UNIVERSIDAD DE EXTREMADURA

FACULTAD DE VETERINARIA

DEPARTAMENTO DE
PRODUCCIÓN ANIMAL Y
CIENCIA DE LOS ALIMENTOS

ÁREA DE TECNOLOGÍA DE LOS ALIMENTOS

Effect of pig feeding and pre-cure freezing raw thighs on physical, chemical and sensory features of Iberian ham

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Efecto de la alimentación de los cerdos y de la congelación de los perniles sobre características físicas, químicas y sensoriales del jamón Ibérico

Tesis Doctoral

Mª Trinidad Pérez Palacios

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Memoria de Tesis Doctoral elaborada por Mª Trinidad Pérez Palacios

Cáceres, Octubre 2009

Fdo.: Mª Trinidad Pérez Palacios

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INFORMA:

Que la Tesis Doctoral presentada por la Lda. Mª Trinidad Pérez Palacios "Influencia de la alimentación de los cerdos y de la congelación de los perniles sobre parámetros físicos, químicos y sensoriales del jamón Ibérico", ha sido realizada baja mi dirección en el Área de Tecnología de los Alimentos de la Facultad de Veterinaria. Hallándose concluida y reuniendo a mi entender las condiciones necesarias, autorizo para su defensa ante el tribunal pertinente.

Cáceres, Octubre 2009

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RESUMEN

Existe un gran interés por parte de diversos sectores del cerdo Ibérico en encontrar métodos de clasificación de la materia prima en función de la alimentación de los cerdos durante el periodo de cebo. Por otra parte, en cuanto al proceso de elaboración del jamón Ibérico, aunque cada vez son más los productores que congelan-descongelan los perniles antes de la salazón, esta práctica no está descrita explícitamente dentro del procesado del jamón Ibérico.

Uno de los objetivos de este trabajo ha sido caracterizar jamones en función de la alimentación de los cerdos Ibéricos durante la etapa de cebo, en montanera o con pienso alto oleico, mediante parámetros físicos, químicos y sensoriales de la materia prima y del jamón curado. El otro propósito consistió en estudiar el efecto de la congelación-descongelación de los perniles sobre los parámetros de calidad de la materia prima, y también durante el procesado del jamón y en el producto final.

El análisis de la composición lipídica, y específicamente el porcentaje de los ácidos grasos araquidónico (C20:4 n-6) y linolénico (C18:3 n-3) y el contenido en neofitadieno y γ-tocoferol, así como el análisis de imágenes de resonancia magnética, permitieron diferenciar jamones Ibéricos procedentes de cerdos cebados en montanera y con pienso alto oleico, tanto en materia fresca como en producto final.

En las características físico-químicas de la materia prima se detectaron diferencias entre los perniles refrigerados y los congelados-descongelados. Los jamones congelados-descongelados presentaron un menor contenido en sal que los refrigerados. Sin embargo, a lo largo del procesado del jamón y en el producto final, el efecto de la congelación-descongelación de los perniles fue poco manifiesto, atribuyéndose las escasas diferencias encontradas a las diferencias en el contenido en sal.

SUMMARY

Nowadays, there is an increasing interest in finding methods for classifying Iberian products as a function of pig feeding during fattening. On the other hand, and as far as the processing of the Iberian ham is concerned, although the procedure of pre-cure freezing raw thighs is progressively more carried out by the Iberian ham industries, it is not described within the processing of Iberian ham accurately.

The first aim of this work was to characterize Iberian pig hams as a function of pig diet (montanera vs. high oleic acid concentrates) by means of physical, chemical and sensory features of raw thighs and dry-cured hams. The other goal was to study the effect of pre-cure freezing raw thighs from Iberian pigs on the quality parameters of fresh ham, through its processing and of dry-cured ham.

Lipid composition analysis, specifically proportions of araquidonic (C20:4 n-6) and linolenic (C18:3 n-3) acids and the content of neophytadiene and γ-tocopherol, as well as magnetic resonance imaging allowed the differentiation of Iberian hams from pigs fattened acorn and grass and oleic acid concentrates, in both raw and dry-cured ham.

Pre-cure freezing Iberian raw thighs influenced the content of salt, being lower in frozen-thawed than in refrigerated hams. This procedure affected physicochemical features of the raw material notably, leading to scarce differences throughout the processing of hams and in the final product, which were more likely due to the differences in the salt content than to the pre-cure freezing procedure.

1. Introducción

1.1. CARACTERÍSTICAS DE CALIDAD DE LA CARNE Y LOS PRODUCTOS DEL CERDO IBÉRICO

Si se compara el cerdo Ibérico con otras razas porcinas, los parámetros productivos no son muy favorables, ya que no presenta una buena conformación cárnica y además sus canales tienen un contenido graso muy elevado, fruto tanto del carácter adipogénico de los animales como de su ciclo productivo (sacrificio a edad y peso elevados). Pero, independientemente de la cantidad de carne producida, la producción del cerdo Ibérico se sostiene por las características excepcionales de su carne, tanto para su venta en fresco como para la producción de productos curados.

El sistema de explotación del cerdo Ibérico en la dehesa se emplea desde muy antiguo, dando lugar a productos únicos y que además atienden a las demandas de los consumidores de los países más avanzados en lo referente al bienestar de los animales, y a la producción de alimentos en condiciones ecológicas. Todo ello permite el sostenimiento de un ciclo productivo que de otra manera sería inviable, ya que no podría competir con los modernos sistemas de producción porcina, en los que se priman aspectos relacionados con el volumen de producción y los rendimientos por encima de la calidad sensorial de los productos obtenidos.

Tanto en la carne fresca de cerdo Ibérico como en los productos elaborados, como el jamón y el lomo, las características más sobresalientes de su aspecto son el elevado grado de veteado y el color intenso. Esto se debe a diversos factores, entre los que sobresale el sistema de explotación, que implica una mayor edad de sacrifico y obliga a los animales a realizar una mayor actividad física en busca del alimento durante el periodo de cebo, o la propia genética del cerdo Ibérico (Carrapiso y col., 2003). Como consecuencia del alto grado de veteado, estos productos también destacan por su jugosidad. Otra característica del aspecto al corte de los productos del cerdo Ibérico es la fluidez de la grasa, resultado de su composición en ácidos grasos, dando lugar a un intenso brillo (Cava y col., 2000; Ruiz y col., 2000). No obstante, lo que más destaca de estos productos es su flavor, muy complejo e intenso, fruto de numerosas transformaciones en su composición química, que

hace que el jamón Ibérico pueda incluirse en el grupo de los productos considerados como "delicatessen".

No cabe duda que, de entre las características implicadas en la calidad de la carne y los productos cárnicos del cerdo Ibérico, la cantidad de grasa es uno de los factores determinantes (Ruiz y col., 2000; Ventanas y col., 2005). De hecho, la Asociación Española de Criadores de Ganado Porcino Selecto Ibérico Puro y Trono Ibérico (AECERIBER) ha incluido este parámetro como criterio de selección porcina.

Así, desde el punto de vista de la importancia de la cantidad de grasa intramuscular, se considera necesario disponer de métodos fiables para la cuantificación de la misma (Iverson y col., 2001). Los métodos más comúnmente empleados para cuantificar la cantidad de grasa en carne y productos cárnicos han sido el método de Soxhlet, que es el método oficial recomendado por la AOAC (Association Of Analytical Chemists, 1990), y aquellos que se basan en el uso de mezclas de cloroformo y metanol como disolvente de extracción, como los descritos por Folch y col. (1957) y Bligh y Dyer (1959).

El método de Soxhlet tiene la ventaja de su automatización, sin embargo, y debido a que el disolvente utilizado es de baja polaridad (hexano, éter de petróleo), no consigue extraer la totalidad de los lípidos polares. Además, la grasa extraída con este método no puede analizarse posteriormente ya que se realiza una hidrólisis previa en medio ácido, lo que producirá modificaciones en los componentes lipídicos.

Varios estudios han demostrado que el empleo de diferentes métodos de extracción de grasa da lugar a diferentes resultados. Manikariza y col. (2001) obtuvieron mejores resultados con el método Soxhlet que con el de Bligh y Dier (1959) para extraer la grasa en conejos, mientras que otros autores encontraron el resultado contrario al estudiar el contenido en grasa en pescado (Ewald y col., 1998). Así, sería necesario establecer qué método de los existentes es el más adecuado para cuantificar la grasa en carne y productos cárnicos.

1.2. ALIMENTACIÓN DEL CERDO IBÉRICO Y SU INFLUENCIA EN LA CALIDAD. SISTEMAS DE CLASIFICACIÓN

Entre los factores que afectan a la calidad de la carne y los productos del cerdo Ibérico, la alimentación parece ser uno de los más influyentes (Cava y col., 2000; Carrapiso y col., 2003). Así, los productos procedentes de cerdos Ibéricos cebados en montanera son los que alcanzan un mayor precio en el mercado, debido a su alta calidad sensorial, la cuál está relacionada, entre otros factores, con el consumo de hierba y bellotas (Rey y col., 1997).

Sin embargo, debido a la marcada estacionalidad de los recursos de la explotación en montanera y al incremento de la demanda de los productos del cerdo Ibérico por parte de los consumidores, también se emplean piensos comerciales para el cebo del cerdo Ibérico. Estos piensos son similares a los empleados en el cebo de cerdos blancos pero su composición se ha ido modificando. En un primer momento, se le incorporaron fuentes ricas en ácido linoleico (C18:2 n-6), originándose así mayores niveles de ácidos grasos poliinsaturados n-6 en los distintos depósitos grasos de los cerdos (Cava y col., 1997; Ruiz y col., 1998a), dando lugar al incremento de los fenómenos de oxidación lipídica, con la consecuente disminución de la calidad sensorial del producto final (Carrapiso y col., 2003; Cava y col., 2000). Desde hace un tiempo, es habitual la incorporación de fuentes ricas en ácido oleico (C18:1 n-9) a los piensos para cerdos Ibéricos, con el objetivo de conseguir un perfil de ácidos grasos en la grasa de los animales y unas características de calidad en los productos cárnicos similares a las de los cerdos Ibéricos de montanera.

Con el objetivo marcar los productos del cerdo Ibérico presentes en el mercado, se aprobó en el año 2001 la Norma de Calidad para el jamón Ibérico, paleta Ibérica y caña de lomo Ibérico elaborados en España (BOE, 2001). Esta norma fija criterios raciales y de alimentación de los cerdos Ibéricos así como las etapas y tiempos mínimos de procesado. El cumplimiento de estos criterios se certificaba mediante procedimientos de control y trazabilidad desde la explotación ganadera hasta su venta al consumidor final, para lo que se crearon las entidades certificadoras.

Desde su aprobación, se han realizado diversas modificaciones a la Norma, lo que conllevó en el año 2007 a la entrada en vigor de una nueva

Norma de Calidad (BOE, 2007). Su ámbito de aplicación se amplía a los productos procedentes del despiece de la canal que se comercializan en fresco. Esta nueva Norma también tiene por objeto favorecer el mantenimiento de la dehesa, protegiendo consecuentemente este ecosistema. Así, la nueva Norma de Calidad identifica aquellos municipios con recursos existentes en parcelas de dehesa, y limita la carga ganadera máxima de dichas parcelas para garantizar la conservación y sostenibilidad de las mismas.

Considerando la alimentación suministrada al cerdo en el periodo inmediatamente anterior al sacrificio se distinguen actualmente 4 designaciones en relación con el tipo de alimentación (Tabla 1).

La anterior Norma de Calidad aprobada en 2001 permitía establecer las designaciones de calidad en función de la alimentación de los cerdos basándose en el perfil de ácidos grasos del tejido subcutáneo de las canales de cerdos Ibéricos determinados mediante cromatografía de gases (BOE, 2004). La clasificación se realizaba en base a los porcentajes obtenidos de los cuatro ácidos grasos mayoritarios: ácido palmítico (C16:0), ácido esteárico (C18:0), ácido oleico (C18:1 n-9) y ácido linoleico (C18:2 n-6). Para cada campaña, la Asociación Interprofesional del Cerdo Ibérico (ASICI) proponía unos valores analíticos para cada uno de estos cuatro ácidos grasos, en función de los cuales los cerdos se clasificaban en los diferentes grupos de alimentación. Sin embargo, en los últimos años este método ocasionó un número elevado de falsos positivos (cerdos cebados con pienso clasificados como de bellota) y de falsos negativos (cerdos criados en montanera que no presentaban el perfil de ácidos grasos para clasificarse como de bellota) ya que muchos de los cerdos clasificados en el grupo de alimentación de bellota eran en realidad animales cebados con piensos enriquecidos en ácido oleico (C18:1 n-9), de forma que no era posible diferenciar el perfil de ácidos grasos de la grasa subcutánea de estos animales del de los animales cebados en régimen de montanera.

Así pues, la nueva Norma de Calidad ha establecido que el control de la alimentación de los cerdos se lleve a cabo únicamente mediante entidades de inspección que visitan las explotaciones ganaderas, y sólo indica el análisis de ácidos grasos en los casos en los que este control se lleve a cabo por

entidades de inspección no acreditadas, lo que ha motivado el descontento de algunas partes del sector, ya que se considera necesario algún método analítico que permita objetivamente establecer una clasificación correcta.

	De bellota o terminado en montanera	**De recebo o terminado en recebo**	**De cebo de campo**	**De Cebo**
Alimentos consumidos	Bellota, hierba y otros recursos naturales	Bellota e hierba y terminado con piensos	Piensos	Piensos
Estancia durante el cebo	Dehesa	Dehesa	Fase final en campo, mínimo 60 días	
Peso entrada montanera	92-115 kg	92-115 kg	92-115 kg	
Fecha entrada montanera	1 Octubre a 15 de Diciembre	1 Octubre a 15 de Enero		
Reposición en montanera	46 Kg. durante 60 días mínimo	29 Kg. durante 60 días mínimo		
Periodo de sacrificio	15 Diciembre a 15 de Abril	15 Diciembre a 15 de Mayo		
Edad mínima sacrificio	14 meses	14 meses	12 meses	10 meses
Peso canal mínimo al sacrificio	117 kg	117 kg	117 kg	117 kg
Carga ganadera total de la dehesa	2 cerdos/ha	2 cerdos/ha	15 cerdos/ha	

Tabla 1. Requisitos de cada una de las designaciones de calidad de los productos del cerdo Ibérico en función de la alimentación consumida por los animales durante el periodo de cebo (BOE, 2007).

En la actualidad se está realizando un estudio coordinado por el Centro del Cerdo Ibérico a través de un proyecto INIA (RTA 2008-00026-C07-07) para proponer uno o más métodos que complementen al de los ácidos grasos para poder clasificar los productos del cerdo Ibérico en función de la alimentación.

Varios estudios han demostrado que el perfil de ácidos grasos tanto de la grasa subcutánea como de la intramuscular se ve afectado por el cebo de los cerdos Ibéricos con pienso enriquecidos en ácido oleico (Muriel y col., 2002a; Muriel y col., 2002b; Daza y col., 2005; Ventanas y col., 2007; González y col., 2007). Sin embargo, hasta ahora no se ha planteado la viabilidad de usar el procedimiento basado en el análisis de ácidos grasos mayoritarios y

minoritarios para diferenciar cerdos Ibéricos alimentados con pienso alto oleico de aquellos cebados en montanera.

Los niveles de otros componentes de la fracción insaponificable en la dieta, como neofitadieneo y α- y γ-tocoferol, también están íntimamente relacionados con su contenido en los diferentes tejidos animales (Tulliez y col, 1978; Tejeda y col., 1999; Daza y col., 2005; Rey y col., 2006). Además, existen evidencias de que estos compuestos de la fracción insaponificable podrían ser herramientas útiles a la hora de la clasificación (Lopez-Bote y col., 1998; Tejeda y col., 2005).

Como ya se ha comentado anteriormente, la composición en ácidos grasos de los productos del cerdo Ibérico tiene una gran importancia sobre las características sensoriales de fluidez de la grasa, brillo y flavor. Los componentes de la fracción insaponificable, y más concretamente los tocoferoles, también influyen sobre la calidad de estos productos, especialmente en su aroma, debido a su actividad antioxidante (Ruiz y col., 2002).

Se han propuesto otras medidas instrumentales para la clasificación de cerdos Ibéricos con distinta alimentación, como el análisis de isómeros de ácidos grasos (Sánchez González y col., 2007), de isótopos (Recio, 2007), la tecnología de espectroscopía en el infrarrojo cercano (NIR) (de Pedro y col., 2007) así como en técnicas de ultrasonidos (Niñoles y col., 2007) y olfactómetricas (Santos y col., 2004; González-Martín y col., 2000). Y aunque no se han publicado, se conoce que el análisis de los triglicéridos y el desarrollo de un sensor químico son también técnicas planteadas para este fin (Ventanas y col., 2009). El principal inconveniente de la mayoría estas técnicas es que son destructivas.

En este sentido y en relación con la clasificación de los cerdos Ibéricos en función de su alimentación durante la fase de cebo (montanera vs. piensos alto oleico), se está considerando otro tipo de técnicas como la de imágenes de resonancia magnética, que tiene la ventaja de ser no destructiva, no invasiva además de no radiante e inocua. De hecho, en la actualidad se están empleando imágenes de resonancia magnética junto con distintas técnicas de visión por computador para el estudio de distintos productos cárnicos. Mediante este procedimiento se ha conseguido clasificar lomos

Ibéricos en función de la raza (Cernadas y col., 2001), del contenido en grasa intramuscular y de ciertos atributos sensoriales (Antequera y col., 2003). Se han logrado reconocer los músculos bíceps femoral y semimembranoso aplicando imágenes de resonancia magnética junto con otras técnicas computacionales (Contornos Activos) (Caro y col., 2001), así como calcular su contenido en grasa intramuscular (Ávila y col., 2005) y determinar el peso y la cantidad de humedad del jamón en distintas fases del proceso de maduración (Antequera y col., 2007).

1.3. EL JAMÓN IBÉRICO

La producción del jamón Ibérico es especialmente importante en el suroeste de España, donde el cerdo Ibérico se cría en su hábitat natural, la dehesa. El aprovechamiento y la revalorización de estas superficies han permitido la pervivencia de una zona de más de dos millones de hectáreas de gran valor ecológico.

En los últimos años, se ha producido un incremento en la producción de jamones y paletas Ibéricos, debido al aumento de la comercialización en el mercado nacional y también a las exportaciones a países de la Unión Europea, que se iniciaron en el año 2003, y a países terceros, que comenzaron en 2005 (Tabla 2) (M.A.R.M., 2009).

Este crecimiento en la demanda del jamón Ibérico ha ido acompañado de una importante renovación y desarrollo del sector, tanto desde un punto de vista de la producción porcina como del desarrollo tecnológico en la elaboración de los diferentes productos cárnicos. Como consecuencia de esto, se ha ido mejorando la producción de jamones y por lo tanto el rendimiento económico.

	Mercado Nacional	Unión Europea	Países Terceros	Total
Año 2002	250.529	0	0	250.529
Año 2003	299.332	2.100	0	301.432
Año 2004	473.272	0	0	473.272
Año 2005	603.497	3.500	2.000	608.997
Año 2006	643.040	4.800	2.000	649.840

Tabla 2. Número de piezas (jamones y paletas) comercializadas por las Denominaciones de Origen Protegidas de jamón Ibérico (M.A.R.M., 2009).

1.4. EL PROCESADO DEL JAMÓN IBÉRICO

En la actualidad, la elaboración del jamón Ibérico ha adquirido un indiscutible carácter industrial, sin embargo, hasta hace poco tiempo, su elaboración se basaba en los conocimientos adquiridos empíricamente y trasmitidos de padres a hijos.

La elaboración del jamón Ibérico se fundamenta en un aporte de sal y su distribución por la pieza a temperatura de refrigeración y un proceso posterior de desecación. Estas bases pretenden la estabilización inicial de la materia prima y la consecución de cambios físicos y químicos esenciales para conseguir la elevada calidad sensorial del producto. Las condiciones del proceso de elaboración varían de una zona geográfica a otra, como consecuencia de las diferencias climáticas existentes entre ellas. Estas distintas condiciones junto con el intento de particularizar los protocolos de las diferentes zonas han dado lugar a las distintas Denominaciones de Origen Protegidas del jamón Ibérico (D.O.P. Dehesa de Extremadura, D.O.P. Jamón de Guijuelo, D.O.P. Jamón de Jabugo y D.O.P. Jamón de los Pedroches).

De forma general, el proceso de elaboración se basa en cuatro etapas (salado, post-salado, secado y bodega), precedidas de un previo acondicionamiento de las piezas (preparación de los perniles y selección y clasificación de la materia prima).

Preparación de los perniles

Antes de comenzar el procesado de los jamones debe medirse el pH de los perniles. A los 45 minutos tras el sacrificio el pH debe ser superior a 6,2 y a las 24 horas debe oscilar entre 5,6 y 6,2. Por encima y por debajo de estos límites las carnes son defectuosas y no deben ser destinadas al proceso de elaboración del jamón curado. A continuación, las piezas cárnicas que vayan a ser procesadas se someten a las operaciones de perfilado, corte en la piel en forma de "V" a nivel de la articulación tibiofemoral, y sangrado, que consiste en una presión, manual o mecánica, siguiendo la trayectoria de la arteria femoral y la vena safena para extraer restos de sangre, previniendo así putrefacciones internas. Después, los perniles se mantienen en cámaras de refrigeración a 0-3 ºC durante 1-2 días para bajar la temperatura interna del pernil.

Selección y clasificación

Tras la preparación de los perniles, éstos son sometidos a un nuevo proceso de selección. En esta ocasión, además del pH, que debe estar entre 5,6 y 6,2, se tienen en cuanta como criterios de selección la temperatura, no se admitirán perniles que no se hayan mantenido a temperatura de 3-5 ºC o inferiores, y el aspecto general del pernil, eliminando los que presenten fracturas, grietas o hematomas.

Una vez seleccionados los perniles se clasifican por pesos y por el nivel de engrosamiento en lotes similares.

Salazonado

El salazonado de los jamones Ibéricos se lleva a cabo mediante el sistema tradicional de salado en seco, generalmente en pilas de sal, según describen Andrés y col. (2001a). En primer lugar se frota la superficie magra de los jamones con las sales nitrificantes. Seguidamente, los perniles son introducidos en pilas constituidas por capas alternativas de sal, aunque cada vez es más común el empleo de recipientes de acero inoxidable.

Otras técnicas de salazonado empleadas en diferentes tipos de jamón curado podrían representar una alternativa al salazonado tradicional de los jamones Ibéricos. La técnica del aporte limitado de sal, utilizada corrientemente en los jamones de Parma, consiste en depositar sobre la superficie magra del pernil la cantidad justa de sal en función del peso. Los

jamones se sitúan uno al lado del otro en estantes o bandejas (Andrés y col., 2001a). El salado por inmersión en salmuera es una nueva variante de salado, utilizada hasta ahora a nivel de investigación en jamones blancos (Barat y col., 2004) e Ibéricos (Albarracín, 2009).

Durante la etapa de salado se pretende que el pernil adquiera un contenido en sal suficiente para que una vez distribuida por toda la pieza en las etapas posteriores y en combinación con la paulatina deshidratación, se inhiba el desarrollo de microorganismos alterantes y potencialmente patógenos para el consumidor. Asimismo se persigue la consecución de un ligero sabor salado en el producto final y regular la actividad enzimática endógena y las reacciones químicas durante la maduración.

El tiempo de salazón de los jamones Ibéricos se encuentra en torno a 1día por kg de jamón. Las condiciones termohigrométricas durante esta fase son un factor muy importante a tener en cuenta, tanto en la velocidad de penetración de sal en la pieza como en su estabilidad microbiológica. En este sentido, la humedad relativa debe ser alta (>75%), para facilitar la solubilización de la sal y su penetración y difusión en la pieza, y la temperatura debe mantenerse entre 1 y 4 ºC, para evitar el desarrollo de microorganismos.

Una vez acabado el proceso de salazón, se elimina la sal superficial de los perniles mediante lavado y cepillado de las piezas, siendo también cada vez más corriente la mecanización de este proceso mediante frotado mecánico y aspersión de agua templada a presión. Después del lavado, se efectúa un secado rápido a unos 20 ºC durante 12 a 24 horas.

Por último antes de iniciarse la etapa de post-salado, se procede al alimonado de los jamones, que consiste en aplicar masaje a la pieza, manual o mecánicamente, y así darle una forma más cilíndrica y alargada. Este proceso iría encaminado a favorecer la pérdida homogénea de humedad.

Post-salado

En la fase de post-salado, el control de la temperatura y la humedad relativa son especialmente importantes, debiendo mantenerse en torno a 3-6 ºC y 70-80 %, respectivamente, durante 60 a 90 días. Puede finalizarse con un aumento gradual de la temperatura, denominándose este periodo post-salado en caliente.

Durante esta etapa se persigue la estabilización de la pieza mediante la difusión de la sal en el interior de la masa muscular. Paralelamente el jamón se va deshidratando y disminuyendo su actividad de agua (a_w), lo que favorece la inhibición de los microorganismos.

Secadero

En esta fase del procesado, las temperaturas se elevan hasta los 25-30 ºC mientras que la humedad relativa desciende hasta un 60%. En estas condiciones permanecen durante 2 a 5 meses. Esta etapa se desarrolla generalmente en secaderos naturales, aunque actualmente es frecuente la existencia de secaderos artificiales.

El secado de los perniles es esencial para la estabilización total de la pieza, ya que continúa la deshidratación de la misma al ir elevándose la temperatura (Gou y col., 1997). En esta fase también se producen mayoritariamente los cambios que afectan a los componentes de la materia prima: proteolisis, lipolisis y oxidación de lípidos (Antequera y col., 1992; Córdoba y col., 1994).

Maduración en bodega

Durante la última fase del procesado, la bodega, los perniles son sometidos a temperaturas más bajas que en la etapa anterior, oscilando entre 10 y 15 ºC en invierno y entre 15 y 22 ºC en verano cuando se trata de bodegas naturales. La humedad relativa suele mantenerse entre 60-80%. El tiempo de permanencia en bodega depende del peso de los jamones y de la cantidad de grasa que poseen, al dificultar ésta la pérdida de agua, de modo que este periodo puede alargarse de 12 a 24 meses. Al final de la maduración las pérdidas de peso del jamón están normalmente entorno a 30-32% (Ventanas y col., 2001).

En esta etapa continúan los procesos lipolíticos y proteolíticos y la oxidación de lípidos, así como degradaciones de Strecker y reacciones de condensación entre aminoácidos y compuestos carbonilos (García y col., 1991; Ventanas y col., 1992; Ruiz y col., 1998b).

Las reacciones bioquímicas que tienen lugar durante el procesado están relacionadas con la calidad del producto curado (Toldrá y col., 1998).

Las reacciones de lipolisis constituyen el primer paso en la oxidación de los ácidos grasos, los cuales dan lugar a numerosos compuestos volátiles

responsables del flavor en el producto curado (García y col., 1991; Ruiz y col., 1998b; Andrés y col., 2007). Estos fenómenos lipolíticos producen cambios en la composición de ácidos grasos de las diferentes fracciones lipídicas, especialmente en la de fosfolípidos (Andrés y col., 2005). Así, los ácidos grasos liberados durante el procesado del jamón Ibérico proceden principalmente de esta fracción lipídica (Martín y col., 1999; Andrés y col., 2005). Los fosfolípidos también se caracterizan por tener una alta sensibilidad a la oxidación, debido a que poseen un alto porcentaje de ácidos grasos poliinsaturados y a su posición en la fase acuosa en la célula muscular, cercana a los catalizadores de la oxidación lipídica (Ruiz y col., 2009).

Los fenómenos de proteolisis tienen también una gran influencia en la calidad del jamón Ibérico curado, ya que son una fuente importante de compuestos responsables del sabor (aminoácidos libres y pequeños péptidos). Además, los compuestos volátiles procedentes de aminoácidos a través de la reacciones de Maillard y Strecker influyen de forma importante en el flavor del jamón curado (Carrapiso y col., 2002). Las reacciones proteolíticas también afectan a la textura del producto final (Parolari, 1996).

Asimismo, las características físico-químicas del jamón Ibérico influyen sobre la calidad del producto final y están relacionadas con factores dependientes de la materia prima (Andrés y col., 1999; Andrés y col., 2001b) y con el procesado (Ruiz y col., 1998c; Ruiz y col., 1999). Entre las características de apariencia del jamón curado, el color es una de las más destacadas (Gandemer, 2002), influyendo sobre la elección de compra de los consumidores. Las características de textura son también uno de los principales atributos percibidos por los consumidores, y ejercen un gran efecto sobre la calidad del jamón curado (Szczesniak, 2002).

1.5. CONGELACIÓN DE PERNILES IBÉRICOS

Aunque aún no se ha descrito de forma explícita la congelación y posterior descongelación de perniles Ibéricos como fase previa a la etapa de salazonado, cada vez son más los productores de jamón Ibérico que realizan esta práctica. Por otro lado, ninguna de las cuatro D.O.P del jamón Ibérico contempla la congelación-descongelación de los perniles antes del proceso de curación. En concreto, la D.O.P. "Dehesa de Extremadura" especifica que una vez obtenidos los perniles y extremidades anteriores y previamente al proceso de elaboración, éstos se mantendrán de 36 a 48 horas a temperatura de refrigeración (1-4 ºC) (M.A.R.M., 2009). Además, existe la idea generalizada de que la congelación previa al procesado podría afectar negativamente a la calidad del producto final. Hasta ahora no se han encontrado estudios científicos que muestren el efecto de la congelación de los perniles Ibéricos sobre la calidad del producto. Por otro lado, el almacenamiento de los perniles Ibéricos a temperaturas de congelación supondría una serie de ventajas tecnológicas y económicas, como el poder procesar las piezas con un peso más homogéneo y por lo tanto producir jamones con menor variabilidad, evitar la estacionalidad así como los cambios de precio en el mercado, mayor independencia en el ritmo de trabajo de los mataderos y flexibilidad en la producción. Asimismo, el almacenamiento a congelación de los perniles Ibéricos implicaría la necesidad de disponer en la cadena de elaboración de equipos de frío para congelar y/o mantener el producto congelado así como de cámaras de descongelación. También sería necesario establecer tiempos y condiciones de congelación ya que aunque actualmente esta práctica se está empleando no está regulada.

La congelación de la carne y productos cárnicos puede promover modificaciones en la características físicas (pérdidas de humedad, cambios de textura), químicas (lipolisis, oxidación, desnaturalización y agregación proteica, cambios en el color) y sensoriales. La magnitud de estos efectos depende de las características de la materia prima y de las condiciones en que se realice la congelación y posterior descongelación (Carballo y col., 2001).

Entre la literatura científica se han encontrado algunos trabajos que muestran la influencia de la congelación en jamones blancos (Arnau y col., 1994; Motilva y col., 1994; Bañón y col, 1999; Wang, 2001; Flores y col., 2006). Recientemente, Grau y col. (2008) y Flores y col. (2009) han mostrado los primeros estudios sobre la congelación de perniles Ibéricos.

En jamones de cerdo blanco se ha observado que el proceso de congelación y posterior descongelación de los perniles favorece la penetración de sal hacia el interior de la pieza (Bañón y col., 1999; Wang, 2001). Este hecho se ha relacionado con el alto contenido en agua libre en los jamones congelados-descongelados, lo que favorece la solubilización de la sal en la superficie del jamón, que es el factor más determinante en la regulación de la difusión de la sal al interior del pernil (Sorheim y col, 1986). Como consecuencia, se acorta el tiempo de permanencia en salazón de los jamones congelados-descongelados (Poma, 1987; Bañón y col., 1999).

Los estudios llevados a cabo en jamón blanco mostraron que el tratamiento de congelación y descongelación de los perniles no afectaba al color, a las características sensoriales ni a la aceptabilidad del producto final (Motilva y col., 1994; Bañón y col., 1999). Sin embargo, esta práctica acentuaba la proteolisis e incrementaba los niveles de compuestos volátiles a lo largo del procesado de jamones blancos (Bañón y col., 1999; Wang, 2001; Flores y col., 2006). Con respecto a los fenómenos lipolíticos, Motilva y col. (1994) y Flores y col. (2006) encontraron en los jamones congelados-descongelados un mayor contenido en ácidos grasos libres que en los frescos, pero solamente en las etapas iniciales del proceso. También se ha observado un incremento en la incidencia de precipitados blancos, formados principalmente por cristales de tirosina, en los jamones congelados-descongelados (Arnau y col., 1994; Bañón y col., 1999).

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2. Planteamiento y Objetivos

El equipo de investigación de la unidad de Tecnología de los Alimentos de la Universidad de Extremadura ha realizado un gran número de estudios relacionados con los productos del cerdo Ibérico. Se ha estudiado la influencia de diferentes factores (cruce, líneas genéticas, alimentación, procesado) sobre distintos parámetros relacionados con la calidad, analizando principalmente muestras de jamón y de lomo pero también de otras localizaciones como grasa subcutánea, vísceras e incluso distintos músculos de forma individualizada.

En nuestro laboratorio se han venido observando algunos problemas relacionados con la cantidad de grasa extraída de los distintos productos cárnicos analizados, así como gran variabilidad de resultados y cantidad de lípidos totales considerablemente más baja de la esperada.

En relación con los sistemas de clasificación de los productos del cerdo Ibérico en función de la alimentación, y debido sobre todo a la utilización de piensos enriquecidos en ácido oleico, en la actualidad se plantea la necesidad de sistemas de clasificación que sean eficaces. En trabajos anteriores se observaron diferencias significativas en ácidos grasos poliinsaturados de cadena larga entre lomos de cerdo Ibérico cebados en montanera y con pienso alto oleico. Otros estudios han detectado que algunos compuestos de la fracción insaponificable solo se encuentran en jamones que proceden de animales cebados en montanera. Por lo tanto, el perfil completo de ácidos grasos y el contenido en neofitadieno y tocoferoles, así como la utilización de análisis estadísticos basados en técnicas multivariantes empleando los componentes anteriores, podrían ser útiles para clasificar correctamente la muestras de cerdos Ibéricos cebados de forma diferente (montanera y pienso alto oleico).

Muchos estudios llevados a cabo en jamón Ibérico se han centrado en estudiar la influencia de la alimentación en la composición en ácidos grasos de los fosfolípidos, pero no en el contenido ni el perfil de ácidos grasos de cada clase de fosfolípidos. Sin embargo, la determinación de cada clase de fosfolípido así como su composición en ácidos grasos de forma individualizada podría ser reseñable ya que la mayor parte de los ácidos grasos liberados durante la maduración del jamón Ibérico proceden de esta fracción lipídica.

En colaboración con el Departamento de Ingeniería y Sistemas Informáticos y Telemáticos de esta universidad, desde hace algunos años se viene estudiando el uso de imágenes de resonancia magnética junto con técnicas de visión por computador para analizar lomo y jamón Ibérico. En este trabajo también se abordó el estudio de este método no destructivo para intentar diferenciar los productos procedentes de cerdos Ibéricos cebados en montanera y con pienso alto oleico.

Con respecto al proceso de maduración del jamón Ibérico, tradicionalmente se ha considerado que consta de cuatro etapas: salado, post-salado, secadero y bodega. Sin embargo, en las industrias cada vez se practica más la congelación y posterior descongelación de los perniles como etapa previa al salazonado, aplicándose en la mayoría de los casos las mismas pautas que en jamón blanco. Hasta ahora no se han realizado estudios que muestren el efecto de la congelación-descongelación de perniles de cerdo Ibérico sobre las características físico-químicas y sensoriales de este producto. Además, la instauración de este procedimiento en las industrias de jamón Ibérico supondría una serie de ventajas tanto tecnológicas como económicas.

Teniendo en cuenta estos planteamientos, los objetivos de esta tesis doctoral fueron los siguientes:

1. Evaluar la eficacia de distintos métodos de extracción de grasa para cuantificar la cantidad total de lípidos en productos cárnicos, y particularmente en el jamón.
2. Caracterizar jamones Ibéricos frescos y curados procedentes de cerdos cebados con diferente alimentación (montanera vs. piensos alto oleico) mediante el análisis computacional de las imágenes de resonancia magnética, el perfil lipídico y los atributos sensoriales.
3. Estudiar la influencia de la alimentación del cerdo Ibérico (montanera vs. piensos alto oleico) sobre el contenido y la composición en ácidos grasos de las distintas clases de fosfolípidos.
4. Analizar el efecto de la congelación-descongelación de perniles Ibéricos sobre los parámetros de calidad en la materia prima, a lo largo del procesado del jamón y en el producto final.

Taking into account these approaches, the aims of this Doctoral thesis were the following:

1. Evaluating the efficiency of different lipid extraction methods for quantification of total lipid content in different meat products, and specifically in hams.
2. Characterizing fresh and dry-cured Iberian hams from pigs fattened different diets (acorn and grass vs. oleic acid enriched concentrates) by means of Magnetic Resonance Imaging, lipid profile and sensory traits.
3. Studying the effect of Iberian ham feeding (acorn and grass vs. oleic acid enriched concentrates) on the quantity and fatty acid composition of phospholipid classes.
4. Analyzing of the influence of pre-cure freezing Iberian thighs on quality parameters of the raw meat, throughout ham processing and in the final product.

3. Diseño Experimental

Los diferentes capítulos incluidos en la presente Tesis Doctoral se encuadran en dos secciones.

SECCIÓN I

En el capítulo I.1. de esta sección se planteó evaluar la eficacia de distintos métodos de extracción de grasa para cuantificar la cantidad total de lípidos en carne y productos cárnicos. Para ello se emplearon diferentes productos cárnicos, tal y como se indica en el material y métodos de este capítulo.

Para la realización del resto de los capítulos de esta sección se partió de 50 cerdos Ibéricos, divididos en dos lotes en función de la alimentación recibida durante el periodo de cebo. Uno de los grupos (AG) fue cebado en montanera, en una explotación de 30 ha, a base de bellota y de hierba. El otro grupo de animales (HO) se cebó en una estancia de 1 ha y con libre acceso a la hierba, pero siendo su principal alimento un pienso enriquecido en ácido oleico. Así, y de acuerdo con la Norma de Calidad de 2007, el grupo de cerdos AG sería clasificado como "de bellota o terminado en montanera" y el HO como "de cebo de campo".

Tras un periodo de cebo de 110 días, todos los cerdos fueron sacrificados con un peso medio de 160 kg cada uno. En el momento del despiece, se realizó la toma de muestras de la grasa subcutánea (en la zona de la rabadilla), de acuerdo a la normativa entonces vigente (ORDEN PRE/3844/2004 de 18 de Noviembre), para analizar su composición lipídica. Además se tomó el pernil izquierdo de cada animal.

De los 25 perniles de cada lote, 15 fueron analizados en fresco y los 10 restantes fueron procesados en una industria de elaboración de jamones siguiéndose las condiciones del procesado tradicional del jamón Ibérico. Tras el perfilado y el sangrado de los jamones, éstos fueron salados en pilas a razón de 1 día/kg, a 3 ºC y 80% de humedad relativa. La etapa de post-salado duró 80 días, a 4º ± 1 ºC y 75% de humedad relativa. En la siguiente etapa, secadero, que duró 130 días, la temperatura aumentó hasta los 28 ºC y la humedad relativa fue disminuyendo hasta un 50%. Finalmente, los jamones

pasaron a la etapa de bodega, con temperaturas y humedad relativa entre 10-25 ºC y 65-80%, respectivamente, durante 14 meses.

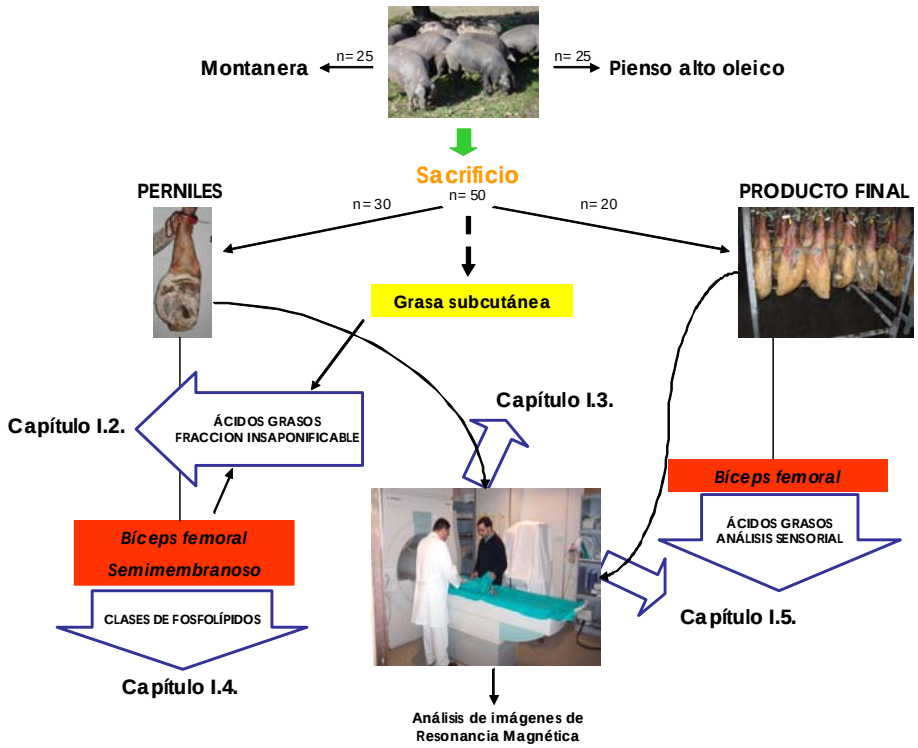
A todos los perniles se le realizó una resonancia magnética, y posteriormente se disecaron los músculos bíceps femoral y semimembranoso para analizar su composición lipídica.

A los jamones curados también se les realizó una resonancia magnética y seguidamente se disecó el músculo bíceps femoral para determinar su perfil de ácidos grasos y realizar el análisis sensorial.

Las imágenes de resonancia magnética fueron analizadas mediante una aplicación informática diseñada en el Departamento de Ingeniería y Sistemas Informáticos y Telemáticos de la Universidad de Extremadura.

Para la realización de los trabajos I.2., I.3. y I.4. se empelaron los datos obtenidos de los jamones frescos mientras que el capítulo I.5. recoge los resultados de los análisis realizados sobre los jamones curados.

Figura 1. Diseño experimental de la sección I.



SECCIÓN II

Los capítulos de la sección II se realizaron a partir de 24 perniles procedentes de cerdos Ibéricos de cebo. Estos jamones frescos fueron divididos en dos grupos en función del tratamiento recibido antes de comenzar el proceso de maduración. Un grupo de jamones (F) se congeló a una temperatura de -20 ºC durante tres meses. El proceso de descongelación se realizó a 3-4 ºC durante 4 días. El otro grupo de jamones (R) fue obtenido 2 días antes de la descongelación de los jamones F, y se mantuvo a 4 ªC durante 36-48 horas hasta que comenzó su proceso de maduración. Estos jamones R procedían de cerdos Ibéricos con las mismas características genéticas y de alimentación que los cerdos de los que se obtuvieron los jamones F.

De cada lote de 12 jamones, 6 se utilizaron para realizar el análisis de la materia prima mientras que el resto fue procesado para la obtención de jamones Ibérico curados. El proceso de maduración se llevó a cabo en una industria cárnica, siendo las condiciones de procesado las mismas para los dos grupos de jamones, excepto el tiempo de permanencia en sal, que fue 1 día por kg de jamón para los jamones R y 0.7 día por kg de jamón para los F. Después de la etapa de salado los jamones se mantuvieron durante 70 días a 4-8 ºC y 73-75 % humedad (etapa de post-salado). La etapa de secadero se realizó en condiciones controladas y duró 120 días, en los cuales la temperatura se fue aumentando de 8 a 20 ºC mientras que la humedad relativa fue progresivamente disminuyendo hasta un 65%. Finalmente, durante la etapa de bodega, los jamones se mantuvieron a 20±2 ºC y 55-65% humedad relativa durante 16 meses.

La toma de muestras se realizó al inicio del procesado (materia fresca), al final de cada una de las etapas del proceso (secadero, post-salado y bodega) y al final del proceso de curación (producto final). Las muestras de jamón obtenidas al final de las etapas de salado, post-salado y secadero fueron tomadas con un sacabocados, de dimensiones 10 x 2.5 cm (Figura 2). Estas muestras incluían principalmente el músculo bíceps femoral. Para la obtención de las muestras correspondientes a la materia prima y al producto

final se disecó el músculo bíceps femoral de cada jamón. Todas la muestras fueron envasadas a vacío y congeladas a -80 ºC hasta su posterior análisis.

La Figura 3. ilustra las determinaciones realizadas en cada toma de muestras y su estructuración en los diferentes capítulos de esta sección.

Figura 2. Toma de muestras con el sacabocados.

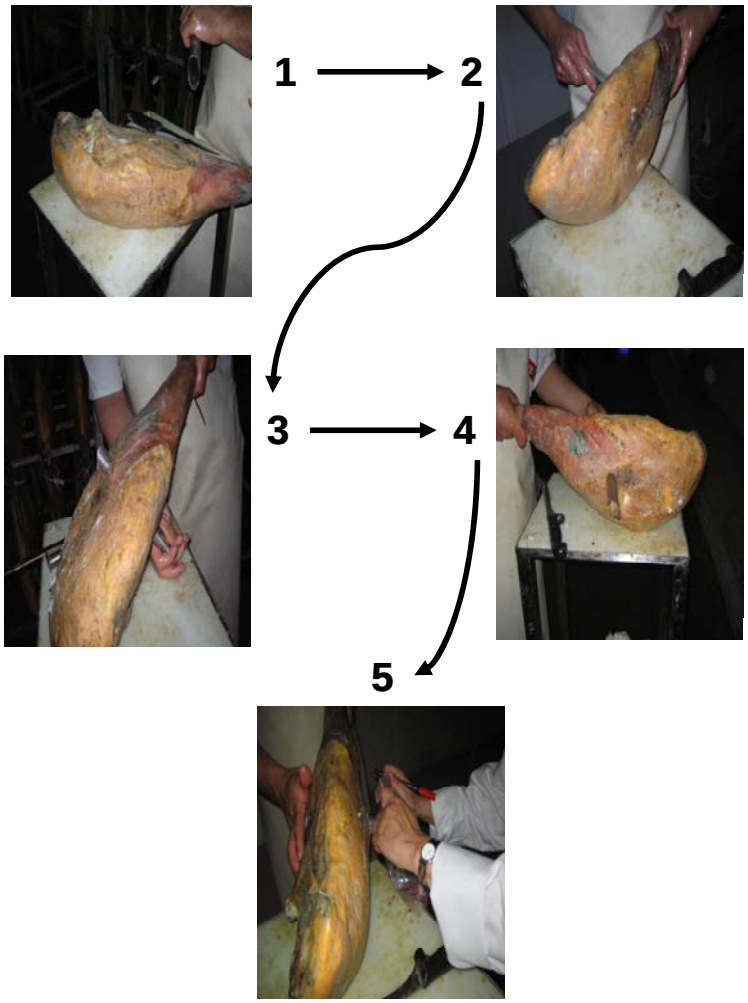
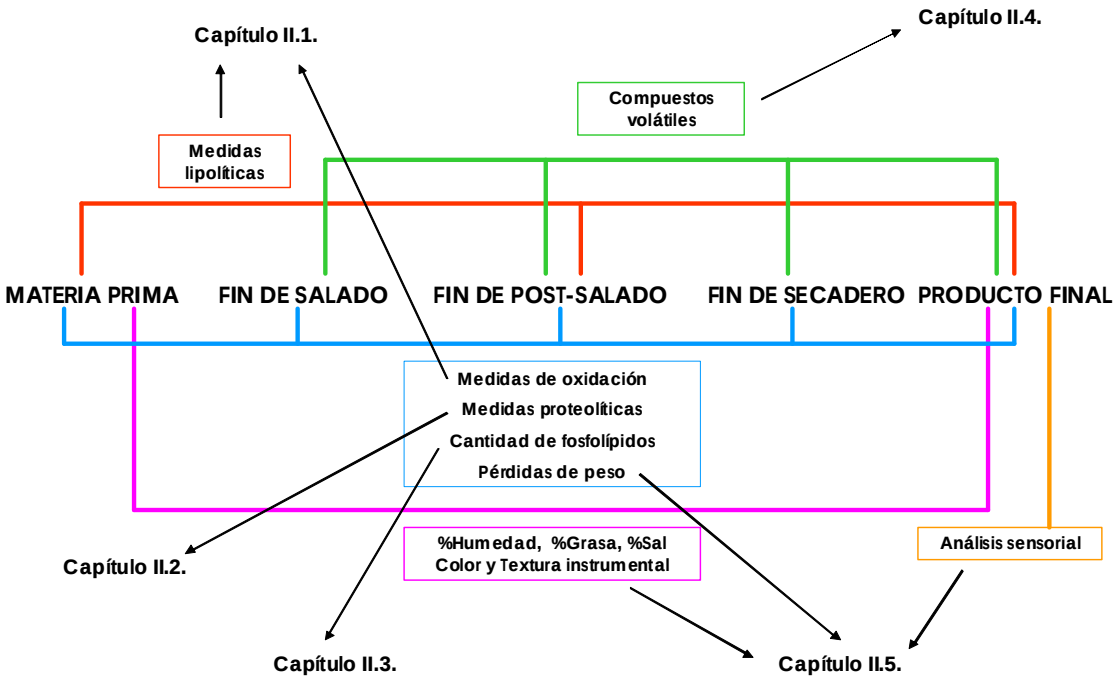


Figura 3. Determinaciones realizadas en las diferentes etapas del procesado de los jamones Ibéricos de la sección II.



4. Capítulos

Capítulo I.1.

Comparison of different methods for total lipid quantification in meat and meat products

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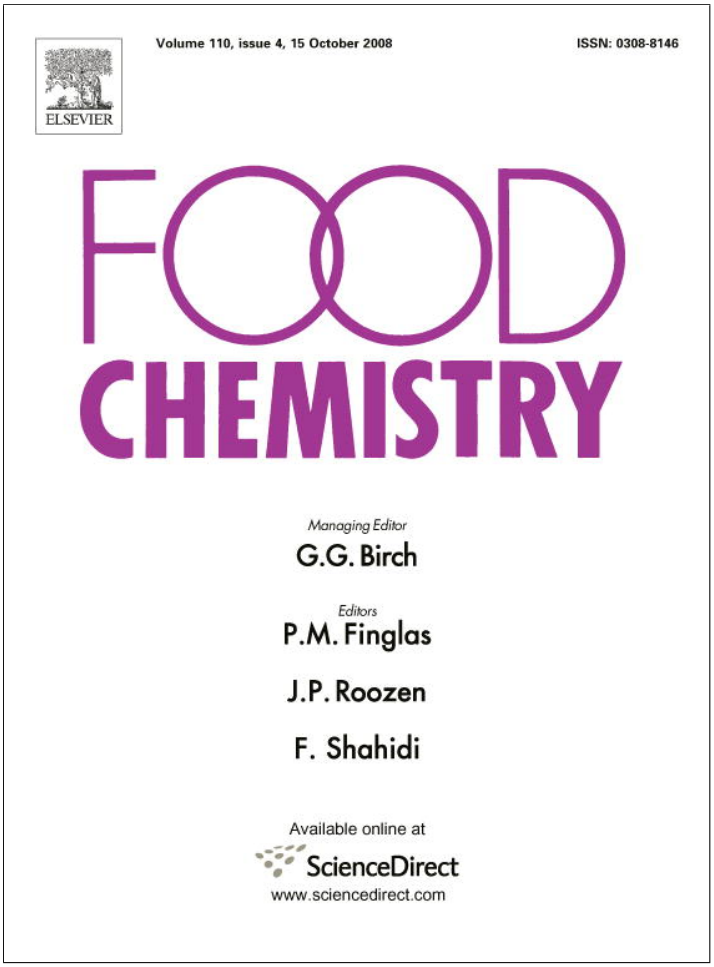

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Analytical Methods

Comparison of different methods for total lipid quantification in meat and meat products

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ABSTRACT

This study was aimed to evaluate the efficiency of six extraction methods for the quantification of total lipid content in meat and meat products: standard Soxhlet method (with and without previous acid hydrolysis), continuous Soxhlet method (with and without previous acid hydrolysis), and those methods based in the use of a mixture of chloroform and methanol, and described by Folch, Less, and Sloane (1957) and Bligh and Dyer (1959). Lipid content was determined in nine different meat products with different fat contents and physico-chemical features: cooked turkey breast, fresh pork loin, cooked ham, dry-cured ham, mortadella, beef burger, fresh sausage, dry-cured sausage and salami. The most effective methods for determining fat content in the studied meat products were the method described by Folch et al. (1957) and the Soxhlet with previous acid hydrolysis method. The Soxhlet method without previous acid hydrolysis adequately extracted lipids only in those meat products with very high fat content. The use of the method described by Bligh and Dyer (1959) gave rise to the lowest lipid contents in all the studied meat products.

1. Introduction

The total lipid content of meat and meat products is an important quantity used in numerous studies. Thus, reliable methods for the quantitative extraction of lipids from this type of foodstuffs are of critical importance (Iverson, Lang, & Cooper, 2001). Lipids in muscle foods are a mixture of nonpolar components (mainly acylglycerides and cholesterol), free fatty acids, and more polar lipids, such as phospholipids or sphingolipids (Ruiz, Antequera, Andres, Petron, & Muriel, 2004). Chemical and physical treatments used for lipid extraction must remove them from their binding sites with cell membranes, lipoproteins and glycolipids. Moreover, the solvents used for extracting fat should have a high solubility for all lipid compounds and be sufficiently polar (Smedes & Askland, 1999).

Several methods have been developed for total lipid extraction, the most used in meat and meat products being the Soxhlet method (SOX), which is the official AOAC-recommended method (Association of Analytical Chemists, 1990) and those based in the use of a mixture of chloroform and methanol, and described by Folch et al. (1957) (FOL) and Bligh and Dyer (1959) (B&D). Moreover, new Soxhlet extraction systems offer different modes of extraction which could improve the extraction procedure, reducing the extraction time and the solvent volume.

Since the B&D and FOL methods were published, there have undoubtedly been several modifications to both procedures in order to improve their lipid recovery efficiency in meat and meat products. However, in many publications where these methods have been used, modifications have been neither described nor validated. In other cases, researchers stated that lipids were quantified "according to" one or the other method, but they did not indicate whether any modifications were made implying that the methods were applied basically according to the original procedures (Iverson et al., 2001).

It has been demonstrated that the use of different methods results in different lipid recoveries in biological samples. Indeed, results varied widely due to differences in extraction methodology, as confirmed by an intercomparative study (Baily, Wells, de Boer, & Delbeke, 1994). However, most used lipid extraction methods for muscle foods have been scarcely compared for different types of meat products. In addition, depending on the study, different results have been reported. Thus, some authors have found a better extraction of total lipids with the SOX method than using the B&D in chicken (Manirakiza, Covaci, & Schepens, 2001), while others have found the opposite result studying the fat content of fish muscle (Ewald, Bremle, & Karlsson, 1998), and other researchers found no differences between these two methods when analysing the total lipid content of pork loin (Ragland, Christian, & Baas, 1996). Iverson et al. (2001) compared the B&D and the FOL methods for extracting fat from marine tissue with different fat contents. Their results showed that, for samples containing more

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than 2% lipids, the B&D method gave rise to significant lower lipid contents than the FOL method, and this underestimation was greater with increasing lipid content of the sample.

Nowadays, lots of meat products with different fat contents and different physical and chemical features (protein network, moisture content, ingredients, additives and so on) are being manufactured. The quantification of total lipids and its characterization is a basic requirement for testing these products (Manirakiza et al., 2001). In order to choose the most appropriate method for extracting lipids in these products, it should be considered that the efficiency of the extraction procedure could be influenced by the nature of the product matrix (Christie, 1993; Young, Frost, West, & Braggins, 2001). In the scientific literature, both the B&D and the FOL methods have been extensively used for lipid quantification in meat and meat products. However, there are not established criteria for choosing the most appropriate one.

The present study was aimed to evaluate the efficiency of different lipid extraction methods (standard Soxhlet with and without previous acid hydrolysis, continuous Soxhlet with and without previous acid hydrolysis, extraction with a chloroform:methanol mixture following the methods described by Bligh and Dyer (1959) and Folch et al. (1957)) for quantification of total lipid content in nine meat products differing in their fat contents and physico-chemical features.

2. Materials and methods

2.1. Sampling

Nine meat products with different composition, processing technology and fat contents were studied in this work. Products were grouped in terms of fat content: low fat content group (1–5%) (cooked turkey breast, fresh pork loin, cooked ham); intermediate fat content group (5–15%) (dry-cured ham, mortadella, beef burger); high fat content group (15–30%) (fresh sausage, dry-cured sausage); very high fat content group (30–50%) (salami). All products were purchased in a local supermarket. Reference values for lipid content of these nine products were provided by an authorized official laboratory (Official Laboratory for Analysis of Agricultural, Foods and Residues of Extremadura, register number 10-006) and by the label provided by the manufacturer in the product. Table 1 shows reference values provided by the label, which were in agreement with those provided by the official laboratory. Each meat product (300 g) were ground using a commercial grinder and stored at −80 °C until analysis. Homogeneous samples (5 g) were taken for fat extraction.

2.2. Chemicals

All solvents used in this study were of analytical grade and obtained from Scharlau (Barcelona, Spain) or Panreac (Barcelona, Spain).

2.3. Methods

Six different lipid extraction methods were followed: standard SOX (ST-SOX) (with and without previous acid hydrolysis), continuous SOX (CN-SOX) (with and without previous acid hydrolysis), B&D (Bligh & Dyer, 1959) and FOL (Folch et al., 1957). The weight of the sample was 5 g for the six extraction methods. Six replicates for each meat product were performed in all the tested methods.

2.4. Soxhlet

Standard and continuous SOX extraction methods (with and without previous acid hydrolysis) were performed on a Büchi Universal Extraction System B-811 (Flawil, Switzerland). The ST-SOX method keeps the sample in contact with the solvent for a longer time, while the CN-SOX method implies a higher number of sample washings with the solvent. The used solvent was petroleum ether, fraction 40–60 °C, and the parameters for extraction were those recommended by Büchi laboratories (BÜCHI Labortechnik AG 1998, Flawil, Switzerland). The initial volume of solvent was 120 ml, being necessary to add a little more (40–80 ml) during the extraction process. Extraction was performed for 2 h.

Acid hydrolysis was performed on a Büchi Hydrolysis Unit B-411 (Flawil, Switzerland). Samples (5 g) were boiled in 3 N hydrochloric acid (100 ml) with 5 g of celite during 45 min. Thereafter, the mixture was filtered through celite (5 g) and quartz sand (50 g). The filter (celite and quartz sand) with the retained sample was subsequently washed with 250 ml of water (40 °C). In this way, lipids were retained by the filter. Finally, the glass vessels with the wet celite and quartz sand residue, which contained the lipids, were dried in a conventional oven (100 °C for 6 h).

In both SOX with previous acid hydrolysis methods, the dried glass vessels were placed in the extraction system and lipids were extracted from the celite and quartz sand residue. Both in the ST-SOX and CN-SOX methods without previous acid hydrolysis methods, samples (5 g) were wrapped inside paper envelopes, which

Table 1
Total lipid content (%) in nine different meat products as obtained by six different lipid extraction methods[A]

Fat content	Food	Methods						p	Reference value[H] (%)
		ST-SOX[B]	Hyd ST-SOX[C]	CN-SOX[D]	Hyd CN-SOX[E]	B&D[F]	FOL[G]		
Low	Cooked turkey breast	$0.61^{bc} \pm 0.01$	$0.84^{b} \pm 0.04$	$0.44^{c} \pm 0.01$	$0.78^{bc} \pm 0.03$	$0.67^{bc} \pm 0.04$	$1.38^{a} \pm 0.01$	<0.001	1
	Fresh pork loin	$1.27^{c} \pm 0.02$	$3.21^{a} \pm 0.05$	$1.44^{bc} \pm 0.13$	$3.26^{a} \pm 0.01$	$1.65^{b} \pm 0.01$	$3.41^{a} \pm 0.07$	<0.001	3
	Cooked ham	$1.8^{d} \pm 0.03$	$2.91^{b} \pm 0.01$	$2.33^{c} \pm 0.01$	$2.85^{b} \pm 0.06$	$1.58^{e} \pm 0.06$	$3.91^{a} \pm 0.06$	<0.001	3.5
Intermediate	Dry-cured ham	$4.16^{bc} \pm 0.27$	$6.27^{a} \pm 0.05$	$4.39^{bc} \pm 0.25$	$5.88^{a} \pm 0.22$	$3.29^{c} \pm 0.33$	$5.36^{ab} \pm 0.35$	<0.001	6.4
	Mortadella	$7.72^{b} \pm 0.14$	$10.17^{a} \pm 0.05$	$7.22^{b} \pm 0.04$	$7.95^{b} \pm 0.13$	$6.02^{c} \pm 0.25$	$9.66^{a} \pm 0.23$	<0.001	9.5
	Beef burger	$4.8^{c} \pm 0.27$	$10.27^{a} \pm 0.18$	$4.54^{c} \pm 0.25$	$10.33^{a} \pm 0.56$	$2.51^{d} \pm 0.18$	$8.67^{b} \pm 0.32$	<0.001	10
High	Fresh sausage	$9.89^{b} \pm 0.64$	$20.16^{a} \pm 0.53$	$8.15^{bc} \pm 0.43$	$19.64^{a} \pm 0.20$	$6.33^{c} \pm 0.30$	$19.62^{a} \pm 0.64$	<0.001	19.5
	Dry-cured sausage	$20^{a} \pm 0.53$	$22.56^{a} \pm 0.81$	$21.4^{a} \pm 0.28$	$20.62^{a} \pm 0.81$	$13.51^{b} \pm 0.73$	$22.84^{a} \pm 0.24$	<0.001	25
Very high	Salami	$49.82^{a} \pm 0.25$	$41.67^{b} \pm 1.32$	$49.8^{a} \pm 0.50$	$45.48^{b} \pm 0.77$	$33.25^{c} \pm 0.86$	$49.39^{a} \pm 0.72$	<0.001	48.6

[A] Mean values ± standard error of the mean. Means with different superscripts differ significantly ($p < 0.05$).
[B] Soxhlet standard without hydrolysis.
[C] Soxhlet santandar with hydrolysis.
[D] Soxhlet continuous without hydrolysis.
[E] Soxhlet continuous with hydrolysis.
[F] Bligh and Dyer.
[G] Folch.
[H] Reference values provided by the label of the product.

were subsequently placed into the extraction system in order to directly extract lipids from the samples. The extracted lipids were collected in beakers. Lipid content was then determined gravimetrically after total solvent evaporation.

2.5. Bligh and Dyer method

Extractions following the B&D method were performed as originally outlined by Bligh and Dyer (1959). Briefly, 5 g of sample were mixed with 15 ml of chloroform:methanol (1:2, v/v). The mixture was homogenized for 2 min in a Sorvall Omnimixer homogenizer, centrifuged (10 min, 3000 rpm) and filtered. The residue was rehomogenized with 5 ml of chloroform, centrifuged (10 min, 3000 rpm), filtered and collected together with the previous filtrate. This filtrate was mixed with 5 ml of distilled water and shaken vigorously. The final biphasic system was allowed to separate by centrifugation (10 min, 3000 rpm). The upper aqueous phase was eliminated. The lower chloroformic phase was filtered through anhydrous sodium sulphate and collected. Lipid content was then determined gravimetrically after chloroform was evaporated using a rotary evaporator under vacuum followed by further drying under nitrogen.

2.6. Folch method

Lipid extractions following the Folch et al. (1957) were performed using the original extraction ratio of 20 parts chloroform:methanol (2:1, v/v) to 1 part sample. Briefly, 5 g of sample were mixed with 100 ml of chloroform:methanol (2:1, v/v). The mixture was homogenized, centrifuged (10 min, 3000 rpm) and filtered. Subsequently, 5 ml of distilled water was added to the filtrate and the new mixture was shaken vigorously. The final byphasic system was allowed to separate by centrifugation (10 min, 3000 rpm). The upper aqueous phase was eliminated. The lower chloroformic phase was filtered through anhydrous sodium sulphate and collected. Lipid content was then gravimetrically determined after chloroform was evaporated with a rotary evaporator under vacuum and the solvent was further evaporated under nitrogen.

2.7. Statistical analysis

Differences between lipid content within each meat product by the six studied methods were compared by one way ANOVA using the General Linear Model of SPSS (v.12.0). When a significant effect ($p < 0.05$) was detected, the comparative analyses were conducted using a Tukey test.

3. Results

The six extraction methods significantly differed ($p < 0.001$) in total extracted lipids for the nine analysed meat products (Table 1). For almost all the products, the methods that extracted a higher amount of lipids were the FOL, the CN-SOX and the ST-SOX with previous acid hydrolysis, followed by the CN-SOX and the ST-SOX without acid hydrolysis, and the B&D method showing the lowest levels of total extracted lipids.

The FOL and the SOX with previous acid hydrolysis methods differed in total extracted lipids for some products. In cooked turkey breast, cooked ham and salami, the FOL method extracted significantly ($p < 0.05$) more lipids (1.38%, 3.41% and 49.39%, respectively) than the CN-SOX (0.78%, 2.85% and 45.48%, respectively) and the ST-SOX (0.84%, 2.91% and 41.67%, respectively) with previous acid hydrolysis methods. However, in beef burger, the total lipid content using the FOL method (8.67%) was lower ($p < 0.05$) than that obtained using the CN-SOX (10.33%) and ST-SOX (10.27%) methods with previous acid hydrolysis.

Both SOX methods without previous acid hydrolysis extracted less total lipids than the FOL and the SOX methods with previous acid hydrolysis in most analysed meat products. However, in salami, both the CN-SOX and the ST-SOX methods without acid hydrolysis and the FOL method extracted a higher amount of lipids (49.80%, 49.82% and 49.39%, respectively) than the CN-SOX and the ST-SOX methods with previous acid hydrolysis (45.48% and 41.67%, respectively). In dry-cured sausage, these five methods gave rise to similar lipid contents (22.84%, 20.62%, 22.56%, 21.4% and 20.00% for the FOL, CN-SOX and ST-SOX with and without previous acid hydrolysis methods, respectively).

Differences between the CN-SOX and the ST-SOX methods were only found in two meat products. In cooked ham, the CN-SOX method without previous acid hydrolysis extracted more lipids than the ST-SOX method without previous acid hydrolysis (2.33% *vs* 1.80%). In mortadella, the lipid content obtained when extracting with the ST-SOX method with previous acid hydrolysis was lower than that obtained with the CN-SOX method with previous acid hydrolysis (7.95% *vs* 10.17%).

The B&D method extracted less lipids than any of the other five methods in all the considered meat products except for fresh pork loin, in which the ST-SOX method without previous acid hydrolysis, gave rise to a lower ($p < 0.05$) total lipid content than the B&D method (1.65% *vs* 1.27%).

4. Discussion

There is little scientific information about the more convenient method for lipid extraction in each type of meat product. This is important, since features such as fat and moisture content, the nature of the protein network, the presence of several additives, the technological process or the physical and chemical interactions between lipids and proteins, could influence the performance of each lipid extraction method. Taking the previous discussion into consideration, the CN-SOX and ST-SOX methods with and without previous acid hydrolysis, and the methods described by Folch et al. (1957) and Bligh and Dyer (1959) were carried out for determination of total lipid content in nine meat products whit different features and fat content.

The studied methods which extracted the highest amount of total lipids in the nine meat products were the FOL and the CN-SOX and ST-SOX methods with previous acid hydrolysis. However, some differences were found between these methods which could be related to the features and fat content of the studied meat products, or even to the sample weight. In two products of the low fat content group (cooked turkey breast and cooked ham) the FOL method extracted a higher amount of lipids than both SOX with previous acid hydrolysis methods, while these latter methods gave rise to a similar fat content in the other meat product of this group (fresh pork loin). All these products are whole muscle meat products, in which the muscle structure, including the integrity of muscle fibres, of connective tissue and of other tissues included in meat (such as vascular, nervous and lymph), have not suffered a disruption due to a grinding step. Due to this, the strong links between fat and proteins are not destroyed during elaboration of these products, leading to a more difficult extraction of those lipids strongly linked to proteins, such as some membrane lipids. Both, acid hydrolysis or the use of solvents with different polarities, are aimed to solve this problem. However, it seems that acid hydrolysis was not effective enough in two of these products. The manufacture of cooked turkey breast and cooked ham involves a cooking phase at temperatures usually above 70–72 °C. This leads to protein denature, forming a protein gel network and insoluble

aggregates. These physical modifications could explain the decrease in extraction when using the two SOX methods with previous acid hydrolysis.

The total lipid content of salami using the FOL method was also higher than with both SOX methods with previous acid hydrolysis. Most of the fat content of this product is added lard, and thus, it is easily extracted. The lower amount of lipids extracted with both SOX methods with previous acid hydrolysis could be partly due to the inefficacy of the celite and quartz sand to retain the high amount of fat of this product. In fact, both SOX methods without previous acid hydrolysis were able to extract a similar amount of fat to that obtained with the FOL method, the only difference with the SOX methods with previous acid hydrolysis being the acid digestion and the retention of the digested sample in the celite and quartz sand steps. In this sense, it would be recommendable to adjust sample weights when performing the SOX method with previous acid hydrolysis.

Strangely, a lower amount of total lipids in beef burger was obtained when using the FOL method than using any of the SOX methods with previous acid hydrolysis. This result was not in agreement with those obtained for the other studied meat products. This might be partially due to the ingredients used for burger production. Some of these ingredients, such as carbohydrates and non-meat protein materials, are aimed to improve the connection between the particles of meat obtained after grinding by forming a network. It could be that some of these ingredients could have impaired fat extraction by increasing the strength of the links between the lipids and the rest of the components of the burger, or by physically making the fat less accessible to solvents.

There was a general trend towards higher lipid extraction by the SOX methods when a previous acid hydrolysis was carried out, except for the two products with the highest fat content (dry-cured sausage and salami) (Table 1). In fact, when carrying out the SOX extraction without previous acid hydrolysis, the total lipid content obtained was considerably far from that of the reference method for most meat products. As explained before, the strong links between some lipid components and proteins in muscle foods is the main cause explaining the lower extraction of lipids by the SOX methods without previous acid hydrolysis. On the other hand, the lower amount of lipids determined in salami when performing the SOX method with previous acid hydrolysis could be related to the insufficient capacity of celite and quartz sand to retain such a high amount of lipids, as it has been previously explained. A similar behaviour was observed for dry-cured sausage, which was the product with the second highest fat content (25% fat), although not to the same extent as in salami. This fact strengthens the need of lower sample weights when performing SOX extractions with acid hydrolysis of high fat content muscle foods.

Mortadella was the only product in which differences in the amount of total lipids obtained between the ST-SOX and the CN-SOX methods with previous acid hydrolysis were detected. In addition, the total lipid content of cooked ham obtained by the ST-SOX and the CN-SOX methods without previous acid hydrolysis was also significantly different. Therefore, it seems that both modes of SOX extraction are perfectly valid for lipid extraction in most meat and meat products. However, given that the CN-SOX method needs lower solvent volumes, it might be recommended the use of such method due to environmental and economic reasons.

For all the analysed products, the B&D method showed the lowest amount of extracted lipids, except for those products containig 1–3% fat, in which the obtained amount was lower for the SOX methods without previous acid hydrolysis of the sample. Other authors have also found a worse extraction with the B&D method as compared to the SOX method (Brooks, Ratnayake, Lampi, & Hollywood, 1998). Moreover, Iverson et al. (2001) studying the total lipid content in muscles from fish with different fat contents, have observed differences in total lipid extraction between the FOL and the B&D methods. Indeed, these authors found similar results with both methods in samples below 2% fat content, but increased underestimation of fat content with the B&D method with increasing fat levels, in a similar trend to the one found in our study.

The B&D method is commonly used for extracting total lipids from many different foodstuffs. However, the original B&D method was initially developed for fish samples with lipid contents below 1%, water contents around 80% and a high level of phospholipids. Although the authors (Bligh & Dyer, 1959) stated that their method could be applied to other biological tissues, they advised that samples with a high fat content may require modifications of the method. Even in samples containing 2–10% lipids (which is common for many marine fish and invertebrates), underestimation will still be a significant problem, and this has likely been neglected. Both, in whole animals and in specific tissues, an increase in total lipid content is mainly due to an increase in the triacylglycerol content. The reduced efficiency of the B&D method could be related to the limited solubility of the predominantly non polar lipids, such as triacylglycerols, in the relatively polar solvent solution (chloroform:methanol 1:2 v/v) employed in this method.

Smedes and Thomansen (1996) found that the absorption of the organic phase by the tissue was one of the main causes of incomplete lipid yield when extracting lipids with chloroform:methanol. Relatively constant amounts of the organic phase are absorbed by the tissue; therefore, using greater volumes of organic phase solvents proportionally reduces the fraction of the organic phase that is lost in this way. Thus, investigators which have found reliable results with the B&D method might have modified the extraction procedure using an increased solvent/sample ratio. In this sense, it is important that researchers specify modifications of the procedures, especially the precise solvent/sample ratio used.

Taking the results obtained in this study into consideration, the FOL and the SOX methods with previous acid hydrolysis could be recommended to extract the total lipid content in meat products. Although the SOX methods take shorter analysis time and they are less laborious than the FOL method, when the lipid fraction must be further characterized after extraction, the former methods should not be used, because exposure to heat and acid hydrolysis promote lipid oxidation, phospholipid hydrolysis and other chemical lipid modifications. Therefore, for lipid characterization purposes, the FOL method should be chosen. In order to extract the total lipid content in meat and meat products using the B&D method, the original solvent/sample ratio (3:1) should be optimize.

5. Conclusion

Among the studied methods for total lipid quantification in meat and meat products, the method described by Folch et al. (1957) is suitable for meat and meat products with low, intermediate, high and very high lipid content. The Soxhlet method with previous acid hydrolysis is also proper for lipid quantification in this kind of products, except for meat products with very high lipid content. In this case, the Soxhlet without previous acid hydrolysis should be chosen.

The highly used lipid extraction method described by Bligh and Dyer (1959) underestimate total lipid content in most meat and meat products, being necessary a correct adjustment of the solvent to sample ratio for each meat product.

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Capítulo I.2.

Subcutaneous and intramuscular lipid traits as tools for classifying Iberian pigs as a function of their feeding background

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Subcutaneous and intramuscular lipid traits as tools for classifying Iberian pigs as a function of their feeding background

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ABSTRACT

The present work was aimed to study the feasibility of classifying Iberian pigs fattened with two different diets, acorns and grass (AG) and oleic acid enriched concentrate (HO), by means of comparing the fatty acid profile and the content of different compounds of the unsaponifiable lipid fraction (neophytadiene and α- and γ-tocopherol) of subcutaneous (SCF) and intramuscular (IMF) fat of *Biceps femoris* and *Semimembranosus* muscles. The proportions of palmitic (C16:0), stearic (C18:0) and linoleic (C18:2 *n*-6) acids of the three studied tissues did not show a consistent behaviour as influenced by the feeding. The proportion of oleic acid (C18:1 *n*-9) was significantly affected by the diet in SCF and IMF of *Semimembranosus* muscle but not in IMF of *B. femoris*. Some minor fatty acids, such as arachidonic (C20:4 *n*-6) and linolenic (C18:3 *n*-3) acids, showed the best ability for the classification of the animals according to their feeding background and the obtained values showed significant differences caused by the diet in the three studied tissues. Neophytadiene and γ-tocopherol contents were statistically higher in AG than in HO pigs, while there were not differences in the α-tocopherol levels between experimental groups. Thus, it seems that procedures based on the quantification of arachidonic (C20:4 *n*-6) and linoleic (C18:3 *n*-3) acids as well as neophytadiene and γ-tocopherol levels would be useful to differentiate Iberian pigs fattened outdoors on acorns and grass from those fed MUFA enriched diets.

1. Introduction

The high quality of meat and meat products from Iberian pigs is the consequence of several factors, including genetics, crossbreeding, rearing system and processing conditions. Within the factors included under the rearing system, feeding seems to be the key one influencing Iberian products quality (Carrapiso, Bonilla, & Garcia, 2003; Cava, Ventanas, Ruiz, Andrés, & Antequera, 2000). In order to avoid commercial fraud and to guarantee consumers rights, the Spanish Ministry of Agriculture, Fishery and Food enacted a law to regulate the market of Iberian meat products (Boletín Oficial del Estado, 2007). This regulation establishes, as a function of the feeding background during the fattening period of the pigs, four commercial categories for Iberian dry-cured meat products, including pigs reared outdoors with free access to natural resources (acorns and grass), animals fed on acorns and grass but supplemented with concentrates, Iberian pigs fed outdoors with concentrates, or animals reared in confinement and fed commercial diets. Iberian meat products from animals fed outdoors on natural resources reach the highest prices in the market because of its quality characteristics, which are mainly attributed to the outdoor rearing, which implies the consumption of acorns and grass (Rey, López-Bote, & Sanz Arias, 1997). Moreover, traditional products of high quality such as those obtained from free-range reared animal are highly demanded (Kernmeyer, 1993). In fact, Iberian ham is not only commercialised in Spain, but also exported to Europe, Japan and USA. On the other hand, the free-range rearing is a sustainable production system, increasing the value and preserving the production areas, which have high ecological value.

Acorns and grass production is seasonal and restricted, and given that demand for Iberian pig products is quite high, farmers have to use concentrates to feed Iberian pigs to fulfil market requirements, which on the other hand implies lower quality and market acceptance (Carrapiso et al., 2003; Cava et al., 2000; Garcia et al., 1991; López et al., 1992). Currently, monounsaturated fatty acids (MUFA) enriched diets, through the inclusion of high oleic acid (C18:1 *n*-9) sunflower oil, are being used to fed Iberian pigs in order to imitate the FA profile of those animals fattened on acorns.

Rearing conditions during fattening strongly affect the FA profile of pig tissues (Petrón, Muriel, Pérez-Palacios, & Antequera, 2005; Rey, Daza, López-Carrasco, & López-Bote, 2006; Ruiz et al., 1998; Tejeda, Gandemer, Antequera, Viau, & Garcia, 2002), which

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in turn is related to the quality characteristics of Iberian pig meat products (Antequera et al., 1992; Carrapiso et al., 2003). Moreover, the relationship between individual FA and sensory characteristics in Iberian ham has been also found (Carrapiso et al., 2003; Ruiz, Ventanas, Cava, Andrés, & García, 2000).

Last decade, the proportions of major FA (palmitic (C16:0), stearic (C18:0), oleic (C18:1 *n*-9) and linoleic (C18:2 *n*-6) acids) of subcutaneous fat (SCF) have been used to classify Iberian pigs as a function of their feeding background during fattening (Boletín Oficial del Estado, 2004). This procedure was useful when animals were fed on traditional concentrates, which were not enriched in MUFA. Nevertheless, with the current skill of feeding Iberian pigs with MUFA enriched concentrates, this kind of classification could not be feasible for Iberian pigs.

The effect of MUFA enriched concentrates on the FA profile of SCF and IMF of Iberian pigs has been studied (Daza, Rey, Ruiz, & López-Bote, 2005; González & Tejeda, 2007; Muriel, Antequera, & Ruiz, 2002; Muriel, Ruiz, Ventanas, & Antequera, 2002; Tejeda et al., 2002; Ventanas, Ventanas, Tovar, Garcia, & Estévez, 2007). However, it has not been analyzed the feasibility of using the procedure based on the FA profile for distinguishing Iberian pigs fattened with MUFA enriched diets from those fed on acorns and grass.

There is evidence that some unsaponifiable lipid components associated with the feeding regimen might also be useful as classifying tools (Rey, Isabel, Cava, & López-Bote, 1998; Tejeda, Antequera, Martín, Ventanas, & Garcia, 2001). It has been shown that the dietary level of α- and γ-tocopherol is directly related to their content in different pig tissues (Daza et al., 2005; Rey et al., 2006). Muscles from Iberian pigs fed α-tocopherol supplemented diets and from those fattened outdoors on acorns and grass showed similar α-tocopherol contents (Cava, Ruiz, Ventanas, & Antequera, 1999; Daza et al., 2005) whereas γ-tocopherol were higher in free-range pigs (Daza et al., 2005).

The presence of other components of the unsaponifiable fraction, such as linear and branched hydrocarbons, in different animal lipid depots, has been also linked to their presence in the diet (Berdagué & Garcia, 1990; Tejeda et al., 1999; Tulliez & Bories, 1978). Particularly, neophytadiene is a common component in grass whereas it is not present in acorn and concentrates (Tejeda et al., 2001). Consequently, neophytadiene has only been found in samples from Iberian pigs fed on acorns and grass but not in pigs fed in confinement with a commercial diet (Petrón, Tejeda, Muriel, Ventanas, & Antequera, 2005; Tejeda et al., 2001).

The objective of the present study was to asses the feasibility of the FA profile of SCF and IMF, together with the content of α- and γ-tocopherol and neophytadiene, as tools for classifying Iberian pigs fed outdoors on acorns and grass and with MUFA enriched diets.

2. Material and methods

2.1. Experimental design

This study was carried out with 30 pure Iberian pigs, which were divided into two groups according to the feeding regime during the fattening period prior to slaughter. One group of pigs (AG) ($n = 15$) was reared outdoors in a 30 Ha extension land with free availability of acorns (*Querqus ilex*, *Querqus rotundifolia* and *Querqus suber*) and grass. The other group of pigs (HO) ($n = 15$) was also fattened outdoors in a 1 Ha extension land, with free availability of grass but mainly fed with an oleic acid enriched concentrate. All the animals were fattened for 110 days and slaughtered at an average weight of 160 kg. The chemical composition of diets (acorns, grass and concentrates) was determined according to the Association of Official Analytical Chemists (AOAC, 2000): moisture (reference 935.29), crude protein (reference 954.01), crude fat (reference 920.39), crude fibre (reference 962.09) and ash (reference 942.05). Feed analysis is shown in Table 1.

2.2. Samples

Pigs were slaughtered by electrical stunning and exsanguination at a local slaughterhouse. Sampling was carried out within the hour following slaughter. Backfat samples were taken 10 cm above the tail following the procedure established by the Spanish Ministry of Agriculture, Fishery and Food (Boletín Oficial del Estado, 2004). Thus, samples were pieces of 3 × 3 cm and contain skin, fatty and a small portion of lean. The whole *Biceps femoris* and *Semimembranosus* muscles of the left ham of each animal were also taken.

2.3. Fat Extraction

Backfat samples were divided into portions (2–5 mm). The skin and the lean were removed to obtain the fat, which was ground using a commercial grinder. A portion of 5 g of the sample were placed in a microwave-resistant glass container and heated in a domestic microwave at 200 W for 8 min (Boletín Oficial del Estado, 2004).

Muscles were ground using a commercial grinder immediately before fat extraction. Lipids were extracted with chloroform/methanol (2:1, v/v) according to the method described by Folch, Less, and Sloane (1957).

Table 1
Chemical, fatty acid and tocopherol compositions of feeds consumed by studied Iberian pigs.

	Diets		
	Acorn	Grass	Concentrate feed
Chemical composition			
Moisture	31.66	72.6	9.97
Lipids[a]	6.70	2.61	4.57
Crude protein[a]	3.81	13.78	18.11
Crude fiber[a]	6.5	22.9	4.2
NFE[a,b]	79.9	50.6	69.2
Ash[a]	2.2	10.4	5.8
Fatty acids[c]			
C16:0	14.16	19.86	7.89
C16:1	0.28	1.92	0.30
C17:0	0.13	0.19	0.07
C17:1	0.07	0.43	0.06
C18:0	3.68	2.98	3.41
C18:1 *n*-9	60.44	11.92	55.97
C18:2 *n*-6	18.67	12.34	28.67
C18:3 *n*-3	0.97	49.97	1.63
C20:0	0.43	nd	0.32
C20:1	0.63	nd	0.42
C20:5 *n*-3	0.19	nd	0.73
C24:0	0.07	nd	0.24
SFA[d]	18.46	23.03	11.92
MUFA[e]	61.35	13.84	56.69
PUFA[f]	19.64	62.31	30.31
α-tocopherol[g]	8.14	296.91	106
γ-tocopherol[g]	50.87	nd	nd

nd, Non detected.
[a] Expressed as percentage of dry matter.
[b] Nitrogen-free extractives.
[c] Values are means expressed as percentage of total fatty acid methylesters.
[d] SFA, total amount of saturated fatty acids.
[e] MUFA, total amount of monounsaturated fatty acids.
[f] PUFA, total amount of polyunsaturated fatty acids.
[g] Expressed as mg kg^{-1}.

2.4. Fatty acid methyl ester preparation and analysis

Fatty acid methyl esters (FAME) from obtained lipid tissues were prepared by trans-esterification in presence of sodium metal (0.1 N) and sulphuric acid in methanol (Sandler & Karo, 1992). Fatty acid methyl esters were analyzed by gas chromatography, using a Hewlett-Packard HP-5890-II gas chromatograph, equipped with a on-column injector and a flame ionization detector (FID). Separation was carried out on a polyethylenglycol capillary column (60 m long, 0.32 mm id, 0.25 mm film thickness) (Supelcowax-10; Supelco, Bellafonte, PA, USA) maintained at 230 °C for 60 min. Injector and detector temperatures were 230 °C. The carrier gas was nitrogen at a flow rate of 0.8 ml/min. Individual compounds were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO, USA).

2.5. Neophytadiene analysis

Neophytadiene determination was carried out following the method described by Tejeda et al. (2001) with slight modifications. Samples (4 g) of SCF were saponified by refluxing for 2 h with 70 ml of 15% KOH in ethanol (w/v). The warm solution was transferred to a separating funnel, and 70 ml of distilled water added and the unsaponifiable fraction extracted with 70 ml of hexane. The organic layer was washed three times with 50 ml of distilled water, then dried over anhydrous sodium sulphate and concentrated to 2 ml. The extract was then transferred onto a chromatography column (1.5 cm i.d.) that had been prepared by adding successively 2 g of silica gel and 8 g of anhydrous sodium sulphate. Hydrocarbons were eluted with 50 ml of hexane. After evaporating to dryness under vacuum, the residue was dissolved in 200 μl of hexane for gas chromatographic analysis.

Branched hydrocarbons were analyzed by gas chromatography on an Agilent 6890 chromatograph, equipped with a FID and a Hewlett-Packard capillary column HP5MS-5% phenylmethylsiloxane (30 m × 0.25 mm × 0.33 μm) with a stationary phase of methylsiloxane. Helium was the carrier gas at a flow rate of 4.1 ml/min.

The oven program was from 100 to 196 °C at 6 °C/min, from 196 to 280 °C at 30 °C/min and 15 min at 280 °C. The split ratio was 1:25, inlet pressure 14 psi, and the volume injected 2 μl.

Neophytadiene was identified by gas chromatography-mass spectrometry, using a Agilent 5973 Network mass selective detector. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV, a multiplier voltage of 1756 V and collected data at 1 scan s^{-1} over a m/z range from 400–300. Spectra were compared with those of the standard and spectra from NIST library.

Quantitative determination was performed by adding to the final extract an appropriate amount of nonadecane (Sigma) as internal standard.

2.6. α- and γ-tocopherol analysis

α-Tocopherol and γ-tocopherol in muscle were determined following the method described by Rey, López-Bote, Soares, and Isabel (1996), in which 0.8 g muscle were homogenized in 6 ml of Na_2 HPO_4 0.054 M buffer adjusted to pH 7 with HCl. After mixing with absolute ethanol and hexane, the upper layer containing tocopherols was evaporated to dryness and solved in 200 μl of ethanol prior to analysis by reverse phase HPLC (Agilent 1100 Series, with a Diode Array detector). Separation was made on an Agilent Technologies Lichrospher RP-C18 column (250 mm × 4 mm i.d. 5 μm particle size), the mobile phase was methanol/water (97:3 v/v) at a flow rate of 2 ml/min and peaks were registered at 292 nm. Peaks were identified and quantified upon calibration with standards of α- and γ-tocopherols (Sigma Chemical Co., St. Louis, USA).

2.7. Statistical analysis

An individual animal was the experimental unit for analysis of all data. The effect of diet on the FA composition was compared by one-way analysis of variance (ANOVA) using the General Linear Model of SPSS (v.15.0). A principal components analysis (PCA) of the composition of SCF and IMF was carried out using the UMSCRAMBLER software (v.5.0, CAMO).

3. Results and discussion

3.1. Diets

The chemical and FA composition of the diets are shown in Table 1. Acorns and grass, the two basic food resources for AG pigs, showed similar composition to those found in previously published works (Muriel, Antequera, et al., 2002; Tejeda et al., 2002). Acorn had high nitrogen-free extractives and low protein content, whereas grass showed high fibre and ash contents. As expected, acorn and grass were characterized by their high contents in oleic acid (C18:1 *n*-9) (60.44%) and linolenic acid (C18:3 *n*-3) (49.97%), respectively. In the oleic acid enriched concentrate feed, oleic acid (C18:1 *n*-9) was the major FA (55.97%), reaching almost those levels found in acorn, followed by linoleic acid (C18:2 *n*-6) (28.67%). The FA profile of conventional mixed diets, which were commonly used for feeding Iberian pigs several years ago, was quite different to the current high oleic acid concentrates, such as that used in this study. In fact, linoleic acid (C18:2 *n*-6) usually showed the highest proportion in conventional diets (Tejeda et al., 2002).

3.2. Fatty acid profile of SCF and IMF from Iberian pigs fed with different diets

The FA composition of SCF and IMF of *B. femoris* and *Semimembranosus* muscles is shown in Tables 2–4, respectively. Most detected FA showed statistical differences between the two experimental groups.

Total saturated FA (SFA) were higher in HO than in AG Iberian pigs ($P < 0.001$) in SCF (32.55% vs. 28.73%) and in the IMF of *Semimembranosus* (32.68% vs. 30.71%). The levels of palmitic acid (C16:0) were also higher ($P < 0.001$) in HO than in AG pigs in the SCF (19.69% vs. 17.32%) and in the IMF of *Semimembranosus* (21.90% vs. 19.87%) while stearic acid (C18:0) showed statistical differences only in the SCF ($P < 0.001$) (11.32% vs. 10.04% in HO and AG pigs, respectively). However, feeding different diets did not influence the total SFA levels of IMF of *B. femoris*. In spite of it, palmitic (C16:0) and stearic (C18:0) acid significantly differed between experimental groups in this muscle ($P < 0.01$ and $P < 0.001$, respectively). Pigs fed high oleic acid diets showed higher levels of palmitic acid (C16:0) (21.06% vs. 20.60%) and lower of stearic acid (6.90% vs. 8.06%) than AG animals. González and Tejeda (2007) also found lower SFA contents in *Longissimus dorsi* from pigs fed on acorns and grass, whereas neither Muriel, Ruiz, et al. (2002) in *L. dorsi* nor Ventanas et al. (2007) in *B. femoris* found any effect of oleic acid enriched diets on the profile of SFA. These different results could be explained by the fact that a high proportion of SFA are from de novo synthesis and only a small proportion of total SFA are directly accumulated from dietary fatty acids (Monahan, Buckley, Morrissey, Lynch, & Gray, 1992).

Monounsaturated fatty acids showed statistical differences between the two groups of animals of this study in SCF ($P < 0.001$) and in the IMF of *Semimembranosus* ($P < 0.001$) but not in the IMF of *B. femoris*. The levels of MUFA were higher in AG than in HO pigs in SCF (59.79% vs. 56.89%) and in the IMF of *Semimembranosus* muscle (58.91% vs. 55.10%) because of the higher content

Table 2
Fatty acid composition of subcutaneous fat from Iberian pigs fed different diets.

	AG[a]	HO[b]	*P*
C12:0	0.02 ± 0.003	0.02 ± 0.007	ns
C14:0	0.74 ± 0.052	0.89 ± 0.077	***
C15:0	0.03 ± 0.005	0.04 ± 0.009	ns
C16:0	17.32 ± 0.899	19.69 ± 0.682	***
C16:1	1.58 ± 0.128	1.90 ± 0.193	***
C17:0	0.32 ± 0.041	0.30 ± 0.036	ns
C17:1	0.27 ± 0.041	0.29 ± 0.038	ns
C18:0	10.04 ± 0.998	11.32 ± 0.720	***
C18:1 *n*-9	56.21 ± 1.134	52.91 ± 1.006	***
C18:2 *n*-6	9.32 ± 0.574	8.92 ± 0.360	*
C18:3 *n*-6	0.01 ± 0.005	0.02 ± 0.006	ns
C18:3 *n*-3	1.03 ± 0.210	0.51 ± 0.051	***
C20:0	0.18 ± 0.019	0.24 ± 0.025	***
C20:1	1.71 ± 0.234	1.77 ± 0.247	ns
C20:2	0.58 ± 0.097	0.66 ± 0.094	*
C21:0	0.01 ± 0.009	0.01 ± 0.009	ns
C20:3 *n*-6	0.07 ± 0.007	0.08 ± 0.011	***
C20:4 *n*-6	0.11 ± 0.014	0.14 ± 0.017	***
C20:3 *n*-3	0.28 ± 0.054	0.17 ± 0.027	***
C20:5 *n*-3	0.01 ± 0.016	0.01 ± 0.018	ns
C22:1	0.01 ± 0.012	0.01 ± 0.011	ns
C22:2	0.03 ± 0.013	0.02 ± 0.020	ns
C24:0	0.10 ± 0.028	0.08 ± 0.030	ns
SFA[d]	28.73 ± 1.625	32.55 ± 1.264	***
MUFA[e]	59.79 ± 1.286	56.89 ± 1.163	***
PUFA[f]	11.32 ± 0.776	10.38 ± 0.405	***

Values are means (expressed as percentage of total fatty acid methylesters) ± standard error of the mean.
ns, $P > 0.05$; $^{*}P > 0.05$; $^{**}P > 0.01$; $^{***}P > 0.001$.
[a] AG, Iberian pigs fed with acorns and grass.
[b] HO, Iberian pigs fed with an oleic acid enriched concentrate.
[d] SFA, total amount of saturated fatty acids.
[e] MUFA, total amount of monounsaturated fatty acids.
[f] PUFA, total amount of polyunsaturated fatty acids.

Table 3
Fatty acid composition of intramuscular lipids of *B. femoris* from Iberian pigs fed different diets.

	AG[a]	HO[b]	*P*
C12:0	0.04 ± 0.01	0.04 ± 0.01	ns
C14:0	0.98 ± 0.13	1.08 ± 0.06	***
C14:1	0.03 ± 0.03	0.04 ± 0.04	ns
C15:0	0.03 ± 0.04	0.04 ± 0.05	ns
C15:1	0.08 ± 0.10	0.11 ± 0.12	ns
C16:0	20.60 ± 0.90	21.06 ± 0.71	**
C16:1	3.79 ± 0.79	4.45 ± 0.35	***
C17:0	0.15 ± 0.03	0.13 ± 0.02	**
C17:1	0.24 ± 0.06	0.27 ± 0.06	**
C18:0	8.06 ± 1.82	6.90 ± 1.15	***
C18:1 *n*-9	56.23 ± 1.75	56.10 ± 1.75	ns
C18:2 *n*-6	6.41 ± 0.62	6.36 ± 0.63	ns
C18:3 *n*-6	0.02 ± 0.00	0.02 ± 0.00	ns
C18:3 *n*-3	0.46 ± 0.19	0.32 ± 0.05	***
C20:0	0.17 ± 0.02	0.17 ± 0.03	ns
C20:1	1.13 ± 0.11	1.12 ± 0.12	ns
C20:2	0.26 ± 0.03	0.26 ± 0.03	ns
C21:0	0.03 ± 0.02	0.02 ± 0.02	ns
C20:3 *n*-6	0.11 ± 0.02	0.12 ± 0.02	**
C20:4 *n*-6	0.82 ± 0.27	0.98 ± 0.21	***
C20:3 *n*-3	0.13 ± 0.09	0.14 ± 0.12	ns
C20:5 *n*-3	0.08 ± 0.05	0.07 ± 0.06	ns
C22:1	0.02 ± 0.01	0.02 ± 0.01	ns
C22:2	0.04 ± 0.02	0.03 ± 0.02	**
C24:0	0.08 ± 0.08	0.15 ± 0.02	***
SFA[d]	30.14 ± 2.00	29.59 ± 1.76	ns
MUFA[e]	61.53 ± 2.05	62.11 ± 1.92	ns
PUFA[f]	8.28 ± 0.85	8.25 ± 0.86	ns

Values are means (expressed as percentage of total fatty acid methylesters) ± standard error of the mean.
ns, $P > 0.05$; $^{*}P > 0.05$; $^{**}P > 0.01$; $^{***}P > 0.001$.
[a] AG, Iberian pigs fed with acorns and grass.
[b] HO, Iberian pigs fed with an oleic acid enriched concentrate.
[d] SFA, total amount of saturated fatty acids.
[e] MUFA, total amount of monounsaturated fatty acids.
[f] PUFA, total amount of polyunsaturated fatty acids.

of oleic acid (C18:1 *n*-9) in AG than in HO animals (56.21% vs. 52.91% in SCF and 54.43% vs. 49.34% in IMF of *Semimembranosus*). Nevertheless, the levels of oleic acid (C18:1 *n*-9) of IMF of *B. femoris* levels were very similar in the two groups of animals. The results found by González and Tejeda (2007) and Muriel, Antequera, et al. (2002) in *L. dorsi* and by Ventanas et al. (2007) in *B. femoris* reported comparable results for MUFA compositions of Iberian pigs fed on acorns and grass and oleic acid enriched diets, whereas Daza et al. (2005) found significantly higher levels of oleic acid (C18:1 *n*-9) in Iberian pigs reared outdoors than in those reared in confinement and fed MUFA enriched diets.

The apparently different influence of the diet on the oleic acid (C18:1 *n*-9) content in the studied muscles in this work could be explain by the reported effects of muscle fibre types and anatomical location on the fatty acid composition (Andrés, Ruiz, Ventanas, Tejeda, & Mayoral, 1999; Muriel et al., 2002).

Following the trend described for SFA and MUFA, the levels of polyunsaturated FA (PUFA) showed statistical differences between groups ($P < 0.001$) in SCF and in the IMF of *Semimembranosus*, but not in the IMF of *B. femoris*. In SCF, the content of PUFA were higher in AG than in HO pigs (11.32% vs. 10.38%, respectively) while in the IMF of *Semimembranosus* AG animals showed lower content of PUFA than HO pigs (10.28% vs. 12.14%, respectively). These results were the consequence of the proportion of linoleic acid (C18:2 *n*-6). In SCF, the levels of this fatty acid were higher in AG (9.32%) than in HO (8.92%) pigs, whereas in the IMF of *Semimembranosus* muscle AG animals showed lower proportion of linoleic acid (C18:2 *n*-6) (7.85%) than HO pigs (8.66%). Finally, similar values for this fatty acid in the two groups of animals were found in the IMF of *B. femoris*.

Therefore, the differences found in the proportions of linoleic acid (C18:2 *n*-6) between animals fed the different diets are not easily attributable to a single diet effect. The biosynthesis of arachidonic acid (C20:4 *n*-6) involves the desaturation and elongation of the dietary linoleic acid (C18:2 *n*-6) (Valette, Croset, Prigent, Mesdini, & Lagard, 1991). In fact, the proportion of arachidonic acid (C20:4 *n*-6) was significantly higher ($P < 0.001$) in HO than in AG pigs in SCF (0.14% vs. 0.11%, respectively) and in the IMF of *B. femoris* (0.98% vs. 0.82%, respectively) and *Semimembranosus* (2.07% vs. 1.09%, respectively), which could be the consequence of the linoleic acid (C18:2 *n*-6) levels in diets. However, González and Tejeda (2007) and Muriel, Antequera, et al. (2002) found higher levels of linoleic acid (C18:2 *n*-6) and similar proportion of arachidonic acid (C20:4 *n*-6) in the IMF of *L. dorsi* from pigs fed on acorns and grass compared to animals fattened with oleic acid enriched concentrates, while the obtained results by Ventanas et al. (2007) did not show differences in these two FA between Iberian pigs fed similar diets to those in this study.

Significant differences in the proportion of some minor FA between the two experimental Iberian pig groups were also found. As it can be observed in Tables 2–4, AG pigs had significant higher levels of linolenic acid (C18:3 *n*-3) than HO animals in the three studied tissues (1.03% vs. 0.51%, 0.46% vs. 0.32%, 0.61% vs. 0.36%, in SCF and IMF of *B. femoris* and *Semimembranosus*, respectively), which could be due to the high content of linolenic acid (C18:3 *n*-3) in grass. This effect has been previously reported (Muriel et al., 2002; Rey & López-Bote, 2001).

For an Iberian pig to be considered as being from the group of those reared outdoors on acorns and grass, the official norm for classifying Iberian pigs establishes the maximum and the minimum percentage of several fatty acids from the SCF that a sample should fulfil. The established levels are: palmitic acid (C16:0):

Table 4
Fatty acid composition of intramuscular lipids of *Semimembranosus* from Iberian pigs fed different diets.

	AG[a]	HO[b]	*P*
C12:0	0.03 ± 0.00	0.04 ± 0.00	***
C14:0	0.83 ± 0.07	0.70 ± 0.38	ns
C14:1	0.03 ± 0.03	0.08 ± 0.06	**
C15:0	0.03 ± 0.00	0.04 ± 0.02	ns
C15:1	0.06 ± 0.07	0.35 ± 0.27	***
C16:0	19.87 ± 0.47	21.90 ± 0.65	***
C16:1	3.04 ± 0.24	3.95 ± 0.34	***
C17:0	0.18 ± 0.02	0.17 ± 0.04	ns
C 17:1	0.22 ± 0.05	0.39 ± 0.16	***
C18:0	9.34 ± 0.55	9.59 ± 0.79	ns
C18:1 *n*-9	54.43 ± 1.02	49.34 ± 1.91	***
C18:2 *n*-6	7.85 ± 0.65	8.66 ± 1.05	*
C18:3 *n*-6	0.02 ± 0.00	0.03 ± 0.01	***
C18:3 *n*-3	0.61 ± 0.13	0.36 ± 0.06	***
C20:0	0.18 ± 0.01	0.18 ± 0.05	ns
C20:1	1.10 ± 0.27	0.98 ± 0.25	ns
C20:2	0.35 ± 0.25	0.34 ± 0.16	ns
C21:0	0.04 ± 0.08	0.05 ± 0.09	ns
C20:3 *n*-6	0.21 ± 0.19	0.40 ± 0.17	**
C20:4 *n*-6	1.09 ± 0.42	2.07 ± 0.44	***
C20:3 *n*-3	0.11 ± 0.02	0.25 ± 0.44	ns
C20:5 *n*-3	0.12 ± 0.05	0.09 ± 0.03	*
C22:1	0.02 ± 0.01	0.00 ± 0.01	ns
C22:2	0.02 ± 0.05	0.04 ± 0.02	ns
C24:0	0.21 ± 0.07	0.01 ± 0.06	***
SFA[d]	30.71 ± 0.75	32.68 ± 1.44	***
MUFA[e]	58.91 ± 1.17	55.10 ± 2.50	***
PUFA[f]	10.28 ± 0.97	12.14 ± 1.77	**

Values are means (expressed as percentage of total fatty acid methylesters) ± standard error of the mean.
ns, $P > 0.05$; *$P > 0.05$; **$P > 0.01$; ***$P > 0.001$.
[a] AG, Iberian pigs fed with acorns and grass.
[b] HO, Iberian pigs fed with an oleic acid enriched concentrate.
[d] SFA, total amount of saturated fatty acids.
[e] MUFA, total amount of monounsaturated fatty acids.
[f] PUFA, total amount of polyunsaturated fatty acids.

⩽22%, stearic acid (C18:0): ⩽10.5%, oleic acid (C18:1 *n*-9): ⩾53% and linoleic acid (C18:2 *n*-6): ⩽10.5%. In spite of the statistical differences found in SCF for these four FA between AG and HO Iberian pigs, according to the established values, both groups of animals could be considered as animals reared outdoors and with free access to natural resources, which was not the actual situation.

Official classification methods for Iberian pigs do not consider the analysis of the fatty acid profile of muscle tissue, but only of SCF. However, muscle constitutes the main edible part of pig carcasses, and its composition is closely related to the final quality. Therefore, it is also of interest to study the effect of diet on the features of this tissue and its potential use as the chosen tissue for classification.

In the two muscles studied in this work, there were not a direct relationship between palmitic (C16:0), stearic (C18:0) and linoleic (C18:2 *n*-6) acids and the diet. Consequently, the proportion of these FA in muscle does not seem to be suitable as markers for classifying Iberian pigs. The proportion of oleic acid (C18:1 *n*-9) in *B. femoris* was rather similar between experimental groups, whereas in *Semimembranosus* the level of this FA allowed to distinguish AG and HO pigs.

The proportion of arachidonic (C20:4 *n*-6) and linolenic (C18:3 *n*-3) acids in the three studied tissues showed the best ability for distinguishing the feeding background of the pigs. Linolenic acid (C18:3 *n*-3) is directly accumulated from the diet, while arachidonic acid (C20:4 *n*-6) derived from dietary linoleic acid (C18:2 *n*-6). As a chief consideration, it could be pointed out that percentages of arachidonic (C20:4 *n*-6) and linolenic (C18:3 *n*-3) acids could be a really trustworthy procedure to classify Iberian pigs fattened on acorns and grass and with oleic acid enriched diets.

3.3. Unsaponifiable lipids of SCF and IMF of B. femoris from Iberian pigs fed with different diets

Table 5 shows the proportion of neophytadiene of SCF and α- and γ-tocopherol contents of the IMF of *B. femoris* from the two groups of Iberian pigs considered in this study.

The levels of neophytadiene in SCF were more than ten times higher ($P < 0.001$) in AG (10.55 UAA) than in HO pigs (1.24 UAA). This reflected the composition of the diets, since AG Iberian pigs had free access to grass, which was the only feeding source in which this compound was detected (136.23 UAA), while it was not detectable in acorns and concentrates. As explained in the material and methods section, Iberian pigs fed on HO concentrates also had available grass, but given that the land in which they were reared was much smaller, the amount of available grass was almost absent. These results are in agreement with those found by Petrón et al. (2005) and Tejeda et al. (2001) in the IMF of *B. femoris*. Furthermore, it has also been described a higher proportion of unsaponifiable material and *n*-alkanes in SCF of hams from Iberian pigs fed on acorns and grass than those from pigs receiving concentrate pigs (Tejeda et al., 1999).

Although α-tocopherol levels in oleic acid enriched concentrates (106 mg/kg) were lower than the levels found for this compound in grass (296.91 mg/kg), AG and HO pigs did not show differences in α-tocopherol contents of *B. femoris*, which could be due to the amount of grass consumed by AG pigs, which was most likely lower than the amount of concentrates consumed by HO animals. The levels of γ-tocopherol were significantly higher ($P < 0.001$) in AG (1.34 mg/kg) than in HO pigs (0.38 ppm), reflecting the higher content of γ-tocopherol in acorns (50.87 mg/kg) than in concentrates (not detected). The low but detectable levels of γ-tocopherol in muscles from Iberian pigs fattened with concentrates have also been described by Ventanas et al. (2007). This could be strange, since none of the feeding sources for these pigs showed detectable levels of γ-tocopherol in our study. However Daza et al. (2005), did find low levels in grass. At any rate, the presence of γ-tocopherol in pig muscles is almost restricted to tissues from pigs fed on acorns (Cantos et al., 2003; López-Bote, 1998).

Thus, based on present results, it seems that α-tocopherol content of muscle is not adequate for classifying AG and HO Iberian pigs, given that the inclusion of this compound in the concentrates would make indistinguishable samples from both types of animals. However, both neophytadiene and γ-tocopherol seem to allow such classification. In fact, there are two different patented procedures, differencing Iberian pigs according to their feeding background, based on neophytadiene (Tejeda, Garcia, Antequera, & Ventanas, 2005) and tocopherol and other lipid soluble compounds (López-Bote & Rey, 1998). Previous studies in bovine tissues have also proposed the use of neophytadiene content as markers of diet (Bernardini, Boniforti, Citti, & Mosini, 1982; Lintas, Balduzzi, Bernardini, & Di Muccio, 1979). However, the analysis of the hydrocar-

Table 5
Neophytadiene content in subcutaneous fat and α- and γ-tocopherol concentrations in *B. femoris* from Iberian pigs fed different diets.

	AG[a]	HO[b]	*P*
Neophytadiene[c]	10.55 ± 2.90	1.24 ± 0.45	***
α-tocopherol[d]	10.56 ± 2.86	12.21 ± 3.45	ns
γ-tocopherol[d]	1.34 ± 0.46	0.34 ± 0.18	***

Values are means ± standard error of the mean.
ns, $P > 0.05$; *$P > 0.05$; **$P > 0.01$; ***$P > 0.001$.
[a] AG, Iberian pigs fed with acorns and grass.
[b] HO, Iberian pigs fed with an oleic acid enriched concentrate.
[c] Neophytadiene, expressed as peak of peak area x100/internal standard peak area.
[d] α- and γ-tocopherol, expressed as mg kg^{-1}.

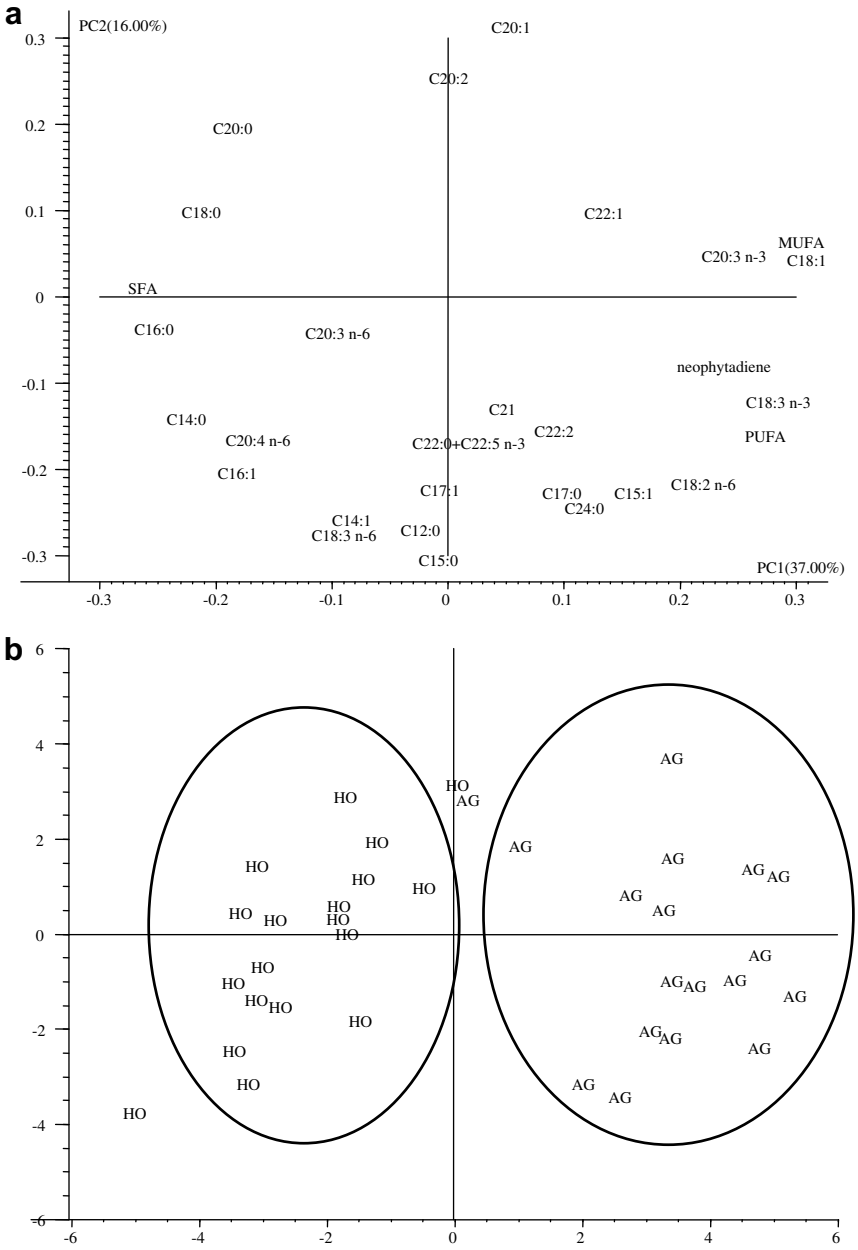

Fig. 1. Principal components analysis of subcutaneous fat: (a) fatty acid profile and neophytadiene plot, (b) score plot for subcutaneous fat from Iberian pigs fattened with different diets (AG, Iberian pigs fed with acorns and grass; HO, Iberian pigs fed with an oleic acid enriched concentrate).

bons of the unsaponifiable fraction is complex, as they are at very low concentrations, and the analytical methods are expensive and time consuming. Thus, its use should be considered as a complementary method.

3.4. Principal components analysis

Principal components analysis was performed using the data obtained from the lipid composition of SCF and the IMF of *B. femoris* and *Semimembranosus* in order to study the relationship among studied variables, which could be useful to further improve the methods for classification of Iberian pigs fattened with different diets. Figs. 1, 2 and 3a show the similarity map of the measured parameters defined by the two first Principal Components (PC1 and PC2) accounted for 37% and 16% of the total variance in SCF, 28% and 17% in the IMF of *B. femoris*, and 36% and 16% in the IMF of *Semimembranosus*.

In SCF, MUFA, oleic (C18:1 *n*-9) and eicosatrienoic (C20:3 *n*-3) acids showed high positive values for PC1, and were placed close to the neophytadiene, PUFA and linolenic acid (C18:3 *n*-3). In the opposite location, SFA and palmitic acid (C16:0) showed high negative values for PC1 (Fig. 1a). The projection of the samples of SCF (Fig. 1b) onto the PCs space showed that AG pigs were located in the upper and lower right quadrants, in the area corresponding to MUFA, PUFA, neophytadiene, oleic (C18:1 *n*-9), eicosatrienoic (C20:3 *n*-3) and linolenic (C18:3 *n*-3) acids. On the contrary, HO pigs were in the upper and lower left quadrants, which are defined by SFA, myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1) stearic (C18:0), eicosanoic (C20:0) and arachidonic (C20:4 *n*-6) acids.

Principal components analysis of lipid composition of IMF from *B. femoris* showed that MUFA and oleic acid (C18:1 *n*-9) were situated in the positive right quadrant, whereas γ-tocopherol and linolenic acid (C18:3 *n*-3) were found in the middle of the positive left quadrant and SFA and stearic acid (C18:0) were in the negative left

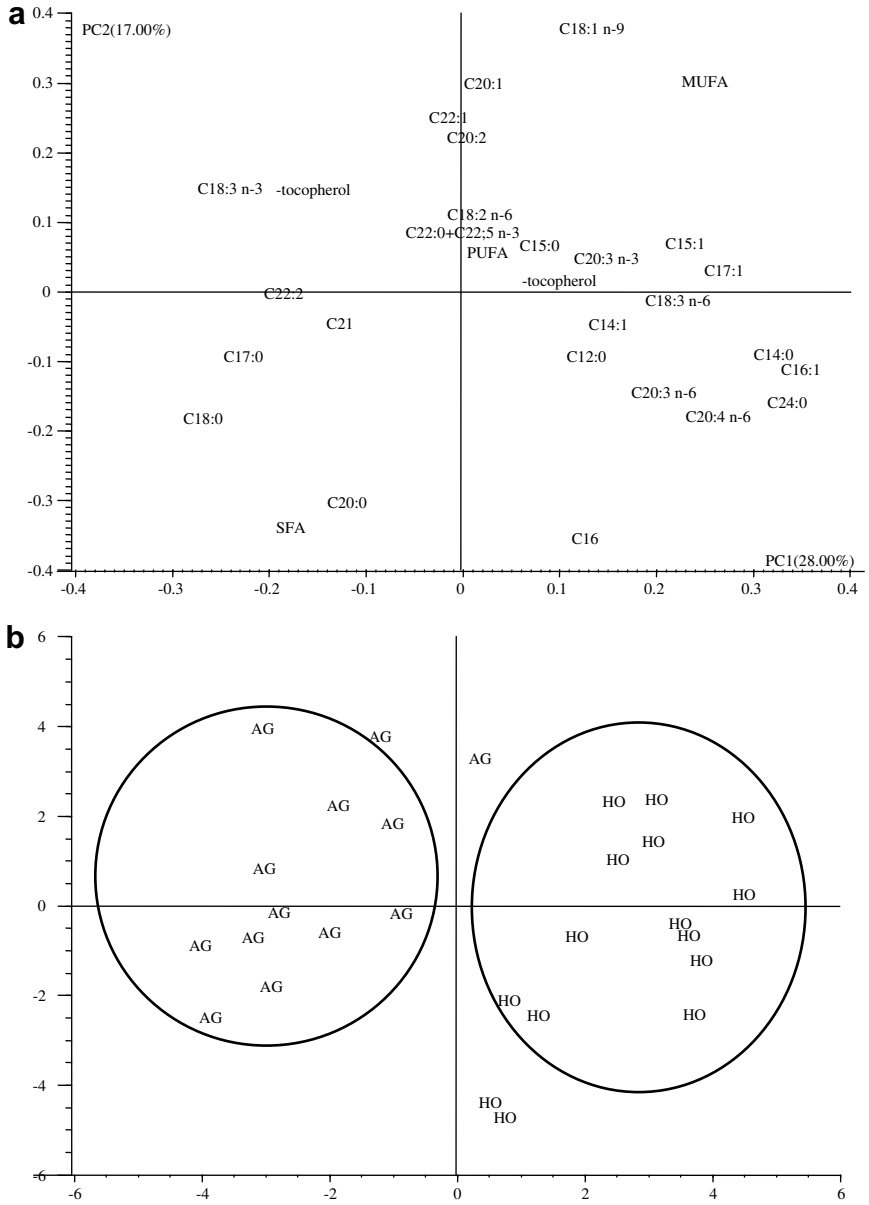

Fig. 2. Principal components analysis of intramuscular fat of *B. femoris* muscle: (a) fatty acid profile and α- and γ-tocopherol plot, (b) score plot for subcutaneous fat from Iberian pigs fattened with different diets (AG, Iberian pigs fed with acorns and grass; HO, Iberian pigs fed with an oleic acid enriched concentrate).

quadrant (Fig. 2a). The samples of *B. femoris* from AG Iberian pigs of this study were situated in the upper and lower left quadrants, defined by γ-tocopherol, linolenic acid (C18:3 *n*-3) and other minor FA such as heptadecanoic (C17:0), eicosadienoic (C20:2), heneicosanoic, erucic (C22:1) and docosadieonic (C22:2) acids. Samples of *B. femoris* from HO group of animals were located in the upper and lower right quadrants, in the area corresponding to α-tocopherol, arachidonic acid (C20:4 *n*-6) and several minor FA.

With respect to the PCA carried out using the results of the fatty acid composition of *Semimembranosus* muscle Fig. 3a, shows that PUFA and linoleic acid (C18:2 *n*-6) were in the positive right quadrant, far from the origin and axis and close to SFA, miristoleic (C14:1), stearic (C18:0), eicostrienoic (C20:3 *n*-3) and arachidonic (C20:4 *n*-6) acids. In the opposite location, linolenic (C18:3 *n*-3), erucic (C22:1) and lignoceric (C24) acids were situated in the left upper quadrant whereas MUFA and oleic acid (C18:1 *n*-9) were found in the negative left quadrant. The projection of the samples of the IMF of *Semimembranosus* muscle (Fig. 3b) onto de PC space showed that HO Iberian pigs were situated in the upper and lower right quadrants, in the plane area corresponding to SFA, pentadecanoic (C15:0), palmitic (C16:0), linoleic acid (C18:2 *n*-6), eicosatrienoic (C20:3 *n*-6), arachidonic (C20:4 *n*-6) and docosadienoic (C22:2) acids. Samples of IMF of *Semimembranosus* from AG pigs were in the upper and lower left quadrants, defined mainly by oleic (C18:1 *n*-9), linolenic (C18:3 *n*-3), erucic (C22:1) and lignoceric (C24:0) acids.

Taking into consideration the statistical differences in the lipid composition as well as the results of PCA of this study it can be observed that linolenic (C18:3 *n*-3) and arachidonic (C20:4 *n*-6) acids showed statistical differences in the three analyses tissues between the two group of animals of this study. Moreover, this two compounds were the only FA found in the area in which AG pigs (C18:3 *n*-3) and HO pigs (C20:4 *n*-6) of SCF and IMF of *B. femoris* and *Semimenbranousus* were located in the representation of the scores in the PC axis. Neophytadiene and γ-tocopherol were statistical different between AG and HO Iberian pigs being also lipid

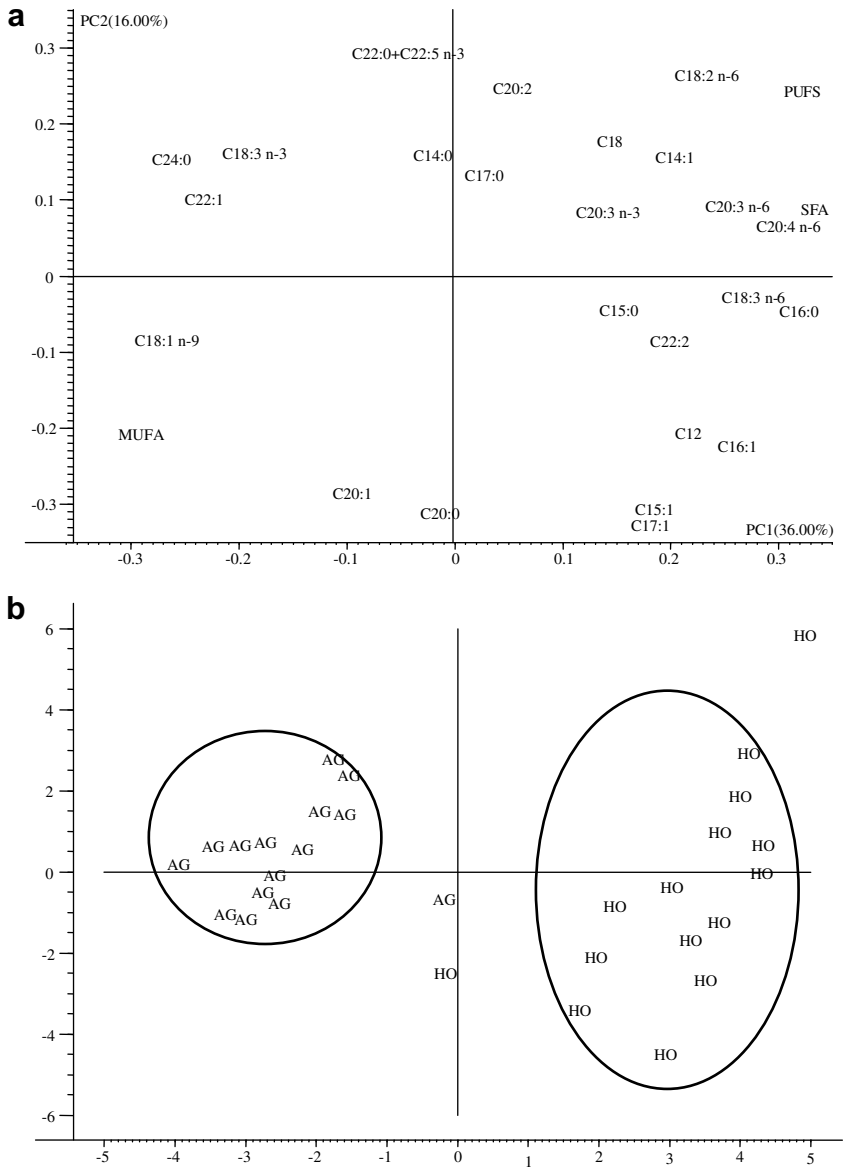

Fig. 3. Principal components analysis of intramuscular fat of *Semimembranosus* muscle: (a) fatty acid profile plot, (b) score plot for subcutaneous fat from Iberian pigs fattened with different diets (AG, Iberian pigs fed with acorns and grass; HO, Iberian pigs fed with an oleic acid enriched concentrate).

components which defined the plane area of AG pigs onto the PC space.

4. Conclusions

After the study of the lipid fraction of SCF and IMF of *B. femoris* and *Semimembranosus*, it can be pointed out that procedures based on the proportion of arachidonic (C20:4 *n*-6) and linolenic (C18:3 *n*-3) acids as well as the content of neophytadiene and γ-tocopherol, could be used for correctly classifying Iberian pigs fed either on acorn and grass or with oleic acid enriched concentrates.

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Capítulo I.3.

MRI-based analysis of diet effect on fresh Iberian ham

Enviado a: *Food Chemistry* (Marzo, 2009)

TITLE

MRI-based analysis of diet effect on fresh Iberian ham

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RUNNING TITLE MRI-based analysis of diet effect on Iberian ham

ABSTRACT

This paper describes the Magnetic Resonance Imaging (MRI) contrastive analysis of Iberian hams from pigs that have been fattened only with acorns and grass (AG) and those that have been fattened with high oleic acid concentrates (HO). Based on computer vision techniques, the implemented methods of image processing allowed for the automatic recognition of the *Biceps femoris* muscle as well as for computational texture feature detection in the muscle. The data collected was then checked against physical-chemical composition. The MRI sequence obtained displayed markedly visual differences in terms of the grey hue for both muscle (darker in AG-fed hams) and inter-muscular and intra-muscular fat (darker in HO hams). Most computational texture characteristics proved to be statistically different between the AG and HO image sets. Two groups (G1 and G2) of texture features were derived. G1 was closely related to linolenic acid (C18:3 n-3) and γ-tocopherol, defining Iberian ham of AG pigs, while G2 was associated with HO pigs. Thus MRI-based analyses enable visual distinction of Iberian hams according to the feeding background. This distinction is also achieved computationally via the examination of the texture features in the muscles.

KEY WORDS

MRI, computer vision techniques, lipid composition, Iberian ham, diet.

INTRODUCTION

Iberian ham is an expensive Spanish dry-cured meat product, characterized by a high intramuscular fat and its fatty acid (FA) composition (high levels of monounsatured FA (MUFA)), which influence on its high sensorial quality and made Iberian ham different from other types of hams [1](Cava, Ventanas, Ruiz, Andrés & Antequera, 2000).

Iberian ham from animals fed outdoors on acorns and grass reach the highest prices in the market because of its quality characteristics. However, these pigs may also be fattened with concentrates, which causes lower quality and less market acceptance [](Carrapiso, Bonilla & García, 2003; Cava et al., 2000; López, de la Hoz, Cambero, Gallardo, Reglero & Ordóñez, 1992; Garcia, Berdagué, Antequera, López-Bote, Córdoba & Ventanas, 1991). Nowadays, MUFA enriched diets, through the inclusion of high oleic acid (C18:1 n-9) sunflower oil, are been used to feed Iberian pigs in order to imitate the FA profile of acorn-fed animals.

Likewise, features of the pigs fattening phase, with acorn and grass or with concentrate feeds, affect the FA profile (Petrón, Muriel, Pérez-Palacios & Antequera, 2005; Tejeda, Gandemer, Antequera, Viau & García, 2002; Cava, Ruiz, Ventanas & Antequera, 1999). Moreover, some unsaponifiable lipid components, such as neophytadiene and α- and γ-tocopherol, are also associated with the feeding regimen (Rey, Daza, López-Carrasco & López-Bote, 2006; Daza, Rey, Ruiz & López-Bote, 2005; Tejeda, Antequera, Martín, Ventanas & Garcia, 2001).

The FA composition of subcutaneous and intramuscular fat of *Biceps femoris* and *Semimembranosus* muscles as well as some components of the unsaponiable fraction, neophytadiene and γ-tocopherol, may be regarded as useful means for the classification of different pig feeding diets (Pérez-Palacios, Ruiz, Tejeda & Antequera, 2009).

Magnetic Resonance Imaging (MRI) constitutes a non-destructive and non-invasive technique. The combination of MRI and computer vision techniques can present decisive capabilities for the characterisation of muscle structures. Different MRI applications have demonstrated the feasibility of this technique for studying meat and meat products. Beavallet and Renou (1992), for example, use MRI to study lipid distribution in meat, while other authors

characterise muscle structure by relying on such MRI devices (Bonny, Zanca, Boespflug-Tanguy, Dedieu, Joandel & Renou, 1998; Bonny, Laurent, Labas, Taylor, Berge & Renou, 2000). Some researchers are interested in the MRI-based analysis of NaCl diffusion in meat (Hansen, van der Berg, Ringgaard, Stodkilde-Jorgensen & Karlsson, 2008), whereas other studies employ MRI and computational texture features to classify Iberian loins as a function of cross-breeding (Cernadas, Antequera, Rodríguez, Durán, Gallardo & Villa, 2001) and in terms of intramuscular fat content and certain sensory attributes (Antequera, Muriel, Rodríguez, Cernadas & Ruiz, 2003). The calculation of intramuscular fat levels of Iberian ham *Biceps femoris* and *Semimembranosus* muscles can also result from MRI applications (Ávila, Durán, Antequera & Gallardo, 2005). The implementation of active contours in MRI can be used to explore the *Biceps femoris* and *Semimembranosus* muscles in Iberian hams. These techniques enabled the measurement of ham weight and moisture so that an optimal ripening time period may be determined for Iberian hams (Caro, Rodríguez, Cernadas, Durán, Muriel, Antequera & Villa, 2001; Antequera, Caro, Rodríquez & Pérez, 2007).

The main goal of this research was to be able to discriminate fresh Iberian hams from pigs fattened with different diets (acorn and grass vs. high oleic acid concentrates). The means for the analysis are MRI-based active contours and computational texture features. The relationship between the MRI-based texture characteristics and physical-chemical composition was also examined.

MATERIAL AND METHODS

Experimental design

This study was based on testing done with 30 Iberian pigs, divided into two groups according to the two feeding diets applied during the fattening period. The group of pigs labelled as AG (n=15) corresponds to those animals reared outdoors (on a 30 Ha-field) with free access to acorns (*Querqus ilex*, *Q. rotundifolia* and *Q. suber*) and grass. The other group of pigs (HO) (n=15) also matched outdoors breeding criteria (on a 1 Ha piece of land), also had free access to grass, but, in contrast, mainly fed on oleic acid enriched concentrates. After the fattening period of 110 days, all the pigs were slaughtered, weighing an average of 160 kg each. The slaughtering was done by electrical stunning and exsanguination at a local slaughterhouse. One ham was taken from each animal, and individual weights were recorded.

MRI acquisition

Magnetic resonance sequences enabled the exploration of *Biceps femoris* muscles in Iberian hams via computer vision techniques. MR images were stored on a database acquired at the ''Infanta Cristina'' University Hospital (Badajoz, Spain). The images were currently withdrawn by using a MRI (Philips Gyroscan NT Intera 1.5 T) scanner. The ''body'' antenna was used, according to sequences of T1 with the following parameters: 120 x 85 mm for field-of view (FOV), 20 ms for echo time (TE), 500 ms for repetition time (TR), 2 mm of thickness for slices, 90º for flip angle, 0.23 x 0.20 mm for pixel resolution, and 60 as the number of slices for each ham. There were a total of 1800 images on the database. All the images were in DICOM, with a 512 x 512 resolution, and converted into the GIF format with the same resolution and 256 grey level.

Computer-Aided MRI Analysis

A software application containing three modules was used for the analysis (Figure 1). The initial module aimed to detect the *Biceps femoris* muscle by using Active Contours according to the method described by Antequera et al. (2007) (Figure 1a). The second module consisted in the selection procedure for the Region of Interest (ROI) on each image; this selection drew up the

maximum rectangular area on the muscle (Figure 1b). The third and last module included the analysis of the ROIs by applying the three most common methods in computational texture analysis, which require the use of rectangular images. All three methods integrated matrices based on second order statistics (Cernadas, Rodriguez, Muriel & Antequera, 2005; Antequera et al., 2003): The first one, Grey Level Coocurrence Matrix (GLCM), was constructed with information of the complete ROI, and presents five features: Energy, Entropy, Haralicks Correlation, Inverse Difference Moment, and Inertia. Second, the so-called Neighbouring Grey Level Dependence Matrix (NGLDM) gathered information from square neighbourhoods inside the ROI, providing five features: Small Number Emphasis, SNE; Long Number Emphasis, LNE; Number Nonuniformity, NNU; Second Moment, SM; Entropy, ENT. Third, the Grey Level Run Length Matrix (GLRLM) only accounted for information about lineal segments of the ROI and it gave five features: Long Run Emphasis, LRE; Short Run Emphasis, SRE; Grey Level Nonuniforminty, GLNU; Run Length Nonuniformity, RLN; Run Percentage, RPC (Figure 1c).

Physical-chemical analysis

The entire *Biceps femoris* muscles of the hams were dissected and then weighed. Moisture content was registered by observing gravimetric water loss according to AOAC (2000) (reference 935.29).

Lipids were extracted with chloroform:methanol (2:1, v/v) according to the method described in Pérez-Palacios, Ruiz, Martin, Muriel and Antequera (2008). Fat content was then determined gravimetrically.

FA methyl esters (FAME) obtained from lipid tissues were assembled by transesterification in the presence of sodium metal (0.1 N) and sulphuric acid within methanol (Sandler & Karo, 1992). FAME were analysed by gas chromatography, using a Hewlett-Packard HP-5890-II gas chromatograph, equipped with an on-column injector and a flame ionization detector (FID). Separation was done on a polyethylenglycol capillary column (60 m long, 0.32 mm id, 0.25 mm film thickness) (Supelcowax-10; Supelco, Bellafonte, PA, USA) maintained at 230 ºC for 60 min. The injector and detector temperatures were kept at 230 ºC. The carrier gas was nitrogen, at a flow rate of 0.8 ml/min rate. The individual compounds were identified as a result of the comparison made

between their retention times and standard retentions (Sigma, St. Louis, MO, USA).

α-tocopherol and γ-tocopherol were determined within each *Biceps femoris* by following the method in Rey, López-Bote, Soares and Isabel (1996). Muscle tissue (0.8 g) was homogenized in 6 ml of Na_2HPO_4 0.054M buffer adjusted to pH = 7 with HCl. After mixing with absolute ethanol and hexane, upper layers containing tocopherols were evaporated to dryness and solved in 200 µl of ethanol prior to analysis by reverse phase HPLC (Agilent 1100 Series, with a Diode Array detector). Separation was done on an Agilent Technologies Lichrospher RP-C18 column (250 mm x 4 mm i.d., 5 µm particle size). The mobile phase consisted in methanol/water (97:3 v/v) at a 2 ml/min flow rate, and peaks were registered at 292 nm. The peaks were identified and quantified upon calibration with standards of α- and γ-tocopherols (Sigma Chemical Co., St. Louis, USA).

Statistical analysis

The statistical application used was the one-way analysis of variance (ANOVA). Both the Pearson correlation and Principal Component Analysis (PCA) were applied to evaluate the relationships between the computational texture characteristics obtained and physical-chemical composition. Analyses were done by using the SPSS package (v.15.0).

RESULTS AND DISCUSSION

Influence of feeding background on MRI and texture characteristics in fresh Iberian hams.

Figure 2 shows two MRI sequences, one corresponding to AG hams (a, b, c), and the other to HO hams (d, e, f). Differences between AG and HO images were quite clear. The grey colour representing the muscle has a distinctly darker hue in AG hams than in HO hams. Then, the white colour illustrating intramuscular and intermuscular fat was brighter in AG samples than in the HO ones, where the colour was greyish. Visually, the effect of different diets on the pig products can be easily detected by means of MRI. Indeed, MRI techniques from T1 allow for the detection of Hydrogen and other features like fat fluidity and water retention, which lengthen the T1 relaxation time (Lufkin, 1998). In fact, HO hams do have a higher degree of moisture than AG hams (unpublished data). Fat fluidity is related to fatty acid composition, influenced by the difference in diets (Pérez-Palacios et al., 2009). Thus, changes in fatty acid profiles derived from the use of different diets may modify T1 and lead to differing MRI.

In statistical terms, most computational texture characteristics of the three methods used for analysing MRI showed significant differences between images from AG and HO Iberian hams (Table 1). With the GLCM method, two of the five texture features, Energy and Entropy, showed statistical differences, as their values were higher in the HO hams than in the other group. The two characteristics directly refer to image homogeneity. Secondly, the NGLDM method application led to statistical distinctions among all five characteristics. While SNE, LNE, NNU and SM displayed higher rates for AG samples than for HO ones, ENT had a lower level in AG hams than in HO products. SNE and LNE yield measurement of image fineness, NNU and ENT encapsulate thickness, and SM does so for image homogeneity.

Finally, in relation to the GLRLM method, all five texture features demonstrate important differences from one ham group to the other. In fact, AG images displayed higher levels of LRE, GLNU and RLNU, whereas the opposite occurred with SRE and RPC, as their values were lower in AG hams. The image properties of roughness, smoothness, and fineness are found in LRE,

SRE and RPC, respectively. Image homogeneity is given by GLNU and RLNU, since the former measures grey level variability, and the latter determines length uniformity. Homogeneity thus seems to play a major role in MRI measurements in order to discriminate the diet source of Iberian pigs. A second major observation was that both the NGLDM and GLRLM methods seem to fulfill more effectively (than GLCM) the goal of MRI-based ham identification from different diet-fed Iberian pigs.

Relationships between MRI texture features and physical-chemical compositions.

The Pearson correlation coefficient (Table 2) and PCA (Figure 3) were run to measure the relationship between MRI computational texture characteristics and physical-chemical composition in the two groups of Iberian hams. Given the results above, all five features in the NGLDM (SNE, LEN, NNU, SM and ENT) and GLRLM (LRE, SRE, GLNU, RLNU and RPC) methods, plus two other texture characteristics in the GLCM method (Energy and Entropy) were correlated with some physical-chemical components (Table 2). Thus, Haralicks Correlation, Difference Inverse Moment, and Inertia (from the GLCM method) were left out in the correlation analysis, as, in fact, the three computational texture features were the only ones not showing statistical differences between AG and HO groups (Table 1).

Two sets of computational texture characteristics were derived from their correlation coefficients with the physical-chemical components. The G1 group (Energy, SNE, LNE, NNU, SM, LRE, GLNU and RLNU) positively correlated with ham weight, Biceps femoris weight, stearic (C18:0) and linolenic (C18:3 n-3) acids, and γ-tocopherol. In turn, G1 results in a negative correlation coefficient with miristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), eicosatrienoic (C20:3 n-6) and arachidonic (C20:4 n-6) acids. The G2 group (Entropy, ENT, SRE and RPC) significantly correlated with the same G1 physical-chemical components, but it does so in the opposite way.

A PCA test was then conducted with the data from the MRI texture characteristics and the physical-chemical components of *Biceps femoris* hams. This analysis was found useful to further improve the methods for Iberian ham classification based on the different diets. Figure 3a illustrates with a similarity

map the measured parameters, defined by two principal components (PC1 and PC2) accounting for 47% and 14%, respectively, of the total variance. Linolenic acid (C18:3 n-3) and γ-tocopherol showed high positive values for PC1, and were found close to Energy, NMU, SNE, GLNU, RLNU, LRE and LNE. The positions agreed with the positive and significant correlation coefficients found between the variables ($p < 0.01$). On the opposite side, mirisitic (C14:0), palmitoleic (C16:1), eicosatrieonic (C20:3 n-6), and arachidonic (C20:4 n-6) acids, Entropy, SRE and RPC all featured high negative values for PC1, a result that also matched the correlation findings among the variables ($p < 0.01$).

By projecting the samples onto the areas where the main components were found (Fig. 3b). AG hams were located on the right, both at the upper and lower quadrants. These locations correspond to γ-tocopherol, linolenic acid (C18:3 n-3), and texture characteristics of Energy, NNU, SNE, GLNU, RLNU, LRE and LNE. In contrast, HO samples were found on the opposite side of the graph, at both upper and lower quadrants, defined by miristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), eicosatrienoic (C20:3 n-6) and arachidonic (C20:4 n-6) acids, as well as by the texture features of Entropy, ENT, SRE and RPC. Such results entirely matched those in Pérez-Palacios et al. (2009), where the γ-tocopherol and linolenic acids (C18:3 n-3) define the AG ham area, while the arachidonic acid (C20:4 n-6) appears in the HO area.

CONCLUSIONS

The feeding diet of the Iberian pig (either linked to acorn and grass or high oleic acid concentrates) can be figured out visually by means of MRI techniques, and computationally by means of muscle-driven texture feature analysis.

The group of texture features composed by Energy, NNU, SNE, GLNU, RLNU, LRE and LNE showed a markedly relationship with linolenic acid (C18:3 n-3) and γ-tocopherol, and define Iberian hams from pigs fattened with acorns and grass. However, Entropy, ENT, SRE and RPC were the texture features associated to hams from pigs with high oleic acid concentrates.

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Table 1. Values of MRI-based texture characteristics for hams from Iberian pigs fattened with different diets: acorn and grass (AG) and high oleic acid enriched concentrates (HO).

		AG	HO	*p*
GREY LEVEL COOCURRENCE MATRIZ (GLCM)	Energy	$0.1.10^{-4} \pm 4.10^{-5}$	$8.10^{-4} \pm 9.10^{-5}$	0.001
	Entropy	3.15 ± 0.01	3.27 ± 0.05	0.001
	Haralicks correlation	$6.10^{-4} \pm 6.10^{-5}$	$7.10^{-4} \pm 1.10^{-4}$	0.164
	Inverse Different Moment	0.06 ± 2.10^{-3}	0.06 ± 4.10^{-3}	<0.324
	Inertia	587.17 ± 68.14	628.65 ± 88.10	0.433
NEIGHBOURING GREY LEVEL DEPENDENCE MATRIX (NGLDM)	SNE[a]	4.13 ± 0.44	2.65 ± 0.28	0.001
	LNE[b]	10.36 ±1.36	5.92 ± 0.76	<0.001
	NNU[c]	19186.39 ± 3439.22	8738.23 ± 933.98	0.001
	SM[d]	669.17 ± 117.21	217.05 ± 31.80	0.001
	ENT[e]	-10.16 ± 1.64	-5.18 ± 0.74	0.001
GREY LEVEL RUN LENGTH MATRIX (GLRLM)	LRE[f]	1.11 ± 8.10^{-4}	1.09 ± 6.10^{-3}	0.003
	SRE[g]	0.97 ± 2.10^{-4}	0.98 ± 1.10^{-3}	0.003
	GLNU[h]	175.09 ± 13.91	89.41 ± 9.01	<0.001
	RLNU[i]	5311.80 ± 443.11	3270.76 ± 151.18	<0.001
	RPC[j]	0.96 ± 2.10^{-4}	0.97 ± 1.10^{-3}	0.003

[a]SNE: small number emphasis; [b]LNE: large number emphasis; [c]NNU: number nonuniformity; [d]SM: second moment: [e]ENT: entropy; [f]LRE: long run emphasis; [g]SRE: short run emphasis; [h]GLNU: gray level nonuniformity; [i]RLNU: run length nonuniformity; [j]RPC: run percentage
$p < 0.05$: means differ significantly

Table 2. Pearson´s correlation coefficients between MRI-based texture features and physical-chemical compositions of *Biceps femoris* muscles from fresh Iberian hams in pigs fattened with different diets: acorns and grass, and high oleic acid enriched concentrates.

	GREY LEVEL COOCURRENCE MATRIX (GLCM)					NEIGHBOURING GREY LEVEL DEPENCENDE MATRIX (NGLDM)					GREY LEVEL RUN LENGTH MATRIX (GLRLM)				
	Energy	Entropy	Haralicks correlation	Diference Inverse Moment	Inertia	SNE[a]	LNE[b]	NNU[c]	SM[d]	ENT[e]	LRE[f]	SRE[g]	GLNU[h]	RLNU[i]	RPC[j]
Ham weight	0.54*	-0.54*	-0.17	-0.060	-0.26	0.38	0.4	0.34	0.37	-0.39*	0.52*	-0.53*	0.43*	0.39*	-0.52*
Biceps femoris weight	0.46*	-0.45*	-0.37	-0.290	-0.09	0.51*	0.50*	0.46*	0.47*	-0.50*	0.46*	-0.46*	0.51*	0.51*	-0.46*
Moisture (%)	-0.35	0.34	-0.07	-0.080	0.35	-0.26	-0.27	-0.25	-0.27	0.27	-0.3	0.28	-0.29	-0.26	0.29
Lipid content (% DM[k])	0.35	-0.32	-0.23	-0.190	-0.08	0.33	0.33	0.3	0.3	-0.33	0.32	-0.3	0.34	0.33	-0.31
C14:0	-0.72**	0.71**	0.46*	0.370	0.23	-0.79**	-0.79**	-0.78**	-0.80**	0.80**	-0.63**	0.65**	-0.81**	-0.79**	0.64**
C16:0	-0.60**	0.59**	0.09	-0.030	0.45*	-0.56**	-0.58**	-0.53**	-0.55**	0.57**	-0.57**	0.58**	-0.60**	-0.57**	0.57**
C16:1	-0.78**	0.76**	0.44	0.330	0.27	-0.88**	-0.89**	-0.87**	-0.87**	0.89**	-0.74**	0.75**	-0.89**	-0.88**	0.75**
C18:0	0.49*	-0.48*	-0.51*	-0.48*	0.07	0.64**	0.64**	0.67**	0.66**	-0.65*	0.46*	-0.46*	0.63**	0.64**	-0.46*
C18:1 n-9	0.28	-0.26	0.11	0.180	-0.41	0.21	0.22	0.17	0.19	-0.21	0.21	-0.22	0.24	0.21	-0.22
C18:2 n-6	0.19	-0.20	0.02	0.070	-0.04	0.04	0.07	0.02	0.03	-0.05	0.29	-0.27	0.07	0.05	-0.28
C18:3 n-6	-0.12	0.11	0.12	0.180	0.05	-0.3	-0.28	-0.31	-0.28	0.29	-0.12	0.09	-0.26	-0.3	0.11
C18:3 n-3	0.69**	-0.68**	-0.35	-0.290	-0.21	0.72**	0.73**	0.71**	0.71**	-0.72**	0.68**	-0.67**	0.73**	0.72**	-0.68**
C20:3 n-6	-0.61**	0.57**	0.24	0.130	0.33	-0.65**	-0.66**	-0.63**	-0.65**	0.66**	-0.52*	0.52*	-0.67**	-0.66**	0.52**
C20:4 n-6	-0.64**	0.61**	0.43	0.370	0.16	-0.71**	-0.71**	-0.69**	-0.70**	0.71**	-0.57**	0.55**	-0.72**	-0.71**	0.56**
SFA[l]	0.05	-0.04	-0.35	-0.390	0.31	0.19	0.18	0.23	0.21	-0.19	0.04	-0.03	0.16	0.19	-0.04
MUFA[m]	-0.07	0.08	0.31	0.340	-0.29	-0.18	-0.17	-0.21	-0.19	0.18	-0.11	0.1	-0.15	-0.17	0.11
PUFA[n]	0.09	-0.10	0.15	0.180	-0.08	-0.05	-0.03	-0.06	-0.06	0.04	0.2	-0.19	-0.03	-0.05	-0.2
α-tocopherol	-0.28	0.27	0.06	0.030	0.08	-0.27	-0.29	-0.29	-0.29	0.28	-0.32	0.3	-0.28	-0.27	0.31
γ-tocopherol	0.72**	-0.72**	-0.39	-0.320	-0.23	0.71**	0.73**	0.70**	0.71**	-0.72**	0.69**	-0.68**	0.74**	0.72**	-0.69**

[a]SNE: small number emphasis; [b]LNE: large number emphasis; [c]NNU: number nonuniformity; [d]SM: second moment: [e]ENT: entropy; [f]LRE: long run emphasis; [g]SRE: short run emphasis; [h]GLNU: gray level nonuniformity; [i]RLNU: run length nonuniformity; [j]RPC: run percentage; [k]DM: dry matter; [l]SFA: total amount of saturated fatty acids; [m]MUFA: total amount of monounsaturated fatty acids; [n]PUFA: total amount of polyunsaturated fatty acids

* significant correlation at the level of 0.05; ** significant correlation at the level of 0.01

Figure 1. Processing modules in the computer-aided MRI analysis of Iberian hams: a) muscle detection; b) region of interest selection; c) region of interest analysis by using the three different methods of texture features.

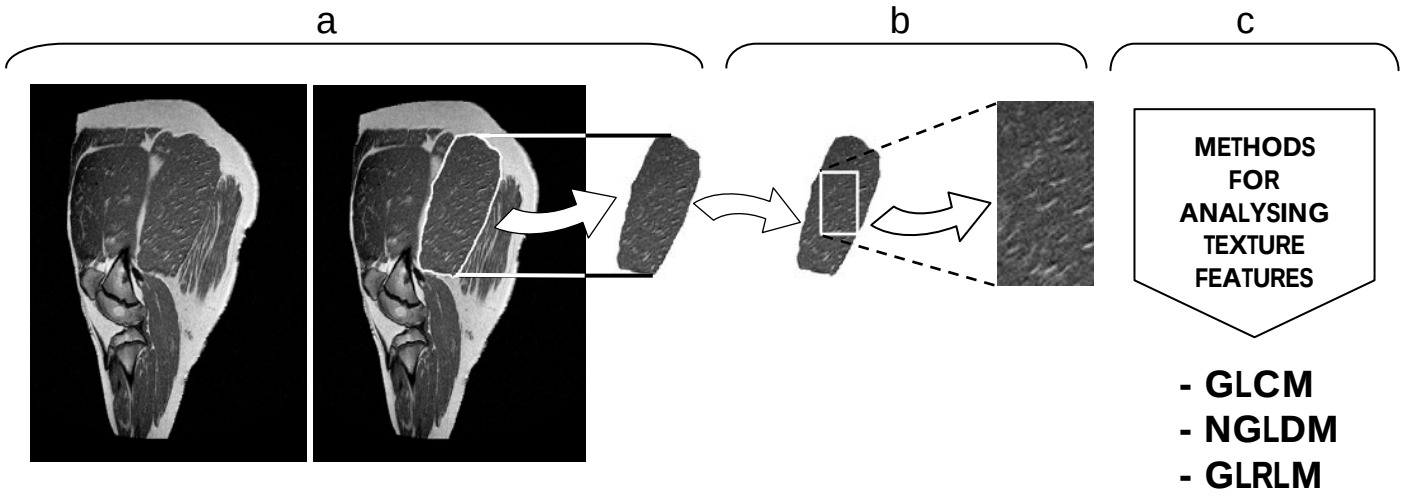


Figure 2. MRI-based sequence of three hams from Iberian pigs fattened with different diets: acorn and grass (a, b, c) and high oleic acid enriched concentrates (d, e, f).

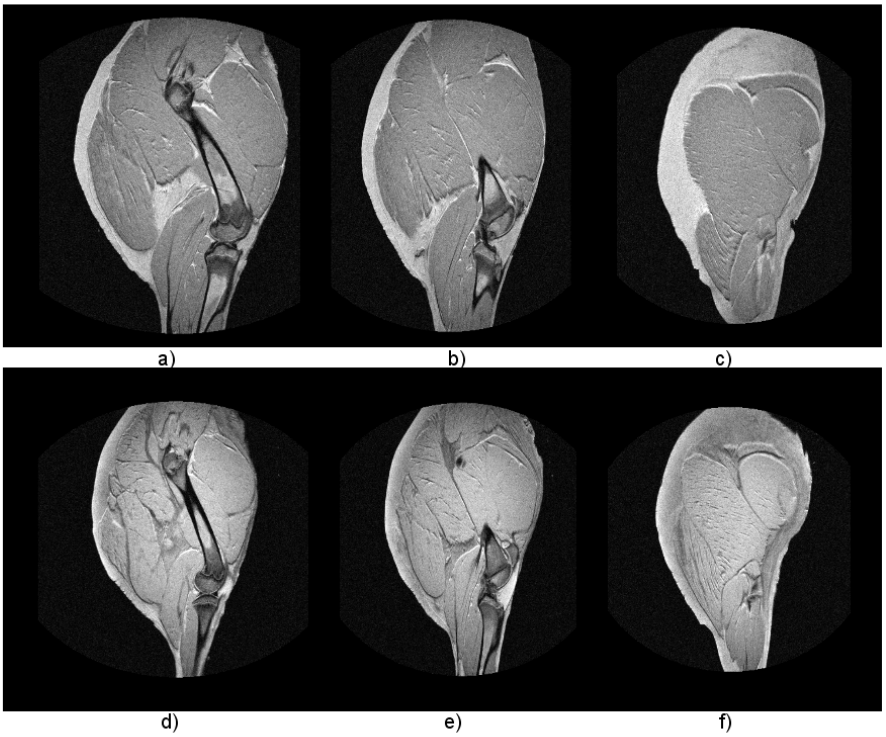
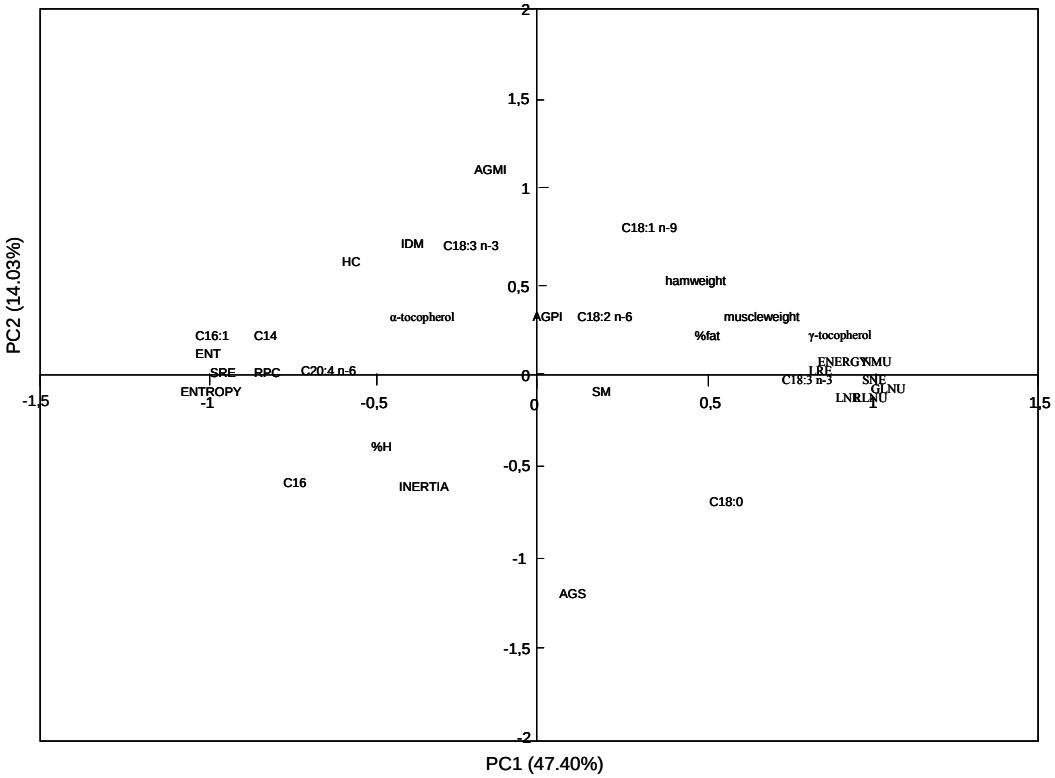
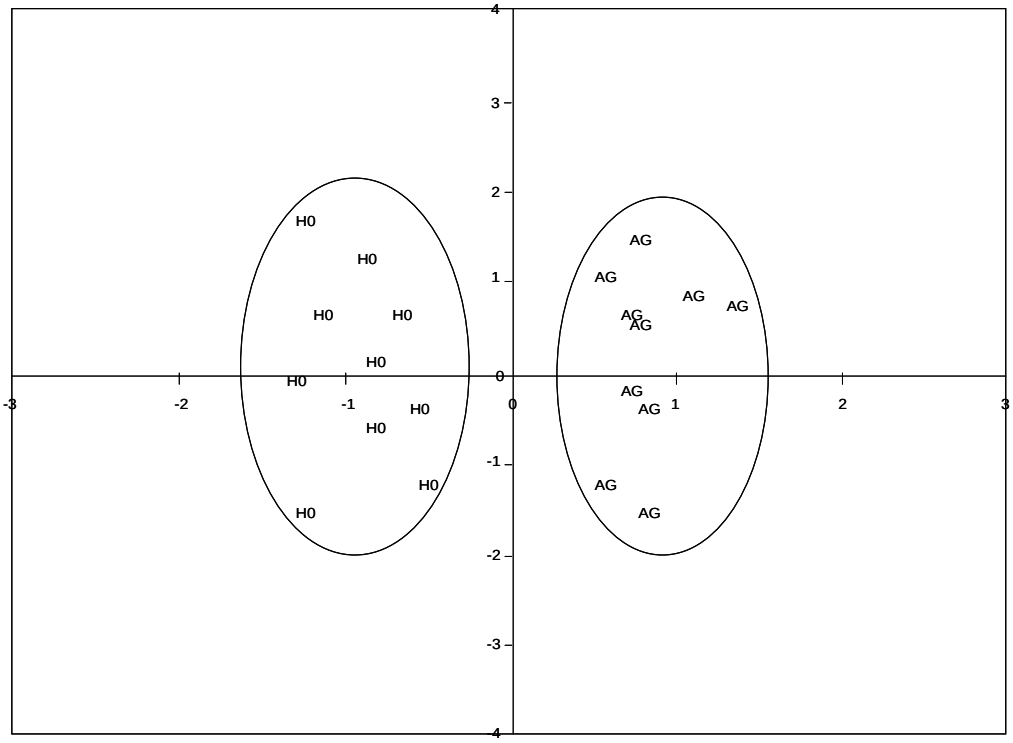


Figure 3. Principal component analysis: MRI-based texture characteristics and physical-chemical composition: (a) variables plot, (b) score plot of Iberian pigs fattened with different diets. (AG: acorns and grass; HO: high oleic acid enriched concentrates).

a



b



Capítulo I.4.

Quantity and lipid composition of individual phospholipid classes from Iberian thighs as a function of the feeding background

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TITLE

Quantity and lipid composition of individual phospholipid classes from Iberian thighs as a function of the feeding background

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RUNING TITLE

Phospholipid classes of Iberian ham affected by diet

ABSTRACT

The aim of this work was to study the effect of feeding Iberian pigs with different diets, acorn and grass (AG) and high oleic acid enriched concentrates (HO), on the quantity and fatty acid (FA) and dimethylacetal (DMA) composition of individual phopholipid (PL) classes of raw thighs. The quantity of PC, PE and PI was higher in HO thighs than in AG ones. There were differences in the FA and DMA profile between the individual PL classes. Thus, the highest proportions of saturated FA (SFA) were found in phophatidylserine (PS) and phosphatidylinositol (PI), monounsaturated FA (MUFA) showed the highest percentage in PS, whereas PC and PE contained the highest proportion of polyunsaturated FA (PUFA). DMA were only found in PC and PE, the later showing the highest proportion of these compounds. The pig feeding influences the FA and DMA profile of PL classes, which reflected consumed FA: PC and PE from AG thighs had higher content of oleic acid (C18:1 n-9), whereas those from HO ones showed higher proportions of linoleic (C18:2 n-6) and arachidonic (C20:4 n-6) acids in PC, PE and PS. The DMA profile of PC and PE was also modified by the animal feeding, but not following the same trend in these two PL: PC showed a higher percentage of DMA in HO than in AG raw thighs, while in PE there were higher proportions of DMA in thighs from AG than from HO Iberian pigs.

KEY WORDS

Phospholipid classes; quantity; lipid composition; Iberian ham; different feeding.

INTRODUCTION

Phospholipids (PL) are the key components of all biological membranes. Each tissue exhibits its own pattern of PL classes; phosphatidlycholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) are the more representative classes in mammal skeletal muscle (Olsson & Salem, 1997). Each PL class shows a pattern of acyl and alkyl chains in the sn-1 and sn-2 positions (Pérez-Palacios, Antequera, Muriel & Ruiz, 2006). In animal tissues, PC tends to contain lower proportion of arachidonic (C20:4 n-6) and docosahexanoic (C22:6 n-3) acids and higher proportions of C18 unsaturated fatty acids than PE. The fatty acid composition of animal PI stands out for a high content of stearic (C18:0) and arachidonic (C20:4 n-6) acids (Christie, 2005).

It is generally accepted that the FA composition of PL can be altered due to nutritional or environmental factors (Stubbs & Smith, 1984). In fact, several works have demonstrated that the FA composition of muscle PL from pig, beef, or chicken is strongly influenced by the FA composition of dietary FA (Asghar, Lin, Gray, Buckley, Booren & Flegal, 1990; Muriel, Ruiz, Ventanas & Antequera, 2002; Dannenberger, Nuernberg, Scollan, Ender & Nuernberg, 2007). Moreover, the effect of feeding diets showing a different FA composition on the FA profile of individual PL classes from different rat tissues was show by Sánchez and Lutz (1998). Similarly, the proportion of dimethylacetals (DMA) in different PL classes has been shown to be influenced by dietary FA composition (Barceló-Coblijn et al., 2003).

Iberian meat products from animals fed outdoors on natural resources reach the highest prices in the market because of its quality characteristics, which are mainly attributed to the outdoor rearing, which implies the consumption of acorns and grass (Cava, Ventanas, Ruiz, Andrés & Antequera, 2000; Carrapiso, Bonilla & García, 2003).

Acorns and grass production are seasonal and restricted, and thus farmers have to use concentrates to feed Iberian pigs, which implies lower quality and market acceptance (Carrapiso et al., 2003; Cava et al., 2000). Currently, monounsaturated FA (MUFA) enriched diets, through the inclusion of high oleic acid (C18:1 n-9) sunflower oil, are being used to fed Iberian pigs in order to imitate the FA profile of those animals fattened on acorns.

Most studies in Iberian ham focusing on the effect of the diet on the FA composition have been devoted to study mainly the FA acid profile of neutral lipids, free fatty acids and PL, whereas its influence on the content of individual PL classes of Iberian ham or the FA profile of each PL class have not been studied yet. However, the determination of PL classes as well as the FA composition of each PL class is important because these compounds are subject to degradation throughout the processing. In fact, FA released during the processing in Iberian ham come mainly from this fraction (Andrés, Cava, Martín, Ventanas & Ruiz, 2005; Martín, Córdoba, Ventanas & Antequera, 1999). In addition, the rate and extension of the lipolysis that takes place during the ripening of Iberian ham is related to the features of the raw material (Andrés et al., 2005; Martín et al., 1999).

Thus, this studied was aimed to study the effect of feeding Iberian pigs with different diets (acorn and grass vs. oleic acid enriched concentrates) on the quantity and lipid composition of individual PL classes from raw thighs.

MATERIAL AND METHODS

Experimental design

This study was carried out with 30 pure Iberian pigs, which were divided into two groups according to the feeding regime during the fattening period prior to slaughter. One group of pigs (AG) (n=15) was reared outdoors in a 30 Ha extension land with free availability of acorns (Querqus ilex, Q. rotundifolia and Q. suber) and grass. The other group of pigs (HO) (n=15) was also fattened outdoors in a 1 Ha extension land, with free availability of grass but mainly fed with an oleic acid enriched concentrate. All the animals were fattened for 110 days and slaughtered at an average weight of 160 kg by electrical stunning and exsanguination at a local slaughterhouse. One ham of each animal was taken and their *Biceps femoris* and *Semimembranosus* muscles were disected. The quantity of each PL class was analyzed in both muscles whereas the *Biceps femoris* was the only muscle used for studying the FA and DMA composition of each PL class.

Intramuscular fat extraction

Samples were ground using a commercial grinder immediately before fat extraction. Intramuscular total lipids were extracted with chloroform/methanol (2:1, vol/vol), according to the method described by Pérez-Palacios, Ruiz, Martin, Muriel and Antequera (2008).

Fractionation of phospholipid classes

The PL fractionation was carried out following the method described by Rombaut, Camp and Dewettinck (2005) with slight modifications. Lipids (24 mg) were dissolved in 1.6 ml of chloroform:methanol solvent (88:12, vol/vol). Analysis was carried out using an HPLC Shimadzu (LC-20AT prominence liquid chromatography) instrument equipped with a pump (DGU-20A5 prominence degasser) and a SIL-20AC autosampler. The analytical column (150 mm × 30 mm I.D.) was packed with a silica normal-phase Prevail Silica 3u (GRACE) thermostated in an oven (Shimadzu CTO-20AC prominence column over) at 40 °C. The chromatographic separation was carried out using a linear gradient according to the following scheme: t = 0 min, 87.5%A 12%B 0.5%C; t = 12 min,

2%A 90%B 8%C for 2 min. The mobile phase was brought back to the initial conditions at t = 16 min and the column was allowed to equilibrate until the next injection at t = 25 min. Eluent A consisted of chloroform, eluent B of methanol and eluent C of triethylamine buffer (pH 3, 1 M formic acid). The flow was maintained at 0.7 ml/min. The injection volume was 10 µL. HPLC was coupled with an evaporative light scattering detector (ELSD) (Alltech 3300). The nebulising gas was N2, at a flow rate of 1.6 L/min, and a nebulising temperature of 65 °C. The gain was set at 1.

Fatty acid methyl ester preparation and analysis

For analysing the FA composition of each PL class, the PL fractionation was carried out in NH2-aminopropyl minicolumns (500 mg) from Varian (Harbor City, CA). Briefly, minicolumns were activated with 7.5 mL of hexane. Twenty milligrams of lipids dissolved in 150 µL of hexane:chloroform:methanol (95:3:2, v/v/v) was loaded onto the column. Neutral lipids were eluted with 5 mL of chloroform and free FA with 5 mL of diethyl ether:acetic acid (98:2, v/v) (Ruiz, Antequera, Andres, Petrón & Muriel, 2004). In this way, minicolumns retained the PL, being further separated into PL classes in the same minicolumn in which they had been retained, following the method used for muscle PL fractionation into PC, PE, PS, and PI described by Pérez-Palacios, Antequera, Muriel and Ruiz (2006). PC, PE, PS, and PI were eluted with 30 mL of acetonitrile:n-propanol (2:1, v/v), 10 mL of methanol, 7.5 mL of isopropanol:3 N methanolic HCl (4:1, v/v), and 17.5 mL of chloroform:methanol:37% HCl (200:100:1, v/v/v), respectively. The vaccum was adjusted to generate a flow of 1 mL/min.

Fatty acid methyl esters (FAME) from obtained lipid tissues were prepared by transesterification in presence of sodium metal (0.1 N) and sulfuric acid in methanol (Sandler & Karo, 1992). FAME were analyzed by gas chromatography, using a Hewlett-Packard HP-5890-II gas chromatograph, equipped with a on-column inyector and a flame ionization detector (FID). Separation was carried out on a polyethylenglycol capillary column (60 m long, 0.32 mm id, 0.25 mm film thickness) (Supelcowax-10; Supelco, Bellafonte, PA, USA) maintained at 230 °C for 60 min. Injector and detector temperatures were 230 °C. The carrier gas was nitrogen at a flow rate of 0.8 ml/min. Individual

compounds were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO, USA).

Statistical analysis

The effect of pig feeding, muscle and the interaction on each PL content from raw thighs was analyzed by using a two-way analysis of variance with interaction by the General Linear Model procedure. The effect of pig feeding the FA and DMA composition of each individual PL class from raw thighs was analysed by one-way analysis of variance (ANOVA) using the General Linear Model. Statistic analysis were performed by means of the SPSS (v.15.0).

RESULTS AND DISCUSSION

Quantity of each individual PL class from raw thighs of Iberian pigs

Data from the raw thighs from AG Iberian pigs were considered as the control group because this group of animals was fattened following the usual procedure.

Four different PL classes were determined in the *Biceps femoris* and *Semimembranosus* muscles from the raw thighs of the Iberian pigs of this study (Table 1). The major PL was PC, followed by PE, PS and PI being the minor PL, which is in agreement with the result found by other authors studying different meats (Wang, Xu, Xu, Zhou, Zhu & Li, 2009; Xu, Xu, Zhou, Wang & Li, 2008; Boselli, Pacetti, Curzi & Frega, 2008; Hernández, Navarro & Toldrá, 1999; Pérez-Palacios et al., 2006; Fernández, Monin, Talmant, Mourot & Lebret, 1999; Kesava Rao & Kowale, 1991).

The effect of the muscle did not lead to significant difference in any PL classes. However, it could be observed the tendency of higher content of PC (p=0.143) and PE (p=0.146) in the *Semimembranosus* than in the *Biceps femoris* muscle, which could be due to the higher IMF in the *Biceps femoris* (29.07 g/100 g muscle dry matter) than in the *Semimembranosus* muscle (17.08 g/100 g muscle dry matter). In fact, the increase in IMF level is mostly due to an increase in triglycerides content, and a relative decrease of the majority PL (Fernández et al., 1999). Moreover, muscles containing a lower IMF level showed higher content of PC and PE than those with a higher content of lipids (Weihrauch & Son 1971).

The effect of Iberian pig diet on each PL class of *Biceps femoris* and *Semimembranosus* muscles of raw thighs is shown in Table 1. The quantity of PC, PE and PI was significantly higher (p<0.001, p=0.017 and p<0.001, respectively) in HO than in AG *Biceps femoris* (1444.33, 499.44 and 86.66 vs. 976.10, 352.71 and 58.91 mg/100 g muscle DM, respectively) and *Semimembranosus* muscles (1683.62, 608.82 and 80.20 vs. 1215.57, 524.61 and 63.73 mg/100 g muscle DM, respectively), whereas PS was not influenced by pig feeding. As a consequence, the sum of the content of the four PL classes was also statistically higher (p<0.001) in HO than in AG *Biceps femoris* (2061.12 vs. 1529.20 mg/100 g muscle dry matter, respectively) and *Semimembranosus* muscles (2409.73 vs.

1903.96 mg/100 g muscle DM, respectively). The amount of phospholipid has been related to the type, the diameter and the content of mitochondria of the muscular fibres (Leseigneur-Meynier & Gandemer, 1991), which could explain the different individual PL classes levels between AG and HO Iberian pigs. In fact, although both groups of Iberian pigs were fattened outdoors, the extension land of AG pigs was higher than that of HO ones. Thus, AG Iberian pigs would have done more exercise than HO pigs, which may influence muscle fibre characteristics.

Different from the present study, Petrón, Muriel, Timón, Martín & Antequera (2004) and Tejeda (2002) found similar content in the whole fraction of PL in muscles from Iberian pigs fed on different diets. Other studies have shown that the relative percentage of PL classes was not influence by the diet in either fish (Soudant, Moal, Marty & Samain, 1997) or mammary tissue and erythrocytes from rats (Williams & Maunder, 1992).

Fatty acid and dimethylacetal composition of each individual PL class from raw thighs of Iberian pigs

The percentage of the sum of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and DMA in the four PL classes from the *Biceps femoris* muscle of raw thighs is shown in Figure 1. The highest proportion of SFA was found in PS (47.55%) and PI (41.45 %), being lower in PC (31.14) and PE (28.94 %). Thus, PS showed a high content of palmitic acid (C16:0) (30.30 g/100g muscle DM) (Table 4) whereas high levels of stearic acid (C18:0) were found in PI (23.89 %) (Table 5). Nevertheless, in muscle rat the highest content of palmitic (C16:0) and stearic (C18:0) acids were found in PC and PS, respectively (Pérez-Palacios et al., 2006).

The highest content of MUFA was found in PS, followed by PC and PI, and PE showing the lowest proportion of this family of FA. Thus, the highest levels of oleic acid (C18:1 n-9) were observed in PS (32.72 %), which is in agreement with the results found by Pérez-Palacios et al. (2006) in muscle rat.

PC and PE showed the highest levels of PUFA (33.84% and 37.25%, respectively), which is due to the high levels of linoleic acid (C18:2 n-6) in PC (27.13 %) (Table 2), and the high content of both linoleic (C18:2 n-6) and arachidonic acids (C20:4 n-6) in PE (15.28 and 19.53 %, respectively) (Table 3).

Similarly, Alasnier and Gandemer (1998) and Cambero et al. (1991) showed a higher content of arachidonic acid (C20:4 n-6) in PE than in PC of muscle rabbit. On the contrary, PI showed the highest proportion of arachidonic acid (C20:4 n-6) in *Longissimus dorsi* of rat (Pérez-Palacios et al., 2006).

PE showed the highest proportion of DMA, followed by PC, while these compounds were not found in PS and PI. Hexadecanaldimethylacetal (C16:0 DMA), octadecanaldimethylacetal (C18:0 DMA) and octadecenaldimethylacetal (C18:1 n-9) appeared in both PC (1.79, 0.30 and 0.53 %, respectively) (Table 2) and PE (4.07, 4.23 and 3.26 %, respectively) (Table 3). Thus, the low content of SFA and MUFA in PC and PE could be, at least in part, due to the found levels of DMA, since one of the pathways for the biosynthesis of plasmalogens involves a desaturation process of the FA sterified in the analogue PL. According to the results of this study, Pérez-Palacios et al. (2006) showed that PE contained the highest levels of DMA in *Longissimus dorsi* of rats. However, these authors also found DMA in PC, PS and PI of this rat tissue.

To our knowledge, the present study is the only one in the available scientific literature dealing with the FA and DMA profile of individual PL from Iberian hams.

The effect of pig diet on the FA and DMA composition of PC, PE, PS and PI from *Biceps femoris* muscle from raw thighs of AG and HO Iberian pigs is shown in Tables 2-5, respectively.

The effect of feeding Iberian pigs with different diets did not lead to changes in the SFA of PC and PE of raw thighs. On the other hand, PS and PI showed statistically higher total SFA percentage in AG (47.55 and 41.45 %, respectively) than in HO thighs (29.90 and 37.08 %, respectively), as a consequence of the significant higher proportion of palmitic (C16:0) and stearic (C18:0) acids in AG than in HO raw thighs in both PS and PI. These differences reflected the FA of feeding, oleic acid enriched concentrates showing lower SFA content than acorns and grass (Pérez-Palacios et al., 2009). Moreover, the incorporation of FA into each PL class is a selective process (Sánchez & Lutz, 1998; Galli, Mosoni & Marangoni, 1992), which can be explained by specific differences in the acylation process for each individual PL (William et al., 1992).

The percentage of MUFA was influence by the feeding background but showing a variable behaviour depending on the PL classes. Thus, PC and PE

from thighs of AG Iberian pigs showed higher percentages of total MUFA (28.27 and 17.73 %, respectively) and oleic acid (C18:1 n-9) (22.51 and 14.78 %, respectively) than those from HO pigs (23.56, 14.59, 17.47 and 11.18 %, respectively), despite of the scarce differences in the content of oleic acid (C18:1 n-9) between the feeds consumed by AG (60.44% in acorns) and HO pigs (55.97% in high oleic enriched concentrate) (Pérez-Palacios et al., 2009). On the other hand, PS and PI of raw thighs from HO Iberian pigs showed higher MUFA proportions than those of AG ones, as a consequence of a higher content of vaccenic (C18:1 n-7) and palmitoleic (C16:1) acids in PS and PI of AG thighs. The high levels of palmitolec acid (C16:1) in PS and PI from thighs of AG Iberian pigs could be attributed to the increased substrate (C16:0), which would stimulate the activity of the Δ^9 desaturase (Ruiz Gutierrez, Molina & Vazquez, 1990). In fact, as described above, oleic acid enriched concentrate had higher content of palmitic acid (C16:0) than acorn and grass, whereas PS and PI of HO raw thighs showed lower content of palmitic acid (C16:0) than those of AG ones, which could be a consequence of the desaturation of this FA to palmitoleic acid (C16:1).

In PC, PE and PS, the proportion of total PUFA was significantly higher in HO (38.06, 55.80 and 17.92 %, respectively) than in AG raw thighs (33.84, 37.25 and 13.02 %, respectively), as a result of the higher levels of arachidonic acid (C20:4 n-6) (in PC, PE and PS) and linoleic acid (C18:2 n-6) (only in PS) in HO than in AG thighs. These results are in agreement with the FA of feeding, the high oleic enriched concentrate showing a higher content of linoleic acid (C18:2 n-6) than acorns and grass (Pérez-Palacios et al., 2009). The pathway for biosynthesis of arachidonic acid (C20:4 n-6) involves desaturation and elongation of the dietary linoleic acid (C18:2 n-6) (Valette, Croset, Prigent, Mesdini & Lagard, 1991). On the other hand, PI showed statistically higher proportion of total PUFA in AG than in HO thighs, due to the presence of eicosapentanoic (C20:5 n-3), erucic (C22:1) and docosadienoic (C22:2) acids in thighs from AG Iberian pigs, whereas these FA were not found in HO ones. However, no differences in linoleic (C18:2 n-6) and arachidonic (C20:4 n-6) acids were found in PI between AG and HO Iberian thighs. In fact, other authors, studying the influence of the diet on the FA and DMA composition of PL classes from animal muscles, showed that PI was the less affected PL (Williams et al., 1992; Dannenberger et

al., 2007; Pérez-Palacios et al., 2007), which could be related to the role of PI as a second messenger in cell signal transduction mechanism and also to the fact that the maintenance of the FA composition of PI is an important feature of membrane homeostatic mechanisms (Williams et al., 1992).

The effect of the diet on the content of DMA did not follow the same trend in PC and PE. Higher levels of hexadecanaldimethyldacetal (C16:0 DMA), octadecanaldimethylacetal (C18:0 DMA), octadecenaldimethylacetal (C18:1 DMA) and consequently of total DMA were found in PC of HO (3.60, 0.72, 1.34 and 5.66 %, respectively) than in that of AG raw thighs (1.79, 0.30, 0.53 and 2.62 %, respectively). On the other hand, in PE the percentages of octadecanaldimethylacetal (C18:0 DMA), octadecenaldimethylacetal (C18:1 DMA) and total DMA were higher in AG (4.23, 3.26 and 12.76 %, respectively) than in HO thighs (1.48, 0.55 and 5.18 %, respectively). The different influence of the feeding background on the content of DMA between PC and PE is difficult to explain. This could be related to particular PL characteristics, such as the abundance and situation of the PL in membrane, the physiological function of a particular PL, or the role as precursor of several FA for the biosynthesis of biologically active compounds.

Accordingly, several authors have also shown the effect of the diet on FA composition of PL classes in different mammal muscles (Cambero et al., 1991; Williams et al., 1992; Soriguer et al., 2000; Pérez-Palacios et al., 2007). In addition, Muriel, Ruiz, Ventanas & Antequera (2002), studying feeding Iberian pigs on acorn and grass or with high oleic acid enriched concentrate, also found differences in the FA profile of total PL from the *Longissimus dorsi* muscle.

Since PL are very sensitivity to oxidation, which is mainly due to its high proportion of long chain polyunsaturated fatty acids and its close contact with catalysts of lipid oxidation in the aqueous phase of the muscle cell (Ruiz, Muriel, Pérez-Palacios & Antequera, 2009), raw thighs from HO Iberian pigs would have poor oxidative stability because their higher quantity of PC, PE and PE and of PUFA content in each PL class.

CONCLUSIONS

Iberian pig feeding (acorn and grass vs. high oleic acid concentrates) lead to differences in both the quantity of individual phospholipid classes and the fatty acid and dimentylacetal composition of each phospholpid class, which reflects the consumed fatty acid rather accurate. Thus, these determinations, quantity and lipid composition of individual phospholipid classes, seem to be able to differentiate raw thigh from Iberian pigs fattened outdoors with different diets.

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Table 1. Phospholipid classes content (expresses as mg phospholipid/100 g muscle dry matter) in the *Biceps femoris* and *Semimembranosus* muscles of fresh Iberian hams from pigs fattened with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

	Biceps femoris		***Semimembranosus***		***p***		
	AG	**HO**	**AG**	**HO**	F[z]	M[y]	F * M
PC	976.10 ± 108.05	1444.33 ± 80.16	1215.57 ± 70.06	1683.62 ± 181.65	<0.001	0.143	0.180
PE	352.71 ± 61.03	499.44 ± 41.16	524.61 ± 36.45	608.82 ± 14.59	0.017	0.146	0.710
PS	76.96 ± 6.73	81.70 ± 8.09	79.53 ± 9.16	75.29 ± 11.30	0.894	0.697	0.740
PI	58.91 ± 2.59	86.66 ± 5.33	63.73 ± 6.04	80.20 ± 6.82	<0.001	0.361	0.120
Σ PL	1529.20 ± 82.65	2061.12 ± 51.06	1103.96 ± 21.12	2409.73 ± 157.05	<0.001	0.114	0.229

[z]F: feeding effect; [y]M: mucle effect.

Table 2. Fatty acid and dimethyl acetal composition (% of total FAME and DMA detected) of phosphatidylcholine of the Biceps femoris mucle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

	AG	**HO**	***p***
C16:0	21.47 ± 0.71	21.63 ± 0.79	0.748
C16:1	1.45 ± 0.11	1.26 ± 0.20	0.102
C18:0	8.81 ± 0.82	8.85 ± 0.48	0.936
C18:1 n-9	22.51 ± 0.90	17.47 ± 0.94	<0.001
C18:1 n-7	4.15 ± 0.22	4.53 ± 0.38	0.089
C18:2 n-6	27.13 ± 1.04	27.22 ± 1.05	0.901
C18:3 n-6	0.19 ± 0.01	0.20 ± 0.01	0.193
C18:3 n-3	0.84 ± 0.12	0.46 ± 0.03	<0.001
C20:3 n-6	0.89 ± 0.02	0.92 ± 0.05	0.226
C20:4 n-6	4.17 ± 0.80	7.38 ± 0.52	<0.001
C20:3 n-3	0.47 ± 0.23	0.61 ± 0.16	0.286
C20:5 n-3	0.23 ± 0.03	0.50 ± 0.14	0.003
C22:1 n-9	0.16 ± 0.02	0.29 ± 0.07	0.003
C22:2	0.19 ± 0.09	0.18 ± 0.01	0.786
C24:0	0.86 ± 0.09	0.69 ± 0.07	0.010
C22:6 n-3	0.46 ± 0.15	0.60 ± 0.09	0.113
ΣSFA[v]	31.14 ± 1.19	31.16 ± 0.94	0.977
ΣMUFA[x]	28.27 ± 0.92	23.56 ± 1.42	<0.001
ΣPUFA[y]	33.84 ± 0.89	38.06 ± 1.29	<0.001
C16:0 DMA	1.79 ± 0.15	3.60 ± 1.04	0.005
C18:0 DMA	0.30 ± 0.02	0.72 ± 0.05	<0.001
C18:1 DMA	0.53 ± 0.05	1.34 ± 0.10	<0.001
ΣDMA[z]	2.62 ± 0.16	5.66 ± 1.02	<0.001

v: total amount of saturated fatty acids
x: total amount of monounsaturated fatty acids
y: total amount of polyunsaturated fatty acids
z: total amount of dimethyl acetals

Table 3. Fatty acid and dimethyl acetal composition (% of total FAME and DMA detected) of phosphatidylethanolamine of the Biceps femoris mucle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

	AG	**HO**	***p***
C16:0	11.43 ± 2.91	5.70 ± 0.79	0.003
C16:1	0.81 ± 0.51	1.20 ± 0.23	0.161
C18:0	15.67 ± 1.09	18.66 ± 0.63	0.001
C18:1 n-9	14.78 ± 2.96	11.18 ± 0.63	0.029
C18:1 n-7	2.00 ± 0.49	1.79 ± 0.14	0.376
C18:2 n-6	15.28 ± 1.22	16.10 ± 0.77	0.239
C18:3 n-3	0.54 ± 0.09	0.30 ± 0.06	0.001
C20:3 n-6	1.05 ± 0.02	1.30 ± 0.13	0.003
C20:4 n-6	19.53 ± 2.18	35.26 ± 0.77	<0.001
C20:3 n-3	0.21 ± 0.02	0.38 ± 0.11	0.007
C20:5 n-3	0.52 ± 0.14	0.85 ± 0.14	0.006
C22:1 n-9	0.26 ± 0.07	0.43 ± 0.12	0.026
C22:2	0.56 ± 0.08	0.93 ± 0.06	<0.001
C24:0	3.31 ± 0.42	2.93 ± 0.44	0.198
C22:6	0.76 ± 0.16	0.68 ± 0.05	0.289
ΣSFA[v]	28.94 ± 1.53	27.83 ± 0.59	0.172
ΣMUFA[x]	17.73 ± 1.10	14.59 ± 0.84	0.001
ΣPUFA[y]	37.25 ± 1.51	55.80 ± 0.91	<0.001
C16:0 DMA	4.07 ± 1.33	3.15 ± 1.32	0.301
C18:0 DMA	4.23 ± 1.16	1.48 ± 0.09	0.001
C18:1 DMA	3.26 ± 1.38	0.55 ± 0.13	0.002
ΣDMA[z]	12.76 ± 2.09	5.18 ± 1.24	<0.001

v: total amount of saturated fatty acids
x: total amount of monounsaturated fatty acids
y: total amount of polyunsaturated fatty acids
z: total amount of dimethyl acetals

Table 4. Fatty acid and dimethyl acetal composition (% of total FAME detected) of phosphatidylserine of the Biceps femoris mucle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

	AG	**HO**	***p***
C16:0	30.30 ± 0.77	17.57 ± 1.24	<0.001
C16:1	4.47 ± 0.77	12.79 ± 1.24	<0.001
C18:0	16.71 ± 1.12	12.33 ± 0.42	<0.001
C18:1 n-9	32.72 ± 3.14	29.99 ± 0.59	0.093
C18:1 n-7	5.33 ± 0.37	7.02 ± 0.48	<0.001
C18:2 n-6	5.30 ± 1.05	6.80 ± 0.43	0.018
C18:3 n-3	0.23 ± 0.01	0.36 ± 0.00	<0.001
C20:3 n-6	0.26 ± 0.00	0.29 ± 0.11	0.511
C20:4 n-6	1.48 ± 0.29	2.26 ± 0.57	0.025
C20:3 n-3	1.22 ± 0.82	3.46 ± 1.13	0.007
C20:5 n-3	2.69 ± 0.99	3.69 ± 0.68	0.099
C22:1 n-9	0.99 ± 0.23	1.95 ± 0.54	0.006
C22:2	2.64 ± 1.47	2.76 ± 1.24	0.897
ΣSFA[v]	47.55 ± 0.47	29.90 ± 1.58	<0.001
ΣMUFA[x]	42.67 ± 1.99	51.76 ± 1.66	<0.001
ΣPUFA[y]	13.02 ± 1.74	17.92 ± 0.17	<0.001

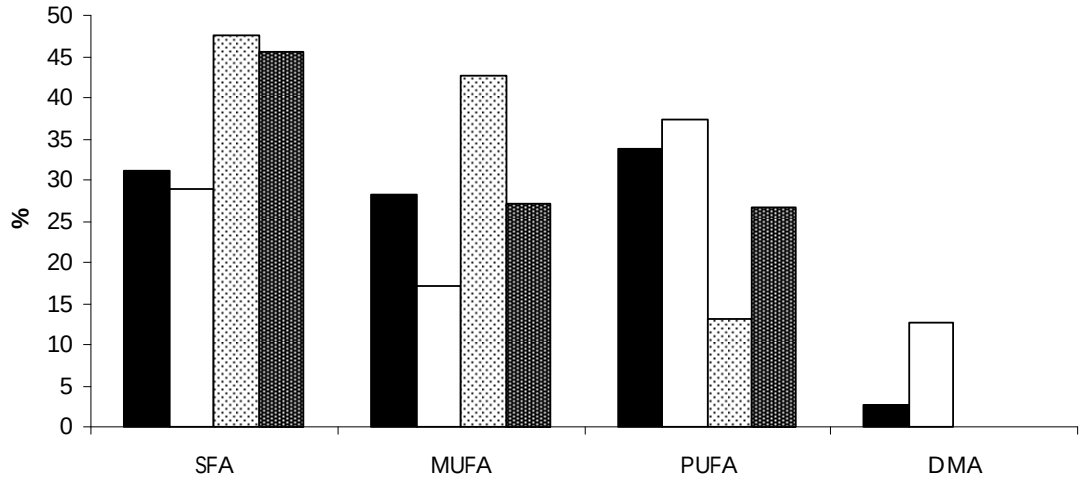
v: total amount of saturated fatty acids
x: total amount of monounsaturated fatty acids
y: total amount of polyunsaturated fatty acids
z: total amount of dimethyl acetals

Table 5. Fatty acid and dimethyl acetal composition (% of total FAME detected) of phosphatidylinositol of the Biceps femoris mucle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

	AG	HO	*p*
C16:0	18.33 ± 0.07	16.66 ± 0.89	0.020
C16:1	8.82 ± 0.65	18.47 ± 0.23	<0.001
C18:0	23.89 ± 2.13	20.19 ± 1.38	0.023
C18:1 n-9	10.98 ± 2.28	12.11 ± 2.50	0.550
C18:1 n-7	7.19 ± 0.47	10.29 ± 1.59	0.019
C18:2 n-6	11.42 ± 0.27	12.38 ± 1.57	0.352
C20:4 n-6	7.23 ± 0.06	7.04 ± 1.45	0.835
C20:5 n-3	1.46 ± 0.47	-	
C22:1 n-9	2.95 ± 1.21	-	
C22:2	5.46 ± 1.22	-	
ΣSFA[v]	41.45 ± 1.57	37.08 ± 1.66	0.011
ΣMUFA[x]	27.04 ± 6.35	41.70 ± 2.54	0.003
ΣPUFA[y]	26.71 ± 4.74	19.92 ± 1.48	0.021

v: total amount of saturated fatty acids
x: total amount of monounsaturated fatty acids
y: total amount of polyunsaturated fatty acids
z: total amount of dimethyl acetals

Figure 1. Percentage of dimethyl acetals (DMA) and saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively) in phosphatydilcholine (■), phosphatydilethanolamine (□), phosphatydilserine (▒) and phosphatydilinositol (▓) from fresh Iberian hams.



Capítulo I.5.

MRI-bases analysis, lipid composition and sensory traits for studying Iberian dry-cured hams from pigs fed with different diets

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TITLE

MRI-based analysis, lipid composition and sensory traits for studying Iberian dry-cured hams from pigs fed with different diets.

ABREVIATED RUNNING HEAD

Chemical, sensory and MRI features of Iberian dry-cured hams as affected by pig feeding

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ABSTRACT

The present work was aimed to study Iberian dry-cured hams from pigs reared outdoors and either fed with acorns and grass (AG) or with oleic acid enriched concentrates (HO), by means of their fatty acid profile, sensory traits and Magnetic Resonance Imaging (MRI). The level of total saturated fatty acids (SFA) was higher in HO than in AG hams, whereas total monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) showed higher proportions in AG than in HO Iberian dry-cured hams. Besides, lower levels of the n-6/n-3 ratio were found in AG than in HO dry-cured hams. Sensory analyses only showed differences between AG and HO Iberian dry-cured hams in pastiness (higher in AG hams, p=0.039) and marbling (lower in AG ham, p=0.002). Pig feeding also leads to differences in two computational texture features obtained by MRI-based analysis. Thus, analysis of the fatty acid profile and MRI-based analysis might be used for classifying dry-cured hams from pigs fattened with different diets.

KEY WORDS

Lipid composition, MRI, sensory features, Iberian dry-cured hams, pig feeding.

INTRODUCTION

Iberian dry-cured hams are highly rated by consumers because of their unique sensory features, which are consequence of both the characteristics of the raw material, especially lipid-related ones, and the particular processing conditions (Ventanas, Ventanas, Ruiz & Estévez, 2005). The most appreciated and expensive hams are those from Iberian pigs reared outdoors and fed on natural resources, mainly acorns and grass. However, Iberian pigs are also fed on mixed diets, which seems to imply lower quality and market acceptance (Carrapiso, Bonilla & García, 2003; Cava, Ventanas, Ruiz, Andrés & Antequera, 2000).

Nowadays, monounsaturated fatty acids (MUFA)-enriched diets, achieved through the inclusion of high oleic acid (C18:1 n-9) sunflower oil, are being used to feed Iberian pigs in order to obtain hams containing quality characteristics similar to those of hams from pigs fattened on acorn and grass and, consequently, of a higher quality than hams from pigs fed with common concentrates. The influence of MUFA enriched concentrates on the lipid composition of subcutaneous and intramuscular fat of Iberian pigs has been studied (Tejeda, Gandemer, Antequera, Viau & García 2002; Muriel, Ruiz, Ventanas & Antequera, 2002a; Muriel, Ruiz & Antequera, 2002b; Pérez-Palacios, Ruiz, Tejeda & Antequera, 2009). Besides, it has been pointed out that this feeding strategy seems to be successful for improving the sensory and technological quality of dry-cured products from Iberian pigs fed on concentrates (Muriel, Ruiz, Petrón, Andrés & Antequera, 2003; Ventanas, Estevez, Tejeda & Ruiz, 2006).

In order to regulate the market of Iberian meat products, last decade, the proportions of major fatty acid (FA) (palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids) of subcutaneous fat have been used to classify Iberian pigs as a function of their feeding background during fattening (Boletín Oficial del Estado, 2004). This procedure was useful when animals were fed on traditional concentrates, which were not enriched in MUFA. Nevertheless, with the current use of MUFA enriched concentrates for feeding Iberian pigs, this procedure for classification could not be feasible for Iberian pigs. On the other hand, it seems that other lipid components, specially arachidonic (C20:4 n-6) and linolenic acids (C18:3 n-3), and the content of γ-tocopherol seem to be suitable tools for distinguishing fresh Iberian hams from

pigs fattened with acorns and grass or with oleic acid enriched concentrate (Pérez-Palacios et al., 2009a).

In comparison with these analytical methods, Magnetic Resonance Imaging (MRI) is non-destructive, and moreover non-invasive, non-radiant and innocuous. MRI implemented with computer vision and pattern recognition techniques have been used to evaluate quality characteristics of raw and ripened products from Iberian pigs. Thus, it has been allowed the classification of Iberian loins as a function of cross-breeding (Cernadas, Antequera, Rodríguez, Durán, Gallardo & Villa, 2001) and in terms of intramuscular fat content and certain sensory attributes (Antequera, Muriel, Rodríguez, Cernadas & Ruiz, 2003) and the recognition of the *Biceps femoris* and *Semimembranosus* muscles in Iberian ham (Caro, Rodrígue, Ávila, Antequera & Palacios, 2004; Antequera, Caro, Rodríguez and Pérez, 2007). Recently, a procedure based on MRI joined to computational texture analysis also achieve the differentiation of fresh meat from Iberian pigs as a function of the feeding background (Pérez-Palacios, Ruiz, Ávila, Sánchez, & Antequera, 2009b).

The aim of this work was to study the MRI-based texture characteristics, the lipid composition and the sensory traits of dry-cured Iberian hams from pigs fed with different diets (acorn and grass vs. high oleic acid). The relationship between these variables was also examined.

MATERIAL AND METHODS

Experimental design

This study was carried out using 20 Iberian pigs divided into two groups according to the feeding during the fattening period. The group of pigs labelled AG (n=10) corresponded to those reared outdoors (on a 30 Ha-field) with free access to acorns (*Querqus ilex*, *Q. rotundifolia* and *Q. suber*) and grass. The other group of pigs (HO) (n=10) was also reared outdoors (on a 1 Ha piece of land) with free access to grass, but were fed on oleic acid enriched concentrates. After the fattening (110 days), all the pigs were slaughtered by electrical stunning and exsanguination at a local slaughterhouse, weighing an average of 160 kg. One ham was taken from each animal, and processed following the usual procedure (Antequera et al., 2007). Once finished, the *Biceps femoris* muscle of the dry-cured hams was dissected and analyzed for chemical and sensory analysis.

MRI acquisition

Magnetic resonance sequences enabled the exploration of *Biceps femoris* muscles in dry-cured Iberian hams via computer vision techniques. MRI were stored on a database acquired at the ‘‘Infanta Cristina’’ University Hospital (Badajoz, Spain). The images were routinely withdrawn by using a MRI (Philips Gyroscan NT Intera 1.5 T) scanner. The ‘‘body’’ antenna was used, according to sequences of T1 with the following parameters: 120 x 85 mm for field-of view (FOV), 20 ms for echo time (TE), 500 ms for repetition time (TR), 2 mm of thickness for slices, 90º for flip angle, 0.23 x 0.20 mm for pixel resolution, and 60 as the number of slices for each ham. There were a total of 1200 images on the database. All the images were in DICOM format, with a 512 x 512 resolution, and converted into the GIF format with the same resolution and 256 grey levels. Figure 1 shows three images of the MRI sequence for the AG and HO dry-cured hams.

Computer-Aided MRI Analysis

A software application containing three modules was used for the analysis of MRI (Figure 2). The initial module aimed to detect the *Biceps femoris* muscle

by using Active Contours according to the method described by Antequera et al. (2007). The second module consisted in the selection procedure for the Region of Interest (ROI) on each image; this selection drew up the maximum rectangular area on the muscle. The third and last module included the analysis of the ROIs by applying three common methods in computational texture analysis, which require the use of rectangular images. All three methods used matrices based on second order statistics (Cernadas, Rodriguez, Muriel & Antequera, 2005; Antequera et al., 2003). The first one, Grey Level Coocurrence Matrix (GLCM), was constructed with information of the complete ROI, and includes five features: Energy, Entropy, Haralicks Correlation, Inverse Difference Moment, and Inertia. The second one, the so-called Neighbouring Grey Level Dependence Matrix (NGLDM), gathered information from square neighbourhoods inside the ROI, providing five features: Small Number Emphasis (SNE), Long Number Emphasis (LNE), Number Nonuniformity (NNU), Second Moment (SM) and Entropy (ENT). The third one, the Grey Level Run Length Matrix (GLRLM), only accounted for information about lineal segments of the ROI and it gave five features: Long Run Emphasis (LRE), Short Run Emphasis (SRE), Grey Level Nonuniforminty (GLNU), Run Length Nonuniformity (RLN) and Run Percentage (RPC).

Fatty acid methyl ester preparation and analysis

Lipids were extracted with chloroform:methanol (2:1, v/v) according to the method described in Pérez-Palacios, Ruiz, Martin, Muriel and Antequera (2008). Fatty acid methyl esters (FAMEs) obtained from lipid tissues were assembled by transesterification in the presence of sodium metal (0.1 N) and sulphuric acid within methanol (Sandler & Karo, 1992). FAMEs were analysed by gas chromatography, using a Hewlett-Packard HP-5890-II gas chromatograph, equipped with an on-column injector and a flame ionization detector (FID). Separation was done on a polyethylenglycol capillary column (60 m long, 0.32 mm id, 0.25 mm film thickness) (Supelcowax-10; Supelco, Bellafonte, PA, USA) maintained at 230 ºC for 60 min. The injector and detector temperatures were kept at 230 ºC. The carrier gas was nitrogen, at a flow rate of 0.8 ml/min rate. The individual compounds were identified as a result of the comparison made

between their retention times and standard retentions (Sigma, St. Louis, MO, USA).

Sensory analysis

Dry-cured hams of this work were assessed by a trained panel of fourteen members. Eighteen sensory attributes of dry-cured Iberian (Ruiz, Ventanas, Cava, Timón & García, 1998) ham grouped in appearance and texture of subcutaneous fat (yellow and pink colour, hardness and oiliness), appearance and texture of lean (red colour, brightness, marbling, hardness, juiciness and pastiness), taste (salty, sweet and bitter), aroma (odour intensity) and flavour (intensity, cured, rancid and persistence) were analyzed. Analyses were developed in tasting rooms with the conditions specified in UNE regulation. All sessions were conducted at room temperature in a sensory room equipped with white fluorescent lighting. The software used to record scores in the sensory sessions was FIZZ Network (version 2.20: Biosystemes, France). The hams were cut into 1.5 mm thick slices, with a slicing machine. Slices were served on plates to panellists. The panel sessions were held mid-morning, about 4 h after breakfast. Panellists evaluated the different sensory traits by means of a quantitative-descriptive analysis in a non structured scale 0-10. Three samples randomly presented to the panellist were analyzed in each session. About 200 ml of water at room temperature was provided to the panellists. In each session, the panel average for each sample was recorded.

For the acceptability sensory study, untrained (243) subjects (consumers) chosen at random rated the Iberian dry-cured hams following a mixed hedonic scale (very good, good, average, poor and very poor) in a sensorial test carried out in accordance with UNE 87004 (1979).

Statistical analysis

The statistical application used was the one-way analysis of variance (ANOVA). A Principal Component Analysis (PCA) was applied to evaluate the relationships between the computational texture characteristics obtained, the lipid composition and the sensory traits. Data from the acceptability test were analysed using the Friedman non-parametric statistic test, in order to find out whether consumers considered AG and HO samples different at each hedonic

scale rank. Analyses were done by using the SPSS package (v.15.0) and the Unscramble (CAMO, V.9.2).

RESULTS AND DISCUSSION

MRI-based analysis

The computational analysis of the images showed statistical differences as a function of pig feeding in the texture features Haralicks Correlation and Inertia (of the GLCM method). AG hams had higher values of Haralicks Correlation and lower of Inertia than HO ones, whereas there were no differences in the characteristics of the NGLDM and GLRLM methods (Table 1). These two computational texture features are related to the homogeneity of the images (Tamura, Mori & Yamawaki, 1978). Thus, it seems that ham identification from Iberian pigs fed different diets could be achieved by MRI-bases analysis. In the *Semimembranosus* muscle from fresh Iberian hams, most computational texture characteristics showed significant differences between HO and AG hams (Pérez-Palacios et al., 2009b). Thus, it could be pointed out the notable influence of the ripening process, leading to a decrease in the initial differences in computational texture features.

Fatty acid profile

The FA composition of the intramuscular lipids from AG and HO dry-cured Iberian hams is shown in Table 2. Total saturated FA (SFA) were significantly (p=0.004) higher in HO than in AG cured hams, which was due to the levels of miristic (C14:0), palmitic (C16:0) and stearic acids (C18:0). Total monounsaturated FA (MUFA) as well as oleic acid (C18:1 n-9) showed higher proportions in AG hams than in HO ones. With respect to polyunsaturated FA (PUFA), linoleic (C18:2 n-6), linolenic (C18:3 n-3), eicosatrienoic (C20:3 n-3) and eicosapentanoic (C20:5 n-3) acids and consequently total PUFA had statistically higher levels in AG than in HO cured hams. However, Ventanas, Ventanas, Tovar, García and Estévez (2007a) only found significant higher levels of linolenic (C18:3 n-3) and eicosatrieonic (C20:3 n-3) acids in dry-cured hams from pigs fattened on acorns and grass than in those from pigs reared indoors and fed with high oleic acid enriched concentrates. In comparison with fresh Iberian hams (Pérez-Palacios et al., 2009a), *Biceps femoris* muscle of dry-cured hams showed more differences in the fatty acid composition between AG and

HO groups. Thus, in dry-cured Iberian hams there were differences in SFA, MUFA and PUFA but not in fresh ones.

There were significant higher values of the sum of both *n-6* and *n-3* fatty acids in AG (8.27 and 0.95, respectively) than in HO dry-cured hams (7.25 and 0.38, respectively), whereas the *n-6*/*n-3* ratio was statistically (p<0.001) lower in AG (8.89) than in HO dry-cured hams (19.10). This is likely the consequence of the high content of linolenic acid (C18:3 n-3) in the grass consumed by AG Iberian pigs (Pérez-Palacios et al., 2009a). Muriel et al. (2002a) and Ventanas et al. (2007a) also showed higher levels of n-3 fatty acids and lower *n-6/n-3* ratio in dry-cured Iberian hams from pigs reared outdoors fattened on acorns and grass than in those hams from Iberian pigs fed in confinement with enriched oleic acid concentrates.

Sensory analysis

Figure 3 shows mean scores for appearance and texture of subcutaneous fat (Figure 3.a), appearance and texture of lean (Figure 3.b), and taste and flavour from AG and HO dry-cured Iberian hams (Figure 3.c). There were significant differences (p<0.05) in the appearance and texture of the lean between the studied batches. Unexpectedly, AG dry-cured Iberian hams had higher scores for pastiness and lower for marbling than HO ones. However, Ventanas, Ventanas and Ruiz (2007b) did not find differences in sensory features between dry-cured Iberian loins from pigs fattened on acorns and grass with those from pigs reared indoors and fed with high oleic acid enriched concentrates.

García-Garrido, Quiles-Zafra, Tapiador and Luque de Castro (2000) found higher moisture content in pasty hams than in those not showing defective texture, but this was not likely the reason for the found differences in pastiness in the present study, since there were no differences in moisture content between the studied batches of Iberian hams. Nevertheless, others studies in Iberian hams have not shown the influence of moisture on pastiness (Andrés, Cava, Ventanas, Thovar & Ruiz, 2004) neither on other texture characteristics (Ruiz-Carrascal, Ventanas, Cava, Andrés & García, 2000). In Parma hams, defective texture pieces were found to contain increased peptide and free amino acid concentrations (Parolari, Rivaldi, Leonelli, Bellati & Bovis, 1988), which has been

related to increased cathepsin B activity values (Virgili, Parolari, Schivazappa, Soresi Bordini & Borri, 1995). Moreover, Enfält et al. (1993) have recorded higher enzymatic activities in exercised pigs when compared to non-exercise ones.

Previous papers have shown either a higher marbling of Iberian hams from pigs fattened on acorn and grass than in those from pigs fed on concentrates (González & Tejeda, 2007) or similar values (Ventanas et al., 2007a), mainly due to the higher IMF content of the formers, which has been shown as the main factor determining the marbling degree (Muriel, Ruiz, Martin, Petrón & Antequera, 2004; Ruiz-Carrascal et al., 2000). In the present study, hams from Iberian pigs fed HO diets showed higher marbling scores than AG ones. However, there were not statistical differences in the levels of IMF between AG and HO Iberian dry-cured hams (Table 2). On the other hand, appearance attributes are affected by the spatial distribution of intramuscular fat streaks (Cernadas, Durán & Antequera, 2002).

Results from the acceptability analysis are shown in Figure 4. Most consumers considered AG and HO Iberian hams to be between average and good. Ventanas et al. (2007a) found that the use of high oleic acid concentrates for feeding Iberian pigs was nearly to attain the quality characteristics of Iberian dry-cured hams from Iberian pigs fed acorns and grass. These authors found lower IMF levels in hams from pigs fed in confinement with high oleic acid concentrates, which they considered a serious drawback, since IMF influences essential sensory features of Iberian hams. However, there were not statistical differences in IMF between HO and AG Iberian dry-cured hams of the present study.

Relationships between MRI texture feature, lipid composition and sensory traits in dry-cured Iberian hams.

A PCA test (Figure 5) was run to measure the relationship between MRI computational texture features, physical-chemical composition and sensory traits in the two groups of dry-cured Iberian hams. Figure 3a shows the similarity map of the measured parameters defined by two principal components (PC1 and PC2) accounted for 38% and 21%, respectively, of the total variance. Linoleic (C18:2 n-6), linolenic (C18:3 n-3), eicosatrienoic (C20:3 n-3), eicosapentanoic (C20:5 n-3) and nervonic (C24:1) acids and the sum of n-3 FA

showed high negative values for PC1 and were found close to PUFA, pastiness and the computational texture feature GLNU. On the opposite side, miristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic acid (C18:0), vaccenic (C18:1 n-7) and eicosanoic (C20:0) acids, SFA, lean hardness and flavour and odour intensity featured high values for PC1 and were close to flavour persistency and marbling.

The projection of the samples onto the areas where the main components were found is shown in Figure 3b. AG hams were located on the upper, close to the positive axis of PC1, and lower left quadrants. These locations correspond to oleic (C18:1 n-9), linolenic (C18:3 n-3), eicosatrienoic (C20:3 n-3) and eicosapentanoic (C20:5 n-3), nervonic (C24:1) and linoleic (C18:2 n-6) acids, MUFA, sum of n-3 FA, pastiness and the computational texture features GLNU, LNE and SM. Thus, it could be pointed out that AG dry-cured hams are defined preferentially by n-3 FA. In contrast, HO samples were found on the opposite side of the graph, at the upper and lower right quadrants, defined by myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic acid (C18:0), vaccenic (C18:1 n-7) and eicosanoic (C20:0) acids, SFA, lean hardness, flavour and odour intensity, marbling and the computational texture feature Inertia.

In comparison with others similar works, in this study there were found more differences in the fatty acid composition and sensory traits, which could be due to other variables such as availability of acorn and grass or pig exercise. Linolenic (C18:3 n-3) and eicosatrieonic (C20:3 n-3) acids showed statistical differences in the present and also in the previous works (Muriel et al., 2002a; Ventanas et al., 2007a), which seems to indicate that these fatty acids could be useful means for differentiating dry-cured hams from pigs fattened different diets, acorn and grass versus oleic acid enriched concentrates.

CONCLUSIONS

The computational texture features of MRI and the fatty acid profile could be useful tools for distinguishing dry-cured hams from pigs fattened different diets (acorn and grass vs. high oleic acid concentrates). On the other hand, these two pig feedings lead to scarce differences in sensory traits.

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Table 1. Values of MRI-based texture characteristics for dry-cured hams from Iberian pigs fattened with different diets: acorn and grass (AG) and high oleic acid enriched concentrates (HO).

		AG	HO	*p*
GREY LEVEL COOCURRENCE MATRIZ (GLCM)	Energy	$7.10^{-4} \pm 5.10^{-5}$	$7.10^{-4} \pm 1.10^{-4}$	0.55
	Entropy	3.31 ± 0.04	3.35 ± 0.09	0.40
	Haralicks Correlation	$7.10^{-4} \pm 6.10^{-5}$	$5.10^{-4} \pm 9.10^{-5}$	0.03
	Inverse Diference Moment	0.06 ± 1.10^{-3}	0.06 ± 4.10^{-3}	0.08
	Intertia	679.90 ± 62.56	874.11 ± 162.29	0.04
NEIGHBOURING GREY LEVEL DEPENDENCE MATRIX (NGLDM)	SNE[a]	3.18 ± 0.60	2.64 ± 0.46	0.14
	LNE[b]	6.86 ± 1.30	5.61 ± 0.82	0.11
	NNU[c]	12876.34 ± 4007.66	8858.44 ± 3260.62	0.12
	SM[d]	298.22 ± 88.54	193.70 ± 53.23	0.05
	ENT[e]	-6.49 ± 1.44	-5.00 ± 0.96	0.09
GREY LEVEL RUN LENGTH MATRIX (GLRLM)	LRE[f]	1.09 ± 3.10^{-3}	1.09 ± 7.10^{-3}	0.81
	SRE[g]	0.98 ± 8.10^{-4}	0.98 ± 1.10^{-3}	0.74
	GLNU[h]	95.68 ± 18.23	74.96 ± 8.31	0.05
	RLNU[i]	3720.49 ± 681.24	3097.44 ± 506.13	0.14
	RPC[i]	0.97 ± 1.10^{-3}	0.97 ± 2.10^{-3}	0.78

[a]SNE: small number emphasis; [b]LNE: large number emphasis; [c]NNU: number nonuniformity; [d]SM: second moment: [e]ENT: entropy; [f]LRE: long run emphasis; [g]SRE: short run emphasis; [h]GLNU: gray level nonuniformity; [i]RLNU: run length nonuniformity; [i]RPC: run percentage
$p < 0.05$: means differ significantly

Table 2. Moisture (expressed as g/100g muscle), lipid content (expressed as g/100g muscle dry matter) and fatty acid composition (% total of FAME detected) of intramuscular lipids of dry-cured hams from Iberian pigs fed with different diets: acorn and grass (AG) and oleic acid enriched concentrates (HO).

	AG	**HO**	*p*
Moisture	53.84 ± 0.86	51.92 ± 2.13	0.147
Lipid content	14.14 ± 2.05	16.81 ± 3.44	0.189
C14:0	0.91 ± 0.05	1.21 ± 0.02	<0.001
C14:1	0.06 ± 0.00	0.05 ± 0.01	0.055
C16:0	18.94 ± 1.02	20.80 ± 0.59	0.008
C16:1	2.59 ± 0.18	3.47 ± 0.21	<0.001
C18:0	9.54 ± 0.71	11.55 ± 0.77	0.003
C18:1 n-9	53.25 ± 0.99	48.84 ± 1.76	0.001
C18:1 n-7	3.70 ± 0.25	4.71 ± 0.35	0.001
C18:2 n-6	6.96 ± 0.49	5.75 ± 0.27	0.001
C18:3 n-6	0.05 ± 0.01	0.05 ± 0.00	0.177
C18:3 n-3	0.64 ± 0.17	0.27 ± 0.03	0.002
C20:0	0.16 ± 0.02	0.24 ± 0.02	<0.001
C20:1	1.09 ± 0.11	1.07 ± 0.11	0.723
C20:2	0.30 ± 0.02	0.27 ± 0.02	0.065
C20:3 n-6	0.15 ± 0.03	0.16 ± 0.04	0.842
C20:4 n-6	1.10 ± 0.14	1.29 ± 0.36	0.301
C20:3 n-3	0.14 ± 0.02	0.06 ± 0.00	<0.001
C20:5 n-3	0.12 ± 0.01	0.04 ± 0.01	<0.001
C22:0	0.01 ± 0.00	0.02 ± 0.01	0.021
C22:1 n-9	0.03 ± 0.00	0.03 ± 0.01	0.059
C24:0	0.21 ± 0.05	0.16 ± 0.04	0.096
C22:6 n-3	0.01 ± 0.00	0.01 ± 0.01	0.849
C24:1	0.04 ± 0.01	0.02 ± 0.01	0.014
SFA[a]	29.40 ± 1.47	33.34 ± 1.59	0.004
MUFA[b]	60.76 ± 1.03	58.18 ± 1.77	0.023
PUFA[c]	9.44 ± 0.86	7.91 ± 0.48	0.008
Σ n-6	8.27 ± 0.58	7.25 ± 0.46	0.016
Σ n-3	0.95 ± 0.13	0.38 ± 0.02	<0.001
n-6 / n-3	8.89 ± 1.16	19.10 ± 1.47	<0.001

Values are means (expressed as percentage of total fatty acid methylesters) ± standard error of the mean.
[a]SFA: total amount of saturated fatty acids
[b]MUFA: total amount of monounsaturated fatty acids
[c]PUFA: total amount of polyunsaturated fatty acids
$p < 0.05$: means differ significantly

Figure 1. MRI-based sequence of three Iberian dry-cured hams from pigs fattened with different diets: high oleic acid enriched concentrates (a, b, c) and acorn and grass (d, e, f).

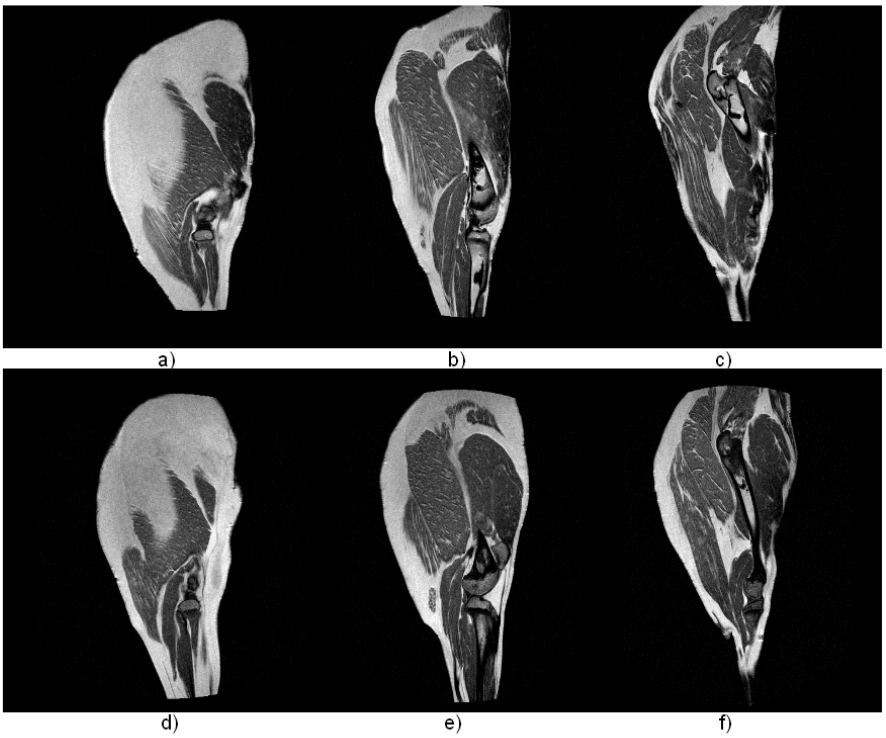


Figure 2. Processing modules within the computer-aided MRI analysis of Iberian hams: a) muscle detection; b) region of interest selection; c) region of interest analysis by using three different methods of texture features.

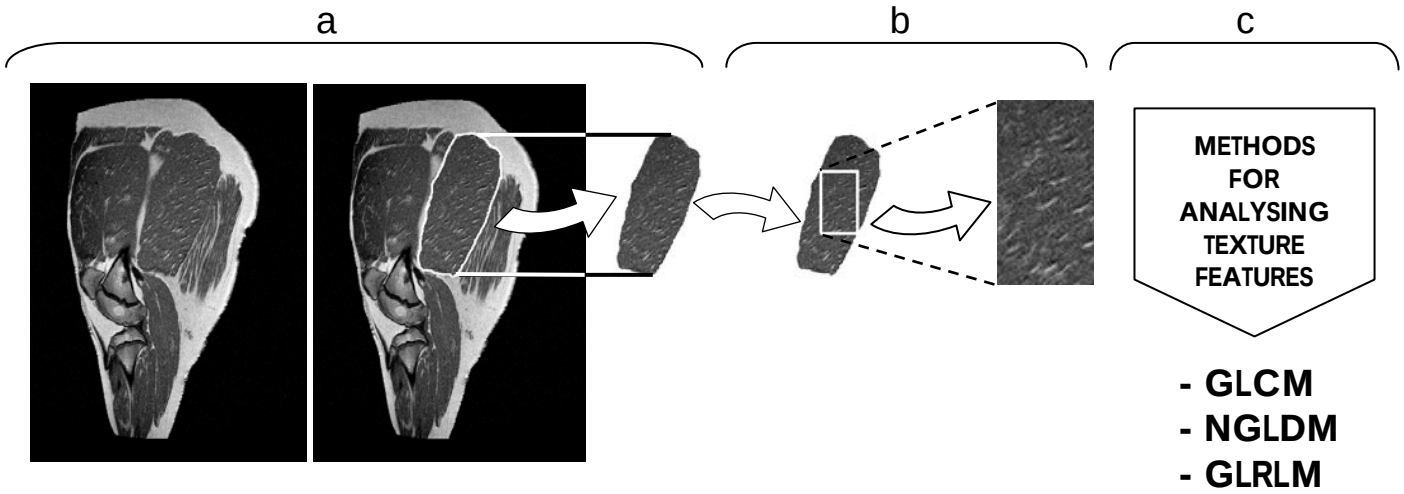
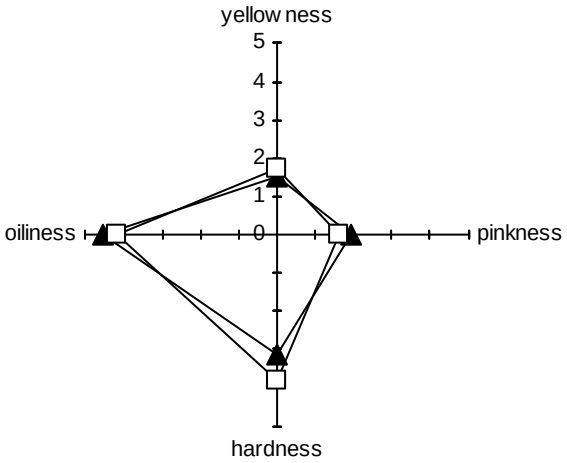
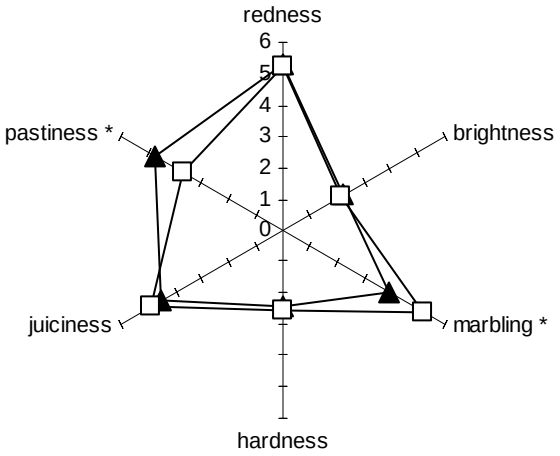


Figure 3. Sensory analysis of dry-cured Iberian hams from pigs fattened with different diets: acorn and grass (▲) and oleic acid enriched concentrates (□). 1a., appearance and texture of ham backfat; 2b., appearance and texture of lean; 2c., taste and flavour.
* significative effect ($p<0.05$)

a



b



c

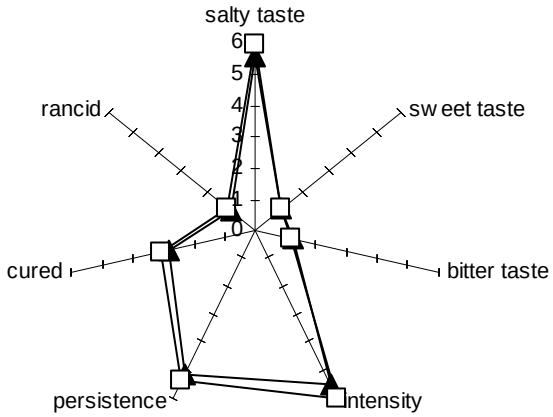


Figure 4. Acceptability analysis of dry-cured Iberian hams from pigs fattened with different diets: acorn and grass (□) and oleic acid enriched concentrares (■).

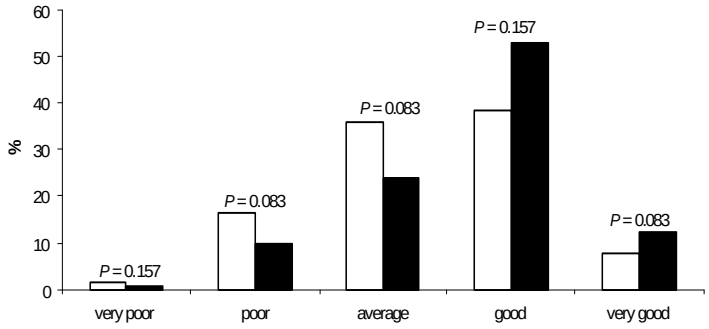
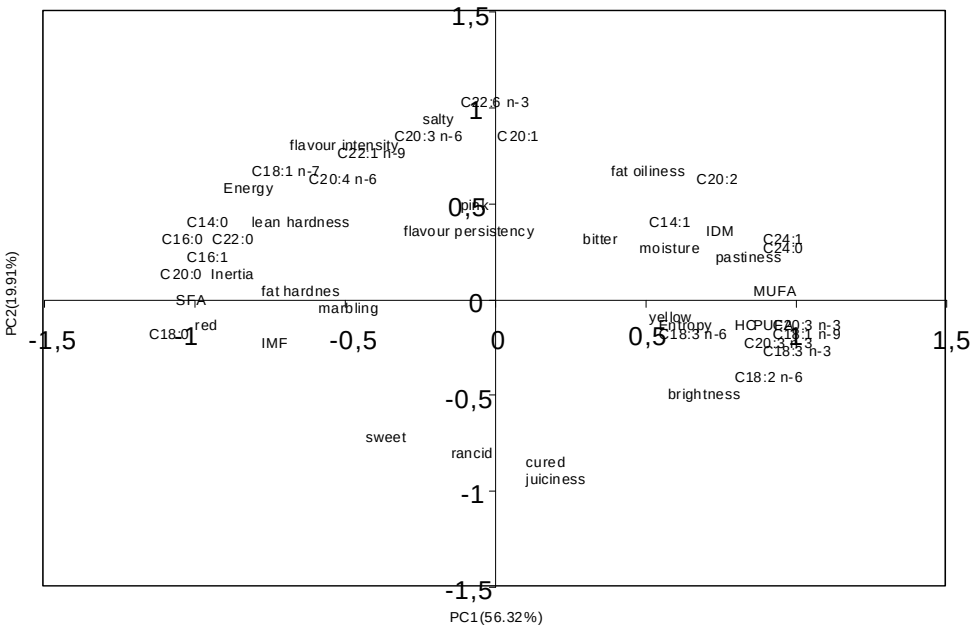
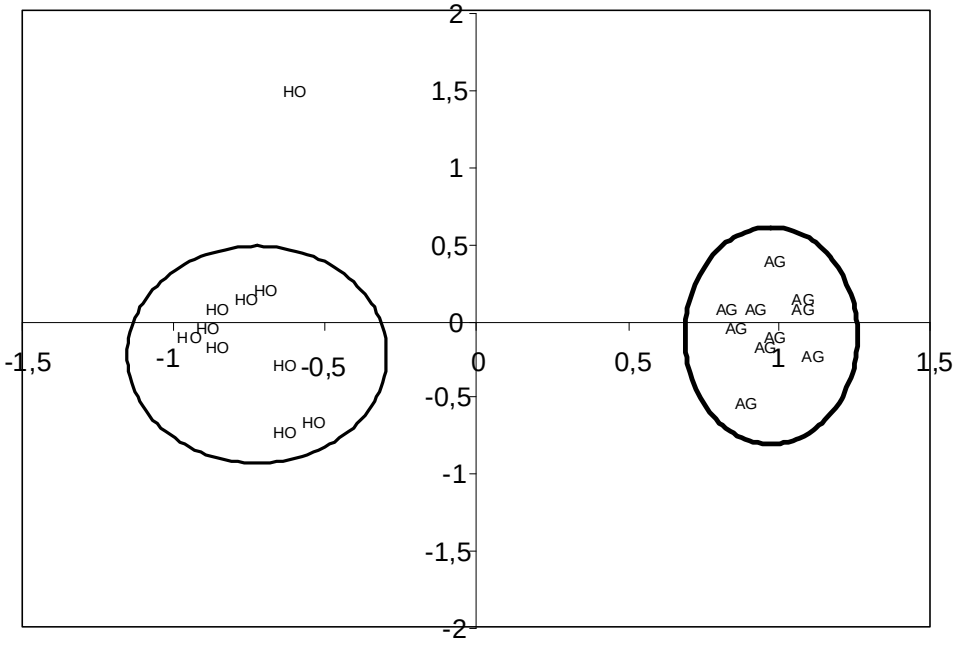


Figure 5. Principal component analysis: MRI-based texture characteristic, physical-chemical composition and sensory traits of Iberian dry-cured hams: (a) variables plot, (b) score plot of Iberian pigs fattened with different diets. (AG: acorns and grass; HO: high oleic acid enriched concentrates).

a



b



Capítulo II.1.

Influence of pre-cure freezing Iberian ham on lipolytic changes and lipid oxidation

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TITLE

Influence of pre-cure freezing of Iberian hams on lipolytic changes and lipid oxidation

RUNNING TITLE

Lipolysis in pre-cure frozen Iberian hams

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KEY WORDS

pre-freezing, lipolysis, oxidation, evolution, Iberian ham

ABSTRACT

The purpose of this work was to study the influence of pre-cure freezing of Iberian hams on lipolysis and lipid oxidation during the ripening of the dry-cured meat product. At the green stage, the levels of fatty acids (FA) in the free fatty acids (FFA) fraction were higher in pre-cure frozen (F) than in refrigerated (R) hams, whereas in the polar lipid (PL) fraction, FA and dimethyl acetals (DMA) values were higher in R than in F Iberian hams. These results point out the existence of lipolysis phenomena during the freezing storage. At the end of post-salting and at the final stage, both R and F hams showed similar FA and DMA profiles. The effect of pre-cure freezing of hams influenced lipolysis evolution throughout the processing. The development of lipid oxidation was similar in R and F hams, but TBARS values were significantly higher in F hams at the initial stage and at the end of post-salting and drying steps. Despite all these differences, at final stage the FA and DMA composition as well as TBARS levels were quite similar in R and F hams.

INTRODUCTION

Meat products from Iberian pigs are the most valuable in the market, both in Spain and several other countries, due to their exceptional sensory attributes (García el al., 1996). These are a consequence, in part due to the lipolytic and oxidative phenomena that take place during the ripening process (Antequera, et al., 1992; Andrés et al., 2005). Lipolytic processes constitute the first step to free fatty acid (FFA) auto-oxidation, which in turn gives rise to numerous volatile compounds, which are responsible to a large extent for the Iberian dry-cured ham flavour features (García et al., 1991; Ruiz et al., 1998; Andrés et al., 2007). In addition, lipolysis also leads to changes in the fatty acid (FA) composition of the different lipid fractions, particularly polar lipids (PL) (Andrés et al., 2005). Fatty acids released during the processing in Iberian ham come mainly from this fraction (Martín et al., 1999; Andrés et al., 2005). The rate and extension of the lipolysis that takes place during the ripening of Iberian ham is related to the processing conditions and the features of the raw material (Martín et al., 1999; Andrés et al., 2005).

Freezing of raw thighs and frozen storage of the raw material before Iberian dry-cured ham processing is not a common strategy. Dry cured ham producers believe that this procedure leads to lower quality dry cured Iberian hams. The Specific Designation of Origin that protects the production of dry-cured Iberian hams, specifically exclude freezing as a procedure for raw material storage. Nevertheless, there is not scientific evidence that pre-cure freezing has any effect on the quality of the derived Iberian dry-cured meat products. Such procedure could show several technological and economical advantages, such as the possibility of salting hams with more homogeneous weights or the processing of hams to avoid the fluctuations in seasonal availability and market price.

Freezing could promote changes in physical (drip loss, texture modifications), chemical (lipolysis and FA oxidation, protein denaturation and aggregation, changes in colour), and sensory properties of meat, depending on the characteristics of the fresh meat, further processing of the meat, and frozen conditions (Carballo & Jiménez, 2001). During frozen storage, the oxidation processes are slowed, but not completely hindered. Some lipid soluble radicals may even be more stable at the lower temperature and

thereby propagate oxidation (Kanner, 1994). As far as lipolityc reactions are concerned, products from enzymatic reactions (lipase-catalyzed hydrolysis) have been found in frozen samples (Parducci & Fennema, 1978).

Although there are numerous reports of the influence of freezing on several parameters related to meat quality, only a few studies have been done to show the effect of using frozen and subsequently thawed raw material on the quality of dry-cured hams (Arnau et al., 1994; Motilva et al., 1994; Bañón et al., 1999; Wang, 2001; Flores et al., 2006). We have not found any such report on Iberian hams in the scientific literature.

In dry-cured hams processed using frozen and subsequently thawed raw pieces, salt penetration is favoured, which has been related to the higher free water content of thawed hams as compared to fresh ones (Bañón et al., 1999; Wang, 2001). This appears to increase the amount of solubilized salt on the surface of the ham, which is the main factor regulating its diffusion to inside the piece (Sorheim & Gumpen, 1986). Thus, the salting time for thawed hams is shorter than that used for fresh ones (Poma, 1987; Bañón et al., 1999).

Studies have not found differences at the end of ripening in lipolysis, colour, sensory features and acceptability scores between dry-cured hams produced using fresh and thawed raw materials (Motilva et al., 1994; Bañón et al., 1999). Other results have shown that pre-cure freezing increased the proteolysis phenomena throughout the ripening (Bañón et al., 1999; Flores et al., 2006) and the levels of FFA and volatile FA (Flores et al., 2006; Wang, 2001). As a consequence, dry-cured hams from thawed raw material usually show a high incidence of white precipitates, formed mainly by tyrosine crystals (Arnau et al., 1994; Bañón et al., 1999).

The aim of this work was to study the influence of pre-cure freezing of Iberian hams on the lipolyitic changes of different intramuscular lipid fractions and on the lipid oxidation during the ripening.

MATERIAL AND METHODS

Experimental design

Twenty-four hind limbs were obtained from Iberian pigs fattened in confinement and fed with a commercial diet. These hind limbs were divided into two groups, refrigerated (R) and pre-cure frozen (F) hams. The F hams were frozen (at -20 ºC) and thawed (4 days at 3-4 ºC) three months later. The R hams were obtained later from pigs of the same breeding and fed the same diet as those used for obtaining the F hams, but slaughtered two days before the frozen hams were totally thawed. These R hams were kept at 4 ºC during 36-48 hours until processing. Six hind limbs of each group were used to obtain the data for the green stage. The others were processed for dry-cured hams. The processing conditions were the same for the two groups of hams, except for the salting time, which was 1 day/kg for the R hams and 0.7 day/kg for the F hams. After salting, the salt from the surface was brushed and, the hams were processed as follows. Iberian hams were held at 4-8 ºC and 73-75% relative humidity for 70 days (post-salting step). During the drying stage the hams were kept in a room under controlled conditions for 120 days, temperature was increased from 8 to 20 ºC, while relative humidity was progressively reduced to 64%. Finally, hams were left to mature for 16 months (cellar stage) at 20-25 ºC and relative humidity 55-65%.

Sampling

Sampling was carried out at the green stage (raw meat) and at the end of each processing step. Samples taken at the end of salting, post-salting and drying steps were obtained by extracting a cylindrical piece of ham (10 x 2.5 cm) using a stainless steel tube with a cutting edge. These samples mainly included the *Biceps femoris* muscle. Samples taken at the beginning and at the end of processing were obtained by dissecting the *B. femoris* muscle of each ham. Samples were vacuum-packaged and kept frozen at -80 ºC until analysed. Lipolytic changes were studied on the samples taken at the green stage, at the end of post-salting and at the end of the processing, while for the study of the lipid oxidation, samples taken at the five steps were used. Sodium chloride content was analyzed at the end of salting and in the final product.

Intramuscular fat extraction

Samples were ground using a commercial grinder immediately before fat extraction. Intramuscular total lipids were extracted with chloroform/methanol (2:1, vol/vol), according to the method described in Perez-Palacios et al (2008).

Fractionation of lipids

Lipid extracts were separated into lipid classes in NH_2-aminopropyl minicolumns (500 mg) (Varian Corp., Harbor City, CA, USA), following the method described by Ruiz et al (2004). Briefly, minicolumns were activated with hexane (7.5 ml). Lipids (10 mg) dissolved on 150 ml of hexane/chloroform/methanol (95:3:2, vol/vol/vol) were loaded onto the column. Neutral lipids (NL) were eluted with 5ml of chloroform, FFA with 5 ml of diethyl ether:acetic acid (98:2, vol/vol) and 2.5 ml of methanol:chloroform (6:1) following by 2.5 ml of sodium acetate in methanol:chloroform (6:1) were employed to elute the PL fraction.

Fatty acid methyl esters (FAME) and dimethyl acetals (DMA) preparation and analysis

FAME from acyl chains and DMA from alkenyl chains were prepared by acidic trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992). FAME were analyzed by gas chromatography, using an Agilent 6890N gas chromatograph, equipped with a flame ionization detector (FID). Separation was carried out on a polyethyleneglycol capillary column (60 m long, 0.32 mm id, and 0.25 mm film thickness) (Supelcowax-10, Supelco, Bellafonte, PA). Oven temperature programming started at 180 °C. Immediately, it was raised 5 °C min^{-1} to 200 °C, held for 40 min at 200 °C, increased again at 5 °C min^{-1} to 250 °C, and held for the last 21 min at 250 °C. Injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO). To confirm identification, selected samples were subjected to gas chromatography coupled to mass spectrometry (GC-MS) in a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector (HP-5971 A, Hewlett-Packard). FA and DMA were separated using the same

column as that used for GC-FID, with helium operating at 41.3 KPa of column head pressure, resulting in a flow of 1.45 ml min^{-1} at 180 °C. The injector and oven program temperatures were the same as for the GC-FID analysis. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of 1 scan s^{-1} over the m/z range of 30-500. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries (Wiley 7/NIST 05).

Measurement of lipid oxidation

Thiobarbituric acid-reactive substances (TBARS) were measured by following the extraction method described by Salih et al (1978). Sample (2.5 g) was homogenized for 2 min with 7.5 ml of 3.86% perchloric acid and 0.5 ml of butylated hydroxytoluene. Tubes were kept in ice to avoid heat degradation. This homogenate was filtered and centrifuged (3 min, 3500 rpm). The supernatant (2 ml) was mixed with 2 ml of 97% 1,1,3,3-tetraethoxypropane (TEP). Immediately, the mixture was heated to 90º C for 30 min, cooled and centrifuged again (2 min, 3500 rpm). Absorbance was measured at 532 and 600 nm on a spectrophotometer (Hitachi U-2000, Tokio). The measurement at 600 nm is considered contamination and it was subtracted to the other measurement to obtain the final absorbance. The concentration of malonaldehyde (MDA) was calculated from a standard curve, which was developed simultaneously with the samples using solutions of TEP (Merck, Schchardt). TBARS were expressed as mg MDA kg^{-1} muscle.

Determination of the sodium chloride content

The analysis of the sodium chloride content of was performed according to the Association of Official Analytical Chemists (AOAC, 2000): Carpentier-Volhard method (reference 971.19).

Statistical Analysis

The effect of pre-cure freezing of Iberian hams on the fatty acid composition from intramuscular lipid fractions and TBARS during the processing were analysed by one-way analysis of variance (ANOVA) using the General

Linear Model of SPSS (v.15.0). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test.

RESULTS

The effect of pre-cure freezing of Iberian hams on FA composition (expressed as mg FAME g^{-1} of intramuscular fat (IMF)) in NL, FFA and PL was studied at three different stages of the process: at the green stage, at the end of the post-salting phase and at the end of the processing. Fat content of studied hams ranged between 17.44 % DM for R hams and 16.12 % DM for F ones, showing no significant differences.

Table 1 shows the FA composition of NL of R and F Iberian hams throughout the ripening process. At the green stage, no significant differences were found between F and R hams in most detected FA, nor in the total SFA, MUFA and PUFA. The same trend was observed at the end of post-salting and in the final product. With respect to the changes seen in the different fatty acids in this lipid fraction due to the process, no significant differences were found in the fatty acids between the two groups of hams in this study.

The FA composition of FFA from R and F Iberian hams is shown in Table 2. Initially, at green stage, significant differences were found for MUFA (p=0.041), being higher in F (11.21 mg/g IMF) than in R hams (8.32 mg/g IMF). However, individual MUFA did not show statistical differences. At the end of the post-salting phase, there were not significant differences in any of the FA of the FFA fraction. At the final stage, SFA were statistically lower (p=0.014) in F (20.93 mg/g IMF) than in R hams (26.86 mg/g IMF), due to the amount of palmitic (C16:0) and stearic (C18:0) acids. Throughout the ripening process, most FA in the FFA fraction of R hams showed a significant increase. Thus, the levels of SFA, MUFA and PUFA rose statistically (p<0.001) from the green stage (10.47, 8.32 and 6.23 mg/g IMF, respectively) to the end of post-salting (14.06, 11.05, and 12.47 mg/g IMF, respectively) and over again to the end of processing (26.86, 31.86 and 16.47 mg/g IMF, respectively). Fatty acid evolution of FFA from F hams did not follow exactly the same trend as in R hams. SFA, MUFA and PUFA rose significantly (p=0.014, p<0.001 and p=0.049, respectively) along the processing in R Iberian hams whereas in F hams these sums did not vary from the green stage (14.20, 11.21, and 10.85 mg/g IMF, respectively) to the end of post-salting (14.07, 10.36 and 11.67 mg/g IMF, respectively). Nevertheless, there happened to be an increase until the end of processing (20.93, 26.60 and 19.02 mg/g IMF, respectively). Thus, only six FA showed a significant increase throughout the

processing (palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6), linolenic (C18:3 n-3) and eicosenoic (C20:1) acids) in F hams while most FA detected statically increased during the processing in R hams.

Table 3 shows the FA composition of PL from R and F Iberian hams during the processing. At green stage, values for SFA, MUFA and PUFA were significantly higher (p=0.034, p=0.023 and p=0.037, respectively) in R hams (20.96, 18.05 and 29.72 mg/g IMF, respectively) than in F hams (15.41, 14.25 and 20.13, respectively). However, there were not significant differences between R and F Iberian hams in the FA composition of PL neither at the end of the post-salting step nor at the final stage. During the processing of both R and F Iberian hams of this study, a decrease in the quantity of total FA of PL was observed. Nevertheless, similarly to the FFA fraction, the two groups of hams did not follow the same trend. In R hams, SFA, MUFA and PUFA significantly decreased (p<0.001) from the green stage (20.96, 18.05 and 29.72 mg/g IMF, respectively) to the end of post-salting (14.31, 13.59 and 18.13 mg/g IMF, respectively) and again to the final stage (11.42, 11.16 and 10.89 mg/g IMF, respectively). F hams also showed in SFA, MUFA and PUFA a statistical decrease (p=0.040, p=0.023 and p=0.013) mainly from the end of post-salting (16.36, 15.00 and 18.04 mg/g IMF, respectively) to the final stage (11.09, 10.89 and 10.90mg/g IMF, respectively).

The DMA composition of PL of R and F Iberian hams throughout the process is shown in Table 4. Four DMA were identified in the two groups of hams. The most abundant was hexadecanaldimethylacetal (C16:0 DMA), which agrees with previous data concerning muscle from other mammals (Pérez-Palacios et al., 2007; Pérez-Palacios et al., 2006). Higher levels of DMA were found in R (14.39 mg/g IMF) than in F (7.59 mg/g IMF) hams at the green stage, while there were no statistical differences at the end of the post-salting and the final stages. With regard to the DMA evolution throughout the ripening, there was an observed decrease in R hams, mainly from the initial stage (14.39 mg/g IMF) to the end of post-salting (8.41 mg/g IMF) and a subsequent steady state until the end of ripening, while in F hams the DMA composition did not vary with the processing.

The influence of pre-cure freezing of Iberian hams on lipid oxidation (measured as TBARS and expressed as mg MDA/kg muscle) was studied at five

different stages of the process. They were at the green stage, at the end of the salting, the post-salting and the drying steps and in the final product (figure 1). The means for TBARs between R and F Iberian hams were statistically different at the green stage (0.12 *vs* 0.26 mg MDA/kg muscle, respectively), at the end of the post-salting step (1.36 *vs* 2.10 mg MDA/kg muscle, respectively) and at the end the of drying phase (0.74 *vs* 0.52 mg MDA/kg muscle, respectively). Despite these differences, the behaviour of the TBARs levels along the process was quite similar in both R and F hams, showing an increase from the green stage to the end of the post-salting, and falling thereafter until the final stage.

DISCUSSION

Influence of pre-cure freezing of Iberian hams on FA and DMA composition of lipid fractions at different processing steps

The pre-cure freezing effect caused differences in the FA composition of studied lipid fractions at the green stage. The levels of SFA, MUFA and PUFA in F hams were higher in FFA (Table 2) and lower in PL (Table 3) than in R hams, while no differences were found for NL (Table 1), indicating that during the frozen storage of Iberian hams a noticeable lipolysis took place. Accordingly, Motilva et al. (1994) found a higher quantity of total FFA at the beginning of the process in Serrano hams processed using thighs previously frozen and thawed than in those processed using fresh material. The results of our study suggest that during the frozen storage the PL were more hydrolyzed than NL, with the PL being the main contributor to the formation of FFA. This hypothesis is supported by the results obtained by Igene et al (1981) in beef and chicken and Hernández et al (1999) in pig loin, reporting a decrease in the proportion of PUFA from PL while total FA from NL remained unchanged during the frozen storage. In buffalo meat, freezing caused a decrease in the levels of both MUFA and PUFA of PL (Kesava & Kowale, 1991).

The FA composition of NL, FFA and PL was quite similar in R and F Iberian hams at the end of the post-salting stage and at the end of the processing. The only difference found being that in FFA, the levels of SFA were higher in R than in F hams at the final stage. These results are in concordance with those obtained in Serrano hams, in which no differences between hams processed

using either fresh or frozen raw material were found until the second month of the processing (Motilva et al., 1994). Similarly, no significant differences were found in the FFA content in boneless dry-cured hams made with frozen/thawed or chilled meat after salting and at the end of maturing (Wang, 2001). Contrary to this, Flores et al. (2006) found higher FFA in pre-cure frozen Serrano hams than in hams processed using fresh raw material.

The influence of pre-cure freezing was also noticeable in the DMA composition of PL (Table 4), showing a similar effect than that observed in the FA composition of this fraction. The level of DMA was significantly higher in R hams than in F ones at the green stage, which was mainly due to the hexadecanaldimethylacetal (C16:0 DMA) content. These results reinforce the hypothesis of the hydrolysis process during the frozen storage in Iberian hams. Estévez and Cava (2004) also found a decrease in the proportion of this compound during the refrigerated storage of pork. These results could be attributable to a specific activity of a muscle phospholipase enzyme for the phospholipids in which hexadecanaldimethylacetal (C16:0 DMA) is linked, as previously was described for palmitic acid (C16:0) (Morcuende et al., 2003).

Influence of pre-cure freezing of Iberian hams on evolution of FA and DMA composition throughout the processing

Although the FA evolution in NL, FFA and PL during the processing of the hams of this study is similar to that found in Serrano (Buscailhon et al., 1994, Motilva et al., 1994; Larrea et al., 2007), Iberian (Martín, et al., 1999; Andrés, et al., 2005) and even Chinese hams (Yang et al., 2005), there are a few variations, mainly in the NL fraction. There were no changes in the FA composition of NL (Table 1) throughout the ripening of the Iberian hams in this study. Contrarily, Andrés et al. (2005) found that SFA, MUFA and PUFA of NL decreased during Iberian ham processing. In NL from Chinese hams the proportion of PUFA decreased and that of MUFA increased while SFA remained unchanged during the process (Yang et al., 2005). Antequera et al. (1992) and Díaz and García-Regueiro (1991) found (in Iberian and Serrano hams, respectively) that NL decreased markedly, mainly in the early processing stages. As far as FFA are concerned, SFA, MUFA and PUFA increased during the processing of the Iberian hams studied in the present work, which is in agreement with data published by

Andrés et al. (2005) and Motilva et al. (1994), but not with those from Martín et al. (1999), who reported that in the FFA fraction only SFA increased, while MUFA and PUFA decreased. Buscailhon et al. (1994) also found that SFA raised in the FFA fraction throughout the processing, while MUFA remained unchanged and PUFA decreased. In Chinese hams, total FA of the FFA did not vary during the processing (Yang et al., 2005).

The decrease in the FA of the PL fraction has been observed in most studies dealing with lipolysis in dry-cured ham. Some of them, as in the present study, found that SFA, MUFA and PUFA changed during the processing (Andrés et al., 2005; Martín et al., 1999), while other only showed that MUFA and PUFA were affected (Larrea et al., 2007), and Buscailhon et al. (1994) and Yang et al. (2005) only reported changes in the PUFA in the PL fraction.

With respect to the evolution of DMA composition throughout the ripening of Iberian hams, no study in the scientific literature has been found considering this variable. The results obtained in this work showed that the levels of DMA decreased from the green stage to the end of the post-salting phase, and remaining unchanged thereafter up to the final stage. Nevertheless, Muriel et al (2005) found that the proportion of DMA increased during the ripening of dry-cured Iberian loin, explained by the lack of phospholipase A_1 activity against the ether linkage.

Pre-cure freezing caused differences in the FA evolution in the FFA and PL fractions but not in the NL. In both R and F hams, FA significantly raised in the FFA fraction and decreased in the PL, reaching similar values at the end of the processing for both group of hams in each fraction, but following a different trend. While in R hams occurred from the green stage to the end of the post-salting step and from this point to the final stage, in F hams changes were only noticeable from the end of the post-salting step to the final stage. In this sense, it has been described that there are two phases of greater lipolysis intensity, the first one occurring immediately after salting and the second one during the drying step (Antequera et al., 1992). This agrees with results found for the FA evolution described for the R hams of this study but not with those found for F hams, in which lipolysis seems to occur only during the drying step. Although there are no data about the evolution activities of the hydrolysis PL enzymes (phospholipases and lysophospholypases), Gandemer (2002) has postulated

that these enzymes remain active because the proportion of long chain PUFA in the FFA fraction increases throughout the processing, giving evidence of PL hydrolysis (Buscailhon et al., 1994). However, in this study there was no evidence of PL hydrolysis in F hams from the green stage to the end of the post-salting step, which might be related to the salt content, being statistically lower in F (0.71% dry matter) than in R (1.20% dry matter) hams at the end of the salting phase. In fact, Andrés et al. (2005) reported a promoting effect of sodium chloride on Iberian ham lipolysis. Nevertheless, Motilva et al. (1994) found the same trend in lipolysis for Serrano hams processed using fresh and pre frozen raw material.

In relation to the effect of freezing of Iberian hams on the DMA evolution, following the same trend observed for FA of PL, DMA remained unchanged throughout the processing in F hams, which might also be related to the lower salt content found in this group of hams, causing a lesser extent of lipolysis.

Influence of pre-cure freezing of Iberian hams on FA oxidation throughout the processing

Figure 1 shows the values for TBARs at the end of the different steps of the processing of R and F Iberian hams. Pre-cure freezing influenced lipid oxidation. Thus, the values of TBARs were higher in F than in R hams at the initial stage and at the end of the post-salting and drying steps, whereas there were no differences at the end of the processing. In Serrano hams, Motilva et al. (1994) also found similar differences but only at the green stage. However, Sakata et al (1995) and Hansen et al (2004) showed similar TBA levels for frozen and fresh *Longissimus dorsi* muscle. On the other hand, the oxidation process during the ripening followed the same tendency in both R and F Iberian hams of this study, reaching the highest levels at the end of post-salting, also in agreement with Antequera et al., (1992) but not with Martín et al. (1999) or Andrés et al (2004) who detected the oxidation peak during the drying step.

CONCLUSIONS

The effect of pre-cure freezing of Iberian hams influenced the FA profile of FFA and PL and the DMA composition of PL, mainly at the green stage. Lipolysis phenomena and lipid oxidation were also influenced by using either fresh or pre-frozen raw material. However, at the end of the processing, pre-cure freezing of Iberian hams does not show any significant effect.

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Table 1. Fatty acid composition (expressed as mg FAME/g intramuscular fat) of neutral lipid fraction throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	GREEN STAGE			END OF POST-SALTING			FINAL STAGE			*p* (evolution)	
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]
C14:0	6.67 ± 0.19	6.27 ± 0.45	0.201	7.03 ± 0.06	7.26 ± 0.57	0.527	7.27 ± 0.87	7.37 ± 1.45	0.914	0.412	0.156
C14:1	1.84 ±0.11	1.79[b] ± 0.11	0.577	2.00 ± 0.28	2.11[a] ± 0.14	0.495	1.92 ± 0.12	1.84[b] ± 0.07	0.268	0.382	0.005
C16:0	92.17 ± 6.62	84.91 ± 8.87	0.270	100.78 ± 3.50	106.34 ± 12.85	0.498	116.59 ± 16.13	108.53 ± 29.59	0.679	0.068	0.138
C16:1	19.05 ± 1.02	19.36 ± 3.26	0.883	21.42 ± 1.26	22.17 ± 2.54	0.653	19.16 ± 1.15	21.58 ± 4.71	0.424	0.076	0.436
C18:0	35.00 ± 12.03	28.51 ± 5.61	0.326	33.28 ± 3.20	41.29 ± 11.65	0.295	49.68 ± 5.60	44.17 ± 16.88	0.609	0.081	0.135
C18:1 n-9	221.94 ± 6.43	220.03 ± 24.53	0.902	251.44 ± 11.49	269.82 ± 22.60	0.236	260.00 ± 31.55	261.33 ± 50.02	0.968	0.121	0.081
C18:1 n-7	27.34 ± 0.81	26.39 ± 3.77	0.691	29.48 ± 2.42	30.37 ± 2.88	0.661	25.86 ± 1.11	29.25 ± 5.16	0.312	0.084	0.288
C18:2 n-6	15.91 ± 0.60	15.79 ± 3.92	0.963	18.58 ± 0.70	20.18 ± 3.73	0.499	19.37 ± 2.87	19.85 ± 3.42	0.842	0.110	0.134
C18:3 n-6	0.86 ± 0.06	0.75 ± 0.08	0.042	0.93 ± 0.25	0.84 ± 0.03	0.379	0.90 ± 0.06	0.80 ± 0.08	0.034	0.785	0.161
C18:3 n-3	1.62 ± 0.12	1.44 ± 0.21	0.134	1.68 ± 0.10	1.68 ± 0.20	0.975	1.80 ± 0.23	1.68 ± 0.21	0.375	0.197	0.134
C20:0	1.89 ± 0.25	1.68 ± 0.14	0.161	1.76 ± 0.07	1.89 ± 0.20	0.323	2.14 ± 0.17	1.96 ± 0.29	0.365	0.106	0.139
C20:1	5.54 ± 0.16	4.89[b] ± 0.31	0.020	5.29 ± 0.44	6.06[a] ± 0.15	0.020	6.04 ± 0.41	5.86[a] ± 0.60	0.668	0.099	0.005
C20:4 n-6	4.17 ± 1.42	3.34 ± 1.22	0.351	4.09 ± 0.46	3.74 ± 0.89	0.419	3.80 ± 0.77	3.63 ± 0.57	0.659	0.789	0.760
C20:3 n-3	2.41 ± 0.98	2.62 ± 0.90	0.735	2.78 ± 0.24	2.94 ± 0.70	0.602	2.70 ± 0.37	2.94 ± 0.66	0.454	0.573	0.727
C24	1.30[a] ± 0.02	1.16 ± 0.03	<0.001	1.11[b] ± 0.06	1.20 ± 0.09	0.162	1.13[b] ± 0.02	1.15 ± 0.03	0.382	0.001	0.365
C22:6 n-3	0.60 ± 0.13	0.53 ± 0.00	0.222	0.53 ± 0.00	0.53 ± 0.00	1.000	0.53 ± 0.00	0.53 ± 0.00	1.000	0.152	1.000
C24:1	0.75 ± 0.12	0.76 ± 0.17	0.897	0.62 ± 0.00	0.67 ± 0.10	0.341	1.05 ± 0.68	0.65 ± 0.07	0.188	0.223	0.289
SFA[v]	136.91 ± 18.52	122.51 ± 13.03	0.239	144.20 ± 6.67	144.87 ± 12.79	0.938	177.12 ± 22.83	139.30 ± 39.19	0.201	0.063	0.378
MUFA[x]	276.23 ± 4.72	273.13 ± 31.38	0.875	312.64 ± 16.40	328.59 ± 33.18	0.485	317.59 ± 37.21	289.62 ± 46.47	0.433	0.143	0.127
PUFA[y]	26.54 ± 1.03	24.47 ± 4.74	0.498	28.85 ± 1.52	27.59 ± 3.43	0.583	29.74 ± 4.72	27.40 ± 3.97	0.507	0.435	0.469

s: Mean values ± standard error of the mean. Means with different superscripts differ significantly throughout ripening ($p<0.05$).
t: refrigerated Iberian hams; u: pre-cure frozen Iberian hams
v: total amount of saturated fatty acids; x: total amount of monounsaturated fatty acids; y: total amount of polyunsaturated fatty acids

Table 2. Fatty acid composition (expressed as mg FAME/g intramuscular fat) of free fatty acid fraction throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	GREEN STAGE			END OF POST-SALTING			FINAL STAGE			*p* (evolution)	
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]
C14:0	1.63[b] ± 0.02	2.49 ± 1.85	0.331	1.69[b] ± 0.02	1.70 ± 0.05	0.687	2.21[a] ± 0.24	1.99 ± 0.14	0.071	<0.001	0.441
C16:0	3.88[b] ± 0.54	5.09[b] ± 2.21	0.270	6.01[b] ± 0.80	5.90[b] ± 1.48	0.879	14.08[a] ± 2.26	9.90[a] ± 2.66	0.015	<0.001	0.005
C18:0	2.65[c] ± 0.34	4.12 ± 2.27	0.189	3.95[b] ± 0.69	4.00 ± 1.03	0.915	7.99[a] ± 0.49	6.43 ± 1.44	0.030	<0.001	0.037
C18:1 n-9	4.72[b] ± 0.26	5.64[b] ± 2.25	0.392	6.95[b] ± 1.31	6.18[b] ± 1.33	0.340	24.28[a] ± 5.43	19.81[a] ± 3.10	0.110	<0.001	<0.001
C 18:1 n-7	2.21[b] ± 0.02	4.02 ± 3.18	0.239	2.65[b] ± 0.29	2.52 ± 0.31	0.492	5.50[a] ± 0.98	4.92 ± 0.61	0.248	<0.001	0.090
C18:2 n-6	2.63[c] ± 0.61	5.32 ± 4.62	0.233	6.16[b] ± 2.52	5.64 ± 2.56	0.733	9.40[a] ± 1.52	10.89 ± 3.73	0.388	<0.001	0.037
C18:3 n-6	0.67[b] ± 0.00	0.75 ± 0.09	0.063	0.72[ab] ± 0.06	0.71 ± 0.05	0.708	0.77[a] ± 0.02	0.76 ± 0.05	0.516	0.002	0.378
C18:3 n-3	0.64[c] ± 0.00	0.73[b] ± 0.11	0.081	0.77[b] ± 0.08	0.74[b] ± 0.08	0.591	0.91[a] ± 0.06	0.90[a] ± 0.06	0.766	<0.001	0.007
C20:0	1.19[b] ± 0.00	1.25 ± 0.11	0.251	1.20[b] ± 0.04	1.22 ± 0.04	0.482	1.30[a] ± 0.01	1.27 ± 0.02	0.001	<0.001	0.463
C20:1	0.68[b] ± 0.01	0.66[b] ± 0.09	0.711	0.74[b] ± 0.04	0.81[b] ± 0.32	0.599	1.24[a] ± 0.23	1.14[a] ± 0.08	0.343	<0.001	0.005
C20:4 n-6	1.01[b] ± 0.20	2.59 ± 1.91	0.104	3.52[a] ± 1.38	2.68 ± 1.44	0.330	4.04[a] ± 1.11	5.13 ± 2.08	0.284	0.001	0.055
C20:3 n-3	0.75[b] ± 0.00	0.87 ± 0.24	0.308	0.75[b] ± 0.00	1.34 ± 1.43	0.341	0.82[a] ± 0.01	0.81 ± 0.03	0.641	<0.001	0.533
C24	1.10[b] ± 0.04	1.25 ± 0.13	0.054	1.20[b] ± 0.11	1.24 ± 0.12	0.554	1.27[a] ± 0.07	1.35 ± 0.17	0.357	0.016	0.392
C22:6 n-3	0.53 ± 0.00	0.58 ± 0.09	0.209	0.55 ± 0.04	0.55 ± 0.03	0.998	0.53 ± 0.00	0.54 ± 0.04	0.341	0.245	0.486
C24:1	0.71 ± 0.03	0.89 ± 0.20	0.334	0.72 ± 0.06	0.84 ± 0.19	0.140	0.83 ± 0.14	0.73 ± 0.07	0.122	0.061	0.309
SFA[v]	10.47[c] ± 0.90	14.20[b] ± 4.90	0.132	14.06[b] ± 1.53	14.07[b] ± 2.58	0.991	26.86[a] ± 2.67	20.93[a] ± 4.13	0.014	<0.001	0.014
MUFA[x]	8.32[b] ± 0.30	11.21[b] ± 2.55	0.041	11.05[b] ± 1.58	10.36[b] ± 1.51	0.457	31.86[a] ± 6.65	26.60[a] ± 3.67	0.121	<0.001	<0.001
PUFA[y]	6.23[b] ± 0.80	10.85 ± 6.49	0.153	12.47[a] ± 3.81	11.67 ± 4.40	0.742	16.47[a] ± 2.51	19.02 ± 5.83	0.347	<0.001	0.049

s: Mean values ± standard error of the mean. Means with different superscripts differ significantly throughout ripening ($p<0.05$).
t: refrigerated Iberian hams; u: pre-cure frozen Iberian hams
v: total amount of saturated fatty acids; x: total amount of monounsaturated fatty acids; y: total amount of polyunsaturated fatty acids

Table 3. Fatty acid composition (expressed as mg FAME/g intramuscular fat) of polar lipid fraction throughout the processing of refrigerated and pre-cure frozen Iberian hams[s]

	GREEN STAGE			END OF POST-SALTING			FINAL STAGE			*p* (evolution)	
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]
C14:0	2.48[a] ± 0.21	1.97 ± 0.38	0.055	1.90[b] ± 0.34	1.94 ± 0.29	0.823	1.60[b] ± 0.02	1.60 ± 0.04	0.739	<0.001	0.096
C14:1	2.08[a] ± 0.13	1.80 ± 0.23	0,077	1.78[b] ± 0.21	1.78 ± 0.16	0.990	1.62[b] ± 0.09	1.58 ± 0.02	0.398	<0.001	0.086
C16:0	8.82[a] ± 0.95	5.60[ab] ± 2.06	0.030	5.29[b] ± 0.82	5.91[a] ± 2.14	0.557	3.06[c] ± 0.19	2.84[b] ± 0.26	0.170	<0.001	0.026
C16:1	1.94[a] ± 0.06	1.75 ± 0.17	0.076	1.70[b] ± 0.11	1.77 ± 0.14	0.390	1.60[b] ± 0.02	1.60 ± 0.01	0.578	<0.001	0.104
C18:0	5.79[a] ± 0.59	4.47 ± 1.46	0.144	3.81[b] ± 0.37	5.12 ± 1.25	0.051	3.61[b] ± 0.17	3.49 ± 0.27	0.412	<0.001	0.086
C18:1 n-9	7.63[a] ± 0.35	4.86[ab] ± 1.53	0.012	4.57[b] ± 0.33	5.77[a] ± 2.11	0.245	2.96[c] ± 0.26	2.75[b] ± 0.22	0.203	<0.001	0.024
C18:1 n-7	3.16[a] ± 0.14	2.56[a] ± 0.74	0.164	2.33[b] ± 0.12	2.40[a] ± 0.37	0.701	1.77[c] ± 0.04	1.74[b] ± 0.07	0.452	<0.001	0.032
C18:2 n-6	14.62[a] ± 1.87	8.62[a] ± 4.27	0.042	7.39[b] ± 1.08	7.40[ab] ± 2.99	0.997	2.70[c] ± 0.36	2.60[b] ± 0.43	0.719	<0.001	0.017
C18:3 n-6	1.68[a] ± 0.03	1.59 ± 0.03	0.011	1.59[b] ± 0.01	1.59 ± 0.02	0.783	1.56[c] ± 0.00	1.56 ± 0.01	0.699	<0.001	0.110
C18:3 n-3	1.82[a] ± 0.05	1.64[a] ± 0.05	0.003	1.64[b] ± 0.03	1.64[a] ± 0.05	0.931	1.57[c] ± 0.01	1.56[b] ± 0.01	0.248	<0.001	0.016
C20:0	1.57[ab] ± 0.0	1.56[b] ± 0.01	0.523	1.56[b] ± 0.00	1.58[a] ± 0.01	<0.001	1.57[a] ± 0.00	1.57[ab] ± 0.00	0.867	<0.001	0.020
C20:1	1.68 ± 0.09	1.69 ± 0.05	0.919	1.65 ± 0.03	1.71 ± 0.04	0.035	1.64 ± 0.04	1.65 ± 0.01	0.566	0.530	0.080
C20:3 n-6	2.12[a] ± 0.07	1.82[a] ± 0.18	0.022	1.79[b] ± 0.03	1.84[a] ± 0.14	0.531	1.60[c] ± 0.01	1.59[b] ± 0.02	0.782	<0.001	0.017
C20:4 n-6	8.37[a] ± 1.05	5.09[a] ± 2.13	0.033	4.37[b] ± 0.45	4.27[a] ± 1.01	0.832	1.97[c] ± 0.13	2.08[b] ± 0.27	0.435	<0.001	0.009
C20:3 n-3	1.57 ± 0.0*	1.60 ± 0.07	0.485	1.57 ± 0.02	1.58 ± 0.06	0.618	1.55 ± 0.01	1.55 ± 0.00	0.427	0.090	0.332
C24:0	2.30[a] ± 0.12	1.81[a] ± 0.15	0.002	1.75[b] ± 0.06	1.81[a] ± 0.09	0.230	1.59[c] ± 0.02	1.59[b] ± 0.02	0.919	<0.001	0.004
C22:6 n-3	1.67[a] ± 0.05	1.58[a] ± 0.02	0.017	1.57[b] ± 0.01	1.57[ab] ± 0.01	0.969	1.55[b] ± 0.00	1.55[b] ± 0.00	0.266	<0.001	0.015
C24:1	1.57 ± 0.01	1.59 ± 0.02	0.115	1.56 ± 0.02	1.57 ± 0.04	0.539	1.57 ± 0.04	1.56 ± 0.02	0.540	0.790	0.309
SFA	20.96[a] ± 1.34	15.41 ± 3.83	0.034	14.31[b] ± 1.40	16.37 ± 3.75	0.277	11.42[c] ± 0.36	11.09 ± 0.56	0.292	<0.001	0.040
MUFA	18.05[a] ± 0.29	14.25[ab] ± 2.49	0.023	13.59[b] ± 0.59	15.00[a] ± 2.76	0.298	11.16[c] ± 0.32	10.89[b] ± 0.30	0.199	<0.001	0.023
PUFA	29.72[a] ± 3.00	20.13[a]± 6.54	0.037	18.13[b] ± 1.26	18.04[ab] ± 4.04	0.962	10.89[c] ± 0.49	10.90[b] ± 0.69	0.977	<0.001	0.013

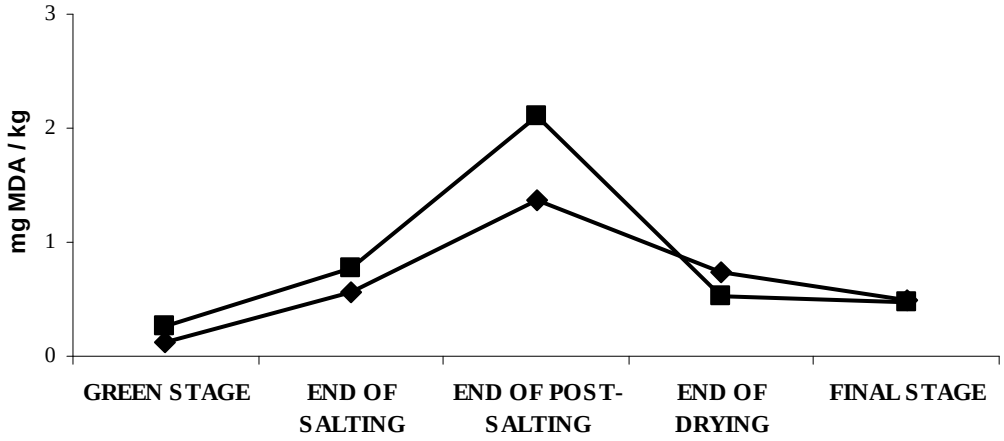
s: Mean values ± standard error of the mean. Means with different superscripts differ significantly throughout ripening (p<0.05).
t: refrigerated Iberian hams; u: pre-cure frozen Iberian hams
v: total amount of saturated fatty acids; x: total amount of monounsaturated fatty acids; y: total amount of polyunsaturated fatty acids

Table 4. Dimethyl acetal composition (expressed as g DMA/kg intramuscular fat) of polar lipid fraction throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	GREEN STAGE			**END OF POST-SALTING**			**FINAL STAGE**			*p* (evolution)	
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]
C16:0 DMA	6.43[a] 0.41	2.34[ab] ± 0.88	0.002	2,88[b] ± 1.03	3.18[a] ± 0.91	0.643	1.78[b]± 0.11	1.79[b] ± 0.11	0.878	<0.001	0.033
C16:1 DMA	2.19[a]± 0.09	1.79 ± 0.25	0.024	1.70[b] ± 0.15	1.80 ± 0.25	0.466	1.57[b] ± 0.01	1.57 ± 0.01	0.515	<0.001	0.173
C18:0 DMA	2.96[a]± 0.44	1.83 ± 1.46	0.011	1.91[b] ± 0.23	2.03 ± 0.39	0.573	1.63[b] ± 0.05	1.62 ± 0.04	0.888	<0.001	0.103
C18:1 DMA	2.44[a]± 0.31	1.73± 0.18	0.018	1.90[b] ± 0.31	1.92 ± 0.24	0.943	1.59[b]± 0.02	1.59 ± 0.10	0.719	0.001	0.040
Σ DMA[z]	14.39[a] ± 0.54	7.59 ± 1.43	0.020	8.41[b] ± 1.64	8.71 ± 1.86	0.785	6.59[b]± 0.19	6.58 ± 0.18	0.469	<0.001	0.083

s: Mean values ± standard error of the mean. Means with different superscripts differ significantly throughout ripening (p<0.05).
t: refrigerated Iberian hams; u: frozen Iberian hams
z: total amount of dimethyl acetals

Figure 1. Evolution of lipid oxidation (expressed as mg malonaldehyde/kg muscle) throughout the processing of refrigerated (◆) and pre-cure frozen (■) Iberian hams.



Capítulo II.2.

Muscle individual phospholipid classes throughout the processing of dry-cured ham: influence of pre-cure freezing

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TITLE

Muscle individual phospholipid classes throughout the processing of dry-cured ham: Influence of pre-cure freezing

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RUNNING TITLE

Phospholipid classes of fresh and pre-cure frozen Iberian hams.

ABSTRACT

This paper aims to study the profile of phospholipid (PL) classes of Iberian ham throughout its processing and the changes it underwent due to the influence of the pre-cure freezing treatment. The general profile of each PL class did not vary during the ripening stage. Phosphatidylcholine (PC) showed the highest proportion, followed by phosphatidyletanolamine (PE) and phosphatidylserine (PS) and phosphatidylinositol (PI) being the minor PL. The four PL classes were highly hydrolysed during the salting stage and their degradation continued during the rest of the processing. Pre-cure freezing of Iberian ham influenced the levels of the four PL classes at the initial stage, all of them being higher in refrigerated (R) than in pre-cure frozen (F) hams. Moreover, the pattern of hydrolysis was not the same in these two groups.

KEY WORDS

Phospholipid classes, Iberian ham, ripening process, pre-cure freezing.

INTRODUCTION

The phospholipid (PL) fraction is an important group of lipids in meat and meat products from a qualitative and quantitative point of view because of its high sensitivity to oxidation, which is mainly due to its high proportion of long chain polyunsaturated fatty acids and its close contact with catalysts of lipid oxidation in the aqueous phase of the muscle cell (Ruiz, Muriel, Pérez-Palaicos & Antequera, 2009). The more representative PL in mammal muscle are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) (Pérez-Palcios, Antequera, Muriel & Ruiz, 2006). PC is usually the major PL in animal tissues, often amounting to almost 50% of the total (Christie, 2005). PE is generally the second most abundant PL in animals, while PS usually constitutes less than 10% of the total PL (Christie, 2008) and PI is often below 4% in animal tissues (Genge, Licia, Wuthier & Wutier, 2003).

The determination of PL classes in cured meat products is important because these compounds are subject to degradation throughout the processing, due to either the activity of endogenous or microbial phospholipases (Ordóñez, Hierro, Bruna & de la Hoz, 1999; Andrés, Cava, Martín, Ventanas & Ruiz, 2005) or to the in-situ autoxidation of membrane PL (Muriel, Andrés, Petrón, Antequera & Ruiz, 2007).

In studies conducted on Iberian hams, the focus has been mostly on the fatty acid profile of total polar lipids. Andrés et al. (2005) and Martín, Córdoba, Ventanas and Antequera (1999) found a decrease in the quantity of fatty acids of the PL fraction during the ripening process. The overall lipolysis taking place during the ripening of these products seem to affect the PL more intensely than the neutral lipids fraction (Andrés et al., 2005).

Freezing raw thighs and frozen storage of the raw material before the processing of dry-cured Iberian ham is not a common strategy nowadays. In fact, dry-cured ham producers believe that this procedure leads to lower quality. Moreover, some of the Specific Designation of Origin that protect the production of dry-cured Iberian hams, specifically exclude freezing as a procedure for raw material storage. Nevertheless, there are no scientific evidences demonstrating that pre-cure freezing has any consequence on the quality of the derived Iberian dry-cured meat products. Besides, such

procedure could show several technological and economical advantages, such as the possibility of salting hams with more homogeneous weights or the processing of hams avoiding seasonal availability and market price fluctuations.

Although there are numerous works about the influence of freezing on several parameters related to meat quality, only a few studies have been devoted to show the effect of using frozen and subsequently thawed raw material on the quality of dry-cured hams (Arnau, Pere & Guerrero, 1994; Motilva, Toldrá, Nadal & Flores, 1994; Bañón, Cayuela, Granados & Garrido, 1999; Wang, 2001; Flores, Soler, Aristoy & Toldrá, 2006) and no one dealing with such topic in Iberian ham has been found in the scientific literature.

While most studies concerning PL in Iberian ham have been devoted to the analysis of their fatty acid composition, neither PL classes of Iberian ham nor their evolution during the ripening have been studied yet.

Thus, the main goal of this paper was to analyze the PL classes throughout the ripening process of Iberian hams. The effect of pre-cure freezing Iberian hams on the different PL classes during the processing was also studied.

MATERIAL AND METHODS

Experimental design

Twenty-four hind limbs were obtained from Iberian pigs fattened in confinement and fed with a commercial diet. These hind limbs were divided into two groups, refrigerated (R) and pre-cure frozen (F) hams. The F hams were frozen (at -20 ºC) and thawed (4 days at 3-4 ºC) three months later. The R hams were obtained later from pigs of the same genetic and fed with the same diet as those used for obtaining the F hams, but slaughtered two days before the frozen hams were totally thawed. The R hams were kept at 4 ºC during 36-48 hours until the start of their processing. Six hind limbs of each group were used for obtaining the data of the green stage. The others were processed for obtaining dry-cured hams. The processing conditions were the same for the two groups of hams, except for the salting time, which was 1 day/kg for the R hams and 0.7 day/kg for the F hams because salt penetration is favoured in frozen/thawed raw pieces. After salting, the salt from the surface was brushed and the hams were processed as follows: hams were held at 4-8 ºC and 73-75% relative humidity for 70 days (post-salting step); during the drying stage, the hams were kept in a chamber under controlled conditions for 120 days where the temperature was increased from 8 to 20 ºC, while relative humidity was progressively reduced to 64%. Finally, hams were ripened for 16 months (cellar stage) at 20-25 ºC and 55-65% relative humidity.

Sampling

Sampling was carried out at the green stage (raw meat) and at the end of each processing step. Samples taken at the end of salting, post-salting and drying steps were obtained by extracting a cylindrical piece of ham (sized 10 x 2.5 cm) using a stainless steel tube with a cutting edge. These samples mainly included the *Biceps femoris* muscle. Samples taken at the beginning and at the end of processing were obtained by dissecting the *Biceps femoris* muscle of each ham. Samples were vacuum-packaged and kept frozen at -80 ºC until analysis.

Intramuscular fat extraction

Samples were ground using a commercial grinder immediately before fat extraction. Intramuscular total lipids were extracted with chloroform/methanol solvent (2:1, vol/vol), according to the method described by Pérez-Palacios, Ruiz, Martin, Muriel and Antequera (2008).

Fractionation of phospholipid classes

The PL fractionation was carried out following the method described by Rombaut, Camp and Dewettinck (2005) with slight modifications. Lipids (24 mg) were dissolved in 1.6 ml of chloroform:methanol solvent (88:12, vol/vol). Analysis was carried out using an HPLC Shimadzu (LC-20AT prominence liquid chromatography) instrument equipped with a pump (DGU-20A5 prominence degasser) and a SIL-20AC autosampler. The analytical column (150 mm × 30 mm I.D.) was packed with a silica normal-phase Prevail Silica 3u (GRACE) thermostated in an oven (Shimadzu CTO-20AC prominence column over) at 40 °C. The chromatographic separation was carried out using a linear gradient according to the following scheme: t = 0 min, 87.5%A 12%B 0.5%C; t = 12 min, 2%A 90%B 8%C for 2 min. The mobile phase was brought back to the initial conditions at t = 16 min and the column was allowed to equilibrate until the next injection at t = 25 min. Eluent A consisted of chloroform, eluent B of methanol and eluent C of triethylamine buffer (pH 3, 1 M formic acid). The flow was maintained at 0.7 ml/min. The injection volume was 10 µL. HPLC was coupled with an evaporative light scattering detector (ELSD) (Alltech 3300). The nebulising gas was N_2, at a flow rate of 1.6 L/min, and a nebulising temperature of 65 °C. The gain was set at 1.

Statistical analysis

The effect of the ripening process and the influence of pre-cure freezing on PL classes of Iberian hams were analysed by one-way analysis of variance (ANOVA) using the General Linear Model of SPSS (v.15.0). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test.

RESULTS AND DISCUSSION

Phospholipid classes of Iberian ham throughout the ripening process

Data from the R Iberian hams were examined for studying the PL classes in Iberian hams throughout the processing, considering these hams as the control group because they were ripened following the usual procedure.

Four different PL classes were determined in the *Biceps femoris* muscle from the Iberian hams of this study (Figure 1). The major PL was PC, followed by PE, PS and PI being the minor PL, which is in agreement with the result found by other authors studying different meats (Wang, Xu, Xu, Zhou, Zhu & LI, 2009; Xu, Xu, Zhou, Wang & Li, 2008; Boselli, Pacetti, Curzi & Frega, 2008; Hernández, Navarro & Toldrá, 1999; Kesava Rao & Kowale, 1991). Figure 2 shows the proportion of each PL at the end of every stage of the processing. In fresh ham and during the salting stage, the percentages of the four PL did not change notably, being around 70%, 20%, 5% and 5% for PC, PE, PS and PI, respectively. During the post-salting, drying and cellar stages, there was an increase in the percentage of PC and a decrease in those of PE, PS and PI. At the final stage the proportions of PC, PE and PI were 89.23%, 8.92% and 2.15%, respectively, while PL was not detected.

The content of each PL class, expressed as mg PL/100 g muscle dry matter, throughout the processing of Iberian hams is shown in Table 1. The quantity of each PL class was decreased significantly (p<0.001) during the ripening of hams, indicating that the four PL classes underwent hydrolysis during the processing of Iberian ham. It was found that the content of total PL was higher in fresh than in dry-cured Iberian hams (Tejeda, Gandemer, Antequera, Viau & García, 2002; Petrón, Muriel, Timón, Martín & Antequera, 2004). In Chinese Xuanwei ham, Yang, Ma, Qiao, Song and Du (2005) have also shown a decrease in the total PL content during the processing of this meat product.

The percentage of accumulated losses of each PL class at the end of every processing stage of Iberian ham is shown in Figure 3. The hydrolysis of PC, PS and PI classes took part principally during the salting step (38.38%, 53.72% and 52.00% of losses, respectively), whereas PE was slightly degraded during this stage (16.41%). At the post-salting phase, PC, PS and PI were less hydrolyzed than in the salting phase while PE was substantially degraded (31.01% of losses).

At the drying and cellar stages, PL hydrolysis continued but at lesser extent than in the previous phases. Acid phospholipases A_1 and A_2 catalyse the hydrolysis of PL at position *sn*-1 and *sn*-2, respectively, at the water-lipid interface (Yuan, Quinn, Sigler & Gelb, 1990). Moreover, salt plays an important role in controlling enzyme activity. Neutral lipases are inhibited by salt whereas acid lipases are activated at low salt concentrations (Motilva & Toldrá, 1992). Actually, the salt concentration of the Iberian hams of this study at the end of salting step was lower than 2% (unpublished data), which might have favoured the acid phospholipases activity.

Hydrolysis throughout the ripening stage was not exactly the same for the four PL classes. During the salting step, PS and PI underwent higher hydrolysis than PC and PE (53.72% and 52.04% of accumulated losses vs. 38.38% and 16.41%, respectively). PE was the more hydrolyzed PL during the post-salting stage, increasing its percentage of accumulated losses from the end of the salting to the end of the post-salting stages (16.41% and 57.42%, respectively). During the drying stage hydrolysis was more marked in PI and was less notable in PC (the percentage of accumulated losses increased from 55.17% to 86.14% and from 46.32% to 57.63%, respectively). During the cellar stage, PI was the least hydrolyzed PL whereas PS was the most degraded one.

At the end of the processing, PC was the least hydrolyzed PL, followed by PE and PI, whereas PS was totally degraded (87.32%, 94.56%, 95.35% and 100% of accumulated losses, respectively). Similar results were found in Nanjing dry-cured duck (Xu et al., 2008). These authors indicated that PE was more susceptible to hydrolysis or oxidation than PC. Hernández et al. (1999) ascribed the greater susceptibility of PE to degradation to its high content of polyunsaturated fatty acids and plasmalogen, which are very sensitive to hydrolysis. Furthermore, phospholipase A_2 enzymes hydrolyze preferentially phospholipids esterified with arachidonic acid (C20:4 n-6) in the *sn*-2 position as well as those PL which have a dimethylacetal in the *sn*-1 position (Yang, Farooqui & Horrocks, 1996). It has been described that in animal tissues, PC tends to contain lower proportions of arachidonic (20:4 n-6) and docosahexaenoic (22:6 n-3) acids and higher proportions of C18 unsaturated fatty acids than PE. Also, in the *Longissumus dorsi* muscle of mammals, the highest proportions of dimethylacetals were found in PE (Pérez-Palacios et al.,

2006). PI stands out for a high content of arachidonic acid (20:4 n-6) (Christie, 2008), which could also explain the high percentage of accumulated losses of this PL in dry-cured ham. The fatty acid composition of animal PS varies from tissue to tissue (Christie, 2008). The fatty acid profile of individual PL classes of Iberian hams has not been found in literature. Consequently, it is difficult to explain that PS of Iberian ham showed the highest susceptibility to hydrolysis.

Influence of pre-cure freezing Iberian ham on phospholipid classes

At the initial, salting, post-salting and drying stages, pre-cure freezing Iberian hams did not influence the general PL profile: PC was the major PL, followed by PE, and being PS and PI the minor PL in both F and R Iberian hams (Figure 2). However, at the end of the processing, in dry-cured F hams PS and PI were not found while in R ones PS was the only PL that was not present.

Moreover, pre-cure freezing Iberian hams influenced the content of the four PL classes and the PL hydrolysis pattern throughout the processing. In fresh ham the quantity of PC, PE and PI was significantly lower in F (1426.72, 318.43 and 110.59 mg/100 g muscle DM, respectively) than in R hams (2214.97, 624.68, 168.84 mg/100 g muscle DM, respectively) (Table 1), which could indicate the occurrence of PL hydrolysis during the freezing storage. Indeed , phospholipase activity at frozen temperatures has been detected in mammal muscle (Hernández et al., 1999) and fish (El-Sebaiy, Metwalli & Khalil, 1987). Other authors have also shown that frozen storage significantly decrease the levels of PC and PE of pork (Hernández et al., 1999), buffalo (Kesava Rao et al., 1991), fish (El-Sebaiy et al., 1987), beef and chicken meat (Igene, Pearson & Grey, 1981).

Figure 3 shows the percentage of accumulated losses of each PL class during the ripening of R and F Iberian hams. The four detected PL classes in R and F Iberian hams were hydrolyzed throughout the ripening process but this hydrolysis did not follow the same trend. In F Iberian hams, PC and PE were not hydrolyzed during the salting step (0% of losses), while PS and PI showed 52.00% and 16.41% losses, respectively. On the other hand, the four PL classes of R hams were hydrolyzed during this stage with PS and PI showing the highest percentages of loss (53.72 and 52.04 %, respectively), followed by PC (38.38%) and PE (16.41%). Consequently, the levels of the four PL classes were statistically

($p < 0.001$) higher in F than in R hams at the end of the salting step (Table 1). Throughout the post-salting step, the four PL classes of F hams were hydrolyzed with PC, PE and PI hydrolyzed to a higher degree. In R hams PC, PS and PI were slightly hydrolyzed during this phase, whereas PE was highly degraded. These results could be related to the found lower content of salt in the *Biceps femoris* of R (1.20%) than in F hams (0.71%) at the end of salting (Pérez-Palacios, Ruiz, Grau, Flores & Antequera, *in press*), since salt diffusion proved to be associated with increased activities of both acid and neutral lipases acid lipases (Vestergaard, Schivazappa & Virgili, 2000). The lower concentration of salt in F hams during the salting stage might have lead to a lower phospholipase activity in comparison with R hams, giving a great PL hydrolysis in F hams at this stage.

During the last stages namely, drying and cellar, the four PL classes of R and F hams went on being hydrolysed, but not to the same extent for each PL class in each group of Iberian hams. In dry-cured ham, the hydrolysis underwent by PC and PS was similar in both groups of Iberian hams (the percentages of accumulated losses were around 85% and 100%, respectively). PI was totally hydrolyzed in F dry-cured hams while it showed 95.35% of accumulated losses in R ones. However, in F hams, PE was not as hydrolyzed as in R ones (84.97% vs. 94.56% of accumulated losses, respectively), being the content of PE significantly higher in F (29.98 mg/100 g muscle DM) than in R dry-cured hams (16.77 mg/100 g muscle DM). Thus, in dry-cured F hams, PC and PE were hydrolysed to the same extent whereas in R hams, PE underwent higher degradation. As explained above, the greater susceptibility of PE to hydrolysis has been related to its high content of polyunsaturated fatty acids and plasmalogen (Hernández et al., 1999; Ngah, Alasnier & Gandemer, 1994). The fatty acid profile profile of the PL fraction of the fresh Iberian hams of the present study showed a lower content of plasmalogen and polyunsaturated fatty acids in F than in R ones (unpublished data), which could explain the lower hydrolysis undergone by PE of F Iberian hams than that of R ones.

CONCLUSION

It can be concluded that the profile of PL classes of fresh Iberian hams consists of PC, which is the major PL, followed by PE and PS and PI. The four PL classes were hydrolysed throughout the processing but mainly at the salting step. At the end of the processing, PS is totally degraded while PC was least hydrolyzed. Besides, pre-cure freezing Iberian hams influences the content of the four PL classes mainly at the initial stage, being lower in pre-cure frozen hams than in refrigerated hams, and also affects the pattern of hydrolysis throughout the ripening.

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Table 1. Phospholipid classes content (expressed as mg phospholipid/100 g muscle dry matter) throughout the processing of refrigerated (R) and pre-cure frozen (F) Iberian hams[†].

	Fresh ham			End of salting			End of post-salting			End of drying			Cured ham			Evolution	
	R	F	*p*	R	F	*p*	R	F	*p*	R	F	*p*	R	F	*p*	*p* (R)	*p* (F)
PC[v]	2214.97[a] ± 685.58	1426.72[a] ± 139.06	*	602.02[b] ± 31.89	1598.61[a] ± 158.33	***	659.73[b] ± 63.97	468.79[b] ± 41.42	***	367.59[b] ± 72.36	280.80[bc] ± 120.80	ns	167.05[b]± 25.46	144.72[c] ± 20.75	ns	***	***
PE[w]	624.68[a] ± 64.84	318.43[b] ± 43.90	***	162.66[b] ± 8.49	486.76[a] ± 31.82	***	113.02[b] ± 42.86	135.01[c] ± 13.05	ns	32.95[c] ± 4.13	50.27[d] ± 3.43	***	16.77[c] ± 2.39	29.98[d] ± 1.43	***	***	***
PS[x]	165.89[a] ± 19.91	187.28 ± 45.04	ns[t]	43.75[b] ± 8.34	90.03 ± 20.59	***	57.36[b] ± 7.05	41.60 ± 6.63	**	11.83[c] ± 0.76	18.14 ± 1.01	***	Nd[u]	nd	-	***	***
PI[y]	168.84[a] ± 8.02	110.59 ± 6.33	***	37.73[b] ± 3.10	90.87 ± 13.62	***	18.10[c] ± 6.25	17.76 ± 6.45	ns	8.87[d] ± 1.11	7.28 ± 0.80	*	4.05[d] ± 0.63	nd	-	***	***
ΣPL[z]	3174.38[a] ± 676.28	2043.02[a] ± 180.30	**	846.16[b] ± 37.96	2265.28[a] ± 190.16	***	848.21[b] ± 75.46	663.16[b] ± 54.86	**	421.25[bc] ± 74.03	353.52[c] ± 122.58	ns	187.87[c] ± 25.39	174.22[c] ± 20.86	ns	***	***

[†]Means with different letters differ significantly at the different stages of the processing. ns[t]: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; nd[u]: no detected
[v]PC: phosphatidylcholine; [w]PE: phosphatidylethanolamine; [x]PS: phosphatidylserine; [y]PI: phosphatidylinositol; [z]ΣPL: total amount of phospholipid classes

Figure 1. High performance liquid chromatogram of PL classes in Iberian hams.

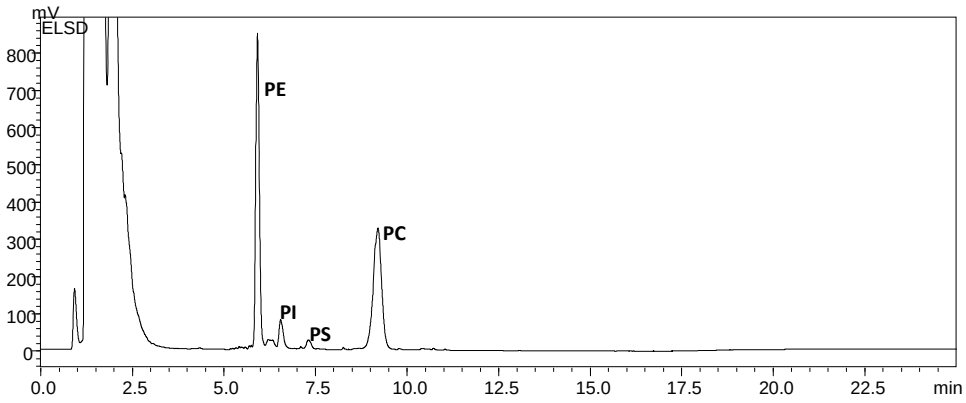


Figure 2. Percentage of phospholipid classes (phosphatidylcholine ■, phosphatidylethanolamine □, phosphatidylserine ⁙ and phosphatidylinositol ▩) throughout the processing of refrigerated (R) and pre-cure frozen (F) Iberian hams.

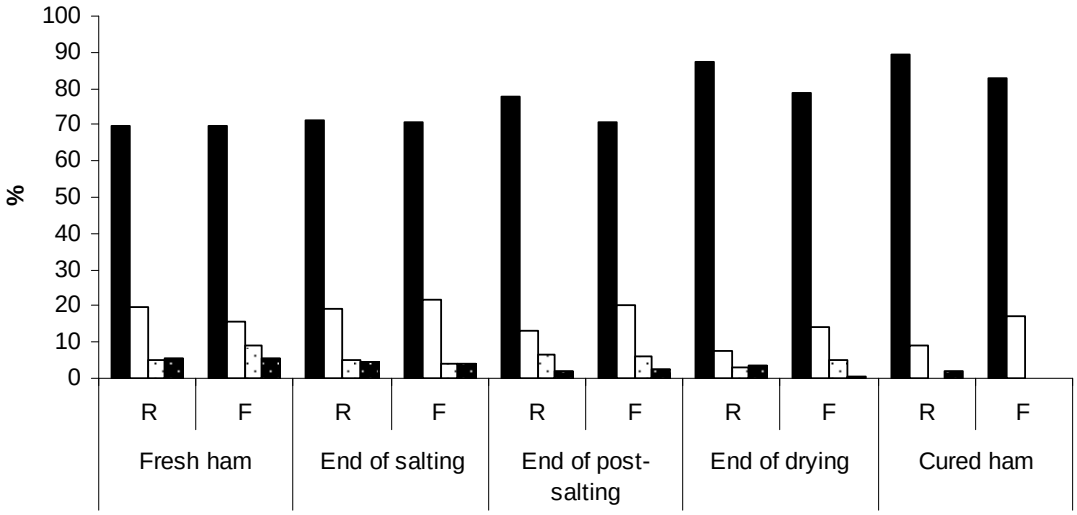
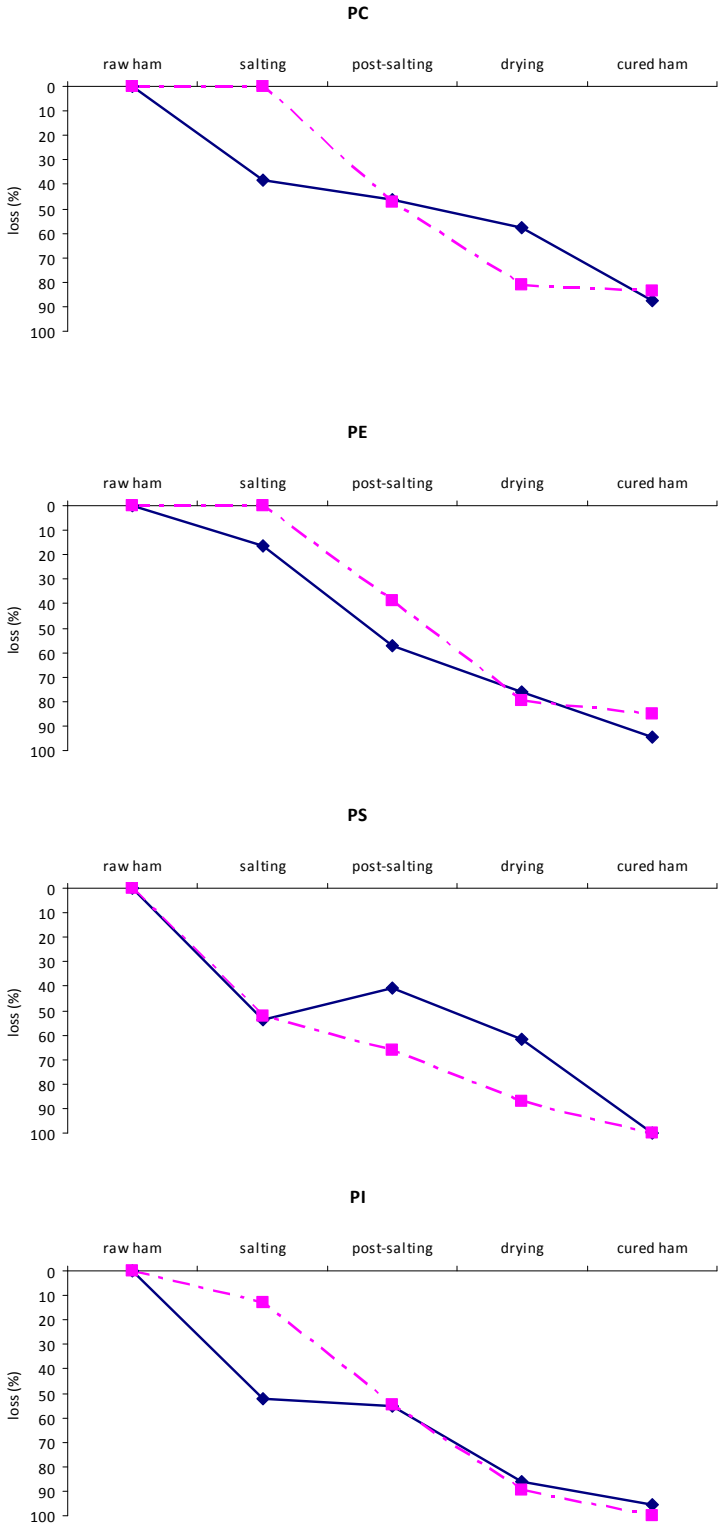


Figure 3. Percentage of accumulated losses of phospholipid classes (PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine, PI, phosphatidylinositol) throughout the processing of refrigerated (◆) and pre-cure frozen (■) Iberian hams.



Capítulo II.3.

Influence of pre-cure freezing Iberian ham on proteolytic changes throughout the ripening process

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TITLE

Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process.

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RUNNING TITLE

Proteolysis and amino acids in pre-cure freezing Iberian hams

ABSTRACT

This work was aimed to investigate the effect of pre-cure freezing Iberian hams on proteolysis phenomena throughout the ripening process. Non-protein nitrogen (NPN), peptide nitrogen (PN) and amino acid nitrogen (AN) as well as amino acid and dipeptide evolution followed the same trend in both refrigerated (R) and pre-cure frozen (F) Iberian hams during the processing. At the different stages of the ripening, there were no differences in the content of NPN and NA while F dry-cured hams showed higher levels of NP than R ones at the final step, which seems to be more related with the salt content (lower in F than in R hams) than with the pre-cure freezing treatment. Most amino acids and dipeptides detected showed higher concentration in F than in R Iberian hams at the green stage, being rather similar at the intermediate phases. At the final stage, the effect of pre-cure freezing Iberian hams was not well defined, higher levels of some amino acids and dipeptides were found in R than in F Iberian hams whereas other amino acids showed lower levels in R than in F hams.

KEY WORDS

Pre-freezing, proteolysis, amino acids, processing, Iberian ham.

INTRODUCTION

Dry-cured meat products from Iberian pigs are highly rated by Spanish consumers, because of their unique sensory features, which are consequence of the characteristics of the raw material and of the particular processing (Ventanas, Ventanas, Ruiz, & Estévez, 2005). Proteolysis has a great influence on the quality characteristics of Iberian hams, being an important source of flavour compounds (free amino acids, small peptides). Moreover, volatile compound coming from amino acids throughout the Maillard and Strecker reactions (Ventanas, Estévez, Andrés & Ruiz, 2008) are considered to have a great importance for the flavour of Iberian ham (Carrapiso, Jurado, Timón & García, 2002). Such changes in compounds released from proteins are notable, because the overall acceptance of meat products depends to a large extent on their flavour, which is mainly determined by taste and odour compounds (Ruiz, Muriel & Ventanas, 2002). The proteolysis phenomena also affect ham texture (Parolari, 1996) and leads to an increase in non-protein nitrogen (NPN) (Martín, Córdoba, Antequera, Timón & Ventanas, 1998; Córdoba, Antequera, Ventanas, López-Bote, García & Asensio, 1994a), free amino acid (Jurado, García, Timón & Carrapiso, 2007; Martín, Antequera, Ventanas, Benítez-Donoso & Córdoba, 2001; Ruiz, García, Díaz, Cava, Tejeda & Ventanas, 1999; Córdoba, Antequera, García, Ventanas, López-Bote & Asensio, 1994b) and peptide content (Martín et al., 2001; Ruiz et al., 1999; Flores, Aristoy , Spanier & Toldrá, 1997). Thus, high NPN levels have been found in doughy hams (Ventanas et al., 1998). Besides, the proteolytic processes are highly dependent on the salt content (Martín et al., 1998).

The use of frozen/thawed thighs is not a common strategy within the processing of Iberian ham nowadays. In fact, producers consider that this practise is detrimental to the quality of this product. Furthermore, some of the Specific Designation of Origin that protect the production of dry-cured Iberian hams, keep out the procedure of freezing and subsequently thawing raw material before Iberian ham processing. On the other hand, there are not scientific studies showing the effect of pre-cure freezing on the quality of the derived Iberian dry-cured meat products. Nevertheless, such procedure could constitute technological and economical advantages e.g. processing hams

with more homogeneous weight, avoiding the seasonal availability and the changes of the market price.

Changes in physical (drip loss, texture modifications), chemical (lipolysis and FA oxidation, protein denaturation and aggregation, changes in color), and sensory properties of meat could be promoted by freezing. Their extended influence depends on raw meat characteristics, processing and above all the closer to the optimum frozen conditions (Carballo & Jiménez, 2001).

However, only a few researches studying the freezing effect on hams are available in the scientific literature (Flores, Soler, Aristoy & Toldrá, 2006; Arnau, Gou & Guerrero, 1994; Motilva, Toldrá, Nadal & Flores, 1994; Bañón, Cayuela, Granados & Garrido, 1999; Wang, 2001), and none in the case of Iberian hams.

Salt penetration is favoured in frozen/thawed hams, due to the higher free water content of those hams (Bañón et al., 1999; Wang, 2001), increasing the amount of solubilised salt on the surface of the ham, which is the main factor regulating its diffusion within the piece (Sorheim & Gumpen, 1986). As a consequence, the salting time of thawed hams should be shorter than that of fresh ones (Poma, 1987; Bañón et al., 1999).

Studies carried out on Serrano hams have shown that the pre-cure freezing treatment seems not to have an influence on colour, sensory features and acceptability scores (Motilva et al., 1994; Bañón et al., 1999), although it increased proteolysis (Flores et al., 2006; Bañón et al., 1999). On the other hand, there were contradictory results about the effect of the frozen process on lipolysis. Flores et al. (2006) found higher lipolytic activity in frozen hams during salting and post-salting stages while Motilva et al. (1994) did not show differences. Pre-cure freezing Serrano hams increased the incidence of white precipitates, formed mainly by tyrosine crystals (Arnau et al., 1994; Bañón et al., 1999)

This work was aimed to study the influence of pre-cure freezing Iberian hams on the proteolytic changes and on the evolution of amino acids and dipeptides during the ripening.

MATERIAL AND METHODS

Experimental design

Twenty-four hind limbs were obtained from Iberian pigs fattened in confinement and fed with a commercial diet. These hind limbs were divided into two groups, refrigerated (R) and pre-cure frozen (F) hams. The F hams were frozen (at -20 ºC) and thawed (4 days at 3-4 ºC) three months later. The R hams were obtained later from pigs of the same genetic and fed the same diet as those used for obtaining the F hams, but slaughtered two days before that the frozen hams were totally thawed. These R hams were kept at 4 ºC during 36-48 hours until starting their processing. Six hind limbs of each group were used to obtaining the data for the green stage. The others were processed for obtaining dry-cured hams. The processing conditions were the same for the two groups of hams, except for the salting time, which was 1 day/kg for the R hams and 0.7 day/kg for the F hams. After salting, the salt from the surface was brushed and, the hams were processed as follows. Iberian hams were held at 4-8 ºC and 73-75% relative humidity for 70 days (post-salting step). During the drying stage the hams were kept in a room under controlled conditions for 120 days, temperature was increased from 8 to 20 ºC, while relative humidity was progressively reduced to 64%. Finally, hams were left to mature for 16 months (cellar stage) at 20-25 ºC and relative humidity 55-65%.

Sampling

Sampling was carried out at the green stage (raw meat) and at the end of each processing step. Samples taken at the end of salting, post-salting and drying steps were obtained by extracting a cylindrical piece of ham (sized 10 x 2.5 cm) using a stainless steel tube with a cutting edge. These samples mainly included the *Biceps femoris* muscle. Samples taken at the beginning and at the end of processing were obtained by dissecting the *Biceps femoris* muscle of each ham. Samples were vacuum-packaged and kept frozen at -80 ºC until being analysed.

Non-protein nitrogen fractions

In order to analyze NPN, amino acid nitrogen (AN) and peptide nitrogen (PN), a muscle extract was prepared following the method described by De Ketelaere, Demeyer, Vandekerckhove and Vervaeke (1974). Briefly, 5 g of ground sample were mixed with 50 ml of perchloric acid 0.6 N. The mixture was homogenized for 3 min in a Sorval Omnimixer homogenizer, and subsequently centrifuged (10 min, 5000 rpm) and filtered through Whatman No. 54 filter paper. The residue was rehomogenized with 10 ml of perchloric acid 0.6 N, centrifuged (10 min, 5000 rpm), filtered and collected together with the previous filtrate. This filtrate was adjusted to pH 6 with potassium hydroxide, chilled, filtered and filled up to 100 ml with distilled water.

Non-protein nitrogen was analyzed following the Johnson (1941) method. Muscle extract (0.2 ml) was taken into a tube and dried (1h, 100 ºC). The dried sample was hydrolyzed with sulphuric acid (0.2 ml) on hot sand (120º C) until it was transparent. Then, it was mixed with distilled water (4.8 ml), Nessler reactive (2 ml) and sodium hydroxide 4 N (3 ml). This mixture was shaken and keep in dark for 10 min. Absorbance was measured at 490 nm on a spectrophotometer (Hitachi U-2000, Tokio). Concentration of NPN was calculated from a standard curve, which was developed simultaneously with the samples using solutions of ammonic sulphate 0.1 mg N/ml.

Amino acid nitrogen was determined according to Moore and Stein (1954). The muscle extract (10 ml) was mixed with 10 ml of sulphosalicilic acid 10%. The mixture was kept at 0-1 ºC during 17 h. After that, it was adjusted to pH 6 with sodium hydroxide 4 N, filtered and filled up to 50 ml with distilled water. 0.5 ml of this solution were mixed with 1.5 ml of ninhydrin reactive. The tubes were shaken, heated for 20 min in boiling water and chilled. Then, 8 ml of n-propanol 50% were added to the solution, which was again shaken and settled for 10 min. Absorbance was measured at 570 nm on a spectrophotometer (Hitachi U-2000, Tokio). Concentration of AN was calculated from a standard curve, which was developed simultaneously with the samples using solutions of leucine 0.1 mg N/ml.

Peptide nitrogen was calculated following the Moore and Stein (1954) method. 10 ml of chlorhydric acid 6 N were added to 3 ml of the muscle extract. The mixture was kept on hot sand (120 ºC) for 24h. It was adjusted to pH

6 with sodium hydroxide 30%, filtered and filled up to 50 ml with distilled water. The absorbance measuring and standard curve were the same as AN. PN was quantified by the difference between the absorbance values obtained for this last solution and those previously determined for AN.

Amino acid analysis

Amino acid content was determined following the procedure described by Flores et al. (1997). Sample was prepared by homogenizing 5 g of the ground ham sample, diluted 1:5 with chlorhydric acid 0.1 N, in a Sorval Omnimixer for 8 min and cooled by submerging the extract in ice. The homogenized samples were centrifuged (20 min, 10000 rpm) and the supernatant material was filtered through glass wool prior to further analyses. 100 µl of this extract were mixed with nor-leucine (50 µl), as internal standard, being deproteinized by adding 2.5 volume of acetonitrile (Aristoy & Toldrá, 1991) and centrifuged (3 min, 10000 rpm). Amino acid derivatization was carried out with phenyl isothiocyanate (PITC) according to the method of Bidlingmeyer, Cohen, Tarvin and Frost (1987). Supernatant (200 µl) was dried, mixed with 15 µl of methanol: sodium acetate 1M:triethylamine (2:2:1 vol,vol,vol) and dried, doing this procedure one more. Then, 15 µl of methanol:water: triethylamine:PTIC (7:1:1:1 vol,vol,vol,vol) was added, settling for 20 min and drying. The residue was dissolved in 200 µl of 0.005 M phosphate buffer, pH 7.4. Amino acid content was determined by high performance liquid chromatography (Hewlett-Packard model 1050) with a photodiode array detector (254 nm). The solvent system consisted of two eluents: (A) 0.07 M sodium acetate adjusted to pH 6.55 with acetic acid 10% and acetonitrile 2.5%; (B) acetonitrile:water:methanol (45:40:15 vol,vol,vol). The flow rate was 1 ml/min and the solvent gradient was: initial 0% B, 13.5 min linear change to 3% B, 3 min linear change to 3.1% B, 2.5 min linear change to 3.5% B, 2 min to 4.5% B, 3 min to 6% B, 1 min to 6.9% B, 1 min to 8% B, 2 min to 8.8% B, 2.5 min to 9% B, 20 min to 34% B and maintained 10 min at 34% B, then 2 min to 100% B and maintained for 8 min. Identification was based on the retention times of reference compounds (Sigma). Dipeptides anserine and carnosine were also identified supported by standars (Sigma) whereas isolated balenine from pork muscle was used as reference for this dipeptide (Aristoy, Soler & Toldrá, 2004).

Statistical Analysis

The effect of pre-cure freezing Iberian hams on the non-protein nitrogen fractions and on the profile of amino acid and dipeptides throughout the processing were analysed by one-way analysis of variance (ANOVA) using the General Linear Model of SPSS (v.15.0). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test.

RESULTS AND DISCUSSION

Non-protein nitrogen throughout the processing of refrigerated and pre-cure frozen Iberian hams

The NPN content of Iberian hams throughout the processing is shown in Table 1. Values of NPN rose during the ripening, reaching the highest content at the end of the final stage, which indicate the occurrence of proteolysis. These results agree with those obtained by Córdoba et al. (1994a) and Martín et al. (1999), in which two maxima in the daily increase of NPN fraction were found, the first and highest during salting and the second during drying. These authors related the increase in NPN during the drying step to the high temperatures (up to 30ºC) reached during this stage. It has been shown that temperature in the usual range of the drying process (22 and 30ºC) leads to a substantial proteolytic enzyme activities (around 40-50 and 80%, respectively) (Toldrá, et al., 1992b). Toldrá, Cerveró and Part (1993) indicated that these enzymes showed a maximum activity at around 35 ºC. However, NPN values described by Córdoba et al. (1994a) and Martín et al. (1999) in Iberian ham are higher than those observed in this work, which could be due to the lower temperature reached in the present study during the drying stage (up to 20 ºC) in comparison with those achieved in the previous works (25-30 ºC). In this sense, higher amounts of NPN have been reported in hams ripened at higher temperature (Flores, Bermell, Nieto & Costell, 1984; Virgili, Parolari, Schivazappa, Soresi Bordini & Borri, 1995).

The effect of pre-cure freezing did not lead to differences in NPN content throughout the processing (Table 1). These results are in agreement with those found by Flores et al. (2006), who did not show differences between frozen/thawed and traditional fresh processing Serrano hams in NPN index during the salting and the post-salting stages. On the contrary, Wang (2001) obtained higher NPN content in Taiwanese ham prepared with chilled meat than that made with frozen/thawed meat during the ripening process. Bañón et al. (1999) showed higher levels of NPN/total nitrogen in pre-cured frozen than in refrigerated dry-cured Serrano hams, which were related to protein modifications during freezing, providing a more favourable environment for muscle proteases (Bañón et al., 1999).

It has been described that salt has a powerful inhibitory effect on proteinases (Sárraga, Gil, Aranou & Monfort, 1989; Toldrá, Rico & Flores, 1992a). In fact, higher content of NPN has been associated to lower content of salt in Iberian (Martín et al., 1998) and Taiwanese hams (Wang, 2001). However, although the salt content was higher in R than in F Iberian hams of this study at final stage (6.58% vs. 5.21%, respectively), the values of NPN did not showed differences between these two groups of hams at the end of the processing. In fact, Córdoba et al. (1994a) considered that salt concentrations in the range 1.5-6% do not markedly affect NPN levels.

Peptide nitrogen throughout the processing of refrigerated and pre-cure frozen Iberian hams

The PN levels of Iberian hams throughout the processing are shown in Table 1. The PN content did not change during the first stages of the processing. It mainly increased during the cellar stage, reaching the highest levels at the final stage. Salt strongly inhibits cathepsin activity, but it affects more pronouncedly cathepsins H and D activities, which release large fragments from proteins (Rico, Toldrá & Flores, 1991, 1990). Nevertheless, these enzymes are very active in the temperature range of 20-30º C, which is reached at cellar stage (Rico et al., 1991). However, results found in this study are not totally in concordance with those showed by Martín et al. (1999) who also found an increase in PN values during the drying but not at the cellar stage in which PN levels decreased or remained constant, depending on the processing conditions.

There were significant differences in the PN content between R and F Iberian hams at the final stage (3.57 vs 6.47 mg/g muscle dry matter, respectively), whereas PN levels were very similar in the previous phases. This difference could be explained by the significant higher salt content in R (6.58%) than F Iberian hams (5.21%). In fact, Martín et al. (1999) related high PN content in Iberian ham with low salt levels. Thus, according to the NPN and PN results of this study, it could be pointed out that minor variation in the levels of salt influenced PN content but not the levels of NPN at the end of the processing of Iberian hams.

Amino acid nitrogen throughout the processing of refrigerated and pre-cure frozen Iberian hams

Table 1 shows the AN content of Iberian hams during the ripening process . The levels of AN kept constant from the initial step to the end of the post-salting stage, whereas it experimented enhancement during the drying and cellar steps, attaining the highest AN content at the end of the processing. Córdoba et al. (1994b) and Martín et al. (2001, 1999) found the larger daily increases of NA at the drying stage. Such an increase in AN during cellar has been only previously found in Iberian hams with high salt content (around 6%) and temperatures close to 25 ºC, conditions similar to those found in the hams of this study, but not in hams processed with cellar temperature below 20 ºC and with 4% NaCl (Martín et al., 1999).

Throughout the processing of both R and F Iberian hams of this study, the levels of NA only showed differences at the end of the drying step, being statistically higher (p=0.038) in F (5.82 mg/g muscle dry matter) than in R hams (4.74 mg/g muscle dry matter). However, there were no differences in NA content at the final stage. Virgili, Parolari, Soresi-Bordini, Schivazappa, Cornet and Monin (1999) found that free amino acid content was negatively correlated with salt content, which could explain the results of AN content at the drying stage but not at the end of the processing. Thus, it seems that the differences in salt content between R and F Iberian ham were not so large to influence NA levels during the final step, being more important the process conditions. In fact, Martín et al. (1998) pointed out that temperature is the main parameter regulating AN formation and that high temperature during the cellar stage allows the accumulation of free amino acid only in suitable salted hams.

Amino acid and dipeptide evolution throughout the processing of refrigerated and pre-cure frozen Iberian hams

Content of free amino acids and dipeptides throughout the processing of Iberian hams in this study is shown in Table 2. Twenty five peaks were identified in the chromatograms, 22 of them being amino acids and the other three being didpeptides. ß-alanine, taurine and ornithine, which have a non-protein origin, as well as the dipeptides carnosine, anserine and balenine have not been previously reported in Iberian ham (Córdoba et al., 1994b; Ruiz et al.,

1999; Martín et al., 2001; Jurado et al., 2007) but they were identified in other types of dry-cured ham (Buscailhon, Gandemer & Monin, 1994; Flores et al., 1997; Toldrá, Aristoy & Flores, 2000). Cysteine, a free amino acid coming from proteolysis, was not detected, which agrees with the above cited studies. Levels of most free amino acid detected at the green stage raised significantly throughout the processing, even during the cellar stage, in agreement with Córdoba et al. (1994b). However, other studies showed an increase in free amino acid content between green stage and the end of the drying but no changes during the last step (Jurado et al., 2007; Martín et al., 2001; Toldrá et al., 2000; Ruiz et al., 1999). Several circumstances reduce aminopeptidase activities along the dry-curing process. Salt is an effective inhibitor, while pH has a lower effect due to its narrow range of variation during the process (Flores et al., 1997). The accumulation of free amino acids in the hams also produces a feedback inhibition on aminopeptidases (Flores, Aristoy & Toldrá, 1998). Enzyme activities are also influenced by moisture and water activity, which diminish as drying progresses, leading to a reduction in the overall proteolityc phenomena (Toldrá et al., 1992b). Dry-cured hams of the present study showed higher moisture content (52.02 %) than Iberian hams from the studies cited above, in which amino acid content did not vary during cellar (45-48% of moisture content) (Jurado et al., 2007; Martín et al., 2001). Thus, such higher moisture content in the hams of the present study could in part explain the progressive increase of amino acid during the cellar step compared to previous studies.

Among non protein origin amino acids, ornithine increased above all at the final stages, similarly to what Buscailhon et al., (1994) and Toldrá et al. (2000) described in other dry–cured hams, whereas the content of ß-alanine and taurine increased throughout the ripening, which is not in agreement with Toldrá et al. (2000), who reported that these amino acids remained constant, neither with Buscailhon et al. (1994), whose results showed that these compounds decreased during the cellar stage. Levels of Carnosine, anserine and balenine started to decrease at the post-salting stage and continued diminishing during the rest of the processing. Thus, the lowest content of these dipeptides were found at the end of the processing. This result is in concordance with Toldrá et al. (2000) and Buscailhon et al. (1994).

On the other hand, there were significant differences in the content of these amino acids and dipeptides between R and F hams at the end of the different stages of the processing, mainly in the initial step, but also in the last phases. Most amino acids and dipeptides showed higher levels in F than in R hams at green stage. The higher proteolysis rate in F hams could be attributed to an enhanced release of these cathepsins from lysosomes due to the physical change caused by ice crystals (Flores et al., 2006). However, these authors found lower cathepsin activity in the frozen/thawed Serrano hams than in traditional fresh processing ones during salting and post-salting steps. Khan (1986) also reported that the free amino acid and peptide content increased during frozen storage, suggesting the subsistence activity of cathepsins. Kristensen, Christensen and Ertbjerg (2006) pointed out that calpain and calpastatin are stable during frozen storage of meat.

At the end of salting and post-salting there were scarce differences between F and R Iberian hams in amino acid and dipeptide content. However, Flores et al. (2006) found higher concentration of free amino acids in frozen/thawed than in refrigerated hams during salting and post-salting stages of the manufacturing of Serrano hams. At the end of drying, glutamic acid, asparagine, glutamine, leucine and phenylalanine showed higher levels in F than in R hams. These results are in concordance with those of AN content and could be related to the higher salt content in R than in F hams, as it has been explained above. Although there were statistical differences in individual amino acid and dipeptides content between R and F hams at the final stage, the effect of pre-cure freezing does not seem to be well defined. Taurine, arginine, proline, tyrosine, leucine, phenylalanine and triptophane showed higher levels in F than in R Iberian hams. However glutamine, ornithine and dipeptide balenine showed higher levels in R than in F hams, and no differences were found in the rest of compounds. In contrast to the obtained results in this work, Arnau et al. (1994) did not observe statistical differences in tyrosine content between refrigerated and thawed hams at the end of the ripening in dry-cured Serrano hams. In Taiwanese dry-cured ham, Wang (2001) found a higher free amino acid content in samples from chilled meats than in frozen ones, which the author related to the lower salt content in pre-cure frozen hams.

CONCLUSIONS

Pre-cure freezing Iberian hams does not seem to markedly influence the content of NPN, PN and AN in the different stages of the processing. The only significant effect was the higher content of NP in refrigerated than in frozen/thawed hams at the end of the ripening, which seem to be related to the salt level more than to the pre-cure freezing.

On the other hand, pre-cure freezing of Iberian ham seems to influence the content of most free amino acids and dipeptides at the initial stage, being higher in refrigerated than in pre-cure frozen Iberian hams. However, this effect was not that clear in the final stage, where differences in these compounds between Iberian dry-cured hams processed under these two different technologies being did not follow a well defined pattern.

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Table 1. Non-protein nitrogen (NPN), peptide nitrogen (PN) and amino acid nitrogen (NA) content (expresses as mg /g muscle dry matter) throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	NPN			NP			NA		
	R[t]	**F[u]**	*p*	**R[t]**	**F[u]**	*p*	**R[t]**	**F[u]**	*p*
Green stage	$4.26^{c} \pm 0.311$	$4.64^{c} \pm 0.434$	0.495	$2.92^{ab} \pm 0.23$	$3.02^{b} \pm 0.35$	0.820	$1.63^{c} \pm 0.160$	$1.68^{c} \pm 0.251$	0.877
Salting	$5.55^{c} \pm 0.377$	$5.08^{b} \pm 0.427$	0.425	$1.82^{b} \pm 0.245$	$2.49^{b} \pm 0.317$	0.135	$1.53^{c} \pm 0.115$	$1.62^{c} \pm 0.190$	0.385
Post-salting	$8.21^{bc} \pm 0.521$	$7.55^{b} \pm 0.816$	0.512	$1.53^{b} \pm 0.316$	$1.87^{b} \pm 0.236$	0.420	$2.32^{c} \pm 0.038$	$2.15^{c} \pm 0.101$	0.156
Drying	$12.42^{b} \pm 2.38$	$15.72^{a} \pm 1.36$	0.24	$2.15^{ab} \pm 0.425$	$2.85^{b} \pm 0.198$	0.175	$4.74^{b} \pm 0.283$	$5.70^{b} \pm 0.273$	0.038
Final stage	$17.49^{a} \pm 1.10$	$17.94^{a} \pm 0.830$	0.75	$3.57^{a} \pm 0.490$	$6.47^{a} \pm 0.478$	0.004	$8.96^{a} \pm 0.417$	$8.70^{a} \pm 0.235$	0.596
p (evolution)	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

s: Mean values ± standard error of the mean. Means with different superscripts differ significantly throughout ripening ($p<0.05$).
t: refrigerated Iberian hams; u: pre-cure frozen Iberian hams

Table 2. Content of amino acid and dipeptide (expresses as mg /100g muscle dry matter) throughout the processing of refrigerated and pre-cure frozen Iberian hams[s].

	GREEN STAGE			SALTING			POST-SALTING			DRYING			FINAL STAGE			evolution	
	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	R[t]	F[u]	*p*	*p* (R)	*p* (F)
Asp	6.89^{c} ± 2.72	7.27^{c} ± 1.65	0.807	15.60^{c} ± 5.44	24.89^{bc} ± 9.03	0.202	63.75^{b} ± 2.81	65.19^{bc} ± 10.32	0.828	93.31^{b} ± 23.73	86.26^{b} ± 27.29	0.729	255.75^{a} ± 27.77	236.22^{a} ± 58.78	0.563	<0.001	<0.001
Glu	25.05^{c} ± 0.80	42.10^{d} ± 2.78	<0.001	52.21^{c} ± 9.06	67.29^{d} ± 2.22	0.058	255.29^{b} ± 14.46	177.44^{c} ± 10.32	<0.001	246.57^{b} ± 23.59	300.70^{b} ± 17.91	0.034	371.10^{a} ± 56.25	387.67^{a} ± 56.38	0.716	<0.001	<0.001
Ser	27.64^{c} ± 4.51	38.79^{d} ± 5.14	0.048	54.25^{bc} ± 9.68	64.39^{cd} ± 9.71	0.229	127.33^{bc} ± 3.80	139.08^{bc} ± 21.69	0.327	175.40^{ab} ± 25.82	207.57^{b} ± 23.40	0.185	266.23^{a} ± 95.02	332.11^{a} ± 71.59	0.344	<0.001	<0.001
Asn	10.61^{c} ± 1.98	20.03^{c} ± 1.05	0.001	23.82^{c} ± 4.82	27.62^{bc} ± 2.91	0.247	56.43^{b} ± 5.37	55.21^{abc} ± 8.96	0.823	43.18^{b} ± 6.78	84.07^{ab} ± 9.73	0.002	111.39^{a} ± 13.66	107.57^{a} ± 54.17	0.912	<0.001	<0.001
Gly	38.01^{d} ± 4.84	43.38^{d} ± 5.58	0.230	60.10^{d} ± 10.64	62.72^{cd} ± 7.32	0.713	114.30^{c} ± 7.22	130.67^{bc} ± 16.40	0.117	158.79^{b} ± 18.38	193.58^{b} ± 19.92	0.090	580.66^{a} ± 16.72	622.24^{a} ± 67.87	0.279	<0.001	<0.001
Gln	102.64^{b} ± 9.37	106.81^{bc} ± 14.42	0.645	153.81^{a} ± 25.12	143.88^{a} ± 14.08	0.530	152.17^{a} ± 7.93	142.32^{ab} ± 23.68	0.404	68.77^{c} ± 1.73	101.74^{c} ± 4.56	<0.001	28.86^{d} ± 2.79	22.12^{d} ± 0.79	0.016	<0.001	<0.001
Tau	168.93^{b} ± 22.60	179.06^{bc} ± 24.55	0.566	206.73^{b} ± 74.88	223.76^{bc} ± 21.44	0.724	214.29^{b} ± 18.38	196.25^{bc} ± 31.15	0.357	215.74^{c} ± 10.80	267.00^{b} ± 37.30	0.073	364.22^{a} ± 6.15	451.55^{a} ± 41.88	0.006	<0.001	<0.001
His	16.00^{d} ± 1.80	20.65^{c} ± 3.40	0.044	42.94^{d} ± 6.89	48.44^{c} ± 3.29	0.280	99.93^{c} ± 1.52	113.35^{b} ± 19.06	0.210	134.13^{b} ± 15.44	154.27^{b} ± 31.97	0.381	408.03^{a} ± 24.43	401.49^{a} ± 37.05	0.778	<0.001	<0.001
Thr	21.06^{b} ± 2.38	27.51^{d} ± 3.20	0.018	47.37^{b} ± 9.30	54.62^{cd} ± 7.14	0.293	121.48^{b} ± 4.94	125.40^{c} ± 19.00	0.703	178.34^{b} ± 21.87	230.19^{b} ± 26.72	0.060	375.82^{a} ± 126.83	514.81^{a} ± 67.05	0.090	<0.001	<0.001
Ala	116.61^{c} ± 19.39	122.98^{b} ± 21.96	0.679	137.52^{c} ± 36.46	170.88^{b} ± 16.36	0.222	230.82^{b} ± 7.06	226.63^{b} ± 32.12	0.807	307.78^{b} ± 47.20	368.13^{b} ± 30.53	0.136	1338.96^{a} ± 54.18	1626.94^{a} ± 320.77	0.193	<0.001	<0.001
Arg	30.21^{c} ± 4.17	45.95^{b} ± 5.91	0.005	49.69^{c} ± 16.35	71.22^{b} ± 8.06	0.067	134.90^{b} ± 7.99	157.62^{a} ± 24.66	0.090	175.84^{a} ± 33.26	210.26^{a} ± 28.00	0.242	39.46^{c} ± 18.38	152.29^{a} ± 46.06	0.017	<0.001	<0.001
Pro	16.29^{d} ± 5.79	17.89^{c} ± 1.64	0.614	36.09^{cb} ± 5.46	44.59^{c} ± 9.94	0.244	115.15^{c} ± 14.61	127.22^{c} ± 12.26	0.253	274.65^{b} ± 31.16	294.51^{b} ± 28.84	0.463	1039.75^{a} ± 68.24	1213.00^{a} ± 113.13	0.039	<0.001	<0.001
Tyr	19.91^{d} ± 2.87	35.71^{d} ± 3.50	<0.001	45.23^{d} ± 8.83	49.14^{d} ± 2.81	0.506	118.23^{c} ± 12.37	95.20^{c} ± 13.08	0.030	191.69^{b} ± 26.10	226.12^{b} ± 16.70	0.127	351.54^{a} ± 54.78	455.87^{a} ± 28.90	0.032	<0.001	<0.001
Val	28.50^{c} ± 4.49	41.43^{c} ± 2.71	0.007	56.35^{c} ± 8.06	60.47^{c} ± 6.92	0.538	134.16^{c} ± 4.48	124.58^{c} ± 20.30	0.392	291.39^{b} ± 53.81	306.57^{b} ± 23.69	0.678	1076.41^{b} ± 106.83	1201.18^{a} ± 130.71	0.168	<0.001	<0.001
Met	15.24^{d} ± 3.70	27.47^{c} ± 1.90	0.004	33.67^{d} ± 5.66	37.02^{c} ± 1.73	0.382	67.95^{c} ± 6.37	65.44^{c} ± 9.92	0.685	111.88^{b} ± 15.38	141.71^{b} ± 11.49	0.055	620.60^{a} ± 16.20	598.07^{a} ± 41.32	0.420	<0.001	<0.001
Ile	20.26^{c} ± 1.76	37.40^{c} ± 6.13	0.002	43.94^{c} ± 8.08	46.10^{bc} ± 3.21	0.689	97.39^{bc} ± 6.14	84.66^{bc} ± 5.44	0.026	232.09^{b} ± 46.92	252.17^{b} ± 19.78	0.532	1007.34^{a} ± 120.88	1149.50^{a} ± 155.63	0.179	<0.001	<0.001
Leu	36.29^{d} ± 1.75	68.00^{c} ± 3.03	<0.001	88.51^{d} ± 14.42	92.83^{c} ± 5.19	0.652	225.17^{c} ± 26.46	198.73^{c} ± 5.12	0.093	382.12^{b} ± 55.28	525.40^{b} ± 28.77	0.016	2203.18^{a} ± 49.43	2598.49^{a} ± 245.55	0.044	<0.001	<0.001
Phe	20.98^{d} ± 2.13	43.84^{c} ± 0.93	<0.001	47.65^{cd} ± 9.37	49.06^{c} ± 2.63	0.781	108.46^{c} ± 11.10	118.74^{c} ± 24.99	0.481	245.22^{b} ± 16.55	335.34^{b} ± 46.07	0.033	1398.65^{a} ± 56.74	1627.17^{a} ± 105.31	0.020	<0.001	<0.001
Lys	42.29^{c} ± 10.94	63.10^{c} ± 3.20	0.026	103.09^{c} ± 18.19	117.20^{c} ± 17.42	0.345	227.92^{b} ± 20.46	214.79^{b} ± 43.60	0.599	249.56^{b} ± 44.37	292.07^{b} ± 68.60	0.418	645.17^{a} ± 76.78	602.36^{a} ± 18.63	0.398	<0.001	<0.001
B-ala	6.97^{b} ± 2.34	7.51^{b} ± 0.40	0.715	8.85^{b} ± 1.36	11.29^{ab} ± 1.32	0.063	9.01^{b} ± 2.11	8.04^{ab} ± 2.01	0.530	9.91^{b} ± 0.68	9.58^{ab} ± 0.17	0.465	13.68^{a} ± 0.54	12.11^{d} ± 2.53	0.213	<0.001	<0.001
Trp	<0.001	<0.001	-	<0.001	<0.001	-	<0.001	<0.001	-	60.60 ± 13.26	74.97 ± 10.00	0.208	226.42 ± 46.81	315.28 ± 10.10	0.025	0,002	<0.001
Orn	<0.001	<0.001	-	<0.001	<0.001	-	<0.001	<0.001	-	6.54 ± 2.33	2.80 ± 1.36	0.058	128.02 ± 21.93	99.30 ± 4.22	0.046	<0.001	<0.001
Carnosine	2642.69^{a} ± 340.77	2216.38^{a} ± 353.05	0.110	2654.64^{a} ± 228.42	2641.30^{a} ± 191.19	0.942	1457.79^{b} ± 117.92	1475.16^{b} ± 255.17	0.906	1125.27^{b} ± 87.84	1162.11^{b} ± 358.92	0.872	979.31^{b} ± 117.26	850.58^{b} ± 136.75	0.203	<0.001	<0.001
Anserine	121.45^{ab} ± 13.57	146.83^{a} ± 17.53	0.044	131.85^{a} ± 28.81	153.64^{a} ± 15.58	0.313	86.34^{bc} ± 9.31	79.96^{b} ± 7.96	0.337	69.10^{c} ± 9.68	79.92^{b} ± 33.35	0.618	68.44^{c} ± 10.69	65.75^{b} ± 7.33	0.738	<0.001	<0.001
Balenine	168.29^{b} ± 21.08	205.12^{a} ± 22.94	0.041	224.59^{a} ± 32.77	201.30^{a} ± 8.90	0.301	127.84^{bc} ± 14.26	119.71^{b} ± 10.62	0.377	99.91^{c} ± 15.46	97.02^{bc} ± 26.82	0.863	98.68^{c} ± 4.26	75.54^{c} ± 7.56	0.010	<0.001	<0.001
Σ amino acid	762.63^{c} ± 12.16	991.10^{c} ± 112.85	0.025	1163.34^{c} ± 250.44	1259.37^{c}± 77.91	0.597	2539.46^{b} ± 48.93	1869.85^{bc} ± 232.26	0.001	3173.12^{b} ± 642.65	4611.76^{b} ± 655.69	0.085	12165.86^{a} ± 927.29	14001.76^{a} ± 655.69	0.005	<0.001	<0.001
Σ dipeptide	2741.82^{a} ± 74.15	2360.14^{a} ± 409.70	0.180	2911.65^{a} ± 329.65	2996.24^{ab} ± 209.33	0.727	1673.37^{b} ± 140.13	1674.82^{bc} ± 270.21	0.993	1280.10^{bc} ± 101.24	1294.81^{c} ± 396.92	0.953	1090.61^{c} ± 149.02	956.54^{c} ± 117.51	0.207	<0.001	<0.001

s: Mean values ± standard error of the mean. Means with different letter superscripts differ significantly throughout ripening (p<0.05).
t: refrigerated Iberian hams; u: pre-cure frozen Iberian hams

Capítulo II.4.

Influence of pre-cure freezing on volatile compounds throughout the processing of Iberian ham

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TITLE

Influence of pre-cure freezing on volatile compounds throughout the processing of Iberian hams.

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RUNNING TITLE

Volatile compound in pre-cure freezing Iberian hams

ABSTRACT

The effect of pre-cure freezing on the profile of volatile compounds throughout the processing of Iberian hams was studied. Aldehydes was the major group of volatile compounds throughout the processing, alcohols being the second most abundant family. Esters were only found at the end of the ripening. Straight chain aldehydes, alcohols and ketones, which are originated from the oxidation of fatty acids, were firstly generated during the post-salting step, decreased thereafter during the drying step and rose again at the end of the processing. Branched and aromatic aldehydes, branched alcohols, branched acids, esters, furans and pirazines was firstly detected in the drying stage and showed a marked increase at the cellar step.

The influence of pre-cure freezing Iberian hams on volatile compound generation during the processing was not very marked. At the final stage, the levels of 2-methyl butanal, 2-methyl-1-butanol, 2,3-butanediol and 2-heptanol were significantly higher in pre cure-frozen (F) than in refrigerated (R) hams, whereas the content of most detected esters were statistically lower in F than in R hams.

KEY WORDS

Pre-cure freezing, volatile compounds, Iberian ham, processing

INTRODUCTION

The particular flavour of Iberian dry-cured hams is the consequence of volatile compound generation during ham ripening, involving enzymatic reactions (proteolysis and lipolysis) and chemical processes (lipid autooxidation, Strecker and Maillard reactions) of the main components of raw meat (protein and lipids) (Antequera et al., 1992; Ventanas et al., 1992).

There is scientific information about the volatile aldehyde formation in Iberian ham (Martín, Timón, Petrón, Ventanas & Antequera, 2000), the changes in some individual volatile compound of Iberian ham in a shortened processing (Andrés, Cava & Ruiz, 2002; Andrés, Cava, Ventanas, Muriel & Ruiz, 2007) and in the last steps of processing (Ruiz, Ventanas, Cava, Andrés & García, 1999;) as well as studies showing the flavour development in other types of dry cured ham (Huam, Zhou, Zhao, Xu & Peng, 2005; Flores, Grimm, Toldrá & Spanier, 1997; Bolzoni, Barbieri & Virgili, 1996; Hinrichsen & Andersen, 1995). Ruiz, Muriel and Ventanas (2002) have outlined a scheme of the formation of volatile compounds throughout processing of Iberian ham using all this information. Besides, it has been described the changes in some volatile compounds during the ripening of Iberian hams (Jurado, García, Timón & Carrapiso, 2007; Jurado, Carrapiso, Ventanas & García, 2009).

The procedure of freezing storage of raw thighs and thawing before Iberian ham processing is not commonly used nowadays. Besides, dry cured ham producers believe that this practise could have detrimental consequences on the quality of dry cured Iberian hams. Moreover, some of the Specific Designation of Origin that protect the production of dry-cured Iberian hams, specifically exclude freezing as a procedure for raw material storage. However, there are not scientific data confirming the detrimental effect of using frozen/thawed raw material on the quality of the derived Iberian dry-cured meat products. Moreover, such procedure could show several technological and economical advantages, such as the possibility of salting hams with more homogeneous weights or the processing of hams avoiding seasonal availability and market price fluctuations.

Frozen storage could lead to changes in physical (drip loss, texture modification), chemical (lipolysis and FA oxidation, protein denaturation and aggregation, changes in colour), and sensory properties of meat, depending

on the characteristics of fresh meat, further processing of the meat, and frozen condition. (Carballo & Jiménez, 2001).

Although there are numerous works about the influence of freezing on several parameters related to meat quality, only a few studies have been devoted to show the effect of using frozen and subsequently thawed raw material on the quality of dry-cured hams (Flores, Soler, Aristoy & Toldrá, 2006; Arnau, Gou & Guerrero, 1994; Motilva, Toldrá, Nadal & Flores, 1994; Bañón, Cayuela, Granados & Garrido, 1999; Wang, 2001) and no one dealing with such topic in Iberian ham has been found in the scientific literature.

Some researches have shown no differences between dry-cured hams produced using fresh and frozen/thawed raw material in color, sensory analysis and acceptability scores (Motilva et al., 1994; Bañón et al., 1999), whereas others found a higher proteolysis in frozen hams (Flores et al., 2006; Bañón et al., 1999). On the other hand, there were contradictory results about the effect of this procedure on lipolysis. Flores et al. (2006) found higher lipolytic activity in hams from thawed raw material at the salting and post-salting stages while Motilva et al. (1994) did not show differences. Besides, a high incidence of white precipitates has been found in dry-cured hams produced with frozen/thawed pieces (Arnau et al., 1994; Bañón et al., 1999).

Thus, the aims of this work were to describe the generation of volatile compounds during the processing of Iberian hams and to study the influence of pre-cure freezing Iberian hams on the profile of volatile compounds at each stage of the ripening.

MATERIAL AND METHODS

Experimental design

Twelve hind limbs were obtained from Iberian pigs fattened in confinement and fed with a commercial diet. These hind limbs were divided into two groups, refrigerated (R) and pre-cure frozen (F) hams. The F hams were frozen (at -20 ºC) and thawed (4 days at 3-4 ºC) three months later. The R hams were obtained at the moment of thawing the F hams, and kept at 4 ºC during 36-48 hours until starting their processing. The two groups of Iberian hams were processed following the same conditions, except for the salting time, which was 1 day/kg for the R hams and 0.7 day/kg for the F hams. After salting, the salt from the surface was brushed and, the hams were processed as follows. Iberian hams were held at 4-8 ºC and 73-75% relative humidity for 70 days (post-salting step). During the drying stage the hams were kept in a room under controlled conditions for 120 days, temperature was increased from 8 to 20 ºC, while relative humidity was progressively reduced to 64%. Finally, hams were left to mature for 16 months (cellar stage) at 20-25 ºC and relative humidity 55-65%.

Sampling

Sampling was carried out at the end of each processing step. Samples taken at the end of salting, post-salting and drying steps were obtained by extracting a cylinder (sized 10 x 2.5 cm) using a stainless steel tube with a cutting edge. These samples mainly included the *Biceps femoris* muscle. Samples taken at the end of processing were obtained by dissecting the *Biceps femoris* muscle of each ham. Samples were vacuum-packaged and kept frozen at -80 ºC until being analysed.

Volatile compound analysis

A microextraction solid phase (SPME) fibre coated with Carboxen-polydimethylsiloxane-divinylbenzene (50µm thickness, Supelco Co., Bellefonte, USA) was used to extract the volatiles compounds. Prior to analysis, the SPME fibre was preconditioned at 250 ºC for 60 min in the gas chromatography (GC) injection port. Samples were ground with a commercial grinder. 1 g was weighed into a 4 ml vial, which was screw-capped with a laminated Teflon-

rubber disk. The vial was kept in a thermostatized water bath at 37 ºC for 15 min to equilibrate the sample. Then, the fibre was inserted into the sample vial through the septum and exposed to headspace for 30 min at 37 ºC. After, the SPME fibre was inserted and desorbed during 30 min. The injection port was in the splitless mode. The chromatographic analysis was performed using a Agilent 6890 series gas chromatograph (Agilent, Avondale, USA) coupled to a mass selective detector (Agilent 5973, Agilent, Avondale, USA). Volatiles were separated using a 5% Phenyl-Methyl Silicone (HP-5) bonded phase fused silica capillary column (Hewlett-Packard, 50 m x 0.32 mm i.d., film thickness 1.05 µm), operating at 45 kPa of column head pressure, resulting in a flow of 1.3 ml/min at 40 ºC. The oven temperature program was isothermal for 10 min at 40 ºC, raised to 190 ºC at the rate of 5ºC min^{-1} and then raised at 250 ºC at 20 ºC min^{-1}, and held for 5 min. The transfer line to the mass spectrometer was maintained at 280ºC. The mass spectra were obtained by electronic impact at 70 eV, with a multiplier voltage of 1756 V, collecting data at a rate of 1 scan s^{-1} over the *m/z* range of 30-500. *n*-Alkanes (Sigma, St. Louis, MO, USA) were run under the same chromatographic conditions to calculate the linear retention indices (LRI). Volatile compounds were identified by comparing their mass spectra with those contained in the NIST and Wiley libraries and by comparing their LRI with those reported in the literature (García-Esteban, Ansorena, Astiasarán and Ruiz, 2004; Muriel, Antequera, Petrón and Andrés, 2004) and in the NIST database (NIST, 2007, http//:webbook.nist.gov/chemistry/name-ser.html). Data of volatile compounds were expresses as abundance (total area counts x 10^{-5}).

Statistical Analysis

The effect of pre-freezing Iberian hams on the evolution of volatile compound during the processing were analysed by one-way analysis of variance (ANOVA) using the General Linear Model of SPSS (v.15.0). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test.

RESULTS AND DISCUSSION

Volatile compounds profile of both R and F Iberian dry-cured hams at the end of each processing step is shown in Table 1. A total of 61 volatile compounds were tentatively identified: 28 compounds were found at the end of the salting step, 30 at the end of the post-salting, 38 at the end of the drying and 55 at the end of the processing. The identified volatile compounds were clustered in the following chemical families: acids, aliphatic and aromatic hydrocarbons, alcohols, aldehydes, ketones, esters, furans and terpenes.

Figure 1 shows the proportion of the different chemical families at each processing step. In the four considered stages, aldehydes were the most abundant family of volatile compounds, followed by alcohols, while the other groups of volatile compounds (acids, aliphatic and aromatic hydrocarbons, ketones and furans) showed lower proportions. The family of esters was not found at the salting, post-salting and drying stage, only being at the last step. For cured Iberian ham, the major chemical classes found in this study agree with those reported in the literature (García et al., 1991; López et al, 1992; Ruiz et al., 1999). Huan et al., 2005 and Hinrichsen et al., 1995 in Chinese Jinhua and Parma hams, respectively, showed a similar outline of chemical families of volatile compounds as in the present study. However, Bolzoni et al. (2005), who also studied Parma hams, found that esters was the major group at 6 and 9 months of processing and aldehydes and ketones at the end of ripening.

At the salting, postsalting and drying steps the major volatile compound was hexanal whereas this compound was the second most abundant at the final stage, in which benzeneacetaldehyde was the principal volatile compound. In previous studies dealing with Iberian dry-cured ham, hexanal was the major volatile compound, while benzeneacetaldehyde was even not detected in some of them (García et al., 1991; López et al., 1992; Ruiz et al., 1999). This variation may be due to the different analytical method used. In fact, Jurado et al. (2007), using a SPME fibre coated with carboxen-polydimethysiloxane, found high values of benzeneacetaldehyde at the final stages of the Iberian dry-cured ham processing. Moreover, García-Esteban, Ansorera, Astiasarán and Ruiz (2004) extracted high levels of this compound when using a carboxen-polydimethylsiloxane-divinylbenzene fibre, which was the one used in the present study. It is worth to point out then phenylalanine is

the precursor of benzenacetaldehyde. Masson, Hinrichsen, Talon & Montel (1999) have postulated that this conversion is mediated by microbial action, although the Strecker degradation at the temperatures followed by the hams during the processing has been also demonstrated (Ventanas, Estevez, Delgado & Ruiz, 2007).

Pre-cure freezing Iberian hams did not seem to influence the aldehydes generation throughout the processing. Moreover, there were not notably differences in the content of individual aldehydes throughout the different stages of the ripening. However, at the end of the processing the levels of 2-methylbutanal were statistically higher in F (5.47) than in R (3.67) hams. Isoleucine is the precursor of this 2-methylbutanal. Thus, this result could be related with the higher content of this amino acid found in F than in R hams of this study at the initial and post-salting stages (unpublished data).

Although there were not statistical differences among the four analysed steps in total aldehydes, it could be observed that most individual aldehydes showed significant differences along the stages of the processing. Hexanal, nonanal and other minor straight chain aldheydes reached their highest content at the end of the post-salting stage, decreasing throughout the rest of the processing. These linear aldehydes arise from fatty acids oxidation; specifically hexanal is generated from linoleic and arachidonic acids (Tamura, Kitta & Shibamoto, 1991) and its decrease could be due to the high temperature reached in the drying and cellar stages and also to condensations with amino acids during these last stages (Ventanas et al., 1991; Antequera et al., 1992). Accordingly, the highest values of oxidation of the Iberian hams of this study were found at the end of the post-salting phase (unpublished data). A similar trend was found in previous studies dealing with Iberian dry-cured hams, both for volatile aldehydes and TBARs (Andres, Cava, Ventanas, Muriel & Ruiz, 2004; Andres, Cava, Ventanas, Muriel & Ruiz, 2007). 3- and 2-methylbutanal, benzaldehyde and benzeneacetaldehyde were not found at the salting and post-salting steps, reaching their highest contents at the end of the processing. The main formation route for branched (2 and 3-methylbutanal) and aromatic aldehydes (benzaldehyde and benzeneacetaldehyde) seems to be the Strecker degradation reactions of isoleucine, leucine, phenilglycine and phenylalanine, respectively (García et al., 1991; Belitz & Grosh, 1997). Other

studies have also found that the highest content of these amino acids are shown at the last stages of the processing of Iberian dry-cured hams (Ruiz et al., 1999), and their origin has been corroborated by the loss of their precursor amino acids during the last stages of the processing (Ventanas et al., 1991), and their formation at room temperature in model systems (Ventanas et al., 2007).

The procedure of using frozen/thawed raw material for processing dry-cured Iberian hams did not influence alcohols generation along the ripening. However, there were differences between R and F Iberian hams in some individual alcohols. The content of 1-hexanol was significantly higher in F than in R hams at the end of the salting (10.62 *vs* 3.94) and post-salting stages (4.34 *vs* 2.56) while at the end of the drying step the levels of this volatile compound were higher in R than in F Iberian hams (1.89 *vs* 0.78), and no differences were found at the end of the processing. Moreover, at the end of the processing the values of 2-methyl-1-butanol, 2,3- butanediol and 2-heptanol were statistically higher in F than in R hams. These results could be explained by the same reasons explained above for aldehydes, higher content of their precursors at the previous phases of the processing.

Total alcohols were statistically different among processing steps (p<0.003), their levels raising from the salting to the post-salting steps, decreasing thereafter at the end of the drying step and increasing again to the highest contents at the end of the processing. This evolution is the consequence of a similar behaviour for most detected individual alcohols, that rose at the end of the processing. These results are according to the volatile compound evolution described for Parma and Iberian hams (Hinrichsen et al., 1995; Jurado et al., 2007). However, in Chinese Jinhua hams, this chemical family predominated at the green stage (Huan et al., 2005). Straight chain aliphatic alcohols are most likely oxidative decomposition products of lipids, while branched alcohols are probably derived from the Strecker degradation of amino acids through the oxidation of their respective Strecker aldehydes (2- and 3-methylbutanal) (Ruiz et al., 2002). In fact, both pairs of volatile compounds followed the same trend during the Iberian dry-cured ham ripening in this study.

The influence of pre-cure freezing Iberian hams was significant for esters. The content of eight out of nine esters detected was higher in R than in F hams at the final stage, which could be explained by the fact that esters seems to be generated more easily by undamaged cells (Belitz et al., 1997), and the formation of ice crystals during frozen storage may damage muscle cells. In dry-cured hams, higher content for esters has been related to high amount of alcohols (Sabio et al., 1997), since esters are formed by esterification of carboxylic acids and alcohols (Mottram, 1991), and also to dry-cured hams processed without adding nitrate (Flores et al., 1997). The high content of ester in Parma hams has been attributed to the lack of nitrate use in its processing (Parolari, 1996). In fact, Irfan Aksu et al. (2005) found that the content of nitrite of pastrima (a traditional Turkish meat product using nitrite and/or nitrate in the process of manufacturing) samples made with frozen/thawed meat was higher than that of the pastirma made from fresh meat at both 0 days and at the end of the processing (150 days).

Esters were only found at the final stage of the processing. In Parma and Chinese Jinhua hams these compounds were detected throughout ripening, but the highest values were also detected at the end of the processing (Hinrichsen et al., 1995; Huan et al., 2005). Esters are formed by the interaction of free fatty acids and alcohols generated by lipid oxidation in the intramuscular tissues (Shahidi et al., 1986). Flores et al., (1997) discarded a microbial origin for these compounds in dry-cured hams, due to the low microbial counts found (Baldini et al., 1992), while Andrade, Córdoba, Sánchez, Casado and Rodríguez (2009) considered that microorganisms could be responsible in part of esters formation, because of the high counts of yeasts, moulds and bacteria found in the surface of the hams during ripening (Núñez, Rodríguez, Bermúdez, Córdoba & Asensio, 1996). Flores et al., (1997) also believed nitrate could be related to the lowest concentration of esters found in Iberian ham, because of the greater amounts of these compounds found in Italian hams, which are basically nitrate-free (Barbieri et al., 1992; Parolari, 1996). However, Sabio, Vidal-Aragón, Bernalte & Gata (1998) have showed similar contents for esters in Italian and Iberian dry-cured hams, while French and Serrano hams showed the highest levels of these volatile compounds, which the authors related to the high amount of ethanol found in these hams.

The effect of freezing and subsequently thawing Iberian hams before processing on the chemical families of acids, aliphatic and aromatic hydrocarbons, ketones and furans, was not remarkable, the levels and the evolution of these volatile compounds being similar in R and F Iberian hams, and no qualitative difference being detected

Statistical differences (p=0.016) in total aliphatic hydrocarbons throughout the ripening were found. Their highest content was found at the post-salting and drying steps and the lowest levels at the end of the processing, which is mainly due to the hexane content variations. The rest of aromatic hydrocarbons detected did not show important differences during the processing. In Chinese Jinhua hams the lowest levels of alkanes and alkenes were found at the final stage (Huan et al., 2005) while in Parma hams these volatile compounds increased during the ripening and showed their highest contents at the end of the processing (Hinrichsen et al., 1995). Straight chain aliphatic hydrocarbons arise mainly from lipid oxidation (Ruiz et al, 2002) while the oxidation of methyl branched fatty acids has been porposed as a possible formation for branched aliphatic hydrocarbons (Ruiz et al., 1999). Nevertheless, hydrocarbons could also arise from the accumulated unsaponificable fraction from vegetals fed to pigs (Shahidi, Rubin & D´Souza, 1986). Lower aliphatic hydrocarbons were detected in the hams of this study at the end of the processing in comparison with initial investigation about Iberian ham volatiles (García et al., 1991; López et al., 1992; Ruiz et al., 1999). The different diets employed for feeding Iberian pigs (acorns and pasture *versus* concentrated diets) might explain these differences. Iberian pigs of this study were fed with concentrated while the animals studied by García et al. (1991), López et al. (1992) and Ruiz et al. (1999) had acorns and grass. In fact, some aliphatic hydrocarbons are directly accumulated in the fat depots from the feeding (Tejeda et al., 1999; Ruiz et al., 2002).

Total aromatic hydrocarbons were significantly higher (p=0.045) at the end of drying step than in any of the other stages. In Parma hams, Hinrichsen et al. (1995) found that the major detected aromatic hydrocarbons decreased during the processing, while Bolzoni et al. (2005) showed lower proportion of these volatile compounds at 9 months of processing than at 6 months and at the final stage. These volatile compounds seem to come from animal feeding

(Berdagué, Monteil, Montel & Talon, 1993) and it is difficult to explain their changes throughout the ripening of the hams. The levels of major volatile compound families could influence on the minor volatile groups.

Although the changes of total acids throughout the processing were not significant, some compounds of this family showed statistical differences. The highest levels of butanoic acid were found at the end of the post-salting and drying steps, while 2- and 3-methylbutanoic acids were only detected at the end of the processing. In Chinese Jinhua hams, acids increased throughout the ripening (Huan et al., 2005), while in Parma hams, it has not been described important differences in this chemical family during the processing (Hinrichsen et al., 1995; Bolzoni et al., 2005). Some of the straight chain carboxylic acids detected could derive from the hydrolysis of triglycerides and phospholipids, but most are formed during oxidation of unsaturated lipids. On the other hand, 2- and 3-methylbutanoic acids derive from their respective branched aldehydes (2- and 3-methylbutanal) (Ruiz et al., 2002).

Statistical differences (p=0.033) in ketones throughout the ripening of Iberian dry-cured hams were found. This chemical family increased from the salting to the post-salting step, decreased thereafter at the end of the drying step and increased again at the final stage. Thus, the lowest levels were found at the end of the salting and drying steps and the highest at the end of the post-salting and at the final stages. However, individual ketones showed a different evolution. The major one at the end of salting step, 2,3-octadienone, decreased to the lowest levels at the final stage. 3-hydroxi-2-butanone and 2-heptanone reached their highest contents at the end of the processing. 2-butanone, 2-pentanone, 2-octanone and 2-nonanone were only detected at the end of the processing. A similar trend was shown in other kind of hams (Bolzoni et al., 2005; Huan et al., 2005). However, in Iberian ham with a shortened process Andrés et at. (2002) found increasing levels of 2-heptanone during the first part of the cellar phase and decreasing amounts thereafter. This volatile compound family arises more likely from oxidation of unsaturated lipids (Shahidi et al., 1986).

Most detected furans and dihydro-2(3H)-furanone were found at the end of the processing, while in Parma and Chinese Jinhua hams, these volatile compounds increased during the processing and reached the highest contents

at the final stage (Hinrichsen et al., 1995; Huan et al., 2005). These compounds are also formed through oxidation of unsaturated fatty acids (Ruiz et al., 2002) and have been linked to heating processes in other foodstuffs (Muriel et al., 2004). Pyrazines are products from Maillard reactions and are extensively generated during meat cooking (Mottram and Edwards, 1983). During the dry-cured process the temperature reahed is not as high as in cooking, tis being the most likely reason for the few pyrazines found (Flores et al., 1997).

CONCLUSIONS

The effect of pre-cured freezing Iberian ham on the profile of volatile compound along the ripening was not very noticeable. However, in dry-cured Iberian hams there were differences in some aldehydes and alcohols and in most detected esters.

The volatile compounds of Iberian hams were generated throughout the whole processing, being straight chain aldehydes, straight chain alcohols and some ketones mainly formed during the post-salting step while branched and aromatic aldehydes, branched alcohols, most ketones, esters and furans were principally produced at the last stages.

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Table 1. Abundance (total area counts x 10^{-5}) of the volatile compounds of refrigerated (R) and pre- cure frozen Iberian hams at the end of the different processing steps.

IK[a]	Rel[b]		END OF SALTING			END OF POST-SALTING			END OF DRYING			FINAL STAGE				
			R	F	*p*	R	F	*p*	R	F	*p*	R	F	*p*	*p* (R)	*p* (F)
605	MS. IK	Acetic acid	nd	nd^{b}	.-	nd	nd^{b}	-.	nd	nd^{b}	.-	nd	2.28^{a}	0.006	.	<0.001
785	MS. IK	Butanoic acid	1.51^{ab}	2.30^{a}	0.101	2.66^{a}	2.49^{a}	0.806	2.74^{a}	1.93^{ab}	0.055	0.44^{b}	0.83	0.319	0.012	0.002
838	MS. IK	3-Methyl butanoic acid	nd^{b}	nd^{b}	.-	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	.-	0.68^{a}	0.33	0.284	0.007	0.053
847	MS. IK	2-Methyl butanoic acid	nd^{b}	nd^{b}	.-	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	.-	0.46^{a}	0.29^{a}	0.401	<0.001	0.014
971	MS. IK	Hexanoic acid	<0.01	0.51	0.104	0.64	0.25	0.182	0.41	0.38	0.677	0.47	0.29	0.375	0.227	0.558
		Σ ACIDS	1.51	2.81	0.06	3.3	2.75	0.539	2.47	2.31	0.852	2.05	3.64	0.852	0.38	0.171
567	MS. IK	2-Methyl pentane	nd	nd^{b}	.-	0.41	0.57^{ab}	0.529	0.41	0.44^{b}	0.89	0.37	1.65^{a}	0.051	0.264	0.004
588	MS. IK	2-Methyl pentane	nd	0.54	0.12	0.29	0.41	0.417	0.42	0.44	0.934	0.32	1.48	0.098	0.148	0.068
600	MS. IK	Hexane	2.41^{b}	18.28	0.004	13.99^{a}	14.85	0.857	16.51^{a}	13.39	0.536	2.68^{b}	5.21	0.536	0.012	0.131
700	MS. IK	Heptane	0.30^{b}	0.16	0.488	nd^{b}	0.35	0.161	0.68^{a}	0.45	0.149	nd^{b}	0.27	0.141	<0.001	0.706
		Σ ALIPHATIC HYDROCARBONS	2.71^{b}	11.67	0.212	12.36^{ab}	13.64	0.812	18.03^{a}	7.92	0.093	2.71^{b}	5.13	0.093	0.016	0.294
	MS	Ethanol	1.00^{b}	1.71^{b}	0.206	1.96^{b}	3.55^{ab}	0.014	3.32^{ab}	4.05^{ab}	0.379	7.14^{b}	5.47^{a}	0.379	0.006	0.043
555	MS. IK	1-Propanol	0.95^{b}	nd^{b}	0.031	nd^{b}	nd^{b}	-.	0.89^{b}	0.78^{b}	0.648	6.77^{a}	7.07^{a}	0.829	<0.001	<0.001
624	MS. IK	3-Methyl-1-butanol	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	0.74^{b}	nd^{b}	0.129	1.12^{a}	1.57^{a}	0.072	0.005	<0.001
739	MS. IK	2-Methyl-1-butanol	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	.-	0.32^{a}	0.55^{a}	0.002	<0.001	<0.001
771	MS. IK	1-Pentanol + Methylbenzene	3.94^{ab}	4.88^{ab}	0.56	5.49^{a}	5.57^{a}	0.9	1.70^{b}	1.08^{c}	0.058	3.90^{ab}	2.98^{bc}	0.389	0.017	<0.001
781	MS. IK	2.3-Butanediol	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	.-	1.90^{a}	5.82^{a}	0.038	<0.001	<0.001
870	MS. IK	1-Hexanol	2.06	10.63^{a}	0.039	2.56	4.34^{b}	0.026	1.89	0.78^{c}	0.008	2.5	2.46^{bc}	0.919	0.744	<0.0010
899	MS. IK	2-Heptanol	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	.-	0.22^{a}	0.41^{a}	0.02	<0.001	<0.001
905	MS. IK	2-Butoxyethanol	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	0.19^{b}	0.18^{b}	0.909	2.65^{a}	3.51^{a}	0.116	<0.001	<0.001
979	MS. IK	1-Octen-3-ol	5.41^{b}	8.68^{a}	0.172	10.09^{a}	11.11^{a}	0.557	3.19^{bc}	1.99^{b}	0.006	0.69^{c}	0.87^{b}	0.356	<0.001	<0.001
1070	MS. IK	1-Octanol	nd^{b}	0.27^{a}	0.065	0.20^{a}	0.16^{ab}	0.496	nd^{b}	nd^{b}	.-	nd^{b}	nd^{b}	.-	0.001	0.001
1121	MS. IK	Benzenethanol	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	.-	0.56^{a}	1.12^{a}	0.443	0.004	0.011
		Σ ALCOHOLS	11.02^{b}	21.92^{ab}	0.222	19.06^{ab}	23.22^{a}	0.261	9.97^{b}	8.85^{b}	0.567	25.31^{a}	26.90^{a}	0.567	0.003	0.011
		Acetaldehyde	5.07^{a}	4.88^{b}	0.896	1.04^{b}	4.97^{b}	0.01	5.22^{a}	9.97^{a}	0.011	0.26^{b}	2.23^{b}	0.168	0.004	0
648	MS. IK	3-Methyl butanal.	nd^{b}	nd^{b}	-.	nd^{b}	nd^{b}	-.	0.57^{b}	0.53^{b}	0.681	14.49^{a}	17.42^{a}	0.478	<0.001	<0.001

IK[a]	Rel[b]		END OF SALTING			END OF POST-SALTING			END OF DRYING			FINAL STAGE				
			R	F	*p*	R	F	*p*	R	F	*p*	R	F	*p*	*p* (R)	*p* (F)
658	MS. IK	2-Methyl butanal	nd[b]	nd[b]	-.	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	3.67[a]	5.37[a]	0.04	<0.001	<0.001
697	MS. IK	Pentanal	1.08	0.77[b]	0.538	1.15	4.64[a]	0.015	0.52	0.14[b]	0.054	0.94	0.61[b]	0.232	0.17	<0.001
800	MS. IK	Hexanal	60.60[ab]	89.19	0.356	69.00[a]	82.31	0.418	29.51[bc]	9.9	0.031	22.33[c]	22.68	0.96	0.004	<0.001
901	MS. IK	Heptanal	1.78	1.18[ab]	0.401	2.14	2.65[a]	0.402	1.5	1.15[c]	0.304	1.35	1.33[ab]	0.947	0.25	0.023
966	MS. IK	Benzaldehyde	nd[b]	nd[b]	-.	nd[b]	nd[b]	-.	0.10[b]	0.14[b]	0.378	0.99[a]	0.94[a]	0.919	0.008	0.001
1002	MS. IK	Octanal	1.61[b]	2.42[b]	0.266	3.02[ab]	3.19[ab]	0.723	2.68[b]	2.04[b]	0.192	4.85[a]	4.70[a]	0.192	0.01	0.017
1051	MS. IK	Benceneacetaldehyde	nd[b]	nd[b]	-.	nd[b]	nd[b]	-.	0.34[b]	0.25[b]	0.667	58.11[a]	95.78[a]	0.415	<0.001	0.001
1105	MS. IK	Nonanal	5.41[b]	10.08[a]	0.098	9.03[a]	8.75[ab]	0.827	5.74[b]	4.84[bc]	0.273	2.33[c]	2.26[c]	0.844	<0.001	<0.001
1164	MS. IK	2-Nonenal	0.35[a]	0.65[a]	0.184	0.63[a]	0.62[a]	0.836	0.44[a]	0.35[b]	0.411	nd[b]	nd[b]	-.	<0.001	<0.001
1204	MS. IK	Decanal	0.28[a]	0.42[a]	0.246	0.42[a]	0.39[a]	0.615	0.43[a]	0.31[a]	0.249	nd[b]	nd[b]	-.	<0.001	<0.001
1369	MS. IK	2-Undecenal	1.02[ab]	1.17[a]	0.733	1.49[a]	1.19[a]	0.405	1.25[a]	1.03[a]	0.561	nd[b]	nd[b]	-.	0.006	<0.001
1412	MS. IK	Dodecanal	1.29[a]	0.90[a]	0.387	0.68[ab]	0.64[ab]	0.842	0.74[ab]	0.68[a]	0.887	nd[b]	nd[b]	-.	0.011	0.006
		Σ ALDEHYDES	56.26	88.96	0.432	73.22	93.4	0.419	43.5	30.05	0.064	49.61	52.05	0.064	0.45	0.061
	MS	2-Propanone	nd[b]	nd[b]	.-	1.34	0.54[b]	0.409	0.77	0.13[b]	0.473	1.46	3.85[a]	0.19	0.595	0.002
612	MS. IK	2-Butanone	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	0.46	0.74[a]	0.582	0.153	0.015
684	MS. IK	2-Pentanone	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	0.20[b]	0.28[b]	0.37	1.44[a]	0.99[a]	0.087	<0.001	<0.001
711	MS. IK	3-Hydroxy-2-butanone	0.27[b]	0.56	0.357	0.46[b]	0.55[b]	0.729	nd[b]	nd[b]	.-	3.83[a]	3.27[a]	-.	<0.001	0.002
891	MS. IK	2-Heptanone	0.24[b]	0.31	0.515	0.60[b]	0.55[b]	0.792	0.64[b]	0.45[b]	0.033	1.93[a]	1.89[a]	0.936	<0.001	0.001
983	MS. IK	2.3-Octadieone	3.27[a]	9.62[a]	0.142	5.87[a]	9.28[a]	0.206	0.86[b]	0.24[b]	0.132	nd[b]	nd[b]	-.	0.02	<0.001
987	MS. IK	2-Octanone	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	0.18[a]	nd[b]	0.033	0.29[a]	0.24[a]	0.554	<0.001	<0.001
1093	MS. IK	2-Nonanone	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	0.18[a]	0.13[a]	0.548	0.002	0.006
		Σ KETONES	2.69	8.45[ab]	0.194	8.26	10.82[a]	0.356	2.64[b]	0.95	0.064	8.27	8.42[ab]	0.064	0.033	0.038
615	MS. IK	Acetic acid ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	5.19[a]	4.43[a]	0.646	<0.001	<0.001
716	MS. IK	Propanoic acid ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	0.76[a]	0.18[a]	0.129	0.039	0.024
762	MS. IK	Propanoic acid. 2- methl ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	0.41[a]	0.18[a]	0.025	<0.001	<0.001
802	MS. IK	Butanoic acid ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	1.88[a]	0.55[a]	0.039	<0.001	0.005
853	MS. IK	Butanoic acid. 2-methyl ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	1.04[a]	0.29[a]	0.031	0.001	<0.001
856	MS. IK	Butanoic acid. 3-methyl ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	1.58[a]	0.36[a]	0.025	0.001	<0.001

IK[a]	Rel[b]		END OF SALTING			END OF POST-SALTING			END OF DRYING			FINAL STAGE				
			R	F	*p*	R	F	*p*	R	F	*p*	R	F	*p*	*p* (R)	*p* (F)
997	MS. IK	Hexanoic acid ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	4.15[a]	0.82[a]	0.000	<0.001	<0.001
1195	MS. IK	Octanoic acid ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	1.00[a]	0.38[a]	0.004	<0.001	<0.001
1394	MS. IK	Decanoic acid ethyl ester	nd[b]	nd[b]	.-	nd[b]	nd[b]	-.	nd[b]	nd[b]	.-	1.08[a]	0.351[a]	0.003	<0.001	<0.001
		Σ ESTERS	nd[b]	nd[b]	-	nd[b]	nd[b]	-	nd[b]	nd[b]	-	14.13[a]	7.57[a]	0.01	<0.001	<0.001
913	MS. IK	Dihydro-2(3H)-furanone + 2,5-dimethylpirazine	nd[b]	nd[b]	.-	nd[b]	nd[b]	.-	0.08[b]	nd[b]	0.062	0.86[a]	0.65[a]	0.164	<0.001	<0.001
1059	MS. IK	5-Ethyldihydro- 2(3H)-furanone.	nd[b]	nd[b]	.-	nd[b]	nd[b]	.-	nd[b]	nd[b]	.-	0.30[a]	0.20[a]	0.371	<0.001	0.007
1262	MS. IK	5-butyldihydro-2(3H)-furanone-	0.81[ab]	1.12[a]	0.382	1.31[a]	1.10[a]	0.473	1.08[ab]	1.01[a]	0.873	0.14[b]	0.13[b]	0.802	0.012	0.001
1374	MS. IK	5-pentyldihydro-2(3H)-furanone-	nd[b]	nd[b]	.-	nd[b]	nd[b]	.-	nd[b]	nd[b]	.-	0.18[a]	0.17[a]	0.804	<0.001	<0.001
		Σ FURANS	0.54	0.89	0.431	1.09	0.91	0.643	0.87	1.01	0.743	1.48	1.14	0.743	0.226	0.841
866	MS. IK	Ethyl benzene	0.28[b]	0.28[b]	0.973	0.35[b]	0.31[b]	0.329	0.52[a]	0.72[a]	0.059	0.37[b]	0.60[ab]	0.168	0.002	0.003
874	MS. IK	1,4-Dimethyl benzene	1.00[b]	1.04[b]	0.625	1.42[b]	1.06[b]	0.044	2.22[a]	3.11[a]	0.057	1.33[b]	2.14[ab]	0.211	<0.001	0.001
895	MS. IK	ethenylbenzene	nd[b]	nd[b]	.-	nd[b]	1.03[b]	0.07	3.99[a]	3.68[a]	0.886	1.13[ab]	0.42[b]	0.076	0.015	<0.001
897	MS. IK	1.2-Dimethyl benzene.	0.96	0.39	0.456	0.38	0.05	0.2	0.15	nd	0.134	0.08	0.25	0.155	0.407	0.123
		Σ AROMATIC HYDROCARBONS	2.24	1.72[b]	0.487	2.09	2.44[b]	0.559	5.87	7.50[a]	0.487	2.91	3.41[b]	0.487	0.045	<0.001
1036	MS. IK	l-Limonene	nd[b]	nd[b]	.-	nd[b]	0.12[b]	0.029	0.13[a]	0.20[b]	0.231	0.12[ba]	0.56[a]	0.053	0.008	0.002

nd: non detected; LRI[a]: linear retention index; ID[b]: method of identification. MS, mass spectrum agrees with those reported in the NIST and Wiley library. LRI: linear retention index agree with those reported in the literature and in the NIST database (http://webbook.nist.gov/chemistry/name-ser.html)

Figure 1. Conditions of temperature (■) and relative humidity (▲) registered throughout the processing of the refrigerated and frozen Iberian hams.

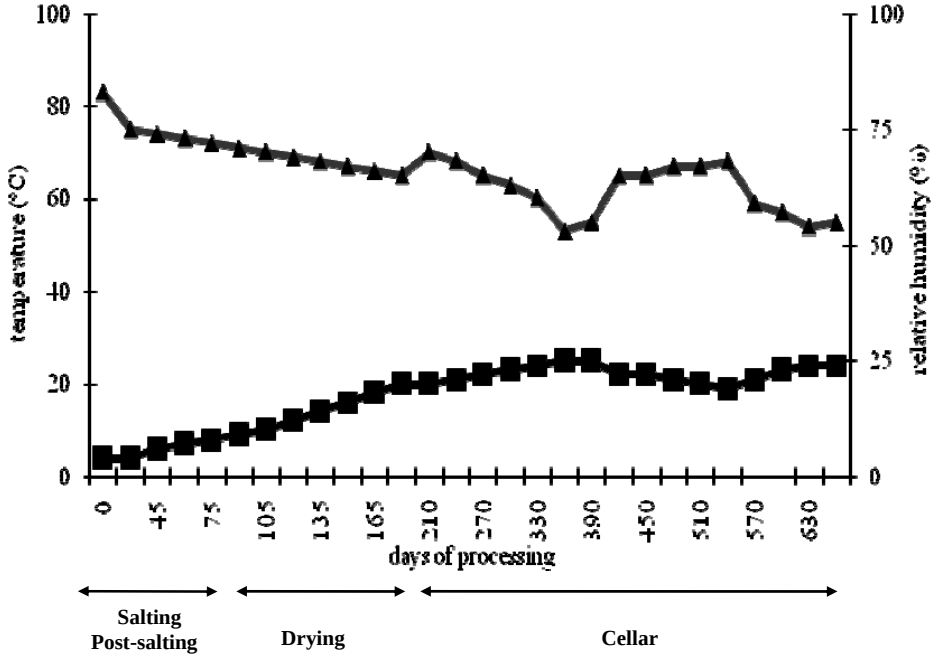
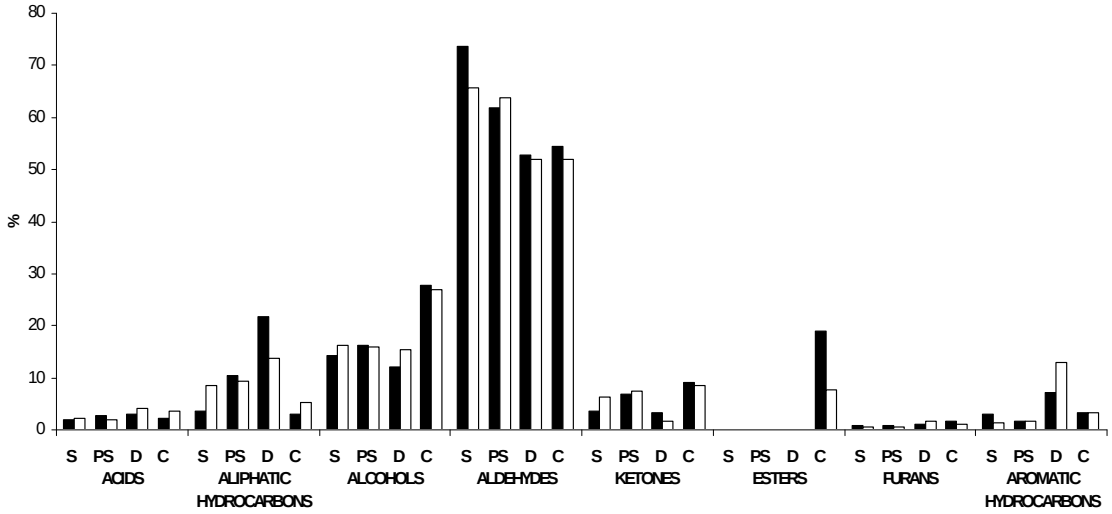


Figure 2. Contents of chemical volatile compound families (expressed as percentage of the total chromatographic area) extracted at the end of the differentes processing steps from refrigerated (□) and frozen (■) Iberian hams



S: end of salting step: PS: end of post-salting step; D: end of drying step: C: final stage.

Capítulo II.5.

Pre-cure freezing effect on physicochemical, texture and sensory characteristics of Iberian ham

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TITLE

Pre-cure freezing effect on physicochemical, texture and sensory characteristics of Iberian ham

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RUNNING TITLE

Quality characteristics in pre-cure frozen Iberian hams

ABSTRACT

The aim of this work was to investigate the effect of pre-cure freezing on the physicochemical, texture and sensory characteristics of raw and dry-cured hams.. Both refrigerated (R) and pre-cure frozen (F) hams showed the same weight losses during the processing. At the green stage F Iberian hams had less moisture, higher values of a* and chroma, lower hardness and chewiness and higher adhesiveness and springiness than R ones. However, at the end of the processing R and F Iberian hams only were different in salt content, F hams being less salted than R ones. Sensory analysis of dry-cured Iberian ham did not show differences in salty taste. Panelist detected a higher fat hardness and lean pastiness in F than in R hams. The overall acceptability for both groups of dry-cured hams was between average and good.

KEY WORDS

Pre-cure freezing; Iberian ham; physicochemical analysis; texture analysis; sensory analysis

INTRODUCTION

Iberian ham is a traditional dry cured product greatly appreciated because of its unique sensory characteristics such as the intense red colour, marbling and cured aroma (Ruiz, García, Muriel, Andrés & Ventanas, 2002). Sensory characteristics of Iberian ham are related to its physicochemical composition, which is influenced by factors related to the raw meat (Andres, Ruiz, Ventanas, Tejeda & Mayoral, 1999; Andres, Cava, Mayoral, Tejeda, Morcuende & Ruiz, 2001) and the processing (Ruiz, Ventanas, Cava, Timon, Garcia, 1998; Ruiz, Garcia, Diaz, Cava , Tejeda & Ventanas, 1999).

Colour is an important characteristic of meat and meat products (Risvik, 1994; Resurreccion, 2003). In dry-cured ham, colour is among the most outstanding characteristics of appearance (Gandemer, 2002), and it could influence consumers choice of sliced and packaged ham in the supermarket. Texture also constitutes one of the main attributes influencing consumer preference for Iberian ham, being mainly determined by the technological parameters and the characteristics of the raw material (Guerrero, Gou & Arnau, 1999).

The use of frozen/thawed thighs is not a common strategy within the processing of Iberian ham nowadays. In fact, producers consider that this practise is detrimental to the quality of this product. Furthermore, some of the Specific Designation of Origin that protect the production of dry-cured Iberian hams, keep out the procedure of freezing and subsequently thawing raw material before Iberian ham processing (M.A.R.M., 2009). On the other hand, there are not scientific studies showing the effect of pre-cure freezing on the quality of the derived Iberian dry-cured meat products. Nevertheless, such procedure could constitute technological and economical advantages, e.g. processing hams with more homogeneous weight, avoiding the seasonal availability and the changes of the market price.

Changes in physical (drip loss, texture modifications), chemical (lipolysis and FA oxidation, protein denaturation and aggregation, changes in color), and sensory properties of meat could be promoted by freezing. Their extended influence depends on raw meat characteristics, processing and above all the closer to the optimum frozen conditions (Carballo & Jiménez, 2001).

Although there are numerous works studying the influence of the frozen storage on several parameters related to meat quality, only a few researches deal with such topic in dry-cured hams (Flores, Soler, Aristoy & Toldrá, 2006; Arnau, Gou & Guerrero, 1994; Motilva, Toldrá, Nadal & Flores, 1994; Bañón, Cayuela, Granados & Garrido, 1999; Wang, 2001), whereas there are no studies about the effect of using frozen/thawed raw material on the quality of Iberian hams.

As a consequence of freezing and thawing hams before processing, salt penetration is favoured, caused by the higher free water content of frozen/thawed hams as compared to fresh ones (Arnau et al., 1994; Bañón et al., 1999; Wang, 2001). This effect could increase the amount of solubilized salt on the surface of the ham, which is the main factor regulating its penetration (Sorheim & Gumpen, 1986). Thus, the salting time used for pre-cure frozen hams is shorter than that for refrigerated ones (Poma, 1989; Bañón et al., 1999).

Some studies have not found differences at the end of the processing in lipolysis, colour, sensory features and acceptability scores between dry-cured hams from fresh and frozen/thawed pieces (Motilva et al., 1994; Bañón et al., 1999). However, Flores et al. (2006) and Bañón et al. (1999) showed a higher proteolysis phenomena in pre-cure frozen than in refrigerated hams. The lipolityc activity was also more accentuate in hams processed using frozen and subsequently thawed raw material at the beginning of ripening (Flores et al., 2006; Motilva et al., 1994), whereas these differences were not detected at the final stage (Motilva et al., 1994).

Therefore, setting the limits of new processing conditions that do not compromise the sensory quality of Iberian ham is essential, the aims of this work was to analyze the effect of pre-cure freezing Iberian hams on physicochemical, texture and sensory characteristics in raw and dry-cured meat.

MATERIAL AND METHODS

Experimental design

Twelve hind limbs were obtained from Iberian pigs fattened in confinement and fed with a commercial diet. These hind limbs were divided into two groups, refrigerated (R) and pre-cure frozen (F) hams. The F hams were frozen (at -20 ºC) and thawed (4 days at 3-4 ºC) three months later. The R hams were obtained at the moment of thawing the F hams, and kept at 4 ºC during 36-48 hours until starting their processing. Six hind limbs were used to obtain the data for the green stage. The others were processed for obtaining dry-cured hams. The processing conditions were the same for the two groups of hams, except for the salting time, which was 1 day/kg for the R hams and 0.7 day/kg for the F hams. After salting, the salt from the surface was brushed and, the hams were processed as follows. Iberian hams were held at 4-8 ºC and 73-75% relative humidity for 70 days (post-salting step). During the drying stage the hams were kept in a room under controlled conditions for 120 days, temperature was increased from 8 to 20 ºC, while relative humidity was progressively reduced to 64%. Finally, hams were left to mature for 16 months (cellar stage) at 20-25 ºC and relative humidity 55-65%.

Sampling was carried out by dissecting the *Biceps femoris* muscle of each ham.

Physicochemical analysis

Moisture and salt content were determined according to the Association of Official Analytical Chemists (AOAC, 2000) (moisture reference 935.29; salt content reference 971.19). The intramuscular fat content was analysed gravimetrically with chloroform/methanol (2:1, vol/vol), according to the method described in Pérez-Palacios, Ruiz, Martín, Muriel and Antequera (2008).

Instrumental color was measured across the cut surface of *Biceps femoris* muscle. The following colour coordinates were determined: lightness (L*), redness (a*) and yellowness (b*). Colour parameters were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The colorimeter was standardized before use with a white tile having the following values:

L*=93.5, a*=1.0 and b*=0.8. In addition, hue angle, which describes the hue or colour was calculated (H = arctg(b*/a*)*(360/6.28)) as well as the saturation index or chroma (C*) (C = (a*2 + b*2)0.5), which describes the brightness or vividness of colour.

Texture analysis

Texture analysis was performed in a TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). For determination of the texture profile analysis (TPA) uniform portions of the *Biceps femoris* muscle were cut into 1 cm^3 cubes. Samples were axially compressed to 50% of their original height with a flat plunger 50 mm in diameter (P/50) at a crosshead speed of 2 mm/s through a 2-cycle sequence. The following texture parameters were measured from the force–deformation curves (Bourne, 1978): Hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); adhesiveness (N x s) = work necessary to pull the compressing plunger away from the sample; springiness (dimensionless) = height that the sample recovers during the time that elapses between the end of the first compression and the start of the second; cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required for the first compression and A2 the total energy required for the second compression); chewiness (N) = the work needed to chew a solid sample to a steady state of swallowing (hardness x cohesiveness x springiness). For Warner Bratzler shear force (WBSF) analyses, samples were cut into 1 x 10 x 1 cm slices (thickness x length x width). Samples were cut with a Warner–Bratzler blade (HDP/BS) perpendicularly to the muscle fibers. Determinations were repeated ten times per sample and were averaged.

Sensory analysis

Fourteen trained panelists formed the tasting panel. Eighteen sensory attributes of dry-cured Iberian ham grouped in appearance of fat and lean, texture fat and lean, aroma, taste and flavour were analyzed (Table 1). Analyses were developed in tasting rooms with the conditions specified in UNE regulation. All sessions were conducted at ambient temperature in a sensory room equipped with white fluorescent lighting. The software used to record scores in the sensory sessions was FIZZ Network (version 1.01: Biosystemes,

France). The hams were cut into 1.5 mm thick slices, with a slicing machine. Then, slices were served on plates to panellists. The panel sessions were held mid-morning, among 12-13h in the morning. Panelists evaluated the different parameters by means of a quantitative-descriptive analysis in a non structured scale 0-10. Three samples randomly presented to the panellist were analyzed in each session. About 200 ml of water at room temperature was provided to the panellists. In each session, the panel average for each sample was recorded.

For the acceptability sensory study, untrained (193) subjects (consumers), including university students, professors and staff recruited at the School of Veterinary Sciences (Extremadura University, Cáceres, Spain) rated the Iberian dry-cured hams following a mixed hedonic scale (5: very good; 4: good; 3: average; 2: poor; 1: very poor).

Statistical analysis

The effect of pre-cure freezing Iberian hams on its physicochemical, texture and sensory were analysed by one-way analysis of variance (ANOVA) using the General Linear Model. Data from the acceptability test were analysed using the Friedman non-parametric statistic test, in order to find out if consumers considered R and F samples different at each hedonic scale rank. Analyses were done by using the SPSS package (v.15.0).

RESULTS AND DISCUSSION

Effect of pre-cure freezing on physicochemical characteristics of Iberian ham

Salt (NaCl) content was significantly higher (p=0.018) in R (6.58 g/100 g DM) than in F (5.21 g/100 g DM) dry-cured Iberian hams (Table 2). The salting time for the Iberian hams of this study was that established by the industry: 1 day/kg and 0.7 day/kg for R and F Iberian hams, respectively, according to empirical knowledge and previous studies on others dry-cured hams (Poma, 1989; Bañón et al., 1999), which recommend reducing the salting time for frozen/thawed hams. In agreement with the results of the present work, Grau, Albarracín, Toldrá, Antequera and Barat (2007) also found higher salt content in pre-cure frozen hams than in refrigerated ones. Thus, it seems that the influence of freezing/thawing on the diffusion of the salt within the ham is different in hams from Iberian pigs and those from commercial pigs, which Grau et al. (2007) have related to the higher fat content of the Iberian hams, which could act as the controlling phase in the mass transport process.

Figure 1 shows the accumulated weight losses along the processing of R and F Iberian hams. After thawing, F hams had 0.94% (w/w) of weight losses. There were not statistical differences in weight losses between R and F hams along the ripening, reaching both groups around 28% of weight losses at the end of the processing. Contrarily, Andrés et al. (2005) found higher weight losses in Iberian hams processed with a higher salt content at the salting and post-salting stages.

Moisture values of raw hams were lower in F (71.23 g/100g) than in R (73.55 g/100g) Iberian hams (Table 2), which could be related to the water losses during the frozen storage (Jalang'O, Saul & Lawrie, 1987). In fact, F hams showed 0.94% of weight losses after thawing. However, R and F Iberian ham had similar moisture content in the final product (52.02 and 51.57 g/100 g, respectively).

Dry-cured Iberian hams of this study showed lower total accumulated weight losses and higher moisture than those found by other researches (Martín, Córdoba, Antequera, Timón & Ventanas, 1998; Córdoba, Antequera, Ventanas, López-Bote, García & Asensio, 1994; Andrés et al., 2005; Carrapiso & Garcìa, 2008), which could be related to the time and conditions of the

processing. In fact, the temperature during the drying stage was lower (up to 20 ºC) as compared to those in previous works (25-30 ºC).

The procedure of pre-cure freezing Iberian hams influenced the instrumental color variables at green stage but not in the final product. The values of a* and chroma were significantly higher in F (19.07 and 19.50, respectively) than in R hams (14.46 and 14.69, respectively) (Table 2). However, the other studied colour coordinates (L*, b* and Hue) did not show statistical differences. Chroma and a* have been related to desiccation (Arnau, Gou & Comaposada, 2003). Thus, the higher moisture content in F than in R hams could explain the differences in Chroma and a* between these two groups of Iberian hams. The greater water losses in F hams could lead to a higher pigment concentration, leading to the redder and more vivid color. Sakata, Oshida, Morita & Nagata (1995) also found higher a* values in frozen/thawed *Longissimus dorsi* from pork than in fresh muscle. Nevertheless, in dry-cured Iberian hams of this study there were not differences in colour parameters between R and F hams. In fact, Carrapiso and García (2005) described that ham processing markedly decreased the variability of instrumental colour data caused by crossbreeding and rearing system. However, Bañón et al. (1999) did no found differences of L* between dry cured hams from frozen/thawed raw material and those from fresh thighs, whereas a slight lowering was observed in a* and b* in the frozen hams compared to the refrigerated ones.

Effect of pre-cure freezing on texture characteristics of Iberian ham

Texture profile analysis and WBSF of the R and F fresh and dry-cured Iberian hams are shown in Table 3. As instrumental color, the procedure of freezing/thawing Iberian hams influenced texture characteristics at the initial stage but not in dry-cured hams. Pre-cure frozen Iberian hams had significantly lower hardness (11.77 vs. 22.73), chewiness (1.86 vs. 4.05) and springiness (0.45 vs. 0.45) and higher adhesiveness (0.34 vs. 0.30) than R ones, while no differences were found in cohesiveness. Contrarily, WBSF analysis showed that more force was needed to shear the samples from F than from R Iberian hams at green stage. It has been described that the greatest myofibril fragmentation in *Longissimus dorsi* from pig (Sakata et al., 1995) and beef (Rahelic, Puac & Gawwad, 1985) occurs at around -20º C, which could explain the lower

hardness in the *Biceps femoris* muscle from F than from R hams. Besides, Ramírez et al. (2007) found negative correlations between moisture content and WBSF and between intramuscular fat content and hardness, chewiness and WBSF. Thus, the lower moisture values in the F fresh hams of our study could influence the higher WBSF values of this group of hams. Moreover, the proteolysis phenomena also affect ham texture (Parolari, 1996), thus the higher proteolysis rate found in F than in R raw hams (Pérez-Palacios, Ruiz, Grau, Aristoy & Antequera, 2009) could also contribute to the lower hardness in F hams.

Despite there were differences in texture parameters at the green stage between R and F hams, dry-cured Iberian hams show similar texture profile. Thus, it seems that the ripening levels texture differences found in the raw material. It has been also described that dry-cured muscles with lower salt content showed lower hardness, cohesiveness and springiness (Ruiz-Ramírez, Arnau, Serra & Gou, 2005), which was not detectable in this study, despite of the lower salt conten in F than in R Iberian hams.

Sensory analysis of refrigerated and pre-cured frozen dry-cured Iberian hams

Figure 2 shows mean scores for appearance and texture of fat and lean, aroma, taste and flavour from R and F dry-cured Iberian hams. Appearance of subcutaneous fat was not significantly affected by pre-cure freezing whereas significantly higher scores of fat hardness were shown in F than in R Iberian hams (Figure 1.a). This result could be related to the fat cristalization as a consequence of the cooling effect. In fact, the crystallization process leads to harder lard (Campos, Narine & Marangoni, 2002). On the other hand, Carrapiso, Bonilla and García (2003) showed that subcutaneous fat hardness was highly related to fatty acid composition of subcutaneous fat, being palmitic (C16:0), stearic (C18:0) (Davenel, Riaublanc, Marchal, & Gandemer, 1999) and oleic (C18:1 n-9) acids (Flores, Birón, Izquierdo & Nieto, 1988) closely related to solid fat content.

With respect to the lean texture, there were statistical differences in pastiness, being higher in F than in R Iberian hams. Texture defects are currently becoming frequent in the dry-cured ham industry, being soft and pasty textures the most common texture problems (García-Garrido, Quiles-Zafra, Tapiador & Luque de Castro, 2000). These anomalies have been related to low salt

concentrations (Martín et al., 1998; Virgili, Parolari, Schivazappa, Soresi, Bordini & Borri, 1995). In Parma hams, defective texture pieces were found to contain increased peptide and free amino acid concentrations (Parolari, Rivaldi, Leonelli, Bellati & Bovis, 1988), which has been related to increased levels of cathepsin B (Virgili et al., 1995). In fact, F dry-cured hams of the present study had lower salt content and higher peptide nitrogen levels than R ones (unpublished data). However, other texture traits of lean such as hardness and juiciness did not show differences between F and R dry cured hams.

No effect of pre-cure freezing Iberian hams on aroma, taste and flavour was detected. In white hams, the sensory evaluation also showed that the procedure of freezing/thawing did not affect final sensory quality, except for a more intense salty taste in frozen/thawed hams due to a higher salt concentration (Motilva et al., 1994). Andrés et al. (2004) also found that hams with a higher salt content were considered saltier by the sensorial panel. Nevertheless, there were not differences in salty taste between the two groups of Iberian hams of this study, despite the higher salt content in F than in R hams. Salt is not the only factor determining salty taste. This attribute can also be influenced by other kind of compounds such as aminoacids or nucleotides exhibiting salty taste (Careri, Mangia, Barbieri, Bolzoni, Virgili & Parolari, 1993). Other works have neither shown differences in the sensory evaluation between frozen/thawed and refrigerated meat from pig and caw (Méndez, 1999). Sausages of mutton, pork and beef from frozen/thawed raw material did not show sensorial differences as compared to sausages processed with refrigerated meat (Verma, Alarcon Rojo, Ledward, Lawrie, 1985).

Results from the acceptability analysis are shown in Table 4. Most untrained panelists considered R and F Iberian hams to be between average and good. Although there were no statistical differences between R and F dry-cure Iberian hams at any hedonic scale ranks, it can be observed a better acceptability tendency for R hams. Bañón et al., (1999) also found that both refrigerated and frozen/thawed dry-cured Serrano hams were considered to be between average and good.

CONCLUSION

Pre-cure freezing Iberian hams influences moisture, color and texture parameters at initial stage whereas in dry-cured ham salt content is the only parameter influenced, being lower in F than in R hams. Nevertheless, there are differences in sensory traits only affecting hardness of subcutaneous fat and pastiness of lean, both higher in F cured ham, while traits of taste and flavor are not negatively influenced by the pre-cure freezing procedure.

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Table 1. Sensory attributes of dry-cured hams.

Sensory trait	Definition
Appearance of subcutaneous fat	
Yellowness	Intensity of yellow colour in the ham backfat (less-much)
Pinkness	Intensity of pink colour in the ham backfat (less-much)
Texture of subcutaneous fat	
Hardness	Firmess perception during pressing with hand
Oiliness	Level of liquid fat (complete solid-very oily)
Appearance of lean	
Red colour	Intensity of red colour in the lean (less-much)
Brightness	Intensity of bright
Marbling	Level of visible intramuscular fat (very lean-intense marbling)
Texture of lean	
Hardness	Firmness perception during chewing (very tender-very firm)
Pastiness	Impression of pastiness during chewing (not doughy-very doughy)
Juiciness	Impression of juiciness during chewing (not juicy–very juicy)
Aroma	
Odour	Intensity of odour before eating (odourless-very intense odour)
Taste	
Salty taste	Intensity of salt taste (not salty-very salty)
Sweet taste	Intensity of sweet taste (not sweet-very sweet)
Bitter taste	Intensity of bitter taste (not bitter-very bitter)
Flavour	
Flavour intensity	Intensity of overall flavor (flavourless-very intense flavor)
Flavor persistence	Time you are perceiving flavor (persistenceless-very persistence flavor)
Cured flavor	Intensity of cured flavor (not cured-very cured)
Rancid	Intensity of rancid flavor (not rancid-very rancid)

Table 2. Moisture (g/100 g), intramuscular fat content (g/100 dry matter), pH, instrumental color and salt content (g/100g dry matter) (mean ± standard error of the mean) of refrigerated (R) and pre-cure frozen (F) Iberian hams at green and final stages.

	Green stage			**Final stage**		
	R	F	*p*	R	F	*p*
Moisture	73.25 ± 1.45	71.23 ± 2.28	0.028	52.02 ± 5.57	51.57 ± 2.33	0.861
Intramuscular fat	15.11 ± 2.23	16.12 ± 1.88	0.654	24.23 ± 2.20	22.20 ± 1.34	0.129
Instrumental color						
Cie L^*	43.85 ± 2.15	43.60 ± 3.25	0.894	39.405 ± 2.37	36.71 ± 2.71	0.097
Cie a^*	14.46 ± 2.72	19.07 ± 1.21	0.009	19.68 ± 1.99	18.44 ± 1.42	0.246
Cie b^*	2.46 ± 0.78	3.37 ± 0.46	0.082	8.192 ± 1.14	7.05± 0.90	0.087
Chroma	14.69 ± 2.78	19.50 ± 1.00	0.007	21.332 ± 2.18	19.77 ± 1.43	0.174
Hue	10.28 ± 1.40	9.78 ± 1.17	0.605	22.56 ± 2.02	20.95 ± 2.61	0.260
Salt	0.0	0.0		6.58 ± 0.84	5.210 ± 0.12	0.018

Table 3. Texture profile analysis of refrigerated (R) and pre-cure frozen (F) Iberian hams at green and final stages.

	Green stage			**Final stage**		
	R	F	*p*	R	F	*p*
WBSF (N)	13.58 ± 1.331	15.05 ± 2.332	0.037	20.00 ± 2.44	19.65 ± 7.437	0.923
Texture profile text						
Hardness (N)	22.73 ± 0.905	11.77 ± 1.114	<0.001	1126.98 ± 321.378	1149.40 ± 542.359	0.932
Adhesiveness (N s)	0.30 ± 0.016	0.34 ± 0.015	0.008	0.44 ± 0.038	0.416 ± 0.566	0.379
Springiness	0.55 ± 0.051	0.45 ± 0.014	0.005	0.52 ± 0.042	0.45 ± 0.119	0.228
Cohesiveness	0.04 ± 0.014	0.04 ± 0.162	0.591	24.11 ± 12.95	21.43 ± 5.53	0.652
Chewiness (N)	4.05 ± 0.376	1.86 ± 0.135	<0.001	266.69 ± 102.90	226.27 ± 140.52	0.582

Figure 1. Percentage of accumulated weight losses of refrigerated (□) and pre-cure frozen (■) Iberian hams throughout the processing.

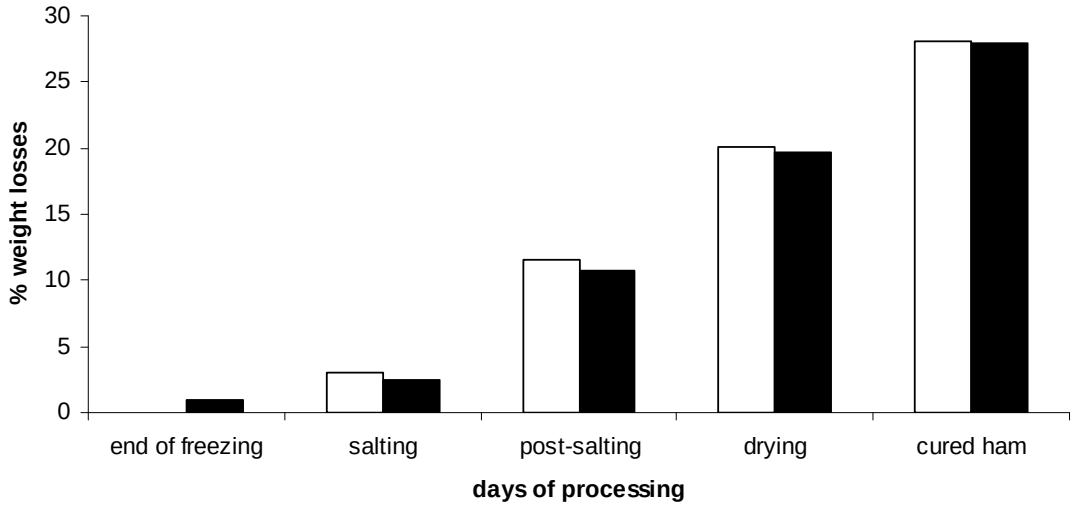
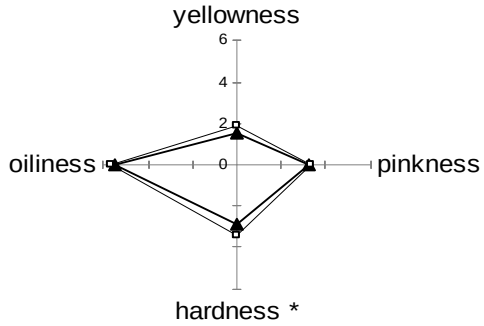


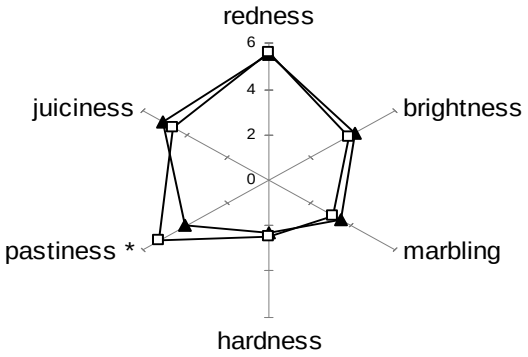
Figure 2. Sensory analysis of refrigerated (▲) and pre-cure frozen (□) dry-cured Iberian hams. 2a., appearance and texture of ham subcutaneous fat; 2b., appearance and texture of lean; 2c., odour, taste and flavor.

* significative effect ($p<0.05$)

a.



b.



c.

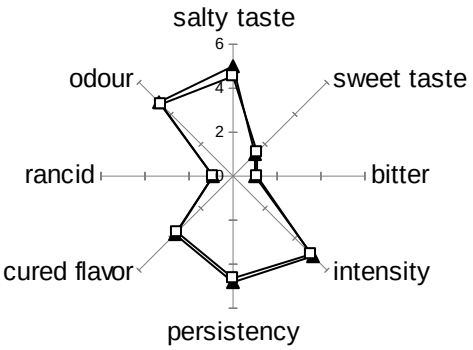
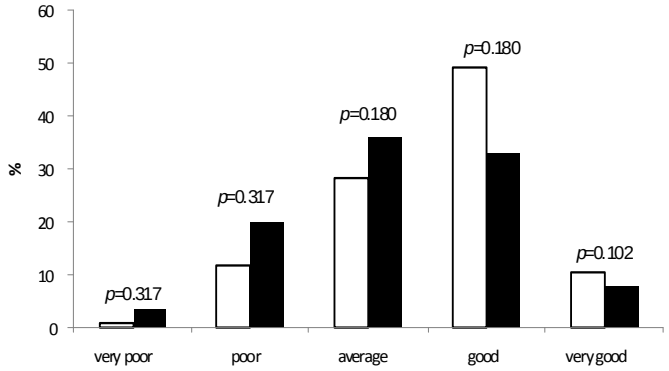


Figure 3. Acceptability analysis of refrigerated (□) and pre-cure frozen (■) dry-cured Iberian hams.



5. Discusión

SECCIÓN I

I.1. Comparación de diferentes métodos para cuantificar la cantidad de lípidos totales en carne y productos cárnicos.

Teniendo en cuenta la problemática observada en cuanto a la variabilidad de resultados en la cantidad de grasa extraída en carne y productos cárnicos dependiendo del método empleado, así como la importancia de la determinación de la cantidad de lípidos totales y la utilización de la grasa extraída para realizar análisis posteriores, se planteó, como paso previo de esta tesis doctoral, la comparación entre los métodos más comúnmente utilizados para la extracción y cuantificación de lípidos en productos cárnicos, Folch (Folch y col., 1957), Bligh y Dyer (Bligh y Dyer, 1957) y Soxhlet (AOAC, 1990) (Capítulo I.1.). Los mejores resultados se obtuvieron con el método de Folch, que consiguió extraer prácticamente la totalidad de los lípidos en todos los productos cárnicos analizados. El método de Soxhlet con hidrólisis mostró resultados similares al de Folch, excepto cuando la cantidad de grasa de los productos era muy elevada (próxima al 50%), en estos casos sería más adecuado realizar el Soxhlet sin hidrólisis previa. El método de Soxhlet es el único automatizado y además es el método oficial de análisis para extracción de grasa recomendado por la AOAC. Sin embargo, este método presenta la desventaja de no permitir el análisis posterior de la grasa una vez extraída, ya que debido a la hidrólisis ácida los lípidos sufren alteraciones. Además, debido a la baja polaridad del disolvente empleado con este método, éter de petróleo, parte de los lípidos polares de la muestra no se pueden extraer. El método de Folch utiliza clorofomo:metanol (2:1), cuya polaridad es mas alta que la del éter de petróleo, por lo que la extracción de compuestos polares es mayor.

El método de Bligh y Dyer ha sido ampliamente utilizado para extraer los lípidos de la carne y productos cárnicos (Brooks y col., 1998), sin embargo este método fue el que peores resultados mostró en gran parte de los productos cárnicos estudiados. Otros autores también han encontrado la menor eficacia del método de Bligh y Dyer en comparación con el Soxhlet y el de Folch (Brooks y col., 1998; Iverson y col., 2001). Esta dificultad para extraer la totalidad de la grasa en productos cárnicos con el método de Bligh y Dyer podría estar

relacionada con la polaridad de la mezcla de disolventes que utiliza, cloroformo:metanol (1:2), en el que la solubilidad de los triglicéridos es limitada. Además, cuando se extraen lípidos con la mezcla de disolventes cloroformo:metanol, la muestra absorbe cantidades constantes de la misma (Smedes y col., 1996), reduciéndose así la cantidad de disolvente disponible para extraer los lípidos. Por lo tanto, la relación disolvente:muestra del método Bligh y Dyer (3:1) debería ser optimizada para cuantificar adecuadamente la cantidad de grasa en carne y productos cárnicos. De hecho, este método fue desarrollado y descrito inicialmente para extraer grasa de muestras de pescado con un contenido lipídico inferior al 1%, porcentaje de humedad en torno al 80% y altos niveles de lípidos polares, e incluso los autores aconsejaron modificar este método para ser utilizado en muestras con un contenido en grasa elevado.

I.2. Diferenciación de jamones Ibéricos en función de la alimentación (montanera vs. piensos alto oleico)

La alimentación de los cerdos Ibéricos durante el periodo de cebo, en montanera (AG) o con piensos alto oleico (HO), afectó al perfil de ácidos grasos de la grasa subcutánea y de la grasa intramuscular de los perniles, concretamente de los músculos semimembranoso y bíceps femoral (Capítulo I.2.). Los animales HO presentaron valores de ácido palmítico (C16:0) significativamente más altos que los cerdos AG en los tres tejidos estudiados, lo que refleja el mayor contenido de este ácido graso en la hierba y en la bellota que en el pienso alto oleico. El porcentaje de ácido esteárico (C18:0) fue similar en el músculo bíceps femoral mientras que presentó diferencias en la grasa subcutánea, más elevado en los HO, y en el músculo semimembranoso, en el que se observó un mayor porcentaje de este ácido graso en los animales AG. Resulta difícil explicar este diferente efecto de la dieta en función del músculo, y más teniendo en cuenta que las dos alimentaciones contenían porcentajes similares de ácido esteárico (C18:0). De hecho, la mayor parte de los ácidos grasos saturados proceden de la síntesis "de novo" y solo una pequeña porción se acumula directamente de la dieta (Monahan y col., 1992). Otros trabajos no han mostrado el efecto de la alimentación con piensos alto oleico sobre el porcentaje de estos ácidos grasos saturados en los

músculos longissimus dorsi (Muriel y col., 2002) y bíceps femoral (Ventanas y col., 2007a).

A pesar del alto contenido en ácido oleico (C18:1 n-9) del pienso empleado en este estudio (casi igual que el de la bellota), se encontraron diferencias significativas en los valores de este ácido graso entre los dos grupos de cerdos Ibéricos en la grasa subcutánea y en el músculo semimembranoso, siendo más altos en los cerdos AG, pero no se encontraron diferencias en el músculo bíceps femoral. Los estudios realizados por González y col., (2007) y Muriel y col. (2002), en el músculo longissimus dorsi, y Ventanas y col. (2007a), en el bíceps femoral, han mostrado similares porcentajes de ácido oleico (C18:1 n-9) en cerdos de montanera y cebados con pienso alto oleico, mientras que Daza y col. (2005) encontraron un mayor contenido de este ácido graso en los cerdos de montanera.

En la grasa subcutánea, el porcentaje de ácido linoleico (C18:2 n-6) fue significativamente mayor en los cerdos AG que en los HO, mientras que en el músculo semimembranoso los valores de este ácido grasos fueron más altos en los animales HO, no encontrándose diferencias en el músculo bíceps femoral. Así, las diferencias encontradas en este ácido graso no son fácilmente atribuibles a la dieta. De hecho, a partir del ácido linoleico (C18:2 n-6) se sintetizan otros ácidos grasos cadena larga como el araquidónico (C20:4 n-6) (Valette y col., 1991). Así, en los tres tejidos analizados en este trabajo, el porcentaje de ácido araquidónico (C20:4 n-6) fue significativamente mayor en los cerdos HO que en los AG, lo que sí refleja la cantidad de ácido linoleico (C18:2 n-6) de la dieta, mayor en el pienso que en la bellota y en la hierba. Sin embargo, otros estudios similares no han encontrado diferencias significativas en el ácido araquidónico (C20:4 n-6) (Muriel y col., 2002; González y col., 2007; Ventanas y col., 2007a).

Al analizar el perfil completo de ácidos grasos se encontraron diferencias estadísticamente significativas en algunos de los ácidos grasos minoritarios de los tres tejidos estudiados. Entre ellos, los más abundantes son el ácido araquidónico (C20:4 n-6), que ya se ha comentado anteriormente, y el ácido linolénico (C18:3 n-3), cuyo porcentaje fue mayor en los cerdos AG que en los HO en los tres tejidos analizados, debido posiblemente al elevado contenido de este ácido graso en la hierba.

Con respecto a la fracción insaponificable, las cantidades de neofitadieno y γ-tocoferol también mostraron diferencias estadísticamente significativas entre los dos grupos cerdos Ibéricos de este estudio, presentado ambos compuestos niveles más altos en los animales AG. De hecho, existen dos procedimientos patentados para la diferenciación de cerdos Ibéricos en función de su alimentación basados en el análisis del neofitadieno (Tejeda y col., 2005) y del γ-tocoferol y otros compuestos lipídicos solubles (López-Bote y col., 1998).

El perfil de ácidos grasos de los cerdos HO podría considerarse de montanera si se compara con las muestras de otros estudios (Ventanas y col., 2007a). Sin embargo, existen diferencias significativas entre los jamones AG y HO de este estudio. Por otra parte, con el sistema de clasificación basado en el porcentaje de los cuatro ácidos grasos mayoritarios de la grasa subcutánea, no se conseguiría una correcta clasificación de los cerdos ibéricos de este estudio.

Sin embargo, cuando se utilizaron todos los parámetros analizados (perfil completo de ácidos grasos y componentes de la fracción insaponificable) mediante un Análisis de Componentes Principales para intentar clasificar las muestras, se pudo observar una clara diferenciación entre los cerdos Ibéricos AG y HO (Capítulo I.2.). En los tres tejidos estudiados, este análisis mostró a los dos grupos de cerdos perfectamente separados y definidos. Los compuestos lipídicos que definieron a cada grupo de cerdos no fueron siempre los mismos en las tres localizaciones. Entre los ácidos grasos, dos de ellos fueron comunes en los tres tejidos analizados: el ácido linolénico (C18:3 n-3), que se situó en el área correspondiente a los perniles procedentes de los cerdos AG, y el ácido araquidónico (C20:4 n-6), que estaba en el área de los jamones HO. En cuanto a la fracción insaponificable, neofitadieno y γ-tocoferol estaban en el área correspondiente a los cerdos AG mientras que los HO estuvieron definidos por el α-tocoferol, lo que se explica por el mayor contenido de neofitadieno y γ-tocoferol en la bellota y la hierba, respectivamente, y de α-tocoferol en el pienso alto oleico empleado en este estudio, que estaba enriquecido en este compuesto.

El análisis de las clases de fosfolípidos reveló la influencia de la alimentación sobre estos compuestos lipídicos, afectando a la cantidad de las

clases individuales de fosfolípidos: fosfatidilcolina (PC), fosfatidiletanolamina (PE), fosfatidilserina (PS) y fosfatidilinositol (PI) de los perniles, así como a la composición lipídica de cada una de ellas (Capítulo I.4.). La cantidad de PC, PE y PI fue significativamente mayor en los jamones HO que en los AG. Por el contrario, otros estudios realizados sobre muestras de pescado (Soudant y col., 1997) y rata (Williams y col., 1992) no encontraron diferencias en el porcentaje de las diferentes clases de fosfolípidos debido a la dieta. Leseigneur-Meynier y col. (1991) han relacionado la cantidad de fosfolípidos musculares con el tipo, el diámetro y la cantidad de mitocondrias de las fibras musculares. Los dos grupos de cerdos de este estudio se cebaron en extensivo, pero los AG dispusieron de una mayor extensión de terreno que los HO, determinando un ejercicio más intenso en los animales AG, lo que podría influir sobre las características de las fibras musculares.

La composición en ácidos grasos y dimetilacetales de las cuatro clases de fosfolípidos detectadas también se vio afectada por la alimentación. En los perniles AG, PC y PE presentaron un mayor contenido de ácido oleico (C18:1 n-9) y dimetilacetales, mientras que en PC, PE y PS la cantidad de los ácidos linoleico (C18:2 n-6) y araquidónico (C20:4 n-6) fue superior en los jamones frescos HO. Según este estudio los perniles AG y HO podrían diferenciarse tanto por la cantidad de las clases individuales de fosfolípidos como por la composición lipídica de las mismas.

Además del análisis de los compuestos lipídicos para clasificar los cerdos Ibéricos, también se ha estudiado la posibilidad de analizar las muestras mediante imágenes de resonancia magnética (Capítulo I.3.). Las imágenes de los perniles AG y HO mostraron diferencias visualmente apreciables. La imágenes procedentes de los jamones frescos AG presentaban un color grisáceo, correspondiente a las estructuras musculares, más oscuro que los jamones HO. El color blanco, correspondiente a la grasa intermuscular e intramuscular, fue más brillante en los perniles AG que en los HO. Parámetros como la cantidad de agua y la fluidez de la grasa podrían modificar las características de las imágenes, ya que las técnicas de imágenes de resonancia magnética se basan en la actitud de los hidrógenos ante la presencia de un campo magnético (Lufkin, 1998). De hecho, los perniles HO presentaron un mayor porcentaje de humedad que los AG. La fluidez de la

grasa está relacionada con la composición en ácidos grasos. Por lo tanto, las diferencias en el perfil de ácidos grasos entre los jamones AG y HO podría dar lugar a las diferencias observadas en las imágenes. Además, las imágenes fueron analizadas mediante métodos basados en características computacionales de textura. Estas características están relacionadas con propiedades de las imágenes como homogeneidad, finura, suavidad y rugosidad (Mitchel y col., 2000). Entre los perniles AG y HO se encontraron diferencias significativas en trece de las quince características de textura analizadas. Gran parte de estas características están relacionadas con la homogeneidad de la imagen, la cuál parece tener un papel importante en la diferenciación de los jamones AG y HO mediante imágenes de resonancia magnética.

El hecho de poder diferenciar perniles procedentes de cerdos Ibéricos con distinta alimentación mediante técnicas de imágenes de resonancia magnética es destacable ya que esta técnica tiene la ventaja de ser no destructiva, no invasiva, no radiante e inocua. La técnica de imágenes de resonancia magnética podría ser adaptada para implantarla "*on line*" en las cadenas de procesado de las industrias y/o mataderos por sus múltiples aplicaciones.

Para establecer la posible relación entre la composición lipídica del músculo bíceps femoral y sus características computacionales de textura, éstos fueron analizados mediante Correlación de Pearson y Análisis de Componentes Principales (Capítulo I.3.). En los resultados obtenidos pudieron diferenciarse dos grupos de características computacionales de textura, G1 (Energy, SNE, LNE, NNU, SM, LRE, GLNU y RLNU) y G2 (Entropy, ENT, SER y RPC). Los jamones AG estuvieron definidos principalmente por el grupo de texturas computacionales G1, los ácidos grasos esteárico (C18:0) y linolénico (C18:3 n-3) y el γ-tocoferol, todos ellos correlacionando entre sí positivamente. El grupo G2 y los ácidos grasos mirístico (C14:0), palmítico (C16:0), palmitoleico (C16:1), eicosatrienoico (C20:3 n-6) y araquidónico (C20:4 n-6), que se relacionaron de forma positiva, y definieron los jamones HO.

Para analizar si las diferencias encontradas en la materia prima se seguían manteniendo en los jamones curados se llevó a cabo un estudio sobre el producto final (Capítulo I.5.).

La alimentación de los cerdos Ibéricos durante el cebo también afectó al perfil de ácidos grasos de los jamones curados. El sumatorio de ácidos grasos saturados fue superior en los jamones HO que en los AG, como consecuencia de las diferencias en los ácidos grasos mirístico (C14:0) y palmítico (C16:0). El sumatorio de ácidos grasos monoinsaturados así como el ácido oleico (C18:1 n-9) presentaron niveles superiores en los jamones AG que en los HO. Con respecto a los ácidos grasos poliinsaturados, el porcentaje de los ácidos grasos linoleico (C18:2 n-6), linolénico (C18:3 n-3), eicosatrienoico (C20:3 n-3) y eicosapentanoico (C20:5 n-3) y como consecuencia, el total de ácidos grasos poliinsaturados, fueron superiores en los jamones AG. Ventanas y col. (2007a) solo encontraron niveles más altos de los ácidos grasos linolénico (C18:3 n-3) y eicosapentanoico (C20:5 n-3) en jamones curados procedentes de cerdos Ibéricos cebados en montanera que en aquellos de cerdos alimentados con pienso alto oleico.

Las imágenes de resonancia magnética de los jamones curados AG y HO no mostraron diferencias visualmente perceptibles. Sin embargo, algunas de las características computaciones de textura sí fueron significativamente diferentes entre estos dos grupos de jamones, lo que permitiría su diferenciación mediante esta técnica. No obstante, en comparación con la materia prima, en la que se encontraron diferencias significativas en la mayoría de las características de textura analizadas, en producto final las diferencias fueron menores. Esto hecho parece indicar que el procesado del jamón influye de manera notable atenuando las diferencias encontradas en los perniles, posiblemente debido a las pérdidas de agua durante la maduración.

Para comprobar si las diferencias existentes en composición lipídica y en las características computacionales de las imágenes de resonancia magnética se manifestaban en la calidad final, se realizó un análisis sensorial cuantitativo-descriptivo. Los panelistas consideraron los jamones curados AG significativamente más pastosos y con menos veteado que los HO. En jamones de Parma con defectos de textura, Parolari y col. (1998) encontraron altas concentraciones de péptidos y aminoácidos, lo que ha sido relacionado con elevados niveles de enzimas proteolíticas (Virgili y col., 1995). Por otra parte, Enfält y col. (1993) hallaron una mayor actividad enzimática en músculos de cerdos que hacían más ejercicio. Así, y como se ha explicado anteriormente,

aunque los dos grupos de cerdos de este estudio se cebaron en extensivo, los AG dispusieron de una mayor extensión de terreno que los HO, lo que permitiría a los cerdos AG realizar más ejercicio.

En cuanto al atributo veteado, que obtuvo mayor puntuación en los jamones HO que en los AG, se ha relacionado de forma positiva con la cantidad de grasa intramuscular (Muriel y col., 2004; Ruiz-Carrascal y col., 2000). Sin embargo, la cantidad de grasa intramuscular los jamones AG y HO de este estudio fue similar. Por otra parte, la distribución de las vetas de grasa intramuscular en el jamón afecta a su apariencia, y en consecuencia a la apreciación visual del veteado (Cernadas y col., 2002). Esta diferencia en el aspecto podría influir en las características computacionales de textura.

El test de aceptabilidad no mostró diferencias significativas entre los jamones AG y HO. En un estudio previo, Ventanas y col. (2007b) encontraron escasas diferencias en cuanto a calidad sensorial entre jamones procedentes de cerdos cebados en montanera y con pienso alto oleico. Este hecho lo atribuyeron a las diferencias encontradas en la cantidad de grasa entre estos dos lotes de jamones, menor en los de pienso alto oleico.

En los jamones curados también se llevó a cabo un Análisis de Componentes Principales para evaluar la posible relación entre la composición lipídica, las características de computacionales de textura y los atributos sensoriales. Este análisis mostró los dos grupos de jamones curados perfectamente separados y definidos (Capítulo I.5.).

Aunque la composición lipídica y las características de textura permiten diferenciar entre los dos grupos de jamones de este estudio, AG y HO, estas diferencias no se manifiestan en los atributos sensoriales del producto final, al menos en los relacionados con el aroma, el sabor y el flavor del jamón curado.

SECCIÓN II

En la sección II de esta tesis doctoral se estudió el efecto de la congelación-descongelación de perniles Ibéricos sobre parámetros físicos, químicos y sensoriales en materia fresca, durante el procesado del jamón y en el producto final.

II.1. Efecto de la congelación-descongelación de perniles Ibéricos sobre parámetros físico-químicos de la materia fresca.

Los perniles refrigerados (R) presentaron un contenido en humedad más elevado que el de los congelados-descongelados (F) en materia prima (Capítulo II.5.). Este hecho podría explicarse por las pérdidas de agua experimentadas durante la descongelación (Jalang'O y col., 1987). De hecho, los jamones F presentaron un 0,97 % de pérdidas de peso tras la descongelación.

En las medidas de color instrumental también se observaron algunas diferencias significativas entre los jamones R y F (Capítulo II.5.). Concretamente, los perniles F mostraron valores más altos de a* y Chroma que los perniles R. Estos dos parámetros han sido relacionados con las pérdidas de agua (Arnau y col., 2003), por lo tanto, las diferencias de color observadas podrían ser debidas a las diferencias en el contenido en humedad existentes entre los dos grupos de jamones, siendo menor en los jamones F. La pérdida de agua sufrida por los perniles F durante la congelación-descongelación supondría una mayor concentración de los pigmentos, dando lugar a una carne fresca más roja y con un color más vivo. Además, estos resultados concuerdan con los mostrados por Sakata y col. (1995) que también encontraron mayores valores de a* en lomo de cerdo congelado-descongelado que en lomo refrigerado.

La congelación-descongelación de los jamones Ibéricos también afectó a los parámetros de textura instrumental de la materia fresca (Capítulo II.5). En la prueba de Warner Bratzler, la dureza al corte fue mayor en los jamones frescos F que en los R. En el análisis del perfil de textura (TPA) los perniles F obtuvieron valores significativamente más bajos en la dureza, en la masticabilidad y en la elasticidad que los perniles R. Esta medida de dureza instrumental podría explicarse como turgencia. Así, las diferencias encontradas

podrían estar relacionadas con el menor porcentaje de humedad en los jamones F que en los R. De manera que, a mayor contenido en humedad mayor turgencia (dureza) y menor dureza al corte. El hecho de que los perniles F presenten una menor dureza instrumental podría deberse a la ruptura de las miofibrillas musculares durante la congelación. En carne de cerdo y ternera se ha descrito que esta rotura ocurre de forma importante en torno a los -20º C (Sakata y col., 1995; Rahelic y col., 1985).

La congelación-descongelación de los perniles influyó sobre la composición en ácidos grasos de la materia prima (Capítulo II.1). En los jamones F, los sumatorios de ácidos grasos saturados, monoinsaturados y poliinsaturados presentaron niveles más altos en la fracción de ácidos grasos libres y más bajos en la fracción de lípidos polares que en los jamones R, indicando la existencia de lipolisis durante la congelación-descongelación de los perniles. El sumatorio de dimetilacetales en los lípidos polares también mostró niveles más altos en los jamones R que en los F. Sin embargo, no se manifestaron diferencias en el perfil de ácidos grasos de los lípidos neutros. Esto parece indicar la existencia de una mayor hidrólisis en los lípidos polares que en los neutros durante la congelación-descongelación de los jamones Ibéricos. También se observó una mayor oxidación en los perniles F que en los R, como consecuencia del desarrollo de las reacciones lipolíticas durante la congelación. En jamón blanco, Motilva y col. (1994) también mostraron un mayor contenido en ácidos grasos libres y una mayor oxidación en los perniles congelados-descongelados que en los refrigerados.

En relación con la composición lipídica, en este estudio se describe por primera vez el contenido de las diferentes clases de fosfolípidos en jamón Ibérico, en materia prima y a lo largo del proceso de maduración (Capítulo II.2.). Esta determinación se llevó a cabo mediante cromatografía líquida de alta resolución y detector "evaporative light scattering" (HPLC-ELSD). Así, se identificaron cuatro clases de fosfolípidos: fosfatidilcolina (PC), que es la más abundante (70%), fosfatidiletanolamina (PE), la segunda en importancia cuantitativa (20%), fosfatidilserina (PS) y fosfatidilinositol (PI) (en torno al 5% cada una). La congelación-descongelación de los jamones Ibéricos afectó a la cantidad de las distintas clases de fosfolípidos en materia prima, observándose en los perniles F menores cantidades de PC, PE y PI que en los R,

lo que de nuevo indica que se produce una hidrólisis apreciable de los fosfolípidos durante la congelación-descongelación de los jamones Ibéricos. De forma similar, estudios realizados con carne de cerdo, búfalo, ternera, pollo y pescado también han mostrado la disminución de los niveles de PC y PE durante el almacenamiento a congelación (Hernández y col., 1999; Kesava-Rao y col., 1991; El-Sebaiy y col., 1987; Igene y col., 1981). De hecho, se ha detectado actividad de las enzimas fosfolipasas a temperaturas de congelación en músculos de mamíferos y peces (Hernández y col., 1999; El-Sebaiy y col, 1987).

En cuanto a los parámetros relacionados con la actividad proteolítica (Capítulo II.3.), aunque no se encontraron diferencias significativas en las cantidades de nitrógeno no proteico, nitrógeno peptídico y nitrógeno aminoacídico entre los jamones frescos R y F, la mayoría de los aminoácidos y dipéptidos detectados mostraron mayores niveles en los perniles F que en los R. Estos resultados indican la existencia de proteolisis durante la congelación-descongelación de los jamones ibéricos, lo que podría atribuirse a una mayor liberación de las enzimas catepsinas (Flores y col., 2006), debido posiblemete al daño causado en los lisosomas por los cristales de hielo. Khan (1986), que también observó el incremento en la cantidad de aminoácidos y péptidos durante el almacenamientos a congelación, sugirió la subsistencia de la actividad de las enzimas catepsinas durante la congelación. Además, Kristensen y col. (2006) apuntaron que las enzimas calpainas y calpastatinas eran estables durante el almacenamiento de la carne a congelación.

Como ya se ha comentado, los perniles F presentaron una menor dureza que los R, lo que también podría ser debido a la mayor proteolisis.

II.2. Efecto de la congelación-descongelación de perniles Ibéricos sobre parámetros físico-químicos durante el procesado del jamón.

El porcentaje de mermas a lo largo del proceso de maduración fue similar para los jamones R y F (Capítulo II.5.). El contenido en sal al final de la etapa de salado fue superior en los jamones R que en los F. El tiempo de estancia en sal de los jamones de este trabajo fue establecido por la industria (1 día/kg y 0,7 día/kg para los jamones R y F, respectivamente), siguiendo la misma recomendación que para los jamones de cerdo blando de disminuir el tiempo

de salado de los jamones congelados-descongelados (Poma, 1989; Bañón y col., 1999). Coincidiendo con nuestros resultados, Grau y col. (2008) también encontraron un menor contenido en sal en jamones Ibéricos congelados-descongelados que en los refrigerados. Estos resultados parecen indicar que el efecto de la congelación-descongelación sobre la difusión de la sal hacia el interior de las piezas es diferente en jamones blancos e Ibéricos. Este hecho es posiblemente debido al mayor contenido en grasa en los jamones Ibéricos (Grau y col., 2008), haciendo que la difusión de la sal hacia el interior del jamón sea mas lenta que en jamones con menos cantidad de grasa. Esta consideración hace pensar que en el caso de utilizar jamones congelados-descongelados de cerdo Ibérico, las pautas de tiempo de salado deberían ajustarse si se quiere llegar a un contenido salino similar al obtenido cuando se procesan jamones refrigerados.

La evolución de los ácidos grasos a lo largo del proceso de curación fue diferente en los dos grupos de jamones de este estudio (Capítulo II.1.). En los jamones R se observó que la cantidad de ácidos grasos disminuyó en la fracción de lípidos polares y aumentó en la de ácidos grasos libres durante las etapas de post-salado y secadero. Sin embargo, en los jamones F estos cambios solo ocurrieron durante la etapa de secadero. Estos resultados podrían estar relacionados con el menor contenido en sal en los jamones F que en los R. Según los resultados obtenidos por Vestergaar y col. (2000), la actividad de las enzimas lipasas incrementa conforme la sal difunde al interior del jamón. Por su parte Andrés y col. (2005) también encontraron un efecto promotor de la sal sobre las reacciones lipolíticas del jamón Ibérico. Por tanto, la mayor concentración de sal en los jamones R supondría una mayor actividad de las enzimas lipasas y consecuentemente una mayor lipolisis en comparación con los jamones F. Asimismo, los niveles de oxidación lipídica de los jamones F fueron superiores que la de los R en las etapas de post-salado y secadero, posiblemente debido a las diferencias en los valores iniciales que sugiere una mayor lipolisis en los perniles F que en los R.

La congelación-descongelación de los jamones Ibéricos también afectó a la evolución de cada una de las clases de fosfolípidos (Capítulo II.2.). Durante la etapa de salado, PC, PE, PS y PI fueron hidrolizados en los jamones R mientras que PC y PE no sufrieron hidrólisis en los jamones F durante esta fase.

Este hecho podría también estar relacionado con la menor cantidad de sal en los jamones F al final de la etapa de salado. En las etapas de post-salado y secadero, las cuatro clases de fosfolípidos siguieron hidrolizándose siguiendo una tendencia similar en los dos grupos de jamones.

Con respecto a los fenómenos proteolíticos durante el procesado (Capítulo II.3.), no se observaron diferencias importantes en los valores de nitrógeno no proteico, nitrógeno peptídico y nitrógeno aminoacídico entre los jamones R y F, lo que concuerda con los resultados obtenidos por Flores y col. (2006) en jamón blanco. Sin embargo, Wang (2001) observó un mayor contenido de nitrógeno no proteico a lo largo del procesado de jamones procedentes de materia prima congelada-descongelada que en los procedentes de perniles refrigerados. El contenido en aminoácidos y dipéptidos mostró diferencias importantes entre los jamones R y F en la materia prima, mientras que a lo largo del proceso de maduración éstas fueron escasas. Flores y col. (2009) tampoco detectaron un efecto significativo de la congelación-descongelación de los perniles Ibéricos sobre el contenido en aminoácidos en las etapas de salado y post-salado del jamón Ibérico.

En cuanto a la influencia de la congelación-descongelación de los perniles Ibéricos sobre la evolución de los compuestos volátiles (Capítulo II.4.), los resultados encontrados han mostrado escasas diferencias entre los jamones R y F en el contenido de estos compuestos al final de las etapas de salado, post-salado y secadero. Sin embargo, sí se observaron diferencias en el producto final, como se expone en el siguiente apartado.

II.3. Efecto de la congelación-descongelación de perniles Ibéricos sobre parámetros físicos, químicos y sensoriales del producto final.

Como cabía esperar, el contenido en sal de los jamones curados F fue significativamente más bajo que el de los R. Sin embargo, el porcentaje de mermas y de humedad fue similar en los dos grupos de jamones al final del procesado (Capítulo II.5.).

En cuanto a las medidas de color y textura instrumental (Capítulo II.5.), los jamones R y F no presentaron diferencias significativas en ninguno de estos parámetros en el producto final, aunque sí mostraron diferencias significativas en materia prima. Carrapiso y col. (2005) también observaron diferencias en los

parámetros de color instrumental debido al efecto de la raza y al sistema de explotación en materia prima pero no en jamón curado.

Al final del proceso de maduración la composición en ácidos grasos de las tres fracciones lipídicas estudiadas (lípidos polares, ácidos grasos libres y lípidos polares) así como los valores de TBA fueron similares en ambos grupos de jamones (Capítulo II.1.). La única diferencia se encontró en los niveles del total de ácidos grasos saturados de la fracción de ácidos grasos libres, siendo superior en los jamones curados R que en los F. Estos resultados concuerdan con los obtenidos en otros estudios en jamón blanco (Motilva y col., 1994; Wang, 2001).

Sin embargo, en el caso de las clases de fosfolípidos (Capítulo II.2.), las diferencias observadas durante la maduración de los jamones se observaron también en producto final. Así, en los jamones curados R se detectaron PC, PE y PI, pero no PS, mientras que el los jamones F solo fueron encontradas PC y PE. En cuanto al porcentaje de pérdidas acumuladas de las distintas clases de FOSFOLÍPIDOS en producto final, el de PC y PS fue similar en los dos grupos de jamones mientras que se observaron diferencias en PE y PI. En los jamones curados F, PE mostró un menor porcentaje de pérdidas que en los R, lo que podría estar relacionado con el alto contenido en ácidos grasos poliinsaturados y dimetilacetales de este fosfolípido, haciéndole más susceptible a la hidrólisis (Hernández y col., 1999; Ngah y col., 1994). De hecho, en materia fresca se observaron diferencias en el contenido de dimetilacetales y de ácidos grasos poliinsaturados de los lípidos polares, encontrándose niveles superiores en los jamones R que en los F.

En cuanto a las medidas de proteolisis en jamón curado (Capítulo II.3.), se observó que el contenido de nitrógeno peptídico fue significativamente superior en los jamones F que en los R, mientras que los niveles de nitrógeno no proteico y nitrógeno aminoacídico fueron similares en los dos grupos de jamones. En varios trabajos se ha relacionado un bajo contenido en sal de los jamones con altos niveles de nitrógeno no proteico (Martín y col., 1998; Wang, 2001), de nitrógeno peptídico (Martín y col., 1998) y de nitrógeno aminoacídico (Virgili y col., 1999). Sin embargo, y según los resultados de este estudio, las diferencias en el contenido en sal de los jamones curados afectarían únicamente al nitrógeno peptídico. De hecho, Córdoba y col. (1994)

señalaron que concentraciones de sal entre 1.5% y 6%, entre las que se encuentran los jamones de este trabajo, no afectan de forma importante los niveles de nitrógeno no proteico. Por otra parte, los resultados obtenidos por Martín y col (1998) parecen indicar que la temperatura es el factor que tiene más influencia sobre los niveles de nitrógeno aminoacídico. Se observaron diferencias en algunos de los aminoácidos y péptidos detectados en producto final. Sin embargo, estas diferencias no siguieron una tendencia definida, lo que puede sugerir que la congelación-descongelación de los perniles no afecta de forma clara sobre la composición final de aminoácidos y péptidos.

En relación con los compuestos volátiles en producto final (Capítulo II.4.), se encontraron diferencias en algunos de estos compuestos entre los dos grupos de jamones de este estudio. Los niveles de 2-metilbutanal, 2,3-butanediol y 2-heptanol fueron significativamente más altos en los jamones curados F que en los R, lo que podría explicarse por un mayor contenido de los precursores de estos compuestos volátiles en alguna de las etapas del procesado en los jamones F. De hecho, la isoleucina, que es el precursor del 2-metilbutanal, mostró un mayor contenido en los jamones F que en los R en materia fresca y al final de la etapa de post-salado. Por otro lado, ocho de los diez ésteres detectados mostraron niveles significativamente más altos en los jamones R que en los F. En jamones curados, la presencia de cantidades apreciables de ésteres se ha relacionado con un alto contenido en alcoholes (Sabio y col., 1997). En los estudios realizados en el jamón Ibérico parece que la contribución de los ésteres al aroma no es importante (Ruiz y col., 2000). Sin embargo, en jamón blanco se ha descrito que estos compuestos, junto con las cetonas, los hidrocarburos aromáticos y las pirazinas están relacionados de forma importante con el aroma (Flores y col., 1997). En jamón de Parma, los ésteres influyen de manera positiva en el flavor (Careri y col., 1993).

En el análisis sensorial cuantitativo-descriptivo se encontraron diferencias en la dureza de la grasa y la pastosidad del magro entre los jamones R y F (Capítulo II.5.). Así, los jamones F presentaron una grasa más dura y fueron más pastosos que los R. La mayor dureza de la grasa de los jamones F podría ser debida la congelación. En este sentido, Campos y col. (2002) han descrito que la cristalización de la grasa, como consecuencia de las bajas temperaturas, da lugar a un incremento en su dureza. Así, la dureza de la grasa estaría más

relacionada con las propiedades físicas de la grasa que con su composición química en este caso. La pastosidad y la textura blanda son los principales problemas de textura en el magro (García-Garrido y col. 2000), los cuales se han asociado con un bajo contenido en sal (Martín y col., 1998; Virgili y col., 1995). Se considera que jamones sometidos a menos de 1 día de salado/kg los valores de sal pueden estar por debajo del 3%, comprometiendo la correcta formación del gel proteico (Ventanas y col., 1998). Asimismo, bajos contenidos en sal se han relacionado con altos valores de nitrógeno no proteico y con la aparición de consistencia pastosa (Martín y col, 1998; Ventanas y col., 1998). En jamones de Parma con problemas de textura, Paralori y col. (1988) encontraron elevadas concentraciones de aminoácidos y de nitrógeno peptídico, es decir una proteolisis elevada. En nuestro estudio, la menor cantidad de sal de los jamones F junto con los mayores niveles de nitrógeno peptídico podrían explicar que sean considerados más pastosos. Sin embargo, y pese a las diferencias detectadas en el contenido de sal y en algunos aminoácidos y compuestos volátiles, los atributos sensoriales relacionados con el olor, sabor y flavor obtuvieron similares puntuaciones en ambos grupos de jamones. En el test de aceptabilidad, no se encontraron diferencias significativas entre los jamones R y F.

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6. Conclusiones

1. Los métodos de Folch y Soxhlet con hidrólisis previa permiten la adecuada cuantificación del contenido lipídico en carne y productos cárnicos. Para extraer la totalidad de los lípidos del jamón, el método de Folch puede considerarse el más adecuado.
2. Para cuantificar los lípidos totales en carne y productos cárnicos con el método de Bligh y Dyer, se debe ajustar adecuadamente la relación entre el volumen de disolvente y cantidad de muestra utilizados.
3. El porcentaje de los ácidos grasos araquidónico (C20:4 n-6) y linolénico (C18:3 n-3) y el contenido de neofitadieno y γ-tocoferol así como las técnicas de imágenes de resonancia magnética basadas en el análisis de las características computacionales de textura parecen ser herramientas adecuadas para diferenciar perniles Ibéricos procedentes de cerdos cebados con diferente alimentación (montanera vs. pienso alto oleico).
4. El efecto de la alimentación de los cerdos Ibéricos (en montanera o con piensos alto oleico) sobre la composición lipídica de los jamones es notable, pero no influye de forma importante en los atributos sensoriales.
5. La congelación-descongelación de perniles Ibéricos previa a su salazón influye de forma considerable sobre las características físico-químicas de la materia fresca, siendo este efecto menos notable durante el procesado del jamón y en el producto final.
6. El tiempo de salado para perniles Ibéricos congelados-descongelados debe ajustarse si se pretende obtener jamones con una concentración salina similar a la de los jamones refrigerados.
7. La congelación-descongelación de los perniles Ibéricos podría incluirse como práctica habitual en el protocolo de elaboración del jamón Ibérico sin afectar significativamente a las características físicas, químicas y sensoriales del producto.

1. The Folch and Soxhlet with acid hydrolysis methods are appropriate for quantifying the total lipids in meat and meat products. For extracting the total lipid content of hams, the Folch method is the most suitable.
2. The ratio solvent:sample used in the original Bligh and Dyer method should be optimized for quantifying the total lipids in meat and meat products properly.
3. The proportion of araquidonic (C20:4n-6) and linolenic (C18:3 n-3) acids and the content of neophytadiene and γ-tocopherol as well as Magnetic Resonance Imaging techniques based on the analysis of computational texture characteristics, seem to be useful tools for differentiating thighs from Iberian pigs fattened different diets (acorn and grass vs. oleic acid enriched concentrates).
4. The effect of Iberian pig feeding (acorn and grass or oleic acid enriched concentrates) on the lipid composition of hams is noticeable but, it does not markedly influence on sensory traits.
5. Pre-cure freezing Iberian thighs significantly influences physicochemical characteristics of raw thighs, while this effect is less marked during the processing of hams and in the final product.
6. The salting time for frozen-thawed Iberian thighs should be adjusted for obtaining hams with similar salt content to refrigerated hams.
7. Pre-cure freezing thighs could be carried out within the typical processing of Iberian hams without markedly affecting physical, chemical and sensory features.

7. Anexo

Perfil de ácidos grasos de la grasa subcutánea e intramuscular de cerdos ibéricos cebados en montanera y con pienso "alto oleico"

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Se presenta un estudio cuyo objetivo es diferenciar los cerdos ibéricos alimentados con piensos "alto oleico" de los alimentados y criados en montanera, mediante el análisis del perfil de ácidos grasos tanto en la grasa subcutánea como en la grasa intramuscular.

Introducción

Los factores asociados a la calidad de los productos del cerdo ibérico son variados. Así, las características finales de estos productos son el resultado de la combinación de factores ligados a la raza, al sistema de explotación y al proceso de maduración.

Dentro del sistema de explotación del cerdo ibérico, la alimentación es sin duda, uno de los factores con mayor influencia sobre la calidad de los productos elaborados (Cava y cols., 2000; Carrapiso y cols., 2003).

De hecho, la Norma de Calidad del ibérico (Real Decreto 1469/2007, de 2 de noviembre; BOE núm. 264 de 3 de noviembre de 2007) contempla la existencia de diferentes categorías comerciales ("de bellota", "de recebo" "de cebo de campo" y "de cebo") en función de diferentes sistemas de alimentación (cebado en montanera, cebado en montanera y terminado con pienso, cebado con pienso con o sin estancia en campo), de forma que cada una de estas categorías alcanzaría un precio diferente en el mercado.

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Pérez-Palacios, T.; Antequera, T.; Ruiz, J.

MONTANERA *vs* PIENSOS ALTO OLEICO: RELACIÓN CON LA COMPOSICIÓN EN ÁCIDOS GRASOS DE LA GRASA SUBCUTÁNEA DEL CERDO IBÉRICO

Este trabajo ha sido realizado dentro del ámbito del proyecto "Métodos de clasificación de jamones ibéricos. Correlación entre parámetros físico-químicos y de imágenes de resonancia magnética" (EXP: 3PR05B027) correspondiente al III Plan Regional de Investigación, Desarrollo Tecnológico e Innovación, financiado por la Junta de Extremadura.

COMUNICACIÓN ORAL

IV Congreso Mundial del Jamón, Salamanca, España, 2007

EFECTO DE LA CONGELACIÓN SOBRE LAS CARACTERÍSTICAS SENSORIALES DE JAMONES IBÉRICOS

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INTRODUCCIÓN

La elaboración del jamón ibérico se realiza generalmente a partir de materia prima fresca. Sin embargo, se están empezando a utilizar perniles congelados con el fin de optimizar el ritmo de trabajo en las industrias y poder establecer lotes homogéneos de materia prima, lo que permitiría producir jamones con menor variabilidad entre ellos.

Hasta ahora no existen estudios que muestren el efecto de la congelación de jamones ibéricos sobre el proceso de maduración ni en las características sensoriales del producto final.

Dada la estrecha relación entre las características sensoriales del jamón ibérico y su sistema de elaboración, cualquier modificación en el sistema de procesado podría modificar sus características sensoriales.

MATERIAL y MÉTODOS

Se procesaron dos lotes de jamones ibéricos de cebo, 12 procedentes de perniles frescos y otros 12 procedentes de perniles congelados.

Los dos lotes fueron salados mediante el sistema tradicional en pilas de sal (>75% H y T<4ºC), a razón de 1 día/kg para los perniles frescos y 0,7 día/kg para los perniles congelados.

Una vez curados los jamones, se extrajo el músculo *Bíceps femoris* que se cortó en lonchas finas para realizar el **análisis sensorial**:

-**ANÁLISIS CUANTITATIVO-DESCRIPTIVO**: Panel entrenado

-**TEST DE ACEPTABILIDAD**: 100 consumidores

Tabla 1. Resultados obtenidos del test de aceptabilidad de los jamones de cerdo ibérico de cebo procedentes de jamones frescos y congelados.

ATRIBUTO	Jamones frescos	Jamones congelados	*p*
Aspecto	5.1	6.26	**<0.0001**
Aroma	6,92	6,76	0,2807
Sabor	7,09	6,94	0,2923
Dureza	7.14	6.68	**0,0047**
Jugosidad	7,19	7,09	0,4613
Calidad global	7,00	6,86	0,2996

CONCLUSIONES

La congelación de jamones de cerdo ibérico parece no afectar a las características sensoriales del producto final ni al grado de aceptabilidad por los consumidores, ya que las diferencias de textura observadas entre los jamones procedentes de materia prima fresca y congelada podrían ser debidas al menor tiempo de salazonado al que se someten los perniles congelados con respecto a los frescos, y por consiguiente a la captación de sal.

OBJETIVOS

Evaluar las posibles diferencias entre las características sensoriales de jamones ibéricos procedentes de materia prima fresca y congelada.

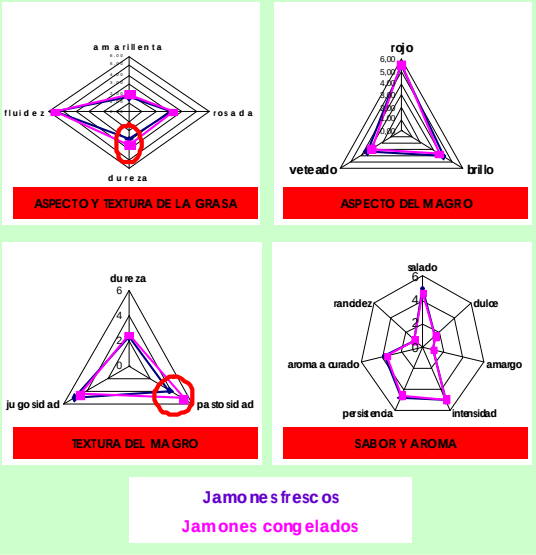

Figura 1. Resultados obtenidos del análisis cuantitativo-descriptivo de los jamones de cerdo ibérico procedentes de perniles frescos y congelados.

RESULTADOS Y DISCUSIÓN

El análisis cuantitativo-descriptivo reveló valores de pastosidad del magro superiores en los jamones congelados (5,18) que en los frescos (3,92). La dureza de la grasa obtuvo puntuaciones superiores en los jamones congelados (3,42) que en los frescos (2,86). El test de aceptabilidad mostró valores más altos en la apariencia (6,23) y más bajos en la dureza (6,68) en los jamones procedentes de perniles congelados que los procedentes de perniles frescos (5,10 y 7,14, respectivamente). La menor dureza que detectaron los consumidores en los jamones congelados podría estar relacionada con los elevados valores de pastosidad que el panel de catadores entrenados dio a estos jamones. Se considera que jamones sometidos a menos de 1 día de salado/kg los valores de sal pueden estar por debajo del 3%, comprometiendo la correcta formación del gel proteico (Ventanas y cols., 1998). Asímismo, bajos contenidos en sal se han relacionado con altos valores de nitrógeno no proteico y con la aparición de consistencia pastosa (Martín y cols, 1998; Ventanas y cols., 1998).

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Este trabajo ha sido realizado dentro del ámbito del proyecto "Desarrollo de una tecnología basada en el salado y descongelación en salmuera saturada, con y sin empleo de la impregnación a vacío, para la obtención de jamones curados a partir de perniles congelados procedentes de cerdo ibérico" (REF: PTR-1995-0754-OP-0303). Proyecto PETRI financiado por el Ministerio de Ciencia y Tecnología.

PÓSTER

IV Congreso Mundial del Jamón, Salamanca, España, 2007

5th Euro Fed Lipid Congress – Oils, Fats and Lipids: From Science to Applications

Gothenburg, 16-19 September 2007

QUANTIFICATION OF FAT CONTENT IN MEAT PRODUCTS BY DIFFERENT EXTRACTION METHODS

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COMUNICACIÓN ORAL

5th Euro Fed Lipid Congress-Oil, Fats and Lipids: From Science to Applications
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Fatty acid and dimethylacetal composition of the sn-1 and sn-2 position of phosphatidylcholine and phosphatidylethanolamine as affected by diet in rat muscle

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INTRODUCTION

The fatty acid (FA) and dimethylacetal (DMA) profile of the sn-1 and sn-2 position in phospholipids (PL) from animal muscle may have several important consequences, both *in vivo* and in the derived foodstuffs. In animal tissues, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant PL. Several works have shown that the FA and DMA composition of PL from meat is influenced by the dietary fat. However, the influence of dietary FA composition on the FA and DMA distribution in the sn-1 and sn-2 position of different muscle PL classes remains unstudied.

✓ The aim of this study was to investigate the effect of feeding diets enriched in different oils on the FA and DMA composition of the sn-1 and sn-2 position of PC and PE from *Longissimus dorsi* of rats

MATERIALS AND METHODS

- Muscle samples (*Longissimus dorsi* from 3 groups of 6 rats)
- Control diet (C), sunflower oil enriched diet (SO), olive oil enriched diet (OO)
- PC and PE extraction by solid phase extraction (Pérez-Palacios et al., 2007)
- PL hydrolysis with Phospholipase A_2

RESULTS AND DISCUSSION

Diets enriched in SO and OO showed the highest levels of linoleic acid (C18:2 n-6) and oleic acid (C18:1 n-9), respectively. The FA composition of PC reflects consumed FA better than that of PE, which could be related to the abundance and situation of PC in membranes. In the group fed a OO enriched diet, oleic acid (C18:1 n-9) content significantly increased in both positions of PC, and in the group fed a SO enriched diet, the proportion of arachidonic acid (C20:4 n-6) was the highest in the sn-1 position of PC. Contrarily, values of oleic acid (C18:1 n-9) in the sn-1 position of PE were lower in group OO than in groups C and SO, which could be related with the higher levels of C18:1 n-9 DMA found in PE in this specific position, since one of the pathways for the biosynthesis of plasmalogens involves a desaturation process of the FA sterified in the sn-1 position of the analogue PL. The DMA content was affected by diet only in PE. Proportions of octadecenal DMAs (18:1 n-9 DMA, 18:1 n-7 DMA) and total DMA were higher in rats fed an OO enriched diet than in C and SO rats. In both positions of PC and PE, the proportion of docosahexaenoic acid (C22:6 n-3) increased in the OO group. As a consequence, the level of total n-3 FA was higher and the ratio n-6/n-3 was lower in group OO than in groups C and SO.

Table 1. DMA composition (% total FA methyl ester and DMA detected) in the sn-1 position of PE of *Longissimus dorsi* from rats fed different diets.

	feeding groups				
DMA	C	SO	OO	SEM	*p*
C16:0 DMA	8.8	5.8	10.7	0.910	0.109
C16:1 DMA	1.5	2.3	1.2	0.350	0.501
C18:0 DMA	0.8	0.6	1.2	0,110	0,150
C18:1 n-9 DMA	3,5	2,6	6,8	0,570	0,040
C18:1 n-7 DMA	2,7	1,2	4,8	0,460	0,006
Σ DMA	17,3	12,6	24,3	1,830	0,049

➔ It can be concluded that dietary FA influence the FA profile of the sn-1 and sn-2 position of PC and PE and only the DMA composition of PE.

PÓSTER

5th Euro Fed Lipid Congress-Oil, Fats and Lipids: From Science to Applications
Gothenburg, Sweden, 2007

EFECTO DE LA CONGELACIÓN SOBRE EL CONTENIDO EN ÁCIDOS GRASOS DE JAMONES IBÉRICOS

3ª SESIÓN:
INSTALACIONES Y PROCESOS INDUSTRIALES

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INTRODUCCIÓN

Las reacciones de lipolisis y oxidación que tienen lugar durante el proceso de curación de los jamones Ibéricos tienen gran importancia en su elevada calidad sensorial, estando los procesos lipolíticos muy relacionados con las condiciones del procesado y con las características de la materia prima.

La congelación de la carne promueve cambios físicos, químicos y sensoriales en la misma, y estos dependen en gran medida de las características de la carne y de las condiciones de congelación.

El procesado tradicional de los jamones Ibéricos no contempla la congelación, y posterior descongelación, de los perniles antes de comenzar su proceso de curación. Sin embargo, esta práctica podría favorecer tecnológica y económicamente el procesado de estos productos. No obstante, hasta ahora no se ha estudiado si el empleo de perniles congelados-descongelados afecta a los parámetros de calidad del jamón Ibérico.

OBJETIVO

Estudiar el efecto de la congelación-descongelación de perniles Ibéricos sobre el contenido en ácidos grasos de los jamones en materia fresca y en producto final.

MATERIAL Y MÉTODOS

- Jamones Ibéricos refrigerados (n=12) y congelados-descongelados (n=12).
- Extracción de lípidos totales con cloroformo:metanol (1:2) (Pérez-Palacios y cols., 2008) y su fraccionamiento (Ruiz y cols., 2004).
- Metilación de ácidos grasos (Sandler y Karo, 1992).
- Determinación y cuantificación de ésteres metílicos: CG-FID

RESULTADOS Y DISCUSIÓN

La congelación y posterior descongelación de los perniles Ibéricos antes del comienzo del proceso de maduración solo afectó a la composición en ácidos grasos de los lípidos polares en materia fresca. Los valores de AGS, AGMI y AGPI fueron superiores en los jamones refrigerados que en los congelados-descongelados (Tabla 1), apuntando que durante las etapas de congelación y descongelación tiene lugar una intensa lipolisis. Sin embargo, en producto final no se encontraron diferencias significativas en el contenido de ácidos grasos, lo que podría indicar que durante el largo proceso de maduración del jamón Ibérico las diferencias encontradas al inicio del procesado se fueron reduciendo hasta desaparecer. Estos resultados son similares a los encontrados por Motilva y cols. (1994) en jamón blanco.

Tabla 1. Composición en ácidos grasos saturados (AGS), monoinsaturados (MUFA) y poliinsaturados (PUFA) (expresados como mg de ésteres metílicos/100 g de grasa intramuscular) de los lípidos neutros y polares procedentes de jamones Ibéricos refrigerados y congelados-descongelados en materia fresca y en producto final.

		MATERIA FRESCA			PRODUCTO FINAL		
		Refrigerados	Congelados Descongelados	*p*	Refrigerados	Congelados Descongelados	*p*
Lípidos Neutros	AGS	136,91	122,51	0,239	177,12	139,30	0,201
	AGMI	276,23	273,13	0,875	317,59	289,62	0,433
	AGPI	26,54	24,47	0,498	29,74	27,40	0,507
Lípidos Polares	AGS	20,96	15,41	0,034	11,42	11,09	0,292
	AGMI	18,05	14,25	0,023	11,16	10,89	0,199
	AGPI	29,72	20,13	0,037	10,89	10,90	0,977

CONCLUSIONES

La influencia de la congelación-descongelación de perniles Ibéricos afectó al contenido en ácidos grasos de los lípidos polares en materia fresca. Sin embargo, en la composición lipídica de los jamones curados este efecto no fue manifiesto.

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Este trabajo ha sido realizado dentro del ámbito del proyecto "Desarrollo de una tecnología basada en el salado y descongelación en salmuera saturada, con y sin empleo de la impregnación a vacío, para la obtención de jamones curados a partir de perniles congelados procedentes de cerdo ibérico" (REF: PTR-1995-0754-OP-0303). Proyecto PETRI financiado por el Ministerio de Ciencia y Tecnología. Los autores agradecen a la Junta de Extremadura- Consejería de Economía, Comercio e Innovación y FEDER (Ayuda a grupos de investigación GRU09148) su financiación para participar en este congreso.

PÓSTER

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CONTENIDO EN AMINOÁCIDOS Y DIPÉPTIDOS DURANTE EL PROCESADO DE JAMONES IBÉRICOS PROCEDENTES DE MATERIA PRIMA REFRIGERADA Y CONGELADA-DESCONGELADA

3ª SESIÓN:
INSTALACIONES Y PROCESOS INDUSTRIALES

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INTRODUCCIÓN

La proteolisis es uno de los procesos bioquímicos más importantes que ocurren durante el proceso de maduración del jamón Ibérico, ya que da lugar a la liberación de compuestos relacionados con el flavor (aminoácidos y pequeños péptidos), influyendo también en la textura del producto final. Entre los factores que pueden influir en el desarrollo de la proteolisis se encuentran los relacionados con las características de la materia prima y con el sistema de procesado.

En relación a la materia prima, la utilización de perniles congelados y descongelados antes del salazonado para la elaboración de jamones ibéricos se ha incrementado considerablemente en la última década. Este procedimiento tiene ventajas desde el punto de vista industrial, sin embargo, el efecto de la congelación-descongelación de jamones Ibéricos sobre sus diferentes parámetros de calidad no ha sido estudiado.

OBJETIVO

Estudiar la influencia del empleo de perniles congelados-descongelados sobre la evolución del contenido en aminoácidos y dipéptidos durante las diferentes etapas del procesado.

MATERIAL Y MÉTODOS

- Jamones Ibéricos refrigerados (n=12) y congelados (n=12).
- Desproteinización de la muestra con acetonitrilo (Aristoy y Toldrá, 1991).
- Derivatización de aminoácidos y péptidos con fenil isotiocianato (Bidlingmeyer y cols., 1987).
- Determinación de aminoácidos y péptidos: HPLC-detector photodiode array.

Tabla 1. Contenido total de aminoácidos (expresado como mg aa/100 g muestra extracto seco) durante el procesado de jamones Ibéricos procedentes de materia prima refrigerada y congelada.

	REFRIGERADOS	CONGELADOS DESCONGELADOS	*p*
Jamón fresco	762,63^{c}	991,10^{c}	0,025
Final de salado	1186,34^{c}	1259,37^{c}	0,597
Final de post-salado	2539,46^{b}	1869,85^{bc}	0,001
Final de secadero	3173,12^{b}	4611,76^{b}	0,085
Jamón curado	12165,86^{a}	14001,45^{a}	0,050
p (evolución)	<0,001	<0,001	

Tabla 2. Contenido total de dipéptidos (expresado como mg dipéptido/100 g muestra extracto seco) durante el procesado de jamones Ibéricos procedentes de materia prima refrigerada y congelada.

	REFRIGERADOS	CONGELADOS DESCONGELADOS	*p*
Jamón fresco	2741,82^{a}	2360,14^{a}	0,180
Final de salado	2911,65^{a}	2996,24^{ab}	0,727
Final de post-salado	1673,37^{b}	1674,82^{bc}	0,993
Final de secadero	1280,10^{bc}	1294,81^{c}	0,953
Jamón curado	1090,61^{c}	956,54^{c}	0,207
p (evolución)	<0,001	<0,001	

RESULTADOS Y DISCUSIÓN

En producto fresco, los jamones congelados-descongelados mostraron un mayor contenido de aminoácidos totales que los refrigerados (Tabla 1), indicando la existencia de proteolisis durante la congelación-descongelación de los perniles. Este hecho podría estar causado por la liberación de enzimas catepsinas de los lisosomas debido al daño ocasionado por los cristales de hielo que se forman durante la congelación. Por otra parte, al final del post-salado el contenido en aminoácidos de los jamones refrigerados fue mayor que en los jamones congelados-descongelados, lo que contrasta con los resultados encontrados por Flores y cols. (2006) en jamón blanco. Sin embargo, en producto curado ambos grupos de jamones presentaron similar contenido en aminoácidos totales. Con respecto al contenido en péptidos (Tabla 2), no se encontraron diferencias significativas entre los jamones refrigerados y los congelados-descongelados en ninguna de las fases del proceso de maduración.

Por otra parte, el contenido de aminoácidos y de péptidos mostró el mismo patrón de cambios en ambos grupos de jamones. La cantidad de aminoácidos fue aumentando durante el proceso de curación, alcanzando los niveles más altos en producto curado. Sin embargo, los valores más altos de dipéptidos se encontraron al final de la etapa de post-salado y fueron disminuyendo durante el resto de las etapas del procesado, mostrando los valores más bajos en los jamones curados.

CONCLUSIONES

La congelación-descongelación de perniles Ibéricos afectó al contenido en aminoácidos en las etapas iniciales del procesado pero no en producto final. Sin embargo, este efecto no fue manifiesto en el contenido en dipéptidos en ninguna de las etapas del proceso de maduración.

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Analyzing the *Semimembranosus* muscle by MRI for classifying Iberian hams as a function of the diet

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Effect of brine thawing/salting Iberian hams on sensory features

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INTRODUCTION

A simultaneous brine thawing/salting operation has been proposed for the processing of frozen meat products which are salted after thawing [1]. Frozen hams constitute one of the products that can be processed through the simultaneous brine thawing/salting operation as recently reported [2, 3]. The results from these studies indicated a reduction in the salting time when using the brine thawing/salting method, which was performed together with vacuum impregnation. Besides, the post-salting stage could be reduced from the 50 days in the traditional fresh raw material salting, to 25 days when using frozen hams brine thawed/salted. However, no influence of the use of vacuum impregnation during the salting stage was observed in the post-salting period. From the microbiological point of view, no significant differences were observed among the hams processed by the different treatments (pile salting, brine thawing/salting operation) [4]. Moreover, the brine thawing/salting process resulted in similar or even better sensory preferences than hams produced through the traditional method [5]. Iberian ham is a traditional dry cured product characterized by its long time of processing. Although the different stages of the processing of Iberian hams are totally established, not considering even freezing the hams, taking into account the positive conclusion of the using of the brine thawing/salting procedure in white hams, it could be considered to try this procedure in Iberian hams.
Therefore, setting the limits of new processing conditions that do not compromise the sensory quality of Iberian ham is essential, THE AIM OF THIS WORK WAS TO ANALYZE THE EFFECT OF BRINE THAWING/SALTING ON SENSORY CHARACTERISTICS OF IBERIAN HAMS

MATERIAL AND METHODS

- Eighteen Iberian hams: 6 frozen/thawed and pile salted (T); 6 brine/thawed salted in saturated brine at atmospheric pressure (B); 6 brine/thawed in saturated brine applying vacuum pulse (BV).

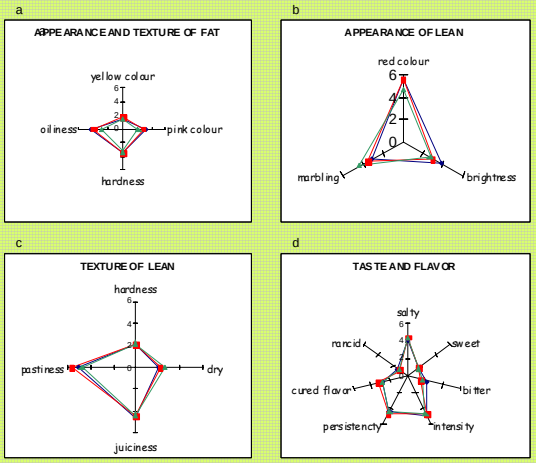
- After processing samples were obtained by dissecting the *Biceps femoris* muscle of each dry-cured ham.

- Sensory analysis: quantitative-descriptive analysis (14 panelists) and acceptability test (193 consumers).

RESULTS AND DISCUSSION

Figure 1 shows the mean scores for appearance and texture of fat and lean, taste and flavor from the T, B and SV dry-cured Iberian hams of the present study. Sensory traits related to appearance of fat and lean were significantly affected by the brine thawing/salting practice. T Iberian hams showed higher scores of fat oiliness and lean brightness and lower of marbling than B and BV ones. Besides, BV hams obtained higher scores of red color of lean than the other groups of hams. However, there were no differences in the texture of fat and lean, taste and flavor among T, B and BV dry-cure Iberian hams. Flores et al. [5] also found differences in the appearance, obtaining higher scores Iberian hams salted traditionally than those processed with both procedures, brine thawing/salting with and without vacuum pulse.
Results from the acceptability did not reflect differences among the three groups of hams of this study. Most panelists considered T, B and BV dry-cured Iberian hams to be between good and average. Nevertheless, in white hams the sensory analysis showed that the consumers preferred the brine thawed/salted hams than those processed traditionally [5].

Figure 1. Sensory analysis of dry cured Iberian hams salted traditionally in pile (◆) and brine thawed /salted without (■) and with vacuum pulse (▲). 1a., appearance and texture of fat; 1b., appearance of lean; 1c. texture of lean; 1d., taste and flavor.



CONCLUSIONS

The brine thawing/salting procedure influences fat and lean appearance of Iberian dry-cured hams, affecting fat oiliness, brightness, marbling and red color of lean, whereas there was no influence on sensory traits concerning texture, taste and flavor neither on overall acceptability.

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