TESIS DOCTORAL

Características físico-químicas, microbiológicas y estructurales de la carne de cordero cocinada al vacío

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Physical-chemical, microbiological and structural characteristics of vacuum cooked lamb meat

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Expresan su conformidad para la tramitación de la presente tesis doctoral.

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Resumen/Summary

RESUMEN

El cocinado de los alimentos al vacío es una técnica que está cobrando cada vez más relevancia en la alta cocina, pues tanto los cocineros como los consumidores aprecian los resultados obtenidos mediante este tipo de procesado culinario. Concretamente, el cocinado al vacío de la carne de cordero empleando temperaturas bajas (en torno a 60ºC) y tiempos de cocinado prolongados (hasta 24 horas) da lugar a un producto de unas características sensoriales extraordinarias. Sin embargo, la información científica sobre las características de las carnes cocinadas en esas condiciones es muy escasa y ampliar estos conocimientos podría permitir explorar el uso de este tipo de cocinado en la industria del catering o de los platos preparados. Por ello, uno de los objetivos de este trabajo ha sido estudiar el efecto de diferentes combinaciones de temperatura y tiempo sobre las características físico-químicas, estructurales y microbianas en lomos de cordero, así como el efecto sobre el perfil de compuestos volátiles de los mismos. Además, se llevó a cabo el estudio de la conservabilidad de este tipo de producto durante su almacenamiento en refrigeración. El otro propósito consistió en estudiar los efectos de estrategias culinarias que tienen como objetivo mejorar las características sensoriales de la carne de cordero cocinada a la vacío, como son el uso de fosfatos para mejorar la textura, y la adición de azúcares para potenciar el desarrollo de notas aromáticas a asado.

Se ha podido constatar la gran influencia de la temperatura de cocinado sobre los cambios físico-químicos y estructurales en la carne de cordero cocinada al vacío, siendo mayores las mermas durante el cocinado cuanto mayores eran las temperaturas y los tiempos de cocinado, aunque este segundo parámetro tuvo menor importancia. Los cambios estructurales en carne de cordero cocinada al vacío se resumen en una granulación del colágeno endomisial a las temperaturas más bajas de cocinado (60 ºC), y una formación de un gel (gelatina) más clara a temperaturas superiores (80 ºC). Las medidas de oxidación lipídica se vieron afectadas de manera diferente por las condiciones de cocinado: mientras que en los TBARS, las muestras sometidas a menores temperaturas de cocinado presentaron valores mayores, que luego fueron disminuyendo a lo largo del período de almacenamiento, en los dienos

conjugados ocurrió justo lo contrario. El tratamiento de cocinado fue más que suficiente para conseguir una pasterización efectiva de las muestras estudiadas, extendiendo la vida útil de la carne así cocinada y almacenada a refrigeración a más de un mes. El análisis del perfil de compuestos volátiles mostró que el cocinado al vacío utilizando combinaciones de temperaturas y tiempos suaves (60 ºC durante 6 y 24 h, 80 ºC durante 6 h) favorecen la formación de compuestos volátiles procedentes de la oxidación lipídica, mientras que una combinación temperatura y tiempo más intensa (80 ºC durante 24 h) estimula la formación de compuestos volátiles procedentes de reacciones en las que participan aminoácidos.

Finalmente, el uso de fosfatos y de sustancias precursoras de las reacciones generadoras de aroma provocaron cambios sustanciales en las características de la carne de cordero cocinada a vacío en condiciones similares a las empleadas en restauración, con un incremento en el agua retenida en el primero de los casos, y un ligero aumento de las reacciones de Maillard en el segundo.

SUMMARY

Vacuum cooking of foods is a technique that is becoming more and more important in haute cuisine, since chefs and consumers appreciate the results obtained by this type of culinary treatment. Specifically, vacuum cooking of lamb meat at relatively low temperatures (around 60ºC) and considerably long times (even 24 hours) gives rise to a product showing exquisite sensory features. However, the available scientific information of this type of cooking is scarce and improving this knowledge could allow a further exploration of the use of this type of cooking for the catering or the ready to eat food industries. Therefore, the first aim of this work was to study the effect of different time-temperature combinations on the physico-chemical, structural and microbial characteristics of vacuum cooked lamb loins, as well as the effect on the volatile compound profile. In addition, it was carried out the study of this type of product shelf-life during its storage in refrigeration. The other goal was to study the effect of culinary strategies to improve the sensory properties of vacuum cooked lamb meat, such as the use of phosphates to improve the texture and sugars addition to enhance the development of roasted aromatic notes.

It has been confirmed the great influence of cooking temperature on the physic-chemical and structural changes in vacuum cooked lamb meat. The higher cooking temperatures and times the greater weight losses, although the cooking time affected in a lesser degree. The structural changes in vacuum cooked lamb loins are summarized in an endomysial collagen granulation at the lowest cooking temperatures (60 ºC), and a gel formation at higher temperatures (80 ºC). The lipid oxidation measures were affected in a different way by the cooking conditions: samples subjected to lower temperatures showed higher TBARS values, which then gradually decreased over the storage period, while the conjugated dienes showed the opposite effect. The cooking treatments were more than enough for an effective pasteurization of the studied samples, extending their shelf-life at more than one month. The volatile compound profile analysis showed that the volatile compounds arising from lipid oxidation are the major ones when cooking at milder cooking conditions (60 ºC for 6 and 24 h, 80 ºC for 6 h), while a more intense time and temperature

combination (80 ºC for 24 h) resulted on a higher concentration of volatile compounds formed from amino acid-involved reactions.

Finally, the use of phosphates and precursors of flavor-generating reactions caused substantial changes in the characteristics of lamb meat cooked in similar conditions to those used in restaurants, with an increase in water retention in the first case, and a light increase of Maillard reactions in the second one.

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1. Introducción

1.1 GENERALIDADES SOBRE EL SECTOR OVINO

El ganado ovino ha sido históricamente el principal pilar de la ganadería española dadas nuestras particularidades climatológicas, orográficas y edafológicas. Es un sector muy complejo debido, en gran medida, a la gran diversidad de sistemas de producción que coexisten y que están condicionados por la gran dependencia del sector al medio en que se desarrolla su actividad productiva.

1.1.1 Censo y producción

Dentro de las producciones ganaderas, el sector ovino–caprino de carne es el sexto en importancia en España por detrás del sector del porcino, vacuno de leche, vacuno de carne, aves y huevos, representando aproximadamente el 6 % de la producción final ganadera (MAGRAMA, 2012) (Figura 1).

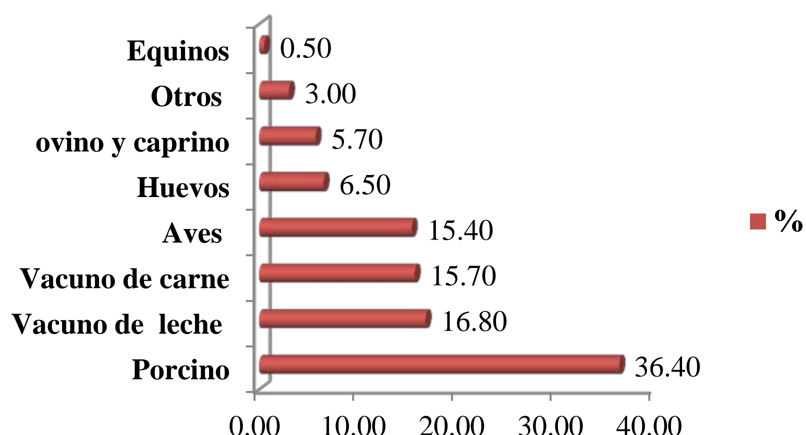

Figura 1. Distribución de la producción final ganadera en España – 2011 (Fuente: MAGRAMA, 2012).

Se trata de un sector que ha sabido adaptarse en los últimos años a los nuevos retos que imponen la apertura creciente de los mercados agrarios y la fuerte competencia exterior, gracias a mejoras tecnológicas y estructurales, que le ha permitido convertirse en un sector especializado y competitivo.

La distribución territorial del censo ovino dentro de la geografía española abarca a más del 80% de los municipios, aunque se concentra de manera mayoritaria en cinco Comunidades Autónomas (C.C.A.A.): Extremadura, Castilla y León, Castilla-La Mancha, Andalucía y Aragón; las cuales agrupan aproximadamente el 82% de todo el censo ovino de carne en nuestro país: (MAGRAMA, 2013) (Figura 2).

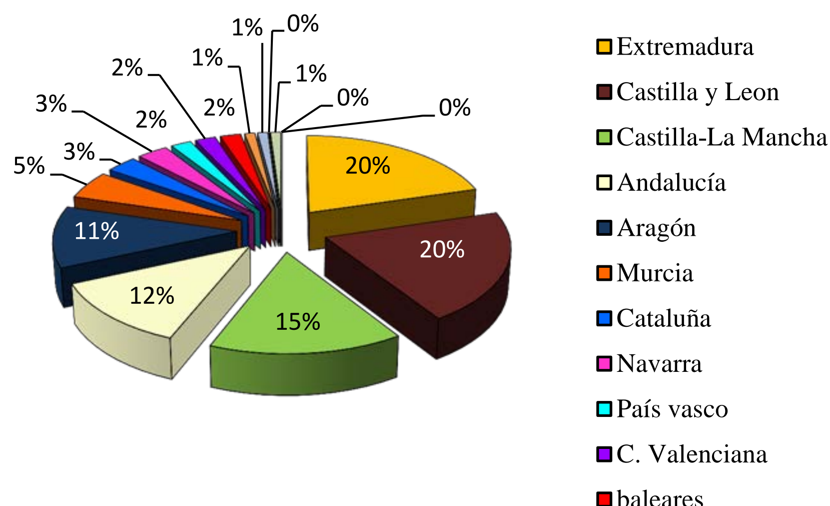

Figura 2. Distribución del censo ovino por CCAA en el año 2012. (Fuente: MAGRAMA, 2013).

Sin embargo, durante los últimos 10 años, el censo de ovino en España ha experimentado una disminución de un 30 % aproximadamente, pasando de casi 23,5 millones de cabezas en el año 2003, a situarse en torno a 16,5 millones de cabezas en 2013 (MAGRAMA, 2013). Igualmente, cabe destacar que en los últimos 8 años, la producción de carne de cordero se ha reducido en más de un 50%, pasando de 231.463 toneladas (t) en el año 2004, a 122.752 t en el año 2012 (MAGRAMA, 2012). Aproximadamente el 70 % de la producción total de carne de ovino en nuestro país se encuentra distribuida entre las C.C.AA. de Castilla y León (con 31.043 t aprox.), Castilla-La Mancha (20.138 t aprox.), Cataluña (17.540 t aprox.) y Aragón (13.161 t aprox.). Extremadura, a pesar de presentar uno de los mayores censos de ovino de todo el país, su participación en la producción nacional de carne de ovino es ciertamente baja (3739 t aprox.) (MAGRAMA, 2013) (Figura 3).

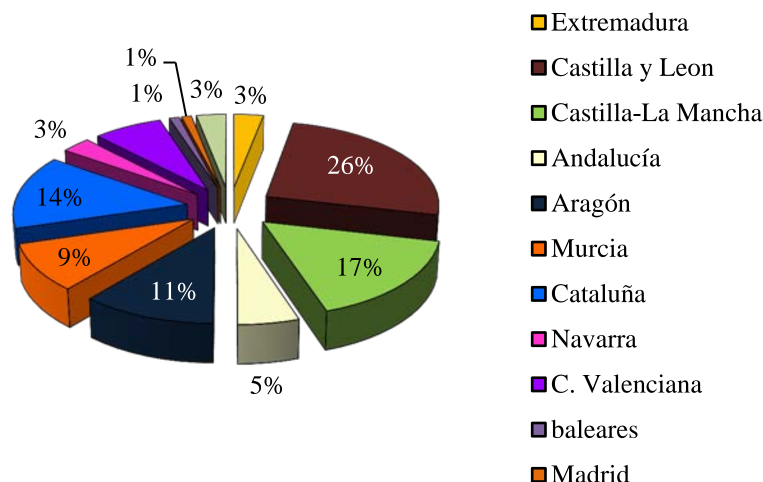

Figura 3. Distribución de la producción total de ovino por CCAA en el año 2012 (Fuente: MAGRAMA, 2013).

1.1.2. Consumo de carne de cordero

El consumo de carne en España es de 52,65 kg por habitante y año, valor medio del que tan solo 2,08 kg al año corresponderían a carne de ovino (MAGRAMA, 2012a). En general, el consumo per cápita de carne de cordero es bajo en comparación con el resto de carnes, observándose además una disminución en la demanda de este tipo de carnes en la última década (Figura 4).

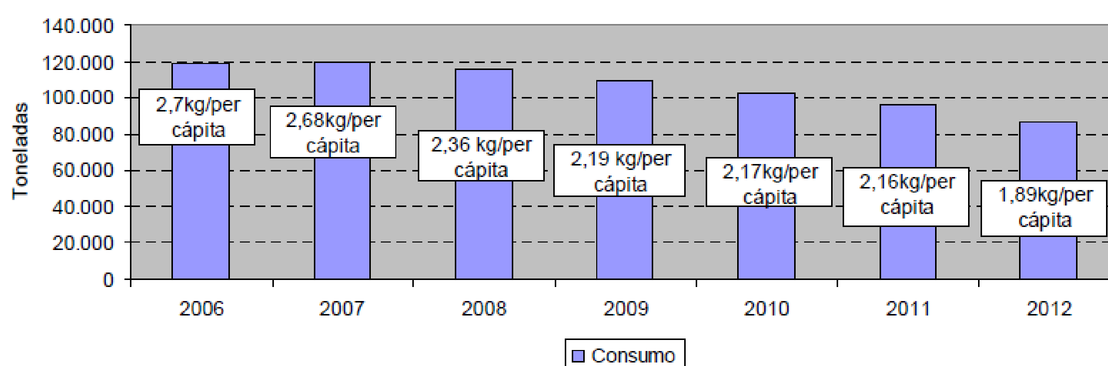

Figura 4. Evolución del consumo de carne fresca de ovino y caprino en los hogares españoles (Fuente: MAGRAMA, 2012a).

La causa a esta continua disminución se puede asociar a varios factores: por un lado, el precio de este producto, ya que suele ser más caro que otras carnes, lo que ha desviado el consumo de proteínas de origen animal hacia producciones económicamente más competitivas como son los huevos, y la carne de pollo y cerdo; y por otro lado, a los cambios socio-

culturales que se están produciendo en nuestra sociedad, con nuevos ritmos de vida y pautas de alimentación en las que priman otro tipo de alimentos, especialmente alimentos precocinados y alimentos con una vida útil más larga (envasados en atmósfera modificada, loncheados, etc.), presentaciones poco habituales para la carne de cordero, que sigue siendo muy tradicional (Cruz, 2013). Por tanto, las formas tradicionales del cocinado de la carne cordero, como son el asado, el estofado o la brasa, que necesitan largos tiempos de preparación, tiene poca cabida en la sociedad actual.

No obstante, desde mediados de la pasada década, con el objetivo claro de añadir valor a la carne de cordero y acercar más este tipo de carne a los consumidores, muchas de las principales empresas cárnicas de este sector han iniciado su apuesta por la comercialización de platos preparados o precocinados a base de carne de cordero, de manera que, junto al despiece tradicional del cordero, también comercializan platos de IV y V gama (Cruz, 2013). Por tanto, el desarrollo de nuevas estrategias que incrementen la vida útil de este producto en el mercado reduciendo, por una parte, el desarrollo de la carga microbiana y, por otra, la oxidación lipídica y el deterioro del color que se produce como consecuencia de ella (p. ej., incluyendo antioxidantes en la ración de los animales, nuevos empaquetados…), permitiría prolongar el periodo de tiempo a lo largo del cual esta carne podría ser comercializada y consumida, lo que constituiría, sin duda, un estímulo económico para este sector agropecuario tan castigado por las circunstancias actuales.

Además, se están realizando grandes esfuerzos para diferenciar la carne de cordero y hacerla más competitiva frente a carnes de otras especies y sobre todo, frente a carne de corderos procedentes de otras regiones o países. Para ello, se han creado diferentes Denominaciones de Calidad, existiendo actualmente en España nueve figuras de calidad relacionadas con la carne de cordero: Indicación geográfica protegida (I.G.P.) Ternasco de Aragón, I.G.P. Lechazo de Castilla y León, I.G.P. Cordero Manchego, I.G.P Cordero de Navarra, I.G.P. Cordero de Extremadura, I.G.P. Cordero Segureño, marca de garantía (M.G.) Lechazo de la Meseta Castellano-Leonesa, M.G. Cordero Selecto Certificado y M.G. Cordero Lechal del País Vasco (Bernués, Ripoll, & Panea, 2012; Dimara, Petrou, & Skuras, 2004).

1.2 EL COCINADO AL VACÍO

La cocción al vacío, también llamada *sous-vide*, fue introducida a manos de un chef francés llamado George Pralus en 1974, y desde entonces, su popularidad ha ido en aumento siendo su uso habitual en la preparación de alimentos en restaurantes, catering y procesos industriales. Este método de cocinado consiste en envasar los productos en bolsas de plástico con baja permeabilidad al oxígeno y resistentes a las altas temperaturas. En el caso de las carnes se sigue el proceso denominado *cocción indirecta*, en el que el producto se cocina durante un período normalmente muy prolongado en un baño termostatizado o un horno de convección, para refrigerarse inmediatamente después del cocinado (Ruíz, 2010). Uno de los aspectos que caracteriza el uso de esta técnica en los restaurantes es el empleo de temperaturas sensiblemente inferiores a las empleadas tradicionalmente. Así, las temperaturas recomendadas para el cocinado de las carnes a vacío suele oscilar entre los 65 y 90 ºC (Hrdina-Dubsky, 1989), las cuales se aplicarán durante largos períodos de tiempos dependiendo de la composición y tamaño de las piezas y de la calidad final deseada. Concretamente, para cocinar al vacío carne de ternera, cerdo o cordero, los chefs recomiendan combinaciones de temperatura y tiempo alrededor de 58-63 ºC durante 10-48h (Myhrvold, Young, & Bilet, 2011), aunque las temperaturas de cocinado de carne de cerdo en catering, pueden llegar a alcanzar los 75-80 ºC (Armstrong, 2000). Durante todo el procesamiento de los alimentos, la temperatura de cocinado se encuentra justo por encima de la temperatura final deseada en el interior del producto, y gracias a este ajuste de la temperatura se consigue evitar el exceso de cocinado de los alimentos (Baldwin, 2009). La aplicación controlada de bajas temperaturas y largos tiempos (LT-LT) permite cocinar las carnes en unas condiciones que maximizan la reducción de la dureza, ya que permiten la desnaturalización del colágeno, sin llegar a alcanzar las temperaturas a las que empieza a producirse la retracción de proteínas (Ruíz, 2010).

Las ventajas de la tecnología *sous-vide* han permitido su empleo en diferentes sectores (Creed, 2001). Por ejemplo, en los restaurantes, el cocinado al vacío ofrece una serie de ventajas organizativas con respecto al cocinado tradicional, ya que permite una mayor optimización de las operaciones

culinarias y una mejor organización de la cocina con la consecuente menor pérdida de tiempo a la hora de preparar platos cuyo proceso de elaboración es prolongado. Además, es un medio de diversificación de los productos culinarios, ya que al utilizar temperaturas y tiempos de cocción muy diferentes de los utilizados en la cocina tradicional, permite obtener resultados sensiblemente distintos de los que se consiguen tras el cocinado tradicional de los alimentos. Por otro lado, esta técnica facilita la manipulación de los alimentos ya cocinados, ya que evita la contaminación microbiana de los mismos tras el tratamiento térmico, alargando así la vida útil de los productos (Armstrong, 2000). Así, en el caso de alimentos que tras su cocinado son enfriados y almacenado en refrigeración podemos destacar una ralentización del crecimiento de aquellos microorganismos alterantes que se encuentren aún en el alimento (Nyati, 2000), ya que no se produce re-contaminación y el tratamiento culinario supone muchas veces la pasterización del alimento (Sánchez del Pulgar, 2007).

El cocinado *sous-vide* también ofrece ventajas relativas a las características del alimento como son, por ejemplo, la conservación de la estructura del alimento a consecuencia de quedar comprimido por la película plástica, especialmente importante en alimentos de estructura frágil. Además, este tipo de cocinado reduce las pérdidas de humedad durante el cocinado (Schellekens, 1996), y conlleva a una mejor conservación de los aromas generados durante el mismo, preservando la calidad sensorial de los alimentos (Baird, 1990). Otra ventaja del cocinado *sous-vide* es que permite la impregnación del alimento con los condimentos y especias disueltos en el líquido que lo rodea por efecto del vacío (Andrés, Salvatori, Albors, Chiralt, & Fito, 2001).

Sin embargo, este tipo de cocinado también presenta alguna desventaja, ya que este método no potencia el desarrollo de las reacciones químicas responsables de los aromas típicos de la carne cocinada debido a las bajas temperaturas utilizadas. Por ello, una vez terminado el cocinado al vacío, se suele proceder a un acabado de la misma por un sistema tradicional con el objetivo de conseguir una superficie endurecida a través de la formación de una costra externa crujiente, sabrosa y de color parda (Ruíz, 2010).

1.3 MODIFICACIONES FÍSICO-QUÍMICAS, SENSORIALES Y MICROBIOLÓGICAS DURANTE EL COCINADO AL VACÍO

El calentamiento es una parte crucial del procesado de las carnes, ya que casi todas las carnes son calentadas previamente a su consumición. El cocinado, en mayor o menor grado, afecta a la terneza, a la jugosidad y al aroma de la carne a través cambios físicos y bioquímicos producidos en las proteínas, hidratos de carbono, lípidos y otros componentes minoritarios, y que son inducidos por el aumento de temperatura. El alcance de estos cambios dependerá en gran medida de la temperatura y tiempo de cocinado, así como del método de cocinado aplicado y el estado (contraído o relajado) del músculo (Obuz, 2003).

1.3.1 Color

En general, los consumidores basan sus decisiones de compra en la percepción de la calidad de la carne (Troy & Kerry, 2010). En el caso de las carnes rojas, el color se convierte en sinónimo de calidad (Renerre & Labas, 1987) pasando a ser el principal factor en la decisión de compra de este tipo de carnes, ya que los consumidores asocian la decoloración a una falta de frescura y valor nutricional de la misma. En estudios realizados en carne de ternera, se ha observado que el consumidor puede llegar a rechazarla cuando presenta un 20 % de metamioglobina (MMb) (MacDougall, 1982).

En la carne cocinada, el color de la misma viene determinado por el grado de desnaturalización de la mioglobina (Mb). En un primer momento, cuando se aplica calor a la carne, la Mb tiende a oxidarse dando lugar a la formación de MMb; sin embargo, si esta aplicación de calor continúa, la Mb se desnaturaliza dando lugar a un pigmento de color marrón llamado globina-hemicromógeno (King & Whyte, 2006). Por tanto, el color final de la carne cocinada dependerá de la cantidad de ferrihemocromo formado, que a su vez, dependerá de la proporción inicial de Mb que haya en el músculo y de la concentración final de oximioglobina (OMb) y deoximioglobina (DMb) sin desnaturalizar (King & Whyte, 2006). La desnaturalización de la Mb comienza a los 55-65 ºC en carnes, produciéndose la mayor parte de la desnaturalización entre los 75-80 ºC (King & Whyte, 2006).

La carne cocinada al vacío utilizando bajas temperaturas (por debajo de 65

ºC) y prolongados tiempos (LT-LT), presenta una menor desnaturalización de la Mb y muestra un color más rojizo en comparación con las carnes cocinadas mediante métodos tradicionales (Garcia-Segovia, Andres-Bello, & Martinez-Monzo, 2007; Sánchez Del Pulgar, Gázquez, & Ruiz-Carrascal, 2012). Según Schafheitle (1990), el proceso *sous-vide* minimiza la pérdida de color en mayor proporción que otros métodos de cocinado y empaquetado convencionales. A efectos prácticos, este hecho puede resultar muy interesante ya que los chefs valoran mucho el hecho de que el tiempo de cocción no afecte al color rojizo de la carne cocinada a temperaturas moderadas, incluso cuando son cocinadas durante tiempos muy prolongados.

El color de la carne puede ser medido de varias formas: visual, normalmente utilizando panelistas entrenados que asignan valores de acuerdo a una escala descriptiva que contiene unos valores ya preestablecidos; química, midiendo la concentración de Mb en las muestras; o instrumental, midiendo la reflexión de la luz procedente de las muestras (King & Whyte, 2006). Actualmente se utiliza el espacio CIELab, especificado por la Commision Internationale de l'Eclairage (CIE), que consiste en definir el color en función de tres parámetros: L*, que determina la luminosidad del cuerpo coloreado; a*, que mide la desviación rojo-verde; y b*, que indica la desviación amarillo-azul. A partir de estas mediciones se pueden calcular otros valores ópticos adicionales tales como el ángulo de Hue, que determina el tono de color y la cromaticidad, saturación o croma, que indica la intensidad del color (Warriss, 2000).

1.3.2 Textura y Estructura

Muchas de las características tecnológicas, sensoriales o nutricionales de la carne, tales como la textura, su comportamiento tras la cocción, conservación y la pérdida de jugos de la carne, están estrechamente ligadas a la estructura proteica del músculo, habiéndose contrastado ampliamente la preferencia de los consumidores por las carnes tiernas (Koohmaraie, 1994; Nishimura, 2010; Wheeler & Koohmaraie, 1994).

La textura de la carne depende de las características zootécnicas del animal, tales como la raza, la edad y el sexo, de las características anatómicas, tales como el tipo de músculo, o del método de cocinado utilizado (Nikmaram,

Yarmand, Emamjomeh, & Darehabi, 2011).
Por otro lado, la textura de la carne también está influenciada por el grado de contracción del músculo, y éste, a su vez, por la temperatura a la cual se instaura el *rigor mortis*. Así, cuando éste se produce a temperaturas inferiores a 15 ºC los filamentos de actina y miosina se entrecruzan provocando un acortamiento de los músculos. Este proceso es conocido como *acortamiento por frío*, lo que resulta en un gran incremento de la dureza de la carne (Locker & Hagyard, 1963).

El cocinado de los productos cárnicos es esencial para lograr productos seguros y agradables al paladar (Tornberg, 2005). Durante el calentamiento, los componentes del músculo que se afectan principalmente son las proteínas, las cuales se desnaturalizan provocando cambios estructurales en la carne. Dependiendo de la temperatura de cocinado alcanzada, el efecto sobre la estructura de las proteínas será diferente, y consecuentemente, también lo será el efecto sobre la textura de la carne (Díaz Molins, 2009).

A temperaturas de 54-58 ºC, comienzan a producirse cambios en la estructura de la miosina (Martens & Vold, 1976; Wright, Leach, & Wilding, 1977), por encima de los 60 ºC, comienza a verse afectada la estructura del colágeno (Martens & Vold, 1976; Stabursvik & Martens, 1980) y las proteínas sarcoplásmicas (Wright et al., 1977), y por último, temperaturas de 80-83 ºC modifican la estructura de la actina (Wright et al., 1977). Así, cuando la carne es cocinada por encima de los 60-62 ºC el tejido conectivo comienza a desnaturalizarse (Bechtel, 1986), lo que conlleva a un ablandamiento de la carne, y continúa gradualmente con el tiempo (Beilken, Bouton, & Harris, 1986). Por tanto, en lo referente a los cambios producidos en el colágeno por acción del calor, el tiempo juega un papel igual de importante como la temperatura. A nivel estructural, la desnaturalización del tejido conectivo por acción del calor de lugar a fibras musculares con un aspecto granuloso (Garcia-Segovia et al., 2007).

Además de la desnaturalización del colágeno, este incremento en la terneza de la carne a temperaturas en torno a los 60 ºC también es debido a que las proteínas sarcoplasmáticas se agregan formando un gel, el cual facilita la masticación de la misma. Este hecho tiene lugar en el intervalo de temperaturas comprendido entre los 40 y 60 ºC (Hamm, 1977), aunque en

ocasiones se puede extender hasta los 90 ºC (Díaz Molins, 2009). En cambio, la desnaturalización de las proteínas miofibrilares provoca un endurecimiento de la carne, lo que comienza a producirse por debajo de los 60 ºC, llegando a ser más intenso por encima de los 70 ºC (Laakkonen, Sherbon, & Wellington, 1970; Ruiz, Calvarro, Sánchez del Pulgar, & Roldán, 2013; Tornberg, 2005). Como se comentó anteriormente, estos cambios de textura son debidos a los cambios estructurales que se producen al desnaturalizarse las proteínas, y son bastante notables cuando se cocina la carne a partir de 70 ºC (Garcia-Segovia et al., 2007). Además, la pérdida de agua desde el tejido muscular debido al tratamiento térmico, contribuye también al endurecimiento de la carne durante el cocinado de la misma a estas temperaturas (Ruíz, et al., 2013).

En el caso de las carnes cocinadas mediante el método *sous-vide*, se ha constatado que temperaturas de cocinado de 70-80 ºC dan lugar a carnes con texturas más blandas que las cocinadas a temperaturas más bajas (Garcia-Segovia et al., 2007; Sánchez Del Pulgar et al., 2012). Desde el punto de vista sensorial, la transformación del colágeno en gelatina determinará la elección de la temperatura óptima de cocinado en carnes cocinadas al vacío. Del mismo modo, la cantidad de colágeno establecerá el tiempo de duración del tratamiento térmico, ya que a estas temperaturas de cocinado, cuanto más largos sean los tiempos de cocinado, mayor solubilización del colágeno se conseguirá. Por tanto, las carnes de textura más dura, con mayor contenido en colágeno, necesitan tratamientos térmicos de varias horas para conseguir que todo el colágeno se transforme en gelatina. Por otra parte, las carnes blandas, con menor proporción de colágeno y mayor de proteínas miofibrilares, son cocinadas a mayores temperaturas y durante menores tiempos de cocinados para evitar la pérdida de jugosidad (Lawrie, 1998).

La determinación de la textura en carne puede ser realizada mediante un panel de catadores entrenados o, de forma instrumental mediante un texturómetro, el cual permite medir la resistencia del tejido tanto al corte como a la compresión. Muchos instrumentos se han desarrollado para evaluar la dureza de la carne, siendo el más comúnmente utilizado la Warner- Bratzler (WB) (Nikmaram et al., 2011).

Los cambios en la estructura de la carne durante el cocinado, los cuales influyen en las modificaciones de textura de la misma, pueden ser evaluados

mediante un microscopio electrónico de barrido (SEM). Este instrumento ofrece una visión directa de cómo la estructura de la carne cambia cuando se cocina a diferentes temperaturas internas. Del análisis de la carne mediante el SEM, puede observarse cómo el diámetro de la fibra muscular y la longitud del sarcómero, están estrechamente relacionados con la firmeza de la carne (Kong, Tang, Lin, & Rasco, 2008; Palka & Daun, 1999; Wattanachant, Benjakul, & Ledward, 2005).

1.3.3 Oxidación

La oxidación es la principal causa de deterioro de la calidad de la carne durante el procesado y almacenamiento de la misma (Xiong, 2000). Tanto la temperatura como el tiempo de cocinado tienen un marcado efecto en la producción de radicales libres en carnes que conlleva a la oxidación de lípidos y proteínas, lo que a su vez puede causar indeseables cambios en las características sensoriales, el color y en los valores nutritivos de la misma (Promeyrat, Daudin, & Gatellier, 2013).

1. 3.3.1. *Oxidación Lipídica*

La oxidación lipídica es un proceso complejo a través del cual, hidroperóxidos y otros productos primarios de oxidación son formados a partir de ácidos grasos poliinsaturados (PUFA) (Min & Ahn, 2005). A esta auto-oxidación primaria le siguen una serie de reacciones secundarias que conllevan, por un lado, a la degradación de estos hidroperóxidos y, por otro lado, a la formación de un amplio rango de compuestos, incluyendo las sustancias reactivas del ácido tiobarbitúrico (TBARs) y compuestos volátiles. Entre estos compuestos volátiles, nos encontramos con la formación de aldehídos, tales como el hexanal o el 2,4-decadienal, que son producidos como resultado de la oxidación del ácido linoleico y que son responsables de los sabores desagradables en carnes y productos cárnicos oxidados (Hodgen, 2006). Estos aldehídos son ampliamente utilizados como indicadores de la oxidación lipídica en carnes (Min & Ahn, 2005).

Existen muchos factores que influyen en la oxidación lipídica, tales como la composición y el contenido en fosfolípidos de la carne (Carrapiso, 2007), o la concentración y el estado de los pro- o antioxidantes en la misma (Ma,

Ledward, Zamri, Frazier, & Zhou, 2009). En carnes cocinadas, los factores más importantes de los que depende el grado de oxidación son, la temperatura, el tiempo y el método utilizado para el cocinado (Gandemer, Girard, & Desnoyers, 1983). En general, el calentamiento de la carne incrementa la oxidación lipídica (Broncano, Petrón, Parra, & Timón, 2009), la cual puede verse acelerada a mayores temperaturas y tiempos de cocinados utilizados (Hernández, Navarro, & Toldrá, 1999). Cocinados suaves, en torno a los 70-80 ºC, conlleva a la destrucción de las membranas celulares facilitando la interacción de los catalizadores de la oxidación lipídica con los ácidos grasos insaturados, lo que resulta en la formación de más radicales libres y el desarrollo de estos sabores desagradables (Pearson, Love, & Shorland, 1977). Sin embargo, cuando se cocina la carne a temperaturas más elevadas, por encima de 100 ºC, se observa la disminución del nivel de las TBARS (Brown, Morris, Rhodes, Sinha, & Levander, 1995), y la desaparición de estos sabores extraños (Bailey & Um, 1992) debido a la diminución en la concentración de los aldehídos responsables de los mismos (Zamora, Gallardo, & Hidalgo, 2008).

El hexanal es el principal aldehído producido durante la oxidación lipídica en carne y muchos autores han constatado una buena correlación entre éste y las TBARS en diferentes tipos de carnes (Brunton, Cronin, Monahan, & Durcan, 2000; St. Angelo et al., 1987). Esta disminución en la oxidación lipídica a mayores temperaturas de cocinado se ha visto que es posible debido a que los productos secundarios de la oxidación lipídica reaccionan rápidamente con los grupos aminos de determinados amino ácidos como son, la lisina, cisteína y glutatión (Zamora et al., 2008).

Entre las estrategias comerciales que se utilizan para prevenir la oxidación lipídica, a parte del uso de antioxidantes, se encuentra el envasado de los alimentos al vacío, el cual retarda el desarrollo de la rancidez en carne (Popova, Marinova, Veselka, Gorinov, & Lidji, 2009; Xiao, Zhang, Lee, Ma, & Ahn, 2011). Algunos autores (Sánchez Del Pulgar et al., 2012) han estudiado el efecto del cocinado al vacío de la carne durante largos tiempos y temperaturas moderadas, observando también una reducción de las TBARs a mayores temperaturas y tiempos de cocinado.

1. 3.3.2 *Oxidación Proteica*

La oxidación de las proteínas también puede influir de forma negativa en la calidad final de la carne y productos cárnicos. Por un lado, provoca la fragmentación o agregación de las proteínas, reduciendo su solubilidad, pero además, la oxidación proteica altera la actividad de las enzimas proteolíticas, estando este proceso ligado a la terneza de la carne (Mercier, Gatellier, & Renerre, 2004).

Las proteínas pueden ser oxidadas por los mismos factores que producen la oxidación de lípidos. Así, la oxidación proteica puede ser inducida directamente, a través de las especies reactivas del oxígeno o, indirectamente, a través de la reacción con productos secundarios del estrés oxidativo, como lípidos oxidados u otros agentes pro-oxidantes generados durante el procesado (Estevez, 2011). Estas reacciones pueden provocar múltiples cambios físico-químicos en las proteínas, incluyendo la degradación de aminoácidos, la disminución en la solubilización debido a su polimerización, pérdida de actividad enzimática y disminución en su digestibilidad (Traore et al., 2012).

Uno de los cambios más destacados en las proteínas de carne oxidada es la formación de carbonilos proteicos (Estevez, 2011). En productos cárnicos, la evaluación de la oxidación proteica se lleva a cabo a través del método basado en su reacción con 2,4- dinitrofenilhidrazina (DNPH) para formar 2,4-dinitrofenilhidrazona (Estévez, Morcuende, & Ventanas, 2008). Miembros de nuestro grupo de investigación están utilizando compuestos más específicos para evaluar el daño oxidativo en proteínas de origen cárnico, como son los semialdehídos α-aminoadípico y el γ-glutámico (AAS y GGS, respectivamente) (Estevez, Ollilainen, & Heinonen, 2009; Utrera, Estévez, 2013; Utrera, Morcuende, & Estévez, 2014). Estos semialdehídos se consideran los principales compuestos carbonílicos derivados de la oxidación de las proteínas y se han utilizado con éxito como indicadores de la oxidación de proteínas en una amplia gama de productos cárnicos (Armenteros, 2010).

El cocinado de la carne también afecta al metabolismo de estos compuestos. Así, diferentes estudios han demostrado que a medida que aumenta la temperatura y el tiempo de cocinado se produce un aumento del nivel de carbonilos proteicos (Armenteros, Heinonen, Ollilainen, Toldrá, &

Estévez, 2009; Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008). Por otro lado, se ha demostrado que el envasado de la carne al vacío disminuye la oxidación proteica (Filgueras et al., 2010). Sin embargo, no hay información científica sobre cómo el cocinado de la carne al vacío a temperaturas moderadas y tiempos de cocción largos puede afectar a la formación de los carbonilos proteicos.

1.3.4 Aroma

El aroma de la carne cocinada desempeña el papel más importante en la aceptación de la misma por los consumidores (Van Ba, Hwang, Jeong, & Touseef, 2012). Mientras que la carne cruda tiene poco o ningún aroma y solo un ligero sabor a metálico (Mottram, 1998), en la carne cocinada se han detectado y descrito más de 1000 compuestos volátiles (Pegg & Shahidi, 2004). La carne desarrolla su aroma durante el cocinado a partir de las interacciones de los precursores no volátiles, que incluyen aminoácidos libres, péptidos, azúcares, vitaminas, nucleótidos y ácidos grasos insaturados. Estas interacciones incluyen la reacción de Maillard entre compuestos amino y carbonilo, la oxidación de los lípidos, la degradación térmica de la tiamina y las interacciones entre estas vías (Mottram, 1998a).

Cuantitativamente, la oxidación de los lípidos es la principal fuente de compuestos volátiles en la carne cocinada, especialmente la oxidación de los ácidos grasos insaturados (Cheng & Ho, 1998). La menor contribución del resto de las reacciones al volumen de compuestos volátiles no les resta importancia en el desarrollo del aroma final de la carne cocinada, ya que los compuestos formados por estas vías (compuestos heterocíclicos con aroma cárnico) presentan menores umbrales de detección (Van Ba et al., 2012). Entre los compuestos formados se encuentran furanos, furanonas, piranos, piracinas, tiofenos, tiazoles, tiazolinas, oxazolinas y polisulfuros heterocíclicos, cuya combinación produce el aroma básico e inespecífico de la carne. En cambio, las reacciones de oxidación lipídica producen compuestos aromáticos que parecen ser los responsables del aroma específico de cada tipo de carne (Horstein & Wasserman, 1987).

Es bien sabido que tanto la temperatura como el tiempo de cocinado afectan de forma significativa al desarrollo de los compuestos volátiles, y por

tanto al aroma de la carne cocinada (Domínguez, Gómez, Fonseca, & Lorenzo, 2014). En general, se ha observado que a medida que aumenta la temperatura de cocinado, incrementa la formación de compuestos (Ames, Guy & Kipping, 2001). Por ejemplo, la carne de potro asada a 200 ºC presenta una mayor formación de compuestos volátiles que esa misma carne cocinada mediante otros métodos culinarios que implican temperaturas más bajas, como son el cocinado a la plancha, en el microondas o frito (Domínguez et al., 2014). Además, dependiendo de las condiciones de cocinado utilizadas, la proporción de los diferentes compuestos volátiles será diferente (King, Matthews, Rule & Field, 1995). Así, a temperaturas por encima de 140 ºC, la reacción de Maillard se intensifica, estando favorecida por la deshidratación de la superficie de la carne (MacLeod, Seyyedain-Ardebili, & Chang, 1981). Por otro lado, los compuestos volátiles procedentes de la degradación lipídica predominan en las carnes cocidas o ligeramente asadas (Mottram, 1985). Con relación a los compuestos azufrados, los tiazoles y piridinas, son asociados a las carnes asadas, mientras que los tioles están más relacionados con carnes cocinadas a bajas temperaturas (70 - 100 ºC) (Almela, et al., 2010).

Cuando se cocina la carne al vacío, el uso de temperaturas moderadamente altas (80 ºC) estimula la formación de compuestos volátiles procedentes de las reacciones de degradación de los aminoácidos y las reacciones de Maillard, con la consiguiente formación de compuestos aromáticos con un deseable aroma a carne. Por el contrario, estas temperaturas provocan una disminución en la formación de compuestos procedente de la degradación de los ácidos grasos, normalmente asociados con aromas indeseables en la carne (Sanchez del Pulgar, Roldan, & Ruiz-Carrascal, 2013). Otro autores han observado una menor formación de compuestos volátiles en hamburguesas de cerdos irradiadas y cocinadas al vacío, comparadas con esas mismas hamburguesas irradiadas y cocinadas en presencia de aire (Ahn et al., 1998). Parece ser que el envasado al vacío tiene un escaso efecto en la formación de compuesto aromáticos durante el cocinado de la carne cuando se aplican bajas temperaturas y largos tiempos (LT-LT) (Sanchez del Pulgar et al., 2013).

No obstante, existe poca información científica en relación a cómo afecta el cocinado al vacío sobre el perfil de compuestos volátiles de la carne

cuando se aplican tales condiciones de temperatura y tiempo.

1.3.5 Aspectos microbiológicos

Al mismo tiempo que el cocinado debe conseguir que la carne tenga una textura tierna y jugosa, no hay que olvidar que el principal objetivo es proporcionar alimentos seguros y con una extensa vida comercial. Sin embargo, podría ocurrir que las temperaturas necesarias para conseguir una textura adecuada en el alimento puedan no ser capaces de asegurar la conservación del alimento durante su almacenamiento. En este sentido, la calidad microbiológica de la materia prima es fundamental para garantizar la seguridad de los productos *sous-vide*, ya que la supervivencia de patógenos durante el tratamiento depende de la carga inicial (Nyati, 2000).

La tecnología *sous vide* ha sido asociada a diversos casos de intoxicaciones alimentarias a gran escala (Rhodehamel, 1992). El principal riesgo que puede encontrarse es *Clostridium perfringens*, aunque otros microorganismos anaeróbicos esporulados, tales como *Bacillus cereus* y *Clostridium botulinum*, podrían aparecer (Borch & Arinder, 2002). La proliferación de *C. perfringens* en este tipo de productos está asociado condiciones de refrigeración y de recalentamiento inadecuadas ya que no prolifera a temperaturas inferiores a 10 °C. Algunos microorganismos psicrotrofos no esporulados como son *Listeria monocytogenes*, *Staphylococcus auresus*, *Salmonella spp.* y *Escherichia coli* O157:H7, pueden también proliferar, aunque son inactivados usando temperaturas de cocinado moderadas (Sutherland & Porrit, 1997).

Así, es indispensable establecer una relación temperatura/tiempo óptima con el fin de alcanzar un equilibrio entre la seguridad y la calidad sensorial y nutricional de los platos cocinados. La temperatura empleada debe superar los 65 °C con el fin de inactivar células vegetativas y destruir la microflora inicialmente presente en el alimento (*Pseudomonas* spp., *Enterobacteriaceae*, *lactobacillus* spp. y otras bacterias potencialmente patógenas no formadoras de esporas) (Díaz Molins, 2009). Se recomienda que el tratamiento térmico aplicado a este tipo de productos garantice la reducción de 6 unidades logarítmicas de *L. monocytogenes* o bien temperaturas de 70 °C en el interior del producto para una vida útil de 3

semanas en condiciones de refrigeración (Brown, 1991; Rhodehamel, 1992). Schellekens (1996) indica que el tratamiento térmico debe asegurar 6 reducciones decimales en los recuentos de esporas de la variedad no proteolítica de *C. botulinum* y de los patógenos vegetativos *Listeria*, *Salmonella* y *Escherichia coli*. Otros autores señalan la necesidad de que el tratamiento térmico asegure una reducción de 12-13 unidades logarítmicas del microorganismo *Streptococcus faecalis*, garantizando la pasterización el producto (Simpson et al, 1994).

No obstante, Baldwin (2012) contempla procesos de cocción para productos *sous-vide* donde la combinación de la temperatura y el tiempo está fuera de estas recomendaciones (Tabla 1), si bien limita su vida útil tras su cocinado a las 4 horas estipuladas por la FDA para alimentos no pasterizados (Food Code, 2013).

Tabla 1: Tiempo aproximado (horas: minutos) para calentar y cocinar la carne de vacuno refrigerada en función de su grosor.

Grosor	Temperatura del baño de agua °C											
(mm)	55	56	57	58	59	60	61	62	63	64	65	66
5	1:16	0:54	0:38	0:28	0:21	0:17	0:14	0:12	0:09	0:08	0:07	0:06
10	1:24	1:02	0:47	0:38	0:31	0:27	0:23	0:21	0:19	0:18	0:16	0:15
15	1:37	1:15	1:00	0:51	0:44	0:39	0:35	0:32	0:30	0:28	0:26	0:25
20	1:54	1:32	1:17	1:06	0:59	0:53	0:49	0:45	0:42	0:39	0:37	0:35
25	2:08	1:46	1:31	1:20	1:11	1:05	1:00	0:56	0:52	0:49	0:47	0:44
30	2:23	2:00	1:44	1:33	1:24	1:17	1:11	1:07	1:03	0:59	0:56	0:54
35	2:38	2:15	1:58	1:46	1:36	1:29	1:23	1:18	1:13	1:09	1:06	1:03
40	2:53	2:29	2:12	1:59	1:49	1:41	1:34	1:29	1:24	1:20	1:16	1:13
45	3:15	2:51	2:32	2:18	2:07	1:58	1:51	1:45	1:39	1:34	1:30	1:27
50	3:39	3:13	2:54	2:39	2:27	2:17	2:09	2:02	1:56	1:50	1:46	1:41
55	4:04	3:37	3:17	3:01	2:48	2:37	2:28	2:20	2:13	2:07	2:02	1:57
60	4:31	4:03	3:42	3:24	3:10	2:58	2:48	2:40	2:32	2:26	2:20	2:14
65	4:59	4:30	4:07	3:49	3:34	3:21	3:10	3:00	2:52	2:45	2:38	2:32
70	5:30	4:59	4:34	4:14	3:58	3:44	3:32	3:22	3:13	3:05	2:58	2:51

Baldwin (2012)

Por otro lado, durante almacenamientos prolongados en refrigeración

los productos *sous vide* preparados usando procesos de cocción moderados presentan un ambiente donde cepas no proteolíticas (tolerantes al frío) de *C. botulinum* son un peligro evidente. El envasado al vacío que restringe el crecimiento de las bacterias aeróbicas, pero favorece el crecimiento de bacterias anaerobios; mientras que el tratamiento térmico moderado elimina gran parte de las células vegetativas de las bacterias, pero no inactiva las esporas bacterianas. La mayoría de los problemas de seguridad alimentaria sobre este tipo de productos se han centrado en el potencial de crecimiento y de producción la toxina por parte de estas cepas (Peck, 1997). Las recomendaciones a este respecto de la Comisión Consultiva en Seguridad Microbiológica de Alimentos del Reino Unido (ACMSF, 1992) se resumen en:

- almacenamiento a <3,3 ºC.
- almacenamiento a <5 °C con una vida útil de <10 días.
- almacenamiento a 5-10 ºC con una vida útil de <5 días.
- almacenamiento a temperatura de refrigeración en combinación con un tratamiento térmico de 90 ºC durante 10 min o equivalentes.
- almacenamiento a temperatura de refrigeración en combinación con un valor de pH <5 en el producto.
- almacenamiento a temperatura de refrigeración en combinación con una concentración de sal > 3,5% en el producto.
- almacenamiento a temperatura de refrigeración en combinación con una $a_w < 0,97$ en el producto.
- almacenamiento a temperatura de refrigeración en combinación con combinaciones de tratamiento térmico y otros factores de conservación que garanticen la inhibición del crecimiento y la producción de toxinas de *C. botulinum*.

Otro patógeno a tener en cuenta en productos *sous-vide* bajo condiciones de refrigeración durante periodos prolongados es *L. monocytogenes*. Este microorganismo es uno de los patógenos no esporulados transmitidos por los alimentos más resistentes al calor que también es capaz de crecer a temperaturas de refrigeración. Aunque la listeriosis es una enfermedad rara, su gravedad y la frecuente implicación de alimentos procesados en brotes de listeriosis, hacen de esta enfermedad una de las más importantes de las transmitidas por los alimentos desde el punto de vista de

impacto social y económico (OMS, 2004). Son varios los autores que han estudiado la posible proliferación de este microorganismo en productos *sous-vide* a partir de células con lesiones subletales tras el tratamiento térmico (Knabel et al., 1990; Hansen y Knøchel, 2001).

Por otro lado, el deterioro causado por microorganismos alterantes en los productos cárnicos cocinados conservados en refrigeración se traduce en defectos en la calidad sensorial de los mismos, como puede ser la formación de sabores y olores extraños, decoloración, cambios texturales, producción de gas o formación de limo en superficie (Hu, Zhou, Xu, Li, & Han, 2009). Nyati, (2000) estudiaron la microbiología del varios productos cárnicos cocinados a vacio (70 °C/2min) y almacenado a diferentes temperaturas de refrigeración (3 y 8 ºC) durante 5 semanas, obteniendo recuentos microbiológicos bajos incluso durante todo el tiempo de almacenamiento cuando la temperatura fue de 3 ºC. No obstante, este autor detectó problemas microbiológicos cuando la temperatura de almacenamiento fue 8 °C, con recuentos altos de bacterias anaerobias mesófilas (6,6 log ufc/ g), bacterias ácido lácticas (5,6 log ufc/g) y *Pseudomonas* (6 log ufc/g). Recuentos importantes de las bacterias anaerobias mesófilas (hasta 4 ufc/g) y bacterias ácido lácticas (más de 3 log ufc/g) también se observaron en los espaguetis y salsa de carne (65 °C/15 min y 75 °C/37 min ; 40 días a 5 °C) (Simpson et al, 1994 .) y alas de pollo (75 °C/24 min o 90 °C/13 min; 7 semanas en 2 °C) (Wang y col., 2004). Según estos últimos autores, la incidencia (%) de microorganismos anaerobios en las alas de pollo fue del 15% cocos Gram negativos, 49 % bacilos Gram positivo, 23 % bacilos Gram negativo y el 13% levaduras. Por el contrario, Hansen y col., (1995) no detectaron anaerobios mesófilos en carne de vacuno asada (62 °C/16 min, 5 semanas a 2 °C). Igualmente, Díaz y col. (2008) cocinaron carne de cerdo a 70 ºC durante 12h obteniendo un producto microbiológicamente seguro incluso después de 10 semanas de almacenamiento a 2 ºC.

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2. Planteamiento y Objetivos

El presente proyecto pretende profundizar en los conocimientos sobre los cambios que acontecen en la carne de cordero cuando es cocinada al vacío a temperaturas moderadas y durante tiempos prolongados. Este tipo de cocinado es empleado con asiduidad hoy en día por un gran número de cocineros de alta restauración. Los datos que existen respecto a los efectos que este tipo de cocinado tienen sobre la carne son fundamentalmente empíricos, aunque el equipo de investigación de la unidad de Tecnología de los Alimentos de la Universidad de Extremadura ha realizado estudios previos utilizando estas condiciones de cocinado sobre la carne de cerdo, existiendo en ambos casos una unanimidad en las repercusiones positivas que tiene sobre la terneza y jugosidad de la carne. No obstante, a parte de la textura instrumental, apenas se dispone de información científica sobre otros aspectos tales como el efecto sobre la generación de compuestos volátiles, la conservabilidad, el efecto sobre la viabilidad de determinados microorganismos, o sobre la microestructura del músculo. Por otra parte, se quiere intentar extender los conocimientos generados sobre este tipo de cocinado al vacío a temperaturas relativamente bajas (en torno a 60ºC), más vinculado a la restauración, para intentar aplicarlos a la carne cocinada y envasada en la industria, tanto del catering como de comidas preparadas, para de esta manera intentar mejorar las características de este tipo de productos.

Se ha elegido la carne de cordero, por dos razones; en primer lugar, se trata de un producto que se adapta a este tipo de cocinado, y de hecho, existe un gran número de restaurantes que en la actualidad han variado el tipo de cocinado tradicional en horno, para adaptarse a un cocinado a vacío durante tiempos prolongados a temperaturas moderadas. Por otra, en la actualidad existe una gran crisis del sector productor del ganado ovino, con dificultades evidentes en la obtención de márgenes económicos, y disminución año tras año en los niveles de consumo per cápita. La investigación en líneas de comercialización diferentes con un alto valor añadido, como son los platos preparados, podría facilitar en el futuro una válvula de escape para este tipo de producción, ya que el consumidor actual demanda con cada vez más frecuencia productos fáciles de preparar o ya preparados. De hecho, el consumo de platos preparados, según datos del

Ministerio de Agricultura, Alimentación y Medio Ambiente del año 2012, ha crecido de 10,1 kilos en el año 2005 a 11,9 en 2011.

Si bien el cocinado de la carne al vacío presenta evidentes ventajas desde el punto de vista de la textura de la misma, también presenta una serie de inconvenientes bien definidos que podrían dificultar el éxito en su aplicación a la elaboración de platos preparados. Así, no existen muchos datos científicos sobre el efecto de tratamientos térmicos a temperaturas moderadas (aprox. 63 ºC) y tiempos muy prolongados (más de 12 horas) sobre la viabilidad de determinados microorganismos patógenos y alterantes. Dicha información es crucial si se pretende adaptar este tipo de proceso a platos que van a ser almacenados y distribuidos. Por otro lado, el almacenamiento a refrigeración de la carne cocinada a vacío lista para el consumo durante tiempos comerciales (bastante prolongados para poder tener viabilidad económica) puede suponer un desarrollo importante de las reacciones de oxidación lipídica, como de hecho pasa en la gran mayoría de platos preparados.

Finalmente, en los últimos años se está desarrollando una importante colaboración entre cocineros y científicos, que ha dado lugar a la creación de numerosas herramientas novedosas en el mundo da la restauración. En lo referente al cocinado de la carne, se han propuesto estrategias importadas de la industria alimentaria, tales como el uso de aditivos que aumentan la capacidad de retención de agua de la carne (y por lo tanto su jugosidad) como son los fosfatos, o el uso de diferentes azúcares (que actúan como precursores en las reacciones de Maillard) para modificar el aroma final de la carne cocinada. Sin embargo estas estrategias se han probado de una manera puntual y empírica, existiendo una total falta de datos científicos en su uso para carne de cordero cocinada.

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

1. Estudiar el efecto que tienen diferentes combinaciones de temperatura y tiempo sobre las características físico-químicas, texturales, estructurales y microbiológicas de lomos de cordero cocinados al vacío.
2. Evaluar el efecto que tienen diferentes combinaciones de temperatura y tiempo sobre los productos primarios y secundarios procedentes de la oxidación lipídica y proteica en lomos de corderos cocinados al vacío.
3. Analizar la influencia que tienen diferentes combinaciones de temperatura y tiempo sobre el color instrumental y las características oxidativas, texturales y microbiológicas de lomos de corderos cocinados al vacío y almacenados en refrigeración.
4. Estudiar el efecto de diferentes combinaciones de temperatura y tiempo sobre la generación de compuestos volátiles en lomos de corderos cocinados al vacío.
5. Determinar la influencia de diferentes niveles de fosfatos sobre las características físico-químicas y calidad sensorial de lomos de corderos cocinados mediante dos tratamientos culinarios diferentes: asado al horno y cocinado al vacío.
6. Investigar el efecto de la adición de diferentes precursores del flavor (glucosa, ribosa, cisteína y tiamina) sobre las características sensoriales y el desarrollo de la reacción de Maillard, en lomos de corderos cocinados mediante dos tratamientos culinarios: asado al horno y cocinado al vacío.

Taking into account the aforementioned approaches, the aims of this Doctoral Thesis were the following:

1. Studying the effect of sous-vide cooking of lamb loins at different temperature–time combinations on different physico-chemical, textural, microbiological and structural features.
2. Evaluating the effect of different time–temperature combinations on primary and secondary products from lipid and protein oxidation in sous-vide cooked lamb loins.
3. Analyzing of the influence of different temperature-time combinations on instrumental color and oxidative, textural and microbial features of sous-vide cooked lamb loins stored under refrigeration.
4. Studying the effect of different combination of time and temperature of sous-vide cooking lamb loins on the generation of volatile compounds.
5. Determining the influence of different phosphate levels on the physico-chemical characteristics and sensorial quality of lamb loins cooked using two different culinary procedures: roasting in the oven or sous-vide cooking.
6. Researching the effect of addition of several flavor precursors (glucose, ribose, cysteine and thiamin) on sensory features and development of Maillard reaction in either sous-vide cooked or roasted lamb loins.

3. Diseño Experimental

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

SECCION I

En esta sección se incluyen los capítulos 1.1, 1.2, 1.3 y 1.4 que tuvieron como objetivo estudiar el efecto del cocinado al vacío a temperaturas moderadas y tiempos prolongados sobre las características físico-químicas, texturales, estructurales y microbianas de la carne de cordero. Así como su estabilidad oxidativa, perfil de compuestos volátiles y su conservabilidad en refrigeración.

Para ello, nueve combinaciones diferentes de temperatura (60, 70 y 80 °C) y tiempo (6, 12 y 24 h) se utilizaron para cocinar 45 lomos de corderos (5 muestras por cada lote). Además, 5 lomos sin cocinar se usaron como control, y en ellos se realizaron los mismos análisis que en los cocinados. Todos los lomos de cordero procedieron del mismo lote de producción, con una media de 26 kg de peso vivo y 90 días de edad. Las muestras fueron pesadas, envasadas en bolsas de plástico (bolsas de nylon/polietileno; termo-resistentes de −40 °C/+120 °C, permeabilidad al oxígeno de 9 cm^3/m^2 cada 24 h a 4 °C/80 % de humedad relativa y permeable al vapor de agua de 1.2 g/m^2 cada 24 h) (Joelplas SL, Barcelona, Spain) y cocinadas en baños termostatizados a diferentes combinaciones de temperatura y tiempo. La temperatura interna fue monitoreada usando un registrador de datos Testo735-2 (Testo, Lenzkirch, Germany) equipado con una sonda termopar. Una vez que el proceso de cocinado finalizó, las bolsas fueron sumergidas en agua fría (2 ºC) durante 1 h, tras lo cual, los lomos envasados fueron guardados en refrigeración durante toda la noche. Al día siguiente, se llevaron a cabo los diferentes análisis: pesaje, contenido de humedad, color instrumental, características de textura instrumental y análisis sensorial. Además, se cogieron muestras para llevar a cabo la microbiología y el análisis microscópico. El sobrante de cada lomo fue envasado al vacío y guardado en el congelador a -80ºC para analizar posteriormente el análisis de la oxidación lipídica y proteica de los mismos, así como el análisis del perfil de volátiles.

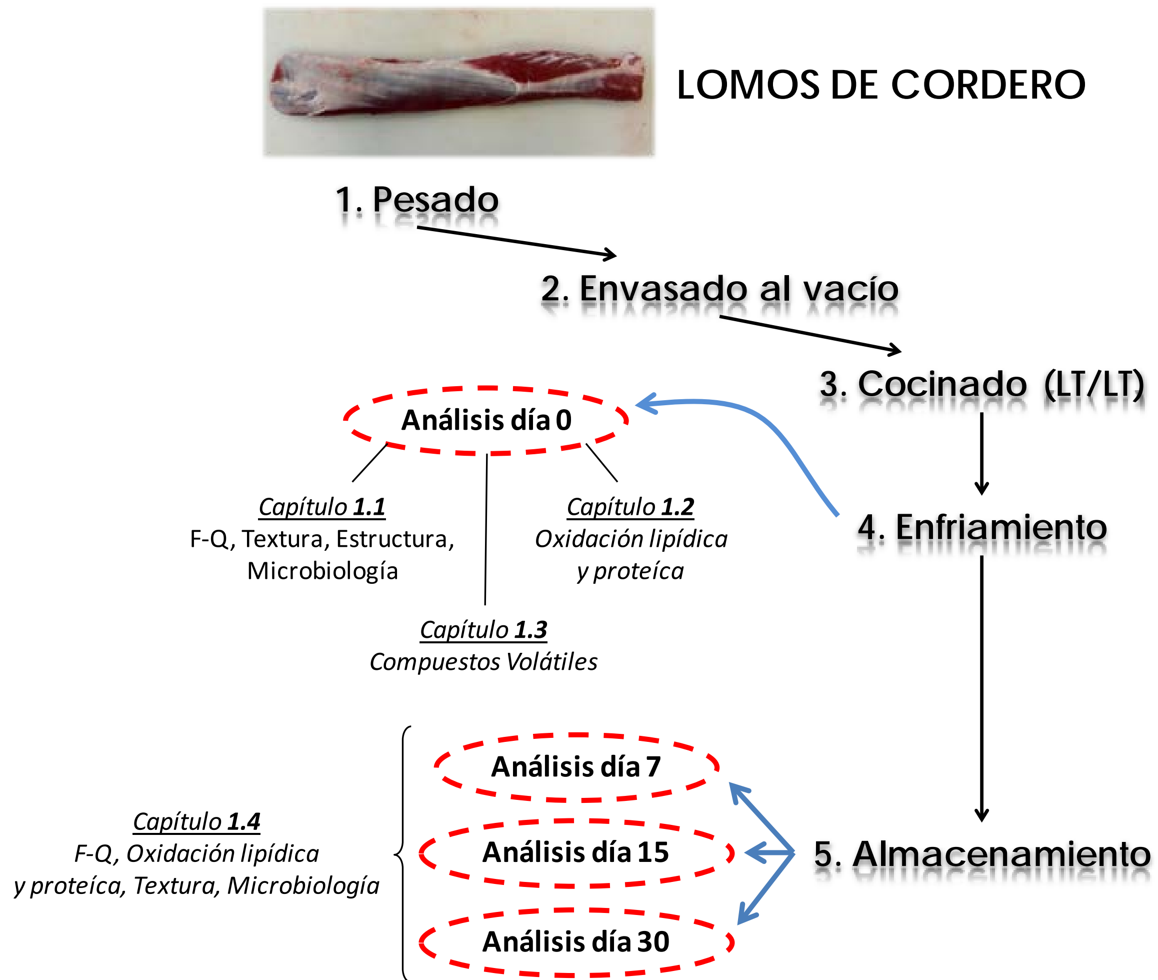

Figura 1. Diseño experimental de la sección I

SECCION II

Los capítulos de la sección II tuvieron como objetivo estudiar diferentes estrategias para mejorar las características sensoriales de la carne de cordero cocinada al vacío. Entre estas estrategias se encuentran el uso de fosfatos para mejorar la capacidad de retención de agua de la carne y con ello la textura de la misma (cap. 2.1) o, el uso de azúcares para potenciar el desarrollo de notas aromáticas a asado (cap. 2.2).

Al igual que en los capítulos de la sección I, todos los lomos de corderos utilizados en esta sección procedieron de un mismo lote de producción, con una media de 26 kg de peso vivo y 90 días de edad.

En el capítulo 2.1 se partieron de 48 lomos, los cuales fueron divididos en tres grupos, pesados individualmente e inyectados con una salmuera hasta alcanzar el 110% de su peso inicial. La salmuera inyectada estaba formada por una solución de Trifosfato de Sodio (STPP) y Pirofosfato de Tetrasodio (TSPP) a tres niveles: 0% (se inyectó agua destilada), 2% y 4%. Una vez inyectada la salmuera, los lomos de cordero fueron envasados al vacío y sometidos una hora de "tumbling" para conseguir una distribución homogénea de la salmuera por todo el lomo. Tras esto, los lomos fueron desempaquetados, pesados nuevamente y almacenados en refrigeración durante toda la noche. Al día siguiente, la mitad de los lomos de cada grupo inyectado con un nivel de fosfatos diferente fue envasado al vacío y cocinado en un baño termostatizado a 60ºC durante 12 h, mientras que la otra mitad de los lomos de cada grupo fueron cocinados en un horno a 180ºC hasta alcanzar una temperatura interna de 73 ºC. Tras el cocinado, se procedió al enfriamiento de los lomos en un baño con agua fría (2 ºC) hasta llevar a cabo el análisis sensorial.

En el capítulo 2.2 se partió de 20 lomos de corderos, los cuales fueron divididos en dos grupos y pesados individualmente. Un grupo fue inyectado con agua destilada hasta alcanzar el 110% de su peso inicial, mientras que el otro grupo fue inyectado, en las mismas condiciones que el grupo anterior, con una solución potenciadora del aroma compuesta por azúcares, aminoácidos y vitaminas. A partir de aquí, los lomos siguieron el mismo procedimiento que los lomos del capítulo anterior, siendo sometidos a "tumbling" y cocinados mediante dos tratamientos culinarios diferentes. Al

igual que en el capítulo anterior, tras el cocinado se procedió al enfriamiento de los lomos y se llevaron a cabo los diferentes análisis.

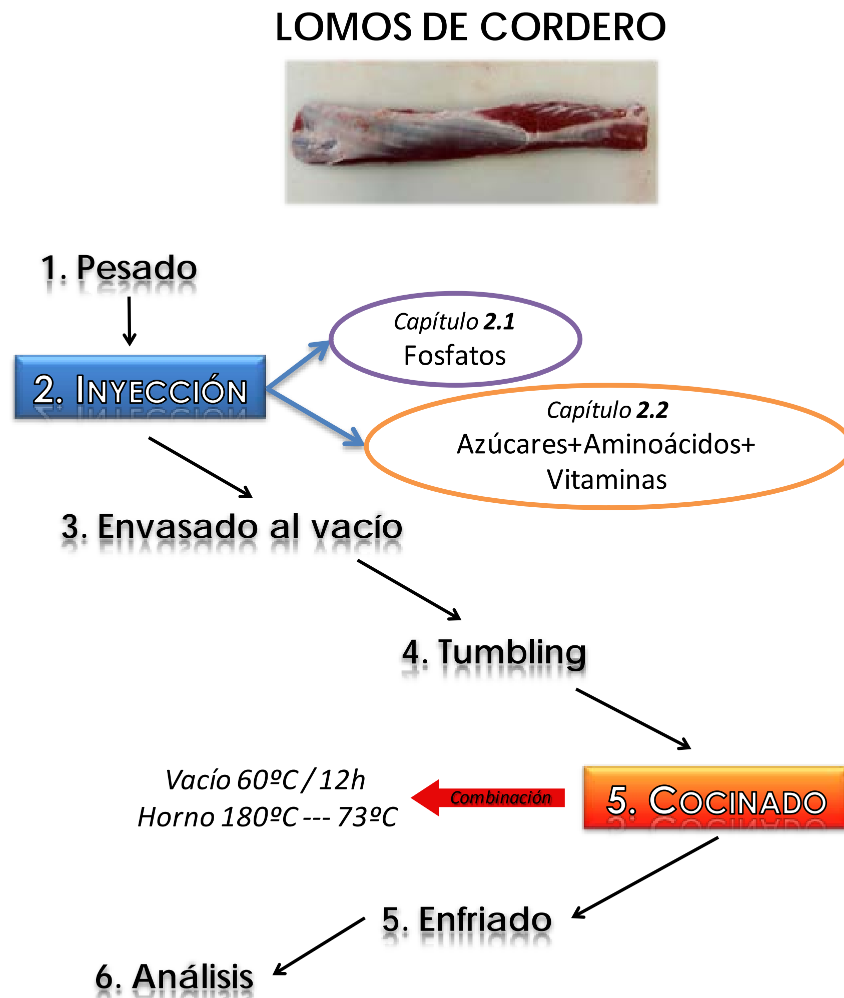

Figura 2. Diseño experimental de la sección II

4. Capítulos

Capítulo 1.1

Effect of different temperature-time combinations on physicochemical, microbiological, textural and structural features of *sous-vide* cooked lamb loins

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Effect of different temperature–time combinations on physicochemical, microbiological, textural and structural features of sous-vide cooked lamb loins

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ABSTRACT

Lamb loins were subjected to sous-vide cooking at different combinations of temperature (60, 70, and 80 °C) and time (6, 12, and 24 h). Different physicochemical, histological and structural parameters were studied. Increasing cooking temperatures led to higher weight losses and lower moisture contents, whereas the effect of cooking time on these variables was limited. Samples cooked at 60 °C showed the highest lightness and redness, while increasing cooking temperature and cooking time produced higher yellowness values. Most textural variables in a texture profile analysis showed a marked interaction between cooking temperature and time. Samples cooked for 24 h showed significantly lower values for most of the studied textural parameters for all the temperatures considered. Connective tissue granulation at 60 °C and gelation at 70 °C were observed in the SEM micrographs. The sous-vide cooking of lamb loins dramatically reduced microbial population even with the less intense heat treatment studied (60 °C–6 h).

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1. Introduction

Sous-vide cooking can be defined as the cooking of raw materials under controlled conditions of temperature and time, inside heat-stable vacuumized pouches or containers followed by rapid cooling (Baldwin, 2012; García-Segovia, Andrés-Bello, & Martínez-Monzo, 2007). This technique is nowadays used in restaurants, catering and industrial processing because of its ease and appropriateness for the management of prepared foodstuff, providing the manipulation of the already cooked food after the thermal treatment with no risk of microbial contamination, and thus, increasing food shelf-life (Armstrong, 2000). The sous-vide cooking conditions suggested by chefs for different types of meat are very different to those used for traditional cooking methods or in catering. Thus, commonly recommended combinations of temperature and time by chefs for beef, pork or lamb are around 58–63 °C for 10–48 h (Myhrvold, Young, & Bilet, 2011) while temperatures for pork in catering most likely reaches 75–80 °C (Armstrong, 2000).

Both, temperature and cooking time have a large effect on the eating quality of meat (Christensen, Ertbjerg, Aaslyng, & Christensen, 2011). As a consequence of heating, several changes take place in meat, such as protein denaturation, fiber shrinkage or collagen solubilization (Tornberg, 2005). While the latter has a tenderizing effect, most changes affecting the myofibrillar proteins during cooking cause an increase in toughening (Laakkonen, Wellington, & Sherbon, 1970; Nikmaram, Yarmand, Emamjomeh, & Darehabi, 2011).

There is only limited information about the effect of low temperature–long time (LT–LT) combinations on the characteristics of sous-vide cooked meats It seems that under these conditions, there occurs an intense collagen solubilization, which in turn leads to a great formation of gelatin, while the myofibrillar based toughening is still not very intense (Sánchez del Pulgar, Gázquez, & Ruiz-Carrascal, 2012). Other authors have reported the effect of such LT–LT sous-vide cooking treatments on instrumental color, water losses, moisture content, and instrumental texture of microbial counts of different meats (Christensen et al., 2011; Díaz, Nieto, Garrido, & Bañón, 2008; García-Segovia et al., 2007; Hansen, Knøchel, Juncher, & Bertelsen, 1995). However, despite the fact that lamb meat is frequently sous-vide cooked at this LT–LT conditions in many restaurants and catering (Myhrvold et al., 2011), as far as our knowledge, there is not scientific information available for sous-vide cooked lamb meat. On the other hand, the lamb meat sector has traditionally been a mainstay of the economy of several regions in Spain. However, in the last 10–20 years, there has been a decrease in the per capita consumption of lamb meat, due to several factors, one of them being the long time that preparation and cooking of traditional lamb recipes involve. The production of sous-vide, ready to eat, lamb meat based dishes could be an interesting alternative for the commercialization of this livestock.

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On the other hand, the use of such cooking temperatures might not be enough for destruction of some spore-forming pathogenic bacteria and other spoilage microorganisms, like lactic acid bacteria (Díaz, Garrido, & Bañón, 2010; Vaudagna et al., 2002). However, there is not much scientific information about the effect of these temperatures for such long cooking times on counts for different microbial groups.

Thus, this research was aimed to study the effect of sous-vide cooking of lamb loins at different temperature–time combinations on different physico-chemical, textural, microbiological and structural features.

2. Materials and methods

2.1. Experimental design

Lamb loins sous-vide cooked at different temperature–time combinations were studied. The *Longissimus dorsi* muscle was chosen because it is a top quality primal that is frequently sous-vide cooked both in restaurants and in the catering industry.

Nine different combinations of time (6, 12 and 24 h) and temperature (60, 70 and 80 °C) were used for cooking 45 lamb loins ($n=5$ for each batch). In addition, 5 lamb loins were used for performing analyses of fresh meat. All loins were from a homogeneous production batch of lamb averaging 26 kg live weight and 90 days of age.

Samples were weighed, packaged in vacuum plastic bag (nylon/polyethylene pouches; heat resistance of −40 °C/+120 °C, O_2 permeability of 9 cm^3/m^2 per 24 h at 4 °C/80% HR and water steam permeability of 1.2 g/m^2 per 24 h) (Joelplas SL, Barcelona, Spain) and cooked in thermostatized water baths at different temperature–time combinations. The internal temperature was monitored using a data logger Testo735-2 (Testo, Lenzkirch, Germany) equipped with a needle thermocouple. Once the cooking process had finished, the pouches were removed from the water bath and submerged in iced cold water (2 °C) for 1 h. Subsequently, the packaged loins were kept under refrigeration (2 °C) overnight.

The day after the cooking process, weight, moisture content, instrumental color, and instrumental texture features were measured. In addition, samples for microbiology and microscopy were taken. The rest of the sample was kept at −80 °C until analysis.

2.2. Moisture content and water losses

Cooking losses were calculated by difference of weight before and after cooking and moisture content was determined by drying the samples (5 g) at 102 °C (A.O.A.C., 2000).

2.3. Instrumental color measurement

Color was measured across the cut surface of the cooked loin after chilling. L* value (lightness), a* value (redness) and b* value (yellowness) were obtained using a Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) programmed to used the built-in internal illuminant D65. Means of readings on three locations on each sample were determined. Before each series of measurements, the instrument was calibrated using a white ceramic tile.

2.4. Instrumental texture analyses

Texture analyses were performed in a texturometer TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). For the determination of the texture profile analysis (TPA), uniform portions of the cooked loins were cut into 1 cm^3 cubes. For each sample, eight cubes were obtained and analyzed. They were axially compressed to 50% of the original height with a flat plunger of 50 mm in diameter (P/50) at a crosshead speed of 2 $mm \cdot s^{-1}$ through a 2-cycle sequence. The following texture parameters were measured from force deformation curves (Bourne, 1978): Hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); Adhesiveness (N·s) = work necessary to pull the compressing plunger away from the sample; Springiness (cm) = height that the sample recovers during the time that elapses between the end of the first compression and the start of the second; Cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required to for the first compression and A2 the total energy required for the second compression); and Chewiness (N·cm) = the work needed to chew a solid sample to a steady state of swallowing (hardness × cohesiveness × springiness).

Shear force analysis on cooked samples was performed using a Warner–Bratzler blade (3 × 1 × 1 cm), which sheared the specimen perpendicularly to the muscle fibers at a constant speed of 1 $mm \cdot s^{-1}$ and then pushed through the slot. The maximum force (N) required to shear the sample was measured. Six determinations were performed for each cooked sample.

2.5. Microbiology

In order to carry out the counts, 10 g of the sample of the loins were homogenized in 90 ml sterile 0.1% peptone in a Stomacher (Lab Blender, Model 4001, Seward Medical, London, UK) for 30 s. Appropriate dilutions were made with 0.1% peptone broth and 1 ml was plated onto the culture media under the following conditions. Total mesophilic and psychrotrophic counts on Plate Count Agar (PCA, Oxoid, Unipath, Basingstoke, UK) for 72 h at 30 °C and 7 days at 7 °C, respectively; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBG, Oxoid) for 24 h at 37 °C; Coliforms on Violet Red Bile Agar (VRBA, Oxoid) for 24 h at 37 °C; lactic acid bacteria, LAB, on MRS Agar (Oxoid) in anaerobic conditions for 72 h at 30 °C; Gram-positive Catalase-positive cocci on Mannitol Salt Agar (MSA, Oxoid) after 72 h at 37 °C; sulfite reducing clostridia on Sulfite-Polymyxin-Sulfadiazine (SPS) agar incubated anaerobically for 72 h at 37 °C; intestinal enterococci on Slanetz and Bartley agar (S&B, Oxoid) for 24 h at 37 °C; and *Brochothrix thermosphacta* on Streptomycin Thallous acetate actidione agar (STAA, Oxoid) for 72 h at 20 °C. Typical colonies for each selective media were counted in plates from the dilution with 10–100 colonies.

Listeria and *Salmonella* were also researched. For these pathogens, 25 g sample was homogenized in 225 ml of primary enrichment broth (buffered peptone water) and incubated at 30 °C for 24 h. Then, 1 ml of these primary enrichments were transferred to 10 ml of *Listeria* Enrichment Broth (LEB, Merck) and incubated at 30 °C for 24 h. The enrichments were subcultured by streaking onto PALCAM *Listeria* selective agar (Merck) supplemented with PALCAM *Listeria* selective supplement (Merck) and incubated at 30 °C for 24 h. Detection of *Salmonella spp.* was carried out according to the International Standard Organization protocol (ISO 6579, 2002).

2.6. Microscopy analysis

Samples of 1 cm^3 each were immediately immersed in buffered formaldehyde solution (10% v/v), followed by dehydration in ethanol solution (15 min each) at increased concentrations (50–100% v/v). Then, immersed in ethanol:xylol solution (50:50 vol:vol) for 15 min at 4 °C, and finally included in paraffin. Transversal sections, 10 μm thick were cut using a microtome LEICA RM 2255 and allowed to air-dry. Sections were stained with hematoxilin–eosin solution. From these stained samples, muscular fiber diameters were measured in around 200 muscle fibers per sample, using an optical microscope NIKON ECLIPS 80i and equipped with a digital camera NIKON DXM 1200F. 2.2.

2.7. Cryo-scanning electron microscopy (Cryo-SEM)

Cryo-SEM was performed with Cryostage CT-1500C equipment from Oxford Instruments, coupled to a Jeol JSM 5410 Scanning Electron Microscope. Samples were immersed in slush Nitrogen at temperature less or equal to minus 210 °C and were rapidly transferred to a Cryostage at 10^{-2} bar, where it was fractured and gold-coated (2 mbar, 2 mA, 4 min). The observation in the scanning electron microscope was carried out at 15 kV, at a temperature below minus 130 °C, and at a working distance of 15 mm.

2.8. Statistical analysis

The effect of cooking time (6, 12, and 24 h) and cooking temperature (60, 70, and 80 °C) was analyzed by a two-way analysis of variance (3 cooking times × 3 cooking temperatures) together with their interaction using the GLM procedure (SPSS 15.0). The Tukey's test was used at the 5% level to make comparisons between sample means when pertinent.

3. Results and discussion

3.1. Cooking weight losses and moisture content

Results for cooking losses and moisture content are shown in Table 1. Both weight losses and moisture content were affected by cooking temperature ($P<0.001$ and $P=0.019$ respectively). As expected, there was an increase in weight losses as temperature rose. These results are basically in accordance with those reported by Vaudagna et al. (2002), Palka (2003) and García-Segovia et al. (2007) for beef or those obtained by Sánchez del Pulgar et al. (2012) for pork. Myofibrillar proteins hold most of the water retained within the muscle. Increasing temperatures cause denaturation and shrinkage of such proteins in the range of 40 °C–90 °C (Tornberg, 2005; Vaudagna et al., 2002) and also shrinkage of collagen in the range of 56–62 °C (Larick & Turner, 1992; Tornberg, 2005). Up until 60 °C the muscle fibers shrink transversely and widen the gap between fibers, but above this temperature the muscle fibers shrink longitudinally and cause substantial water loss and the extent of this contraction increases with temperature. The water losses observed in our samples fit with these behavior of myofibrillar proteins during heating.

Despite the clear effect of cooking temperature on weight losses, this factor just led to limited differences ($P<0.019$) in moisture content of sous-vide cooked lamb loins, that were not enough to reach differences between groups in the post-hoc test. Nevertheless, the trend was consistent with that found in the weight losses, samples cooked at 80 °C showing lower moisture content than those cooked at 60° and 70 °C.

Cooking time significantly affected cooking losses ($P=0.012$), but did not have a significant effect upon moisture content of sous-vide cooked lamb loins ($P=0.463$). There is not much information about the effect of such long cooking times on the moisture retained by lamb meat upon cooking, but the obtained results basically agree with those by Vaudagna et al. (2002) and Christensen et al. (2011), who detected higher weight losses with longer cooking times at different temperatures. From a practical point of view, it seems that increasing sous-vide cooking lamb temperatures till 70 °C and above would lead to higher cooking losses than keeping longer times at 60 °C, which agrees with empirical knowledge reported by chefs (Myhrvold et al., 2011).

3.2. Color of cooked meat

Table 1 shows the obtained instrumental color parameters lightness (L^*), redness (a^*) and yellowness (b^*) in lamb loin samples sous-vide cooked under the different experimental conditions studied. All instrumental color parameters were significantly affected by cooking temperature ($P=0.021$ for L^*, and $P<0.001$ for a^* and b^*). However, differences in the L^* values were not enough to reach statistical significance in the post-hoc test between means. Anyway, samples cooked at 60 °C showed slightly higher L^* values than those cooked at 70 °C or 80 °C. Other authors have detected just the opposite: higher L^* values with increasing temperatures (Christensen et al., 2011; García-Segovia et al., 2007), probably due to 1) a higher moisture content in meat cooked at lower temperature, which would permit a deeper penetration of light in the tissue, and thus producing a darker meat appearance (Bojarska, Batura, & Cierach, 2003); and 2) increasing cooking temperature would lead to higher denaturation and aggregation of sarcoplasmic and myofibrillar proteins, which would increase light scattering (Christensen et al., 2011; Nikmaram et al., 2011). However, Sánchez del Pulgar et al. (2012), also obtained higher L^* values in pork samples cooked at 60 °C than at 80 °C, which was attributed to the higher amount of free water which remains impregnating the surface of the sliced sample before color measurement. In this sense, we empirically observed that those samples cooked at lower temperatures (which retained a higher amount of water), released some water to the surface during slicing for color measurement, meanwhile those samples that lost a higher amount of water during cooking seemed to have a lower amount of exuded water on the surface.

The intensity of the a^* parameter of cooked meat is inversely related to the degree of denatured myoglobin. Such process takes place between 55 °C and 65 °C although continues till 75 °C or 80 °C (King & Whyte, 2006). Accordingly, lamb loin samples were significantly affected by cooking temperature and by the interaction between this parameter and cooking time ($P<0.001$ for both), showing a more intense red color (higher a^* values) those samples cooked at 60 °C than those cooked at 70 °C and 80 °C. These results indicate higher myoglobin degradation as cooking temperature increased. This loss of redness with increasing cooking temperature was in accordance with the results obtained by García-Segovia et al. (2007), who cooked beef samples at 60 °C–80 °C for 15–60 min, and Sánchez del Pulgar et al. (2012), who cooked pork samples at 60 or 80 °C for times as long as 12 h.

There was a significant increase of b^* values as a consequence of both cooking temperature and cooking time (($P<0.001$ for both). This

Table 1
Weight losses, moisture content and instrumental color parameters of sous-vide cooked lamb loins at different temperature–time combinations.

Temperature (°C)	60			70			80			SEM[1]	P (T^a)	P (t)	P (T^a×t)
Time (h)	6	12	24	6	12	24	6	12	24				
Weight loss (%)	20.77^{d}	24.72^{cd}	28.78^{bcd}	30.67^{bc}	33.42^{ab}	32.80^{abc}	35.22^{ab}	35.61^{ab}	39.41^{a}	2.40	<0.001	0.012	0.411
Moisture (%)	66.42	66.57	65.26	67.83	65.07	66.38	64.18	62.56	62.30	1.50	0.019	0.463	0.841
L* (lightness)	66.18	67.47	66.95	67.18	65.82	64.92	63.96	65.07	64.95	0.92	0.021	0.845	0.483
a* (redness)	15.15^{a}	13.41^{ab}	13.42^{ab}	12.45^{bc}	12.43^{bc}	12.76^{bc}	11.21^{c}	13.00^{b}	13.35^{b}	0.41	<0.001	0.732	<0.001
b* (yellowness)	8.65^{d}	9.20^{de}	10.09^{bcde}	9.89^{cde}	10.40^{bcd}	10.96^{bc}	11.17^{bc}	11.64^{ab}	13.05^{a}	0.38	<0.001	<0.001	0.613

T^a: Temperature; t: time.
Different superscript letters within the same row mean significant differences between the different temperature × time ($P<0.05$).
[1] SEM: Standard error of the mean.

was most likely due to the formation of metmyoglobin and further heat-denaturation of this protein, giving rise to a brownish color. Higher b* values as a consequence of increasing sous-vide cooking temperatures have also been detected by other authors (Christensen et al., 2011; García-Segovia et al., 2007).

3.3. Instrumental texture parameters

Obtained values for the different textural variables included in the TPA analysis and for shear force (SF) of sous-vide cooked lamb loins are shown in Table 2. Springiness, cohesiveness and chewiness were significantly affected by temperature ($P=0.001$, $P<0.001$ and $P=0.013$ respectively), cooking time ($P<0.001$ for springiness and cohesiveness and $P=0.005$ for chewiness) and by the interaction between temperature and time ($P<0.001$ for all the three parameters). On the other hand, hardness, gumminess and SF values were affected by cooking time ($P=0.003$ for hardness and $P=0.004$ for gumminess and SF) and by the interaction between temperature and time ($P=0.007$, $P=0.003$ and $P=0.004$ respectively). Adhesiveness was not affected by any of the studied factors.

Changes in meat tenderness during cooking are associated with heat-induced alteration of myofibrillar proteins and connective tissue, since heat solubilizes the connective tissue and this leads to meat tenderization, while denaturation of myofibrillar proteins causes meat toughening (Laakkonen, Wellington, et al., 1970). Water losses from the muscle tissue upon heating also contribute to this meat toughening. But also the change from a viscoelastic to an elastic material influences the changes in texture during heating (Baldwin, 2012): raw meat is tougher because of the viscous flow in the fluid-filled channels between the fibers and fiber bundles; heating up to 65 °C increases tenderness because the sarcoplasmic proteins aggregate forming a gel and makes it easier to fracture the meat with the teeth; over 65 °C and up to 80 °C, the meat becomes tougher because the elastic modulus increases and requires larger tensile stress to extend fractures (Tornberg, 2005). Moreover, Laakkonen, Sherbon, and Wellington (1970) also detected a residual collagenolytic activity at 60 °C after 6 h, compared to that of raw meat or meat at 37 °C for 6 h. Baldwin (2012) suggests that this residual collagenase activity at 60 °C could be one of the factors explaining the tenderness of meat cooked at such temperatures for long times. However, in the same work by Laakkonen, Sherbon, et al. (1970), the activity for the muscle collagenase at 60 °C was around four times lower than at 37 °C. If such collagenolytic at 60 °C effect were real, then just keeping the meat at 37 °C for even a shorter time would achieve a similar effect.

With all this processes taking place, it results very difficult to elucidate the causes behind the detected changes in texture parameters as a consequence of cooking time and temperature. Palka and Daun (1999) and Palka (2003), who studied the textural characteristics of beef cooked at different internal temperatures, reported higher values for hardness in samples cooked at 70 °C and 80 °C than in those cooked to an internal temperature of 60 °C. They explained these results on the basis of myosin (55–60 °C) and actin (≈80 °C) denaturation and collagen contraction (56–65 °C). Nevertheless, they used shorter cooking times than those used in our study and the collagen in samples cooked at 70 °C and 80 °C did not probably have enough time to solubilize completely and counteract the hardness caused by myofibrillar protein shrinkage. Christensen et al. (2011) observed a decrease in shear force values with increasing temperatures from 48 to 63 °C, while García-Segovia et al. (2007) detected a slight increase in SF values in sous-vide cooked beef cooked at temperatures between 60 and 80 °C.

In our study, there was a trend to a decrease, both in hardness and in SF values with cooking time at any given cooking temperature. This could be due to a greater collagen solubilization with longer cooking time, while myofibrillar shrinkage would have reached its maximum even with the shortest cooking time (6 h) and thus, it would have not further increased with longer times. The significant lower hardness values for loin lamb samples sous-vide cooked at 80 °C for 24 h could be caused by an extensive disintegration of the perimysium around muscle bundles (Baldwin, 2012). This fact was empirically observed, giving rise to a very fragile cooked meat structure, which was very difficult to manipulate. This was not detected in the SF test, since in this case the measured force is that needed to transversely cut the meat fibers, while the hardness parameter in the TPA refers to the force to compress the sample to 50% of its height, which is clearly affected by the binding force between bundles. Moreover, the measurement of cohesiveness in the TPA test indicated such generation of a fragile structure as a consequence of long cooking time at high temperature, since samples cooked at 80 °C for 24 h were significantly less cohesive than those from any other combination of time and temperature.

3.4. Fiber diameter and microstructure

The microstructure of sous-vide cooked lamb *Longissimus dorsi* muscle is shown in the SEM micrographs of Figs. 1 and 2. On the transverse sections, it can be observed that the gaps between muscle fibers were clearly visible in samples cooked at 60 °C, and also in those cooked at 80 °C, while at 70 °C, the structure of the lamb meat became denser and with more compact fiber arrangements. No clear influence of cooking time on this fiber arrangement was observed, although at 60 °C, the gaps tended to be wider with longer cooking times, while at 80 °C, the structure tended to be more compact.

At higher magnification (Fig. 2), in samples cooked at 60 °C the formation of granular deposits in the spaces between muscle fibers is visible in the images. At 70 °C these granular deposits do not disappear, but are less evident; the connective tissue is denatured with the subsequent formation of a gel that fills the space between muscle fibers and the endomysium and between fiber bundles. Samples cooked to

Table 2
Instrumental texture parameters (TPA and shear force values) of sous-vide cooked lamb loins at different temperature–time combinations.

Temperature (°C)	60			70			80			SEM[1]	P (T^a)	P (t)	P ($T^a \times t$)
Time (h)	6	12	24	6	12	24	6	12	24				
Hardness (N)	18.59^{abc}	16.74^{abc}	15.97^{bc}	16.83^{abc}	18.86^{ab}	14.37^{bc}	24.79^{a}	16.94^{abc}	10.65^{c}	1.73	0.857	0.003	0.007
Adhesiveness (Ns)	−0.06	−0.04	−0.03	−0.03	−0.04	−0.03	−0.05	−0.05	−0.06	0.01	0.077	0.817	0.195
Springiness (cm)	0.64^{ab}	0.69^{a}	0.60^{abc}	0.65^{ab}	0.58^{bc}	0.52^{cd}	0.59^{bc}	0.53^{cd}	0.47^{d}	0.02	0.001	<0.001	<0.001
Cohesiveness (N/mm^2)	0.48^{a}	0.44^{ab}	0.39^{bc}	0.42^{abc}	0.38^{bcd}	0.32^{de}	0.36^{cd}	0.32^{c}	0.25^{e}	0.01	<0.001	<0.001	<0.001
Gumminess (N/cm^2)	7.83^{ab}	10.00^{a}	6.80^{ab}	8.07^{ab}	7.09^{ab}	4.63^{ab}	9.14^{a}	5.48^{ab}	2.53^{b}	1.18	0.073	0.004	0.003
Chewiness (N·s)	4.92^{ab}	7.03^{a}	4.55^{abc}	5.32^{ab}	4.09^{abc}	2.36^{bc}	5.46^{ab}	3.03^{bc}	1.19^{c}	0.78	0.013	0.005	<0.001
Shear force (N)	27.03^{ab}	25.27^{ab}	23.30^{ab}	32.74^{a}	35.01^{a}	16.26^{b}	28.43^{ab}	26.25^{ab}	23.98^{ab}	2.98	0.613	0.004	0.004

T^a: Temperature; t: time.
Different superscript letters within the same row mean significant differences between the different temperature × time ($P<0.05$).
[1] SEM: Standard error of the mean.

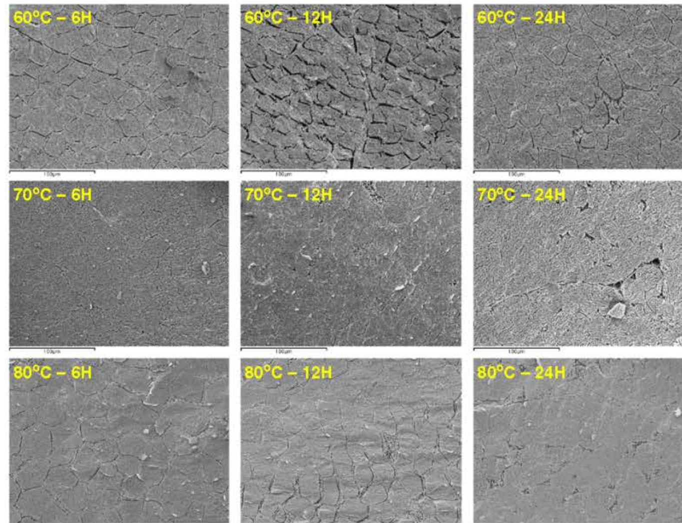

Fig. 1. Microstructure of sous-vide cooked *Longissimus dorsi* muscle of lambs at three different temperature–time combinations (500× magnification).

80 °C still show this granular deposit filling up the space between the fibers and endomysium tubes and between the bundles of muscle fibers. Wu, Dutson, and Smith (1985) observed a similar behavior: no changes in the structure of epimysium when cooking beef muscles for 1 h at 60–80 °C, but the perimysium and endomysium became granular at 60 °C and gelatinized at 80 °C.

Palka (1999) and Li, Zhou and Xu (2010), who cooked beef muscle in the range of 60 °C–80 °C, observed an intense granulation at 70 °C, probably due to the sarcolemma distortion. In addition, Palka (1999) reported at 80 °C compression in the cells. However, in our study, samples cooked at that temperature did not show a volume reduction but appear swollen, as compared to samples cooked at 60 °C (Fig. 2). This was confirmed by the measurement of muscle fiber diameters, which were significantly smaller in the samples cooked at 60 °C compared to those cooked at either 70 °C or 80 °C or the raw ones (data not shown). Wattanachant, Benjakul, and Ledward (2005a) also observed a similar effect when cooking poultry meat, with a reduction of the fiber diameter at a cooking temperature of 60 °C and a subsequent increase upon heating until 80 °C. They ascribed this shrinkage to the thermal denaturation of intramuscular collagen, which was found at temperatures between 60.7 and 61.7 °C (Wattanachant, Benjakul, & Ledward, 2005b), while further heating leads to the formation of a

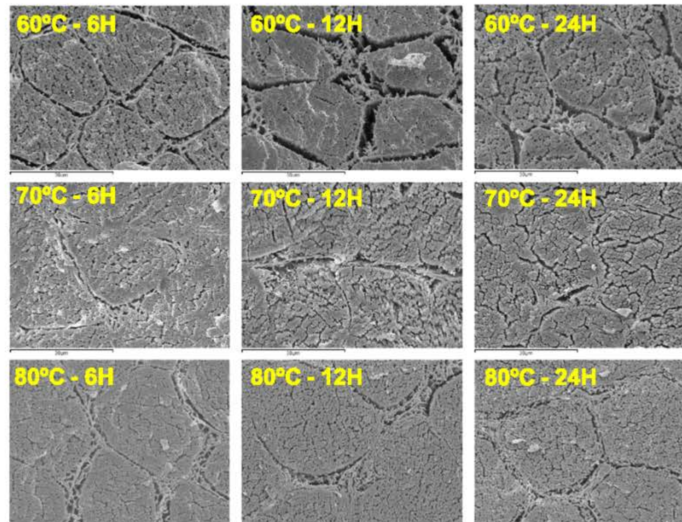

Fig. 2. Microstructure of sous-vide cooked *Longissimus dorsi* muscle of lambs at three different temperature–time combinations (2000× magnification).

Table 3
Microbial counts in raw lamb loins (initial time) and after sous-vide cooking treatments at different temperature–time combinations.

Microbial groups	*Initial counts*	Counts after heat treatment												
		60 °C			70 °C			80 °C			SEM[1]	P (Tª)	P (t)	P (Tª×t)
		6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h				
Mesophilic counts	4.6	1.3	1.7	1.7	0.9	2.7	1.4	1.4	1.2	1.6	0.23	0.923	0.515	0.667
Psychrotrophic counts	4.2	<1	<1	<1	<1	<1	<1	<1	<1	<1	0.15			
Lactic acid bacteria	3.3	<1	<1	<1	<1	<1	<1	<1	<1	<1	0.07			
Enterobacteriaceae	3.6	<1	<1	<1	<1	<1	<1	<1	<1	<1	0.07			
Coliforms	3.3	<1	<1	<1	<1	<1	<1	<1	<1	<1	0.13			
Gram positive cocci	3.2	<1	<1	<1	<1	<1	<1	<1	<1	<1	0.10			
Enterococci	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1				
Clostridium sp.	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1				
Bacillus sp.	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1				
B. thermosphacta	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1				
S. typhimurium	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1				
L. monocytogenes	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1				

Tª: Temperature; t: time.
[1] SEM: Standard deviation.

denatured collagen gel around each muscle fiber, which could help in maintaining the cell shape despite the water losses.

3.5. Microbiology

The microbial counts at initial time and after heat treatments are shown in Table 3. Samples of raw loins showed counts for mesophilic and psychrotrophic bacteria higher than 4 log CFU/g; whereas for LAB, GPC, *Enterobacteriaceae*, and coliforms the counts ranged from 3.23 to 3.58. The prevalence in the raw samples was near to 100% for all these microbial groups. These results are within the Food Quality Standards for fresh meat (EC Regulation No. 2073/2005). In respect to the pathogens, *B. thermosphacta*, and the remaining microbial groups researched, only *Clostridium* was found sporadically.

After the different heat treatments, the results found in the batches were very similar. For all microbial groups the counts were not detectable or were lower than log 1 CFU/g, except for the mesophilic bacteria. These microorganisms may be resistant *Bacillus* forms which are able to grow in a nonspecific and nutrient-rich culture media such as PCA (Moerman, Mertens, Demey, & Huyghebaert, 2001). In this case, analysis of variance of the data indicated a no significant effect among the heat treatments studied with mean counts ranging from 1 to 1.7 CFU/g. In concordance to these results, Díaz et al. (2008) found absence or minor mean microbiological counts for sous-vide cooked pork loins treated at 70 °C for 12 h. In a recent review by Baldwin (2012), pasteurization times for sous-vide cooked meat under the rules of the FDA (FDA, 2011) were provided, confirming that even the shortest of the time–temperature combinations considered in the present study (60 °C–6 h) were far enough to pasteurize meat, which explains the absence of differences in the analyzed microbial groups between treatments.

4. Conclusion

Cooking temperature and cooking time seem to play a major role on the characteristics of sous-vide cooked lamb loins. While the former strongly affects weight losses, moisture content, instrumental color and some instrumental texture features, the interaction between time and temperature determines most sous-vide cooked lamb meat textural parameters. The microstructure of the muscle fibers after sous-vide cooking is also affected by both cooking time and temperature. Thus, the connective tissue granulation starts at 60 °C and a gelatin based protein gel is formed in the endomysial space at 70 °C. The studied combination of time and temperature treatments for sous-vide cooking of lamb loins produced a dramatic reduction of the microbial population, even with the less intense treatment (60 °C–6 h).

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Capítulo 1.2

Effect of different temperature-time combinations on lipid and protein oxidation of *sous-vide* cooked lamb loins

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Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins

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ABSTRACT

Forty-five lamb loins were subjected to sous-vide cooking at different combinations of temperature (60, 70 and 80 °C) and time (6, 12 and 24 h) to assess the effect on the oxidative stability of lipids and proteins. Heating induced both lipid and protein oxidation in lamb loins. Higher cooking temperature–time combinations increased conjugated dienes and decreased thiobarbituric reactive substances (TBARS) values and hexanal. Total protein carbonyls increased throughout time at all cooking temperatures considered, while α-aminoadipic (AAS) and γ-glutamic semialdehydes (GGS) increased when cooking at 60 °C but not at 80 °C. Links between the decrease in secondary compounds from lipid oxidation due to cooking at higher temperatures and for longer times with the increased levels of 3-methylbutanal and greater differences between total protein carbonyls and AAS plus GGS were hypothesised.

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1. Introduction

The most common cooking techniques for meat are probably roasting, stewing, grilling and pan-frying; all of them are carried out at very high temperatures and with the presence of oxygen. On the other hand, sous-vide cooking of vacuum packaged meats at relatively low temperatures and long cooking times has emerged as a popular technique during the last two decades, and it has been extensively adopted by catering services and food processing to provide foods of superior sensory quality with a longer shelf-life compared with conventional cook-chill technologies (Sanchez del Pulgar, Gazquez, & Ruiz-Carrascal, 2012). Although there have been a considerable number of studies dealing with the chemical changes underwent during meat cooking, the effects of low temperature–long time sous vide cooking on the oxidative stability of lipids and proteins of meats has not been studied in detail.

Both, temperature and length of cooking have a large effect on the production of free radicals in muscle foods that lead to the oxidation of lipids and proteins, which in turn may cause undesirable changes in colour, sensorial and nutritive values (Estevez, 2011).

Lipid oxidation is a rather complex process whereby hydroperoxides and other primary products of the oxidation are formed from polyunsaturated fatty acids (PUFA) (Shahidi & Zhong, 2010). The primary autoxidation is followed by a series of secondary reactions which lead to the degradation of hydroperoxides and the formation of a wide range of compounds, including thiobarbituric acid reactive substances (TBARS) and volatile compounds, amongst which some volatile aldehydes such as hexanal or 2,4-decadienal, produced as a consequence of the oxidation of linoleic acid, impart off-flavours in oxidised meat and meat products, and have also become widely used as indicators of lipid oxidation (Shahidi & Zhong, 2010).

The development of lipid oxidation reactions in cooked meat depends on many factors. Heating increases lipid oxidation in meat (Broncano, Petron, Parra, & Timon, 2009; Conchillo, Ansorena, & Astiasaran, 2003), and overall, the rate of oxidation increases with the temperature (Karastogiannidou & Ryley, 1994), although the development of this type of reactions also depends on the method and time of cooking. Broncano et al. (2009) reported huge differences in lipid oxidation values in pork meat between different cooking methods. Sanchez del Pulgar et al. (2012) studied the effect of cooking temperature, cooking time and vacuum in sous-vide pork cheeks and reported lower TBARS in samples cooked at higher temperatures and for longer times. However, there is a lack of scientific knowledge about the development of lipid oxidative reactions in meats cooked for long times at moderate temperatures.

Proteins from animal tissues are also targets for oxygen radical attack in vivo and in foods (Estevez, 2011). Protein oxidation is

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induced either directly by reactive oxygen species or indirectly by reaction with secondary products of oxidative stress, like oxidised lipids or other pro-oxidants generated during processing (Estevez, 2011). This may cause multiple physico-chemical changes in proteins including amino acid degradation, decreases in protein solubility due to protein polymerisation, loss of enzyme activity and impaired protein digestibility (Traore et al., 2012). One of the most prominent changes in meat oxidised proteins is the formation of protein carbonyls (Estevez, 2011). The quantification of the total protein carbonyls for the assessment of protein oxidation in muscle foods is routinely carried out by the 2,4-dinitrophenylhydrazine (DNPH) method (Estevez, 2011). This method makes possible an overall assessment of protein oxidation (Estevez, 2011). More specific methods to determine particular carbonyl derivatives from specific amino acids have been recently used to evaluate the extent of protein oxidation in meat. In 2009, Estevez, Ollilainen and Heinonen identified two specific carbonyl compounds in oxidised myofibrillar proteins: α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively). Both AAS and GGS have already been successfully used as indicators of protein oxidation in a wide range of meat products (Armenteros, Heinonen, Ollilainen, Toldrá, & Estévez, 2009).

Higher levels of protein carbonyls and other indicators of protein oxidation have been shown as a consequence of heating and longer cooking times (Armenteros et al., 2009; Sante-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008). However, there is no scientific information on how the sous-vide cooking of meats at moderate temperatures and for long cooking times may affect the formation of protein carbonyls.

Thus, the aim of the present study was to elucidate the effect of different time–temperature combinations in sous-vide cooked lamb loins on primary and secondary products from lipid oxidation and total protein carbonyls and specific protein carbonyls, such as α-aminoadipic and γ-glutamic semialdehydes.

2. Materials and methods

2.1. Experimental design

The study was carried with 45 lamb loins which were weighed (weight range: 445–503 g), vacuum packaged and cooked at nine different combinations of time (6, 12 and 24 h) and temperature (60, 70 and 80 °C) in thermostatized water baths ($n = 5$ for each batch). In addition, 5 raw lamb loins were also analysed as a control. Once the cooking process had finished, the packages were removed from the water bath and submerged in cold water for 1 h. Subsequently, the packaged loins were kept under refrigeration (2 °C) overnight. Thereafter, loins were sliced transversal to the longitudinal axis (slices of approximately 1 cm thickness and 15–25 g weight), individually wrapped in plastic film, vacuum packaged and frozen at −80 °C until analysis.

Conjugated dienes (CD), thiobarbituric reactive substances (TBARS), protein carbonyl content and content of AAS and GGS were analysed at each time–temperature combination. Selected volatile aldehydes from lipid oxidation (pentanal, hexanal, heptanal, octanal and nonanal) and Strecker degradation of amino acids (3-methylbutanal) were analysed in four of the different combinations of time (6 and 24 h) and temperature (60 and 80 °C).

2.2. Analytical procedures

2.2.1. Determination of conjugated dienes

The measurement of CD was determined according to the procedure described by Juntachote, Berghofer, Siebenhandl, and Bauer (2006). Frozen lamb loin samples were thawed and thereafter, they were cut with a knife in small cubes, immersed in liquid nitrogen and minced. Subsequently, 0.5 g were suspended in 5 ml of distilled water and homogenised to form smooth slurry. A 0.5 ml aliquot of this suspension was mixed with 5 ml of extracting solution (3:1 (v/v) hexane/isopropanol) for 1 min. After centrifugation at 3500 rpm for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of CD was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1} and the results were expressed as mmol per kg of sample.

2.2.2. TBARS measurement

TBARS determination in lamb loins was carried out using the method described by Salih, Smith, Price, and Dawson (1987) with some modifications: 5 g of sample, previously minced following the same procedure described for CD analysis, were homogenised in polypropylene tubes of 50 ml using a ultra-turrax homogeneizer (Ultra-Turrax T25, IKA-Werke GmbH & Co. KG, Stafen, Germany) with 15 mL 3.86% perchloric acid and 0.50 mL BHT (butylated hydroxytoluene) for 30 s and centrifuged at 4000 rpm for 2 min. During homogenisation tubes were kept in ice to avoid heating. The blended sample was filtered through Watman No.1 filter paper. The filtrate was adjusted to 25 mL by adding 3.86% perchloric acid. Next, 2 mL aliquots of the filtrate were mixed in glass tubes with 2 mL of 0.02 M TBA and shaked. The solution was heated at 100 °C for 30 min, cooled and again centrifuged. The absorbance was measured at 530 and 600 nm on a spectrophotometer (Model U-2000, Hitachi, Tokyo) against a blank containing 3 mL of perchloric acid (3.86%) and 3 mL of TBA reagent. The concentration of TBARS was calculated from a standard curve in triplicate using solutions of 1,1,3,3-tetraethoxypropane (TEP) (Merck, Schchardt). Results were expressed as mg malonaldehyde (MDA) equivalents per kg of meat sample.

2.2.3. Determination of total carbonyls content by the DNPH Method

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatisation with dinitrophenylhydrazine (DNPH) according to the method described by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987) with slight modifications. Lamb meat was minced with liquid nitrogen just like in the other procedures and then, 1 g of meat was homogenised 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturrax homogeniser for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 mL) and subsequently centrifuged for 5 min at 5000 rpm. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed twice with 1 mL ethanol/ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 5031 g to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using a BSA standard curve. The amount of carbonyls was expressed as nmol of carbonyl per milligram of protein using an absorption coefficient of 21.0 $nM^{-1} \times cm^{-1}$ at 370 nm for protein hydrazones.

2.2.4. HPLC-FLD analysis of AAS and GGS

Samples were derivatized with 50 mM aminobenzoic acid (ABA) and subsequently hydrolysed with 6 M HCl according to the procedure described by Utrera, Morcuende, Rodriguez-Carpena, and Estévez (2011) with minor modifications. An aliquot of meat homogenate suspensions (200 μL) was dispensed in 2 mL

screw-capped eppendorf tubes. Proteins were precipitated with 2 mL cold 10% TCA and subsequent centrifugation at 2000 rpm for 30 min. The resulting pellets were treated again with 2 mL cold 5% TCA and proteins precipitated after centrifugation at 5000 rpm for 5 min. Pellets were then treated with 0.5 mL of 250 mM 2-(N-morpholino) ethanesulphonic acid (MES) buffer pH 6.0 containing 1% sodium dodecyl sulphate (SDS) and 1 mM diethylenetriaminepentaacetic acid (DTPA), 0.5 mL of 50 mM ABA in 250 mM MES buffer pH 6.0 and 0.25 mL of 100 mM NaBH3CN in 250 mM MES buffer pH 6.0. The derivatization was completed by allowing the mixture to react for 90 min while tubes were immersed in a water bath at 37 °C and stirred regularly. All solutions employed for the derivatization procedure were freshly made at sampling days. The derivatization reaction was stopped by adding 0.5 mL of cold 50% TCA followed by a centrifugation at 5031g for 5 min. Pellets were then washed twice with 1 mL of 10% TCA and 1 mL of ethanol-diethyl ether (1:1, v/v). Centrifugations at 5031g for 5 min were performed after each washing step. Protein hydrolysis was performed at 110 °C for 18 h in the presence of 6 M HCl. Hydrolysates were finally dried *in vacuo* at 40 °C using a Savant speed-vac concentrator. Hydrolysates were finally reconstituted with 200 μL Milli-Q water and filtered through hydrophilic polypropylene GH Polypro (GHP) syringe filters (0.45 μm pore size, Pall Corporation, Ann Arbor, MI, USA) for HPLC analysis. Samples were injected in a HPLC equipped with a COSMOSIL 5C18-AR-II RP-HPLC column (5 μm, 150 × 4.6 mm) and a guard column (10 × 4.6 mm) filled with the same material. The Shimadzu "Prominence" HPLC apparatus (Shimadzu Corporation, Kyoto, Japan) was also equipped with a quaternary solvent delivery system (LC-20AD), DGU-20AS on-line degasser, SIL-20A auto-sampler, RF-10A XL fluorescence detector, and CBM-20A system controller. 50 mM sodium acetate buffer pH 5.4 (Eluent A) and acetonitrile, ACN (Eluent B) were used as eluents. A low pressure gradient program was used, varying B concentration from 0% (min 0) to 8% (min 20). The volume injection was 1 μL, the flow rate was kept at 1 mL/min and the temperature of the column was maintained constant at 30 °C. Excitation and emission wavelengths were set at 283 and 350 nm, respectively. Identification of the derivatized semialdehydes in the FLD chromatograms was carried out by comparing their retention times (Rt) with that from a standard AAS and GGS, injected and analysed in the above-mentioned conditions. The peaks corresponding to AAS-ABA and GGS-ABA were manually integrated from FLD chromatograms and the resulting areas plotted against an ABA standard curve (ranged from 0.1 to 0.5 mM). Regression coefficients greater than 0.99 were obtained. The estimation of the quantities of AAS-ABA and GGS-ABA through an ABA standard curve was accomplished by assuming that the fluorescence emitted by 1 mol of ABA is equivalent to that emitted by 1 mol of derivatized protein carbonyl. Results are expressed as nmol of AAS or GGS per mg of protein.

2.2.5. Volatile aldehydes

Volatile aldehydes were analysed following the method developed by Garcia-Esteban, Ansorena, Astiasaran, and Ruiz (2004) with small modifications. The visible fat of each lamb loin was previously removed and then, a piece of the sample was ground after being frozen with liquid nitrogen and 5 g were weighted into a 20 mL glass flask sealed with an aluminium cap and a septum. Sealed vial was conditioned in a thermostatized water bath at 37 °C for 30 min. Solid phase micro extraction (SPME) was carried out by using a cross-linked divinylbenzene/carboxen/polydimethylsiloxane fibre, 50/30 μm thick and 2 cm long (Supelco, Bellefonte, PA, USA), conditioned prior to use by heating in the injection port of a GC system under the conditions recommended by the manufacturer. Analyses were performed using a Hewlett–Packard 6890 series II gas chromatograph coupled to a mass selective detector (Hewlett–Packard HP 5973) (Wilmington, DE, USA). Volatiles were separated using a 5% phenyl-methyl silicone HP-5) bonded-phase fused silica capillary column (Hewlett–Packard, 50 m × 0.32 mm i.d., film thickness 1.05 μm), operating at 6 psi of column head pressure, resulting in a flow of 1.3 ml min^{-1} at 40 °C. The SPME fibre was desorbed and maintained in the injection port at the temperature and for the time suggested by manufacturers. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 °C, raised to 200 °C at a rate of 5 C min^{-1} and then raised to 250 °C at a rate of 20 C min^{-1} and held for 5 min. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of 1 scan s^{-1} over the m/z range of 30–550 u.m.a. Compounds were tentatively identified by comparing their retention times and mass spectra with those of standards (Sigma–Aldrich, St. Louis, MO, USA). Results from volatile analyses are provided in total area counts.

2.3. Statistical analysis

The effect of cooking temperature (60, 70 and 80 °C) and cooking time (6, 12 and 24 h) and their respective interaction (T° × t) on CD, TBARS, total protein carbonyls, AAS and GGS in sous-vide cooked lamb loins was evaluated by a two-way ANOVA using the general linear models procedure of SPSS (V.15.0). When the effect of any of the factors was significant ($P < 0.05$), differences between groups were analysed by using Tukey's posthoc test. For the volatile aldehydes, only two temperatures (60 and 80 °C) and two cooking times (6 and 24 h) were tested using also a two-way ANOVA. Similarly, the Tukey's test ($P < 0.05$) was used to reveal differences between means.

3. Results

3.1. Effect of temperature–time combination on lipid oxidation

The effect of cooking at low temperatures and for long times on CD values and TBARS is shown in Fig. 1. The concentration of CD and TBARS were significantly affected by cooking temperature ($P < 0.001$ for both variables) and time ($P = 0.006$ and $P < 0.001$, respectively) and the interaction between both parameters ($P < 0.001$ for both variables). While CD values kept more or less constant throughout time (from 5.9 mmol kg^{-1} in raw meat to 7 mmol kg^{-1} after 24 h) in samples cooked at 60 °C and increased slightly in those cooked at 70 °C (from 5.9 to 8.6 mmol kg^{-1}), they showed a dramatic increase with time in those cooked at 80 °C, reaching final values almost 4 times higher (22.9 mmol kg^{-1}) than those in raw samples or three times higher than the final values in samples cooked at 60 °C.

As shown in Fig. 1, there was an initial increase in TBARS from raw samples (0.4 mg MDA equivalents·kg^{-1}) at all cooking temperatures up until 6 h cooking time. Nevertheless, the increase in TBARS was much higher in lamb loin samples sous-vide cooked at 60 and 70 °C (2.3 and 2.45 mg MDA·equivalents kg^{-1}, respectively) than in those cooked at 80 °C (1.14 mg MDA equivalents kg^{-1}). Thereafter, there was a decrease in TBARS from 6 h until 24 h cooking, which was much more marked in samples cooked at 80 °C (0.26 mg MDA equivalents kg^{-1}), followed by those cooked at 70 °C (0.68 mg MDA equivalents kg^{-1}), while the decrease in those cooked at 60 °C (1.79 mg MDA equivalents kg^{-1}) was not statistically significant.

Other volatile aldehydes formed as a consequence of the degradation of lipid hydroperoxides have been also commonly used as indicators of lipid oxidation in meat (Shahidi & Zhong, 2010). In

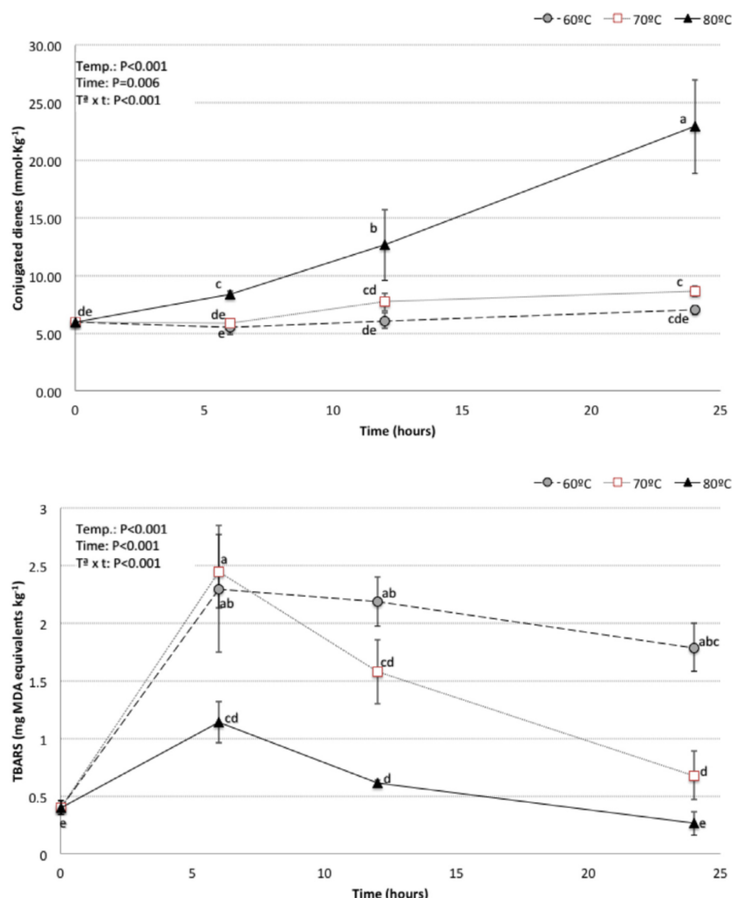

Fig. 1. Conjugated dienes (mmol kg^{-1} sample) and TBARS (mg MDA equivalents kg^{-1} sample) in sous-vide cooked lamb loins cooked at different time–temperature combinations. Values are means ± SD of five samples. Mean values with different letters indicate significant differences ($P < 0.05$) in the Tukey's test.

our study, the five selected aldehydes (pentanal, hexanal, heptanal, octanal and nonanal) were detected in all lamb loin samples sous-vide cooked at the different considered temperature–time combinations (Table 1). Amongst them, hexanal showed the highest chromatographic areas followed by pentanal, both of them comprising most of the chromatographic area of the volatile aldehydes extracted from the headspace of sous-vide cooked lamb. These 5 aldehydes were strongly influenced by cooking temperature and time ($P < 0.05$ for all of them); there was a sharp increase from raw to 6 h cooking time, and thereafter, values kept or slightly decrease when cooking at 60 °C, while there was a dramatic decrease when cooking at 80 °C. Octanal was the only exception in following the described trend, since it showed a similar chromatographic area in all cooking combinations.

3-methylbutanal (3 MB) was selected amongst the volatile compounds extracted by using SPME and analysed by GC/MS as an indicator of the development of Strecker degradation of amino acids, since this compound is derived from the degradation of

Table 1
Volatile aldehydes (Arbitrary Area Units 10^6) in sous-vide cooked lamb loins cooked at different time–temperature combinations. Values are means ± SD of five samples. Mean values in each row with different superscripts indicate significant differences ($P < 0.05$) in the Tukey's test.

Temperature (C°)	Raw	60		80		P temp.	P time	P Tª × t
Time (h)		6	24	6	24			
Pentanal	$1.69^{c} \pm 1.16$	$32.91^{a} \pm 2.79$	$26.01^{b} \pm 3,67$	$30.19^{ab} \pm 0.82$	$3.88^{c} \pm 1.59$	0.001	<0.001	<0.001
Hexanal	$8.49^{c} \pm 0.44$	$34.26^{a} \pm 5.14$	$34.69^{a} \pm 4.33$	$25.38^{b} \pm 1.56$	$4.28^{c} \pm 2.70$	<0.001	0.040	<0.001
Heptanal	$0.56^{d} \pm 0.36$	$3.03^{a} \pm 0.49$	$2.53^{ab} \pm 0.70$	$1.88^{bc} \pm 0.13$	$0.88^{cd} \pm 0.43$	<0.001	0.004	<0.001
Octanal	$0.38^{b} \pm 0.09$	$1.81^{a} \pm 0.60$	$1.81^{a} \pm 0.36$	$1.30^{a} \pm 0.13$	$1.02^{a} \pm 0.63$	<0.001	<0.001	<0.001
Nonanal	$0.51^{c} \pm 0.05$	$3.26^{a} \pm 1.18$	$2.04^{ab} \pm 0.71$	$1.58^{bc} \pm 0.11$	$0.83^{bc} \pm 0.51$	0.001	0.024	0.002
3-Methylbutanal	ND	$0.56^{b} \pm 0.22$	$1.12^{b} \pm 0.43$	$0.66^{b} \pm 0.17$	$3.16^{a} \pm 0.21$	0.027	0.004	<0.001

leucine and it is commonly found in cooked meat (Elmore, Mottram, Enser, & Wood, 1999). The chromatographic area of 3 MB in sous-vide cooked lamb loins was also significantly affected by cooking temperature ($P = 0.027$), cooking time ($P = 0.004$) and their interaction ($P < 0.001$), but unlike for volatile aldehydes derived from lipid oxidation, there was an increase in the detected chromatographic area with the time, especially in those samples cooked at 80 °C, in which there was an almost 5-fold increase from 6 to 24 h cooking time.

3.2. Effect of temperature–time combination on protein oxidation

In this study, the measurement of protein carbonyl content by the DNPH method was used as an indicator of protein oxidative damage. However, this method is limited to the measurement of the total amount of a variety of carbonyl derivatives formed through unspecific pathways during the oxidative process (Armenteros et al., 2009). In addition, more specific indexes of protein oxidation like AAS and GGS semialdehydes (Estevez, Ollilainen, & Heinonen, 2009) were also assessed. The results for the detected amount of these two semialdehydes and for the total amount of protein carbonyls by the DNPH method obtained for lamb loins sous-vide cooked at different time–temperature combinations are shown in Table 2. Cooking temperature, time and their interaction had a significant effect on total protein carbonyls (DNPH), AAS and GGS ($P < 0.005$ for all of them).

The total amount of protein carbonyls steadily increased throughout cooking time for all cooking temperatures considered, reaching levels around 3.5 times higher than those detected in raw samples. Although there was a significant interaction between cooking time and temperature, the pattern of protein carbonyls accumulation throughout cooking time was very similar for all the studied cooking temperatures.

Similarly to total protein carbonyls, the levels of GGS and AAS were much lower in raw samples (0.13 and 0.02 nmol mg prot^{-1} for AAS and GGS, respectively) than in any of the heated ones. The amount of these two semialdehydes increased with cooking time in those samples cooked at 60 °C (from 0.25 and 0.05 nmol mg prot^{-1} at 6 h cooking to 0.67 and 0.19 nmol mg prot^{-1} at 24 h cooking, for AAS and GGS, respectively). However, there was not such an increase when cooking at 70 °C (from 0.38 and 0.12 nmol mg prot^{-1} at 6 h cooking to 0.42 and 0.15 nmol mg prot^{-1} at 24 h cooking, for AAS and GGS, respectively) and 80 °C (from 0.39 and 0.15 nmol mg prot^{-1} at 6 h cooking to 0.37 and 0.05 nmol mg prot^{-1} at 24 h cooking, for AAS and GGS, respectively). In fact, unlike the other oxidation protein markers (AAS and DNPH), the amount GGS decreased with cooking time at 80 °C.

4. Discussion

Formation of CD occurs during the early stages of lipid oxidation, due to the rearrangement of double bonds of hydroperoxides generated from polyunsaturated fatty acids (Shahidi & Zhong, 2010) and hence, CD are good indicators of early stages of lipid oxidation. Some secondary compounds from lipid oxidation also contain conjugated double bonds, but their potential contribution to the CD values is not relevant. Obtained results reflected much higher levels of CD with increasing cooking temperatures and times (Fig. 1). Other authors have also shown an increase in CD values as a consequence of heating meat (Andreo, Doval, Romero, & Judis, 2003). Similarly, increased CD values throughout time when heating pork meat at 80 °C have also been reported (Juntachote et al., 2006).

Contrarily to CD, the level of TBARS is related to the content of secondary lipid oxidation compounds, mainly aldehydes like MDA. As for other lipid oxidation markers, heating usually produces higher TBARS values in meat, and thus, many authors have observed higher levels of TBARS in different types of cooked meat as compared to raw meat (Conchillo et al., 2003). Nevertheless, the decreased TBARS with cooking time was not unexpected (Fig. 1). In fact, these results agree with those obtained by Andreo, Garro, and Judis (2000), who cooked meat emulsions at 60, 80 and 100 °C for 8 h and reported a decrease of TBARS in samples cooked at higher cooking temperatures and for longer times. Sanchez del Pulgar et al. (2012) also reported a decrease in TBARS in sous-vide cooked pork cheeks at 80 °C for 5 and 12 h compared with those cooked at 60 °C. The rate of the lipid oxidative processes in meat during cooking depends upon the temperature of thermal processing (Shahidi, Yun, Rubin, & Wood, 1987) and therefore, it might be expected that higher cooking temperatures and times would lead to higher TBARS, as was shown for CD. However, MDA is very prone to react with other compounds present in meat that contain primary amino groups such as proteins, phospholipids, DNA or amino acids (Ventanas, Estevez, Delgado, & Ruiz, 2007). This leads to a decrease in the amount of MDA and other reactive lipid carbonyls available to react with TBA and consequently produces a decrease in the TBARS values. It would be then reasonable to think that at higher temperatures, MDA would further react at a higher rate with other compounds, explaining the decrease from 6 to 12 h or 24 h of cooking time at 70 °C and especially at 80 °C. In fact, other authors (Adams, De Kimpe, & Van Boekel, 2008) have observed a positive effect of temperature on a faster decrease in MDA and increase in compounds from the reaction of MDA with milk proteins in model systems.

The level of selected aldehydes from lipid oxidation in sous-vide cooked lamb loins, especially hexanal, basically agreed with the trend found for the TBARS (Table 1). Hexanal is the main aldehyde produced during lipid oxidation of meat (Shahidi & Zhong, 2010) and good correlations between hexanal content and TBARS values in cooked ground pork (Shahidi et al., 1987), roasted beef (St. Angelo et al., 1987) and cooked turkey (Brunton, Cronin, Monahan, & Durcan, 2000) have been reported in the scientific literature. In fact, both of them have been widely used as indicators to follow lipid oxidation in meat. The results for volatile aldehydes obtained in the present study point out to a promoting effect of heating on a rapid oxidation of polyunsaturated fatty acids, which in turn increases the number of free radicals capable of attacking other fatty acids less susceptible to oxidation, such as oleic acid, promoting the formation of heptanal, octanal and nonanal, amongst other aldehydes (Elmore et al., 1999). However, aldehydes readily react with the amine groups of lysine, cysteine and glutathione (Zamora, Gallardo, & Hidalgo, 2008). Thus, the decrease in their concentrations at higher cooking temperatures for longer cooking times might indicate the involvement of these aldehydes in further reactions after their formation, similarly to what has been described for MDA, giving rise to other volatile and non-volatile products (Zamora et al., 2008).

Protein oxidation is also a free radical chain reaction that can lead to protein carbonyl compounds generation, formation of protein polymers and peptide scissions (Estevez, 2011). Heating is known to enhance protein carbonylation as a consequence of a number of effects, such as disruption of cellular compartmentalisation, the release of free catalytic iron and the formation and cleavage of hydroperoxides. These oxidative modifications of protein in cooked meat may lead to different effects, from a decrease in protein digestibility or amino acid availability, to reduced protein functionality, such as impaired texture or decreased water retention (Estevez, 2011).

In the present study, heating led to a great increase in total protein carbonyls (Table 2). The values for total carbonyls compound were of the same order than those described for other cooked meat

Table 2
Total protein carbonyls according to the DNPH-method and amount of AAS and GGS (nmol mg prot^{-1}) in sous-vide cooked lamb loins cooked at different time–temperature combinations. Values are means ± SD of five samples. Mean values in each row with different superscripts indicate significant differences ($P < 0.05$) in the Tukey's test.

Temperature (°C)	Raw	60			70			80			PT°	Pt	P T° × t
Time (h)		6	12	24	6	12	24	6	12	24			
DNPH (nmol carbonyls/mg prot)	$2.58^{c} \pm 1.23$	$4.46^{bc} \pm 1.01$	$8.87^{ab} \pm 2.09$	$9.99^{a} \pm 2.46$	$5.97^{abc} \pm 1.76$	$6.98^{abc} \pm 2.12$	$9.25^{ab} \pm 2.09$	$5.92^{abc} \pm 0.76$	$7.59^{ab} \pm 3.52$	$9.70^{a} \pm 2.62$	0.003	<0.001	<0.001
AAS (nmol/mg prot)	$0.13^{c} \pm 0.06$	$0.25^{bc} \pm 0.02$	$0.42^{ab} \pm 0.29$	$0.67^{a} \pm 0.14$	$0.38^{bc} \pm 0.09$	$0.36^{bc} \pm 0.04$	$0.42^{ab} \pm 0.10$	$0.39^{bc} \pm 0.06$	$0.37^{bc} \pm 0.06$	$0.37^{bc} \pm 0.02$	0.002	<0.001	<0.001
GGS (nmol/mg prot)	$0.02^{e} \pm 0.01$	$0.05^{de} \pm 0.02$	$0.14^{ab} \pm 0.02$	$0.19^{a} \pm 0.04$	$0.12^{bc} \pm 0.02$	$0.11^{bcd} \pm 0.01$	$0.15^{ab} \pm 0.02$	$0.15^{ab} \pm 0.06$	$0.06^{cde} \pm 0.01$	$0.05^{e} \pm 0.06$	0.001	0.004	<0.001

products (Armenteros et al., 2009; Gatellier, Kondjoyan, Portanguen, & Sante-Lhoutellier, 2010). The levels of total protein carbonyls reached similar final values regardless the cooking temperature. Similarly, Promeyrat, Daudin, and Gatellier (2013) detected only a slight effect of temperature (over a range from 45 to 90 °C) on the total protein carbonyl formation in myofibrilar mimetic models. The same group detected a positive effect of heating time on protein carbonyl formation (Gatellier et al., 2010). The effect was not marked at 60 °C, but it should be considered that in such study, samples were heated only for 300 s, while in the present one heating time ranged from 6 to 24 h.

Estevez et al. (2009) suggested the measurement of GGS and AAS in oxidised myofibrillar proteins as more convenient indicators of protein carbonylation, since DNPH values might be affected by lipid carbonyls bound to proteins. The same group of authors (Armenteros et al., 2009) has used these semialdehydes as indicators of protein oxidation in meat products. In our study AAS was found in larger quantities than GGS, just like other authors have reported in meat and meat model systems (Akagawa et al., 2006; Armenteros et al., 2009; Utrera et al., 2011) probably due to the fact that AAS comes from lysine which is one of the most abundant amino acids in meat (Armenteros et al., 2009).

Similarly to total protein carbonyls, heating enhanced the detected levels of these two semialdehydes (Table 2). However, while samples cooked at 60 °C showed an increased in AAS and GGS throughout cooking time, their amounts remained more or less constant when cooking at 70 °C, and there was even a decrease in GGS when cooking at 80 °C. The promoting effect of heating on the formation of these two protein carbonyls in meat, meat products and meat systems has been previously highlighted (Utrera et al., 2011). Moreover, it has also been proved that once formed, these compounds may be involved in different types of reactions, which may lead to their degradation (Estevez, Ventanas, & Heinonen, 2011). According to Requena, Levine, and Stadtman (2003), AAS could react with other components like non-modified amino acid residues forming cross-links and Schiff bases. Estevez et al. (2011) support the Strecker-type reaction amongst the carbonyl group from AAS and GGS and free amines and, Utrera, Rodriguez-Carpena, Morcuende, and Estevez (2012) found that in more severe oxidation conditions as those achieved during cooking, this semialdehyde can transform in α-aminoadipic acid (AAA). In our study, it seems that this involvement in further reactions is clearly linked to cooking temperature, and thus, while DNPH values increased throughout time regardless the cooking temperature, the amount of AAS and GGS did not follow this pattern when cooking at higher temperatures. In fact, the enhanced involvement of AAS and GGS in Strecker degradation of amino acids with higher temperatures agrees with the increase in 3 MB in samples cooked at 80 °C for 24 h.

Formation of protein carbonyls may alter the overall electrical arrangement of muscle proteins, which in turn may worsen protein functionality, leading to a loss of water holding capacity and impaired texture traits (Estevez, 2011). In our study, the increase in AAS and GGS with longer cooking times in samples cooked at 60 °C was parallel to higher cooking losses, as shown in a previous study carried out with the same samples (Roldan et al., 2013). However, it results difficult to ascribe this effect to the higher levels of protein oxidation, since a number of other concomitant factors, such as protein denaturation, may also affect the ability of muscle proteins to retain water.

Compounds formed during lipid oxidation, like free radicals and hydroperoxides, can react with susceptible amino acids to yield carbonyl moieties (Estevez, 2011), but some lipid carbonyls can also interact with amino acids inducing their Strecker degradation and leading to the formation of Strecker aldehydes and other Maillard-like volatile compounds (Zamora et al., 2008).

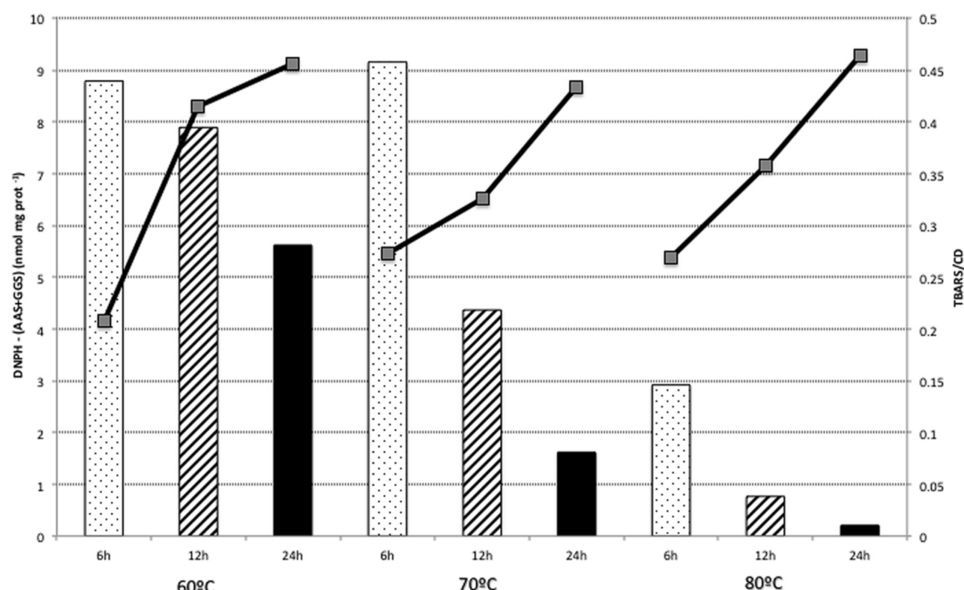

Fig. 2. Lines represent the difference between mean values for total protein carbonyls and the sum of AAS and GGS (left axis: nmol mg^{-1} prot) in sous-vide cooked lamb loins cooked at different time–temperature combinations. Bars show the ratio between the values for CD and TBARS for the same groups (right axis).

The diverse protein oxidation indicators used in this study showed different trends as a consequence of cooking times and temperatures. The black lines in Fig. 2 show the difference between total carbonyls by the DNPH method and the sum of AAS and GGS (expressed in nmol mg^{-1} prot), such a difference being greater with longer cooking times. AAS and GGS usually constitute around 60% of the total DNPH-carbonyls (Requena et al., 2003). Our results are in agreement with those obtained by Armenteros et al. (2009) who also found a lack of consistency between both measurements in meat products. This is most likely due to an overestimation of the total amount of protein carbonyls by the DNPH method, probably as a consequence of the low specificity of this method for measuring only protein carbonyls. In fact, part of the overestimation of protein carbonyls in the DNPH method could be caused by the reaction of dicarbonyls from lipid oxidation with proteins (Utrera et al., 2011). This would lead to the presence of free carbonyl groups from lipid oxidation compounds bound to proteins, which would be quantified as protein carbonyls (Armenteros et al., 2009).

Bars in Fig. 2 shows a numerical ratio between primary (CD) and secondary (TBARS) compounds from lipid oxidation. This ratio could constitute a way to measure the degradation or further reaction of secondary compounds from lipid oxidation. Thus, if this numerical ratio is low, it would reflect that most of the formed secondary compounds from lipid oxidation are being degraded or bound to other compounds. The decrease in this ratio CD/TBARS with longer cooking times regardless the temperature is parallel to the increase in the difference between total carbonyls by the DNPH method and the sum of AAS and GGS (black lines in Fig. 2), which would support the binding of lipid derived dicarbonyls to proteins.

The decrease in secondary compounds from lipid oxidation with longer cooking times was more marked in samples cooked at 80 °C, whereas the difference between total carbonyls and the sum of AAS and GGS did not increase more markedly with cooking time at higher cooking temperatures (Fig. 2). This could mean that there was not a higher amount of carbonyls from lipid oxidation bound to proteins with higher cooking temperatures, despite to the fact that there was a greater loss of secondary compounds from lipid oxidation.

Interestingly, those samples cooked at 80 °C showed much higher levels of 3 MB (Table 2), which can be originated from the attack of lipid derived carbonyls to leucine (Zamora et al., 2008). In our study, the detected higher rate of formation of the Strecker aldehyde 3 MB, could partially explain the drop in secondary compounds from lipid oxidation. Moreover, not only carbonyls from lipid oxidation, but also AAS and GGS, have been described as potential sources of the aldehyde moieties that would react with the amino group of amino acids to form a Schiff base in the initial step of the Strecker degradation of amino acids (Estevez et al., 2011). Accordingly, our results showed a decrease in GGS amounts and steady levels of AAS with longer cooking times in samples cooked at 80 °C, which could also be related to the higher levels of 3 MB detected in these samples.

5. Conclusion

In conclusion, heating induced lipid and protein oxidation of sous-vide cooked lamb loins, including the formation of AAS and GGS. Cooking at increasing temperatures (from 60 to 80 °C) led to a greater fall of secondary compounds from lipid oxidation. This could in turn explain the higher formation of Strecker aldehydes at such temperatures. In addition, longer cooking times caused a greater amount of protein carbonyls different to AAS and GGS. All these lipid and protein oxidative changes could have consequences on cooked meat flavour and texture.

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Capítulo 1.3

Volatile compound profile of *sous-vide* cooked lamb loins at different temperature-time combinations

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TITLE

Volatile compound profile of *sous-vide* cooked lamb loins at different temperature-time combinations

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ABSTRACT

Lamb loins were subjected to *sous-vide* cooking at different combinations of temperature (60 and 80 ºC) and time (6 and 24 h) to assess the effect on the volatile compound profile. Major chemical families in cooked samples were aliphatic hydrocarbons and aldehydes. The volatile compound profile in *sous-vide* cooked lamb loin was affected by the cooking temperature and time. Volatile compounds arising from lipid oxidation presented a high abundance in samples cooked at low or moderate cooking conditions (60 ºC for 6 and 24 h, 80 ºC for 6 h), while a more intense time and temperature combination (80 ºC for 24 h) resulted on a higher concentration of volatile compounds formed from Strecker degradations of amino acids, as 3-methyl-propanal and 2-methyl butanal. Therefore, *sous-vide* cooking at moderately high temperatures for long times would result in the formation of meaty flavour and roast notes in lamb meat.

KEY WORDS

Volatile compounds, lamb loin, *sous-vide*, time-temperature combination

INTRODUCTION

Sous-vide cooking consist in cooking raw materials or raw materials with intermediate food under controlled conditions of temperature and time inside heat-stable vacuumized pouches (Ruiz, Calvarro, Sánchez del Pulgar, & Roldán, 2013). Low temperature-long time (LT-LT) (usually under 70 ºC and for even more than 24 h) are typical for this type of cooking, which has been extensively adopted in the last two decades by catering services, food processing and chefs to provide foods of superior sensory quality with a longer shelf-life as compared to those cooked using conventional cook-chill technologies (Ruiz, et al., 2013). Nowadays, in Spain, *sous-vide* LT-LT cooking is one of the most used methods for whole lamb primal cuts in restaurants and in the catering industry.

It is generally accepted that the aroma of cooked meat is mainly developed upon heating treatment, with more than 1000 volatile compounds described (Pegg & Shahidi, 2004). However, only some of them play a significant role in the overall flavour characteristics of cooked meat. Most volatile compounds in cooked meat are generated as a result of two main reactions: the Maillard reactions, between amino acids and reactive carbonyls, and seems to be the responsible for the formation of the typical meaty flavour, and the thermal degradation of lipids, which seems to provide the characteristic flavours of the different spices (Elmore & Mottram, 2006). Both routes contribute significantly to the formation of the typical and desirable aroma of meat. Overall, the odour threshold for the flavour lipid-derived compounds are much higher than those for some of the sulfur and nitrogen-containing heterocyclic compounds that are formed from the water-soluble precursors via the Maillard reaction (Mottram, 1998a). Strecker degradations of amino acids and thermal degradation of thiamine are also responsible for the generation of flavour volatile compounds in heated meat (Resconi, Escudero, & Campo, 2013). Quantitatively, lipid oxidation is the major source of volatile compounds in cooked meat, specially the oxidation of unsaturated fatty acids (Cheng & Ho, 1998). This reaction is also associated with the development of unpleasant rancid flavours in raw and stored cooked meat (Mottram, 1998a).

The cooking conditions are crucial in the formation of flavour volatiles in meat. The Maillard reactions intensify at temperatures above 140 ºC, being favored

by surface meat dehydration (MacLeod, Seyyedain-Ardebili, & Chang, 1981), while lipid degradation products have been found to dominate the volatile extracts in boiled and lightly grilled or roasted meat (Mottram, 1985). Sulfur compounds, such as thiazols and pyridines, are associated to roasted meat, while thiols are more related to meat cooked at low temperature (70 - 100 ºC) (Almela, et al., 2010).

Very limited scientific information is available about the effect of LT-LT cooking on the volatile profile of *sous-vide* cooked meat (Sanchez del Pulgar, Roldan, & Ruiz-Carrascal, 2013).

Volatile final profiles is very important for determine the aroma in cooked meat. Aroma is the sensory attribute that best identifies cooked lamb meat, and it strongly contributes to its acceptance (Young & Braggins, 2004). Therefore, the aim of this work was to study the effect of different combination of time and temperature of *sous-vide* cooking lamb loins on the generation of volatile compounds.

MATERIAL AND METHODS

Experimental design

The volatile compounds profile was analyzed in lamb loins cooked under vacuum at different temperature-time combinations. The study was carried out using a completely randomized 2 × 2 design, with four combinations of temperature (60 or 80 °C) and time (6 or 24 h) which were used to cook 20 lamb loins (n = 5 for each batch). In addition, 5 lamb loins were used for performing the analysis in fresh meat. All loins were from a homogeneous production batch of lamb averaging 26 kg live weight and 90 days of age. Each lamb loin was packaged individually at the University of Extremadura's meat pilot plant in a vacuum plastic bag (nylon/ polyethylene pouches; heat resistance of −40 °C/+120 °C, O_2 permeability of 9 cm^3/m^2 per 24 h at 4 °C/80 % RH and water steam permeability of 1.2 g/m^2 per 24 h) (Joelplas SL, Barcelona, Spain) and cooked in thermostatized water baths by applying the time-temperature combinations described above. The internal temperature was monitored using a data logger Testo 735-2 (Testo, Lenzkirch, Germany) equipped with a needle thermocouple for meat. Once the cooking process finished, the packaged loins were removed from the water bath and submerged in iced cold water (2 °C) for 1 h. Subsequently, they were kept under refrigeration (2 °C) overnight. Once the bags were open in order to perform the analyses described in a previous paper (Roldán, Antequera, Martín, Mayoral, & Ruiz, 2013), a piece of each loin was taken, vacuum packaged and kept at −80 °C until volatile compound analysis.

Volatile compounds analysis

Volatile compounds were analyzed by headspace solid phase microextraction (HS-SPME) following the method described by (Garcia-Esteban, Ansorena, Astiasaran, & Ruiz, 2004) with some modifications. The visible fat of each lamb loin was removed and a piece of the sample was ground after freezing with liquid nitrogen. 5 g were weighted into a 20 mL glass flask sealed with an aluminium cap and a septum. Sealed vial was conditioned in a thermostatized water bath at 37 ºC for 30 minutes. SPME was carried out by using a cross-linked divinylbenzene/carboxen/polydimethylsiloxane fibre, 50/30 µm thick and 2 cm long (Supelco, Bellefonte, PA, USA), conditioned prior to use

by heating in the injection port of a gas chromatograph (GC) system under the conditions recommended by the manufacturer (at 270 ºC for 1 h). The fibre was then inserted into the sample vial through the septum and exposed to the headspace for 30 min at 37 ºC. The SPME fibre was desorbed and maintained in the injection port for 30 min. Analyses were performed using a Hewlett–Packard 6890 series II GC coupled to a mass selective (MS) detector (Hewlett–Packard HP 5973) (Wilmington, DE, USA). Volatiles were separated using a 5% phenyl-methyl silicone (HP-5) bonded-phase fused silica capillary column (Hewlett–Packard, 50 m x 0.32 mm i.d., film thickness 1.05 μm), operating at 6 psi of column head pressure, resulting in a flow of 1.3 mL/min at 40 ºC. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 ºC, rose to 200 ºC at a rate of 5 ºC/min and then rose to 250 ºC at a rate of 20 ºC/min and held for 5 min. The transfer line to the mass spectrometer was maintained at 280 ºC. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of 1 scan/s over the *m/z* range of 30–550 u.m.a. n-Alkanes (Sigma R-8769) were analyzed under the same conditions to calculate the linear retention indices (LRI) for the volatile compounds. Compounds were identified by comparison with the mass spectrum and RI of commercial reference compounds (Sigma-Aldrich; Steinhein, Germany), by comparison of mass spectrum with mass spectral database (NIST and Wiley libraries) and by comparison of their RI with those available in the literature (Elmore & Mottram, 2006; Madruga, Dantas, Queiroz, Brasil, & Ishihara, 2013; Sanchez del Pulgar, et al., 2013). Results from volatile analyses are provided in area units (AU).

Statistical Analysis

A two-way analysis of variance, with cooking time (6 h or 24 h) and cooking temperature (60 ºC or 80 ºC), together with their interaction was carried out on the quantitative data for each compound using the General Linear Model (GLM) procedure (SPSS 15.0). When significant differences were found, the Tukey´s test at 5 % level was used to make pair wise comparisons.

RESULTS AND DISCUSSION

A total of 57 volatile compounds were tentatively identified in lamb loins *sous-vide* cooked at different time-temperature combinations, all of them being clustered in the following chemical families: aldehydes, aliphatic and aromatic hydrocarbons, ketones, alcohols, esters, furans and sulfur-containing compounds (Table 1). Volatile compounds were also analysed in raw meat, only finding 11 of them in these samples (unpublished data), while *sous-vide* cooked lamb loins presented a higher number. The cooking effect was really notable on the generation of volatile compounds. These results are in agreement with literature, since uncooked meat has little or no aroma, while cooking of meat results in the production of a large number of volatile compounds (Mottram, 1998a). Aldehydes was the major chemical family (67.7 %) in raw lamb loins, followed, in decreasing order, by sulfur compounds, alcohols, ketones and furans (17.3 %, 11.4 % 2.2 % and 1.4 %, respectively), while esters and aliphatic and aromatic hydrocarbons were not found in this batch. As expected, in *sous-vide* cooked samples the profile of chemical families of volatile compounds was different.

Figure 1 shows the proportion of each chemical family of volatile compounds in lamb loin *sous-vide* cooked at the different temperature-time combination. Aldehydes and aliphatic hydrocarbons showed the highest proportion in samples cooked at 60 ºC for 6 h (30.7 % and 28.9 %, respectively) and 24 h (29.5 % and 35%, respectively), and in those cooked at 80 ºC for 6 h (33 % and 40.9 %, respectively). These chemical families were followed in abundance by ketones (20.5 % for the batch 60 ºC-6 h; 18.2 % for 60 ºC - 24 h and 11.4 % for 80 ºC - 6 h), alcohols (12.2 % for 60 ºC - 6 h, 9.6 % for 60 ºC - 24 h and 5.4 % for 80 ºC - 6 h) and sulfur compounds (4.5 % 60 ºC - 6 h, 4.9 % for 60 ºC - 24 h and 6.1 % for 80 ºC - 6 h). The lowest proportions corresponded to furans (with around 2 % for each batch), esters (with less than 0.5 % for each batch) and aromatic hydrocarbons families (with around 1% for each batch). A different profile of chemical families was observed in samples cooked at 80 ºC for 24 h, evidencing the influence of cooking temperature and time on the profile of volatile compounds formed during *sous-vide* cooking. In this batch, aliphatic hydrocarbons were the major chemical family (64 %), followed by sulfur compounds and aldehydes (19.4 % and 9.5 %, respectively). Furans,

ketones, esters, alcohols and aromatic hydrocarbons showed minor proportions (3.2 %, 1.7 %, 1.1 %, 0.8 % and 0.6 %, respectively).

The major chemical families of volatile compounds found in this study are quite in agreement with those reported in the literature for cooked meat, (Madruga, et al., 2013; Madruga, Elmore, Oruna-Concha, Balagiannis, & Mottram, 2010; Mottram, 1998a). In a previous study (Sanchez del Pulgar, et al., 2013), in which the profile of volatile compounds of *sous-vide* cooked pork cheeks was studied, higher levels of volatile compounds derived from fatty acid degradation, such as aldehydes, in samples cooked at softer cooking combinations (60 ºC for 6 and 12 h, and 80 ºC for 5 h) were detected. On the other hand, samples cooked at more severe cooking combination (80 ºC for 12 h) presented higher levels of volatile compounds from degradation of amino acids and/or thiamine, the sulfur-containing compounds being the most representative family in those samples.

The effect of *sous-vide* cooking temperature, as well as time x temperature interaction, was significant in a high number of individual volatile compounds, while the influence of time was not so remarkable (Table 1). In relation to aldehydes, all volatile compounds, except acetaldehyde and butanal, were significantly affected by the combined effect of cooking temperature and time. In general, the abundance of most aldehydes derived from lipid oxidation (pentanal, hexanal, heptanal, nonanal and the sum of total aldehydes) decreased with both time and temperature of cooking, so that the lowest values were found in lamb loin *sous-vide* cooked at 80 ºC for 24 h. Such a decrease in volatile compounds from fatty acid oxidation cooked at higher temperatures for longer times may be due to the further interaction among these carbonyl compounds with the amine groups of lysine, cysteine and glutathione (Zamora, Gallardo, & Hidalgo, 2008), suggesting their implication in the formation of other volatile compounds (Mottram, 1998a). Sanchez del Pulgar, et al. (2013) also reported a similar trend in most detected aldehydes from lipid oxidation. Such linear aldehydes, arising from fatty acid oxidation, show low odour threshold values and may play an important role in the flavour of cooked samples (Elmore, Mottram, Enser, & Wood, 1999). However, it is also important to take into account that when oxidative rancidity starts to develop, the products of this process, such as aldehydes, give rise to unpleasant odours

and tastes which reduces the acceptability of the meat to consumers (Mottram, 1998a).

The opposite effect was observed for 2-methylpropanal and 3-methylbutanal, which showed the highest levels in samples cooked at 80 ºC for 24 h. The main formation route for branched adehydes is the Strecker degradation of amino acids; more specifically, 2-methylpropanal and 3-methylbutanal, which are formed from the degradation of valine and leucine, respectively (Huang & Ho, 2001). These results point out to a positive effect of long cooking times when cooking at moderate high temperatures on the levels of volatile compounds originated from amino acids and thiamine, since the involved reactions are favored by the temperature (Mottram, 1998b), but also they seem to be favored by cooking time, since this kind of compounds has been found in long-ripened meat products (Ruiz, Ventanas, Cava, Andrés, & García, 1999). In this regard, the Strecker degradation of different amino acids has been shown to strongly depend upon temperature and time (Cremer & Eichner, 2000). A similar trend in these aldehydes was found in previous studies dealing with pork cooked under vacuum using almost the same cooking conditions than in our study (60 and 80 ºC for 5 and 12 h) (Sanchez del Pulgar, et al., 2013).

Similarly to linear aldehydes, straight-chain aliphatic hydrocarbons mainly derive from lipid oxidation (Mottram, 1998a) and are the largest class of volatile identified in cooked meats (Mottram, 1991). The individual effect of time and temperature of *sous-vide* cooking on aliphatic hydrocarbon was only remarkable in some of these compounds. Likewise, the combined effect between both parameters also affected significantly some volatile compounds of this chemical family. These compounds showed higher levels in lamb loins cooked at 80 ºC for 24 h in comparison to the other three batches, except to pentane and decane, which showed the opposite trend. Of the hydrocarbons identified, heptane was the most abundant one regardless the cooking temperature and time combination applied, presenting the greatest proportion in samples cooked at more severe cooking conditions. Hydroperoxides can be decomposed by heating in numerous secondary derivatives to generate a huge range of volatile compounds, such as hydrocarbons (e.g. alkane, alkene) (Min & Ahn, 2005). In addition, slow heating and high temperatures stimulate

the lipid peroxidation (Min & Ahn, 2005) and, therefore, it makes sense to expect that higher cooking temperatures increase the level of lipid peroxidation that would result in an increase of hydrocarbons compounds in these samples. In addition, those aliphatic hydrocarbons with longer chains are found accumulated in the fat depots of the muscle animal, resulting probably from feeding (Meynier, Novelli, Chizzolini, Zanardi, & Gandemer, 1999). Thus, it can indicate a potential balance during the cooking process between the formation of some aliphatic hydrocarbons, the loss of other through evaporation or decomposition, as well as the stability of other of these volatile compounds. With respect to aromatic hydrocarbons, neither time nor temperature of cooking affected the abundance of these volatile compounds. They seem to come from animal feeding (Tejeda, Antequera, Ruiz, Cava, & Ventanas, 1999).

Ketones and alcohols were significantly influenced by the interaction between cooking temperature and time. 3-octanone and 1-octen-3-ol were, by far, the most abundant compounds in each group. In general, the proportion of these volatile compounds decreased as time and temperature increased. Thus, lamb loin *sous-vide* cooked at 60 ºC for 6 h showed the highest values, followed by 60 ºC - 24 h and 80 ºC - 6 h batches and finding the lowest values in the samples cooked at 80 ºC for 24 h, as occurred for aldehydes. Straight-chain aliphatic alcohols and ketones are most likely oxidative decomposition products of lipids, giving some of them interesting aromatic notes (Mottram, 1998a). Nevertheless, 3-hydroxy-2-butanone was only detected in samples *sous-vide* cooked at 80 ºC for 24 h. Hydroxiketones are sugar degradation products from the Maillard reaction, what could explain its occurrence at the highest cooking time and temperature. These active intermediates may undergo secondary reactions with amines to form heterocyclic aroma compounds (Xi, Huang, & Ho, 1998). Thus, during thermal processing, 3-hydroxy-2-butanone reacts with ammonia to generate α-aminoketones and then form pyrazines, as well as, oxazoles, oxazolines, thiazoles or thiazolines, all of them being final compounds of the Maillard reaction (Mottram, 1998b; Xi, et al., 1998). In the samples of this study, these compounds have not been detected, probably due to the temperature used, which is not so intense to generate them.

Time and/or temperature of *sous-vide* cooking lamb loins also influenced significantly some furan and sulfur-containing compounds. In addition, some of them, such as 2-methylfuran, 2-pentylfuran and carbon disulfide, were also affected by the interaction between both parameters, with higher values in samples cooked at 80 ºC for 24 h than in the other three batches. Literature data indicate multiple sources of furan formation and its substituted derivatives. Thus, furans have been linked to high heating processes in food (Pérez-Palacios, Petisca, Melo, & Ferreira, 2012), but they are also formed through oxidation of unsaturated fatty acids, namely from α-linolenic and gamma-linolenic acid (Elmore, et al., 1999). Furans can be formed from various precursors naturally present in the food, such as ascorbic acid, carbohydrates, amino acids, fatty acids and carotenoids via complex steps, which mainly involve oxidation or the Maillard reaction (Nie, et al., 2013). Therefore, it seems that the formation of furans in cooked meat at more severe cooking conditions could derive from Maillard reaction, while the furans formation from unsaturated fatty acids could occur at more moderate temperatures.

Sulfur-containing compounds are known to be formed from degradation of amino acids and/or thiamine degradation. Methionine is the source of the sulfur-containing intermediate methional (Toldrá & Flores, 2006). This aldehyde breaks down easily into methanethiol, which is the direct precursor of numerous sulfur compounds such as dimethyl disulphide (Macleod, 1994; Madruga, et al., 2013). Sulfur-containing compounds play a fundamental role in the aroma of meat, since their very low odour thresholds make them potent aroma compounds and important contributors to the aromas of cooked meat (Mottram, 1998a).

Therefore, volatile compounds derived from fatty acids oxidation were higher at soft or moderate combinations of time and temperature of *sous-vide* cooking (60 ºC for 6 h, 60 ºC for 24 h and 80 ºC for 6 h) and decreased at more intense conditions (80 ºC for 24 h). This is in agreement with the results reported by Sanchez del Pulgar, et al. (2013) for pork cheeks cooking under vacuum and using similar cooking conditions than in our study. But, in addition, these results are in concordance with the oxidation measurements in the same samples to those of the present study described in a previous paper, which showed a decrease in the thiobarbituric acid reactive substances (TBARS) values in the

lamb loins *sous-vide* cooked at higher temperatures for longer times (Roldan, Antequera, Armenteros, & Ruiz, 2014). In the same study, conjugated dienes were also measured and samples cooked at more severe cooking conditions (80 ºC – 24 h) presented the highest proportions. These results suggest that at these cooking conditions lipid oxidation is still occurring and the decrease of lipid-derived volatile compounds in samples cooked at 80 ºC for 24 h was probably related to the interactions between carbonyl compounds with the amine groups of lysine, cysteine and glutathione that are promoted by high temperatures (Zamora, et al., 2008). Thus, cooking of lamb loins under vacuum at higher temperature and longer time seem to have a positive influence on the development of volatile compounds from degradation of amino acids and/or thiamine degradation. A similar trend was found in chicken meat cooked to different end-point temperatures (Ang, Liu, & Sun, 1994) and, once more, in pork cheeks cooked under vacuum at 60 ºC and 80 ºC for 5 h and 12 h (Sanchez del Pulgar, et al., 2013). However, other volatile compounds from Maillard reactions are scarce, such as 3-hydroxy-2-butanone, or not detected, such as pyrazines, in the samples of this study. The quantities of Maillard-derived compounds increased as cooking temperature and time increased and the moisture levels decreased (Huang & Ho, 2001). Thus, pyrazines was the major class of volatiles in well-done grilled meat (Mottram, 1998a). Mussinan and Walradt (1974) studied the volatile constituents of pressure cooked pork liver at 163 ºC and reported that, quantitatively over 70 % of the total amount of the volatiles were furans and pyrazines. However, the cooking temperatures applied in our study were milder and the moisture content of surface is not reduced during *sous-vide* cooking (Roldán, et al., 2013) in comparison to these studies, which would explain the low abundance of volatile compounds derived from the Maillard reaction. In fact, in boiled meat these type of volatile compounds present low levels (Mottram, 1998a). In addition, previous studies carried out in the samples of this work showed small amounts of Maillard reaction markers (under review data).

CONCLUSIONS

The volatile compound profile of *sous-vide* cooked lamb loin is mainly affected by cooking temperature and by its interaction with cooking time, such an effect being notable in linear aldehydes, ketones, alcohols and sulfur-containing compounds. In general, volatile compounds arising from lipid oxidation are the major one when cooking at milder cooking conditions (60 º C for 6 and 24 h, 80 ºC for 6 h), while more intense time and temperature combination (80 ºC for 24 h) promoted the formation of volatile compounds from Strecker degradations. It could be indicated that *sous-vide* cooking at moderately high temperatures for long times stimulates the formation of volatile compounds from amino acid-involved reactions that would result in the formation of desirable meaty flavour and roast notes in samples.

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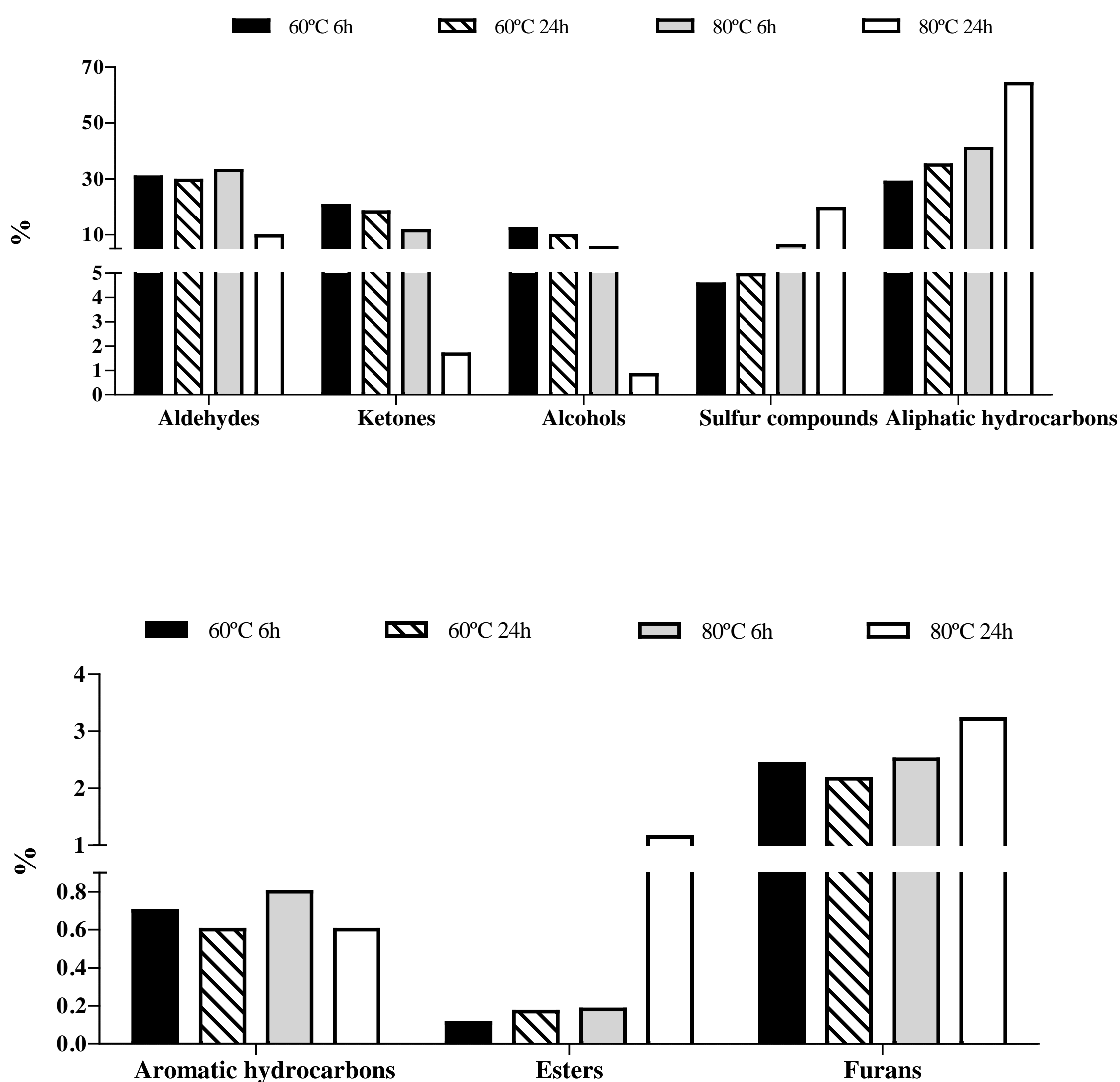

Figure 1. Chemical families of volatile compounds (expressed as percentage of total chromatographic area) in lamb loins *sous-vide* cooked at different temperature-time combinations.

Table 1. Selected volatile compounds (expressed in area units x 10^{-6}) in lamb loins *sous-vide* cooked at different combinations of temperature (T) and time (t).

	LRI[1]	ID[2]	60ºC		80ºC		SEM[3]	P(T)	P(t)	P(Txt)
			6h	24h	6h	24h				
Aldehydes										
Acetaldehyde	<500	B	3.1	4.8	4.4	4.4	0.4	ns	ns	ns
2-Methypropanal	550	A	0.5^{b}	0.6^{b}	1.1^{b}	1.9^{a}	0.2	**	ns	***
Butanal	591	A	1.2	1.3	1.3	nd	0.1	ns	ns	ns
3-Metylbutanal	649	A	0.6^{b}	1.1^{b}	0.7^{b}	3.2^{a}	0.2	*	**	***
Pentanal	698	A	32.9^{a}	26.0^{b}	30.2^{ab}	3.9^{c}	1.2	**	***	***
Hexanal	800	A	34.3^{a}	34.7^{a}	25.4^{b}	4.3^{c}	1.9	***	*	***
Heptanal	901	A	3.0^{a}	2.5^{ab}	1.9^{bc}	0.9^{cd}	0.3	***	**	***
Octanal	1004	A	1.8^{a}	1.8^{a}	1.3^{a}	1.0^{a}	0.3	***	***	***
Nonanal	1101	A	3.3^{a}	2.0^{ab}	1.6^{bc}	0.8^{bc}	0.4	**	*	*
Total			80.1^{a}	74.2^{a}	67.9^{a}	15.7^{b}	3.3	**	*	***
Aliphatic hydrocarbons										
Pentane	500	A	13.7^{a}	10.2^{ab}	11.9^{ab}	6.9^{b}	1.6	ns	*	*
2-Methylpentane	558	A	0.4^{b}	0.5^{ab}	0.6^{ab}	2.1^{a}	0.4	*	ns	*
3-Methylpentane	577	A	0.4	0.4	3.2	2.5	0.9	**	ns	ns
1-Hexene	586	A	0.3^{b}	0.4^{b}	0.4^{b}	0.8^{a}	0.1	**	*	**
Methylcyclopentane	624	A	0.2^{b}	0.3^{b}	0.3^{b}	0.7^{a}	0.1	ns	ns	*
3-Methylhexane	672	A	1.4^{b}	1.1^{b}	1.7^{ab}	5.5^{a}	0.9	*	ns	*
3-Ethylpentane	683	A	nd	nd	0.5	0.9	0.1	-	*	-
1,3-Dimethylcyclopentane	684	A	nd	nd	nd	0.75	nd	-	-	-
1-Heptene	691	A	0.9	0.9	0.9	1.8	0.3	ns	ns	ns
Heptane	700	A	13.4^{b}	16.5^{b}	15.6^{b}	32.6^{a}	3.11	*	*	**
2,2,3,3-Tetramethylbutane	724	A	2.3	2.3	2.2	2.9	0.4	ns	ns	ns
Methylcyclohexane	726	A	3.6^{b}	3.5^{b}	4.3^{b}	12.9^{a}	1.2	*	ns	***
2,5-Dimethylhexane	731	A	4.3	4.1	4.8	6.9	1.0	ns	ns	ns
2,4-Dimethylhexane	734	A	8.7	9.9	9.9	12.8	1.7	ns	ns	ns
Ethylcyclopentane	739	A	2.2^{b}	2.2^{b}	2.3^{b}	5.4^{a}	0.7	*	ns	**
1,2,3-Trimethylcyclopentane	752	A	1.0	0.9	1.0	1.8	0.2	*	ns	*
2,3,4-Trimethylpentane	753	A	0.5^{b}	0.6^{b}	0.6^{b}	1.0^{a}	0.1	*	ns	**

	LRI[1]	ID[2]	60ºC		80ºC		SEM	*p*(T)	*p*(t)	*p*(Txt)
			6h	24h	6h	24h				
Aliphatic hydrocarbons (continued)										
2,3,3-Trimethylpentane	759	A	nd	nd	nd	0.53	-	-	-	-
2-Methylheptane	768	A	11.2^{b}	13.7^{b}	14.6^{b}	21.7^{a}	1.6	*	*	**
1-Ethyl-3-methylcyclopentane	791	A	1.2	0.9	0.7	1.3	0.1	ns	ns	ns
1-Ethyl-2-methylcyclopentane	794	A	0.7	0.6	0.5	0.7	0.1	ns	ns	ns
4-Octene	806	A	0.5	0.4	0.5	0.6	0.1	ns	ns	ns
2-Octene (Z)	808	A	nd	12.3	nd	5.2	1.1	-	-	-
2-octene (E)	816	A	6.4	5.1	5.1	6.1	1.1	ns	ns	ns
Ethylcyclohexane	840	A	0.6	0.6	0.6	0.8	0.1	ns	ns	ns
Decane	1000	A	1.4^{a}	0.9^{ab}	0.9^{ab}	0.8^{b}	0.1	ns	ns	*
Dodecane	1200	A	0.2	nd	0.3	0.5	0.1	ns	*	ns
Total			61.6	75.6	63.6	114.4	15.6	ns	ns	ns
Aromatic hydrocarbons										
Benzene	661	A	1.3^{a}	1.3^{ab}	0.7^{b}	nd	0.1	*	ns	*
Ethylbenzene	866	A	nd	nd	nd	0.3	-	-	-	-
1,4-Dimethylbenzene	874	A	0.6	0.4	0.4	0.6	0.1	ns	ns	ns
1,2-Dimethylbenzene	897	A	nd	nd	0.6	0.5	0.2	-	ns	-
Total			1.3	1.3	1.3	0.9	0.3	ns	ns	ns
Ketones										
2-Pentanone	686	A	0.5	0.4	0.5	nd	0.1	ns	ns	ns
2,3-Pentanedione	696	A	2.8	1. 9	nd	nd	0.4	-	ns	-
3-Hydroxy,2-butanone	720	A	nd	nd	nd	0.6	0.1	-	-	-
2-Heptanone	890	A	1.3	1.4	0.9	1	0.2	ns	ns	ns
3-Octanone	984	A	49.2^{a}	42.6^{ab}	21.9^{bc}	2.0^{c}	5.3	***	ns	***
Total			43.0^{a}	45.4^{a}	23.2^{ab}	2.6^{b}	6.7	***	ns	**

	LRI[1]	ID[2]	60ºC		80ºC		SEM	*p*(T)	*p*(t)	*p*(Txt)
			6h	24h	6h	24h				
Alcohols										
1-Penten-3-ol	682	A	3.7^{a}	2.3^{ab}	1.6^{bc}	nd	0.4	*	*	**
1-Hexanol	870	A	3.9^{a}	2.9^{ab}	1.3^{b}	nd	0.6	**	ns	**
1-octen-3-ol	980	A	24.4^{a}	19.1^{ab}	8.2^{bc}	1.7^{c}	2.7	***	ns	***
Total			30.4^{a}	23.8^{ab}	11.1^{bc}	1.7^{c}	3.9	***	ns	**
Furans										
2-Methylfuran	606	A	0.4^{b}	nd	0.6^{ab}	1.8^{a}	0.3	ns	**	*
2-Ethylfuran	703	A	2.7	1.9	1.9	1.5	0.4	ns	*	ns
2-Butylfuran	894	A	0.2	0.2	0.2	0.2	0.03	ns	ns	ns
2-Pentylfuran	995	A	3.1^{a}	3.4^{a}	2.4^{ab}	3.4^{a}	0.6	**	**	*
Total			5.6^{a}	5.5^{a}	3.4^{ab}	6.1^{a}	1.0	ns	ns	ns
Sulfur-containing compounds										
Methanethiol	<500	B	nd	nd	2.8	3.4	0.3	-	ns	-
Dimethyl sulfide	517	A	2.3	2.1	3.1	3.3	0.4	**	ns	ns
Carbon disulfide	534	A	9.7^{b}	10.4^{b}	6.2^{b}	34.4^{a}	2.9	*	*	***
Dimethyl disulfide	750	A	nd	nd	0.4	0.6	0.1	-	ns	-
Total			4.6^{b}	12.5^{b}	10.9^{b}	35.1^{a}	2.5	*	**	***
Esters										
Ethyl ester acetic acid	613	A	0.3	0.4	0.4	2.5	0.7	ns	ns	ns
Total			0.3	0.4	0.4	2.5	0.7	ns	ns	ns

[1] Linear retention index in the DB-5 column.
[2] Reliability of identification: A, mass spectrum and retention index in agreement with databases and literature data; B, tentative identification by mass spectrum.
[3]SEM: Standard error of the mean.
T^{a}: Temperature; t: time.
Different superscript letters within the same row mean significant differences between the different temperature × time ($p < 0.05$). nd: not detected. ns: No significance. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Capítulo 1.4

Influence of different temperature-time combinations in lamb loins cooked under vacuum during storage

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TITLE

Influence of different temperature-time combinations in lamb loins cooked under vacuum during storage

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ABSTRACT

In previous papers, the effect of cooking temperature and time on different physico-chemical and microbiological parameters of *sous-vide* cooked lamb loins was addressed. In the present one the effect of such cooking factors on these variables over storage time was studied. Milder cooking treatments led to lower weight losses, although this effect was not reflected in the moisture content. No changes were observed in secondary lipid oxidation products (TBARs) and only a decrease in conjugated dienes in those samples cooked at the strongest cooking conditions (80 ºC-24h) was observed. Total protein carbonyls values increased at higher cooking temperatures during the first week of storage to decrease at the end of the same. Similar values of L* and a* were obtained during all storage period, however b* values showed a very variable behavior, although the loins cooked at the mildest cooking treatment (60ºC-6h) had always the lowest values. Remarkable texture changes emerged in those samples subjected to the strongest cooking treatment (80 ºC-24h) with consistently lower values of hardness or cohesiveness during the whole chilled storage. The intensity of heat treatments suggests a total inactivation of vegetative forms of spoilage bacteria during the storage of the samples at 2ºC and, therefore, the contribution of microorganisms to the changes found in the analytical parameters researched may be considered very limited, even after 30 days of storage under refrigeration conditions.

INTRODUCTION

In *sous vide* cooking, meat is cooked in its own juice inside pouches for longer times and lower temperatures compared to those used in conventional cooking. These cooking conditions lead to a more tender and juicier texture, extended shelf-life and a better preservation of sensory properties of the products during storage (Ruiz, Calvarro, Sánchez del Pulgar, & Roldán, 2013). The precise combination of temperature and time is critical in the final meat quality characteristics of *sous vide* cooked meat (Roldán, Antequera, Martín, Mayoral, & Ruiz, 2013; Sánchez Del Pulgar, Gázquez, & Ruiz-Carrascal, 2012). Thus, cooking temperature and time strongly affect the color of *sous-vide* cooked meat; higher cooking temperatures lead to a less intense red color due to a higher myoglobin denaturation (Roldán et al., 2013). Consequently, when cooking *sous-vide* at temperatures around 60 °C for long times, meat usually shows a more intense red color than that cooked by traditional methods, regardless the length of the cooking treatment (Sánchez Del Pulgar et al., 2012). However, little is known about how meat color changes subsequently throughout the chilling storage of meat *sous-vide* cooked at different combinations of time and temperature.

Thermal processing causes the oxidation of lipids and proteins, which could finally result in a decrease of meat quality and acceptability (Filgueras et al., 2010; Renerre & Labadie, 1993). Lipid and protein oxidation are complex processes, but it seems that increasing temperatures and cooking times lead to a different ratio between primary and secondary compounds from lipid oxidation in *sous-vide* cooked meat (Roldán et al., 2013; Sánchez Del Pulgar et al., 2012). Once meat has been cooked, oxidative changes continue during shilling storage. However, there is scarce scientific information about this further development of oxidative phenomena during the storage. Considering that free radicals from lipid oxidation may induce further oxidation of fatty acids and proteins, such knowledge results crucial in the development of ready to eat meat meals produced using low temperature-long time (LTLT) *sous-vide* cooking and that it is intended to be stored for several weeks.

The low oxygen partial pressure in the *sous-vide* container prevents the growth of aerobic spoilage micro-organisms, increasing the time that cooked meat can be preserved (Creed, 1995). However, some authors have pointed

out the potential microbiological hazards associated to *sous-vide* technology, such as thermal-stable exotoxins, obligate psychro-tolerant and some facultative anaerobes. In a previous work, the effect of the LTLT *sous-vide* cooking of lamb loins was studied, with a dramatic reduction of the microbial population after the application of different cooking treatments, even after the less intense one (60 °C–6h) (Roldán et al., 2013). How the different cooking treatment affects the subsequent microbial growth during the chilled storage is decisive to establish the real self-life of the cooked product.

Changes in meat tenderness and juiciness during cooking strongly depend upon the cooking temperature and time applied (Laakonen, Wellington, & Sherbon, 1970). Long cooking times at a high enough temperature seems to enhance collagen solubilization, which in turn would reduce meat toughness (Christensen et al., 2013; Sánchez Del Pulgar et al., 2012). On the other hand, cooking temperatures above 70 ºC very much enhance water loss, leading to lower meat juiciness (Roldán et al., 2013; Sánchez Del Pulgar et al., 2012). However, the subsequent changes in textural traits during the chilled storage of *sous-vide* cooked lamb meat as affected by the cooking conditions remain mostly unknown.

Therefore, there is not much available information about how cooking temperature and time conditions can affect the changes produced in LTLT *sous-vide* cooked meat during the subsequent chilled storage. It seems that, in general, minor changes in microbiological, sensory and physical-chemical (lipid oxidation, color, cooking losses…) characteristics of the *sous vide* cooked beef and pork muscles, were observed around 3 – 5 weeks of chilled storage. However, longer periods of storage result in a substantial sensory deterioration of meat and, therefore, a significant loss of acceptance thereof (Pedro Díaz, Gema Nieto, María Dolores Garrido, & Sancho Bañón, 2008a), Moreover, no data have been published on lamb meat in this respect. Therefore, our objective was to study the effect of different temperature-time combinations on instrumental color and texture, oxidative related deterioration and microbiology of lamb loins *sous-vide* cooked at different combinations of temperature and time and subsequently stored under refrigeration.

MATERIAL AND METHODS

Experimental design

Four different combinations of temperature (60 ºC - 80 ºC) and time (6h – 24h) were used for cooking sixty lamb loins (n=5 for each batch). All loins were from a homogeneous production batch of lambs averaging 26 kg live weight and 90 days of age. Samples were weighed, packaged in vacuum plastic bag (nylon/polyethylene pouches; heat resistance of −40 °C/+120 °C, O2 permeability of 9 cm3/m2 per 24h at 4°C/80% HR and water steam permeability of 1.2 g/m2 per 24h) (Joelplas SL, Barcelona, Spain) and cooked in thermostatized water baths at different temperature–time combinations. The internal temperature was monitored using a data logger Testo735-2 (Testo, Lenzkirch, Germany) equipped with a needle thermocouple. Once the cooking process had finished, the pouches were removed from the water bath and submerged in iced cold water (2 °C) for 1 h. Subsequently, the packaged loins were kept under refrigeration (2 °C) and sampled on days 0, 7, 15 and 30 days. After that, they were kept at - 80 ºC until analysis. For every selected day of storage time, samples were evaluated for level of lipid and protein oxidation level (conjugated dienes (CD), thiobarbituric acid reactive substances (TBARS), and protein carbonyl content), instrumental color, instrumental texture and microbiology. The samples from day 0, together with other *sous-vide* cooked lamb samples from other cooking conditions, were considered elsewhere for evaluating the changes taking place during the cooking of meat (Roldan, Antequera, Armenteros, & Ruiz, 2014; Roldán et al., 2013). In the present paper these samples were taken as the initial point of the chilling storage.

Analytical procedures

Moisture content and water loss

Cooking loss was calculated by difference of weight before and after the cooking treatment on days 0, 7, 15 and 30 days of storage. Moisture content was determined by drying the samples (5 g) at 102 °C (A.O.A.C., 2000). Samples were analyzed in duplicate.

Conjugated dienes

The measurement of CD was determined according to the procedure

described by (Juntachote, Berghofer, Siebenhandl, & Bauer, 2006). Frozen lamb loin samples were thawed and thereafter, they were cut with a knife in small cubes, immersed in liquid nitrogen and minced. Subsequently, 0.5 g were suspended in 5 ml of distilled water and homogenised to form smooth slurry. A 0.5 ml aliquot of this suspension was mixed with 5 ml of extracting solution (3:1 (v/v) hexane/isopropanol) for 1 min. After centrifugation at 3500 rpm for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of CD was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1} and the results were expressed as mmol per kg of sample.

Thiobarbituric acid reactive substances

TBARS determination in lamb loins was carried out using the method described by (Salih, Smith, Price, & Dawson, 1987) with some modifications: 5 g of sample, previously minced following the same procedure described for CD analysis, were homogenized in polypropylene tubes of 50 ml using a ultra-turrax homogeneizer (Ultra-Turrax T25, IKA-Werke GmbH & Co. KG, Stafen, Germany) with 15 mL 3.86 % perchloric acid and 0.50 mL BHT (butylated hydroxytoluene) for 30 s and centrifuged at 4000 rpm for 2 min. During homogenisation tubes were kept in ice to avoid heating. The blended sample was filtered through Watman No.1 filter paper. The filtrate was adjusted to 25 mL by adding 3.86 % perchloric acid. Next, 2 mL aliquots of the filtrate were mixed in glass tubes with 2 mL of 0.02 M TBA and shaked. The solution was heated at 100 ºC for 30 min, cooled and again centrifuged. The absorbance was measured at 530 and 600 nm on a spectrophotometer (Model U-2000, Hitachi, Tokyo) against a blank containing 3 mL of perchloric acid (3.86 %) and 3 mL of TBA reagent. The concentration of TBARS was calculated from a standard curve in triplicate using solutions of 1,1,3,3-tetraethoxypropane (TEP) (Merck, Schchardt). Results were expressed as mg malonaldehyde (MDA) equivalents per kg of meat sample.

Protein carbonyls

Protein oxidation in lamb loins was followed by measuring the formation of protein carbonyls by converting them to 2,4 dinitrophenylhydrazones (DNPH), and the derivatives were measured spectrophotometrically according to method outlined (Oliver, Ahn, Moerman, Goldstein, & Stadtman, 1987) with

slight modifications. Lamb meat was minced with liquid nitrogen just like in the other procedures and then, 1 g of meat was homogenised 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturrax homogeniser for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL eppendorf tubes. Proteins were precipitated by cold 10 % TCA (1 mL) and subsequently centrifuged for 5 min at 5000 rpm. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10 % TCA (1 mL) and washed twice with 1 mL ethanol/ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 5031 g to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using a BSA standard curve. The amount of carbonyls was expressed as nmol of carbonyl per milligram of protein using an absorption coefficient of 21.0 nM^{-1} x cm^{-1} at 370 nm for protein hydrazones.

Instrumental color

Color was measured across the cut surface of the cooked loin after chilling. L* value (lightness), a* value (redness) and b* value (yellowness) were obtained using a Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) programmed to used the built-in internal illuminant D65. Means of readings on three locations on each sample were determined. Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Instrumental Texture Analysis

Instrumental texture analyses were performed in a texturometer TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). For the determination of the texture profile analysis (TPA), uniform portions of the cooked loins were cut into 1 cm^3 cubes. For each sample, eight cubes were obtained and analyzed. They were axially compressed to 50% of the original height with a flat plunger of

50 mm in diameter (P/50) at a crosshead speed of 2 mm·s^{-1} through a 2-cycle sequence. The following texture parameters were measured from force deformation curves (Bourne, 1978): Hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); Cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required to for the first compression and A2 the total energy required for the second compression).
Shear force (SF) analysis on cooked samples was performed using a Warner-Bratzler blade (3x1x1 cm), which sheared the specimen perpendicularly to the muscle fibers at a constant speed of 1 mm·s^{-1} and then pushed through the slot. The maximum force (N) required to shear the sample was measured. Six determinations were performed for each cooked sample.

Microbiology

In order to carry out the counts, 10g of the sample of the loins were homogenized in 90 ml sterile 0.1% peptone in a Stomacher (Lab Blender, Model 4001, Seward Medical, London, UK) for 30s. Appropriate dilutions were made with 0.1% peptone broth and 1 ml was plated onto the culture media under the following conditions. Total mesophilic and psychrotrophic counts on Plate Count Agar (PCA, Oxoid, Unipath, Basingstoke, UK) for 72 h at 30 °C and 7 days at 7 ºC, respectively; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBG, Oxoid) for 24h at 37 °C; Coliforms on Violet Red Bile Agar (VRBA, Oxoid) for 24h at 37 °C; lactic acid bacteria, LAB, on MRS Agar (Oxoid) in anaerobic conditions for 72 h at 30 °C; Gram-positive Catalase-positive cocci on Mannitol Salt Agar (MSA, Oxoid) after 72 h at 37 °C; sulfite reducing clostridia on Sulfite-Polymyxin-Sulfadiazine (SPS) agar incubated anaerobically for 72 h at 37 °C; intestinal enterococci on Slanetz and Bartley agar (S&B, Oxoid) for 24h at 37 °C; *B. thermosphacta* on Streptomycin Thallous acetate actidione agar (STAA, Oxoid) for 72 h at 20 ºC. Typical colonies for each selective media were counted in plates from the dilution with 10-100 colonies.

Listeria and *Salmonella* were also researched. For these pathogens, 25 g sample was homogenized in 225 ml of primary enrichment broth (buffered peptone water) and incubated at 30 ºC for 24h. Then, 1 ml of these primary enrichments were transferred to 10 ml of *Listeria* Enrichment Broth (LEB, Merck)

and incubated at 30 ºC for 24h. The enrichments were subcultured by streaking onto PALCAM *Listeria* selective agar (Merck) supplemented with PALCAM *Listeria* selective supplement (Merck) and incubated at 30 ºC for 24h. Detection of *Salmonella spp*. was carried out according to the International Standard Organization protocol (ISO6579, 2002).

Statistical analysis

The effect of cooking temperature (60 and 80 ºC), cooking time (6 and 24h) and their respective interaction (Tª-t) on each storage day on CD, TBARS, PC, instrumental color, instrumental texture and microbiology in *sous-vide* cooked lamb loins was evaluated by a two-way analysis of variance. The effect of storage time on measured parameters of cooked lamb loins within each combination of cooking time and temperature was analyzed by a one-way analysis of variance. In both cases the analyses were performed using the general linear models procedure of SPSS (V.15.0). When the effect of any of the factors was significant ($p \leq 0.05$), differences between groups were analyzed by using Tukey's posthoc test.

RESULTS

Effect of temperature-time combination on weight loss and moisture content

Results for weight loss and moisture content of lamb loins cooked at different temperature-time combinations and stored under refrigeration for 30 days are shown in Figure 1a and 1b, respectively. Both physical parameters were significantly affected by the interaction between cooking temperature and time in all storage days, except for moisture content on day 0 of chilled storage. Overall, samples cooked at 60 ºC showed higher moisture contents and lower weight loss percentages compared to samples cooked at 80 ºC throughout the whole storage period.

Regarding weight loss (Figure 1a), samples cooked at all temperature-time combinations except for those cooked at 80ºC for 24h, showed a slight decrease at the end of storage. This was not entirely reflected in the moisture content of the different types of samples, since only lamb samples cooked at 60ºC for 6h showed a significant increase. All samples except those from this group showed similar moisture levels after 30 days of chilled storage.

Effect of temperature-time combination on oxidative changes during storage

Lipid oxidation during chilled storage in lamb loins *sous-vide* cooked at different time-temperature combinations was measured by quantification of CD and TBARS. As a consequence of the thermal processing, there were noticeable oxidative changes in the meat. The level of CD and TBARS were significantly affected by the interaction between cooking temperature and time in all storage days. Regarding CD (Figure 2a), samples cooked at 80 ºC for 24h showed the highest values throughout the whole storage period, while those cooked at 60 ºC for 6h showed the lowest. In samples cooked at 60 ºC, either for 6 or 24h, just like at 80 ºC for 6h, values of CD kept quite constant throughout all storage time. However, when samples were cooked at 80 ºC for 24h, the content in CD underwent a significant decrease from day 7 to day 15, although they still were significantly higher than those from the other groups.

Results for TBARs showed the opposite trend to CD ones (Figure 2b). In this case, samples cooked at 80 ºC for 24h showed the lowest values throughout the whole chilled storage, while those cooked at 60 ºC for 6h showed the highest. On the other hand, the chilled storage time showed a limited effect,

that was significant only in samples cooked at 60 ºC for 24h and those cooked at 80 ºC for 6h.

Results for protein oxidation of lamb loins cooked at different temperature-time combinations and stored under refrigeration are shown in Figure 2c. The level of DNPH was significantly affected by the interaction between cooking temperature and time in the first two weeks of storage, those samples cooked at higher temperatures for longer times showing higher protein carbonyl values. However, at the end of storage, none of the cooking factors considered had a significant effect on DNPH values and all lamb loin samples showed similar values. On the other hand, storage time had a significant effect on DNPH values in all groups except for those cooked at 60 ºC for 24h, in which no significant differences in DNPH levels during the 30 days of chilling storage was detected. In samples cooked at 60 ºC for 6h a slight increase in DNPH levels after the first week of storage followed by a decrease at the end of the storage period was evidenced. Contrarily, samples cooked at 80 ºC showed an increase during the first week of storage, followed by a decrease until the end of the storage period.

Effect of cooking temperature-time combination on instrumental color during storage

Table 1 shows the obtained instrumental color parameters, lightness (L*), redness (a*) and yellowness (b*) in lamb loin samples *sous-vide* cooked under the different experimental conditions studied and stored for 30 days at chilling temperature. All three color coordinates were significantly affected by the interaction between cooking temperature and time in all storage days, except for L* values the first of chilled storage and the a* values after 30 days of storage. The highest values for L* were observed in samples cooked at 60 ºC for 24h during all storage, while similar values were observed for the rest of cooking treatments. Only those samples cooked at 80 ºC for 6h showed significant changes in L* values during storage, but the variations were very limited.

Samples cooked at 60 ºC for 6h showed a more intense red color throughout the whole storage, while those cooked at 80 ºC for 6h showed the lowest values. No effect of the storage time on a* values was observed.

Finally, the highest b* values were obtained in samples cooked at 80 ºC for

24h during the first two weeks of chilled storage. However, after 30 days of storage, samples cooked at 80 ºC for 6h showed the highest values. On the other hand, storage time had a significant effect on b* values in all cooking treatments except for samples cooked at 60 ºC for 6h. The rest of the temperature-time combinations showed different trends: samples cooked at 60 ºC for 24h and at 80 ºC for 6h presented a higher yellowness at the end of storage, while samples cooked at 80 ºC for 24h showed the lowest values for this parameter after 30 days of storage under refrigeration.

Effect of temperature-time combination on texture during storage

Obtained mean values for the hardness and cohesiveness from the TPA, and for the SF values in the Warner-Blatzer of *sous-vide* cooked lamb loins throughout 30 days of chilled storage are shown in Figure 3. The interaction between cooking temperature and cooking time had a significant effect on the two TPA parameters at all storage days. Overall, samples cooked at 80 ºC for 24h showed the lowest values for both TPA parameters throughout the whole chilled storage period. Hardness (Figure 3a) was significantly affected by the storage time in samples cooked at 80ºC, so that there was a decrease in measured values during the first week of chilled storage, followed by an trend to increase until the end of the storage. On the other hand, samples cooked at 60ºC for 24h showed quite constant levels of hardness during the first two weeks of chilled storage, followed by a decrease at day 30. And samples cooked at 60ºC for 6h did not show any significant changes in hardness during the whole storage. Changes in cohesiveness (Figure 3b) followed a similar pattern, but were subtler, so that only samples cooked at 80ºC for either 6h or 24h showed significant changes throughout the chilled storage.

Values for SF (Figure 3c) were also affected by the interaction between cooking temperature and time but only at the end of the chilled storage, since during the first two weeks similar values for this attribute were detected. After 30 days of storage, samples cooked at the more severe cooking conditions (80 ºC for 24h) showed significantly lower SF values compared to samples cooked with the mildest treatment. On the other hand, the effect of the storage time was not clear, and only samples cooked at 60 ºC for either 6h or 24h showed a

significant effect, with a decrease during the first week of storage and steady values or slight increase thereafter.

DISCUSSION

In previous papers, the effect of cooking temperature and time on different physico-chemical and microbiological parameters of *sous-vide* cooked lamb loins was addressed (Roldan et al., 2014; Roldán et al., 2013). In the present one we will focus on the effect of such cooking factors on how different variables change over storage time.

Weight loss and moisture content

Higher cooking temperatures in our study led to higher weight loss of lamb meat right after cooking (Figure 1a), most likely due to a stronger denaturation and shrinkage of myofibrilar proteins and collagen (Sánchez Del Pulgar et al., 2012). These results are in agreement with those reported by (Palka & Daun, 1999) who observed a relationship between changes in sarcomere length and cooking loss. However, during the subsequent chilled storage, there was a slight decrease in the weight loss, which was significant in three of the cooking temperature-time combinations. This could have been due to a slight increase in the ability of hold water as a consequence of an increase in meat pH. Indeed, other authors (Pedro Díaz, G. Nieto, M. D. Garrido, & S. Bañón, 2008b) have observed increased pH values of sous-vide cooked pork during the chilled storage.

On the other hand, despite such changes in weight loss during storage, moisture was not affected in the same extent (Figure 1b), and only those samples from the 60ºC-6h group showed a significant increase in moisture content during the chilled storage.

Oxidative changes during storage

TBARs values represent the content of secondary lipid oxidation products, mainly aldehydes (or carbonyls), which may contribute to off-flavors in oxidized meat and meat products (Teets, Sundararaman, & Were, 2008). In general, TBARs values increase with cooking and storage time in meat (Broncano, Petrón, Parra, & Timón, 2009). Therefore, it would make sense to expect an increase of the final lipid oxidation products as storage time draws on. However, this did not happen in our study, since the TBARs values (Figure 2b) were similar at the beginning and at the end of the storage period. MDA is

very prone to react with other compounds present in meat, specially those containing primary amino groups such as proteins, phospholipids, DNA or amino acids (Ventanas, Estevez, Delgado, & Ruiz, 2007). This may lead to decreasing levels of MDA and other reactive lipid carbonyls available to react with TBA and consequently produces a decrease in the TBARS values (Sánchez Del Pulgar et al., 2012). This fact seems to be promoted at higher cooking temperatures for longer times (Roldan et al., 2014). The absence of significant changes in TBARs during the whole chilled storage might point out to a balance between the ongoing lipid oxidation and the chemical reactions between MDA and other components.

CD (Figure 2a) are formed at the early stages of lipid oxidation and, just as TBARs, the rate of formation of CD depends of cooking conditions, increasing with higher cooking temperatures and longer times (Juntachote et al., 2006; Roldan et al., 2014). These compounds are decomposed to secondary products; therefore, a decrease in CD during the chilled storage might be expected. However, as shown in Figure 2a, only those samples cooked at the strongest cooking conditions (80ºC-24h) followed this trend. It seems that after the heating treatment the rate of formation of CD decreases to a great extend, but they continue decomposing to secondary lipid oxidation products. Other authors have detected steady or decreasing levels of CD during the chilled storage of cooked meat, although in this case this was paralleled with increasing levels of TBARs (Peña-Ramos & Xiong, 2003). Different factors could affect how the overall lipid oxidative phenomena takes place, from the fatty acid composition of the meat to the release of free heme during cooking or the presence and amount of compounds available for reacting with carbonyls from lipid oxidation (Ladikos & Lougovois, 1990).

Higher cooking temperatures in our study led to an increase of protein carbonyls groups during the first week of storage compared to samples cooked at lower temperatures. Heating degrades myoglobin causing the release of iron which is believed to increase its pro-oxidant potential in cooked meats (Ganhao, Morcuende, & Estevez, 2010); this fact, together with the promoting effect of higher cooking temperatures on protein oxidation, could explain the higher DNPH values obtained in samples cooked at 80 ºC compared to samples cooked at 60 ºC during the first weeks after cooking.

According to (Requena, Levine, & Stadtman, 2003), around 60% of the total carbonyls value in oxidized proteins comes from specific protein carbonyls, α-aminoadipic semialdehyde (AAS) and γ-glutamic semialdehydes (GGS). Utrera, Rodríguez-Carpena, Morcuende, and Estévez (2012) reported that lysine is modified by oxidation during meat cooking and the subsequent chilled storage to yield AAS, which could be involved in further reactions during meat processing and storage (Requena, Chao, Levine, & Stadtman, 2001). Thus, the decrease in DNPH values at the end of storage in samples cooked at 80ºC could be due to the involvement to the AAS in reactions with the amino groups from the side chain of proteins via Schiff base formation promoted by cooking and subsequent chilled storage (Utrera et al., 2012).

Effect of cooking temperature-time combination on instrumental color during storage

Higher cooking temperatures in our study led to lower lightness of lamb meat right after cooking, most likely due to a higher denaturation and aggregation of sarcoplasmic and myofibrillar proteins, which would lead to increased light scattering (Christensen, Bertram, Aaslyng, & Christensen, 2011). However, this effect changed during the chilling storage, so that at the end of the storage the lowest L* values were from samples of the 60ºC-6h cooking treatment (Table 1). Nevertheless, variations during the storage were only significant for the 80ºC-6h group, and detected differences in L* value seems to be too short to have a real influence on perception. Such changes in L* values could be related to the observed decrease in water loss and related slight increase in moisture content in lamb loin samples cooked at 60ºC for 6h.

As far as the redness is concerned, as expected, the mildest cooking treatments applied in the current study entailed a lower myoglobin degradation, with higher values for a* (Table 1). Cooking results in the denaturation of soluble Mb, and heat-induced Mb denaturation is responsible for the dull-brown color of cooked meats (King & Whyte, 2006). Redness was very stable during the whole storage period, with no significant change in any of the groups. Our results are in agreement with those reported by (Díaz et al., 2008b) who also found no significant differences in the mean color values during storage of pork loins processed by the *sous-vide* method and at 70ºC for

12h. Thus, apparently no color reversion was detected. In beef, poultry and pork cooked to temperatures up to 80 ºC, color reversion in chilled storage has been described (Mancini & Hunt, 2005), but no information about lamb about this fact has been found in the literature. The long heating time in *sous-vide* could inactivate the mechanisms involved in color reversion, explaining the absence of such a process even though the cooking conditions were quite mild in temperature.

On the other hand, b* values showed a very variable behavior during the storage depending on the cooking conditions, although the lowest values were always those from the samples cooked at the mildest ones (60 ºC-6h).

Effect of cooking temperature-time combination on texture during storage

Changes in meat tenderness during cooking are associated with heat-induced alteration of myofibrillar proteins and connective tissue, since heat solubilizes the connective tissue, which in turn leads to meat tenderization, while denaturation of myofibrillar proteins causes meat toughening (Laakonen et al., 1970). In the current study, remarkable texture changes emerged in those samples subjected to the strongest cooking treatment (80 ºC-24h) with consistently lower values of hardness or cohesiveness during the whole chilled storage (Figure 3). In fact, these samples have the appearance of pulled pork or beef, with fiber bundles easily falling apart, while those cooked at 60 ºC remained more cohesive. There were significant changes during the storage affecting 80ºC-6h cooked lamb loins, which showed a marked decrease in hardness after one week of chilled storage. Finally, after 30 days of storage, the effect of cooking time on instrumental hardness was much more evident than that of cooing temperature, event though still those cooked at 80ºC were closer to a pulled meat, while those cooked at 60ºC were more compact, as can be observed in the cohesiveness (Figure 3 b). Even more difficult to address are the changes affecting SF values during the chilled storage. In general, there was a trend to decreasing values of SF during the storage, although this was significant only for samples cooked at 60ºC. It could be that, after the cooking treatment at 60ºC, some proteolysis activity could remain active.

Effect of temperature-time combination on microbiology during storage

The microbial parameters found in the raw loins used in this work were within the Food Quality Standards for fresh meat (EC Commission Regulation, 2005)). The counts found after the different heat treatments in the specific media (<1 log CFU/g) confirmed that even the shortest of the time–temperature combinations considered in the present study (60°C–6 h) were far enough to pasteurize meat (Nyati, 2000). The latter author found that LAB and *Pseudomonas* species were dominant in the microbial population of spoilt sous vide pork loins heat treated at 70ºC for 2 min and stored during 5 weeks at the temperature of 8°C. However, the same samples were capable of extending their shelf-life to four weeks at 3°C without any significant microbial growth. In our study, the intensity of heat treatments (minimal 60ºC for 6h) together with the raw material quality, suggest a total inactivation of vegetative forms of spoilage bacteria during the storage of the samples at 2ºC. (Díaz et al., 2008) studied the microbiological quality of cooked pork loin processed by the sous vide method at an internal temperature of 70ºC for 11 h and stored up to ten weeks at 2 ºC. These author detected counts below 1 log CFU/g of LAB, psychrotrophs, and *Enterobacteriaceae* in any control week.

In the case of the microorganisms isolated from PCA medium, they may be resistant *Bacillus* forms that are able to grow in a nonspecific and nutrient-rich culture media such as PCA (Moerman, Mertens, Demey, & Huyghebaert, 2001). For *Bacillus cereus*, Turner, Foegeding, Larick, and Murphy (1996) established mild–moderate heat treatment in combination with refrigeration temperatures below 10 °C during storage and distribution as safe conditions for a shelf life of 28 days in sous vide chicken breast. Anyway, based on the PCA counts found during the storage, the contribution of microorganism to the changes found in the analytical parameters researched of the vacuum-packed loins heat-treated may be considered very limited, even after 30 days of storage under refrigeration conditions.

CONCLUSIONS

In the present work, the effect of different cooking temperature-time combinations on physico-chemical and microbiological parameters over storage time of *sous-vide* cooked lamb loins was measured. Milder cooking treatments led to lower weight losses, although this effect was not reflected in the moisture content. On the other hand, no changes were observed in secondary lipid oxidation products (TBARs) and only a decrease in conjugated dienes in those samples cooked at the strongest cooking conditions (80 ºC-24h) was showed. Total protein carbonyls values increased at higher cooking temperatures during the first week of storage to decrease at the end of the same. Similar values of instrumental color were obtained during all storage period, however b* values showed a very variable behavior, although the loins cooked at the mildest cooking treatment (60ºC-6h) had always the lowest values. Remarkable texture changes emerged in those samples subjected to the strongest cooking treatment (80 ºC-24h) with consistently lower values of hardness or cohesiveness during the whole chilled storage. The intensity of heat treatments suggests a total inactivation of vegetative forms of spoilage bacteria during the storage of the samples at 2ºC and, therefore, the contribution of microorganisms to the changes found in the analytical parameters researched may be considered very limited, even after 30 days of storage under refrigeration conditions.

Overall, these cooking conditions only caused little changes on these studied variables throughout the chilling period, which suggests that these cooking treatments are appropriated to keep the quality of sous-vide cooked lamb loins even after 30 days of storage.

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Table 1. Instrumental color measured in lamb loins *sous-vide* cooked at different temperature-time combinations and subsequent chilled storage.

	Temperature (°C)	**60**		**80**		**SEM^{A}**	**PT**	**Pt**	**PTxt**
	Time (h)	**6**	**24**	**6**	**24**				
	Storage time (d)								
L*	**0**	66.18	66.95	63.96$^{1-2}$	64.95	0.96	0.040	0.475	0.188
	7	64.57^{b}	68.64^{a}	61.33^{b,2}	64.45^{b}	0.92	0.010	0.011	0.001
	15	65.46^{ab}	67.71^{a}	65.51^{ab,1}	63.19^{b}	0.97	0.050	0.977	0.037
	30	63.52^{b}	66.76^{a}	64.91$^{ab,1-2}$	65.67^{ab}	0.78	0.871	0.022	0.060
	P Storage	0.267	0.528	0.035	0.147				
	SEM^{B}	0.96	0.98	0.93	0.75				
a*	**0**	15.15^{a}	13.42^{ab}	11.51^{b}	13.93^{a}	0.55	0.63	0.863	0.003
	7	15.70^{a}	13.47^{ab}	12.47^{b}	14.50^{ab}	0.56	0.162	0.677	0.006
	15	15.36^{a}	13.92^{ab}	12.18^{b}	14.39^{a}	0.52	0.055	0.602	0.004
	30	15.36	13.80	13.03	13.13	0.65	0.038	0.334	0.081
	P Storage	0.943	0.865	0.350	0.342				
	SEM	0.63	0.50	0.58	0.57				
b*	**0**	8.65^{c}	10.09$^{bc,1-2}$	10.86^{ab,2}	12.55$^{a,1-2}$	0.50	0.001	0.035	<0.001
	7	7.79^{c}	9.41$^{bc,1-2}$	10.79^{b,2}	12.75$^{a,1-2}$	0.40	<0.001	0.060	<0.001
	15	8.64^{b}	8.74^{b,2}	10.99^{b,2}	15.07^{a,1}	0.72	<0.001	0.130	<0.001
	30	8.92^{b}	10.96^{ab,1}	12.99^{a,1}	9.24^{b,2}	0.69	0.239	0.394	0.002
	P Storage	0.456	0.010	0.002	0.004				
	SEM	0.51	0.41	0.39	0.95				

Values with different letter (a-b) within a row of the same storage day are significantly different (P<0.05). Values with a different number (1-2) within a column of the same batch are significantly different (P<0.05).

^{A} Standard error of the mean within the same storage day (n=20)

^{B} Standard error of the mean within the same batch (n=20)

Table 2. Mesophilic bacteria counts in lamb loins *sous-vide* cooked at different temperature-time combinations and subsequent chilled storage.

Days[1]	Hours[2]	60 ºC				80 ºC			
		%[3]	Mean	SD	Max	%	Mean	SD	Max
0 day	6 h	40	1.29	1.84	3.98	60	1.24	1.15	2.40
	24 h	60	1.70	0.96	2.28	80	1.41	1.71	3.48
7 days	6 h	40	0.91	1.26	2.57	40	0.89	1.23	2.46
	24 h	20	0.53	1.19	2.67	20	0.43	0.95	2.12
30 days	6 h	80	2.19	1.42	3.72	80	2.00	1.49	4.14
	24 h	60	1.34	1.34	3.11	80	1.86	1.09	2.76

[1]Days under refrigeration
[2]Cooking time
[3]Prevalence

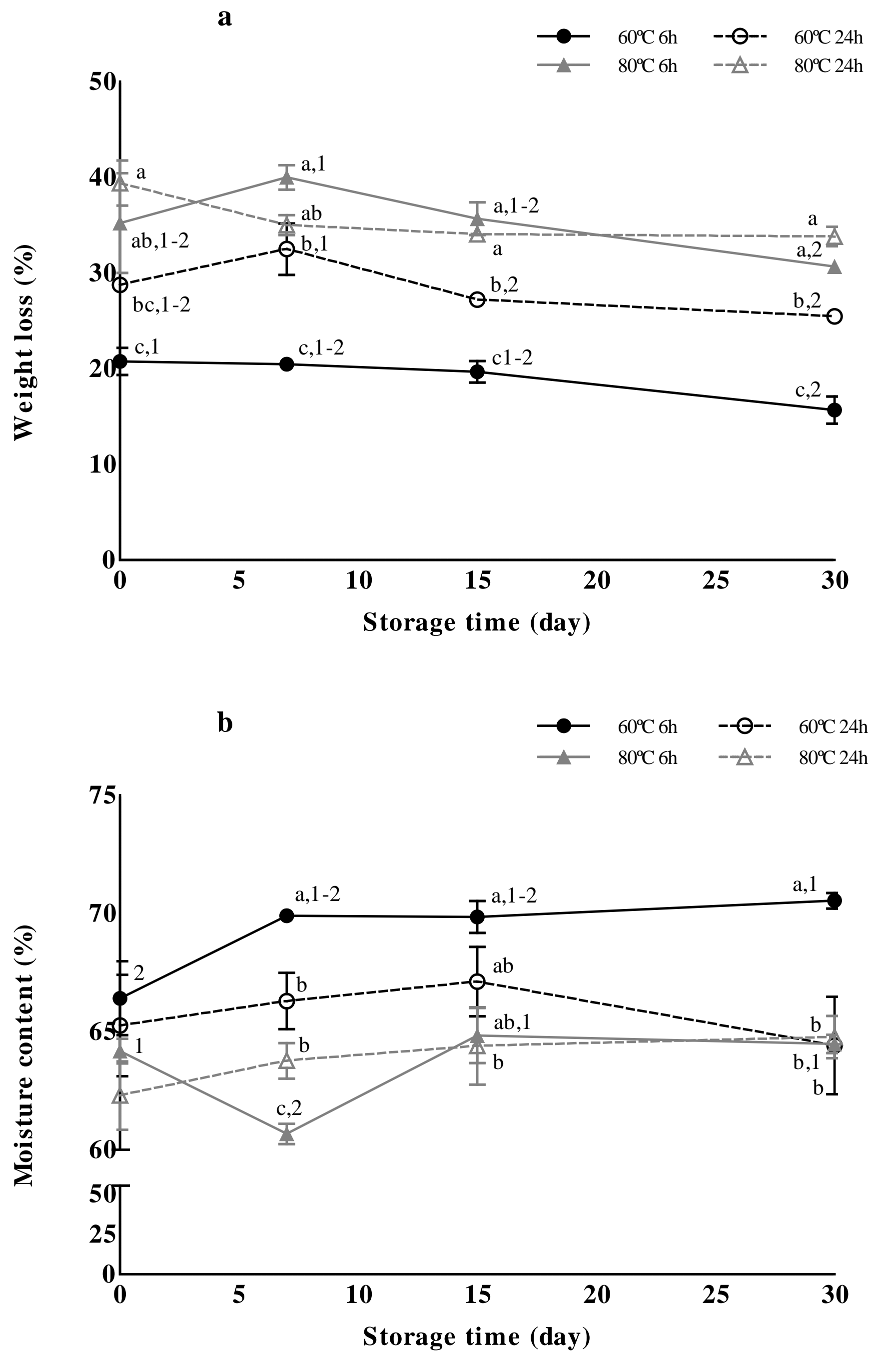


Figure 1. Weight losses (%) and moisture content (%) values in *sous-vide* cooked lamb loins cooked at different time–temperature combinations and stored in refrigeration.

Values are means ± SD of five samples. Mean values with different letters (a-c) indicate significant differences ($P < 0.05$) in the Tukey's test for the cooking conditions, while mean values with different letters (1-2) indicate significant differences ($P < 0.05$) in the Tukey's test among the storage days.

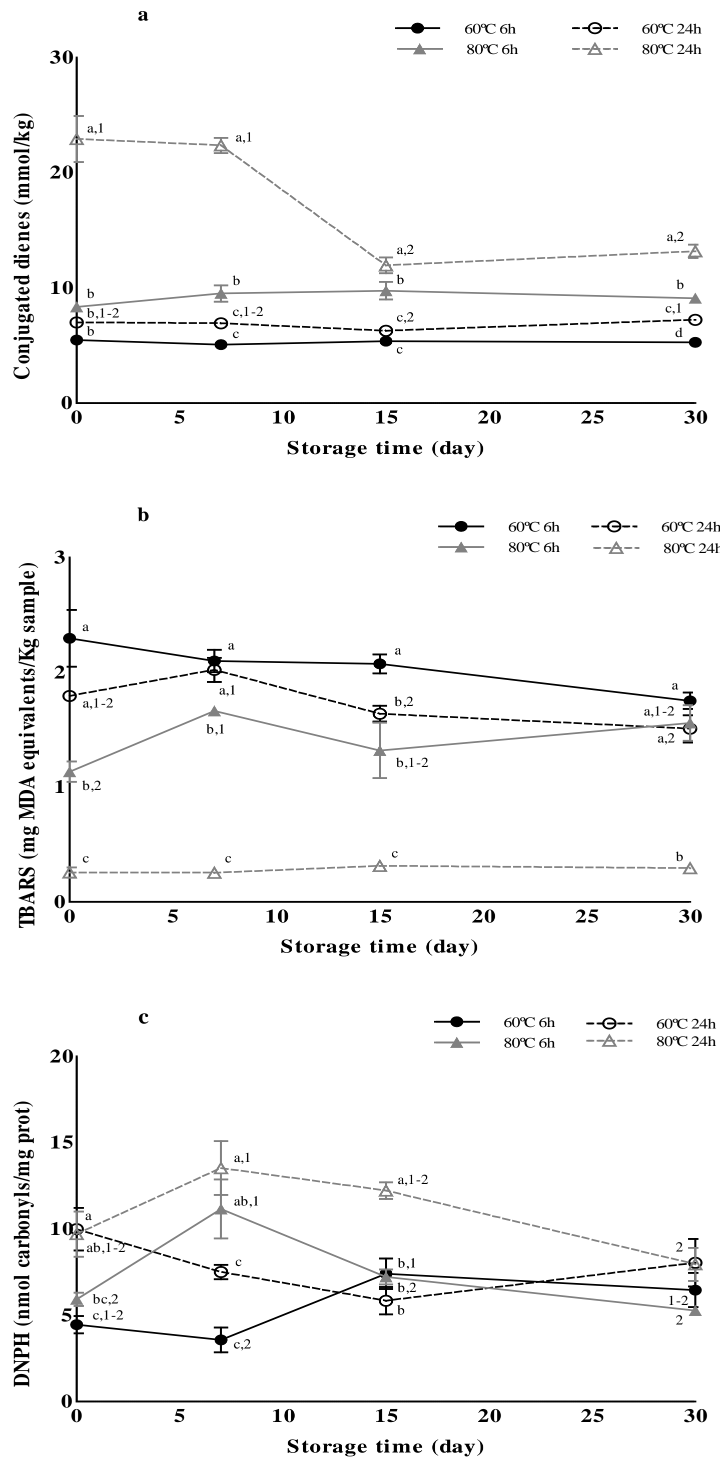


Figure 2. Conjugated dienes (mmol/kg sample), TBARS (mg MDA equivalents/kg sample) and DNPH (nmol carbonyls/mg prot) values in *sous-vide* cooked lamb loins cooked at different time–temperature combinations and stored in refrigeration.

Values are means ± SD. Mean values with different letters (a-d) indicate significant differences ($P < 0.05$) in the Tukey's test for the cooking conditions, while mean values with different letters (1-2) indicate significant differences ($P < 0.05$) in the Tukey's test among the storage days..

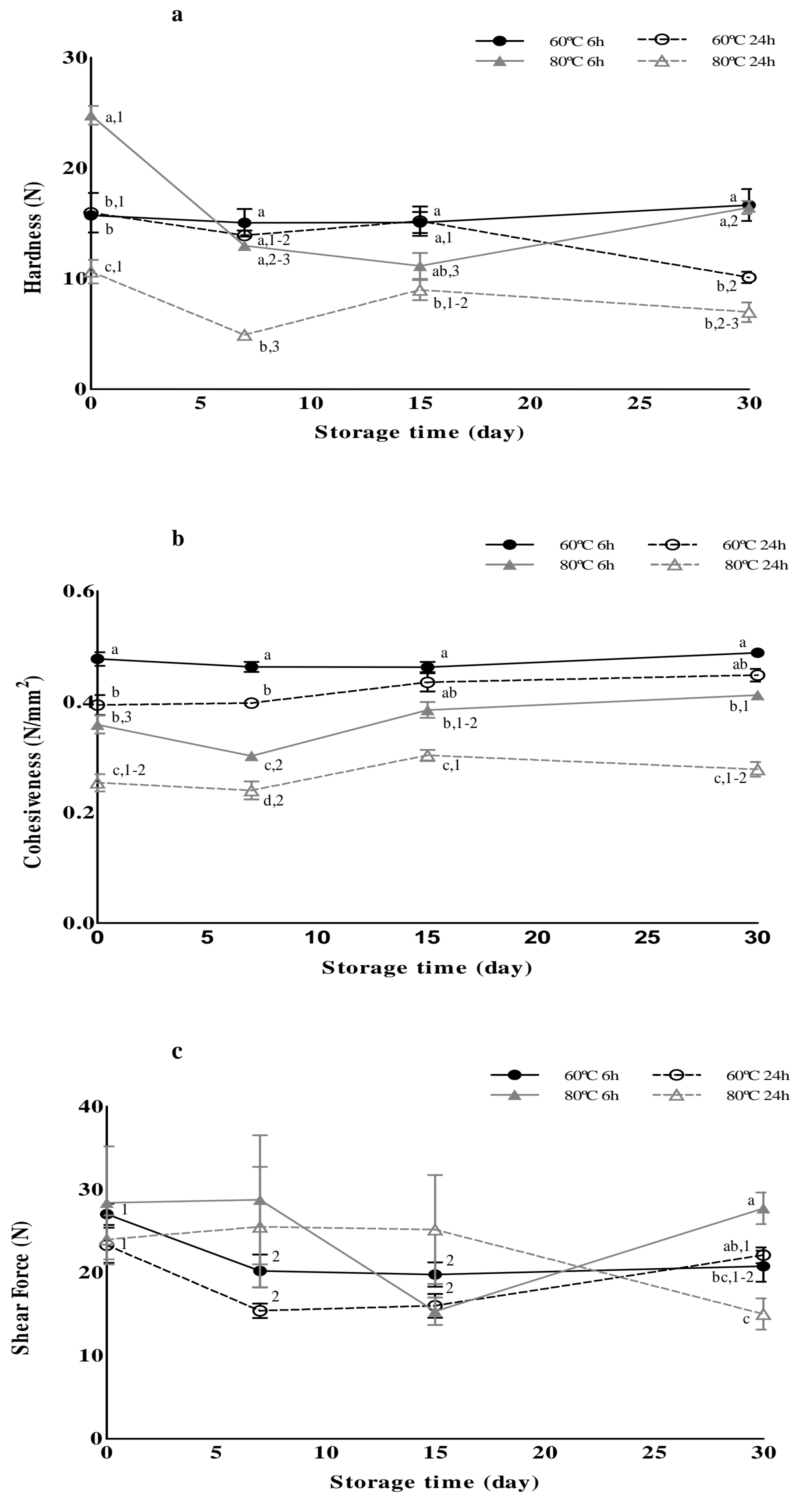
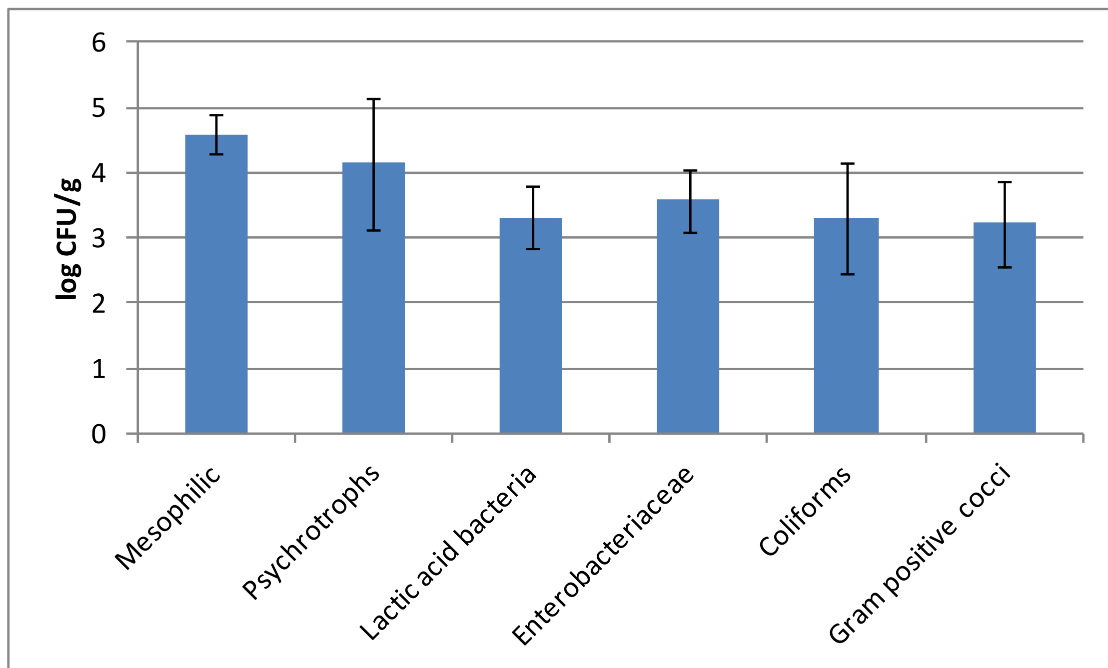


Figure 3. Hardness (N), Shear Force (N) and Cohesiveness (N/mm^2) values in *sous-vide* cooked lamb loins cooked at different time–temperature combinations and stored in refrigeration.

Values are means ± SD of five samples. Mean values with different letters (a-d) indicate significant differences (P < 0.05) in the Tukey's test for the cooking conditions, while mean values with different letters (1-3) indicate significant differences (P < 0.05) in the Tukey's test among the storage days.

Figure 4. Microbial counts in raw lamb loins.



Values are means ± SD.

Capítulo 2.1

Effect of added phosphate and type of cooking method on physico-chemical and sensory features of cooked lamb loins

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Effect of added phosphate and type of cooking method on physico-chemical and sensory features of cooked lamb loins

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ABSTRACT

This study evaluated the effect of brining with phosphates on the physico-chemical and sensory features of sous-vide and roasted cooked lamb. Lamb loins (n = 48) were injected with either 10% w/w of distilled water or a solution containing 0.2% or 0.4% (w/v) of a mixture of phosphate salts. After injection, samples were either sous-vide cooked (12 h—60 °C) or oven roasted (180 °C until 73 °C of core temp.). Expressible moisture, cooking loss, instrumental color, pH, water holding capacity, instrumental texture and sensory properties were evaluated. Brining with phosphates led to lower cooking loss in both sous-vide and oven roasted samples, but only the former showed significantly higher moisture content. Phosphates increased instrumental hardness and shear force values in sous-vide samples, while this effect was not as evident in roasted ones. Toughness was reduced and juiciness was improved as a consequence of phosphate addition. Overall, injection of a phosphate solution appears as a potential procedure for improving sensory textural features of cooked lamb whole cuts.
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1. Introduction

Roasting in the oven and sous-vide cooking are probably the two most used cooking methods nowadays in Spain for whole lamb primal cuts in restaurants and in the catering industry. While roasting implies oven temperatures between 180 and 200 °C, which usually lead to a core temperature of around 68–72 °C, sous-vide cooking temperatures recommended by chefs for lamb meat are around 62–65 °C, but for much longer times than those for roasting (Ruiz, Calvarro, Sánchez del Pulgar, & Roldán, 2013).

The rise in temperature during cooking leads to dramatic changes in the meat, such as protein denaturation, muscle fiber shrinkage, water loss, changes in color, lipid oxidation, and development of Maillard reactions, as more remarkable ones (Bejerholm & Aaslyng, 2004). The nature and extent of these changes will greatly influence the final eating quality of cooked meat: while some changes lead to desirable features (i.e. collagen solubilization or development of Maillard reactions), some others could ruin the sensory quality of meat if they are not carefully controlled. Among the latter, the loss of meat juices is one of the key factors influencing the quality of cooked meat: If water loss is too high, the meat will become tough and fibrous, while keeping water loss to a minimum will produce juicier cooked meat (Heymann, Hedrick, Karrasch, Eggeman, & Ellersieck, 1990).

Myofibrillar proteins hold most of the water retained within the muscle. Increasing temperatures during cooking cause denaturation and shrinkage of such proteins in the range of 40 °C–90 °C and also shrinkage of collagen in the range of 56–62 °C (Tornberg, 2005). Up until 60 °C muscle fibers shrink transversely and widen the gap between fibers, but above this temperature they shrink longitudinally and cause substantial water loss and the extent of this contraction increases with temperature.

Injection of brines followed by tumbling is a widely used procedure to minimize water loss during the subsequent heating in the processing of different meat products (Mills, 2004). Such brines usually contain phosphate salts, due to the ability of these additives for improving meat water holding capacity, which in turn enhances products' textural properties such as juiciness and tenderness by reducing cooking loss (Alvarado & McKee, 2007; Sheard & Tali, 2004). This effect is mainly a consequence of shifting the pH of the meat from the isoelectric point of the muscle proteins (Bianchi, Petracci, & Cavani, 2009).

The use of phosphates has been proposed for improving cooked lamb chops and retail cuts quality (Murphy & Zerby, 2004; Sawyer, Brooks, Apple, & Fitch, 2009). However, no published study has dealt with the use of phosphates for improving cooked meat quality features when whole lamb cuts are roasted in the oven or sous-vide cooked, aimed for restaurant or catering.

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Thus, the objective of this study was to determine the influence of different phosphate levels on the physico-chemical characteristics and sensorial quality of lamb loins cooked using two different culinary procedures: roasting in the oven or sous-vide cooking.

2. Material and methods

2.1. Experimental design

Forty-eight lamb loins were randomly assigned to one of six groups according to a 3 × 2 factorial design, with three levels of phosphates in the injected brine (0%, 0.2% or 0.4% (w/v)) of a sodium triphosphate (STPP) and tetrasodium pyrophosphate (TSPP) (ANVISA, Madrid, Spain) solution and two different cooking treatments (either sous-vide cooked or roasted in the oven).

All loins were from a homogeneous production batch of male lambs averaging 26 kg of live weight and 90 days of age, slaughtered at a local abattoir. Whole fresh boned out lamb loins, trimmed of subcutaneous fat, were individually weighed and their pH was measured by inserting the spear-head electrode of a Testo 205 pH-meter (Testo, Lenzkrich, Germany) equipped with an automatic temperature compensation probe, in three different locations of the muscle, in order to obtain a representative averaged pH. The pH-meter was calibrated using buffers of pH 4 and 7 and the pH of brine solutions was also measured.

Then, loins were injected to a target of 110% of their initial weight with the previously cited solutions by using a culinary syringe of 250 ml, and they were subsequently vacuum-packaged and tumbled (L-100 tumbler, Mimasa, Spain) intermittently for 1 h at 8 rpm in order to improve brine spreading within the muscle. After tumbling, loins were unpacked, weighed, subjected to pH measurement and kept at 2 °C overnight. Then, half the loins (n = 8) of each phosphate level were vacuum-packaged and sous-vide cooked in a thermostatized water bath at 60 °C during 12 h, which were the temperature-time conditions selected in a previous study (Roldán, Antequera, Martín, Mayoral, & Ruiz, 2013). The rest of the loins of each phosphate level (n = 8) were cooked in an oven at 180 °C with dry air until reaching an internal temperature of 73 °C. Temperature was monitored during cooking using a digital probe thermometer (112 thermocouple Testo735-2, Lenzkirch, Germany). After cooking all loins were immediately chilled and kept refrigerated at 2 °C until sensory analysis. Just before re-heating for sensory analysis, the loins were weighed, and the pH and color of the cut surface were measured.

2.2. Moisture content and water loss

Moisture content was determined by drying the samples (5 g) at 102 °C (AOAC, 2000). Samples were analyzed in duplicate. Cooking loss was calculated by difference of weight before (after injection and tumbling) and after cooking, while total loss was calculated by difference of raw weight and weight after cooking.

2.3. Instrumental color measurement

Color was measured across the cut surface of the cooked loin after chilling, 10 min after slicing and before re-heating for sensory analysis. L^*-value (lightness), a^*-value (redness) and b^*-value (yellowness) were obtained using a Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) programmed to use the built-in internal illuminant D65. Means of readings on three locations on each sample were determined. Before each series of measurements, the instrument was calibrated using a white ceramic tile.

2.4. Water holding capacity

Water holding capacity was measured using the centrifugation method described by Jauregui, Regenstein, and Baker (1981), with minor modifications. Samples (1.5 g) were taken with care to exclude external surfaces and obvious masses of connective tissue and fat. Fifteen grams of 4 mm glass beads were placed in the bottom of a 50 ml disposable centrifuge tube, with one meat sample added on top of the beads before centrifuging at 2200 rpm for 15 min. After centrifugation, the meat sample was removed from the tube and reweighed. Expressible moisture (%) was calculated as the proportion of weight lost after centrifugation to the initial sample weight. Water holding capacity was also calculated as the ratio of moisture retained in the sample to the initial moisture content. Samples were analyzed in duplicate.

2.5. Texture profile analysis

Texture analysis was performed in a texturometer TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). For the determination of texture profile analysis (TPA) uniform portions of the cooked loins were cut into 1 cm^3 cubes. For each sample, eight cubes were obtained and analyzed. They were axially compressed to 50% of the original height with a flat plunger of 50 mm in diameter (P/50) at a crosshead speed of 2 mm/s through a 2-cycle sequence. The muscle fibers were placed parallel to the compression plates. The following texture parameters were measured from force deformation curves (Bourne, 1978): Hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); Cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required to for the first compression and A2 the total energy required for the second compression).

Shear force (SF) analysis on cooked samples (3 cm length × 1 cm width × 1 cm thickness) was performed using a Warner-Bratzler blade, which sheared the specimen perpendicularly to the muscle fibers at a constant speed of 1 mm/s and then pushed through the slot. The maximum force (N) required to shear the sample was measured. Six determinations were performed for each cooked sample.

2.6. Sensory analysis

Sous-vide cooked and oven roasted lamb loins were assessed by a trained panel of 12 members, using a descriptive analysis method. The sensory traits, their definitions and extremes are explained in Table 1. Questions were presented to assessors in the normal perception order, as follows: visual analysis, texture, taste and flavor.

Panelists were selected from faculty, staff and researchers of the University using individual taste, flavor and aroma recognition thresholds. Selected subjects underwent further training in meat and meat products sensory characteristics over five years, and have subsequently participated in several panels for cooked meat sensory analysis.

Three lamb loins from different groups were evaluated in each session. Sample order was randomized. The sessions were held 3 h after breakfast. During each session, two slices (1 cm thickness) of lamb loins from each batch were separately served warm to each panelist. A glass of water was provided for each assessor. All sessions were done in a six-booth sensory panel room at 22 °C equipped with white fluorescent lighting (220–230 V, 35 W). Twelve traits concerning sensory characteristics of cooked lamb (Table 1), grouped under appearance, texture, taste and flavor were assessed by the panelists in a 10 cm unstructured line, ranging from "less" to "more". FIZZ Network (version 1.01, Biosystemes, France) program was used for the session performances and the recording of all data obtained.

2.7. Statistical analysis

To test the effect of phosphates addition (0.2%, 0.4% or distilled water) and cooking treatment (sous-vide vs oven) on the

Table 1
Sensory attributes, definitions and extremes of each attribute scored in an unstructured line of 10 cm.

Sensory trait	Definition	Extremes
Appearance		
Brightness	Intensity of brightness on the lean surface	Dull to very bright
Color intensity	Intensity of the color in the slice surface	Pale to dark brown
Texture		
Toughness	Effort required to bite through lean and to convert the sample to a swallowable state	Very tender to very firm
Chewiness	Work required to masticate the sample before swallowing	Very low to very high
Juiciness	Impression of lubricated food during chewing	Dry to very juicy
Pastiness	Pasty feeling inside the mouth during chewing	Very low to very high
Taste		
Saltiness	Level of salt taste	Unsalty to very salty
Umami	Intensity of umami taste	Very low to very high
Flavor		
Flavor intensity	Level of overall flavor	Flavorless to very intense flavor
Lamb flavor	Intensity of sheep flavor	Very low to very high
Cooked meat flavor	Intensity of typical cooked meat flavor	Very low to very high
Rancidity	Intensity of rancid flavor	Very low to very high

physico-chemical variables considered in this study, all variables were submitted to an analysis of variance considering two fixed factors (3 brine solutions × 2 cooking treatment) with the following model:

$$Y_{ik} = + P_i + C_j + P_i \times C_j + e_{ijk}$$

where Y_{ik} = values for each considered variable; μ = least square means; P_i = fixed effect due to phosphate content; C_j = fixed effect due to cooking procedure; $P_i \times C_j$ = effect due to the interaction between added phosphates and cooking procedure; e_{ijk} = random residual effect.

Whenever the interaction between the two main effects was significant, the p values for each individual main effect were shown as not testable in the tables, since they are not meaningful separately.

For the data reported by the panelists, a mixed analysis of variance was used, with the two fixed effects (phosphate addition and cooking procedure), plus a random factor for the tasting session within panelist.

All models were fitted using the GLM procedure (SPSS 15.0). The Tukey's test was used at the 5% level to make comparisons between sample means.

3. Results and discussion

3.1. Meat pH

Meat pH before and after injection and after cooking is shown in Table 2. In our research, the pH after tumbling and after cooking was significantly affected by phosphate addition ($p < 0.001$ and $p = 0.018$ respectively). Thus, samples injected with phosphates showed higher pH values than those injected with distilled water. Such an effect of phosphate injection was not affected by phosphate level in cooked samples, since samples injected with either 0.4 or 0.2% phosphate brine showed no significant differences between them. The main effect of phosphate addition on pH was most likely due to the fact that both STPP and TSPP are alkaline salts and their addition to the slightly acidic meat leads to an increase in pH. In fact, the pH of the brines was quite similar for both the 0.2% and the 0.4% solutions (around 8.3–8.5), while the distilled water solution showed an almost neutral pH value. Such an effect of phosphate addition on meat pH has been previously reported in different types of meat and meat products, such as pork (Sheard & Tali, 2004), turkey breast (Ergezer & Gokce, 2011), poultry (Bianchi et al., 2009), lamb (Murphy & Zerby, 2004) or beef (Pietrasik & Janz, 2009).

The increase in lamb loins pH values as a consequence of cooking (Table 2) could be ascribed to a reduction in available carboxylic groups of proteins, but also to the release of calcium and magnesium ions from proteins (Ergezer & Gokce, 2011). On the other hand, the type of cooking treatment did not show any significant effect on the measured pH values.

3.2. Weight loss, moisture content and water holding capacity

Results for cooking loss, total weight loss and moisture content of phosphate added and phosphate free lamb loins, either sous-vide or oven cooked, are shown in Table 2. Cooking loss and total weight loss

Table 2
Effect of phosphate based brine at three levels (H2O = injected with plain water; 0.2% P: injected with a 0.2% (w/v) phosphate brine; 0.4% P: injected with a 0.4% (w/v) phosphate brine) and cooking (SV: sous-vide; O: oven roasting) on pH, cooking losses, moisture content, expressible moisture, WHC and instrumental color of cooked lamb loins.

	H2O	0.2% P	0.4% P	SV	O	SEM	p phosphate	p cooking	p cook × phos
pH initial	5.75					0.02			
pH after tumbling	5.74^{a}	5.91^{b}	6.01^{c}			0.03	<0.001		
pH after cooking	6.10^{b}	6.22^{a}	6.20^{a}	6.17	6.20	0.02	0.018	NS	NS
Cooking weight losses (%)	22.53^{a}	15.40^{b}	13.05^{c}	17.8	28.9	1.08	<0.001	<0.001	NS
Total weight losses (%)	28.38^{a}	22.51^{b}	20.49^{b}	10.5	22.5	1.29	<0.001	<0.001	NS
WHC (%)	76.6	73.3	76.4	78.2	72.4	0.85	NS	0.001	NS
L*	66.4^{a}	64.4^{ab}	63.6^{b}	66.1	63.2	0.35	<0.001	<0.001	NS
a*	13.9	13.6	13.3	12.5	14.7	0.28	NS	<0.001	NS
b*	10.3^{a}	9.4^{b}	10.1^{a}	9.5	10.3	0.16	0.039	0.016	NS

	H2O		0.2% P		0.4% P					
	SV	O	SV	O	SV	O		p phosphate	p cooking	p cook × phos
Moisture (%)	64.3^{ab}	60.8^{b}	65.5^{ab}	61.2^{b}	68.4^{a}	61.1^{b}	0.55	NT	NT	<0.001
Expressible moisture (%)	14.57^{b}	19.60^{a}	15.02^{ab}	18.83^{ab}	16.2^{ab}	17.3^{ab}	0.51	NT	NT	0.037

SEM: standard error of the mean.
Different superscript letters within the same row mean significant differences between batches ($p < 0.05$).
NS: not significant.
NT: not testable.

were significantly affected by phosphate addition ($p < 0.001$ for both variables) and by the type of cooking treatment ($p < 0.001$ for both variables). Contrarily, moisture content values were affected by the interaction between phosphates and the type of cooking ($p < 0.001$), so that sous-vide cooked samples with upward amounts of added phosphates showed increasing levels of moisture, while the moisture content of oven roasted samples remained unaffected by the addition of phosphate content. Water-holding capacity (WHC) was affected by the type of cooking treatment ($p < 0.001$), while expressible moisture was affected by the interaction between cooking treatment and phosphate addition ($p = 0.037$). Surprisingly, there was no significant effect of phosphate addition on water holding capacity.

The lower cooking loss and total weight loss of samples injected with phosphates was most likely the consequence of the described effect of phosphates on meat pH. It is known that increasing meat pH shifts the isoelectric point of myofibrillar proteins creating larger gaps between actin myofilaments because of electrostatics repulsive forces, which eventually give rise to an increase in the amount of water retained by meat (Alvarado & McKee, 2007; Young, Zhang, Farouk, & Podmore, 2005). Moreover, the increased ionic strength as a consequence of phosphate addition might also enhance actomyosin solubility, leading to a greater swelling of the filaments (Ergezer & Gokce, 2011). In addition, phosphates promote the depolymerization of myosin filaments and weaken the binding of myosin heads to actin enabling the dissociation of actomyosin to allow a limited expansion of the filament lattice permitting to the phosphates-treated loins taking up and retaining more added water than untreated ones (Erdogdu, Erdogdu, & Ekiz, 2007).

Despite this effect of phosphates on water loss, no significant differences in moisture content between samples with different levels of injected phosphates were found, which could be partially due to the fact that the pH in both brines (that for 0.2% and the one for the 0.4% phosphates) and in both type of cooked samples was very similar, although the pH after tumbling and before cooking was higher in 0.4% added phosphates samples. Vaudagna et al. (2008) also detected a positive effect of injecting sodium triplyphosphate brine on cooking loss of sous-vide cooked beef, and similarly, they found negligible difference in this parameter between 0.25 and 0.5% tripolyphosphate injected samples. Massafra (2006) neither observed any significant increases in WHC between 1.25% and 2.5% phosphate injected pork loins.

Overall, it seems that phosphate addition could be a potential approach for reducing cooking loss in whole muscle lamb cuts, for both sous-vide and roasting cooking methods. In fact, by adding phosphates, the cooking loss was reduced around 70% in sous-vied cooked lamb loins and 35% in oven roasted ones.

There was a significant interaction between the type of cooking and the amount of phosphates injected ($p < 0.001$) for the moisture content, so that while in sous-vide cooked samples the injection of phosphates led to higher, but not significant, levels of moisture, in oven cooked samples the differences in moisture content between groups were very small.

The detected interaction between the effect of phosphate injection and that of cooking type in expressible moisture was due to the higher values for sous-vide cooked samples as a consequence of phosphate addition, and the opposite behavior for oven roasted ones. At any rate, the detected differences between groups were not significant in a Tukey's test.

Sous-vide cooked samples showed lower water loss and higher moisture content than oven cooked ones at all phosphate contents tested. This was most likely due to the lower core temperature reached in sous-vide samples (60 °C vs 73 °C), despite the fact that the cooking time was also much longer in sous-vide cooked samples. Several authors have also detected greater weight loss as a consequence of higher cooking temperature (García-Segovia, Andrés-Bello, & Martínez-Monzo, 2007; Palka & Daun, 1999). Such an effect is most likely due to the increased longitudinal shrinking of muscle fibers above 60 °C. Additionally, in the oven roasted samples, the flow of dry air would also cause surface water evaporation during cooking, as has been evidenced previously (Modzelewska-Kapituła, Dąbrowska, Jankowska, Kwiatkowska, & Cierach, 2012).

3.3. Instrumental color

Table 2 shows the values obtained for the instrumental color parameters lightness (L^*), redness (a^*) and yellowness (b^*) of sous-vide and oven cooked lamb loins with different amounts of added phosphates. Values for L^* and b^* were affected by both the cooking treatment ($p < 0.001$ and $p = 0.016$, respectively) and phosphates addition ($p < 0.001$ and $p = 0.039$, respectively). On the other hand, a^* values were only affected by the type of cooking treatment ($p < 0.001$). Thus, brining with phosphates resulted in a decrease in lightness of cooked lamb loins, probably due to the effect of phosphates on pH and ionic strength. At higher pH and ionic strengths, such as in lamb loins brined with phosphates, myofibrillar muscle proteins swell, which alters light reflection, since a swollen muscle protein structure would permit a deeper penetration of light in the tissue, leading to a darker appearance (Bojarska, Batura, & Cierach, 2003; Lawrie, 1985). Such an effect of phosphates on meat has been documented by other researches (Önenc, Serdaroglu, & Abdraimov, 2004; Pohlman, Stivarius, McElyea, & Waldroup, 2002).

As far as the cooking effect on L^* values, the prolonged sous-vide treatment might have led to a more intense protein denaturation and aggregation, despite the fact that roasted samples were cooked to final higher temperatures, but for a much shorter time. In turn, this higher protein denaturation would increase light scattering (Christensen, Ertbjerg, Aaslyng, & Christensen, 2011; Nikmaram, Yarmand, Emamjomeh, & Darehabi, 2011).

Similarly, the higher a^* values in oven roasted samples were most likely due a consequence of a lower degree of denatured myoglobin in this samples. Such process is temperature dependent, starting at 55 °C and continuing till 80 °C (King & Whyte, 2006), but it also depends on the time subjected to a certain temperature (Roldán et al., 2013).

Injection of a brining solution with phosphates showed a variable effect on the yellowness of cooked lamb meat samples. In fact, even though the ANOVA showed a significant effect due to phosphate injection, the only group detected as different ($p < 0.05$) by the Tukey's test was that of the lamb loins with 0.2% of added phosphate, while lamb loins with no added phosphate or with 0.4% of added phosphates showed higher levels for this variable. Nevertheless, detected differences for the b^* color parameter could be considered negligible. Other authors have pointed out no effect on this parameter (Önenc et al., 2004) or a slight increase in yellowness (Villamonte, Simonin, Duranton, Chéret, & de Lamballerie, 2014).

Oven roasted samples showed a more intense yellowness. Such higher b^* values have been ascribed to the formation of a higher proportion of metmyoglobin and further heat-denaturation of this protein, giving rise to a brownish color. However, in this case, a^* values, which are inversely related to the denaturation of myoglobin, were also higher in these samples. Nevertheless, yellowness has also been suggested as an indicator of the development of Maillard reactions (Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010), which would develop faster and more intensely in samples cooked at higher temperatures.

3.4. Instrumental texture parameters

Obtained values for hardness and cohesiveness in a TPA analysis and for shear force (SF) of lamb loin samples injected with different amounts of phosphates and subjected to either sous-vide cooking and oven roasting are shown in Table 3. The interaction between cooking method and phosphate addition significantly affected all three instrumental texture parameters studied ($p < 0.001$).

Overall, phosphate addition increased the hardness of cooked lamb loin samples, such an effect being more marked in sous vide cooking than in oven roasting. Despite the effect of myofibrillar and collagen heat denaturation on cooked meat texture, water loss from the muscle tissue upon heating also play a key role in meat toughening, so that the greater the water lost during cooking, the higher the cooked meat toughness (Bertram, Aaslyng, & Andersen, 2005; Palka & Daun, 1999). However, the hardness parameter in the TPA basically assesses how a meat cube may withstand compression; thus, the more swollen the meat becomes due to the addition of phosphates, the greater the force that is needed to compress it. A similar tendency was shown for SF values of sous vide cooked lamb loins, in which swollen samples due to the injection of phosphates showed higher SF values than those injected with water (Table 3). However this effect was not detected in oven roasted samples. This fact could be due to the greater enhance in moisture content due to phosphate injection in the sous-vide cooked samples (Table 2), but also to the fact that in oven roasted samples, due to the slightly higher temperature and longer cooking times, the shrinkage of myofibrillar proteins is most likely more intense than in sous vide cooked ones, while the degradation of collagen is not as intense, leading to a higher overall SF values. In such a situation, the relative contribution of water retention to the texture would be lower than in sous vide cooked samples, in which the impact of both myofibrillar proteins and connective tissue on SF would be lower (Li, Zhou, & Xu, 2010; Roldán et al., 2013).

In fact, when comparing SF values between cooking methods, samples subjected to sous-vide treatment presented lower values than samples roasted in the oven, probably due to the higher denaturation of collagen argued previously. Moreover, the dehydrated crust formed on the surface of oven roasted samples could also contribute to such higher SF values.

A more intense collagen degradation due to the longer cooking time was most likely the reason explaining the less cohesive structure of sous-vide cooked samples as compared to oven roasted ones, given that collagen is the main component of the connective tissue constituting the endomysium and perymissium, which are the main structures bonding muscle fibers and bundles (Bailey & Light, 1989).

3.5. Sensory analysis

The effects of brining with phosphates and the type of cooking treatment on different sensory attributes of lamb loins are reported in Table 4. Brightness, color intensity, toughness, chewiness and juiciness were significantly affected by the interaction between both parameters ($p = 0.001$ for brightness, color intensity, chewiness, $p < 0.001$ for toughness and juiciness), and a trend ($p = 0.059$) was detected for pastiness. The rest of sensory attributes were not affected by any of the studied factors.

Brining with phosphates promoted a brighter appearance of meat surface, despite the type of cooking procedure considered, although such an effect was more evident in oven roasted lamb loin samples. This enhanced brightness was most likely due to the higher water retention as a consequence of phosphates, which in turn would lead to a moister surface upon reheating for sensory analysis. Similarly, sous-vide cooked samples showed a higher brightness when compared to their oven-roasted counterparts at any of the phosphate levels tested, most likely due to the same reason argued before: the higher water content kept in sous-vide cooked samples.

Panelist detected a decrease in the intensity of the color of the meat cut surface as a consequence of higher phosphate levels, which could be related to the lower lightness of lamb loin samples as a consequence of phosphate injection, which as explained previously, was most likely due to the deeper penetration of light in the tissue as a consequence of a more swollen myofibrillar structure.

Cooking procedure showed a significant interaction with phosphate content for the intensity of meat cut surface color, although the differences between both coking types within each phosphate level were not significant in the Tukey's test.

All sensory texture parameters were influenced by the addition of phosphates. Thus, for both types of cooking procedures, panelists found less tough the phosphate added lamb loin samples than those without phosphates. Similar results have been reported by Gorsuch and Alvarado (2010) in poultry fillets injected with a combination of NaCl and phosphates and cooked in an oven to an internal temperature of 73 °C, or by Sheard, Nute, Richardson, Perry, and Taylor (1999) in pork loins with added phosphates and cooked by grilling to an internal temperature of 72.5 °C. Polyphosphate has two main effects that may allow a certain expansion of the filament lattice: Promoting the depolymerisation of myosin filaments and weakening the binding of the myosin heads to actin, thus promoting the dissociation of actomyosin (Offer & Trinick, 1983). This, together with their effect on shifting the meat pH explained previously, would permit polyphosphate-treated meat to take up and retain more added water than untreated meat. Thus, the increased tenderness of polyphosphate-treated samples could be attributed to the weakened muscle structure and also to the higher water content of the cooked samples (Sheard et al., 1999). This tendency is totally the opposite to that found for instrumental texture, in which phosphate added samples showed overall higher hardness and SF values, revealing the lack of consistency between instrumental texture parameters and sensory ones (Peachey, Purchas, & Duizer, 2002).

Although the same tendency in toughness was detected for lamb loin samples with added phosphates and subjected to sous-vide cooking, in this case the Tukey's test did not show significant differences between groups. This could be partly ascribed to the long cooking time considered, which caused such an effect on meat toughness that that of the brining with phosphate could be somehow masked. Szerman et al. (2012) obtained significant differences in tenderness values of samples injected with a combination of phosphates and sodium chloride and sous-vide cooked at 70 °C, but only during a maximum of 2 min.

The effect of phosphate brining on water retention was most likely the reason behind the lower chewiness and higher juiciness of phosphate added cooked lamb loins, such an effect being more evident in the juiciness of sous-vide cooked loin lambs. Similarly, pastiness was also significantly affected by the injection of phosphates, although in this parameter the Tukey's test did not detected significant differences between groups.

Neither the type of cooking procedure, not the injection of phosphates showed any effect on the taste or the flavor characteristics of lamb loins. Accordingly, other authors have neither detected any significant influence of phosphate inclusion on cooked meat taste or flavor

Table 3
Effect of phosphate based brine at three levels (H2O = injected with plain water; 0.2% P: injected with a 0.2% (w/v) phosphate brine; 0.4% P: injected with a 0.4% (w/v) phosphate brine) and cooking (SV: sous-vide; O: oven roasting) on textural parameters of cooked lamb loins.

	H2O		0.2% P		0.4% P		SEM	p phosphate	p cooking	p cook × phos
	SV	O	SV	O	SV	O				
Hardness (N)	13.26^{bc}	11.32^{c}	15.80^{ab}	12.38^{bc}	17.83^{a}	13.54^{abc}	0.52	NT	NT	<0.001
Cohesiveness (N/mm^2)	0.42^{b}	0.50^{a}	0.45^{b}	0.51^{a}	0.45^{b}	0.49^{a}	0.01	NT	NT	<0.001
Shear force (N)	6.73^{d}	13.01^{a}	8.55^{cd}	11.66^{a}	9.17^{bc}	11.04^{ab}	0.35	NT	NT	<0.001

SEM: standard error of the mean.
Different superscript letters within the same row mean significant differences between batches ($p < 0.05$).
NT: not testable.

Table 4
Effect of phosphate based brine at three levels (H2O = injected with plain water; 0.2% P: injected with a 0.2% (w/v) phosphate brine; 0.4% P: injected with a 0.4% (w/v) phosphate brine) and cooking (SV: sous-vide; O: oven roasting) on sensory features of cooked lamb loins.

	H2O		0.2% P		0.4% P		SEM	p phosphate	p cooking	p cook × phos
	SV	O	SV	O	SV	O				
Brightness	$4.9^{a,b}$	3.9^{b}	$4.7^{a,b}$	4.0^{b}	5.3^{a}	$5.0^{a,b}$	0.11	NT	NT	0.001
Color intensity	$4.8^{a,b}$	5.6^{a}	$4.7^{a,b}$	$5.0^{a,b}$	4.3^{b}	4.2^{b}	0.09	NT	NT	0.001
Toughness	3.0^{bc}	4.1^{a}	2.7^{bc}	3.2^{b}	2.0^{c}	3.2^{b}	0.09	NT	NT	<0.001
Chewiness	$3.9^{a,b}$	4.7^{a}	$3.6^{a,b}$	$3.6^{a,b}$	2.8^{b}	$3.8^{a,b}$	0.11	NT	NT	0.001
Juiciness	4.1^{b}	4.0^{b}	$4.3^{a,b}$	$4.3^{a,b}$	5.4^{a}	$5.1^{a,b}$	0.11	NT	NT	<0.001
Pastiness	3.4^{a}	3.4^{a}	3.5^{a}	$3.2^{a,b}$	2.6^{b}	2.7^{b}	0.12	NT	NT	0.059

	H2O	0.2% P	0.4% P	SV	O	SEM	p phosphate	p cooking	p cook × phos
Saltiness	1.9	1.8	2.1	1.9	1.9	0.11	NS	NS	NS
Umami	2.9	2.7	2.7	2.7	2.8	0.15	NS	NS	NS
Flavor intensity	4.9	4.7	4.7	4.7	4.8	0.11	NS	NS	NS
Lamb flavor	4.0	3.7	3.5	3.8	3.6	0.12	NS	NS	NS
Cooked meat flavor	4.4	4.2	4.3	4.4	4.2	0.13	NS	NS	NS
Rancidity	1.1	0.9	0.9	0.9	0.9	0.06	NS	NS	NS

0–10 cm line scale. 0 = low. 10 = high.
SEM: standard error of the mean.
Different superscript letters within the same row mean significant differences between batches ($p < 0.05$).
NS: not significant.
NT: not testable.

(Rowe, Pohlman, Brown, Baublits, & Johnson, 2009; Sheard et al., 1999). However, Sawyer et al. (2009) found soapy flavors and off-flavors in phosphate enhanced cooked meats, even using lower levels of added phosphates than those of the present study.

4. Conclusions

Injecting phosphate brines appears as an interesting approach for enhancing textural sensory features of either sous-vide or oven roasting whole muscle lamb cuts through an increase in their water retention during cooking without detrimental effects on flavor or taste. While in sous-vide cooked lamb loins the effect of phosphate addition enhancing the juiciness is more evident, a decrease in toughness is the more relevant effect on sensory textural parameter in oven roasting lamb loins.

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Capítulo 2.2

Advanced glycation end products, physico-chemical and sensory characteristics of cooked lamb loins as affected by culinary procedures and addition of flavor precursors

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TITLE

Advanced glycation end products, physico-chemical and sensory characteristics of cooked lamb loins as affected by culinary procedures and addition of flavor precursors

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ABSTRACT

The influence of the addition of a flavor enhancer solution (FES) (D-glucose, D-ribose, L-cysteine and thiamin) and of *sous-vide* cooking or roasting on moisture, cooking loss, instrumental color, sensory characteristics and formation of Maillard reaction (MR) compounds in lamb loins was studied. FES reduced cooking loss and increased water content in *sous-vide* samples. FES and cooking method showed a marked effect on browning development, both in the meat surface and within. FES led to tougher and chewier texture in *sous-vide* cooked lamb, and enhanced flavor scores of *sous-vide* samples more markedly than in roasted ones. FES added meat showed higher contents of furosine; 1,2-dicarbonyl compounds and 5-hydroxymethylfurfural did not reach detectable levels. N-ε-carboxymethyllysine amounts were rather low and not influenced by the studied factors. Cooked meat seems to be a minor dietary source of MR products, regardless the presence of reducing sugars and the cooking method.

KEY WORDS

AGEs, lamb meat, sensory, color, protein glycation

INTRODUCCIÓN

An increase in consumer demand for high quality food products has caused a growing interest in the improvement of cooking methodologies capable of producing meals of distinguished and unique quality. In the case of lamb meat, oven roasting and *sous-vide* cooking are probably the two most used cooking methods nowadays in Spain for whole lamb primal cuts in restaurants and in the catering industry. The temperature and length of the cooking process have a large effect on the physical and chemical properties of meat and its eating quality (Roldan et al. 2013). While roasting implies oven temperatures between 180-200 ºC, which usually leads to a core temperature of around 68-72 ºC, *sous-vide* cooking temperatures recommended by chefs for lamb meat are around 60-65 ºC, but for much longer times than those for roasting (Ruiz et al. 2013).

Flavor is one of the most important quality parameters of cooked meat and develops during cooking by complex reactions between natural components present in raw meat (Sanchez del Pulgar et al. 2013). These precursors may include reducing and phosphorylated sugars, amino acids, thiamin, and lipids (Mottram 1991; Farmer et al. 1999). The addition of some of these precursors is a promising strategy for increasing consumer acceptance of cooked meat (Aliani et al. 2013).

The development of the flavor and surface color in cooked meat mainly results from the Maillard reaction (MR) and lipid degradation, but also from the interactions between both reaction pathways (Adams et al. 2011). Cooking of meat generates many hundreds of volatile compounds, but relatively few make a key contribution to the odor and flavor of cooked meat (Aliani et al. 2013), MR being the main route for their formation (Mottram 1991; Meinert et al. 2009b). Some authors have reported the higher flavor-generating potential of some sugars like ribose (Farmer et al. 1999; Mottram 1998; Lauridsen et al. 2006), ribose 5-phosphate (Farmer et al. 1999; Mottram 1998), glucose (Farmer et al. 1999; Lauridsen et al. 2006), and glucose 6-phosphates (Farmer et al. 1999). On the other hand, sulfur compounds undoubtedly play a significant role in meat flavor due to their interesting olfactory properties and generally low odor and taste thresholds. Thus, the sulfur-containing amino acids like cysteine and cystine as well as thiamin (vitamin B1) are important precursors for the resulting

roasted or cooked meat aroma (Guentert et al. 1990). For example, heterocyclic compounds formed due to reaction of cysteine and ribose (Van den Ouweland and Peer 1975; Hofmann and Schieberle 1998) cysteine, (Cerny and Davidek 2003) or the degradation of thiamin (Güntert et al. 1992; Cerny 2007), provide savory, meaty, roast and boiled flavors (Cerny and Davidek 2003).

In the course of the MR different species of sugar- and protein-derived products are formed. These products are responsible for the flavor of heated foods (Mottram 1998) but some authors have also highlighted their health risks (Delgado-Andrade et al. 2009). To evaluate the development of the reaction cascade, reaction products from different stages are useful. Because Amadori products (indirectly analyzed as furosine) generally arise before sensory changes become noticeable. They are used as early indicators for quality changes caused by glycation reactions (Erbersdobler and Somoza 2007). Amadori compounds are degraded to highly reactive α-dicarbonyls, such as glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosulose (3-DG), during the advanced stage of glycation. In particular 3-DG and MGO, are known to occur in considerable amounts in some foods (Degen et al. 2012) and are precursors of aroma-active compounds (Weenen and Tjan 1994; Bravo et al. 2008) and of advanced glycation end products (AGEs) like the N-ε-carboxymethyllysine (CML), a stable advanced Maillard product (Bastos et al. 2012). Hydroxymethylfurfural (HMF) is a thermodynamically controlled product formed in the course of Maillard reaction and during degradation of hexoses at high temperatures and at acid conditions (Arribas-Lorenzo and Morales 2010). It is formed on the surface of fried or roasted food products (Danowska-Oziewicz 2009) and its presence in foods is the result of excessive temperatures during heat treatment as well as inappropriate and long-term storage (Vorlová et al. 2006).

The formation of Maillard reaction products (MRP) depends directly on the processing temperature and time and is greatly heightened by long exposure to high heat (Hardy et al. 1999). MRP content in foods is affected by its composition, the method and conditions of the industrial or culinary preparation, as well as to possible reheating (Li et al. 1994). (Chao et al. 2009)) reported that culinary treatments such as frying or baking have a greater

impact on the formation of MRP than boiling.

In the present study, the effect of addition of several flavor precursors (glucose, ribose, cysteine and thiamin) on cooking loss, moisture content, instrumental color, sensory features and development of MR in either *sous-vide* cooked or roasted lamb loins was investigated.

MATERIAL AND METHODS

Experimental design

Twenty lamb loins were randomly assigned to one of the four groups according to a 2x2 factorial design, with two levels of flavor precursor addition (either a flavor enhancer solution (FES) or distilled water -control-), and two different cooking treatments (either *sous-vide* cooked or roasted in the oven). The FES was composed of sugars, amino acids and vitamins in the following concentrations: D-glucose: 1.376 M; D-ribose: 0.060 M; L-cysteine: 0.075 M and Thiamin: 0.034 M (D-glucose, L-cysteine and thiamin were obtained from Acofarma, S.C.L., Madrid, Spain, and D-ribose from Suplementos Solgar, S. L., Madrid, Spain). All of them were food grade.

All loins were from a homogeneous production batch of male lambs averaging 26 kg live weight and 90 days of age, slaughtered at a local abattoir. Whole fresh boned out lamb loins, trimmed of subcutaneous fat, were individually weighed. Then, loins were injected to a target of 110% of their initial weight with the previously cited solutions by using a culinary syringe of 250 ml, and they were subsequently vacuum-packaged and tumbled (Dorit VV-T-10 Killwangen tumbler, Germany) intermittently for one hour at 8 rpm in order to improve flavor precursors spreading within the muscle.

After tumbling, half the loins (n=5) of each FES level were vacuum-packaged and *sous-vide* cooked in a thermostatized water bath at 60 ºC during 12 h, which were the temperature-time conditions selected in a previous study (Roldan et al. 2013). The rest of the loins of each FES level (n=5) were cooked in an oven at 180 ºC with dry air until reaching an internal temperature of 73 ºC. Temperature was monitored during cooking using a digital probe thermometer (112 thermocouple Testo735-2, Lenzkirch, Germany). After cooking, all loins were immediately chilled and kept refrigerated at 2 ºC until sensory analysis, a maximum of 48 h after cooking. Just before re-heating for sensory analysis, the loins were weighed, and the color of the loin surface and the cut surface were measured. A sample for moisture content was also taken. The rest of the loin was vacuum packaged and kept frozen at -80 °C until analysis.

Analytical procedures

Moisture and cooking loss

Moisture content was determined by drying the samples (5 g) at 102 ºC (A.O.A.C. 2000). Samples were analyzed in duplicate. Total cooking loss was calculated by difference of weight after injection and after cooking.

Instrumental color measurement

Color was measured across the external surface and the cut surface of the cooked loins after chilling. L* value (lightness), a* value (redness) and b* value (yellowness) were obtained using a Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) programmed to used the built-in internal illuminant D65. Means of readings on three locations on each sample were determined. Before each series of measurements, the instrument was calibrated using a white ceramic tile.
Browning index (BI) was calculated using Hunter L, a, and b values (Maskan 2001) as

$$BI = \frac{[100 \text{ x } (\text{x} - 0.31)]}{0.17}$$

with

$$x = \frac{(\text{a} + 1.75 \text{ x } \text{L})}{(5.645\ x\ L + a - 3.012\ x\ b)}$$

Sensory analysis

Sous-vide cooked and oven roasted lamb loins were assessed by a trained panel of 12 members, using a descriptive analysis method. The sensory traits, their definitions and extremes are explained in Table 1. Questions were presented to assessors in the normal perception order, as follows: visual analysis, texture, taste and flavor.

Panelists were selected from faculty, staff and researchers of the university using individual taste, flavor and aroma recognition thresholds. Selected subjects underwent further training in meat and meat products sensory characteristics over five years, and have subsequently participated in several panels for cooked meat sensory analysis.

Four lamb loins from different groups were evaluated in each session. Sample order was randomized. The sessions were held 3 h after breakfast.

During each session, two slices of lamb loins (1 cm thickness) from each batch were separately served warm to each panelist. A glass of water was provided for each assessor. All sessions were done in a six-booth sensory panel room at 22 ºC equipped with white fluorescent lighting (220-230 V, 35 W). Thirteen traits concerning sensory characteristics of cooked lamb (Table 1), grouped under appearance, texture, taste and flavor were assessed by the panelists in a 10 cm unstructured line, ranging from "less" to "more". FIZZ Network program (version 1.01, Biosystemes, France) was used for the session performances and the recording of all data obtained.

Quantification of hydroxymethylfurfural (HMF)

The sample preparation for the HMF determination was based on that described by (Degen et al. 2012), with some modifications. Prior to the extraction stage, meat samples were ground using a mincer and liquid nitrogen. Then, 500 ± 50 mg of grounded sample from each batch were suspended in 3 mL of water (HPLC grade) into a 6 mL centrifuge tube. The tubes were shaken vigorously for 1 min by vortexing and then left to rest for 1h. For protein precipitation 3 mL of methanol (HPLC grade) were added to the tubes and then they were shaken and kept in the fridge (-18 ºC) for 2 h. Finally, the resulting mixture was centrifuged at 5000 rpm for 30 min at 15 °C. The supernatant was filtered (0.45 μm, hydrophilic polypropylene (Pall, Craisheim, Germany) into vials for HMF analysis. The analysis of HMF was carried out by HPLC-UV according to the method by (Weigel et al. 2004) and quantification was realized via external calibration. All analyses were performed in duplicate and the results expressed as mg HMF/kg sample.

1,2-Dicarbonyl compounds analysis by HPLC-UV

Samples preparation for the analysis of 1,2-dicarbonyl compounds followed the same procedure described previously in the HMF analysis. After that, the analysis of these compounds was achieved by RP-HPLC of the corresponding quinoxalines resulting from derivatization with o-phenylenediamine (OPD). To this end, 500 μL of the supernatant was mixed with 150 μL of 0.5 M sodium phosphate buffer (pH 7.0) and 150 μL of a 0.2 % (w/v) OPD solution containing 18.5 mM DETAPAC (diethylenetriaminepentaacetic

acid). The mixture was kept in the dark overnight and membrane filtered (0.45 µm) before chromatographic analysis. The HPLC analyses were performed using a high pressure gradient system from Amersham Pharmacia Biotech (Uppsala, Sweden), consisting of a pump P-900 with an online degasser (Knauer, Berlin, Germany) and a UV detector UV-900. Peaks were evaluated using the software UNICORN V 4.00. Quinoxalines were separated on a stainless steel column filled with Prontosil 60 Phenyl material (250mm x 4.6mm, 5 µm; Knauer, Berlin, Germany) with an integrated guard column (5 mm x 4 mm) filled with the same material and an online filter (3 µm) between the sample loop and the column. The mobile phases were 0.075 % acetic acid (solvent A) and a mixture of 80 % methanol and 20 % solvent A (solvent B). The flow rate was 0.7 mL/min and the separation was performed at room temperature. Fifty microliters of the derivatized solutions were injected. The gradient started with 10 % solvent B and was changed linearly to 50 % B over a period of 25 min. After 5 min at 50 % B, the proportion of solvent B was elevated to 70 % within 4 min, held there for 10 min, and then changed to 10% in 4 min. The column was equilibrated with 10 % solvent B for 12 min at a flow rate of 0.7 mL/min. UV detection was performed at 280 and 312 nm, simultaneously. For external calibration, a stock solution of quinoxalines in water was prepared (c=0.87-1.22 mmol/L), which was diluted to the appropriate concentrations. The limits of detection (LOD) and quantification (LOQ) were calculated as the concentrations of the analyte necessary to show a peak at a signal-to-noise ratio of 3 and 10, respectively. The recovery of 3-DG, GO, MGO and 3-DPs (3-deoxypentosone) was determined via comparison of the recovered amounts of substance with added amounts of substance after spiking meat samples each with four ascending concentrations (3-DG: 16-79 µM, 3-DPs: 2.5-12 µM, GO: 8.5-41 µM, MGO: 9.0-43 µM) of dicarbonyl compounds, following sample preparation and derivatization with *o*-phenylendiamine. After adding the dicarbonyl compounds the sample was allowed to stand 1 h at room temperature prior to sample work-up.

Quantification of furosine via amino acid analyzer

Furosine was determined according to (Wellner et al. 2012) with some modifications. 12 mg of each sample were hydrolyzed for 23 h with 3 mL of

hydrochloric acid (8 mol/L) at 110ºC in screw-cap tubes. After hydrolysis, samples were filtered (white ribbon 589/2, ashless, diameter 40.5 mm, Whatman Inc., Maidstone, UK) and a volume of 1 mL of filtrate was used to remove hydrochloric acid under vacuum. The residue was resolved in 500 µL of sample buffer for amino acid analysis. Finally, samples were shaken in an ultra sonic bath and filtered (0.45 µm) into HPLC vials.

The quantitation of furosine and amino acids was carried out by ion-exchange chromatography and postcolumn derivatization with ninhydrin according to (Henle et al. 1991). An amino acid analyzer S433 (Sykam, Fürstenfeldbruck, Germany) and a PEEK column filled with the cation exchange resin LCA K07/Li (150 x 4.6 mm, 7 µm) was used. Lithium buffers ready for use were purchased from Sykam (Fürstenfeldbruck, Germany) and employed for different gradient programs according to the manufacturer's instructions. Furosine was detected at 280 nm with an additional K-2501 UV-detector (Knauer, Berlin, Germany). For amino acids postcolumn derivatization with ninhydrin was applied and VIS detection was performed with an integrated two-channel photometer simultaneously working at 440 and 570 nm, respectively.

Quantification of N-ε-carboxymethyllysine (CML) via liquid chormatography coupled to tanden mass spectrometry

The analysis of CML was performed according to (Hegele et al. 2008). Cooked lamb loins were cut into small pieces and minced with dry-ice. Then, 6 mg of ground sample were weighted mixed with 3 mL of sodium borate buffer (0.2 M, pH 9.5) and 2 mL of sodium borohydride solution (1 M in 0.1 M NaOH) in screw-cap tubes to carry out the reduction step. Samples were shaken by vortex and reduced overnight at room temperature. After addition of 1 mL of hydrochloric acid 6 mol/L and 5 mL of hydrochloric acid 12 mol/L, samples were hydrolyzed for 23 h at 110 ºC. 1 mL of the hydrolyzates were dried under vacuum and reconstituted in 750 µL of sample buffer (consisting of 37.6 ml 0.02 M HCl, 11.7 ml 2 M Tris buffer pH 8.2 and 0.725 ml distilled water) prior to the clean up by solid phase extraction (SPE). An aliquot of 345 µL of the sample solution was diluted with 1045 µL of water, 100 µL of nonafluorpentanoic acid (NFPA) and 10 µl internal standard (D2-CML 4 ng/µL) resulting in a total volume

of 1,5 mL.. For solid phase extraction cartridges (Oasis HLB 6cc, 200 mg, Waters, Milford, MA) were washed twice with each 2 mL of methanol, 2 mL methanol/NPFA 10 mM (50+50, v+v) and 2 x 2 mL NFPA 10 mM. After that, samples were applied and cartridges were washed with 2 x 2 mL NFPA 10 mM and 2 mL methanol/NPFA 10 mM (5+95, v+v) to remove most interfering compounds. The CML-containing fractions were eluted with 2 mL of methanol/NPFA 10 mM (50+50, v+v), collected and dried using an evaporator with a stream of nitrogen gas. Finally, the dried fractions were resolved in 200 µL of the initial mobile phase (5 mM NFPA in distilled water), vortexed for 10 s, and then filtered into injection vials through a 0.2 µm membrane filter prior to analysis. 1 µL volume was injected into the LC–MS/MS system for analysis. The analyses were performed on a HPLC 1200 series (Agilent Technologies, Waldbronn, Germany) coupled to a triple quadrupole mass spectrometer Agilent 6410 with an ESI ion source. The peak area ratio of the CML standard was compared to the (D_2)-CML internal standard and used to establish the standard calibration curve. (D_2)-CML was spiked into all samples to account for matrix effects. Quantification of samples was achieved using the internal standard and by comparing the sample with standards in terms of relative retention time and relative abundance of the two selected product ions.

Statistical analysis

The effect of FES addition (control *vs.* addition) and cooking treatment (*sous-vide vs.* oven) on the physic-chemical variables considered in this study was analyzed by a two-way analysis of variance (2 FES levels x 2 culinary treatment) together with their interaction following this model

$$Yik = \mu + Pi + Cj + Pi \times Cj + eijk$$

where

Yik = values for each considered variable; µ= least square means; Pi= fixed effect due to the addition or not of a PES content; Cj= fixed effect due to cooking procedure; Pi x Cj= effect due to the interaction between FES addition and cooking procedure; eijk= random residual effect.

For the data reported by the panelists, a mixed analysis of variance was used, with the two fixed effects (FES addition and cooking procedure) and their interaction, plus a random factor for the tasting session within panelist.

All models were fitted using the GLM procedure (SPSS 15.0). The Tukey´s test was used at the 5 % level to make comparisons between means.

RESULTS AND DISCUSSION

Moisture and water lost

Results for physico-chemical analysis are shown in Table 2. Both weight loss and moisture content were affected by cooking method ($p<0.001$). Those samples cooked *sous-vide* showed lower weight loss and accordingly, significantly higher moisture content, than oven roasted samples. This was most likely due to the lower core temperature reached in *sous-vide* samples (60 ºC *vs.* 73 ºC), despite to the fact that the cooking time was much longer for these samples. Several authors have also detected greater weight loss as a consequence of higher cooking temperature (Roldan, Antequera, Martin, Mayoral, & Ruiz, 2013; Sanchez del Pulgar, Gazquez, & Ruiz-Carrascal, 2012). Such effect is most likely due to the increased longitudinal shrinking of muscle fibers above 60 ºC. Additionally, in the oven roasted samples, the flow of dry air would also cause surface water evaporation during cooking, as has been evidenced previously (Modzelewska-Kapituła, Dąbrowska, Jankowska, Kwiatkowska, & Cierach, 2012).

The addition of a FES did not show a significant effect itself on these either the weight loss or the moisture content of cooked lamb loins, but it showed a significant interaction with the type of cooking type ($p<0.001$ and $p=0.004$, respectively). Thus, cooking loss in *sous-vide* cooked samples decreased and moisture content increased with the addition of a FES, while the effect was just the opposite in the oven roasted ones. There is no clear explanation for this effect, but some authors have shown an increase in fish myosin solubilization as a consequence of glycation caused by the addition of reducing sugars (Tanabe & Saeki, 2001). Such a higher solubility would enhance the protein gel formation during low temperature cooking, which in turn might increase the water holding capacity of meat. In fact, a higher thermal stability and water hydration capacity has been shown in glycated proteins of fish (Wahyuni, Ishizaki, & Tanaka, 1999).

However, such effect was not detected in oven roasted samples, perhaps because the cooking time was not long enough for allowing a significant increase in the solubilization of myosin. Nevertheless, other authors have shown just the opposite effect of sugars on protein solubility (Chen, Liu, & Chen, 2002). Further research is needed to elucidate whether protein glycation

induced by addition of reducing sugars might increase protein solubility in cooked meat and meat products, and potential influence of different factors (i.e., temperature, additives, protein oxidation), would be of the highest interest for the meat processing industry.

Instrumental color and browning index measurement

Results for instrumental color measurements are shown in Table 2. As far as the surface of the loin is concerned, *sous-vide* cooked samples showed significantly higher L* values (p=0.003) and lower b* values (p<0.001) than oven roasted ones, while the a* values were not significantly affected by the cooking procedure. The addition of FES did not show any significant effect on L* or a* values of lamb loin surface itself. But it showed a significant interaction with the cooking procedure (p=0.049 and p= 0.006, for L* and a* values, respectively). Addition of a FES caused a significant increase (p=0.004) of b* values. As far as the browning index (BI) is concerned, the cooking procedure significantly affected this parameter (p<0.001), and in fact, the obtained values were much higher in oven roasted than in sous-vide cooked samples. There was also a significant (p=0.014) effect of the addition of a FES on the browning of the surface of lamb loins, with higher browning in those loins that were added the FES, regardless the cooking method.

The influence of the cooking method on the instrumental color values of the loin surface, and especially the enhancement of the development of the brownish color in oven roasted samples as compared to *sous-vide* cooked ones, is most likely due to 1) the more intense dehydration of the loin surface, 2) a higher surface metmyoglobin formation and further heat-denaturation of this protein due to higher temperatures (Roldan, Antequera, Martin, Mayoral, & Ruiz, 2013) and 3) the enhanced formation of brown compounds in those lamb loins roasted in the oven, as a consequence of the higher temperatures during cooking and the use of a dry air oven (Chiavaro, Rinaldi, Vittadini, & Barbanti, 2009).

More interestingly, the addition of a FES containing glucose and ribose, led to higher b* values and browning indexes on the lamb loin surface, regardless of the cooking method. The presence of higher amounts of reducing sugars in meat is most likely the reason explaining this effect, leading to the

development of more intense MR. Other authors have also pointed out increased browning as a consequence of added higher amounts of sugars in cooked meat products (Chen, Liu, & Chen, 2002). As far as the instrumental color of the cutting surface is concerned, L* values were affected by the cooking method, the addition of FES and their interaction (p=0.006, p<0.001 and p=0.01, respectively). This parameter showed lower values as a consequence of FES addition in both types of cooking methods, but much more marked in *sous-vide* cooked samples. On the other hand, a* values were not affected by any of the factors studied, while the b* scores were significantly affected by the cooking method (p<0.001). Both the cooking method and the addition of a FES showed a significant effect (p=0.036 and p=0.009) on the BI, so that both roasting and FES addition caused an increase in the BI of the cutting surface of lamb loins.

The effect of cooking method on instrumental color values was most likely due to the lower temperatures reached in the core of *sous-vide* cooked samples (60 °C in *sous-vide* vs. 73 °C in oven roasting), despite being cooked for a much longer time. As a consequence, the myoglobin thermal degradation in oven roasted samples would be higher (Roldan, Antequera, Martin, Mayoral, & Ruiz, 2013), and development of browning would be enhanced (Chiavaro, Rinaldi, Vittadini, & Barbanti, 2009).

The addition of a FES showed a similar but milder browning enhancement effect as that found in the lamb loin surface. Again, this was most likely due to the increased development of non-enzymatic browning reactions as a consequence of the higher amounts of reducing sugars in the FES added samples.

Therefore, from a practical point of view, the addition of reducing sugars to lamb meat previous to cooking could be an interesting approach for improving surface browning, especially in *sous-vide* cooked meats, in which the development of such darker surface is milder or inexistent. However, in some types of meat, in which keeping a reddish color note of the cutting surface is desirable, such as roasted beef type preparations, the increasing browning of the core could perhaps be a drawback.

Sensory analysis

The effects of FES addition and the type of culinary treatment on different sensory attributes of lamb loins are shown in Table 3. Cooked lamb loins injected with the solution containing D-glucose, D-ribose, thiamin and L-cysteine showed a less bright and more intense color appearance and a tougher, more chewy and less soggy texture (p<0.001 for all parameters). They also showed a more intense umami taste (p=0.048), and higher scores in all flavor parameters (p<0.001), as compared to control samples. On the other hand, *sous-vide* preparation of lamb loins led to a less bright color (p=0.037), a less chewy (p=0.019), less juicy (p=0.009) and a more soggy (p<0.001) texture, a lower sweet taste (p<0.001), and a less intense flavor (p=0.002). Nevertheless, some of these parameters showed significant interaction between both studied factors, which will be described and discussed within each groups of parameters.

The appearance attributes were much more affected by the addition of a solution containing sugars, thiamin and L-cysteine than by the different heat treatment. This is not surprising in the case of the color intensity, since this is mainly due to the denaturation of myoglobin and partially to the occurrence of brown compounds from Maillard reactions (King & Whyte, 2006). The temperature reached in the core of the lamb loin was high enough to promote the degradation to myoglobin to produce the brown pigment ferrihemochrome in both types of cooking procedures. However, it seems that the slightly higher temperature in those samples cooked in the oven was not enough to cause noticeable differences in the formation of browning compounds through Maillard reactions. Accordingly, we have previously shown that above 70 ºC of cooking temperature, there is not much variation in the cutting surface color of cooked lamb loin (Roldan, Antequera, Martin, Mayoral, & Ruiz, 2013). Interestingly, the addition of reducing sugars produced a much more intense formation of colored compounds, which probably indicates a more intense development of MR, which is in agreement with other studies in which reducing sugars were added to meat before cooking (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009). In fact, this highly agrees with the results obtained for instrumental color measurement in the cutting surface of the lamb

loins shown previously. Probably the reason explaining the lower brightness of these FES added samples is also the higher formation of browning compounds.

It was somehow surprising that the addition of a FES, containing reducing sugars, thiamin and L-cysteine, showed an effect as strong as the cooking method on sensory texture traits. From the obtained sensory results, it seemed that the presence of these compounds produced cooked lamb loins that showed an overall firmer texture, reflected in a tougher, chewier and less soggy score. The trend was similar for both *sous-vide* and oven roasting, although some differences were reflected in the significance of the interaction of FES addition with the cooking method in all of them. This effect on texture could be due to the increased solubility of glycated myofibrilar proteins caused by the action cooking loss (Sanmartin, Arboleya, Villamiel, & Moreno, 2009). A higher protein solubility produced by sugar might lead to the formation of a stronger protein gel, which would cause such a change in texture features. Further research on the potential effect of protein glycation on texture parameters of meat is needed.

Sous-vide cooking produced less tough and chewy cooked lamb 419 loins, but surprisingly they resulted less juicy and soggier. Low temperature-long time cooking has been shown to produce more tender cooked meat as compared to other cooking methods, due to a more intense solubilization of collagen and to a lower water loss (Roldan, Antequera, Martin, Mayoral, & Ruiz, 2013; Sanchez del Pulgar, Gazquez, & Ruiz-Carrascal, 2012), and some authors have also suggested an increased proteolytic activity as another potential factor (Christensen, Ertbjerg, Løje, Risbo, van den Berg, & Christensen, 2013). This could also explain the soggier detected texture in *sous-vide* cooked lamb loins, since the whole structure of the muscle results less cohesive (Roldan, Antequera, Martin, Mayoral, & Ruiz, 2013).

An interesting interaction between cooking procedure and addition of a FES was detected for all texture parameters, so that the addition of a FES solution somehow counteracted the effects of *sous-vide* cooking, producing a more similar texture to oven roasted lamb loins. As mentioned before, we think that the addition of the two reducing sugars present in the FES could have favored protein glycation, which in turn could have led to the formation of a stronger protein gel network.

While saltiness was not affected by either the cooking method or the addition of a FES, umami taste was more intense in those samples with added FES, even though none of the compounds included in the solution (glucose, ribose, thiamin and cysteine) show umami taste. It could be that the presence of these compounds led to the formation of flavor compounds that could enhance the overall flavor meat notes, and the assessors perceived this also as an increase in umami taste. As far as sweetness is concerned, the addition of the FES containing D-glucose and D-ribose, led to a more intense sweet taste in oven roasted lamb loins, while the same amount of added sugars did not change sweet perception in *sous-vide* cooked samples. Both sugars show a sweet taste, and the added amount was above their detection threshold, since the calculated final concentration in the aqueous phase was around 0.2 mol/L for 445 D-glucose and 0.01 mol/L for D-ribose, while their detection thresholds are around 0.065 mol/L and 0.2 mol/L, respectively. It could be that chemical reactions contributed to the degradation of these two sugars or their binding to other compounds present in meat, were more intense in *sous-vide* cooked samples, due to the much longer cooking time. In fact, (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009) detected an almost total withdrawal of added ribose and ribose 5-phosphate to pork samples after 2 hours storage at 4 ºC, and this disappearance was attributed to enzymatic activity. Aliani and Farmer (2005) did not show any effect of ribose or glucose addition to chicken on sweet taste either.

All flavor traits showed higher scores as a consequence of a FES addition, except for rancidity, which points out to an increase in the formation of flavor compounds due to the presence of the compounds included in the solution injected. Other researchers have shown that the addition of reducing sugars (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009) enhanced the formation of volatile compounds generated in the course of Maillard reaction in cooked pork. Upon cooking, reducing sugars added to meat could react with free amino acids and generate volatile compounds affecting the overall aroma and flavor of cooked meat (Farmer, Hagan, & Paraskevas, 1999). In fact, Aliani and Farmer (2005) also showed that the addition of ribose, glucose, thiamin or cysteine to chicken before cooking led to an increase in the scored of several flavor descriptors.

The effect of added a FES on flavor traits was much more marked in *sous-vide* cooked samples, leading to a significant interaction with the cooking method. In fact, the addition of a FES led to similar or even higher scores in "flavor intensity" and "cooked meat flavor" in the *sous-vide* cooked and the oven roasted ones. Again, it seems that the longer cooking time, although at lower temperatures, could have allowed a greater formation of compounds from MR in *sous-vide* cooked samples.

Maillard reaction products

To evaluate the development of the MR cascade, products from different stages are useful. Thus, furosine (ε-*N*-(furoylmethyl)- L-lysine) formation is an indirect indicator of the formation of Amadori products from Lysine, and accordingly, is related to the early stage of the MR (Erbersdobler & Somoza, 2007). Furosine is formed during acid hydrolysis of the Amadori compounds (fructosyl-lysine, lactulosyl-lysine and maltulosyl-lysine) produced by the reaction of the ε-amino groups of lysine with glucose, lactose and maltose (Henle, 2008).

On the other hand, these Amadori reaction compounds are degraded to highly reactive 1,2-dicarbonyl compounds (Thornalley, Langborg, & Minhas, 1999), such as 3-deoxyglucosone (3-DG), 3-deoxypentosone (3-DPs), glyoxal (GO) or methylglyoxal (MGO), which were analyzed in this study. These compounds are thus considered intermediates in a complex reaction cascade, where they are prone to react with mainly the N-terminal and lysine and arginine side chains of proteins leading to AGEs (Silvan, van de Lagemaat, Olano, & del Castillo, 2006). In particular, 3-DG and MGO are known to occur in considerable amounts in foods (Degen, Hellwig, & Henle, 2012) and are known to be precursors of aroma-active compounds (Bravo, et al., 2008). HMF is also an intermediate compound of the MR, formed as an end product of 3-DG (Henle, 2008). Finally, reactions of these dicarbonyl compounds with lysine and arginine residues (free or side chains of proteins) lead to stable AGEs, among which CML has been widely used as an indicator (Henle, 2008).

Results from furosine determination in lamb loins injected with a FES and cooked under two different cooking treatments are shown in Table 4. The cooking conditions had no significant effect by itself on furosine formation

(p=0.122). However, the addition of a FES solution, containing D-glucose and D-ribose, and the interaction between the type of cooking treatment and the addition of such a FES, showed a significant effect on the amount of furosine ($P<0.001$). In samples without FES addition, comparable amounts, around 30 mg furosine/100 g protein, could be measured regardless the cooking conditions applied. The addition of the FES resulted in a dramatic increase of the furosine concentration in samples cooked following any of the procedures. But this effect was much more marked in oven roasted samples (almost 4 times higher levels than in not injected ones), which were subjected to a more severe heat treatment. This was not unexpected, since the extent of the formation of Amadori compounds is enhanced by heating (Martins, Jongen, & van Boekel, 2000). In fact, (Pompei & Spagnolello, 1997) also observed a higher formation of furosine in pork meat as a consequence of increasing heating temperature. In our study, both temperature and time played a role, so that the formation of furosine at the core cooking temperature of oven roasting (final temperature of 73 ºC) is faster than in *sous-vide* samples (60 ºC), but the cooking time is much shorter. Pompei and Spagnolello (1997) also showed a reduction in the activation energy of furosine formation as a consequence of the dextrose content of the enhancement solution injected, which would explain the much higher levels of furosine in samples injected with the FES solution. None of the compounds derived from the degradation of Amadori products measured in this study (HMF and 1,2-dicarbonyl compounds) reached detectable limits in any of the samples (Table 4 and Figure 1). This type of compounds usually reach higher levels in foods which are rich in glucose or fructose, while their concentration in meat is rather low, even though some of our samples were spiked with glucose. The literature on the HMF formation in meat during cooking is very limited. Danowska-Oziewicz, Karpińska-Tymoszczyk, and Borowski (2007) obtained higher HMF values in cooked samples following more intense cooking methods, but the samples were coated with egg and bread crumbs.

Concerning the dicarbonyl compunds (Figure 1), all samples showed concentrations lower than the detection limit (1 mg/kg), regardless of the cooking process or the addition of a FES solution. As far as our knowledge, there is no information about the content of these compounds in meat or meat products in the scientific literature.

The absence of these compounds could indicate that there was no important degradation of the Amadori reaction compounds. Nevertheless, it could be that, due to their high reactivity, generated dicarbonyl compounds would have readily reacted with matrix components like arginine and lysine side chains of proteins, giving rise to the formation of adducts (Henle, Walter, & Klostermeyer, 1994). And another possible cause for their absence in cooked lamb loin samples could be related to the meat specific dipeptide carnosine, which has been shown to form adducts with reactive aldehydes, acting as an effective anti-glycating agent, inhibiting the formation of protein glycation and cross links induced by reducing sugars and other reactive aldehydes e.g. malondialdehyde and methylglyoxal (Hipkiss, Preston, Himsworth, Worthington, Keown, Michaelis, et al., 1998). Thus, 1,2-dicarbonyl compounds seems not to be suitable indicators for predicting/evaluating the extent of the MR in lamb meat. Nevertheless, the potential effect of the complex meat matrix on hindering these compounds should not be dismissed; in fact, a similar problem was found when analyzing coffee samples (Degen, Hellwig, & Henle, 2012).
Therefore, from the results of our study and with respect to current discussion about possible antinutrive properties of glycation compounds (Henle, 2008), cooked meat can be regarded as a negligible source of 1,2-dicarbonyl compounds such as methylglyoxal.

Concerning the formation of AGEs, table 4 shows that the CML content of cooked lamb loins was not affected by the addition of a FES, neither by the type of cooking treatment applied, nor by their interaction. All samples showed the same low CML concentration, around 4 mg CML/100g proteins, what implied that advanced MR have only occurred to a small extend in our samples. Similar results have been found in the literature for different types of cooked meats (Hull, Woodside, Ames, 549 & Cuskelly, 2012).

Nevertheless, these results are unexpected, since those samples containing added amounts of reducing sugars did not show higher CML values, as has been previously shown in meat cooked in sauces containing this type of sugars (Chao, Hsu, & Yin, 2009).

CONCLUSIONS

Overall, relatively small amounts of Maillard reaction markers are formed during cooking of lamb loin, even after the addition of reducing sugars. Sous-vide or oven roasted cooked meat, therefore, seems to be only a minor dietary source of Maillard reaction products. Nevertheless, the addition of reducing sugars and other flavor precursors, such as thiamin or L-cysteine, seems to be a suitable and easy approach for enhancing browning and flavor formation in cooked lamb. And even more interesting, the presence of reducing sugars together with long cooking times at moderately high temperatures induce physical changes in the cooked meat structure, leading to reduced water loss and a firmer texture. In our experiments this occurs together with an increase in early glycation measured as furosine, probably resulting in functional changes.

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Table 1. Sensory attributes, definitions and extremes of each attribute scored in an unstructured line of 10 cm.

Sensory trait	Definition	Extremes
Appearance		
Brightness	Intensity of brightness on the lean surface	Dull to very bright
Color intensity	Intensity of the color in the slice surface	Pale to dark brown
Texture		
Toughness	Effort required to bite through lean and to convert the sample to a	Very tender to very firm
Chewiness	Work required to masticate the sample before swallowing	Very low to very high
Juiciness	Impression of lubricated food during chewing	Not to very juicy
Sogginess	Soggy feeling inside the mouth during chewing	Very low to very high
Taste		
Saltiness	Level of salt taste	Not to very salty
Umami	Intensity of umami taste	Not to very high
Sweetness	Intensity of sweet taste	Not to very sweet
Flavor		
Flavor intensity	Level of overall flavor	Flavorless to very intense
Lamb flavor	Intensity of sheep flavor	Very low to very high
Cooked meat	Intensity of typical cooked meat flavor	Very low to very high
Rancidity	Intensity of rancid flavor	Very low to very high

Table 2. Effect of addition of a flavor enhancer solution (FES) and of the cooking method on moisture content, cooking losses and instrumental color of the external surface and the cutting surface of cooked lamb loins.

	Sous-vide		Oven roasting					
	Control	FES[1]	Control	FES	SEM[2]	*p* cook	*p* FES	*p* cook x FES
Weight Loss (%)	23.7^{c}	16.0^{d}	35.7^{b}	40.6^{a}	2.26	<0.001	0.119	<0.001
Moisture (%)	68.1^{b}	71.8^{a}	65.9^{bc}	64.7^{c}	0.71	<0.001	0.100	0.004
External surface								
L*	62.2^{a}	58.3^{ab}	54.5^{b}	56.4^{b}	0.90	0.003	0.462	0.049
a*	6.3^{b}	9.0^{a}	6.9^{ab}	6.2^{b}	0.36	0.058	0.081	0.006
b*	8.1^{c}	9.3^{c}	14.9^{b}	17.4^{a}	0.91	<0.001	0.004	0.243
Browning index	21.1^{b}	28.3^{b}	40.8^{a}	44.3^{a}	1.92	<0.001	0.014	0.330
Cutting surface								
L*	$64. 2^{a}$	57.4^{b}	65.1^{a}	$62. 2^{a}$	0.80	0.006	<0.001	0.010
a*	14.7	15.4	13.8	13.4	0.39	0.072	0.878	0.477
b*	6.4^{b}	5.9^{b}	8.6^{a}	9.1^{a}	0.36	<0.001	0.875	0.168
Browning index	26.2^{b}	29.6^{ab}	28.9^{ab}	31.0^{a}	0.91	0.036	0.009	0.491

Means with different superscript showed significant differences in the Tukey's test ($p<0.05$)

[1] FES= Flavor enhancer solution

[2] SEM= Standard error of the mean

Table 3. Effect of the addition of a flavor enhancer solution (FES) and of the cooking method on sensory traits of cooked lamb loins.

	Sous-vide		Oven roasting					
	Control	FES[1]	Control	FES	SEM[2]	*p* cook	*p* FES	*p* cook x
Brightness	4.5^{a}	3.0^{b}	4.9^{a}	3.9^{ab}	0.28	0.037	<0.001	<0.001
Color intensity	3.5^{b}	5.9^{a}	3.9^{b}	5.8^{a}	0.26	0.513	<0.001	<0.001
Toughness	0.9^{c}	4.2^{a}	2.2^{b}	4.1^{a}	0.20	0.053	<0.001	<0.001
Chewiness	1.4^{c}	4.4^{a}	3.0^{b}	4.2^{a}	0.19	0.019	<0.001	<0.001
Juiciness	2.9^{b}	3.2^{ab}	4.2^{a}	3.7^{ab}	0.30	0.009	0.827	0.033
Sogginess	5.3^{a}	2.0^{b}	2.5^{b}	2.2^{b}	0.28	<0.001	<0.001	<0.001
Saltiness	1.3	1.4	1.2	1.0	0.21	0.335	0.853	0.731
Umami	1.9	2.5	1.8	2.2	0.26	0.396	0.048	0.195
Sweetness	0.7^{c}	0.7^{bc}	1.5^{ab}	2.2^{a}	0.23	<0.001	0.162	<0.001
Flavor intensity	2.5^{b}	3.9^{a}	3.5^{a}	4.1^{a}	0.24	0.025	<0.001	<0.001
Lamb flavor	2.4^{b}	3.9^{a}	2.8^{b}	3.1^{ab}	0.28	0.557	0.002	0.004
Cooked meat flavor	1.9^{b}	4.2^{a}	2.4^{b}	3.9^{a}	0.24	0.902	<0.001	<0.001
Rancidity	1.0	0.9	1.0	0.7	0.21	0.498	0.184	0.512

Means with different superscript showed significant differences in the Tukey's test ($p<0.05$)

[1] FES= Flavor enhancer solution

[2] SEM= Standard error of the mean

Table 4. Effect of addition of a flavor enhancement solution (FES) and of the cooking method on hidroxymethylfurfural (HMF), furosine and carboxymethyllysine (CML) content in cooked lamb loins.

	Sous-vide		Oven roasting					
	Control	FES[1]	Control	FES	SEM[2]	*p* cook	*p* FES	*p* cook x FES
Furosine (mg /100g protein)	36.8[c]	72[b]	29.4[c]	122.2[a]	7.33	0.122	<0.001	<0.001
HMF (mg HMF/kg sample)	n.d.[3]	n.d.	n.d.	n.d.	-	-	-	-
CML (mg /100g protein)	4.0	4.1	4.1	4.6	0.30	0.404	0.228	0.454

Means with different superscript showed significant differences in the Tukey's test ($p<0.05$)

[1] FES= Flavor enhancer solution

[2] SEM= Standard error of the mean

[3] n.d.= not detectable

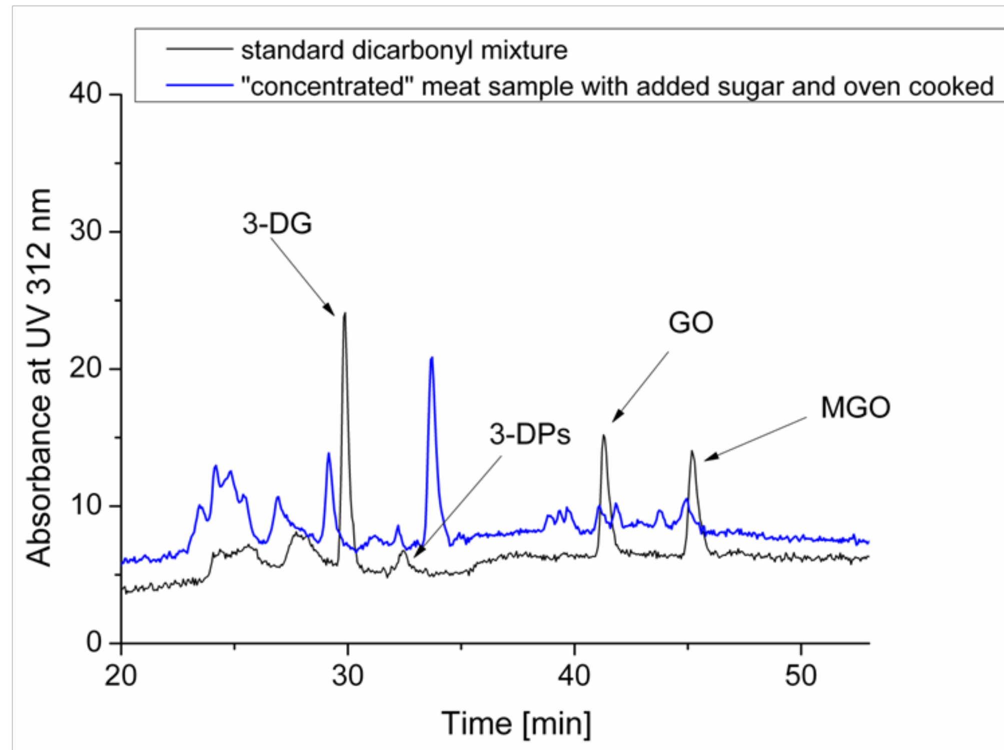

Figure 1. Chromatograms of (black) a standard dicarbonyl mixture (containing 3-DG, 3-DPs, GO and MGO) and (blue) an oven roasted lamb loin sample with added FES, after sample preparation (ration of sample and extractant was changed) and derivatization with o-phenylendiamine acquired by RP-HPLC with UV detection (312 nm). Arrows indicate the peaks of the quinoxalines of 3-deoxyglucosone (3-DG), 3-deoxypentosone (3-DPs), glyoxal (GO) and methylglyoxal (MGO).

7. Discusión

7.1 Efecto de la temperatura y el tiempo de cocinado sobre las características de la carne de cordero cocinada al vacío

Características Físico-químicas

Se ha constatado que el cocinado al vacío de la carne de cordero en restaurantes de alto nivel, empleando temperaturas bajas (en torno a 60ºC) y tiempos de cocinado prolongados (hasta 24 horas) da lugar a un producto de unas características sensoriales extraordinarias. Sin embargo la información científica sobre las características de las carnes cocinadas en esas condiciones es muy escasa. Por tanto, el primer objetivo de este trabajo fue estudiar cómo estas condiciones de cocinado afectan a otras características de la carne de cordero (Capítulos 1.1, 1.2 y 1.3), así como a su conservabilidad (Capítulo 1.4), con el fin de poder extender este tipo de cocinado hacia otras industrias tales como la del catering o los platos preparados.

Se ha observado que tanto las bajas temperaturas como los largos tiempos de cocinado utilizados en este trabajo juegan un papel importante en las características físico-químicas y sensoriales de los lomos de corderos cocinados al vacío. En relación a las propiedades físico-químicas de la carne de cordero (Capítulo 1.1), se ha observado que tanto la temperatura como el tiempo de cocinado afectan de forma sustancial a las pérdidas por cocinado y al color de la carne cocinada. Sin embargo, ninguno de estos parámetros tuvo un gran efecto en el contenido de humedad de las muestras. Así, aquellos lomos cocinados a mayores combinaciones de temperatura y tiempo presentaron mayores mermas durante el mismo, causado posiblemente por una mayor desnaturalización y acortamiento de las proteínas miofibrilares (Vaudagna et al., 2002). Por otro lado, el tiempo de cocinado también afectó a las pérdidas por cocinado, aumentando éstas en los tratamientos más largos. Estos resultados estuvieron acorde con los estudios realizados por Christensen, Bertram, Aaslyng y Christensen (2011) y Vaudagna et al. (2002).

A pesar del claro efecto que la temperatura y el tiempo de cocinado tuvieron sobre las mermas, el contenido de humedad no mostró diferencias significativas, de forma que los lotes estudiados presentaron valores similares, independientemente de la combinación de temperatura y tiempo aplicados. No obstante, se pudo observar una tendencia acorde con las mermas; de

manera que las muestras cocinadas a 80 ºC presentaron un nivel de humedad ligeramente menor comparada con las muestras cocinadas a 60 ºC. Por tanto, desde un punto de vista práctico, parece ser que el cocinado de lomos de corderos al vacío utilizando temperaturas de hasta y por encima de los 70 ºC, conllevaría a mayores pérdidas por cocinado que si esos mismos lomos fueran cocinados a 60 ºC y utilizando mayores tiempos, lo que estaría en consonancia con los conocimientos empíricos aportados por los chefs (Myhrvold, Young, & Bilet, 2011).

Al analizar el comportamiento de los lomos cocinados a vacío, y almacenados a refrigeración durante 7, 15 y 30 días, pudo observarse una disminución significativa en los valores de las pérdidas por cocinado desde el comienzo del almacenamiento hasta el final del mismo en aquellas combinaciones de cocinado más suaves (60 ºC durante 6 y 24 y 80 ºC durante 6h). Sin embargo, no hubo diferencias en el porcentaje de mermas en aquellas muestras sometidas al tratamiento térmico más severo (80 ºC durante 24h). Estos resultados estuvieron en consonancia con el porcentaje de humedad durante en el almacenamiento solamente en las muestras cocinadas mediante el tratamiento más suave (60 ºC durante 6h), ya que el resto de muestras no presentaron diferencias de humedad durante el almacenamiento.

El análisis del color instrumental mostró mayores valores de L* en las muestras cocinadas a 60 ºC comparado con las muestras cocinadas a 70 u 80 ºC. Sánchez Del Pulgar, Gázquez y Ruiz-Carrascal (2012), obtuvieron también mayores brillos en las muestras cocinadas a 60 ºC comparada con las de 80 ºC, lo que se atribuiría a una mayor cantidad de agua libre que se encuentra impregnando la superficie de corte de las muestras, lo que fue observado ampliamente durante el análisis experimental. Sin embargo, otros autores han detectado justamente lo contrario, es decir, mayores valores de L* a mayores temperaturas de cocinado (Christensen et al., 2011; García-Segovia, Andrés-Bello, & Martínez-Monzó, 2007).

Por otro lado, los lomos de cordero que presentaron menores valores de a* y b*, fueron los que se cocinaron a mayores combinaciones de temperatura y tiempo, lo que podría atribuirse a un mayor grado de desnaturalización de la mioglobina (King & Whyte, 2006) y a una menor formación y posterior desnaturalización de metamioglobina. Tales resultados han sido han sido

previamente obtenidos por otros autores en carne de res (García-Segovia et al., 2007) y cerdo (Christensen et al., 2011).

Durante el almacenamiento a refrigeración de los lomos cocinados a vacío, el color instrumental presentó solo ligeras variaciones. Así, los valores de L* y a* se mantuvieron prácticamente contantes durante los 30 días de almacenamiento. Sin embargo, los valores de b* mostraron un comportamiento muy variable dependiendo de las condiciones de cocinado, aunque los valores más bajos fueron siempre obtenidos en aquellas muestras cocinadas con el tratamiento más suave (60 ºC-6h).

En relación a la textura instrumental, tanto la dureza instrumental (TPA) como la dureza al corte (SF) analizadas en los lomos de corderos cocinados al vacío, presentaron una significativa disminución con el tiempo de cocinado independientemente de la temperatura usada. Estos resultados podrían ser debidos, por un lado, a una mayor solubilización del colágeno con mayores tiempos de cocinado (Sánchez del Pulgar et al., 2012), y de hecho, la significativa disminución de la dureza instrumental obtenida en aquellos lomos cocinados a 80 ºC durante 24h, podría ser debido a una extensa desintegración del perimisio alrededor de los haces musculares (Baldwin, 2012), lo que estaría en consonancia con los menores valores de cohesividad que se obtuvieron en estas muestras. Pero por otro lado, esta disminución de la dureza instrumental y dureza al corte podría atribuirse a la posibilidad de que las miofibrillas pudieran haber alcanzado su máximo nivel de acortamiento incluso con el tiempo de cocinado más corto (6h), lo que explicaría que la dureza no aumente con tiempos de cocinados más largos (12 y 24h). Estos resultados fueron opuestos a los obtenidos por Palka (2003) y Palka y Daun (1999), quienes detectaron mayores valores de dureza instrumental en aquellas muestras cocinadas a mayores temperaturas, posiblemente debido a los tiempos más cortos utilizados en su estudio, los cuales no fueron suficientes para una completa solubilización del colágeno, pero sí suficientes para el acortamiento de las proteínas miofibrilares.

En cuanto a los cambios en la estructura del músculo (observado mediante un microscopio electrónico de barrido (SEM)), producidos por las temperaturas y tiempos de cocinado, podemos resumirlos en una mayor granulación del colágeno endomisial en aquellas muestras cocinadas con

tratamiento térmico más suave (60 ºC durante 6h), y la formación de un gel a mayores temperaturas (80 ºC). Un comportamiento similar fue observado por Wu, Dutson y Smith (1985) en carne de res.

Con respecto a la evolución de la textura instrumental a lo largo del periodo de almacenamiento, no se apreciaron cambios muy significativos. Por tanto, la textura deseable que se logra a través del cocinado al vacío, se mantiene durante el almacenamiento en refrigeración durante al menos 30 días.

En cuanto a los recuentos de los grupos microbianos estudiados en los lomos de corderos cocinados al vacío con las diferentes combinaciones de temperatura-tiempo, todos ellos fueron menores a 1 log UFC /g o no se detectaron, lo que está en consonancia con los resultados obtenidos por Díaz, Nieto, Garrido y Bañón (2008), quienes detectaron una ausencia o recuentos mínimos en lomos de cerdo cocinados al vacío a 70 ºC durante 12h. Se pudo constatar, por tanto, que el cocinado al vacío utilizando incluso combinaciones de temperatura-tiempo menores (60 ºC-6h), es suficiente para acabar con microorganismos patógenos, tales como *Salmonella enteritidis* o *E. Coli*. La intensidad de los tratamientos térmicos junto con la calidad microbiológica con la que parten estos lomos de cordero crudos, sugieren una inactivación total de las formas vegetativas de bacterias causantes del deterioro de la carne durante el almacenamiento de las muestras a 2 ºC. Esto, junto con los recuentos obtenidos del PCA, apuntan a una contribución muy limitada de los microorganismos sobre los cambios observados en los parámetros analíticos estudiados de los lomos de corderos cocinados a vacío, incluso después de 30 días de almacenamiento en condiciones de refrigeración.

Oxidación lipídica y proteica. Perfil de compuestos volátiles

Al estudiar el comportamiento de los lomos cocinados a vacío frente a la oxidación lipídica (Capítulo 1.2), se observó que temperaturas más elevadas y tiempos de cocinado más prolongados dieron lugar a una mayor formación de dienos conjugados (CD), así como una disminución significativa de los valores de los compuestos secundarios de oxidación, medidos como las TBARs, y otros compuestos volátiles procedentes de la oxidación lipídica, tales como

el pentanal, hexanal o heptanal. Este aumento de los valores de los CD a mayores temperaturas y tiempos de cocinados sugirieron que a estas condiciones de cocinado la oxidación de los lípidos continúa produciéndose. En cuanto a la disminución de los valores de las TBARs, así como del resto de aldehídos y otros compuestos volátiles procedentes de la oxidación lipídica, podrían atribuirse a reacciones entre los compuestos carbonilos procedentes de la oxidación lipídica con los grupos aminos de la lisina, cisteína y glutatión (Ventanas, Estevez, Delgado, & Ruiz, 2007). Estas reacciones podrían producirse debido a las altas temperaturas de cocinado (Zamora, Gallardo & Hidalgo, 2008). Estos resultados están en concordancia con aquellos obtenidos por otros autores en carne de cerdo cocinada al vacío (Sánchez Del Pulgar et al., 2012; Sanchez del Pulgar, Roldan, & Ruiz-Carrascal, 2013).

Por otro lado, se observó un incremento significativo de la oxidación de las proteínas, medida a través de la cuantificación de carbonilos totales mediante el método del DNPH, con el tiempo de cocinado independientemente de la temperaturas utilizada. Sin embargo, estos resultados no estuvieron en consonancia con aquellos obtenidos para los dos semialdehídos analizados: α-aminoadípico (AAS) γ-glutámico (GGS). Así, las muestras presentaron un incremento significativo en la concentración de estos semialdehídos a medida que aumentaba el tiempo de cocinado, manteniéndose más o menos constantes a 70 ºC y disminuyendo incluso el GGS a 80 ºC. Esta disparidad observada entre los resultados obtenidos al medir los carbonilos totales mediante el método DNPH y los semialdehídos analizados fue observada previamente por Armenteros, Heinonen, Ollilainen, Toldrá y Estévez (2009), y puede ser debida a que el método DNPH mide carbonilos totales, pudiendo haber dado una sobreestimación de la oxidación proteica en las muestras. Por otro lado, mayores concentraciones del AAS fueron obtenidas en todas las combinaciones de temperatura y tiempo de cocinado, hecho observado también por otros autores en carne y sistemas modelos cárnicos (Akagawa et al., 2006; Utrera, Morcuende, Rodriguez-Carpena, & Estévez, 2011). Estos resultados podrían deberse probablemente a que el AAS procede de la lisina, que es el aminoácido más abundante en carne (Armenteros et al., 2009).

Además de disminución de los compuestos volátiles procedentes de las

reacciones de oxidación lipídica (Capítulo I.3), la mayor formación de 3-metilbutanal (3MB), producto de la degradación de la leucina a través de la reacción de Strecker (Elmore Mottram, Enser & Wood, 1999), en las muestras cocinadas a 80 ºC durante 24h, pone de manifiesto una intensificación de las reacciones entre los grupos carbonilos procedentes de la oxidación lipídica y grupos aminos procedente de los aminoácidos a mayores temperaturas y tiempos de cocinado. Por tanto, estos resultados, por un lado, apoyarían los resultados obtenidos en la oxidación lipídica, explicando, en parte, la disminución de los compuestos secundarios de oxidación, pero también la disminución del GGS, ya que este semialdehído ha sido descrito como una fuente potencial de fracciones aldehídicas que podrían reaccionar con los grupos aminos para formar bases de Schiff en la etapa inicial de la degradación de Strecker de los aminoácidos (Estevéz, Ventanas & Heinonen, 2011). Además, este aumento de las reacciones de Strecker a mayores temperaturas de cocinado, podría explicar también la mayor formación de otros compuestos volátiles tales como es el 2-metilbutanal, procedente de la degradación de la isoleucina (Elmore et al., 1999). Resultados similares han sido observados previamente por otros autores en carne de pollo cocinado a diferentes temperaturas finales (Ang, Liu, & Sun, 1994) y en carne de cerdo cocinada al vacío (Sánchez Del Pulgar et al., 2012).

Finalmente, la escasa formación de compuestos volátiles procedentes de las reacciones de Maillard en las muestras de cordero cocinadas al vacío, pudo deberse a las suaves temperaturas utilizadas en este estudio y a los altos contenidos de humedad que presentaron los lomos, lo que evitó que se deshidratara la superficie de los mismos y se desarrollaran estas reacciones (Myhrvold et al., 2011).

Durante el almacenamiento a refrigeración, la oxidación lipídica y proteica presentaron comportamientos muy variables. Así, los valores de CD de las muestras cocinadas a 80 ºC durante 24h sufrieron una diminución significativa durante la segunda semana de almacenamiento para luego mantenerse constantes hasta el final del mismo. El resto de las muestras presentaron valores constantes durante todo el almacenamiento. Estos resultados podrían ser debido a que las reacciones de oxidación lipídica se ralentizan y los compuestos primarios de oxidación, medidos a través de CD se

degradan para formar compuestos secundarios de oxidación (Shahidi & Zhong, 2005). Sin embargo, mínimos cambios fueron observados en los valores de TBARs durante todo el periodo de almacenamiento, siendo las muestras cocinadas a 80 ºC durante 24h las que presentaron menores valores de TBARs en todos los días analizados. Esta reducción de la oxidación lipídica tanto en los CD y TBARs pudo ser debida a que el cocinado se realiza en ausencia de oxigeno (Ahn, Wolfe, Sim, & Kim, 1992).

En relación a la oxidación proteica durante el almacenamiento a refrigeración de los lomos, se observó que aquellas muestras cocinadas a 80 ºC sufrieron una mayor oxidación durante la primera semana de almacenamiento para luego ir disminuyendo hasta el final del mismo. Estos resultados podrían atribuirse a una mayor desnaturalización de la mioglobina a temperaturas de cocinado más elevadas, lo que daría lugar a una mayor liberación de hierro, el cual se ha demostrado como un catalizador de la oxidación proteica en carne cocinada (Ganhao, Morcuende, & Estevez, 2010). Sin embargo, en las muestras cocinadas a 60 ºC no se observaron modificaciones significativas de la oxidación proteica durante el almacenamiento a refrigeración.

7.2 Efecto de la temperatura y el tiempo de cocinado sobre las características de la carne de cordero cocinada al vacío y al horno tras la adición de fosfatos y precursores del aroma

La adición de fosfatos provocó un aumento significativo del pH en los lomos de cordero tras el *tumbling* y el cocinado de los mismos, independientemente del nivel de fosfato inyectado o el tipo de cocinado utilizado, en comparación a las muestras inyectadas con agua destilada. Este aumento del pH tras la adición de fosfatos ha sido previamente observado por otros autores en carne de cerdo (Sheard & Tali, 2004), pechuga de pavo (Ergezer & Gokce, 2011), aves de corral (Bianchi, Petracci, & Cavani, 2009), carne de res (Pietrasik & Janz, 2009) y cordero (Murphy & Zerby, 2004); pudiendo atribuirse al hecho de que tanto el tripolifosfato de sodio (STPP) como el pirofosfato tetrasodio (TSPP) son sales alcalinas y su adicción a la carne conlleva a un aumento del pH de la misma. Por otro lado, el incremento

del pH como consecuencia del cocinado podría ser tribuido a una reducción en la disponibilidad de grupos carboxilos de las proteínas, pero también, a la liberación de iones calcio y magnesio procedente también de las mismas (Ergezer & Gokce, 2011).

En relación a las pérdidas de peso y pérdidas por cocinado, la adición de fosfatos provocó una reducción significativa en los valores de ambos parámetros en los lomos de corderos cocinados tanto a vacío como al horno, en comparación con las muestras inyectadas con agua destilada. Estos resultados podrían atribuirse al aumento de pH previamente descrito, el cual podría desplazar el punto isoeléctrico de las proteínas miofibrilares aumentando así la cantidad de agua retenida en la carne (Alvarado & Mckee, 2007). Vaudagna et al. (2008) también obtuvieron menores pérdidas por cocinado tras la adición de una salmuera compuesta por tripolifosfato de sodio en carne de res cocinada a vacío. Por otro lado, las muestras cocinadas a vacío presentaron valores significativamente más bajos de pérdidas de peso y pérdidas por cocinado comparados con los lomos cocinados al horno, lo que estaría en consonancia con los mayores valores de humedad obtenido en estas muestras. Estos resultados podrían atribuirse a la menor temperatura de corazón alcanzada en las muestras cocinadas a vacío (60 ªC *vs* 73 ºC). De hecho, otros autores han detectado mayores pérdidas de peso y menores contenidos de humedad a mayores temperaturas de cocinado (García-Segovia et al., 2007; Palka & Daun, 1999), probablemente debido a un incremento en la contracción longitudianl de las fibras musculares por encima de los 60 ºC.

El contenido de humedad solo se vio significativamente afectado por la interacción entre la adición de fosfatos y el tipo de cocinado, de manera que aquellas muestras cocinadas a vacío y que fueron inyectadas con una mayor concentración de fosfatos (0,4 %) presentaron un mayor contenido de humedad, mientras que los valores de humedad de las muestras cocinadas al horno no se vieron afectadas por la adición de fosfatos. Por otro lado, la capacidad de retención de agua solo se vio significativamente afectada por el tipo de cocinado presentando mayores valores aquellas muestras cocinadas a vacío. Sin embargo, no se observaron diferencias significativas entre las muestras inyectadas con diferentes niveles de fosfatos en ninguno de

estos parámetros, lo que podría ser debido, en parte, al hecho de que el pH en ambas salmueras (pH=8,37 para el nivel de fosfatos de 0,2 %; pH= 8,52 para 0,4 %) y el pH en las muestras de ambos tipos de cocinados (pH=6,17 para vacío; pH= 6,20 para horno) fueron muy parecidas. Nuevamente, Vaudagna et al. (2008) encontraron insignificantes diferencias en el contenido de humedad entre las muestras inyectadas con tripolifosfato de sodio a un nivel de 0,25 o 0,5 % de esta salmuera. Massafra (2006) tampoco observó ningún incremento significativo en la capacidad de retención de agua entre muestras inyectadas con fosfatos a un nivel de 1,25 o 2,5 % en lomos de cerdos.

En relación al color instrumental, la adición de fosfatos provocó una disminución de los valores de L* de los lomos, siendo más patente en las muestras inyectadas con el nivel de fosfatos más alto (0,4 %). Estos resultados se podrían atribuir al incremento de pH previamente descrito, y a la fuerza iónica de la disolución, que conllevarían a un hinchamiento de las fibras musculares provocando una mayor penetración de la luz (Bojarska, Batura, & Cierach, 2003). Sin embargo, el cocinado al vacío de los lomos dio lugar a mayores valores de L*, probablemente debido a una mayor desnaturalización y agregación de las proteínas, lo que daría lugar a una mayor dispersión de la luz y por tanto, mayores valores de L* (Christensen et al., 2011; Nikmaram, Yarmand, Emamjomeh, & Darehabi, 2011). Por otro lado, las muestras cocinadas al horno mostraron mayores valores de a* y b*, probablemente debido a un menor grado de desnaturalización de la mioglobina y a una mayor formación y desnaturalización de metamioglobina, lo que indicaría también un mayor desarrollo de las reacciones de Maillard en estas muestras sometidas a un tratamiento más severo (Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010).

En cuanto al análisis del perfil de textura, las muestras inyectadas con fosfatos presentaron valores más altos de dureza instrumental (TPA) y menores de dureza al corte (prueba de Warner Bratzler) que las muestras inyectadas con agua destilada, siendo este efecto más marcado en las muestras cocinadas al vacío que en las cocinadas al horno. Esta medida de dureza instrumental podría explicarse como turgencia. Así, las diferencias encontradas podrían estar relacionadas con el menor porcentaje de humedad en las muestras cocinadas al horno que en las cocinadas a vacío. De manera que, a

mayor contenido en humedad mayor turgencia (dureza) y menor dureza al corte. Si comparamos ambos tipos de cocinados, el cocinado al vacío dio lugar a menores valores de SF, probablemente debido a una mayor desnaturalización del colágeno causado por los mayores tiempos de cocinados. Esta mayor desnaturalización del colágeno pudo ser la causa de los menores valores de cohesividad en estas muestras.

Tras el análisis sensorial de los lomos de corderos, se observó que aquellos atributos relacionados con la apariencia y la textura sensorial de la carne fueron significativamente afectados por la interacción entre la adición de fosfatos y el tipo de cocinado. Así, aquellas muestras cocinadas a vacío e inyectadas con fosfatos dieron lugar a superficies más brillantes comparadas con las muestras inyectadas con agua destilada, probablemente debido a una mayor retención de agua. Por otro lado, mayores niveles de fosfatos dieron lugar a una disminución significativa de la intensidad de color en la superficie de corte de la carne, siendo más marcado este efecto en las muestras cocinadas al horno, Por otro lado, los panelistas encontraron más tiernas aquellas muestras tratadas con fosfatos comparadas con las que fueron inyectadas con agua, siendo más marcado este efecto en las muestras cocinadas al vacío, lo que podría atribuirse a una mayor solubilidad del colágeno y también a la mayor retención de agua de las muestras cocinadas (Sheard, Nute, Richardson, Perry, & Taylor, 1999). Menores valores de dureza fueron también obtenidos por Gorsuch y Alvarado (2010) en filetes de ave de corral tras ser inyectados con una combinación de NaCl y fosfatos y cocinados en un horno hasta un temperatura interna de 73 ºC, y por Sheard et al. (1999) en lomos de cerdo inyectados con fosfatos y asados a la parrilla hasta un temperatura interna de 72,5 ºC. Sin embargo, esta tendencia fue completamente opuesta a la encontrada en la textura instrumental, en la cual, las muestras inyectadas con fosfatos presentaron mayores valores de dureza instrumental (TPA), revelando una falta de consistencia entre los parámetros de textura instrumental y los sensoriales. El efecto de la adición de fosfatos en la retención de agua fue probablemente la principal razón de los menores valores de masticabilidad y mayores de jugosidad obtenidos en aquellas muestras inyectadas con estas salmueras, efecto que fue más notable

en las muestras inyectadas con el mayor nivel de fosfatos (0,4 %) y cocinadas a vacío.

Sin embargo, ni la adición de fosfatos, ni el tipo de tratamiento culinario utilizado, ni la interacción entre ambas variables tuvo ninguna influencia en el sabor o aroma característico de los lomos de cordero. De igual forma, otros autores tampoco han detectado ningún efecto de la adición de fosfatos sobre estos atributos en carne cocinada (Rowe, Pohlman, Brown, Jr., & Johnson, 2009; Sheard et al., 1999). No obstante, Sawyer, Brooks, Apple y Fitch (2009) detectaron sabores jabonosos y desagradables en carnes cocinadas e inyectadas con fosfatos, incluso con niveles más bajos de fosfatos que los utilizados en el presente estudio.

Como ya se ha comentado en capítulos anteriores, se ha evaluado la adición de precursores del sabor (FES). La adición de una disolución compuesta por glucosa, ribosa, cisteína y tiamina no afectó de forma significativa a las pérdidas por cocinado y contenido de humedad, sin embargo, sí lo hizo la interacción entre la adición de esta solución y el tipo de cocinado. Así, las pérdidas por cocinado en las muestras cocinadas a vacío disminuyeron y el contenido de humedad incrementó con la adición de la FES, mientras que el efecto fue completamente opuesto para las muestras cocinadas en el horno. No hay una explicación clara para estos resultados aunque algunos autores han sugerido que podría ser debido a un incremento en la solubilidad de la miosina glicada como se ha observado previamente en peces (Tanabe & Saeki, 2001), lo que podría dar lugar a la formación de un gel proteico a bajas temperaturas de cocinado, el cual a su vez podría incrementar la capacidad de retención de agua de la carne (Wahyuni, Ishizaki, & Tanaka, 1999). El efecto opuesto mostrado por las muestras cocinadas al horno probablemente se debió a que los tiempos de cocinado no fueron suficientemente largos para permitir una suficiente solubilización de la miosina. Sin embargo, otros autores han detectado justamente el efecto opuesto de los azúcares sobre la solubilidad proteica (Chen, Liu, & Chen, 2002). Por tanto, futuros estudios son necesarios para dilucidar el efecto de los azúcares sobre la glicación proteica.

Al igual que ocurrió en la adición de fosfatos, aquellas muestras cocinadas a vacío presentaron menores pérdidas de peso y

consecuentemente, mayores contenidos de humedad en comparación con las muestras cocinadas en el horno, probablemente debido a las mismas razones que se explicaron previamente.

En relación al color instrumental, las muestras cocinadas al vacío mostraron significativamente mayores valores de L* y menores de b* que las muestras cocinadas al horno, mientras que los valores de a* no fueron significativamente afectadas por el procedimiento culinario. Por otro lado, la adición de la FES no causó ningún efecto significativo sobre los valores de L* y a* de la superficie de los lomos por sí misma, pero sí causó un incremento significativo sobre los valores de b*. En relación al índice de pardeamiento (BI), el tipo de cocinado afectó significativamente a este parámetro, y de hecho, los valores obtenidos en las muestras cocinadas al horno fueron mayores a las obtenidas por el cocinado a vacío. Pero también, la adición de la FES tuvo un efecto significativo sobre el BI, obteniéndose mayores valores de este parámetro en aquellas muestras tratadas con esta solución, independientemente del tipo de cocinado utilizado. La influencia del método de cocinado sobre los valores de color instrumental de la superficie de los lomos, y especialmente el aumento del desarrollo del color pardo en las muestras cocinadas en el horno comparado con las muestras cocinadas a vacío, son probablemente debidos a las mayores temperaturas de cocinado alcanzadas en el horno y el flujo de aire seco utilizado durante el mismo, lo que provocaría una mayor deshidratación de la superficie de estas muestras, una mayor formación y desnaturalización de la metamioglobina, y un mayor desarrollo de las reacciones de Maillard con la consecuente formación de compuestos pardeados en la superficie de la carne (Chiavaro, Rinaldi, Vittadini, & Barbanti, 2009). Por otro lado, los mayores valores obtenidos de b* y de BI con la adición de la FES, fue probablemente debido a la mayor presencia de azucares reductores en carne. De hecho, otros autores han detectado un incremento en el pardeamiento de la superficie de productos cárnicos cocinados como consecuencia de la adición de mayores cantidades de azúcares (Chen et al., 2002).

En relación a la superficie de corte de las muestras, la adición de la FES sí afecto de forma significativa a los valores de L* dando lugar a una disminución de los mismos en ambos tipos de cocinados, siendo más notable

este efecto en las muestras cocinadas a vacío. Los valores de a* no fueron afectados por ninguna de las variables estudiadas, mientras que los valores de b* solo se vieron afectados por el tipo de cocinado, incrementándose en las muestras cocinadas al horno. El efecto del método de cocinado, así como el efecto de la adición de la FES, fueron probablemente debidos a las mismas causas explicadas previamente para la superficie de las muestras.

Tras el análisis sensorial, se observó que la adición de la FES tuvo un efecto más marcado sobre los atributos sensoriales relacionados con la apariencia que el tipo de cocinado utilizado. Así, la adición de la FES produjo un incremento significativo en la intensidad de color, probablemente debido a una mayor desnaturalización de la mioglobina y la aparición de compuestos pardos procedentes de las reacciones de Maillard (King & Whyte, 2006). Estos resultados están en consonancia con los resultados obtenidos en otros estudios en los que se añadieron azúcares reductores a la carne previamente a su cocinado (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009).

Con respecto a las características sensoriales de textura, en general los lomos de cordero presentaron una textura más firme, que se vio reflejada en una mayor dureza y masticabilidad, y una menor pastosidad. Esta tendencia fue similar para todas las muestras independientemente del tipo de cocinado utilizado. Este efecto en la textura se debió probablemente a una mayor solubilidad de las proteínas glicadas que conduciría a la formación de un gel de proteínas más fuerte, responsable de estas características de textura. Si comparásemos ambos tipos de cocinados, las muestras cocinadas al vacío presentaron una menor dureza y masticabilidad con respecto a las muestras cocinadas en el horno, pero también una menor jugosidad y pastosidad de las mismas. Esta menor dureza de la carne podría ser atribuida a las mismas razones que se explicaron previamente para la adición de fosfatos. Y estas mismas razones podrían también explicar el incremento de la pastosidad detectada en los lomos cocinados al vacío, los cuales presentaron una estructura muscular menos cohesiva.

Por otro lado, la adición de la FES dio lugar a un sabor más dulce en los lomos de corderos cocinados al horno, mientras que la misma solución no provocó ningún cambio en la percepción de este sabor en las muestras cocinadas al vacío. Estos resultados podrían atribuirse a que las reacciones

químicas que contribuyeron a la degradación de los azúcares presentes en la solución o su unión a otros compuestos presentes en la carne, fueron más intensas en las muestras cocinas al vacío debido a los mayores tiempos de cocinado. De hecho, Meinert et al. (2009) detectaron una desaparición casi total de la ribosa y la ribosa-5-fosfato añadida a muestras de cerdo después de 2h almacenadas a 4 ºC, lo que se atribuyó a la actividad enzimática. Por otro lado, Aliani y Farmer (2005) no detectaron ningún efecto de la adición de ribosa o glucosa sobre el sabor dulce en carne de pollo.

Todos los atributos relacionados con el sabor de la carne de cordero estuvieron significativamente afectados por la adición de la FES, presentando mayores valores tras la adición de la misma, excepto el atributo "rancidez", siendo más marcada esta diferencia en las muestras cocinadas al vacío. Este aumento en los valores de estos atributos parece indicar que las concentraciones utilizadas de los compuestos que componen la FES son suficientes para provocar cambios significativos en los atributos sensoriales analizados, a través de una mayor formación de compuestos aromáticos. Estos resultados están en consonancia con aquellos obtenidos por Meinert et al. (2009), quienes obtuvieron una mayor formación de compuestos volátiles tras la adición de azúcares reductores en la carne de cerdo cocinada.

Como indicadores de la reacción de Maillard, se analizaron por RP-HPLC-UV las concentraciones de furosina, 1,2-Dicarbonyl compounds, HMF y CML. La cantidad de furosina presentó un incremento significativo en las muestras inyectadas con la FES independientemente del tipo de tratamiento aplicado, aunque este efecto fue mucho más marcado en aquellas muestras cocinadas al horno, las cuales estuvieron sometidas a mayores temperaturas de cocinado. La formación de los compuestos de Amadori está potenciada por el calentamiento (Martins, Jongen, & van Boekel, 2000) y de hecho, Pompei y Spagnolello (1997) también encontraron un incremento en la formación de furosina en carne de cerdo como consecuencia del incremento de la temperatura de calentamiento.

Por otro lado, ninguno de los compuestos procedentes de la degradación de los compuestos de Amadori medidos en este estudio, como son el Hidroximetilfurfural (HMF) y los compuestos α-dicarbonilos, alcanzaron niveles detectables en ninguna de las muestras. Este tipo de compuestos

normalmente se presentan en mayores cantidades en alimentos ricos en glucosa o fructusa, pero su concentración en la carne es muy baja. La información científica sobre la formación de HMF durante el cocinado de la carne es muy limitada; Danowska-Oziewicz, Karpińska-Tymoszczyk y Borowski (2007) obtuvieron mayores valores de HMF en muestras sometidas a un tratamiento térmico más intenso, pero las muestras fueron recubiertas con huevo y migas de pan previamente al cocinado. Con respecto a los compuestos α-dicarbonilos, todas las muestras presentaron concentraciones menores a las del límite de detección (1 mg/kg), independientemente del tipo de cocinado y de la adición de la FES. Sin embargo, no existe información científica sobre la formación de estos compuestos en carne o productos cárnicos. La ausencia de estos compuestos podría indicar que no hubo una importante degradación de los compuestos de Amadori. Pero también podría ser debido a la alta reactividad de estos productos, que pudieron generar compuestos dicarbonilos que reaccionaron rápidamente con aminoácidos de las cadenas laterales de las proteínas, dando lugar a un aumento de la formación de aductos (Henle, Walter, & Klostermeyer, 1994). Aunque otra posible causa de la ausencia de estos compuestos en los lomos cocinados podría estar relacionada con la formación de aductos entre la carnosina y aldehídos reactivos, lo que inhibiría la glicación proteica (Hipkiss et al., 1998). Por esta razón, los compuestos α-dicarbonilos no parecen ser indicadores adecuados para predecir o evaluar la reacción de Maillard en lomos de cordero. Por tanto, a partir de los resultados obtenidos en este estudio y con respecto a la presente discusión, la carne cocinada puede ser considerada como una insignificante fuente de compuestos α-dicarbonilos tales como el metilglioxal.

En relación a la formación de los productos finales de glicación avanzada (AGEs), se obtuvieron concentraciones similares de carboximetillisina (CML) en todas las muestras (alrededor de 4 mg CML/100 g de proteínas) independientemente del tipo de cocinado utilizado, de la adición o no de la FES y de la interacción de ambas variables. Similares resultados han sido encontrados en la bibliografía en diferentes tipos de carne cocinada (Hull, Woodside, Ames, & Cuskelly, 2012). Sin embargo, estos resultados no estuvieron en consonancia con las mayores concentraciones de CML obtenidos por otros

autores previamente en carne cocinada en salsa, la cual contenía este tipo de azúcares (Chao, Hsu, & Yin, 2009).

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8. Conclusiones

1. El aumento de las temperaturas y tiempos de cocinados provoca mayores pérdida por cocinado y una disminución en el color rojo de la carne. Por otro lado, mayores tiempos de cocinados provocan una mayor desnaturalización del colágeno y en consecuencia, disminuye la dureza de la carne. Esta desnaturalización del colágeno aparecerá en forma de pequeños gránulos a temperaturas de cocinado de 60 ºC, y formará un gel a temperaturas de 80 ºC. Todas combinaciones de temperatura-tiempo estudiadas causaron una intensa disminución en la población microbiana de los lomos de cordero cocinados al vacío, incluida la combinación más suave (60 ºC-6h).
2. Mayores temperaturas de cocinado dieron lugar a una gran disminución en la cantidad de compuestos secundarios procedentes de la oxidación lipídica, lo que podría explicar a su vez el incremento en la formación de aldehídos de Strecker. Mayores tiempos de cocinados causaron un incremento en la formación de carbonilos proteicos diferentes a AAS y GGS.
3. Los compuestos volátiles procedentes de la oxidación lipídica fueron mayoritarios cuando se cocinaron los lomos de corderos utilizando las combinaciones de temperatura-tiempo más suaves (60 º C durante 6 y 24 h, 80 ºC durante 6 h), mientras que combinaciones de cocinado más intensas (80 ºC durante 24 h) estimulan la formación de compuestos volátiles procedentes de reacciones en las que participan aminoácidos, lo que resultaría en la formación del deseable aroma a carne cocinada en las muestras.
4. El almacenamiento en refrigeración de los lomos de cordero cocinados al vacío no provocó grandes cambios en las características de los mismos, permitiendo mantener la calidad de los lomos durante un mes.
5. La inyección de fosfatos parece mejorar las características sensoriales de textura tanto en lomos de cordero cocinados al vacío como al horno a través del incremento en la retención de agua de los mismos durante el cocinado sin detrimento alguno en el sabor o aroma.
6. Los lomos de corderos cocinados al vacío o al horno parecen ser una fuente menor de marcadores de la reacción de Maillard. Sin embargo, la adición de azúcares reductores y otros compuestos precursores del sabor,

parece ser una estrategia adecuada y fácil para potenciar el color pardo y el sabor en lomos de corderos, que además reduce las pérdidas por cocinado y da lugar a una carne con una textura más firme.

1. Cooking at increasing temperatures and times led to higher water loss and a decrease of meat redness. On the other hand, higher cooking times led to a higher collagen solubilization and, consequently, the hardness of meat decreased. This collagen solubilization appeared as a connective tissue granulation at 60 ºC and as a protein gel at 80 ºC. The studied combination of time and temperature treatments for *sous-vide* cooking of lamb loins produced a dramatic reduction of the microbial population, even with the less intense treatment (60 °C–6 h).
2. Cooking at increasing temperatures led to a greater fall of secondary compounds from lipid oxidation that it could in turn explain the higher formation of Strecker aldehydes at such temperatures. Longer cooking times caused a greater amount of protein carbonyls different to AAS and GGS.
3. Volatile compounds arising from lipid oxidation are the major one when cooking at milder cooking conditions (60 º C for 6 and 24 h, 80 ºC for 6 h), while more intense time and temperature combination (80 ºC for 24 h) promoted the formation of volatile compounds from Strecker degradations. It could be indicated that *sous-vide* cooking at moderately high temperatures for long times stimulates the formation of volatile compounds from amino acid-involved reactions that would result in the formation of desirable meaty flavor and roast notes in samples.
4. The storage under refrigeration did not caused great changes on the characteristics of the lamb loins cooked under vacuum, which allows them to keep its quality for at least a month.
5. Injecting phosphate brines appears as an interesting approach for enhancing textural sensory features of either *sous-vide* or oven roasting whole muscle lamb cuts through an increase in their water retention during cooking without detrimental effects on flavor or taste.
6. The addition of reducing sugars to lamb meat previous to cooking could be an interesting approach for improving surface browning, especially in *sous-vide* cooked meats, in which the development of such darker surface is milder or inexistent. However, in some types of meat, in which keeping a reddish color note of the cutting surface is desirable, such as roasted beef

type preparations, the increasing browning of the core could perhaps be a drawback.

9. Anexo

TEXTURA Y ESTRUCTURA DE LA CARNE DE CORDERO COCINADA AL VACÍO

Roldán, M.; Jiménez, E.; Calvarro, J. y Ruíz, J.

COMUNICACIÓN ORAL

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TEXTURA Y ESTRUCTURA DE LA CARNE DE CORDERO COCINADA AL VACÍO

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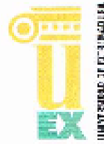
El cocinado al vacío de los alimentos aplicando tiempos prolongados de cocinado y temperaturas moderadas, es una técnica cada vez más empleada en la alta cocina y es especialmente importante en el caso de la carne. Sin embargo, hay pocos estudios que expliquen el efecto de dicha técnica culinaria sobre la textura instrumental o sobre la microestructura del músculo. Para este trabajo se partió de 50 lomos de cordero que fueron cocinados a diferentes combinaciones de temperatura (60, 70 y 80ºC) y tiempo (6, 12 y 24 horas). Se observó que a medida que aumenta la temperatura de cocinado, aumentan las mermas (*P=0,000*) y disminuye la humedad *(P=0,0197*) de la carne de cordero. Además, se ven afectados gran parte de los parámetros de textura instrumental, así, la dureza y la fuerza al corte disminuyen a medida que lo hace el tiempo de cocinado (*P=0,0079* y *P=0,0300* respectivamente). La desnaturalización del colágeno se observa a partir de 60ºC, cuando los tiempos de cocinado son prolongados (12 y 24 horas). La formación de un gel proteico de gelatina aparece a partir de 70ºC dando lugar a un aumento en los diámetros de las fibras. A partir de 60ºC y tiempos de cocinado prolongados comienza la solubilización del colágeno y se consiguen texturas más tiernas.



II Jornadas Veterinarias de Estudiantes y I de Ciencias de la Salud

24-26 Marzo 2011

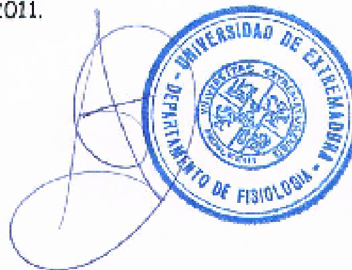
Organiza: Área de Toxicología y Fisiología, Facultad de Veterinaria
Universidad de Extremadura

Dr. Antonio González Mateos, Director del Comité Organizador de las "II Jornadas Veterinarias de Estudiantes y I de Ciencias de la Salud" celebradas en Cáceres durante los días 24, 25 y 26 de marzo de 2011

CERTIFICA:

Que la comunicación presentada en formato oral titulada **"TEXTURA Y ESTRUCTURA DE LA CARNE DE CORDERO COCINADA AL VACÍO"** de la que son autores *M. Roldán Romero*, *E. Jiménez Martín*, *J. Calvarro Labrador* y *J. Ruiz Carrascal* ha sido premiada por su calidad científica en dichas jornadas.

Y para que conste a los efectos oportunos, firmo el presente certificado en Cáceres, a 26 de marzo de 2011.



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EFECTO DEL TIEMPO Y TEMPERATURA DE COCINADO SOBRE LA TEXTURA Y ESTRUCTURA DE LA CARNE DE CORDERO COCINADA AL VACÍO

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INTRODUCCIÓN

El cocinado al vacío de los alimentos aplicando tiempos prolongados de cocinado y temperaturas moderadas es una técnica cada vez más empleada en la alta cocina y es especialmente importante en el caso de la carne, ya que permite la solubilización del colágeno y la consiguiente formación de gelatina, consiguiéndose texturas más tiernas y homogéneas. Los datos empíricos de los que se dispone muestran que este tipo de cocinado repercute positivamente sobre la terneza y la jugosidad de la carne. No obstante, apenas se dispone de información científica relativa a aspectos tales como el efecto sobre la textura instrumental o sobre la estructura del músculo.

Tabla 1. *Valores de mermas durante el cocinado, humedad y variables de textura instrumental en muestras de cordero cocinadas a diferentes combinaciones de tiempo temperatura.*

Temperatura (ºC)		**60**			**70**			**80**				
Tiempo (horas)	**6**	**12**	**24**	**6**	**12**	**24**	**6**	**12**	**24**	**P (temp)**	**P (tiempo)**	**P (Tª x t)**
Mermas (%)	20.77^{c}	24.72^{bc}	28.78^{abc}	26.94^{bc}	33.42^{ab}	33.01^{ab}	39.86^{a}	35.61^{ab}	39.41^{a}	0.0000	0.1039	0.2368
Humedad (%)	66.42	66.18	64.84	67.83	65.07	66.38	64.18	62.56	62.30	0.0197	0.3405	0.9088
Dureza (N/cm^3)	18.59^{abc}	16.74^{abc}	15.97^{bc}	16.83^{abc}	18.86^{ab}	14.37^{bc}	24.79^{a}	16.94^{abc}	10.65^{c}	0.8573	0.0003	0.0079
Fracturabilidad (N/cm^2)	0.09	0.09	0.09	0.10	0.09	0.09	0.09	0.10	0.10	0.2585	0.4774	0.4954
Adhesividad (N·s)	-0.064^{ab}	-0.035^{ab}	-0.031^{ab}	-0.038^{ab}	-0.026^{a}	-0.039^{ab}	-0.034^{ab}	-0.078^{b}	-0.053^{ab}	0.0776	0.8172	0.0195
Elasticidad (cm)	0.64^{ab}	0.69^{a}	0.60^{abc}	0.65^{ab}	0.58^{bc}	0.52^{cd}	0.59^{bc}	0.53^{cd}	0.47^{d}	0.0000	0.0000	0.0259
Cohesividad (N/mm^2)	0.48^{a}	0.44^{ab}	0.39^{bcd}	0.42^{abc}	0.38^{bcde}	0.32^{ef}	0.36^{cde}	0.32^{f}	0.25f	0.0000	0.0000	0.9832
Gomosidad (N/cm^2)	7.83^{ab}	8.98^{a}	5.98^{ab}	8.07^{ab}	7.09^{ab}	4.63^{ab}	9.14^{a}	5.48^{ab}	2.53^{b}	0.1790	0.0008	0.2754
Masticabilidad (N·s)	4.92^{ab}	6.36^{a}	4.06^{abc}	5.32^{ab}	4.09^{abc}	2.36^{bc}	5.46^{ab}	3.03^{abc}	1.19^{c}	0.0230	0.0006	0.1453
Fuerza al corte (N)	27.03^{ab}	25.27^{ab}	23.30^{ab}	32.74^{a}	35.01^{a}	16.26^{b}	29.36^{ab}	26.25^{ab}	23.98^{ab}	0,4850	0,0020	0,0300

MATERIAL Y METODOS

Se envasaron al vacío 50 lomos de cordero y se cocinaron en baños termostatizado a diferentes combinaciones de temperatura (60, 70 y 80ºC) y tiempo (6, 2 y 24h), realizándose lotes de 5 muestras por cada combinación de temperatura-tiempo. Tras ser cocinados, los lomos se sumergieron en recipientes con agua muy fría y se almacenaron a refrigeración. Al día siguiente se determinó la humedad de las muestras, las mermas durante el cocinado, el análisis del perfil de textura y la toma de muestra para microscopía. El perfil de textura se realizó con un texturómetro TA-XT2i, siguiendo el método previamente descrito por García-Segovia y col. (2007). Las muestras fueron observadas mediante la técnica de cryo-SEM con un microscopio modelo JEOL JSM-5410.

CONCLUSIONES

Temperaturas y tiempos prolongados de cocinado suponen mayores pérdidas de humedad y mayores mermas en la carne de cordero cocinada al vacío. Además, gran parte de los parámetros de textura instrumental de la carne de cordero cocinada al vacío se ven afectados por la temperatura y tiempo de cocinado. Por otro lado, la formación del característico granulado en el endomisio como consecuencia de la desnaturalización del colágeno, comienza a ocurrir por encima de 60ºC y a partir de 70ºC se forma un gel proteico de gelatina en el espacio endomisial.

OBJETIVOS

Analizar las posibles modificaciones de los atributos de textura y las posibles diferencias estructurales que se producen durante el cocinado a vacío de la carne de cordero pascual de raza Merina, concretamente el músculo *Longissimus dorsi*, utilizando tres combinaciones diferentes de temperatura-tiempo.

RESULTADOS Y DISCUSIÓN

En la Tabla 1 se observa que a medida que aumenta la temperatura de cocinado, aumentan las mermas y disminuye la humedad de la carne de cordero. Este hecho podría ser debido, por un lado, a la evaporación de agua retenida físicamente en la carne, y por otro lado, a la retracción de las proteínas miofibrilares y del colágeno por acción del calor, lo que supone una disminución en la capacidad de retención de agua de la carne. Además, la mayor parte de los parámetros de textura se vieron afectados, disminuyendo a medida que aumentaban la temperatura y tiempo de cocinado. La dureza y la fuerza al corte no se vieron afectadas por la temperatura, pero disminuyeron a medida que lo hizo el tiempo de cocinado. Los cambios que tuvieron lugar en la estructura de la carne de cordero cocinada al vacío durante el calentamiento de las muestras se observan en la Figura 1. A 60ºC y 80ºC se pueden diferenciar claramente unas células musculares de otras, mientras que a 70ºC, el boceto de las mismas es menos claro debido a la formación de un gel proteico por acción del calor. Además, se puede apreciar la formación de unos depósitos granulares en los espacios existentes entre las fibras musculares, debidos posiblemente a la desnaturalización por calor del colágeno endomisial, así como del sarcolema.

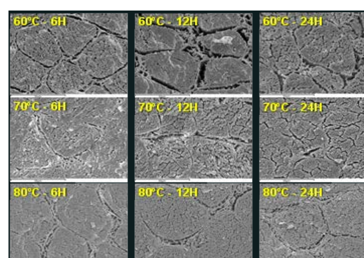

Figura1. *Cambios en la microestructura del músculo Longissimus dorsi cocinado a vacío utilizando tres combinaciones diferentes de temperatura-tiempo (magnificación 2000x).*

AGRADECIMIENTOS

Este trabajo ha sido financiado por el proyecto AGL2008-00890/ALI de la Junta de extremadura. Mar Roldán Romero agradece a la Junta de Extremadura la concesión de su beca predoctoral (PRE09057).

VI congreso nacional de Ciencia y tecnología de los alimentos.
Valencia, 8-10 de junio de 2011

PÓSTER

VI congreso nacional de Ciencia y tecnología de los alimentos, Valencia, España, 2011

EFECTOS DE LA ADICIÓN DE FOSFATOS SOBRE LAS CARACTERÍSTICAS SENSORIALES DE LA CARNE DE CORDERO COCINADA MEDIANTE DOS TRATAMEINTOS CULINARIOS

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INTRODUCCIÓN

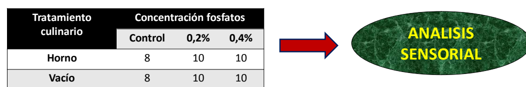
El asado en horno y el cocinado al vacío son probablemente los dos métodos de cocinado más utilizados en restaurantes y la industria del catering para las principales piezas de carne ovina. Durante el cocinado, el incremento de temperatura conlleva a una serie de cambios en la carne, siendo la pérdida de jugosidad uno de los factores claves que tiene mayor influencia en la calidad final de la carne cocinada (Jama, Muchenje, Chimonyo, Strydom, Dzama & Raats, 2008). La inyección de salmueras en la carne y el subsiguiente "tumbling" de la misma es un procedimiento ampliamente utilizado para minimizar las pérdidas durante la cocción. Concretamente, los fosfatos mejoran las características texturales de los productos cárnicos tales como la jugosidad y la terneza reduciendo las pérdidas durante el cocinado (Sheard & Tali, 2004) mediante el incremento de la capacidad de retención de agua en la carne, consecuencia de la extracción de proteínas musculares y del desplazamiento del punto isoeléctrico de las mismas (Bianchi, Petracci & Cavani, 2009).

OBJETIVOS

Determinar la influencia de diferentes niveles de fosfatos en la calidad sensorial de lomos de cordero cocinados mediante dos procedimientos culinarios diferentes: asado en el horno o cocinado al vacío.

MATERIAL Y METODOS

Se partió de 56 lomos de corderos y se llevó a cabo un diseño experimental 3x2 utilizando 3 soluciones de salmuera diferente y 2 tratamientos culinarios distintos. Se inyectaron los lomos con una solución de fosfatos hasta alcanzar un 110% de su peso inicial, se envasaron y se sometieron a un tratamiento de volteo ("tumbling"). Después, la mitad fueron envasados al vacío y cocinados en un baño termostatizado a 60ºC durante 12h, y el resto fue cocinado en un horno a 180ºC hasta alcanzar una temperatura interna de 73ºC. Tras el cocinado, las muestras se guardaron en refrigeración durante la noche llevándose a cabo el análisis sensorial al día siguiente.



Tratamiento culinario	Concentración fosfatos		
	Control	0,2%	0,4%
Horno	8	10	10
Vacío	8	10	10

Figura 1. Esquema de la distribución de las muestras en función del nivel de fosfatos inyectado y el tipo de tratamiento culinario recibido.

Para el análisis sensorial se empleó un panel de catas formado por 12 panelistas entrenados. Los atributos relacionados con la apariencia, textura en boca y flavor, se evaluaron mediante un análisis cuantitativo descriptivo (ACD) utilizando una escala descriptiva de 10 puntos, siendo 0=poco y 10=mucho. El efecto de la adición de fosfatos y del tratamiento culinario aplicado sobre los diferentes atributos sensoriales, fueron analizados mediante un ANOVA utilizando el programa SPSS v15.0, considerando como factor el nivel de fosfatos inyectado en las muestras. Cuando el factor mostró una influencia significativa ($P<0.05$), se llevó a cabo un test de Tukey para comparar las medias utilizándose un nivel de significancia del 5%.

RESULTADOS Y DISCUSIÓN

En la Figura 2 se muestra el perfil sensorial de lomos de cordero inyectados con fosfatos y cocinados mediante los dos tratamientos culinarios establecidos. En la tabla 1 se incluye la significancia de cada uno de los parámetros estudiados (adición de fosfatos y cocinado) sobre los diferentes atributos sensoriales.

Los fosfatos no afectaron a los parámetros relacionados con el sabor o el aroma, pero provocaron un aumento de la terneza y la jugosidad de la carne. Esta disminución de la dureza de las muestras inyectadas con fosfatos y cocinadas en horno es probablemente debido a que los fosfatos provocaron la disociación del complejo actomiosina originando la relajación de la matriz proteica y permitiendo que la carne retuviese más agua (Erdogdu et al., 2007). El efecto de los fosfatos no fue tan claro con respecto a la terneza de las muestras cocinadas al vacío probablemente debido a los prolongados tiempos de cocinado, que provocaron una importante desnaturalización del colágeno en todas las muestras enmascarando así el efecto de los fosfatos sobre las mismas.

Sin embargo, los panelistas sí encontraron diferencias significativas en relación a la jugosidad de las muestras cocinadas al vacío, otorgando una mayor puntuación a aquellas muestras inyectadas con fosfatos. Además, las muestras inyectadas con fosfatos presentaron mayor brillo pero menos coloración rojiza.

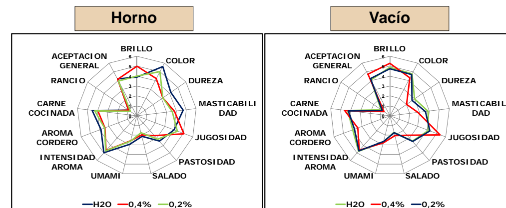


Figura 2. Perfil sensorial de lomos de cordero inyectado con fosfatos y cocinados al horno y al vacío.

ATRIBUTOS	Brillo	Color	Dureza	Masticabilidad	Jgosidad	Pastosidad	Salado	Umami	Intensidad aroma	Aroma cordero	Aroma carne cocinada	Rancidez	Aceptabilidad general
P cocinado	0,003	0,066	<0,001	0,01	0,508	0,731	0,658	0,931	0,675	0,389	0,683	0,829	0,29
P fosfoatos	0,009	0,001	0,001	0,003	<0,001	0,006	0,516	0,907	0,783	0,524	0,909	0,427	0,383
SEM	0.11	0.09	0.09	0.11	0.11	0.12	0.11	0.15	0.11	0.12	0.13	0.06	0.12

Tabla 1. Significancia de la adición de fosfatos y posterior cocinado sobre cada unos de los atributos sensoriales estudiados.

CONCLUSIONES

Los resultados de este estudio muestran que la adición de fosfatos en lomos de cordero cocinados al vacío o al horno, tiene efectos positivos sobre el brillo, dureza, jugosidad y la masticabilidad de este tipo de carne sin detrimento de su sabor o aroma. Sin embargo, en este estudio se observa que la adición de fosfatos tiene también un efecto negativo sobre el color de la carne, haciéndola más susceptible a la pérdida del mismo.

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AGRADECIMIENTOS

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VII congreso español de ingeniería de los alimentos, Ciudad Real, España,

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3rd MS Food Day
Divisione di Spettrometria di Massa
FONDAZIONE EDMUND MACH
October 9-11 , 2013 - Trento (Italy)

Effect of the addition of a flavor enhancer solution on the aldehyde content from lamb loin samples cooked by two different culinary treatments

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Este trabajo ha sido financiado por el proyecto **AGL2008-00890/ALI de la Junta de Extremadura**. Mar Roldán Romero agradece a la Junta de Extremadura la concesión de su beca predoctoral (**PRE09057**).

COMUNICACIÓN ORAL

3rd MS Food Day, Trento, Italy, 2013

EFFECT OF THE ADDITION OF A FLAVOR ENHANCER SOLUTION ON THE ALDEHYDE CONTENT FROM LAMB LOIN SAMPLES COOKED BY TWO DIFFERENT CULINARY TREATMENTS

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20 lamb loins of *Longissimus dorsi* muscles were injected with a flavor enhancer solution (FES) composed of Glucose: 1.376 M; Ribose: 0.060 M; Cysteine: 0.075 M and Thiamine: 0.034 M, or with distilled water for control samples. Samples were vacuum-packaged and sous-vide cooked in a thermostatized water bath at 60ºC for 12h, or in an oven at 180ºC until reaching an internal temperature of 73ºC. Samples added with the FES showed lower aldehyde content than control samples. In turn, higher aldehyde content was observed in samples cooked in the oven than in samples cooked under vacuum.

TECHNISCHE UNIVERSITÄT DRESDEN

Furosine and N^{ε}-Carboxymethyllysine in Cooked Lamb Meat

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Introduction

Maillard reaction products (MRPs) are useful parameters to control food processing. Their physiological role is discussed controversially, as the relevance of dietary MRPs for the total "MRP-load" in vivo and related pathophysiological consequences is not clear.

Background

In the present investigation the content of MRPs in lamb meat samples was studied. The lamb meat was either cooked in an oven traditionally (180 °C, 1,2 hours) or sous-vide (60 °C, 12 hours, vacuum). Vacuum cooking results in tender texture [1], but also in low flavor. With the objective to generate the characteristic flavor and color of roasted meat, glucose was added.

The progress in the Maillard reaction between sugars and amino acids can be monitored by the formation of specific amino acid derivatives. The formation of Amadori rearrangement products (ARPs) of lysine was analyzed after acidic hydrolysis as furosine. ARPs can undergo further degradation reactions to CML (N^{ε}-carboxymethyllysine). CML represents a stable end product of the advanced glycation reaction (AGE).

Conclusion

Very small amounts of ARPs and CML are formed during cooking of native lamb meat. An addition of sugar (glucose) resulted in slightly increased contents of furosine in all samples and increased levels in samples with higher cooking temperature.

For CML, no increase even after addition of glucose could be found. Cooked meat, therefore, is only a minor dietary source of Maillard reaction products.

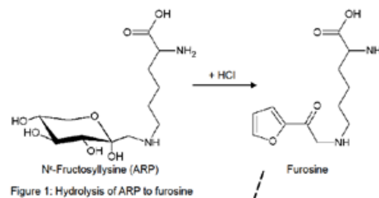
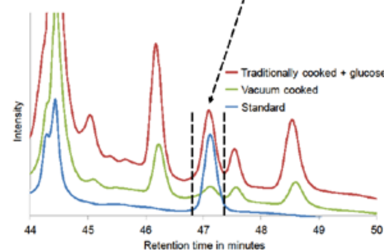


Figure 1: Hydrolysis of ARP to furosine



Furosine

Sample preparation

Hydrolysis of homogenized meat in hydrochloric acid

Analysis

Amino acid analyzer [2]

Results

Concentration of furosine was the same with different cooking conditions, but higher after addition of glucose.

→ independent of cooking conditions
→ dependent of glucose addition

Figure 2: Chromatogram of two samples (traditionally cooked with addition of glucose or vacuum cooked without glucose) and a furosine standard analyzed by amino acid analyzer

Table 1: Concentration of furosine and CML in meat samples

	vacuum cooked	vacuum cooked + glucose	traditionally cooked	traditionally cooked + glucose
Furosine [mg/100 g protein]	34 ± 13	87 ± 33	29 ± 4	122 ± 42
CML [mg/100 g protein]	4.0 ± 0.8	4.1 ± 0.8	4.1 ± 0.9	4.8 ± 0.7

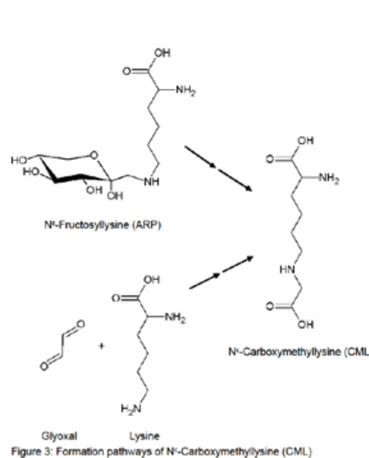


Figure 3: Formation pathways of N^{ε}-Carboxymethyllysine (CML)

N^{ε}-Carboxymethyl-lysine (CML)

Sample preparation

Reduction of ARPs with sodium borohydride in homogenized meat,
Hydrolysis in hydrochloric acid,
Solid phase extraction (SPE)

Analysis

RP-HPLC-ESI-MS/MS [3]
(MRM - multiple reaction monitoring)

Results

Concentration of CML was the same in all lamb meat samples

→ independent of cooking conditions
→ independent of glucose addition

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[1] M. Roldán, Meat Science, 2013, 93, 572-578; [2] T. Henle, Int. Dairy J. 1991, 1, 125-135; [3] J. Hegele, Ann N. Y. Acad. Sci. 2008, 1126, 300-306; [4] G.L.J. Hull, Food Chemistry 2012, 131, 170-174

PÓSTER

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TECHNISCHE UNIVERSITÄT DRESDEN

Dicarbonyl Compounds in Meat - Effect of Cooking Processes

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Background

In the course of the **Maillard reaction**, various sugar- and protein-derived products are formed. Among them are 1,2-dicarbonyl compounds, which are **reactive intermediates** in a complex reaction cascade. 1,2-Dicarbonyl compounds, in particular **3-deoxyglucosone** and **methylglyoxal**, occur in considerable amounts in foods[1] and are precursors of **aroma-active compounds**[2,3] and advanced glycation endproducts.

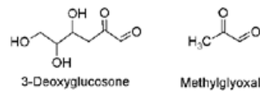


Fig. 1: The 1,2-dicarbonyl compounds 3-deoxyglucosone and methylglyoxal.

Motivation

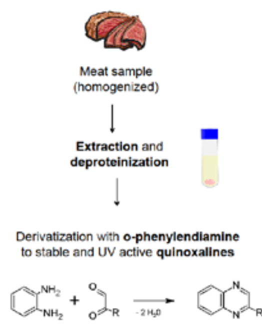
Quantitative data about the occurrence of these sugar degradation products in prepared meat are scarce.

The aim of our investigations, therefore, was to assess the **content of 1,2-dicarbonyl compounds** in **meat** samples, especially considering the **effect of cooking process**. A new meat cooking technique (**low temperature** under **vacuum** to get a more tender meat) was investigated.

In order to generate the **characteristic taste** and **color of roasted meat**, monosaccharides like **glucose** or **ribose** are added before vacuum cooking to enhance non-enzymatic browning, known as the Maillard reaction.

Methodological Aspects: Determination of 1,2-dicarbonyl compounds in meat

Sample preparation[1]



HPLC analysis[4]

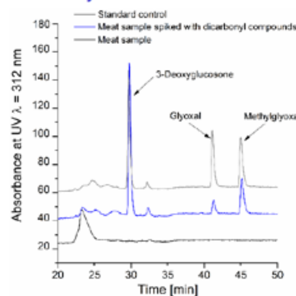


Fig. 2: Chromatograms of a standard dicarbonyl mixture (containing 3-DG, glyoxal and methylglyoxal), a spiked meat sample and a meat sample after sample preparation and derivatization with o-phenylendiamine acquired by RP-HPLC with UV detection (312 nm).

Results of quantitative analysis

- Successful application of sample preparation and analytical method to the matrix meat.
- Concentration of 1,2-dicarbonyl compounds in all meat samples was **lower than 1 mg/kg**.
- **No dependency of cooking process** (low *versus* high temperature) and added sugars.

Evaluation of the results

Due to their **reactivity**, we assume that generated dicarbonyl compounds have **readily reacted** with **arginine** and **lysine** side chains of proteins.

Another possible reactant is the meat **specific dipeptide carnosine** (β-alanyl-L-histidine) which is discussed to form adducts with reactive aldehydes[5].

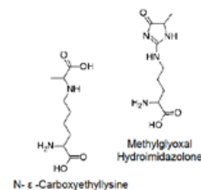


Fig. 3: Structures of lysine and arginine modidications of methylglyoxal: N-ε-carboxyethyllysine and metyhlglyoxal hydroimidazolone.

Discussion

It seems more possible to detect **protein-bound glycation end-products** than dicarbonyl compounds in a **protein-rich matrix** like meat (see poster 778, Jürgen Löbner).

1,2-Dicarbonyl compounds are **no suitable indicator** for predicting/evaluate the **extent of flavor and color** generation in prepared meat samples.

With respect to current discussion about possible antinutrive properties of glycation compounds[6], **cooked meat** is a **negligible source** of 1,2-dicarbonyl compounds.

References

[1] J. Degen, J Agr Food Chem **2012**, 60:7071-9; [2] A. Bravo, J Agr Food Chem **2008**, 56: 4134-44; [3] Y. Wang, Chem Soc Rev **2012**, 41:4140-9 [4] M. Hellwig, J Agr Food Chem **2010**, 58:10752-60; [5] A. Hipkiss, Mech Ageing Dev **2005**, 126:1034-9; [6] H. Vlassara, Curr Diab Rep **2007**, 7:235-41.

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