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Marc Bellés Safont

Optimización de distintas estrategias para mantener la calidad de la carne de cordero desde el campo a la mesa.

Departamento
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Tesis Doctoral

OPTIMIZACIÓN DE DISTINTAS ESTRATEGIAS PARA MANTENER LA CALIDAD DE LA CARNE DE CORDERO DESDE EL CAMPO A LA MESA.

Autor

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UNIVERSIDAD DE ZARAGOZA

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FACULTAD DE VETERINARIA

DEPARTAMENTO DE PRODUCCIÓN ANIMAL Y CIENCIA DE LOS ALIMENTOS

Optimización de distintas estrategias para mantener la calidad de la carne de cordero desde el campo a la mesa

Memoria para optar al grado de doctor por la Universidad de Zaragoza presentada por:

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D. José Antonio Beltrán Gracia y Dña. Verónica Alonso Martín,

Certifican:

Que la tesis doctoral titulada “Optimización de distintas estrategias para mantener la calidad de la carne de cordero desde el campo a la mesa”, de la que es autor Marc Bellés Safont, ha sido realizada bajo su dirección, su contenido corresponde con el proyecto de tesis aprobado en su momento y cumple las condiciones requeridas para optar al grado de doctor por la Universidad de Zaragoza.

En Zaragoza, a 30 de mayo de 2018

Fdo.: Dr. José Antonio Beltrán Gracia

Fdo.: Dra. Verónica Alonso Martín

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RESUMEN

El consumo de carne de cordero está muy arraigado en los países mediterráneos, donde este alimento se considera una exquisitez. España es uno de los principales países productores de la Unión Europea, solo superado por el Reino Unido. No obstante, el consumo de este tipo de carne, y por tanto su producción, ha disminuido desde el inicio de la crisis económica, siendo necesaria la adopción de medidas que consigan revertir esta tendencia. Una de las apuestas por parte del sector ha sido la apertura al mercado exterior. De hecho, las exportaciones de carne de cordero han aumentado notablemente en los últimos años, representado en la actualidad más del 25 % del total de la carne de cordero producida en España. Por otro lado, existen consumidores dentro de nuestras fronteras que demandan productos de gran calidad y lo que es más importante, están dispuestos a asumir su coste. La mejora de la calidad del producto es una de las principales estrategias comerciales para incrementar la competitividad de las empresas, siendo especialmente importante en el caso del cordero, en el que su calidad es un factor diferencial. No obstante, la carne de cordero presenta una corta vida útil, disminuyendo su calidad prácticamente desde el momento del sacrificio. Es por ello que los esfuerzos del sector deben ir dirigidos a frenar los procesos de deterioro que sufre la carne de cordero a lo largo de la cadena de comercialización. Bajo este contexto, el objetivo de esta tesis doctoral fue optimizar distintas estrategias de conservación para mantener la calidad inicial de la carne de cordero desde el campo a la mesa.

Los estudios se realizaron con corderos pertenecientes a la raza Rasa Aragonesa, una raza local de aptitud cárnica de la que se obtienen canales de tipo ligero (8-14 kg). Los animales, criados en condiciones intensivas, se alimentaron con leche materna y, posteriormente, con concentrado y paja *ad libitum* hasta su sacrificio con aproximadamente tres meses de edad. Los corderos se sacrificaron en las instalaciones de Mercazaragoza, realizando el despiece de las canales en las instalaciones de Casa de Ganaderos y Franco y Navarro SA. Únicamente se utilizaron las piernas en los estudios de esta tesis doctoral.

Entre las estrategias de conservación de la carne aplicadas antes del sacrifico del animal destaca la suplementación de los corderos con dosis supra-nutricionales de vitamina E. La revisión realizada sobre los efectos de esta práctica en la calidad de la carne fresca puso de manifiesto la actividad dosis-dependiente del tocoferol en la inhibición de los procesos oxidativos. El incremento de la concentración de vitamina E

en el músculo mejora su estabilidad oxidativa, inhibiendo las reacciones de oxidación lipídica y proteica y retrasando la pérdida del color. Además, estas modificaciones afectan indirectamente al desarrollo del aroma de la carne de cordero. La concentración de tocoferol muscular necesaria para frenar estos procesos depende de las condiciones de conservación de la carne, influyendo todos aquellos factores capaces de modificar el equilibrio entre compuestos antioxidantes y prooxidantes. Por el contrario, la suplementación con vitamina E no tiene ningún efecto sobre los parámetros productivos ni inhibe la multiplicación de los microorganismos.

En relación a su efecto en la carne congelada, el primer estudio de esta tesis doctoral investigó la actividad de la vitamina E tras mantener la carne hasta 9 meses a -20 °C. La suplementación con 1000 mg de DL-α-tocoferol incrementó la concentración de este antioxidante en el músculo, mejorando la estabilidad oxidativa de la carne durante su almacenamiento. El tocoferol protegió a los ácidos grasos poliinsaturados de la oxidación e inhibió significativamente la formación de malondialdehído y la conversión de la mioglobina en metamioglobina. Por tanto, se demostró que la vitamina E mantiene su efecto tras la congelación de la carne. Además, este estudio sirvió para evaluar la viabilidad, desde el punto de vista de la calidad, de la comercialización de carne descongelada, envasada en atmósfera protectora y mantenida en expositores refrigerados. Los resultados de este experimento mostraron la ausencia de diferencias significativas entre la oxidación de la carne fresca y descongelada, mientras que la pérdida del color en esta última ocurrió a mayor velocidad. Las muestras descongeladas también presentaron un oscurecimiento significativo de la médula ósea. Por el contrario, la congelación-descongelación de la carne inhibió el crecimiento microbiano. En conclusión, la venta de carne descongelada bajo las condiciones de comercialización habituales sería viable desde el punto de vista de la calidad, siendo recomendable la utilización de una estrategia antioxidante para prolongar su vida útil.

Tras el sacrificio del animal, la refrigeración de las canales es uno de los procedimientos con mayor incidencia en la vida útil de la carne. La revisión de los datos existentes evidenció el efecto inhibitorio de la ultra refrigeración de las canales de cordero sobre el crecimiento microbiano. Además, esta técnica presenta ventajas logísticas al reducir a 7 horas el tiempo necesario para enfriar las mismas. Una vez finalizada la refrigeración de las canales, habitualmente se procede a su despiece y fileteado. El segundo estudio presentado en esta tesis doctoral investigó el efecto de los sistemas de corte más comunes (sierra y cizalla) en la vida útil y la aceptabilidad de las chuletas de cordero a lo largo de su conservación en condiciones comerciales. No se encontraron diferencias significativas entre la vida útil de las chuletas cortadas por sierra

o cizalla; no obstante, las primeras presentaron una mayor aceptabilidad en el análisis visual, posiblemente debido a las esquirlas que genera la cizalla al impactar en el hueso. Estas diferencias visuales podrían tener repercusión en la intención de compra de los consumidores.

El análisis de la bibliografía ha permitido evidenciar cómo la refrigeración de la carne a temperaturas justo por encima de su punto de congelación *(superchilling)* consigue prolongar significativamente la vida útil de la carne al inhibir el crecimiento microbiano y las reacciones químicas y enzimáticas. Por otro lado, las atmósferas protectoras son el sistema de envasado más extendido para la venta minorista de la carne, no obstante, las reacciones oxidativas y el crecimiento microbiano progresan rápidamente bajo estas condiciones. La utilización del envasado a vacío tipo *skin* o la adición de compuestos antimicrobianos y antioxidantes (envase activo) se vislumbran como posibles alternativas a la utilización de las atmósferas protectoras.

Precisamente, el tercer trabajo de investigación evaluó el efecto combinado del *superchilling* (refrigeración a -1 °C) con el envasado (en atmósfera protectora y a vacío tipo *skin*) sobre la calidad de la carne de cordero. La refrigeración a -1 °C redujo significativamente el crecimiento microbiano, independientemente del sistema de envasado. Sin embargo, su efecto en la oxidación lipídica y la estabilidad del color estuvo influenciado por el envasado. Mientras mejoró la estabilidad del color en las muestras envasadas a vacío tipo *skin*, promovió la oxidación lipídica y la pérdida del color de las chuletas conservadas en atmósfera protectora. Por otro lado, el envasado a vacío tipo *skin* inhibió eficazmente la oxidación lipídica tanto a 4 °C como a -1 °C. La combinación del *superchilling* con el envasado a vacío tipo *skin* consiguió duplicar la vida útil de la carne de cordero alcanzada bajo las condiciones comerciales habitualmente empleadas. En cambio, la refrigeración a temperaturas cercanas al punto de congelación de la carne se mostró desaconsejable cuando la carne se envasó en una atmósfera rica en O_2, debido a la rápida progresión de las reacciones de oxidación.

Los dos últimos estudios presentados en esta tesis doctoral analizaron los efectos de la utilización de extractos vegetales sobre la calidad de la carne de cordero. En el primero de ellos se investigó el efecto de un extracto acuoso de semillas de borraja y de un extracto de té sobre la vida útil de chuletas de cordero. Ambos extractos exhibieron un efecto dependiente de la concentración, siendo necesaria una concentración mínima de polifenoles de 2,08 mg EAG (equivalentes de ácido gálico)/100 cm^2 de carne para reducir significativamente las reacciones oxidativas. Los extractos también inhibieron la oxidación de la mioglobina, retrasando la pérdida del

color. Estos efectos se lograron sin modificar las propiedades sensoriales de la carne de cordero, siendo estos extractos imperceptibles para un panel entrenado. Finalmente, se concluyó que las concentraciones recomendables para prolongar la vida útil de las chuletas de cordero serían 0,5 % y 10 % para los extractos de té y borraja, respectivamente. Estas concentraciones consiguieron prolongar la vida útil de la carne de cordero de 8 a 11 días.

En el último estudio se determinó la capacidad de un extracto de té y del carvacrol para sustituir a los sulfitos como conservante del *burger meat*. Ambos extractos inhibieron significativamente la oxidación lipídica a una concentración de 300 ppm, sin embargo, únicamente el carvacrol consiguió retrasar la pérdida del color y el crecimiento microbiano cuando se utilizó a una concentración de 1000 ppm. Mediante este terpeno se alcanzó una vida útil similar a la obtenida mediante la utilización de 400 ppm de sulfitos, no obstante, el análisis sensorial reveló modificaciones significativas en las propiedades sensoriales de la carne de cordero.

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Introducción

1. Introducción

1.1. Situación del sector ovino

1.1.1. Situación mundial y en la Unión Europea

La producción mundial de carne ha aumentado notablemente en los últimos años, alcanzando la cifra récord de 311 millones de toneladas en 2015. Además, las previsiones para los próximos años señalan una continuidad en esta tendencia, proyectando una producción de 376 millones de toneladas para el año 2026. Este aumento también se ha constatado en el sector ovino, en el que a pesar del receso observado en el periodo comprendido desde 2007 a 2010, se ha experimentado un incremento de aproximadamente un millón de toneladas entre los años 2006 y 2016 (figura 1) (FAOSTAT, 2016). A pesar de este incremento, la aportación del ovino al sector cárnico representa solo el 2,77 % del total (FAOSTAT, 2015). No obstante, presenta una importancia destacada debido a que su producción y consumo se distribuyen por todos los continentes, aunque con importantes diferencias entre ellos (figura 2). Actualmente Asia domina la producción mundial con aproximadamente el 50 % del total, debido principalmente al volumen de China. Además, la producción en este continente sigue en crecimiento desde el año 2000, al igual que en África, que se sitúa como segundo máximo productor (19 %).

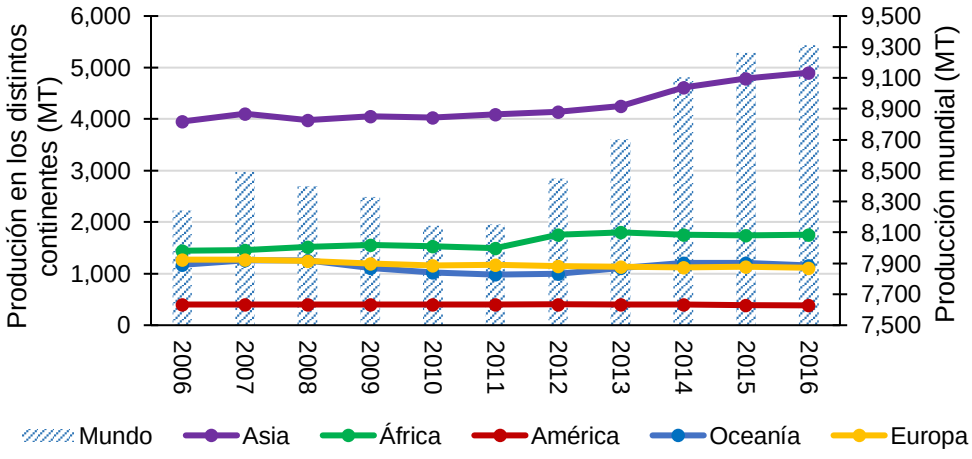

Figura 1. Evolución mundial de la carne de cordero.
(Fuente: FAOSTAT, 2006-2016; elaboración propia)

Una tendencia diferente se observa en el resto de continentes; en Europa la producción de carne de cordero se ha reducido progresivamente, representado actualmente el 12 % del total. Unas cifras similares se obtuvieron en Oceanía, cuya producción (12 %), al igual que en América (4 %), se ha mantenido constante.

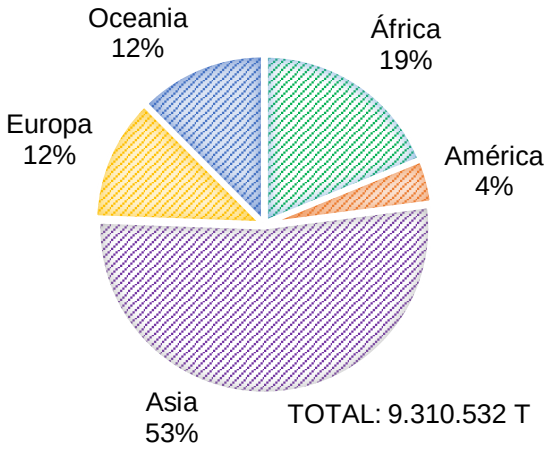

Figura 2. Producción mundial de carne de ovino en 2016.
(Fuente: FAOSTAT, 2016; elaboración propia)

En cuanto a los principales países productores, China domina la producción, cerrando 2016 con más de 2 millones de toneladas. Muy por debajo se encuentran Australia y Nueva Zelanda, que con 678.812 y 480.607 T se sitúan como los segundos y terceros productores mundiales. Otros países con una producción importante de carne de ovino son Sudán, Turquía, India y Reino Unido, siendo este último el mayor productor europeo (figura 3) (FAOSTAT, 2016).

Como se ha comentado anteriormente, la producción europea se ha reducido en la última década. Las razones de este declive son variadas, destacando los bajos ingresos de esta actividad, el envejecimiento de la mano de obra, la ausencia de mejoras en la productividad, así como el descenso del consumo interno (Garnier, 2010). Entre los países más sensibles a este descenso destacan España y la República de Irlanda, segundo y cuarto productores europeos. Hay que mencionar además la focalización de la producción en el área mediterránea, donde el conjunto formado por España, Grecia, Francia, Rumanía e Italia agrupa casi el 50 % de la producción. No obstante, el Reino Unido lidera la producción con 290.000 T en el año 2016 (figura 4) (EUROSTAT, 2016).

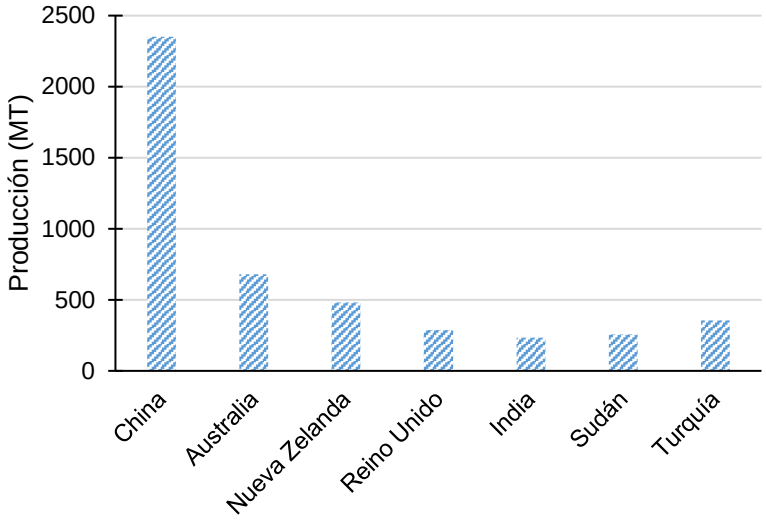

Figura 3. Principales países productores de carne de cordero en 2016.
(Fuente: FAOSTAT, 2016; elaboración propia)

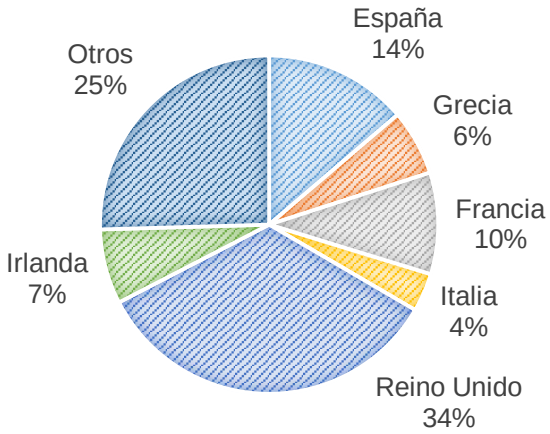

Figura 4. Producción de carne de cordero en la UE en el año 2016.
(Fuente: EUROSTAT, 2016; elaboración propia)

1.1.2. Situación nacional

La cría de ovino tiene una gran tradición en el territorio español. Esta especie ha sabido adaptarse a las zonas más desfavorecidas, donde otras especies ganaderas no han podido explotarse. Sin competir en recursos con la agricultura, el ganado ovino ha constituido una fuente importante de ingresos en las zonas rurales. Su importancia va más allá de lo económico, puesto que esta actividad tiene un destacado papel social y medioambiental al permitir fijar a las personas, evitando la despoblación, y al favorecer la conservación del paisaje con su forma tradicional de pastoreo (MAPAMA, 2008). De esta forma, con una producción cárnica de 118.261 T, España constituye una potencia

europea en esta actividad (FAOSTAT, 2015). Sin embargo, su distribución en el territorio nacional es muy heterogénea, existiendo importantes diferencias entre comunidades. Castilla y León presenta la mayor producción (25,13 % del total) seguida por Cataluña (13,98 %), Castilla la Mancha (13,24 %), Aragón (10,72 %) y Murcia (9,23 %), mientras que el resto de comunidades solo suponen un 27,7 % del total de la producción (MAPAMA, 2016).

Además de este problema, otra dificultad a la que se enfrentan productores y comercializadores es la centralización de las ventas en los últimos meses del año, así como en determinadas festividades religiosas. Por otro lado, también existen importantes variaciones en el consumo entre comunidades, lo que dificulta aún más la actividad comercial. Aragón se sitúa en la cabeza (datos del 2012) con un consumo per cápita de 4,70 kg/persona/año, seguida por Castilla y León y la Rioja con 3,54 y 3,48 kg/persona/año. En el otro extremo se encuentra Canarias con un consumo de 0,23 kg/persona/año, muy alejada del resto (MAPAMA, 2012).

A pesar de mantener todavía un volumen significativo, la producción y el consumo de carne de ovino en España se ha reducido drásticamente en los últimos años. En el periodo comprendido entre 2005 y 2015 la producción ha pasado de 224.126 T a 115.864 T, lo que supone un descenso de casi el 50 % (FAOSTAT, 2015). Esta disminución fue especialmente acusada entre 2007 y 2009, cuando se produjo una caída del 39 % respecto de la anualidad anterior (figura 5).

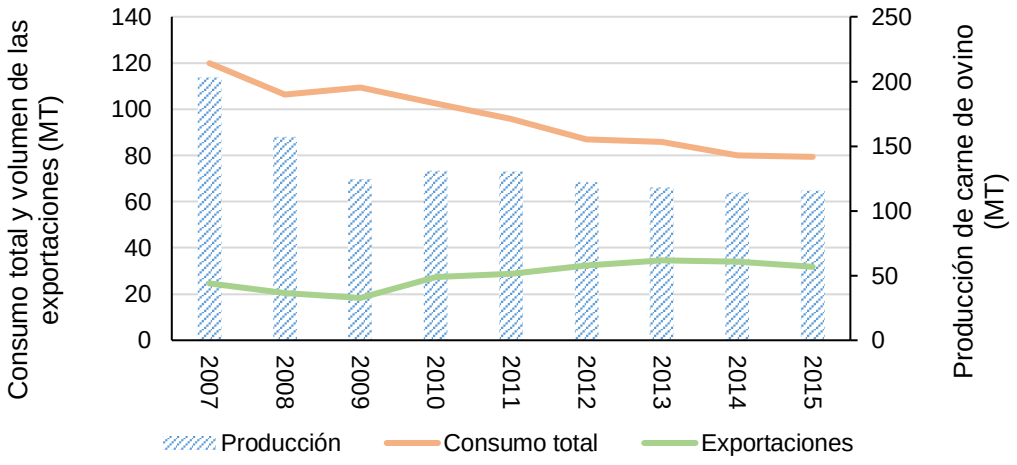

Figura 5. Evolución del sector ovino en España.
(Fuente: FAOSTAT, 2007-2015; elaboración propia)

1.2. La calidad de la carne

1.2.1. El concepto de calidad de carne

Durante casi seis décadas ha habido un intenso debate sobre la definición más adecuada del término “calidad de la carne”, habiéndose propuesto diferentes definiciones pero sin llegar a un acuerdo sobre la más adecuada. Una descripción simplista define la calidad como "lo que quieren los consumidores" (Hammond, 1952), mientras que la FAO propone que la calidad de la carne comprende tanto la composición química y los factores de palatabilidad como la apariencia visual, el olor, la firmeza, la jugosidad, la terneza y el sabor. Una definición más detallada incluye factores higiénicos, de composición, nutricionales, sensoriales y tecnológicos (Nardone y Valfré, 1998). Sin embargo, no existe ninguna duda de la gran cantidad de factores que afectan a la calidad de la carne. Sañudo, Muela y Campo (2013) clasificaron los factores que afectan a la calidad de la siguiente manera:

- Factores intrínsecos: son aquellos relacionados directamente con el animal (raza, sexo, castración y edad de sacrificio).
- Extrínsecos: afectan indirectamente al animal (sistema de producción, dieta, manejo e instalaciones).
- Pre-sacrificio y sacrificio: transporte y aturdimiento.
- Conservación: temperatura, envasado, higiene y corte.
- Cocinado y consumo: Temperatura, aditivos y forma de cocinado.

1.2.2. Disminución de la calidad de la carne tras el sacrificio

La carne de cordero es un producto altamente perecedero debido a su composición (Wulf *et al*., 1995; Zhou, Xu y Liu, 2010). La alteración de las características organolépticas de la carne empieza prácticamente desde el sacrificio del animal; tan pronto es desangrado, los mecanismos de defensa antimicrobianos y antioxidantes casi desaparecen (Bianchi y Feed, 2010). Como consecuencia de las modificaciones que sufre la carne durante su almacenamiento, se produce un descenso continuo de la calidad de la misma, hasta llegar al fin de su vida útil. Podemos definir la vida útil como el periodo de tiempo hasta la alteración del producto. Este punto final puede ser consecuencia de un nivel microbiológico inaceptable, del desarrollo de olores o sabores desagradables o por una apariencia inadecuada (Borch y Kant-Muemansb, 1996). Los principales procesos causantes del deterioro de la carne de cordero son el crecimiento microbiano, las reacciones de oxidación y la pérdida del color.

Deterioro microbiano de la carne

Como se ha comentado anteriormente, la composición química de la carne ofrece unas condiciones muy favorables para el crecimiento microbiano, siendo esta una de las principales causas del deterioro de la carne fresca. El origen de estos microorganismos puede ser interno (bacterias propias de la canal) o externo (por contaminación). En consecuencia, la calidad microbiológica de la carne depende de numerosos factores como el estado fisiológico del animal en el momento de sacrificio, las condiciones higiénicas en las que se realiza el faenado y el procesado de la carne, así como de las condiciones de conservación (Nychas, Skandamis, Tassou y Koutsoumanis, 2008).

Como se muestra en la tabla 1, se han identificado una gran cantidad de géneros microbianos en la carne durante su almacenamiento, no obstante, los investigadores asocian los procesos de deterioro a un número limitado de géneros microbianos que se presentan de forma mayoritaria bajo las condiciones habituales de conservación (Casaburi, Piombino, Nychas, Villani y Ercolini, 2015; Nychas *et al*., 2008). En condiciones aerobias, *Pseudomonas* spp. es el principal género bacteriano responsable del deterioro de la carne, siendo *Pseudomonas fragi*, *Pseudomonas fluorescens* y *Pseudomonas lundensis* las tres especies más importantes (Esmer, Irkin, Degirmencioglu y Degirmencioglu, 2011). No obstante, otros géneros son capaces de crecer en ambientes aerobios: Fernandes *et al*. (2014) y Osés *et al*. (2013) encontraron recuentos significativos de bacterias acidolácticas mientras que Sheridan *et al*. (1997) observaron un crecimiento destacable de *Brochotrix thermospacta*. En cambio, las bacterias acidolácticas suelen predominar cuando la carne se envasa en anaerobiosis, siendo *Leuconostoc* spp. y *Carnobacterium* spp. los principales géneros aislados bajo estas condiciones (Jones, Hussein, Zagorec, Brightwell y Tagg, 2008).

Durante su multiplicación los microorganismos utilizan compuestos de la carne como glucosa, ácido láctico, compuestos nitrogenados y aminoácidos libres, liberando al medio ácidos orgánicos, ácidos grasos volátiles, compuestos azufrados, cetonas, aldehídos, alcoholes, amoniaco, etc. Estos compuestos resultantes del metabolismo microbiano son los responsables de las principales alteraciones organolépticas que se relacionan con el crecimiento de los microorganismos: los olores y sabores desagradables, la formación de limosidad superficial y la pérdida del color rojo (Borch y Kant-Muemansb, 1996; Gram y Huss, 1996).

Tabla 1. Principales géneros bacterianos identificados en la carne.

Gram +	Atmósfera			Gram -	Atmósfera		
	A	MAP	V		A	MAP	V
Bacillus	+		+	*Achromobacter*	+		
Brochothrix	+	+	+	*Acinetobacter*	+	+	+
Carnobacterium	+	+	+	*Aeromonas*	+		+
Corynebacterium	+			*Alcaligenes*	+	+	+
Clostridium			+	*Alteromonas*	+	+	+
Enterococcus	+	+		*Campylobacter*	+		
Kocuria	+			*Chromobacterium*	+		
Kurthia	+			*Citrobacter*	+	+	
Lactobacillus	+	+	+	*Enterobacter*	+	+	
Lactococcus	+			*Escherichia*	+		
Leuconostoc	+	+	+	*Flavobacterium*	+		
Listeria	+	+		*Hafnia*	+	+	+
Microbacterium	+	+	+	*Klebsiella*	+		
Micrococcus	+	+		*Vibrio*	+		
Paenibacillus	+			*Moraxella*	+		
Staphylococcus	+	+	+	*Proteus*	+	+	
Streptococcus	+	+		*Pseudomonas*	+	+	+
Weisella	+	+	+	*Serratia*	+	+	+
				Shewanella	+		
				Yersinia	+		+

MAP: Atmósfera protectora, A: Envasado en aíre, V: Envasado a vacío. Fuente: Casaburi *et al.* (2015)

Las reacciones de oxidación

La oxidación es un proceso bioquímico que implica la transferencia de átomos de oxígeno, hidrógeno o de electrones entre dos compuestos manteniendo una carga final neta. En biología, el compuesto donante se llama prooxidante mientras que el aceptor es el antioxidante (Morrissey, Sheehy, Galvin, Kerry y Buckley, 1998). En la carne fresca se producen principalmente dos tipos de reacciones de oxidación, la autooxidación de los lípidos y la oxidación de las proteínas (Gray, Gomaa y Buckley, 1996; Zhang, Xiao y Ahn, 2013).

La oxidación de los lípidos arranca en la membrana fosfolipídica de las células musculares, dando lugar a una serie de reacciones en cadena comúnmente divididas en tres etapas: iniciación, propagación y terminación (Cheng, 2016) (figura 6). Para comenzar este proceso se necesita un iniciador, un papel frecuentemente desempeñado por la luz, metales de transición o especies reactivas de oxígeno (ROS). Estos compuestos reactivos atacan un ácido graso, le arrebatan un átomo de hidrógeno y como consecuencia, se forma un radical lipídico. Este último radical reacciona con el

oxígeno para formar un radical peroxilo (ROO·) que, en una etapa posterior, ataca de nuevo a un ácido graso. A causa de esta última reacción se generan dos compuestos diferentes: un radical alquilo y un hidroperóxido. Llegados a este punto, el proceso se propaga a través de una cascada de reacciones en la que cada nueva molécula que se genera acelera la velocidad del proceso (propagación). La única vía para detener estas reacciones es la generación de compuestos no reactivos. Esta fase, llamada terminación, aparece cuando la concentración de radicales es lo suficientemente alta como para que reaccionen entre sí, generando compuestos no reactivos (Blanksby y Ellison, 2003).

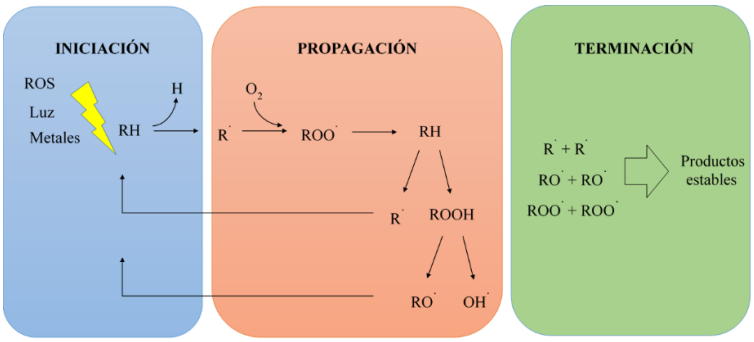

Figura 6. Etapas en la autooxidación de los lípidos.
(Elaboración propia)

Respecto a la oxidación de las proteínas, esta puede tener lugar por distintas vías. Las ROS pueden atacar múltiples sitios de la cadena de aminoácidos, originando modificaciones en la estructura proteica. Por otro lado, los productos secundarios de la oxidación lipídica también pueden interaccionar con los aminoácidos de las proteínas modificando su estructura. Los cambios en la estructura proteica comprometen su funcionalidad y en consecuencia, causan modificaciones importantes en la calidad de la carne (Faustman, Sun, Mancini y Suman, 2010; Zhang *et al*., 2013).

Los procesos oxidativos en la carne dependen tanto del equilibrio entre la concentración de compuestos prooxidantes y antioxidantes como de las interacciones entre estos (Rock, Fada, Jacob y Bowen, 1996). Son muchos los factores que pueden alterar este equilibrio, no obstante, la composición de la carne es una de las principales variables que influyen en la susceptibilidad de esta a oxidarse. El porcentaje de grasa intramuscular es un factor crítico debido a que su incremento favorece el desarrollo de los procesos oxidativos (Estévez, Ventanas y Cava, 2005; Park *et al*., 2008). Respecto al tipo de ácido graso, los ácidos grasos poliinsaturados son más susceptibles a oxidarse (Díaz *et al*., 2011; Wood *et al*., 2003). En el caso de la carne roja, su elevado contenido

en hierro favorece el desarrollo de las reacciones de oxidación, ya que este actúa como catalizador (Tang, Kerry, Sheehan, Buckley y Morrissey, 2001). Por otro lado, la presencia de antioxidantes naturales en el músculo, como la vitamina E o los compuestos fenólicos, previene la oxidación de los lípidos y las proteínas. Las diferencias en la susceptibilidad de los distintos músculos a oxidarse se relacionan con el tipo de fibras que predomina en cada uno de ellos. Los músculos con mayor contenido en fibras musculares de tipo I presentan un contenido más elevado de mioglobina y un mayor consumo de oxígeno, lo que favorece la oxidación de la mioglobina y la pérdida de color (Jeong, Kim, Yang y Joo, 2011). Otro factor intrínseco como el pH de la carne ejerce una notable influencia en los procesos oxidativos. Cuando el pH es bajo, el grupo hemo de la mioglobina está más expuesto a las condiciones ambientales, comprometiendo su estabilidad. Además, estas condiciones favorecen la protonación del oxígeno unido a la mioglobina, estimulando la formación del anión superóxido (Yin y Faustman, 1993).

Entre los factores extrínsecos con influencia en los procesos oxidativos destacan la luz, la temperatura y la composición de la atmósfera. La luz puede excitar tanto los ácidos grasos como el oxígeno, favoreciendo de ambas formas la formación de hidroperóxidos. Su capacidad de actuar como prooxidante depende tanto de su longitud de onda como de su intensidad. De entre los sistemas utilizados habitualmente para iluminar la carne los fluorescentes son más oxidantes que la luz incandescente, mientras que los nuevos sistemas de iluminación LED parecen reducir la pérdida de color, aunque podrían favorecer ligeramente la oxidación lipídica (Cooper *et al*., 2016; Martínez, Cilla, Beltrán y Roncalés, 2007; Steele *et al*., 2016).

El efecto de la temperatura en la cinética de las reacciones químicas es ampliamente conocido. De acuerdo con la regla VRT (velocidad de reacción - temperatura), un descenso térmico de unos 10 ºC reduce la velocidad de las reacciones a la mitad o un tercio del valor inicial (Jasper y Plazek, 1994). En el caso de la conservación de la carne, la refrigeración tiene un efecto protector sobre los procesos oxidativos, que se acentúa cuando la carne se mantiene en congelación (Jakobsen y Bertelsen, 2000; Yin y Faustman, 1993).

Con relación al efecto de la atmósfera que rodea al producto, el oxígeno juega un papel clave. De hecho, los procesos oxidativos comúnmente arrancan debido a la acción de las ROS (Blanksby y Ellison, 2003; Zhang *et al*., 2013). Se ha demostrado que la velocidad de oxidación de los lípidos aumenta con la concentración de oxígeno de la atmósfera (Clausen, Jakobsen, Ertbjerg y Madsen, 2009; Jakobsen y Bertelsen,

2000). El dióxido de carbono también puede actuar como prooxidante cuando se encuentra a concentraciones superiores al 30 %, principalmente debido al descenso del pH de la carne por la formación de ácido carbónico (Sorheim, Erlandsen, Nissen, Lea y Haryem, 1997). Por otro lado, no se ha observado ningún efecto prooxidante del monóxido de carbono, sin embargo la formación de carboximioglobina previene la oxidación de este pigmento (Martínez *et al*., 2005). En cambio, otros gases como el nitrógeno o el argón no parecen ejercer ningún efecto sobre los procesos oxidativos (Bellés, Alonso, Roncalés y Beltrán, 2017c).

El impacto de los procesos oxidativos en la calidad de la carne se produce a tres niveles: sensorial, nutricional y de seguridad alimentaria. Los hidroperóxidos son compuestos inodoros e insípidos, no obstante, tienden a descomponerse en cientos de compuestos volátiles y no volátiles que modifican las propiedades sensoriales de la carne. De entre todos ellos, los aldehídos son los máximos responsables del desarrollo de la rancidez en la carne, ya que son abundantes en la carne oxidada y presentan unos umbrales de detección muy bajos. Otros compuestos como las cetonas, los alcoholes o los furanos también tienen un papel importante en la modificación de las propiedades sensoriales de la carne. La acumulación de estos compuestos intensifica la rancidez, a la vez que dificulta la percepción de los sabores y olores característicos de la carne (Resconi, Escudero y Campo, 2013).

De entre los distintos tipos de ácidos grasos, los poliinsaturados son los más susceptibles a los procesos oxidativos, especialmente los n-3 (Mottram, 1998). La oxidación de este tipo de ácidos grasos supone una disminución de su contenido y con ello, una pérdida del valor nutricional (Wood *et al*., 2003, 2008). Además, los radicales libres y los hidroperóxidos favorecen la degradación de Strecker de los aminoácidos en aldehídos, α-cetoácidos y aminas, reduciendo de esta forma su biodisponibilidad (Hidalgo y Zamora, 2016). Otros micronutrientes como las vitaminas A, C y E y los flavonoides podrían también verse reducidos como consecuencia de los procesos oxidativos.

Por otro lado, recientemente se ha señalado que los compuestos resultantes de la oxidación de la carne podrían poseer citotoxicidad y genotoxicidad, habiéndose relacionado el consumo frecuente de carne oxidada con enfermedades inflamatorias, cáncer, arterioesclerosis, etc. (Vieira, Zhang y Decker, 2017).

La pérdida del color

El color es una de las propiedades sensoriales más importantes de la carne de cordero, ya que tiene un enorme impacto en la decisión de los consumidores en el momento de la compra. De hecho, el color rojo brillante se considera tradicionalmente como un aspecto positivo, ya que se asocia con frescura y un producto de calidad superior (Berruga, Vergara y Gallego, 2005).

El color de la carne depende principalmente del contenido de su principal pigmento, la mioglobina, su estado químico y las propiedades de dispersión de la luz en la carne. La mioglobina es una proteína sarcoplásmica que contiene un grupo hemo (anillo porfirínico con un átomo de hierro en el centro). En condiciones biológicas en el músculo y en la carne fresca, el hierro se encuentra en la mioglobina en forma de ion ferroso. Este puede tener asociada una molécula de oxígeno, formando entonces la denominada oximioglobina, de color rojo brillante. Mientras que en ausencia de oxígeno se encuentra en forma de desoximioglobina, mostrando un color rojo púrpura más intenso y oscuro que el de la oximioglobina (estas dos formas son intercambiables, ver figura 7). La oxidación del hierro a Fe^{3+} supone la conversión del pigmento en metamioglobina, y con ello el paso de un color rojo deseable a un color marrón o pardo que es rechazado por los consumidores (Bianchi y Feed, 2010).

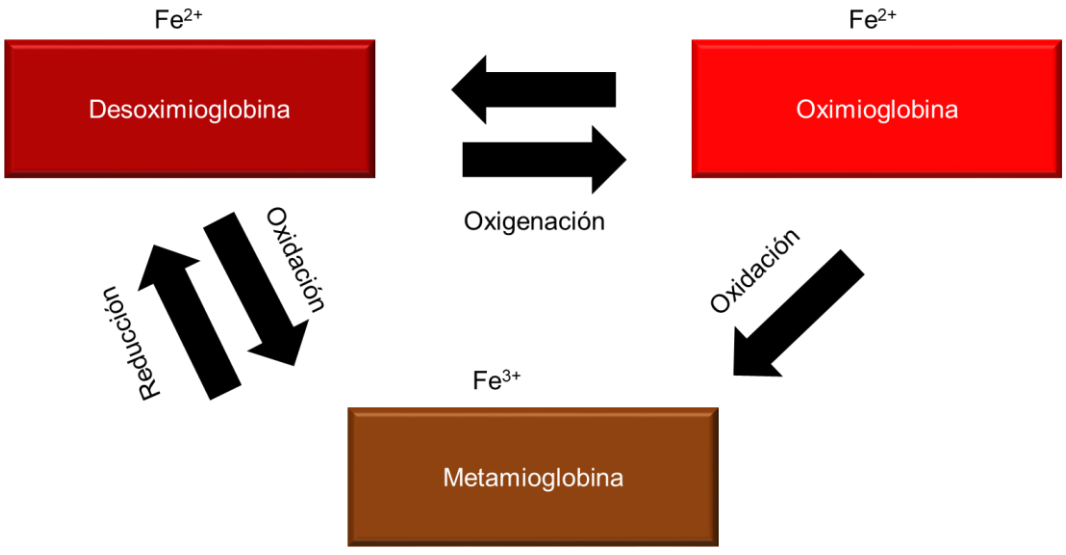

Figura 7. Estados de la mioglobina
(Elaboración propia)

Es por ello que los sistemas de conservación actuales para la venta minorista tratan de potenciar la oxigenación de la mioglobina con el fin de otorgar a la carne un color rojo brillante muy atractivo para los consumidores. El envasado en atmósfera modificada permite alcanzar este objetivo mediante la inclusión de un gran porcentaje de oxígeno en su composición (Bellés *et al*., 2017a). Se ha demostrado que la presencia de altas concentraciones de oxígeno en la atmósfera del envase resulta en un color óptimo al promover la formación de oximioglobina y además, favorece su estabilidad (Jeremiah, 2001). Por estas razones, las atmósferas empleadas para el envasado de la carne de cordero contienen en torno a un 70-80 % de oxígeno (Bellés *et al*., 2017a). En cambio, otros sistemas como el envasado a vacío tratan de eliminar el oxígeno del interior del envase, lo que favorece la formación de desoximioglobina, mostrando la carne un color rojo púrpura menos atractivo para el consumidor. Por otro lado, la adición de un pequeño porcentaje de monóxido de carbono promueve la formación de carboximioglobina, un estado de la mioglobina en el que la carne muestra un color rojo muy atractivo y a la vez muy estable (Martínez *et al*., 2005). No obstante, su utilización comercial no está permitida en un gran número de países.

La pérdida del color deseable de la carne es simultánea a la conversión de la mioglobina en metamioglobina. De acuerdo con Djenane, Sánchez-Escalante, Beltrán y Roncalés (2002), los consumidores rechazan la carne de vacuno cuando el porcentaje de metamioglobina supera el 40 %. Esta trasformación gradual de la mioglobina en metamioglobina se ve favorecida por distintos procesos. En primer lugar, cabe destacar la oxidación lipídica. Es bien conocido que la oxidación lipídica y proteica están interrelacionadas, de forma que los productos de ambos procesos se estimulan y retroalimentan (Faustman *et al*., 2010). Por tanto, aquellas condiciones que favorezcan la oxidación de los lípidos terminarán estimulando indirectamente la oxidación de la mioglobina y en consecuencia, la pérdida del color. El crecimiento microbiano también puede favorecer la acumulación de metamioglobina. Abdallah, Marchello y Ahmad (1999) propusieron que el metabolismo microbiano podría reducir la disponibilidad de oxígeno en la superficie de la carne, estimulando de esta forma la conversión de la mioglobina en metamioglobina. Además, determinados géneros bacterianos producen sulfuro de hidrógeno durante su multiplicación, el cual convierte el pigmento en sulfomioglobina, otorgando a la carne un color verdoso (Egan, Shay y Rogers, 1989).

1.3. Estrategias de conservación de la carne

La elección de un adecuado método de conservación es fundamental para prolongar la vida útil de un producto altamente perecedero como es el cordero. Existe un amplio abanico de métodos para conservar la carne, no obstante, debido a la creciente demanda de productos frescos, libres de conservantes químicos sintéticos y al mismo tiempo seguros y estables, muchas opciones quedan descartadas. Resulta complicado conseguir estos objetivos mediante un único método de conservación. Por ello, se necesita un nuevo enfoque basado en la combinación de procesos. La sinergia que se consigue al combinar distintos métodos de conservación está ampliamente descrita en la bibliografía (Leistner y Gorris, 1995) y constituye la base bajo la que se plantean las distintas estrategias para conservar la carne presentadas en esta tesis doctoral. Aunque todas las combinaciones deben ir enfocadas a mantener la calidad de la carne desde el campo a la mesa, para favorecer su estudio se han dividido en dos grandes bloques en función de su momento de aplicación: las estrategias productivas y las tecnológicas.

1.3.1. Estrategias productivas

Con el término de estrategias productivas nos referimos a todas aquellas que se aplican antes del sacrificio del animal. Aunque son muy numerosas, esta tesis doctoral se ha centrado en el estudio de la suplementación de la dieta de los corderos con vitamina E.

Suplementación con vitamina E

La vitamina E es el principal antioxidante liposoluble en las células de los mamíferos. Esta se sitúa entre los ácidos grasos de la membrana fosfolipídica, donde bloquea las reacciones oxidativas en la etapa de iniciación (Rigotti, 2007). Bajo el término “vitamina E” se agrupan una serie de compuestos con una actividad antioxidante similar (Jensen y Lauridsen, 2007). Esta acción se debe a su grupo hidroxilo, que es capaz de trasferir un átomo de hidrógeno a un radical peroxilo, convirtiéndolo de esta manera en un hidroperóxido. Por otro lado, la vitamina E se convierte en un radical con una actividad muy baja que puede volver a ser funcional si es reducido por algún otro antioxidante como la vitamina C. Mediante este mecanismo, la vitamina E es capaz de inactivar un gran número de especies reactivas como el oxígeno singlete, radicales alcoxi, peroxinitrito, dióxido de nitrógeno, ozono y el radical superóxido (Eitenmiller y Lee, 2004).

Como se ha comentado anteriormente, las reacciones oxidativas dependen del equilibrio entre los compuestos prooxidantes y antioxidantes, es por ello que el incremento de la concentración de vitamina E en el músculo favorece su estabilidad frente a la oxidación (González-Calvo, Ripoll, Molino, Calvo y Joy, 2015; Kasapidou *et al*., 2012). Se ha demostrado que la administración de dosis supra-nutricionales de vitamina E antes del sacrificio estimula su deposición en el músculo, transfiriendo posteriormente sus propiedades antioxidantes a la carne (Bellés *et al*., 2018; De la Fuente *et al*., 2007; Jose, Jacob, Pethick y Gardner, 2016; Lauzurica *et al*., 2005; Muela, Alonso, Campo, Sañudo y Beltrán, 2014).

Los efectos descritos de la suplementación de los corderos con vitamina E en la calidad de la carne incluyen una disminución de las reacciones oxidativas, una mayor estabilidad del color, una mejora de las propiedades sensoriales y como consecuencia, una prolongación de su vida útil (Álvarez *et al*., 2008; Bellés *et al*., 2018; De la Fuente *et al*., 2007; Kasapidou *et al*., 2012; Lauzurica *et al*., 2005; Muela *et al*., 2014).

1.3.2. Estrategias tecnológicas

Bajo esta denominación se agrupan todos aquellos métodos que tratan de mantener la calidad de la carne desde el sacrificio del animal hasta su consumo.

Conservación por el descenso de la temperatura

La aplicación de frío es el procedimiento utilizado tradicionalmente para la conservación de la carne. Se trata de uno de los primeros métodos de conservación de los alimentos desarrollados por la humanidad. Muchos siglos atrás ya se utilizaba el hielo natural para conservar alimentos, almacenándolos en grutas u hoyos incluso en los meses de verano. No obstante, fue el desarrollo de las máquinas frigoríficas en el siglo XIX el momento en el que se impuso este método de conservación, dando un gran impulso al crecimiento de la industria cárnica (Jasper y Plazek, 1994). La gran ventaja de este método de conservación respecto a otros (salazón, desecación, calentamiento y ahumado) es la mejor conservación del estado fresco original de la carne (Jasper y Plazek, 1994).

A medida que desciende la temperatura se enlentece el crecimiento microbiano, así como las reacciones químicas y enzimáticas (Carballo y Jiménez-Colomero, 2001). Según este principio, el proceso de alteración de los productos conservados por el frío se reduce ostensiblemente a medida que desciende la temperatura (Jasper y Plazek, 1994). En consonancia, los métodos de conservación por el frío se han

clasificado según su intensidad en los siguientes: refrigeración, *superchilling* y congelación.

Refrigeración

Generalmente la refrigeración se define como el proceso que abarca tanto la operación de hacer descender la temperatura de las canales hasta el nivel óptimo de conservación, como el mantener esta temperatura, junto con otros parámetros, en unos niveles que consigan aumentar al máximo su vida útil, siempre sin inducir la formación de cristales de hielo en su estructura (Carballo y Jiménez-Colomero, 2001). La refrigeración permite mantener la calidad del producto pero no mejorarla, por ello, es fundamental para lograr el mayor efecto posible seguir tres reglas básicas conocidas como "Trípode frigorífico de Monvoisín", cuyas premisas son: aplicarlo a carne poco contaminada, aplicarlo precozmente y continuar su aplicación sin interrupción durante toda la vida útil del producto (Moreno, 2006). En consecuencia, el mantenimiento de una adecuada cadena de frío es fundamental para asegurar la calidad higiénica, organoléptica y la inocuidad del producto.

La refrigeración es el método más empleado para la conservación de la carne fresca de cordero. Habitualmente las temperaturas de refrigeración oscilan entre 0 y 4 °C, en función de la naturaleza de la carne y el tiempo de conservación previsto (Bellés *et al*., 2017a). Diversos autores han señalado la temperatura de 4 °C como el límite máximo para la refrigeración de productos cárnicos, siendo recomendable la utilización de temperaturas más bajas (Laguerre, Derens y Palagos, 2002). La aplicación de estas temperaturas persigue enlentecer las reacciones químicas, enzimáticas y el crecimiento microbiano. No obstante, la refrigeración no solo selecciona el número de microorganismos, sino también el tipo predominante, favoreciendo el crecimiento de los psicrótrofos (*Pseudomonas* spp. en aerobiosis y *Lactobacillus* en anaerobiosis, principalmente). Estos microorganismos son capaces de multiplicarse a las temperaturas de refrigeración, siendo los responsables de las alteraciones microbianas de la carne (limosidad superficial, olores desagradables, decoloración o pardeamiento) que ocasionan el fin de su vida útil (Nychas *et al*., 2008). Es por ello que la carne refrigerada presenta una corta vida útil, siendo este método inapropiado cuando se requieren tiempos de almacenamiento y distribución más prolongados.

Superchilling

La tecnología conocida como *superchilling* o *deep chilling* se ha desarrollado durante los últimos 15 años. Este método consiste en la conservación de la carne a

temperaturas comprendidas entre -0,5 y -2,8 °C, justo por encima de su punto de congelación (Beaufort, Cardinal, Le-Bail y Midelet-Bourdin, 2009).

El mantenimiento del producto a estas temperaturas persigue una doble finalidad, por un lado se busca obtener los beneficios de la conservación a bajas temperaturas mientras que por otro lado, se pretende convertir una cierta cantidad de agua en hielo, disminuyendo de este modo su disponibilidad para los procesos de deterioro. No obstante, la formación de hielo debe limitarse a la superficie, puesto que una formación de cristales de hielo en el interior del producto podría alterar gravemente la calidad del mismo (Kaale y Eikevik, 2014). La formación de cristales de hielo está altamente relacionada con la temperatura aplicada y la naturaleza del producto. Un descenso lento de la temperatura unido a oscilaciones térmicas durante el periodo de conservación incrementa el número y el tamaño de los cristales, ejerciendo un impacto negativo en la calidad final del producto (Kaale y Eikevik, 2014). Es por tanto primordial definir la intensidad del tratamiento, es decir, las combinaciones de tiempo y temperatura apropiadas para lograr una vida útil suficientemente larga sin suponer un descenso en la calidad del producto ni un incremento excesivo de los costes. Esta optimización debe hacerse para cada producto, puesto que los alimentos son una matriz compleja donde las diferentes cantidades de agua, proteínas, carbohidratos y grasas ofrecen distintas velocidades en la eliminación del calor así como puntos de congelación diferentes (Magnussen, Haugland, Torstveit Hemmingsen, Johansen y Nordtvedt, 2008).

El principal efecto del *superchilling* en la conservación de la carne es la gran inhibición que ejerce en el crecimiento microbiano. El descenso rápido de la temperatura en la superficie del producto detiene inmediatamente el crecimiento microbiano, mientras que su mantenimiento a bajas temperaturas ralentiza la multiplicación de los microorganismos durante el almacenamiento (Lan, Shang, Song y Dong, 2016; Zhang, Wang, Li, Wu y Xu, 2016). Como consecuencia de la inhibición del crecimiento microbiano, diversos autores han descrito un incremento de la vida útil de entre 1,4 y 4 veces superior a la obtenida bajo la refrigeración convencional (Magnussen *et al*., 2008).

Además de las ventajas directas en la conservación del producto, esta tecnología presenta numerosos beneficios en el procesado, almacenamiento, transporte y distribución de la carne (Magnussen *et al*., 2008). El descenso de la temperatura de la carne hasta la temperatura de refrigeración es tradicionalmente una de las etapas del procesado que genera mayores complicaciones en la industria. Esta operación requiere un tiempo prolongado cuando se realiza a la temperatura de refrigeración convencional, lo que demanda unas instalaciones de gran volumen o supone un cuello de botella en

el faenado. La aplicación del *superchilling* consigue reducir eficazmente el tiempo requerido en esta etapa, siendo necesario un menor espacio y ofreciendo una mayor velocidad de procesado (Magnussen *et al*., 2008). Igualmente, el mantenimiento de la carne a temperaturas cercanas al punto de congelación podría facilitar diversas operaciones de procesado posteriores. Una de las operaciones más críticas en el faenado de la carne es el fileteado, siendo la temperatura del producto un punto clave en el desarrollo da la misma. La carne conservada a temperaturas cercanas a 4 °C carece de la rigidez necesaria para llevar a cabo esta operación, por lo que previamente a filetear la carne se le da un "golpe de frío", es decir, un descenso rápido de la temperatura superficial con objeto de aumentar la rigidez del producto. La carne refrigerada en *superchilling* presenta ya una rigidez óptima para ser fileteada, eliminando por tanto una etapa en el procesado de la carne (Magnussen *et al*., 2008). Como se ha comentado anteriormente, uno de los objetivos de aplicar temperaturas cercanas al punto de congelación es la formación de una delgada capa de hielo superficial. Esta capa de hielo puede ayudar a compensar pequeñas variaciones en la cadena del frío, ya que reduciría las variaciones de temperatura en el interior del producto durante pequeñas oscilaciones térmicas (Magnussen *et al*., 2008).

Por otro lado, esta tecnología podría suplir eficazmente la congelación cuando se requieren tiempos de vida útil intermedios, como por ejemplo en empresas con distribución nacional o en productos con una ligera estacionalidad en el consumo. La aplicación del *superchilling* en lugar de la congelación supondría un ahorro de energía muy importante, además de una menor alteración en la calidad del producto. No hay que olvidar tampoco la mayor aceptación de la carne fresca por parte del consumidor en detrimento de la congelada, lo que tendría también una incidencia en el precio final de venta (Lagerstedt, Enfält, Johansson y Lundström, 2008).

A pesar de los múltiples beneficios del *superchilling* tanto en la conservación de la carne como en su procesado, recientemente se han descrito una serie de alteraciones derivadas de su utilización cuyos efectos en la calidad final de la carne aún no están bien definidos. Lan *et al*. (2016) observaron diferencias en la estructura muscular de carne de conejo refrigerada a -2 y 4°C. Estas consistieron en la aparición de roturas en las fibras musculares así como la aparición de espacios entre ellas en las muestras mantenidas en *superchilling*, mientras que la carne refrigerada a la temperatura convencional mantuvo la integridad muscular intacta. Estas alteraciones se han asociado a la formación de cristales de hielo y podrían repercutir directamente en la calidad de la carne.

Esta tecnología está ampliamente estudiada para la conservación de pescado, donde ha mostrado unos resultados muy prometedores (Duran-Montgé, Permanyer y Belletti, 2015; Kaale y Eikevik, 2014). No obstante, los estudios acerca de sus efectos en la calidad y la vida útil de la carne son escasos, siendo necesaria una mayor descripción de sus efectos para la aplicación a nivel industrial.

Congelación

La utilización de la congelación para conservar la carne se remonta a muchos años atrás, pudiéndose encontrar referencias de la aplicación de este método a nivel industrial desde finales del siglo XIX. La congelación consiste en el descenso de la temperatura de un alimento hasta conseguir que la mayor parte del agua de su composición esté en forma de hielo (Bejarano, 2001). De acuerdo con la FAO, un producto puede considerarse completamente congelado cuando la temperatura en el centro del mismo es igual o inferior a -12 °C, no obstante, las temperaturas habitualmente empleadas para el mantenimiento de la carne en congelación son algo menores, estando comprendidas entre -18 y -30 °C.

El efecto protector de la congelación se fundamenta en dos acciones: la reducción de la velocidad de las reacciones químicas y microbianas, debido al descenso de la temperatura, y la disminución de la actividad de agua (a_w). Mientras que la a_w en la carne fresca se encuentra comprendida entre 1 y 0,98, en la carne congelada (-20 °C) es de 0,82. Este descenso no solo disminuye la velocidad de las reacciones químicas y enzimáticas, sino que además constituye una limitación para el crecimiento microbiano (Bejarano, 2001). Adicionalmente se han descrito diversos mecanismos mediante los cuales la congelación ejercería daño a los microorganismos. Esta tecnología podría dañar a las células microbianas a través la formación y el aumento del tamaño de los cristales de hielo, así como debido a la recristalización durante el proceso de descongelación. Por otro lado, la deshidratación de las células, causada por la concentración de solutos en la carne, podría suponer también un grave inconveniente para la viabilidad de los microorganismos (Archer, 2004). No obstante, estas últimas teorías generan controversia, y existe un intenso debate acerca del efecto real de la congelación en las células microbianas. Leygonie, Britz y Hoffman (2012) defienden que ni la congelación ni la descongelación dañarían a las células bacterianas, que permanecerían latentes durante el almacenamiento de la carne en congelación. Posteriormente, los microorganismos podrían multiplicarse con normalidad tras incrementarse la temperatura. En cualquier caso, la congelación consigue reducir en gran medida los procesos alterantes de la carne, lo que posibilita que esta pueda conservarse durante periodos muy prolongados sin comprometer su vida útil comercial

(Bellés *et al*., 2018; Muela, Monge, Sañudo, Campo y Beltrán, 2015; Muela, Sañudo, Campo, Medel y Beltrán, 2010, 2012).

Por tanto, no es de extrañar que la congelación sea el método elegido para la conservación de la carne durante periodos prolongados de tiempo (Pietrasik y Janz, 2009), ya que ofrece una gran flexibilidad tanto para los productores como para los comercializadores (Wheeler, Miller, Savell y Cross, 1990). Además, la congelación permite estabilizar la oferta (Pietrasik y Janz, 2009) y de esta forma estabilizar la variabilidad estacional, lo cual es una ventaja para un producto como la carne de ovino que, como se ha comentado en apartados anteriores, presenta una enorme variabilidad en el consumo a lo largo del año.

No obstante, durante el mantenimiento en congelación la carne experimenta diversas modificaciones que podrían llegar a comprometer su calidad:

- Aumento de las pérdidas por exudación (Añón y Calvelo, 1980; Ngapo, Babare, Reynolds y Mawson, 1999).
- Disminución del color rojo de la carne (Farouk y Swan, 1998; Lanari, Schaefer, Cassens y Scheller, 1995; Lanari y Zaritzky, 1991; Leygonie, Britz y Hoffman, 2011; Otremba, Dikeman y Boyle, 1999).
- Oxidación lipídica y proteica (Akamittah, Brekke y Schanus, 1990; Benjakul y Bauer, 2001; Hansen *et al*., 2004; Vieira, Díaz, Martínez y García-Cachan, 2009).

La intensidad de estas alteraciones depende tanto del procedimiento empleado para descender la temperatura del producto hasta su congelación como de las condiciones durante el almacenamiento. Durante la primera etapa el aspecto clave es la velocidad de congelación. Mediante una congelación rápida se consigue disminuir el número y el tamaño de los cristales de hielo, con lo que se minimiza la rotura de las células (Ballin y Lametsch, 2005). En cuanto al mantenimiento en congelación, es muy importante reducir al máximo las oscilaciones de la temperatura con tal de evitar los fenómenos de recristalización (Jasper y Plazek, 1994).

Conservación mediante el envasado

De forma general se define el envasado como la operación destinada a proteger el producto mediante el empleo de una envoltura o recipiente denominado envase. El envasado de los productos ofrece numerosos beneficios respecto a un producto no envasado, entre las que destacan los siguientes (McMillin, 2008; Yam, Takhistov y Miltz, 2005; Zhou *et al*., 2010):

- Protege al producto de los procesos de deterioro.
- Contiene el producto y constituye una barrera frente a la contaminación externa.
- Identifica al producto y permite su etiquetado.
- Facilita el transporte y el almacenamiento.
- Constituye una herramienta de *marketing*.

Durante las últimas décadas se ha producido un gran cambio en los hábitos de los consumidores. Los sistemas tradicionales de venta de carne al corte en pequeños comercios han sido poco a poco sustituidos por la venta de carne envasada y mantenida en grandes lineales refrigerados en los supermercados. Además, la globalización de la economía ha estimulado la aparición del comercio internacional, y con ello el desarrollo de empresas con producción centralizada pero con un sistema de comercialización universal (Gill, 1995). Con objeto de dar respuesta a los nuevos requerimientos de los consumidores y de los productores, los sistemas de envasado han evolucionado enormemente, en gran medida gracias a los nuevos materiales desarrollados por la industria química (Brody, Bugusu, Han, Sand y McHugh, 2008).

En la actualidad, el plástico es el material más empleado para el envasado de la carne. Los materiales plásticos presentan una elevada resistencia a la tracción, al desgarro y al impacto, unas buenas propiedades ópticas (transparencia), pueden ser termoformados y termosellados y presentan una permeabilidad al agua y a los gases variable (Jenkins y Harrington, 1991). Los polímeros comúnmente empleados en el envasado de los alimentos son el polietileno de baja densidad, el polietileno de alta densidad, el polipropileno, el politetrafluoroetileno y la poliamida, así como los polímeros de barrera EVOH y PVDC. No obstante, la utilización de una lámina de un solo material no reúne todas las propiedades necesarias para poder utilizarse en el envasado de la carne, es por ello que suelen emplearse combinaciones de estos materiales para aunar las diferentes propiedades de estos, siendo los materiales multicapa los más frecuentes (Jenkins y Harrington, 1991). El desarrollo en los materiales de envasado ha permitido la evolución del envasado desde un simple *film* plástico hasta los complejos envases activos. Actualmente los sistemas de envasado más empleados en la conservación de la carne de cordero son las atmósferas protectoras, el envasado a vacío y los envases activos (Bellés *et al*., 2017a).

Envasado en atmósfera protectora

El envasado en atmósfera protectora consiste en la sustitución de la atmósfera que rodea al producto por otra conveniente y el posterior sellado del envase mediante

materiales impermeables (McMillin, 2008). Aunque se realizaron estudios con anterioridad, el gran desarrollo de las atmósferas protectoras se produjo a partir de 1974, cuando una empresa francesa empezó a comercializar carne fresca envasada en atmósferas modificadas. A partir de este momento creció el interés por este sistema de envasado, siendo actualmente el más empleado para la venta directa de carne fresca (Bejarano, 2001).

Debido a las características del sistema actual de comercialización, el consumidor solo puede efectuar una evaluación visual del producto y por tanto, el color del mismo determina su decisión de compra (Jeremiah, 2001). Como se ha comentado anteriormente, los consumidores asocian el color rojo brillante del músculo con una mayor frescura y una mayor calidad (Berruga *et al*., 2005). En consecuencia, los últimos avances de la investigación en atmósferas protectoras se han centrado en encontrar la mezcla correcta de los gases que maximice el color rojo brillante, la estabilidad del color y la vida útil de la carne, minimizando el crecimiento microbiano, la oxidación de lípidos y el espacio de cabeza gaseoso (Mancini y Hunt, 2005). Diversos estudios señalaron que las concentraciones elevadas de oxígeno en el interior del envase favorecen la formación de oximioglobina, observándose además una mayor estabilidad del color en carnes rojas (Jeremiah, 2001). Por otro lado, el oxígeno limita el crecimiento de microorganismos anaerobios estrictos, entre los que se encuentran algunos causantes de toxiinfecciones alimentarias como *Clostridium perfringens* y *Clostridium botulinum* (Bejarano, 2001). El empleo de CO_2 se fundamenta en su actividad antimicrobiana, que depende de la concentración (Dixon y Kell, 1989). De acuerdo con Gill (1996), una concentración de CO_2 en el envase del 20 % es suficiente para inhibir el crecimiento de los microorganismos psicrótrofos un 50 %. Su efecto antimicrobiano en la conservación de la carne de cordero está ampliamente demostrado (Berruga *et al*., 2005; Sheridan *et al*., 1997; Vergara y Gallego, 2001). Otros gases como el nitrógeno o el argón también se utilizan habitualmente para el envasado de la carne de cordero en atmósfera protectora (Bellés, Alonso, Roncalés y Beltrán, 2017b). Ambos gases son inertes, inodoros, incoloros e insípidos, limitándose su función a actuar como relleno y evitar el colapso del envase en los productos que, como la carne, absorben CO_2 (Zhou *et al*., 2010). Las atmósferas empleadas para la conservación de la carne de cordero contienen habitualmente un 20-30 % CO_2 y 70-80 % O_2 con objeto de maximizar el color rojo de la carne y limitar el crecimiento microbiano (Bellés *et al*., 2017a), no obstante se pueden encontrar mezclas muy variadas en la bibliografía.

Envasado a vacío

El envasado a vacío fue uno de los primeros métodos utilizados comercialmente. Este sistema consiste en la eliminación del aire del interior del envase con el fin de crear un ambiente anóxico (Jeremiah, 2001). La eficacia del envasado a vacío depende del porcentaje de aire residual, siendo más eficaz cuando menor sea el contenido de oxígeno dentro del envase. Por tanto, se necesita un contacto íntimo entre la película de envasado y la superficie del producto, ya que los huecos (arrugas) que queden tras el envasado supondrán un reservorio de aire para las reacciones oxidativas y el crecimiento de microorganismos aerobios (Lagerstedt, Ahnström y Lundström, 2011). Además, estas arrugas favorecerán la acumulación de exudado en las mismas, disminuyendo la aceptación visual de la carne (Stiles, 1990).

La ausencia de oxígeno en el envase minimiza la oxidación de la mioglobina y en consecuencia, la formación de metamioglobina (Bellés, Alonso, Roncalés y Beltrán, 2017c). En el envasado a vacío la mioglobina se encuentra como desoximioglobina, presentando un color rojo oscuro. No obstante, la mioglobina se oxigena rápidamente tras abrir el envase y exponer la carne al oxígeno, mostrando entonces un color rojo intenso, fenómeno conocido como *blooming*. Por otro lado, el ambiente anóxico limita el crecimiento de la flora aerobia (Zhou *et al*., 2010). Sin embargo, la intensidad de estos efectos está limitada por el grado de vacío logrado, que depende de la tecnología utilizada.

El primer sistema de envasado a vacío consistía en la utilización de bolsas de plástico fabricadas con materiales con baja permeabilidad al oxígeno y termosellables (Stiles, 1990). En este sistema, la película de envasado se pliega alrededor del alimento a causa del descenso de la presión interna frente a la atmosférica. Dicho material debe presentar una permeabilidad muy baja a los gases, incluido el vapor de agua (Jenkins y Harrington, 1991). Mediante esta tecnología el contenido de oxígeno residual durante el almacenamiento es solamente del 1 %. No obstante, esta pequeña cantidad es suficiente para promover la formación de metamioglobina (Hood, 1984). Con objeto de reducir al máximo el porcentaje de aire residual surgió el envasado a vacío tipo *skin*. En este sistema de envasado el *film* superior se calienta hasta temperaturas superiores a los 200 °C, lo que permite su perfecta adaptación a la superficie del producto, para posteriormente evacuar el aire y termosellar el *film* a la bandeja inferior. Por efecto del calor la lámina se retrae adaptándose a la superficie del producto, lo que posibilita una unión más íntima del *film* con la carne, que impide la formación de bolsas de aire, y con ello se reduce el porcentaje de oxígeno residual (Vázquez *et al*., 2004). El envasado a vacío tipo *skin* reduce la cantidad de exudado presente en el envase en comparación

con el envasado en bolsas de plástico, mejorando por tanto la aceptabilidad visual (Kameník *et al*., 2014; Lagerstedt *et al*., 2011). Además, el menor contenido de exudado junto con las temperaturas alcanzadas durante el proceso de envasado podrían reducir el crecimiento microbiano (Lagerstedt *et al*., 2011).

El envasado a vacío ha sido ampliamente utilizado para la conservación de cortes primarios. Mediante el envasado a vacío la vida útil de estas piezas de carne puede prolongarse hasta 4 veces respecto a la obtenida en productos sin envasar (Gill, 1996). Este aumento en la vida útil supone una gran flexibilidad para productores y comercializadores, lo que convierte a este sistema de envasado en el preferido para el almacenamiento y conservación de los cortes primarios (Jeremiah, 2001; Sañudo *et al*., 2013; Zhou *et al*., 2010). No obstante, esta tecnología presenta limitaciones para su utilización en carnes destinadas a la venta directa. La formación de desoximioglobina en condiciones de anaerobiosis otorga a la carne un color rojo mate, que resulta poco atractivo para los consumidores (Jeremiah, 2001; Sañudo *et al*., 2013).

Envasado activo

En los últimos años se ha desarrollado otra línea de investigación en materiales de envasado denominada envasado activo. El envasado activo supone una revolución del concepto clásico según el cual el envase debe ser un material inerte que no interaccione con su contenido. Un envase se vuelve activo cuando desempeña otro papel deseado además del de barrera, generalmente orientado hacia mejoras en la calidad y seguridad del alimento (Hutton, 2003; Kerry, O'Grady y Hogan, 2006). Ejemplos de envases activos aplicados en la industria cárnica son los de capacidad absorbente (de oxígeno, humedad, luz ultravioleta, etc.), emisora (CO_2, etanol, antioxidantes, etc.) e incluso de propiedades antimicrobianas (Brody *et al*., 2008). Los compuestos activos pueden ser aplicados de diferentes formas (Zhou *et al*., 2010):

- Mediante una bolsita depositada en el interior del envase.
- Incorporados en el *film* durante la formación del mismo.
- Compuestos con capacidad de polimerización.

Como se ha comentado en apartados anteriores, el crecimiento microbiano y las reacciones de oxidación son las principales causas de deterioro de la carne de cordero, es por esto que los estudios actuales se centran en desarrollar envases activos que consigan frenar estos procesos. Uno de los aspectos esenciales de la investigación en esta área es la búsqueda de compuestos con propiedades antimicrobianas o antioxidantes. No obstante, existen grandes restricciones legales en cuanto al uso de antibióticos y compuestos químicos sintéticos, a lo que hay que sumar el creciente

rechazo de los consumidores hacia los aditivos sintéticos y su preferencia por productos más “naturales” (Smid y Gorris, 1999). Bajo este contexto, las investigaciones se han centrado en la búsqueda de compuestos de origen natural con propiedades interesantes en la conservación de los alimentos.

Conservación mediante la utilización de aceites esenciales y extractos de plantas y hierbas

Las plantas, especias y sus compuestos derivados se han utilizado desde la antigüedad para mejorar las características sensoriales de los alimentos, como conservantes e incluso con el fin de aprovechar sus beneficios para la salud (Gyawali e Ibrahim, 2014). En los últimos años se ha renovado el interés por los extractos naturales y aceites esenciales, dedicando grandes esfuerzos a identificar nuevos compuestos que puedan aplicarse a la tecnología de los alimentos y particularmente, a la conservación de la carne y los productos cárnicos. Las propiedades antimicrobianas y antioxidantes de los extractos y aceites vegetales se deben a una gran variedad de compuestos químicos, tradicionalmente agrupados en las siguientes categorías: polifenoles, flavonoides, taninos, alcaloides, terpenos, isotiocianatos, lecitinas y polipéptidos (Negi, 2012). Estos compuestos se han buscado en un gran número de plantas, pudiéndose extraer de las hojas, los tallos, las raíces, los frutos e incluso las semillas. La extracción y el aislamiento de los compuestos de interés se realizan mediante el empleo de distintas técnicas, como la extracción por calor, la destilación, los ultrasonidos, los pulsos eléctricos, los fluidos supercríticos, etc. La tecnología empleada, así como la naturaleza del solvente, determinará el tipo de compuestos aislados.

La destilación es la técnica comúnmente empleada para la obtención de aceites esenciales. Los aceites esenciales son líquidos aromáticos aceitosos que se obtienen de diferentes partes de las plantas como flores, brotes, semillas, hojas, corteza, hierbas, madera, frutos, raíces, etc. (Burt, 2004). Son volátiles, solubles en lípidos y disolventes orgánicos, y de menor densidad que el agua (Bakkali, Averbeck, Averbeck e Idaomar, 2008). Un aceite esencial puede contener más de 16 compuestos individuales, aunque alguno de estos puede suponer hasta el 85 % de la composición del mismo (Burt, 2004). Este compuesto principal va a determinar la acción antimicrobiana del aceite esencial, que puede ser incrementada por la acción sinérgica de los demás compuestos minoritarios (Marino, Bersani y Comi, 1999). El efecto antimicrobiano de estos compuestos no puede asociarse a un solo mecanismo de acción sino que actúan sobre distintas dianas de la célula bacteriana. En general, pueden degradar la pared celular, la membrana citoplasmática y dañar las proteínas de la membrana, incrementando la

permeabilidad de la membrana celular y en consecuencia la pérdida de componentes celulares (Jayasena y Jo, 2013). En este sentido, se ha demostrado la acción antimicrobiana de los aceites esenciales frente a diversos microorganismos grampositivos y gramnegativos asociados al deterioro de la carne. Entre los aceites esenciales más estudiados para su utilización en carne y derivados cárnicos destacan el aceite de orégano, romero, ajo, tomillo, hinojo y jengibre (Goulas y Kontominas, 2007; Karabagias, Badeka y Kontominas, 2011). Además, los aceites esenciales presentan actividad antioxidante. Su capacidad para inhibir las reacciones oxidativas se fundamenta en la presencia de compuestos fenólicos en su composición (Karabagias *et al*., 2011). Cabe destacar la acción antioxidante del aceite de romero (Vital *et al*., 2016), orégano (Chouliara, Karatapanis, Savvaidis y Kontominas, 2007; Goulas y Kontominas, 2007; Vital *et al*., 2016) y tomillo (Baydar, Sagdiç, Özkan y Karadogan, 2004; Karabagias *et al*., 2011) durante el almacenamiento de la carne.

Además de los aceites esenciales también se han evaluado las propiedades de extractos hidrosolubles, solubles en disolventes polares e incluso de componentes vegetales sin tratar. Como puede observarse en la tabla 2, entre los extractos aplicados a la conservación de la carne de cordero destacan los de té, orégano, tomillo, borraja y romero. Estos extractos poseen grandes cantidades de compuestos fenólicos, responsables de sus efectos antimicrobianos (mediante el aumento de la permeabilidad de la membrana celular y la formación de hidroperóxidos) y antioxidantes. Los compuestos fenólicos poseen propiedades antioxidantes gracias a su actividad redox, ejerciendo su acción mediante la adsorción y la neutralización de radicales libres (Falowo, Fayemi y Muchenje, 2014). La aplicación de los aceites esenciales y los extractos vegetales a la conservación de la carne puede realizarse principalmente a través de tres estrategias diferentes:

- Suplementando la dieta de los corderos con los compuestos de interés (Nieto, Díaz, Bañón y Garrido, 2010; Ortuño, Serrano y Bañón, 2015; Ortuño, Serrano y Bañón, 2016; Ortuño, Serrano, Jordán y Bañón, 2014).
- Incorporando los compuestos a los materiales de envasado (envase activo) (Camo, Beltrán y Roncalés, 2008; Camo, Lorés, Djenane, Beltrán y Roncalés, 2011; Lorenzo, Batlle y Gómez, 2014).
- Adicionándolos directamente a la carne o producto cárnico (Alp y Aksu, 2010; Bellés *et al*., 2017b; Djenane *et al*., 2002; Latoch y Stasiak, 2015; Mastromatteo, Lucera, Sinigaglia y Corbo, 2009; Sánchez-Escalante, Djenane, Torrescano, Beltrán y Roncalés, 2003).

Las principales ventajas de los aceites esenciales y los extractos de plantas respecto de los conservantes químicos sintéticos son su presencia en la lista GRAS (*Generally Recognized As Safe*) de la Administración de Alimentos y Medicamentos de Estados Unidos y su mayor aceptación por los consumidores (Smid y Gorris, 1999). No obstante, también presentan una serie de limitaciones:

- La adición de extractos vegetales directamente sobre la carne fresca implica inmediatamente su categorización como preparado de carne de acuerdo con lo dispuesto en el Real Decreto 1376/2003, de 7 de noviembre, por el que se establecen las condiciones sanitarias de producción, almacenamiento y comercialización de las carnes frescas y sus derivados en los establecimientos de comercio al por menor (Gobierno de España, 2003).
- En general, existen diferencias importantes en la magnitud del efecto cuantificado en los ensayos *in vitro* y el observado en la matriz alimentaria (Martínez, González, Cabellero, Santaella y Frontela, 2015).
- Las concentraciones de aceites y extractos naturales necesarias para obtener un efecto significativo suelen ser tan elevadas que acarrean una modificación notable de las propiedades sensoriales de la carne (Martínez *et al*., 2015).

Tabla 2. Extractos vegetales utilizados para la conservación de la carne de cordero.

Fuente vegetal	Obtención	Aplicación	Referencia
Tomillo (*Thymus*)	Aceite esencial	Directa	Karabagias *et al*., 2011
Orégano (*Origanum vulgare*)	Aceite esencial	Directa	Karabagias *et al*., 2011
Borraja (*Borago officinalis*)	Extracto de semillas	Directa	Bellés *et al*., 2017b
Té verde (*Camellia sinensis*)	Extracto de hojas	Directa	Bellés *et al*., 2017b
Romero (*Rosmarinus officinalis*)	Extracto de hojas	Envase activo	Camo *et al*., 2008
Orégano (*Origanum vulgare*)	Extracto de hojas	Envase activo	Camo *et al*., 2008
Orégano (*Origanum vulgare*)	Extracto de hojas	Directa	Fernandes *et al*., 2016
Pu-erh té (*Camellia sinensis*)	Extracto de hojas	Directa	Hu *et al*., 2010
Romero (*Rosmarinus officinalis*)	Extracto de hojas	Dieta	Ortuño *et al*. 2014
Tomillo (*Thymus*)	Hojas	Dieta	Nieto *et al*. 2010

Justificación y objetivos

2. Justificación y objetivos

Como se ha comentado en el capítulo anterior, la producción y el consumo de carne de cordero en España se han reducido en la última década. Esta situación hace necesaria la búsqueda de soluciones para mejorar las cifras de venta y con ello, frenar la disminución de la producción. Una de las apuestas por parte del sector ha sido la apertura al mercado exterior. El incremento de las exportaciones queda reflejado en las cifras: en el año 2004 se vendió fuera de nuestras fronteras un 12,74 % de la carne de ovino producida (160.252 T) mientras que en 2012 se exportó el 26,46 % del volumen total (121.999 T) (figura 5). Los principales países importadores son de la Unión Europea (Italia, Francia y Reino Unido) mientras que China es el principal destino de la producción española fuera de la UE, seguido de países árabes (Datacomex, 2016). Por tanto, existen consumidores fuera de nuestras fronteras que demandan carne de cordero y, lo que es más importante, con capacidad para asumir su coste. Esta tendencia parece que seguirá al alza puesto que el aumento de las exportaciones no solo permite compensar el descenso en el consumo interno, sino que ayuda a los grupos productores y comercializadores a superar la estacionalidad del consumo de la carne de ovino en España.

La vida útil de la carne fresca no es suficiente para su comercialización fuera de nuestras fronteras, siendo necesaria su congelación. No obstante, en los últimos años se han producido cambios importantes en la sociedad. Existe una creciente demanda de productos listos para el consumo o con escasa preparación que no sería satisfecha con la venta de carne congelada (Cruz, 2013). Sin embargo, la comercialización de carne descongelada envasada en atmósfera protectora se adaptaría tanto a las exigencias de productores y comercializadores como a las de los consumidores. La aplicación de esta estrategia podría suponer un descenso de la calidad de la carne, principalmente debido a las reacciones oxidativas, por tanto, la aplicación de algún compuesto antioxidante podría ser necesario.

Por otro lado, dentro de las nuevas demandas de los consumidores destaca la búsqueda de productos de gran calidad y libres de productos químicos sintéticos (Jayasena y Jo, 2013). La mejora de la calidad del producto es una de las principales estrategias comerciales para incrementar la competitividad de las empresas (Besik y Nagurney, 2017; Verbeke *et al.*, 2010). No hay que olvidar que la carne de cordero se considera un producto de alta calidad en los países mediterráneos, especialmente en

España (Karabagias *et al.*, 2011; Vieira y Fernández, 2014). Entre las razones que justifican esta valoración destacan las siguientes (MAPAMA, 2008):

- Existe una elevada seguridad en el consumo de este tipo de carne.
- Este alimento nunca ha sido vinculado a ningún tipo de crisis alimentaria.
- Esta especie se asocia a una producción ligada al medio natural y lejana a sistemas de producción intensivos así como a tratamientos hormonales.
- Desde el punto de vista nutricional, el cordero es visto por la mayoría de los consumidores como un alimento saludable.
- El sabor de este producto es muy valorado, siendo el principal motivo de su compra.

En consecuencia, se puede afirmar que se parte de un producto de elevada calidad. No obstante, la cadena de distribución de los productos alimentarios difiere del resto de productos debido a que la calidad de los alimentos disminuye con el tiempo, especialmente en el caso de la carne de cordero que posee una corta vida útil (Yu y Nagurney, 2013). Es por ello que los esfuerzos del sector deben ir dirigidos a mantenerla desde el campo a la mesa. Las estrategias de conservación empleadas para mantener la calidad deben responder a las exigencias de los consumidores, entre las que destaca la ausencia de compuestos químicos sintéticos. La refrigeración a temperaturas cercanas al punto de congelación, el envasado a vacío tipo *skin* y la utilización de extractos y aceites vegetales podrían reducir notablemente los procesos de deterioro que afectan a la carne de cordero.

Bajo este contexto, el objetivo de esta tesis doctoral fue optimizar distintas estrategias de conservación para mantener la calidad inicial de la carne de cordero a lo largo de la cadena de comercialización.

Objetivos específicos:

1. Analizar y sintetizar los datos publicados sobre la suplementación de los corderos con vitamina E con el fin de consolidar el conocimiento existente e identificar nuevas áreas de investigación.
2. Investigar la capacidad antioxidante de la vitamina E administrada en la dieta sobre la calidad de la carne de cordero mantenida en congelación.
3. Evaluar la viabilidad, desde el punto de vista de la calidad del producto, de la comercialización de carne de cordero descongelada, envasada en atmósfera protectora y mantenida en expositores refrigerados.

4. Estudiar el efecto de los sistemas de corte más habituales (sierra y cizalla) en la vida útil y en la aceptabilidad de las chuletas de cordero a lo largo de su conservación en condiciones comerciales.
5. Describir los métodos utilizados y las nuevas tendencias para la refrigeración de las canales y la conservación de la carne fresca de cordero.
6. Analizar el efecto combinado del *superchilling* y el envasado en la vida útil de la carne de cordero.
7. Investigar el efecto de la aplicación de extractos de té y borraja en la conservación de chuletas de cordero.
8. Evaluar la capacidad del extracto de té y el carvacrol para sustituir a los sulfitos como conservantes del *burger meat*.

La tabla 3 muestra los objetivos, las hipótesis y los capítulos de esta tesis doctoral en los que se desarrollan.

Tabla 3. Relación entre los objetivos, las hipótesis y los capítulos donde se abordan.

Objetivo	Hipótesis	Capítulo
Analizar y sintetizar los datos publicados sobre la suplementación de los corderos con vitamina E con el fin de consolidar el conocimiento existente e identificar nuevas áreas de investigación.	La concentración de tocoferol en el músculo necesaria para inhibir completamente los procesos oxidativos, y por tanto la estrategia de suplementación utilizada, podría depender de las condiciones de conservación.	Capítulo 4.1.1. Supranutritional doses of vitamin E to improve lamb meat quality.
Investigar la capacidad antioxidante de la vitamina E administrada en la dieta sobre la calidad de la carne de cordero mantenida en congelación.	Durante la congelación se generan compuestos primarios que aceleran la oxidación lipídica al descongelar la carne. La suplementación de los corderos con vitamina E reduciría notablemente los procesos oxidativos durante el mantenimiento de la carne en congelación.	Capítulo 4.1.2. Effect of dietary vitamin E on physicochemical and fatty acid stability of fresh and thawed lamb.
Evaluar la viabilidad, desde el punto de vista de la calidad, de la comercialización de carne de cordero descongelada, envasada en atmósfera protectora y mantenida en expositores refrigerados.	La comercialización de carne de cordero descongelada y envasada en atmósfera protectora podría satisfacer los requerimientos de los consumidores y comercializadores. La suplementación de la dieta de los corderos con vitamina E o la aplicación de un extracto de borraja con propiedades antioxidantes podría frenar la pérdida de color y los procesos oxidativos en la carne descongelada.	Capítulo 4.1.3. Display stability of fresh and thawed lamb supplemented with vitamin E or sprayed with an antioxidant borage seed extract.
Describir los métodos utilizados y las nuevas tendencias para la refrigeración de las canales y la conservación de la carne fresca de cordero.	La ultra refrigeración de las canales, el mantenimiento en *superchilling*, el envasado a vacío tipo *skin* y el envasado activo serían las estrategias más prometedoras para conservar la carne fresca de cordero.	Capítulo 4.2.1. A review of fresh lamb chilling and preservation.

Continúa en la siguiente página.

Tabla 3. Relación entre los objetivos, las hipótesis y los capítulos donde se abordan (Continuación).

Objetivo	Hipótesis	Capítulo
Estudiar el efecto de los sistemas de corte más habituales (sierra y cizalla) en la vida útil y en la aceptabilidad de las chuletas de cordero a lo largo de su conservación en condiciones comerciales.	El sistema de corte tendría un efecto significativo en la vida útil de la carne, bien al favorecer la contaminación microbiana o al liberar compuestos prooxidantes desde el hueso. Además, podrían existir diferencias visuales que dirigirían la decisión de compra del consumidor.	Capítulo 4.2.2. ¿Afecta el sistema de corte a la calidad y aceptabilidad de la carne de cordero?
Analizar el efecto combinado del *superchilling* y el envasado en la vida útil de la carne de cordero.	El mantenimiento de la carne en *superchilling* incrementaría significativamente su vida útil, no obstante, podría incidir negativamente en su calidad.	Capítulo 4.2.3. The combined effects of superchilling and packaging on the shelf life of lamb.
Investigar el efecto de la aplicación de extractos de té y borraja en la conservación de chuletas de cordero.	Los extractos de té y borraja reducirían la oxidación, el crecimiento microbiano y la pérdida de color de las chuletas de cordero durante su almacenamiento.	Capítulo 4.2.4. Effect of borage and green tea aqueous extracts on the quality of lamb leg chops displayed under retail conditions.
Evaluar la capacidad del extracto de té y el carvacrol para sustituir a los sulfitos como conservantes del *burger meat*.	Los efectos antioxidantes y antimicrobianos del extracto de té y el carvacrol conseguirían un incremento de la vida útil del *burger meat* suficiente para sustituir a los sulfitos.	Capítulo 4.2.5. Sulphite-free lamb burger meat: antimicrobial and antioxidant properties of green tea and carvacrol.

Material y métodos

3. Material y métodos

3.1. Materia animal

Los animales utilizados en todos los estudios pertenecen a la raza Rasa Aragonesa, una raza rústica de tamaño medio y aptitud cárnica autóctona de la región. Los animales, que fueron criados bajo condiciones intensivas, se alimentaron con leche materna hasta los 40 días de edad y posteriormente, con concentrado y paja de cereal *ad libitum* hasta que alcanzaron un peso comprendido entre 20 y 25 kg. Los corderos se sacrificaron con aproximadamente tres meses de edad en el matadero de Mercazaragoza, siguiendo los protocolos estándar de la UE. Tras aproximadamente 15 minutos de faenado, las canales, con un peso comprendido entre 8 y 12 kg, se transportaron a las instalaciones de Casa de Ganaderos y Franco y Navarro SA, donde se refrigeraron durante 24 h (4 ± 0,5 °C, 90 % HR, 1-2 m/s). El número de canales seleccionado para cada experimento se estableció en función de las necesidades del mismo. Tras la refrigeración de las canales se procedió a su despiece, utilizando únicamente las piernas para los estudios de esta tesis doctoral. Estas condiciones de cría y muestreo se siguieron para todos los experimentos excepto para el que evaluó el efecto de la suplementación de la dieta de los corderos con vitamina E. Los detalles de este estudio se explican en el diseño experimental del mismo.

3.2. Envasadoras y materiales de envasado

3.2.1. Envasado en atmósfera protectora

El envasado en atmósfera protectora se realizó mediante una envasadora ULMA-SMART-500 (ULMA Packaging, S. Coop., Oñati, España). Se utilizaron bandejas de poliestireno y el sellado de las mismas se realizó con un *film* laminado de polietileno y poliamida. El *film* tenía un grosor de 30 µm, una permeabilidad al oxígeno a 23 °C de 15 cm^3/m^2/24 h/0 % HR y una permeabilidad al vapor de agua a 23 °C de 7 g/m^2/24 h/85 % HR (Linpac Packaging SL, Pravia, España). Los gases utilizados fueron adquiridos de Abelló-Linde SA, Puçol, España.

3.2.2. Envasado a vacío convencional

El envasado a vacío de tipo convencional se realizó mediante una envasadora Tecnotrip EV-13-L-CD-SC (Tecnotrip SA, Terrassa, España) utilizando bolsas de polietileno-poliamida y sellado mediante etil vinil acetato. Las bolsas tenían un grosor de

90 µm, una permeabilidad al vapor de agua a 23 °C de 2,8 g/m^2/24 h/85 % HR y una permeabilidad al O_2 a 23 °C de 50 cm^3/m^2/24 h/75 % HR (Eurobag y Film SL, Málaga, España).

3.2.3. Envasado a vacío tipo *skin*

Para el envasado a vacío tipo *skin* se utilizó una envasadora Multivac R570 CD (MULTIVAC Sepp Haggenmüller SE y Co. KG, Wolfertschwenden, Alemania). El *film* de sellado (Cryovac® VST 0250 *SKIN* TOP WEB, Sealed Air SL, Charlotte, EEUU) tenía un grosor de 100 µm, una permeabilidad al oxígeno a 23 °C de 1,5 cm^3/m^2/24 h/0 % HR y una permeabilidad al vapor de agua a 38 °C de 6 g/m^2/24 h/90 % HR, mientras que el *film* inferior (Cryovac® EGA 008, Sealed Air SL, Charlotte, EEUU) tenía un grosor de 200 µm y una permeabilidad al oxígeno a 23 °C de 21 cm^3/m^2/24 h/atm/0 % HR.

3.3. Análisis fisicoquímicos

3.3.1. Medida del pH

La medición del pH se realizó mediante un electrodo de punción (Crison PH 25, Crison instruments, Barcelona, España), tomando tres medidas en cada muestra. El medidor del pH fue calibrado antes de cada sesión y limpiado entre muestras con agua destilada.

3.3.2. Medida instrumental del color

Se midió el color en la superficie de la carne mediante un espectrofotómetro de reflectancia (Minolta CM-2002, Osaka, Japón), realizando 10 medidas en cada muestra. Se utilizó un iluminante estándar (D65) y una apertura de luz de 30 mm. Antes de cada sesión de medida se calibró el espectrofotómetro mediante un estándar blanco y negro, y se limpió la lente con etanol entre cada muestra. Los parámetros registrados fueron: *L** (luminosidad), *a** (índice de rojo), *b** (índice de amarillo) y las reflectancias en las longitudes de onda comprendidas entre 400 y 750 nm. A partir de estos valores se calcularon los índices de color y el porcentaje relativo de las distintas formas de la mioglobina.

Índice 630/580

El índice 630/580 mide la pérdida del color rojo de la carne, por tanto cuanto más bajo sea el mismo, peor será la calidad. Este índice se calculó dividiendo la reflectancia obtenida a 630 nm entre la medida a 580 nm (AMSA, 2012).

Índice *a*/*b*

Se trata de otro índice para medir el color rojo de la carne y en consecuencia, la estabilidad del color. Un descenso en sus valores se relaciona con una pérdida del color rojo de la carne. Se obtuvo dividiendo el valor de la coordenada *a** entre el de la *b** (AMSA, 2012)

Hue y Chroma

Los parámetros *Hue* y *Chroma* se calcularon siguiendo las directrices marcadas por la AMSA (2012).

$$Hue = tan^{-1}\left(\frac{b^*}{a^*}\right)$$

$$Chroma = \sqrt{\frac{a^{*2}}{b^{*2}}}$$

3.3.3. Contenido relativo de los distintos estados de la mioglobina

El contenido relativo de los distintos estados de la mioglobina se calculó a partir de la curva de reflectancia según Krzywicki (1979) utilizando 690 nm (mayor longitud de onda del instrumento). Puesto que el espectrofotómetro solo mide la reflectancia en intervalos de 10 nm, las longitudes de onda 473, 525 y 572 se calcularon mediante interpolación lineal.

$$A = log\left(\frac{1}{reflectancia}\right)$$

$$\%\ Metamioglobina = \left(1{,}395 - \frac{(A572 - A730)}{(A525 - A730)}\right) x\ 100$$

$$\%\ Desoximioglobina = \left(2{,}375\ x\ \left(1 - \frac{(A473 - A730)}{(A525 - A730)}\right)\right) * 100$$

$$\%\ Oximioglobina = 100 - (\%\ MMb + \%\ DMb)$$

3.3.4. Medida de la oxidación lipídica

La medida de la oxidación lipídica se llevó a cabo por la determinación de las sustancias reactivas al ácido tiobarbitúrico (TBARS) de acuerdo con el método descrito por Pzalgraf, Frigg y Steinhart (1995). Para ello, se homogeneizaron 10 g del músculo *Semimembranosus* con 20 ml de ácido tricloroacético (TCA) (VWR International Eurolab

SL, Barcelona, España) al 10 % durante 90 segundos a 11.000 revoluciones por minuto (rpm) mediante un Ultra-Turrax (T-25 basic, IKA-WERKE, Staufen, Alemania). Posteriormente, las muestras se centrifugaron (30 minutos, 4.000 rpm, 10 °C) en una centrífuga refrigerada (Heraeus Megafuge 1.0R, Kendro, Alemania) y se realizó el filtrado del sobrenadante mediante papel de filtro (Machere-Nagel, Düren, Alemania). Después de la filtración, se tomaron 2 ml del homogeneizado y se añadieron a un tubo de ensayo con 2 ml de una solución de ácido tiobarbitúrico (TBA) 20 mM (Sigma-Aldrich, San Luis, EEUU). Tras homogeneizar las muestras en un rotatubos (Heidolph REAX 2000, Apeldoorn, Holanda) durante 30 segundos, se incubaron a 97 °C durante 20 minutos en un baño de agua termostático (Grant W14, Cambridge, Reino Unido). Una vez transcurrido el tiempo de incubación, los tubos se enfriaron en agua con hielo para detener la reacción y a continuación, se procedió a la lectura de la absorbancia mediante un espectrofotómetro a 532 nm (Unicam 5625 UV/VIS, Cambridge, Reino Unido).

El cálculo de los resultados se realizó usando una recta patrón de 1,1,3,3 tetrametoxipropano (TMP) (Sigma-Aldrich, San Luis, EEUU). Se empleó dicha sustancia debido a que el malondialdehído (MDA) puede obtenerse por hidrólisis ácida del TMP en una reacción equimolecular. Las medidas fueron expresadas como sustancias reactivas al ácido tiobarbitúrico (TBARS) en mg de MDA/kg de muestra.

3.3.5. Análisis del perfil de ácidos grasos intramuscular de la carne

El perfil de ácidos grasos se determinó mediante cromatografía de gases de acuerdo con el método descrito por Bligh y Dyer (1959). Esta metodología consta de 3 fases diferentes:

Extracción de la grasa

Diez gramos del músculo *Semimembranosus* se picaron en un tubo de centrífuga, anotando el peso exacto. A continuación, se añadieron 10 ml de cloroformo ($CHCl_3$) (Merck, Darmstadt, Alemania) y 20 ml de metanol (CH_3OH) (Merck, Darmstadt, Alemania) homogeneizando el contenido durante 2 minutos a 4000 rpm con un Ultra-Turrax (T-25 basic, IKA-WERKE, Staufen, Alemania). Posteriormente, se adicionaron 10 ml de $CHCl_3$, 10 ml de cloruro potásico (KCl) (Panreac Quimica SLU, Castellar del Vallès, España) al 0,88 % en agua y 4 ml de agua destilada, realizando una segunda homogeneización bajo las mismas condiciones que la primera. Una vez homogeneizadas, las muestras se centrifugaron (Hermle Z320, Apeldoorn, Holanda) durante 10 minutos a 4.000 rpm y a continuación, se trasvasó el cloroformo (con la grasa disuelta) a unos viales de vidrio, a los cuales se les añadió BHT (Sigma Aldrich, San

Luis, EEUU) (10 µl aproximadamente) como antioxidante. Posteriormente, se evaporó el cloroformo de los viales en una atmósfera de nitrógeno (N_2), utilizando para ello un baño de arena (Bunsen, Madrid, España) a una temperatura de 55 °C. Una vez evaporado todo el cloroformo se almacenó la grasa en congelación (-20 °C) hasta su metilación.

Metilación

Para el proceso de metilación se utilizó la grasa obtenida en la etapa anterior. Se depositaron 0,03 ± 0,005 g de grasa en un tubo de vidrio de 5 ml y se añadieron 100 µl de patrón interno disuelto en cloroformo (C19:0, Sigma Aldrich, San Luis, EEUU), dejando el tubo abierto para que se evaporase el cloroformo. Una vez evaporado se adicionaron 2 ml de hexano (Merck, Darmstadt, Alemania), agitando el tubo ligeramente para conseguir la disolución total de la grasa, y tras ello, se pipeteó 1 ml de hidróxido potásico (KOH) (Panreac Quimica SLU, Castellar del Vallès, España) en metanol (CH_3OH) (Merck, Darmstadt, Alemania) a concentración sobresaturada. Se cerró el tubo con un tapón de goma, se agitó vigorosamente y se dejó reposar para conseguir la separación del contenido en dos fases. Una vez la separación de las fases fue visible, se extrajo la fase superior (hexano) y se depositó en un vial de 2 ml. En el hexano se encontraban disueltos los recién formados ésteres metílicos de los ácidos grasos (FAME). Con todo el proceso completado se encapsuló el vial.

Cromatografía de gases

Todos los viales se analizaron en un cromatógrafo de gases HP-6890 II (Hewlett-Packard, Waldbronn, Alemania) con una columna capilar SP-2380 (100 m × 0,25 × 0,20 µm) y el siguiente programa de temperaturas de horno: la columna fue ajustada a una temperatura de 140 °C, a parir de aquí la rampa de subida de la temperatura fue de 3 °C/min de 130 a 158 °C y de 1 °C/min hasta 165 °C, manteniendo este temperatura durante 10 minutos. Después se elevó 5 °C/min hasta 220 °C y se mantuvo constante durante 50 minutos. La temperatura de entrada se mantuvo a 230 °C y el detector a 240 °C. El inyector en modo de fraccionamiento tenía un ratio de 1/32. El nitrógeno fue usado como gas portador con un flujo constante de 0,8 ml/min con un volumen de inyección de 1 µl. Los FAME fueron identificados mediante la comparación de sus tiempos de retención con los obtenidos con una mezcla estándar (Supelco® 37 Component FAME Mix, Sigma Aldrich, San Luis, EEUU). Los datos obtenidos de la composición de los FAME fueron expresados como el porcentaje del área total de los FAME identificados.

3.3.6. Determinación del porcentaje de grasa intramuscular

Se realizó la extracción de la grasa mediante cloroformo y metanol según la metodología de Bligh y Dyer (1959). Se pipeteó un mililitro de cloroformo (con la grasa ya disuelta en él) en una cápsula de aluminio y se dejó evaporar en una estufa a 100 °C. Posteriormente, se pesó la cápsula con la grasa en el interior y por diferencia de pesadas se halló el contenido de grasa (resultados expresados en gramos de grasa/100 g de carne).

3.3.7. Cuantificación del contenido total de compuestos fenólicos

La determinación del contenido total de compuestos fenólicos se realizó siguiendo la metodología descrita por Matthäus (2002) con ligeras modificaciones. Los extractos se mezclaron con 0,6 ml de HCl al 0,3 % (Panreac Quimica SLU, Castellar del Vallès, España) hasta completar un volumen total de 1 ml. Posteriormente, se añadieron 100 µl de esta mezcla a 2 ml de Na_2CO_3 al 2 % (Merck, Darmstadt, Alemania) y a continuación, se adicionaron 100 µl del reactivo Folin Ciocalteau (Sigma-Aldrich, San Luis, EEUU) (diluido con metanol 1:1). Tras 30 minutos de incubación en oscuridad, se midió la absorbancia a 750 nm usando un espectrofotómetro (Unicam 5625 UV/VIS, Cambridge, Reino Unido). Se utilizó ácido gálico (Sigma-Aldrich, San Luis, EEUU) como estándar, expresando los resultados como mg de ácido gálico/ml o gramo de extracto.

3.3.8. Cuantificación del contenido de tocoferol en el músculo

La cuantificación del contenido de tocoferol del músculo se realizó en el Wageningen Institute of Animal Sciences, Wageningen University and Research Centre, Wageningen, Países Bajos. Se empleó la metodología descrita por Kasapidou *et al*. (2012) y Zhou *et al*. (2010). Las muestras se analizaron con un HPLC de fase inversa (LiChrospher RP-18 column, 4,60 x 150 mm, 5 lm, 100 Å) con una fase líquida de metanol (grado isocrático) (Merck, Darmstadt, Alemania), un flujo constante de 1,2 ml/min y un detector de fluorescencia (λ excitación = 295 nm y λ emisión = 330 nm; Agilent Series 1100). El volumen de inyección fue de 20 µl y el tiempo de ejecución de 9 minutos. Para la identificación y la cuantificación de los picos se utilizó (±)-α-tocopherol como patrón externo. Los resultados se expresaron como mg vitamina E/kg carne.

3.4. Análisis microbiológicos

3.4.1. Toma de muestras

Recuentos en superficie

La toma de muestras se realizó mediante el hisopado de una superficie de 10 cm^2 delimitada con una ventanilla metálica estéril. Se emplearon hisopos de algodón (152,4 mm, VWR International Eurolab SL, Barcelona, España), llevando a cabo el primer hisopado con un hisopo mojado en agua de peptona al 0,1 % (Merck, Darmstadt, Alemania) y el segundo en seco, realizando este hisopado varias veces y en direcciones diferentes. A continuación, los hisopos se llevaron a un tubo de ensayo con 10 ml de agua de peptona estéril al 0,1 % (Merck, Darmstadt, Alemania) y se agitaron vigorosamente en un vórtex (Heidolhp REAX 2000, Apeldoorn, Holanda).

Recuentos en masa

Se tomaron 25 g de carne y se mezclaron con 225 ml de agua de peptona al 0,1 % en un homogeneizador (Masticator Classic 400 ml, IUL SA, Barcelona, España). Posteriormente, se realizaron diluciones decimales en agua de peptona al 0,1 % y se procedió a la siembra.

3.4.2. Siembra e incubación

Los medios y las condiciones de incubación de los microorganismos investigados se resumen en la tabla 4.

Microorganismos aerobios viables totales

El recuento de microorganismos aerobios viables totales se realizó mediante la técnica de siembra en masa. Para ello, 1 ml de la dilución correspondiente se depositó en una placa de Petri, sobre la que se añadieron 15 ml de agar PCA (Plate Count Agar, Merck, Darmstadt, Alemania) como medio de cultivo. Tras la homogeneización de las placas, estas se incubaron durante 48 horas a 37 °C en una estufa (Incudigit, modelo 2001248, J.P. Selecta, Barcelona, España) cuando se hizo el recuento de microorganismos mesófilos, o a 10 °C durante 96 horas en una estufa refrigerada (FTC 90i. Refrigerated incubator, VELP Scientífica, Usmate Velate MB, Italia) en el caso de los microorganismos psicrótrofos. Los recuentos obtenidos se expresaron como unidades formadoras de colonias (UFC) por centímetro cuadrado o gramo de carne.

Microorganismos de la familia *Enterobacteriaceae*

En este caso, la siembra por homogeneización en masa se realizó en agar VRBD (Violet Red Bile Dextrose Agar, Merck, Darmstadt, Alemania). A continuación, las placas se incubaron durante 48 horas a 37 °C (Incudigit, modelo 2001248, J.P. Selecta, Barcelona, España), y transcurrido dicho tiempo se llevó a cabo el recuento de las colonias características, expresando el resultado como UFC por centímetro cuadrado o gramo de carne.

Recuento de *Pseudomonas* spp.

La siembra se realizó por extensión en superficie en agar CFC (Cephalothin-Sodium Fusidate-Cetrimide Agar, Merck, Darmstadt, Alemania). Para ello, se inocularon 100 µL de la muestra sobre la superficie del agar y se extendieron con un asa de Digralsky (VWR International Eurolab SL, Barcelona, España). Las placas se incubaron durante 24 horas a 25 °C. Transcurrido dicho tiempo se llevó a cabo el recuento de las colonias características, expresando el resultado como UFC por centímetro cuadrado o gramo de carne.

Recuento de *Brochotrix thermospacta*

La siembra se realizó inoculando 100 µL de la muestra sobre la superficie del agar STAA (Streptomycin-Thallous Acetate-Actidione, Merck, Darmstadt, Alemania) con suplemento selectivo STAA, extendiendo el inóculo posteriormente con un asa de Digralsky (VWR International Eurolab SL, Barcelona, España). Las placas se incubaron durante 72 horas a 25 °C. A continuación, se llevó a cabo el recuento de las colonias características, expresando el resultado como UFC por centímetro cuadrado o gramo de carne.

Recuento de bacterias acidolácticas

El recuento de bacterias acidolácticas se llevó a cabo mediante la siembra en masa. Se depositó 1 ml de la muestra en una placa de Petri y se vertieron 15 ml de agar MRS (Man, Rogosa and Sharpe, Merck, Darmstadt, Alemania). Tras agitar las placas y dejarlas enfriar, se incubaron durante 96 horas a 37 °C en anaerobiosis. Para crear las condiciones de anaerobiosis se utilizaron jarras herméticas y un kit generador de anaerobiosis (Anaerocult A, Merck, Darmstadt, Alemania), así como tiras indicadoras (Gazpack®, Zierikzee, Países Bajos). Posteriormente, se llevó a cabo el recuento de las colonias características, expresando el resultado como UFC por centímetro cuadrado o gramo de carne.

Tabla 4. Medios de cultivo y condiciones de crecimiento de los microorganismos investigados.

Microorganismo	Medio de cultivo	Temperatura	Horas de incubación	Atmósfera
Mesófilos aerobios viables totales	PCA	37 °C	48	Aerobia
Psicrótrofos aerobios viables totales	PCA	10 °C	96	Aerobia
Enterobacteriaceae	VRBD	37 °C	48	Aerobia
Pseudomonas spp.	CFC	25 °C	24	Aerobia
Brochotrix thermospacta	STAA	25 °C	72	Aerobia
Bacterias acidolácticas	MRS	37 °C	96	Anaerobia

PCA: Plate Count Agar; VRBD: Violet Red Bile Dextrose Agar; CFC: Cephalothin-Sodium Fusidate-Cetrimide Agar; STAA: Streptomycin-Thallous Acetate-Actidione; MRS: Man, Rogosa and Sharpe.

3.5. Análisis sensorial

3.5.1. Carne fresca

El análisis sensorial de la carne sin cocinar consistió en un análisis visual. Las muestras de carne fueron evaluadas por un panel entrenado formado por nueve miembros, que había sido previamente entrenado en análisis sensoriales (ISO 8586-1, 1992) y estaba familiarizado con la evaluación visual del cordero. Se realizó un entrenamiento especial para garantizar que los panelistas pudieran reconocer los atributos antes de comenzar el proceso de evaluación de las muestras de cordero. Los panelistas usaron una escala de ocho puntos para cuantificar el color (1 = color rojo cereza muy brillante , 2 = rojo brillante, 3 = rojo opaco, 4 = rojo oscuro, 5 = marrón 6 = ligeramente verdoso, 7 = moderado marrón verdoso , 8 = verde), una escala de cinco puntos para evaluar la decoloración de la superficie (% de metamioglobina) (1 = 0 %, 2 = 1 a 20 %, 3 = 21 a 40 %, 4 = 41 a 60 %, 5 = 61 a 100 %) y una escala de seis puntos para cuantificar el color de la médula ósea (1 = color rojizo-rosa a rojo brillante, 2 = color rosado-rojo sin brillo, 3 = rojo grisáceo, 4 = moderadamente gris, 5 = negro grisáceo, 6 = decoloración negra). Estas escalas fueron diseñadas siguiendo las recomendaciones de la AMSA (2012) con algunas modificaciones. En cada día de muestreo, los panelistas evaluaron las muestras en las mismas condiciones. Esta se realizó en una vitrina expositora refrigerada simulando las condiciones comerciales habituales (1200 lx, 14 horas diarias de luz).

3.5.2. Carne cocinada

Cocinado

El cocinado de las muestras se realizó sin aditivos en un *grill* industrial de doble placa (Samic GRD10, Guipúzcoa, España) a 200 °C con las muestras envueltas en papel de aluminio, considerando el fin del cocinado una temperatura interna de la chuleta de 72 °C. Una vez cocinadas se troceó la carne en porciones equivalentes, descartando los extremos y cualquier resto visible de tejido conjuntivo y grasa. Posteriormente, las porciones fueron envueltas en papel de aluminio y mantenidas en caliente (60 °C) hasta su degustación (máximo 10 minutos).

Evaluación

El análisis sensorial se realizó con un panel entrenado (ISO 8586-1:1192). Los miembros del panel recibieron un entrenamiento previo a cada estudio con el objetivo de familiarizarse con los descriptores sensoriales evaluados. Las sesiones se realizaron en la sala de catas de la Planta Piloto de la Facultad de Veterinaria de la Universidad de Zaragoza (ISO 8589:1988). Para enmascarar el color de la carne, las sesiones se realizaron con luz roja. En cada cabina los panelistas dispusieron de un plato caliente, una botella de agua mineral a temperatura ambiente, un vaso para el agua, pan, lápiz, goma y estadillos. Se estableció un diseño de bloques completos no equilibrados, es decir, los catadores evaluaron todas las muestras pero no en todas las ordenaciones posibles. A cada muestra se le asignó un código de 3 cifras, variando este en cada sesión. El orden de evaluación de las muestras fue al azar y se emplearon escalas estructuradas.

3.6. Análisis estadístico

Los datos obtenidos en esta tesis doctoral han sido analizados estadísticamente mediante el modelo lineal general (GLM) del paquete estadístico SPSS, versión 19.0 (IBM SPSS, 2010) (IMB, Nueva York, EEUU). El modelo evaluó los efectos principales, los efectos aleatorios y las interacciones entre ellos. Las diferencias se consideraron significativas si $P \leq 0,05$. Cuando las diferencias fueron significativas se aplicó el test de Tukey para comparar la media de los valores entre los tratamientos analizados. Otro de los métodos utilizado fue el análisis de componentes principales (PCA), que se elaboró empleando el programa estadístico XLSTAT 2016 (Addinsoft España, Sardenya, España). Los datos del análisis estadístico se presentan con mayor detalle en cada uno de los estudios.

Resultados

4. Resultados

Manuscritos:

4.1. Estrategias productivas:

4.1.1. Supranutritional doses of vitamin E to improve lamb meat quality

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4.1. Estrategias productivas

4.1.1. Supranutritional doses of vitamin E to improve lamb meat quality

Supranutritional doses of vitamin E to improve lamb meat quality

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Shelf life

ABSTRACT

Vitamin E is a major fat-soluble antioxidant deposed within the highly unsaturated phospholipid bilayer of cell membranes, where it exerts a potent chain-breaking antioxidant effect. Moreover, the antioxidant activity of vitamin E-like compounds is transferred to meat after slaughter. The deposition of tocopherol in the muscle depends on the dosage and the period of supplementation, so different dosage-time combinations have been developed. Dietary vitamin E does not affect productive parameters but it could modify tissue fatty acid profile. During meat display, vitamin E protects PUFA from degradation, reducing lipid oxidation and therefore, delaying colour fading. Furthermore, the action of vitamin E would indirectly affect the development of lamb aroma by reducing protein and lipid oxidation. The present paper reviews scientific studies focused on vitamin E supplementation of lambs in order to discuss its effects on meat quality, comprehensively summarizing the main factors that influence it to facilitate producers and retailers optimizing the supplementation rate.

1. Introduction

What is meat quality? For almost six decades there has been intense debate about the most suitable answer to this question and different definitions have been proposed without an agreement about the most suitable. The FAO proposes that meat quality encompasses chemical composition and palatability factors such as visual appearance, smell, firmness, juiciness, tenderness, and flavour, whereas a more detailed definition includes hygienic, compositional, nutritional, sensory and technological quality traits.

Lamb is defined as a high quality product and it is considered a delicacy in Mediterranean countries (Karabagias, Badeka, & Kontominas, 2011; Vieira, & Fernández, 2014). Visual appearance is the chief lamb sensory property during retail display because of consumer purchasing decision relies on meat appearance (Fasutman et al., 2010). Consumers associate a bright red colour with freshness and superior meat quality. Therefore, fresh lamb for retail display is commonly packaged in oxygen enriched atmospheres to satisfy consumers demand for fresh, tender, tasty and attractive appearance to meat (Bellés, Alonso, Roncalés, & Beltrán, 2017).

High concentrations of oxygen in the gas mixture have been demonstrated to result in optimum colour in red meats by promoting oxymyoglobin formation. However, the high level of oxygen in the package enhances oxidative reactions which lead to discoloration, development of off flavours, formation of toxic compounds, nutrient and drip losses that compromises meat quality. Oxidation is one of the main causes of lamb deterioration, which explains the great efforts that have been taken on to solve this problem (Bellés et al. 2017). The addition of compounds having antioxidant activity in the feed or in the package has emerged as a promising strategy to inhibit oxidative reactions and therefore, to offer a product with a higher quality an extended shelf life.

Vitamin E is the main fat soluble antioxidant in mammalian cells preventing oxidative reactions at the start site and it has been demonstrated that its antioxidant effect is transferred to meat after slaughter. A large number of studies have observed that the administration of supranutritional doses of vitamin E before slaughter favours the deposition of tocopherol in the muscle increasing the stability of meat against oxidation (Álvarez et al., 2008; Bellés et al., 2018; de la Fuente et al., 2007; Jose, Jacob, Pethick, & Gardner, 2016; Kasapidou et al., 2012; Kerry, O'Sullivan, Buckley, Lynch, & Morrissey, 2000). Dietary vitamin E has shown to have a great potential in struggling the negative effect of oxidative reactions on lamb quality, so it is vital to optimize this feeding strategy in order to obtain the maximum benefit. The following review will briefly present the biochemical properties of vitamin E focusing on the effect of dietary vitamin E on physicochemical, microbiological and sensory quality of lamb.

2. Biochemical characterisation

2.1 Chemical structure

The generic term vitamin E includes several compounds having a similar antioxidant activity (Jensen, & Lauridsen, 2007). These compounds comprise both tocopherols and tocotrienols, which are characterised by possessing a hydroquinone nucleus and an isoprenoid side chain. The chemical difference between them is located in the isoprenoid side chain; tocopherols have a saturated side chain while tocotrienols have an unsaturated side chain containing three double bonds. Moreover, four different tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ) are differentiated depending on the position of methyl (CH_3) groups at positions 5, 7 or 8 of the chroman ring (Colombo, 2010). Table 1 shows the chemical structure of vitamin E-like compounds.

Among all of them, α-tocopherol shows the highest biological activity (Jensen, Nørgaard, & Lauridsen, 2006). Alpha-tocopherol is a chiral molecule, which means that it is non-superposable on its mirror image. Each one of the mirror images of a chiral molecule is called enantiomer or optical isomer. Chirality is often produced due to the presence of an asymmetric carbon centre. Indeed, α-tocopherol presents three chiral centres at positions 2C, 4C and 8C of the phytyl tail. At each chiral centre two configurations are possible, which are named R or S, so there are eight different stereoisomers of α - tocopherol (Dersjant-Li, & Peisker, 2010; Jensen, & Lauridsen, 2007). Figure 1 presents the chemical structure of α-tocopherol stereoisomers.

Alpha-tocopherol obtained from natural sources consists of a single stereoisomer presenting a RRR configuration, which means that it presents a configuration of R at the three positions (2C, 4C and 8C). In contrast, chemically synthetised vitamin E comprises an equimolar mixture of the eight different α-tocopherol stereoisomers (RRR, RRS, RSS, RSR, SRR, SSR, SRS, and SSS) (Dersjant-Li & Peisker, 2010). According to IUPAC recommendations (1982), vitamin E obtained from natural sources is called RRR-α-tocopherol while that which is chemically synthetised should be named all-rac-α-tocopherol in order to describe accurately the differences in chemical composition between both sources.

Both natural and synthetised vitamin E free forms are easily degraded during the processing, manufacturing and storage of finishing feeds, which make difficult their use as additives in feedstuffs. To overcome these problems more stable forms have been developed. Since esters are less susceptible to oxidation, the phenol group of α - tocopherol is commonly converted to an ester by using acetic or succinic acid, so vitamin E is commercialised as α - tocopheryl-acetate or α-tocopheryl-succinate. These forms are very stable to in vitro oxidation but they need to be hydrolysed in the animal gut to show antioxidant activity (Vagni, Saccone, Pinotti, & Baldi, 2011). The all-rac α-tocopheryl acetate is the form of vitamin E most used to supplement animal feeds due to its high stability and lower cost.

2.2 Bioavailability and biopotency

Bioavailability and biopotency are close-knit concepts. The first one is defined as the absorbed proportion of a substance after its administration while biopotency refers to the ability of a compound to exert an effect in a biological system (Dersjant-Li & Peisker, 2010). It is obvious that a substance needs to be absorbed prior to act in an organism.

Therefore, knowing the mechanism of absorption of vitamin E is the key to maximize its benefits in meat quality, even more in ruminants whose digestive anatomy and physiology is markedly different and more complex than that of monogastric animals. Several studies have been carried out to assess the effect of ruminal microbiota and fermentations on vitamin E absorption. Weiss, Smith, Hogan and Steiner (1995) observed that vitamin E was not degraded during *in vitro* ruminal fermentation. In agreement, Hymøller and Jensen (2010) noted neither *in vitro* nor *in vivo* hydrolysis of α-tocopherol in the rumen. Taking into account that α-tocopherol esters need to be hydrolysed before their absorption, little if any absorption of vitamin E in the rumen should be expected.

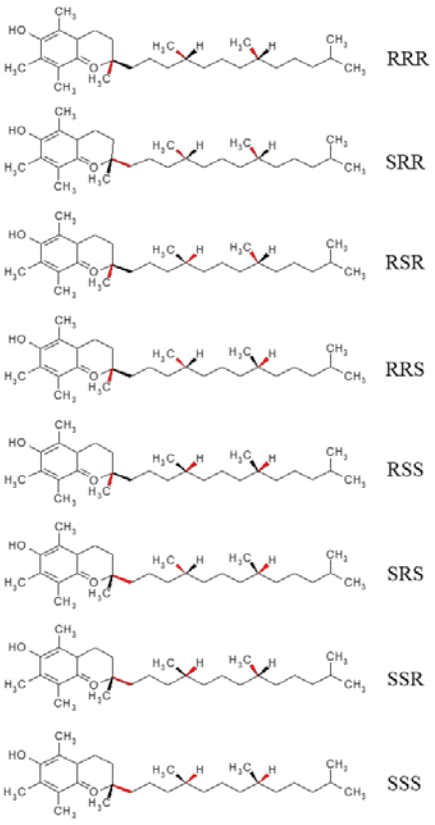


Figure 1. Steroisomers of α-tocopherol.

In contrast, tocopherol esters are largely hydrolysed in the intestinal lumen, where they are then absorbed in combination with lipid micelles. Once in the enterocytes, vitamin E is packed into chylomicrons and delivered to the liver trough circulation (Lauridsen, & Jensen, 2012). In the liver, the hepatic α-tocopherol transfer protein, α-TTP, binds to the vitamin E to facilitate its incorporation into nascent VLDL and its secretion from hepatocytes. This lypoprotein has a central role in vitamin E metabolism as it regulates the body-wide levels of α-tocopherol (Rigotti, 2007). Furthermore, the α-TPP seems to be a major cause of the differences found among the bioavailability of the different vitamin E-like compounds. Both tocopherols and tocotrienols are transported into the chylomicrons to the liver but at this point, the α-TPP exerts a selective transport. Hosomi et al. (1997) demonstrated in vitro differences in the affinity of α-TPP to the different types of tocopherols and tocotrienols. Relative affinities (RRR -α - tocopherol = 100%) were as follows: RRR-ß-tocopherol, 38%; RRR-y-tocopherol, 9%; RRR-δ-tocopherol, 2%; α-tocopherol acetate, 2%; α-tocopherol quinone, 2%; SRR - α - tocopherol, 11%; α-tocotrienol, 12%. Therefore, α-TPP not only has a higher affinity for tocopherols but it is also able to sort out among isomers. The different affinity of α-TPP to α-tocopherol stereoisomers seems to be the major cause of their differences in activity. RRR-α-tocopherol is generally accepted to have the highest bioactivity but the equivalence of the different isomers is still a point of concern. The first study about this topic assigned a value of 1 IU mg−1 for 2-ambo-α-tocopheryl acetate (a mixture of RRR-and SRR-α-tocopherol) while a value of 1.36 $IUmg^{-1}$ was proposed for RRR-α-tocopheryl acetate (Harris & Ludwig, 1949). This ratio was later corroborated by Weiser and Vecchi (1982), who also associated a relative biopotency to each α-tocopherol stereoisomer. The results obtained were: RRR = 1, RRS = 0.9, RSS = 0.73, SSS = 0.6, RSR = 0.57, SRS = 0.37, SRR = 0.31 and SSR = 0.2. Despite both studies used clinical endpoints based on the physiological activity of vitamin E during the gestation on rats to determine the bioequivalence (RRR-α-tocopheryl acetate = 1.36 $IUmg^{-1}$), it was accepted by the United States Pharmacopeia

(USP) and widely used as the conversion factor either for human or livestock.

Nevertheless, an intense debate about the adequacy of this ratio for livestock is growing among researchers. Jensen and Lauridsen (2003) observed that cows have a higher selectivity than rats to utilise the different stereoisomers; so, while cows mostly preferred the RRR form, rats were able to utilize all 2R forms. Moreover, it has been noted that vitamin E bioavailability is also influenced by dietary dosage, feeding period, age and tissue (Dersjant-Li & Peisker, 2010; Jensen et al., 2006; Jensen, & Lauridsen, 2007). All these data support the need of calculating the equivalence between stereoisomers more accurately, taking into account both species and age. Equivalences in large animals cannot be determined with clinical endpoint markers due to ethical and practical aspects, so they are calculated by bioavailability studies. It has been demonstrated a higher bioavailability of RRR-a-tocopherol in cattle (Meglia, Jensen, Lauridsen, & Waller, 2006; Weiss, Hogan, & Wyatt, 2009), dairy cows (Weiss et al., 2009), sows (Lauridsen, Engel, Jensen, Craig, & Traber, 2002), piglets (Lauridsen et al., 2002) and finishing swine (Yang et al., 2009). Table 2 shows some ratios for livestock. Unfortunately, there is a lack of studies about this topic in lambs.

2.3 Antioxidant activity

Vitamin E is described to be a potent chain-breaking antioxidant, protecting cell membranes from free radical attack. All vitamin E compounds show a similar mechanism of action, being the hydroxylic group at position 6 in the chroman ring their functional group (Brigelius-Flohé, 2006). The hydroxilic group is able to transfer a hydrogen atom to a peroxil radical, which becomes into a lipid hydroperoxide, while vitamin E becomes a low activity radical, the α-tocopheroxyl radical. This radical is more stable and does not take part in propagation reactions. Tocopherols can inactivate different kinds of high reactive molecules such as singlet oxygen, alkoxyl radicals, peroxynitrite, nitrogen dioxide, ozone and superoxide. Moreover, it could be reduced to tocopherol by other antioxidants like vitamin C, showing again antioxidant activity (Fennema, Whitaker, Davidson, & Hartel, 2004).

Table 1. Chemical structure of vitamin E-like compounds

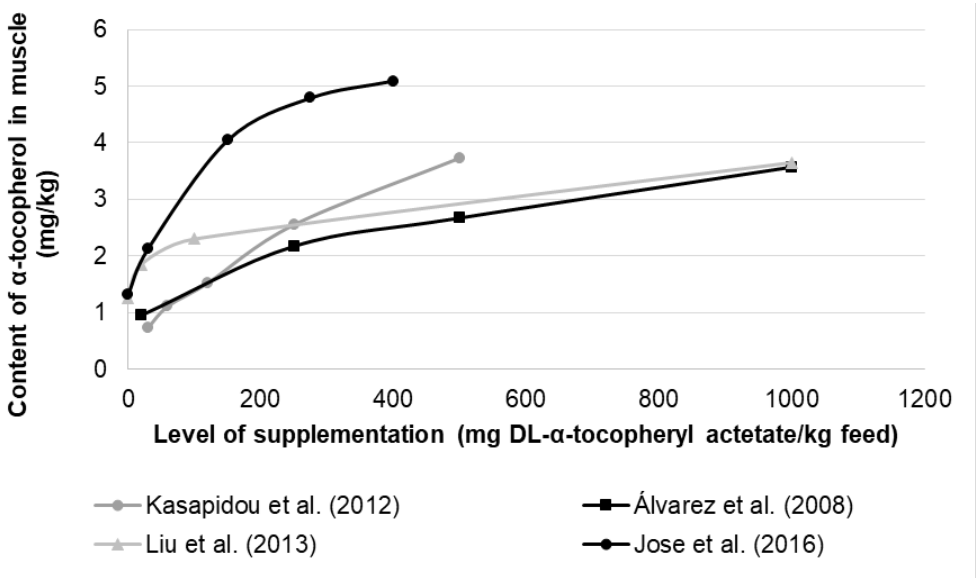
		Ring position		
Trivial name	Chemical name	R^1	R^2	R^3
α-tocopherol	5,7,8 -Trymethiltocol	CH_3	CH_3	CH_3
β-tocopherol	5,8-Dymethiltocol	CH_3	H	CH_3
γ-tocopherol	7,8-Dymethiltocol	H	CH_3	CH_3
δ-tocopherol	8-Methyltocol	H	H	CH_3
α-tocotrienol	5,7,8 -Trymethiltocotrienol	CH_3	CH_3	CH_3
β-tocotrienol	5,8-Dymethiltotrienol	CH_3	H	CH_3
γ-tocotrienol	7,8-Dymethiltotrienol	H	CH_3	CH_3
δ-tocotrienol	8-Methyltocotrienol	H	H	CH_3

From: Fennema,Whitaker, Davidson, & Hartel (2004)

Table 2. Bioequivalence between RRR-α-tocopherol and all-rac-α–tocopherol in some livestock species

Species	Bio-availability RRR-α-tocopherol/ all-rac- α -Tocopherol	Reference
Dairy cows	2	Weiss, Hogan, & Wyatt (2009)
Piglets	2	Lauridsen et al. (2002)
Sows	1.5	Lauridsen et al. (2002)
Swine	2	Lauridsen et al. (2002)

Figure 2. Content of α-tocopherol in the muscle *Semimembranosus* after slaughter obtained in different studies.



3. Effect on productive parameters

Vitamin E is an essential nutrient which plays a significant role in many biological functions like reproduction or immunity. A dosage of 20 mg vitamin E/kg feed has been recommended in lamb for the normal function of the body (NRC, 2001). Under this supplementation level a decrease of livestock production parameters could be expected, besides the appearance of some diseases related to vitamin deficiency. Regarding to the effect of supra nutritional dosages on productive parameters, Maiorano et al. (2007) stated that this strategy may improve some productive parameters in very young animals. They observed a higher average daily gain (ADG) in suckling lambs receiving 150 IU/wk. intramuscular for eight weeks in comparison to those which only receive vitamin E from milk. A feasible explanation to these results could be the key role of vitamin E in the development of the immune system of young animals. Vitamin E concentration in milk mainly depends on diet, so the non-supplemented lambs might have consumed very low levels of vitamin E. The lack of this vitamin may make difficult the correct growth of these lambs. Anyway, further studies are needed to clarify the effect of vitamin E on productive parameters of very young lambs.

In contrast, conclusions in older lambs seem to be clearer. A large number of studies (Berthelot, Broudiscou, & Schmidely, 2014; Lauzurica et al., 2005; Liu, Ge, Luo, Yue, & Yan, 2013;Leal et al., 2018; Zhao et al., 2013) have demonstrated that supranutritional intakes of tocopherol do not exert any effect on average dairy gain, live weight, feed intake or feed efficiency. Moreover, neither carcass conformation nor fatness are affected by dietary vitamin E. Therefore, any modification of growth performance and carcass characteristics could be expected by increasing the content of tocopherol of the feed if the minimum requirements for normal growth and health are already satisfied.

4. Effect on meat quality

4.1 Muscle composition

The National Research Council recommends a content of 20 mg of DL-α-tocopherol/kg feed in order to satisfy the minimum nutritional requirements for normal growth and health of sheep (NRC, 2001). According to the existing bibliography, the concentration of α-tocopherol in muscle at this level of supplementation commonly ranges between 0.50 and 1.3 mg/kg (Figure 2).

The deposition of vitamin E in muscle depends on the length and the level of supplementation. Jose et al. (2016) measured the evolution of α-tocopherol concentration in muscle at different levels of supplementation (30, 150, 275, 400 IU vitamin E) through the feeding period (up to 8 weeks), registering a

continuous increase with time in each treatment, except in the lambs supplemented with 30 IU. This dosage satisfactorily prevented lambs from nutrition related diseases, but it was not enough to stimulate the deposition of tocopherol in muscle. The deposition rate of vitamin E depended on the level of supplementation, the more dosage, the faster deposition in the muscle. In fact, the content of α-tocopherol in muscle after 6 weeks was 2 fold higher in the lambs supplemented with 150 IU than in those receiving 30 IU, and it was even 3 fold higher in the animals that were fed at the highest level of supplementation (400 IU). The deposition of tocopherol through the feeding period showed a linear increase, the slope depending on the dosage; however, a plateau was reached after 5 weeks at the highest level of supplementation, which suggests the existence of a saturation point. At this time, the content of tocopherol in muscle was comprised between 5 and 6 mg/kg (Jose et al., 2016).

It is obvious that the higher dosage, the higher accumulation of tocopherol in muscle at the same period of supplementation. Nevertheless, visual inspection of the published α-tocopherol concentrations in muscle after slaughter (Figure 2) suggests a nonlinear relationship between vitamin E dose and α-tocopherol concentration in M. *longissimus*. The concentration of α-tocopherol in muscle reaches a plateau as the level of supplementation increases. Therefore, there is a point above which the rate of deposition of tocopherol in muscle decreases. Reaching this saturation level depends on either the period or the level of supplementation, so both parameters should be taken into account to establish a feeding strategy. A target concentration of α-tocopherol in muscle could be reached by different dosage-time combinations. Kasapidou et al. (2012) registered 3.73 mg α-tocopherol/kg muscle by a moderate level of supplementation (500 mg/kg) during 63 days. Increasing the level of supplementation to 1000 mg/kg allowed obtaining a similar concentration of tocopherol in muscle with a reduced time of supplementation. Álvarez et al. (2008) and De la Fuente et al. (2007) reached a muscle concentration of 3.57 mg α-tocopherol after 37 days while Bellés et al. (2018) quantified 3.91 mg even in a shorter period of supplementation (14 d). Vitamin E has a high cost, so optimizing a rate of supplementation is a key feature to achieve the maximum improvement in product quality with a lower cost.

Special attention has been given to reduce fat content and produce healthier meat in the recent years. One of these strategies consists of modifying meat fatty acid profile to obtain a higher content of polyunsaturated fatty acid (PUFA) and conjugated linoleic acid. Vitamin E has been described to modify both of them by acting at two different levels, ante-mortem and post-mortem. Vitamin E seems to play a role in rumen metabolism although it is not completely clear the mechanism and the specific modifications. Recent studies pointed at a modification of biohydrogenation processes, which would involve ruminal fatty acid hydrogenation. The modification of ruminal microbiota might be a possible mechanism by which vitamin E would affect ruminal processes (Hou, Wang, Wang and Liu, 2013). The addition of supranutritional doses of vitamin E to the feed could result in changes of specific fatty acids. Demirel et al. (2014) observed an increase in the proportions of C18:2 *n*-6, C20:5 *n*-3 and C22:6 *n*-3 together with a decrease of the percentages of C16:1 and C18:1 *n*-9, while Berthelot et al. (2014) only registered an increase in the proportion of C18:1 10*t*. Similarly, Hou et al. (2013) determined a higher percentage of C18:1 10*t* and C18:1 11*t* while the proportions of C18:1 *n*-9, C18:2 *n*-6 and C18:3 *n*-3 were lower. Nevertheless, the effect of vitamin E on modifying total percentage of saturated, monounsaturated or polyunsaturated fatty acids in lamb tissue seems to be low (Chen, Mao, Lin, and Liu, 2008) or even inexistent (Demirel et al., 2014; Kasapidou et al., 2009; Berthelot et al., 2014). Oxidative processes may modify fatty acid profile during display, depending meat susceptibility to oxidation on its fatty acid composition. As it is widely known, PUFA are more prone to oxidation than monounsaturated and saturated fatty acids due to their higher degree of unsaturation, decreasing the proportion of this kind of fatty acids during aerobic display (Álvarez et al., 2009, Bellés et al., 2018). Many studies focused on the effect of dietary vitamin E on fatty acid evolution trough display have registered a higher percentage of PUFA in meat from supplemented than from control

lambs after several days of aerobic display (Bellés et al. 2018, Alvarez et al., 2009; Chen et al., 2008). Supranutritional dosages of vitamin E stimulate the deposition of tocopherol into cell membranes, where it offers an enhanced protection of PUFA from oxidative degradation during display and therefore, it allows to preserve lamb nutritional value. In contrast, published data suggest the lack of effect of dietary vitamin E on modifying the percentage of intramuscular fat (Bellés et al., 2018; Kasapidou et al., 2012, Zhao et al. 2013).

4.2 Oxidative reactions

Meat is highly susceptible to oxidative deterioration because of the high content of unsaturated lipids, heme pigments, metal catalysts and a range of oxidizing compounds in the muscle tissue (Falowo, Fayemi, & Muchenje, 2014). Meat oxidation comprises both lipid and protein oxidative reactions. Lipid oxidative reactions start at the membrane level in the phospholipid fractions as a free-radical autocatalytic chain mechanism in which prooxidants interact with unsaturated fatty acids leading to the release of free radicals and the propagation of the oxidative chain. Regarding protein oxidation, it could be promoted by either the interaction with reactive oxygen species or indirect reactions with secondary products of oxidative stress (Faustman, Sun, Mancini, & Suman, 2010; Zhang, Xiao, & Ahn, 2013).

Vitamin E is a major *in vivo* antioxidant that prevents tissues from oxidative damage. This effect is transferred onto the meat after slaughter. The effect of vitamin E on lipid stability increases with dosage and therefore, with its concentration in muscle (Kasapidou et al., 2012; González-Calvo, Ripoll, Molino, Calvo, & Joy, 2015). Indeed, it has been observed a strong correlation (higher than -0.7) between the content of α-tocopherol in muscle and its stability against lipid oxidative reactions (Álvarez et al., 2008). Nevertheless, there is a threshold concentration above which any improvement against lipid oxidation could be noted. Álvarez et al. (2008) evaluated the effect of increasing dietary dosages of vitamin E (20, 270, 520 and 1020 mg vitamin E/kg feed) on lamb oxidation, without registering any additional improvement in lipid stability when α-tocopherol in muscle overpassed 2.26 mg/kg. Similarly, Lauzurica et al. (2005) and De la Fuente et al. (2007) did not observe any further improvement by increasing the concentration of α-tocopherol above 2.17 mg/kg meat. However, a lower threshold has been proposed by González-Calvo et al. (2015) (0.61-0.90 mg α-tocopherol/kg meat) and Kasapidou et al. (2012) (1.52 mg α-tocopherol/kg meat).

Disagreements in the target concentration could be related with differences in meat susceptibility to oxidation, which depends on the balance between pro-oxidants and antioxidants. Lipid oxidation is highly affected by several parameters such as muscle type, time, illumination and atmosphere composition during display, which could affect significantly the balance between meat prooxidants and antioxidants and therefore, influence the concentration of α-tocopherol needed to avoid oxidative processes. Differences in muscle susceptibility to oxidation have been associated to their content and activity of antioxidant enzymes, which vary among muscle types (Renerre, Dumont, & Gatellier, 1996). Packaging storage conditions are widely known to have a large effect on lipid and protein stability. The content of oxygen in the package atmosphere has a major role in oxidative reactions development; while oxygen enriched atmospheres provide an adequate environment to lipid oxidation progress, these reactions are highly inhibited when O_2 is removed from the package such as in vacuum or in CO_2 enriched atmospheres (Bellés et al., 2017). According to existing data, a concentration between 1.52 and 2.26 mg α-tocopherol/kg meat may be enough for completely inhibiting lipid oxidation when lamb is packaged in an enriched oxygen atmosphere and maintained in common display conditions (González-Calvo et al., 2014; Kasapiudou et al., 2012; Lauzurica et al., 2005), while the target concentration could be lower if the storage conditions are less favourable for lipid oxidation development (Kerry et al., 2000). Table 3 summarises the effect of muscle α-tocopherol on inhibiting lipid and protein oxidation during display.

Freezing extends meat shelf life by reducing microbial and chemical reactions rate. However, the formation of ice crystals during frozen storage involves a mechanical damage to muscle tissue as well as the breakage of cell membranes, releasing

compounds which enhance oxidative reactions. The deposition of tocopherol in cell membranes seems to be an effective means of stabilizing meat against lipid oxidation during frozen storage as well as through display of thawed lamb. A significant antioxidant effect of muscle tocopherol has been described even after 9 months (Bellés et al., 2017). Thus, the minimum concentration of vitamin E to obtain the maximum effect against lipid oxidation depends on several factors that modify the balance between pro-oxidants and meat antioxidants.

Oxidation of meat lipids and proteins widely contribute to the deterioration in flavour of meat products (Campo et al., 2006). TBARS has been described to be a good indicator of the development of rancid off-flavours. Campo et al. (2006) have related TBARS with human perception of rancid compounds, concluding that beef flavours were overpowered when TBARS values exceeded a value of 2 mg/kg. Ortuño, Serrano and Bañón (2015) registered values under this threshold in supplemented lamb maintained in common retail conditions even after 18 days, while it was widely exceeded in controls. The strong inhibition of oxidative reactions reduced the perception of rancid odour in chops from supplemented lambs, registering also a more intense meaty odour and as a result, a higher freshness. Similarly, Guerra-Rivas et al. (2016) and Muíño et al. (2014) pointed out the great effect of dietary vitamin E on preserving lamb sensory properties, since dietary vitamin E was an effective tool to preserve freshness and overall liking of fresh lamb by reducing the formation of rancid odours and flavours.

Dietary vitamin E also seems to exert a significant effect on delaying protein oxidation. Protein oxidation involves amino acid destruction, a decrease in protein solubility, loss of enzyme activity, and formation of carbonyls, resulting in a reduction of tenderness and juiciness, flavour deterioration, and discoloration (Zhang et al., 2013). A great reduction of carbonyl formation during storage in lamb chops containing 3.95 mg α-tocopherol/kg (Ortuño et al., 2015) and a delay of protein oxidation in lamb meat with a concentration of α-tocopherol of 2.42 mg/kg (Muíño et al., 2014) have been observed. Nevertheless, these levels are not high enough to completely inhibit carbonyl formation throughout display. Lipid and protein oxidation are cross linked reactions by which they could exacerbate each other (Faustman et al., 2010); therefore, the target concentration of α-tocopherol in muscle to completely inhibit oxidative reactions should be determined taking into account either lipid or protein oxidation processes.

4.3 Colour

Meat colour mainly depends on the quantity and the chemical state of its principal pigment, myoglobin. When this sarcoplasmic protein is associated with an oxygen molecule forms a complex called oxymyoglobin, which shows a bright red colour. In the absence of oxygen, it remains as the deoxymyoglobin form, having a purplish red colour (oxymyoglobin and deoxymyoglobin forms are interconvertible). Oxidation of Fe^{2+} to Fe^{3+} implies the conversion of the pigment into metmyoglobin, resulting in the change of a desirable red to a brown colour.

Myoglobin may be oxidised to metmyoglobin by different chemical pathways. Primary and secondary products of lipid oxidation have been identified as major causes of myoglobin oxidation, especially in muscles with high amounts of PUFA. Unsaturated fatty acids are known to be more susceptible to oxidation, leading to the release of free radicals that enhance meat discolouration. On the other hand, heme proteins could favour lipid oxidation. Nevertheless, lipid and protein oxidation are not always tightly interconnected. In low pO_2 atmospheres oxymyoglobin is rapidly converted to metmyoglobin while these conditions provide a high stability to lipids (Faustman et al., 2010). Therefore, myoglobin may oxidise either by interacting directly with reactive oxygen species or indirectly with secondary products of lipid oxidation.

A better appearance of meat from supplemented lambs through display can be explained by the effect of vitamin E on delaying the formation of metmyoglobin. The mechanism by which vitamin E inhibits the conversion of myoglobin to its oxidised form is not completely clear. The direct antioxidant action of α-tocopherol on protecting membrane lipids would reduce the formation of primary and secondary compounds of lipid oxidation, delaying indirectly myoglobin oxidation. On the other hand, vitamin E could

prevent the formation of metmyoglobin by acting against the propagation of free radicals. By both mechanisms, vitamin E has been demonstrated to reduce the conversion of myoglobin to metmyoglobin and therefore to protect lamb from colour fading (Bellés et al., 2018; Kerry et al., 2000; Lauzurica et al., 2005; Leal et al., 2018; Ripoll et al., 2011). Table 4 summarises the effects of dietary vitamin E on meat colour. Data from instrumental (Álvarez, et al., 2008; Bellés et al., 2018, Ripoll, Joy and Muñoz, 2011, Kerry et al., 2000) and sensory (Muela, Alonso, Campo, Sañudo, & Beltrán, 2014) colour analyses indicate the existence of a concentration-dependent effect; the higher α-tocopherol concentration in muscle, the higher colour stability. Nevertheless, it seems to exist a concentration above which any improvement in meat colour could be expected. Recent studies suggested a concentration of 3.5-4.0 mg α-tocopherol/kg tissue as the threshold for obtaining an improvement in lamb colour (Jose et al. 2016; Hopkins, Lamb, Kerr, Van der Ven, & Ponnampalam, 2013).

4.4 Aromatic compounds

The aroma of meat is the result of a very complex process. The primary reactions involved in the formation of aroma compounds are the oxidation of lipids, the degradation of thiamine, the Strecker reaction, and the Maillard reaction (Resconi, Escudero, & Campo, 2013). The aroma of meat is mainly developed upon heating treatment, where the precursors of aroma compounds (thiamine, glycogen, glycoproteins, nucleotides, nucleosides, free sugars/phosphate, amino acids, peptides, amines, organic acids and lipids) participate in through those primary reactions in forming intermediates, which can continue to react with other degradation products to form a complex mixture of volatiles, including those that are responsible for the aroma of meat (Imafidon & Spanier, 1994).

The action of vitamin E would indirectly affect the development of lamb aroma by reducing protein and lipid oxidation. The inclusion in the diet of 300 IU (Rivas-Canedo et al., 2013) is able to reduce the formation of 2-heptanone, 2-penten-1-ol, 2-octen-1-ol, pentane and heptane, deriving from lipid oxidation, and ethylbenzaldehyde, deriving from Strecker degradation. Aldehydes and ketones have a key impact in sensory perception due to their lower threshold. As an example, 2-heptanone has been associated to lamb flavour (Resconi et al., 2010).

Most studies in volatile compounds have focused on the effect that lipid composition has in aroma formation. The different ingredients in the diet have a clear effect in the fatty acid composition (Wood et al., 2003). Together with a higher PUFA composition, especially from *n*-3 PUFA, pasture based diets show increased levels of antioxidants. The effect of antioxidants in these diets is difficult to separate from the effect of the fatty acid composition, since the different fatty acids are able to develop different volatile compounds, in increasing number as the unsaturation of the fatty acid increases (Elmore et al., 2002). This increased number of aroma volatiles also implies a higher number of odour notes (Campo et al., 2003), including fishy notes that only appear when *n*-3 PUFA are involved.

4.5 Microbial growth

Microbial growth is one of the main causes of fresh meat spoilage. Commonly, bacteria responsible of this process are *Brochotrix thermospacta*, *Carnobaterium*, *Enterobacteriaceae*, *Lactobaciluus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens*. Microbial growth involves the consumption of meat specific compounds such as glycogen, glucose, lactic acid, amino acids or proteins and its conversion into a wide variety of metabolites responsible of the characteristic off odours and off flavours of spoiled meat. Moreover, other defects like discolouration, gas or slime production tend to appear in fresh meat when microbial counts reach 10^{7-8} ufc/g (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

Vitamin E is not likely to exert any effect against bacteria multiplication as no feasible bacteriostatic or bactericide mechanism has been described. Neither the addition of α-tocopherol directly on meat products (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007) nor the supplementation of lamb diet with DL- α-tocopherol resulted in lower total viable, *Enterobacteriaceae* or lactic acid bacteria counts trough display (De la Fuente et al., 2007; Muíño et al., 2014). Therefore, despite there is a limited number

of studies in which the effect of α-tocopherol on microbial growth has been evaluated, it could be concluded that vitamin E lacks of antimicrobial activity.

5. Conclusions

Vitamin E acts as a chain breaking antioxidant at the start site, in the lipid bilayer of muscle cells. This effect depends on concentration, which may be increased by supranutritional dosages, either by high dosages during short periods of time or low dosages during an extended period. Increasing dietary vitamin E does not affect growth performance or carcass characteristics if the minimum requirements for normal growth and health are already satisfied. Nevertheless, it could produce modifications in ruminal processes which seem to modify fatty acid hydrogenation, resulting in changes in the proportion of specific fatty acids in tissue. Increasing the content of muscle tocopherol is an effective means to protect PUFA and proteins from oxidation, therefore decreasing colour fading and off-odour formation in lamb meat.

A key feature is to reach a minimum muscle concentration enough to effectively inhibit oxidative reactions, which unfortunately depends on the different factors that affect the balance between meat pro-oxidants and antioxidants such as muscle type, packaging or temperature.

6. Conflict of interests

None to declare

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Table 3. Effect of muscle tocopherol on lipid and protein oxidation during display.

Muscle	Content of tocopherol (mg/kg)	MAP composition	Display	Effect on selected traits	Reference
Lipid oxidation					
Semimembranosus	3.91	70% O_2-30% CO_2	9 days at 4 °C	Vitamin E supplementation reduced at least half the content of MDA found in control samples (1.21 vs 2.83 mg MDA/kg)	Bellés et al. 2018
Longissimus thoracis et lumborum	3.95	70% O_2-30% CO_2	18 days at 2 °C	Tocopherol decreased TBARS values compared to control (1.03 vs 8.15 mg MDA/kg)	Ortuño et al., 2015
Longissimus dorsi	2.42	70 % O_2-30% CO_2	12 days at 2 °C	Dietary vitamin E inhibited MDA formation	Muiño et al., 2014
Semimembranosus	1.90	75% O_2-25% CO_2	6 days at 4 °C	This level seemed to be minimum to inhibit lipid oxidation	Kasapidou et al., 2012
Semimembranosus	0.90	75% O_2-25% CO_2	6 days at 4 °C	This concentration of tocopherol in muscle appeared inactive in preventing lipid oxidation	Kasapidou et al., 2012
Longissimus dorsi	2.17	70% O_2-30% CO_2	28 days at 2 °C	Muscle tocopherol reduced significantly lipid oxidation compared to control (1.5 vs 8 mg MDA/kg)	Lauzurica et al., 2005
Longissimus dorsi	3.57	70% O_2-30% CO_2	28 days at 2 °C	This concentration of tocopherol avoided lipid oxidation	Lauzurica et al., 2005
Longissimus dorsi	5.30	70% O_2-30% CO_2	10 days at 4 °C	Supplemented lamb had lower TBARS values than non-supplemented (3 vs 9 mg MDA/kg)	Kerry et al., 2000
Protein oxidation					
Longissimus thoracis et lumborum	3.95	70% O_2-30% CO_2	18 days at 2 °C	Muscle tocopherol prevented protein oxidation (3.16 vs 6.96 nmol carbonyl g^{-1} protein)	Ortuño et al., 2015
Longissimus dorsi	2.42	70% O_2-30% CO_2	12 days at 2 °C	Vitamin E samples showed lower carbonyl content than the controls	Muiño et al., 2014

MAP: Modified atmosphere packaging; MDA: Malondialdehyde; TBARS: Thiobarbituric reactive substances.

Table 4. Effect of muscle tocopherol on meat colour during display.

Muscle	Content of tocopherol (mg/kg)	MAP composition	Display	Effect on selected traits	Reference
Semimembranosus	3.91	70% O_2-30% CO_2	9 days at 4 °C	Vitamin E decreased MMb formation (12 %), obtaining a higher value of the 630/580 ratio (1.41 vs 1.18)	Bellés et al. 2018
Longissimus thoracis et lumborum	3.95	70% O_2-30% CO_2	18 days at 2 °C	Supplemented sampled showed higher chroma values and a better appearance	Ortuño et al., 2015
Semimembranosus	3.73	75% O_2-25% CO_2	6 days at 4 °C	Dietary vitamin E prevented meat from discolouration, resulting in higher chroma and a* values than control	Kasapidou et al., 2012
Longissimus dorsi	3.57	70% O_2-30% CO_2	28 days at 2 °C	A dosage of 1000 mg α-tocopherol/kg feed reduced MMb formation by half	Lauzurica et al., 2005
Longissimus dorsi	5.30	70% O_2-30% CO_2	10 days at 4 °C	Dietary vitamin E enhanced colour stability (lower proportions of MMb and higher a* values)	Kerry et al., 2000
Semimembranosus	3.5-4.0	Wrapping with chloride cling wrap	96 hours at 4 °C	No added benefit in meat colour was noted above this concentration	Jose et al., 2016

MAP: modified atmosphere packaging; MMb: metmyoglobin.

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4.1.2. Effect of dietary vitamin E on physicochemical and fatty acid stability of fresh and thawed lamb

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Effect of dietary vitamin E on physicochemical and fatty acid stability of fresh and thawed lamb

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ABSTRACT

This study evaluated the effect of dietary vitamin E supplementation (1000 mg of DL-α-tocopheryl acetate/kg of basal diet) on physicochemical and fatty acid stability of fresh and thawed lamb leg chops, frozen stored for 3, 6 and 9 months. Legs were chopped, modified atmosphere packaged (70% O_2/30% CO_2) and maintained under retail conditions (4 ± 0.5 °C, with 14 h fluorescent light) for 9 days. Muscle α-tocopherol concentration was over 3.5-fold higher in supplemented samples than in control lambs. The effect of dietary vitamin E was independent of frozen storage, so these effects were analysed separately. Vitamin E supplementation reduced lipid oxidation ($P \leq 0.001$) and decreased metmyoglobin formation, leading to a more attractive colour of meat. Moreover, supplementation led to a higher percentage of polyunsaturated fatty acids. Therefore, vitamin E supplementation could be recommended for preserving either fresh or thawed lamb.

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1. Introduction

Colour is the main sensory property of lamb used by consumers to select a product at the time of purchase (Jeremiah, 2001). A bright red colour is traditionally considered a positive aspect since it is associated with freshness and superior product quality (Berruga, Vergara, & Gallego, 2005). Fresh lamb for retail sale is therefore commonly packaged in an oxygen enriched atmosphere with the aim of enhancing this colour development (Bellés, Alonso, Roncalés, & Beltrán, 2017). A disadvantage of these packaging conditions is the development of lipid oxidation, which leads to a decrease in meat quality, concerning colour, aroma, flavour, texture and even fatty acid composition (particularly polyunsaturated) (Rodríguez-Carpena, Morcuende, & Estévez, 2011).

The inclusion of vitamin E in the basal compound feed of lambs during the last days before slaughter has been proposed to overcome these problems, obtaining promising results (Jose, Jacob, Pethick, & Gardner, 2016; Lauzurica et al., 2005; Ripoll, Joy, & Muñoz, 2011). Vitamin E is not degraded in the rumen (Leedle, Leedle, & Butine, 1993) and then, it accumulates in cell membranes and lipid depots, where it shows antioxidant activity (Liu, Scheller, & Schaefer, 1996). It has been satisfactorily applied to reduce lipid oxidation of fresh meat, showing better colour stability and reducing off odours and flavours (Kerry, O'Sullivan, Buckley, Lynch, & Morrissey, 2000; Ripoll et al., 2011). However, the knowledge about the effect of vitamin E on lamb quality after long-term frozen storage is scarce. Freezing is commonly used for long-term preservation as it results in a greater flexibility for distributors and retailers. Nonetheless, the possible negative effect of freezing and thawing on meat quality is still a point of concern. Primary compounds of lipid oxidation are formed during frozen storage, which lead to lipid oxidation secondary radicals after thawing. This phenomenon produces adverse changes on colour, odour, flavour and nutritional value (Owen & Lawrie, 1975). Thus, dietary vitamin E may be envisaged as a very suitable tool for inhibiting deteriorative oxidation of thawed lamb. The aim of this work was therefore to investigate the effect of dietary vitamin E on colour, lipid oxidation and fatty acid stability of fresh lamb and thawed lamb after frozen storage for three, six or nine months, maintained under retail conditions.

2. Materials and methods

2.1. General

The lambs used for this trial were reared in accordance with the guidelines from the Spanish Ministry of Agriculture (Boletín Oficial del Estado (BOE), 2007).

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2.2. Slaughtering, storage, packaging and refrigerated storage

Sampling procedure is shown in Fig. 1. Ninety-six male Rasa Aragonesa lambs, a medium wool breed that is reared for meat purpose in Spain, with an average body weight of 22.3 ± 0.25 kg were randomly allocated in 8 different pens (12 lambs per pen). Four pens were fed with a basal diet containing 30 mg/kg feed of DL-α-tocopheryl acetate (control feed, C), while the other four received the basal diet plus 1000 mg/kg feed of DL-α-tocopheryl acetate (high supplemented feed, HS). Ingredients and chemical composition of the concentrates are presented in Table 1. Lambs were offered *ad libitum* access to the experimental compound feed and wheat straw, for 14 days before slaughter. Thereafter, lambs were slaughtered at a local slaughterhouse with a body weight of 25.8 ± 0.71 kg and the carcasses were chilled during 24 h at 4 °C.

The right leg of each carcass was removed and assigned (12 carcasses of each feed treatment) to one of the four frozen storage durations: 0 days (fresh meat), 3, 6 and 9 months. Prior to freezing, lamb legs were vacuum packaged (−900 mbar of pressure) in polyethylene-polyamide bags with ethyl vinyl acetate sealant layer (30 × 25 cm, 90 μm thickness, water vapour transmission rate at 23 ± 1 °C of 2.8 g/m^2/24 h 85 ± 2% RH, an O_2 transmission rate at 23 ± 1 °C of 50 cm^3/m^2/24 h/bar 75 ± 2% RH and a CO_2 transmission rate at 23 ± 1 °C of 150 cm^3 /m^2/24 h/bar; Eurobag & Film S.L., Spain) using a Tecnotrip EV-13-L-CD-SC machine (Tecnotrip S.A., Spain) to prevent freezer burn and water losses. Frozen storage conditions (−20 °C) were monitored with a data logger Testo 175-H2 (Testo S.A., Spain).

After the corresponding frozen storage duration (0 d, 3, 6 or 9 months) legs were sliced in 20 mm chops in the facilities of Casa Ganaderos (Zaragoza, Spain) and then, chops were transported to the pilot plant of the Faculty of Veterinary (University of Zaragoza), where they were modified atmosphere packaged (70% O_2 and 30% CO_2) with a product to gas ratio of 1:3 (ULMA-SMART-500). Polystyrene trays were used and sealing was done with a polyethylene and polyamide laminate film (30 μm of thickness, water vapour transmission rate at 23 °C of <7 g/m^2/24 h/bar/85% relative humidity (RH), an O_2 transmission rate at 23 °C of <15 cm^3/m^2/bar 0% R.H. and a CO_2 transmission rate at 23 °C of <75 cm^3/m^2/24 h/bar 0% R.H.; Linpac Packaging S.L., Spain). Samples were displayed under retail conditions (4 °C ± 0.5 °C, with 14 h fluorescent light) during 9 days of storage. Chops from frozen legs thawed within the package during the display period at 4 °C.

Table 1
Ingredients and chemical composition of the concentrates.

	Control feed	High vit e supplemented feed
Raw material (%)		
Barley grain	28.5	28.5
Corn grain	32.0	32.0
Wheat grain	6.0	6.0
Toasted soybean meal	26.0	26.0
Beet molasses	2.0	2.0
Powder milk serum	1.0	1.0
Animal fats	1.0	1.0
Fatty acid's calcium salts	1.0	1.0
Calcium carbonate	2.0	2.0
Salt	0.5	0.5
Chemical composition (%)		
Crude protein	17.0	17.0
Crude fibre	3.9	3.9
Crude fat	4.0	4.0
Ash	6.0	6.0
Salt	0.2	0.2
Aditives		
Vitamin A	13000 UI/kg	13000 UI/kg
Vitamin D_3	3000 UI/kg	3000 UI/kg
DL-α-tocopheryl acetate	30 mg/kg	1000 mg/kg
Total oligoelements	199.05 mg/kg	199.05 mg/kg
Butilhidroxitoluene (BHT)	30 mg/kg	30 mg/kg

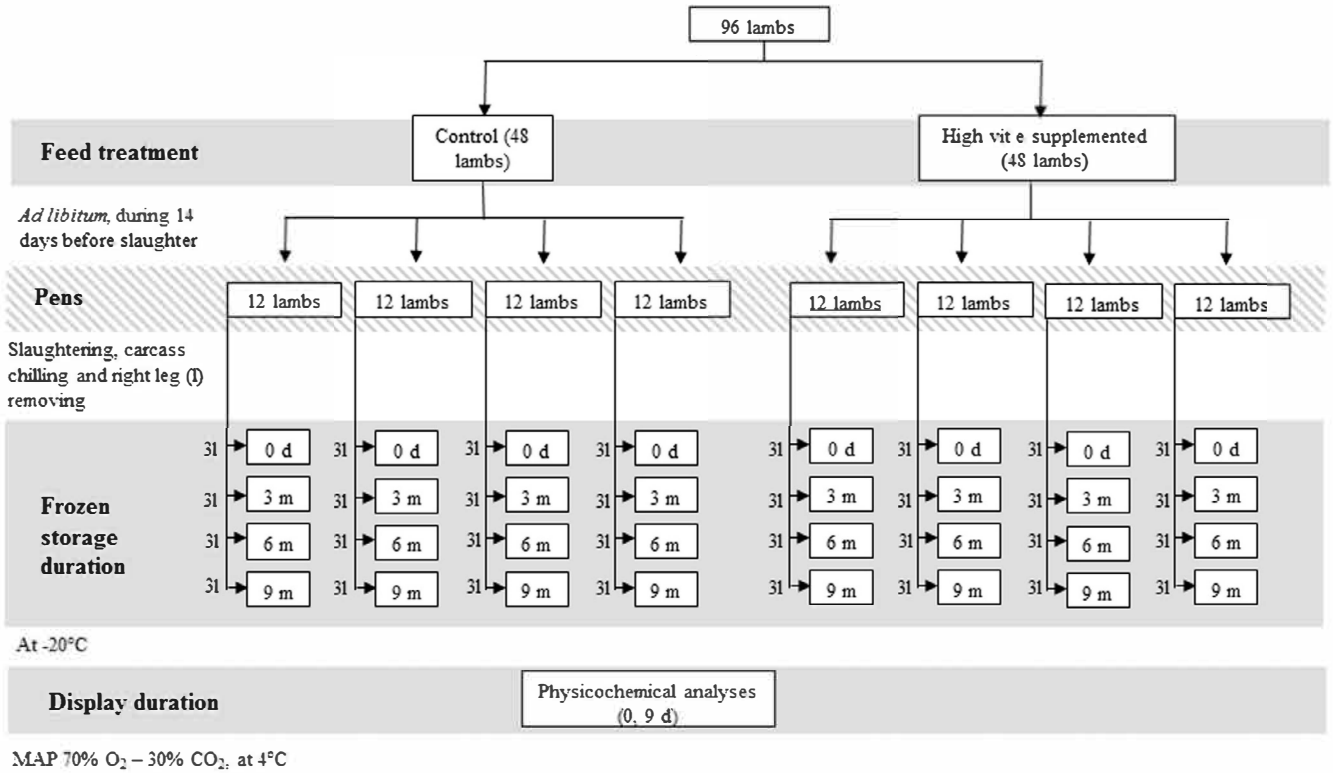

Fig. 1. Sampling procedure.

The content of α-tocopherol was measured in the muscle *semimembranosus* after carcass chilling. Instrumental colour, lipid oxidation and fatty acid profile analyses were performed at 0 and 9 days after packaging using only the muscle *semimembranosus*.

2.3. Content of α-tocopherol in semimembranosus

The content of α-tocopherol in tissue was determined following the methodology described by Kasapidou et al. (2012) and Liu et al., 1996. Samples were analysed by reverse phase HPLC (LiChrospher RP-18 column, 4.60 × 150 mm, 5 μm, 100 Å) with a mobile phase of isocratic methanol 100% at a constant flow rate of 1.2 ml/min and fluorescence detection (λexcitation = 295 nm and λemision = 330 nm; Agilent Series 1100) The injection volume was 20 μl and 9 min of run time. For peak identification and quantification (±)-α-tocopherol was used as external standard. Results were expressed as mg vitamin E/kg fresh meat.

2.4. Instrumental colour

A Minolta CM-2002 (Osaka, Japan) spectrophotometer was used for measuring colour at the surface of a 20-mm-thick chop just after opening the tray. The parameters registered were L^* (lightness), a^* (redness) and b^* (yellowness) values. A D65 illuminant was used at an observation angle of 10° and with a cell opening of 30 mm. Equipment was previously calibrated using a white and black standard. Ten measurements were done on each sample.

The hue angle (H^*) and chroma (C^*) indexes were calculated as: $H^* = \tan^{-1}(b/a)$ expressed in degrees, and $C^* = \sqrt{(a^2 + b^2)}$. The ratio 630/580 values were calculated dividing the percentage of light reflectance at wavelength 630 nm by the percentage of light reflectance at wavelength 580 nm. The relative content of metmyoglobin was calculated from the reflectance curve according to Krzywicki (1979) using 690 nm (the highest wavelength of the instrument).

2.5. Lipid oxidation

Lipid oxidation was determined following the methodology described by Alonso et al. (2015).

2.6. Intramuscular fat and fatty acid analysis

The muscle *semimembranosus* was ground and 10 g were used to extract the fat in chloroform-methanol (1:1 v/v), with 2,6-di-*tert*-butyl-4-methylphenol (BHT) (1 g/10 ml methanol) as antioxidant (Bligh & Dyer, 1959). One millilitre of chloroform phase was used to assess the percentage of intramuscular fat (IMF) by drying at 100 °C for 20 min; the results were expressed as the weight percentage of wet muscle. The rest was evaporated in a sand bath under nitrogen gas at 50 °C. The fatty acid methyl esters (FAMES) were formed using a KOH solution in methanol and collected in hexane for analysis by gas chromatography following the methodology described by Carrilho, López, and Campo (2009). The FAMES were analysed in a gas chromatograph HP-6890 II (Hewlett-Packard, Waldbronn, Germany) using a capillary column SP-2380 (100 m × 0.25 mm × 0.20 μm), and oven temperature programming as follows: column temperature was set at 140 °C, then raised at a rate of 3 °C/min from 130 to 158 °C, and 1 °C/min to 165 °C, kept for 10 min, raised at 5 to 220 °C and kept constant for 50 min. Nitrogen was used as a gas carrier at a constant flow rate of 0.8 ml/min with an injected volume of 1 μl. The methyl esters were identified using retention times of Supelco® 37 Component FAME Mix. Data regarding FAMES composition was expressed as area percentage of total identified FAMES. Fatty acid C19:0 was used as the internal standard for quantification. All chemicals were supplied by Sigma-Aldrich.

2.7. Statistical analysis

The effects of feed treatment (FT), frozen storage duration (FSD), display duration (D), and their interactions on meat quality traits, were assessed using the GLM procedure of the SPSS statistical package, version 19.0 (IBM SPSS, 2010), with pen as random effect. The model is as follows:

$$Y_{ijkl} = \mu + FT_i + FSD_j + D_k + A_l + (FT_i \times FSD_j) + (FT_i \times D_k) + (FSD_j \times D_k) + (FT_i \times FSD_j \times D_k) + e_{ijkl}$$

where Y_{ijkl} is the dependent variable; μ is the population average; FT_i is the fixed effect of the feeding treatment (C, HS); FSD_j is the fixed effect of frozen storage duration (0 d, 3, 6, 9 months); D_k is the fixed effect of display duration (0, 9 d); A_l is the random effect of pen; $(FT_i \times FSD_j)$ is the interaction effect of feed treatment and frozen storage duration; $(FT_i \times D_k)$ is the interaction effect of feed treatment and display duration; $(FSD_j \times D_k)$ is the interaction effect of frozen storage duration and display duration; $(FT_i \times FSD_j \times D_k)$ is the interaction effect of feed treatment, frozen storage duration and display duration; and e_{ijkl} is the aleatory error. Differences were declared significant when $P \leq 0.05$.Tukey's post hoc test was used to assess differences between mean values when $P \leq 0.05$.

There was not any interaction between the effects of frozen storage conditions and feed treatment on the variables measured, so both effects were analysed separately. However, significant interactions ($P \leq 0.001$) between each one of them and display time were noted for lipid oxidation and colour, so a second model was designed for these variables where the effect of frozen storage conditions and feed treatment were analysed within the day of display and vice versa. This model determined the quality differences among treatments in each day of study and it also assessed changes of each treatment over the days of study.

Fatty acid profile was not affected by the interactions among the fixed effects so the effects of frozen storage conditions, feed treatment and display duration on fatty acid profile were analysed separately.

3. Results and discussion

3.1. Content of α-tocopherol in muscle

Table 2 shows the content of α-tocopherol in muscle at slaughter. Following the recommendations of the Agricultural Research Council (ARC, 1980), the basal diet contained 30 mg of DL-α-tocopherol/kg in order to satisfy the minimal nutritional requirements for normal growth and health of sheep. The content of α-tocopherol in *semimembranosus* at this vitamin E level was 1.057 mg/kg meat, which agreed with previously published data. Both Alvarez et al. (2009) and Lauzurica et al. (2005) quantified 0.95 mg of α-tocopherol per kg of muscle in lambs fed with a basal diet. Kasapidou et al. (2012) also registered a similar concentration in muscle at slaughter (0.73 mg/kg). Compared with the concentration of vitamin E obtained with the basal diet, the muscle deposition of vitamin E strongly increased with the supplementation of lambs with 1000 mg/kg of DL α-tocopheryl acetate ($P < 0.001$). As a result, muscle vitamin E concentration at slaughter was over 3.5-fold higher in supplemented than in control lambs.

The deposition of vitamin E in muscle depends on the length and the level of supplementation (González-Calvo, Ripoll, Molino, Calvo, & Joy, 2015; Jose et al., 2016). Kasapidou et al. (2012) obtained a concentration in muscle (3.73 mg α-tocopherol/kg muscle) similar to that we found using a lower level of vitamin E (500 mg/kg) but extending the period of supplementation to 63 days. In contrast, Alvarez et al. (2009) and Lauzurica et al.

Table 2
M. semimembranosus α-tocopherol concentration after slaughter of high (1000 mg of DL-α-tocopheryl acetate/kg of feed) and low supplemented (30 mg of DL-α-tocopheryl acetate/kg of feed) (control) lambs.

	C	HS	SEM	*P*
Treatments[1]				
α-tocopherol (mg/kg)	1.057[a]	3.908[b]	0.201	<0.001

C = Control feed; HS = high supplemented feed.
Values within a row with different superscript (a, b) are significantly different ($P < 0.05$).
[1] Means; SEM = standard error of the mean.

(2005) observed a similar deposition with a supplementation length of 37 days and a level of vitamin E of 1000 mg/kg.

Jose et al. (2016) established a concentration of 3.5–4.0 mg α-tocopherol/kg tissue as the threshold above which no added benefit of vitamin E on meat colour can be expected, while Álvarez et al. (2008) did not observe any difference in inhibiting lipid oxidation when muscle α-tocopherol concentration overpasses 1.87 and 2.37 mg/kg meat. Our results showed that these concentrations of vitamin E in muscle may be reached already after 14 days of high vitamin E level supplementation, which would represent a significant economical saving. Either natural or artificial vitamin E has a high cost, so optimizing a rate of supplementation is the key to achieve the maximum improvement in product quality without an unnecessary cost (González-Calvo et al., 2015).

3.2. Instrumental colour

3.2.1. Effect of dietary vitamin E

Dietary vitamin E supplementation did not modify Hue, Chroma, metmyoglobin (MMb) content or the 630/580 index before display but its effect on colour after 9 days was significant (Table 3). Increasing the rate of supplementation resulted in lower values for Hue and higher for the ratio 630/580 than those measured in control samples at 9 days post-packaging ($P \leq 0.001$). Similar results were obtained by Ripoll et al. (2011) when they evaluated the effect of vitamin E on lamb colour. These results could be explained by the different content of metmyoglobin noted in control and vitamin E supplemented samples after display ($P \leq 0.001$). In fact, higher contents of metmyoglobin are related to changes in colorimetric parameters, such as an increase of Hue or a decrease of the 630/580 ratio (Liu et al., 1996). According to Lawrie (1998), a brownish discolouration can be perceived onto meat surface when 60% of meat pigment is oxidised to metmyoglobin. This percentage was exceeded in control samples after 9 days of display, while dietary vitamin E allowed maintaining a better colour for a longer period of time. However, Ripoll et al. (2011) observed that consumers rejected lamb when the value of the index 630/580 fell below 2.23, which was observed either in control or supplemented lamb. Therefore, despite the great effect of vitamin E on preventing discolouration, the concentration of α-tocopherol reached in muscle was unable to maintain a desirable colour during 9 days of display.

3.2.2. Effect of frozen storage

As is shown in Table 3, significant differences were not found in H* and 630/580 values between fresh and thawed lamb either before or after display. Concerning the content of metmyoglobin found in fresh lamb before display, it was similar to values previously registered in fresh meat (Muela, Alonso, Campo, Sañudo, & Beltrán, 2014; Ripoll et al., 2011). These initial values did not differ significantly from those measured in thawed meat. Moreover, frozen storage, prior to thawing, did not result in an increase of metmyoglobin content after display. At this time, differences were only found in Chroma, showing fresh lamb the highest values ($P \leq 0.05$).

3.3. Lipid oxidation

3.3.1. Effect of dietary vitamin E

The effect of dietary vitamin E on TBARS index is shown in Table 3. There was no difference between both treatments before

Table 3
Effect of dietary vitamin E supplementation and frozen storage time (0 d, 3, 6, and 9 months) on physicochemical properties of *semimembranosus* muscle from lamb displayed for 0 or 9 d.

[1]Physicochemical parameters	Day of display	Frozen storage time						Dietary vitamin E			
		Fresh (0d)	3 m	6 m	9 m	SEM	$P_{frozen\ storage}$	C	HS	SEM	P_{feed}
C*	0	16.53^{y}	14.81	15.47^{y}	15.88^{y}	0.273	0.061	14.84^{y}	15.51^{y}	0.47	0.332
	9	14.68^{bx}	12.51^{a}	13.30^{ab,x}	13.35^{ab,x}	0.22	0.009	13.74^{x}	13.18^{x}	0.34	0.252
	$P_{display}$	<0.001	0.651	0.007	0.013			<0.001	<0.001		
H*	0	45.27^{x}	43.73^{x}	42.69^{x}	44.16^{x}	0.483	0.301	46.86^{x}	45.56^{x}	0.83	0.282
	9	69.72^{y}	66.96^{y}	70.76^{y}	72.19^{y}	1.38	0.592	74.48^{b,y}	65.34^{a,y}	1.70	<0.001
	$P_{display}$	<0.001	<0.001	<0.001	<0.001			<0.001	<0.001		
% MMb	0	17.27^{x}	16.99^{x}	19.23^{x}	19.86^{x}	0.475	0.121	19.71^{x}	20.97^{x}	0.81	0.282
	9	59.64^{y}	53.83^{y}	60.37^{y}	59.03^{y}	1.75	0.545	64.13^{b,y}	52.31^{a,y}	2.15	<0.001
	$P_{display}$	<0.001	<0.001	<0.001	<0.001			<0.001	<0.001		
630/580	0	2.78^{y}	2.56^{y}	2.85^{y}	2.85^{y}	0.057	0.260	2.68^{y}	2.84^{y}	0.08	0.182
	9	1.26^{x}	1.35^{x}	1.24^{x}	1.33^{x}	0.040	0.724	1.18^{a,x}	1.41^{b,x}	0.05	<0.001
	$P_{display}$	<0.001	<0.001	<0.001	<0.001			<0.001	<0.001		
mg MDA/kg of meat	0	0.07^{a,x}	0.10^{b,x}	0.11^{b,x}	0.11^{b,x}	0.11	<0.001	0.13^{x}	0.12^{x}	0.13	0.429
	9	1.75^{y}	2.25^{y}	2.26^{y}	1.80^{y}	0.30	0.442	2.83^{b,y}	1.21^{a,y}	0.11	<0.001
	$P_{display}$	<0.001	<0.001	<0.001	<0.001			<0.001	<0.001		

% MMb = percentage of metmyoglobin; MDA = malondialdehyde; C = Control feed; HS = High supplemented feed.
[a,b,c] Values within a row with different superscript are significantly different (within treatment);
[x,y,z]Values within a column with different superscript are significantly different (within days of display) ($P < 0.05$).
[1] Means; SEM = standard error of the mean.

display, which may be due to a residual activity of muscle enzymes post-mortem (Renerre, Dumont, & Gatellier, 1996) and the presence of cell antioxidant compounds. However, control samples showed a higher malondialdehyde content after 9 days ($P \leq 0.001$). The large amount of malondialdehyde measured in controls at this time agreed with bibliography (Lauzurica et al., 2005) and it could be related to the high percentage of O_2 in the atmosphere used for this trial, which has been described to enhance lipid oxidation (O'Grady, Monahan, Burke, & Allen, 2000). In contrast, oxidative reactions were significantly inhibited by the inclusion of vitamin E in the basal feed ($P < 0.001$). Dietary vitamin E supplementation reduced at least half the content of MDA found in control samples after 9 days of display.

The effectiveness of vitamin E on reducing lipid oxidation in fresh lamb had been already described. Ripoll et al. (2011) reported that treatments with vitamin E kept low values of MDA (0.1–0.23 mg MDA/kg) while control treatments reached values greater than 1.5 mg/kg. Lauzurica et al. (2005) and Muela et al. (2014) also found a strong inhibition of lipid oxidation by adding vitamin E in the basal feed. According to Álvarez et al. (2008), a concentration of α-tocopherol, comprised between 1.87 and 2.37 mg α-tocopherol / kg meat, may be enough for optimum improvement against lipid oxidation, while González-Calvo et al. (2015) suggested that it could be even lower (0.61–0.90 mg α-tocopherol/kg muscle). Nevertheless, our results showed a development of lipid oxidation reactions with a higher content of α-tocopherol in muscle (3.908 mg α-tocopherol/kg muscle). These differences may have been related to different storage conditions, which pointed out that the minimum concentration of α-tocopherol in muscle for completely inhibiting lipid oxidation depends on storage conditions and it may be higher under common retail conditions (high oxygen content atmospheres and 14 h of fluorescent light). In fact, oxidation affects colour, odour, flavour, texture and nutritional value, and it finally compromises lamb shelf life. Products of lipid oxidation have been associated with off flavours and off odours (Jeremiah, 2001), which decrease meat quality by overpowering its characteristic flavour and odour. TBARS has been described to be a good indicator of the development of rancid off-flavours. Several researchers have related TBARS values with human perception of rancid compounds. Greene and Cumuze (1981) observed that untrained panellists accepted beef with TBARS values up to 2 mg/kg. Similarly, Campo et al. (2006) noted that beef flavours were overpowered when TBARS values exceeded a value of 2 mg/kg, so this point was proposed as the maximum level for positive sensory perception of beef. In our trial, control samples widely exceeded the threshold for the acceptability of oxidized beef of 2 mg MDA/kg; meanwhile a dietary dosage of 1000 mg of α-tocopherol/kg was enough to maintain oxidation levels under the limit of acceptance after display. Therefore, supplemented lamb is expected to keep better characteristics of odour and flavour, obtaining a good acceptance of consumers.

3.3.2. Effect of frozen storage

Table 3 also shows the effect of frozen storage duration before thawing on lipid oxidation of lamb. TBARS values before display were lower in fresh than in thawed lamb ($P \leq 0.001$). Initial differences between fresh and thawed lamb had been already described (Muela, Sañudo, Campo, Medel, & Beltrán, 2010) probably because of damage caused to cellular structure during freezing, frozen storage and thawing or as a result of MDA accumulation during frozen storage. Lipid oxidation has been widely described to accelerate after thawing, as peroxidation (primary lipid oxidation) is not stopped during frozen storage giving rise to rapid and severe secondary lipid oxidation (thiobarbituric acid forming), which results in increased TBARS values (Owen & Lawrie, 1975). In this study, however, there were no significant differences in MDA content among treatments after 9 days of display. Similar to our results, Alonso et al. (2016) and Leygonie, Britz, and Hoffman (2012) did not register differences in TBARS values among fresh and thawed meat after frozen storage during different periods of time. Muela et al. (2010) neither registered any significant difference between fresh and 3 months frozen storage meat after 10 days of display but differences became significant when thawed meat had been kept frozen up to 6 months. The lack of a higher content of MDA in thawed samples could be explained due to its consumption in other chemical pathways. According to Leygonie et al. (2012) the interaction between lipid and protein oxidation presumably leads to a lower TBARS value as malondialdehyde(MDA) acts as a substrate in one of the pathways of protein oxidation.

3.4. Intramuscular fatty acid profile

3.4.1. Effect of dietary vitamin E

Vitamin E supplementation did not modify the content of intramuscular fat (Table 4), which was in agreement with the findings of Kasapidou et al. (2012) and Zhao et al. (2013). Nevertheless, there were some significant effects of the supplementation with vitamin E on fatty acid composition of intramuscular fat in *semimembranosus*.

Supplementation with α-tocopherol resulted in a higher percentage of PUFA ($P \leq 0.001$) (Table 4), which was previously reported by Alvarez et al. (2009) and Chen, Mao, Lin, and Liu (2008). The higher percentage of PUFA found in vitamin E added samples may be related to its antioxidant activity. A relationship among *in vivo* low tissue concentrations of vitamin E and lower amounts of both n-6 and n-3 PUFA has been demonstrated (Kasapidou, Wood, Sinclair, Wilkinson, & Enser, 2001), which suggests that PUFA decrease appears *in vivo* when antioxidant concentrations are low. Unsaturated fatty acids are known to be more susceptible to oxidation so the inclusion of dietary vitamin E in the basal diet may have protected PUFA from these reactions, obtaining a higher PUFA percentage in these samples than in controls. According to Alvarez et al. (2009) the supplementation with 250 mg acetate-R- tocopherol/kg feed is enough to prevent PUFA from oxidation, while Demirel et al. (2004) noted significant differences in intramuscular PUFA proportions when the concentration of α-tocopherol in muscle increased from 0.27 mg/g to 0.52 mg/g. Regarding individual PUFA percentages, supplemented samples presented significant higher proportions of C18:2 n-6, C20:3 n-6 and C20:4 n-6. In a similar way, Zhao et al. (2013) registered a higher proportion of C18:2 and C20:3 in vitamin E supplemented lambs while Alvarez et al. (2009) also found a significant effect of vitamin E on preserving C18:2 n-6. This fatty acid is considered essential to humans (Beare-Rogers, 1988) and vitamin E supplementation seems to protect it against degradation.

The oxidation of PUFA in control samples may have led to a relative increase of the percentage of total SFA ($P \leq 0.001$), as well as a significant higher percentage of some individual saturated fatty acids (C10:0, C16:0, C18:0). Regarding the percentage of C18:1 n-9 fatty acid, as it was previously noted by Zhao et al. (2013), a lower content was found in vitamin E supplemented than in control samples ($P = 0.039$). This result could be explained by the findings of Hou, Wang, Wang, and Liu (2013), who reported that vit E supplementation may accelerate the ruminal biohydrogenation of C18:1 unsaturated fatty acids *in vitro*. In contrast, no effect on fatty acid profile was reported by Berthelot, Broudiscou, and Schmidely (2014) and Kasapidou et al. (2012), which suggests the need of more studies about this topic.

3.4.2. Effect of frozen storage

As it is widely known, PUFA are particularly susceptible to oxidative processes and enzymatic reactions are not completely

Table 4
Effect of dietary vitamin E supplementation, frozen storage time (0d, 3, 6 and 9 months) and display (0 or 9 d) on intramuscular fatty acid profile (percentage of total identified fatty acids) and intramuscular fat content of *semimembranosus* muscle from lamb (g fat/100 g of meat).

Fatty acid[1]	Frozen storage time						Dietary vitamin E				Display			
	0 d	3 m	6 m	9 m	SEM	*P*-value	C	HS	SEM	*P*-value	0d	9d	SEM	*P*-value
C10:0	0.13^{b}	0.08^{a}	0.11^{b}	0.12^{b}	0.03	<0.001	0.12^{b}	0.10^{a}	0.03	0.019	0.11	0.11	0.04	0.340
C12:0	0.18^{ab}	0.14^{a}	0.26^{b}	0.23^{b}	0.01	0.001	0.22	0.19	0.01	0.237	0.20	0.20	0.11	0.917
C14:0	2.28^{ab}	1.96^{a}	2.64^{b}	2.60^{b}	0.06	0.001	2.47	2.26	0.06	0.135	2.35	2.39	0.07	0.798
C15:0	0.42^{a}	0.47^{ab}	0.49^{b}	0.48^{b}	0.01	0.009	0.47	0.45	0.01	0.401	0.46	0.47	0.08	0.512
C16:0	20.52^{b}	19.01^{a}	20.11^{b}	20.05^{b}	0.123	<0.001	20.34^{b}	19.51^{a}	0.12	0.002	19.65^{a}	20.20^{b}	0.13	0.036
C16:1	1.99^{b}	1.64^{a}	2.05^{b}	1.98^{b}	0.03	<0.001	1.93	1.90	0.07	0.647	1.90	1.93	0.03	0.635
C17:0	1.47^{a}	1.83^{b}	1.73^{ab}	1.69^{ab}	0.04	0.007	1.68	1.67	0.04	0.918	1.67	1.69	0.04	0.819
C18:0	11.97	12.64	12.19	12.27	0.13	0.338	12.59^{b}	11.94^{a}	0.13	0.014	12.28	12.26	0.13	0.946
tC18:1 n-9	6.27^{ab}	6.01^{a}	5.97^{a}	7.38^{b}	0.17	0.015	6.33	6.48	0.17	0.663	6.35	6.46	0.18	0.775
C18:1 n-11	0.13^{a}	0.30^{c}	0.26^{bc}	0.21^{ab}	0.01	<0.001	0.22	0.24	0.11	0.296	0.22	0.23	0.01	0.702
C18:1 n-9	33.80	31.48	32.30	31.23	0.26	0.103	32.91^{b}	31.49^{a}	0.26	0.009	31.67	32.74	0.27	0.062
C18:2 n-6	8.52^{a}	10.57^{b}	9.25^{a}	9.43^{ab}	0.17	0.001	8.74^{a}	10.14^{b}	0.18	<0.001	9.89^{b}	9.00^{a}	0.18	0.018
C20:1	0.16	0.18	0.17	0.16	0.01	0.432	0.16	0.17	0.00	0.072	0.17	0.16	0.01	0.653
C18:3 n-3	0.57	0.55	0.57	0.52	0.01	0.425	0.57	0.53	0.02	0.277	0.59	0.52	0.02	0.101
C20:3 n-6	0.23^{a}	0.29^{b}	0.26^{ab}	0.25^{ab}	0.01	0.011	0.24^{a}	0.28^{b}	0.01	0.005	0.28^{b}	0.23^{a}	0.07	0.001
C20:4 n-6	2.32^{a}	3.23^{b}	2.72^{b}	2.55^{ab}	0.09	0.008	2.34^{a}	3.07^{b}	0.13	<0.001	3.03^{b}	2.38^{a}	0.09	0.001
C20:5 n-3	0.28	0.29	0.34	0.26	0.00	0.617	0.30	0.28	0.03	0.600	0.34^{b}	0.24^{a}	0.02	0.027
C22:6 n-3	0.20	0.23	0.22	0.18	0.00	0.402	0.19	0.23	0.02	0.135	0.25^{b}	0.17^{a}	0.01	<0.001
C22:5 n-3	0.60	0.72	0.59	0.54	0.02	0.085	0.58	0.64	0.02	0.310	0.68^{b}	0.54^{a}	0.22	0.003
∑SFA	37.84	37.21	38.51	38.30	0.12	0.125	38.86^{b}	37.08^{a}	0.47	<0.001	37.72	38.22	0.21	0.237
∑MUFA	44.99	43.69	43.67	43.86	0.25	0.063	44.31	43.31	0.27	0.073	43.15^{a}	44.65^{b}	0.33	0.001
∑PUFA	13.75	16.51	14.48	14.27	0.34	0.125	13.49^{a}	16.01^{b}	0.37	<0.001	15.88^{b}	13.62^{a}	0.29	0.002
∑n-6	12.07	14.64	12.71	12.72	0.09	0.068	11.79^{a}	14.28^{b}	0.46	<0.001	13.96^{b}	12.10^{a}	0.07	0.009
∑n-3	1.68	1.88	1.77	1.55	0.08	0.495	1.70	1.74	0.40	0.815	1.92^{b}	1.52^{a}	0.07	0.015
IMF (%)	2.78	2.48	2.78	2.70	0.07	0.418	2.82	2.85	0.10	0.057	2.75	2.62	0.12	0.450

C = Control feed; HS = High supplemented feed; ∑SFA = saturated fatty acids summation; ∑MUFA = monounsaturated fatty acids summation; ∑PUFA = polyunsaturated fatty acids summation; ∑n-6 = polyunsaturated n-6 fatty acids summation; ∑n-3 = polyunsaturated n-3 fatty acids summation; IMF (%) = percentage of intramuscular fat.
[a,b,c]Values within a row with different superscripts are significantly different ($P < 0.05$).
[1] Means (percentage of total fatty acids); SEM = standard error of the mean.

inhibited during frozen storage. Therefore, it was expected a decrease in the percentage of PUFA due to lipolysis and oxidative reactions, producing a relative increase in the percentages of MUFA and SFA. In fact, this is what Alonso et al. (2016) found in pork after long-term frozen storage. Hernández, Navarro, and Toldrá (1999), who reported a significant increase in free fatty acids during frozen storage, identified phospholipids hydrolysis as the major cause of this phenomenon. Alonso et al. (2016) also considered oxidative reactions a significant cause of the reduction of PUFA during frozen storage. Contrary to what was expected, frozen storage did not modify the percentage of total SFA, MUFA or PUFA (Table 4). Similar to our results Zymon, Strzetelski, Pustkowiak, and Sosin (2007) did not find any effect of frozen storage on meat fatty acid profile. A feasible explanation to our results could be that legs were vacuum packaged prior to being frozen, so it may have inhibited oxidative reactions (Table 3).

3.4.3. Effect of display

Display led to a significant decrease in the percentage of C20:5 n-3, C22:6 n-3, C22:5 n-3, C18:2 n-6, C20:3 n-6, C20:4 n-6, as well as in the summary of n-3, n-6 and total polyunsaturated fatty acids (Table 4). As it has been previously mentioned, PUFA are more susceptible to degradation, especially long chain fatty acids with a higher degree of unsaturation, so oxidative reactions throughout display may have been responsible of this decrease. In fact, malondialdehyde content was significantly higher after the storage period. Alvarez et al. (2009) studied the changes in fatty acid profile of vitamin E supplemented lamb packaged in modified atmosphere and displayed during 28 days, registering great changes in PUFA percentage. They noted a reduction in the percentage of n-3 and n-6 fatty acids of non-supplemented samples throughout display, which was attributed to lipid oxidation. In contrast, no changes were measured in the vitamin E high supplemented samples (1000 mg acetate-R-tocopherol/kg feed), which demonstrated the relationship between antioxidant content, oxidative reactions and PUFA content.

4. Principal components analysis

The first two components of the principal component analysis (PCA) explained 56.98% of the variability of the results (Fig. 2). The first component (PC1), which was able to explain 33.65% of the variation of the whole study, was characterised by the variables metmyoglobin, Hue, and thiobarbituric acid reactive substances (TBARS) in the positive side and ∑PUFA, ∑n-6, and the index 630/580 in the negative side. This component divided the observations in two main groups depending on the day of display. The centroids of the observations at 0 days of storage were mainly on the left quadrants of the figure, closely related to ∑PUFA, ∑n-6, ∑n-3 and the index 630/580; meanwhile, the observations at day 9 were located on the right side, characterized by the % of metmyoglobin, ∑SFA, Hue and TBARS. Display significantly affected colour, lipid oxidation (Table 3), ∑PUFA, ∑n-6 and ∑n-3 (Table 4). A significant positive correlation was found between lipid oxidation and the percentage of metmyoglobin (0.393) and significant strong correlations were also registered among the latter and H* (0.94) and the index 630/580 (−0.910).

Enriched oxygen atmospheres have been described to favour lipid oxidation, which increased throughout display in all conditions evaluated in this trial (Table 3). Primary and secondary products of lipid oxidation enhance myoglobin oxidation, leading to its conversion into metmyoglobin and resulting in meat discolouration (Faustman, Sun, Mancini, & Suman, 2010). Polyunsaturated fatty acids are known to be more susceptible to oxidative reactions which could explain its decrease during display (Ródriguez-Cárpe na et al., 2011). Similarly, Álvarez et al. (2009) noted a reduction in the percentage of n-3 and n-6 fatty acids throughout display, which was attributed to lipid oxidation. In fact, a significant nega-

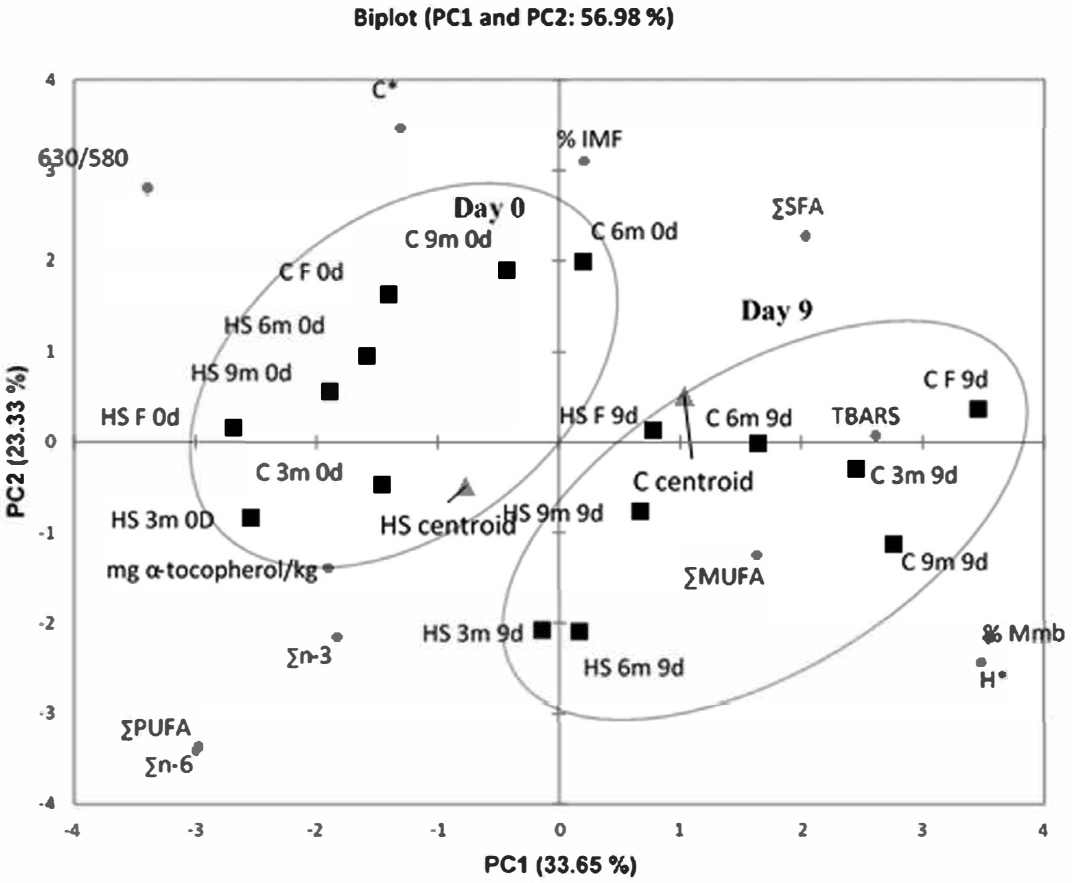

Fig. 2. Projection in the plane of physicochemical parameters, fatty acid groups and the centroids of the observations defined by two principal components.HS: High rate of vitamin E supplementation (1000 mg α-tocopherol/kg), C: Control feed (30 mg α-tocopherol/kg), Frozen storage duration: F (fresh meat), 3 m (3 months), 6 m (6 months), 9 m (9 months), Days of display after frozen storage: 0 d or 9 d. % MMb: percentage of metmyoglobin, TBA: Thiobarbituric acid reactive substances, ∑SFA: saturated fatty acids summation; ∑MUFA: monounsaturated fatty acids summation; ∑PUFA: polyunsaturated fatty acids summation; ∑n-6:polyunsaturated n-6 fatty acids summation; ∑n-3: polyunsaturated n-3 fatty acids summation; IMF (%): percentage of intramuscular fat.

tive correlation was observed between TBARS and ∑PUFA (−0.326).

Moreover, a discrimination between high and low vitamin E supplemented samples within each day could be noted. The observations of the animals supplemented with 1000 mg α-tocopherol/kg were at the left of those which were supplemented with 30 mg at both days. As a result, the centroid of the animals with a high level of supplementation was located in the lower left-hand quadrant, which was related with ∑PUFA, ∑n-6, ∑n-3 and the concentration of α-tocopherol in muscle. The centroid of the animals supplemented with a dosage of 30 mg α-tocopherol/kg was on the opposed quadrant, defined by the percentage of intramuscular fat (%IMF), ∑SFA and the TBARS.

Significant negative correlations among the concentration of α-tocopherol in muscle TBARS and ∑SFA (−0.341, −0.425) were noted, while the content of tocopherol was positively correlated with ∑PUFA (0.341) and ∑n-6. Vitamin E supplementation significantly reduced lipid oxidation during display (Table 3), which could have protected PUFA from degradation. Therefore, SFA are expected to increase if the percentage of PUFA decrease. The correlation between tissue antioxidant status and fatty acid stability has been previously reported (Kasapidou et al., 2001).

5. Conclusions

The effect of dietary vitamin E on lamb quality was independent from freezing and frozen storage duration. Muscle α-tocopherol concentration was over 3.5-fold higher in supplemented samples than in control lambs. At this vitamin E level, lipid oxidation was significantly reduced, leading to a lower metmyoglobin formation and a better colour maintenance (a lower value for Hue and a higher for the 630/580 ratio). However, a higher concentration of α-tocopherol in muscle is required to completely inhibit lipid oxidation and discolouration. Vitamin E addition also had an effect on fatty acid profile. The antioxidant effect of vitamin E protected PUFA from oxidative reactions during display, showing higher values in these samples than in controls. In conclusion, dietary vitamin E could be recommended either for fresh or frozen thawed meat, resulting in a better colour maintenance, a lower lipid oxidation and a higher nutritional value.

Conflict of interests

There is not any conflict of interests.

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4.1.3. Display stability of fresh and thawed lamb supplemented with vitamin E or sprayed with an antioxidant borage seed extract

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Display stability of fresh and thawed lamb supplemented with vitamin E or sprayed with an antioxidant borage seed extract

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Abstract

BACKGROUND: The commercialization of thawed lamb packaged in modified atmosphere and maintained on display could serve as an alternative capable of satisfying the requirements of both customers and distributors. However, previous studies have suggested that lipid oxidation may accelerate post-thawing because peroxidation occurs during frozen storage, thereby leading to rapid and severe secondary lipid oxidation. The addition of an antioxidant compound either in the lamb diet or in the packaged meat could resolve this problem. Therefore, the present study aimed to compare the effect of dietary vitamin E (1000 mg of DL-α-tocopheryl acetate per kg of basal diet) and the spraying of borage seed aqueous extract (10% p/v) on the quality of fresh and thawed lamb leg chops.

RESULTS: Both borage extract and vitamin E improved colour (as measured via instrumental and visual assessment of colour) and lipid stability (thiobarbituric acid reactive substances) of fresh and thawed lamb throughout display, although neither of them had any antimicrobial effect. Freezing/thawing accelerated bone marrow darkening and reduced redness but delayed microbial growth.

CONCLUSION: Both of these antioxidant strategies would be very profitable for the preservation of lamb meat, allowing thawed meat packaged in a modified atmosphere to be commercialized. However, additional studies should be carried out to determine how bone darkening in thawed chops can be avoided.
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Keywords: bone marrow darkening; lipid oxidation; colour; freezing; display; modified atmosphere packaging

INTRODUCTION

Lamb for retail sale is usually packaged in modified atmosphere (MAP) and refrigerated at 4 °C to satisfy consumer demand for display-ready meat. High concentrations of oxygen in the gas mixture produce optimum colour in red meats by promoting oxymyoglobin formation; carbon dioxide (CO_2), on the other hand, is employed because of its antimicrobial activity, which allows meat shelf life to be extended.[1] However, the attainable shelf life hardly surpasses 21 days; therefore, when a more extended life is required, a different strategy needs to be applied.

Fresh lamb exported to distant markets is commonly preserved in anoxic atmospheres at −1.5 °C, reaching a shelf life of 60–70 days. It is then packaged in MAP for retail selling. Freezing is also used for long-term distribution: the extended shelf life of frozen meat provides distributors and retailers with greater flexibility.[2] Although the sale of thawed meat is currently not allowed in some countries, the commercialization of thawed lamb packaged in MAP (with high oxygen content) and maintained on display could serve as an alternative capable of satisfying the requirements of customers (display-ready meat) as well as distributors (flexibility). However, knowledge about the quality and shelf life of lamb subjected to this process is scarce and so new studies need to be carried out in order to investigate its potential use.

Lipid oxidation is one of the main causes of lamb deterioration, leading to off-odour and discolouration.[3] Several strategies have been proposed to deal with this problem. The supplementation of basal compound feed with vitamin E during the final days prior to slaughter has emerged as one of the most promising strategies for the reduction of lipid oxidation. The effect of dietary vitamin E on fresh meat has been thoroughly investigated, although its benefits on frozen/thawed meat are still a point of concern.[4–6] Natural herbs and extracts are also being studied for this purpose. Borage (*Borago officinalis* L.) seeds have been shown to exert antioxidant properties *in vitro*, based on their high content of rosmarinic, syringic and sinapic acids.[7,8] Previous studies carried out by our research group have reported the effectiveness of borage seed meal in the reduction of lipid oxidation in beef patties and fresh pork sausages.[9,10] However, the borage seed meal led to a greyish colour in the product surface, which was detected by panelists. To address this problem, a new aqueous extract of borage seeds has been developed. Recent data from our research group have established a 10% (p/v) aqueous extract as the optimum concentration for reducing lipid oxidation at the

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same time as ensuring that the sensory properties of fresh lamb are not detectably modified.[11]

Both of these antioxidant strategies could serve as profitable methods to improve the quality of thawed lamb chops maintained under retail conditions. Therefore, the present study aimed to investigate the effect of dietary vitamin E supplementation and of the spraying of borage seed aqueous extract on the quality of fresh and thawed lamb leg chops throughout their display. The outcomes of the present study may serve as a basis for assessing the viability of commercializing thawed lamb chops packaged in MAP, as well as the usefulness of applying antioxidants.

MATERIALS AND METHODS

The lambs used for this trial were reared in accordance with the guidelines established by the Spanish Ministry of Agriculture.[12]

Borage seed extract preparation

Borage seeds were purchased from a local market (Semillas Fitó, Spain). Borage meal was obtained as described by Sánchez-Escalante *et al.*[9] with some modifications. Seeds were ground in a coffee grinder, and the husk was separated from endosperm using a 1-mm sieve. The endosperm was recovered and defatted by stirring continuously with hexane (1:5 w/v, 5 min, three times) in a magnetic agitator. After each extraction, the combination of ground seeds and solvent was centrifuged in a refrigerated centrifuge (model CR-4.11; Jouan, Saint-Herblain, France) at 2300 × *g* for 15 min at 10 °C. Hexane was eliminated by evaporation at ambient temperature overnight in a fume hood. Dried meal was dissolved in distilled water in a crystal flask (10 g of meal in 100 mL of distilled water). To aid the dissolution of phenolic compounds in water, the flask was placed into a water bath at 65 °C (Grant W14; Grant Instruments, Cambridge, UK) for 45 minutes with continuous agitation. The solution was subsequently filtered with filter paper (number 43; Machery-Nagel, Düren, Germany), sterilized using a 0.2-μm cellulose acetate sterile syringe filter (VWR International Ltd, Lutterworth, UK) and placed in a sterile commercial applicator. The resulting solution was kept frozen at −20 °C until its utilization, prior to which it was thawed at ambient temperature.

Sampling protocol

The sampling procedure is presented in Fig. 1. Seventy-two male Rasa Aragonesa lambs with an mean ± SD body weight of 22.3 ± 0.25 kg were randomly allocated to eight different pens (nine lambs per pen) and assigned to one of the two feeding treatments: Control feed (four pens) (30 mg kg^{-1} DL-α-tocopheryl acetate) (CF) and 1000 mg kg^{-1} CF of DL-α-tocopheryl acetate (four pens) (vitamin E). Lambs were offered *ad libitum* access to the experimental compound feed along with wheat straw for 14 days before slaughter. They were then slaughtered at a local slaughterhouse with a body weight of 25.8 ± 0.71 kg. Carcasses were chilled for 24 h at 4 °C, and three carcasses from each pen (four control feed pens and four vitamin E-supplemented pens) were assigned to one of the three storage conditions tested: fresh and thawed after 3 or 9 months of frozen storage (12 control and 12 vitamin E-supplemented lambs per storage condition). Subsequently, two legs were removed; those which were assigned to frozen storage treatment were vacuum packaged in polyethylene-polyamide bags with ethyl vinyl acetate sealant layer (30 × 25 cm, 90 μm thickness, a water vapour transmission rate of 2.8 g m^{-2} 24 h^{-1}/85 ± 2% relative humidity (RH) at 23 ± 1 °C, an O_2 transmission rate of 50 cm^3 m^{-2} 24 h^{-1}/75 ± 2% RH at 23 ± 1 °C and a CO_2 transmission rate of 150 cm^3 m^{-2} 24 h at 23 ± 1 °C; Eurobag & Film SL, Málaga, Spain) using a Tecnotrip EV-13-L-CD-SC machine (Tecnotrip SA, Terrassa, Spain) and maintained at −20 °C for 3 or 9 months. Frozen storage conditions were monitored with a Testo data logger 175-H2 (Testo SA, Barcelona, Spain). Both fresh and frozen legs (after corresponding storage times) were cut into eight 20-mm thick chops from the top end; chops were subsequently packaged in modified atmosphere (70% O_2 and 30% CO_2) with a product-to-gas ratio of 1:3 (ULMA-SMART-500; Ulma Packaging, Oñati, Spain). Polystyrene/ethylene vinyl alcohol/polyethylene trays were used, and a polyethylene and polyamide laminate film was used for sealing (30 μm thickness, water vapour transmission rate of <7 g m^{-2} 24 h^{-1}/85% RH at 23 °C, an O_2 transmission rate of <15 cm^3 m^{-2}/0% RH at 23 °C, and a CO_2 transmission rate of <75 cm^3 m^{-2} 24 h/0% RH at 23 °C; Linpac Packaging SL, Pravia, Spain). The right leg chops from control-fed lambs were assigned to the control treatment, whereas the left leg chops were used to evaluate the borage extract. Only the right leg chops from the vitamin E-fed animals were used. Prior to packaging, chops subjected to borage treatment were sprayed with the aqueous extract using a commercial applicator (1 mL of solution per 100 cm^2 of meat approximately). Samples were displayed under retail conditions (4 ± 0.5 °C, with 14 h of fluorescent light) for 9 days. Chops thawed within the package during display. Analyses were performed at 0, 3, 6 and 9 days of display using only the *Semimembranosus* muscle.

Content of α-tocopherol in the *Semimembranosus* muscle

The content of α-tocopherol in tissue was determined in accordance with the methodology described by Kasapidou *et al.*[13] and Liu *et al.*[14] Samples were analyzed by reverse phase high-performance liquid chromatography (LiChrospher RP-18 column; Merck, Darmstadt, Germany; 4.60 × 150 mm, 5 μm, 100 Å) with a mobile phase of isocratic methanol 100% at a constant flow rate of 1.2 mL min^{-1} and fluorescence detection ($\lambda_{excitation}$ = 295 nm and $\lambda_{emision}$ = 330 nm; Agilent Series 1100). The injection volume was 20 μl with a run time of 9 minutes. For peak identification and quantification, (±)-α-tocopherol was used as external standard. Results were expressed as mg vitamin E/kg fresh meat.

Instrumental colour

A CM-2002 (Minolta, Osaka, Japan) spectrophotometer was used for measuring colour at the surface of a 20-mm thickchop just after opening the tray. The parameters registered were *L** (lightness), *a** (redness) and *b** (yellowness). A D65 illuminant was used at an observation angle of 10° and with a cell opening of 30 mm. Equipment was previously calibrated using a black-and-white standard. Measurements were conducted in the *Semimembranosus* muscle, where each value represents the mean of ten observations on the same chop.

The *a**/*b** value ratio was calculated by dividing *a** by *b** values. The relative content of metmyoglobin was calculated from the reflectance curve according to Krzywicki[15] using 690 nm (i.e. the instrument's highest wavelength). Because the reflectance spectrophotometer only measures reflectance at 10-nm intervals, the wavelengths 473, 525 and 572 nm were calculated via linear interpolation.

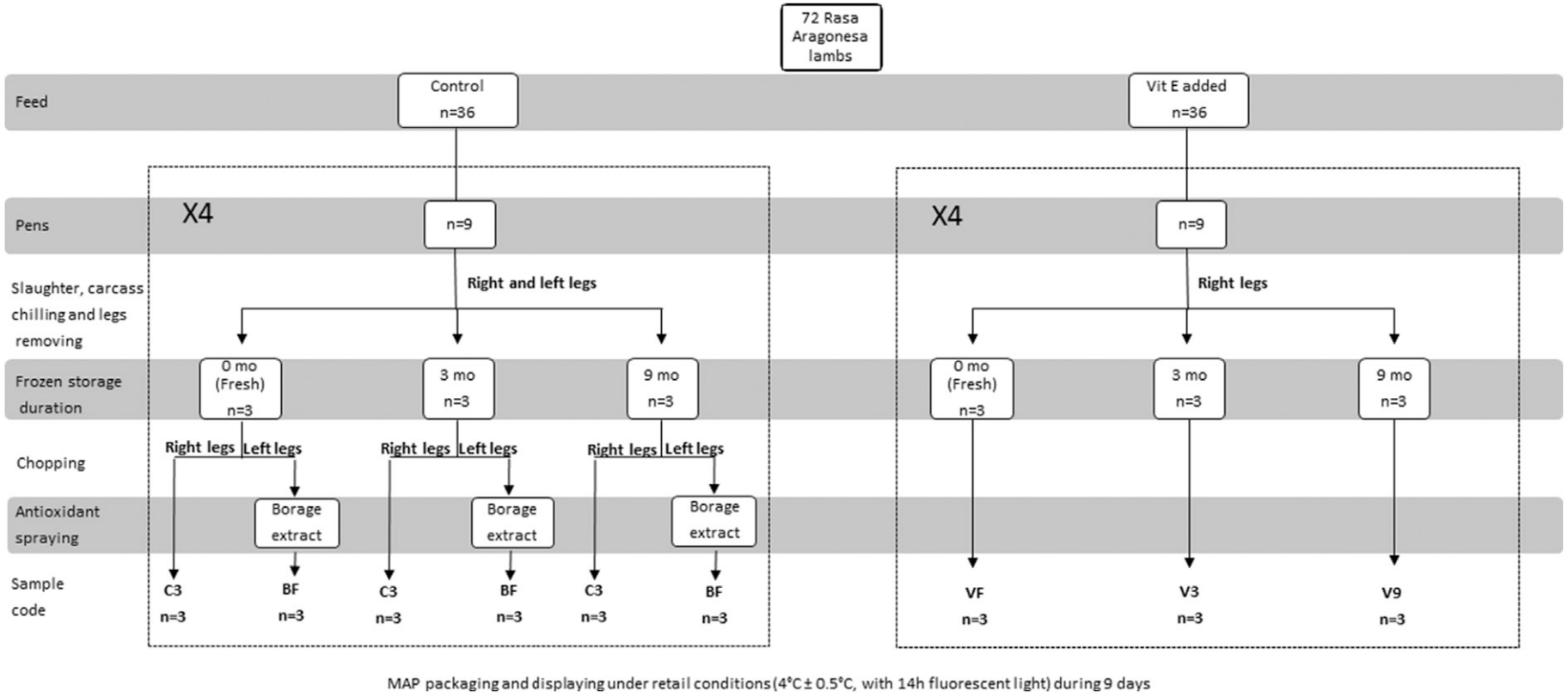

Figure 1. Sampling procedure.

Lipid oxidation

Lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in accordance with the method described by Alonso *et al.*[16]

Microbial analysis

Samples were aseptically collected from the chops by swabbing an area of 10 cm^2 delimited with a sterile aluminium template (10 cm^2). Each sample was homogenized in 0.1% peptone water (Biolife, Milan, Italy) and serial dilutions were carried out. Then, 1 mL of the appropriate dilution was plated in the following manner: aerobic total viable counts (ATVC) on Plate Count Agar (Merck) at 37 °C for 24 h; *Enterobacteriaceae* on violet red bile dextrose agar (Merck) at 37 °C for 48 h and *Pseudomonas* spp. on Cephalothin-Sodium Fusidate-Cetrimide Agar (Merck) at 20 °C for 24 h. Counts were expressed as the base 10 logarithm of colony-forming units per cm^2 of surface area (log CFU cm^{-2}).

Visual assessment of lamb meat

Meat samples were evaluated by a panel of nine members who had been trained in sensory analyses (ISO 8586-1, 1992) and were familiar with the visual assessment of lamb. Special training was undertaken to ensure that panelists could recognize attributes before starting to evaluate lamb samples. Panelists used an eight-point scale to quantify colour (1 = extremely bright cherry-red; 2 = bright cherry red; 3 = dull red; 4 = dark red; 5 = brown; 6 = slightly greenish brown; 7 = moderate greenish brown; 8 = green), a five-point scale for surface discolouration (% of metmyoglobin) (1 = 0%; 2 = 1 to 20%; 3 = 21 to 40%; 4 = 41 to 60%; 5 = 61 to 100%) and a six-point scale for bone marrow colour (1 = bright reddish–pink to red; 2 = dull pinkish–red; 3 = grayish red; 4 = moderately grey; 5 = grayish black; 6 = black discolouration). These scales were designed on the basis of those recommended by AMSA with some modifications.[17] On each sampling day, panelists evaluated the samples under the same conditions and in the same display cabinet. Nine samples per treatment were evaluated in each session (27 samples per session).

Statistical analysis

Data were statistically analyzed by the general linear model procedure of SPSS, version 19.0 (IBM Corp., Armonk, NY, USA). The model included the addition of antioxidants (vitamin E, borage extract and control), total frozen storage time (fresh, 3 and 9 months) and display time (as fixed effects) and their interaction, plus the random effect of pen. A significant interaction between the effects of antioxidant inclusion and total frozen storage time on lipid oxidation and instrumental colour was found: a second model was therefore performed in which total frozen storage time and antioxidant addition were analyzed together in nine different treatments: control plus fresh lamb (CF), control plus thawed lamb after 3 months of frozen storage (C3), control plus thawed lamb after 9 months of frozen storage (C9), fresh vitamin E-supplemented-lamb (VF), vitamin E-supplemented lamb thawed after 3 months of frozen storage (V3), vitamin E-supplemented lamb thawed after 9 months of frozen storage (V9), fresh lamb sprayed with borage extract (BF), thawed lamb after 3 months of frozen storage sprayed with borage extract (B3) and thawed lamb after 9 months of frozen storage sprayed with borage extract (B9). An interaction between treatments and days of study was noted, so a second model was performed, in which treatment was analyzed within the day of study. Conversely, microbial growth was not significantly affected by the interactions among the effects of antioxidant inclusion and frozen storage time and so those effects were analyzed separately on each sampling day.

Differences were considered significant when $P \leq 0.05$.Tukey's post-hoc test was used to assess differences between mean values when $P \leq 0.05$.

For sensory analyses, a similar model was used, including 'panelist' and 'session' as fixed effects and their interactions. 'Panelist' resulted in a significant effect ($P \leq 0.001$) on all of the sensory parameters evaluated. By contrast, 'session' had no significant effect. A significant interaction was found between the effects of antioxidant inclusion and of frozen storage time on the attributes analyzed by sensory evaluation: the effects were therefore analyzed together, leading to nine treatments. Differences among means were evaluated with Tukey's post-hoc test ($P \leq 0.05$).

RESULTS

Content of α-tocopherol in the *Semimembranosus* muscle

The content of α-tocopherol in the *Semimembranosus* muscle at slaughter was 1.057 ± 0.45 and 1.080 ± 0.44 mg kg^{-1} meat in control and borage-sprayed samples respectively, whereas the α-tocopherol concentration reached 3.908 mg kg^{-1} meat in lambs supplemented with 1000 mg kg^{-1} of DL-α-tocopheryl acetate: differences among those concentrations were statistically significant ($P \leq 0.001$).

Instrumental colour

Table 1 shows the results for instrumental colour measurements of each treatment throughout display. Initially, differences in a^* values among treatments were not evident, although they became significant after 3 days of display, when fresh lamb (CF, BF, VF) displayed higher values than thawed meat treatments (C3, C9, B3, B9, V3, V9) ($P \leq 0.001$). From this time onward, lower values were registered in control (CF, C3, C9) than in borage-sprayed (BF, B3) and in vitamin E-supplemented (VF, V3, V9) chops ($P \leq 0.001$). The values of the a^*/b^* ratio during storage were significantly different among groups. Prior to packaging, treatments of thawed lamb kept frozen during 9 months showed the lowest values ($P \leq 0.001$), whereas differences among the other treatments were not evident. Similar to the a^* index, fresh lamb treatments showed higher a^*/b^* values after 3 days, whereas control treatments displayed the lowest values from 6 days post-packaging to the end of the experimental period ($P \leq 0.001$). Therefore, both antioxidant strategies resulted in higher values than controls of either a^* or a^*/b^* after 6 days of display. Differences in surface metmyoglobin formation (% MMb) among treatments were not found at 0 and 3 days of display, although metmyoglobin content was significantly higher in control than in antioxidant treatments at 6 days after packaging ($P \leq 0.001$). The post-hoc analysis at 9 days of display revealed that VF led to the lowest MMb formation, without significant differences within the group including BF, B3, B9 and V3, whereas control treatments resulted in the highest proportion of metmyoglobin ($P \leq 0.001$).

Lipid oxidation

Mean TBARS values were not significantly different among treatments before display ($P = 0.065$), although differences among them were observed after 3 days, when those supplemented with vitamin E resulted in a significantly lower content of

Table 1. Physicochemical parameters of lamb leg chops subjected to different treatments throughout display

Parameter	Day of display	Treatments										
		BF	B3	B9	CF	C3	C9	VF	V3	V9	SEM	*P*-value
*L**	0	39.34 b	35.60 ab	36.71 ab	41.27 b	37.22 ab	37.99 ab	40.02 b	38.58 ab	33.85 a	0.44	0.010
	3	39.25	38.33	39.80	41.06	36.08	39.80	39.13	38.37	38.12	0.33	0.063
	6	36.97 a	38.85 ab	40.68 ab	40.69 ab	41.72 ab	43.74 b	37.05 a	37.22 ab	39.21 ab	0.46	0.011
	9	41.49 ab	40.09 ab	39.17 a	48.72 c	42.07 ab	45.28 bc	41.81 ab	43.39 abc	39.46 ab	0.44	≤ 0.001
*a**	0	11.78	12.16	10.48	11.56	11.36	10.45	11.73	12.11	10.91	0.19	0.065
	3	13.96 b	9.74 a	8.93 a	13.91 b	8.85 a	9.04 a	12.80 b	8.46 a	10.92 a	0.23	≤ 0.001
	6	11.15 c	9.80 b	6.30 a	5.50 a	5.20 a	4.24 a	10.37 b	8.80 b	8.62 b	0.17	≤ 0.001
	9	8.65 c	7.68 bc	5.98 b	3.49 a	3.52 a	2.59 a	6.49 bc	6.39 bc	6.37 bc	0.27	≤ 0.001
*a**/*b**	0	1.03 b	1.16 b	0.83 a	1.08 b	1.15 b	0.84 a	1.01 b	1.14 b	0.81 a	0.01	≤ 0.001
	3	1.09 c	0.85 ab	0.74 a	1.05 bc	0.78 a	0.73 a	1.10 c	0.71 a	0.90 ab	0.02	≤ 0.001
	6	1.01 c	0.81 c	0.63 b	0.49 ab	0.42 a	0.34 a	0.99 c	0.88 c	0.77 c	0.02	≤ 0.001
	9	0.76 d	0.67 cd	0.62 cd	0.25 a	0.28 a	0.21 a	0.59 cd	0.48 bc	0.54 bc	0.02	≤ 0.001
%MMb	0	23.17	19.93	24.37	21.83	17.56	22.65	22.53	19.23	23.07	0.69	0.365
	3	25.59	27.08	30.76	26.74	32.58	28.66	26.25	34.39	35.62	1.17	0.572
	6	28.44 a	31.53 ab	37.53 b	60.47 c	50.70 c	57.64 c	29.51 a	30.18 a	34.49 ab	0.74	≤ 0.001
	9	36.67 ab	37.57 ab	43.07 ab	67.71 d	61.31 cd	67.66 d	31.01 a	46.82 abc	50.94 bcd	1.28	≤ 0.001
mg MDA kg^{-1}	0	0.02	0.04	0.04	0.07	0.01	0.07	0.01	0.04	0.02	0.01	0.065
	3	0.99 b	1.02 b	0.60 ab	1.91 c	1.04 ab	0.70 b	0.35 a	0.34 a	0.24 a	0.05	≤ 0.001
	6	1.52 b	1.22 ab	1.23 ab	3.40 c	2.28 c	2.52 c	0.44 a	0.79 ab	0.60 ab	0.07	≤ 0.001
	9	1.41 a	1.24 a	1.28 a	2.74 b	3.10 b	2.50 b	0.77 a	1.40 a	1.10 a	0.08	≤ 0.001

Data are the means. SEM, standard error of the mean.
Control plus fresh lamb (CF), control plus thawed lamb after 3 months of frozen storage (C3), control plus thawed lamb after 9 months of frozen storage (C9), fresh vitamin E supplemented lamb (VF), vitamin E supplemented lamb thawed after 3 months of frozen storage (V3), vitamin E supplemented lamb thawed after 9 months of frozen storage (V9), fresh lamb sprayed with borage extract (BF), thawed lamb after 3 months of frozen storage sprayed with borage extract (B3) and thawed lamb after 9 months of frozen storage sprayed with borage extract (B9).
% MMb, percentage of metmyoglobin; MDA, malondialdehyde.
Values within a row with different lowercase letters are significantly different (within treatment) ($P \leq 0.05$).

malondialdehyde (MDA) than control, BF and B3 treatments (Table 1). From this time onward, TBARS values were higher in control than in borage-sprayed as well as dietary-vitamin E-supplemented samples ($P \leq 0.001$). Inhibition of lipid oxidation was more pronounced in VF than in BF at 6 days post-packaging, although differences among antioxidant-adding treatments were not evident after 9 days of display.

Microbial counts

The effect of dietary vitamin E and borage spraying on microbial growth of lamb leg chops during display is shown in Fig. 2. *Enterobacteriaceae* and *Pseudomonas* spp. counts remained below the limit of detection until 6 days after packaging, whereas initial ATVC were between 1.75 and 1.84 log CFU cm^{-2}. Significant differences in microbial counts among vitamin E-supplemented, borage-extract-sprayed and control samples were not registered for any microorganism at any sampling day. By contrast, frozen storage of lamb prior to thawing, both 3 and 9 months, resulted in a significant reduction of microbial growth (Fig. 3). Indeed, ATVC were lower in thawed than in fresh lamb during display ($P \leq 0.001$). Differences in ATVC between samples kept frozen for 3 and 9 months were not observed on any sampling day. Growth of *Enterobacteriaceae* and *Pseudomonas* spp. was only detected in thawed lamb after 9 days of display, although the counts of both microorganisms were also lower than those registered in fresh samples ($P \leq 0.001$).

Visual evaluation

The results obtained for visual evaluation of colour, surface discolouration and bone marrow colour of lamb chops displayed during 9 days are shown in Table 2. Prior to packaging, treatments including fresh meat showed lower values for the three attributes evaluated than those that were frozen stored during 9 months ($P \leq 0.001$). Differences between frozen storage times were also significant for colour stability and bone marrow colour on that sampling day: the chops frozen stored during a longer period of time registered higher scores. The effect of antioxidants on the preservation of colour was evident from 6 days post-packaging onwards, when control treatments were accorded the highest values for colour stability and surface discolouration (note that '1' was the best grade) ($P \leq 0.001$). Moreover, panelists detected significant differences in bone marrow colour among borage-sprayed and vitamin E-supplemented thawed samples after 3 days of display: the former obtained lower scores than the latter. However, differences in bone marrow colour among those two kinds of samples were not evident in treatments that included fresh meat. Bone marrow darkening was enhanced by freezing/thawing, with thawed meat displaying higher values for that parameter from 3 days post-packaging onwards ($P \leq 0.001$).

DISCUSSION

Colour

According to Djenane *et al.*,[18] consumers reject beef when the percentage of metmyoglobin exceeds 40%. As has been demonstrated, both borage spraying and vitamin E supplementation reduced the formation of metmyoglobin in fresh lamb, as well as in thawed lamb, maintaining the content of MMb below the rejection limit for 6 days of display. Ripoll *et al.*[19] and Albertí

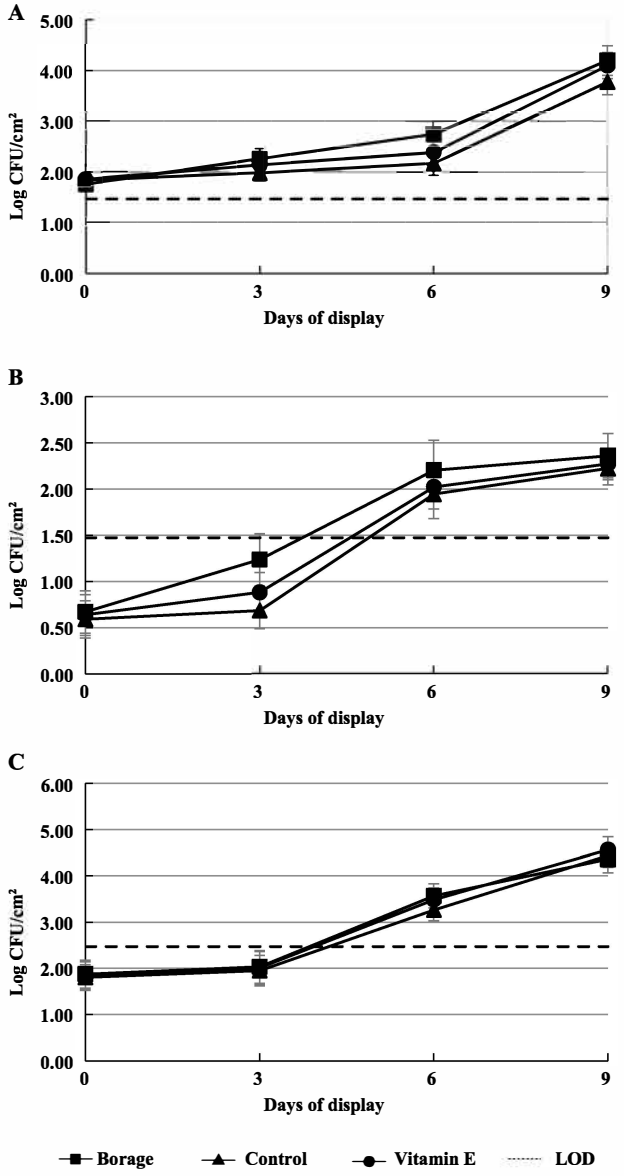

Figure 2. Effect of dietary vitamin E (vitamin E) and 10% borage aqueous extract spraying (borage) on growth (mean ± SEM) of aerobic total viable counts (A), *Enterobacteriaceae* (B) and *Pseudomonas* spp. (C) for lamb leg chops displayed at 4 °C in modified atmosphere packaging. The limit of detection (LOD) was 1.48 log CFU cm^{-2} for aerobic total viable counts and *Enterobacteriaceae* and 2.48 log CFU cm^{-2} for *Pseudomonas* spp.

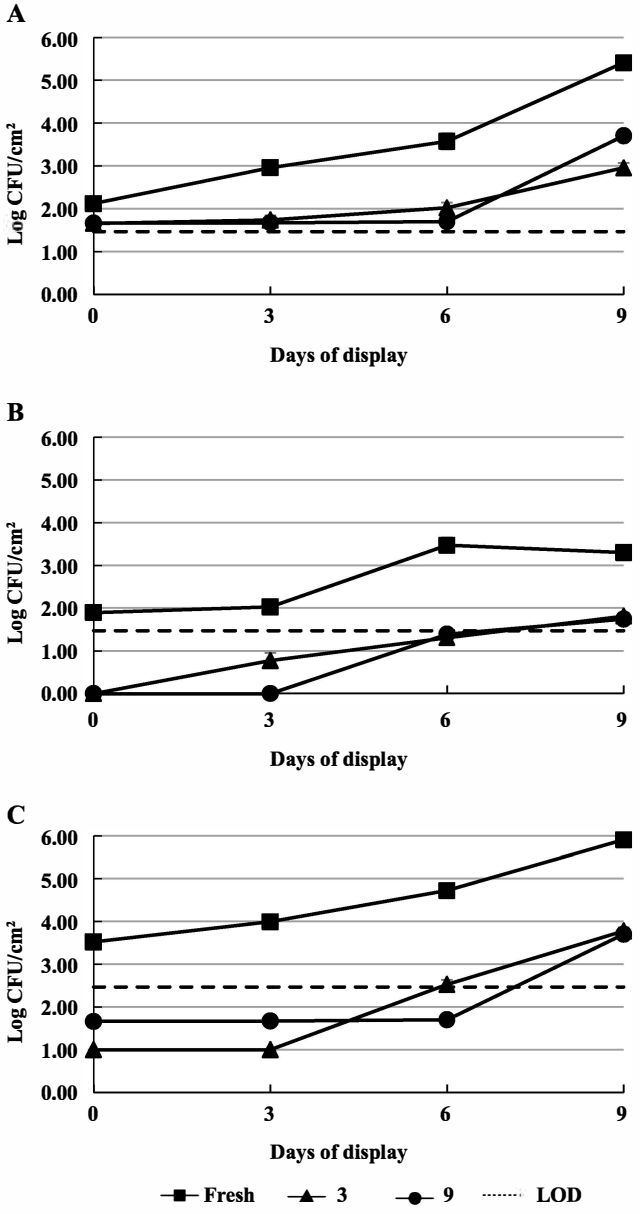

Figure 3. Growth (mean ± SEM) of aerobic total viable counts (A), *Enterobacteriaceae* (B) and *Pseudomonas* spp. (C) for fresh (fresh) and thawed lamb leg chops kept 3 (3) and 9 months (9) in frozen storage and displayed at 4 °C in modified atmosphere packaging. The limit of detection (LOD) was 1.48 log CFU cm^{-2} for aerobic total viable counts and *Enterobacteriaceae* and 2.48 log CFU cm^{-2} for *Pseudomonas* spp.

et al.[20] also noted a lower formation of metmyoglobin and a better maintenance of redness in vitamin E-supplemented fresh lamb and beef packaged in MAP, whereas the capacity of borage extract to reduce colour fading had been previously observed by Sánchez-Escalante *et al.*[9] in beef patties. The results of the present study thus corroborate the potential held by both of these antioxidant strategies with respect to helping to reduce colour deterioration and also demonstrate that colour is maintained after thawing. The effectiveness of these two antioxidants in preventing lamb chops from discolouration throughout display could be related to their great capacity to reduce lipid oxidation, which was indeed significantly inhibited. However, neither borage extract, nor vitamin E supplementation yielded thawed chops with the typical red colour of fresh lamb. As can be seen in Tables 1 and 2, fresh chops showed higher a^* and a^*/b^* values, as well as lower scores for colour stability, than thawed lamb at 3 days of display, independently of antioxidant addition. Lipid oxidation could not explain these differences because MDA content was not lower in fresh than in thawed samples. A feasible explanation for these results is the relationship between blooming and metmyoglobin reducing activity. This enzyme is more active in fresh meat, in which metmyoglobin, as it is formed, is quickly converted to deoxymyoglobin and oxygenated back to oxymyoglobin, thereby retaining the bright red (bloom) colour. By contrast, metmyoglobin accumulates more rapidly in thawed meat, thereby causing discolouration to appear sooner.[21,22] Moreover, physical disruption of muscle fibres during freezing/thawing might

Table 2. Sensory visual scores of lamb chops subjected to different treatments throughout MAP display period

Sensory attributes	Day of display	Treatments										
		BF	B3	B9	CF	C3	C9	VF	V3	V9	SEM	*P*-value
Colour[a]	0	2.25 a	2.63 a	3.14 b	2.32 a	2.40 a	3.03 b	2.30 a	2.46 a	3.14 b	0.04	≤ 0.001
	3	1.68 a	3.14 b	3.51 bc	2.12 a	3.95 c	4.06 c	1.80 a	3.60 c	2.85 b	0.06	≤ 0.001
	6	2.96 a	3.43 a	4.81 b	7.10 d	6.63 c	7.42 d	3.2 a	5.17 bc	4.82 b	0.06	≤ 0.001
	9	5.63 b	4.36 a	5.44 b	7.82 d	8.00 d	8.00 d	5.83 bc	5.86 bc	6.50 c	0.06	≤ 0.001
Surface discolouration (% MMb formation)[b]	0	1.44 a	1.88 abc	2.42 d	1.56 a	1.63 ab	2.07 cd	1.47 a	1.90 abc	2.18 cd	0.04	≤ 0.001
	3	1.38 a	2.25 bc	2.65 cd	1.94 b	2.58 cd	2.99 d	1.40 a	2.36 bc	2.04 b	0.04	≤ 0.001
	6	1.97 a	2.14 a	3.21 b	4.56 d	4.51 d	4.71 d	2.20 a	3.35 b	3.21 b	0.04	≤ 0.001
	9	3.71 b	2.72 a	3.51 b	4.90 d	5.00 d	6.00 e	3.95 b	3.72 b	4.22 c	0.04	≤ 0.001
Bone marrow colour[c]	0	1.86 a	2.10 a	3.22 b	2.18 a	2.03 a	3.57 b	1.80 a	2.19 a	3.08 b	0.04	≤ 0.001
	3	1.74 a	3.67 d	3.93 d	2.65 b	4.73 e	5.54 f	1.82 a	5.22 ef	5.21 ef	0.06	≤ 0.001
	6	2.04 a	4.00 c	4.94 d	2.93 b	5.15 de	5.49 e	2.21 a	5.25 de	5.44 e	0.04	≤ 0.001
	9	2.96 a	4.90 b	5.25 c	3.21 a	6.00 d	6.00 d	3.36 a	5.68 d	5.74 d	0.03	≤ 0.001

Data are the means. SEM, standard error of the mean.
Control plus fresh lamb (CF), control plus thawed lamb after 3 months of frozen storage (C3), control plus thawed lamb after 9 months of frozen storage (C9), fresh vitamin E supplemented lamb (VF), vitamin E supplemented lamb thawed after 3 months of frozen storage (V3), vitamin E supplemented lamb thawed after 9 months of frozen storage (V9), fresh lamb sprayed with borage extract (BF), thawed lamb after 3 months of frozen storage sprayed with borage extract (B3) and thawed lamb after 9 months of frozen storage sprayed with borage extract (B9).
[a] 8-point scale (1 = extremely bright cherry-red, 2 = bright cherry red, 3 = dull red, 4 = dark red, 5 = brown, 6 = slightly greenish brown, 7 = moderate greenish brown, 8 = green); [b] 5-point scale (1 = 0%, 2 = 1 to 20%, 3 = 21 to 40%, 4 = 41 to 60%, 5 = 61 to 100%); [c] 6-point scale (1 = bright reddish–pink to red, 2 = dull pinkish–red, 3 = grayish red, 4 = moderately grey, 5 = grayish black, 6 = black discolouration).
Values within a row with different lowercase letters are significantly different (within treatment) ($P \leq 0.05$).

release enzymes into the sarcoplasm, which would enhance metmyoglobin formation.[23] Therefore, freezing/thawing may have enhanced myoglobin oxidation independently of lipid oxidation.

Darkened bones are sometimes found at the supermarket: very little literature has been published on this subject, and the factors that promote the darkening process are not clear. Bone darkening does not affect meat flavour or odour, although it leads to an undesirable appearance that limits consumer acceptance. Although borage extract spraying delayed this process, large differences in bone darkness were found between treatments including fresh or thawed lamb. Freezing promoted this process and resulted in a higher degree of darkening than in fresh meat. This phenomenon appears to be a result of hemoglobin oxidation, which leads to a change in bone marrow colour from attractive red to undesirable black.

Despite having a similar degree of lipid oxidation, borage-sprayed chops maintained their colour slightly better than those from vitamin E supplemented lambs. These differences may be related to the cell location where these respective processes are acting. Vitamin E is deposited in cell membranes, where it protects polyunsaturated fatty acids from oxidation, whereas borage extract, being water soluble, is more likely to permeate the cytoplasm and thus protect water-soluble myoglobin from oxidation.

Lipid oxidation

Previous studies have demonstrated that high concentrations of oxygen in the gas mixture negatively affect lipid stability.[24] In accordance with those findings, lipid oxidation increased rapidly in control treatments, resulting in TBARS values above the threshold for consumer rejection of oxidized beef as proposed by Campo *et al.*[25] (2 mg MDA kg^{-1}) after 6 days of display. Similar values were reported by Camo *et al.*[26] and Gutiérrez *et al.*[27] in fresh lamb, as well as by Guidera *et al.*[28] in thawed lamb.

Regarding the effect of freezing/thawing, several investigations have suggested that frozen storage could affect lipid oxidation, which may accelerate during the post-thawing stage: peroxidation occurs during frozen storage, leading to rapid and severe secondary lipid oxidation (thiobarbituric acid forming).[29,30] Hansen *et al.*[31] noted accelerated lipid oxidation in frozen–thawed pork subjected to a refrigerated shelf life; a study by Benjakul and Bauer[32] associated this phenomenon with the release of pro-oxidant compounds as a result of cellular damage. Nevertheless, this effect was not found in our trial. Indeed, a higher content of MDA was registered in fresh than in thawed control treatments after 3 days. In agreement with these findings, Jeong *et al.*[33] registered higher TBARS values in fresh than in frozen–thawed beef. The results of the present study showed that lipid oxidation develops in fresh and thawed lamb in a similar way, which might represent a limiting factor for the commercialization of both. Therefore, an antioxidant treatment on both fresh and thawed lamb appears to hold great advantages because both borage extract spraying and vitamin E supplementation led to a great reduction of lipid oxidation, with values significantly lower than those registered in control treatments at 6 and 9 days post-packaging. In those groups, MDA content did not reach the threshold for acceptability of oxidized beef as reported by Campo *et al.*[25] on any sampling day. The effectiveness of vitamin E in reducing lipid oxidation in fresh lamb has been already demonstrated.[19,34] Nevertheless, its effect on the reduction of lipid oxidation of frozen–thawed lamb maintained in display has not yet been described. No differences in MDA content between fresh and thawed vitamin-E-supplemented lamb were registered in this trial; therefore, no evidence of a reduction in the effect of vitamin E on inhibiting lipid oxidation during frozen storage was found. This strategy is thus equally recommendable when lamb is expected to be frozen-stored.

To the best of our knowledge, borage seed extract has never been used to preserve fresh lamb, although it has been shown to

reduce lipid oxidation in beef patties and fresh pork sausages.[9,10] The effect of this extract on the inhibition of lipid oxidation has been attributed to its phenolic composition, mainly rosmarinic, sinapic and syringic acids.[7] These compounds are able to quench reactive oxygen species, which would explain the ability of borage seed extracts to limit oxidative reactions.[35] Moreover, no differences were noted between the effect of borage aqueous extract on the reduction of lipid oxidation in either fresh and thawed lamb, such that it could be satisfactorily applied in both.

Microbial growth

According to Insausti *et al.*,[36] microbial spoilage of meat appears when microbial counts exceed 7 log CFU g^{-1}. In the present study, this value was not reached for any genus in any treatment and so it did not represent a limit for lamb shelf life. As previously reported by Lauzurica *et al.*[34] and Sanchez-Escalante *et al.*,[9] neither Vitamin E supplementation, nor borage spraying significantly reduced microbial growth in either lamb or beef patties, thereby demonstrating the lack of any antimicrobial effect.

It is well known that microbial growth and chemical and enzymatic reaction rates are reduced when temperature decreases: microbial growth is practically inhibited during frozen storage.[37] However, the effect of freezing/thawing on microbial growth during meat storage is still a point of concern. Leygonie *et al.*[38] reported that neither freezing, nor thawing would be capable of inactivating viable microbial cells present in meat. During frozen storage, microorganisms would only be dormant, and their growth rate would increase after thawing. By contrast, studies also report mechanisms via which freezing and thawing could injure microbial cells.[39] Freezing may damage microorganisms mainly as a result of crystal formation, concentration of solutes and low temperature, whereas the effect of thawing on microbial cells would be a result of recrystallization.[40] After thawing, injured cells could repair their damages and remain fully viable (sublethal damage) or they would not be able to multiply (lethal damage).[39] Therefore, freezing/thawing damages combined with the antimicrobial effect of the high CO_2 percentage of the atmosphere used (30% CO_2) would explain the lower counts found in thawed lamb.

CONCLUSIONS

Both borage extract spraying and dietary vitamin E improved lipid and colour stability of fresh and thawed lamb, although only borage spraying exerted a significant effect on the delay of bone marrow darkening. Neither borage, nor vitamin E showed any antimicrobial effect. By contrast, freezing/thawing led to a great delay in microbial growth; however, it exerted an adverse effect on meat colour, with thawed lamb showing less blooming, as well as a greater degree of bone marrow darkening. Therefore, both antioxidant strategies would be recommended to achieve a delay in deterioration of either fresh or thawed lamb: they would thus allow commercialization of thawed lamb packaged in MAP. New studies should nevertheless be carried out aiming to identify methods that could help avoid the undesirable effects of freezing/thawing on bone marrow darkening and blooming.

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4.2. Estrategias tecnológicas

4.2.1. A review of fresh lamb chilling and preservation

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A review of fresh lamb chilling and preservation

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ABSTRACT

The aim of this review was to summarise the methodologies used nowadays for lamb chilling and new improvements for extending fresh lamb shelf-life. The time required for lamb carcasses chilling is reduced to 7 h by ultra-rapid chilling, allowing distributors to dispatch carcasses the same day. Refrigeration just above the freezing point, known as superchilling, extends meat shelf-life due to reduction of microbial growth and enzymatic reactions. Refrigerated storage is commonly combined with modified atmosphere packaging for providing a desirable colour to meat adequate for retail selling, although display life is limited by microbial growth and lipid oxidation. These problems may be solved by adding plant extracts onto the package film, which is the basis of active packaging. In contrast, vacuum packaging can be used for wholesale or storage, but it has an undesirable colour for retail selling. New vacuum skin packaging might improve the benefits of vacuum packaging.

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1. Introduction

World meat consumption reached 42 kg per capita in 2011, predominating pork, poultry and beef. Far away, mutton and goat consumption, which has remained invariable since 2004 (1.90 kg/person/year), only represents 4.5% of the total and it is characterised by large variations among continents. Lamb consumption is led by Oceania with 12 kg per capita, whereas America shows the lowest levels (0.60 kg per capita) (FAOSTAT, 2015). Moreover, it is mainly concentrated in a few areas: China, Australia, New Zealand, Islamic countries and Europe (Garnier, 2010). In spite of the low consumption of this product, it is defined as a high quality product and it is considered a delicacy in Mediterranean countries (Karabagias et al., 2011; Vieira and Fernández, 2014).

Consumers demand for fresh, tender, tasty and attractive appearance meat may be satisfied by storage or display under refrigeration, which results in a good keeping of sensory properties during short-term periods (Carballo and Jiménez, 2001). Meat preservation by low temperatures involves two stages, carcass chilling and low temperature maintenance throughout display. Lamb preserved only by refrigeration has a limited shelf-life, so it is usually combined with packaging to extend its lifespan either for storage or display (Vieira and Fernández, 2014; Berruga et al., 2005). Despite this is a relevant concern to meat industry, only limited information is currently existent on the available and feasible procedures to allow maintaining optimum quality of lamb throughout storage or display. Reviews on this topic up to now dealt only with carcass chilling and seem to be out of date. Comprehensive reviews focusing on current options for lamb refrigerated storage, discussing advantages and disadvantages of each method and designing the most appropriate strategy to provide fresh lamb with the longest shelf or display life are indeed lacking.

With this aim in mind this review deals with the methods currently used for lamb chilling and keeping under refrigeration and describes optimal conditions to obtain quality fresh lamb with enlarged shelf or display life. New preservation trends such as superchilling and active packaging provide a basis for technological and scientific innovations on this topic. Economical and safety aspects should be also covered, besides quality, to provide suitable guidelines for meat industry.

2. Carcass chilling regimes

After slaughter, carcasses are chilled to reduce microbial growth and chemical and enzymatic reactions. In fact, an internal leg temperature of 7 °C is required before a carcass may be commercialised in the EU (Commission Regulation (EC) 853/2004). Besides maintaining food safety and meat quality, producers are interested in reducing the time needed for decreasing temperature in lamb carcasses since it means an increase in distribution time (McGeehin et al., 2002). Heavier carcasses are preferred in northern Europe, while in southern Europe carcasses are from milk fed animals or

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young lambs (Beriain et al., 2000). This results in different weights and types of carcasses, which should be taken into account when a cooling rate is chosen (Muela et al., 2010).

A cooling rate may be defined by four parameters: temperature, relative humidity, air velocity and time (McGeehin et al., 2002). Variations in any factor during chilling could reduce the time needed for accomplishing EU requirements; however, it has been described that severe variations could have negative effects on meat quality (Muela et al., 2010). For this reason, different cooling rates are applied depending on carcass weight, time and quality requirements.

Traditionally, lamb carcasses were chilled with a room temperature of 2–4 °C combined with an air speed of 0.2 m/s and a relative humidity higher than 90% for 24 h. This regime is known as conventional chilling (McGeehin et al., 1999). Lamb muscle core temperature reaches 7 °C after approximately 16 h with conventional chilling, requiring carcasses to be maintained in the abattoir overnight (McGeehin et al., 2002). It means that a large chilling room is needed because carcasses need to be stored for almost 24 h. Several chilling protocols have been developed with the aim of solving the drawbacks of conventional chilling. The most common alternatives are ultra-rapid and spray chilling. The first one consists of cooling lamb carcasses in an ambient temperature of −20 °C at an air speed of 1.5 m/s for 3.5 h (McGeehin et al., 1999), after that carcasses are moved to a room at an ambient temperature of 4 °C and an air speed of 0.2 m/s. Therefore, ultra-rapid chilling allows to dispatch carcasses the same day since chilling time is reduced to 7 h (McGeehin et al., 2002). The second one has been used for poultry and beef chilling in commercial abattoirs of North America since 1987 (Jones and Robertson, 1988; Mead, 2004). In spray chilling carcasses are sprayed intermittently with cold water to maintain wet their surface during the chilling process. Mass transfer of heat and evaporative cooling are improved by this chilling regime, meanwhile carcass weight losses are decreased (Savell et al., 2005).

2.1. Effect of chilling regimen on weight loss

Lamb is fairly susceptible to excessive evaporative weight loss due to the high surface-to-volume ratio (Brown et al., 1993). It is a serious economic problem to meat industry that has to be taken account when a chilling regime is chosen. In fact, this is a drawback for conventional chilling in which carcasses show too high weight losses (about 2% in weight). As can be seen in Table 1, this problem has been satisfactorily solved by either ultra-rapid or spray chilling. The first one reduces evaporative losses due to a faster decrease of carcass surface temperature, improving the profitability for meat industry (McGeehin et al., 1999). Accordingly, McGeehin et al. (1999) found 1.48% weight loss at 24 h postmortem for conventional chilling and 0.57% for ultra-rapid chilling. Sheridan et al. (1998) and McGeehin et al. (2002) also found significant differences among weight losses in chilled meat under those rates; being approximately 0.50% lower with ultra-rapid than conventional chilling. Fernández and Vieira (2012) described higher weight losses in conventional than in ultra-rapid chilled meat (2.57% vs. 1.59%) than those found by other cited researchers.

The purpose of spray chilling is the reduction of carcass weight losses (Allen et al., 1987). Numerous studies have confirmed that this chilling regime minimises shrinkage dramatically in beef (Kinsella et al., 2006; Greer and Jones, 1997). In lamb, Brown et al. (1993) compared two spray chilling regimes to conventional chilling with similar conclusions. As can be observed in Table 1, weight losses obtained after 24 h post sacrifice in conventional chilling were improved in both spray chilling regimes tested, being significantly lower in multiple than in double spray chilling regimen. Spray chilling allows to reduce evaporative losses by maintaining a wet carcass surface.

2.2. Effect of chilling regimen on meat colour

Colour is the main lamb sensory property valued by consumers at the time of purchase. Bright red colour is traditionally considered as a positive aspect since it is associated with freshness and superior quality product (Berruga et al., 2005). Several trials have studied the effect of chilling on colour with no remarkable differences between conventional and ultra-rapid chilling (Fernández and Vieira, 2012; Vieira and Fernández, 2014). Regarding spray chilling, no significant differences between colour determinations of *longissimus dorsi* muscles from sprayed and conventional chilled carcasses were found. However, an increase in yellowness of fat and external lean was recorded in sprayed lamb carcasses (Brown et al., 1993)

2.3. Effect of chilling regimen on tenderness

Sarcomere length and shear force value are commonly used for measuring this quality parameter. A relationship between sarcomere length and meat toughness has been described with short sarcomeres resulting in less tender meat (Wheeler et al., 2000). According to Jaime et al. (1992) the initial mean sarcomere length value for *M. longissimus thoracis et lumborum* in lamb is 1.86 μm. As shown in Table 1, sarcomere length could decrease throughout the chilling period depending on the chilling rate. Some researchers

Table 1
Carcass chilling regimes for lamb carcasses.

Carcass type	Carcass weight (kg)	T (° C)/air speed (m/s)/RH (%)/water spraying	Sarcomere length at 24 h (μm)	Shear force at 24 h (N)	Weight loss at 24 h (%)	References
Light	10.5 ± 0.9	2 ± 2/2/*/no	1.5	70	2.7	Fernández and Vieira (2012)
		−20 ± 2/2/*/no	1.1	76	1.59	
Light	10.5 ± 0.9	2 ± 2/2/*/no	1.5	62	*	Vieira and Fernández (2014)
		−20 ± 2/2/*/no	1.4	76	*	
Heavy	16.5–25.7	4/0.2/90/no	1.72	53.6	1.89	Sheridan et al. (1998)
		−20/1.5/90/no	1.64	53.2	1.50	
Heavy	16–23	4/0.2/90/no	1.76	33.6	1.48	McGeehin et al. (1999)
		−20/1.5/90/no	1.71	35.6	0.57	
Heavy	17.5–24.4	4/0.2/90/no	1.67	64.8	*	Redmond et al. (2001)
		−20/1.5/90/no	1.58	77.3	*	
Heavy	16–26	4/0.2/90/no	*	63.6	1.80	McGeehin et al. (2002)
		−20/1.5/90/no	*	88.8	1.15	
Heavy	15.63–19.96	10/0.8-1.2/95/no	*	27.93	2.20	Brown et al. (1993)
		10/0.8-1.2/95/multiple spray with water at 10 °C	*	26.75	0.86	
		10/0.8-1.2/95/double spray (2, 10 h) with water at 10 °C	*	22.25	1.20	

* Data not available.

(Vieira and Fernández, 2014; Fernández and Vieira, 2012; Redmond et al., 2001; Sheridan et al., 1998) found significant differences in sarcomere length among chilling rates. In these trials ultra-rapid chilling led to shorter muscle sarcomeres than those obtained by conventional chilling. However, these differences were not reflected in tenderness. Panellists described ultra-rapid chilled meat as tender as that conventionally chilled.

Shear force value is very commonly used to measure meat toughness; high shear force values are significantly associated with tough meat (Hwang et al., 2003). McGeehin et al. (2002) and Vieira and Fernández (2014) recorded significant differences among meats subjected to different chilling rates, being ultra-rapid higher than conventional. Nevertheless, McGeehin et al. (1999), Sheridan et al. (1998), Redmond et al. (2001) and Fernández and Vieira (2012) did not find any significant difference between these chilling methods. Regarding to spray chilling, Brown et al. (1993) noted lower shear force values in multiple spray than in conventional chilled lamb but there were no differences between the last one and double spray chilled meat. More studies about this topic are needed to clarify the effect of spray chilling on lamb tenderness.

An explanation for these results could be that the sarcomeres from ultra-rapid chilled meat were shorter due to cold shortening, which is induced by low temperatures and results in an increase of meat toughening (Honikel et al., 1983). However, cold shortening itself appears to be insufficient to result in tougher meat. Regarding this, it has been described that meat subjected to ultra-rapid chilling can be as tender as that which has been cooled with conventional chilling (Vieira and Fernández, 2014; Fernandez and Vieira, 2012; McGeehin et al., 2002; Sheridan et al., 1998; McGeehin et al., 1999). These results agree with Jaime et al. (1992), who removed lamb *longissimus dorsi* muscles and brought them to internal temperatures of either 0, 4, 10, 15, 20, 36 °C within 3–4 h post-mortem, afterwards they were allowed to age at 4 °C for 7 days. In this experiment, meat chilled at 0 °C had the highest sensory scores and it also showed a very intense proteolysis despite suffering the most intense cold shortening. The ability of ultra-rapid chilling to provide tender meat despite suffering an intense cold shortening was explained by a higher muscle pH combined with an increase of the release of Ca^{2+} from the sarcoplasmic reticulum to the myofibrils, leading to a proteolysis stimulation capable of overcoming toughness induced by cold shortening (Jaime et al., 1992). This means that ultra-rapid chilling could balance the cold shortening effect; consequently, it would provide meat as tender as meat from conventional chilling.

A possible solution for avoiding the cold shortening phenomenon is electrical stimulation. It was firstly used in New Zealand and then exported to Australia with the aim of avoiding toughness linked to cold shortening (Hwang et al., 2003). It consists on passing an electric current through the carcass immediately after slaughtering, which triggers muscular contraction and increases the rate of glycolisis, resulting in a quicker pH fall and an early rigor mortis development (Hwang et al., 2003). Application of an electrical current lower than 100 V is known as low-voltage electrical stimulation whereas a higher current is named high-voltage stimulation (Cetin et al., 2012). Low voltage is preferred under commercial conditions as it is less dangerous for operators and the requirements imposed by governments are less stringent (Polidori et al., 1999) but high voltage electrical stimulation has a better efficacy (Cetin et al., 2012). Medium voltage treatments (≈300 V) have been developed with the aim of solving both disadvantages, leading to interesting improvements in meat tenderness and eating quality (Shaw et al., 2005; Smith et al., 2016). Moreover, Pearce et al. (2009) suggested an increase in medium voltage electrical stimulation response when alternating frequencies are used.

Therefore, it is well known that meat tenderness improves when lamb carcasses have been stimulated (Cetin et al., 2012; Toohey et al., 2008; Polidori et al., 1999; Shorthose et al., 1986). Nevertheless, this is not only due to cold shortening reduction, as electrical stimulation also accelerates proteolysis and increases physical disruption of the muscle fibers (Hwang et al., 2003). However, the effect of electrical stimulation on meat colour is not clear. Some researchers (Pouliot et al., 2012; Cetin et al., 2012) found differences in lamb meat instrumental colour measurements (L*, a*, b*) with electrical stimulated carcasses having higher values for L* and b* than control. In contrast, others did not find any evidence of colour variation (Channon et al., 2005; Moore and Young, 1991; Toohey et al., 2008), which suggests the need for more studies about this topic. Electrical stimulation together with ultra-rapid chilling is an alternative to provide for faster meat distribution than conventional systems without quality decrease.

2.4. Effect of chilling rate on microbiology

There is a concern about the effects of spray chilling on the microbiology quality of lamb carcasses. Spray chilling prevents from desiccation and maintains wet the surface of carcasses for a long time, which could enhance microbial growth. However, it has been demonstrated that there are not significant differences between microbial counts of sprayed and conventional chilled carcasses. This conclusion has been obtained either in lamb (Brown et al., 1993) or beef (Greer and Jones, 1997; Strydom and Buys, 1995). In contrast, total viable counts have been described lower in ultra-rapid than in conventional chilled lamb carcasses (Fernández and Vieira, 2012). Carcass temperature decreases faster in ultra-rapid chilling, resulting in a later development of unfavourable conditions to microbial growth. This is a great advantage for meat industry since a low initial microbial load is a key factor for extending lamb shelf-life.

2.5. Economical aspects

Besides meeting meat quality, shelf-life and/or legal aspects, a chilling rate should be suitable and profitable for industry. From an economic point of view, there are interesting differences between the chilling rates that may influence the chilling method adopted by producers.

As it has been explained elsewhere, weight loss is dramatically reduced by spray chilling. According to Allen et al. (1987) shrinkage in beef has been reduced at least 1.5% by the replacement of conventional with spray chilling in North America. A lower but significant reduction of shrinkage is expected in ultra-rapid chilling. The reduction of evaporative weigh losses is the main reason to apply these chilling regimes in the slaughterhouse procedures since it would have a great impact on profitability. However, carcasses should be dispatched or packaged immediately after chilling to maintain the benefits, as evaporation losses would increase if carcasses are left during longer periods in the chillers (Bowater et al., 1997). Other benefits of ultra-rapid chilling are the reduction of the initial microbial load and the time needed to chill carcasses, leading to an increase of meat shelf-life. An extended lamb shelf-life may be a great advantage for distribution, allowing to export this product to distant markets. Moreover, smaller chilling rooms are required in ultra-rapid chilling due to the fact that carcasses are chilled faster, so the cost of equipment is reduced. Regarding spray chilling, its implementation to existing slaughterhouses is relatively inexpensive (Brown et al., 1993). So, the cost of equipments is low in both chilling procedures. However, ultra-rapid chilling increases plant capital as a result of a higher electrical consumption, which is its principal disadvantage.

In brief, industries should calculate inputs and outputs of each methodology and choose the most satisfactory regimen according to quality, security and economical requirements.

3. Refrigerated storage

After carcass chilling, the optimal preservation temperature should be maintained to delay microbial growth and enzymatic reactions. A current challenge for the meat industry is to extend meat shelf-life while keeping its initial quality. Fresh meat shelf-life end-point appears mainly due to microbial growth and lipid and protein oxidation. Oxidation has a significant role in refrigerated meat deterioration as it affects colour, odour, flavour, texture and nutritional value. Products of lipid oxidation are related to rancid odours and flavours while myoglobin oxidation results in meat discolouration. Moreover, there is a relationship among lipid and myoglobin oxidation according to which primary and secondary products of lipid oxidation enhance myoglobin oxidation, leading to meat discoloration. On the other hand, heme proteins, which are abundant in muscle, could enhance lipid oxidation (Faustman et al., 2010; Zakrys et al., 2008). High concentrations of iron and myoglobin are related to greater rates of lipid oxidation (Faustman et al., 1992). Myoglobin becomes metmyoglobin when the central iron atom is oxidised, changing the colour from a red fresh appearance to a less desirable brown colour and decreasing consumers acceptance. As already stated, colour is the main property valued by consumers at the time of purchase; so discoloration resulting from oxidative reactions will mark the end of lamb shelf life. Djenane et al. (2002) reported that a consumer panel rejected beef samples with a percentage of metmyoglobin greater than 40%.

Regarding microbial growth, its rate depends on initial bacterial counts, the predominant flora and the preservation conditions. Initial bacterial counts on lamb are highly variable. In a recent survey on fresh lamb meat in Australian abattoirs, the initial total viable counts means were 2.02 and 2.29 log CFU/cm^2 for leg and shoulder cuts, respectively (Phillips et al., 2013). These values were similar to those obtained by Fernandes et al. (2014) for lamb loins, which were close to 2.5 log CFU/g of sample. However, Berruga et al. (2005) registered higher initial microbial levels (3.3 log CFU/cm^2) in portioned lamb. The predominant flora responsible for spoilage in refrigerated meat consists of *Pseudomonas* spp., *Enterobacteriaceae,* Lactic Acid Bacteria and *Brochotrix thermosphacta* (Borch et al., 1996).

Nowadays, low temperature together with packaging is the most common methodology used to preserve fresh lamb for retail sale. The effect of refrigeration applied to meat preservation is well known, as when temperature decreases the microbial growth and the chemical and enzymatic reaction rates are reduced (Carballo and Jiménez, 2001). The effect of low temperature was reflected in a trial done by Sheridan et al. (1997), who packed lamb primals in several atmospheres and stored them under different temperatures, showing a microbial inhibitory effect when lamb was stored at 0 °C compared to those stored at 5 °C. Usually, the temperatures used to preserve meat range between 1 and 4 °C; however, meet keeping at lower temperatures, which is known as superchilling, is being studied. This consists of meat storage at temperatures below 0 °C, near to the freezing point, without crystal ice formation (Ando et al., 2004). Superchilling improves the effects of conventional refrigeration resulting in an intense microbial growth delay and an extension of the shelf-life (Einarsson, 1988). There is also lower structural damage and protein degradation than in freezing, leading to better quality maintenance (Gallart-Jornet et al., 2007). Youssef et al. (2014) stored vacuum packaged boneless and bone-in beef cuts at 2 °C and −1.5 °C with initial total aerobic bacteria counts of 3.2 vs. 3.4 log CFU/cm^2. Counts reached a maximum number of 7.5 log CFU/cm^2 after 30 days of storage at 2 °C in bone-in cuts, whereas cuts stored at −1.5 °C had 7 log CFU/cm^2 after 70 days. On the other hand, counts reached 7.8 log CFU in boneless cuts after 50 days of storage at 2 °C and 7 log CFU after 90 days at −1.5 °C. In this study, refrigeration at temperatures below 0 °C resulted in a dramatic delay of microbial growth. Moreover, a reduction of undesirable odours and flavours was also observed. In spite of the benefits of superchilling, it is rarely used in meat preservation and more developments are to be expected.

3.1. Refrigerated storage and vacuum packaging

Vacuum packaging was the first packaging methodology used commercially. It consists of air removal from the package with the aim of providing an anoxic environment. Then, the scarce residual oxygen in the package is converted to carbon dioxide by meat respiratory activity (Bell et al., 2001), so oxygen in the pack is totally eliminated. Due to the lack of O_2, bacterial microflora is selected, aerobic microorganisms growth is retarded whereas anaerobic and facultative bacteria grow slowly. Lactic acid bacteria are usually the dominant flora in vacuum packaged lamb, mainly *Leuconostoc* spp. and *Carnobacterium* spp. (Jones et al., 2008). Other bacteria have been also isolated: some environmental species of psychrotrophic *Enterobacteriaceae*, *Serratia spp., Hafnia alvei, Rahnella aquatilis*, avirulent members of the *Yersinia enterocolitica* group and specific spoilage organisms such as *Brochothrix thermosphacta, Shewanella putrefaciens* and psychrophilic "blown-pack" *Clostridium* spp. (e.g. *Clostridium estertheticum*, *Clostridium gasigenes*). However, their proliferation is rare and it is associated with high pH meat which has been temperature abused (Brightwell et al., 2007; Pennacchia et al., 2011).

Large pieces of lamb are commonly vacuum packaged. Ageing is not stopped by vacuum during display, resulting in more tender meat (Lagerstedt et al., 2011). Moreover, microbial growth rate is lower in whole cuts than in vacuum packaged chops leading to an extended shelf-life. Regarding this, Fernandes et al. (2014) recorded 7–8 log CFU/g of aerobic psychrotrophic microorganism after 28 days of storage when whole vacuum packaged lamb loins where maintained at 1 °C. Similar results were registered by Sheridan et al. (1997). When lamb primals were refrigerated at 5 °C mesophilic counts reached 7.8 CFU/g but when they were stored at 0 °C, counts reached 6.3 CFU/g after 28 days.

Besides this, lipid oxidation is dramatically reduced too. Malonaldehyde values for lamb loins packaged in vacuum plastic bags did not reach 1 mg/kg after 28 days of storage at 2–4 °C, which demonstrated that vacuum was very effective in lipid oxidation control (Fernandes et al., 2014). Vacuum packaged large pieces could be also portioned and re-packaged using various types of packaging more adequate for retail selling. Vacuum packaging is less common for retail display, due to the purple colour of meat and the visible purge loss in the bag (Lagerstedt et al., 2011). But microbial growth and lipid oxidation are also significantly inhibited. TBARS values of lamb chops did not achieve 1 mg/kg after 28 days of display at 2 °C, showing the effectiveness of vacuum in reducing lipid oxidation (Berruga et al., 2005)

Nevertheless, oxidative reactions might not be completely inhibited, neither in whole cuts nor in chops, which could have been due to low levels of residual oxygen into the plastic bags enough to promote lipid oxidation reactions (Berruga et al., 2005). But the content of oxygen surrounding meat can be dramatically reduced with skin packaging (VSP), a relative new vacuum packaging development. It consists of placing meat in trays, after that the upper film is heated and placed tightly around the meat. Then, when vacuum is applied the upper film is adhered to the tray performing a tightly disposition which prevents the formation of crevices and improves microbial and sensory meat shelf-life (Lagerstedt

et al., 2011; Strydom and Hope-Jones, 2014). It is known that VSP minimises wrinkles in the pack compared to plastic bag vacuum packaging (Lagerstedt et al., 2011; Kamenik et al., 2014). Moreover, purge loss collected in the pack, which is very common in traditional vacuum packaging (TVP), is reduced, leading to an improvement of meat consumer acceptance. The reduction of purge loss together with the heating of the upper film could reduce microbial growth (Lagerstedt et al., 2011). According to this, Vázquez et al. (2004) showed lower aerobic mesophilic counts in VSP than in TVP when beef was stored at 4 °C for 40 days. The difference recorded between both packaging systems was 1.65 log CFU/g. Moreover, an average difference between VSP and TVP of 1.47 log CFU/g for coliforms was also reported.

Regarding colour maintenance, there is not an agreement about the effect of VSP in beef redness preservation. Li et al. (2012) described VSP to be more effective in redness maintenance, which was attributed to the fact that residual oxygen was lower in VSP than in TVP. However, Lagerstedt et al. (2011) and Kamenik et al. (2014) did not find any significant difference between these packaging systems, which might be due to the low oxygen content achieved by both. Although purple colour of vacuum packaged lamb retail cuts changes to bright red colour when meat is bloomed, this is the main drawback for selling red meat packaged under anaerobic conditions.

Unfortunately, studies using VSP for lamb meat and its acceptance at retail are limited so there are not enough data available. Despite this, VSP is commonly used in beef preservation with highly promising results.

3.2. Refrigerated storage and modified atmosphere packaging

Modified atmosphere packaging (MAP) consists of the removal and replacement of gases surrounding the meat and sealing with barrier materials to maintain a constant package environment during storage (Resconi et al., 2012; Zhou et al., 2010). MAP combined with refrigeration has been considered a useful technology to maintain quality and extend fresh meat shelf-life (Bejarano, 2001), providing an attractive reddish meat highly desirable for retail display (Carpenter et al., 2001).

But the filling atmosphere has to be chosen carefully. High concentrations of oxygen in the gas mixture have been demonstrated to result in optimum colour stability in red meats by promoting oxymyoglobin formation (Jeremiah, 2001). However, the presence of oxygen in the package may promote lipid oxidation, and ultimately myoglobin oxidation (OíGrady et al., 2000). Carbon dioxide (CO_2) is also employed in MAP due to its antimicrobial activity (Dixon and Kell, 1989), which depends on concentration. According to Gill (1996), a concentration of 20% of CO_2 is enough to inhibit psychrotrophic microorganism growth by 50%. Several studies recorded a positive relationship among the percentage of CO_2 present in the atmosphere and microbial growth inhibition (Sheridan et al., 1997; Berruga et al., 2005; Vergara and Gallego, 2001). Other gases such as nitrogen or argon are also commonly used for meat modified atmosphere packaging. Nitrogen is an inert gas that does not react with meat so it is used for preserving package from collapse by its presence in the headspace (Zhou et al., 2010). For red meat preservation 20–30% CO_2 + 70–80% O_2 atmospheres are commonly used for retail selling (Esmer et al., 2011).

Deterioration of modified atmosphere packaged lamb is due to microbial spoilage and/or lipid oxidation. *Pseudomonas* spp. is the predominant genus in aerobic storage and it is involved in off-odour production (Esmer et al., 2011). Other gena are able to grow too; Osés et al. (2013) and Fernandes et al. (2014) registered high counts of lactic acid bacteria and Sheridan et al. (1997) found significant growth of *Brochotrix thermosphacta* in lamb packaged in aerobic conditions. For large pieces, Sheridan et al. (1997) registered 7.2 log CFU/g of total viable counts in 80% O_2/20% CO_2 atmosphere after 28 days at 0 °C and similar results were obtained by Fernandes et al. (2014) in comparable trial conditions. Counts reached 8.5 log CFU/g in lamb primals refrigerated at 5 °C after the same days of storage (Sheridan et al., 1997). On the other hand, 8 log CFU/cm^2 of total viable counts were found in modified atmosphere packaged (80% CO_2/20% O_2) lamb loin chops after 21 days of storage at 2 °C (Berruga et al., 2005).

Regarding oxidative rancidity, it was poorly inhibited under these packaging conditions. TBARS values in lamb chops increased throughout display, reaching 5 mg malonaldehyde/kg after 28 days under 80% CO_2/20% O_2 atmosphere (Berruga et al., 2005). High malonaldehyde values were also obtained by Camo et al. (2008) for lamb steaks packaged under two different atmospheres, 70% O_2/20% CO_2/10% N_2 and 50% O_2/30% CO_2/20% Ar. Malonadehyde values registered after 12 days post-packaging were 4 and 3 mg/kg meat respectively, showing the effect of O_2 in promoting lipid oxidation. Karabagias et al. (2011) observed lower development of oxidative rancidity (2 mg malonaldehyde/kg meat after 12 days of chilling), but the atmosphere used did not contain O_2 (80% CO_2/20% N_2). In conclusion, these results show that MAP is less efficient in controlling lipid oxidation than vacuum packaging.

3.3. Refrigerated storage and active packaging

Since the display life of MAP is limited by microbial growth and lipid oxidation it might be extended by incorporating antimicrobial and/or antioxidant agents within the package. In fact, this is the basis of active packaging (AP). Active packaging emerges due to these demands, so the majority of researchers developing active packages for lamb preservation have used essential oils and extracts obtained from plants or spices to the package, with promising results (Camo et al., 2008; Karabagias et al., 2011). In contrast to chemical additives, which are rejected by consumers, essential oils are included in the Generally Recognised As Safe (GRAS) list of the American Food and Drug Administration. Essential oils and herb extracts contain substances such as thymol, linalool, carvacrol and many others which show antioxidant and inhibitory effects against numerous pathogenic and spoilage bacteria (Kuorwel et al., 2011). Several active packages have been tested in beef (Camo et al., 2011; Barbosa-Pereira et al., 2014; Vargas et al., 2015) with successful results. The advantage of AP is that essential oils and the extracts are not added directly to the meat surface, they are indeed incorporated onto the internal side of the package material.

Despite the benefits of active packaging, there are only a few studies in lamb meat. In one of these, Karabagias et al. (2011) found a significant reduction of bacterial growth in leg lamb chops packaged in MAP containing 0.1% thyme essential oil. This active packaging extended 2–3 days the shelf-life over that reached with conventional MAP. Camo et al. (2008) also reported similar results adding to the package film a varnish containing 4% oregano (*Origanum vulgare*) or rosemary extracts (*Rosmarinus officinalis*). In this case, lamb steaks were chilled at 1 °C for 13 days and meat showed a significantly lower lipid oxidation, a better bright red colour and lower bacterial counts than the control. The presence of oregano extract into the active package resulted in an extension of fresh odour and colour from 8 to 13 days.

In contrast, the use of essential oils and/or extracts may have the disadvantage of adverse sensory effects. This is due to its intense odour which modifies the original odour of the product and may lead to consumer rejection. Nevertheless, rejection depends on the concentration added (Camo et al., 2011).

4. Critical research areas

New improvements are required with the aim of satisfying new demands of fresh meat with an extended shelf-life. A critical point in this field is the reduction of the initial microbial load without adding any chemical compound. In this context, either High Pressure Processing (HPP) or Ultra-Violet Light (UVL) have shown promising results and are both "consumer friendly". HPP results in a better microbiological stability of meat by inactivating microorganisms with minimal modifications of taste or nutritional values (Balasubramaniam and Farkas, 2008). It has been commercially applied in ready-to-eat meat products but its use in fresh meat is still limited due to the effects on meat colour, texture or lipid oxidation. These undesirable changes depend on the processing parameters: temperature, time and pressure (Bajovic et al., 2012), which could be optimised to minimise the effects on meat quality. Several trials have been done with this aim in other species. In lamb, McArdle et al. (2013) studied the effects of HPP on meat quality attributes and their stability during storage; showing promising results. However, there is room to improve this technology for being used in fresh lamb preservation, so new studies are required.

UVL is easy to apply in meat industries and is relatively inexpensive. It has been tested in raw chicken, showing great effects against several pathogen microorganisms such as *Campylobacter jejuni*, *Listeria monocytogenes*, and *Salmonella enterica* serovar Typhimurium (Lyon et al., 2007; Chun et al., 2010) without severe alterations of meat quality. This technology could be satisfactorily applied for fresh lamb decontamination but more studies focused on optimising its application and describing the effects on meat quality are necessary for its future use.

5. Conclusions

The main procedures for lamb chilling have been presented in this review. After discussing pros and cons of each one, it could be concluded that both superchilling and ultra-rapid chilling are the most advantageous technologies for the meat industry, especially when selling fresh meat to distant markets. Rapid chilling rates extend meat shelf-life with retention of sensory properties by reducing time needed to chill carcasses and initial microbial load. Moreover, the implementation of this procedure in existing slaughterhouses would not be difficult and the increase in plant capital costs might be balanced by reduced evaporative losses. Subsequent maintenance at temperatures of $\leq 4\,^{\circ}C$ together with vacuum skin packaging would result in the best stability for long term storage due to strong inhibition of lipid oxidation. Unfortunately, vacuum provides an undesirable colour to meat, which prevents its use for retail display. MAP is currently the most common packaging method for this purpose although it presents several drawbacks that could be overcome by using antimicrobial and/or antioxidant natural agents. New studies should focus on active packaging, which has already shown promising results. This, together high pressure and ultra-violet light, seem to be promising fields of development which would signify a step forward to meat industry.

Conflict of interest

There is not conflict of interest.

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4.2.2. ¿Afecta el sistema de corte a la calidad y aceptabilidad de la carne de cordero?

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¿Afecta el sistema de corte a la calidad y aceptabilidad de la carne de cordero?

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Para el fileteado del cordero se utilizan normalmente dos sistemas de corte: la sierra o la cizalla. El objetivo de este estudio fue evaluar el efecto de ambos sistemas de corte en la vida útil y en la apariencia de chuletas de cordero a lo largo de su conservación en condiciones comerciales.

Resumen

En la actualidad el cordero es fileteado generalmente mediante dos sistemas de corte, la sierra o la cizalla. Se presuponen diferencias en la vida útil y/o la aceptabilidad de las chuletas en función del sistema utilizado, pero son pocos los estudios que lo evalúan, pudiendo tener una gran repercusión en la decisión de compra de los consumidores. Es por esto que el objetivo de este estudio fue evaluar el efecto del sistema de corte en la vida útil de las chuletas de cordero y la aceptación visual de las mismas.

Para este objetivo se emplearon 7 canales de cordero procedentes de un mismo día de sacrificio, seleccionando ambas piernas de cada canal y asignándolas a dos sistemas de corte (sierra y cizalla). Las chuletas resultantes se envasaron en atmósfera modificada (40% O_2 - 30% CO_2 - 30% Ar) y se mantuvieron refrigeradas a 4°C con iluminación artificial durante 14h. Se llevaron a cabo análisis físico-químicos (pH, color, TBARs), microbiológicos (recuento de microorganismos aerobios mesófilos viables totales, enterobacterias, *Pseudomonas spp.* y bacterias ácido lácticas) y sensoriales (análisis visual). Los resultados se analizaron mediante el paquete estadístico SPSS versión 19.0 considerando las diferencias significativas si $P \leq 0,05$.

No se encontraron diferencias significativas entre la vida útil de las chuletas cortadas por sierra o cizalla; no obstante, las fileteadas por cizalla presentaron mayores valores en los índices de rojo (a* y 630/580) a los dos días de almacenamiento. El corte con sierra originó chuletas con una mayor aceptabilidad en el análisis visual, posiblemente debido a las esquirlas que genera la cizalla al impactar en el hueso.

En conclusión, el sistema de corte no afectó a la vida útil de las chuletas de cordero. Sin embargo, el corte con sierra produjo chuletas con mayor aceptabilidad visual, lo que podría tener repercusión en la intención de compra de los consumidores.

Introducción

La carne, y concretamente el cordero, es un producto altamente perecedero y cualquier método que incremente su vida útil es bien recibido por distribuidores y consumidores (Zhou, Xu y Liu, 2010). De hecho, la corta vida útil de la carne de cordero es uno de los factores que más preocupa al sector (Wulf *et al.,* 1995). Existen una gran cantidad de factores que pueden afectar a la calidad general del producto, de los cuales, algunos no han sido todavía bien estudiados o tienen una influencia controvertida, mientras que otros son conocidos y controlados. Este hecho hace necesario definir y controlar el proceso completo desde el campo a la mesa. Principalmente, estamos hablando de la higiene del proceso, la temperatura de conservación y el tipo de envasado, relacionados también entre sí (Sañudo *et al.,* 2013).

El sistema de corte podría tener un efecto en la vida útil de la carne y las diferencias visuales tener un impacto importante en la decisión de compra del consumidor

Además, hay que prestar especial atención a las demandas de los consumidores, cuyos patrones de consumo están en constante evolución. De hecho, los productos cárnicos se adquieren cada vez más en las grandes superficies, en detrimento del comercio tradicional. Esta nueva tendencia ha generado un incremento en la demanda de carne en bandeja y de carne envasada en atmósfera protectora. En consecuencia, los principales factores que determinan actualmente la intención de compra son el precio y aspecto externo, seguidos de la marca y el diseño del envase, el origen local y la trazabilidad del producto (MAGRAMA, 2008).

En el caso de las chuletas de cordero, su aspecto visual resulta de la integración del aspecto del músculo y del aspecto del hueso. El aspecto del músculo depende principalmente del color del mismo. El color es la principal propiedad sensorial del cordero valorada por los consumidores en el momento de la compra. El color rojo brillante se considera tradicionalmente como un aspecto positivo, ya que se asocia con frescura y un producto de calidad superior (Berruga, Vergara y Gallego, 2005).

Con objeto de potenciar el color de la carne, el envasado en atmósfera protectora (MAP) en combinación con bajas temperaturas se ha erigido como la principal tecnología para el mantenimiento de los estándares de calidad y la vida útil de la carne fresca destinada a la venta minorista (Bellés *et al.,* 2017a). El envasado en atmósfera protectora consiste en la eliminación del aire que rodea la carne y su reemplazo por una mezcla de gases adecuada, con un posterior sellado del envase con materiales de barrera para mantener un ambiente constante durante el almacenamiento (Resconi *et al.,* 2012; Zhou *et al.,* 2010).

Se ha demostrado que las concentraciones altas de oxígeno en la mezcla de gases dan como resultado una estabilidad óptima del color en las carnes rojas al promover la formación de oximioglobina (Jeremiah, 2001), y por lo tanto, la carne envasada en estas condiciones presenta un color rojo brillante deseable en el momento de la compra. La carne de cordero se envasa habitualmente en atmósferas con un 20-30% de CO_2 y un 70-80% de O_2 aunque otros gases como el nitrógeno o el argón también se usan con frecuencia (Bellés *et al.,* 2017a).

En relación al aspecto del hueso, su aceptabilidad depende principalmente de la homogeneidad del corte y del color del mismo. La homogeneidad del corte viene definida por el desprendimiento de fragmentos y por el astillado del mismo, mientras que en el color del hueso las principales diferencias se presentan en la médula ósea. Inicialmente esta presenta un color rojo brillante, similar al color de la sangre, observándose un pardeamiento a lo largo del periodo de conservación. Ocasionalmente se observan huesos ennegrecidos en los supermercados, siendo uno de los problemas que más preocupa al sector cárnico. No se conocen muy bien las causas que originan este fenómeno oxidativo, no obstante se intuye un efecto del sistema de corte sobre este proceso de deterioro.

Mientras que las condiciones de conservación y el envasado son temas recurrentes en la investigación, menos estudios se han centrado en los procesos que componen el faenado de la carne. Uno de estos procesos es el fileteado, el cual podría tener una gran importancia en la vida útil de la carne y la aceptabilidad por parte del consumidor.

Actualmente se utilizan principalmente dos sistemas para filetear la carne de cordero, la sierra y la cizalla. Las sierras empleadas en la industria alimentaria, en especial en el sector cárnico, consisten en una hoja o lamina de acero inoxidable cuyos bordes tienen el filo dentado que al aplicar una fuerza mecánica es capaz de cortar músculos y huesos de la carne de cordero al pasar sobre ella. En cambio, la cizalla consiste en una cuchilla helicoidal de acero que ejerce su acción sobre la carne mediante una fuerza cortante tangencial. Aun-

que actualmente se están desarrollando nuevas alternativas de corte como la utilización del láser, estos dos sistemas son todavía los más habituales en el ovino.

El sistema de corte podría tener un efecto en la vida útil de la carne, bien al favorecer o disminuir la contaminación microbiana, así como al liberar compuestos prooxidantes desde el hueso que incrementarían la velocidad de las reacciones de oxidación. Además, las diferencias visuales podrían tener un impacto importante en la decisión de compra del consumidor. Bajo este contexto, el objetivo de este estudio fue evaluar el efecto de ambos sistemas de corte en la vida útil y en la apariencia de chuletas de cordero a lo largo de su conservación en condiciones comerciales.

Material y métodos

Muestreo y diseño experimental

Siete canales de cordero se escogieron aleatoriamente y se refrigeraron durante 24 horas (-1,5 – 0,5 ˚C). A continuación, las piernas derechas se filetearon con una sierra vertical mientras que las izquierdas se filetearon con una cizalla. Las chuletas resultantes se envasaron en una atmósfera protectora (40% O_2/ 30% CO_2/ 30% Ar) y los envases se refrigeraron a 4 ± 0,5 ˚C con ciclos de 14 horas de luz artificial durante 8 días. Se realizaron análisis antes del envasado y tras 2, 5 y 8 días de almacenamiento refrigerado.

Análisis físico-químicos

La determinación del pH se realizó por duplicado sobre el músculo *semimembranosus* mediante un electrodo de punción SENSION+ PH1 (Hach, Reino Unido).

Se realizó la medida del color instrumental mediante un espectrofotómetro Minolta CM-2002 (Oaka, Japón), realizando 10 réplicas sobre la superficie de cada chuleta. El ratio 630/580 se calculó dividiendo el porcentaje de luz reflejada a la longitud de onda de 630 nm ratio por la reflejada a 580 nm, mientras que el porcentaje de metamioglobina se calculó a partir de las reflectancias mediante la ecuación propuesta por Krwinzky (1979).

La oxidación lipídica se calculó como sustancias reactivas al ácido tiobarbitúrico (TBARS) siguiendo la metodología descrita en Bellés *et al.,* (2017b).

Análisis microbiológicos

Los recuentos microbianos se obtuvieron mediante técnicas de microbiología clásica. Tras la recogi-

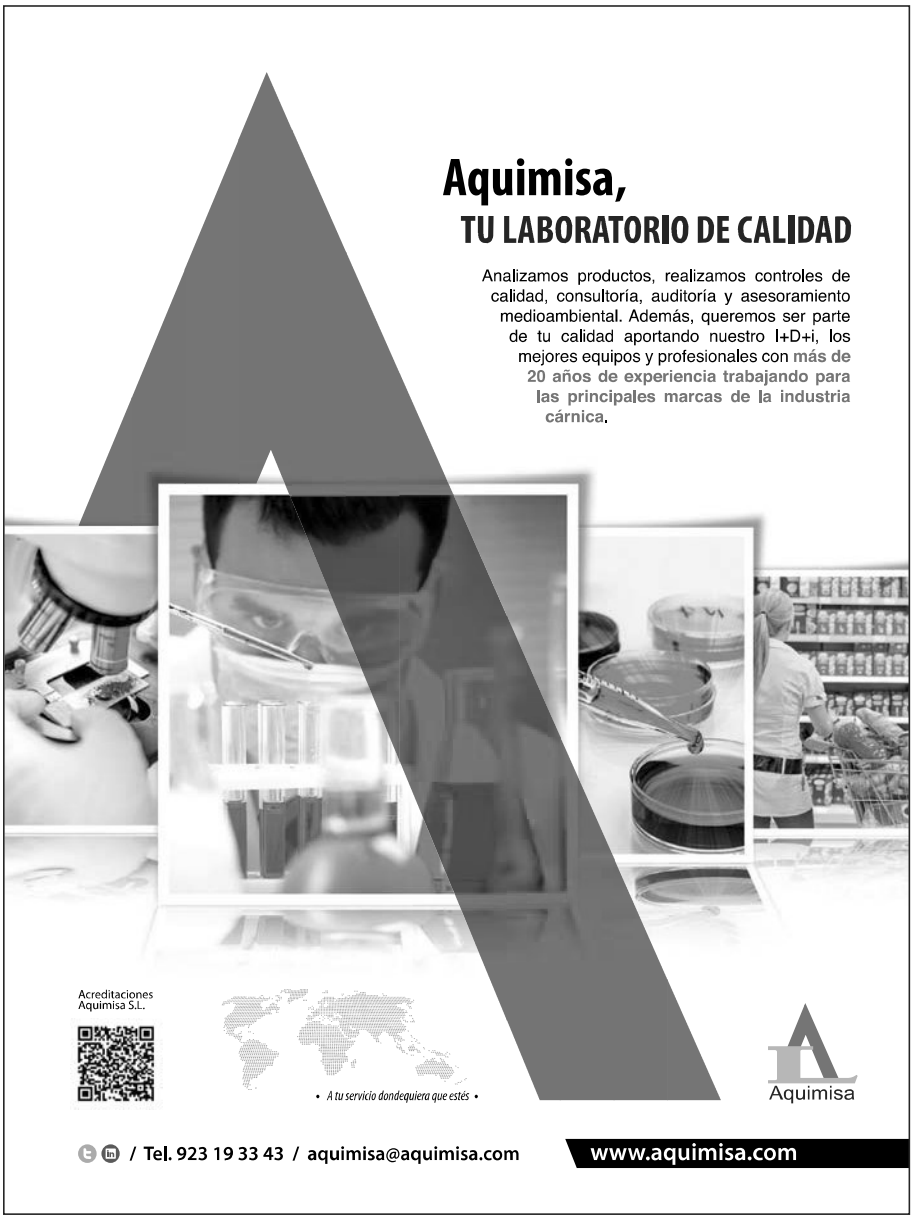
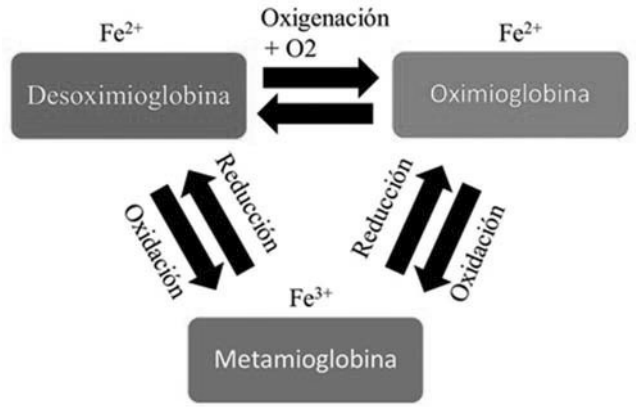


Tabla 1. Efecto del sistema de corte en la evolución de los valores de pH de las chuletas de cordero envasadas en MAP

Corte	Días de conservación				
	0	2	5	8	P
Cizalla	5,70±0,09	5,65±0,14	5,68±0,07	5,74±0,11	0,577
Sierra	5,66±0,07	5,67±0,08	5,70±0,06	5,69±0,08	0,821
P	0,487	0,749	0,597	0,469	–

Los valores en las celdas muestran la media y la desviación estándar.

Figura 1. Estados de la mioglobina en la carne



da de las muestras por hisopado, se realizaron diluciones en agua de peptona para realizar la siembra en masa en el caso de los mésofilos aerobios viables totales (MAVT) y *Enterobacteriaceae* y en superficie para *Pseudomonas spp.* Se utilizó agar PCA (Merck) para los recuentos de MAVT, agar VRBD (Merck) para los microorganismos de la familia *Enterobacteriaceae* y agar CFC (Merck) para las *Pseudomonas spp.* Los recuentos de MAVT se realizaron tras 24h de incubación a 37 °C, los de enterobacterias tras 48h a 37 °C y los de *Pseudomonas spp.* después de 24h a 25 °C.

Análisis visual

El efecto del sistema de corte en la aceptación visual se estudió utilizando un panel no entrenado equilibrado por sexo y edad (n=50). Los panelistas evaluaron la aceptabilidad del músculo, la aceptabilidad del hueso y la aceptabilidad global mediante una escala estructurada hedónica de 9 puntos desde 1 (me desagrada extremadamente) a 9 (me agrada extremadamente), donde el punto central (ni me agrada ni me desagrada) había sido eliminado.

Análisis estadístico

Todos los datos fueron analizados mediante el modelo lineal general del programa estadístico IBM SPSS versión 19 (IBM SPSS, 2010). El modelo incluyó el efecto del sistema de corte y el tiempo de refrigeración como efectos principales, además de su interacción. Para el análisis visual se utilizó el mismo modelo, incluyendo el panelista como efecto aleatorio. Las diferencias se consideraron significativas si $P \leq 0,05$.

Resultados y discusión

Valores de pH

Sobrinho, Purchas, Kadim y Yamamoto (2005) propusieron que los valores normales de pH de la carne de ovino se encuentran comprendidos entre 5,5 y 5,8, con pequeñas variaciones en función de la raza y el músculo elegido. Como se muestra en la **tabla 1**, todos los valores obtenidos se encontraron dentro de este rango. Nuestro estudio no mostró diferencias significativas en los valores de pH de las chuletas cortadas por cizalla y sierra. Además, tampoco se observó una evolución del pH a lo largo del periodo de estudio. En ocasiones se puede observar un descenso del pH en la carne de cordero durante el periodo de conservación, debido principalmente al crecimiento de las bacterias ácido lácticas (Cayré, Vignolo y Garro, 2003) y a la disolución de CO_2 en el músculo, que también podría contribuir a una reducción del pH debido a la formación de ácido carbónico. No obstante, este fenómeno no se observó en este estudio.

Medida instrumental del color

Como se ha comentado anteriormente, el color de la carne es uno de los parámetros de calidad más importantes. El color de la carne depende principalmente del contenido de su principal pigmento, la mioglobina, su estado químico y las propiedades de dispersión de la luz en la carne. La mioglobina es una proteína sarcoplásmica que contiene un grupo hemo (anillo porfirínico con un átomo de hierro en el centro). En condiciones biológicas en el músculo y en la carne fresca, el hierro se encuentra en la mioglobina en forma de ión ferro-

so. Éste puede tener asociada una molécula de oxígeno, formando entonces lo que se denomina la oximioglobina, de color rojo brillante. Mientras que en ausencia de oxígeno se encuentra en forma de desoximioglobina, mostrando un color rojo púrpura más intenso y oscuro que el de la oximioglobina (estas dos formas son interconvertibles). La oxidación del hierro a Fe^{3+} supone la conversión del pigmento en metamioglobina, y con ello el paso de un color rojo deseable a un color marrón o pardo rechazado por los consumidores (Bianchi y Feed, 2010) (**figura 1**).

La medida instrumental del color de la carne se expresa habitualmente mediante las coordenadas CIELAB. El espacio CIELAB permite especificar estímulos de color en un espacio tridimensional. El eje *L es el de luminosidad o claridad y va de 0 (negro) a 100 (blanco). Los otros dos ejes de coordenadas son a* y b*, y representan variación entre rojizo-verdoso, y amarillento-azulado, respectivamente. Además, el índice 630/580 también se emplea habitualmente para representar la pérdida del color rojo de la carne a lo largo del periodo de conservación. De acuerdo con Hunt (1980), una caída en los valores de este índice se puede asociar a un cambio en el color de la carne debido a la formación de metamioglobina.

Como puede observarse en la **tabla 2**, no se apreció ningún efecto significativo del sistema de corte en la luminosidad de las chuletas, mostrando valores similares durante todo el periodo de estudio. En cambio, sí que se encontraron diferencias significativas entre ambos sistemas en los valores de a* y del índice 630/580. Estas diferencias se identificaron tras dos días de refrigeración, mostrando las chuletas cortadas por cizalla mayores valores en ambos índices. Por tanto, el sistema de corte por cizalla resultó en una carne más roja y más atractiva para el consumidor. Las diferencias pudieron deberse al aumento en los valores de a* y del índice 630/580 que se observó en las chuletas fileteadas por

Tabla 2. Efecto del sistema de corte en la medida instrumental del color de la carne de cordero envasada en MAP

	Corte	Días de conservación				
		0	2	5	8	P
L*	Cizalla	39,77±1,27 xy	36,98±1,42 x	39,86±2,77 xy	44,18±4,08 y	0,005
	Sierra	39,47±3,34	39,88±2,68	39,33±3,73	42,39±3,16	0,43
	P	0,859	0,065	0,806	0,46	–
a*	Cizalla	11,69±0,57 y	17,61±0,4 b,z	10,23±3,85 xy	6,65±2,7 x	<0,001
	Sierra	12,39±2,48 y	13,92±2,21 a,y	10,54±3,55 xy	6,62±2,76 x	0,005
	P	0,56	0,006	0,898	0,989	–
b*	Cizalla	12,75±0,49 x	17,15±0,32 b,z	14,47±0,39 b,y	13,54±1,04 xy	< 0,001
	Sierra	12,78±1,04	14,89±1,61 a	13,56±0,46 a	13,89±1,44	0,092
	Total	0,956	0,015	0,011	0,664	
630/580	Cizalla	2,66±0,12 yz	3,44±0,16 a,z	1,88±0,60 xy	1,44±0,33 x	< 0,001
	Sierra	2,78±0,44 y	2,70±0,37 b,y	1,98±0,47 y	1,35±0,24 x	< 0,001
	P	0,722	0,006	0,716	0,682	

Los valores en las celdas muestran la media y la desviación estándar.
Letras diferentes en la misma columna (a, b) indican diferencias significativas entre los sistemas de corte (P ≤ 0,05).
Letras diferentes en la misma fila (x, y, z) indican diferencias significativas entre los distintos días de estudio dentro de un mismo sistema de corte (P ≤ 0,05).

cizalla y que por el contrario, no experimentaron aquellas cortadas por sierra. Este incremento del color rojo, fenómeno conocido como "blooming", se presenta cuando la mioglobina entra en contacto con una atmósfera más rica en oxígeno. Al interaccionar la superficie de la carne con una atmósfera con mayor presión parcial de oxígeno, la desoximioglobina se oxigena en la superficie del filete, formando un complejo con el oxígeno (oximioglobina) y resultando en un color rojo más brillante. Una de las posibles razones que explicaría la ausencia de "blooming" en las muestras cortadas por sierra podría ser el polvo que este sistema genera al cortar el hueso, que se depositaría en la superficie de la chuleta e impediría el desarrollo de este fenómeno.

En ambos sistemas se observó un descenso significativo y continuo de los valores de a* y del índice 630/580 a lo largo del periodo de conservación. Esta decoloración en la carne envasada a lo largo del tiempo ha sido ampliamente descrita en la bibliografía (Bellés *et al.,* 2017b; Ripoll *et al.,* 2011; Berruga *et al.,* 2005). La principal causa de esta evolución del color es la formación de metamioglobina.

Como muestra la **figura 2**, el contenido inicial de metamioglobina se encontraba comprendido entre el 20 y el 30%, unos valores frecuentes en carne fresca de cordero. A partir del día 2 se registró un incremento significativo del porcentaje de metamioglobina, muy

Figura 2. Porcentaje de metamioglobina (% Mmb) de las chuletas envasadas en MAP a lo largo del periodo de estudio

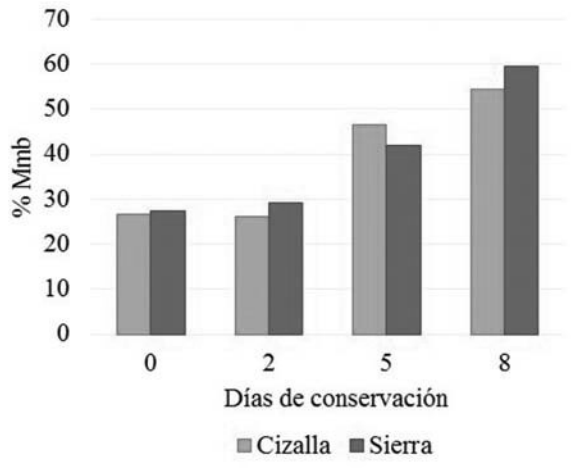
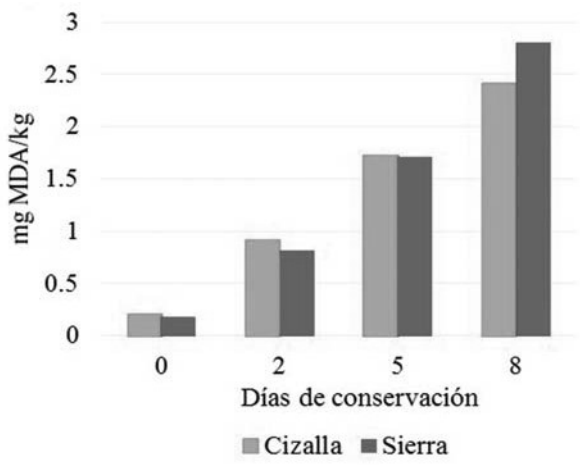


Figura 3. Evolución de la oxidación lipídica de las chuletas de cordero envasadas en MAP a lo largo del periodo de conservación



elevado ya el día 5, y que sería el responsable de la caída de los índices de rojo.

La formación de metamioglobina es una de los principales factores limitantes de la vida útil de la carne de cordero. Es por ello que se han realizado un gran número de investigaciones en torno al efecto que provoca su presencia en la aceptabilidad del consumidor, y a partir de qué contenido de metamioglobina los consumidores rechazarían la carne. En este sentido, Lawrie (1998) estableció un 60% de metamioglobina como el límite de aceptabilidad, no obstante, de acuerdo con Djenane *et al.,* (2002) este porcentaje sería demasiado alto, rechazando los consumidores la carne cuando el contenido relativo de metamioglobina sobrepasase un 40%. Como muestra la **figura 2** y teniendo en cuenta este último límite, parece evidente que las muestras de ambos sistemas se encontraban en el umbral del rechazo tras 5 días de conservación, superando ampliamente el límite de aceptabilidad al final del periodo experimental.

También se observa en la **figura 2** la ausencia de efecto del sistema de corte sobre la oxidación de este pigmento, ofreciendo ambas tecnologías de corte unos valores similares en todos los días de estudio.

Oxidación lipídica

La carne es muy susceptible a las reacciones de oxidación debido a su alto contenido en ácidos grasos insaturados, pigmentos hemo, catalizadores metálicos y un amplio abanico de compuestos prooxidantes presentes en el tejido muscular (Falowo, Fayemi y Muchenje, 2014). Las reacciones de oxidación de los lípidos empiezan en la membrana fosfolipídica, como una reacción autocatalítica en la que los compuestos prooxidantes interaccionan con los ácidos grasos insaturados provocando la liberación de radicales libres, alimentando una seria de reacciones en cadena que amplifican los procesos oxidativos (Labuza, 1971).

Tabla 3. Efecto del sistema de corte en los recuentos microbianos de las chuletas de cordero envasadas en MAP

	Corte	Días de conservación				
		0	2	5	8	P
MAVT	Cizalla	nd	nd	1,78±0,39	2,58±1,22	–
	Sierra	nd	nd	2,11±0,42 x	3,7±1,01 y	< 0,001
	P	–	–	0,229	0,152	
Pseudomonas spp.	Cizalla	nd	nd	nd	nd	–
	Sierra	nd	nd	nd	2,5±0,77	–
	P	–	–	–	–	
BAL	Cizalla	nd	nd	nd	1,74±1,73	–
	Sierra	nd	nd	nd	2,55±0,57	–
	P	–	–	–	0,348	

Los valores en las celdas muestran la media y la desviación estándar.
nd: crecimiento por debajo del límite de detección.

Tabla 4. Efecto de los sistemas de corte en el aspecto visual de las chuletas de cordero envasadas en MAP

	Corte	Días de conservación		
		1	6	P
Aceptabilidad del músculo	Sierra	6,01±1,22 y	4,26±1,6 b,x	<0,001
	Cizalla	5,67±1,39 y	3,81±1,40 a,x	<0,001
	P	0,055	<0,001	–
Aceptabilidad del hueso	Sierra	6,26±1,22 b,y	4,89±1,64 b,x	<0,001
	Cizalla	4,76±1,40 a,y	4,10±1,31 a,x	<0,001
	P	<0,001	<0,001	–
Aceptabilidad global	Sierra	6,25±0,3 b,y	4,53±1,6 b,x	<0,001
	Cizalla	5,53±1,20 a,y	4,00±1,40 a,x	<0,001
	P	<0,001	<0,001	–

Los valores en las celdas muestran la media y la desviación estándar. Letras diferentes en la misma columna (a, b) indican diferencias significativas entre los sistemas de corte (P ≤ 0,05). Letras diferentes en la misma fila (x, y, z) indican diferencias significativas entre los distintos días de estudio dentro de un mismo sistema de corte (P ≤ 0,05). Los atributos se evaluaron con una escala estructurada de 8 puntos desde 1 (me desagrada extremadamente hasta 9 (me agrada extremadamente) donde el punto central (ni me agrada ni me desagrada) había sido eliminado.

La oxidación afecta por tanto a los ácidos grasos, sobre todo a los ácidos grasos poliinsaturados que son altamente inestables y reaccionan fácilmente con el oxígeno, produciéndose el fenómeno conocido como autooxidación. La autooxidación provoca cambios importantes en el sabor, aroma, color, textura, valor nutritivo y condiciona la estabilidad de la carne de cordero durante el tiempo de almacenamiento.

La oxidación de la carne se determina habitualmente con la cuantificación de las sustancias reactivas al ácido tiobarbitútico, principalmente malondialdehído (MDA), unos de los principales compuestos derivados de las reacciones de oxidación. Una de las ventajas de esta técnica es su buena correlación con la detección sensorial de la rancidez de la carne. De acuerdo con Lanari *et al.,* (1995) la rancidez de la carne empieza a ser detectada cuando el contenido en MDA supera 0,5 mg/kg, mientras que Campo *et al.,* (2006) observaron el rechazo de la carne debido a su oxidación lipídica cuando la cantidad de MDA sobrepasaba los 2 mg/kg. Como muestra la **figura 3**, la oxidación lipídica aumentó de forma progresiva a lo largo del periodo de conservación, sin diferencias significativas entre ambos sistemas de corte (P > 0,05). Por tanto, no se observó ningún efecto prooxidante derivado del sistema de fileteado. En el día 5 de estudio se superaron los 0,5 mg de MDA/kg, por lo que las chuletas presentarían ya una rancidez detectable por los consumidores. No obstante, no fue hasta el día 8 cuando las chuletas mostraron valores de MDA por encima del límite de aceptabilidad (2 mg MDA/kg). La oxidación lipídica limitaría por tanto la vida útil de la carne de cordero envasada en MAP a menos de 8 días, independientemente del sistema de corte empleado.

Los altos valores de malondialdehído obtenidos coincidieron con los de otros estudios realizados con anterioridad en los que evaluaban la vida útil de chuletas de cordero envasadas en atmósferas protectoras ricas en O_2 (Bellés *et al.,* 2017b; Camo, Beltrán y Roncalés, 2008). Ambos estudios observaron valores de malondialdheido superiores a 2 mg/kg tras 8 días de refrigeración, identificando las reacciones oxidativas como un punto crítico en la conservación de carne de cordero. El elevado contenido de oxígeno en estas atmósferas se ha señalado como la principal causa de la velocidad de este proceso de deterioro. De hecho, las reacciones oxidativas dejan de ser un parámetro crítico para la conservación de la carne de cordero cuando esta se envasa en ausencia de oxígeno, condiciones ofrecidas por ejemplo con el envasado a vacío.

Además del desarrollo de olores y sabores rancios, la oxidación tiene un efecto determinante en la pérdida del color. Los productos de la oxidación lipídica estimulan la conversión gradual de la oximioglobina en metamioglobina, resultando en la pérdida del color rojo brillante de la carne. De igual forma, diversos productos de la oxidación proteica podrían favorecer el desarrollo de la oxidación lipídica. Se trata por tanto de procesos interconectados (Faustman *et al.,* 2010).

Recuentos microbianos

Los recuentos microbianos obtenidos en ambos sistemas de corte a lo largo del periodo de estudio se presentan en la **tabla 3**.

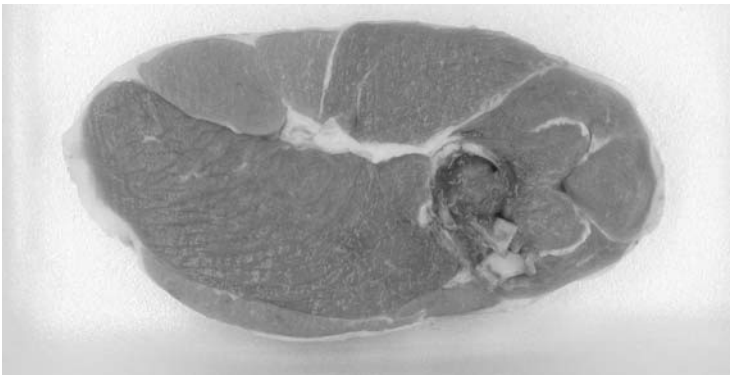
Los recuentos iniciales para los dos sistemas de corte y para todos los grupos de microorganismos se encontraron por debajo de nuestros límites de detección, lo que demuestra que tanto el faenado como el sacrificio en el matadero se llevaron a cabo bajo unas óptimas condiciones higiénicas.

No se obtuvieron recuentos de microorganismos de la familia *Enterobacteriaceae* durante el periodo experimental, mientras que el crecimiento de microorganismos de aerobios mesófilos viables totales (AMVT) se detectó a partir de los 5 días y el de las bacterias ácido lácticas (BAL) y *Pseudomonas spp.* tras 8 días de refrigeración. Como era de esperar los recuentos más altos se dieron el último día de estudio.

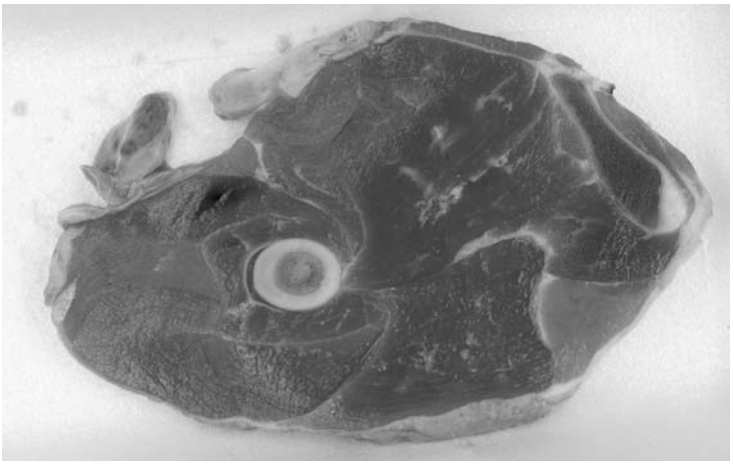
Aunque se obtuvieron mayores recuentos de AMVT, BAL y *Pseudomonas spp.* en las chuletas cortadas por sierra que en cizalla, estos no difirieron significativamente ($P > 0,05$), lo que demuestra que ningún tipo de corte favorece la dispersión microbiana.

Todos los recuentos fueron inferiores a 7 unidades logarítmicas/cm^2, que es el valor establecido por muchos autores como el límite de aceptación (Jeremiah, 2001). Por tanto, el crecimiento microbiano no supuso un límite para la vida útil de las chuletas de pierna de cordero envasadas en MAP.

Fotografía 1. Chuleta de pierna de cordero fileteada por cizalla



Fotografía 2. Chuleta de pierna de cordero fileteada por sierra



Análisis visual

La **tabla 4** presenta los resultados obtenidos en el análisis visual de las chuletas. Como puede comprobarse, los panelistas no encontraron diferencias significativas en el aspecto inicial del músculo, pero en cambio, sí que señalaron diferencias en el aspecto del hueso, obteniendo las chuletas cortadas por sierra una mayor valoración ($P < 0,001$) Estas diferencias en la aceptabilidad del hueso se mantuvieron tras 6 días de conservación ($P < 0,001$), siendo las valoraciones en este punto 4,89 y 4,10 para las chuletas cortadas por sierra y cizalla, respectivamente. La menor aceptabilidad del hueso obtenida por las chuletas cortadas por cizalla pudo deberse a las astillas que este sistema genera al impactar la cuchilla en el hueso. Como se observa en la **fotografía 1** estas son evidentes, en cambio, el aspecto del hueso es mucho más homogéneo en el corte por sierra (**fotografía 2**).

Estas diferencias en la aceptabilidad del hueso tuvieron repercusión en la aceptabilidad global de las chuletas. Los panelistas otorgaron mejores puntuaciones a las chuletas cortadas por sierra, tanto el día 1 como el 6 ($P < 0,001$)

Además, se observó una disminución de la aceptabilidad de los tres parámetros a lo largo del periodo de conservación. Este descenso pudo deberse a los procesos de deterioro, principalmente a la pérdida del color rojo y al oscurecimiento del color del hueso. Como se ha comentado anteriormente, el aspecto visual es un factor decisivo para la intención de compra del consumidor. De esta forma, el sistema de fileteado tendría una gran influencia en la decisión de los consumidores.

Conclusiones

La vida útil de las chuletas de cordero no se vio influenciada por el sistema de corte empleado, no obstante, las chuletas cortadas por cizalla mostraron un color más rojo tras dos días de refrigeración.

Las chuletas envasadas en atmósfera protectora presentaron una vida útil comprendida entre 5 y 8 días, siendo el color y la oxidación lipídica los factores limitantes.

Las chuletas cortadas con sierra mostraron una mayor aceptabilidad del hueso y aceptabilidad global en el análisis visual, probablemente debido al corte más homogéneo y a la ausencia de esquirlas óseas. Estas diferencias podrían tener una gran repercusión en la decisión de compra del consumidor, por tanto, el corte con sierra sería más adecuado para la venta de carne en expositores refrigerados.

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4.2.3. The combined effects of superchilling and packaging on the shelf life of lamb

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The combined effects of superchilling and packaging on the shelf life of lamb

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ABSTRACT

The aim of this study was to evaluate the effect of superchilled storage at −1° on the shelf life of lamb slices packaged in an O_2 enriched (40% O_2/30% CO_2/30% Ar) or in an anaerobic atmosphere (vacuum skin packaging). Physicochemical, microbial and sensory analyses were performed. The effect of superchilled storage on lamb stability differed depending on the atmosphere surrounding the product. Superchilled (−1 °C) slices of lamb showed lower microbial counts than those refrigerated at 4 °C in both packaging conditions. Moreover, meat stored at −1 °C had a higher colour stability under vacuum. Superchilled storage combined with an O_2 enriched atmosphere increased the rate of lipid oxidation, which reduced the shelf life reached by refrigerating at 4 °C. Vacuum skin packaging strongly inhibited lipid oxidation independently of storage temperature. Thus, superchilled storage extended the shelf life at least twice compared to storage at 4 °C under anaerobic conditions while it was disadvantageous when an O_2 enriched atmosphere was used.

1. Introduction

Fresh lamb is a perishable product so it is commonly preserved by refrigeration combined with packaging either for storage or display (Berruga, Vergara, & Gallego, 2005; Vieira & Fernández, 2014). Refrigeration temperatures around 4 °C and modified atmosphere (MAP) or vacuum skin packaging (VSP) are usually used to extend the lifespan of lamb meat. Traditionally the gases used in MAP comprises O_2, CO_2 and N_2 (Bellés, Alonso, Roncalés, & Beltrán, 2017a). Lamb is packaged in enriched O_2 atmospheres (70–80% O_2) to provide a desirable red colour to meat. However, the presence of O_2 could enhance lipid oxidation and ultimately myoglobin oxidation (O'Grady, Monahan, Burke, & Allen, 2000), so an atmosphere including a lower content of O_2 could be more suitable when an extended shelf life is required. Argon (Ar) is an inert, odourless and tasteless noble gas envisaged as a possible alternative to N_2 for MAP application (Heinrich, Zunabovic, Nehm, Bergmair, & Kneifel, 2016). Spencer and Humphreys (2002) suggested that Ar displaces O_2 more efficiently and offers a higher shelf life than N_2 based MAP, while Ruiz-Capillas and Jiménez-Colmenero (2010) and Herbert, Roissant, Khanna, and Kreyenshmidt (2013) noted a better keeping of sensory properties in Ar based MAP. Despite N_2 is still more common, Ar based MAP is increasingly used for lamb retail cuts packaging in Spain since it could have some benefits on lamb preservation.

Regarding vacuum skin packaging, it is a relative new development which achieves a tight disposition of the film on meat surface, reducing the formation of pockets and consequently, decreasing residual O_2. Wrinkles, which are common in traditional vacuum packaging, are also avoided thus improving the visual appearance of packaged meat (Lagerstedt, Ahnström, & Lundström, 2011).

However, the shelf life reached by refrigerating at 4 °C hardly overpasses 21 days (Berruga et al., 2005; Łopacka, Półtorak, & Wierzbicka, 2016), which represents a limiting factor for industries interested in an extended life for distribution or storage. Freezing is commonly used for long-term meat preservation due to the extended shelf life of frozen meat resulting in a greater flexibility for distributors and retailers (Wheeler, Miller, Savell, & Cross, 1990). However, some studies have found that freezing could modify meat tenderness, juiciness and flavour (Kaale, Eikevik, Rustad, & Kolsaker, 2011; Vieira, Díaz, Martínez, & García-Cachán, 2009), and these variations have not gone unnoticed for consumers, who associate frozen meat with a lower quality product than chilled meat (Lagerstedt, Enfält, Johansson, & Lundström, 2008).

In this context, superchilling (also called deep chilling) emerges as a possible solution. It consists of meat storage at temperatures comprised between freezing and chilling, usually between −0.5 and −2.8 °C (Beaufort, Cardinal, Le-Bail, & Midelet-Bourdin, 2009; Kaale et al., 2011). At superchilling temperatures microbial growth is strongly inhibited, extending the shelf life reached with conventional chilling by 1.4–4 times (Magnussen, Haugland, Torstveit Hemmingsen, Johansen, & Nordtvedt, 2008). Superchilling has been extensively used for seafood with promising results (Kaale & Eikevik, 2014) but the studies in meat preservation are scarce. Lan, Shang, Song, and Dong

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(2016) observed an extension of rabbit shelf life in 3–5.5 times by superchilled storage, which was also noted by Zhang, Wang, Li, Wu, and Xu (2016) for broiler meat. Nevertheless, severe myofibrils damages were detected, which may have been due to the formation and growth of ice crystals (Lan et al., 2016). These alterations could favour prooxidants release (Benjakul & Bauer, 2001) and consequently, lipid oxidation and colour deterioration. Superchilling can extend the shelf life of lamb but its effect on meat quality is a matter of concern. Moreover, there is also little knowledge about the effect of superchilling combined with packaging on meat shelf life and, as far as we know, there is not literature published concerning lamb.

Therefore, the aim of this study was to assess the effect of superchilled storage on physicochemical, microbiological and sensory quality of fresh lamb leg slices packaged in an O_2 enriched atmosphere (MAP) and in absence of O_2 (VSP). At the same time, differences in quality throughout storage were used to determine the shelf life reached by superchilling combined with both MAP and VSP.

2. Materials and methods

The lambs used for this trial were cared in accordance with the guidelines from the Spanish Ministry of Agriculture (Boletín Oficial del Estado (BOE), 2007).

2.1. Slaughtering, packaging, refrigerated storage and sampling

Twenty lamb carcasses were randomly chosen among commercial lambs of the Rasa Aragonesa, a medium wool breed that is reared for meat purpose in Spain. Animals were reared under intensive husbandry conditions with natural suckling until 40 days of age and fodder with concentrate and cereal straw ad libitum until they reached a body weight between 20 and 25 kg. The animals, aged about three months, were slaughtered in a commercial slaughterhouse (Mercazaragoza) following standard protocols. Within 15 min of dressing, carcasses were transported to the facilities of Casa de Ganaderos and Franco y Navarro S.A. and chilled for 24 h (-0.5 ± 0.5 °C, 90% RH, 1–2 m/s). The two legs of each carcass were removed and allocated among two packaging systems (MAP and VSP) and two storage temperatures (-1 °C and 4 °C), being the 10 legs of each group from different animals ($n = 10$). Legs were cut into 20-mm-thick slices and transported under refrigeration (4 °C) to the food technology pilot plant of the Faculty of Veterinary Science (University of Zaragoza). After that, slices were either vacuum skin (Multivac R570 CD, MultivacSepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany) or modified atmosphere packaged (40% O_2 + 30% CO_2 + 30% Ar) (ULMA-SMART-500, Ulma S. Coop., Guipúzcoa, Spain) with a product to gas ratio of 1:3 (2 slices per tray). The top film of the vacuum skin packages (VSP) (Cryovac® VST 0250 SKIN TOP WEB, Sealed Air S.L., Abrera, Spain) was 100 μm thick; its O_2 permeability rate at 23 °C was 1.5 $cm^3/m^2/24$ h/0% relative humidity (R.H.) and the water vapour transmission rate at 38 °C was 6 $g/m^2/24$ h/90% R.H. The bottom film (Cryovac® EGA 008, Sealed Air S.L., Abrera, Spain) was 200 μm thick and O_2 permeability rate at 23 °C was 21 $cm^3/m^2/24$ h/atm/0% R.H. For modified atmosphere packaging, polystyrene trays (Linpac packaging S.A.U., Pravia, Spain) were used and sealing was done with a polyethylene and polyamide laminate film. The film was 30 μm thick, its O_2 permeability rate at 23 °C was 15 $cm^3/m^2/24$ h/0% R.H. and the water vapour transmission rate at 23 °C was 7 $g/m^2/24$ h/85% R.H., (Linpac Packaging S.A.U., Pravia, Spain). After packaging, samples were refrigerated at conventional (4 ± 0.5 °C) or superchilling (-1 ± 0.5 °C) temperatures according to the assigned batch.

Specific analyses were performed at 0 (approximately 24 h post mortem), 7, 14, 21 and 28 days post packaging. Samples destined to sensory analyses were vacuum packaged in each sampling day (-900 mbar of pressure) in polyethylene-polyamide bags with ethyl vinyl acetate sealant layer (30 × 25 cm, 90 μm thickness, water vapour transmission rate at 23 °C of 2.8 $g/m^2/24$ h/85% RH, an O_2 transmission rate at 23 ± 1 °C of 50 $cm^3/m^2/24$ h/75% RH; Eurobag & Film S.L., Spain) using a Tecnotrip EV-13-L-CD-SC machine (Tecnotrip S.A., Spain) and frozen stored at -20 °C until the evaluation was performed. All the analyses were performed on the *Semimembranosus* muscle.

2.2. Lipid oxidation

Lipid oxidation was determined as Thiobarbituric Acid Reactive Substances (TBARS) following the method described by Alonso et al. (2015).

2.3. Instrumental colour

A Minolta CM-2002 (Osaka, Japan) spectrophotometer was used to measure colour at the surface of a 20-mm-thick slice after opening the trays and exposing the samples to air for 2 h at 4 °C. The parameters registered were L^* (lightness), a^* (redness) and b^* (yellowness). A D65 illuminant was used at an observation angle of 10° and with an aperture of 30 mm. The instrument was calibrated using a white and black standard.

2.4. Microbial analyses

Samples were aseptically collected from the slices swabbing an area of 10 cm^2 delimited with a sterile aluminium template (10 cm^2). Each sample was homogenized in 0.1% peptone water (Biolife) and serial dilutions were done.

For psychrotrophic total viable counts (PTVC), *Enterobacteriaceae* and lactic acid bacteria (LAB) determination, one milliliter of the correct dilution was inoculated in a Petri Plate and after that approximately 15 ml of the appropriate agar were added. Psychrotrophic total viable counts (PTVC) were investigated using plate count agar (PCA) (Merck) after incubation at 10 °C for 96 h. For *Enterobacteriaceae* counts violet red bile dextrose agar (VRBD) was used, covering plates after solidifying with 3–4 ml of VRBD (double layer) (Merck). Then, they were incubated at 37 °C for 48 h. Plates for LAB investigation were covered with man rogosa and sharpe agar (MRS) (Merck) and they were placed in an anaerobic jar with an anaerobic atmosphere generator kit (Anaerocult A) (Merck) together with an anaerobic conditions indicator strip (GazPack™). Incubation was done for 96 h at 37 °C. Regarding *Pseudomonas* spp. determination, 0.1 ml of the property dilution was inoculated on the surface of Cephalothin-Sodium Fusidate-Cetrimide Agar (CFC) (Merck) and the inoculum was spread using a sterile plastic handle. Plates were counted after incubation at 20 °C for 24 h.

All microbial counts were expressed as base-10 logarithm of colony forming units per cm^2 of surface area (log CFU/cm^2).

2.5. Sensory analyses

Samples were thawed in tap water for 4 h and after breaking vacuum were wrapped in aluminium foil and cooked at 200 °C in a double-plate grill (Sammic GRS-5, Guipúzcoa, Spain) until an internal temperature of 72 °C was reached, which was monitored by an internal thermocouple Jenway 200 (Jenway Scientific, Ston, United Kingdom). After cooking, the muscle *Semimembranosus* was cut in portions (2 cm × 2 cm × 2 cm) wrapped individually in aluminium foil and assigned a single random three digit code. Samples were placed at 60 °C in a warming cabinet until they were tasted (≤ 10 min after being cooked).

A 9-member trained panel (ISO 8586–1, 1992) was used to evaluate the samples. It had been trained in sensory assessment (ISO 8586–1, 1992) and was familiar with sensory assessment of meat. Special training was undertaken in order to recognise attributes before beginning the process of evaluating the samples. Evaluations were done using a quantitative structured scale based on descriptors punctuated from 0

to 10. The descriptors quantified were lamb odour (0 = no odour to 10 = very intense odour), rancidity odour (0 = no rancid odour to 10 = very intense rancid odour), tenderness (0 = very tough to 10 = very tender), juiciness (0 = very dry to 10 = very juicy), lamb flavour (0 = no flavour to 10 = very intense flavour), rancidity flavour (0 = no rancid flavour to 10 = very intense rancid flavour) and acid flavour (0 = no acid flavour to 10 = very intense acid flavour). Twelve samples were evaluated in each session (12 sessions were needed in total), taking a break (approximately 45 min) after the first six eva-luations. The analysis took place in individual cabins illuminated with red light (ISO8589 1998) and breadsticks and water were available to panellists.

2. 6. Statistical analysis

Data were statistically analysed by the General Linear Model (GLM) procedure of SPSS, version 19.0 (IBM SPSS, 2010). The model included temperature, packaging and time of storage (as main effects) and their interaction. Significant interactions ($P \leq 0.001$) between the effects were found on the variables studied. Thus, the effects of packaging and temperature were analysed in four treatments: a) MAP − 1 °C (modified atmosphere packaged slices maintained at −1 °C), b) MAP 4 °C (mod-ified atmosphere packaged slices stored at 4 °C), c) VSP − 1 °C (vacuum skin packaged slices refrigerated at − 1 °C) and d) VSP 4 °C (vacuum skin packaged slices stored at 4 °C). Afterwards, an interaction between the treatments and days of study was noted so a second model was performed where treatment was analysed within day of study effect and vice versa. This model determined the quality differences among treatments for each day of study and changes of one treatment over various days of study. Tukey's post hoc test was used to assess differences between mean values when $P \leq 0.05$.

For the sensory analyses a similar model was used, including "panellist" and "session" as fixed effects and their interactions. "Panellist" resulted in a significant effect ($P \leq 0.001$) on all of the sensory parameters evaluated. In contrast, "session" had not significant effect. No significant interactions were noted. Differences among means were evaluated by using Tukey's post hoc test ($P \leq 0.05$).

3. Results

3.1. Lipid oxidation

The changes of TBARS concentration in the four treatments during storage is presented in Fig. 1. Initially, TBARS values did not differ between storage conditions, ranging from 0.083 to 0.098 mg MDA/kg of meat, but differences among them became significant throughout time. While superchilling did not show any effect on lipid oxidation when meat was maintained under anaerobic conditions, it resulted in a higher rate of increase in modified atmosphere packaged lamb, which showed a higher content of malondialdehyde at − 1 than at 4 °C after 7 and 14 days of display ($P \leq 0.001$). These differences disappeared after 21 days, when lipid oxidation exceeded 4 mg MDA/kg of meat at both temperatures. In contrast, no > 0.275 mg MDA/kg were measured in VSP during storage.

3.2. Instrumental colour

Table 1 shows the results obtained for instrumental colour measurements in each treatment. Superchilled storage resulted in lower L^* values after 21 days in VSP ($P \leq 0.01$); however, differences in lightness related to temperature were not noted in MAP during the experimental period. Finally, samples packaged in VSP and stored at − 1 °C showed the lowest L^* values at 28 days post packaging ($P \leq 0.001$).

The effect of superchilling on maintaining redness differed between VSP and MAP. While no difference was found between MAP samples stored at different temperature, superchilled samples packaged in vacuum skin showed higher a^* values than those refrigerated at 4 °C from 7 day onwards ($P \leq 0.001$). Regarding differences between packaging technologies, VSP resulted in higher a^* values than MAP after 14 days at both temperatures ($P \leq 0.001$).

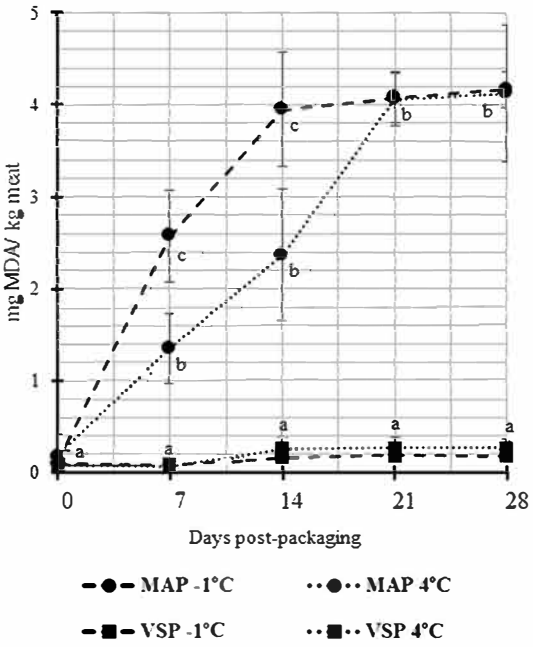

Fig. 1. TBARS values of lamb chops stored at − 1 °C in modified atmosphere packaging (MAP − 1 °C), at 4 °C in modified atmosphere packaging (MAP 4 °C), at − 1 °C in vacuum skin packaging (VSP − 1 °C) and at 4 °C in vacuum skin packaging (VSP 4 °C).
Markers represent the mean of malonaldehyde values (MDA) and the error bars represent standard deviation.
Means with different letters (a, b, c) in the same day of storage indicate significant differences among mean values ($P \leq 0.001$).

Concerning b^* value, an increase was measured in VSP samples ($P \leq 0.001$). Slices vacuum packaged and stored at 4 °C showed lower values than the other treatments at 0 and 7 days of storage ($P \leq 0.001$) but differences disappeared after 14 days of storage.

3.3. Microbial analyses

The means of PTVC, *Enterobacteriaceae*, LAB and *Pseudomonas* spp. counts for each day in each treatment are shown in Fig. 2. Initial growth of *Enterobacteriaceae*, LAB and *Pseudomonas* spp. were below the limit of detection in the four treatments. From this point to the end of storage, counts increased in the four storage conditions ($P \leq 0.001$) with significant differences among the growth rate of each treatment. Generally, superchilled storage showed a great inhibition of microbial growth both in MAP and VSP ($P \leq 0.001$).

Regarding differences in PTVC, it was observed that counts of samples chilled at − 1 °C did not increase up to 7 days. Then, counts increased over time in both packaging methods without significant differences among them until 28 days of study, when PTVC were lower in vacuum than in modified atmosphere packaged meat ($P \leq 0.001$). In samples maintained at 4 °C, PTVC were significantly different depending on the packaging system. Those differences were already found after 7 days, and they remained significant ($P \leq 0.001$) until the end of the experimental period. PTVC were lower in vacuum than in modified atmosphere packaged meat after 28 days at both temperatures ($P \leq 0.001$).

Enterobacteriaceae growth was significantly lower in samples refrigerated at − 1 °C ($P \leq 0.001$). In fact, growth was not measurable at this temperature until 14 days post packaging, when differences between packaging systems were not found. *Enterobacteriaceae* growth at 4 °C was higher in MAP than in VSP the 14th and 21st day of study

Table 1
Means and standard error of the mean (SEM) of lamb instrumental colour.

Variables	Days	0	7	14	21	28	Sig.	SEM
L^*	MAP −1 °C	38.45x,a	39.99x,a	44.67y,c	44.90y,b	45.06y,b	***	0.59
	MAP 4 °C	40.15xy,a	39.83x,a	42.97y,bc	41.28xy,ab	43.28y,b	**	0.39
	VSP −1 °C	40.81x,a	38.94x,a	38.52x,a	39.76x,a	38.73x,a	ns	0.40
	VSP 4 °C	38.75x,a	39.54x,a	41.11xy,ab	44.47y,b	44.32y,b	***	0.59
Sig.		ns	ns	***	**	***		
a^*	MAP −1 °C	12.24y,a	12.34y,ab	8.29x,a	8.50x,a	5.39x,a	***	0.50
	MAP 4 °C	11.90z,a	10.84yz,a	7.94x,a	8.14xy,a	8.28xy,a	***	0.40
	VSP −1 °C	11.83x,a	14.23y,b	15.78y,b	15.54y,c	15.29y,c	***	0.30
	VSP 4 °C	10.45x,a	10.51x,a	13.18y,b	11.73xy,b	10.64x,b	***	0.26
Sig.		ns	***	***	***	***		
b^*	MAP −1 °C	13.27x,b	14.79x,b	13.54x,a	13.30x,a	14.24x,a	ns	0.24
	MAP 4 °C	13.25xy,b	14.41y,b	11.98x,a	13.81xy,a	15.80z,a	***	0.27
	VSP −1 °C	13.75x,b	13.63x,b	15.31xy,b	13.69x,a	15.78y,a	**	0.24
	VSP 4 °C	10.99x,a	10.77x,a	13.28y,a	14.40y,a	14.13y,a	***	0.27
Sig.		***	***	***	ns	ns		

MAP −1 °C: modified atmosphere packaging at −1 °C; MAP 4 °C: modified atmosphere packaging at 4 °C; VSP −1: vacuum skin packaging at −1 °C; VSP 4 °C: vacuum skin packaging at 4 °C.
Different letters in the same row (x, y, z) indicate significant differences among days of storage. Different letters in the same column (a, b, c) indicate significant differences among packaging conditions.
ns P > 0.05.
* P ≤ 0.05.
** P ≤ 0.01.
*** P ≤ 0.001.

(P ≤ 0.001) but differences between them disappeared at the final of the experimental period.

Following the general pattern described, LAB counts were lower at −1 °C than at 4 °C in both packaging systems (P ≤ 0.001). Growth could not be detected in samples stored at −1 °C until 21 days after packaging. Differences between MAP and VSP stored at the same

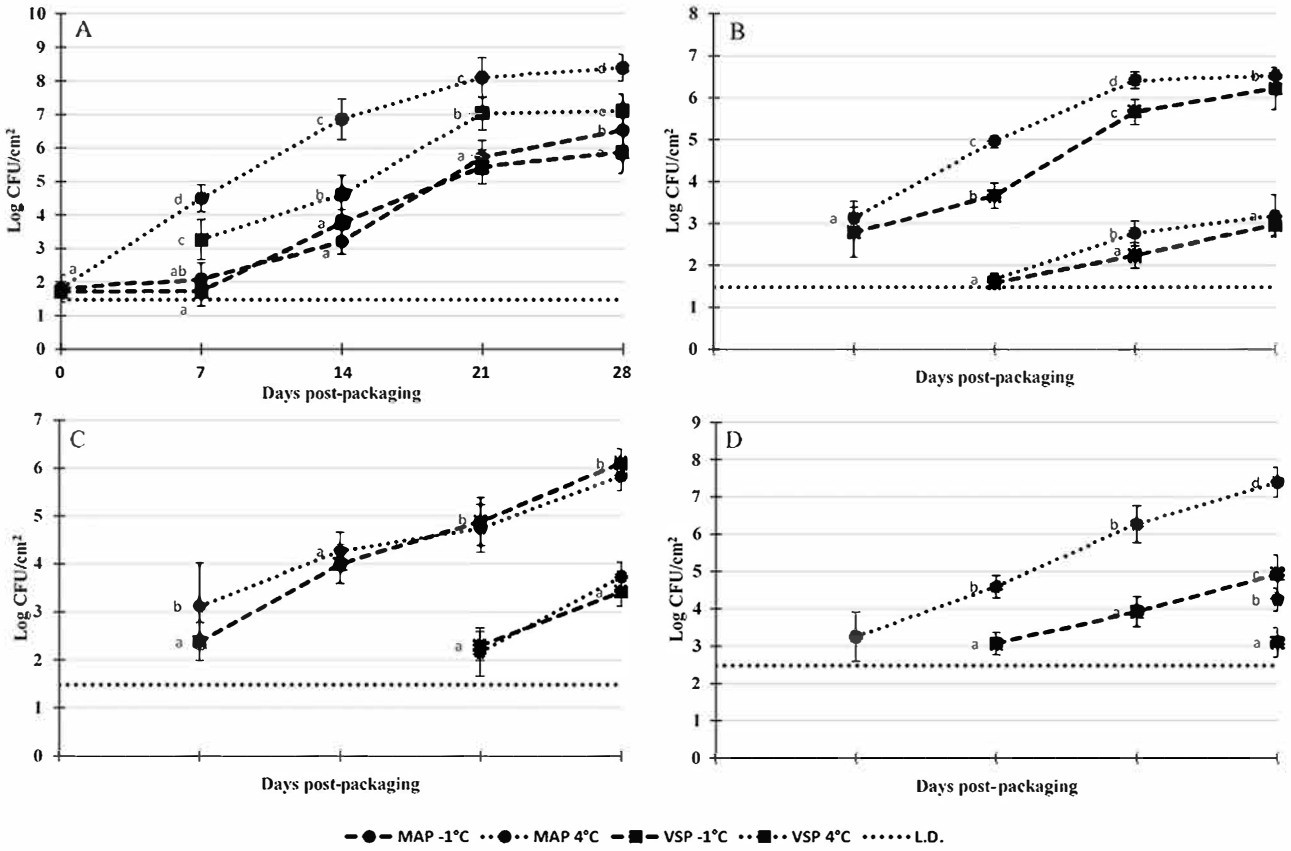

Fig. 2. Growth (mean values and standard deviation) of psychrotrophic total viable counts (A), *Enterobacteriaceae* (B), lactic acid bacteria (C) and *Pseudomonas* spp. (D) for lamb leg chops stored at −1 °C in modified atmosphere packaging (MAP −1 °C), at 4 °C in modified atmosphere packaging (MAP 4 °C), at −1 °C in vacuum skin packaging (VSP −1 °C) and at 4 °C in vacuum skin packaging (VSP 4 °C).
The limit of detection (L.D.) was 1.48 log CFU/cm² for psychrotrophic total viable counts, *Enterobacteriaceae* and lactic acid bacteria and 2.48 log CFU/cm² for *Pseudomonas* spp. Markers represent the mean and the error bars represent standard deviation.
Different letters (a, b, c, d) in the same day of storage indicate significant differences among mean values (P ≤ 0.001).

temperature were only found in samples refrigerated at 4 °C, consisting on a higher LAB growth in MAP than in VSP at 7 days of storage ($P \leq 0.001$).

Significant differences were noted in *Pseudomonas* spp. counts among VSP and MAP maintained at 4 °C from 14th day onwards, obtaining in VSP a lower growth. Regarding samples maintained at −1 °C, counts could not be detected until 28 days of storage, when MAP also registered higher counts than VSP ($P \leq 0.001$).

3.4. Sensory analyses

Neither samples chilled at 4 °C nor those stored in MAP at −1 °C were evaluated after 14 days of study (Table 2). Ones were eliminated due to microbial spoilage and the others as a result of excessive lipid oxidation. In contrast, vacuum skin packaged samples chilled at −1 °C were suitable for tasting even at day 28.

As can be seen in Table 2, VSP obtained lower scores than MAP in the attributes related to rancid odours and flavours. Differences in the intensity of rancidity odours were noted after 14 days of storage ($P \leq 0.001$). MAP at −1 °C showed the highest values, followed by MAP at 4 °C and finally both VSP (at 4 °C and −1 °C). These differences were also registered for rancidity flavour after 7 and 14 days, obtaining therefore the same groups in the post hoc analysis ($P \leq 0.001$).

Characteristic odours and flavours of lamb decreased significantly over time in all packaging conditions. Concerning differences among storage conditions, superchilled MAP showed lower values than the other treatments at 7 and 14 days after packaging. Moreover, MAP at 4 °C also resulted in lower lamb flavour than VSP at both temperatures ($P \leq 0.001$).

Significant differences in tenderness were recorded depending on packaging conditions at 14 days post packaging; VSP at 4 °C was given higher values than MAP while VSP at −1 °C was also more tender than superchilled MAP. Moreover, lamb became more tender over time in VSP at 4 °C ($P \leq 0.001$). Concerning juiciness, differences were registered at 14 days of storage, showing VSP higher scores than MAP at both temperatures ($P \leq 0.001$). Nevertheless, juiciness decreased throughout storage in both packaging systems; MAP samples were less juicy at 14 than 7 days while VSP samples stored at −1 °C were noted less juicy at 21–28 days than at 14 days. Finally, acid flavour was noted to be higher in MAP than in VSP after 14 days; moreover, panellists perceived more intensity of this flavour in MAP samples at 4 °C than those stored at −1 °C ($P \leq 0.001$).

Table 2
Means and standard error of the mean (SEM) for sensory attributes of grill-cooked lamb.

Variables[a]	Days	0	7	14	21	28	Sig.	SEM
Lamb odour	MAP −1 °C	6.70z,a	4.38y,a	2.53x,a	–	–	***	0.141
	MAP 4 °C	6.98z,a	4.67x,a	5.11y,b	–	–	***	0.104
	VSP −1 °C	6.37z,a	6.00yz,b	5.77xyz,c	5.35xy	5.17x	***	0.090
	VSP 4 °C	6.55y,a	5.65x,b	5.62x,bc	–	–	***	0.119
	Sig.	ns	***	***	–	–		
Rancidity odour	MAP −1 °C	0.00x,a	1.75y,b	6.59z,c	–	–	***	0.239
	MAP 4 °C	0.00x,a	0.68x,a	2.07y,b	–	–	***	0.136
	VSP −1 °C	0.72y,b	0.18x,a	0.02x,a	0.08x	0.07x	***	0.051
	VSP 4 °C	0.32x,ab	0.13x,a	0.10x,a	–	–	ns	0.051
	Sig.	***	***	***	–	–		
Tenderness	MAP −1 °C	7.00z,b	6.58y,a	5.99x,a	–	–	***	0.122
	MAP 4 °C	6.47x,a	6.54x,a	6.63x,ab	–	–	ns	0.627
	VSP −1 °C	6.52xy,a	6.45xy,a	7.26y,bc	5.93x	6.12x	***	0.096
	VSP 4 °C	6.50x,a	6.23x,a	7.33y,c	–	–	***	0.117
	Sig.	***	ns	***	–	–		
Juiciness	MAP −1 °C	6.70z,b	5.95y,a	3.77x,a	–	–	***	0.106
	MAP 4 °C	6.35z,a	5.85y,a	3.36x,a	–	–	***	0.098
	VSP −1 °C	6.32yz,a	5.67xy,a	6.53z,b	4.72w	5.18wx	***	0.089
	VSP 4 °C	6.37xy,a	5.85y,a	6.45y,b	–	–	*	0.953
	Sig.	***	ns	***	–	–		
Lamb flavour	MAP −1 °C	6.20z,ab	4.44y,a	1.95x,a	–	–	***	0.154
	MAP 4 °C	6.87z,b	5.42y,b	2.77x,b	–	–	***	0.120
	VSP −1 °C	6.33y,ab	6.07y,b	5.53xy,c	4.87x	5.03x	***	0.103
	VSP 4 °C	6.10y,a	5.80xy,b	5.07x,c	–	–	**	0.140
	Sig.	*	***	***	–	–		
Rancidity flavour	MAP −1 °C	0.00x,a	2.20y,c	6.93z,c	–	–	***	0.236
	MAP 4 °C	0.00x,a	0.85y,b	1.72z,b	–	–	***	0.155
	VSP −1 °C	0.03x,a	0.02x,a	0.00x,a	0.03x	0.07x	ns	0.120
	VSP 4 °C	0.00x,a	0.03x,a	0.02x,a	–	–	ns	0.009
	Sig.	ns	***	***	–	–		
Acid flavour	MAP −1 °C	0.00x,a	0.47y,b	0.42y,b	–	–	***	0.044
	MAP 4 °C	0.00x,a	0.00x,a	3.38y,c	–	–	***	0.126
	VSP −1 °C	0.15x,a	0.03x,a	0.02x,a	0.03x	0.12x	ns	0.130
	VSP 4 °C	0.00x,a	0.00x,a	0.03x,a	–	–	ns	0.111
	Sig.	ns	***	***	–	–		

MAP −1 °C: modified atmosphere packaging at −1 °C; MAP 4 °C: modified atmosphere packaging at 4 °C; VSP −1 °C: vacuum skin packaging at −1 °C; VSP 4 °C: vacuum skin packaging at 4 °C.

– = not determined.

Different letters in the same row (w, x, y, z) indicate significant differences among days of storage. Different letters in the same column (a, b, c) indicate significant differences among packaging conditions.

[a] 10 point scale (0 = low, 10 = high).

ns $P > 0.05$.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

4. Discussion

Several authors have previously reported the effectiveness of vacuum packaging on controlling lipid oxidation (Łopacka et al., 2016; Yang et al., 2016). In accordance, VSP led to a strong inhibition of lipid oxidation at both temperatures, showing no differences between superchilled and conventional stored samples. Actually, lipid oxidation was expected to be very low in VSP due to the tight disposition of the film over the meat surface, which reduces air pockets (Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan, & Guilbault, 2002). In contrast, samples packaged in MAP showed a pronounced increase in TBARS values over time. Modified atmospheres with high O_2 are widely known to increase lipid oxidation resulting in higher TBARS values than those found under anaerobic conditions (Berruga et al., 2005; Łopacka et al., 2016; Yang et al., 2016). Previous studies observed that the substitution of N_2 with Ar in O_2 enriched MAP did not seem to offer any benefit on lipid stability since the values obtained in TBARS assay did not differ between both (Fraqueza & Barreto, 2009; Herbert et al., 2013). Therefore, Ar was unexpected to exert any effect on lipid oxidation development. In fact, the MDA values determined in our trial were similar to those registered in previous studies either using Ar based atmospheres (Camo, Beltrán, & Roncalés, 2008; Bellés, Alonso, Roncalés, & Beltrán, 2017b) or in atmospheres including N_2 (Rubio, Vieira, & Martínez, 2016). Moreover, lipid oxidation highly increased by the combination of superchilled storage and MAP. In contrast, temperature had no effect on lipid oxidation when samples were stored under anaerobic conditions.

Several researchers had previously described mechanical damages to muscle structure during superchilled storage, consisting on gaps and myofibrils breakages (Lan et al., 2016; Liu, Liang, Xia, Regenstein, & Zhou, 2013). Lan et al. (2016) attributed these damages to intracellular and extracellular crystal formation and growth, which could result also in a release of pro-oxidant compounds and consequently, in an increase of lipid oxidation (Benjakul & Bauer, 2001). The liberation of pro-oxidant compounds in presence of O_2 may have promoted lipid oxidation in superchilled MAP samples, resulting in an increased MDA concentration. Although superchilled storage may have also led to crystal formation in VSP samples, the absence of O_2 could have prevented lipid oxidation. However, MAP exceeded the limiting threshold for the acceptability of oxidised beef (2 mg MDA/kg of meat) at both temperatures, as proposed by Campo et al. (2006).

Bright red colour is traditionally considered a positive aspect since it is associated with freshness and superior quality product (Berruga et al., 2005). Superchilling of vacuum packages protected lamb from discolouration but this effect was not shown in modified atmosphere packaged samples, in which redness decreased during storage at both temperatures ($P \leq 0.001$). Several researchers (Berruga et al., 2005; Fernandes et al., 2014) have described a reduction in redness over time in O_2 enriched atmospheres. The presence of a high percentage of O_2 in the pack promotes lipid oxidation, which has been suggested to enhance myoglobin oxidation (Faustman, Sun, Mancini, & Suman, 2010). The gradual conversion of oxymyoglobin to metmyoglobin reduces redness, leading to meat colour deterioration (Insausti et al., 1999). In contrast, VSP removed O_2 from the package, providing an adequate anaerobic environment to prevent pigment oxidation and maintaining the typical purple colour of vacuum packaged lamb for a long time. Moreover, superchilled storage led to higher a^* values than refrigerating at conventional temperatures in VSP. In accordance, Lan et al. (2016) also observed a protective effect of superchilling temperatures against discolouration of fresh rabbit meat. These differences in redness may have been related to the effect of superchilling on delaying microbial growth. Abdallah, Marchello, and Ahmad (1999) observed that microbial growth increased metmyoglobin accumulation. They proposed that microbial metabolism would reduce O_2 availability on the meat surface, which may increase metmyoglobin formation and thus discolouration.

The effect of temperature on microbial growth is well known, as when temperature decreases the microbial rate is reduced. Refrigeration at -1 °C prevented the meat from microbial spoilage, resulting in lower counts for all microorganisms determined in both types of packaging ($P \leq 0.001$). As a result, superchilled samples did not exceed the limit for acceptable quality meat (7 log CFU/cm^2) proposed by ICMFS (1986) during storage, not having microbial spoilage as a limiting factor for the shelf life of superchilled lamb. Previously, several studies pointed out the efficacy of superchilled storage on delaying microbial growth in meat (Zhang et al., 2016). In contrast, microbial growth overpassed this limit in lamb maintained at 4 °C after 21 days either in MAP or VSP. Similar results were found by Zhang et al. (2016) when evaluated microbial growth in broiler stored at -2 and 4 °C; superchilled storage reduced at least 2.5 log UFC/g the microbial total viable counts determined in the samples stored at 4 °C.

According to TBARS results, rancid odours and flavours were detected in MAP but they were not found in VSP ($P \leq 0.001$). MAP meat stored at -1 °C showed higher scores for rancid odours and flavours than the other storage conditions after 7 days ($P \leq 0.001$), when the threshold for a positive sensory perception of beef (TBARS value of 2 mg MDA/kg of meat) was already exceeded in these samples. MAP at 4 °C also registered significant rancid odours and flavours at 14 days after packaging. Rancid odours and flavours may have been expressed more clearly over time by the decrease in the characteristic lamb odour and flavour in MAP, whose values were significantly lower than those detected in VSP at 14 days of storage. The development of off flavours and odours in these samples overpowered meat flavours. Lamb characteristics odours and flavours were within an acceptable range in VSP, as it has to be in account that Spanish consumers reject very intense lamb odour and flavour (Sañudo, Muela, & Campo, 2013). Differences in tenderness between MAP and vacuum packaging have been related to protein modifications. It has been demonstrated that high O_2 MAP promotes protein oxidation and myosin intermolecular cross linking, which results in tougher meat (Clausen, Jakobsen, Ertberg, & Madsen, 2009; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). Moreover, these physical modifications of meat structure have been associated with an increase in drip loss and consequently, a decrease in meat juiciness (Lund et al., 2007). These chemical reactions depend on the presence of O_2 so that VSP may have prevented them, leading to more tender and juicier lamb. A feasible explanation to the decrease of juiciness over time could be the constant increase of drip loss time (data not shown). Similarly, Vergara and Gallego (2001) observed a decrease of juiciness throughout display together with a decrease of water holding capacity, which was associated to changes in protein stability.

Panellists only noted an increase in tenderness of VSP at 4 °C, which could be as a result of meat ageing over time. An increase in tenderness of vacuum packaged beef through time had been previously registered (Kameník et al., 2014).

5. Conclusions

The results of this study pointed out a different effect of superchilled storage on lamb quality depending on the atmosphere surrounding the product. Superchilled storage combined with an O_2 enriched atmosphere resulted in an increased rate of lipid oxidation compared to 4 °C. In contrast, lipid oxidation was not observed under anaerobic conditions, in which it was strongly inhibited. While the limit of microbial acceptability was exceeded in packs maintained at the conventional temperature after 21 days, preservation at -1 °C limited microbial growth. Moreover, superchilling led to a better colour stability in VSP and finally a shelf life of 28 days of this combination was observed. Therefore, the microbial shelf life was at least twice as long with superchilling than traditional chilling under anaerobic conditions, which could be very useful for industries interested in extended time for distribution. Nevertheless, more studies are required to clarify the interaction between superchilled storage and enriched O_2 atmospheres.

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4.2.4. Effect of borage and green tea aqueous extracts on the quality of lamb leg chops displayed under retail conditions

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Effect of borage and green tea aqueous extracts on the quality of lamb leg chops displayed under retail conditions

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ABSTRACT

Different concentrations of two aqueous extracts from green tea leaves and borage seeds with potential antioxidant activity were evaluated in lamb leg chops. Chops were sprayed with 0.005, 0.05, 0.5, 5% (p/v) green tea extracts (T) and 0.5, 5 and 10% (p/v) borage seed extracts (B) and displayed under retail conditions for 13 days. Total polyphenols, TBARS, colour, microbial and sensory analyses were performed. The extracts showed a concentration-dependent action; the minimum concentration of polyphenols which significantly reduced lipid oxidation was 2.08 mg GAE/100 cm^2 of meat. Both 0.5% T and 10% B limited colour deterioration, reducing also metmyoglobin formation. The extracts showed no antimicrobial effect, exceeding microbial counts of 7 log CFU/-cm^2 at 13 days of display. Sensory analyses determined that none of the extracts added herb odours or flavours to lamb. In conclusion, 0.5% T or 10% B extracts extended lamb shelf life from 8 to 11 days, so both would be recommended for lamb chops preservation.

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1. Introduction

Lamb for retail display is currently packaged in modified atmospheres including high percentage of O_2 for providing a desirable red colour to meat. However, the presence of O_2 could enhance lipid oxidation and ultimately oxymyoglobin oxidation, leading to a loss of colour and off flavour and odour development (Sañudo, Muela, & Campo, 2013).

The addition of antioxidant compounds into the package or onto the meat surface may retain product quality by reducing lipid oxidation. Natural antioxidant compounds are being studied for this purpose. In contrast to chemical additives, essential oils and herb extracts, which are included in the Generally Recognised As Safe (GRAS) list of the American Food and Drug Administration and natural compounds, are not rejected by consumers. Several studies have already reported the effectiveness of natural compounds on reducing lipid oxidation of meat (Alp & Aksu, 2010; Wu, Wang, & Chen, 2010; Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011).

Borage (*Borago officinalis* L.) is an annual herb cultivated for medicinal and culinary uses (Asadi-Samani, Bahmani, & Rafieian-Kopaei, 2014). However, since Wettasinghe and Shahidi (1999) demonstrated the antioxidant properties of borage seeds, several studies have focused on its application to food preservation (Giménez, Gómez-Guillén, Pérez-Mateos, Montero, & Márquez-Ruiz, 2011; Sánchez-Escalante, Djenane, Torrescano, Beltran, & Roncalés, 2003). The high antioxidant activity of borage extracts is associated with their high content of phenolic compounds, which are able to quench reactive oxygen species (Wettasinghe & Shahidi, 1999). Rosmarinic, syringic and synapic acids have been determined to be the major compounds present in borage seed extracts (Wettasinghe, Shahidi, Amarowicz, & Abou-Zaid, 2001). Sánchez-Escalante et al. (2003) and Martínez, Cilla, Beltrán, and Roncalés (2006) reported that the inclusion of a defatted borage seed meal in beef patties and fresh pork sausages significantly inhibited lipid oxidation; however, this meal brought about a greyish colour to product. Therefore, the development of a new methodology of extraction, optimization and application to make a profit of borage antioxidant properties is still a point of concern. The antioxidant effect of green tea has been also thoroughly studied. Green tea composition includes catechin, flavones, anthocyan and phenolic acid (Huang, Huang, Liu, Luo, & Xu, 2007). Catechin represents up to 30% of water-soluble solids of the dry weight of green tea (Harbowy & Balentine, 1997) while myricetin, quercetin and kaempferol are the main derivatives of the flavonol group (Colon & Nerín, 2016). Green tea has been used for preserving meat and meat products, significantly reducing lipid oxidation as well as microbial growth (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007; Lorenzo, Sineiro, Amado, & Franco, 2014b).

Both borage and green tea extracts may be very profitable to extend lamb shelf life; however, the most suitable concentrations of each one should be determined. Nevertheless, they should offer a significant benefit without modifying lamb sensory quality. The development of aqueous antioxidant solutions emerged as a possible solution to visual problems reported when the extracts were used as a crude meal. Therefore, the aim of this study was to determine the effect of spraying

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different concentrations of new aqueous extracts of borage seeds and green tea leaves on lamb quality throughout display. The physicochemical, microbiological and sensory data will help to determine the optimum concentrations of each extract for keeping lamb quality during display without modifying lamb characteristic odour and flavour.

2. Materials and methods

The lambs used for this trial were cared in accordance with the guidelines from the Spanish Ministry of Agriculture (Boletín Oficial del Estado (BOE), 2007).

2.1. Sampling

Eighty one lamb carcasses were randomly chosen among commercial lambs of the Rasa Aragonesa, a medium wool breed that is reared for meat purpose in Spain. Animals were reared together under intensive husbandry conditions with natural suckling until 40 days of age and fodder with concentrate and cereal straw ad libitum until they reached a body weight between 20 and 25 kg. The animals, aged about three months, were slaughtered in a commercial slaughterhouse (Mercazaragoza) on three different days (27 lambs each day, 3 lambs per treatment) following standard protocols. Within 15 min of dressing, carcasses were transported to the facilities of Casa de Ganaderos and Franco y Navarro S.A. and chilled for 24 h (4 ± 0.5 °C, 90% RH, 1–2 m/s). The two legs of each carcass were removed and randomly assigned to one of the nine treatments (the legs from 9 carcasses per treatment). The proximal part of each leg was then cut into 20-mm-thick chops and transported under refrigeration (4 °C) to the Food Technology Pilot Plant of the Faculty of Veterinary Science (University of Zaragoza). The treatments consist of two controls (no sprayed chops (C) and chops sprayed with water (WC)) and samples sprayed with the following extracts: 0.5% (p/v) borage aqueous extract (0.5% B), 5% (p/v) borage aqueous extract (5% B), 10% (p/v) borage aqueous extract (10% B), 0.005% (p/v) green tea aqueous extract (0.005% T), 0.05% (p/v) green tea aqueous extract (0.05% T), 0.5% (p/v) green tea aqueous extract (0.5% T), 5% green tea aqueous extract (5% T). Mean leg weight and meat pH were similar across all treatments ($P = 0.727$ and 0.107 respectively; data not shown).

2.2. Extract preparation

Borage seeds were purchased from a local market (Semillas Fitó, España). Borage meal was obtained as described by Sánchez-Escalante et al. (2003), with some modifications. Seeds were ground in a coffee grounder and the husk was separated from endosperm using a 1 mm sieve. The endosperm was recovered and defatted by removing continuously with hexane (1:5 w/v, 5 min, 3 times) in a magnetic agitator. After each extraction, the combination of ground seeds and solvent was centrifuged in a refrigerated centrifuge (Jouan, model CR-4.11, Saint-Herblain, France) at 2300*g* for 15 min at 10 °C. Hexane was eliminated by evaporation at ambient temperature overnight in a fume hood. Dried meal was vacuum packaged and maintained at 4 °C until its utilization.

Borage aqueous solutions (0.5, 5 and 10% p/v) were prepared by dissolving the dried meal in distilled water in a crystal flask (0.5, 5 and 10 g of meal in 100 ml of distilled water). To favor dissolution of phenolic compounds in water, the flask was placed into a water bath at 65 °C (Grant W14, Cambridge, UK) for 45 min in continuous agitation. After that, the solution was filtered through filter paper (Machery-Nagel number 43, Düren, Germany) and then sterilized using 0.2 μm cellulose acetate sterile syringe filter (VWR) and placed in a sterile commercial applicator. The resulting solutions were kept frozen at −20 °C.

Green tea extract Sunphenon 90 MB (GTE) was obtained from TAIYO Europe (Filderstadt, Germany), and the extract contained around 75% total catechins (w/w, HPLC determination provided by the supplier Company). The aqueous extracts (0.005, 0.05, 0.5 and 5% p/v) were prepared by dissolving the dry meal in distilled water (0.005, 0.05, 0.5 and 5 g of the meal in 100 ml of distilled water). Then, aqueous extracts were filtered through filter paper (Machery-Nagel number 43, Düren, Germany) and sterilized by filtration (0.2 μm cellulose acetate sterile syringe filter) (VWR). The resulting solutions were placed in commercial applicators and frozen stored (−20 °C).

Prior to its utilization, the aqueous extracts were completely thawed at 4 °C over 12 h.

2.3. Packaging and storage conditions

Chops were sprayed with the corresponding solution (C, WC, 0.005% T, 0.05% T, 0.5% T, 5% T, 0.5% B, 5% B, 10% B) (1 ml of solution per 100 cm^2 of meat approximately) and modified atmosphere packaged (40% O_2/30% CO_2/30% Ar) (ULMA-SMART-500) with a product to gas ratio of 1:3. Polystyrene trays were used and sealing was done with a polyethylene and polyamide laminate film. The film was 30 μm thick, its oxygen permeability rate at 23 °C was 15 cm^3/m^2/24 h/0% RH and the water vapour transmission rate at 23 °C was 7 g/m^2/24 h/85% RH, (Linpac Packaging S.L., Spain). Afterwards, samples were placed in a commercial refrigerator at 4 ± 0.5 °C with 14 h of artificial light per day over 13 days. A standard supermarket fluorescent tube (Mazdafluor Aviva TF/36w; Philips, Eindhoven, Holland) with an UV-filter plate of polycarbonate was used. The UV-filter plate of polycarbonate allowed transmission of about 80% of the visible light (410 to 710 nm) but it was about 0% below 390 nm (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2001). Light intensity (1000 lx) was measured with a luxometer (Chauvin Arnoux 810; Paris, France).

Specific analyses were performed at 0 (approximately 24 h post mortem), 5, 8, 11 and 13 days post packaging using 36 packages per treatment on each sampling day (4 packages from each lamb, 9 lambs per treatment). One package from each lamb was used for microbial, instrumental colour and TBARS analyses while the other three packages were assigned to sensory analyses. Samples for sensory analyses were vacuum packaged (−900 mbar of pressure) in polyethylene-polyamide bags with ethyl vinyl acetate sealant layer (30 × 25 cm, 90 μm thickness, water vapour transmission rate at 23 °C of 2.8 g/m^2/24 h/85% RH, an O_2 transmission rate at 23 ± 1 °C of 50 cm^3/m^2/24 h/75% RH; Eurobag & Film S.L., Spain) using a Tecnotrip EV-13-L-CD-SC machine (Tecnotrip S.A., Spain) and frozen stored in each sampling day at −20 °C until sensory analysis was performed.

2.4. Total phenolic compounds

Total phenolic compounds were determined in the extracts following an adaptation of the method described by Matthaüs (2002). In brief, 2 ml of the extract was filled with 0.3% HCl (Panreac) to 5 ml. A 100 μl aliquot of the resulting solution was mixed with 2 ml of 2% Na_2CO_3 (Merck) and then 2 ml 100 μl of Folin Ciocalteau reagent (diluted with methanol 1:1) (Sigma-Aldrich) was added. After 30 min of incubation, the absorbance was measured at 750 nm using a spectrophotometer. Gallic acid (Sigma-Aldrich) was used as a standard, expressing the results as milligrams of gallic acid equivalents (GAE) per ml of extract.

2.5. Instrumental colour

A Minolta CM-2002 (Osaka, Japan) spectrophotometer was used to measure colour at the surface of a 20-mm-thick chop after opening the trays and exposing the samples to air for 2 h at 4 °C. The parameters registered were L^* (lightness) and a^* (redness). A D65 illuminant was used at an observation angle of 10° and with a cell opening of 30 mm. Equipment was previously calibrated using a white and black standard. Ten measurements were done on *Semimembranosus*, and averaged. As an indirect measure of metmyoglobin formation, and therefore of colour

stability, the 630/580 ratio was calculated by dividing the percentage of light reflectance at wavelength 630 nm by the percentage of light reflectance at wavelength 580 nm (AMSA, 2012). The relative content of metmyoglobin was calculated from the reflectance curve according to Krzywicki (1979) using 690 nm (the highest wavelength of the instrument). Since the reflectance spectrophotometer only measures the reflectance at 10 nm intervals, the wavelengths 473, 525 and 572 were calculated using linear interpolation.

2.6. Lipid oxidation

Lipid oxidation was determined as Thiobarbituric Acid Reactive Substances (TBARS) following the method described by Pfalzgraf, Frigg, and Steinhart (1995). In brief, 20 ml of tricloracetic acid (TCA 10%) (VWR) were added to 10 g of meat and homogenized using an ultraturrax for 90 s at 2000 rpm (T-25 basic, IKA-WERKE, Staufen, Alemania). Then, it was centrifuged at 4000 rpm for 30 min at 10 °C (Jouan CR 411, USA). Supernatant was filtered (Machere-Nagel, Alemania) and 2 ml were mixed with 2 ml of TBA 20 mM (Sigma-Aldrich). Tubes were vortexed (Heidolph REAX 2000, Schwabach, Germany) and incubated in a thermostatic bath at 97 °C for 20 min (Grant W14, Cambridge, UK). After that, samples were cooled in tap water at ambient temperature (15 °C) and absorbance was measured at 532 nm with a spectrophotometer (Unicam 5625 UV/VIS, Cambridge, UK). The TBA-reactive substances (TBARS), mainly malondialdehyde (MDA), values were calculated from a standard curve of 1, 1, 3, 3-tetramethoxypropane (TMP) (Sigma-Aldrich) because the MDA can be obtained by acid hydrolysis from TMP in an equimolecular reaction, so the lipid oxidation was expressed as the average of two replicates per sample in mg malondialdehyde/kg meat.

2.7. Microbial analyses

Samples were aseptically collected from the chops swabbing an area of 10 cm^2 delimited with a sterile aluminum template (10 cm^2). Each sample was homogenized in 0.1% peptone water (Biolife) and serial dilutions were done.

For psychrotrophic total viable counts (PTVC), *Enterobacteriaceae* and lactic acid bacteria (LAB) determination, 1 ml of the correct dilution was inoculated in a Petri Plate and after that approximately 15 ml of the appropriate agar was added. Psychrotrophic total viable counts (PTVC) were investigated using plate count agar (PCA) (Merck) after incubation at 10 °C for 96 h. For *Enterobacteriaceae* counts violet red bile dextrose agar (VRBD) was used, covering plates after solidifying with 3–4 ml of VRBD (double layer) (Merck). Then, they were incubated at 37 °C for 48 h. Plates for LAB investigation were covered with man rogosa and sharpe agar (MRS) (Merck) and they were placed in an anaerobic jar with an anaerobic atmosphere generator kit (Anaerocult A) (Merck) together with an anaerobic conditions indicator strip (GazPack™). Incubation was done for 96 h at 37 °C. Regarding *Pseudomonas* spp. determination, 0.1 ml of the property dilution was inoculated on the surface of Cephalothin-Sodium Fusidate-Cetrimide Agar (CFC) (Merck) and the inoculum was spread using a sterile plastic handle. Plates were counted after incubation at 20 °C for 24 h.

All microbial counts were expressed as base-10 logarithm of colony forming units per cm^2 of surface area (log CFU/cm^2).

2.8. Sensory analyses

Samples were thawed at 4 °C in a refrigerator for 12 h. After breaking vacuum, chops were wrapped in aluminum foil and cooked at 200 °C in a double-plate grill (Sammic GRS-5, Guipúzcoa, Spain) until an internal temperature of 72 °C was reached, which was monitored by an internal thermocouple JENWAY 200 (Jenway scientific, Ston, United Kingdom). After cooking, the muscle *Semimembranosus* was cut in portions (2 cm × 2 cm × 2 cm) wrapped individually in aluminum foil and assigned a single random three digit code. Samples were placed at 60 °C in a warming cabinet until they were tasted (≤10 min after being cooked).

Sensory evaluations were done using a quantitative structured scale based on descriptors punctuated from 0 to 10. Table 1 shows the definitions and references of the attributes used for the sensory analyses. The descriptors quantified were herb odour (0 = no herb odour to 10 = very intense herb odour), microbial odour (0 = no microbial odour to 10 = very intense microbial odour), oxidation odour (0 = no oxidation odour to 10 = very intense oxidation odour), herb flavour (0 = no herb flavour to 10 = very intense herb flavour), microbial flavour (0 = no microbial flavour to 10 = very intense microbial flavour) and oxidation flavour (0 = no oxidation flavour to 10 = very intense oxidation flavour). Ten samples were evaluated in each session (16 sessions were needed in total), taking a break (approximately 45 min) after the first five evaluations. The analysis took place in individual cabins illuminated with red light (ISO8589 1998) and breadsticks and water were available to panellists to cleanse their palates between samples.

A 9-member trained panel (ISO 8586-1, 1992) was used to evaluate the samples. It had been trained in sensory assessment (ISO 8586-1, 1992) and was familiar with generalized sensory assessment of meat. Special training was undertaken in order to recognise attributes relative to this study. It consisted of 4 sessions of approximately 1 h. For rating rancid odour and flavour olive oil and lamb samples presenting different levels of oxidation were presented. Training for microbial odour and flavour evaluation was performed using lamb chops with different microbial counts, for that purpose several chops were packaged in MAP and stored at 2 °C for different times up to 3 weeks. Infusions of borage and tea leaves at different concentrations were used to rate herb odour and flavour.

2.9. Statistical analysis

Data were statistically analysed by the General Linear Model (GLM) procedure of SPSS, version 19.0 (IBM SPSS, 2010). The model included the fixed effects antioxidant treatment, display duration and their interaction, plus animal as random effect. Day of slaughter was initially included in the statistical model as fixed effect but it was finally excluded owing to the fact that no statistical effect was found. Therefore, the model was as follows:

$$Y_{ijk} = \mu + T_i + D_j + A_k + (T_i \times D_j) + e_{ijk}$$

where Y_{ijk} is the dependent variable; μ is the population average; T_i is the fixed effect of antioxidant treatment; D_j is the fixed effect of display duration; A_k is the random effect of animal; $(T_i \times D_j)$ is the interaction effect of antioxidant treatment and display duration; and e_{ijk} is the aleatory error. Differences were declared significant when $P \leq 0.05$. Tukey's post hoc test was used to assess differences between mean values when $P \leq 0.05$.

Table 1
Description and references of the attributes used for the sensory analyse.

Attribute	Definition	References
Odour		
Rancid	Odour associated with oxidation compounds derived from fat	Rancid seed oil
Herbal	Odour associated with herbs	Tea and borage leaves
Microbial	Putrid odours derived from meat spoilage	Spoiled meat
Flavour		
Rancid	Flavour associated with oxidation compounds derived from fat	Rancid seed oil
Herbal	Flavour associated with herbs	Tea and borage leaves
Microbial	Putrid flavours derived from meat spoilage	Spoiled meat

A significant interaction ($P \leq 0.001$) between the effects antioxidant treatment and display duration on the variables studied was noted, so a second model was tested where treatment was analysed within day of study, and vice versa. Tukey's post hoc test was used to assess differences between mean values when $P \leq 0.05$.

For the sensory analyses a similar model was used, including "panellist" and "session" as fixed effects and their interactions. "Panellist" resulted in a significant effect ($P \leq 0.001$) on all of the sensory parameters evaluated. This is very common in sensory analysis due to the different use of the scale (Rousset-Akrim, Young, & Berdagué, 1997). In contrast, "session" had no significant effect. No significant interactions were noted. Differences among means were evaluated by using Tukey's post hoc test ($P \leq 0.05$).

3. Results and discussion

Results of all treatments are presented for polyphenol content and lipid oxidation but only the results of those that exerted a significant antioxidant activity are shown for colour, microbial and sensory analyses.

3.1. Polyphenol content

Table 2 shows the total phenolic content of the extracts. The 5% green tea extract contained the highest amount of polyphenols, showing 19.97 ± 1.06 mg GAE/ml. Green tea extract has been already described to contain a huge amount of phenolic compounds (Lorenzo et al., 2014b), which are mostly catechins and flavonoids (Perumalla & Hettiarachchy, 2011). The catechins present in green tea extract are mainly epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Perumalla & Hettiarachchy, 2011). As it was expected, a lower content of polyphenols was measured in the less concentrated tea extracts.

Regarding the borage seed extracts, 0.30, 1.38 and 3.29 mg GAE/ml were quantified in 0.5%, 5% and 10% aqueous extracts. The polyphenol content increased with concentration too. Rosmarinic acid has been identified to be the major phenolic acid in borage seeds, followed by sinapic and syringic acids (Mhamdi, Wannes, Bourgou, & Marzouk, 2009; Wettasinghe et al., 2001).

3.2. Lipid oxidation

As seen in Fig. 1, initial malondialdehyde values were similar in all treatments but significant differences were found among them after 5 days. At this time, two different groups were found ($P \leq 0.001$). The group which registered the lowest values was composed of chops sprayed with 0.5% T, 5% T, 5% B and 10% B while 0.005% T and 0.5% B added chops showed the highest MDA content. TBARS values obtained

Table 2
Total phenolic compounds expressed as gallic acid equivalents (GAE).

Aqueous extracts	Total polyphenols (mg GAE/ml extract)[a]
0.005% T	0.15 ± 0.01
0.05% T	0.49 ± 0.04
0.5% T	2.08 ± 0.15
5% T	19.97 ± 1.06
0.5% B	0.30 ± 0.02
5% B	1.38 ± 0.03
10% B	3.29 ± 0.02

0.005% T: 0.005% (p/v) tea aqueous extract; 0.05% T: 0.05% (p/v) tea aqueous extract; 0.5% T: 0.5% (p/v) tea aqueous extract; 5% T: 5% (p/v) tea aqueous extract; 0.5% B: 0.5% (p/v) borage aqueous extract; 5% B: 5% (p/v) borage aqueous extract; 10% B: 10% (p/v) borage aqueous extract.
GAE: gallic acid equivalents.
[a] Means and standard error.

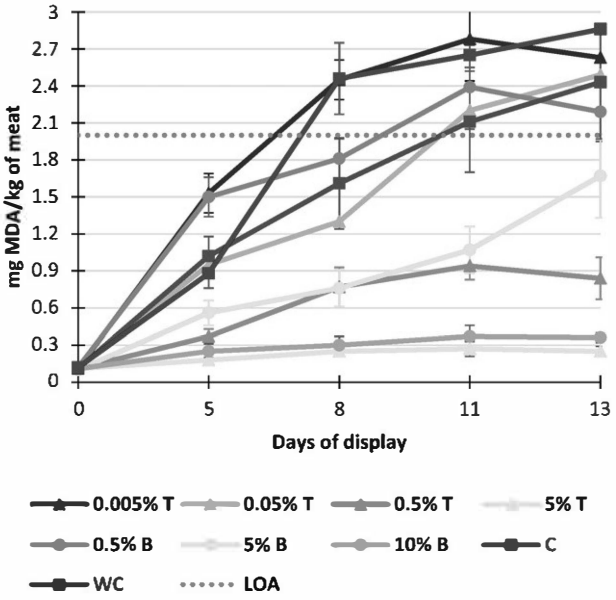

Fig. 1. Lipid oxidation of lamb treated with tea and borage aqueous extracts during display. 0.005% T: 0.005% (p/v) tea aqueous extract; 0.05% T: 0.05% (p/v) tea aqueous extract; 0.5% T: 0.5% (p/v) tea aqueous extract; 5% T: 5% (p/v) tea aqueous extract; 0.5% B: 0.5% (p/v) borage aqueous extract; 5% B: 5% (p/v) borage aqueous extract; 10% B: 10% (p/v) borage aqueous; C = control; WC = water control; LOA = limit of acceptability proposed by Campo et al. (2006). MDA: malondialdehyde. Markers represent the means and bar errors represent standard error of the mean.

in controls (C, WC) and in the treatment 0.05% T did not differ significantly from the latter.

Spraying with either 5% T or 10% B extracts resulted in similarly strong inhibition of lipid oxidation, maintaining the lowest values of MDA throughout storage ($P \leq 0.001$). In fact, these samples reached values well below 0.5 mg MDA/kg at the end of display. Samples sprayed with 0.5% tea and 5% borage aqueous extract also showed significant lower TBARS values than controls (C and WC) at 8 and 11 days of display, but only the extract 0.5% T showed a significant effect after 13 days. The other concentrations tested (0.005% T, 0.05% T, 0.5% B) did not differ from control samples (C) at any sampling day. Both natural extracts showed a concentration-dependent action, showing only the extracts 0.5% T, 5% T and 10% B a significant antioxidant effect at every sampling day. Therefore, a minimum concentration of polyphenols of 2.08 mg GAE/100 cm^2 of meat was required to significantly reduce lipid oxidation. The limit of rejection for oxidized beef proposed by Campo et al. (2006) was not reached in these treatments nor in the samples sprayed with 5% borage aqueous extract during display whereas it was already exceeded at 11 days after packaging in both controls and in the rest of concentrations used.

The effect of tea extracts on inhibiting lipid oxidation throughout meat display has been reported by some authors (Bañón et al., 2007; Lorenzo et al., 2014b). Its antioxidant activity has been mainly attributed to catechins, which are plentiful in green tea extracts, because of its ability to act as free radical scavengers (Carrizo, Taborda, Nerín, & Bosetti, 2016). Regarding borage seeds extract, it has never been used, as far as we know, to preserve fresh meat but has been reported to reduced lipid oxidation in beef patties (Sánchez-Escalante et al., 2003), pork fresh sausages (Martínez et al., 2006) and fish (Giménez et al., 2011). Wettasinghe and Shahidi (1999) demonstrated in vitro that the high content of phenolic compounds in borage seeds, which are able to quench reactive oxygen species, may explain the ability of borage seed extracts to limiting oxidative reactions.

Only the results of the treatments that showed a significant antioxidant activity at every sampling day (0.5% T, 5% T and 10% B) and C are presented in this manuscript for the rest of parameters.

3.3. Instrumental colour

Colour values of lamb leg chops during display are given in Fig. 2. Lightness (L^*) increased in control samples throughout display while a decrease was registered in 5% T treatment ($P \leq 0.001$). In contrast, initial and final L^* values did not differ in 0.5% T and 10% B sprayed samples. Significant differences between treatments were obtained at the end of display, showing control higher L^* values than 5% T and 10% B treatments. The increase of lightness over time has been related to structural changes of meat, especially protein denaturation, which result in greater dispersion of light and as a result, increased lightness (MacDougall, 1982).

Chops spraying did not modify initial a^* values but a different evolution of redness was found among treatments. Control samples suffered a constant decrease in saturation from 8 days post packaging to the final of the experimental period ($P \leq 0.001$), registering a decrease either in a^* value or in the 630/580 ratio. A loss of redness intensity was also measured in 5% T sprayed chops, measuring a fall in both parameters after 11 and 13 days, respectively. The 630/580 ratio decreased in 0.5% T and 10% B treatments throughout display, while initial and final a^* values did not differ significantly in these samples.

Although redness decreased over time in all treatments, significant differences were found between them. The spraying with 0.5% T (2.08 ± 0.15 mg GAE/ml extract), 5% T (19.97 ± 1.06 mg GAE/ml extract) and 10% B (3.29 ± 0.02 mg GAE/ml extract) aqueous extracts resulted in higher a^* values than those found in control samples after 11 days of display ($P \leq 0.001$). Differences among treatments were also registered in the 630/580 ratio. Sprayed chops showed higher values in this ratio after 8 days of display, indicating an increase in metmyoglobin formation, while only those added with 0.5% T and 10% B offered higher values than controls at 13 days post packaging ($P \leq 0.001$).

Both herb extracts brought about a better keeping of colour, which agreed with previous reported effects of tea (Bañón et al., 2007; Lorenzo et al., 2014b) and borage (Sánchez-Escalante et al., 2003) extracts on reducing the loss of redness during display. Colour is the main lamb property valued by consumers at the time of purchase, so the protective effect of aqueous extracts on redness may influence consumers purchase decision.

The percentage of metmyoglobin increased throughout display in the four batches ($P \leq 0.001$) but myoglobin oxidation occurred at different velocity. Either 10% B or 0.5% T exerted a highly significant effect ($P \leq 0.001$) on delaying myoglobin oxidation, which resulted in a lower metmyoglobin content than control samples at the end of display. Concerning 5% T sprayed samples, the metmyoglobin percentage did not differ from that quantified either in control or in 10% B and 0.5% T added chops.

Discolouration of lamb registered during display could be explained because of lipid oxidation, which is known to enhance myoglobin oxidation. Products of lipid oxidation promote the conversion of red oxymyoglobin to brown metmyoglobin, which accumulates in meat surface, leading to meat discoloration (Faustman, Sun, Mancini, & Suman, 2010). Therefore, the antioxidant effect of herb extracts delayed this process during an extended period, maintaining a lower lipid oxidation and a better colour stability.

However, chops sprayed with 5% T did not differ in metmyoglobin content from control samples despite registering a significant inhibition of oxidative reactions. It should be noted that small shadows appeared onto the meat surface, which may explain the decrease of lightness as well as the high metmyoglobin content registered, since both parameters are obtained from colour measurements. Tea catechins, which account for about 75% of the green tea total dry weight, may have precipitated on the meat surface during display in the most concentrated aqueous extract (5% p/v), resulting in unacceptable colour modifications. Indeed, this extract contained 19.97 ± 1.06 mg GAE/ml extract, a concentration approximately seven times higher than that of the other extracts showing an antioxidant effect. Therefore, it may be pointed out that this is the concentration limit for its use in meat preservation.

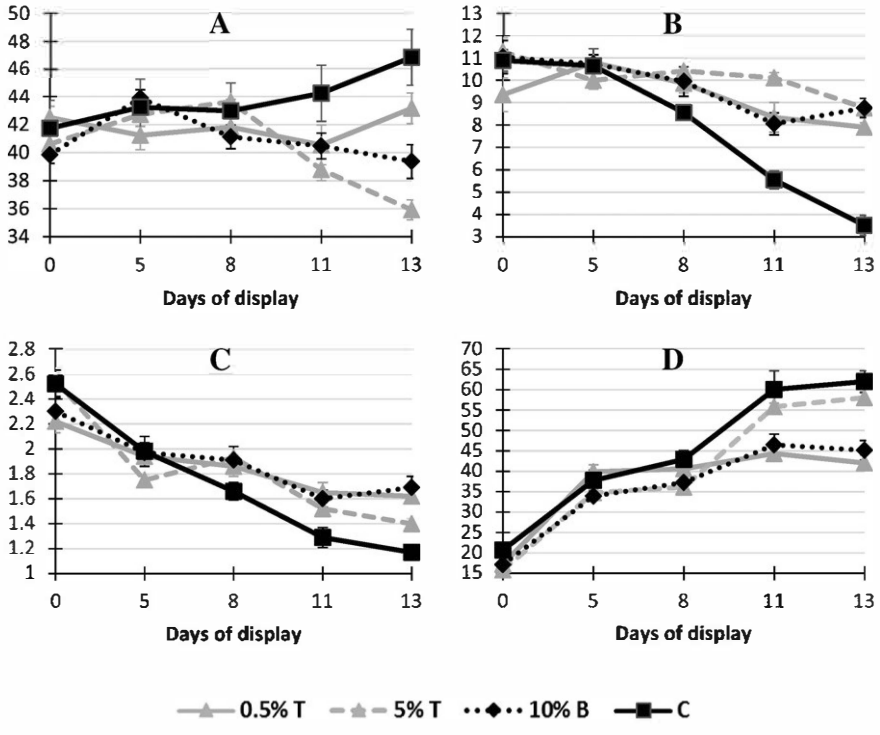

Fig. 2. L^* (A), a^* (B), 630/580 ratio (C) and percentage of metmyoglobin (D) values of lamb treated with tea and borage aqueous extracts during display. 0.5% T: 0.5% (p/v) tea aqueous extract; 5% T: 5% (p/v) tea aqueous extract; 10% B: 10% (p/v) borage aqueous extract; C = control. Markers represent the means and bar errors represent standard error of the mean.

Less concentrated extracts should be used to obtain a significant reduction of lipid oxidation without modifying lamb quality properties.

3.4. Microbial growth

The effect of natural extracts on microbial growth throughout display is presented in Fig. 3. Initial microbial counts did not differ among treatments, being comprised of between 3.12 and 3.49 for *Pseudomonas* spp. and between 2.04 and 2.42 for psychrotrophic total viable counts (PTVC). *Enterobacteriaceae* and lactic acid bacteria (LAB) growth could not be detected in all treatments up to 8 days post packaging. Microbial growth increased significantly in all batches over time, exceeding the limit for microbial spoilage proposed by Jeremiah (2001) (7 log CFU/g) after 13 days of display. This limit was reached at this time by *Pseudomonas* spp. growth in all samples as well as by PTVC in samples sprayed with 0.5% T and 10% B. Therefore, microbial growth limited the shelf life of refrigerated lamb to 11 days. *Pseudomonas* spp. has been described to be the predominant flora when meat is packaged in aerobic conditions such as those found in modified atmosphere packaging containing a high percentage of O_2, since this genus is primarily responsible for microbial spoilage of fresh meat (Osés et al., 2013).

Counts of the genera determined showed mostly non significant differences between treated and untreated lamb chops and among treatments at any sampling day ($P \leq 0.05$). Thus, the extracts used in this trial did not show any significant antimicrobial effect. The absence of a significant reduction of microbial counts in the samples sprayed with borage seed extract was in accordance with the results obtained by Sánchez-Escalante et al. (2003), demonstrating the lack of any antimicrobial effect of borage seed extracts. Lorenzo, Batlle, and Gómez (2014a) had previously reported a significant antimicrobial effect of green tea extract; however, it has not been observed in our study. Differences in activity may be related to differences in extract compositions or because they used an active packaging.

3.5. Sensory analyses

Results of sensory analyses are presented in Table 3. Samples were not evaluated after 11 days of display due to the high microbial counts registered.

Including herb extracts and essential oils into the package may bring about strange odours and flavours to meat, leading to consumers' rejection. Regarding this, Latoch and Stasiak (2015) reported that mint extract overpowered typical pork sausage odours and flavours, compromising its commercial use despite improving colour. In contrast, herb odour and flavours were not detected in any treatment at any concentration in our trial, showing that the extracts used did not provide strange odours or flavours to lamb chops. Similarly, Mitsumoto, O'Grady, Kerry, and Joe Buckley (2005) reported no taste and flavour differences between controls and samples treated with green tea catechins.

Oxidation odours and flavours increased over time either in control or 0.5% T sprayed samples but beyond day 0 remained stable in 5% T and 10% B added chops. At 5 days, panellists already detected significant differences among treatments, when, of all treatments, control samples were given the highest values for both sensory attributes (2.50 and 2.25 for oxidation odour and flavour respectively) while no significant difference was perceived among 0.5% T, 5% T and 10% B sprayed samples. From this point to 11 days of display, post-hoc analysis determined 3 significantly different groups; control samples registered the highest values, followed by 0.5% T sprayed samples and finally 5% T and 10% B treatments. In accordance with Greene and Cumuze (1982), who reported 0.6 mg MDA/kg as the minimum TBARS value detectable in

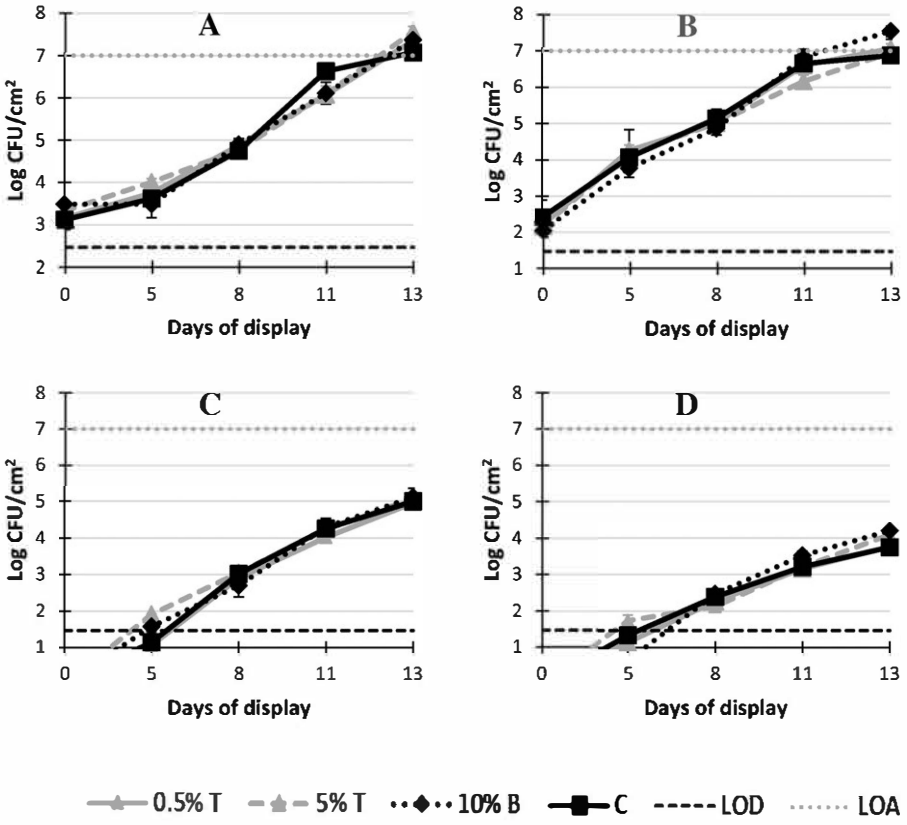

Fig. 3. Growth of *Pseudomonas* spp. (A), psychrotrophic total viable counts (B), lactic acid bacteria (C) and *Enterobacteriaceae* (D) for lamb leg chops treated with tea and borage aqueous extracts. 0.5% T: 0.5% (p/v) tea aqueous extract; 5% T: 5% (p/v) tea aqueous extract; 10% B: 10% (p/v) borage aqueous extract; C = control LOA = limit of acceptability. Markers represent the means and bar errors represent standard error of the mean. The limit of detection (LOD) was 1.48 log CFU/cm^2 for psychrotrophic total viable counts, *Enterobacteriaceae* and lactic acid bacteria and 2.48 log CFU/cm^2 for *Pseudomonas* spp.

Table 3
Means and standard error of the mean (SEM) for sensory attributes of grill-cooked lamb treated with tea and borage aqueous extracts.

Attributes[A]	Days of display	0.5% T	10% B	5% T	C	SEM	$P_{treatment}$
Herb odour	0	0.00	0.00	0.00	0.00	0.00	–
	5	0.00	0.00	0.00	0.00	0.00	–
	8	0.00	0.00	0.00	0.00	0.00	–
	11	0.00	0.00	0.00	0.00	0.00	–
	$P_{display}$	–	–	–	–		
Microbial odour	0	0.00^{x}	0.00^{x}	0.00^{x}	0.00^{x}	0.00	–
	5	2.78^{y}	2.25^{y}	2.38^{y}	2.28^{y}	0.08	0.960
	8	3.00^{y}	3.38^{y}	2.50^{y}	3.13^{y}	0.12	0.258
	11	4.63^{z}	4.50^{z}	4.75^{z}	4.63^{z}	0.16	0.937
	$P_{display}$	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001		
Oxidation odour	0	0.00^{x}	0.00	0.00	0.00^{x}	0.00	–
	5	$0.29^{a,x}$	0.25^{a}	0.15^{a}	$2.50^{b,y}$	0.09	P ≤ 0.001
	8	$2.50^{b,y}$	0.25^{a}	0.13^{a}	$4.88^{c,z}$	0.12	P ≤ 0.001
	11	$2.75^{b,y}$	0.38^{a}	0.38^{a}	$5.50^{c,z}$	0.14	P ≤ 0.001
	$P_{display}$	P ≤ 0.001	0.577	0.238	P ≤ 0.001		
Herb flavour	0	0.00	0.00	0.00	0.00	0.00	–
	5	0.00	0.00	0.00	0.00	0.00	–
	8	0.00	0.00	0.00	0.00	0.00	–
	11	0.00	0.00	0.00	0.00	0.00	–
	$P_{display}$	–	–	–	–		
Microbial flavour	0	0.00^{x}	0.00^{x}	0.00^{x}	0.00^{w}	0.00	–
	5	2.25^{y}	2.00^{y}	2.13^{y}	2.15^{x}	0.10	0.807
	8	2.75^{y}	3.00^{y}	2.75^{y}	3.25^{y}	0.10	0.434
	11	5.13^{z}	5.38^{z}	5.31^{z}	5.18^{z}	0.11	0.834
	$P_{display}$	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001		
Oxidation flavour	0	0.00^{x}	0.00	0.00	0.00^{w}	0.00	–
	5	$0.50^{a,x}$	0.38^{a}	0.13^{a}	2.25^{bx}	0.12	P ≤ 0.001
	8	$1.63^{b,y}$	0.00^{a}	0.25^{a}	$3.63^{c,y}$	0.10	P ≤ 0.001
	11	$3.00^{b,z}$	0.27^{a}	0.15^{a}	$5.50^{c,z}$	0.19	P ≤ 0.001
	$P_{display}$	P ≤ 0.001	0.412	0.656	P ≤ 0.001		

0.5% T: 0.5% (p/v) tea aqueous extract; 10% B: 10% (p/v) borage aqueous extract; 5% T: 5% (p/v) tea aqueous extract; C: control samples.
Different superscripts (a, b, c) within a row indicate significant differences among packaging conditions (P ≤ 0.05). Different superscripts (x, y, z) within a column indicate significant differences among days of display (P ≤ 0.05).
[A] 10 point scale (0 = low, 10 = high); SEM: standard error of the mean; – = not determined.

beef, panellists were not able to detect oxidation odour and flavour in samples with a MDA content lower than 0.6 mg MDA/kg (Fig. 1). However, they could detect differences among 0.5% and both 5% T and 10% B sprayed samples at 8 days after packaging, when TBARS values were 0.77, 0.24 and 0.34 mg MDA/kg for 0.5%, 5% T and 10% B treatments. The control samples, which contained 2.11 mg MDA/kg, were awarded the highest values for oxidation odour and flavours (5.50 and 6.50 respectively) after 11 days of display. Therefore, these results showed a good correspondence with those found in TBARS analyses, agreeing also with the threshold of rejection for oxidized beef (2 mg MDA/kg) proposed by Campo et al. (2006).

Microbial odour and flavour, which were detected after 5 days of display, increased throughout display in all treatments without statistical differences among them. PTVC as well as *Pseudomonas* spp. counts were close to the limit of microbial spoilage proposed by Jeremiah (2001) after 11 days, when panellists scores comprised between 4.50 and 4.74 for microbial odour and 5.13 to 5.38 for microbial flavour. Therefore, microbial growth modified sensory properties of lamb, which was detected by panellists. In fact, it is known that organoleptic spoilage may appear because of microbial growth; microbial consumption of meat nutrients result in the release of volatile metabolites, leading to off odours and flavours development and ultimately consumers' rejection (Ercolini et al., 2010).

4. Conclusions

The quality of retail displayed lamb chops could be improved by spraying natural water extracts, either tea (T) or borage (B). A minimum polyphenol concentration of 2.08 ± 0.15 mg GAE/100 cm^2 of meat was required to reduce significantly malondialdehyde formation. This concentration was reached by spraying lamb chops with 0.5% T, 5% T and 10% B aqueous extracts, showing these samples malondialdehyde values under the limit of acceptability during display. The inhibition of lipid oxidation resulted in a better keeping of redness and a lower metmyoglobin formation in 0.5% T and 10% B sprayed chops but the improvement of colour was not found in 5% T samples, probably as a result of catechin precipitation. The use of 5% (p/v) green tea aqueous extract on lamb chops resulted in unacceptable colour modifications despite reducing lipid oxidation, so lower concentrations must be recommended for meat preservation. No extracts showed antimicrobial effects. Microbial growth exceeded the limit of rejection at 13 days of display in all samples. None of the extracts brought about perceptible strange odours and flavours to meat, thus this is not a limitation for its use; the new aqueous extract of borage seeds seems to be highly suitable for this application. Either 0.5% T or 10% B could be recommended for preserving lamb chops maintained under retail conditions, extending lamb chops shelf life from 8 to 11 days.

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4.2.5. Sulphite-free lamb burger meat: antimicrobial and antioxidant properties of green tea and carvacrol

Sulphite-free lamb burger meat: antimicrobial and antioxidant properties of green tea and carvacrol

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Shelf life

ABSTRACT

Background
Sulphite is commonly used to preserve lamb burger meat in the European Union. Nevertheless, its consumption has been related to certain health problems which have increased consumer demands of free-sulphite products. Natural compounds with antioxidant and antimicrobial properties could be a feasible alternative to preserve lamb burger meat. This study evaluated the antimicrobial and antioxidant properties of carvacrol, green tea and their combination in preserving lamb burger meat. Their effect was also compared with that of 400 ppm sulphite.
Results
Lamb burger meat was mixed with different concentrations of the extracts and packaged aerobically through 8 days of refrigerated display. Total polyphenols, TBARS, colour, microbial and sensory analyses were performed. Green tea and carvacrol avoided lipid oxidation even at 300 ppm while only carvacrol, which showed a concentration-dependent action, decreased discolouration and microbial growth. Carvacrol and green tea also limited the development of oxidation odour and flavour but the former brought about herbal odours and flavours to meat. On the other hand, sulphite reduced discolouration and microbial growth compared to both carvacrol and green tea but presented a higher lipid oxidation.
Conclusion
Carvacrol seems to be a promising alternative to replace sulphite in lamb burger meat while green tea should be combined with an antimicrobial agent.

1. Introduction

The term sulphite comprises several compounds containing the sulphite ion. Sulphite is largely used as preservative in a wide variety of food products such as vegetables, wine, meat and seafood because of its protective effects against oxidative reactions, colour fading and microbial growth. In the European Union, the use of sulphite in meat and meat products is restricted to breakfast sausages and burger meat at a maximum concentration of 450 mg SO_2/kg.[1] Nevertheless, consumer concern about chemical additives continues to grow and sulphite is currently the subject of considerable debate. The consumption of sulphite has been related to certain health problems, including respiratory and allergic reactions, thiamine absorption deficit and disruption of carbohydrate metabolism.[2] For that reason, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) recently delivered a scientific opinion re-evaluating the use of sulphite for food preservation.[3] Indeed, several European projects are being carried out with the goal of reducing and/or replacing sulphite in food products.

Natural compounds emerge as a promising alternative to address consumer demand of clean labelled food without synthetic chemical preservatives. Herb extracts and essential oils are perceived as

natural and safe and are also included in the Generally Recognised As Safe (GRAS) list of the American Food and Drug Administration.[2] Antioxidant and antimicrobial compounds have been searched in different plant materials such as fruits, leaves, oilseeds, cereal crops, spices and herbs. Green tea encompasses several compounds like catechin, flavones, anthocyan and phenolic acids, which are of great interest because of their potential antioxidant and antimicrobial properties.[4,5] Catechins, which constitute 30% of water-soluble solids of the dry weight, are the chief compounds of green tea while myricetin, quercetin and kaempferol are the main derivatives of the flavonol group.[6] Due to its chemical composition, which is rich in polyphenols, green tea extract may be a feasible strategy to reduce oxidation of sulphite-free burger meat. On the other hand, carvacrol (2-metil-5-(1-metiletil) phenol) is a monoterpenic phenol present in many aromatic plants such as oregano and thyme. *In vitro* studies showed that carvacrol inhibits autoxidation in purified triacylglycerols, suggesting its possible use to extend the shelf life of lipid-containing food.[7] Moreover, carvacrol exerts a wide antimicrobial spectrum against both Gram-positive and Gram-negative bacteria isolated from food.[8]

Therefore, the aim of this work was to evaluate the preservative properties of green tea extract and carvacrol for increasing the shelf life of sulphite-free lamb burger meat. The antioxidant and antimicrobial activities of both extracts were compared with those of sulphite. The sensory contribution of both extracts to cooked meat was also studied.

2. Materials and methods

2.1 Sampling

This study used nine carcasses chosen at random among commercial lambs of Rasa Aragonesa breed. Lambs were raised intensively and fed with natural suckling up to 40 days of age and cereal straw *ad libitum* until they weighted between of 20 to 25 kg, when they were killed in a commercial abattoir (Mercazaragoza) according to EU regulation. After dressing (15 min), carcass chilling took place during 24 h (4 ± 0.5 °C, 90% RH, 1-2 m/s) in the facilities of Casa de Ganaderos and Franco y Navarro S.A. Both legs of each carcass were excised and transported at 4 °C to the food technology pilot plant of the Faculty of Veterinary Science (University of Zaragoza), where they were deboned, minced through a 4-mm plate (M-94-32 Gesame, Vic, Spain), divided in five different batches (n = 5, batch is the replicate) and mixed with potato starch (4% w/w) for 5 min using a AVT-50 mixer (Castellvall, Girona, Spain). After that, the mixture of each batch was distributed equally among the following treatments:

- Control (B): with no additives.
- S400: 400 ppm of SO_2 added in the form of sodium metabisulphite.
- T300: 300 ppm of green tea extract.
- C300: 300 ppm of carvacrol.
- C1000: 1000 ppm of carvacrol.l
- T+C300: 300 ppm of green tea extract and 300 ppm of carvacrol.
- T+1000C: 300 ppm of green tea extract and 1000 ppm of carvacrol.

Green tea extract (Sunphenon 90MB, TAIYO Europe, Filderstadt, Germany), sulphite (Merck, Darmstadt, Germany) and carvacrol (Sigma Aldrich, Misouri, USA) were dissolved in sterilized water and the resulting solutions were immediately added to the meat (10 ml of solution for 1 kg minced meat); each sample was thoroughly hand-mixed. The same amount of water was added to the control samples.

Burger meat was distributed in polystyrene/EVOH/polyethylene trays (Linpac packaging S.A.U., Pravia, Spain) and covered with PVC film (O_2 transmission rate at 25 °C of 650-750 $cm^3/m^2/24$ h and 0% RH, Irma S.A., Zaragoza, Spain). One hundred and seventy five trays were maintained under commercial retail conditions (4 °C ± 0.5 °C with 14 h of artificial light per day) during 8 days. Illumination conditions are described in Bellés *et al.*[9] Physicochemical, microbiological and sensory analyses were done at 0 (approximately 24 h post mortem), 1, 3, 6 and 8 of display.

2.2 Total phenolic compounds

Total polyphenols were quantified following the methodology presented by Matthäus[10] with some modifications described in Bellés *et al*.[9]

Table 1. Description and references of the attributes used for the sensory analyses.

Attribute	Definition	References
Odour		
Oxidation	Odour associated with oxidation compounds derived from fat	Rancid seed oil
Herbal	Odour associated with herbs	Tea leaves
Microbial	Putrid odours derived from meat spoilage	Spoiled meat
Flavour		
Oxidation	Flavour associated with oxidation compounds derived from fat	Rancid seed oil
Herbal	Flavour associated with herbs	Tea leaves
Microbial	Putrid flavours derived from meat spoilage	Spoiled meat

2.3 Lipid oxidation

The Thiobarbituric Acid Reactive Substances (TBARS) assay was carried out to determine lipid oxidation of meat samples according to the methodology described by Alonso *et al*.[11]

2.4 Instrumental colour

Instrumental colour was determined on the meat surface using a Minolta CM®2002 reflectance spectrophotometer (Osaka, Japan) with an aperture of 30 mm and a D65 illuminant. The instrument was calibrated at the beginning of each session. Each value was the mean of 10 consecutive determinations. Meat pigment proportions were calculated as described by Krzywicki[12] while the values of the oxymyoglobin/metmyoglobin ratio were determined as the quotient of light reflectance at 630 and 580 nm.

2.5 Microbial analyses

Ground meat (25 g) was aseptically collected from each package, placed in a stomacher bag, diluted with 0.1% peptone water (225 ml) (Biolife) and homogenized for 3 min in a stomacher. Decimal dilutions were carried out using the same diluent. One ml of the appropriate dilution was plated in the following manner: aerobic total viable counts (ATVC) on plate count agar (PCA) (Merck, Darmstadt, Germany) at 37 °C for 24 h, *Enterobacteriaceae* on violet red bile dextrose agar (VRBD) (Merck, Darmstadt, Germany) at 37 °C for 48 h and lactic acid bacteria (LAB) on Man Rogosa Sharpe agar (MRS, Merck, Darmstadt, Germany) at 37 °C for 96 h in anaerobiosis. Anaerobic conditions were generated in an incubator jar using a commercial kit (Anaerocult A) (Merck, Darmstadt, Germany) and checked with an indicator strip (GazPack™). For the investigation of *Pseudomonas spp*. and *Brochotrix thermospacta,* 0.1 ml of the proper dilution was inoculated on the surface of Cephalothin-Sodium Fusidate-Cetrimide Agar (CFC, Merck, Darmstadt, Germany) and streptomycin thallous acetate actidione agar (STAA) (STAA, Oxoid, Unipath Ltd., Basingstoke, UK) and incubated at 20 °C for 24 h and 30 °C for 48 h, respectively. Results were presented as base-10 logarithm of colony-forming units per g of meat (log CFU/g).

2.6 Sensory analyses

Meat for the sensory analyses was thawed at 4 °C during 12 h before cooking and the samples were prepared as described by Bellés *et al*.[9] The panel involved 9 members trained following the methodology proposed in the study cited previously. Panellists rated the samples on a 10-point structured scale in which intensity was from low (0) to high (10). The descriptors, definitions and references of the parameters evaluated in the sensory analyses are shown in Table 1. Sessions occurred in individual booths with red light. Nine sessions of approximately 40 minutes were required to evaluate the samples.

2.7. Statistical analyses

All data were analysed using the General Linear Model (GLM) procedure of SPSS,

version 19.0 (IBMSPSS, 2010) and mean separation was carried out using Tukey's post hoc test with the level for statistical significance set at $P \leq 0.05$. The model was as follows:

$$Yij = \mu + Ti + Dj + (Ti \times Dj) + eij$$

where Yij is the dependent variable; μ is the population average; Ti is the fixed effect of treatment; Dj is the fixed effect of display duration; (Ti ×Dj) is the interaction effect of treatment and display duration; and eij is the aleatory error.

The same model was employed for analyzing the results from the sensory evaluation, including "panellist" and "session" as fixed effects and their interactions. "Panellist" resulted in a significant effect ($P \leq 0.001$) on all of the sensory parameters evaluated but the effect of "session" was not significant. No significant interactions were found.

3. Results

3.1 Polyphenol content

Total content of phenolic compounds expressed as gallic acid equivalents (GAE) was significantly higher in green tea extract (108.25 ± 3.2 mg GAE/g extract) than in carvacrol (77.78 ± 2.8 mg GAE/g of carvacrol) ($P < 0.001$).

3.2 Lipid oxidation

Mean TBARS values of lamb burger meat during display are presented in Figure 1. As it could be expected, oxidation developed significantly in all the batches ($P < 0.001$). However, the propagation of lipid oxidation differed among treatments: the addition of both carvacrol and green tea resulted in significant lower TBARS values than those measured in control and sulphite treatments during display ($P < 0.001$). Differences between tea and carvacrol batches were only found at eight days post-packaging, when the former showed higher values than those observed in the carvacrol and the carvacrol plus green tea groups ($P < 0.001$). Increasing the concentration of carvacrol from 300 to 1000 ppm did not modify significantly the rate of lipid oxidation of lamb burger meat. Otherwise, the addition of sulphite also inhibited the spread of oxidative reactions compared to control; nonetheless, its antioxidant action was weaker than that exerted natural antioxidants ($P < 0.05$).

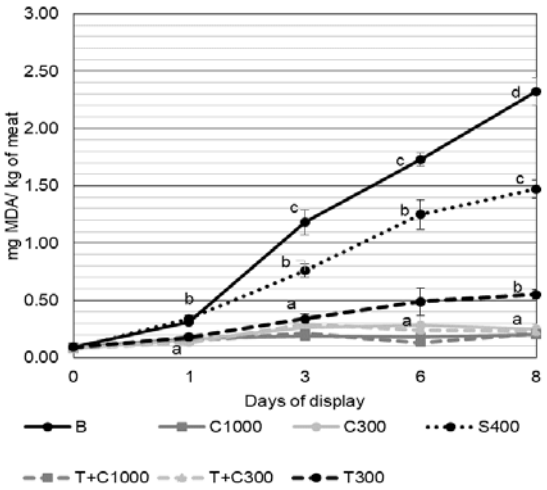

Figure 1. TBARS values of lamb burger meat during display at 4 °C.
Treatments: B -control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; T + 1000C - green tea extract 300 ppm + carvacrol 1000 ppm. Markers represent the mean of malonaldehyde values (MDA) and the error bars represent standard error of the mean. Means with different letters (a, b, c) in the same day of storage indicate significant differences among mean values ($P \leq 0.05$).

3.3 Instrumental colour and meat pigment proportions

The evolution of the ratio 630/580 and meat pigment proportions are presented in Table 2. Natural antioxidants did not modify colour immediately after its application ($P = 0.623$) but gave rise to significant differences during display. The addition of sulphite protected meat from discolouration thereby exhibiting higher values of the ratio 630/580 than control from three days post-packaging onwards ($P < 0.001$). Treatments including 1000 ppm carvacrol (C1000, T+C1000) also differed significantly from control at 3 and 6 days of display while the addition of carvacrol at lower dosage (300 ppm) led only to significant higher values of the ratio 630/580 after 6 days of refrigeration. In contrast, adding 300 ppm of green tea extract did not improve significantly the values of control at any sampling day.

Metmyoglobin accumulated during display in all treatments ($P < 0.001$) (Table

2). Nevertheless, both sulphite (S400) and carvacrol (C300, C1000, T+C300, T+C100) reduced the oxidation of the pigment, therefore leading to a lower percentage of metmyoglobin than control at 6 days of display (P < 0.001). Moreover, metmyoglobin content in S400 was also lower at 3 and 8 days of refrigeration (P = 0.006).Differences in the proportion of deoxymyoglobin among treatments were only observed after 6 days (P > 0.05) when sulphite showed a higher percentage of deoxymyoglobin than control. Similarly, burger meat containing 400 ppm sulphite or 1000 ppm carvacrol had a higher percentage of oxymyoglobin than the control batch after 6 days of display (P = 0.001) (Table 2).

Table 2. Colour and relative content of the different forms of myoglobin of lamb burger meat during display at 4 °C.

	Treatments	Days of display					$P_{display}$	SEM
		0	1	3	6	8		
630/580 ratio	B	2.50^{z}	2.08^{y}	$1.78^{a,x}$	$1.47^{a,w}$	$1.53^{a,wx}$	< 0.001	0.084
	S400	2.57^{xy}	2.48^{x}	$2.72^{c,y}$	$2.79^{c,y}$	$2.56^{b,xy}$	< 0.001	0.067
	T300	2.67^{z}	2.28^{z}	$1.87^{a,y}$	$1.81^{ab,y}$	$1.41^{a,x}$	< 0.001	0.092
	C300	2.63^{z}	2.22^{y}	$2.14^{ab,y}$	$2.04^{b,y}$	$1.43^{a,x}$	< 0.001	0.017
	C1000	2.64^{yz}	2.29^{y}	$2.67^{bc,z}$	$2.22^{b,y}$	$1.67^{a,x}$	< 0.001	0.076
	T+C300	2.58^{z}	2.37^{z}	$1.99^{a,y}$	$1.99^{b,y}$	$1.62^{a,x}$	< 0.001	0.073
	T+C1000	2.63^{z}	2.07^{xy}	$2.72^{c,z}$	$2.18^{b,yz}$	$1.53^{a,x}$	< 0.001	0.124
	$P_{treatment}$	0.623	0.798	< 0.001	< 0.001	0.003	-	-
% MMb	B	21.90^{x}	32.08^{y}	$36.85^{bcd,y}$	$48.05^{d,z}$	$49.62^{b,z}$	< 0.001	2.371
	S400	22.90^{x}	28.25^{yz}	$25.92^{a,y}$	$29.43^{a,z}$	$28.22^{a,yz}$	< 0.001	0.607
	T300	23.45^{w}	30.46^{x}	$41.58^{d,y}$	$43.37^{cd,yz}$	$47.06^{b,z}$	< 0.001	1.897
	C300	21.85^{w}	32.01^{x}	$37.64^{cd,y}$	$37.58^{bc,y}$	$50.66^{b,z}$	< 0.001	0.374
	C1000	21.49^{w}	33.61^{xy}	$29.44^{ab,x}$	$33.66^{ab,y}$	$45.44^{b,z}$	< 0.001	1.542
	T+C300	23.36^{w}	28.48^{x}	$39.63^{d,y}$	$40.60^{c,yz}$	$43.99^{b,z}$	< 0.001	1.666
	T+C1000	22.36^{x}	32.84^{y}	$30.94^{abc,xy}$	$31.86^{ab,xy}$	$50.26^{b,z}$	< 0.001	2.642
	$P_{treatment}$	0.491	0.237	< 0.001	< 0.001	0.006	-	-
% DMb	B	21.79^{y}	22.14^{y}	16.18^{xy}	$11.78^{a,x}$	11.99^{x}	< 0.001	1.208
	S400	23.59^{y}	28.91^{z}	24.29^{y}	$20.12^{b,x}$	22.53^{xy}	< 0.001	0.809
	T300	22.91^{y}	27.87^{y}	19.92^{xy}	$14.06^{ab,x}$	16.09^{x}	< 0.001	1.539
	C300	23.77^{y}	23.59^{y}	18.14^{xy}	$17.07^{ab,xy}$	15.19^{x}	< 0.001	0.193
	C1000	24.12^{y}	19.79^{y}	22.28^{y}	$13.42^{ab,x}$	20.76^{y}	< 0.001	0.905
	T+C300	22.87^{y}	29.45^{z}	20.07^{xy}	$15.49^{ab,x}$	29.53^{z}	< 0.001	2.087
	T+C1000	23.88	20.22	19.21	18.47^{ab}	22.73	0.785	1.080
	$P_{treatment}$	0.502	0.120	0.626	0.046	0.155	-	-
% OMb	B	56.31^{z}	45.78^{y}	46.97^{y}	$40.17^{a,x}$	$38.39^{b,x}$	< 0.001	1.364
	S400	53.51^{y}	42.84^{x}	49.80^{y}	$50.45^{bc,y}$	$49.25^{c,y}$	< 0.001	0.626
	T300	53.64^{y}	41.67^{x}	38.49^{x}	$42.57^{ab,x}$	$36.85^{ab,x}$	< 0.001	1.069
	C300	54.38^{z}	44.41^{y}	44.22^{y}	$45.35^{abc,y}$	$34.15^{ab,x}$	< 0.001	0.274
	C1000	54.39^{z}	46.6^{y}	48.28^{y}	$52.92^{c,yz}$	$33.8^{ab,x}$	< 0.001	1.788
	T+C300	53.77^{z}	42.07^{y}	40.29^{y}	$43.91^{ab,y}$	$26.48^{a,x}$	< 0.001	1.924
	T+C1000	53.76^{z}	46.94^{y}	49.86^{y}	$49.67^{bc,y}$	$27.00^{ab,x}$	< 0.001	2.937
	$P_{treatment}$	0.611	0.104	0.063	0.001	< 0.001	-	-

Treatments: B - control -; S400 - sulphite 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; T + 1000C - green tea extract 300 ppm + carvacrol 1000 ppm -. Means and standard error of the mean (SEM). Different superscripts (a, b, c) within a column indicate significant differences among treatments ($P \leq 0.05$). Different superscripts (x, y, z) within a row indicate significant differences among days of display ($P \leq 0.05$). – = not determined. % MMb: percentage of metmyoglobin; % DMb: percentage of deoxymyoglobin; % OMb: percentage of oxymyoglobin.

3.4 Microbial growth

The means of total aerobic (ATVC), *Enterobacteriaceae*, lactic acid bacteria (LAB), *Pseudomonas spp*. and *Brochotrix thermospacta* counts for each day in each treatment are shown in Figure 2. Counts increased significantly during display in all the batches but with different growth rates ($P < 0.001$). Generally, both sulphite and 1000 ppm carvacrol (C1000, T+C1000) inhibited significantly microbial growth from the 3rd day onwards. In contrast, green tea extract did not exert any antimicrobial effect against the microbial genera determined. Treatments with carvacrol at 300 ppm (C300, T+C300) showed lower counts than those determined in the control batch, nevertheless, differences did not become significant at all sampling days.

Regarding ATVC, counts in S400, C1000, T+C1000, and C300 were lower than those determined in the control treatment at 3 days post packaging ($P < 0.001$), while the effect of sulphite and carvacrol (C1000 and T+C100) on delaying microbial growth was also significant after 6 days of refrigeration. Tukey's post hoc test identified four groups differing significantly the 8th day: burger meat with 400 ppm sulphite presented the lowest ATVC, followed by those included 1000 ppm of carvacrol (C1000 and T+C1000) and 300 ppm of carvacrol (C300). The control batch reached the highest ATVC, while the treatments T300 and T+C300 neither differed from control nor from those with 1000 ppm carvacrol (C1000 and T+C1000) and C300. *Enterobacteriaceae* and *Pseudomonas spp*. growth followed a similar pattern and they only differed in the counts of the treatments with 300 ppm of carvacrol at 6 post-packaging. *Enterobacteriaceae* counts in the T+C300 treatment were significantly lower than those registered in the control batch while *Pseudomonas spp*. growth was lower in both treatments with carvacrol at 300 ppm (C300 and T+C300) than in control.

Lactic acid bacteria and *Brochotrix termosphacta* grew at a lower rate. In fact, growth in the latter could not be detected up to the 3rd day of refrigeration. The addition of sulphite and carvacrol both at 300 and 1000 ppm resulted in lower LAB counts than those quantified in control and T300 batches after 3 days of display ($P < 0.001$). The effect of sulphite and carvacrol at 1000 ppm was also significant the 6th day, while only the former led to lower counts after 8 days ($P < 0.001$). Similarly, the batches containing sulphite and 1000 ppm of carvacrol delayed the growth of *Brochotrix thermosphacta*, thereby registering lower counts than those found in the control treatment both at 6 and 8 days of display ($P < 0.001$).

3.5 Sensory evaluation

Results of sensory evaluation of grill-cooked lamb are presented in Table 3. Carvacrol was perceived by panellists, who detected a higher herbal odour and flavour in the treatments which contained both 300 and 1000 ppm. Moreover, they were capable of discriminating between these concentrations ($P \leq 0.05$). In contrast, green tea was not detected. As it could be expected, microbial odour and flavour increased throughout display in all batches ($P < 0.001$). Nevertheless, differences among treatments arose from 3 days post-packaging onwards: the addition of both sulphite and carvacrol at 1000 ppm decreased the development of microbial odour and flavour without significant differences between them. The addition of natural antioxidants also reduced the development of oxidation odour and flavour thus registering lower scores than the control at 3, 6 and 8 days of display ($P \leq 0.05$). Similarly, sulphite diminished oxidation related odour and flavour although scores attributed to this batch were higher than those recorded in the treatments with carvacrol (from 6 days onwards) or in those with green tea at 8 days post packaging.

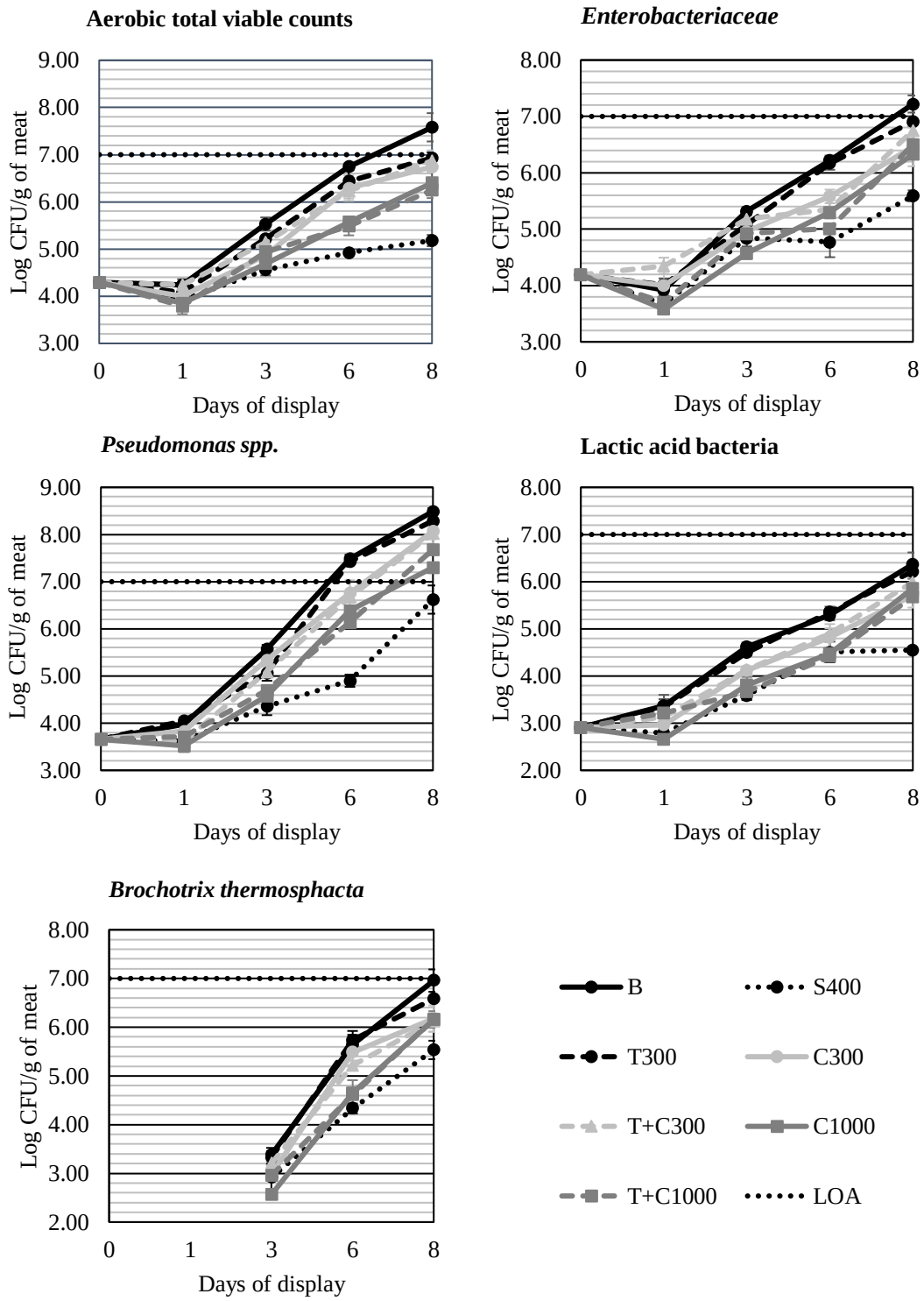

Figure 2. Microbial counts of lamb burger meat during display at 4 °C.

Treatments: B - control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; T + 1000C - green tea extract 300 ppm + carvacrol 1000 ppm -. LOA: Limit of acceptability. Markers represent the mean and the error bars represent standard error of the mean.

4. Discussion

4.1 Lipid oxidation

Total polyphenols quantified in green tea extract were in accordance with data previously reported by Stapornkul *et al*.[13] Green tea is a complex mixture of polyphenols in which the chief compounds are catechins and flavonoids, mainly epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG).[5] Tea polyphenols have been described to possess antioxidant properties by acting as hydrogen donors, reducing agents, nascent oxygen quenchers, and chelating metal ions.[14] These mechanisms would explain the great inhibition of lipid oxidation observed in the batch with 300 ppm of green tea. This concentration was not able to completely avoid lipid oxidation but it seemed enough to maintain lipid oxidation below the limit of acceptability (2 mg MDA/kg) proposed by Campo *et al*.[15] for oxidised beef. In this regard, Mitsumoto *et al*.[16] and Liu *et al*.[17] also concluded that tea catechins at concentrations close to 300 ppm were capable of effectively retarding lipid oxidation. The phenolic compounds, expressed as gallic acid equivalents, quantified in carvacrol were lower than those registered in green tea. Nevertheless, carvacrol reduced lipid oxidation to a similar degree, showing even lower TBARS values than those registered in T300 after eight days of display. The antioxidant properties of an extract are strongly related to its content of polyphenols; nonetheless, the species of phenolic compound is also a key factor, as it determines its mechanism of action.

Carvacrol is an oxygenated monoterpene that possesses a phenol group in its chemical structure. It may inhibit peroxidation of phospholipid liposomes in the presence of iron (III) and ascorbate and has been also described to be a good scavenger of peroxyl radicals.[8] As far as we know, the antioxidant properties of carvacrol had not been tested in meat matrix but an active film containing 4 % of an oregano extract showed a three-fold reduction in the degree of lipid oxidation in lamb.[18] Neither increasing the concentration of carvacrol to 1000 ppm nor the combination with 300 ppm of green tea extract improved the effects reached with 300 ppm of carvacrol, thus the latter concentration would be fairly enough to inhibit lipid oxidation of lamb burger meat under the conditions tested. On the other hand, the addition of 400 ppm sulphite also delayed lipid oxidation compared to control, regardless its effect was lower than that exerted by both carvacrol and green tea. Sulphite acts as secondary antioxidant by decomposing hydroperoxides in a non-radical way.[19] However, data obtained revealed that 400 ppm of sulphite were not able to control lipid peroxidation during eight days of display. Likewise, Bañón *et al*.[20] pointed out noticeable lipid oxidation in low sulphite (100 ppm) beef patties. Therefore, lipid oxidation is likely to be a limiting factor to the shelf-life of sulphite added burger meat.

4.2 Colour

Colour is the chief lamb sensory property during retail display because of consumer purchasing decision relies on meat appearance. Consumers associate a bright red colour with freshness and superior meat quality.[21] Colour is highly related with the quantity and the chemical state of meat myoglobin; but it transforms to metmyoglobin when the central iron atom is oxidised, changing meat colour from a red fresh appearance to a less desirable brown colour and therefore decreasing consumers acceptance. Sulphite is able to reduce the myoglobin haem group, thereby lessening the formation of metmyoglobin and providing a fresh appearance to red meat.[22]. These properties explain the lower amount of metmyoglobin and the higher values of the index 630/580 registered in the treatment including 400 ppm sulphite. Similarly, Bañón *et al*.[20] and Ortuño *et al*.[23] also identified a protective effect of SO_2 against the discolouration of beef and lamb minced meat throughout display. According to Djenane *et al*.[24] consumers reject beef when the percentage of metmyoglobin exceeds 40%. Only sulphite maintained the percentage of metmyoglobin below this threshold after eight days of display, while carvacrol controlled the oxidation of myoglobin up to 6 days post packaging. Otherwise, no effect of green tea on preventing pigment oxidation was detected. Therefore, sulphite maintained the ratio 630/580 values below the acceptability limit (2.23) proposed by Ripoll *et al*.[25] while it was

fairly exceeded in the B, T300, C300 and T+C300 treatments after 3 days of display. The 630/580 ratio has been widely used to describe meat discolouration as larger ratios and differences indicate more redness while a ratio of 1.0 would indicate essentially 100% MMb.[26] Thereafter, sulphite addition resulted in more redness and a better appearance than that of natural extracts.

Meat discolouration may be related, too, to lipid oxidation, since secondary-by-products of lipid oxidation have been described to promote myoglobin oxidation and thereafter colour fading.[27] Nevertheless, both the percentage of metmyoglobin and the values of the ratio 630/580 did not differ between control and T300 treatments despite having different degrees of lipid oxidation. Likewise, C1000 accumulated lower metmyoglobin than T300, regardless of their similar TBARS values. Hence, myoglobin oxidation does not seem to have been mediated by lipid oxidation-by-products. It has been demonstrated that in low pO_2 atmospheres oxymyoglobin is rapidly converted to metmyoglobin while these conditions provide a high stability to lipids.[27] In fact, myoglobin can interact directly with oxygen reactive species and become oxidised. In this regards, it could have been expected that green tea extract, being water soluble, might had been more likely to permeate the cytoplasm and thus would have protected water-soluble myoglobin from oxidation. In contrast, carvacrol, which has a hydrophobic nature, may have had more difficulties to protect myoglobin from direct oxidation. Nevertheless, our results did not confirm this hypothesis. In agreement with our data, Liu *et al*.[17] did not observe any effect of tea catechins on preventing from myoglobin oxidation. Moreover, Lorenzo *et al*.[28] registered that green tea catechins accelerated the accumulation of metmyoglobin in pork patties during display. Mitsumoto *et al*.[16] threw light on this matter, pointing out that tea catechins may cause discolouration by binding with the iron component of myoglobin. Therefore, tea cathechins should not be recommended to prevent meat discolouration.

4.3 Microbial growth

As it could be expected, microbial counts increased throughout display. *Pseudomonas spp.* growth exceeded the limit for acceptable quality meat (7 log CFU/cm^2) proposed by ICMFS[29] in all batches except for that containing 400 ppm sulphite. The effectiveness of sulphite in inhibiting microbial growth was higher than that showed by both green tea and carvacrol for all the microorganisms investigated. Previously, Bañón *et al*.[20] reported a remarkable inhibition of total viable and total coliform counts in low sulphite beef patties (100 ppm). The antimicrobial properties of sulphite are widely known, indeed. The mechanisms by which sulphite inhibits microbial multiplication are very complex since they can interact with many critical components of the microbial cell. Sulphite may modify several bacterium metabolic systems such as energy synthesis, energy production, DNA replication and membrane functions.[30]

Carvacrol also demonstrated relevant antimicrobial properties against the microorganisms determined. The addition of 1000 ppm carvacrol reduced at least 1.00 log CFU/g final total viable counts compared to the control. Moreover, *Enterobacteriaceae* and *Pseudomonas spp* growth followed a similar trend, while the inhibition of LAB and *Brochothrix thermosphacta* was slightly lower. However, the antimicrobial activity of carvacrol seemed to be concentration-dependent since the addition of a lower concentration (300 ppm) did not show significant reductions in most sampling points. According to Lambert *et al*.[31] the minimum inhibitory concentration (MIC) of carvacrol against *Staphylococcus aureus* and *Pseudomonas aeruginosa* are 140 and 385 ppm, respectively. It should be pointed out that these MIC were calculated *in vitro* and hence may be expected to be lower than those required *in vivo*. Therefore, these data support the low inhibition reached with 300 ppm carvacrol. In contrast, Mastromatteo *et al*.[32] registered a reduction of the cell load of about 1–1.5 log CFU/g in poultry patties with 300 ppm carvacrol. A feasible explanation may be the lower temperature or the combined effect of carvacrol and modified atmosphere packaging used in this study. The antimicrobial effect of carvacrol is based on its hydrophobicity: carvacrol accumulates in the cell membrane and thereafter induces its conformational modification which results in cell death.[33]

Otherwise, tea catechins have been described to manifest certain antimicrobial

activity by interacting with bacterial enzymes or proteins.[34] Wu *et al*.[35] reported a noticeable reduction of microbial counts by using 1000 ppm of tea catechins while Lorenzo *et al*.[28] observed also an antimicrobial effect on pork patties using the same concentration. Nevertheless, we did not observe any antimicrobial effect with 300 ppm of green tea extract, which suggests that higher concentration of tea catechins may be required to make profit of green tea antimicrobial properties.

4.4 Sensory properties

In agreement with the results obtained from the physicochemical analyses, panellists detected differences in both oxidation odour and flavour among treatments. Mean values of the treatments C300, C1000, T+C300 and T+C1000 were generally lower than 1 point, which demonstrated the great effect of carvacrol on inhibiting lipid oxidation and agreed with Dunshea *et al*.[36], who proposed 0.5 mg MDA/kg as borderline level for detection of rancidity by trained sensory panellists. The panel also detected low levels of rancidity in the batch including 300 ppm of green tea extract while the scores assigned to the sulphite batch were close to the central value. Panellists reported the highest oxidation odour and flavour in the control samples displayed during eight days (7.33 and 5.80 respectively) agreeing with the results obtained in the TBARS analyses and also with the threshold of rejection for oxidised beef (2 mg MDA/kg) proposed by Campo *et al*.[15]

As it could be expected, microbial odour and flavour increased with bacteria counts. Hence, control and T300 treatments were assigned the highest scores. *Pseudomonas spp*. counts exceeded the limit for acceptable quality meat (7 log CFU/cm^2) proposed by ICMFS[29] after 8 days in all the batches except in that with 400 ppm sulphite, which explains the high values assigned by panellists. It has been demonstrated that microbial metabolism produces volatile compounds which are perceived as off odours and flavours and ultimately result in meat rejection.[37] Treatments including 1000 ppm carvacrol were given scores close to 5 despite having Pseudomonas spp. counts higher than 7 Log CFU/g. A feasible explanation to these results could be the characteristic odour and flavour of carvacrol, which was perceived by panellists. This compound could have overpowered microbial odour and flavour and hence lower scores were assigned to the latter. Moreover, the perception of this compound is likely to be concentration dependent since higher values were attributed to the samples with 1000 ppm of carvacrol compared to those with 300 ppm. Several researchers have previously described a significant impact of essential oils and herb extracts on meat sensory properties. Latoch and Stasiak[38] noticed that mint extract bring about strange odours and flavours to pork sausages which compromise their acceptability. In contrast, herbal odours and flavours were not related to green tea extract, showing that it did not provide strange odours or flavours to burger meat. Similarly, neither Mitsumoto *et al*.[16] nor Bellés *et al*.[9] detected herbal flavours and odours in the samples with green tea.

5. Conclusion

The addition of a preservative to lamb minced meat seems to be required due to its limited shelf-life. Sulphite demonstrated to have strong antimicrobial properties and prevented lamb from discoloration. It also reduced lipid oxidation compared to control but to a lower degree than both green tea and carvacrol. Indeed, these natural additives almost avoided lipid oxidation while only carvacrol showed a significant effect against discolouration and microbial growth. The antimicrobial properties of carvacrol are likely to be concentration-dependent, hence the inhibition obtained using 1000 ppm was significantly higher than that observed at 300 ppm. Moreover, both green tea and carvacrol limited the development of oxidation odour and flavour while the latter also decreased the perception of microbial odour and flavour. Nevertheless, carvacrol gave rise to herbal odour and flavour which were detected by panelists. In contrast, neither green tea nor sulphite were sensorily detected. Therefore, carvacrol seems to be a promising alternative to replace sulphite in lamb burger meat while green tea should be combined with an antimicrobial agent. However, carvacrol may bring about herbal odours and flavours to meat, which must be taken into account.

Table 3. Means and standard error of the mean (SEM) for sensory attributes of grill-cooked lamb.

Attributes	Treatments	Days of display					$P_{display}$	SEM
		0	1	3	6	8		
Oxidation odour	B	0.00^{w}	0.00^{w}	$2.33^{b,x}$	$5.67^{c,y}$	$7.33^{d,z}$	<0.001	0.559
	S400	0.00^{w}	0.00^{w}	$1.27^{ab,x}$	$3.33^{b,y}$	$4.50^{c,z}$	<0.001	0.350
	T300	0.00^{x}	0.00^{x}	$1.17^{ab,xy}$	$2.67^{b,y}$	$1.50^{b,xy}$	0.001	0.262
	C300	0.00	0.00	0.67^{a}	0.67^{a}	0.50^{ab}	0.010	0.089
	C1000	0.00	0.00	0.33^{a}	0.33^{a}	0.00^{a}	0.146	0.063
	T+C300	0.00	0.00	0.50^{a}	0.67^{a}	0.30^{ab}	0.025	0.088
	T+C1000	0.00^{x}	0.00^{x}	$0.40^{a,xy}$	$0.33^{a,xy}$	$0.83^{ab,y}$	0.004	0.088
	$P_{treatment}$	-	-	<0.001	<0.001	<0.001	-	-
Microbial odour	B	0.00^{w}	0.00^{w}	$2.17^{b,x}$	$5.33^{c,y}$	$7.50^{c,z}$	<0.001	0.563
	S400	0.00^{x}	0.00^{x}	$0.50^{a,xy}$	$1.67^{a,y}$	$4.50^{a,z}$	<0.001	0.344
	T300	0.00^{w}	0.00^{w}	$1.17^{ab,x}$	$5.00^{bc,y}$	$7.00^{c,z}$	<0.001	0.541
	C300	0.00^{w}	0.00^{w}	$1.37^{ab,x}$	$3.00^{ab,y}$	$6.17^{bc,z}$	<0.001	0.439
	C1000	0.00^{w}	0.00^{w}	$0.17^{a,w}$	$2.33^{a,x}$	$4.50^{a,y}$	<0.001	0.348
	T+C300	0.00^{x}	0.00^{x}	$1.00^{a,x}$	$3.33^{abc,y}$	$6.00^{abc,z}$	<0.001	0.462
	T+C1000	0.00^{x}	0.00^{x}	$0.10^{a,x}$	$2.67^{a,y}$	$5.00^{ab,z}$	<0.001	0.383
	$P_{treatment}$	-	-	<0.001	<0.001	<0.001	-	-
Herbal odour	B	$0.00^{a,x}$	$0.17^{a,x}$	$0.00^{a,x}$	$0.67^{a,y}$	$0.00^{a,x}$	<0.001	0.069
	S400	0.00^{a}	0.17^{a}	0.00^{a}	0.33^{a}	0.00^{a}	0.222	0.056
	T300	0.67^{ab}	1.17^{ab}	0.50^{a}	0.67^{a}	0.83^{a}	0.604	0.133
	C300	$3.83^{cd,y}$	$2.33^{b,x}$	$2.50^{b,x}$	$3.33^{b,y}$	$3.83^{b,y}$	<0.001	0.145
	C1000	$4.00^{cd,x}$	$4.33^{c,xy}$	$4.67^{c,xy}$	$4.67^{c,xy}$	$6.33^{c,y}$	0.028	0.256
	T+C300	2.33^{bc}	2.00^{b}	2.50^{b}	2.67^{b}	3.33^{b}	0.123	0.140
	T+C1000	$4.25^{d,x}$	$4.33^{c,x}$	$5.67^{c,y}$	$5.87^{c,y}$	$5.33^{c,xy}$	0.005	0.172
	$P_{treatment}$	<0.001	<0.001	<0.001	<0.001	<0.001	-	-
Oxidation flavour	B	0.00^{x}	0.00^{x}	$2.17^{b,y}$	$5.33^{c,z}$	$5.83^{d,z}$	<0.001	0.475
	S400	0.00^{x}	0.00^{x}	$1.33^{ab,y}$	$3.67^{b,z}$	$4.33^{c,z}$	<0.001	0.358
	T300	0.00^{x}	0.00^{x}	$1.67^{ab,xy}$	$3.33^{b,y}$	$1.83^{b,xy}$	<0.001	0.297
	C300	0.00^{x}	0.00^{x}	$1.33^{ab,z}$	$0.33^{a,xy}$	$0.83^{ab,yz}$	<0.001	0.125
	C1000	0.00^{x}	0.00^{x}	$0.67^{a,y}$	$0.67^{a,y}$	$0.17^{a,xy}$	0.004	0.085
	T+C300	0.00^{x}	0.00^{x}	$0.67^{a,y}$	$0.00^{a,x}$	$0.83^{ab,y}$	<0.001	0.085
	T+C1000	0.00	0.00	0.67^{a}	0.00^{a}	0.50^{a}	0.027	0.092
	$P_{treatment}$	-	-	0.015	<0.001	<0.001	-	-
Microbial flavour	B	0.00^{w}	0.00^{w}	$2.37^{b,x}$	$5.33^{c,y}$	$8.50^{e,z}$	<0.001	0.615
	S400	0.00^{x}	0.00^{x}	$0.67^{a,x}$	$2.00^{a,y}$	$4.50^{b,z}$	<0.001	0.328
	T300	0.00^{w}	0.00^{w}	$1.17^{ab,x}$	$5.33^{c,y}$	$7.50^{d,z}$	<0.001	0.578
	C300	0.00^{w}	0.00^{w}	$1.37^{ab,x}$	$4.00^{bc,y}$	$6.00^{c,z}$	<0.001	0.454
	C1000	0.00^{w}	0.00^{w}	$0.33^{a,w}$	$2.00^{a,x}$	$5.00^{b,y}$	<0.001	0.364
	T+C300	0.00^{x}	0.00^{x}	$0.83^{a,x}$	$4.33^{bc,y}$	$6.67^{cd,z}$	<0.001	0.513
	T+C1000	0.00^{x}	0.00^{x}	$0.17^{a,x}$	$3.00^{ab,y}$	$3.00^{a,y}$	<0.001	0.270
	$P_{treatment}$	-	-	<0.001	<0.001	<0.001	-	-
Herbal flavour	B	0.00^{a}	0.00^{a}	0.00^{a}	0.17^{a}	0.17^{a}	0.86	0.069
	S400	0.17^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.861	0.069
	T300	0.67^{ab}	0.83^{a}	0.83^{a}	0.33^{a}	0.27^{a}	0.404	0.133
	C300	$4.17^{c,z}$	$2.67^{b,x}$	$2.17^{b,xy}$	$3.33^{b,yz}$	$3.83^{b,z}$	<0.001	0.177
	C1000	5.67^{c}	4.67^{c}	4.33^{c}	6.00^{c}	5.83^{c}	0.088	0.240
	T+C300	$2.33^{b,x}$	$2.50^{b,xy}$	$2.83^{b,xyz}$	$3.67^{b,z}$	$3.33^{b,yz}$	<0.001	0.126
	T+C1000	$5.00^{c,xy}$	$4.33^{c,x}$	$5.17^{c,xy}$	$5.07^{c,xy}$	$5.83^{c,y}$	0.008	0.139
	$P_{treatment}$	<0.001	<0.001	<0.001	<0.001	<0.001	-	-

B: control; S400: sulphites 400 ppm; T300: green tea extract 300 ppm; C300: carvacrol 300 ppm; T+C300: green tea extract 300 ppm + carvacrol 300 ppm; T + 1000C: green tea extract 300 ppm + carvacrol 1000 ppm. Different superscripts (a, b, c, d) within a column indicate significant differences among treatments. Different superscripts (w, x, y, z) within a row indicate significant differences among days of display. A 10 point scale (0 = low, 10 = high).

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8. Conflict of interests

None.

9. References

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Discusión

5. Discusión general

La mejora de la calidad del producto es una de las principales estrategias comerciales para incrementar la competitividad de las empresas cárnicas, especialmente ante la demanda de los consumidores de productos de gran calidad. El descenso de la calidad de la carne durante su comercialización es uno de los aspectos que más preocupa al sector, especialmente en el caso del cordero, que posee una corta vida útil. Los principales factores que determinan el descenso de su calidad a lo largo de la cadena de comercialización son el crecimiento microbiano, las reacciones de oxidación y la pérdida del color. Por ello, las estrategias planteadas en esta tesis doctoral para mantener la calidad inicial de la carne de cordero hasta el consumidor se han diseñado con objeto de frenar estos tres procesos. A continuación, se presenta una discusión conjunta de los trabajos incluidos en esta tesis doctoral que pretende abordar el efecto de cada una de las estrategias evaluadas sobre el crecimiento microbiano, la oxidación lipídica y el color de la carne.

5.1. Crecimiento microbiano

El tejido muscular de los animales sanos es inicialmente estéril produciéndose la contaminación microbiana durante el procesado de la carne (Dave y Ghaly, 2011), cuyas principales etapas son el sacrificio, el desangrado, el despellejado, el eviscerado, la refrigeración de las canales, el despiece, el fileteado y el envasado. Cualquier deficiencia higiénica durante el desarrollo de las mismas puede incrementar la contaminación microbiana de la carne, lo que compromete su calidad futura (Nastasijević, Lakićević y Petrović, 2017). Entre todas estas etapas, diversos estudios han señalado la evisceración como la operación que presenta un mayor riesgo, debido a la elevada carga microbiana del contenido intestinal y su riesgo de fuga durante la retirada de las vísceras (Cerveny, Meyer y Hall, 2009; Dave y Ghaly, 2011). La piel de los animales se ha identificado también como un reservorio importante de microorganismos, es por ello que el despellejado es una etapa crucial para prevenir la contaminación de las canales (Dave y Ghaly, 2011). Por tanto, la piel y el contenido intestinal son las fuentes internas más importantes de microorganismos contaminantes (Cerveny *et al*., 2009; Petruzzelli *et al*., 2016; Zweifel, Capek y Stephan, 2014). No obstante, la carga microbiana de las canales también puede verse incrementada por fuentes externas. En este sentido, la calidad de las instalaciones, su higiene, la cualificación de los trabajadores y los sistemas de calidad de los mataderos juegan un papel fundamental. De hecho, se han encontrado diferencias significativas entre los

recuentos microbianos de las canales en función de los mataderos donde se han sacrificado los animales (Kaur, Bowman, Porteus, Dann y Tamplin, 2017). También la contaminación cruzada entre las canales puede ser una fuente importante de contaminación microbiana (Petruzzelli *et al*., 2016). Por el contrario, la refrigeración de las canales es una de las principales herramientas para frenar el crecimiento de los microorganismos durante el faenado de la carne (Yalçin, Nizamlioglu y Gürbüz, 2004). Tal y como se describe en el capítulo 4.2.1. de esta tesis doctoral, el sistema de refrigeración empleado tiene un efecto notable sobre la multiplicación de los microorganismos. Mientras que no se han encontrado diferencias significativas entre los recuentos microbianos de las canales refrigeradas de forma tradicional o mediante pulverización, Fernández y Vieira (2012) observaron como la ultra refrigeración de las canales de cordero permitiría obtener unos recuentos finales inferiores con respecto a las combinaciones de tiempo y temperatura utilizadas de forma convencional.

En cambio, existe un menor conocimiento sobre el efecto del proceso de corte de la carne en la carga microbiana de las chuletas y los filetes resultantes. Voloski *et al*. (2016) observaron un incremento de los recuentos microbianos tras las operaciones de corte y deshuesado, demostrando la influencia de estas etapas en la calidad microbiológica de la carne. De forma similar, Widders *et al*. (1995) destacaron el papel del proceso de corte en la diseminación de la contaminación microbiana de las canales. Por tanto, las condiciones en las que se realice este proceso podrían tener impacto en la calidad microbiológica de la carne. Una de las hipótesis que planteó esta tesis doctoral fue la existencia de diferencias significativas entre la carga microbiana de las chuletas de cordero dependiendo del sistema de corte utilizado. Habitualmente dos son los métodos empleados con este propósito en la industria del ovino: la cizalla y la sierra. Como se muestra en el capítulo 4.2.2., los recuentos de microorganismos aerobios viables totales, bacterias acidolácticas y *Pseudomonas* spp. se encontraron por debajo del límite de detección en ambos lotes tras el fileteado, y no fue hasta tras 5 días de refrigeración cuando pudieron ser cuantificados. A partir de este momento, las chuletas cortadas por sierra presentaron mayores recuentos de microorganismos aerobios totales, aunque estas diferencias no fueron significativas (capítulo 4.2.2.).

Inicialmente se esperaban unos recuentos superiores en las muestras cortadas por sierra, debido a que esta tecnología genera pequeñas partículas de músculo y hueso que se depositan sobre la superficie de las chuletas (ilustración 1), lo que podría favorecer la diseminación de la contaminación microbiana. Sin embargo, esta hipótesis no fue demostrada, obteniendo unos recuentos similares en ambos tratamientos. Una posible explicación a estos resultados sería la baja carga microbiana de las piernas

utilizadas en este estudio. Los recuentos de microorganismos aerobios totales en las canales ovinas suelen oscilar entre 1,50 y 3,65 log UFC/cm^2 (Sumner *et al*., 2003; Yalçin *et al*., 2004), mientras que en los cortes destinados a la venta directa son sensiblemente superiores, situándose habitualmente entre 2,50 y 4,00 log UFC/g (Berruga *et al*., 2005; Karabagias *et al*., 2011; Nieto *et al*., 2010). Los recuentos iniciales obtenidos en los estudios de esta tesis doctoral se han situado habitualmente por debajo de estos valores, lo que es indicativo de las buenas prácticas de las empresas proveedoras. Debido a que la diseminación de los microorganismos durante las operaciones de corte depende de la carga microbiana previa de las canales, una baja carga inicial habría impedido obtener diferencias entre ambos sistemas. Estos resultados ponen de manifiesto la importancia de la higiene del proceso en la calidad microbiológica de la carne.

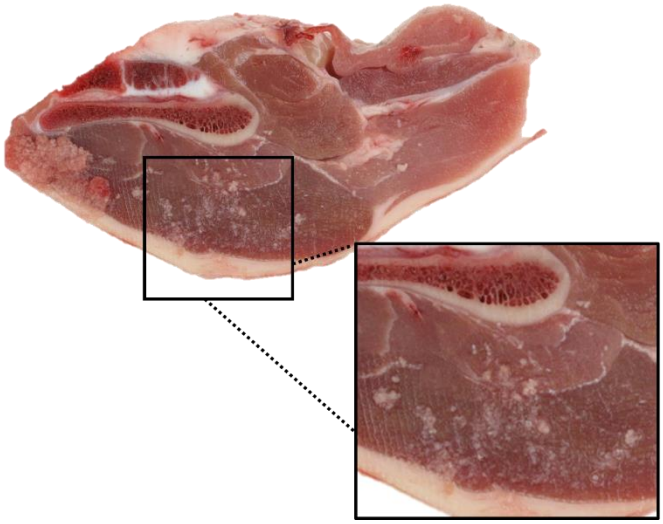

Ilustración 1. Chuleta cortada por sierra.

Debido a su composición, la carne fresca es un medio muy favorable para el desarrollo de los microorganismos. Durante su multiplicación, estos utilizan distintos sustratos como glucosa, lactato, aminoácidos, nucleótidos, etc. y liberan al medio los productos resultantes de su metabolismo, que son los responsables de la percepción del deterioro de la carne. El tipo de sustratos consumidos, y en consecuencia los productos liberados al medio, dependen de los géneros bacterianos predominantes en la carne (Ercolini *et al*., 2010; Dave y Ghali, 2011; Nychas *et al*., 2008). La tabla 5 muestra los principales géneros bacterianos responsables del deterioro y los compuestos asociados a su metabolismo.

Tabla 5. Compuestos responsables del deterioro y microorganismos asociados.

Microorganismo	Compuesto responsable del deterioro
Pseudomonas spp. y *Enterobacteriaceae*	Cisteína, cistina, metionina, sulfuro de hidrógeno, metil sulfuro y metil disulfuro.
Pseudomonas fluorescens	Metilamina, dimetilamina, trimetilamina, etil ésteres
Enterobacteriaceae fragi, Clostridium spp., *Lactobacillus sakei, Hafnia alvei, Shewanella putrefaciens*	Sulfuro de hidrógeno, trimetilamina, metilmercaptano, hipoxantina, dimetilsulfuro
Brochothrix thermosphacta	3-metilbutanol
Photobacterium phosphoreum	Trimetilamina, hipoxantina
Bacterias del deterioro aeróbicas	Amoniaco, ácido acético, butírico y propiónico

Fuente: Dave y Ghaly (2011)

Generalmente, las modificaciones originadas en la carne como consecuencia del crecimiento microbiano se perciben sensorialmente en forma de olores y sabores desagradables, formación de limosidad superficial y modificaciones en el color de la carne (Comi, 2016). Los olores y sabores producidos por el metabolismo microbiano se han asociado a productos como el queso, la mantequilla o la fruta (Casaburi *et al*., 2015; Jones, Wiklund, Zagorec y Tagg, 2010). No obstante, la mayoría de estudios utilizan los descriptores pútrido (Nieto *et al*., 2010; Ortuño *et al*., 2014), olor a deterioro y sabor a deterioro (Ahn *et al*., 1998; Fernandes, Trindade, Lorenzo, Munekata y De Melo, 2016; Martínez *et al*., 2005) para cuantificar sensorialmente las modificaciones producidas por el crecimiento de los microorganismos. La intensidad de las alteraciones aumenta a medida que lo hace la carga microbiana de la carne, y estas modificaciones terminan por limitar su vida útil. La disminución de la calidad de la carne durante el almacenamiento debido al crecimiento microbiano se ha podido constatar en los diversos estudios llevados a cabo en esta tesis doctoral. En el análisis sensorial presentado en el capítulo 4.2.4. se observó cómo el incremento de las valoraciones asignadas por los panelistas a los olores y sabores asociados al crecimiento microbiano aumentó progresivamente durante los 11 días que duró el estudio, coincidiendo las mayores valoraciones con los recuentos microbianos más altos. También se identificó esta tendencia en el análisis sensorial presentado en el capítulo 4.2.5. Además, en este último estudio se pudo observar como la carne con unos recuentos microbianos más bajos (*burger meat* con 1000 ppm de carvacrol o con 400 ppm de sulfitos) desarrolló menores olores y sabores pútridos. Los olores y sabores resultantes del metabolismo microbiano no solamente son desagradables *per se*, sino que además pueden llegar a enmascarar los olores característicos de la carne de cordero. Diversos estudios han tratado de determinar la carga microbiana a partir de la cual se produce el rechazo de la carne por los consumidores. La *International Commission on Microbiological Specifications for Foods* (ICMSF, 1986) estableció 7 log UFC/g como el umbral a partir

del cual los consumidores rechazan la carne como consecuencia del crecimiento microbiano. Mantener el número de colonias por debajo de este límite durante el proceso de comercialización es un objetivo prioritario de las distintas estrategias de conservación de la carne.

Con este objetivo en mente, en esta tesis doctoral se propuso la conservación a bajas temperaturas como una estrategia fundamental para inhibir el crecimiento de los microrganismos y prolongar la vida útil de la carne. En consonancia, se estableció la hipótesis de que el *superchilling* podría aumentar significativamente la vida útil de la carne fresca alcanzada bajo las temperaturas de refrigeración habituales (capítulo 4.2.3). Como puede comprobarse en dicho capítulo, la refrigeración a -1 °C permitió obtener menores recuentos de todos los microorganismos investigados, tanto en la carne envasada en MAP como en *skin* ($P \leq 0{,}001$). Un descenso de la temperatura de refrigeración de 5 °C (desde 4 hasta -1 °C) permitió retrasar el deterioro microbiológico en más de 14 días (capítulo 4.2.3.). De forma similar, Zhang *et al*. (2016) observaron una reducción en los recuentos de microrganismos aerobios totales de casi 2,5 log UFC/g al descender la temperatura de refrigeración de 4 a -2 °C, permitiendo extender la vida útil de la carne de pollo hasta 28 días. El efecto del *superchilling* en la inhibición del crecimiento microbiano también fue descrito por Lan *et al*. (2016), quienes observaron un descenso en los recuentos totales de más de 2 log UFC/g al refrigerar la carne de conejo a -2,5 °C en lugar de a 4 °C. El mecanismo mediante el cual el *superchilling* consigue retrasar el crecimiento microbiano se basa en dos principios. Por un lado, las bajas temperaturas limitan la funcionabilidad de la membrana de la célula microbiana, pudiendo llegar a bloquearla completamente. Además, existen indicios de que estas modificaciones en la membrana podrían limitar la captación de nutrientes por parte de los microorganismos, restringiendo de esta forma su multiplicación (Nedwell, 1999). Por otro lado, a las temperaturas de *superchilling* parte del agua se convierte en hielo, disminuyendo el agua disponible para los microorganismos y en consecuencia, limitando su crecimiento (Kaale y Eikevik, 2014; Kaale, Eikevik, Rustad y Kolsaker, 2011; Nastasijević *et al*., 2017). Tanto nuestros resultados como los de los estudios publicados previamente demuestran el potencial del *superchilling* en la inhibición de los microorganismos alterantes habituales de la carne, confirmando la hipótesis planteada.

El efecto de la temperatura sobre el crecimiento microbiano se intensifica cuando la carne se mantiene en congelación. Las bajas temperaturas unidas a la notable reducción de la actividad del agua, ofrecen unas condiciones donde los microrganismos habituales de la carne son incapaces de multiplicarse (la mayoría de flora alterante no es xerotolerante) (Leygonie *et al*., 2012). En este sentido, Lee y Bae (2018) observaron

cómo los recuentos microbianos de la carne se mantuvieron estables incluso tras 6 meses de almacenamiento a -23 °C, mientras que Fernandes, Freire, Carrer y Trindade (2013) obtuvieron una vida útil de hasta 12 meses mediante la congelación de la carne de cordero. Por tanto, la congelación permite prolongar la vida útil microbiológica de la carne durante largos periodos de tiempo. No obstante, existe un mayor desconocimiento acerca del comportamiento microbiano en la carne descongelada. La descongelación es un proceso más lento y heterogéneo que la congelación, de forma que algunas zonas de la carne pueden quedar expuestas a unas temperaturas más favorables para el desarrollo de los microorganismos. Además, la presencia de exudado, que es rico en proteínas, minerales y vitaminas, favorecería aún más su multiplicación (Akhtar, Khan y Faiz, 2013; Leygonie *et al*., 2012). Bajo este contexto y dado que el objetivo planteado en el capítulo 4.1.3. de esta tesis doctoral fue evaluar la viabilidad de la comercialización de carne de cordero descongelada, se llevó a cabo un estudio del crecimiento de los microorganismos en estas condiciones.

Los resultados de este trabajo arrojaron un comportamiento *a priori* sorprendente. Como puede observarse en el capítulo 4.1.3., el crecimiento microbiano no sólo mostró un periodo de latencia durante la descongelación, sino que bajo estas condiciones se obtuvieron unos recuentos significativamente menores de microorganismos de la familia *Enterobacteriaceae* y de *Pseudomonas* spp. que en la carne fresca ($P < 0{,}001$). Estas diferencias podrían explicarse por un efecto bacteriostático o bactericida de la congelación, que tendría impacto en la actividad futura de los microorganismos. En concordancia, varios investigadores han detectado una reducción de los recuentos bacterianos tras el almacenamiento de la carne en congelación. Coombs, Holman, Collins, Friend y Hopkins (2017) cuantificaron una reducción de hasta 3 unidades logarítmicas en los recuentos de *Brochotrix thermosphacta* tras almacenar la carne de cordero durante 3 meses en congelación a - 18 °C. De forma similar, Medić *et al*. (2018) reportaron un descenso de una unidad logarítmica en el recuento de microorganismos aerobios totales y de hasta dos unidades en el de *Pseudomonas* spp. después de 18 meses de congelación. No obstante, el efecto de la congelación sobre el crecimiento microbiano es todavía una fuente de un intenso debate. Por un lado, algunos investigadores defienden que ni la congelación ni la descongelación podrían dañar las células microbianas, las cuales se encontrarían únicamente inactivas durante el almacenamiento a bajas temperaturas (Leygonie *et al*., 2012). Sin embargo, otros estudios han descrito algunos mecanismos mediante los cuales la congelación y la descongelación inactivarían las bacterias. La congelación podría dañar las membranas celulares como consecuencia de la formación de cristales,

la concentración de solutos y las bajas temperaturas, mientras que el efecto antibacteriano de la descongelación se debería al fenómeno de la recristalización (Archer, 2004). Tras la descongelación, las células dañadas podrían recuperarse y permanecer viables (daño subletal) o ser incapaces de multiplicarse (daño letal). Los resultados obtenidos en nuestros estudios, así como los publicados recientemente, ponen de manifiesto el efecto bacteriostático de la congelación y aportan indicios de la existencia de algún mecanismo mediante el cual la congelación dañaría las células bacterianas. No obstante, esta última teoría no queda demostrada y queda abierta para futuras investigaciones. En cualquier caso, queda reflejado el potencial de las bajas temperaturas para inhibir el crecimiento microbiano y la posibilidad de comercializar carne descongelada desde el punto de vista de la estabilidad microbiológica.

La baja velocidad de multiplicación de los microorganismos observada en la carne descongelada también podría explicarse por el efecto combinado de las bajas temperaturas con la actividad bacteriostática del dióxido de carbono. De acuerdo con Gill (1996), una concentración de CO_2 del 20 % es suficiente para inhibir el crecimiento de los microorganismos un 50 %. El efecto bacteriostático del CO_2 está ampliamente descrito en la bibliografía (Berruga *et al*., 2005; Sheridan *et al*., 1997; Vergara y Gallego, 2001) y es uno de los pilares sobre los que se cimienta el envasado en atmósfera protectora. Las atmósferas empleadas para la conservación de carne de cordero destinada a la venta directa incluyen habitualmente porcentajes de CO_2 cercanos al 30 % (Lauzurica *et al*., 2005; Muíño *et al*., 2014; Ortuño *et al*., 2014).

La acción combinada del envasado y el efecto de las bajas temperaturas sobre el crecimiento microbiano se abordó en mayor profundidad en el capítulo 4.2.3. de esta tesis doctoral. En este apartado se evaluó el efecto del envasado en MAP y a vacío tipo *skin* en combinación con la refrigeración sobre el crecimiento microbiano. El primer resultado de este estudio que debe resaltarse fue la existencia de interacciones significativas entre los efectos fijos sistema de envasado y temperatura de refrigeración ($P \leq 0,05$). El efecto del sistema de envasado sobre el crecimiento microbiano estuvo influenciado por la temperatura de almacenamiento de la carne. Mientras que existieron diferencias notables entre los sistemas de envasado en los recuentos de microorganismos aerobios totales, *Pseudomonas* spp. y bacterias acidolácticas cuando la carne se mantuvo a 4 °C ($P < 0,001$), solo se observaron pequeñas diferencias en algunos días de análisis cuando las muestras se refrigeraron a -1 °C. El envasado a vacío tipo *skin* ejerció una mayor inhibición del crecimiento microbiano que la atmósfera protectora. De acuerdo con estos resultados, Kameník *et al*. (2014) también destacaron la efectividad del vacío tipo *skin* en la inhibición del crecimiento microbiano. El efecto

bacteriostático del envasado a vacío se fundamenta en la ausencia de O_2 en el espacio de cabeza del envase. Debido a las creación de una atmósfera anaerobia, se produce una selección de la microflora bacteriana, impidiendo el crecimiento de los géneros aerobios y retardando el crecimiento de los géneros anaerobios facultativos (Jones *et al*., 2008). La inhibición del crecimiento bacteriano lograda mediante el envasado a vacío se potencia con el envasado a vacío tipo *skin*, debido a que este consigue obtener un porcentaje de oxígeno residual inferior a los que se registran mediante los sistemas de envasado a vacío convencionales (Lagerstedt *et al*., 2011; Strydom y Hope-Jones, 2014). En concordancia, Vázquez *et al*. (2004) observaron una diferencia de alrededor de 1,5 log UFC/g entre los recuentos de microorganismos aerobios totales de las muestras envasadas a vacío tipo *skin* y a vacío convencional. Además, estos resultados también se observaron en los recuentos de microorganismos coliformes. Por tanto, existe un amplio consenso acerca de la efectividad del vacío *skin* para retardar el crecimiento microbiano en la carne refrigerada.

La inhibición de los microorganismos también podría conseguirse mediante el empleo de aceites y extractos de plantas, y esta es la hipótesis bajo la que se plantearon los estudios incluidos en los capítulos 4.2.4. y 4.2.5. Sin embargo, como se muestra en el capítulo 4.2.4., ni el extracto de té ni el de borraja consiguieron inhibir la multiplicación de ninguno de los géneros microbianos investigados. Previamente, Sánchez-Escalante *et al*. (2003) observaron la ausencia de efectos antimicrobianos de la harina de semilla de borraja cuando se incluía en la formulación de hamburguesas de vacuno. Por el contrario, diversos estudios habían señalado ciertas propiedades antimicrobianas en los extractos de té. Wu, Wang y Chen (2010) observaron una notable reducción en el crecimiento de los microrganismos mediante la inclusión de 1000 ppm de catequinas de té en un envase activo para la conservación de carne roja. Lorenzo, Sineiro, Amado y Franco (2014) también describieron una acción antimicrobiana similar al utilizar la misma concentración de extracto de té en hamburguesas de cerdo. De acuerdo con Amarowicz, Pegg y Bautista (2000) la acción antimicrobiana del té se fundamenta en la capacidad de las catequinas (principal componente de los extractos de té) de actuar sobre diversas enzimas y proteínas bacterianas. Kumazawa *et al*. (2004) evidenciaron diversos compuestos fenólicos presentes en la composición del té capaces de interaccionar con la bicapa lipídica, lo que explicaría sus propiedades antimicrobianas. Las diferencias observadas entre estos estudios y nuestros resultados podrían deberse a la concentración empleada y sugerirían la existencia de una acción dependiente de la concentración. En consecuencia, la concentración elegida para nuestro estudió (aproximadamente 500 ppm) sería insuficiente para inhibir el crecimiento microbiano.

En esta línea, Bañón, Díaz, Rodríguez, Garrido y Price (2007) observaron que la inclusión de 300 ppm de extracto de té en la formulación de hamburguesas de vacuno tuvo un efecto antimicrobiano muy limitado. De acuerdo con la bibliografía, podríamos haber registrado un efecto antimicrobiano si hubiéramos aplicado el extracto de té a una concentración mayor. No obstante, esto habría comprometido la calidad sensorial de las chuletas, debido a que ya la concentración empleada (500 ppm) originó modificaciones en la apariencia de la carne. Estos cambios consistieron en la aparición de manchas marrones que se asociaron a la precipitación de las catequinas.

Por el contrario, la incorporación de carvacrol en el *burger meat* ejerció un efecto significativo sobre el crecimiento microbiano (capítulo 4.2.5.). La adición de 1000 ppm de carvacrol consiguió reducir al menos una unidad logarítmica los recuentos de microrganismos aerobios viables totales, *Pseudomonas* spp. y microorganismos de la familia *Enterobacteriaceae*. Sin embargo, el efecto fue mucho menor cuando la concentración empleada fue de 300 ppm. Estos resultados están en concordancia con las concentraciones mínimas inhibitorias (CMI) del carvacrol determinadas por Lambert, Skandamis, Coote y Nychas (2001), que serían de 140 y 385 ppm para *Staphylococcus aureus* y *Pseudomonas aeruginosa*. Hay que tener en cuenta que estas CMI se calcularon en estudios *in vitro* y por tanto, se puede esperar un menor efecto del carvacrol cuando se aplica en la matriz alimentaria. Mastromatteo *et al*. (2009) también observaron una reducción significativa del crecimiento microbiano mediante el empleo del carvacrol (1-1,5 log UFC/g), aunque en este caso este efecto se registró con solo 300 ppm. Las propiedades antimicrobianas del carvacrol se deben a su carácter hidrofóbico: el carvacrol es capaz de disolverse en los lípidos de la membrana lipídica, donde se acumula y genera cambios en su conformación que terminan por provocar la muerte de la célula (Ben Arfa, Combes, Preziosi-Belloy, Gontard y Chalier, 2006).

De acuerdo con los estudios publicados previamente (De la Fuente *et al*., 2007; Lauzurica *et al*., 2005; Muíño *et al*., 2014), la suplementación de la dieta de los corderos con vitamina E no ejerció ningún efecto sobre el crecimiento microbiano, demostrando que el tocoferol carece de propiedades antimicrobianas (capítulo 4.1.3.).

La figura 8 muestra la máxima inhibición lograda por las estrategias evaluadas en esta tesis doctoral con relación al tratamiento control incluido en cada estudio. A pesar de la dificultad de establecer comparaciones debido a las distintas condiciones experimentales bajo las que se han determinado los efectos, se puede observar cómo la inhibición lograda mediante la aplicación de bajas temperaturas fue muy superior a la obtenida mediante los extractos naturales e incluso al beneficio logrado mediante el

envasado. Esta comparativa vuelve a poner de manifiesto la importancia de la refrigeración en la conservación de la carne. No obstante, la aplicación de esta estrategia no es excluyente, pudiendo combinarse con otras tecnologías para lograr el máximo efecto. Este es el principio de la teoría de los procesos combinados, según la cual la combinación de diversos métodos de conservación con distintas dianas en la célula microbiana incrementa la inhibición lograda mediante la aplicación independiente de cada tecnología (Archer, 2004; Zhou *et al*., 2010).

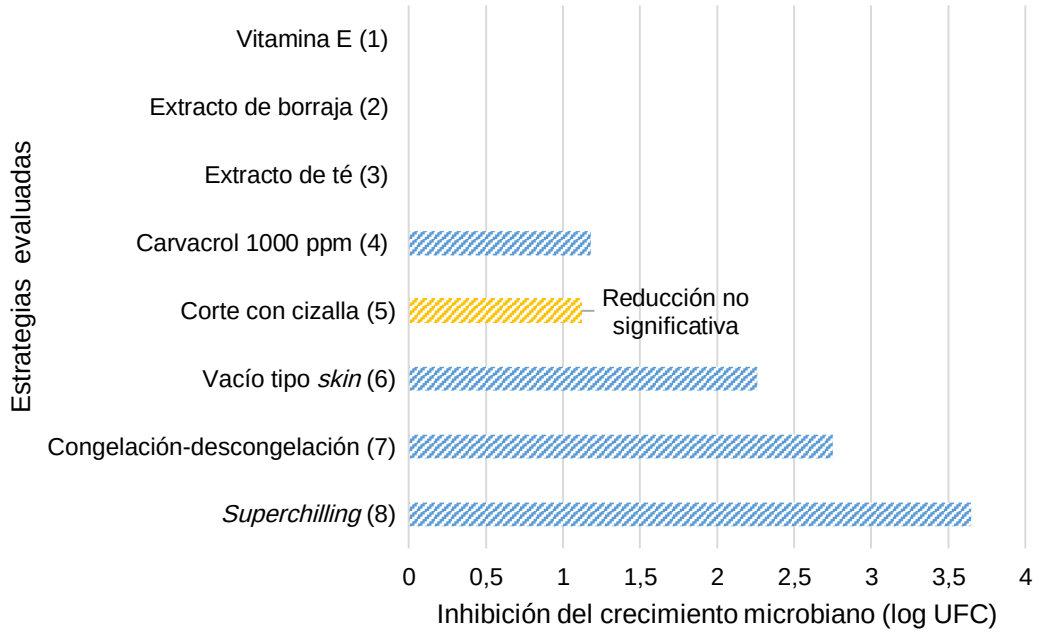

Figura 8. Máxima inhibición obtenida por cada una de las estrategias evaluadas con relación al tratamiento control incluido en cada estudio.
1: Recuentos obtenidos en la carne suplementada con 1000 ppm de vitamina E respecto al control durante la refrigeración a 4 °C.; 2: Recuentos obtenidos en la carne rociada con un extracto de borraja al 10 % frente los de la carne sin aditivos; 3: Recuentos obtenidos en la carne rociada con un extracto de té al 5 % frente a los de la carne sin aditivos; 4: Diferencia entre los recuentos obtenidos en el *burger meat* con 1000 ppm de carvacrol y el control (sin antimicrobianos) tras 8 días de refrigeración a 4 °C; 5: Diferencia entre los recuentos de las chuletas cortadas con cizalla respecto de los obtenidos en aquellas cortadas por sierra tras 8 días de refrigeración 4 °C en MAP; 6: Diferencia entre los recuentos de la carne envasada en vacío tipo *skin* y MAP tras 11 días de refrigeración a 4 °C. 7: Diferencia entre los recuentos de la carne fresca y la carne descongelada (tras 3 meses de congelación) después de 9 días de refrigeración a 4 °C. 8: Diferencia en los recuentos microbianos de la carne refrigerada a -1 °C y la refrigerada a 4 °C tras 14 días.

5.2. Oxidación lipídica

Como se ha expuesto en la introducción, las reacciones oxidativas tienen un gran impacto en la calidad de la carne de cordero y constituyen uno de los principales factores limitantes de su vida útil. Por estas razones, su determinación se incluye en la gran mayoría de estudios centrados en mejorar la conservación de este tipo de carne. La determinación de las TBARS es la técnica de referencia para medir la oxidación lipídica de la carne. Este ensayo colorimétrico cuantifica los compuestos generados por la descomposición de los hidroperóxidos, principalmente MDA, tras su reacción con el TBA (Ghani, Barril, Bedgood y Prenzler, 2017). La determinación del MDA es un buen indicador de la oxidación lipídica debido a que este compuesto se ha identificado como uno de los principales desencadenantes de la percepción de la rancidez en los productos cárnicos (Fernández, Pérez-Álvarez y Fernández-López, 1997). En este sentido, diversos estudios han intentado establecer relaciones entre la percepción sensorial de la oxidación y la concentración de MDA en la carne. Greene y Cumuze (1982) observaron que la detección de la rancidez por parte de un panel de consumidores no se producía hasta alcanzar una concentración de 0,6 mg MDA/kg de carne. Por otro lado, Campo *et al*. (2006) propusieron un valor de 2 mg MDA/kg como el límite de MDA a partir del cual los consumidores rechazarían la carne debido a la oxidación. Ambos estudios establecieron estas relaciones en carne de vacuno, mientras que Ripoll, Joy y Muñoz (2011) propusieron una concentración de 1 mg MDA/kg como el umbral de rechazo en la carne de cordero. A pesar de haberse determinado en vacuno, en esta tesis doctoral se utilizó el límite de 2 mg MDA/kg propuesto por Campo *et al*. (2006) como referencia para determinar la vida útil de la carne de cordero con relación a la oxidación. Optimizar distintas estrategias para mantener la oxidación lipídica por debajo de este umbral ha sido uno de los objetivos principales de los estudios presentados en esta memoria.

El desarrollo de las reacciones oxidativas depende en gran medida del equilibrio entre los compuestos prooxidantes y antioxidantes presentes en la carne. El tocoferol es el principal antioxidante liposoluble de los mamíferos, inhibiendo las reacciones oxidativas en su lugar de origen (Brigelius-Flohe y Traber, 1999). La suplementación de los corderos con dosis supra-nutricionales de vitamina E permitiría incrementar la concentración de tocoferol en el músculo, obteniendo un balance favorable de compuestos antioxidantes y en consecuencia, otorgando a la carne una mayor protección frente a los procesos oxidativos. Bajo esta hipótesis se realizaron los estudios presentados en los capítulos 4.1.2. y 4.1.3. La suplementación de los corderos con 1000 mg α-tocoferol/kg de pienso durante 14 días antes del sacrificio consiguió

incrementar los niveles de vitamina E del músculo desde 1.057 hasta 3.908 mg α-tocoferol/kg de carne. Esta concentración de tocoferol en el músculo redujo significativamente la oxidación lipídica en comparación con las muestras control ($P < 0,001$), presentando unos niveles de malondialdehído por debajo del límite de aceptabilidad incluso tras 9 días refrigeración en condiciones comerciales (MAP 70 % O_2 – 30 % CO_2, 4 °C) (capítulo 4.1.2). El efecto de la suplementación de los corderos con vitamina E frente a la propagación de las reacciones oxidativas en la carne fresca había sido previamente descrito (De la Fuente *et al*., 2007; González-Calvo *et al*., 2015; Lauzurica *et al*., 2005; Muela *et al*., 2014; Ripoll *et al*., 2011). Sin embargo, los datos acerca de la actividad del tocoferol tras mantener la carne en congelación eran más escasos. Durante la congelación se producen roturas en las envolturas celulares, donde se deposita la vitamina E, como consecuencia de la formación de cristales de hielo. Este proceso podría modificar la actividad de esta vitamina frente a la oxidación. No obstante, como se presenta en el estudio 4.1.3., el efecto antioxidante del tocoferol se mantuvo incluso tras 9 meses de congelación, lo que permitió mantener las chuletas de cordero con unos niveles de MDA inferiores a 2 mg/kg durante todo el periodo de refrigeración (9 días). Por tanto, se confirmó la hipótesis planteada, según la cual la suplementación de los corderos con vitamina E reduciría notablemente los procesos oxidativos durante el mantenimiento de la carne en congelación. Además, y en respuesta a uno de los objetivos de esta tesis doctoral, la oxidación no sería un inconveniente para la comercialización de carne descongelada envasada en MAP siempre que se incorpore un agente antioxidante. Desde un punto de vista técnico, y teniendo en cuenta estos resultados, la suplementación con vitamina E podría ejercer esta función.

La vitamina E tiene un coste elevado, por ello, optimizar el régimen de suplementación es un objetivo fundamental del sector ovino. De los estudios de Álvarez *et al*. (2008), Lauzurica *et al*. (2005) y Muela *et al*. (2014) se desprende que el efecto de esta estrategia frente a las reacciones oxidativas depende de la concentración de tocoferol alcanzada en el músculo antes del sacrificio. Como se discute en el capítulo 4.1.1., una concentración de vitamina E en el músculo comprendida entre 1,52 y 2,26 mg/kg podría ser suficiente para inhibir las reacciones oxidativas en la carne de cordero envasada en MAP y mantenida en condiciones de venta comerciales (González-Calvo *et al*., 2015; Kasapidou *et al*., 2012; Lauzurica *et al*., 2005), mientras que el contenido requerido podría ser incluso menor si la carne se conservara bajo unas condiciones menos favorables para el desarrollo de las reacciones oxidativas (Kerry, O'Sullivan, Buckley, Lynch y Morrissey, 2000). Estas concentraciones de tocoferol en el músculo pueden alcanzarse mediante diferentes combinaciones de la dosis

administrada y la duración del periodo de suplementación. Kasapidou *et al*. (2012) registraron una concentración de 3,73 mg de tocoferol/kg de músculo tras suplementar a los corderos con 500 mg DL-α-tocoferol/kg de pienso durante 63 días mientras que Álvarez *et al*. (2008) y De la Fuente *et al*. (2007) obtuvieron un contenido similar mediante una combinación de 1000 mg DL-α-tocoferol/kg de pienso y 37 días de suplementación. En nuestros experimentos se alcanzaron unas concentraciones similares tras un periodo más breve (14 días) pero con una dosis más elevada (1000 mg DL-α-tocoferol/kg de pienso). Son muchas las combinaciones posibles, no obstante, hay que tener en cuenta que la acumulación de tocoferol en el músculo no guarda una relación lineal con la cantidad de vitamina E ingerida, llegando a un punto de saturación. Con el fin de reducir los costes, se debe evitar alcanzar el punto de saturación, ya que en este momento se estará excretando gran parte de la vitamina E administrada.

La oxidación lipídica está influenciada, en gran medida, por el contenido de oxígeno disponible en el envase. Aunque los datos se han obtenido bajo diferentes condiciones experimentales, en la figura 9 se puede observar como la reducción de la concentración de oxígeno en la atmósfera de envasado del 70 al 40 % acarrea un descenso notable de la formación de MDA. En concordancia, Esmer *et al*. (2011) y Martínez *et al*. (2005) observaron cómo la velocidad de las reacciones oxidativas estaba directamente relacionada con la concentración de oxígeno en el espacio de cabeza del envase, disminuyendo notablemente cuando la carne se envasaba bajo una atmósfera con un menor contenido en oxígeno que la empleada tradicionalmente para la conservación de la carne de cordero (70 % O_2 – 30 % CO_2).

La inhibición de la oxidación es aún mayor si la carne se envasa en anaerobiosis. El envasado de la carne a vacío tipo *skin* fue, de entre las estrategias evaluadas en esta tesis doctoral, la que consiguió una mayor reducción de la oxidación lipídica. Como se presenta en el capítulo 4.2.3., los niveles de MDA en la carne envasada en *skin* fueron inferiores a 0,3 mg/kg incluso tras 28 días de refrigeración. La gran inhibición lograda por este sistema de envasado se manifestó en el análisis sensorial, donde los panelistas no detectaron ningún indicio de rancidez en las chuletas almacenadas bajo estas condiciones. Estos resultados concuerdan con el umbral de detección de la oxidación lipídica propuesto por Greene y Cumuze (1982), según el cual se necesita una concentración mínima de 0,6 mg MDA/kg para percibir la oxidación de la carne. Además, también están de acuerdo con los datos obtenidos en estudios publicados con anterioridad: Łopacka, Półtorak y Wierzbicka (2016) cuantificaron en las muestras de vacuno envasadas en *skin* un contenido de malondialdehído menor de 0,6 mg durante el periodo de refrigeración de la carne (12 días), mientras que Kameník *et al*. (2014)

obtuvieron unos valores similares incluso después de 35 días de almacenamiento. La efectividad de este sistema de envasado en la inhibición de la oxidación lipídica se explica por su capacidad para retirar el aire del interior del envase, reduciendo al máximo el oxígeno residual disponible para iniciar estas reacciones indeseadas (Lagerstedt *et al*., 2011). El oxígeno tiene un papel clave en la propagación de los procesos oxidativos que se desarrollan en la carne, debido a que frecuentemente ese tipo de reacciones se inician por la acción de ROS (Cheng, 2016). El envasado *skin* impediría la formación de estos compuestos mediante la eliminación del oxígeno del espacio de cabeza del envase.

Otra estrategia que inhibió eficazmente los procesos oxidativos fue la adición a la carne de extractos y aceites vegetales ricos en polifenoles (capítulos 4.2.4. y 4.2.5.). De hecho, las concentraciones de MDA en la carne tratada con extractos de té, borraja y carvacrol fueron similares a las observadas en la envasada a vacío *skin*. Sin embargo, el mecanismo de acción de estas estrategias es diferente. En lugar de impedir la formación de ROS, los polifenoles son capaces de unirse a estos compuestos e inactivarlos, evitando de esta forma su ataque sobre los ácidos grasos de la membrana celular y en consecuencia, la propagación de las reacciones de oxidación. Además, los compuestos fenólicos pueden actuar como quelantes de diferentes metales que actúan frecuentemente como catalizadores de este tipo de reacciones en la etapa de iniciación (Balasundram, Sundram y Samman, 2006). Los principales compuestos fenólicos identificados en las semillas de borraja son el ácido rosmarínico, siríngico y sinápico (Wettasinghe, Shahidi, Amarowicz y Abou-Zaid, 2001), mientras que en el caso del extracto de té su acción antioxidante depende principalmente del contenido en catequinas, antocianos y ácidos fenólicos (Huang, Huang, Liu, Luo y Xu, 2007). Por otro lado, el carvacrol es el componente principal del aceite de orégano y su acción antioxidante se fundamenta en la presencia de un grupo fenol en su estructura. Aunque el mecanismo de acción no está completamente claro, este compuesto parece inactivar los radicales peroxilo que se forman tras la oxidación primaria de los ácidos grasos (Suntres, Coccimiglio y Alipour, 2015).

Tanto la actividad de los extractos de té y borraja como la del carvacrol dependieron de la concentración. Como se muestra el capítulo 4.2.4., una concentración de 500 ppm de extracto de borraja y de 50 ppm de extracto de té fue suficiente para observar una inhibición significativa de la oxidación lipídica en comparación con las muestras control. Sin embargo, se necesitó alcanzar en la carne una concentración de 1000 ppm del extracto de borraja y 500 ppm del extracto de té para mantener los valores de MDA por debajo del límite de detección sensorial (0,6 mg MDA/kg). En cambio, la

oxidación pudo mantenerse por debajo de este umbral con tan solo 300 ppm de carvacrol, e incluso podría haber sido suficiente una concentración menor (capítulo 4.2.5.). La utilización de estos compuestos redujo el desarrollo de la rancidez en la carne, llegando a ser imperceptible para los panelistas en las muestras tratadas con las concentraciones más elevadas (capítulo 4.2.4 y 4.2.5.). Diversos estudios habían evaluado previamente las capacidades antioxidantes del extracto de té (Liu, Xu, Dai y Ni, 2015; Lorenzo *et al*., 2014; Mitsumoto, O'Grady, Kerry y Buckley, 2005), borraja (Sánchez-Escalante *et al.* 2003) y orégano (Camo *et al*., 2008) obteniendo unas conclusiones similares. Por consiguiente, queda demostrada la capacidad de estos compuestos de inhibir las reacciones oxidativas en la carne y los productos cárnicos. Sin embargo, la adición de compuestos vegetales a la carne conlleva, en muchas ocasiones, la modificación de sus propiedades sensoriales. Latoch y Stasiak (2015) observaron cómo la adición de un extracto de menta, a pesar de reducir notablemente la oxidación, aportaba sabores y olores indeseables que comprometían la calidad del producto. De forma similar, Camo *et al*. (2008) comprobaron que los panelistas también identificaron sabores y olores vegetales en carne de cordero envasada bajo un *film* activo de romero. Uno de los aspectos positivos que demostraron poseer los extractos de té y borraja fue la baja modificación que originaron en los olores y sabores característicos de la carne de cordero, ya que ninguno de estos compuestos fue detectado por los panelistas en el análisis sensorial. Sin embargo, el carvacrol fue percibido por los panelistas, convirtiéndose en el olor y el sabor predominante de las muestras tratadas con este terpeno. La intensidad de los olores y sabores asociados al carvacrol dependió de su concentración, no obstante, los panelistas también lo detectaron fácilmente en la carne con 300 ppm. A pesar del efecto significativo del carvacrol en la inhibición del crecimiento microbiano y las reacciones oxidativas, la modificación que origina en las propiedades sensoriales de la carne de cordero podría comprometer su aceptabilidad por los consumidores, siendo el principal obstáculo para su utilización. Por el contrario, la ausencia de alteraciones en los olores y sabores característicos de la carne es una de las principales ventajas de la vitamina E y los extractos de té y borraja (Muíño *et al*., 2014; Ortuño *et al*., 2015).

La composición del tejido y la médula ósea incluye diversos metales como el hierro o el cobre, algunos de los cuales se han identificado como catalizadores de las reacciones oxidativas (Wiechuła, Jurkiewicz y Loska, 2008). Debido a que el sistema de corte por sierra provoca una diseminación de partículas óseas, y en consecuencia de estos metales sobre la superficie de la carne, se podría esperar una mayor oxidación en las chuletas cortadas con esta tecnología en comparación con las procesadas mediante

el corte de cizalla. Sin embargo, los resultados obtenidos en esta tesis doctoral (capítulo 4.2.2.) pusieron de manifiesto la ausencia de diferencias significativas entre los valores de MDA de las chuletas cortadas mediante estas tecnologías, lo que contradice la hipótesis planteada al inicio del estudio.

Otro resultado inesperado fue el efecto del *superchilling* en la oxidación lipídica. El efecto inhibidor de la temperatura sobre la velocidad de reacciones químicas y enzimáticas es ampliamente conocido (Carballo y Jiménez-Colomero, 2001; Jasper y Plazek, 1994). Por ello, era esperable obtener una menor oxidación lipídica en las chuletas de cordero conservadas a -1 ° C respecto a las mantenidas a 4 ° C. Como se puede observar en el capítulo 4.2.3., el *superchilling* no solo no exhibió ningún efecto inhibidor, sino que aceleró los procesos oxidativos cuando la carne se envasó en atmósfera protectora. Una posible explicación de estos resultados sería la liberación de compuestos prooxidantes en el interior de la célula como consecuencia de los daños mecánicos causados por las bajas temperaturas (Benjakul y Bauer, 2001). De hecho, algunos autores habían descrito previamente daños mecánicos en las fibras musculares de la carne como consecuencia de la aplicación del *superchilling* (Lan *et al*., 2016; Liu, Liang, Xia, Regenstein y Zhou, 2013) que podrían deberse a la formación y el crecimiento de cristales de hielo (Lan *et al*., 2016).

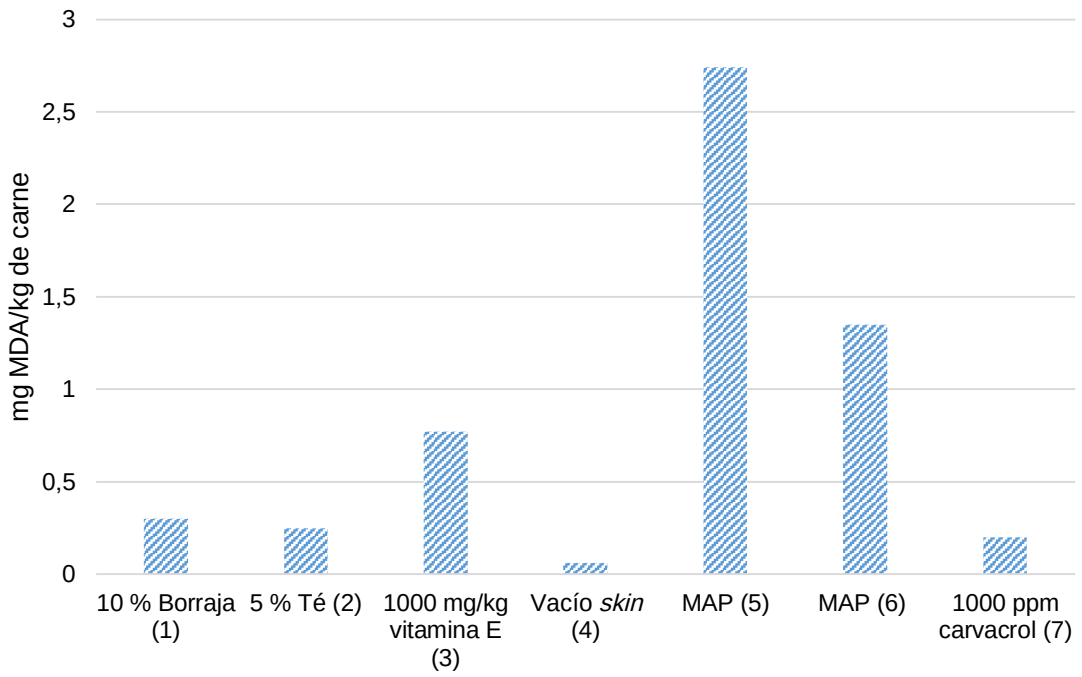

Figura 9. Valores de malondialdehído obtenidos bajo las distintas condiciones evaluadas en esta tesis doctoral.
(1), (2): Medido tras 8 días de refrigeración a 4 °C y envasado en MAP (40% O_2 – 30 % CO_2- 30% Ar); (3): Resultados obtenidos después de 9 días de refrigeración a 4 °C y envasado en MAP (70 % O_2 – 30 % CO_2); (4): Cuantificado tras 7 días de refrigeración a 4 °C; (5): Valores de MDA alcanzados después de 9 días de refrigeración a 4 °C en MAP (70 % O_2 – 30 % CO_2); (6): Resultados obtenidos tras 7 días de refrigeración a 4 °C en MAP (40% O_2 – 30 % CO_2- 30% Ar); (7): Oxidación determinada después de 8 días a 4 °C en aire.

5.3. Color

La carne de cordero se comercializa habitualmente envasada en atmósfera protectora y expuesta en grandes lineales refrigerados con iluminación artificial, donde los consumidores eligen el producto en función de su aspecto. Los consumidores asocian el color rojo brillante con mayor frescura y calidad del producto, discriminando la carne de color púrpura y rechazando aquella que presenta un color marrón (Mancini y Hunt, 2005). Está demostrado que el envasado con niveles elevados de oxígeno favorece la oxigenación de la mioglobina y prolonga la estabilidad del color de la carne: Resconi *et al*. (2012) registraron un color rojo más estable en la carne envasada con un 80 % de O_2 respecto a la mantenida con un 50 % de O_2 durante 8 días, y este mismo comportamiento fue observado por Yang *et al*. (2016). No obstante, como se ha comentado en apartados anteriores, las atmósferas ricas en oxígeno estimulan el desarrollo de procesos oxidativos, que en etapas posteriores favorecerán la formación de metamioglobina y en consecuencia, la pérdida del color. No hay que olvidar que la

oxidación lipídica y la proteica están interrelacionadas, de forma que los productos secundarios de una estimulan a la otra y viceversa (Faustman *et al*., 2010).

Muchas de las estrategias destinadas a mejorar la estabilidad del color de la carne envasada en atmósfera protectora actúan sobre ambas dianas, es decir, sobre la oxidación de los lípidos y de la mioglobina. Un ejemplo de estas estrategias es la suplementación de los corderos con dosis supra-nutricionales de vitamina E. Como se presenta en el capítulo 4.1.3., la suplementación de los corderos con 1000 mg de DL-α-tocoferol consiguió retrasar la formación de metamioglobina y en consecuencia, permitió obtener un color más atractivo durante todo el periodo de refrigeración en comparación con las muestras control (P < 0,001). La mayor estabilidad del color de la carne procedente de corderos suplementados con vitamina E ha sido ampliamente descrita en la bibliografía y se asocia a una inhibición de la conversión de la mioglobina en metamioglobina (Kerry *et al*., 2000; Lauzurica *et al*., 2005; Leal *et al*., 2018; Ripoll *et al*., 2011). El mecanismo a través del cual la vitamina E consigue bloquear esta reacción no está completamente claro. Como se ha comentado en el apartado anterior, la vitamina E inhibe eficazmente las reacciones lipídicas en su lugar de origen, lo que evita la formación de radicales primarios y secundarios y en consecuencia, la interacción de estos con la mioglobina. Por otro lado, la vitamina E puede inhibir la propagación de los radicales libres ya formados.

Sin embargo, la concentración de tocoferol alcanzada en el músculo (3,908 mg/kg) no fue suficiente para frenar completamente la pérdida del color rojo, señalando la necesidad de una mayor concentración para mantener un color atractivo durante todo el periodo de refrigeración. Diversos estudios han intentado establecer la concentración óptima para frenar completamente la pérdida del color. Jose *et al*. (2016) observaron que la estabilidad del color de la carne incrementaba progresivamente con el contenido de tocoferol en el músculo hasta alcanzar 3,5-4 mg α-tocoferol/kg de carne, concentración a partir de la cual no se observó ningún efecto adicional. Esta concentración tampoco evitó la formación de metamioglobina y la consecuente pérdida del color, poniendo de manifiesto la mayor efectividad de la vitamina E en la inhibición de la oxidación lipídica que en el mantenimiento del color. De forma similar, otros estudios observaron como la inhibición completa de la oxidación lipídica mediante la suplementación de la vitamina E no evitó la degradación progresiva del color a lo largo del periodo de refrigeración (Kerry *et al*., 2000; Lauzurica *et al*., 2005; Ripoll *et al*., 2011).

La suplementación de los corderos con vitamina E también permitió reducir la pérdida de color en las chuletas descongeladas (capítulo 4.1.3.). Las chuletas

procedentes de corderos suplementados presentaron un color más atractivo que las muestras control durante su almacenamiento en expositores refrigerados tras la congelación. No obstante, los procesos de congelación y descongelación redujeron la estabilidad del color de las muestras suplementadas con vitamina E. De hecho, las chuletas frescas mostraron mayores valores en los índices de rojo (*a**, *a**/*b**) que las descongeladas tras tres días de refrigeración, independientemente de la suplementación con vitamina E. Estas diferencias entre el color de las muestras frescas y descongeladas no parecen ser debidas a la oxidación lipídica, ya que no se observaron diferencias significativas entre ambos tratamientos. Sin embargo, podrían estar originadas por una menor actividad de la enzima reductora de la mioglobina. Esta enzima es más activa en la carne fresca, en la que tal como se forma la metamioglobina, es transformada en desoximioglobina y posteriormente en oximioglobina. Por el contrario, la metamioglobina se acumula con mayor rapidez en la carne descongelada, acelerando la pérdida del color rojo (Abdallah *et al*., 1999; Livingston y Brown, 1981).

El rociado de las chuletas con un extracto de semillas de borraja mostró ser más efectivo que la suplementación de los corderos con vitamina E para prevenir la pérdida del color en la carne descongelada durante su refrigeración en expositores iluminados ($P < 0,05$) (capítulo 4.1.3.). La diferencia entre ambas estrategias antioxidantes podría deberse al lugar de la célula donde actúa cada una de ellas. El tocoferol, debido a su naturaleza liposoluble, se almacena en las membranas celulares, donde protege a los ácidos grasos de la oxidación. Sin embargo, la mioglobina es una proteína sarcoplásmica, por lo que quedaría lejos de la localización celular de la vitamina E. En cambio, el extracto de borraja es hidrosoluble, siendo capaz de penetrar en el citoplasma y proteger a la mioglobina de la oxidación. De hecho, se ha observado cómo la adición de extractos de plantas a la carne incrementa el contenido de antioxidantes en el citoplasma de las células musculares (Faustman *et al*., 2010). Como se muestra en el capítulo 4.2.4., el extracto de borraja también resultó efectivo para prevenir la pérdida de color de las chuletas frescas de cordero. Tanto el rociado de las muestras con un extracto de borraja al 10 % como el de té al 5 % redujo la conversión de mioglobina en metamioglobina con relación a las muestras control ($P < 0,001$). De esta forma, las chuletas tratadas con estos extractos mostraron mayores valores en los índices de rojo (*a**, 630/580) durante todo el periodo de refrigeración. Estos resultados coinciden con los observados previamente por Bañón *et al*. (2007) y Lorenzo *et al*. (2014) al evaluar un extracto de té y con los registrados por Sánchez-Escalante *et al*. (2003) al utilizar un extracto de borraja para mejorar la conservación de hamburguesas de vacuno. El efecto de los compuestos fenólicos sobre el color de la carne se asocia tanto a su capacidad

de proteger directamente a la mioglobina frente al ataque de radicales libres como a su efecto indirecto al inhibir las reacciones oxidativas (Suman, Hunt, Nair y Rentfrow, 2014). A pesar de su naturaleza liposoluble, el carvacrol inhibió significativamente la formación de metamioglobina en relación con las muestras control, aunque su efecto fue menor que el logrado mediante el empleo de 400 ppm de sulfitos (capítulo 4.3.5). En concordancia, Bañón *et al*. (2007) y Ortuño *et al*. (2016) también describieron el mayor efecto de los sulfitos en comparación con los extractos naturales para reducir la formación de metamioglobina y en consecuencia la pérdida del color.

El color es uno de las causas habituales que limitan la vida útil de la carne fresca bajo las condiciones de comercialización habitualmente empleadas. De acuerdo con Djenane *et al*. (2002), los consumidores rechazan la carne cuando el porcentaje de metamioglobina alcanza el 40 %. Como puede observarse en los resultados presentados en esta tesis doctoral, este porcentaje se alcanzó en menos de 7 días cuando la carne de cordero se conservó en atmósfera protectora (capítulos 4.1.3., 4.2.2. y 4.2.4) o en aire (4.2.5.). Se demostró además el efecto limitado de la suplementación de los corderos con Vitamina E o la adición de extractos vegetales con propiedades antioxidantes en la prevención de la oxidación de la mioglobina. De hecho, ninguna de las estrategias mencionadas consiguió mantener un color adecuado durante más de diez días de refrigeración. Estos resultados destacan la dificultad de combinar un color rojo brillante con una estabilidad suficiente para mantenerlo en el tiempo.

El envasado a vacío parte de una estrategia diametralmente opuesta. A diferencia del envasado en atmósferas ricas en oxígeno, la carne conservada mediante este sistema presenta un color rojo púrpura. Este color se debe a la ausencia de oxígeno en el interior del envase y por ende a la formación de desoximioglobina (Jeremiah, 2001). El color de la carne envasada a vacío es el principal inconveniente para la utilización de este sistema de envasado en la venta minorista, ya que se ha demostrado que es menos atractivo para el consumidor (Carpenter, Cornforth y Whittier, 2001). Sin embargo, la mioglobina se oxigena al entrar en contacto con el oxígeno y otorga a la carne un color rojo brillante, fenómeno conocido como *blooming*. Además, como muestran los resultados presentados en el capítulo 4.2.3., el envasado a vacío tipo *skin* protegió eficazmente a la mioglobina de la oxidación, lo que permitió que la carne almacenada mediante este sistema mostrara unos valores de *a** más altos que la envasada en MAP a partir de los 14 días de almacenamiento ($P < 0{,}001$) (dejando oxigenar a la carne durante 2 horas). De forma similar, Łopacka *et al*. (2016) registraron un contenido de metamioglobina inferior al 5 % en los filetes de ternera envasados a vacío tipo *skin*, frente al 25 % observado en las muestras envasadas en atmósfera

protectora (80 % O_2 – 20 % CO_2) tras 12 días de refrigeración. Kameník *et al*. (2014) también observaron cómo, a partir de los 14 días de refrigeración, el color de las muestras envasadas a vacío tipo *skin* era más atractivo que el de las conservadas en atmósfera protectora (80 % O_2 – 20 % CO_2). Los resultados de esta tesis doctoral ponen de manifiesto la sinergia que se consigue al combinar este sistema de envasado con el *superchilling*, resultando en una mayor estabilidad de la mioglobina en comparación con la refrigeración a temperatura convencional (capítulo 4.2.3.). Este efecto podría explicarse por la mayor inhibición del crecimiento microbiano lograda a -1 °C. Está ampliamente descrito cómo el metabolismo microbiano promueve la oxidación de la mioglobina y en consecuencia favorece el pardeamiento de la carne (Abdallah *et al*., 1999).

Por tanto, queda demostrado el efecto protector del envasado a vacío tipo *skin* sobre el pigmento de la carne y la capacidad de esta de mostrar un color atractivo cuando, tras abrir el envase, entra en contacto con el oxígeno. Sin embargo, en el momento de compra la carne muestra un color poco atractivo para el consumidor. Actualmente diversas líneas de investigación están tratando de diseñar estrategias para que la carne envasada a vacío tipo *skin* muestre un color atractivo durante su almacenamiento en los expositores refrigerados. A pesar de presentar diferentes matices y formas de aplicación, todas ellas apuestan por el uso del monóxido de carbono (Lyu, Shen, Ding y Ma, 2016; Van Rooyen, Allen, Crawley y O ’Connor, 2017). La utilización de este gas no está permitida en la UE, sin embargo, crece el debate en torno a la reevaluación de su uso (Van Rooyen, Allen y O ’Connor 2017).

Además del color del músculo, el aspecto del hueso desempeña un papel importante en la aceptabilidad visual de la carne de cordero. El fenómeno conocido como “hueso negro” ha recibido menor atención que el color del músculo, no obstante, es fácil encontrar casos en los supermercados. Este proceso consiste en el ennegrecimiento de la médula ósea en los cortes con hueso durante su almacenamiento, presumiblemente debido a procesos oxidativos. En la ilustración 2 se puede observar la diferencia entre un hueso ennegrecido (A) y un hueso normal (B).

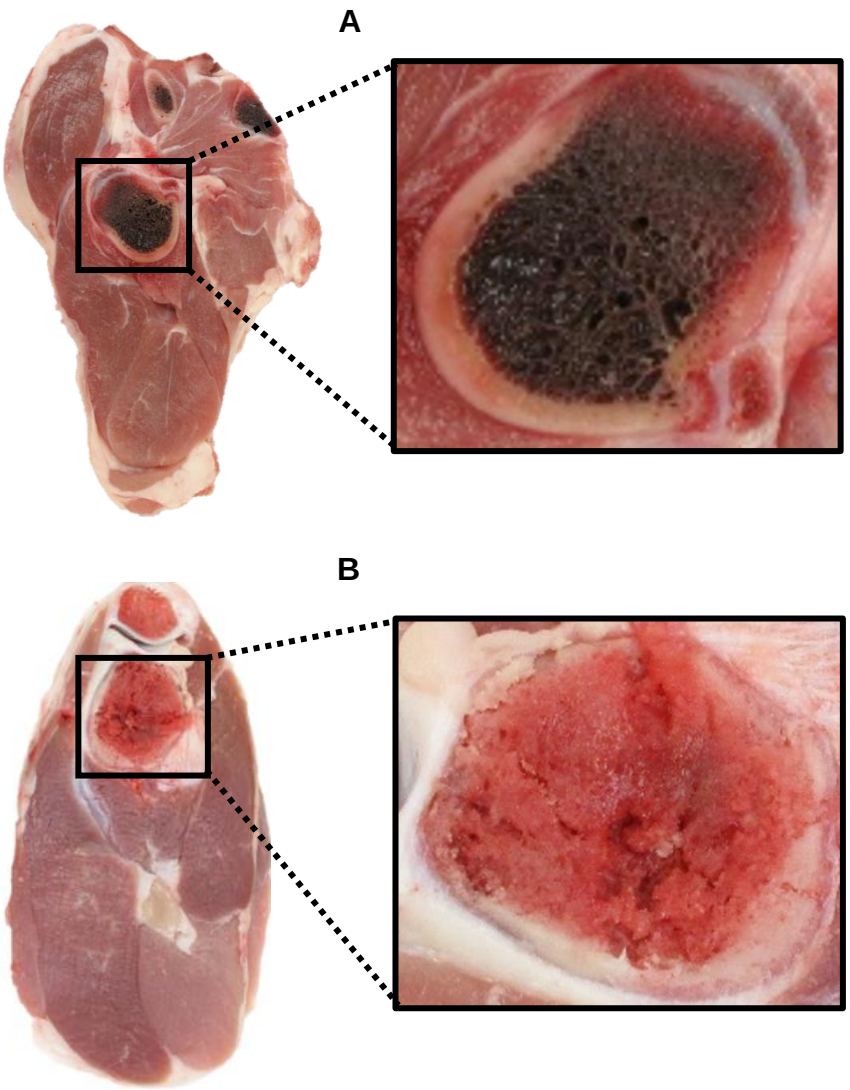

Ilustración 2. Aspecto de un hueso ennegrecido (A) y un hueso normal (B).

El fenómeno del hueso negro se estudió en el trabajo presentado en el capítulo 4.1.3., obteniendo unos resultados interesantes acerca de los factores que influencian su aparición. Como se puede apreciar, la congelación-descongelación de la carne fue el principal desencadenante de este proceso: los panelistas apreciaron unos huesos más oscuros en las chuletas descongeladas que en las frescas a partir del tercer día de refrigeración ($P < 0{,}001$). Nicolade, Stetzer, Tucker, McKeith y Brewer (2006) también observaron un mayor oscurecimiento del hueso en la carne sometida al proceso de congelación y descongelación, encontrando además un mayor contenido en metahemoglobina en la médula ósea de estas muestras en comparación con la carne fresca. Por tanto, estos resultados hacen sospechar de la oxidación de la hemoglobina como factor desencadenante de este fenómeno indeseado. Además, los resultados de nuestro estudio también mostraron cómo el rociado del hueso con un extracto antioxidante de semillas de borraja consiguió disminuir significativamente la intensidad

de este proceso, apuntando una vez más a las reacciones oxidativas como causa originaria. Sin embargo, la suplementación con vitamina E no tuvo efecto en su inhibición.

Otro factor que afecta a la apariencia de los cortes con hueso es el astillado del mismo durante el proceso de corte. El estudio 4.2.2. evaluó las diferencias en el aspecto del hueso en función del sistema de corte utilizado: cizalla o sierra. Los panelistas otorgaron una mayor aceptabilidad a los huesos de la carne cortada por sierra ($P < 0,001$), explicando estas diferencias por la frecuencia de huesos astillados cuando se utiliza la cizalla. Como se observa en la ilustración 3, estas diferencias son fácilmente perceptibles y, como también muestra este mismo estudio, influencian la aceptabilidad de la carne por el consumidor.

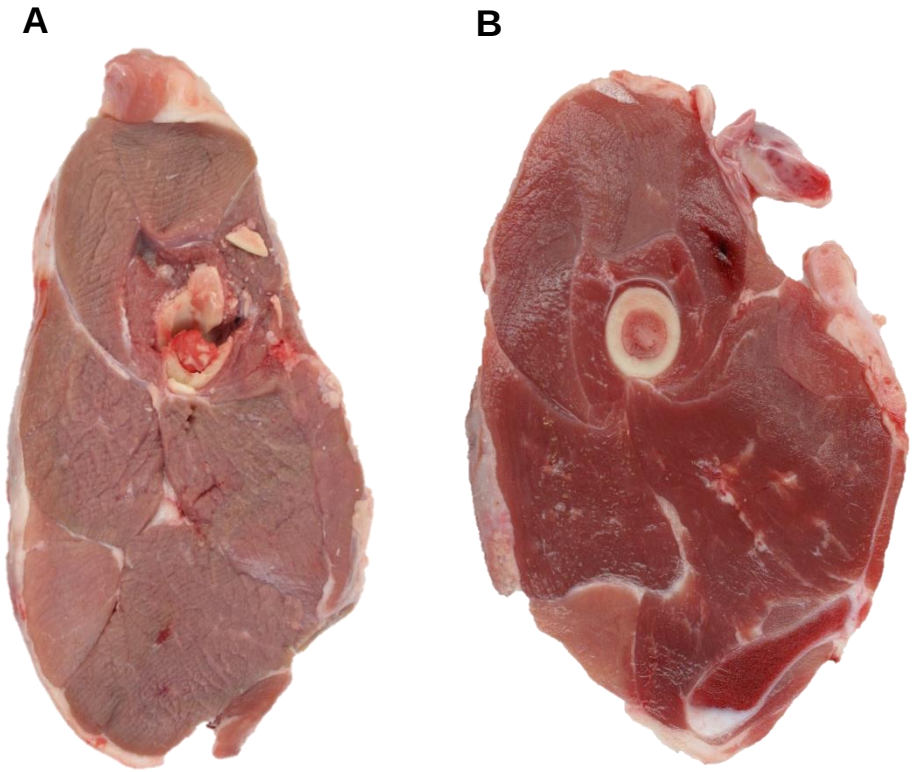

Ilustración 3. Chuletas cortadas por cizalla (A) y por sierra (B).

Conclusiones

6. Conclusiones

Bajo las condiciones experimentales utilizadas en los estudios y con los resultados obtenidos en los mismos se pueden establecer las siguientes conclusiones:

1. La suplementación de la dieta de los corderos con vitamina E protege a los ácidos grasos poliinsaturados, inhibe los procesos oxidativos y reduce la pérdida de color durante el almacenamiento de la carne. Estos efectos dependen tanto de la concentración de tocoferol en el músculo como de las condiciones de conservación.

2. La carne descongelada presentó menores recuentos microbianos que la fresca y similares niveles de oxidación, siendo el color el parámetro más crítico. La suplementación de la dieta de los corderos con vitamina E o el uso de un extracto antioxidante de semillas de borraja retrasó la oxidación lipídica y la pérdida del color. Finalmente, la comercialización de carne de cordero descongelada, envasada en MAP y mantenida en linales refrigerados sería viable desde un punto de vista técnico.

3. La ultra-refrigeración de las canales permite prolongar la vida útil de la carne a la vez que agiliza el procesado en el matadero, todo ello sin disminuir la calidad. Durante el almacenamiento, la refrigeración en combinación con el envasado a vacío tipo *skin* parece ser la estrategia más eficaz para mantener la calidad de la carne de cordero. Los envases activos con compuestos antimicrobianos y antioxidantes podrían solucionar los inconvenientes que presenta el MAP.

4. El sistema de corte no afectó a la vida útil de las chuletas de cordero. Sin embargo, el corte con sierra produjo chuletas con mayor aceptabilidad visual, lo que podría tener repercusión en la intención de compra de los consumidores.

5. El *superchilling* en combinación con el envasado a vacío tipo *skin* consiguió duplicar la vida útil de la carne de cordero refrigerada a 4 °C, al inhibir el crecimiento microbiano, la oxidación lipídica y la pérdida del color. No obstante, su utilización junto con el MAP favoreció las reacciones oxidativas, comprometiendo la calidad de la carne y su vida útil.

6. La aplicación de un extracto de borraja al 10 % o de un extracto de té al 0,5 % inhibió la oxidación lipídica, la formación de metamioglobina y la pérdida del color sin aportar sabores u olores vegetales a la carne. No obstante, ningún extracto mostró efecto antimicrobiano. La utilización de estos compuestos incrementó la vida útil de las chuletas de cordero de 8 a 11 días.

7. Tanto el extracto de té como el carvacrol inhibieron la oxidación lipídica del *burger meat* a una concentración de 300 ppm. Sin embargo, solo el carvacrol, que mostró una actividad dependiente de la concentración, retrasó la pérdida del color y el crecimiento microbiano. La vida útil obtenida con 1000 ppm de carvacrol fue similar a la alcanzada con 400 ppm de sulfitos, no obstante, modificó significativamente las propiedades sensoriales de la carne.

En la tabla 6 se puede observar la relación de estas conclusiones con los objetivos planteados al inicio de esta tesis doctoral.

Tabla 6. Relación entre los objetivos de la tesis doctoral y las conclusiones obtenidas en respuesta a cada uno de ellos.

Objetivo	Conclusión
Analizar y sintetizar los datos publicados sobre la suplementación de los corderos con vitamina E con el fin de consolidar el conocimiento existente e identificar nuevas áreas de investigación.	La suplementación de los corderos con vitamina E protege a los ácidos grasos poliinsaturados, inhibe los procesos oxidativos y reduce la pérdida de color durante el almacenamiento de la carne. Estos efectos dependen tanto de la concentración de tocoferol en el músculo como de las condiciones de conservación
Investigar la capacidad antioxidante de la vitamina E administrada en la dieta sobre la calidad de la carne de cordero mantenida en congelación.	El efecto de la vitamina E se mostró independiente de la congelación, manteniendo su actividad frente a los procesos oxidativos tras la descongelación de la carne.
Evaluar la viabilidad, desde el punto de vista de la calidad, de la comercialización de carne de cordero descongelada, envasada en MAP y mantenida en expositores refrigerados.	La carne descongelada presentó menores recuentos microbianos que la fresca y similares niveles de oxidación, siendo el color el parámetro más crítico. La suplementación de los corderos con vitamina E o el uso de un extracto antioxidante de semillas de borraja retrasó la oxidación lipídica y la pérdida de color. Finalmente, desde un punto de vista técnico, la comercialización de carne de cordero descongelada, envasada en MAP y mantenida en lineales refrigerados sería viable.
Describir los métodos utilizados y las nuevas tendencias para la refrigeración de las canales y la conservación de la carne fresca de cordero.	La ultra-refrigeración de las canales permite prolongar la vida útil de la carne a la vez que agiliza el procesado en el matadero, todo ello sin disminuir la calidad. Durante el almacenamiento, la refrigeración en combinación con el envasado a vacío tipo *skin* parece ser la estrategia más eficaz para mantener la calidad de la carne de cordero. Los envases activos con compuestos antimicrobianos y antioxidantes podrían solucionar los inconvenientes que presenta el envasado en MAP.

Continúa en la página siguiente.

Tabla 6. Relación entre los objetivos de la tesis doctoral y las conclusiones obtenidas en respuesta a cada uno de ellos (continuación).

Objetivo	Conclusión
Estudiar el efecto de los sistemas de corte más habituales (sierra y cizalla) en la vida útil y en la aceptabilidad de las chuletas de cordero a lo largo de su conservación en condiciones comerciales.	El sistema de corte no afectó a la vida útil de las chuletas de cordero. Sin embargo, el corte con sierra produjo chuletas con mayor aceptabilidad visual, lo que podría tener repercusión en la intención de compra de los consumidores.
Analizar el efecto combinado del *superchilling* y el envasado en la vida útil de la carne de cordero.	El *superchilling* en combinación con el envasado a vacío tipo *skin* consiguió duplicar la vida útil de la carne de cordero refrigerada a 4 °C al inhibir el crecimiento microbiano, la oxidación lipídica y la pérdida del color. No obstante, su utilización junto con el envasado en atmósfera protectora favoreció las reacciones oxidativas, comprometiendo la calidad de la carne y su vida útil.
Investigar el efecto de la aplicación de extractos de té y borraja en la conservación de chuletas de cordero.	La aplicación de un extracto de borraja al 10 % o de un extracto de té al 0,5 % inhibió la oxidación lipídica, la formación de metamioglobina y la pérdida del color, sin aportar sabores u olores vegetales a la carne. No obstante, ningún extracto mostró efecto antimicrobiano. La utilización de estos compuestos incrementó la vida útil de las chuletas de cordero de 8 a 11 días.
Evaluar la capacidad del extracto de té y el carvacrol para sustituir a los sulfitos como conservante del *burger meat*.	Tanto el extracto de té como el carvacrol inhibieron la oxidación lipídica del *burger meat* con una concentración de 300 ppm. Sin embargo, solo el carvacrol, que mostró una actividad dependiente de la concentración, retrasó la pérdida del color y el crecimiento microbiano. La vida útil obtenida con 1000 ppm de carvacrol fue similar a la alcanzada con 400 ppm de sulfitos, no obstante, modificó significativamente las propiedades sensoriales de la carne.

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7. Bibliografía

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