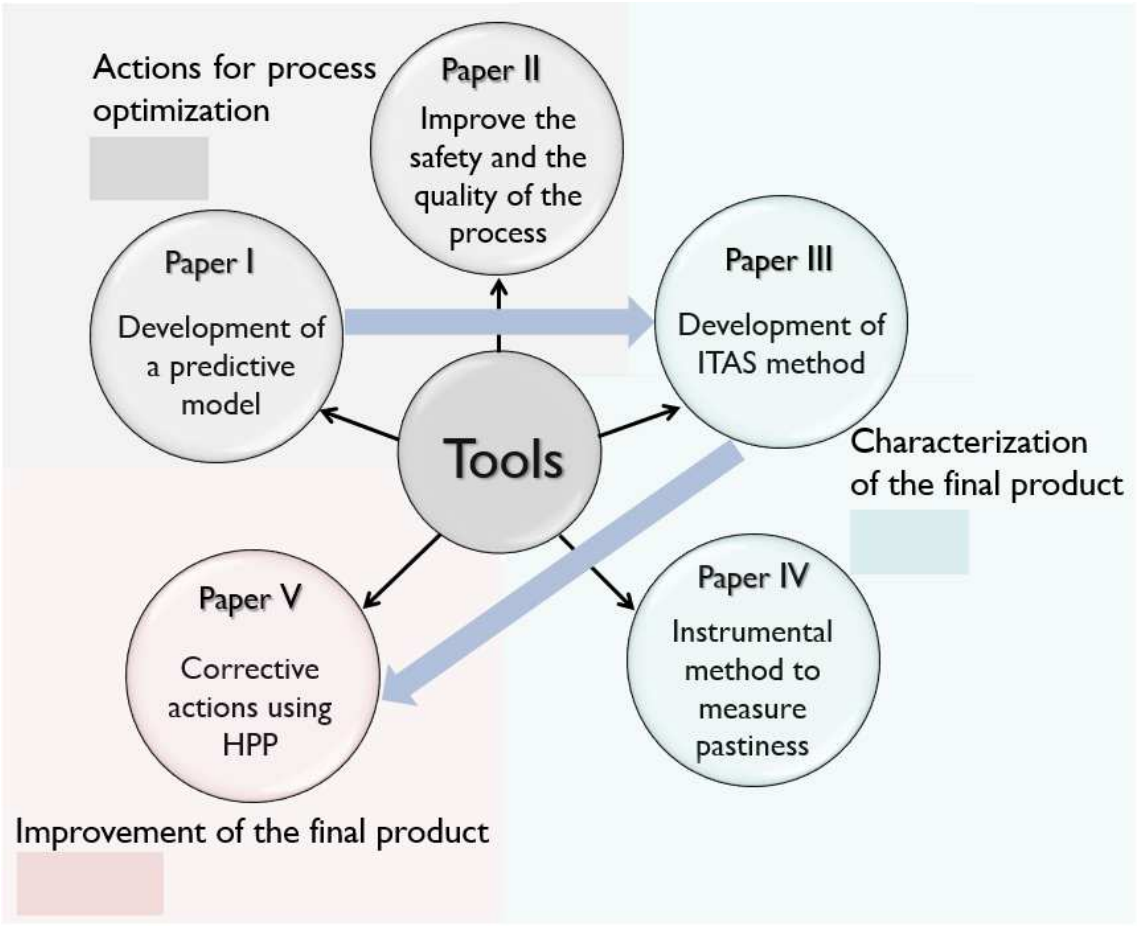

Figure 13. Conceptual diagram that represents the structure of the present Thesis.

4 Results

Contents

Paper I

Coll-Brasas, E., Gou, P., Arnau, J., Olmos, A. & Fulladosa, E. (2021). Processing parameters involved in the development of texture and tyrosine precipitates in dry-cured ham: Modelisation of texture development. *Meat Science*, *172*. 108362.

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Processing parameters involved in the development of texture and tyrosine precipitates in dry-cured ham: Modelisation of texture development

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ABSTRACT

The aim of this study was to quantify the effects of different processing parameters on texture development and the incidence of white film and tyrosine crystals in dry-cured ham. Hams were dry-salted for 0.65, 0.8 or 1.0 days/kg. After drying for 45 days at 5 °C, they were dried at 10, 15 or 20 °C until reaching 33% weight loss and, thereafter, dried at 25 °C until reaching 36 or 40% weight loss. The salting time, drying temperature and target weight loss significantly affected the texture and incidence of white film and tyrosine crystals. A beneficial effect of drying at 20 °C on texture was found, which was especially important for low target weight loss (33%). Besides, hams dried at 20 °C and those with 40% weight loss showed higher incidence of tyrosine crystals. Contour plots and predictive models for texture can be used to define optimal processing parameters

1. Introduction

The texture of dry-cured ham is one of the most important quality criteria for consumer acceptability and is of interest for the industry (Cilla, Martinez, Beltran, & Roncales, 2005; Schivazappa & Virgili, 2020). The main texture defects are excessive softness (Parolari, Virgili & Schivazappa, 1994) and pastiness which are mainly related to raw material characteristics such as pH, genetics and fat content (Candek-Potokar & Skrlep, 2012; Carcò et al., 2019; García-Rey, García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2004; García-Rey, Quiles-Zafra, & Luque de Castro, 2006) and processing conditions such as temperature, time and salt content (Coll-Brasas et al., 2021; Ruiz-Ramírez, Arnau, Serra, & Gou, 2005). A proteolysis activity contributes to texture development by breaking down the muscle structure (Monin et al., 1997). However, when proteolysis is excessive the structure is severely damaged and unpleasant textures appear (Contreras et al., 2020). Proteolysis index (PI = 100 x non-protein nitrogen / total nitrogen) is even used as quality criteria in PDO Parma ham, considering that values on *Biceps femoris* muscle should be below 31% (Consorzio del Prosciutto di Parma, 1992). Tapiador-Farelo and García-Garrido (2003) found an incidence of 12% pastiness defect in hams with a standard salt content (salted 1 day/kg of green ham). This defect increases when salt content is reduced (Tomažin et al., 2020) producing severe problems during slicing because of high adhesiveness (Gou, Morales, Serra, Guàrdia, & Arnau, 2008; Pérez-Santaescolástica, Carballo, Fulladosa, Garcia-Perez José, et al., 2018) and reducing consumer acceptability (Morales, Guerrero, Claret, Guàrdia, & Gou, 2008). For this reason, studies for the development of new strategies and corrective actions to reduce this defect has been carried out (Coll-Brasas et al., 2019; Fulladosa et al., 2021; Pérez-Santaescolástica, Carballo, Fulladosa, Garcia-Perez, et al., 2018).

A high proteolysis index also favours tyrosine precipitates in dry-cured ham as tyrosine crystals or white film (Arnau, Guerrero, Hortós, & García-Regueiro, 1996). These crystals are normally present in dry-cured hams aged for a period longer than 12 months, but they can also be found in hams aged for only 5 months (Arnau et al., 1996). White film on a cut surface appears several days after slicing (Butz, Blumer, Christian, Swaisgood, & Lucas, 1974) or in some cases within a few hours (Arnau, 1991). The main component of the white film and crystals is tyrosine (Arnau et al., 1996; Comi et al., 1981; Silla, Innerarity & Flores, 1985) followed by phenylalanine (Arnau et al., 1996), which both are the result of proteolysis.

In order to reduce textural problems and the development of tyrosine precipitates in dry-cured ham, it is necessary to more deeply study aspects related to quality of raw material and processing conditions such as saltiness, drying level, temperature and their combined effects. The development of mathematical models could help to make the effect of different factors on texture and tyrosine crystals and white film

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formation more clear, allowing modification/adaptation of the processes of elaboration according to the most influencing parameters.

The aim of this work was to study the effect of the raw material pH (pH_{SM24h}) and processing conditions (salting time, drying temperature and target weight loss) on texture development, white film intensity and incidence of tyrosine crystals in dry-cured ham. Texture predictive models aimed at optimising the process of elaboration of dry-cured ham were also developed.

2. Material and methods

2.1. Raw material selection and process of elaboration

One hundred and sixty nine raw hams were obtained from two commercial slaughterhouses, supplied from animals which were lean crosses of Large White and Landrace (L, $n = 151$) and fattier crosses with 50% Duroc breed (F, $n = 18$) in order to obtain a batch of hams with a wide range of fat contents. All animals were slaughtered during winter season. The pH determination was performed with a Crison Basic pH meter (Crison Instruments S.A., Barcelona, Spain) in the *Semimembranosus* muscle at 24 h post mortem (pH_{SM24h}). All hams were weighed (11.9 kg ± 1.1 kg) and salted according to the traditional system with some modifications in order to obtain four salting groups (SG). In brief, hams were manually rubbed with the following mixture (g/kg of raw ham): 0.15 KNO_3, 0.15 $NaNO_2$, 1.0 dextrose, 0.5 sodium ascorbate and 10 NaCl. Thereafter, the hams were pile salted at 3 ± 1 °C and 85 ± 5% RH. F hams were salted for 0.65 days/kg (F0.65) to obtain a reduced salting group. In contrast, L hams were salted for 0.65 days/kg (L0.65), 0.8 days/kg (L0.8) and 1 day/kg (L1.0) for a reduced, standard and high salting group respectively. After salting, the hams were washed with cold water and dried at 3 ± 2 °C and 80 ± 5% RH for 45 days (post-salting period). Thereafter, all of the hams from the L and F breeds continued the drying process at different Drying Temperatures (DT; 10 °C, 15 °C or 20 °C) (Table 1) and at 55–60% RH, until a weight loss of 33% was reached. The processing time to reach 33% weight loss ($t_{33\%}$) was recorded. Thereafter, hams were assigned to different Target Weight Losses: 33% (TWL33), the minimum weight loss accepted for Traditional Speciality Guaranteed of Jamón Serrano; 36% (TWL36), as a standard weight loss; and 40% (TWL40) to highlight the effect of drying on soft texture. After reaching 33% weight loss, hams were dried at 25 °C.

2.2. Sampling procedure

When hams reached the target weight loss, the aitch bone and femur were removed and they were transversally cut at the coxofemoral joint level. The cushion part was trimmed and five slices were obtained: three 2.0 cm thick slices and two 1.5 cm thick slices. *Biceps femoris* (BF) muscles from the first three slices were sampled and used for instrumental texture analysis right after sampling. After texture analysis, BF samples were individually minced, vacuum packed and stored at 4 ± 2 °C until the chemical analyses (moisture content and NaCl content) were performed (within under three weeks). From each minced BF sample, a subsample of 30 g was frozen and stored at −19 ± 1.5 °C until PI analysis (non-protein nitrogen and total nitrogen content) was performed. The 4th and 5th slices were vacuum packed and stored at 4 ± 2 °C for 4 weeks. Thereafter, tyrosine crystals and white film evaluation were performed. All samples were packed in 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).

Table 1
Distribution of the hams ($n = 169$) according to the salting group (SG; L/F: lean/fatty hams, 0.65/0.8/1.0: salting days/kg of ham), target weight loss (TWL; 33%, 36% or 40%) and drying temperature (DT; 10 °C, 15 °C or 20 °C).

Salting group	Target Weight loss	Drying temperature		
		10 °C	15 °C	20 °C
F 0.65	33%	3	2	3
	36%	2	2	1
	40%	1	2	2
L 1.0	33%	6	6	6
	36%	8	7	6
	40%	3	4	4
L 0.8	33%	7	6	7
	36%	7	6	5
	40%	4	3	4
L 0.65	33%	6	6	6
	36%	6	6	6
	40%	5	6	5

2.3. Instrumental texture analysis

According to a previous study (Coll-Brasas et al., 2019), a minimum of five parallelepipeds were cut from each BF muscle with the same dimensions (2 cm × 2 cm × 1.5 cm). The pieces were wrapped in polyvinyl chloride (P.V.C.) film (oxygen permeability of 20,000 cm^3/m^2/24h and water vapour transmission of 200 g/m^2/24h, Macopal S.L., Spain) to reduce drying and kept at 4 ± 2 °C for 24 h for temperature stabilisation in a temperature control cabinet (Model EC-360, Radiber S. A., Barcelona, Spain). A Stress Relaxation test (SR) was performed by using a Universal Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, England) provided with a 30 kg load cell and a 60 mm diameter compression plate. Samples were compressed to 25% of their original height, perpendicular to the muscle fibre bundle direction, at a cross-head speed of 1 mm/s and at a temperature of 4 ± 2 °C.

The force decay or relaxation versus time $Y_{(t)}$ was recorded obtaining a deformation curve and it was calculated as follows:

$$Y_{(t)} = \frac{F_0 - F_{(t)}}{F_0}$$

Where F_0 (N) is the initial force and $F_{(t)}$ is the force recorded after t seconds of relaxation. The force decay at 2 s (Y_2) and 90 s (Y_{90}) were calculated (Morales, Arnau, Serra, & Gou, 2007). For each parameter, the average of the five samples was used for the statistical analyses.

2.4. Chemical analysis

The following chemical analysis was performed. Moisture content was determined by drying at 103 ± 2 °C until a constant weight was reached AOAC (1990). NaCl content on a dry-matter basis (DM%) was determined according to ISO 1841-2. (1996) by using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland). Non-Protein Nitrogen content (NPN) was determined by precipitation of proteins with trichloroacetic acid (Gáspár, 1984) followed by determination of the total nitrogen (TN) in the extract with the Kjeldahl method ISO 937. (1978). The Proteolysis Index (PI) was calculated as a percentage of the ratio between NPN and TN. All the analyses were done in duplicate.

2.5. Evaluation of tyrosine precipitates

Evaluation of the incidence of tyrosine crystals was carried out by counting the number of crystals on the cut surface of the whole ham slice. The evaluation of the intensity of the white film was performed on the surface of the ham slices by using a 0–10 intensity scale, where 0 means no presence of white film and 10 means that the whole slice is covered with white film. Samples were assigned to different groups according to their intensity range: from 0 to 1.25; from 1.25 to 2.5; from 2.5 to 3.75; from 3.75 to 5.0; from 5.0 to 6.25; from 6.25 to 7.5; from 7.5 to 8.75 and from 8.75 to 10) considering mean intensity of 0.625; 1.75; 3.1; 4.4; 5.6; 6.8; 7.8 and 9.1 for each group respectively. Evaluation was carried out by a three-member expert panel trained following ASTM

(ASTM, 1981). The average score of the 3 experts for each sample was used for the statistical analysis.

2.6. Statistical analysis

Processing time, physicochemical characteristics (moisture content, NaCl content, PI) and texture (F_0, Y_2, Y_{90}) for BF muscle and tyrosine precipitates (incidence of tyrosine crystals incidence, white film intensity) in the whole slice were analysed using the GLM procedure of the SAS package (SAS Institute, 2019). The following linear model was fitted:

$$Y_{ijkl} = \mu + b*(pH_{SM24h})_{ijkl} + SG_i + DT_j + TWL_k + (SG*DT)_{ij} + (SG*TWL)_{ik} + (DT*TWL)_{jk} + e_{ijkl}.$$

Where Y_{ijkl} is the observed value (dependent variable); μ and b are constants of the model; $(pH_{SM24h})_{ijkl}$ is the pH on *Semimembranosus* muscle 24 h post mortem (covariate); SG_i is the salting group (i = 1, …,4); DT_j is the drying temperature until 33% weight loss is reached (j: 1,2,3); TWL_k is the target weight loss group (k: 1,2,3) and e_{ijkl} is the random residual.

Counter plots for Y_{90} were plotted on DT and TWL parameters for each salting group using JMP (SAS Institute, 2019). A model for predicting Y_{90} was developed by using these continuous variables that had a significant effect in the previous GLM analysis (SAS Institute, 2019).

3. Results and discussion

LS-means of main factors in the linear model and regression coefficient of PH_{SM24h} used as a covariable are shown in Table 2. Table 3 shows the LS-means of parameters with significant interaction DT*TWL ($p < 0.05$). Interactions SG*DT and SG*TWL were removed from the model since no significant effect on texture or the incidence of precipitates was found.

3.1. Texture of BF muscle

Values of pH_{SM24h} ranged between 5.40 and 6.05 for all the salting treatments. No significant linear effect of pH_{SM24h} on texture was neither detected ($p > 0.05$), although several authors have previously proved the influence of meat pH on the texture of the final product (Guerrero, Gou, & Arnau, 1999; Morales, Guerrero, Serra, & Gou, 2007). The pH_{SM24h} of raw material had a positive relationship with processing time and a negative relationship with NaCl content (Table 2). Previous studies have also found that hams with lower pH dry faster (Guerrero et al., 1999). Hams with low pH are reported to be more prone to develop soft textures (Ruiz-Ramírez, Serra, Arnau, & Gou, 2005). However, this fact was not observed in our results probably because hams with lower pH were processed for less time and had higher NaCl content, which hinders the development of soft textures.

Significant differences in texture between salting groups, drying temperature groups and target weight loss groups were found. A decrease of salting time in L hams resulted in a significant decrease of F_0 and an increase of Y_2 and Y_{90} ($p < 0.05$), as previously described by several authors (Morales, Guerrero, et al., 2007; Ruiz-Ramírez, Arnau, et al., 2005). F hams needed more time to reach a weight loss of 33% and had lower salt content and moisture content than L hams with the same salting time, as expected due to their higher fat content.

Table 3
LS-means of texture analyses (Y_2 and Y_{90}) in *Biceps femoris* muscle and white film intensity in the whole slice according to the interaction Drying temperature x Target Weight loss.

Drying temperature	Target Weight loss	Y_2	Y_{90}	White film intensity
10 °C	33%	0.421^{a}	0.738^{a}	4.43^{b}
	36%	0.338^{cd}	0.637^{c}	7.01^{a}
	40%	0.330^{cd}	0.619^{cd}	5.90^{ab}
15 °C	33%	0.411^{a}	0.720^{a}	6.27^{a}
	36%	0.349^{bcd}	0.644^{c}	6.60^{a}
	40%	0.328^{cd}	0.612^{cd}	5.77^{ab}
20 °C	33%	0.378^{b}	0.679^{b}	6.75^{a}
	36%	0.358^{bc}	0.643^{c}	6.59^{a}
	40%	0.319^{d}	0.595^{d}	4.46^{b}
RMSE		0.0293	0.0304	1.450

a – d means within columns with different letters are significantly different ($p < 0.05$). RMSE: root mean square error of the linear model.

Table 2
Regression coefficients (and standard errors) for pH_{SM24h} in the linear model. LS-means of instrumental texture (F_0, Y_2 and Y_{90}) and chemical parameters on *Biceps femoris* muscle, tyrosine precipitates (tyrosine crystals incidence and white film intensity) in the whole slice, and overall processing time for all the processing conditions.

		Processing time	Chemical parameters				Texture parameters			Tyrosine precipitates	
		Total Processing time (days)	Moisture (%)	NaCl (DM %)	NPN/ Moisture (%)	PI (%)	F_0 (N)	Y_2	Y_{90}	Tyrosine crystals	White film
pH_{SM24h}											
Regression											
Coefficient (Standard error; se)		233* (50.8)	−1.07 (0.99)	−1.62* (0.75)	−0.04 (0.21)	−2.91 (1.93)	1.971 (3.981)	−0.0275 (0.0189)	−0.0221 (0.0196)	−13.22* (6.42)	−2.60* (0.98)
	Salting Group										
	F0.65	564^{a}	58.6^{b}	9.8^{d}	2.7^{a}	31.9^{ab}	21.18^{bc}	0.370^{ab}	0.657^{abc}	3.2^{c}	7.4^{a}
	L0.65	384^{b}	59.9^{a}	13.9^{c}	2.4^{b}	30.9^{ab}	17.26^{c}	0.372^{a}	0.671^{a}	21.2^{a}	5.9^{b}
	L0.8	424^{b}	59.1^{ab}	15.1^{b}	2.6^{ab}	32.0^{a}	20.99^{b}	0.357^{b}	0.655^{b}	20.8^{a}	5.5^{bc}
	L1.0	393^{b}	59.1^{ab}	17.3^{a}	2.4^{b}	30.2^{b}	26.87^{a}	0.338^{c}	0.634^{c}	14.7^{b}	5.1^{c}
	Drying Temperature Group										
LS-means	DT10	459	59.6^{a}	14.1	2.4^{b}	29.6^{b}	22.16	0.363	0.665^{a}	9.5^{b}	5.8
	DT15	436	59.3^{ab}	14.1	2.5^{b}	30.9^{b}	20.59	0.363	0.659^{a}	13.7^{b}	6.2
	DT20	429	58.6^{b}	13.9	2.7^{a}	33.1^{a}	21.97	0.352	0.639^{b}	21.7^{a}	5.9
	Target Weight loss Group										
	TWL33	405^{a}	61.1^{a}	13.3^{c}	2.2^{c}	29.8^{b}	12.45^{c}	0.403^{a}	0.712^{a}	12.0^{b}	5.8^{b}
	TWL36	441^{ab}	59.7^{b}	14.0^{b}	2.5^{b}	31.2^{ab}	21.08^{b}	0.349^{b}	0.641^{b}	13.1^{b}	6.7^{a}
	TWL40	478^{a}	56.7^{c}	14.8^{a}	2.9^{a}	32.7^{a}	31.19^{a}	0.326^{c}	0.608^{c}	19.8^{a}	5.4^{b}
RMSE		78.8	1.53	1.17	0.33	2.99	6.168	0.0293	0.0304	9.84	1.45

a – d means within columns with different letters are significantly different ($p < 0.05$). RMSE: root mean square error of the linear model.
* Regression coefficient significantly different to 0 ($p < 0.05$).

As expected, the processing time decreased when increasing drying temperature. Hams dried at 20 °C dried slightly faster and showed lower moisture content and Y_{90} values, but higher PI in BF muscle than hams dried at 15 or 10 °C. Similarly, the increase in target weight loss reduced the moisture content, Y_2 and Y_{90} and increased F_0, as previously described in the literature (Morales, Guerrero, et al., 2007; Ruiz-Ramírez, Serra, et al., 2005). Hams subjected to this for more time at 25 °C (TWL40) showed higher PI (Arnau, Guerrero, & Gou, 1997).

There was a significant interaction between Drying Temperature (DT) and Target Weight Loss (TWL) for Y_2 and Y_{90} (Table 3). The beneficial effect of drying at 20 °C until reaching 33% weight loss (lower Y_2 and Y_{90} values), in comparison to drying at 10 °C or 15 °C, was less important if hams were subjected to an additional drying at 25 °C (until reaching a final weight loss of 36% or 40%). The additional drying at 25 °C also reduced Y_2 and Y_{90} values, especially in hams that have been dried at 10 °C or 15 °C. In fact, according to the literature, mild thermal treatment (30 °C) of short duration (10 days) can improve texture in BF muscle of dry-cured hams processed at temperatures below 18 °C (Gou et al., 2008). An increase in temperature can produce changes such as unfolding and protein-protein association of the myofibrillar components of the muscle, changing textural properties (Tornberg, 2005).

The higher Y_2 and Y_{90} values have been related to an increase in proteolytic activity (Morales, Arnau, et al., 2007). However, we found significant differences between salting groups for texture, but not for PI. In the same way, the drying temperature group and target weight loss group with higher Y_2 and Y_{90} values showed a lower PI index. It seems that factors other than proteolysis activity (e.g., final moisture and salt contents) might have a significant influence on BF texture or that PI index does not account for all the proteolytic activity related to texture development. The proteolysis indexes found in this study (e.g. in F0.65, L0.8, DT20, TWL36 and TWL40) were higher than the maximum values proposed by the PDO Parma ham (Consorzio del Prosciutto di Parma, 1992).

The total amount of salt in the ham is not expected to change during drying, so the differences found in NaCl (DM%) in BF muscle between the different target weight losses are explained by the differences in moisture content between BF muscle and the rest of the ham (Arnau, Guerrero, Casademont, & Gou, 1995). NaCl/moisture tends to reach an equilibrium in the whole ham; therefore, salt diffuses from the dryer surface to the humid inner parts (such as BF muscle). However, drying conditions and the characteristics of the raw material can influence equalization to a different extend.

The values of PI in BF muscle increased between TWL33 and TWL40. During drying, NPN/moisture increased more in the external parts due to moisture reduction than in BF muscle. This difference is a driving force that moves water soluble NPN from the outer part of ham to the inner part in a similar way as it occurs with NaCl and could partially explain the differences in proteolysis ratio (Gratacós-Cubarsí et al., 2013).

3.2. Tyrosine precipitates

The crystallisation of tyrosine generated by proteolysis is facilitated by structural damage to the bulk of ham to form tyrosine crystals (e.g. if it is frozen before salting or at the end of process) or on the irregularities of cut surface to form white film (Arnau, Gou, & Guerrero, 1994). In this study, the parameters that increased PI also increased the incidence of tyrosine crystals. In this sense, when salting time increased from L0.8 to L1.0 tyrosine crystals incidence decreased (Table 2). Hams dried at 20 °C and those with TWL of 40% showed higher PI and higher incidence of tyrosine crystals than DT10 or DT15 hams, and TWL33 hams respectively ($P < 0.05$). However, F0.65 hams, which had similar PI to L0.65 hams, showed the lowest incidence of tyrosine crystals, but the highest white film intensity. These differences could be due, among other factors, to differences in structural damages that affect the nucleation step and the higher fat content of F0.65. Fat content could slow down tyrosine diffusion inside the ham which would be detrimental to nucleation and crystal growth.

The pH_{SM24h} showed a negative relationship with the incidence of tyrosine crystals incidence and white film intensity, but not with PI. This could be due to the fact that PI was measured in the BF muscle only at the end of the process, whereas the incidence of tyrosine crystals and white film intensity were measured on the whole slice.

White film intensity is also expected to be related to PI, however their formation is affected by tyrosine crystal formation (Arnau, 1991; Butz et al., 1974). Tyrosine that has precipitated previously on the tyrosine crystals will not precipitate as white film on the cut surface when the product is sliced (Arnau et al., 1996). In this sense, TWL40 hams, which showed the highest incidence of tyrosine crystals, showed lower white film intensity than TWL36 hams ($P < 0.05$). In fact, the decrease of white film intensity when hams were dried from 36% weight loss to 40% weight loss was more important in those hams previously dried at 20 °C than in those dried at 10 °C or 15 °C (Table 3). This could be due to the highest incidence of white crystals and perhaps to the reduction of free tyrosine content in the long ageing process (Sforza et al., 2006). White film intensity at 33% weight loss increased significantly ($P < 0.05$) when DT increased from 10 °C to 15 °C, but the increase was not significant ($P > 0.05$) when DT increased from 15 °C to 20 °C (Table 3), probably because of the increase in the incidence of tyrosine crystals.

3.3. Texture at different processing conditions

Because the final texture is dependent on a combination of multiple factors, definition of the optimal processing conditions needs to be evaluated in globally. Contour plots in Fig. 1 represent, for each salting group, variation of Y_{90} according to target weight loss and drying temperature since they are the factors that significantly influenced texture development in this study. Previous studies considered dry-cured ham samples with Y_{90} values above 0.734 as defective or soft (Morales, Serra, et al., 2007). In contrast, hams with Y_{90} values below 0.682 were defined as hams with hard texture (Morales, Guerrero, et al., 2007). Taking this into account, in this study, dry-cured ham samples with $Y_{90} > 0.690$ were considered as hams with soft textures.

Contour plots in Fig. 1 draw optimal processing conditions at intervals to achieve optimal texture. In F0.65 hams, optimal texture was achieved at mild temperatures (20 °C) and weight losses higher than 36%. Optimal textures could be also achieved at low temperatures if target weight loss was higher. In contrast, higher temperatures and weight losses were needed in L0.65 hams to improve the texture. The differences found between L and F hams on the achievement of the optimal textures can be due to both fat and salt contents. Reduction of salt content in lean hams produced hams more prone to develop defective textures. When salt content was not reduced (L0.8 and L1.0) any processing temperature was suitable to achieve an optimal texture if hams were dried to a minimum weight loss of 36%. In the case of L0.80 hams, higher weight losses were needed to achieve an optimal texture, in comparison to L1.0 hams where the higher salt content reduced the incidence of soft textures. The contour plots help to visually understand the effect of processing parameters on texture development.

The Y_{90} behaviour of F hams is different to L hams. Therefore, a model to predict Y_{90} was only developed for L hams, which included Salting Time (ST, days/kg), Drying Temperature (DT, °C) and Weight Loss (WL, %), their quadratic terms and their double interactions as predictor variables. Non-significant terms were excluded from the model.

The fitted model was:

$$Y_{90} = 1.3636404 - 0.089638 \cdot ST - 0.003091 \cdot DT - 0.016857 \cdot WL + 0.0017824 \cdot (WL - 35.833)^2 + 0.0006686 \cdot (DT - 14.9007) \cdot (WL - 35.833).$$

The predictive error and R^2 of the final model were 0.030 and 0.72, respectively. Fig. 2 shows the relationship between the predicted and the analysed texture parameter (Y_{90}). The linear model provides a fairly accurate prediction of Y_{90} in the whole range of Y_{90} values. Therefore,

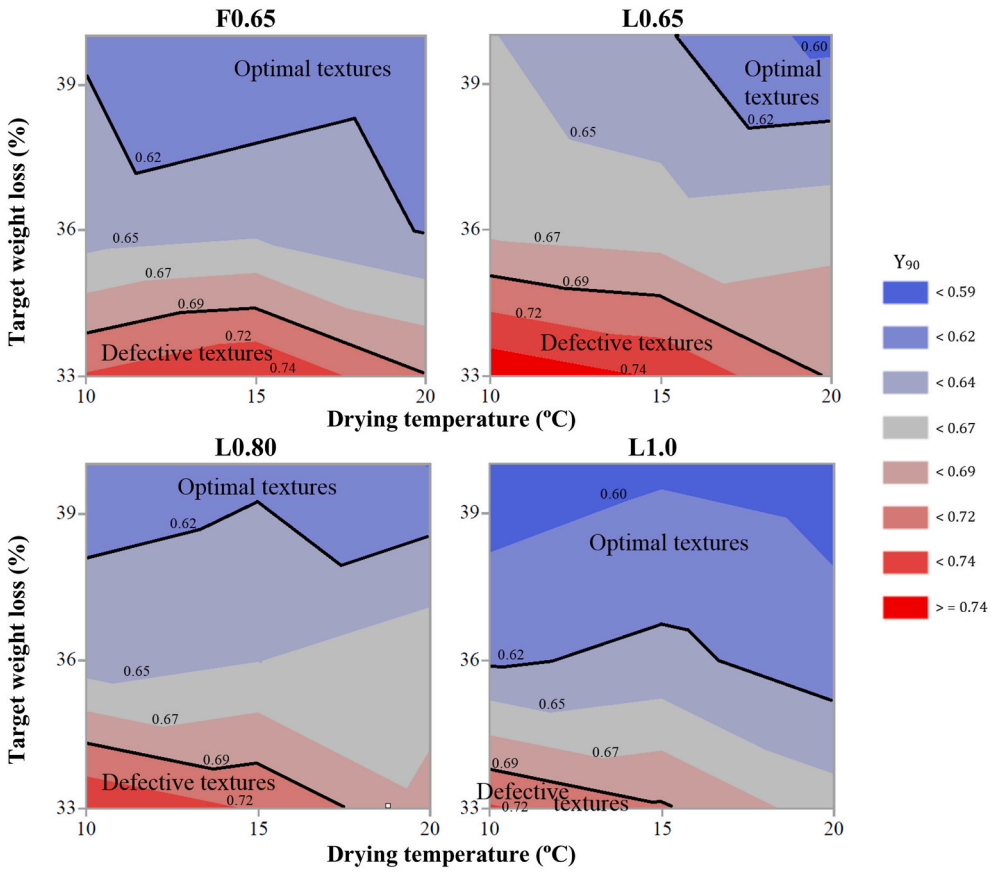

Fig. 1. Estimation of instrumental texture (Y_{90}) using contour plots for each Salting group. Hams with $Y_{90} > 0.690$ were considered as soft textures, whereas hams with $Y_{90} < 0.620$ optimal textures.

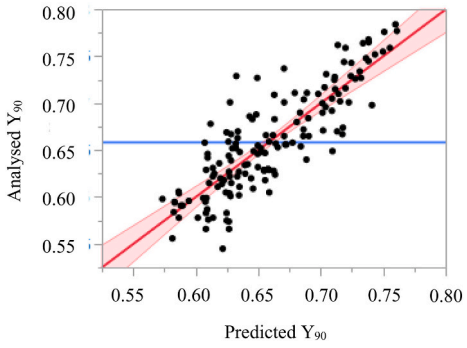

Fig. 2. Relationship between predicted and analysed Y_{90} values on L hams.

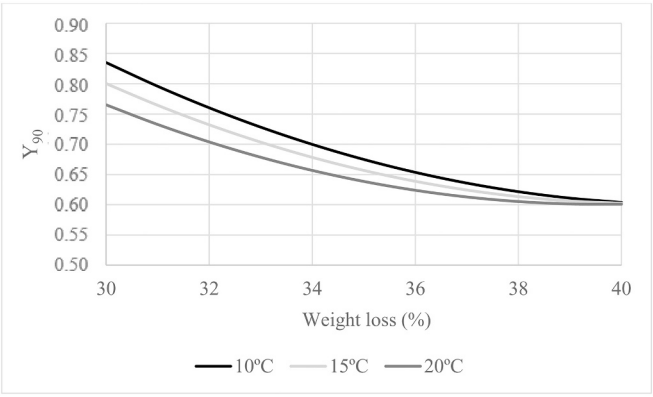

Fig. 3. Predicted Y_{90} at different weight losses and temperatures on L hams for salting time of 0.80 days/kg of ham. (RMSE = 0.030; R^2 = 0.72).

using this predictive model, processing conditions can be set up to reach the desired texture in the final product. Fig. 3 shows an example of predicted Y_{90} at different weight losses and drying temperatures for a specific salting time (0.8 days/kg). Results agree with the interaction DT*WL effect on Y_{90} (Table 3).

There are other relevant parameters that can influence texture development which can be determined at industrial level using non-invasive technologies. Fat content of raw hams (De Prados et al., 2015), NaCl content during processing (Fulladosa, Muñoz, Serra, Arnau, & Gou, 2015; Schivazappa et al., 2017) and internal characteristics of the product such as intramuscular fat (Muñoz, Rubio-Celorio, Garcia-Gil, Guàrdia, & Fulladosa, 2015) might help to optimize texture development. More complex models including the mentioned parameters should be developed before the implementation of a texture optimisation system to improve the texture of dry-cured ham production in the industry.

4. Conclusions

Development of texture and tyrosine precipitates formation is a complex issue that depends on multiple factors such as drying temperature, the achieved weight loss, pH_{SM24h} and NaCl content and needs to be undertaken in a global way. In this sense, texture predictive models based on the information obtained using non-invasive technologies during the process and processing conditions could be useful to optimize processes of elaboration and achieve optimal textures.

Conflict of Interest

Conflict of Interest and Authorship Conformation Form.

CRediT authorship contribution statement

E. Coll-Brasas: Investigation, Formal analysis, Writing - original draft, Visualization. **P. Gou:** Conceptualization, Supervision, Writing - review & editing, Data curation, Formal analysis. **J. Arnau:** Conceptualization, Supervision, Writing - review & editing, Data curation. **A. Olmos:** Investigation, Writing - review & editing. **E. Fulladosa:** Conceptualization, Supervision, Writing - original draft, Formal analysis, Writing - review & editing, Funding acquisition.

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Paper II

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Physicochemical characterisation of restructured Fenalår and safety implications of salt and nitrite reduction

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ABSTRACT

There is a new trend to produce dry-cured ham from lamb in shorter times by boning the ham before salting to later obtain restructured hams that are easier to dry and slice. However, little information about the physicochemical characteristics of Norwegian Fenalårs during the process or the safety implications of their elaboration procedures is reported in the literature. The aim of this study was to characterize the colour, texture and physicochemical properties of restructured Fenalårs when using Standard Salting (SS), Salt Reduced (SR) and a Non-Nitrite Salt Reduced (NNSR) treatments. Microbiological safety implications of the elaboration process when using the different salting treatments were also assessed using predictive microbiology. To do so, sixty Fenalårs were elaborated using a Standard Salting (SS), a Salt reduced (SR) and a Non-Nitrite Salt Reduced (NNSR) treatments. Physicochemical characterization (instrumental colour and texture and Zinc Protoporphyrin content) was performed at the end of the process using thirty Fenalårs. The rest of the Fenalårs were used to characterize the product through the elaboration process (pH and a_w) for the evaluation of microbiological hazards when using the different salting treatments using predictive microbiology. Results showed a significant increase in softness when reducing salt content and a decrease of redness when no nitrite was used, attributed to the formation of ZnPP content instead of nitrosylmyoglobin. In terms of risk assessment, the decrease of a_w through the elaboration process reduced the growth capacity of all the microorganisms evaluated. However, microbiological safety implications in salt reduced Fenalårs are important, especially when no nitrite was added, because the considerable increase of growth potential of *L. monocytogenes*. The increase of growth potential of proteolytic *C. botulinum* is very little and no relevant effect of nitrite on growth potential of *S. aureus* was observed.

Predictive microbiology and optimization of the process to enhance ZnPP formation can help to ensure safety and quality of salt reduced restructured Fenalårs without additives.

1. Introduction

Fenalår is a traditional Norwegian dry-cured product prepared from lamb or mutton leg. Fenalår fra Norge ("Fenalår from Norway") became a legal Protected Geographical Indication (PGI) in Norway in October 2012 (Håseth, Thorkelsson, Puolanne, & Sidhu, 2014), and a PGI in Europe in 2017 (Regulation (EU) No. 1752/2017). According to the traditional elaboration process, the leg is pile salted with the bone inside. However, there is a new trend to remove the bone before salting to later obtain a restructured Fenalår from different meat pieces which can be elaborated in shorter times and easily sliced and sold as a ready-to-eat product. This restructured Fenalår could be as appreciated by consumers as PGI Fenalår. Although long-dry aged products are well appreciated by consumers (Villalobos-Delgado et al., 2014), other authors found that some consumers preferred dry-cured ham from sheep with short maturation times and cheaper prices (De Andrade et al., 2017).

Restructured elaboration procedures have been previously used to elaborate dry-cured products (Fulladosa, Serra, Gou, & Arnau, 2009; Romero de Ávila, Hoz, Ordóñez & Cambero, 2014). Production of restructured Fenalår is also of interest to the Norwegian meat industry, but the safety implications and the quality of the final product need to be evaluated. Besides, the tendency to reduce salt content is also becoming

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more important for Fenalår, which has an above average salt content for dry-cured products, in order to comply with consumers demands and European nutritional recommendations (European Commission, 2020). However, salt content reduction in dry-cured meat products is not straightforward since it can lead to an increase in microbiological safety issues (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017; Taormina, 2010) and cause quality defects in the final product (Costa-Corredor, Serra, Arnau, & Gou, 2009). Reduction or elimination of curing additives to comply with clean label requirements is also emerging. However, nitrite is not only used to achieve the typical cured colour in the final product (Cassens, Greaser, Ito, & Lee, 1979; Honikel, 2008), but also for food safety purposes, i.e. to inhibit the growth of *Clostridium botulinum* (Honikel, 2008). Although dry-cured ham production without curing agents is feasible (Iacumin et al., 2019; Parolari, Aguzzoni, & Toscani, 2016), it must be done with some caution (Buchanan & Phillips, 1990) especially when using restructured hams and salt reduced treatments in which microbiological contamination is more prone to occur (Fulladosa, Sala, Gou, Garriga, & Arnau, 2012).

The microbiological safety implications of lowering the amount of salt and nitrite in restructured Fenalår have not been studied before. *Listeria monocytogenes* and *Salmonella* spp. are often involved in alerts of the Rapid Alert System for Food and Feed (RASFF) concerning dry-cured meat products. The current legislation establishes microbiological criteria for both hazards in ready-to-eat meats (European Commission, 2005). In addition, *E. coli* and *Salmonella* have been described as causative agents of outbreaks associated with the consumption of salted dry-cured meat products (Holck, Axelsson, McLeod, Rode, & Heir, 2017; Omer et al., 2018). *Staphylococcus aureus* is also a pathogen of concern due to its ubiquitous and versatile character and its role in outbreaks linked to the consumption of dry-cured hams (Portocarrero, Newman, & Mikel, 2002; Rajkovic, 2012). Besides, nitrite removal from meat product formulations could increase the probability of the growth of *C. botulinum* in these products (Taormina, 2010).

Predictive microbiology models have been applied by food safety authorities in order to evaluate the microbiological safety consequences of changes in food processing and preservation (Messens, Hempen, & Koutsoumanis, 2018), including salt reduction in Spanish cured meat products (AESAN, 2010). Predictive models are mathematical tools to estimate microbial behaviour in foods, i.e. growth, transfer, survival or inactivation, as affected by a series of intrinsic and environmental factors, such as pH, a_W, nitrite and temperature, without the requirement of time-consuming challenge-tests (Perez-Rodriguez & Valero, 2013). Robust predictive models accounting for the most relevant factors governing microbial behaviour in dry-cured products have been described and can be applied to evaluate safety implications (Zurera-Cosano, Carballeira, Carrasco, Rodríguez, & Díaz, 2011).

The effects of salt reduction or the microbiological safety implications of nitrite elimination have been reported in pork cured meat products (Higuero, Moreno, Lavado, Vidal-Aragón, & Cava, 2020), though no systematic studies for different salt levels in Fenalår can be found in the literature. Similarly, several types of pork based dry-cured meat products, such as PDO Parma ham (Laureati et al., 2014), Bayonne ham (Monin et al., 1997) or TEG Serrano ham (Guàrdia, Aguiar, Claret, Arnau, & Guerrero, 2010) have been extensively characterized. Although some studies dealing with dry-cured products from sheep (De Andrade et al., 2017; Teixeira, Fernandes, Pereira, Manuel, & Rodrigues, 2017) and goat (Ivanovic, Nesic, Pisinov, & Pavlovic, 2016) from different countries have been found in the literature, little information regarding the textural and physicochemical characteristics of bone-in or restructured Norwegian Fenalår is reported.

Thus, the aim of our study was to characterize the colour, texture and physicochemical properties of restructured Fenalårs when using Standard Salting (SS), Salt Reduced (SR) and Non-Nitrite Salt Reduced (NNSR) treatments. Microbiological safety implications of the elaboration procedure when using the different salting treatments were also assessed using predictive microbiology.

2. Material and methods

2.1. Raw material selection and elaboration process of restructured Fenalår

Sixty Norwegian White lambs fed with coastal grazing were obtained. The animals were 4 months old and from the same production system. All the animals were slaughtered in Førde commercial slaughterhouse over the period of 1 day. After carcass dissection, legs were vacuum packed and stored at −20 °C for 7 days. Frozen legs were thawed at 15 °C (room temperature) for 24 h. The ultimate pH in *Semimembranosus* muscle was 5.63 ± 0.04 (pH meter, Mettler Toledo AG 8603 Schwerzenbach, Switzerland). The legs (n = 120) were boned, and the connective tissue and subcutaneous fat removed. For restructured Fenalår production, 2 legs were netted (double rubber 110 net, ScotNet, Scotland) and manually rubbed in fine salt. The netted green hams were vacuum packed in shrink bags (polyamide/EVO/polyethylene; oxygen permeability of 12 cc/m^2/24 h at 23 °C, 0% RH and 1 atm, and a water permeability of 8 g/m^2/24 h at 38 °C, 90% RH and 1 atm; Bemis® Company Inc. USA) together with the corresponding amount of salt inside the plastic bags according to the salting treatments described below.

Three different salting treatments were used: Standard Salting (SS) using 4.8 g of salt/100 g raw meat and 144 ppm nitrite (n = 24); Salt Reduced (SR) using 3.9 g of salt/100 g raw meat and 144 ppm nitrite (8% reduction) (n = 18); and Non-Nitrite Salt Reduced (NNSR) using only 3.9 g of salt/100 g raw meat (n = 18). The salting step occurred in the cold room at 2–4 °C for 42 days. After the salting period, Fenalårs were treated with a 4% potassium-sorbate solution for 1.5 h at 13 °C to avoid mould growth and dried at 18 °C with a relative humidity (RH) of 60% for 48 h. Later, Fenalårs were dried at 13 °C and a RH of 74% for an additional 10 days. Then, Fenalårs were smoked with friction-smoke from beech wood for 6 h before being returned to the chamber at 13 °C for 13 days. Seven days later, the Fenalårs were pressed for 48 h. Once this was finished, Fenalårs were hung up again at 13 °C with a RH of 74% until achieving a weight loss of 36% (16 days). A schematic representation of the restructured Fenalår elaboration process is shown in Fig. 1. During the process, weight loss and temperature were determined twice per week.

2.2. Sampling procedure

Thirty restructured Fenalårs elaborated using SS (n = 10), SR (n = 10) and NNSR (n = 10) salting treatments were sampled at the end of the process (when a weight loss of 36% was reached) (Fig. 1). A 5 cm thick slice was sampled in the central part of the ham and used for instrumental colour determinations. The remaining part of the Fenalår was used for instrumental texture analysis and the determination of salt, water and Zn-protoporphyrin (ZnPP) contents.

To study the microbiological hazards of the restructured Fenalår elaboration procedure, the rest of the Fenalårs (n = 30) were sampled at different steps of the manufacturing process (after 44, 54, 67, 74 and 90 days of processing). At each step of the process, a total of 6 Fenalår from different salting treatments (SS, SR and NNSR) (see Table 2) were sampled in two different areas (the central and the tip area), obtaining 10 cm thick slices in which two or three regions of interest (RoI), depending on the Fenalår, were selected (Fig. 1). The a_w and pH were measured in all the ROIs (n = 166).

2.3. Instrumental colour

A Minolta Spectrophotometer CM 700d colorimeter (Konica Minolta Optics, Inc. Japan) with 2° standard observer and D65 illuminant was used to measure colour in the CIE–LAB space (Commission Internationale de l'Eclairage, 1976): lightness (L*), redness (a*) and yellowness (b*) on the 5 cm thick slice. Colour was measured in triplicate.

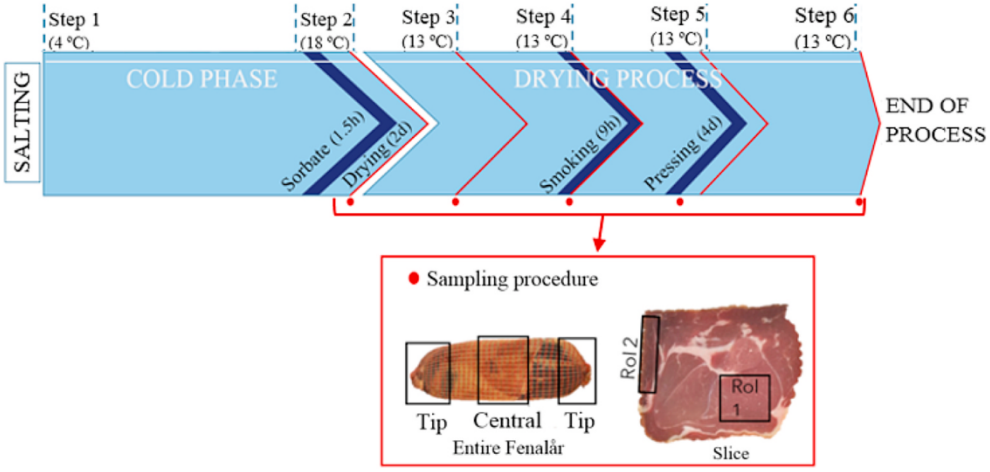

Fig. 1. Schematic representation of restructured Fenalår elaboration process and sampling procedure performed at each step: Step 1 (42 days), Step 2 (2 days); Step 3 (10 days), Step 4 (13 days), Step 5 (7 days) and Step 6 (16 days).

2.4. Instrumental texture

Three or four 2.0 cm thick slices were obtained from which a minimum of five parallelepipeds were cut with the exact same dimensions (2 cm × 2 cm x 1.5 cm) from the different muscles available. The identification of the muscle was not possible since it is a restructured product. The pieces were wrapped in plastic foil to avoid drying and kept at 4 °C ± 2 °C for 24 h for temperature stabilization. A Stress Relaxation test was performed because it allows detection of defective textures (Morales, Serra, Guerrero, & Gou, 2007). A Texture Analyser TA – HD plus 6014 (Stable Micro Systems Ltd, Surrey, England) provided with 30 kg load cell and a 60 mm diameter compression plate (SMS P/45) was used. Samples were compressed to 25% of their original height, at a crosshead speed of 5 mm/s and at a temperature of 4 °C ± 2 °C. The force decay or relaxation versus time $Y_{(t)}$ was recorded obtaining a deformation curve and it was calculated as follows:

$$Y_{(t)} = \frac{F_0 - F_{(t)}}{F_0}$$

where F_0 (kg) is the initial force and $F_{(t)}$ is the force recorded after t seconds of relaxation. The force decay at 2s (Y_2) and 90s (Y_{90}) were calculated.

2.5. Physicochemical analysis

The a_w was measured with an AquaLab™ instrument (AquaLab Series 3, Decagon devices Inc. Pullman, Washington 99163, USA). Chloride content was determined according to (ISO 1841-2, 1996) using a potentiometric corning 926 chloride Analyzer. Moisture content was determined by drying at 103 °C ± 2 °C until a constant weight was reached AOAC (1990). All measurements were done in triplicate.

ZnPP was quantitatively extracted in subdued light conditions with an ethyl acetate/acetic acid/dimethyl sulfoxide solvent mixture (10:2:1, v/v/v) in quadruplicate as described (Bou, Llauger, Arnau, & Fulladosa, 2018). In brief, 2 g of ground sample were weighed into 50 ml capacity centrifuge tubes and homogenized using an UltraTurrax T25 model disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) for 1 min at 9000 rpm with 10 ml of the solvent mixture while the tube was immersed in ice. The sample residues were re-extracted (few second burst) with the same volume of solvent mixture and added to the previous one. After extraction on ice for 20 min and centrifugation (1100 g, 14 min, 4 °C), the supernatant was filtered through a filter paper (grade 1) and collected into a volumetric flask. The solvent extractions were performed until the final volume was attained (typically 20 ml). Two hundred microliters of extracts were transferred to 96-microwell plates and sealed with polyolefin acrylate sealing tape. The samples were then incubated for 2 min at 30 °C and shaken for 30 s before measuring the fluorescence of ZnPP using a Thermo Fisher Scientific Varioskan microplate reader (Waltham, Massachusetts, USA) with the excitation at 416 nm and the emission at 588 nm. Ethyl acetate/acetic acid/dimethyl sulfoxide solvent mixture (10:2:1, v/v/v) was used as a blank. Each sample was analysed four times, and the excitation and emission spectra of the standards and samples were compared. ZnPP content was calculated using a calibration curve prepared with ZnPP standard solutions and expressed on the wet weight (ww) basis and dry matter (dm) basis (ZnPP content DM = ZnPP (mg)/(sample (kg) – water (kg))).

2.6. Assessment of food safety implications associated with different elaboration treatments

The behaviour of *Listeria monocytogenes, Salmonella, Staphylococcus aureus* and *Clostridium botulinum* non-proteolytic and proteolytic in restructured Fenalår elaborated with different salting treatments was assessed and compared by applying the predictive models available in ComBase Predictor (www.combase.cc). For simulations with the predictive models, worse case scenarios were set by using the highest values of pH, a_w and temperature recorded in each step of the restructured Fenalår elaboration process as model inputs (Table 2). Simulations using nitrite concentration of 72 ppm as the input value for the model were performed to consider the uneven diffusion of the added nitrite to the internal parts of Fenalår and the possible transformation to non-active substances such as nitrate (estimation 10–40%), bound to lipids (1–15%) or lost as gas (1–5%) (Cassens et al., 1979; Honikel, 2008). Kinetic parameters, i.e. growth rate ($1/h^{-1}$) resulting from the doubling time (h) provided by the predictive tool was estimated for each sampling step of the production process, i.e. after 42, 44, 54, 67, 74 and 90 days of processing. To assess the impact of the elaboration process of restructured Fenalår, the index time increase (t_{inc}) was used following the approach of the Spanish Agency for Food Safety (Zurera-Cosano et al., 2011). This index is defined as the time required by the microorganism to increase its concentration a given magnitude, e.g. 1 log unit. The impact of the salt and/or nitrite concentration was assessed in relative terms, e.g. the percentage of reduction of t_{inc} in the SR and NNSR products with respect to the SS. The antimicrobial effect of sorbate and the phenolic compounds present in the smoke was not evaluated due to the lack of predictive models including these factors. However, since sorbate and smoking were equally applied in all elaboration treatments,

it was assumed that their influence on microbial behaviour was similar for SS, SR and NNSR products.

2.7. Statistical analysis

A one-way ANOVA was used to evaluate the effect of the salting treatment (SS, SR and NNSR) on chemical, instrumental colour and instrumental texture characteristics. Differences between mean values were tested by means of Tukey's test at $\alpha = 0.05$. All the analysis was performed using the statistical package XLSTAT v.2016.3. (Addinsoft SARL, Paris, France).

3. Results and discussion

3.1. Physicochemical characterization

Chemical characteristics of restructured Fenalårs are shown in Table 1. Mean salt content of SS Fenalårs was 5.8%, being similar to traditional bone-in Fenalår reported to be 5.42% by Petrova, Tolstorebrov, Mora, Toldrá, and Eikevik (2016). "Fenalår fra Norge PGI", which comprises the varieties "Traditional" (minimum 30% desiccation and dry-curing period between three and six months) and "Matured" (minimum 35% desiccation and a dry-curing period between five and nine months) establish a maximum salt content lower than 9% and 7%, respectively (Regulation (EU) No. 1752/2017). However, salt content of Fenalår production can vary greatly, showing values from 5% to 10%, as reported by Håseth et al. (2014). Other lamb/sheep dry-cured products also show a large variation but lower salt contents (Stojković et al., 2015). Teixeira et al. (2017) reported a salt content of 3.8% in goat and 4.7% in sheep cured legs. Ivanovic et al. (2016) found values of 4.5% in goat smoked ham, whereas Paleari, Moretti, Beretta, and Caprino (2006) reported a salt content of 3.53% in dry-cured lamb thighbone from Lamon and Bergamasca breeds. In comparison to pork dry-cured ham on the market, both SS and SR restructured Fenalårs showed similar salt content. A market study (http://www.innovacc.cat/wp-content/uploads/2017/05/Annex_9.2._Informe_final.pdf) showed salt content values of 5.26%, 4.81%, 5.32% and 5.40% for Parma PDO, Alto Addigio PGI, TGE Serrano and Culatello di Zibello PDO, respectively. Tomažin et al. (2020) studied Kraški pršut (Slovenian ham), reporting salt values between 3.61% and 5.68%.

In this study, SR treatment produced a significant decrease of salt content in the final product ($p \leq 0.05$). However, the achieved reduction was only ~15% and therefore the product could not be labelled as a salt reduced product (Official Journal of the European Communities C 371 01.12.1998). Further reduction of the salt in restructured Fenalår production would require additional investigation of product safety. All the salting treatments, SS and SR/NNSR restructured Fenalårs showed a_w lower than 0.90 in the final product, according to the general rule of Fenalår fra Norge stablished in the Regulation (EU) No. 1752/2017. However, variation of pH, a_w and temperature during the elaboration process should be evaluated to study the microbiological safety implications of the salt reduced treatments. Fig. 2 shows fluctuations of pH and a_w during the restructured Fenalår elaboration process.

3.2. Characterization of colour and texture

Colour measurements showed no significant changes between SS and SR treatments for any of the studied parameters (L*, a* and b* values). In contrast, no addition of nitrite (NNSR) produced a significant decrease of redness (a* values) ($p \leq 0.05$). In the case of NNSR, nitrosylmyoglobin is not formed because nitrite has been omitted in the elaboration process. The obtained red colour was in part attributed to ZnPP (Table 1), although other porphyrins such as metmyoglobin could also be present. Given that ZnPP is quite similar to NO-heme, the presence of oxidized forms of myoglobin may contribute to reduced redness values. Wakamatsu, Okui, Ikeda, Nishimura, and Hattori (2004) proved in pork meat that when no nitrites are used, Zn-protoporphyrin (ZnPP) is formed instead of nitrosylmyoglobin, decreasing the redness intensity. To our knowledge, this is the first time that ZnPP formation is reported in lamb dry-cured ham.

Mean ZnPP content in the studied NNSR Fenalår was 23.70 mg/kg on a dry weight basis (Table 1), whereas it was negligible when using nitrite (SS and SR) because of the inhibition that nitric oxide produces on ZnPP formation (Wakamatsu, Hayashi, Nishimura, & Hattori, 2010). Variations found within Fenalårs from the same group (contents between 19 and 31 mg/kg ZnPP dw) can be due to the effect of raw ham pH or salt

Table 1
Mean ± standard deviation of chemical, instrumental colour and instrumental texture parameters of restructured Fenalår at the end of the process using different salting treatments.

	Salting treatments				
	SS	SR	NNSR	RMSE	p-value
n	10	10	10		
Weight loss (%)	36.4 ± 0.9	36.7 ± 0.49	36.5 ± 0.32	0.43	0.543
Physicochemical parameters					
NaCl (%)	5.8 ± 0.4[a]	4.9 ± 0.2[b]	4.8 ± 0.3[b]	0.30	0.001
Moisture (%)	56.8 ± 0.5	56.6 ± 1.6	57.9 ± 3.6	2.38	0.661
pH	5.6 ± 0.0	5.7 ± 0.0	5.6 ± 0.0	0.03	0.179
a_w	0.909 ± 0.007	0.917 ± 0.001	0.918 ± 0.001	0.005	0.066
ZnPP (mg/kg)ww[a]	0.03 ± 0.09[b]	0.18 ± 0.15[b]	10.11 ± 2.9[a]	1.752	<0.0001
ZnPP (mg/kg) dw[b]	0.07 ± 0.16[b]	0.42 ± 0.36[b]	23.70 ± 4.88[a]	2.915	<0.0001
Instrumental colour parameters					
L*	34.9 ± 1.9	33.6 ± 1.0	35.4 ± 2.0	1.88	0.345
a*	12.2 ± 1.1[a]	12.3 ± 1.29[a]	6.9 ± 1.3[b]	1.42	0.000
b*	4.7 ± 0.8	4.8 ± 0.6	4.2 ± 1.5	1.11	0.675
Instrumental texture parameters					
F_0 (kg)	1.73 ± 0.43[a]	1.18 ± 0.32[b]	1.09 ± 0.43[b]	0.391	0.005
Y_2	0.398 ± 0.009[b]	0.426 ± 0.023[a]	0.434 ± 0.027[a]	0.0220	0.005
Y_{90}	0.708 ± 0.014[b]	0.735 ± 0.021[a]	0.744 ± 0.026[a]	0.0210	0.004

[ab]Means within rows with a different letter are significantly different ($p \leq 0.05$). SS: Standard Salting; SR: Salt Reduced; NNSR: Non-Nitrite Salt Reduced. RMSE: root mean square error of the linear model.

[a] Expressed on a wet weight basis.

[b] Expressed on a dry weight basis.

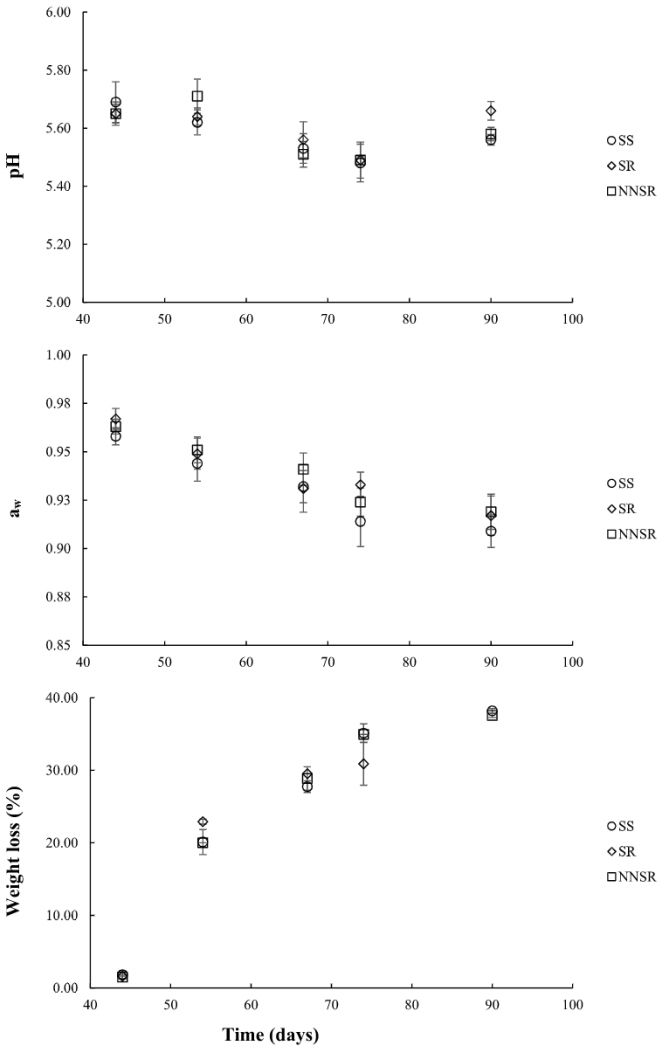

Fig. 2. Evolution of pH, water activity (a_w) and weight loss through the restructured Fenalår elaboration process using different salting treatments. (○) SS: Standard Salting; (◇) SR: Salt Reduced; (□) NNSR: Non-Nitrite Salt Reduced.

content variations which have been reported to influence ZnPP formation (Bou, Llauger, Arnau, Olmos & Fulladosa, 2020; Wakamatsu et al., 2019). Higher concentrations of ZnPP were reported for non-nitrified Parma dry-cured ham (43.8 ± 14 mg/kg on a dw basis) (Bou et al., 2018) and also for Spanish dry-cured ham elaborated without additives (between 67 ± 24 and 95 ± 11 mg/kg on a dw basis) (Bou, Llauger, Arnau Olmos, & Fulladosa, 2020). Wakamatsu, Odagiri, Nishimura, and Hattori (2009) reported ZnPP contents that ranged between 27 and 47 mg/kg on a wet weight basis in three different muscles (*Semimembranosus*, *Semitendinosus* and *Biceps femoris*) of Parma ham. However, Ghadiri Khozroughi, Kroh, Schlüter, and Rawel (2018) mentioned that red meat (lamb and beef) yielded up to four times higher ZnPP concentrations compared to porcine meat muscle, while there was almost no ZnPP quantified in poultry samples. De Maere et al. (2017) also reported differences of ZnPP formation in different *in vitro* meat sources, showing a higher ZnPP formation rate in lamb. However, in this study, this higher formation rate in lamb has not been observed. Lower ZnPP concentration found in lamb Fenalår could be related to the shorter maturation period (4 months) in comparison to the Parma and Spanish dry-cured hams (12–24 months), since processing time is a crucial factor for ZnPP formation (De Maere et al., 2017). These results suggest that redness of NNSR Fenalår could be improved by optimizing elaboration procedures, i.e. increasing ageing times and/or processing temperatures at the different steps of the process or by selecting raw material with characteristics that enhance formation of ZnPP. It must be remarked that elaboration with or without the bone can also produce important differences on a* values. Coll-Brasas, Kåsin et al. (2019) reported that traditional bone-in Fenalår with standard salting treatment had significantly ($p \leq 0.05$) lower redness values ($a^* = 7.41$) than restructured Fenalårs analysed in this study ($a^* = 12.2$). This fact could be explained by the easier diffusion of curing agents inside the product because of the higher nitrified surface of restructured Fenalårs.

In terms of texture, salt reduction decreased F_0 and increased force decay at 2 (Y_2) and 90 s (Y_{90}) (Table 2). These results are in agreement with other studies carried out in pork dry-cured ham. Ruiz-Ramírez, Arnau, Serra, and Gou (2005) found that dry-cured ham muscles from pork with lower NaCl content showed lower hardness, cohesiveness and springiness. Benedini, Parolari, Toscani, and Virgili (2012) found an increase of Y_2 and Y_{90} in low salt class dry-cured ham. Morales et al. (2007) also found that BF muscles from pork dry-cured ham with levels of NaCl lower than 2% were more prone to show soft textures.

Table 2
Results of the simulations applying predictive models in order to evaluate the implications of different Fenalår salting treatments on the behaviour of *Listeria monocytogenes*.

Step of the process	Temperature (°C)[a]	Duration (days)	Type	Maximum a_w	Maximum pH	Growth rate (h^{-1})	Doubling time (h)	Time for 1 log increase (d)	Reduction in comparison to SS (%)
Cold phase	4	42	SS	0.959	5.70	0.004	71.25	10.4	0
			SR	0.969	5.67	0.005	54.98	8.3	20
			NNSR	0.965	5.66	0.007	44.19	6.0	43
Drying	18	2	SS	0.957	5.69	0.042	7.22	1.0	0
			SR	0.964	5.64	0.046	6.57	0.9	9
			NNSR	0.962	5.63	0.061	4.90	0.7	31
Storage	13	10	SS	0.956	5.68	0.020	14.77	2.1	0
			SR	0.960	5.68	0.023	13.24	1.8	13
			NNSR	0.962	5.82	0.035	8.52	1.2	43
Smoking and storage	13	13	SS	0.943	5.62	0.013	23.49	3.2	0
			SR	0.943	5.61	0.013	43.49	3.2	0
			NNSR	0.951	5.57	0.023	13.27	1.8	43
Pressing and storage	13	7	SS	0.932	5.63	0.009	32.70	4.6	0
			SR	0.943	5.63	0.013	23.49	3.2	31
			NNSR	0.933	5.50	0.012	24.56	3.5	25
Final process	13	16	SS	0.918	5.61	0.000	0.000	–	–
			SR	0.927	5.69	0.009	34.80	4.6	–
			NNSR	0.928	5.63	0.011	26.53	3.8	–

SS: Standard Salting; SR: Salt Reduced; NNSR: Non-Nitrite Salt Reduced.

[a] Temperature of the air of the drying room where the hams were stored. It is assumed as a worst-case overestimate (in general representative of the surface of the product) as a lower temperature could be expected in the product in most of the steps.

Optimization of the Fenalår elaboration process and corrective actions using emerging technologies (Coll-Brasas, Arnau et al., 2019; Contreras et al., 2020) could help to yield SR and NNSR Fenalårs with similar textural characteristics to SS Fenalårs.

3.3. Microbial safety implications associated with the different restructured Fenalår salting treatments

The results of the simulations using predictive models indicated that microbial behaviour in restructured Fenalår varies according to the microorganism and the physicochemical characteristics of the products, which are dependent on the salting treatments used (SS, SR and NNSR) and the temperatures at which each manufacturing step is carried out.

During the cold phase, most of the microbiological hazards evaluated would not be able to grow in Fenalår, either because the temperature was below the minimum growth temperature, i.e. *Salmonella*, *S. aureus, E. coli* and proteolytic *C. botulinum*, and/or due to the presence of nitrites (e.g. non-proteolytic *C. botulinum*). At this step, among the microorganisms assessed only *L. monocytogenes* would be able to grow in Fenalår in agreement with its psychrotrophic nature. The t_{inc} of *L. monocytogenes* in SR and NNSR would be affected in comparison with the value of this index in SS (Table 2). The t_{inc} of *L. monocytogenes* in NNSR products would be 43% lower in comparison with the SS product with 72 ppm of active nitrite. This could be attributed to the fact that salt reduction would yield products with physicochemical characteristics that are slightly different in comparison with the SS product, e.g. a higher maximum a_w (Table 2). These differences determine changes in the *L. monocytogenes* growth rate. It was previously reported that under certain circumstances, such as refrigerated storage, nitrite is effective to control *L. monocytogenes* (EFSA, 2003; Tompkin, 2005). The reduction of added nitrite from 144 ppm to 0 ppm in the present study results in products with more favourable conditions for *L. monocytogenes* growth.

Similarly, during drying at 18 °C for 2 days, the salt reduction would result in products with a maximum a_w slightly higher in comparison with SS (Table 2). The t_{inc} for *L. monocytogenes* would be 31% and 9% lower in NNSR and SR products respectively in comparison with SS products, with 72 ppm of active nitrite. Regarding *Salmonella* and *E. coli*, assuming that the inhibitory mechanism of nitrites differs among different species and that it is not considered effective to control Gram-negative bacteria such as these pathogens (EFSA, 2017), the reduction of nitrite would not have a relevant effect on the t_{inc} for NNSR and SR products with respect to SS.

The t_{inc} of *S. aureus* would increase from 12 h during the drying step at 18 °C to 30–63 h during the subsequent manufacturing steps carried out at 13 °C in NNSR products, evidencing the positive correlation between temperature and growth rate. Research on the effects of nitrite removal on the behaviour of *S. aureus* in dry-cured products similar to restructured Fenalår are scarce, but research has demonstrated that nitrite failed to control *S. aureus* growth during the production process of a dry-sausage (Bang, Hanson, & Drake, 2008; Gonzales-Barron et al., 2015). It could also grow in medium-salted bacon, independent of the concentration of nitrite or ascorbate (Crowther, Holbrook, Baird-Parker, & Austin, 1976; Tompkin, 2005). Furthermore, the addition of increasing salt up to 3.64% (w/w) and nitrite at 154 ppm did not affect the growth capacity of *S. aureus* during drying of a pork sausage (Bang et al., 2008). Therefore, based on the available scientific information, the t_{inc} for *S. aureus* in SS, SR and NNSR products would be equivalent, i.e. independent of the salt and nitrite concentrations used in the assessed products.

In general, the decrease of a_w during the steps at 13 °C would drastically reduce the growth capacity of all the microorganisms evaluated. Model simulations indicated that slight differences in t_{inc} of *L. monocytogenes* in SS and SR products would be marked, since the slight differences in the physicochemical parameters of SS and SR Fenalår do not determine changes in growth rate, except after the pressing and storage step (Table 2). *Clostridium botulinum* non-proteolytic would not be able to grow in products in any of the steps of the manufacturing production process, as the minimum a_w enabling its growth is 0.97 (ICMSF, 1996), while during drying at 18 °C, proteolytic *C. botulinum* would not reach 1 log increase in Fenalår (t_{inc} = 14d) for longer than the actual duration of the drying step. Merialdi et al. (2016) detected a slight growth of 2–2.5 Log units of proteolytic *C. botulinum* in 2 out of 9 samples after 7 days of drying of Parma ham (dry ham without nitrite and nitrate) at 20 °C, though the toxin formation was not evidenced until 14 days.

The reduction of nitrite in the Fenalår elaboration is not a determining factor limiting the growth of *Salmonella, E. coli* and *S. aureus*, it increases very little the growth potential of proteolytic *C. botulinum* but considerable increases the growth potential of *L. monocytogenes*, consequently the implementation of complementary antimicrobial hurdles (including a decrease of temperature during the process) would be needed in order to assure the microbiological safety of nitrite-reduced products.

It is worth mentioning that the simulations with the ComBase model, which was developed in growth media, usually provide fail-safe predictions, overestimating the growth that would actually occur in real food matrices. Despite the limitations, the tool provides useful simulations to calculate the t_{inc} for comparing the relative impact of different scenarios (SS, SR and NNSR) of input data.

4. Conclusions

Salt reduced restructured Fenalår is challenging to produce since soft textures, important changes in colour and safety hazards during the elaboration process can occur, especially in non-nitrite salting treatments. The elaboration of Fenalår without nitrite must be cautious as it can increase the growth potential of *L. monocytogenes* and slightly that of proteolytic *C. botulinum*. However, predictive microbiology and the optimization of the process to enhance ZnPP formation can help to ensure the safety and quality of salt reduced restructured Fenalårs without additives.

CRediT authorship contribution statement

E. Coll-Brasas: Investigation, Formal analysis, Writing - original draft, Visualization. **A. Possas:** Data curation, Writing - original draft, Formal analysis. **P. Berg:** Supervision, Funding acquisition. **V. Grabež:** Project administration, Writing - review & editing, Resources. **B. Egelandsdal:** Project administration, Supervision, Writing - review & editing, Resources. **S. Bover-Cid:** Visualization, Supervision, Writing - review & editing, Data curation, Formal analysis. **E. Fulladosa:** Visualization, Supervision, Writing - review & editing, Formal analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107460.

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Paper III

Fulladosa, E., Guerrero, L., Illiana, A., Olmos, A., **Coll-Brasas, E.**, Gou, P. & Arnau, J. (2021). Instrumental texture analysis on the Surface of dry-cured ham to define the end of the process. *Meat Science, 172*. 108334.

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Instrumental texture analysis on the surface of dry-cured ham to define the end of the process

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ABSTRACT

The end of the elaboration process of dry-cured ham is currently decided by product weight loss and/or by an expert who carries out an evaluation of the tactile texture on the surface. The objective of this study was to define the optimal measurement conditions of an instrumental texture analysis on the surface of the dry-cured ham (ITAS), to define the end of process. 120 dry-cured hams were classified by experts into Hard (appropriate) or Soft (non-appropriate) texture groups and used to perform compression tests using different probes on three anatomical positions. Results showed that the small probe in position 2 gave the most discriminant conditions, providing representative information of the internal texture. Although classification using only weight loss was possible with an accuracy rate of 80.4% or 66.7% depending on the weight loss, the maximum classification accuracy was obtained when using ITAS in combination with weight loss. Further studies at industrial level are needed.

1. Introduction

In the dry-cured ham industry, the end of the process is decided by the product weight loss and/or by the evaluation of the texture of the whole hams made by experts on the surface. Weight loss criterion is also used in the Traditional Speciality Guaranteed (TSG) "Jamón Serrano" (Jamón Serrano Foundation, 2007) as a quality standard, requiring a minimum weight loss of 33%. However, weight loss is not always well-correlated to the internal texture because other factors such as salt content, drying temperature and elaboration conditions or raw meat pH can also influence texture development (Harkouss et al., 2015; Morales, Arnau, Serra, & Gou, 2007; Morales, Serra, Guerrero, & Gou, 2007; Tomažin et al., 2020) and therefore, its use alone to decide the end of the process may lead to dry-cured hams with non-appropriate texture characteristics being sent to the market. For this reason, not only a sensory analysis of aspect and flavour, but also of the textural attributes of a small part of the dry-cured ham production is also required in the Jamon Serrano TSG seal. Although some studies to develop faster instrumental methods to evaluate the sensory perception of texture are being investigated, both sensory and instrumental tests are tedious, destructive and can only be performed for a small part of the production. Currently, non-invasive technologies able to determine internal textural characteristics of the product are not available on the market, even though some authors have pointed out the possibility of applying them in the future by using "*in-line*" multi energy X-ray (Fulladosa et al., 2018) or magnetic resonance imaging (García-García, Fernández-Valle, Castejón, Escudero, & Cambero, 2019) devices.

Meanwhile, different strategies could be used to more efficiently decide the end of the process according to texture. Information on the fat content of the raw material and acquired salt content using non-destructive technologies implemented in-line (De Prados et al., 2015; Fulladosa, Munoz, Serra, Arnau, & Gou, 2015; Fulladosa, Santos-Garces, Picouet, & Gou, 2010; Santos-Garcés, Gou, Garcia-Gil, Arnau, & Fulladosa, 2010; Santos-Garcés, Muñoz, Gou, Garcia-Gil, & Fulladosa, 2014; Schivazappa et al., 2017) could provide relevant information for the estimation of the optimal duration of the elaboration process since there is a well-known relationship between the drying time and fat and salt content of the dry-cured ham. Besides, texture development is influenced by salt content (Andrés, Cava, Ventanas, Thovar, & Ruiz, 2004; Benedini, Parolari, Toscani, & R., V., 2012), giving an idea of the final texture of the product. Information on raw meat pH or impedance measured "*in-line*" could also provide further information to predict the final texture of the product (Guerrero et al., 2004; Guerrero, Gou, & Arnau, 1999; Schivazappa et al., 2002). However, because dry-cured ham texture can also be influenced by other processing variables more difficult to control, it would be of interest to measure texture "*in-line*" at the end of the process for classifying hams into Hard (appropriate) or Soft (non-appropriate) texture groups before sending them to the

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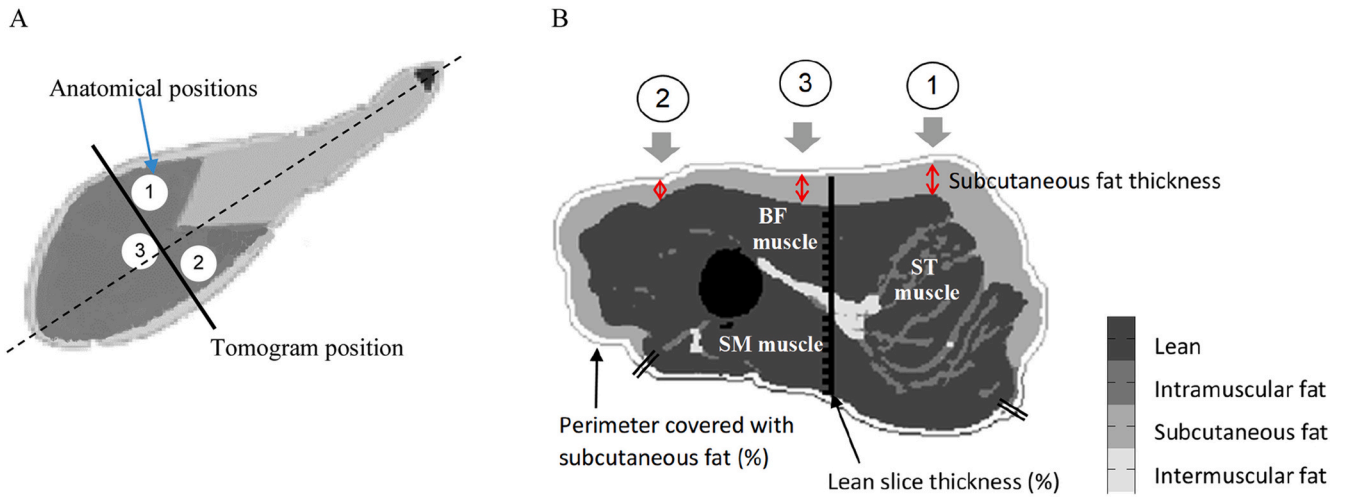

Fig. 1. Anatomical positions (1, 2 and 3) in the whole dry-cured ham (A) and its correspondence to a tomogram (B).

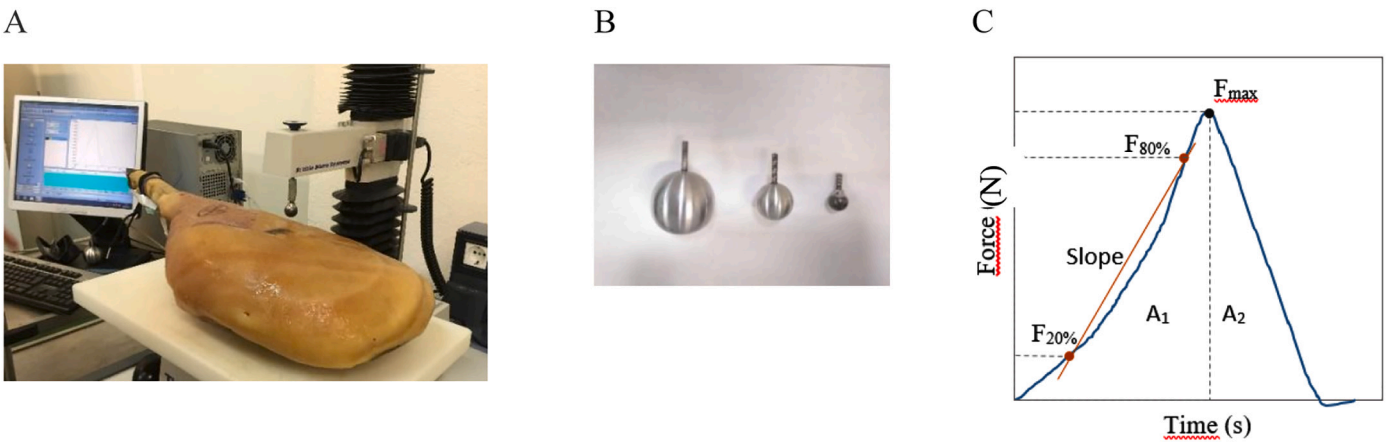

Fig. 2. Instrumental texture analysis on the ham surface (ITAS) (A). Large, medium and small probes used for instrumental texture evaluation on the surface of the ham (B). Example of acquired force/time curve and textural parameters determined (C).

market. An instrumental analysis of the texture from the surface of the ham could reduce workload, subjectivity and also improve the quality of the product marketed.

The objective of this study was to define the optimal measurement conditions (probe size and anatomical location) of a non-destructive instrumental texture analysis on the surface of the dry-cured ham (ITAS), to evaluate whether the product had the appropriate texture to be sent to the market or not. The usefulness of including information about weight loss and subcutaneous fat thickness to improve the prediction of product texture was also evaluated. The representativeness of texture analysis on the surface to define the internal textural characteristics of the ham were also evaluated.

2. Material and methods

2.1. Experimental design

2.1.1. Experiment 1. Setting up the ITAS method

One hundred and twenty dry-cured hams from Duroc, Large White and Landrace breeds crosses skinned with the typical V-shape (Fig.1A), and with an elaboration process time of at least 24 months, were selected according to experts texture evaluation in a dry-cured ham facility owned by Monte Nevado (Segovia, Carbonero el Mayor, Spain). The weight loss of the sampled dry-cured hams ranged between 29% and 42%. Sixty hams were selected for having an appropriate texture for sending to the market after evaluation of the texture on the surface by experts (Hard texture). The rest of the hams ($n = 60$) were selected for having a non-appropriate texture showing too much softness (Soft texture). Classification of hams into Hard or Soft texture groups was carried out by three different experts who routinely perform this activity in the company. They evaluated the texture of the hams in triplicate by pressing with the fingers on the external surface close to *Semimembranosus* (SM) and *Biceps femoris* (BF) as well as on the knuckle muscles. This is the standard procedure in many dry-cured ham companies to estimate the texture of the internal muscles from the surface of the ham. The most common score of the three experts (appropriate/hard texture or not appropriate/soft texture) was considered as the reference value. Triplicates were performed by evaluating each ham on three different days.

These hams were used to define the measurement conditions of an instrumental compression test. In order to minimise any possible damage to the ham, the instrumental texture was only evaluated in the part covered with subcutaneous fat. Three different anatomical positions were selected (1, 2 and 3, as shown in Fig. 1) and three different size spherical probes (large, medium and small, with diameters of 5.5, 3.5 and 1.9 cm respectively, see Fig. 2) were evaluated. Position 1 and 2 were chose because are the areas evaluated by experts at the industry. Position 3 was chosen because it would be the most convenient area of measurement in the case of industrial implementation. Spherical probes were chosen in order to simulate the shape of the human fingers. Dry-cured hams were distributed according to the experimental design defined in Table 1. Subcutaneous fat thickness in each anatomical position

Table 1
Distribution of hams according to texture group and probe size.

Texture group by expert evaluation (Reference values)	Anatomical positions for instrumental test	Probe size		
		Large (ø = 5.5 mm)	Medium (ø = 3.5 mm)	Small (ø = 1.9 mm)
Hard texture	1 2 3	n = 20	n = 20	n = 20
Soft texture	1 2 3	n = 20	n = 20	*n* = 20

and volumetric content of subcutaneous, intermuscular and intramuscular fat (%), the perimeter of the slice covered with subcutaneous fat (%) and the lean slice thickness (%) were determined using computed tomography image analysis (Section 2.3). Salt content of the hams was also estimated using previously developed predictive models based on computed tomography data (Santos-Garcés et al., 2010; Santos-Garcés, Munoz, Gou, Sala, & Fulladosa, 2012).

In order to study the relationship between the textural parameters measured on the surface and those measured in the BF muscle, five hams from the soft texture group and 4 hams from the Hard texture group were randomly selected. A Stress relaxation (SR) and a Texture profile analysis (TPA) test were performed on the BF muscle of these hams as described in Section 2.4.

2.1.2. Experiment 2. The effectiveness of the ITAS method to classify hams into different texture groups

Twenty-seven dry-cured hams with an elaboration process of 24 months and similar weight losses (36.5% ± 2.52) were selected according to their texture from the same dry-cured ham producer as in Experiment 1. This is the mean weight loss of the company production before being sent to market and the weight loss range in which the internal texture prediction is more difficult to assess by experts. Fifteen hams had a hard texture and twelve had a soft texture according to the most common score of the three different experts (reference value). The most discriminant measurement conditions defined in experiment 1 were used to perform the analysis. The discriminant ability of ITAS was assessed using the measured texture parameters on the surface of the ham in combination with the subcutaneous fat thickness and/or weight loss.

2.2. Description of ITAS method

Uniaxial compression tests (samples compressed in one direction and unrestrained in the other two) were performed using the different spherical probes shown in Fig. 2 on the three anatomical positions described in Fig. 1. All the measurements were carried out by using a Universal Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, England) using a load cell of 30 Kg at a constant crosshead speed of 40 mm/s. Crosshead displacement was 10 mm when using the large probe (Ø = 5.5 mm), and 15 mm when using the medium (Ø = 3.5 mm) and the small (Ø = 1.9 mm) probes. These distances were previously calculated to simulate the pressure exerted by a human finger at a maximum force. The displacement was measured automatically by the equipment itself after contacting the sample surface (minimum contact force of 10 g). Anatomical positions 1 and 2 were selected because they fell within the area evaluated by the experts. Anatomical position 3 was centrally located and avoided movement of the ham during ITAS analysis, facilitating in-line evaluation of the hams at the industry without any mounting system. Dry-cured hams were fixed with wedges to prevent any movement during compression. Fig. 2A shows the instrumental texture analysis performed at the ham surface.

The force/time curves were recorded and evaluated by Exponent stable micro Systems software (version 6.1.16.0). The parameters were calculated by macros written using the same software. The following parameters were determined (Fig. 2C): Maximum compression force (F_{max}, N); area under the curve until maximum compression force (A_1, N.mm) representing the total work performed to compress the ham; area under the curve after the maximum compression force (A_2, N.mm) representing sample recovery to its initial state after being compressed (to some extent related to the elasticity of the product), and the slope of the curve between 20% and 80% of the maximum compression force (Slope, N/mm) representing Young's modulus or the modulus of elasticity.

2.3. Computed tomography image analysis and salt content prediction

All the dry-cured hams were scanned at IRTA centre of Monells, using a scanner model HiSpeed Zx/i from General Electric Healthcare (GE Healthcare, Barcelona, Spain). An axial protocol was used with settings 80 kV, 250 mA and rotation time of 2 s. Image size was 512 × 512 voxels and the Displayed Field of View (DFOV) was 461 mm (resolution = 1.1 pixels/mm). Before scanning, the hams were properly aligned in the CT equipment. Then, a 10 mm thick slice was scanned 10 cm from the aitch bone in the distal direction. This was the area where the ITAS was performed. The acquired matrixes of values were analysed using Centricity Radiology RA600 v.7.0 (GE Medical Systems, Barcelona, Spain). From each image, the subcutaneous fat thickness (mm) at each anatomical position (Fig. 1B) was manually measured perpendicular to the tray.

Salt content was estimated in BF muscle by using previously developed predictive models (Santos-Garcés et al., 2010) that used tomograms acquired at 80 kV (RMSE = 0.263%). To do so, a central part of BF muscle was automatically selected, and the predictive model applied with an in-house script written in Matlab. This script used a combination of thresholding and gradient detection algorithms to segment intramuscular fat (Munoz, Rubio-Celorio, Garcia-Gil, Dolors Guardia, & Fulladosa, 2015). Subcutaneous fat and intermuscular fat were segmented applying thresholding after segmentation of the intermuscular fat. Depending on the position of the segmented fat patches, pixels were classified as subcutaneous fat if the fat patch was close to the ham contour, otherwise pixels were classified as intermuscular fat. The content of subcutaneous, intermuscular and intramuscular fat (%) was estimated as the number of pixels corresponding to each class of fat over the total number of pixels of the ham slice (See Fig. 1B). The lean slice thickness (%) was calculated as the percentage of pixels containing lean in a line perpendicular to the tray and at 3 cm from the edge of the bone over the number of pixels containing lean and fat. The perimeter covered with subcutaneous fat (%) was calculated as the percentage of number of pixels of the perimeter of the slice segmented as subcutaneous fat into the total number of pixels of the perimeter (see Fig. 1B).

2.4. Instrumental texture analysis of internal muscles

From each ham selected for internal texture analysis (n = 10), a minimum of twelve parallelepipeds from BF muscle were accurately carved with a scalpel (20 mm × 20 mm × 15 mm, length x width x height) for SR and TPA tests. Before the texture analysis the pieces were stored at 4 °C for 24 h wrapped in plastic film to prevent them from drying out. A SR test was performed using a Universal Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, England), provided with a 30 kg load cell and a 60 mm compression plate. Samples were compressed to 25% of their original height, perpendicular to the muscle fibre bundle direction, at a crosshead speed of 1 mm/s and at a temperature of 4 °C ± 2 °C. The force decay or relaxation versus time $Y_{(t)}$ was calculated as follows:

$$Y_{(t)} = \frac{F_0 - F_{(t)}}{F_0}$$

where F_0 (N) is the initial force and $F_{(t)}$ is the force recorded after t seconds of relaxation. The force decay at 2 s (Y_2) and 90s (Y_{90}) was calculated (Morales, Guerrero, Serra, & Gou, 2007).

The Texture Profile Analysis was performed using a universal Texture Analyser TA.HD.plus (Stable Micro Systems Ltd., Surrey, England) provided with a 250 kg load cell and a 75 mm compression plate. Samples were compressed twice to 75% of their original height (time = 0 s between the two compression cycles), perpendicular to the muscle fibre bundle direction, at a crosshead speed of 1 mm/s and at a temperature of 4 °C ± 2 °C. Force versus time was recorded and the following parameters were calculated: hardness (N), cohesiveness (dimensionless) and springiness (dimensionless). Hardness is defined as the maximum peak force during the first compression cycle; cohesiveness as the ratio between the positive force area during the second compression cycle and that found during the first compression cycle; and springiness as 'the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite (Bourne, 1978). In both SR and TPA tests, for each parameter, the average of the minimum six pieces per ham was used for the statistical analysis.

2.5. Statistical analysis

The reproducibility of each expert was determined by analysing their frequency of successes and failures in the three repetitions performed for each ham compared to the most frequent classification (hard or soft texture) given by the three experts in the three replicates ($n = 9$). The expert was considered to be reproducible when the result of the three repetitions of the same expert was the same and agreed with the most frequent classification given by them all.

Differences of internal texture between Soft and Hard texture groups were analysed using a one-way ANOVA procedure including texture group as fixed effect. Textural parameters measured at the ham's surface (F_{max}, A_1, A_2 and slope) were also analysed by using a one-way ANOVA procedure for each combination of the anatomical position (1, 2 or 3) and each probe size (large, medium and small). The model included the Texture Group defined by the experts (Hard/Soft) as fixed effect. Weight loss and/or subcutaneous fat thickness were also included as covariates in additional models. Differences between mean values were tested by means of Tukey's test ($p < 0.05$).

A discriminant analysis (DA) and a Partial Least Square discriminant analysis (PLS-DA) were applied to classify the dry-cured hams into the different, predefined texture groups (Hard and Soft) on the basis of the textural parameters measured on the surface (Force/time curve or F_{max}, A_1, A_2 and slope), weight loss and subcutaneous fat thickness. The percentage of samples correctly classified according to cross-validation analysis was calculated. Linear correlations between surface and internal texture parameters were calculated in those hams where both measurements (on the surface and on BF muscle) were performed ($n = 9$). All the analyses were performed using the XLSTAT v19.7 (2017) (Addinsoft, Paris).

3. Results and discussion

3.1. Experiment 1: definition of probe size and anatomical measurement position for ITAS

Soft and Hard texture groups showed significant differences in internal texture (F_0 and Y_{90} from the SR test and hardness and cohesiveness from the TPA test) ($p < 0.05$) (Table 2). This analysis was performed to corroborate the ability of experts to predict the internal texture of the ham from the surface when evaluating hams with significantly different weight losses. Besides, the mean reproducibility of experts when classifying the hams into the two different texture groups was 89.1%. This means that experts would classify 89.1% of production into the same group (soft or hard) after evaluating the hams three times, producing an error in classification in 10.9% of the production.

Table 3 shows physicochemical characteristics of the hams used in the experiments performed with the different probe sizes. Hams assigned to the different experiments using large, medium and small probes had similar characteristics ($p > 0.05$). Hard hams showed a significantly ($P < 0.05$) higher salt content, weight losses, lower subcutaneous fat thickness (except in the small probe batch) and lower percentage of perimeter covered with subcutaneous fat than the soft hams. However, no significant differences were found in lean slice thickness, intermuscular fat and intramuscular fat percentage. The higher salt content of hard hams contributed to reduce the activity of Ca-dependent proteases and cathepsin D (Sárraga, Gil, Arnau, & Monfort, 1989) although during the process m-calpain could increase its activity when salt content is up to 0.5 M (Rosell & Toldrá, 1996). An increase of hardness is observed as the water content is reduced during drying (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006).

The higher percentage of the perimeter covered with subcutaneous fat and the higher subcutaneous fat thickness in soft hams than in hard hams slowed down the dehydration process and facilitated proteolysis during the process. Moreover, weight losses had a significant correlation ($P < 0.05$) with the percentage of the perimeter covered with subcutaneous fat ($r = -0.61$) and with the percentage of lean surface ($r = 0.54$), but the correlation with the percentage of intermuscular and intramuscular fat was not significant ($P > 0.05$) ($r = -0.23$ and + 0.14, respectively).

Table 4 shows F values and significance (p-values) of the texture group effect for each of the textural parameters (F_{max}, A_1, A_2 and Slope) when using different probe sizes on the different anatomical positions. All the parameters provided relevant information to classify hams into the two texture groups defined by experts ($p < 0.05$). The highest F values for F_{max} and A_1 were obtained with the small probe in position 2, whereas slightly higher F values for A_2 and slope were obtained with the large probe in position 1. The use of the small probe in position 2 was considered the most discriminant condition and was used for further analysis. The reason could be that the small probe had a smaller surface of contact with the ham. The deformation of the ham surface in the areas surrounding the point of contact between the probe and the ham was lower. Therefore, the small probe was less affected by the areas adjacent to the measurement point, being the more representative measurement of the internal texture of the ham.

For anatomical position 2, the reason could be that, among the three studied, it was the part of the ham with the thinnest subcutaneous fat layer, and beneath this position there are the knuckle muscles and BF, and normally not thick intermuscular fat streaks. However, at anatomical position 2, movements are more prone to occur during the compression because of the shape of the ham. Therefore, a mounting system needs to be designed for deploying this technology in the factory. Anatomical positions 1 and 3 seem less convenient. Beneath position 1 there are the *Gracilis,* SM and *Semitendinosus* muscles, with the thickest subcutaneous fat layer which can produce changes on the results. Beneath position 3, the BF, SM and *Gracilis* muscles are located and intermuscular fat layers can be found.

It must be noted that there was a significant correlation between the textural parameters obtained with the small sensor in position 2 (F_{max} and A_2) and the internal texture measured in the BF muscle (F_0 and hardness), showing significant correlation coefficients between A_2 and F_0 ($r = 0.72$) and between A_2 and hardness ($r = 0.67$) ($p < 0.05$). No significant correlations were found between the other parameters. High correlations found between internal texture and F_{max} and A_2 values measured on the surface of the ham can be explained because both parameters provide relevant textural information on the whole hams. F_{max} represents the maximum force needed to compress the samples. Obviously, the harder the ham, the more force is needed, although

Table 2
Mean ± standard error of weight loss, ham surface measurements (F_{max}, A_1, A_2 and slope using the small probe in position 2) and internal texture in BF muscle (F_0, Y_2 and Y_{90} from SR test and Hardness, cohesiveness and springiness from TPA test) for dry-cured ham experts' texture groups.

Texture group	Weight loss (%)	Measurements at ham surface (small probe - position 2)					Texture in BF muscle (SR test)				Texture in BF muscle (TPA test)		
		n	F_{max} (N)	A_1 (N.mm)	A_2 (N.mm)	Slope (N/mm)	n	F_0 (N)	Y_2	Y_{90}	Hardness (N)	Cohesiveness	Springiness
Soft	34.1 ± 1.78	20	139.25 ^{b} ± 4.98	787.42^{b} ± 29.49	469.71^{b} ± 17.12	11.28^{b} ± 0.40	5	19.88^{b} ± 0.97	0.326 ± 0.005	0.624^{a} ± 0.007	90.22^{b} ± 5.24	0.52^{b} ± 0.013	0.61 ± 0.023
Hard	37.5 ± 1.46	20	195.53^{a} ± 7.10	1137.50^{a} ± 38.92	635.43^{a} ± 26.16	15.49^{a} ± 0.66	4	32.75^{a} ± 0.75	0.299 ± 0.001	0.584^{b} ± 0.001	142.19^{a} ± 4.78	0.60^{a} ± 0.005	0.71 ± 0.007

^{ab} Different letters between texture groups indicate significant differences ($p < 0.05$).

Table 3
Characterisation of hams used in experiments performed with different probes.

Probe size batch	Texture group	N	Weight green ham (kg)		Salt content (%)		Weight loss (%)		Subcutaneous fat thickness (mm)*		Lean slice thickness (%)		Intermuscular fat area (%)		Intramuscular fat area (%)		Perimeter covered with subcutaneous fat (%)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Large	Soft	20	13.0^{a}	0.20	5.3^{b}	0.12	33.6^{a}	0.48	11.5^{a}	1.19	78.0	1.89	4.0	0.30	4.9	0.21	69.6^{a}	0.96
	Hard	20	13.1^{a}	0.18	6.4^{a}	0.17	38.2^{b}	0.42	8.7^{b}	0.94	79.7	1.89	3.4	0.29	5.4	0.24	63.3^{c}	0.88
Medium	Soft	20	13.4^{a}	0.23	5.4^{b}	0.10	32.6^{a}	0.44	13.8^{a}	1.37	83.5	2.10	2.9	0.35	5.4	0.14	69.6^{a}	1.04
	Hard	20	12.9^{b}	0.19	6.3^{a}	0.11	38.4^{b}	0.38	8.7^{b}	0.95	80.9	2.04	3.1	0.25	5.2	0.22	65.3^{bc}	1.03
Small	Soft	20	12.9^{a}	0.15	5.5^{b}	0.09	33.4^{a}	0.35	13.1^{a}	1.15	82.0	1.83	3.0	0.23	5.1	0.20	68.7^{ab}	0.68
	Hard	20	13.2^{a}	0.15	6.4^{a}	0.13	38.1^{b}	0.31	11.2^{a}	1.04	84.7	2.24	2.3	0.38	5.3	0.18	62.6^{c}	0.77

^{ab} Different letters between texture groups within each probe size batch indicate significant differences ($p < 0.05$). The lean slice thickness is the percentage of pixels containing lean in a line perpendicular to the tray and at 3 cm from the edge of the bone over the number of pixels containing lean and fat. The percentage of the perimeter of the slice covered with subcutaneous fat is the number of pixels of the perimeter of the slice segmented as subcutaneous fat into the total number of pixels of the perimeter.

* Mean value for the three anatomical positions.

variability in subcutaneous fat thickness and the percentage of the perimeter covered with fat can interfere on this correlation. A_2 represents the energy that the ham returns when the probe moves back from the ham surface. If the ham is soft inside, the returned energy will be lower, since the sample becomes deformed for longer and does not return to its original position immediately after removing the compression force. These results suggest that textural measurements on the surface of the ham using the small sensor in position 2 could be used to estimate the texture in the BF muscle.

When weight loss (%) and subcutaneous fat thickness (mm) were included in the analysis of variance as covariates (results not shown), a positive significant weight loss effect was observed in all cases ($p \leq 0.05$). Therefore, weight loss was also an important parameter for the classification of hams into different textural groups. This result was expected since hardness increased with weight loss (Serra, Ruiz-Ramírez, Arnau, & Gou, 2005). Weight loss was also positively correlated to F_{max} A_1, A_2 and slope, which were useful for the classification of the hams. In contrast, subcutaneous fat thickness only had a significant effect if weight loss was dropped from the model. The reason for this is the significant negative correlation between these two

Table 4
F values and significance (*p*-values) for differences between texture groups in each textural parameter on the surface of the ham when using different probe sizes in the different anatomical positions (n = 20).

Probe size	Anatomical position		F_{max} (N)	A_1 (N.mm)	A_2 (N.mm)	Slope (N/mm)
Small	1	F-value	152.6	178.7	106.6	120.0
		p-Value	0.000	0.000	0.002	0.001
	2	F-value	407.3	504.9	277.0	290.8
		p-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	3	F-value	240.6	253.5	202.7	190.2
		p-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Medium	1	F-value	248.2	293.7	261.1	166.0
		p-Value	< 0.0001	< 0.0001	< 0.0001	0.000
	2	F-value	302.4	333.7	330.6	290.7
		p-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	3	F-value	227.5	238.0	250.7	184.8
		p-Value	< 0.0001	< 0.0001	< 0.0001	0.000
Large	1	F-value	328.6	309.1	328.9	310.5
		p-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	2	F-value	157.2	143.8	134.4	210.0
		p-Value	0.000	0.000	0.001	< 0.0001
	3	F-value	267.1	286.8	281.2	232.6
		p-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 5
Classification scores (% correctly classified in cross-validation) of dry-cured ham production in soft and hard experts' texture groups using weight loss, textural parameters from the surface of the ham and subcutaneous fat thickness.

Used parameters for classification	Used statistical analysis	n	Whole weight loss range of the production (28 to 42%)	n	Medium weight loss range of the production (34.5 to 37.5%)
Weight loss	DA	136	80.4%	56	66.7%
Textural parameters (F_{max}, A_1, A_2, slope)	PLS-DA	56	80.4%	27	70.4%
Force/time curve	PLS-DA		78.6%		66.7%
Textural parameters (F_{max}, A_1, A_2, slope) + Weight loss	PLS-DA		82.1%		70.4%
Textural parameters (F_{max}, A_1, A_2, slope) + subcutaneous fat thickness	PLS-DA		82.1%		74.1%
Textural parameters (F_{max}, A_1, A_2, slope) + Weight loss + subcutaneous fat thickness	PLS-DA		82.1%		74.1%
Force/time curve + Weight loss	PLS-DA		78.6%		66.7%

Textural parameters were obtained using the small probe in anatomical position 2.

covariates. An increase in subcutaneous fat thickness slows down the drying process and decreases the weight loss. In the case of anatomical positions 1 and 3, correlations between weight loss and subcutaneous fat thickness were around -0.60 whereas a lower correlation ($r = -0.46$) was found in position 2. The reason is the lower representativeness of position 2 with respect to the overall fat content of the ham. Internal differences in texture were also related to other parameters such as salt and fat content (Table 3).

The obtained results drive the development of a multisensor method to more accurately and less destructively identify dry-cured hams with an appropriate texture before being sent to the market. To do so, the use of non-destructive technologies that provide information about the product during the process (De Prados et al., 2015; Pérez-Santaescolástica et al., 2019) and the application of data mining techniques (Peromingo, Caballero, Rodríguez, Caro, & Rodríguez, 2020) in combination with ITAS data could be useful to estimate the remaining processing time to achieve the target texture.

3.2. Classification performance of dry-cured ham production using different data

Results from discriminant analysis showed that classification performance of dry-cured ham production, with weight loss between 28% and 42% (whole weight loss range), into Hard and Soft texture groups using only weight loss was possible with a correctness of 80.4% (Table 5). However, there was still an important part of hams, mainly in a range of weight loss between 34.7 and 37.5% (medium weight loss range), that were misclassified. The rate of feasibility for the classification of hams with a medium weight loss was just 66.7%. This means that, in a company such as the one which provided the hams for this study, in which the main part of the production (54%) had a weight loss between 34.5 and 37.5% before being sent to the market, 36% of the production would be misclassified if only weight loss was used to decide the end of process.

Textural parameters from ITAS provided relevant information for classification, showing similar classification scores to those of using weight loss when hams had a wide range of weight losses and increasing the rate of precision from 66.7% to 70.4% when only hams with a medium weight loss were used. The use of the whole force/time curve for the classification did not provide extra information showing similar classification scores than the textural parameters extracted from the curve. When combining textural parameters and subcutaneous fat thickness information, the maximum classification accuracy was obtained, both when using the whole weight loss range (82.1%) or only the medium weight loss range (74.1%). Therefore, the use of non-destructive measurements (such as individual weight loss, ITAS parameters and subcutaneous fat thickness using computed tomography) provided relevant information for dry-cured ham classification before being sent to the market. Further studies at industrial level with a larger amount of dry-cured hams with different intramuscular and intermuscular fat contents, the ageing process and salt contents are needed to generalise these results.

4. Conclusions

Texture parameters measured on the surface of the ham can discriminate dry-cured hams with appropriate texture from those not ready to be sent to the market. These texture parameters, together with subcutaneous fat thickness, could help to improve classification especially in those hams with a similar weight loss. However, a process validation using different types of hams (with different degrees of proteolysis, intramuscular and intermuscular fat content and ageing conditions) and including relevant information of the product (such as fat content in raw material or salt content after salting) obtained in-line during the process is needed.

Declaration of Competing Interest

None.

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Paper IV

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Evaluation of dry-cured ham pastiness using a rheometer

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Abstract. - Pastiness is a dry-cured ham defect that can be determined by sensory and chemical analysis. Since pastiness produces an increase of saliva viscosity, rheology might be useful to instrumentally evaluate this defect. The aim of this study was to evaluate the rheological behaviour of water extracts obtained *in vitro* mimicking the mastication of dry-cured ham samples with different pastiness intensities analysed at different temperatures. Commercial samples from different groups (non-pastiness (NPG), medium pastiness (MPG) and high pastiness (HPG) groups) were sensorially and rheologically analysed. Effect of temperatures and HPP treatments at 7 °C, 20 °C and 35°C were also evaluated. Results showed that apparent viscosity of the water extracts increased with the increase of pastiness and decreased with temperature. Significant differences were observed between NPG/MPG and HPG samples and between 25 °C and 37 °C in HPG. After HPP, sensory pastiness disappeared in samples from MPG at any temperature and decreased in samples from HPG only when HPP35 was used. Although the increase on viscosity of oral fluid is related to the sensory pastiness of samples, other factors seem to be involved in the mechanism of pastiness perception. Despite this, HPG samples can be distinguished from MPG and NPG samples.

Key words – Defective textures, pastiness, viscosity, rheology, instrumental measurements.

1. Introduction

Development of texture and flavour characteristics of dry-cured ham is due to the lipolysis and proteolysis processes occurring during ripening (Toldrá, 1998). However, an excessive proteolytic activity may produce damages in the structure leading to negative flavour characteristics and defective textures such as pastiness (Arnau, Guerrero & Sárraga, 1998; Toldrá, 2006). Pastiness is a mouth sensation described as the feeling, like a flour-water paste, during the mastication process (Guerrero, Gou & Arnau, 1999) being well-differentiated from softness that is described as a palatable texture in the mouth (Resano, Sanjuán, Cilla, Roncalés & Albisu, 2010).

Pastiness is a defect that has a considerable incidence in dry-cured ham production, *Biceps femoris* being the muscle more prone to developing this defect. Tapiador & García-Garrido (2003) found an incidence of 12% in industrial dry-cured ham production whereas Arnau (2013) in another study found an incidence of 24% in hams with a pH in the *Semimembranosus* muscle at 24 h *post mortem* (pH_{SM24h}) < 5.6 and 10% in hams with a pH_{sM24h} > 5.6. To reduce the incidence of this problem, the use of non-destructive determination of salt and fat contents using devices implemented on the production line

in the industry to readjust salting process (De Prados *et al.*, 2015; Fulladosa, de Prados, *et al.*, 2015; Fulladosa, Muñoz, Serra, Arnau & Gou, 2015; García-García, Fernández-Valle, Castejón, Escudero & Cambero, 2019) as well as the optimization of drying processes (Coll-Brasas *et al.*, 2021) could be useful. Corrective actions of this defect have also been described using mild thermal treatments applied with power ultrasound (Contreras, Benedito, Bon & Garcia-Perez, 2018; Contreras, Benedito, Quiles, Lorenzo, Fulladosa & Garcia-Perez, 2020). The use of High Pressure Processing (HPP) treatments (Coll-Brasas *et al.*, 2019; Fulladosa, Serra, Gou & Arnau, 2009) have also been reported to be effective to this end. HPP, commonly used in industry to eliminate pathogenic microorganisms and extend the product shelf-life (Aymerich, Picouet & Monfort, 2008; Rendueles *et al.*, 2011), can also affect the texture of the product, producing a decrease of pastiness intensity and an increase of hardness and stringiness (Fulladosa *et al.*, 2009; Lorido, Estévez, Ventanas & Ventanas, 2015). Moreover, these changes were found to be different depending on the HPP temperatures used and the initial texture characteristics of the samples (Coll-Brasas *et al.*, 2019).

Defective textures in dry-cured ham can be quantified with instrumental texture tests (Morales, Guerrero, Serra & Gou, 2007) but pastiness has only been measured by sensory analysis. In some studies, pastiness has been related to the proteolysis index (PI), showing this index as a good estimate of this defect (Careri *et al.*, 1993; García-Garrido, Quiles-Zafra, Tapiador & Luque de Castro, 1999; Morales, Arnau, Serra, Guerrero & Gou, 2008; Ruiz-Ramírez, Arnau, Serra & Gou, 2006). However, the cost and the time required for both sensory and chemical analysis show the need for finding simpler and faster techniques for routine analysis.

Sensory viscosity of saliva whilst masticating is highly correlated to pastiness defect (Coll-Brasas *et al.*, 2019). Rheological measurements of the oral dry-cured ham fluid or water whilst masticating, that can be obtained *in vitro* mimicking the mastication, might be useful to instrumentally evaluate pastiness perception. Viscosity analysis of the water extracts using a rheometer might allow for a faster, repeatable and cheaper measurement of pastiness intensity. Chen & Stokes (2012) described rheology studies as effective for overall sensory characterization since rheology dominates texture sensation at the early stage of chewing. Besides, rheology has been previously used to evaluate the mouthfeel of different components in wine (Laguna, Sarkar *et al.*, 2017) and also to compare the relationship between rheological properties of commercial full fat and fat-free/low fat versions of liquid and soft solid colloidal systems (milk, yoghurt, soft cream cheese) with their sensory properties (Laguna, Farrell, Bryant, Morina & Sarkar, 2017). However, quantifying the sensory mouthfeel feelings with an instrumental technique is not easy (Laguna, Sarkar *et al.*, 2017) and the effect of different experimental conditions needs to be evaluated.

The aim of this study was to evaluate if the rheological measurement of viscosity is related with pastiness perception. For that, the rheological behaviour of the water extracts obtained *in vitro* mimicking the mastication of dry-cured ham samples with different pastiness intensities were studied at different temperatures (25 and 37 °C).

Changes in sensory pastiness and in flow behaviour were evaluated in water extracts coming from samples with modified texture after being submitted to HPP at different temperatures (7 °C (HPP7), 20 °C (HPP20) and 35 °C (HPP35)). Characterization of commercial dry-cured ham samples was also performed in terms of sensory pastiness and viscosity of the water extracts using the developed method.

2. Material and methods

2.1 Samples

Twenty whole dry-cured hams from lean crosses of Large White and Landrace breeds and salted according to the traditional system of Serrano ham, were obtained at the end of process from a commercial producer. The sensory pastiness intensity of *Biceps femoris* (BF) muscle was determined by using some slices obtained from each ham and the sensory analyses were performed as described in section 2.4. Among them, three hams were selected with a sensory pastiness qualification of 0, 2.0 and 6.8, in a scale from 0 (absence of pastiness) to 10 (maximum intensity). These samples were considered to correspond to the non-pastiness group (NPG, that considers hams with pastiness intensity <0.5), medium pastiness group (MPG, that considers hams with pastiness intensity between 0.5 and 2.0) and high pastiness group (HPG, that considers hams with pastiness intensity >2.0) respectively as previously defined in Coll-Brasas *et al.*, (2019). Hams with a sensory pastiness of 2.0 were classified as MPG in spite of having less than half of the score on the evaluation scale because in the market the maximum pastiness intensity values are about 6 or 7 not 10.

For each selected ham, BF muscle was completely sliced in 1.5 mm-thick slices. A total of 25 samples of BF muscle (approximately a total of 15 g per sample) were obtained for each ham, vacuum packaged and stored at 4 °C ± 2 °C to be used in further rheological experiments.

Besides, 50 sliced commercial packages of dry-cured ham containing *Biceps femoris* muscle were selected from the market. All packages contained lean type dry-cured ham from different brands including different elaboration procedures. From each commercial package, at least 2 samples of BF muscle (approximately a total of 15 g per sample) were obtained to be used in further rheological experiments.

2.2 Obtaining water extracts and rheological measurements

Water extracts, which represent saliva in the bolus during the mastication process, were obtained *in vitro* using a new procedure that simulates the mastication process. In short, the sample (15 g of BF muscle) was cut into small pieces (0.5 cm x 0.5 cm approximately) and 15 ml of distilled water/liquid was added. Then, samples were sheared using a homogenizer (IKA T18 basic Ultra-Turrax) at low revolutions moving vertically through the ham container 20 times and then adding 10 ml extra of distilled water. After that, the preparation was again sheared in the container 5 times more and the mixture was smashed 25 times using a spatula. Subsequently, the preparation was introduced in a Falcon tube of 50 ml and then centrifuged at 4000 rpm during 3 minutes at a maximum temperature of 15 °C using a centrifuge (Eppendorf 5810 R and a rotor Eppendorf A-4-

62). The obtained supernatant (water extract) was immediately analysed using a rheometer.

Rheological properties of the water extracts were measured in a RheoStress controlled stress rheometer monitored by Rheowin Pro Software v. 3.1 (Haake, Karslruhe, Germany) with a parallel-plates sensor system (60 mm) with a gap between plates of 0.5 mm. Between 2.5 and 3 ml of water extract was placed with a spoon in the pre-heated plate at a defined constant temperature. The temperature was controlled by using a Phoenix P1 Circulator device (Thermo Haake, Karslruhe, Germany); to avoid evaporation, a temperature cover was used.

Flow curves were obtained from stepped shear stress ramp (steady state approximation: 20 s per point). Ranges of shear stresses, in logarithmic distribution, were used to obtain shear rates between 0.05 and 100 s^{-1}. Data from the flow curves were fitted to the Ostwald de Waele fit ($\sigma = K\dot{\gamma}^n$), where *k* (Pa·s) is the consistency index and *n* is the flow index. For each water extract, two measurements at least were performed.

2.3 Experimental protocols

2.2.1 Effect of temperature on rheological behaviour of water extracts

The effect of different temperatures (25 °C and 37 °C) on flow index (*n*), consistency index (*k*) and viscosities at 1, 10 and 100 s^{-1} were evaluated in water extracts from NPG, MPG and HPG. Five extractions from each ham and temperature of analysis were taken. Each extraction was measured in duplicate.

2.2.2 Effect of HPP at different temperatures on rheological and sensory properties of BF muscle of dry-cured ham

The effect of HPP at different temperatures was studied on sensory properties of the dry-cured ham samples with different pastiness intensities and on rheological properties of their water extracts. To do so, samples were submitted to 600 MPa during 6 min in NC Hyperbaric WAVE 6000/120 equipment (NC Hyperbaric, Burgos, Spain) using an initial temperature of 7 °C (HPP7) (n = 9), 20 °C (HPP20) (n = 9) and 35 °C (HPP35) (n = 9). Before application of HPP, samples were kept to the assigned temperature until getting a homogeneous temperature in the entire sample. For each temperature, 6 samples were used for rheological measurements (2 HPG, 2 MPG and 2 NPG) and 3 samples were used for sensory analyses (1 HPG, 1 MPG and 1NPG). Changes in the sensory pastiness intensity and in the rheological behaviour of their water extracts after HPP were analysed using the rheometer at 37 °C.

2.2.3 Characterization of commercial dry-cured ham samples

Sensory pastiness intensity of BF of fifty commercial dry-cured ham samples and viscosity of their water extracts was evaluated using a rheometer at 37 °C.

2.4 Sensory analysis

The evaluation of the pastiness intensity of BF muscle in all the samples was performed using an unstructured scoring scale from 0 (absence of pastiness) to 10 (maximum

intensity) by five-member expert panellists. The average score of the 5 experts for each sample was used for the statistical analysis. Samples were coded with three random numbers and presented to the panellists balancing the first-order and the carry-over effects as much as possible according to (Macfie, Bratchell, Greenhoff & Vallis, 1989). Between all the samples, water was used to rinse the mouth.

For the selection of the three whole hams, a total of 4 sessions were performed with 5 samples per session. In the case of commercial samples, a total of 10 sessions were carried out in which 5 samples per session were randomly selected and evaluated.

All the samples were assigned to the NPG, MPG or HPG as previously defined in Coll-Brasas *et al.*, (2019). In short, NPG included hams with sensory pastiness lower than 0.5, MPG included hams with a sensory pastiness from 0.5 to 2.0 and HPG included hams with a sensory pastiness higher than 2.0.

2.5 Statistical analysis

A two-way ANOVA was used to evaluate the effect of the temperature (25 and 37 °C) on the instrumental viscosity (at 1, 10 and 100 s^{-1}) of samples from different pastiness intensity groups (NPG, MPG and HPG). Similarly, the effect of the HPP treatment at different temperatures (HPP7, HPP20 and HPP35) was also performed on sensory pastiness and instrumental viscosity at 10 s^{-1} on samples with different pastiness intensity (NPG, MPG and HPG). Also, a t-test was performed to evaluate whether viscosity at 10 s^{-1} was significantly different or not from different sensory pastiness groups (NPG, MPG and HPG). Differences between mean values were tested by means of the Tukey test ($p \leq 0.05$). All the analyses were performed using the XLSTAT v2020.1.1 (2020) (Addinsoft, Paris, France).

3. Results and discussion

3.1 Rheological characterization of water extracts of dry-cured ham samples from different pastiness intensity groups

The flow curve parameters of the water extracts from samples assigned to different pastiness intensity groups are presented in Table 1. Parameters showed a shear-thinning behaviour with flow index (*n*) values ranging from 0.07 to 0.36 and a consistency index (*k*) ranging from 0.03 to 0.66 indicating a pseudoplastic behaviour. The *k* and *n* values showed different behaviour for the different pastiness intensity groups and temperatures of analysis. The samples with high pastiness intensity showed higher consistency index and lower flow indexes being farther to the Newtonian behaviour (i.e. $n = 1$). Flow curves of the water extracts were adjusted to the Ostwald de Waele fit showing r ranges from 0.9407 – 0.9991 and 0.9174 – 0.9987 on samples with non-pastiness at 25 and 37 °C, respectively; from 0.8799 – 0.9993 and 0.8359 – 0.9980 on samples from MPG at 25 and 37 °C, respectively; and from 0.9405 – 0.9876 and 0.9812 – 0.9974 on samples from HPG at 25 and 37 °C, respectively.

Table 1. Flow curve parameters (consistency index (*k*; (Pa·s)^n) and flow index (*n*)) of water extracts from dry-cured ham samples with different pastiness intensity.

Temperature	Pastiness intensity groups	n	*k* Mean ± Standard deviation	*n* Mean ± Standard deviation
25 °C	Non-Pastiness (NPG)	5	0.06±0.02	0.36±0.18
	Medium Pastiness (MPG)	5	0.07±0.03	0.20±0.25
	High Pastiness (HPG)	5	0.66±0.21	0.07±0.04
37 °C	Non-Pastiness (NPG)	5	0.05±0.02	0.35±0.17
	Medium Pastiness (MPG)	5	0.03±0.01	0.33±0.18
	High Pastiness (HPG)	5	0.38±0.13	0.15±0.09

Table 2. Viscosity (η) of water extracts from dry-cured ham samples with different pastiness intensity.

Temperature	Pastiness intensity groups	n	η_1 Pa s Mean ± Standard deviation	η_{10} Pa s Mean ± Standard deviation	η_{100} Pa s Mean ± Standard deviation
25 °C	Non-Pastiness (NPG)	5	0.054^c ±0.0153	0.015^c ±0.0027	0.006^c ±0.0008
	Medium Pastiness (MPG)	5	0.067^c ±0.0300	0.012^c ±0.0047	0.006^c ±0.0019
	High Pastiness (HPG)	5	0.590^a ±0.1838	0.094^a ±0.0247	0.023^a ±0.0048
37 °C	Non-Pastiness (NPG)	5	0.045^c ±0.0170	0.010^c ±0.0020	0.005^c ±0.0010
	Medium-Pastiness (MPG)	5	0.029^c ±0.0151	0.007^c ±0.0040	0.004^c ±0.0042
	High-Pastiness (HPG)	5	0.347^b ±0.1169	0.062^b ±0.0169	0.016^b ±0.0034

^{abc}Different letters within columns indicate significant differences ($p \leq 0.05$).

Viscosity of samples with different pastiness intensities when analysed at different temperatures were significantly different (Table 2). An increase of viscosity of the water extracts at 1, 10 and 100 s^{-1} with the increase of the pastiness intensity group was observed ($p \leq 0.05$). However, significant differences were found between the HPG and NPG/MPG (Table 2). These results are in agreement with Coll-Brasas *et al.*, (2019), who found a correlation between the perception of pastiness intensity and the perception of saliva viscosity reported by the expert panellists during mastication (i.e., what water extracts represented) ($r = 0.894$). A high proteolysis in dry-cured ham muscles leads to the generation of smaller fractions of proteins that affects the texture of the sample (Toldrá & Flores, 1998). López-Pedrouso *et al.*, (2018) found that protein fragments increased in samples with a higher proteolysis index, most of the fragments being the result of the hydrolysis of the myosin heavy chain and α-actin myofibrillar proteins. We can hypothesize that during the mastication of HPG dry-cured ham samples, a higher number of protein fragments will be solubilized in water than in NPG and MPG samples, increasing the viscosity of saliva during mastication and also of the water extracts obtained when mimicking this process.

Viscosity values at 1, 10 and 100 s^{-1} decreased with the temperature of the analysis, but significant differences were only found for HPG. Viscosity values were higher at low temperatures (25 °C), because an increase in temperature causes higher kinetic energy in molecules and consequently a fall in viscosity, as described by the Arrhenius equation.

For further experiments, the measurement of viscosity at a shear rate of 10 s^{-1} was chosen because similar viscosity variations were observed at all the studied shear rates but 10 s^{-1} is the closest to the chewing speed (Sharma, Kristo, Corredig & Duizer, 2017). In the same way, although both temperatures provided good discrimination of viscosity between pastiness intensity groups, 37 °C was chosen because it is the temperature of the oral cavity.

3.2 Effect of HPP treatment on sensory pastiness and water extract viscosity of dry-cured ham samples with different pastiness intensity

The effect of HPP treatment at 600 MPa on both sensory pastiness and water extract viscosity at 10 s^{-1} for dry-cured ham samples with different pastiness intensity is shown in Tables 3 and 4. The HPP produced a significant effect on sensory pastiness ($p \leq 0.05$), which was different depending on the HPP temperature used and on the initial pastiness intensity of the sample (Table 4). Pastiness intensity disappeared in samples from MPG ($p \leq 0.05$) after HPP at any of the temperatures. In contrast, when the pastiness intensity was high (HPG), only the HPP treatment at 35 °C produced a significant reduction of pastiness. These results are in agreement with that previously reported by Coll-Brasas *et al.*, (2019) who found that HPP treatments produced changes in texture to a different extent depending on the processing temperature and the initial textural characteristics of the samples. Texture modifications due to HPP can be attributed to the aggregation of myosin molecules, which denature and form disulphide bonds at pressures higher than 400 MPa (Angsupanich, Edde & Ledward, 1999; Orlien 2017; Yamamoto, Yoshida, Morita

& Yasui, 1994), which produces changes in ultrastructure increasing hardness and decreasing pastiness (Garcia-Gil *et al.*, 2014; Picouet *et al.*, 2012).

The decrease of pastiness in samples from HPG could only be achieved when a temperature of 35 °C was used. This fact could be explained because of the more intensive effect of pressure when it is applied together with temperature (Cheftel & Culioli, 1997). It is important to mention that it was estimated that HPP35 samples achieved temperatures above 53 °C during the HPP treatment, because of the adiabatic increase of temperature during pressurization (US Food & Drug Administration, 2014) that depends on the pressure applied but also on the initial temperature and the product composition (Patazca, Koutchma & Balasubramaniam, 2007; Picouet *et al.*, 2016). We can hypothesize that when the structure is damaged and partially denatured, high temperatures are needed to produce changes and to create new rearrangements. In contrast, when the proteins are not so deeply affected by proteolysis and still have the native structure (NPG/MPG), the effect of the HPP treatment is more important. Contreras *et al.*, (2020) attributed the textural changes produced by mild thermal treatments using power ultrasounds to the shrinkage of the myofibrils that increased the hardness and improved the texture.

For the reasons mentioned above, rheological behaviour of water extracts from the same dry-cured ham samples after application of HPP showed lower *k* values (less consistent) and had a more Newtonian behaviour (higher *n* values) (Table 3). After the shrinkage of myofibrils, the release of protein fractions is lower, leading to less consistent water extracts (Tornberg, 2005).

Viscosity of the water extracts at 10 s^{-1} decreased for all the pastiness intensity groups after HPP (Table 4). A significant decrease of instrumental viscosity in NPG samples was found after HPP at any temperature ($p \leq 0.05$) although no decrease of pastiness in sensory analysis (no initial defect was present) was observed. In contrast, although a significant decrease of viscosity in HPG samples was also found after HPP at any temperature ($p \leq 0.05$), sensory changes were only perceived after HPP at 35 °C. It seems that some protein fractions that affect sensory pastiness (during human mastication) are not being detected by instrumental viscosity measurements (in water extracts). Other changes induced by HPP on the ham (such as an increase of hardness) could be also responsible for the lower viscosity values of the extract of these HPP treated hams. Further studies are needed to elucidate the impact of these fractions and other textural attributes of the samples on sensory and rheological properties of pasty hams.

Table 3. Flow curve parameters (consistency index (*k*; (Pa·s)^n) and flow index (*n*)) of water extracts analysed at 37 °C at a shear rate of 10 s^{-1} from dry-cured ham samples with different pastiness intensity submitted to different HPP treatments.

		Non-Pastiness Group		Medium Pastiness Group		High Pastiness Group	
HPP Treatment	**n**	***k*** **Mean ± Standard deviation**	***n*** **Mean ± Standard deviation**	***k*** **Mean ± Standard deviation**	***n*** **Mean ± Standard deviation**	***k*** **Mean ± Standard deviation**	***n*** **Mean ± Standard deviation**
Control	5	0.049±0.0182	0.347±0.1708	0.034±0.0092	0.329±0.1764	0.383±0.1339	0.154±0.0916
HPP7	6	0.013±0.0045	0.549±0.1803	0.016±0.0081	0.561±0.1992	0.018±0.0030	0.633±0.0330
HPP20	6	0.018±0.0092	0.325±0.1203	0.012±0.0043	0.698±0.1211	0.018±0.0030	0.572±0.0602
HPP35	6	0.018±0.0090	0.438±0.2287	0.009±0.0050	0.544±0.1349	0.027±0.0042	0.464±0.0711

Control: No HPP – Treated (samples not submitted to HPP); HPP7: HPP at 7 °C; HPP20: HPP at 20 °C; HPP35 at 35 °C.

Table 4. Effect of HPP at different temperatures on sensory pastiness and viscosity (η) of water extracts analysed at 37 °C at a shear rate of 10 s^{-1} from dry-cured ham samples with different pastiness intensity measured at 37 °C.

		Non-Pastiness Group		Medium Pastiness Group		High Pastiness Group	
HPP Treatment	**n**	**Sensory pastiness Mean ± Standard deviation**	**η (10 s^{-1}) Mean ± Standard deviation**	**Sensory pastiness Mean ± Standard deviation**	**η (10 s^{-1}) Mean ± Standard deviation**	**Sensory pastiness Mean ± Standard deviation**	**η (10 s^{-1}) Mean ± Standard deviation**
Control	5	0.0	0.010±0.0020^a	2.0±0.29^a	0.007±0.0040^a	6.8±0.76^a	0.062±0.0169^a
HPP7	6	0.0	0.004±0.0030^b	0.0^b	0.004±0.0010^{ab}	6.2±1.44^a	0.007±0.0009^b
HPP20	6	0.0	0.004±0.0016^b	0.0^b	0.003±0.0005^b	5.8±1.50^a	0.006±0.0009^b
HPP35	6	0.0	0.005±0.0013^b	0.0^b	0.005±0.0015^{ab}	0.2±0.29^b	0.007±0.0016^b

^{ab}Different letters indicate significant differences ($p \leq 0.05$) within columns. Control: No HPP – Treated (samples not submitted to HPP); HPP7: HPP at 7 °C; HPP20: HPP at 20 °C; HPP35 at 35 °C.

3.3 Pastiness of commercial dry-cured hams and viscosity of their water extracts

The relative frequency of commercial samples from different pastiness intensity groups is shown in Figure 1 (n = 50). Most of the commercial samples had a pastiness intensity lower than 0.5 (44%) whereas 38% and 18% of the samples had a medium and a high pastiness intensity, respectively. It must be noted that pastiness intensities higher than 6 were not found in the market but pastiness intensity higher than 2.0 were already considered defective by the consumers.

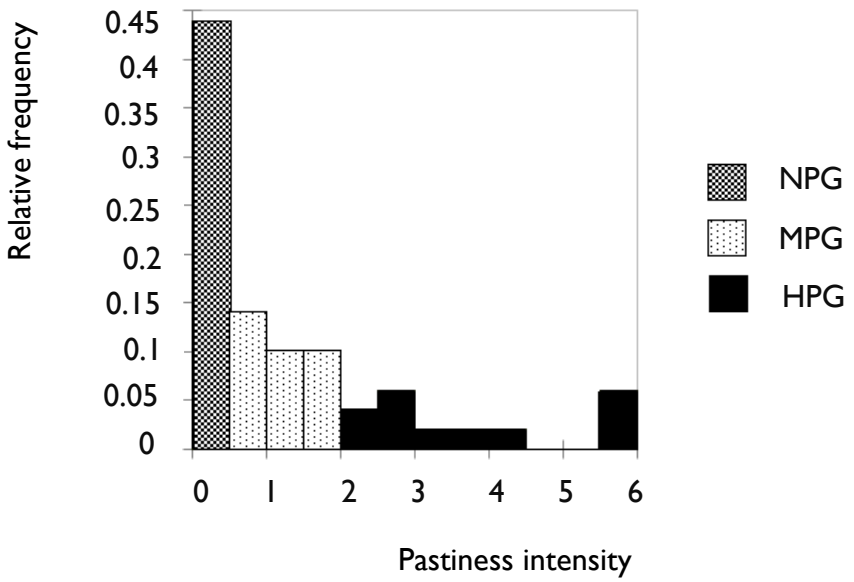

Figure 1. Distribution of commercial sliced dry-cured ham packages according to sensory pastiness intensity. NPG: Non-Pastiness group; MPG: Medium Pastiness group; and HPG: High Pastiness group.

The viscosity of the commercial samples from the three pastiness intensity groups is shown in Table 5. According to results found in section 3.1, results obtained from the commercial samples showed significant differences of viscosity between HPG and MPG/NPG. No significant differences were found between NPG and MPG. Therefore, although using samples from different sources and processed differently, viscosity measurements using the rheometer allows us to distinguish commercial samples with high pastiness intensity from the medium and the non-pastiness ones. However, as expected, samples with medium or non-pastiness intensity cannot be distinguished

Table 5. Sensory pastiness and water extract viscosity at 10 s^{-1} and analysed at 37 °C of commercial samples (n = 50) from the different pastiness intensity groups.

Pastiness intensity groups	Sensory pastiness Mean ± Standard deviation	η_{10} Mean ± Standard deviation	Minimum η_{10}	Maximum η_{10}
Non-Pastiness (NPG) (≤ 0.5)	0.118±0.1513	0.014^{b} ± 0.0149	0.004	0.075
Medium Pastiness (MPG) (0.5 – 2.0)	1.189±0.3720	0.016^{b} ± 0.0131	0.004	0.052
High Pastiness (HPG) (≥ 2.0)	3.636±1.4099	0.111^{a} ± 0.1769	0.010	0.477

[ab]Within columns, different letters indicate significant differences (p ≤ 0.05).

4. Conclusions

In the present study, for the first time, an *in-vitro* approach for measuring changes in oral fluid viscosity has been proposed as a way to instrumentally evaluate pastiness defect in dry-cured ham. Rheological properties of water extracts obtained *in vitro* mimicking the mastication of ham has been characterized showing that the apparent viscosity values at 10 s^{-1} are related to the sensory pastiness intensity. HPP treatment was shown to decrease both pastiness and viscosity values but the different effect observed may indicate that other factors are implied. Further studies are needed to clarify the relationship between protein fractions produced in proteolysis, sensory pastiness and rheological parameters. However, these results show that it is possible to develop a fast, low-cost routine method to instrumentally measure pastiness perception in dry-cured ham.

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Paper V

Coll-Brasas, E., Arnau, J., Gou, P., Lorenzo Rodríguez, J. M., García-Pérez, J. V. & Fulladosa, E. (2019). Effect of high pressure processing temperature on dry-cured hams with different textural characteristics. *Meat Science, 152*, 127-133.

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Effect of high pressure processing temperature on dry-cured hams with different textural characteristics

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ABSTRACT

High pressure processing is mainly used to eliminate pathogenic microorganisms and extend the shelf-life of dry-cured hams, but it also modifies its texture. These changes might be different depending on the initial textural characteristics. In this study, texture, colour and colour stability were evaluated after pressurization at 600 MPa during 6 min at 7 °C, 20 °C and 35 °C in samples with different textural characteristics (no pastiness, medium and high pastiness groups). HP treatments produced an increase of hardness (F_0) and lightness (L*) values and a decrease of softness/pastiness (Y_2 and Y_{90}) and redness (a*) values at any processing temperatures. However, the increase of F_0 and L* values was more pronounced in non–pasty samples. In samples with high pastiness and softness, HP processing at high temperature (35 °C) reduced the intensity of pastiness. However, texture of hams with non-pasty texture might be negatively affected. Therefore, the optimal temperature of HP processing depends on the textural characteristics of dry-cured hams

1. Introduction

Dry-cured ham is appreciated by consumers because of its flavour and texture characteristics. The main texture defects are excessive softness (Arnau, Guerrero, & Sárraga, 1998; Parolari, Virgili, & Schivazappa, 1994; Virgili, Parolari, Schivazappa, Bordini, & Volta, 1995) and pastiness which are mainly related to raw material properties (García-Rey, García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2004; Schivazappa et al., 2002) and processing conditions (Ruiz-Ramírez, Serra, Arnau, & Gou, 2005). Proteolysis activity contributes to texture development by breakdown of the muscle structure (Monin et al., 1997; Sentandreu, Coulis, & Ouali, 2002), to taste through the generation of small peptides and free amino acids and to aroma by further degradation of some free amino acids (Toldrá & Flores, 1998). However, when the extent of proteolysis is exceeded, the structure is severely damaged and unpleasant textures appear (Arnau et al., 1998; Toldrá, 2006). This defect occurs with an incidence of 12% in hams with a standard salt content, and increases when salt content is reduced (Tapiador Farelo & García Garrido, 2003). Some works aimed to characterize and classify sliced ham according to textural characteristics using non-destructive technolgies based on infrared (García-Rey, García-Olmo, Pedro, Quiles-Zafra & Castro, 2005; Ortiz, Sarabia, García-Rey, & Castro, 2006) and X-rays (Fulladosa et al., 2018) have been reported, but more work is need before its implementation at industry level.

Colour is another important attribute which also determines quality and consumer's acceptability. In cured meats, in which curing salts are used, many reactions occur leading to the formation of nitrosyl-myoglobin, the pigment which is responsible of the reddish colour of the cured meat products (Cassens, Greaser, Ito, & Lee, 1979). However, colour modifications of the product during storage or high pressure processing can occur (Clariana et al., 2011; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Ha, Dunshea, & Warner, 2017).

High pressure (HP) treatments are currently being used to eliminate pathogenic microorganisms and to extend the product shelf-life (Aymerich, Picouet, & Monfort, 2008; Garcia-Gil et al., 2014). However, pressure treatments affect quality characteristics such as texture, colour (increasing lightness (L*) values and decreasing redness (a*) values (Clariana et al., 2011; Fuentes, Utrera, Estévez, Ventanas, & Ventanas, 2014; Fuentes et al., 2010; E Fulladosa, Sala, Gou, Garriga, & Arnau, 2012; Ha et al., 2017)) and, potentially, the aroma and taste (increasing rancid odour and saltiness perception) (Andrés, Moller, Adamsen, & Skibsted, 2004; Campus, Flores, Martinez, & Toldrá, 2008; Cheftel & Culioli, 1997; Lorido, Estévez, Ventanas, & Ventanas, 2015;

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Martínez-Onandi, Rivas-Cañedo, Nuñez, & Picon, 2016). A decrease of the incidence and of the intensity of defective textures such as pastiness has been also previously described (Fulladosa, Serra, Gou, & Arnau, 2009; Lorido et al., 2015). These changes are different depending on the product characteristics. For instance, HP processing increased hardness, gumminess and stringiness and decreased adhesiveness and pastiness in dry-cured hams with 30% weight loss, but the effect was lower when the same HP treatment was applied to dryer hams (50% weight loss) (Fulladosa et al., 2012). Serra et al. (2007) also found that lightness observed in the *Biceps femoris* muscle increased when the HP treatment was applied to frozen green hams but not when it was applied to frozen hams at the end of the resting stage. Importance of the processing conditions has also been described. Picouet et al. (2012) found that the application of HP treatments using different pressures 300, 600 and 900 MPa produced different ultrastructural changes on 15 months aged dry cured-hams by transmission electron microscopy (TEM) analysis. Garcia-Gil et al. (2014) also found that the application of HP treatments at 500 MPa produced changes on microstructure of *Biceps femoris* and *Semimembranosus* muscles of dry-cured ham when using micro-computed tomography and scanning electron microscopy. Andrés, Adamsen, Moller, Ruiz, and Skibsted (2006) reported that the application of HP treatment at 400 MPa resulted in discoloration (increase of lightness and a decrease of redness) and in an oxidative degradation of lipids in dry-cured Iberian ham during subsequent illuminated chill storage. Similarly, Campus et al. (2008) found that HP treatments above 300 MPa affected the colour producing an increase of lightness and a decrease of redness on sliced and vacuum packaged dry-cured pork loin.

Previous studies carried out using different HP treatments at 600 MPa in a range of initial temperatures (between 7.5 and 24,4 °C) showed no significant differences on the inactivation of microorganisms with the increase of the initial temperature (Belletti, Garriga, Aymerich, & Bover-Cid, 2013; Bover-Cid, Belletti, Garriga, & Aymerich, 2012). However, the evaluation of the effect of HP processing temperature on texture and colour of samples with different texture characteristics has not been found in literature.

The aim of this work was to study the effect of HP processing at different temperatures (7 °C, 20 °C and 35 °C) on instrumental and sensory texture, colour and colour stability of samples with different textural characteristics(samples with no-pastiness and with medium or high pastiness defect).

2. Material and methods

2.1. Elaboration process

An elaboration process specially designed to induce textural defects of different intensity in dry-cured hams was used. Two hundred raw hams with a pH in the *Semimembranosus* muscle at 24 h *post mortem* lower than 5.5 were obtained from a commercial slaughterhouse supplied with animals from crosses of Large White and Landrace breeds. Hams with this pH were selected because they are more prone to develop texture defects (Tabilo, Flores, Fiszman, & Toldra, 1999). The pH determination was performed with a Crison Basic pH meter (Crison Instruments S.A., Barcelona, Spain). All hams were weighed (11.9 kg ± 1.1 kg) and salted according to the traditional system with some modifications in order to induce pastiness defects. In brief, hams were manually rubbed with the following mixture (g/kg of raw ham): 0.15 of KNO_3, 0.15 of $NaNO_2$, 1.0 of dextrose, 0.5 of sodium ascorbate and 10 of NaCl. Thereafter, the hams were pile salted at 3 ± 2 °C and 85 ± 5% RH during 4 days (n = 50), 6 days (n = 50), 8 days (n = 50) or 11 days (n = 50) in order to obtain hams with different salt contents. After salting, hams were washed with cold water and dried at 3 ± 2 °C and 85 ± 5% RH during 45 days (post-salting period), at 12 ± 2 °C and 70 ± 5% RH until reaching a weight loss of 29%. Hams were then vacuum packaged and kept at 30 °C during 30 days to induce proteolysis. Thereafter, hams continued the drying process at 12 ± 2 °C and 65 ± 5% RH until reaching a weight loss of 34%, vacuum packaged again and kept at 30 °C during 30 days. After this period, hams were dried until the end of process was reached (final weight loss of 36%).

2.2. Sampling procedure

For all the hams (n = 200), the aitch bone, the butt and the femur bone were excised and the cushion part, containing *Biceps femoris* (BF) muscle, was obtained and trimmed. Cushion part was then sampled as follows: Three consecutive1.5 mm-thick slices were prepared for an initial sensory characterization of all the samples. One 2 cm-thick slice of each selected ham was prepared for instrumental texture and chemical characterization before HP treatment (CT-samples). Another consecutive 2 cm-thick slice was prepared for instrumental texture analysis after the specific HP treatment (HP-samples). Seven 1.5 mm thick slices from each selected ham were prepared for sensory analysis and for instrumental colour evaluation: 3 slices before HP treatment (CT-samples), 3 slices after the specific HP treatment (HP-samples) and the last one for evaluation of instrumental colour stability after HP treatment. All slices were vacuum packed in individual plastic bags of polyamide/polyethylene (oxygen permeability of 50 $cm^3/m^2/24$ h at 23 °C and water permeability of 2,6 $g/m^2/24$ h at 23 °C and 85% RH, Sacoliva® S.L., Spain) and stored in a chamber at 4 °C ± 2 °C until the treatment and/or analysis were performed.

2.3. Ham assignment to the different Texture groups and HP treatments

In order to assign hams to the different Texture groups (TG) defined in the experimental design (Table 1), an initial sensory analysis of pastiness of BF muscle from all the hams (n = 200) was performed using the 1.5 mm thick slices. For all the hams, instrumental texture, colour and chemical analysis described in section 2.6 were also performed using the 2 cm thick slices (CT-samples) previously prepared (n = 200).

After this initial characterization, a total of 90 hams were selected and assigned to the three different Texture groups according to their pastiness perception. Texture groups were: no-pastiness group (NPG) (n = 30, sensory pastiness < 0.5, PI < 33.0% and $0.59 < Y_{90} < 0.69$), medium pastiness group (MPG) (n = 30, sensory pastiness between 0.5 and 2.0 and 27.0% < PI < 40.0% and $0.64 < Y_{90} < 0.72$), and high pastiness group (HPG) (n = 30, sensory pastiness > 2.0, 36.0% < PI < 48.0% and $0.66 < Y_{90} < 0.75$). Then, the 30 hams from each Texture group were randomly selected and uniformly distributed to each HP treatment described in the next section (10 for HP7; 10 for HP20 and 10 for HP35). Samples of each ham were analysed before HP treatment (CT-samples) and after the HP treatment. See experimental design in Table 1.

Table 1
Distribution of hams (n = 90) according to different Texture groups and HP treatments.

Texture groups	CT	HP treatment		
		HP7	HP20	HP35
NPG	1 to 30	1 to 10	11 to 20	21 to 30
MPG	31 to 60	31 to 40	41 to 50	51 to 60
HPG	61 to 90	61 to 70	71 to 80	81 to 90

NPG: No pastiness group; MPG: Medium pastiness group; HPG: High pastiness group. CT: Control samples (non HP-treated). HP treatments at different temperatures: 7 °C (HP7); 20 °C (HP20) and 35 °C (HP35).

2.4. High hydrostatic pressure treatments

High-pressure treatments at 600 MPa during 6 min were performed in a NC Hyperbaric WAVE 6000/120 equipment (NC Hyperbaric, Burgos, Spain) at 7 °C (HP7, temperature commonly used in industrial HPP treatments), 20 °C (HP20) or 35 °C (HP35). Samples were tempered to the assigned temperature using a temperature control cabinet (Model EC-360, Radiber S.A., Barcelona, Spain) and submitted to HP treatment using water at the same temperature. Because there is an adiabatic increase of temperature during pressurization from approximately 3 °C/100 MPa (US Food & Drug Administration, 2014), which depends on the pressure applied but also on the initial sample temperature and product composition (Patazca, Koutchma, & Balasubramaniam, 2007; Picouet et al., 2016), it is difficult to determine the achieved temperature for the samples for each treatment. However, it is estimated that HP treated samples achieved temperatures of 23 °C, 36 °C and 53 °C when performing HP treatment at 7 °C, 20 °C and 35 °C, respectively (Koutchma, 2014). Instrumental texture, colour and sensory analysis of the selected hams was performed (n = 90).

2.5. Instrumental texture

From the 2 cm thick slices a minimum of six parallelepipeds were cut from BF muscle with the exact same dimensions (2 cm × 2 cm × 1,5 cm). The pieces were wrapped in plastic film to avoid drying and kept at 4 °C ± 2 °C during 24 h for temperature stabilization in a temperature control cabinet (Model EC-360, Radiber S.A., Barcelona, Spain). A Stress Relaxation test was performed using a Texture Analyser (Zwick/Roell, testXpert II, V3.2, 1996–2010, Zwick GmbH & Co. KG, Ulm, Germany) provided with 30 kN load cell and a 60 mm compression plate. Samples were compressed to 25% of their original height, perpendicular to the muscle fibre bundle direction, at a crosshead speed of 5 mm/s and at a temperature of 4 °C ± 2 °C.

The force decay or relaxation *versus* time $Y_{(t)}$ was calculated as follows:

$$Y_{(t)} = \frac{F_0 - F_{(t)}}{F_0}$$

where F_0 (kg) is the initial force and $F_{(t)}$ is the force recorded after t seconds of relaxation. The force decay at 2 s (Y_2) and 90 s (Y_{90}) were calculated (Morales, Guerrero, Serra, & Gou, 2007). For each parameter, the average of the six samples was used for the statistical analysis (E Fulladosa et al., 2009; Garcia-Gil et al., 2014).

2.6. Chemical analysis

Chemical analysis was performed on the muscle used in instrumental texture analysis (CT-samples). Moisture content was determined by drying at 103 ± 2 °C until a constant weight was reached AOAC (1990). Salt content was also determined according to ISO 1841-2 (1996) using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland) and results were expressed as percentage of NaCl on a dry-matter basis (DM). Non-protein nitrogen content (NPN) was determined by precipitation of proteins with trichloroacetic acid (Gáspár, 1984) followed by determination of the total nitrogen (TN) in the extract with the Kjeldahl method ISO 937 (1978). Proteolysis index (PI) was determined as a percentage of the ratio between NPN and TN (Careri et al., 1993; Schivazappa et al., 2002). All the analyses were done in duplicate.

2.7. Instrumental colour

Lightness (L*), redness (a*) and yellowness (b*) was measured on dry-cured ham slices (CIE, 1976) using a colorimeter (Minolta Chroma Meter CR-400, Tokyo, Japan). The illuminant used was D65 with 2°. Colour was measured in triplicate on the surface of BF muscle avoiding zones with cracks and tyrosine crystals, before and after HP treatment immediately after opening the package. During the colour measurements, the background used was always the same, a white surface. Colour stability of HP treated samples was determined by measuring colour on the same slice immediately after HP treatment (t = 0 h) and after 4, 7, 22 and 28 days of storage at 6 °C ± 0.5 °C in 24 h light exposure, simulating the conditions of the showcase. Samples were vacuum packed immediately after each measure.

2.8. Sensory analysis

For the initial characterization, the pastiness perception in all the hams (n = 200) was performed. Pastiness were scored using an unstructured scale from 0 (very low) to 10 (very high). A total of 30 sessions were carried out by a three-member expert panel trained following ASTM (ASTM, 1981). In each session, six slices randomly selected were evaluated. The average score of the 3 experts for each sample was used for the statistical analysis.

In order to evaluate the effect of HP in the selected batch of hams (n = 90), a profile of different sensory attributes grouped in texture and flavour were assessed on fifteen hams randomly selected from each Texture group, before and after HP treatment. BF muscle of 1.5 mm thick slices was evaluated. The texture attributes were: pastiness (feeling of paste detected in hams with a high proteolytic index, similar to the 'mouthcoating' sensation produced by flour-water paste during the mastication process), saliva viscosity (refers to the feeling of viscous degree of saliva when flowing, after mastication), and stringiness (amount of fibres detected during chewing) as described by Guerrero, Gou, and Arnau (1999). The flavour attribute was saltiness (fundamental taste sensation elicited by NaCl). Attributes were scored using an unstructured scale from 0 (very low) to 10 (very high). A total of thirteen sessions were carried out by a three-member expert panel trained following ASTM (ASTM, 1981). In each session, a pair of samples (control and HP treated) from each HP treatment were randomly selected and evaluated by all the panellists. The sample order, with respect to the HP treatment, was randomized within session, blocking the order of presentation and the first-order carry-over effects (Macfie, Bratchell, Greenhoff, & Vallis, 1989). The average score of the 3 experts for each sample was used for the statistical analysis.

2.9. Statistical analysis

Data were analysed as a randomized unbalanced incomplete block, split-plot design. For all variables except colour stability, the statistical model included as fixed effects the Texture group (2 df), the HP treatment (3 df) and their interaction (6 df) and as random effects the hams nested within the Texture group (36 df; the error term for the main plot; used for testing the Texture group effect) and the residual error (30 df; the error term for the sub-plot; used for testing the HP and TGxHP interaction effects). Therefore, the statistical mixed model was:

$$Y_{ijk} = \mu + (TG)_i + h_{j:i} + (HP)_k + (TGxHP)_{ik} + e_{ijk}$$

where Y_{ijk} is the observed value (dependent variable); μ is the overall population mean; $(TG)_i$ is the fixed effect of the i^{th} texture group; i = 1, 2, 3; $h_{j:i}$ is the random effect of the j^{th} ham nested within the i^{th} texture group; j = 1, …, 10; $(HP)_k$ is the fixed effect of the k^{th} high pressure treatment, k = 1, …, 4; $(TGxHP)_{ik}$ is the fixed effect of the interaction between the i^{th} texture group and the k^{th} high pressure treatment; and e_{ijk} is the random residual. PROC MIXED of SAS release 9.1 was used to solve the mixed effect models.

In order to study colour stability, time of exposure to light was included as fixed effects in the model when analysed separately for each HP treatment and Texture group. Differences between mean values were tested by means of Tukey's test ($p < .05$) used at the 5% level. All the analysis were performed using the ANOVA procedure of SAS 9.3 statistical package (SAS release 9.1).

Table 2
Chemical characteristics (Mean ± standard deviation) of *Biceps femoris* muscle according to the Texture group before HP treatment (Control samples).

	Texture group		
	NPG	MPG	HPG
n	30	30	30
NaCl (%)	4.80 ± 0.72	4.83 ± 0.93	4.71 ± 0.87
Moisture (%)	59.0 ± 0.7	58.7 ± 1.0	58.9 ± 1.1
PI (%)	31.5 ± 2.0^{c}	34.2 ± 2.6^{b}	39.8 ± 2.5^{a}

^{abc} Means within rows without a common letter are significantly different ($p < .05$).
NPG: No pastiness group; MPG: Medium pastiness group; HPG: High pastiness group.

Table 3
Least square means of initial force (F_0, Kg) and force decay at 2 s (Y_2) and 90s (Y_{90}) on *Biceps femoris* muscle according to the interaction HP treatment × Texture group.

	Treatment	n	Texture group		
			NPG	MPG	HPG
F_0	CT	90	1.76^{a}	1.04^{a}	0.53^{a}
	HP7	30	3.08^{b}	2.00^{b}	1.05^{b}
RMSE = 0.413	HP20	30	3.33^{b}	1.89^{b}	1.12^{b}
p < .001	HP35	30	4.24^{c}	2.79^{c}	1.65^{c}
Y_2	CT	90	0.362^{a}	0.408^{a}	0.432^{a}
	HP7	30	0.317^{b}	0.352^{b}	0.398^{b}
RMSE = 0.020	HP20	30	0.312^{b}	0.364^{b}	0.391^{b}
p = .255	HP35	30	0.305^{b}	0.342^{b}	0.389^{b}
Y_{90}	CT	90	0.649^{a}	0.686^{a}	0.697^{a}
	HP7	30	0.616^{b}	0.651^{bc}	0.681^{b}
RMSE = 0.016	HP20	30	0.613^{b}	0.658^{b}	0.679^{b}
p = .084	HP35	30	0.606^{b}	0.638^{c}	0.669^{b}

^{abc} Means within columns without a common letter are significantly different ($p < .05$). NPG: No pastiness group; MPG: Medium pastiness group; HPG: High pastiness group. RMSE: root mean square error of the linear model. P: p value of the interaction (HP treatment × Texture group) effect. CT: Control samples (non HP-treated). All the HP treatments were carried out at 600 MPa during 6 min at different temperatures: at 7 °C (HP7); at 20 °C (HP20) and treatment at 35 °C (HP35).

3. Results and discussion

3.1. Characterization of non-HP treated samples

The BF muscle samples assigned to the different Texture groups showed no significant differences for salt and water contents (Table 2). In contrast, as previously reported (Arnau et al., 1998; Toldrá, 2006), an increase of proteolysis index (PI) with the increase of the pastiness intensity was observed (Table 2). According to PI differences, a decrease of initial force (F_0), and an increase of the force decay (Y_2 and Y_{90} values) with the increase of the pastiness intensity was observed (Table 3), which agrees with Morales et al. (2007). No significant differences for L* (lightness) and a* (redness) between Texture groups were detected (Table 4).

Pastiness was the attribute used for the assignation of the hams to the different Texture groups, therefore, as expected, pastiness increased from the NPG to the HPG (Table 5). Saliva viscosity also increased, while stringiness decreased. The three attributes must be different expressions of the same texture defect. Although there is no significant difference in the NaCl content between Texture groups ($p > .05$), a significant decrease in saltiness was detected in samples with a defective texture (MPG and HPG samples) ($p < .05$) (Table 5). A hypothesis

Table 4
Least square means of L* and a* values colour on *Biceps femoris* muscle according to the interaction HP treatment × Texture group.

	Treatment	n	Texture group		
			NPG	MPG	HPG
L*	CT	90	38.63^{d}	37.44^{c}	38.13^{c}
	HP7	30	41.89^{c}	40.21^{b}	40.55^{b}
RMSE = 1.372	HP20	30	47.19^{a}	44.46^{a}	43.68^{a}
p = .0068	HP35	30	45.18^{b}	43.18^{a}	42.58^{a}
a*	CT	90	20.07^{a}	21.26^{a}	20.82^{a}
	HP7	30	18.23^{bc}	19.37^{b}	19.19^{bc}
RMSE = 1.541	HP20	30	18.33^{b}	18.78^{b}	20.90^{ab}
p = .2101	HP35	30	16.47^{c}	17.95^{b}	18.01^{c}

^{abc} Means within columns without common letter are significantly different ($p < .05$). NPG: No pastiness group; MPG: Medium pastiness group; HPG: High pastiness group. RMSE: root mean square error of the linear model. P: p value of the interaction (HP treatment × defective level group) effect. CT: Control samples (non HP-treated). All the HP treatments were carried out at 600 MPa during 6 min at different temperatures: at 7 °C (HP7); at 20 °C (HP20) and treatment at 35 °C (HP35).

Table 5
Least square means of sensory parameters evaluated (pastiness, saliva viscosity, saltiness perception and stringiness) on *Biceps femoris* muscle according to the interaction HP treatment × Texture group.

	Treatment	Texture group		
		NPG	MPG	HPG
Pastiness	CT	0.0	1.5^{a}	2.5^{a}
	HP7	0.0	0.8^{b}	1.7^{b}
RMSE = 0.461	HP20	0.0	0.4^{b}	1.7^{b}
p = .009	HP35	0.0	0.3^{b}	1.3^{b}
Saliva viscosity	CT	0.47	2.56^{a}	3.44^{a}
	HP7	0.03	1.19^{b}	2.86^{ab}
RMSE = 0.436	HP20	0.07	0.74^{b}	2.29^{bc}
p < .001	HP35	0.07	1.41^{b}	1.69^{c}
Stringiness	CT	2.37^{b}	1.46^{c}	1.31^{b}
	HP7	4.77^{a}	2.59^{b}	2.53^{a}
RMSE = 0.593	HP20	4.83^{a}	3.69^{a}	2.35^{a}
p = .059	HP35	4.77^{a}	3.41^{ab}	3.15^{a}
Saltiness	CT	2.69^{c}	1.96^{b}	1.53^{c}
	HP7	3.51^{ab}	2.19^{b}	1.51^{bc}
RMSE[A] 0.380	HP20	3.09^{bc}	2.43^{ab}	2.28^{ab}
p = .334	HP35	3.77^{a}	2.95^{a}	2.42^{a}

^{abc} Within columns and sensory parameters, means with different letters indicate significant differences ($p < .05$) within each experiment. NPG: No pastiness group; MPG: Medium pastiness group; HPG: High pastiness group.
[A] Root mean square error of the linear model. CT: Control samples (non HP-treated). All the HP treatments were carried out at 600 MPa during 6 min at different temperatures: at 7 °C (HP7); at 20 °C (HP20) and treatment at 35 °C (HP35).

to explain this fact could be the influence of the different saliva viscosity caused by the different texture and proteolysis index on the saltiness perception.

3.2. Effect of HP temperature on texture and sensory properties of samples from different Texture groups

In all Texture groups, an increase of F_0 was produced by the HP treatments, showing a higher increase when processing at high temperatures (Table 3). Ma and Ledward (2004) also found that an increase in pressure up to 400 MPa led to an increase in the instrumental hardness of raw beef. An increase in instrumental hardness, gumminess

and chewiness with increasing pressure (above 150 MPa) was also reported after pressurization of HP-treated raw turkey samples (Villacís, Rastogi, & Balasubramaniam, 2008). Tanzi et al. (2004) observed a significant increase in stringiness and consistency in Parma dry-cured ham after pressurization. These results are also in agreement with Fulladosa et al. (2009), who found that the HP treatment at 600 MPa on restructured dry-cured hams increased hardness and decreased pastiness. Interestingly, in the present study, a significant interaction between HP treatments and Texture group was detected ($P < .05$). Increase of F_0 was more pronounced in NPG samples (showing a mean increase of 1.8 Kg) rather than in medium (MPG) or high (HPG) defective ones (which showed a mean increase of 1.2 and 0.7 Kg, respectively). For example, when submitting samples to high pressure at 7 °C, an increase of F_0 from 1.76 to 3.08 Kg was observed in NPG samples whereas an increase from 0.53 to 1.05 Kg was observed in HPG samples. The reason could be that the effect of pressure is more severe/intensive in those proteins not affected by proteolysis (NPG samples) since they still have the native structure. Higher temperatures are needed to produce changes on proteins partially denatured (HPG samples) to create new rearrangements that increases F_0. It must be remarked that, after HP treatments at 7 °C and 20 °C, similar F_0 values to the NPG control samples were achieved for MPG samples. Therefore, HP treatments at 7 °C and 20 °C could be useful as a corrective action for the MPG samples. In contrast, a temperature of 35 °C was needed to do a corrective action of HPG samples. The more pronounced effect of HP35 treatment on F_0 in comparison to HP7 and HP20 may be due to the more severe changes in the myofibrillar proteins (protein denaturalization) and collagen shrinkage because of the high temperatures reached (Palka & Daun, 1999; Pospiech, Greaser, Mikolajczak, Chiang, & Krzywdzińska, 2002; Tornberg, 2005). It is known that the degree of the myofibrillar protein denaturation varies according to pressure, processing time, temperature, and pH (Garcia-Gil et al., 2014; Huppertz, Fox, & Kelly, 2004). Besides, it has been reported that, when pressure is applied together with heat, modifications of ultrastructure are more intensive than when pressure or temperature are applied alone (Cheftel & Culioli, 1997).

HP produced a significant decrease of Y_2 and Y_{90} ($p < .05$) in all the studied temperatures. These texture modifications have been attributed to the aggregation of myosin molecules, which start to denature at pressures above 100 MPa (Yamamoto, Yoshida, Morita, & Yasui, 1994), and to form disulphide bonds at higher pressures (400 MPa) (Angsupanich, Edde, & Ledward, 1999; Orlien, 2017). The decrease of force decay due to HP treatment was similar at any of the used temperatures, showing a decrease around 0.5 for Y_2 and 0.4 for Y_{90} for all the temperatures used. High pressure produced a similar effect on Y_2/Y_{90} regardless of the initial textural characteristics of the sample. A decrease of pastiness and saliva viscosity in samples subjected to all HP treatments in comparison to control samples for both MPG and HPG samples was found (Table 5). Both attributes were highly correlated ($r = 0.894$, $p < .05$). On the contrary, stringiness increased after the application of high pressure. Defective samples (MPG and HPG) achieved a similar stringiness than NPG samples when HP treatment was applied at 7 °C in MPG samples and at 7 or 20 °C in HPG samples. However, the application of HP treatments in NPG samples doubled the score of stringiness regardless of the temperature applied. This fact was attributed to the non-damaged protein structure of NPG samples in which more important changes are produced during HP processing.

A significant increase of saltiness perception was produced by HP treatment in all the Texture groups, especially at 35 °C. This fact could even enhance taste, especially in salt reduced products. The observed increase agrees with Lorido et al. (2015), who reported that, after the application of HP treatment, saltiness intensity perception was potentiated and the persistence was higher in comparison to control ones in both Serrano and Iberian hams. In the present study, a clear increasing tendency of saltiness with HP temperature is observed, although only significant differences were found in NPG samples treated at 35 °C. Besides, a decrease of saltiness perception with the increase of the defective texture intensity was also found. Characteristics of the meat matrix plays an important role on the speed and amount of salt release to the saliva during chewing. As proteolysis index (highly correlated with pastiness defect) will extensible change protein structure of dry-cured hams, textural characteristics will also play a role in salt perception (Kuo & Lee, 2014; Lawrence et al., 2012; Yucel & Peterson, 2015). Differences on the total volatile compounds depending on the Texture group and the samples proteolysis index were also described by Pérez-Santaescolástica et al. (2018). High defective samples with excessive proteolysis index seem to be associated with negative effects on the taste and aroma of the dry-cured ham. Recent studies showed that moderate thermal treatments using ultrasounds (Pérez-Santaescolástica et al., 2018) or HP processing at different temperatures (Pérez-Santaescolástica et al., 2019) produced a significant reduction of the total volatile compounds content. For all these reasons, a consumer study would be necessary to evaluate the impact of these changes in taste and aroma on the product acceptability.

3.3. Effect of HP temperature on colour and colour stability of samples from different Texture groups

An increase of L* values after HP processing in all the used temperatures was observed what agrees with the previous published studies (Andrés et al., 2004; Fuentes et al., 2010; Ha et al., 2017; Hughes, Oiseth, Purslow, & Warner, 2014) (Table 4). This fact was attributed to the increase of reflected:absorbed ratio of light caused by the new rearrangement of proteins when denatured (Andrés et al., 2004; Clariana et al., 2011; Clariana, Guerrero, Sarraga, & Garcia-Regueiro, 2012; Ottestad, Enersen, & Wold, 2011). A significant interaction between HP treatment and Texture groups was found ($p = .003$). Increase of L* values was found to be more pronounced in NPG samples (mean increase of 6) which have a lower proteolysis index and a less damaged structure than in MPG samples (mean increase of 5) or in HPG samples (mean increase of 3.5) (Hughes et al., 2014; Palka & Daun, 1999; Straadt, Rasmussen, Andersen, & Bertram, 2007). Therefore, HPG samples could be submitted to HP at higher temperatures with similar effect on L* values. This could permit an improvement of texture with a slight increase in the lightness of the product.

The decrease of a* values in HP treated samples was attributed to the partial denaturation of the globin part of nitrosylmyoglobin and to the formation of nitrosyl hemocromogen (NO-Heme) which was attributed to the protein and pigment denaturalization because of the combination of pressure and temperature (Carlez, Veciana-Nogues, & Cheftel, 1995; Chensha, Jingzhi, Lizhen, & Weiqing, 2016; Ha et al., 2017; Pegg & Shahidi, 1996; Sun, Zhou, Xu, & Peng, 2009). As in the case of lightness, redness (a*) was not significantly affected when increasing the HP processing temperature, similarly for all the Texture groups (no significant interaction between Temperature treatment and Texture group, $p > .05$). Therefore, high pressure processing at 20 °C or 35 °C could be useful to improve texture without affecting the colour of the product with respect to processing at 7 °C.

Besides, colour during product shelf life was found to be stable after any of the studied HP treatments. Only a slight decrease of a* values were found between 0 h and 4 days of exposure to light for all the Texture groups and HP treatments. However, no significant differences between 4, 7, 21 and 28 days of exposure were observed ($p > .05$). Fig. 1 shows the evolution of a* for HP treatment at 7 °C, showing the samples HP treated at other temperatures a similar pattern. Cava, Ladero, González, Carrasco, and Ramírez (2009), after processing sliced dry-cured Iberian ham and loin using different HP treatment conditions at 200 MPa and 300 MPa during 15 and 30 min, found a similar tendency in each treatment after 60 and 90 days of exposure to light. Results from this study suggest that neither the texture characteristics of the sample nor the temperature of the HP treatment influenced on the

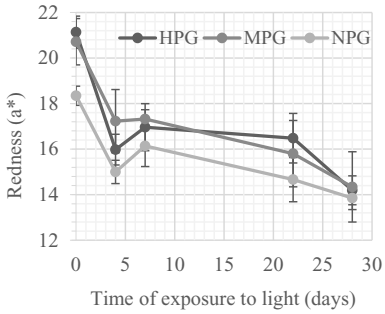

Fig. 1. Colour stability of HP treated samples at 7 °C for the different Texture groups. NPG: Non-pastiness group; MPG: Medium pastiness group; HPG: High pastiness group.

colour stability of sliced dry-cured ham.

Although HP treatments can produce changes on colour and other product characteristics, processing at 7 °C is commonly used by industry to ensure safety and increase shelf life of dry-cured ham, and the resulting product is accepted by the consumers. However, the use of higher temperatures could produce more important changes in the L* values of NPG samples. Due to the different effect of HP processing temperature on saltiness, texture and colour as a function of textural characteristics of the samples, the optimal HP processing temperature will depend on the samples texture. It must be remarked that a previous classification of the samples according to their texture characteristics using non-destructive technologies, as those proposed by Fulladosa et al. (2018) would be a useful tool to apply the optimal HP processing temperature to each product. For soft and pasty textures, a high temperature during HP processing would reduce the intensity of pastiness whereas for non-pasty textures, a low temperature would be recommended to avoid excessive hardness or stringeness.

4. Conclusions

HP treatment produces changes on texture and colour of dry-cured ham samples to a different extent depending on the processing temperature and textural characteristics of the samples. Therefore, textural properties of the product are important to define optimal HP processing conditions to improve texture without deteriorating colour. It would be necessary to study the impact of colour, saltiness perception and texture changes on consumers' acceptability.

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General discussion 5

Although most of the results have been discussed individually in each of the papers enclosed in the previous sections, a global discussion of the results is presented below.

The main objective of this PhD Thesis was to develop and evaluate tools to improve or optimise the manufacturing process and the quality of dry-cured ham from pork and lamb. Texture development of dry cured ham from both pork and lamb is a complex issue since many factors are involved.

To date, different destructive instrumental techniques have been used to characterize the texture of dry-cured ham at the end of the process such as Texture Profile Analysis (TPA) and/or Stress Relaxation (SR) (Morales, Guerrero, *et al.*, 2007) as well as sensory analyses (Guàrdia *et al.*, 2010). However, all of these techniques, although proven to be helpful to characterize the product, cannot be applied *on-line* in industry to evaluate texture development, because they are destructive, expensive and time consuming. A mathematical model to predict the texture of the final product using raw material characteristics and information on processing conditions as the one developed in paper I could be useful. Results of this paper show an important effect of the temperature during processing to improve texture, not only at the end of the process, as previously reported by several authors where moderate thermal treatments were applied (Gou *et al.*, 2008; Morales, Arnau, *et al.*, 2008; Sánchez-Molinero & Arnau, 2014), but also during the elaboration process. However, the appearance of the ham can be modified as the use of high temperatures and increase in weigh losses, there is an increase of proteolysis index also associated with a higher incidence of tyrosine crystals and a lower incidence of white film intensity. Similarly, Arnau *et al.*, (1997) demonstrated the negative effects on the incidence of tyrosine crystals and white film intensity when the temperature is increased at 30 °C, however, processing at 20 or 25 °C would be more adequate, and would minimize the negative effects on the texture and appearance of the product. Because of the complexity and the amount of factors involved in dry-cured ham production, the developed mathematical algorithms would allow to adjust the optimal processing conditions and the appropriate weight loss set up for each ham depending on the raw material characteristics to obtain an optimal texture.

However, not only the quality of the product but also its safety should be considered, because temperature changes at certain steps of the process can increase the incidence of microbiological hazards (Paper II). Also, the variations in environmental factors can facilitate unwanted and uncontrolled growth of mould at the surface of the ham (Rodríguez *et al.*, 1998), often associated with unpleasant appearance, odour and changes in taste and nutritional value (Filtenborg, Frisvad & Thrane, 1996; Papagianni, Ambrosiadis & Filiousis, 2007). The salt content in Fenalår production is higher (ranging from 5% to 10%, Håseth *et al.*, (2014)) than in other cured products such as bone-in dry-cured pork ham (ranging from 4.8% to 5.5% (http://www.innovacc.cat/wp-

content/uploads/2017/05/Annex_9.2._Informe_final.pdf)). In order to reduce the salt and nitrites content without compromising the safety of the product, different salting treatments were evaluated using predictive microbiology models. The obtained results in Paper II showed that the lower the amount of salt, the higher the microbiological risk, especially when no-nitrite was used. Moreover, these modifications of the manufacturing process also resulted in the changes of the final product's appearance. The changes were more important when no nitrite was added, showing Fenalårs with less reddish colour due in part to the formation of Zn-protoporphyrin (ZnPP) (Wakamatsu, Okui, *et al.*, 2004). ZnPP requires longer ageing process to achieve the optimal reddish colour, as time is an important factor in its formation (De Maere *et al.*, 2017; De Maere *et al.*, 2016). This is the case of Italian Parma ham, typically performed without nitrites or nitrates, which have a long curing process between 12 and 24 months. Further studies testing longer ageing times and optimising the duration of the different stages of the process might be useful to improve the red coloration when no nitrites are used. On the other hand, in paper I, only a 15% salt reduction was applied which is in line with the NAOS strategy (AESAN, 2005), but not enough to be labelled as "salt reduced". Therefore, more studies are needed to ensure product safety in the production of Fenalår with reduced salt content (>25%) as the safety of the product can be compromised. In addition, further efforts are needed to reduce the binding problems in restructured Fenalår are necessary since the quality could be negatively affected.

In addition to the models for texture prediction developed in paper I and the use of predictive microbiology models in paper II, new method for *on-line* evaluation of product's texture before its commercialization are still necessary. Currently, this evaluation is carried out by experts or just by classifying the hams according to the weight loss. However, the development of tools to automate this process would ensure that products are sent to the market with an optimal texture. In paper III, a new instrumental tool to discriminate dry-cured hams with appropriate texture from those that are not ready for commercialization has been developed.

The ITAS method allows to define the end of process non-destructively by analysing the texture at the surface of the ham by simulating the tactile texture analysis currently made by the experts. The optimal conditions of ITAS method consist of the use of a 1.9 cm diameter sensor, simulating the shape of the human finger, on the area evaluated by the experts which has a thin subcutaneous fat layer and is beneath the BF muscle. The analysed texture parameters using non-destructive technologies, together with the subcutaneous fat thickness estimation, could contribute to a better classification, especially for hams with weight losses of 34.7 to 37.5%, as they are the ones that are more easily misclassified. The maximum classification accuracy was obtained when combining the textural parameters and the subcutaneous fat thickness (obtaining a classification accuracy of 82.1%). The implementation of this technology at industrial level

would not only improve the quality of the product but also allow economic, energy and space savings, as drying time needed for each ham would be better adapted. However, industrial validation using a larger number of hams of different types (with different degrees of proteolysis, intramuscular and intermuscular fat content and ageing conditions) is required. Including relevant information about the product obtained during the process (such as fat content in raw material (Santos-Garcés *et al.*, 2014) or salt content after salting (García-García *et al.*, 2019)) could also help to better define the end of the process. Moreover, the implementation of this technology in the industry in combination with the predictive model developed in paper I and a pre-classification system, would not only optimise the manufacturing process but also increase the economic, energy and space savings.

However, there are certain internal quality parameters that are difficult to evaluate from the surface of the ham. One of these attributes is the pastiness defect that has an important incidence for the industry (Tapiador & García-Garrido, 2003). Pastiness defect not only affects the texture of the product (increasing pastiness and the adhesiveness between slices (Gou *et al.*, 2008)), but also an increase incidence on bitter taste (Pérez-Santaescolástica *et al.*, 2018b). However, so far, this defect could only be detected by using sensory analysis. Several works were reported on developing non-destructive technologies which would enable *on-line* evaluation of product intrinsic properties such as Magnetic Resonance Imaging (MRI) (García-García *et al.*, 2019) or ultrasounds (US) (Contreras *et al.*, 2020) in the whole ham, or multi energy X-ray (Fulladosa *et al.*, 2018) or Near-Infrared spectroscopy (NIR) (Hernández-Ramos *et al.*, 2020) in sliced dry-cured ham. However, all the mentioned technologies, despite exhibiting considerable potential to evaluate the internal characteristics of the product, are still in experimental phase and not yet applicable in the industry. Meanwhile, in paper IV, a first step was taken to develop a method for evaluating pastiness using a rheometer in order to avoid the tedious sensory analysis. An increase in the viscosity of the water extracts was observed with the increase of pastiness intensity, indicating the possibility to detect this defect with the rheometer. In addition, rheometer results could also be related to changes of texture perceived in sensory analysis. However, there are factors other than viscosity that can affect/alter the results. Furthermore, a rheometer can only discriminate samples with high pastiness intensity from those with medium or non-pastiness defect. The results obtained showed that it seems possible to develop a rapid, inexpensive routine method for the instrumental measurement of pastiness perception of dry-cured ham. Still, further studies need to be conducted using more samples with a larger pastiness range and considering other factors such as fat content. This is of interest to industry and researchers to avoid the costly and time-consuming sensory analysis.

Despite these promising results, it would still be possible that part of the production would enter the market without optimal texture or having defective textures. For this

reason, corrective actions were investigated to resolve or reduce the occurrence of this defect. In Paper V a corrective action using HPP treatments at different temperatures to improve texture and/or reduce the incidence of defective texture in hams with different initial pastiness intensities was investigated. The HPP treatments are widely used in industry to extend the product shelf-life but also to eliminate pathogenic microorganisms (Aymerich, Picouet & Monfort, 2008). Furthermore, pressure treatments also decrease the incidence of soft textures (Fulladosa *et al.*, 2009; Lorido *et al.*, 2015). Results of paper V demonstrated that these textural changes can vary depending on the processing temperature and the initial textural characteristics of the product. Temperatures of 7 °C and 20 °C are effective to correct samples with medium pastiness, while 35 °C is needed for samples with high pastiness as high temperatures are required to produce changes in proteins that are partially denatured to increase hardness. The HPP treatments also affect the aroma and taste of the product and increase the perception of rancid odour and saltiness (Andrés *et al.*, 2004; Campus *et al.*, 2008; Cheftel & Culioli, 1997; Lorido *et al.*, 2015; Martínez-Onandi *et al.*, 2016). While HPP treatments at high temperatures (35 °C) produce intense modifications of free amino acid and volatile profile, at temperatures from 0 to 20 °C the impact is minimized (Pérez-Santaescolástica, Carballo, *et al.*, 2019; López-Pedrouso *et al.*, 2019). The obtained results prove that the HPP treatments are well suited to improve and correct defective texture of dry-cured hams and that initial textural properties of the product are important to define optimal HP processing conditions to improve texture without deteriorating colour and aroma.

Another non-destructive technology is power ultrasound (PuS), which also seems promising for correcting hams with defective textures. However, as PuS causes an increase of hardness this could be excessive for hams that already have an optimal texture (Contreras *et al.*, 2020). In both, HPP and PuS, the treatment has to be limited to hams with defective textures, because the generated temperature increase causes modifications which might be excessive for hams with no pastiness defect. Similarly, as HPP, PuS treatments also affects the aroma and taste of the product (López-Pedrouso *et al.*, 2019; Pérez-Santaescolástica *et al.*, 2018a).

There is a growing interest of industry to find solutions to solving the problem of hams with defective textures. The problem of soft textures affects not only bone-in dry cured hams but also restructured hams, thus affecting products from different animal species. The methods and tools evaluated in this PhD Thesis dissertation to optimise the manufacturing and improve the quality of the final product, are promising tools for the industry. The developed and used mathematical models from the work presented in papers I and II, can be used in industry to optimise the processing and also to control/evaluate the food safety of the process. Applying the ITAS method developed in paper III would allow the industry to more accurately identify the hams that are ready for the market. The combination of different tools could also lead to more efficiency,

energy and space savings in industry. Unfortunately, to date it is not possible to detect *in-line* the hams with defective textures or to apply a corrective action with HPP as described in paper V or using PuS. Several research efforts are focusing on the development of technologies to predict pastiness defect.

The tools evaluated in this PhD Thesis are a first step towards the development of new technologies. Continued research efforts are needed in order to be able to develop these solutions to the level needed for industrial application.

6 Conclusions

6.1 Conclusions in English

According to the general objective and the specific goals of this PhD Thesis, it can be concluded that:

I. Development of texture and tyrosine precipitates formation is a complex issue that depends on multiple factors such as those studied in this Thesis (drying temperature, the achieved weight loss, pH_{SM24h} and NaCl content) and needs to be undertaken in a global way. In this sense, the developed texture predictive models based on the information obtained using non-invasive technologies during the process and processing conditions are useful tools to optimise processes of elaboration and achieve optimal textures.

II. Salt content reduction in restructured Fenalår produces soft textures, important changes in colour and safety hazards during the elaboration process. The elaboration of Fenalår without nitrite must be cautious as it can increase the growth potential of *L. monocytogenes* and slightly that of proteolytic *C. botulinum*. Predictive microbiology is a useful tool to evaluate microbiological hazards and to define conditions to ensure safety when salt and nitrifying agents are reduced on restructured Fenalårs production.

III. The ITAS method discriminates dry-cured hams with an appropriate texture for commercialization, but its maximum accuracy is obtained when combined with the overall ham fat content information obtained using computed tomography.

IV. Changes in oral fluid viscosity, instrumentally evaluated (apparent viscosity values at 10 s^{-1}), are related with pastiness perception intensity in dry-cured ham. Therefore, it is possible to develop a rapid, inexpensive routine method to instrumentally measure pastiness perception in dry-cured ham.

V. The intensity of soft defective texture in dry-cured hams is reduced with HP treatment, but the initial textural properties of the product are important to define the optimal HP processing conditions to optimise the corrective action.

VI. The tools evaluated and/or mentioned in this Thesis are a first step for the development of new methodologies which could be later implemented at the industry at large scale. However, they should first be validated at industry level.

6.2 Conclusiones in Spanish

De acuerdo con el objetivo general y los objetivos específicos descritos en la presente Tesis, se puede concluir que:

I. El desarrollo de la textura y la formación de precipitados de tirosina es un tema complejo que depende de múltiples factores como los estudiados en esta Tesis (temperatura de secado, la merma, el pH_{SM24h} y el contenido de NaCl) y debe abordarse de manera global. Los modelos predictivos de textura desarrollados, basados en la información obtenida mediante tecnologías no invasivas durante el proceso y las condiciones de procesado, son herramientas útiles para optimizar los procesos de elaboración y lograr así texturas óptimas.

II. La reducción del contenido de sal en el Fenalår restructurado no solo produce texturas blandas, sino también cambios importantes en el color y riesgos para la seguridad durante el proceso. La elaboración de Fenalår sin nitrito puede incrementar el potencial de crecimiento de *L. monocytogenes* y ligeramente el de *C. botulinum* proteolítico. La microbiología predictiva es una herramienta útil para garantizar la seguridad cuando se reduce el contenido de sal y de los agentes nitrificantes en la producción de Fenalår restructurado.

III. El método ITAS permite discriminar los jamones curados con una textura adecuada para su comercialización, pero su máxima precisión se obtiene cuando se combina con el contenido total de grasa del jamón obtenida mediante tomografía computarizada.

IV. Los cambios en la viscosidad del fluido evaluados instrumentalmente (valores de viscosidad aparente a 10 s^{-1}), se relacionan con la intensidad de la pastosidad sensorial del jamón curado. Por lo tanto, es posible desarrollar un método rutinario rápido y económico para medir instrumentalmente la percepción de la pastosidad en el jamón curado.

V. La intensidad de los defectos de textura en el jamón curado se reduce con el tratamiento de altas presiones, pero las propiedades iniciales de textura del producto son importantes para definir las condiciones óptimas de las altas presiones para optimizar la acción correctora.

VI. Las herramientas evaluadas y/o mencionadas en esta Tesis son un primer paso para el desarrollo de nuevas metodologías que podrían ser implementadas en la industria a gran escala. Sin embargo, primero deben validarse a nivel industrial.

6.3 Conclusions in Catalan

D'acord amb l'objectiu general i els objectius específics descrits en aquesta Tesis, es pot concloure que:

I. El desenvolupament de la textura i la formació de precipitats de tirosina és un tema complex que depèn de múltiples factors com els estudiats en aquesta Tesis (temperatura d'assecat, la minva, el pH_{SM24h} i el contingut de NaCl) i s'ha de tractar de manera global. Els models predictius de textura desenvolupats, es basen en la informació obtinguda mitjançant tecnologies no invasives durant el procés i les condicions de processat, essent eines útils per optimitzar el procés d'elaboració i aconseguir una textura òptima.

II. La reducció del contingut de sal en la producció de Fenalår reestructurat no només dona lloc a textures toves, sinó també a canvis importants en el color i a riscos per a la seguretat durant el procés. L'elaboració de Fenalår sense nitrit pot incrementar el potencial de creixement de *L. monocytogenes* i lleugerament el de *C. botulinum* proteolític. La microbiologia predictiva és una eina útil per a garantir la seguretat quan es redueixen el contingut de sal i dels agents nitrificants en la producció de Fenalår reestructurat.

III. El mètode ITAS permet discriminar els pernils curats amb una textura adequada per a la seva comercialització, però la seva màxima precisió s'obté quan es combina amb el contingut total de greix del pernil obtinguda mitjançant tomografia computeritzada.

IV. Els canvis en la viscositat del fluid avaluats instrumentalment (valors de viscositat aparent a 10 s^{-1}), es relacionen amb la intensitat de la pastositat en el pernil curat. Per tant, és possible desenvolupar un mètode rutinari ràpid i econòmic per mesurar instrumentalment la percepció de la pastositat en pernil curat.

V. La intensitat dels defectes de textura en el pernil curat es redueix amb el tractament per altes pressions, però les propietats inicials de textura del producte són importants per a definir les condicions òptimes de les altes pressions per a optimitzar l'acció correctora.

VI. Les eines avaluades i/o esmentades en aquesta Tesis són un primer pas per al desenvolupament de noves metodologies que podrien ser implementades en la indústria a gran escala. Primer però, s'han de validar a nivell industrial.

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Technical Paper

Annex 1

Coll-Brasas, E., Arnau, J., Lorenzo, J. M., Purriños, L., García-Pérez, J.V., Benedito, J. & Fulladosa, E.. Cómo evaluar no destructivamente defectos de textura en jamón curado. (2016). *Eurocarne* núm. 246, pg. 92-96.

Cómo evaluar no destructivamente defectos de textura en jamón curado

Cómo evaluar no destructivamente defectos de textura en jamón curado

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El jamón curado constituye un alimento rico en proteínas, vitaminas y minerales muy apreciado por sus características sensoriales y de textura (Arnau, 2006). Desde tiempos inmemoriales ha constituido una forma de conservación de la carne mediante el salado y su posterior secado (Arnau, 2000). Las tendencias actuales del consumo de productos cárnicos se dirigen a productos con un menor contenido en sal, en concordancia con la estrategia NAOS que recomienda reducir el consumo de sal en una dieta cardiosaludable (AESAN, 2005). Debido a la conexión entre la ingesta de elevadas cantidades de sal y las enfermedades cardíacas, existe una concienciación cada vez mayor por parte de los consumidores a demandar productos con bajo contenido en sal (Guàrdia, Guerrero, Gelabert, Gou & Arnau, 2006). La industria alimentaria reconoce la necesidad de responder a la demanda de reducir el nivel de sal en los productos alimenticios ya que, de su innovación y adaptación a las nuevas necesidades del mercado, depende su viabilidad y desarrollo. Sin embargo, esta tendencia implica un aumento de la incidencia de jamones con texturas defectuosas. Esto supone un reto para las empresas productoras: elaborar un producto con un contenido de sal óptimo y con una textura y color adecuados.

Los principales problemas de textura en jamón curado son la formación de una costra dura en la superficie (Flores, 2001; J.A. García-Garrido, Quiles-Zafra, Tapiador & Luque de Castro, 1999) y la textura pastosa y/o excesivamente blanda (ver Figura 1) que normalmente aparece en las partes más internas del jamón (Arnau, Guerrero & Sárraga, 1998; Virgili, Parolari, Schivazappa, Bordini & Volta, 1995). Estos defectos se presentan con una incidencia moderada en jamones con un contenido en sal estándar (Tapiador Farelo & García Garrido, 2003). Sin embargo, cuando se reduce el contenido de sal del producto o se intenta acelerar el proceso de elaboración esta incidencia aumenta

considerablemente, incrementando así la magnitud del problema. Este defecto es más común en la materia prima con un elevado potencial proteolítico, pero está condicionado también por el proceso tecnológico efectuado (Arnau, 2000; García-Rey, García-Garrido, Quiles-Zafra, Tapiador & Luque de Castro, 2004).

Figura 1: Detalle jamón pastoso. Fuente: IRTA Monells. Autora: Elena Coll.

Factores que influyen en el desarrollo de la textura

A lo largo del proceso de elaboración suceden una serie de cambios que condicionan la textura final del jamón (Ouali, 1990). Los factores que principalmente influyen sobre la textura son las características de la materia prima (genética, pH, impedancia, composición y actividad proteolítica), las condiciones del procesado (tipo de salado, condiciones de proceso y tratamientos al final del proceso) y el contenido en sal.

Se ha descrito que los jamones que presentan un pH elevado (pH>6,0), son más susceptibles a presentar texturas anómalas (Arnau *et al.*, 1998). Son jamones más pastosos y adhesivos que los normales, a pesar de tener unos niveles de nitrógeno no proteico normales (Arnau *et al.*, 1998; Guerrero, Gou & Arnau, 1999). Por otro lado, la carne con un pH bajo, también puede ocasionar problemas en la textura del jamón. En este caso se trata de jamones más pastosos que tienen un contenido en nitrógeno no proteico más elevado de lo habitual (García-Rey *et al.*, 2004) debido al aumento de la actividad enzimática de las catepsinas (enzimas implicadas en la degradación de las proteínas) al disminuir el pH (García-Garrido, J. A., Quiles-Zafra, Tapiador & Luque de Castro, 2000).

El tipo de procesado que recibe el jamón y las condiciones del proceso (sobre todo la duración y temperatura de las diferentes etapas) así como también los pre- y postratamientos (como la congelación, tratamientos térmicos suaves en las fases finales del proceso o los tratamientos con altas presiones) pueden influir significativamente en la textura final del jamón (Arnau, 2000; Arnau, Guerrero & Gou, 1997; Parolari, Virgili & Schivazappa, 1994).

La temperatura, el contenido en sal y de agua son factores clave en el proceso que afectan al desarrollo de la proteólisis, la oxidación lipídica, la estructura y la textura final del jamón. La textura se ve influenciada por el contenido de agua, así como también por el índice de proteólisis. Ruiz-Ramírez *et al.*, (2006), describe que cuanto mayor es el contenido de agua y el índice de proteólisis, menor es la dureza (Figura 2). Virgili *et al.*, (1995) también observaron una correlación negativa entre la proteólisis y la dureza en los jamones curados de Parma.

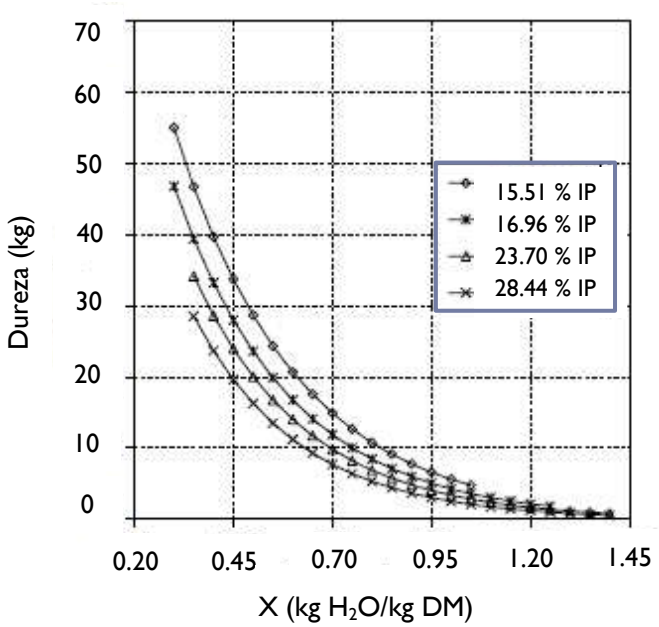

Figura 2: Influencia del contenido de agua y del índice de proteólisis sobre la dureza del jamón (Ruiz-Ramírez, Arnau, Serra & Gou, 2006).

El contenido en sal también tiene un efecto muy importante sobre el desarrollo de la pastosidad. Una reducción del tiempo de salado de 14 a 6 días produce un aumento significativo de la pastosidad y de la dificultad de loncheado sobre todo en jamones de pH bajo. La reducción del contenido de sal produce un retraso en la estabilización del producto, sobre todo en las partes más internas del jamón. El aumento de la temperatura cuando el contenido de sal en las partes más internas no es suficiente, puede suponer un incremento excesivo de la actividad enzimática y la aparición de texturas pastosas. La optimización del tiempo de reposo es clave para reducir la incidencia de este defecto en estos casos.

Otra estrategia para la corrección de los defectos de textura, es el aumento de la temperatura en la fase final de elaboración del jamón. En este sentido, tratamientos térmicos suaves a 30 °C durante 168 horas al final del proceso, disminuyen las texturas blandas, la adhesividad y la pastosidad en el músculo BF sin incrementar la dureza en el músculo SM o afectar a los parámetros físico-químicos (humedad, actividad de agua e índice de proteólisis) (Gou, Morales, Serra, Guàrdia & Arnau, 2008). El incremento de la temperatura de 27 °C a 35 °C, aplicada durante 96 horas al final del proceso en jamones de 8 meses, tampoco produjo ningún cambio negativo en las características del

jamón (Sánchez-Molinero & Arnau, 2014). Sin embargo, el aumento de la temperatura a 30 °C durante el último mes, en un proceso de seis meses de maduración, afectó negativamente la apariencia y la textura (Arnau *et al.*, 1997). Es importante diferenciar las texturas blandas y adhesivas (que aparecen en jamones de pH elevado) de las texturas pastosas (que aparecen debido a una elevada proteólisis en jamones de pH bajo). Las texturas blandas se pueden corregir con el incremento de la temperatura al final de proceso o el aumento del tiempo de secado. Sin embargo, las texturas pastosas debidas a daños en la microestructura por la acción enzimática de las catepsinas, son más difíciles de corregir.

Medición convencional de la textura

La textura en el jamón curado se ha caracterizado hasta el momento mediante análisis instrumentales y sensoriales. En algunos casos, también se relaciona bien con análisis químicos como el índice de proteólisis.

La medida instrumental puede resultar apta para el estudio de la textura debido a su reproducibilidad y facilidad de estandarización (Brady, 1985). Sin embargo, los instrumentos de medida de textura solamente pueden detectar y cuantificar ciertos parámetros físicos que luego deben interpretarse en términos de percepción sensorial (Ruiz-Ramírez, Gou & Arnau, 2003). El análisis del perfil de textura (TPA), es un método instrumental que pretende imitar el proceso que se realiza durante la masticación mediante la aplicación de dos ciclos de compresión-descompresión (Bonilla *et al.*, 2002; Guerrero *et al.*, 1999; Ruiz-Ramirez, Arnau, Serra & Gou, 2005). Por otro lado, el test de relajación (SR), que aplica una compresión mediante una sonda cilíndrica que mantiene la muestra deformada durante un periodo de tiempo establecido, tiene en cuenta la naturaleza viscoelástica de la muestra (Morales, Guerrero, Serra, & Gou, 2007). Morales et al (2007) observaron que la mejor prueba para la detección de texturas defectuosas depende del músculo a analizar. El test de relajación (SR) dio mejores resultados en el músculo *Biceps femoris* (BF) y el análisis de perfil de textura TPA en el músculo *Semimembranosus* (SM).

El análisis instrumental de textura puede complementarse con un análisis sensorial. Éste permite analizar las reacciones humanas frente a las diferentes características de un alimento y como éstas son percibidas con los sentidos de la vista, olfato, gusto y tacto (Moskowitz, 1988). Se realiza con paneles entrenados o de expertos en los que se evalúan atributos como la adhesividad (evaluación de la adhesión que presenta el jamón cuando se comprime con la lengua contra el paladar (3.57, UNE 87-001-94)); la dureza (evaluación de la fuerza necesaria para comprimir el jamón entre los molares durante el primer mordisco (3.50, UNE 87-001-94)); la desmenuzabilidad (facilidad con la que el jamón se descompone en trozos pequeños durante la masticación); la pastosidad (sensación de pasta en la boca similar a la que produce un jamón con un elevado índice de proteólisis); y la fibrosidad (evaluación de la percepción de fibras que se produce durante el proceso de masticación (3.59, UNE 87-001-94)) (Arnau, Guàrdia, Guerrero

& Claret, 2011; Guerrero, Guàrdia & Arnau, 2005). Algunos de estos atributos son difíciles de analizar mediante un análisis de textura instrumental.

En algunos casos, los análisis químicos también se relacionan bien con la textura instrumental y/o sensorial. Parámetros como el índice de proteólisis, definido como la relación entre el nitrógeno no proteico y el nitrógeno total, nos puede dar una idea de la intensidad del defecto de pastosidad. El desarrollo de tecnologías no destructivas o de nuevas medidas instrumentales que se relacionen correctamente con la sensación de pastosidad sería de gran interés.

Medidas no destructivas

En la actualidad, la detección de texturas defectuosas se ha realizado empleando análisis destructivos, ya sea mediante determinaciones instrumentales, sensoriales o químicas. Este tipo de análisis implica la destrucción total o parcial de la muestra, hecho que dificulta su reproducibilidad y supone un coste económico elevado (ya que se requiere de un número elevado de muestras para que el análisis sea robusto), incrementando así el tiempo de análisis y el material necesario. En el marco del proyecto SOLTEXJAM, financiado por el Instituto Nacional de Investigación y Tecnología Agraria y Agroalimentaria (INIA), se está evaluando la capacidad de diversas tecnologías no destructivas para caracterizar y detectar texturas defectuosas como la pastosidad en jamón curado. Estas tecnologías permitirían llevar a cabo las medidas de manera rápida y sin comprometer la integridad del producto.

Hasta el momento, estas tecnologías se han utilizado para evaluar la composición del producto, encontrándose menos trabajos centrados en detectar problemas texturales. La **espectrometría de microondas** (EM), basada en la determinación de las propiedades dieléctricas, se ha utilizado para predecir los contenidos de sal y agua en jamón curado (Bjarnadottir, Lunde, Alvseike, Mason & Al-Shamma'a, 2015; Fulladosa *et al.*, 2013). Además, estudios preliminares apuntan su posible utilidad para determinar defectos de pastosidad en jamón curado (Rubio-Celorio, Fulladosa, Claret, Guàrdia & Garcia-Gil, 2013). La **espectrometría de infrarrojo cercano** (NIR), basada en las vibraciones de las moléculas, ha sido ampliamente utilizada para la determinación de la composición de todo tipo de carnes (Ortiz-Somovilla, España-España, Gaitán-Jurado, Pérez-Aparicio & Pedro-Sanz, 2007). Sin embargo, varios estudios difieren sobre su capacidad para caracterizar parámetros texturales (Liu *et al.*, 2003; Ortiz, Sarabia, García-Rey & Castro, 2006). En jamón curado, los trabajos realizados son escasos (García-Rey, García-Olmo, Pedro, Quiles-Zafra & Castro, 2005) y no se ha abordado la influencia que puede tener la composición de la muestra. Mediante los **ultrasonidos de señal** (UdS), tecnología basada en la medición de la velocidad a la que se desplazan las ondas sonoras en el producto, es posible la monitorización tanto de los cambios que tienen lugar durante el procesado de un producto como su caracterización final (Awad, Moharram, Shaltout, Asker & Youssef, 2012; Koch *et al.*, 2011). Se ha monitorizado con éxito el incremento de dureza asociado a la cristalización de grasas (Niñoles, Mulet,

Ventanas & Benedito, 2010; Santacatalina J.V., Garcia-Perez J.V., Corona. E. & Benedito, 2011). También se ha evaluado la textura de productos cárnicos curados (Llull, Simal, Benedito & Rosselló, 2002; Llull, Simal, Femenia, Benedito & Rosselló, 2002) y el efecto del tratamiento por altas presiones (Corona, García-Pérez, Mulet & Benedito, 2013). En jamón curado, se ha visto que la velocidad ultrasónica aumenta al aumentar la dureza del producto (Corona *et al.*, 2013). En el caso de la **retrodispersión por láser** (Laser Backscattering Imaging, LBI), basada en la dispersión de la luz cuando ésta interacciona con un material, se trata de una tecnología poco utilizada en el ámbito de la tecnología de los alimentos (Adebayo, Hashim, Abdan & Hanafi, 2016; Mollazade, Omid, Akhlaghian & Saeid, 2012). Sin embargo, no se conocen estudios en carne ni en jamón curado. Estudios preliminares indican que esta tecnología permite diferenciar distintas texturas en jamón curado gracias a la dispersión de la luz diferente según la microestructura de la muestra.

En el proyecto SOLTEXJAM se pretende identificar en línea los productos que presentan este defecto mediante las tecnologías no destructivas descritas anteriormente. Una vez identificado, se le aplicará una acción correctora, mediante la aplicación de altas presiones y/o los ultrasonidos de potencia, para disminuir su intensidad. Paralelamente, también se emplearán los ultrasonidos de señal para detectar precozmente este defecto y poder aplicar una acción correctora antes de que éste se desarrolle.

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