Investigating the effect of meat products ingredients and reaction mechanisms for enhancing savory and toasted aromas in dry cured meat products

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Que D. Lei Li, ha realizado bajo su dirección el trabajo que lleva por título "Investigating the effect of meat products ingredients and reaction mechanisms for enhancing savory and toasted aromas in dry cured meat products", que presenta para optar al grado de Doctor en el Programa de Ciencias de la Alimentación de la Universitat de València. Asimismo, certifican haber dirigido y supervisado tanto los distintos aspectos del trabajo como su redacción

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RESUMEN AMPLIO

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En el área mediterránea existen una gran variedad de productos cárnicos curados madurados. La formulación y el proceso de fabricación de estos productos cárnicos varía enormemente según la zona geográfica y las tradiciones gastronómicas locales. Las características organolépticas de la carne y los productos cárnicos influyen en el criterio de elección y en la intención de compra del consumidor. Se cree que el aroma y sabor son los parámetros sensoriales que producen un mayor impacto en la aceptación de los productos cárnicos por parte de los consumidores. El desarrollo del aroma en los productos curados madurados se basa en reacciones químicas complejas (reacción de Maillard, degradación de Strecker y autooxidación de lípidos), así como en reacciones bioquímicas producidas por la microbiota presente (fermentación de carbohidratos, degradación de aminoácidos, β-oxidación de lípidos y actividad esterasa). Estas reacciones ocurren principalmente durante la etapa de maduración y generan una gran cantidad de compuestos volátiles, que contribuyen al sabor característico de los productos cárnicos curados madurados. Los compuestos volátiles derivados de las reacciones de degradación de aminoácidos se consideran la principal fuente de compuestos responsables del aroma a curado-madurado, como el 3-metilbutanal que deriva de la Leu. Además, los compuestos volátiles que contienen nitrógeno y azufre, derivados de los aminoácidos nitrogenados y azufrados, presentan características olfativas específicas y umbrales aromáticos bajos en los productos cárnicos curados madurados, lo que indica un papel importante en el aroma de los productos cárnicos.

Dado que ningún compuesto específico es responsable del aroma distintivo de la carne curada, la presencia de compuestos que producen aromas cárnicos (sabrosos) y tostados en los productos cárnicos puede resultar en un aroma equilibrado, que da como resultado el aroma a curado característico. Actualmente, se sabe que en la carne cocinada las notas olfativas tostadas y cárnicas pueden verse influenciadas por las reacciones de degradación y transformación de los aminoácidos nitrogenados (Pro y Orn), aminoácidos que contienen átomos de azufre como cisteína, cistina y metionina, así como de la vitamina tiamina. Si

bien la contribución de estos compuestos a los aromas cárnicos (sabrosos) en la carne cocinada es conocida, existe una falta de conocimiento sobre el mecanismo de formación de los compuestos aromáticos cárnicos y tostados en los productos cárnicos curados madurados.

Entre los productos cárnicos curados madurados que se elaboran en el área mediterránea podemos encontrar los sometidos a procesos de fermentación como los embutidos fermentados, y los curados-madurados como los lomos y jamones curados. Los mecanismos involucrados en la generación de compuestos aromáticos que producen notas cárnicas y tostadas pueden ser diferentes, dependiendo de las reacciones químicas y bioquímicas existentes en el proceso. Por tanto, como objetivo principal se planteó el estudio de los mecanismos implicados en la generación de compuestos azufrados y nitrogenados en productos cárnicos madurados (embutidos fermentados y lomos curados-madurados), ya que pueden ayudar a comprender la presencia de aromas cárnicos y tostados en dichos productos. En consecuencia, en el proyecto de Tesis Doctoral tanto los embutidos fermentados como los lomos curados-madurados se utilizaron como representantes de productos cárnicos (Capítulos 1, 2 y 3). Además, se emplearon dos sistemas modelo diferentes para estudiar el papel de la reacción de Maillard en la generación de aromas (Capítulo 4) y el efecto de la oxidación de la grasa y de la presencia de la levadura *Debaryomyces hansenii* sobre la biotransformación de aminoácidos (Capítulo 5).

Los objetivos de esta tesis se lograron mediante la utilización de diferentes metodologías. El contenido de precursores aromáticos presentes como los aminoácidos Pro, Orn y Met se analizó mediante cromatografía de gases-detector de ionización de llama (GC-FID) utilizando un kit comercial (Phenomenex, Torrance, CA, EE. UU.). El análisis del precursor tiamina, se realizó por Cromatografía Líquida de Alta Resolución (HPLC) con un detector de fluorescencia.

El análisis microbiológico se realizó utilizando el método de recuento en placa en medios generales y selectivos, para estimar la presencia de bacterias mesófilas totales

(TMB) en agar estándar de recuento (PCA), bacterias ácido lácticas (LAB) en agar Man Rogosa Sharpe (MRS), cocos Gram positivos (GC+) en agar manitol salino (MSA), enterobacterias (EB) en agar violeta rojo bilis glucosa (VRBG) y levaduras y mohos (YM) en agar rosa de Bengala con cloranfenicol (RB).

La cromatografía de gases-espectrometría de masas (GC-MS) con microextracción en fase sólida (SPME) ha demostrado ser una herramienta eficaz para el análisis de compuestos volátiles, considerando que la fibra de extracción debe seleccionarse en función del objetivo del estudio. En este trabajo se seleccionó el recubrimiento de fibra con carboxeno/polidimetilsiloxano (CAR/PDMS), que se ha visto produce los mejores resultados para el análisis de componentes volátiles de bajo peso molecular en productos curados madurados. Aplicando esta metodología se obtuvo la abundancia relativa de los compuestos volátiles presentes en el espacio de cabeza de los productos cárnicos estudiados y en los modelos in vitro (Capítulos 1, 2 y 4). Además, se realizó la cuantificación de los compuestos volátiles (ng/carne o sistemas modelo) mediante SPME-GC-MS empleando curvas de calibración en la matriz cárnica (Capítulo 3) y métodos de calibración externa (Capítulo 5).

El aroma producido por los compuestos volátiles extraídos mediante la técnica de SPME se analizó por GC-olfatometría (GC-O) empleando el método de frecuencia de detección (DF), que mide la intensidad de un aroma según el número de evaluadores que lo detectan. Esta metodología ha demostrado ser efectiva en la detección de aromas en productos cárnicos. Además, esta metodología también permite la selección de compuestos aromáticos clave entre los cientos de compuestos volátiles identificados en las muestras. Asimismo, la cuantificación de los compuestos volátiles en los productos cárnicos utilizando métodos de calibración en la matriz cárnica (Capítulo 3) permitió el cálculo de los valores de actividad aromática (OAV) en términos de la relación entre la concentración del aroma y su umbral aromático. En general, los compuestos con OAV superiores a 1 producen un impacto en el aroma global de los alimentos.

En cuanto a las características sensoriales de los productos cárnicos elaborados, se

seleccionó la técnica Perfil de Libre Elección (FCP). Se eligió esta metodología porque no requiere el entrenamiento de los panelistas, y se realiza en sesiones individualizadas. Además, el beneficio fundamental de FCP es que no requiere la definición de un vocabulario común, complicado y científico, y puede usarse para analizar las diferencias sensoriales entre productos cárnicos.

Con el objetivo de investigar los mecanismos implicados en la degradación química y biotransformación de los aminoácidos (Pro y Orn) y de la tiamina durante el procesado de productos cárnicos curados madurados, se fabricaron lomos curados (Capítulo 1) y embutidos fermentados madurados (Capítulo 2). Ambos productos cárnicos fueron suplementados con precursores de aroma (Pro, Orn y tiamina) y sometidos a un proceso de maduración. Los lomos se analizaron al final del proceso (68 d), mientras que los embutidos se analizaron a diferentes etapas del proceso (8, 34 y 62 d). Además, se emplearon dos sistemas modelo diferentes para estudiar el papel de las reacciones químicas (Capítulo 4) y bioquímicas en la generación de aromas (Capítulo 5).

Los principales resultados obtenidos en cada capítulo son los siguientes:

Capítulo 1 "Aroma enhancement in dry cured loins by the addition of nitrogen and sulfur precursors": En el perfil aromático del lomo curado obtenido mediante SPME resaltó la presencia principalmente de los compuestos 3-metilbutanal, metional, 3-metilbutanoato de etilo, ácido 3-metilbutanoico, 1-octen-3-ol, 2-acetil-1-pirrolina (2AP) y 2-acetilpirrol, que contribuyen con notas aromáticas a moho, patatas cocidas, afrutadas, queso, champiñón, tostadas y cárnicas. La suplementación de los lomos con los aminoácidos Pro y Orn modificó el perfil aromático de los lomos produciendo aromas tostados, mientras que el efecto de la suplementación con tiamina puso de manifiesto la presencia de compuestos derivados de azufre (metional y 2-metil-3-(metiltio)furano (MMTF)), que contribuyen con notas aromáticas de carne curada.

Capítulo 2 "Understanding the impact of nitrogen and sulfur precursors on the aroma of dry fermented sausages": La adición de precursores (Pro, Orn y tiamina)

impactó el aroma, aunque tuvo poco efecto sobre los parámetros microbiológicos y/o químicos de los embutidos. Entre los compuestos aromáticos detectados, únicamente el 2-metil-3-(metiltio)furano (MMTF) verificó el efecto de la suplementación con tiamina y el impacto que produce en el aroma cárnico y con notas a curado detectados mediante el análisis sensorial del perfil de libre elección. En cambio, no se pudo atribuir un efecto claro sobre el aroma (compuestos volátiles) de los embutidos cuando se emplearon Pro y Orn como precursores de aromas.

Capítulo 3 "A comparative study of savory and toasted aromas in dry cured loins versus dry fermented sausages": La concentración de los compuestos volátiles responsables de los aromas cárnicos y tostados se analizó GC-MS, obteniendo curvas de calibrado específicas para cada matriz cárnica. En concreto, se identificaron once compuestos volátiles, nueve en lomos y ocho en embutidos que producían dichas notas aromáticas. En el aroma de lomo curado resaltó la presencia de metional y 2-acetil-1-pirrolina (2AP), que contribuyeron con notas de patata cocida y tostadas; mientras que el aroma del embutido fermentado se vio afectado por la presencia de 2-metil-3-furantiol (MFT), metil 2-metil-3-furil disulfuro (MMFDS), que produjeron aromas a grasa y cárnicos, así como por el compuesto metional. Los resultados pusieron de manifiesto que la contribución del cultivo iniciador empleado en la fabricación parece tener un efecto mayor que la adición de los precursores (Pro, Orn y tiamina) en el desarrollo del aroma del embutido. En cambio, en los lomos curados si se observó un efecto de la adición de precursores. La adición de tiamina en los lomos produjo una mayor concentración de 2-metil-3-(metiltio)furano (MMTF), y de los compuestos 2-acetil-1-pirrolina (2AP) y el 2-acetilpirrol en los lomos suplementados con Pro.

Capítulo 4 "The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions": En este capítulo se evaluó el perfil de compuestos volátiles en sistemas modelo que simulaban las condiciones de curado y maduración en cuanto a parámetros físicos y contenido en aminoácidos libres. Diecisiete compuestos volátiles se identificaron y cuantificaron en los sistemas modelo. Se

detectó una producción significativa de compuestos volátiles de cadena ramificada, azufrados, furanos, pirazinas y compuestos volátiles heterocíclicos. En los sistemas modelo que simulaban la etapa de secado, la temperatura fue el factor principal que afectó la producción de volátiles, seguida de la concentración de aminoácidos y la actividad de agua (a_w). Esta investigación demostró que las condiciones de curado-maduración en donde se emplean temperaturas suaves son suficientes para producir compuestos volátiles a través de la reacción de Maillard y de la presencia de aminoácidos libres. Además, en estas condiciones, la actividad de agua juega un papel importante en la formación de dichos compuestos aromáticos.

Capítulo 5 "Biotransformation of amino acids into aroma compounds in meat model systems affected by fat replacement and yeast inoculation": En este capítulo se determinó el efecto de la oxidación de la grasa y de la presencia de la levadura *Debaryomyces hansenii* sobre la biotransformación de aminoácidos en un sistema modelo sometido a un proceso de incubación a temperatura controlada. El crecimiento de la levadura en el modelo se confirmó hasta los 28 d, aunque el perfil de volátiles cambió hasta los 39 d. Se cuantificaron cuarenta y tres compuestos volátiles y se calcularon sus valores de actividad aromática (OAV). La diferencia en cuanto al perfil de volátiles se relacionó con el tipo de grasa y la presencia de la levadura en el sistema modelo. La producción de aldehídos de cadena ramificada, benzaldehído y metional se vio favorecida por la alta concentración de compuestos carbonilos presentes en la manteca de cerdo al inicio de la incubación. La acción antioxidante producida por *D. hansenii* se relacionó con el retraso en la formación de hexanal en los modelos que contenían grasa de coco, y de heptanal, octanal y nonanal en los que contenían manteca de cerdo. El perfil aromático de los modelos con grasa de coco se vió influenciado por la presencia de hexanal, compuestos ácidos y sus respectivos ésteres, mientras que en los modelos con manteca de cerdo, el aroma se vio afectado por metional (mohoso, patata) y 3-metilbutanal (verde, cacao). La inoculación de levadura potenció la presencia del ácido 3-metilbutanoico (queso) y alcohol feniletílico (floral). En conclusión, el tipo de grasa y la levadura produjeron un efecto

diferencial en el aroma de los modelos.

La relación entre los principales resultados obtenidos en los cinco capítulos de esta Tesis Doctoral puso de manifiesto lo siguiente:

El perfil aromático de los lomos curados y embutidos fermentados obtenidos por olfatometria (GC-FID-O), utilizando el método de frecuencia de detección (Capítulo 1 y 2), permitió la comparación de los compuestos aroma activos en ambos productos cárnicos, ya que se empleó la misma técnica de extracción. El perfil aromático de los productos analizados fue diferente, aunque algunos compuestos están presentes en ambos. El objetivo de este estudio fue dilucidar entre todos los compuestos detectados, la presencia de aromas cárnicos y tostados. En este sentido, los compuestos detectados por olfatometria en los lomos fueron aromas cárnicos (methional (patata cocida), MMTF (cárnico) y metionol (patata, cárnico) y olores tostados 2AP (maíz tostado, tostado), tetrametilpirazina (tostado), 2-acetilpirrol (maíz tostado, nueces, cárnico)) y 2 compuestos desconocidos que producen notas tostadas. En embutidos, los aromas detectados fueron aromas cárnicos (metanotiol (azufrado, verdura cocida) y metional (patata cocida)) y aromas tostados (2,6-dimetilpirazina (tostado) y cinco notas tostadas y cárnicas producidas por compuestos desconocidos). Los aromas tostados desconocidos en lomos y embutidos requieren un examen cuidadoso utilizando técnicas analíticas avanzadas. Además, estos resultados confirman el bajo umbral aromáticos de los aromas tostados y cárnicos en los dos productos cárnicos.

En este estudio, el análisis de los aromas cárnicos y tostados se vio afectado por varios factores como la técnica de extracción SPME, su baja concentración, y la composición de la matriz, que pueden afectar a la concentración de compuestos en el espacio de cabeza, etc. La abundancia de compuestos azufrados y nitrogenados, principales responsables de los olores cárnicos y tostados, se midió en el espacio de cabeza de los embutidos y lomos curados (Capítulos 1 y 2). En algunos casos, se seleccionó un ion específico (m/z) para cuantificar el compuesto debido a la coelución con otros compuestos, y los resultados se

expresaron como unidades de abundancia. No todos los compuestos se detectaron en el espacio de cabeza de ambos productos y, además, su abundancia fue diferente entre productos. Por ejemplo, la abundancia de metanotiol y 2,6-dimetilpirazina fue significativamente mayor en embutidos que en los lomos. Los compuestos 2AP y 2-acetilpirrol se detectaron, por primera vez, en el espacio de cabeza de los lomos curados. Sin embargo, la abundancia de estos dos compuestos no presentó diferencias significativas entre los diferentes lomos, por tanto, no se puedo determinar el efecto de la adición de precursores. En ambos productos, lomos y embutidos, el compuesto MMTF se encontró en mayor abundancia cuando se agregó tiamina como precursor aromático, lo que confirmó el origen del MMTF a partir de la degradación de la tiamina.

Hay que destacar que la abundancia de los compuestos volátiles en el espacio de cabeza de los dos productos analizados no proporcionó una cuantificación precisa, la cual se puede ver afectada enormemente por la composición de la matriz. Sin embargo, esta técnica de medición permitió determinar el efecto de la adición del precursor. Además, la imposibilidad de identificar algunos de los aromas tostados y cárnicos detectados en los productos (Capítulo 1 y Capítulo 2) orientó este estudio al desarrollo de un método de cuantificación preciso, utilizando SPME-GC-MS y el modo de monitorización selectiva de iones (SIM) (Capítulo 3). La cuantificación de los compuestos se expresó como ng de compuestos volátiles/g de productos cárnicos en base seca (Capítulo 3). Para determinar el efecto de la composición de la matriz, la cuantificación se realizó mediante la obtención de curvas de calibración en cada matriz (lomo fresco y masa fresca de embutido). El rendimiento de la extracción mediante la fibra SPME de los compuestos aromáticos cárnicos y tostados fue muy bajo debido a la baja cantidad de estos compuestos presentes en el espacio de cabeza de lomos y embutidos. El contenido de algunos compuestos como el metional se encontró a niveles de ppb a ppm. Este proceso de cuantificación optimizado permitió la cuantificación de DMDS y DMTS en lomos y MFT, DMS, DMTS y MMFDS en embutidos. La mayor concentración de MMTF en lomos se encontró cuando se añadió tiamina, confirmando el origen del compuesto y el efecto de la suplementación con tiamina.

Además, el mayor contenido de 2AP y 2-acetilpirrol en los lomos se encontró después de que la adición de Pro confirmara su efecto sobre la producción de compuestos volátiles tostados, como se indica en el Capítulo 3.

Los compuestos MFT, DMDS y MMFDS, que presentan los valores de hidrofobicidad (Log $P_{o/w}$) más elevados (1,94, 1,77 y 2,28 respectivamente), fueron los que se encontraron en mayor contenido en los embutidos, los cuales contienen más grasa (Capítulo 3**)**. En cambio, los lomos curados poseen valores más elevados de pH, actividad de agua (a_w), contenido de humedad y proteína (capítulo 1 y 2). Teniendo en cuenta que los embutidos fueron fermentados e inoculados con un cultivo iniciador comercial (TRADI-302) compuesto por *Lactobacillus sakei*, *Staphylococcus carnosus* y *Staphylococcus xlyosus*, se confirmaron los mayores recuentos de TMB y LAB en los embutidos que en los lomos (Capítulo 1 y Capítulo 2). Por el contrario, los recuentos bajos de GC+ en embutidos (Capítulo 1 y Capítulo 2) se relacionaron con el pH ácido, mientras que los lomos con un pH más alto, presentaron mayores recuentos de GC+. Estudios recientes en embutidos fermentados han demostrado que la presencia de aroma a palomitas de maíz, identificado como 2AP, puede estar relacionado con la inoculación de *D. hansenii*. Sin embargo, la inoculación de la cepa de levadura *D. hansenii* L1 en la superficie de los embutidos (Capítulo 2) no confirmó la formación de 2AP, que no pudo ser detectado en los embutidos (Capítulos 2 y 3). Este resultado podría explicarse por las posibles vías de formación de 2AP en embutidos fermentados, como la degradación de los aminoácidos Pro y Orn por metabolismo microbiano, o a través de la reacción de Maillard (via química). Además, el origen microbiano de 2AP en embutidos fermentados se ha relacionado con la presencia de *Penicillium nalgiovense*. Por tanto, es necesario profundizar en la relación entre las levaduras y mohos y la formación de 2AP en embutidos fermentados. Además, diversos estudios han demostrado que el compuesto 2AP se puede producir a través de reacción química a pH y temperatura más altos, lo que podría justificar la presencia de 2AP en lomos, los cuales presentaron un pH más elevado que los embutidos (Capítulo 3).

La principal conclusión de los resultados obtenidos en los Capítulos 1, 2 y 3 es que la

concentración de precursores en los productos cárnicos es un factor clave para favorecer la formación de compuestos aromáticos con notas cárnicas y tostadas. Sin embargo, la concentración de los precursores en el producto final varió considerablemente (Capítulo 1 y 2), aunque cada precursor se agregó a la misma concentración en la carne fresca (lomo) o masa cárnica (embutido). La matriz cárnica de lomos y embutidos es esencialmente distinta por el contenido de grasa (5,3 a 7,0 % en lomos y 28,1-30,3 % en embutidos). A concentraciones similares de metionina en lomos y embutidos (Capítulo 1 y 2) , el contenido de los aromas cárnicos resultantes de la degradación de la metionina fue significativamente diferente en lomos y embutidos (Capítulo 3). La razón de estas diferencias podría ser la diferente composición de la matriz de los lomos y embutidos, lo que promueve una diferente estabilidad de estos compuestos con notas cárnicas, debido a la disparidad en el equilibrio redox. En cuanto a los aromas tostados, la concentración de Pro y Orn en los lomos suplementados fue significativamente diferente que en los otros lomos (Capítulo 1), pero cuando se evaluó la abundancia de 2AP y 2-acetilpirrol no se observaron diferencias significativas (Capítulo 1). Sin embargo, al emplear la metodología optimizada para la cuantificación de los aromas (Capítulo 3) se confirmó la existencia de diferencias significativa en la concentración (ng/g de productos cárnicos en extracto seco) de 2AP y 2-acetilpirrol entre los lomos. Por tanto, la cantidad de precursores añadidos afectó a la formación de compuestos aromáticos con notas a tostado lo que sugiere principalmente un origen químico en los lomos. En cambio, el efecto de la adición de Pro y Orn en embutidos requiere de un estudio más profundo para poder confirmar el mecanismo de origen de los aromas tostados.

El contenido de tiamina en los lomos y embutidos suplementados con esta vitamina también fue significativamente diferente al de los productos no suplementados (Capítulo 1 y 2). El compuesto MMTF procedente de la descomposición de la tiamina se detectó en muy baja abundancia (Capítulo 1) y concentración (Capítulo 3) en lomos y embutidos. Esto podría deberse a la baja cantidad de tiamina añadida, pero también podría estar relacionado con la reactividad química de este compuesto. Existen estudios que indican que la

concentración de MMTF aumenta gradualmente con la adición de tiamina en jamones cocidos, y lo mismo se ha observado para MFT. En este proyecto de Tesis Doctoral, la tiamina se suplemento en ambos productos a una concentración de 24 mg tiamina/100 g, y se confirmó la formación de MMTF en lomos, y embutidos aunque en estos últimos no se pudo cuantificar (Capítulo 3). Además, mediante la técnica de olfatometria (GC-O) se detectó que MMTF era el responsable del aroma cárnico en los lomos (Capítulo 1). En cambio, no se encontró correlación entre la concentración de adición de tiamina en lomos y la presencia de MFT, que solo se pudo cuantificar en los embutidos (Capítulo 3). Estos resultados indican que la suplementación con tiamina de los productos cárnicos curados madurados requiere de un mayor estudio para aumentar la concentración de MFT, ya que este compuesto es responsable de notas aromáticas grasas en productos cárnicos.

El análisis sensorial aplicado (FCP) logró distinguir entre muestras, y confirmó el efecto de la suplementación en lomos y embutidos. La suplementación con Pro en lomos se relacionó con los atributos pimiento, tostado y moho, mientras que la suplementación con tiamina impartió notas aromáticas a curado y cárnico en ambos productos, lomos (Capítulo 1) y embutidos (Capítulo 2). El impacto en el aroma de los compuestos volátiles cárnicos y tostados se evaluó mediante el cálculo de los valores de actividad aromática OAV en los productos. Este cálculo, obtenido a partir de la concentración de los compuestos (Capítulo 3), reveló que el aroma de los lomos curados madurados se vio muy afectado por la presencia de metional y 2AP, mientras que el aroma de los embutidos estaba esencialmente modulado por MFT, metional y MMFDS (Capítulo 3). Estos resultados confirmaron, al menos parcialmente, los resultados obtenidos por el panel sensorial utilizando la metodología FCP (Capítulo 1 y 2). El perfil aromático de los lomos suplementados con Pro estaba probablemente relacionado con la presencia y concentración de 2AP, en cambio 2AP no se detectó en embutidos probablemente debido a sus niveles extremadamente bajos. Además, la presencia de MMTF verificó el efecto de la suplementación con tiamina en lomos, pero su impacto en los atributos aromáticos curado y cárnico, no pudo ser confirmado por los OAV calculados.

La complejidad de los productos cárnicos y las reacciones involucradas en la generación de aromas cárnicos y tostados dificultan la obtención de conclusiones. Por ello se planteó el estudio de diferentes sistemas modelo que simulaban el proceso de elaboración de los productos cárnicos. El primer modelo simuló diferentes etapas del proceso de elaboración de embutidos fermentados en términos de composición en aminoácidos libres y condiciones de pH y a_w empleadas. Estos modelos se utilizaron para investigar los mecanismos de conversión de aminoácidos en compuestos aromáticos cárnicos y tostados en los productos cárnicos curados madurados (Capítulo 4). Los sistemas modelo investigaron los resultados de la reacción de Maillard a partir de aminoácidos en presencia de glucosa y a las temperaturas suaves utilizadas durante el proceso (25 °C y 37 °C). Se diseñaron tres modelos para imitar las etapas del proceso en términos de composición de aminoácidos (inicial (I), primer secado (1D) y segundo secado (2D). El segundo sistema modelo (Capítulo 5) fue diseñado para investigar cómo la biotransformación de aminoácidos se puede ver afectada por el proceso de oxidación y el metabolismo de la levadura en presencia de proteínas miofibrilares de cerdo. En este caso los modelos se formularon con diferentes tipos de grasa (manteca de cerdo y grasa de coco) y se inocularon con la levadura *D. hansenii.* Además, los modelos contenían la composición de aminoácidos y aditivos que simulaba la etapa final de secado de los embutidos fermentadas. Los modelos estudiados en el Capítulo 4 y el Capítulo 5 fueron diferentes en función de su composición, pero los resultados pueden compararse.

En los modelos analizados, cinco compuestos (furfural, metilpirazina, tiazol, furano, y 2-butanona) se detectaron únicamente en el sistema modelo 2D-37 °C (Capítulo 4), mientras que 13 compuestos estaban presentes en los modelos 2D-37 °C (Capítulo 4) y Control (C, Capítulo 5). Estos resultaron demostraron que muchos compuestos (2-metilpropanal, 2-metilbutanal, 3-metilbutanal, metional, benzaldehído y bencenoacetaldehído) que derivan de la reacción de degradación de los aminoácidos están presentes en ambos modelos. Por tanto, la reacción de Maillard sería el origen principal de la generación de volátiles, y los principales factores que afectan a la producción de volátiles

a partir de aminoácidos libres son las condiciones de temperatura, seguidas por la concentración de aminoácidos y aw, como se concluyó a partir de los resultados del Capítulo 4. Además de estos factores, se debe considerar el efecto del proceso de oxidación y biotransformación de los aminoácidos por parte de las levaduras, como se puso de manifiesto en el Capítulo 5.

En el Capítulo 5, el contenido de aldehídos de cadena ramificada, benzaldehído y metional aumentó por la alta concentración de manteca de cerdo en los modelos, debido al efecto de compuestos carbonilo derivados de la peroxidación de lípidos. En consecuencia, en los modelos con manteca de cerdo, el aroma se vio afectado por el metional y el 3-metilbutanal, ya que ambos compuestos derivan de la degradación de la metionina y la leucina, respectivamente. En los modelos inoculados con *D. hansenii* se observó un retraso en la formación de hexanal en los modelos que contenían grasa de coco, mientras que en los modelos que contenían manteca de cerdo se observó un retraso en la formación de heptanal, octanal y nonanal, lo que confirmó la acción antioxidante de *D. hansenii*. La presencia de compuestos como los ácidos 3-metilbutanoico y butanoico, así como, alcohol feniletílico, que contribuyeron a las notas de olor a queso y flores, se vio potenciada por la inoculación de la levadura en los modelos con manteca de cerdo. Sin embargo, en los modelos inoculados con *D. hansenii* se detectó únicamente metional como compuesto aromático responsable de notas cárnicas. Diferentes razones pueden haber incidido en la baja generación de compuestos aromáticos cárnicos en los modelos, una puede ser el bajo contenido de precursores como la tiamina (no se añadió en la formulación de los modelos), y otro puede ser la ausencia de otros cultivos iniciadores (LAB y SNC (Coagulase - estafilococos negativos)), que modulan las reacciones de degradación de aminoácidos. Por el contrario, estudios previos realizados en modelos similares, que contenían elevadas cantidades de los aminoácidos metionina y cisteína, inoculados con diferentes cepas *de D. hansenii,* demostraron la capacidad de las mismas para generar aromas cárnicos (compuestos azufrados). Tampoco se detectó 2AP, responsable del aroma tostado, en los modelos inoculados con *D. hansenii*. Este resultado probablemente se debió al bajo

contenido de precursores (Pro y Orn) y a la falta de los mecanismos químicos y bioquímicos responsables de la formación de 2AP. Por lo tanto, la inoculación de *D. hansenii* para promover la formación de aromas cárnicos y tostados requiere de una mayor investigación; aunque, en los sistemas modelo se puso de manifiesto el efecto producido por *D. hansenii* en la degradación de los aminoácidos y en la actividad antioxidante.

En conclusión, los mecanismos de formación de compuestos aromáticos que producen notas cárnicas y tostadas en lomos curados y embutidos fermentados están relacionados con reacciones como la oxidación de lípidos, reacciones de Maillard y degradación de tiamina, aunque se debe considerar el metabolismo microbiano de mohos y levaduras presentes en los embutidos fermentados y en la superficie del lomo curado. En embutidos fermentados, la contribución de los cultivos iniciadores al desarrollo de aromas cárnicos y tostados parece tener un efecto mayor que la adición de precursores aromáticos.

ABBREVIATIONS

2AP: 2-Acetyl-1-pyrroline

ANOVA: Analysis of variance

AU: Units of abundance

BAD2: Betaine aldehyde dehydrogenase 2

BHT: Butylated hydroxytoluene

BHI: Brain Heart Infusion agar

CAR/PDMS: Carboxen/poly-dimethylsiloxane

CBB: Chloramphenicol bromophenol blue agar

CN-Stp: Gram positive coagulase negative staphylococci

CP-Stp: Gram positive coagulase positive staphylococci

CSA: Chloramphenicol Sabouraud Agar

DF: Detection frequency

DG 18: Dichloran Glycerol Agar

DMS: Dimethyl sulfide

DMDS: Dimethyl disulfide

DMTS: Dimethyl trisulfide

EB: Enterobacteriaceae

EIC: Extracted ion current

FAA: Free amino acids

FCP: Free Choice Profile

FID: Flame ionization detector

GC-FID: Gas chromatography-flame ionization detector

GC-FID-O: Gas chromatography-flame ionization detector-olfatometry

GC-MS: Gas chromatography mass spectrometry

GC+: Gram positive cocci

GC-O: Gas chromatography-olfactometry

GLM: Generalized Linear Model

GPA: Generalized Procrustes Analysis

HDT: 2-(l-Hydroxyethyl)-4,5-dihydrothiazole

HPLC: High-performance liquid chromatography

KAA: kanamycin aesculin azide agar

KMBA: 4-methylthio-2-oxobutyric acid

LAB: Lactic acid bacteria

LAMVAB: Lactobacillus anaerobic MRS agar with Vancomycin and Bromocresol

LRI: Linear retention index

MDA: Malonaldehyde

Met: methionine

MEA: Malt Extract Agar

MFT: 2-Methyl-3-furanthiol

MMTF: 2-Methyl-3-(methylthio)furan

MMFDS: Methyl 2-methyl-3-furyl disulfide

MRS: Man Rogosa Sharpe agar

MSA: Mannitol salt agar

MSD: Mass selective detector

MTH: Methanethiol

OAV: Odour-activity values

OGYE: Oxytetracyclin-Glucose-Yeast Extract Agar

Orn: Ornithine

PCA: Principal components analysis

Pro: Proline

RB: Rose Bengal Agar

RH: Relative Humidity

RMSE: Root mean square error

SDA: Sabouraud Dextrose Agar

SIM: Selected ion monitoring

SlaBa: Slanetz and Bartley agar

SPME: Solid phase microextraction

TBARS: Thiobarbituric acid reactive substances

TIC: Total ion current

TMB: Total mesophilic bacteria

YPD: Yeast extract peptone dextrose

VRBG:Violet Red Bile Glucose Agar

YM: yeasts (Y) and molds (M)

INTRODUCTION

INTRODUCTION

1. Dry cured meat products

Meat products are the food products made from meat, including meat preparations, cooked processed meats, and meat-based ingredients. Processed meats are submitted to different treatments such as cooking, curing, smoking, drying, etc. Among these treatments, drying, and fermentation are two of the oldest meat preservation methods (Toldrá & Flores, 2014). Curing was originally used to preserve meat, although it is now used to enhance the aroma and flavor of meat products. Moreover, fermentation is also one of the oldest methods of preserving meat products (Zhang et al., 2010). The dry-cured process uses mainly salt, nitrate, and/or nitrite as curing ingredients without water addition. The dry-curing of minced meats is also used for the manufacturing of fermented sausages (Flores, 1997). Among the variety of dry cured products, those stuffed into casings receive the name of sausages. However, different products are obtained depending on the processing conditions as in the case of dry cured loins and dry fermented sausages.

1.1 Dry cured loins

1.1.1 Definition

Dry-cured loins are made with an entire muscle (*Longissimus thoracis et lumborum*), which is salted, cured, and dried for a long time to reduce moisture and produce a shelf-stable product. The product is typically produced in the southern European countries, where different types of dry cured loins are manufactured depending on raw materials, ingredients, and processing applied.

1.1.2 Processing

Processing starts by rubbing a mixture of curing agents (salt, nitrate, and nitrite) and different spices (Table 1) on the surface of the muscle (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004). The most common spices used in the formulation of dry cured loins are paprika, garlic, and oregano. Initially, the loins remain in the curing chamber at refrigeration temperature (4 °C) for salt equilibration. After that, the loins are stuffed in the casing and let for post-seasoning and drying for 1 to 5 months (Fig. 1).

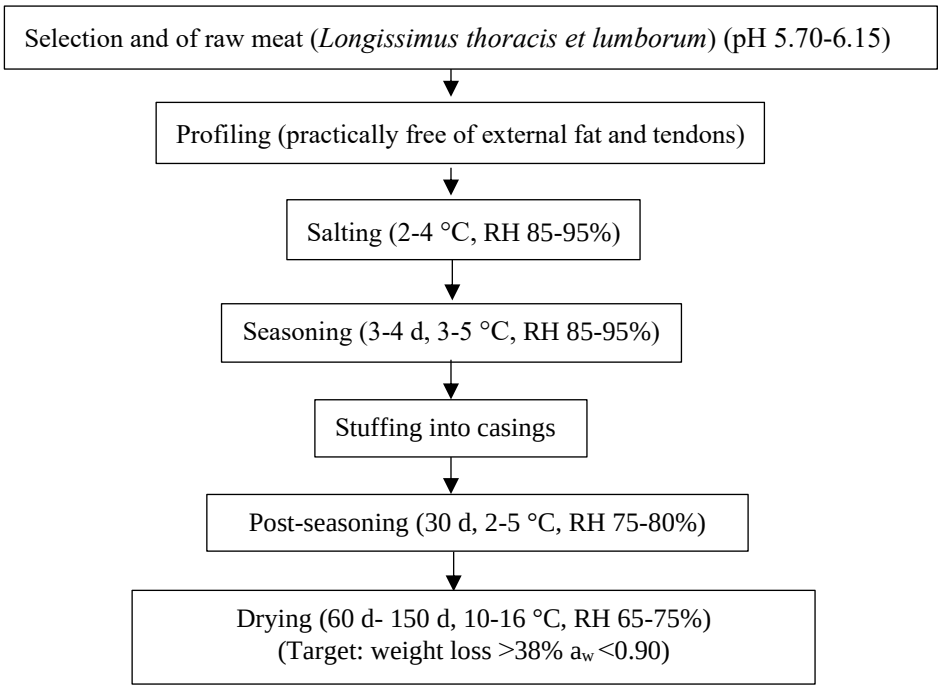

Fig. 1. Diagram of the manufacturing process of dry cured loins.

1.1.3 Product types

Several dry-cured loins produced in different geographical areas and pig breeds are listed in Table 1. The variety of reports about dry cured loins contains the largest number of studies from European countries, mainly from Spain, due to the high consumption of these dry cured products. Nevertheless, there is a high diversity of products based on pig breed and spices used, and the drying and ripening time which can fluctuate from 18 days to 156 days. Spanish dry cured loins are long ripened products, around 2 months for loins made with white pig breeds, or longer drying periods from 3 to 5 months in case Iberian pig breeds are employed. In contrast, heterogeneous dry cured loins manufactured in Poland, Brazil, and China, are characterized by short drying periods of less than 1 month, like in the Chinese product, or around 3 months in a Polish dry loin.

1.1.4 Microbiology

Since dry-cured loins do not need to undergo a fermentation process, there is less research on the microbiota in this product (Table 2). The counts of LAB, Gram positive cocci, and yeast are low in comparison to other sausage products (Perea-Sanz, Montero, Belloch, & Flores, 2018). On the contrary, Polish dry cured loins (Stadnik & Stasiak, 2016) show high counts of lactic acid bacteria in comparison to the Spanish loins because they are manufactured with acid whey aimed to deliver the lactic acid bacteria.

1.1.5 Aroma and flavor characteristics

Among the dry cured loins, those manufactured using Iberian pigs as raw material are very popular within Spanish consumers, because of their unique sensory features including a dry cured aroma and flavor as can be seen in Fig. 2. This Fig. 2 represents, in the form of a word cloud, the sensory descriptors that have been used to describe the aroma (Fig. 2A)

and flavor (Fig. 2B) of dry cured loins in several scientific studies. Most of the descriptors shown in Fig. 2 were obtained applying the sensory technique Quantitative Descriptive Analysis, while others were obtained using the technique Flash Profile (Lorido et al., 2018). The most characteristic descriptors in dry cured loins were the words cured and rancid, which were reported in most of the studies. Other descriptors like spicy and herbal aroma and flavor were linked to the addition of spices in the product (Górska, Nowicka, Jaworska, Przybylski, & Tambor, 2017).

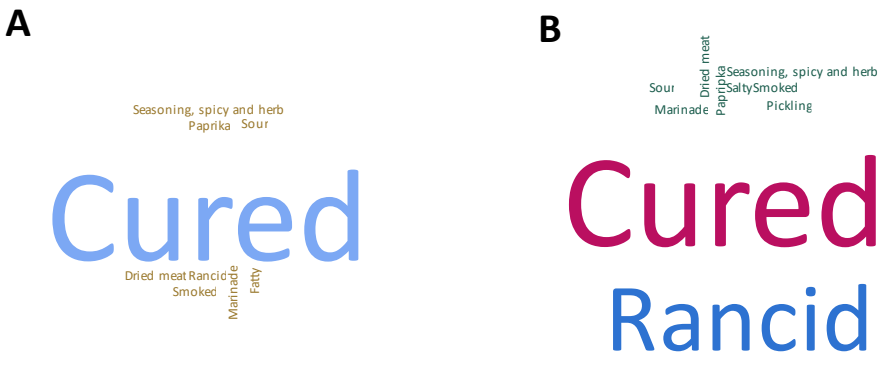

Fig. 2. Word clouds of the sensory descriptors used to describe the aroma (A) and flavor (B) of dry cured loins based on data: 1. Muriel, Ruiz, Martin, Petron, et al. (2004); 2. Ventanas et al. (2007); 3. Salazar et al. (2013); 4. Lorido et al. (2016); 5. Lorido et al. (2018); 6. Gamero-Negrón et al. (2018); 7. Jiménez et al. (2022); 8.Górska et al. (2017).

Table 1. Dry cured loins examples and characteristics.

Geographical area	Breed	Drying time (d)	Moisture content (%)	Water activity (a_w)	pH	Spice[a]	Reference[b]
Spain	(female) crossbred pigs (Landrace × Large White females and Duroc × Pietrain males)	49	58.5	nr	nr	Spanish paprika, garlic, and pepper	1
Spain	Iberian × Duroc pigs	120	33.97-38.66	nr	nr	Spanish paprika, oregano and garlic	2
Spain	Torbiscal Iberian pigs	100	nr	< 0.9	nr	Sweet paprika, hot paprika, powdered garlic, oregano	3
Spain	Landrace × Large White pigs	156	46.1-49.6	nr	nr	-	4
Spain	Landrace × Large White pigs	18	47	nr	nr	-	5
Spain	Torbiscal Iberian pigs	100	nr	< 0.9	nr	Sweet paprika, hot paprika, powdered garlic, and oregano	6
Spain	Chato Murciano; pig crossbreed	60	41.90, 51.80	nr	nr	Paprika and natural spices	7
Spain	Celta breed (Barcina line)	90	34.04	0.841	5.8	Spanish paprika, oregano and garlic	8
Spain	Chato murciano	60	46.01	nr	nr	-	9

Table 1 (continued).

Spain	Iberian	77	nr	0.875-0.883	5.45-5.67	Paprika and powdered garlic	10
Spain	50% Iberian × Duroc pigs	87	42.29	nr	nr	-	11
Spain	Iberian	80	45.7	0.92	5.6	Spanish paprika	12
Spain	Comercial pig	51	51.29-57.31	nr	5.49-5.63	Paprika	13
Spain	Berkshire; Landrace × Yorkshire × Duroc	30	59.60, 53.29	nr	6.17, 5.94	-	14
Poland	Polish Large White	28	53.0	0.929	6.0	-	15
Poland	Puławska × Polish Landrace	118	nr	0.938-0.941	5.82-6.18	-	16
Poland	Crossbreed of Polish Large White and Polish Landrace pigs	nr	52.58	nr	5.96	-	17
Brazil	Landrace × Large White pigs	90	nr	0.79-0.84	nr	Powdered black pepper and garlic	18
China	Comercial chinese pig	18	47.97	0.849	nr[c]	-	19

[a] Scientific names of spices: paprika (*Capsicum annuum*), garlic (*Allium sativum*), black pepper (*Piper nigrum*) and oregano (Origanun vulgare, L.).

[b] Reported in: 1. Hernández et al. (1999); 2. Ramírez & Cava (2007); 3. Soto et al. (2008); 4. Martin et al. (2008); 5. Armenteros et al. (2009); 6. Soto et al. (2009); 7. Salazar et al. (2013); 8. Pateiro et al. (2015); 9. Salazar et al. (2016); 10. Cardoso-Toset et al. (2017); 11. Lorido et al. (2018); 12. Higuero et al. (2020); 13. Belloch et al. (2021); 14. Seo et al. (2021); 15. Stadnik & Dolatowski (2013); 16. Stadnik & Stasiak (2016); 17. Smagowska et al. (2019); 18. Rosario et al. (2020); 19. Zhang et al. (2015).

[c] nr: not reported.

Table 2. Microbial counts (log CFU/g) in dry cured loins.

Geographical area	Breed	TMB[a] PCA[b]	LAB MRS	CN-Stp MSA	YM RB	Reference
Spain	Commercial pig	3.42-4.08	nr	nr	nr	Perea-Sanz et al. (2018)
Spain	Commercial pig	2.77-3.29	2.22-2.81	nr[c]	nr	Aliño et al. (2010)
Spain	Iberian	5.2	nr	nr	4.4	Higuero et al. (2020)
Spain	Commercial pig	5.34	4.12	5.41	5.04	Belloch et al. (2021)
Poland	Puławska × Polish Landrace	7.08-8.28	6.66-7.99	nr	nr	Stadnik & Stasiak (2016)

[a] TMB (Total mesophilic bacteria), LAB (Lactic acid bacteria), CN-Stp (Gram positive coagulase negative staphylococci), YM (yeasts (Y) and molds (M)).

[b] Plate Count Agar (PCA) used for TMB, Man Rogosa Sharpe agar (MRS) used for LAB, Mannitol Salt agar (MSA) used for Gram positive cocci (CN-Stp), Rose Bengal agar (RB) used for YM.

[c] nr: not reported.

1.2 Dry fermented sausages

1.2.1 Definition

Dry fermented sausages are made from ground meat, with salt and other seasonings added, and allowed to ferment and dry over time. Beneficial bacteria, such as lactobacilli, promote fermentation and pH decrease. Sausages loss moisture during the drying process, which helps to preserve it (Toldrá & Hui, 2014). Many countries around the world have a long tradition of manufacturing dry fermented sausages. There are many different types of dry fermented sausages, the most well-known are salami, pepperoni, and chorizo (Toldrá & Hui, 2014). Several studies regarding the physic-chemical and microbial characteristics of several of the most known fermented sausages have been gathered in Tables 3 and 4.

1.2.2 Processing

The detailed processing of dry fermented sausages is listed in Fig. 3. Due to the development of modern techniques for the isolation, preparation, and preservation of microbial strains, those isolated from naturally fermented sausages have been commonly applied for the production of industrial fermented sausages (Baka et al., 2011; Fiorentini et al., 2009; Sawitzki et al., 2009). The addition of different concentrations of sugar and the use of different strains of lactic acid producing bacteria, the production of industrial fermented sausages can be controlled and, different processes including fast and slow fermentations can be applied. The use of spices is very variable, but in the Mediterranean area, three spices (black pepper, garlic, and paprika) are most frequently used in the formulation of dry-fermented sausages (Table 3).

1.2.3 Products types

Depending on the type of processing technique, significant differences can be found in sausages based on the origin of the raw materials, ingredients, use of starters, temperature applied, surface molds, smoking, etc. A summary of dry fermented sausages from different geographical areas and their characteristics are listed in Table 3. Pork meat (lean and back fat) is commonly used for the processing of fermented sausages, while beef is used in Turkish sausages "Sucuk" (Kargozari et al., 2014) and Ethiopian sausages "Wakalim" (Bacha, Jonsson, & Ashenafi, 2010). In the Mediterranean countries, Spain, Portugal, and Italy, longer processing drying times are applied for the production of fermented sausages; although, as seen in Table 3, other characteristics like sausage caliber and production process differ between sausages. In north European countries (Norway and Belgium), sausages are produced in a shorter time, usually less than 1 month. In Asian countries like China, the processing time is shorter than in the Mediterranean products varying from 12 to 23 d. In general, Chinese sausages are stuffed in smaller diameter casings (3-4 cm) such Harbin dry sausages, while dry fermented sausages from the Mediterranean countries are produced using large diameter casings (from 5 to 9 cm), although small caliber sausages are also produced. The pH found in sausages is generally above pH > 5.0 except for several fermented sausages, produced in Belgium and Ethiopia, that show values < 5.0. Another characteristic of the fermented sausages is their low a_w values (< 0.90), which contributes to make sausages a shelf-stable product. Regarding the spices used to modulate the flavor of fermented sausages, a clear distinction can be done depending on habits and cultural traditions (Gagaoua & Boudechicha, 2018). Mediterranean fermented sausages have a distinct flavor, characterized by the use of mainly paprika, black pepper, and garlic, whereas

in Chinese products, Oriental spices such as round cardamom, Chuan Jiao, angelica, aniseed, fennel, Xiang Sha Ren, cassia bark, clove, and pepper are used contributing to the round complex flavor of the sausages.

1.2.4 Microbiology

The microbial counts found in fermented sausages change widely from region to region (Table 4) (Aquilanti, Garofalo, Osimani, & Clementi, 2016). Fermented sausages are characterized by the presence of high counts of lactic acid bacteria (LAB) between 6-8 log cfu/g, and 4-6 log cfu/g Gram positive cocci (CN-Stp), which are the predominant microbiota in fermented sausages. Fermented sausages produced in Belgium have a lower pH (below 5.0) than fermented meat products produced in Spain, Italy, and France. The different counts of Gram positive cocci (CN-Stp) found in these products are likely due to the combination of differences in process technology as well as the starter cultures used (Van Reckem et al., 2019). Yeast counts in different fermented sausages are very variable, which it is due to the type of manufacture and sausage diameter (Encinas, López-Díaz, García-López, Otero, & Moreno, 2000). Moreover, in Chinese products, some reported studies (Table 4) show the presence of Enterobacteriaceae indicating a deterioration process is taking place, which will have a negative impact on the quality and safety of the sausages.

1.2.5 Aroma and flavor characteristics

The aroma and flavor of fermented sausages (such as salchichón from Spain, and salame from Italy) is very complex, and many different descriptors have been used to define it (Fig. 4). Fig 4. represents in the form of a word cloud the sensory descriptors obtained by the Quantitative Descriptive Analysis used to describe the aroma (Fig. 4A) and flavor (Fig. 4B) of dry fermented sausages in several scientific studies. The main sensory

descriptors that define the aroma of dry fermented sausages are rancid, sour, black pepper, spices, mold, and roasted, while flavor is defined by rancid, acid, mold, salty, spicy, maturity, and toasted. Fermented sausages have a distinctly different aroma compared to the predominantly cured aroma of the dry cured loins (Fig. 2). In sausages, the acidification process due to the addition of starter cultures (LAB) reduces the formation of cured aroma compounds (Berdagué, Monteil, Montel, & Talon, 1993). Rancid aromas have been associated with aldehydes, alcohols and alkanes, and the sour aroma with acetic acid (Berdagué et al., 1993).

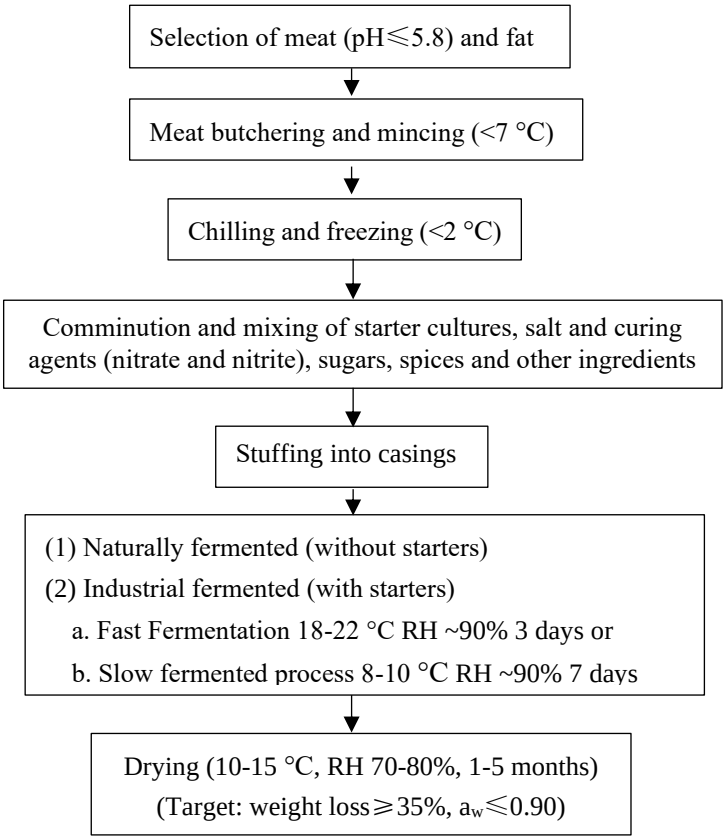

Fig. 3. Diagram of the manufacturing process of dry fermented sausages.

Table 3. Dry fermented sausages types and characteristics.

Geographical area	Examples	Time (d)	Diameter (cm)	Moisture content (%)	Water activity (a_w)	pH	Spice[a]	Reference[b]
Spain	Fuet	53	nr[e]	nr	0.619	5.60	Pepper	1
Spain	Salchichón	90	nr	30.62	0.785	5.27	Black pepper	2
Spain	Salchichón	48	6	31.38	0.89	5.88	White pepper, black pepper	3
Spain	Chorizo	48	3.2-3.4	20.66	0.821	5.58	Sweet paprika, spicy paprika, garlic	4
Spain	Galician chorizo	30	3.6-3.8	nr	0.830	6.13	Sweet paprika, garlic, spicy paprika	5
Spain	Dry fermented sausages	62	9.5	40.6	0.883	5.05	-	6
Portugal	Smoked Fermented Sausage	nr	nr	nr	0.876	5.12	Red pepper, garlic, powder laurel	7
Portugal	Fermented sausages	30-40	7.0	nr	0.85	5.32	Garlic, pepper, bell pepper, black pepper, pepper oleoresin	8
Italy	Sicilian salami	90	nr	26.8	0.81	6.37	Black pepper, wild fennel seeds	9
Italy	Dry fermented sausages	30	4.0	nr	0.794	5.77	Whole black pepper	10
Italy	Vallo di Diano	38	nr	22.42	0.801	6.18	Black pepper, fennel seeds, sweet and hot chili pepper powder	11
Italy	Salame nostrano	21	nr	23.96	0.807	5.66	Pepper, garlic	12
Italy	Low-acid sausages	100	nr	nr	0.874	5.57	Pepper	13
Italy	Felino-type sausages	42	5.0-6.0	nr	0.958	5.4	Black pepper and garlic	14

Table 3 (continued).

Italy	Dry fermented sausages	28	5-6	nr	0.875	4.92	white pepper powder and black pepper whole grain	15
Italy	Dry fermented sausages	60	6.5	nr	0.906	5.77	Black pepper and garlic	16
France	Dry fermented sausages	60	3.0-4.0	nr	0.799	5.32	Black pepper	17
Belgian	Belgian-type salami	21	nr	nr	0.925	4.81	-	18
Belgian	Boulogne sausages	28	nr	nr	0.918	4.74	-	18
Norway	Salami	28	5.0	nr	0.829	5.28	-	19
Turkey	Sucuk	16	3.8	nr	0.813	5.08	Garlic, cumin, red pepper, black pepper, pimento	20
Argentina	Artisanal dry sausages	15	nr	32.81	0.816	5.19	Chopped, garlic, ground red pepper, and ground black pepper	21
Ethiopia	WAKALIM	6	nr	21.85	nr	4.15	Onion, red pepper, Ethiopian cardamom, black cumin, Kemun, Ethiopian mustard and garlic	22
China	Dry fermented sausages	18	nr	nr	0.874	5.43	-	23
China	Harbin dry sausages	12	3.0	25.54	0.784	5.33	Round cardamom, Chuan Jiao, angelica, aniseed, fennel, Xiang Sha Ren, cassia bark, clove and pepper	24
China	Dong fermented pork (Nanx Wudl)	22	nr	nr	0.877	5.22	-	25

Table 3 (continued).

China	Dry fermented sausages	23	nr	nr	nr	5.24	Garlic powder, white pepper,and black pepper	26
China	Dry fermented sausages	12	3.0	nr	nr	5.51	-	27

[a] Scientific names spices: Chuan Jiao (*Pericarpium zanthoxyli*), Xiang Sha Ren (*Amomum villosum*), garlic (*Allium sativum*), pepper (*Capsicum annuum*), black pepper (*Piper nigrum*), powder laurel (*Laurus nobilis L.*), onion (*Allium ascalonicum*), Ethiopian cardamom (*Aframomum corrorima*), black cumin (*Nigella sativa*), Kemun (*Trachyspermum capticum*), Ethiopian mustard (*Brassica nigra*).

[b] 1. Roig-Sagués et al. (1999); 2. Casquete et al. (2011); 3. Ruiz-Moyano et al. (2011); 4. Lorenzo et al. (2013); 5. Fonseca et al. (2013); 6. Perea-Sanz et al. (2019); 7. Dias et al. (2020); 8. Belleggia et al. (2022); 9. Moretti et al. (2004); 10. Patrignani et al. (2007); 11. Casaburi et al. (2007); 12. Cenci-Goga et al. (2008); 13. Spaziani et al. (2009); 14. Tabanelli et al. (2012); 15. Tabanelli et al. (2013); 16. Coloretti et al. (2014);17. Christieans et al. (2018); 18.Janssens et al. (2012); 19. Hagen et al. (1996); 20. Kargozari et al. (2014); 21. Prpich et al., (2015); 22.Bacha et al. (2010); 23. Xiao et al. (2020); 24. Hu et al. (2021); 25. Chen et al. (2021); 26. Liu et al. (2021); 27. Wang et al. (2022).

[c] nr: not reported.

Table 4. Microbial counts (log CFU/g) found in dry fermented sausages at the end of drying.

Geographical area	Examples	TMB[a] PCA[b]	LAB MRS	CN-Stp MSA	CP-Stp BP	EC SlaBa	EB VRBG	YM RB	Reference[c]
Spain	Fuet	8.59	8.30	4.62	nr[d]	nr	nd	nr	1
Spain	Salchichón	nr	7.61	4.52	nr	nr	nr	nr	2
Spain	Salchichón	8.38	8.26 (LAMVAB)	6.78	4.89	nr	3.12	nr	3
Spain	Chorizo	8.59	8.17	nr	nr	nr	nr	6.23 (OGYE)	4

Table 4 (continued).

Spain	Galician chorizo	8.55	8.51	6.38	nr	nr	5.61	nr	5
Spain	Dry fermented sausages	7.1	6.2	1.4	3.2	nr	nr	nr	6
Portugal	Smoked Fermented Sausage	7.26	7.32	4.19	nr	nr	0.86	0.88(Y), nd(M) (DG 18)	7
Portugal	Fermented sausages	nr	6.47	5.59	nr	2.73	nd	nr	8
Italy	Sicilian salami	nr	7.11	6.11	nr	nr	0.15	5.16 (Y) (MEA)	9
Italy	Dry fermented sausages	4.5	8.0	5.6	nr	nr	5.0	3.4 (Y) (SDA)	10
Italy	Vallo di Diano	nr	8.53	7.61	nr	6.55	1.95	4.93	11
Italy	Salame nostrano	8.01	7.00	6.16	nr	6.02	4.18	nr	12
Italy	Low-acid sausages	nr	8.30	4.97	nr	nr	nr	nr	13
Italy	Felino-type sausages	nr	7.50	nr	5.87	5.04	<1	3.13(Y) (SDA)	14
Italy	Dry fermented sausages	nr	7.49	nr	5.62	4.06	nr	nr	15
Italy	Dry fermented sausages	nr	8.55	nr	6.59(CNC)	2.34	3.16	nr	16
France	Dry fermented sausages	nr	6.69	5.32	nr	nr	nr	nr	17
Belgian	Belgian-type salami	8.71	8.71	5.91	nr	2.22 (KAA)	nd	1.58(Y) (MEA)	18
Belgian	Boulogne sausages	8.17	8.36	5.29	nr	1.70 (KAA)	nd	3.02(Y) (MEA)	18
Norway	Salami	nr	7.58	nr	nr	nr	nr	nr	19
Turkey	Sucuk	8.57 (BHI)	8.82	6.49	nr	nr	2.03	nr	20
Argentina	Artisanal dry sausages	nr	8.00	6.71	nr	nr	1.04	2.78 (CSA)	21
Ethiopia	WAKALIM	6.8	9.61	3.83	nr	nr	nd	5.72(Y), nd (M) (CBB)	22

Table 4 (continued).

China	Dry fermented sausages	7.90	6.76	6.48	nr	nr	nr	nr	23
China	Harbin dry sausages	nr	6.82	5.84	nr	nr	nr	nr	24
China	Dong fermented pork (Nanx Wudl)	nr	8.21	6.43	nr	nr	3.21	nr	25
China	Dry fermented sausages	7.97	7.97	6.85	nr	nr	5.25	4.82(Y) (YDP)	26
China	Dry fermented sausages	nr	7.53	6.72	nr	nr	5.52	nr	27

[a] TMB (total mesophilic bacteria), LAB (lactic acid bacteria), CN-Stp (Gram positive coagulase negative staphylococci), CP-Stp (Gram positive coagulase positive staphylococci), EC (enterococci)，EB (Enterobacteriaceae), YM (yeasts (Y) and molds (M)).

[b] Plate Count Agar (PCA) or Brain Heart Infusion agar (BHI) are used for TMB; Man Rogosa Sharpe agar (MRS) or Lactobacillus anaerobic MRS agar with Vancomycin and Bromocresol (LAMVAB) are used for LAB; Mannitol salt agar (MSA) is used for CN-Stp; Baird Parker Agar (BP) is used for CP-Stp; Slanetz and Bartley agar (SlaBa) or kanamycin aesculin azide agar (KAA) used for EC; Violet red bile glucose agar (VRBG) is used for EB; Rose Bengal Agar (RB), Malt Extract Agar (MEA), Oxytetracyclin-Glucose-Yeast Extract (OGYE) Agar, Chloramphenicol bromophenol blue (CBB) agar, Chloramphenicol Sabouraud Agar (CSA) or Dichloran Glycerol Agar (DG 18) are used for YM; Yeast extract peptone dextrose (YPD) or Sabouraud Dextrose Agar (SDA) are used for Yeast.

[c] 1. Roig-Sagués et al. (1999); 2. Casquete et al. (2011); 3. Ruiz-Moyano et al. (2011); 4. Lorenzo et al. (2013); 5. Fonseca et al. (2013); 6. Perea-Sanz et al. (2019); 7. Dias et al. (2020); 8. Belleggia et al. (2022); 9. Moretti et al. (2004); 10. Patrignani et al. (2007); 11. Casaburi et al. (2007); 12. Cenci-Goga et al. (2008); 13. Spaziani et al. (2009); 14. Tabanelli et al. (2012); 15. Tabanelli et al. (2013); 16. Coloretti et al. (2014); 17.Janssens et al. (2012); 18. Hagen et al. (1996); 19. Kargozari et al. (2014); 20. Prpich et al., (2015); 21.Bacha et al. (2010); 22. Xiao et al. (2020); 23. Hu et al. (2021); 24. Chen et al. (2021); 25. Liu et al. (2021); 26. Wang et al. (2022).

[d] nr: not reported, nd: not detected;

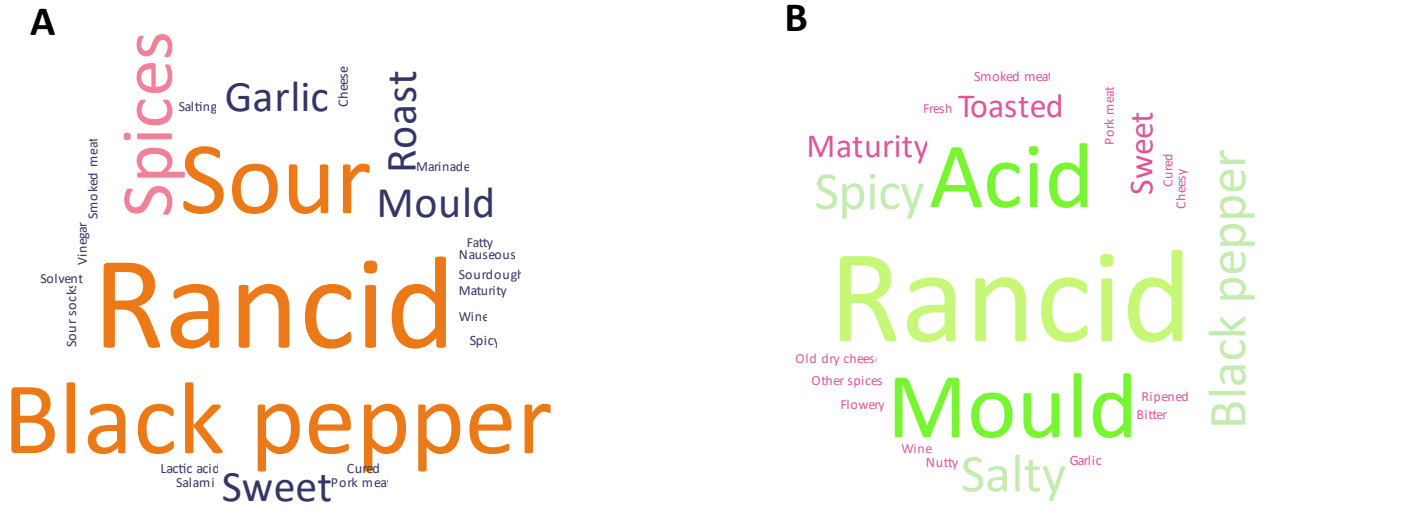

Fig. 4. Word clouds of the sensory descriptors used to describe the aroma (A) and flavor (B) of dry fermented sausages based on data: 1. Pérez-Cacho et al. (2005); 2. Ruiz-Moyano et al. (2011); 3. Stahnke et al. (2002); 4. Casaburi et al. (2007); 5. Cenci-Goga et al. (2008); 6. Spaziani et al. (2009); 7. Rason et al. (2007); 8. Prpich et al. (2015); 9. Hagen et al. (1996); 10. Stahnke (1995); 11. Zinina et al. (2018).

2. **Mechanisms of aroma formation in dry cured meat products**

In dry cured meat products, aroma compounds are generated from the pool of precursors present in raw meat and also during the dry curing process (Toldrá, 1998). Compounds from chemical reactions, biochemical reactions, and spices make up the flavor of dry cured meat products.

2.1 Chemical reactions

In cooked meat, the Maillard reaction is paramount importance in the generation of the characteristic meaty flavor, although other pathways are also known to contribute to meaty flavor such as degradation of thiamine (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2015). In dry cured meat products processed under mild conditions, mechanistic studies of chemical reactions such as the Maillard reaction are still relatively scarce.

2.1.1 Maillard reaction

The Maillard reaction is a chemical reaction between reducing sugars and amino acids that occurs when meat is exposed to high heat during cooking or drying (Parker, 2017). This reaction is responsible for the development of desirable flavors (pyrroles, pyrazines, thiazoles, thiazolines) (Shibamoto, 1989), colors, and textures in for example cooked ham, bacon, etc. The reaction also helps to form a protective barrier on the surface of the cooked meat, reducing the risk of spoilage and extending its shelf life. However the reaction is affected by several factors (Martins, Jongen, & Van Boekel, 2000), including temperature, time, pH, and the presence of water and, additionally, the specific conditions can be adjusted to produce the desired outcome. Among these factors, high temperature (140-160 °C) favors the formation of volatile compounds from the Maillard reaction (Chansataporn, Nopharatana, Samuhasaneetoo, Siriwattanayotin, & Tangduangdee, 2019).

In dry-cured products, which are produced at mild temperature conditions (15-30 °C), free amino acids generated from the hydrolysis of peptides and proteins, and ribose generated from the degradation of ribonucleotides, during the drying process or by the presence of sugars added into the recipe, are the substrates for the Maillard reaction that also occurs but at lower rates (Flores, 2018).

2.1.2 Strecker degradation

In the Strecker degradation, a dicarbonyl from the Maillard reaction combines with the amino group of an amino acid to produce the matching aminoketone and a Strecker aldehyde (aldehyde from the parent amino acid but with one carbon less). Strecker reactions are one of the most significant activities for producing flavor compounds (Flores, 2018). Methionine is an essential amino acid for producing meaty flavor (methional) through the Strecker reaction (Parker, 2017). Moreover, the conversion of amino acids into Strecker aldehydes is favored at lower pH in the presence of oxygen (Hidalgo & Zamora, 2016).

2.1.3 Lipid autooxidation

Autooxidation of unsaturated fatty acids usually occurs by the free radical mechanism. Free radicals consist of superoxide ($O_2^{\bullet -}$), hydroxy (HO•), peroxy (ROO•), hydroperoxy (HOO•), and alkoxy (RO•) anions. The autooxidation process consists of three main stages: initiation, propagation, and termination. Hydroperoxides (ROOH), the primary products of lipid oxidation, are odorless and tasteless. Decomposition of the hydroperoxides (ROOH), which decompose into alkoxy (RO•) and hydroxy (HO•) radicals, is regarded as the initial step in the synthesis of flavor compounds. The reaction of various alkoxy radicals (RO•) produces a diversity of products. As a result of this reaction, an array of secondary products such as aldehydes, hydrocarbons, alcohols, ketones, acids, esters, furans, lactones, epoxy

compounds, as well as polymers, are formed (Jeleń & Wąsowicz, 2011; Shahidi, 2002). A high number of these volatile compounds have been described in fermented sausages (Corral, Salvador, & Flores, 2016). Since they have a low odor threshold value, aldehydes, which are usually described as rancid, green, tallow, oily, and beany are considered as the most intriguing of the volatiles generated from lipids (Jeleń & Wąsowicz, 2011) due to their aroma impact and their chemical reactivity.

2.2 Biochemical reactions

Depending on the processing conditions several biochemical reactions like carbohydrate fermentation, amino acid degradation, lipid β-oxidation, and esterase activity may take part in the flavor profile of dry cured meat products (Flores, 2023).

2.2.1 Carbohydrate fermentation

The raw meat is low in fermentable carbohydrates (Soren & Biswas, 2020), so the added carbohydrates during meat processing serve as the substrate for the growth and development of the microbiota. Lactic acid bacteria (LAB) ferment sugars anaerobically via homofermentation, generating lactic acid. In contrast, heterofermentative starters can produce volatile compounds including acetic acid, ethanol, diethanol, dihydrochloric acid, and others. Many of these compounds have been reported in dry cured meat products, for example, ethanol, acetic acid, and 2,3-butanedione in celta dry cured loins (Pateiro et al., 2015), ethanol, 2-butanol, 2-butanone, acetic acid, 3-hydroxy-2-butanone (acetoin), 2,3-butanediol and butanoic acid in Iberian dry cured loin (Soto et al., 2008) and 2,3-butanedione, 3-hydroxy-2-butanone and acetic acid in fermented sausages (Perea-Sanz, Montero, et al., 2019). The type and amount of added sugar are essential for the control of the fermentation, the final product pH, and the derived volatile compounds in dry fermented

sausages. In this sense, the presence of 2,3-butandione (diacetyl), 3-hydroxy-2-butanone (acetoin), and butanediol have been related to butter and yogurt aromas in fermented sausages (Montanari et al., 2022; Toldrá & Flores, 2006).

2.2.2 Amino acid degradation

Free amino acids accumulate in the matrix of dry cured meat products during maturation mainly by muscle endogenous proteolysis (Toldrá, 1998). The presence of these free amino acids can result in the production of a variety of volatile compounds. Depending on the processing of the dry cured meat products, the transformations of these free amino acids into volatile compounds will occur by the activity of microbial enzymes, as in fermented meat products, or by chemical processes as Strecker degradation (section 2.1.2), as in dry cured products like ham and loin (Flores & Olivares, 2014). In case of the biochemical reactions, their contribution to the aroma will depend on the microbial presence in the products. As seen in Table 2, the microbial load in dry cured loins (section 1.1.4) is low in comparison with the values of LAB and CN present in fermented sausages (Table 4 in section 1.2.4). In this sense, the transformation of amino acids by microbial enzymes will be favored in fermented sausages.

The degradation of leucine (Fig. 5), isoleucine, valine, and phenylalanine, by aminotransferases turns out into the production of 3-methylbutanal, 2-methylbutanal, 2-methylpropanal, and benzeneacetaldehyde, respectively (Hierro, Fernández, & de la Hoz, 2014). 3-Methylbutanal has been found in Iberian dry-cured loins (Soto et al., 2008) and celta dry-cured loins (Pateiro et al., 2015), but also detected as aroma active volatile compounds in fermented sausages (Flores & Olivares, 2014). Marco et al. (2007) defined 3-methylbutanal as contributing to “rancid dry cured ham aroma”, while Olivares et al.

(2011) and Corral et al. (2013) reported as “green odor” in fermented sausages (Fig. 5).

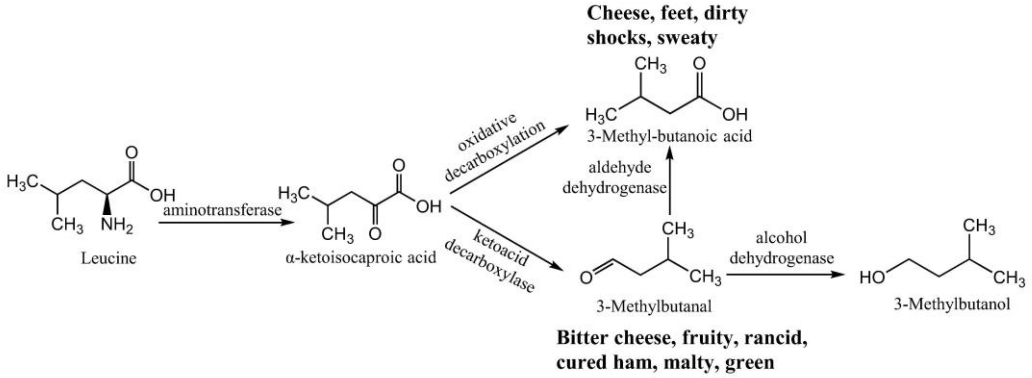

Fig. 5. Pathway for the conversion of leucine into aroma compounds. Modified after Leroy et al. (2006) and Flores & Olivares (2014).

The identification of benzaldehyde and benzeneacetaldehyde in dry cured loins also confirmed that amino acid metabolism occurs during the process (Soto et al., 2008).

2.2.3 Lipid β-oxidation

Saturated fatty acids undergo enzymatic breakdown by β-oxidation to β-ketoacids, which are further degraded to methyl ketones via decarboxylation reactions (Montel, Masson, & Talon, 1998). Different microbial groups have been related to their production. 2-Pentanone and 2-heptanone have been associated with the β-oxidation activity of molds growing on the surface of dry cured loins (Muriel, Antequera, Petrón, Andrés, et al., 2004; Pateiro et al., 2015). *S. carnosus* inoculated into sausages increased the levels of methyl-ketones (Fadda, Lebert, Leroy-Sétrin, & Talon, 2002), and methyl ketones (2-pentanone, 2-heptanone, and 2-nonanone) significantly affect the aroma profile (toasted, fruity, and herbal respectively) of fermented sausages (Flores & Olivares, 2014).

2.2.4 Esterase activity

The esterase activity by microbial metabolism forms ester compounds from the combination of an acid and alcohol. Different microbial groups have been characterized by their esterase activity in dry cured meat products like staphylococci (Stahnke, 1994) and yeast (Cano-García, Rivera-Jiménez, Belloch, & Flores, 2014).

Different ethyl esters (ethyl acetate and ethyl butanoate) have been detected in dry cured loins (Muriel, Antequera, Petrón, Andrés, et al., 2004; Soto et al., 2008) and fermented meat products (Flores & Olivares, 2014). Esters derived from short chain fatty acids exhibit fruity notes (Perea-Sanz et al., 2020; Stahnke, 1994), while esters derived from long chain fatty acids produce fatty odor notes in sausages (Flores, 2018).

2.3 Volatile compounds from spices

The volatile compounds such as monoterpenes, sesquiterpenes, and their oxygenated derivatives are derived from the use of spices (Díaz-Maroto, Pérez-Coello, & Cabezudo, 2002). They provide a particular flavor to dry cured meat products. For example, the use of garlic rubbed on the surface of dry-cured loin contributes to the presence of diallyl disulfide (Lorenzo, 2014; Soto et al., 2008), as well as other allyl compounds detected in chorizo (allyl methyl sulphide, diallylsulphide and diallyl disulphide) (Fonseca et al., 2013). Naphthalene has been identified in dry loins as derived from paprika (Muriel, Antequera, Petrón, Andrés, et al., 2004; Muriel, Antequera, Petrón, Martin, et al., 2004), as well as other terpenes (β-caryophyllene, α-cubene and limonene) in foal dry-cured sausages (Lorenzo, Montes, Purriños, & Franco, 2012). Moreover, a high number of terpenes (α-pinene, 3-carene and D-limonene) in sucuk (Sallan, Kaban, & Kaya, 2021), salchichón (Álvarez, Andrade, García, Rondán, & Núñez, 2020) and Cinta Senese dry-fermented

sausages (Aquilani et al., 2018) have their origin in black pepper, which is an important spice in fermented meat products (Table 3).

3. Volatiles and aroma profile: dry cured loins versus dry fermented sausages

The volatiles and aroma profile depend on the volatile extraction technique used (Flores & Corral, 2017). The volatile profile of dry cured loins and dry fermented sausages can be compared exclusively when the same extraction methods have been utilized. A summary of the studies in dry loins and fermented sausages using the headspace (HS) technique, Solid Phase Micro Extraction (SPME) by gas chromatography (GC-MS) with the adsorbent material CAR/PDMS fiber, allowed to compare the volatile profile in both products (Fig. 6). Fig. 6 represents the number of compounds detected in each group based on their probable origin (as described in section 2) or by their chemical class.

The volatiles profile from dry-cured loins is composed mostly of compounds derived from spices, amino acid degradation reactions, sulfur compounds, and those from unknown origin. On the contrary, the volatile profile of fermented sausages is composed of compounds derived mainly from amino acid degradation, lipid autooxidation, and lipid β-oxidation reactions. In addition, the number of ester compounds in both products is the same. Regarding the chemical class of the compounds reported, alcohols represent an important group in both meat products, while aldehydes are an important group mainly in fermented sausages. In comparison, nitrogen compounds constitute minor groups (Fig. 6).

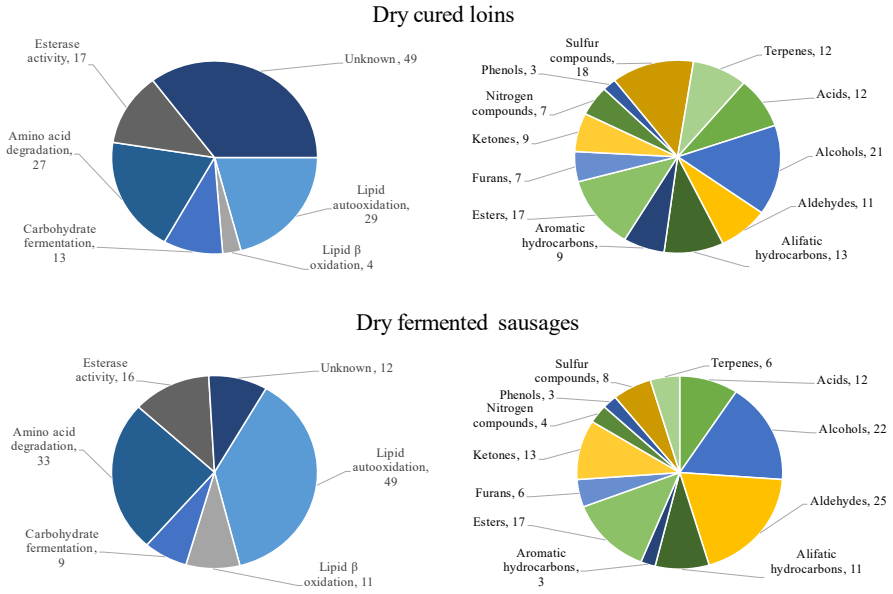

Fig. 6. Volatiles profile (classified according to their origin (left) and chemical class (right)) of dry cured loins (data from Muriel, Antequera, Petrón, Martin, et al. (2004) and Muriel, Antequera, Petrón, Andrés, et al. (2004)) and fermented sausages (data from Corral, Salvador, Belloch, et al. (2015) and Corral, Salvador, et al. (2016)).

In dry cured loins, many volatile compounds have been found and identified (Ventanas, Ventanas, Estévez, & Ruiz, 2010), however, until now no aroma active compounds have been detected by olfactometry analysis (GC-O). In fermented sausages, around 100 aroma active compounds have been identified by different GC-O techniques. The aroma differences between fermented sausages are caused by variations in the concentration of aroma active compounds (Flores & Olivares, 2014). The main aromas in fermented sausages are green, fruity, nutty, savory-meaty, herbal, lactic, and rancid (Flores & Olivares, 2014).

Savory (2-pentyl-furan) and toasted aromas (dimethyl pyrazine, tetramethyl pyrazine,

2AP, 2-acetyl-2-tiazoline) have been reported in dry fermented sausages (Flores & Olivares, 2014). The most likely hypothesis is that savory (meaty) and toasted odors depend on a subtle quantitative balance of different components, because no compound causes the distinct odor of cured meat. However, a systematic study of savory and toasted flavor compounds is necessary in the case of dry cured meat products.

4. Savory (meaty) and toasted aromas formation in dry cured meat products

The concentration of sulfur (savory) and nitrogen (toasted) compounds in dry cured meat products is generally low, in comparison with the ones detected in cooked meat products (Flores, 2018). The reason for the low concentration of these compounds is the mild conditions of processing that restrict their formation. In addition, the small amounts of these compounds, characterized by extremely low flavor-threshold values, can be difficult to isolate and identify (Ramarathnam & Rubin, 1994).

4.1 Savory and toasted aromas

The savory and toasted aromas, with exceptionally low odor threshold values (Table 5), have a high aroma impact on dry cured meat. Savory and toasted aromas have been detected in dry cured meat products (Flores et al., 2021), but their contribution to the aroma has been scarcely studied.

Savory aromas (sulfur compounds) are mainly produced by Maillard reactions between ribose and sulfur-containing amino acids (Mottram, 1998). These compounds have objectionable, sulfur, rotting aromas when sniffed alone, although they play an important role in cooked meat aroma (mainly 9 compounds in Table 5) (Parker, 2017; Thomas et al., 2015). Among these compounds are methanethiol, DMS, DMDS, and DMTS, as well as

methional, which imparts a strong baked or dried potato note. Moreover, these compounds may be involved in interactions that suppress or alter the unpleasant or incongruous aromas or enhance the positive ones (Parker, 2017). Some oxygen-containing heterocyclic compounds also have savory flavor, for example, 2-pentyl-furan has been detected and associated with a savory odor in fermented sausages (Marco et al., 2007) and dry-cured foal loins (Lorenzo & Carballo, 2014).

Toasted aromas are mainly created by nitrogen compounds (pyrroles, pyrazines, thiazoles, thiazolines, etc.), which are produced by Maillard reactions between reducing sugars and nitrogen-containing amino acids. These aromas contribute with roasted, toasted, and cooked meat notes, not only into meat that has been thermally treated, but also to a variety of cooked meals (Parker, 2017). Some oxygen-containing heterocyclic compounds also have toasted flavor, for example, 2-ethyl-furan which has been detected in fermented sausages contributing with toasted odor (Marco et al., 2007).

Moreover, it is important to consider that the presence of proteins and lipids in the meat product matrix affects aroma perception and, therefore, the impact of these minor compounds on the dry cured aroma (Flores, 2023). Evaluating the contribution of savory and toasted volatile compounds to the meat product flavor is a difficult task, which cannot be only based on the use of air or water odor thresholds (Table 5). The use of oil odor thresholds can also be taken into consideration to study the contribution of volatile compounds to the aroma of meat products. This has special significance in fermented sausages, or similar products, which a high fat content. In addition, the mechanisms and factors affecting the formation savory and toasted aromas at mild temperatures are scarcely studied.

Table 5. Thresholds of savory and toasted odors.

	Compounds	Air (mg/m^3)	Water (mg/kg)	Oil (mg/kg)
Savory compounds	Methional	0.000063-0.06	0.0004-0.0018	0.0002
	Methionol	1.71	0.036-0.12323	-
	Methanethiol	0.000000000001-1.1	0.00002-0.004	0.00006
	Dimethyl sulfide (DMS)	0.0003-20.6	0.00008-0.03	0.0012
	Dimethyl disulfide (DMDS)	0.0011-5.6	0.00016-0.012	0.012
	Dimethyl trisulfide (DMTS)	0.00006-0.014	0.00000007-0.00010	0.0025
	2-Methyl-3-furanthiol (MFT)	0.0000025-0.01	0.0000004-0.000007	-
	2-Methyl-3-(methylthio)furan (MMTF)	0.0076-0.2	0.00005-0.025	-
	Methyl 2-methyl-3-furyl disulfide (MMFDS)	0.00002-0.0008	0.000004-0.00001	-
Toasted compounds	2-Acetyl-1-pyrroline (2AP)	0.00002-0.00004	0.000053-0.00012	0.0001
	2-Acetylpyrrole	> 2	58.58525-170	-
	2-Acetylthiazole	0.004-0.0041	0.003-0.01	-
	2-Acetyl-2-thiazoline	0.000016-0.00008	0.00012-0.001	0.0018
	2-Methylpyrazine	> 2	0.06-105	27
	2,3-Diethyl-5-methylpyrazine	0.000009-0.023	0.000031-0.001	0.0005
	2.3-Dimethylpyrazine	0.88-0.90	0.4-2.5	-
	2,5-Dimethylpyrazine	0.17-1.82	0.08-35	2.6-17
	2,6-Dimethylpyrazine	0.25-1.72	0.4-54	1.021-8
	2-Ethyl-3,5-dimethylpyrazine	0.000007-0.00004	0.00004-15	0.0018-0.0075
	2,3,5-Trimethylpyrazine	0.033-0.19	0.023-9	0.29
	Tetramethylpyrazine	0.69-> 2	1.0-10	38

* Data from Van Gemert (2011)

4.2 Production of savory aromas

The savory (sulfur) compounds producing meaty aromas originate via Maillard reactions, specifically from cysteine and ribose; although, there are additional production pathways involving thiamine and methionine (Table 6). The majority of studies involving these Maillard reactions have been done simulating cooking conditions.

There are many savory (sulfur) compounds (Fig. 7) formed in cooked meats (beef, chicken, pork) and other foods (Cerny, 2015). In case of cooked meat, methionol has been found in chicken broths (Jayasena, Ahn, Nam, & Jo, 2013), DMDS in boiled meat (Garbusov, Rehfeld, Wölm, Golovnja, & Rothe, 1976), and MMFDS in roast beef (Rcochat et al., 2007). All these proposed mechanisms have been studied under cooking conditions at high temperatures.

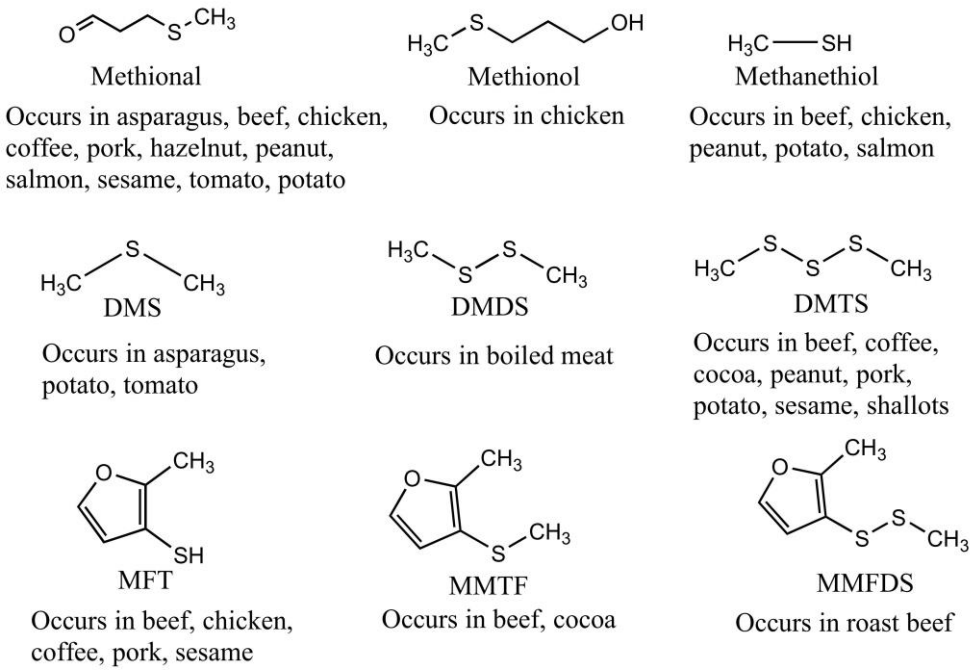

Fig. 7. Chemical structure and distribution of savory aromas in food products.

Table 6. Aroma properties and mechanisms of savory compounds generation.

Compound	Descriptor[a]	Precursor	Reaction type	Reference[b]
Methional	Meaty, savory, brothy	Methionine	Biochemistry or Strecker degradation	1-7
Methionol	Meaty, savory, sulfurous	Methionine	Chemical	4,5
Methanethiol	Sulfurous, savory, meaty	Methionine	Chemical or Biochemistry	1-3,5-7
DMS	Sulfurous, fishy	Methionine	Chemical	3,5
DMDS	Sulfurous, cabbage	Methionine	Chemical	1-3,5-7
DMTS	Sulfurous, savory, meaty	Methionine	Chemical	1-3,5
MFT	Sulfurous, meaty	Thiamine and cysteine	Maillard, degradation or biochemistry	8-11
MMTF	Sulfurous, meaty	Thiamine and cysteine	Chemical	10
MMFDS	Sulfurous, meaty, roasted	Thiamine and cysteine	Chemical	11,12

[a] Odor descriptors from The Good Scents Company database (www.thegoodscentscompany.com).

[b] Reported in: 1. Weimer et al. (1999); 2. Wolle et al. (2006); 3. Schulz & S.Dickschat (2007); 4. Labuda (2009); 5. Varlet & Fernandez (2010); 6. Resconi et al. (2013); 7. Pripi-Nicolau et al. (2000); 8. Tang et al. (2013); 9. Shakoor et al. (2022); 10. Cerny, (2007); 11. Parker, (2017); 12. Thomas et al. (2014).

4.2.1 Chemical and biotransformation reactions from methionine

Methionine degradation, via Strecker reactions (see section 2.1.2) at high temperature, is shown in Fig. 8. Degradation of methionine generates methional, which is then degraded to methanethiol, which easily oxidizes to DMDS. DMDS is transformed into DMTS and DMS. MFT and MMFDS can be also produced by exchange reactions between methanethiol and bis(2-Methyl-3-furyl) disulfide (Parker, 2017).

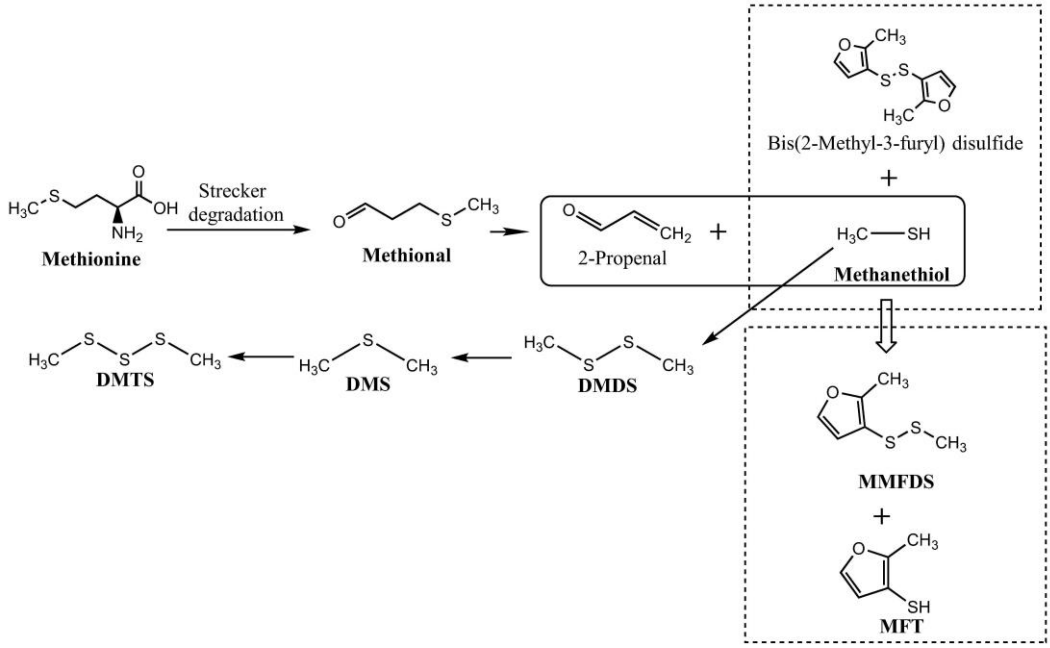

Fig. 8. Formation of MFT from methionine. Adapted from Parker (2017) with the publisher's permission.

Methionine can also produce savory odors through biochemical routes as described in Fig. 9. The action of an aminotransferase converts methionine into 4-methylthio-2-oxobutyric acid (KMBA) (Bonnarme et al., 2001). KMBA is subsequently converted to

methanethiol through a combination of chemical and enzymatic processes; although, KMBA can also be decarboxylated to produce methional. Methional can generate methanethiol by chemical/enzymatic reactions, and methionol by reduction. Oxidation of MTH to produce DMS, followed by DMDS and DMTS has been reported in seafood (Varlet & Fernandez, 2010).

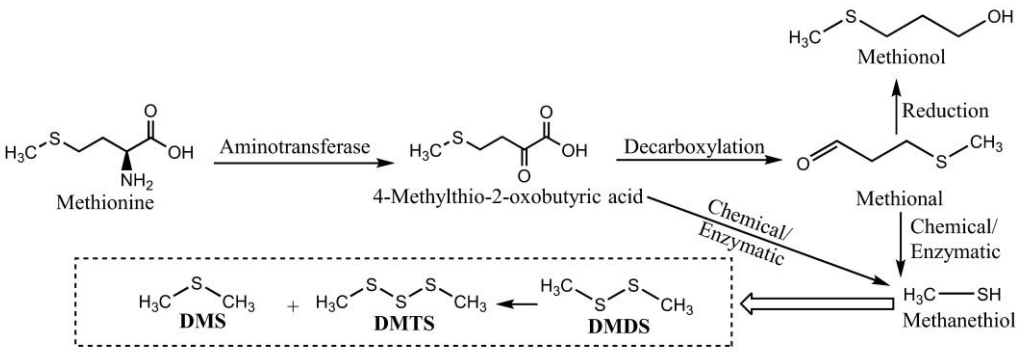

Fig. 9. Generation of meaty odors from enzymatic degradation of methionine. Adapted from Varlet & Fernandez (2010) and Wolle et al. (2006) with the publisher's permission.

4.2.2 Chemical degradation of cysteine

In cooked meat products, cysteine is broken down via Strecker degradation (Resconi et al., 2013), and intermediates (ammonia, hydrogen sulfide, acetaldehyde, and mercaptoacetaldehyde) responsible for producing active meat flavor compounds are produced (Fig. 10).

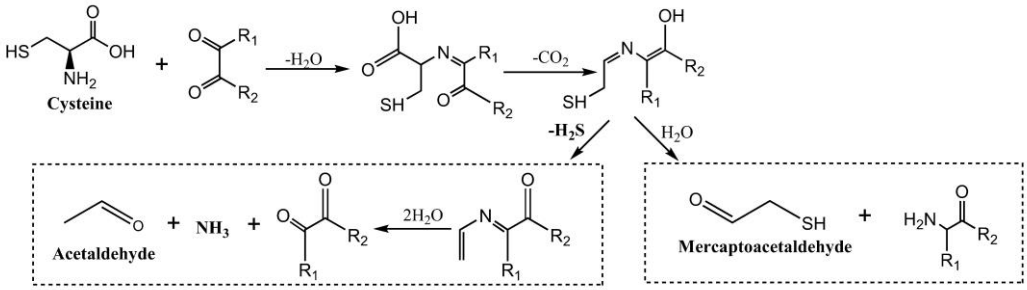

Fig. 10. Strecker degradation of cysteine. Adapted from Resconi et al. (2013) and with the publisher's permission (MDPI under the terms of the CC BY license).

Hydrogen sulfide, which is the product of cysteine degradation, is an important precursor for several savory compounds such as MFT in cooked meat (Fig. 11). A first pathway for MFT generation is the reaction between hydrogen sulfide and norfuraneol leading to MFT generation (Whitfield & Mottram, 1999). The second pathway is an aldol reaction between hydroxyacetaldehyde and mercapto-2-propanone, which is produced between a pyruvaldehyde and hydrogen sulfide, resulting in 4,5-dihydroxy-3-mercapto-2-one, which is then converted to MFT after cyclization and dehydration (Hofmann & Schieberle, 1998b) .

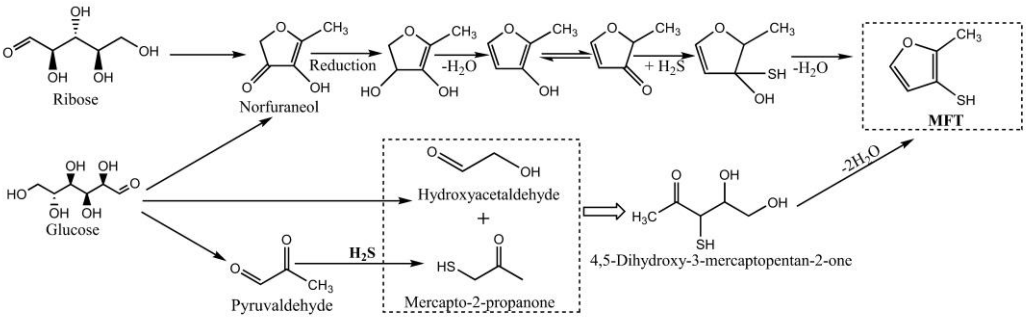

Fig. 11. Formation of MFT from glucose. Adapted from Parker (2017) and with the publisher's permission.

4.2.3 Chemical degradation of thiamine

The thiazole ring of thiamine can be opened, under acidic environment (Parker, 2017) or high temperature conditions (Resconi et al., 2013), releasing 5-hydroxy-3-mercapto-2-pentanone (Fig. 12). The main meaty thiol, MFT, is created by cyclizing 5-hydroxy-3-mercapto-2-pentanone, and after losing water, can then go through an oxidation process (Parker, 2017).

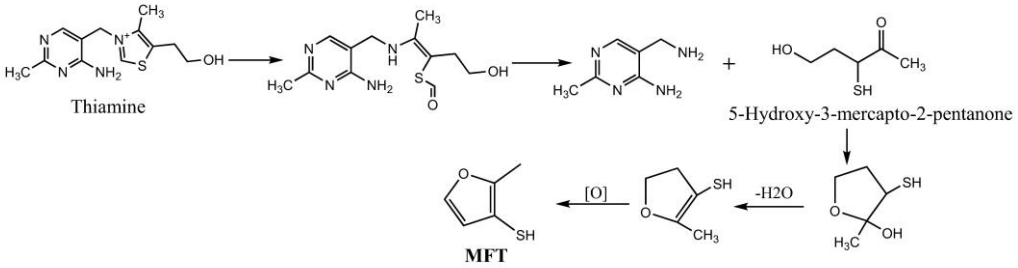

Fig. 12. Chemical degradation of thiamine. Reprinted from Resconi et al. (2013) with the publisher's permission (MDPI under the terms of the CC BY license).

4.3 Production of toasted aromas

Toasted compounds (Table 7) are formed by chemical degradation, via Maillard reaction from several amino acids, mainly Lys, Ala, and Gly, and dipeptides (Shakoor et al., 2022), and biochemical degradation, from the amino acids Pro and Orn (Deshmukh, Khare, & Patra, 2014). All the compounds generated produce odor notes related to nutty, toasted, coffee, and caramel odors (Table 7), although the odor properties among them can vary depending on the chemical structure (Fig. 13). Some toasted compounds can be found in cooked chicken, beef, and Parma ham (Buettner, 2017), whereas pyrazines have been detected in Chinese food (White doufuru, Fried Pork Bundles, Preserved Eggs, Steamed

Quail and Wax Gourd Beverage) (Ho, Zhang, Shi, & Tang, 1989). The compound 2-acetylpyrrole has be found in several food items such as Pan-Fried Steaks (Wei et al., 2022). In order to understand the presence and contribution to meat products of these aromas it is essential to know which mechanisms take part in their formation.

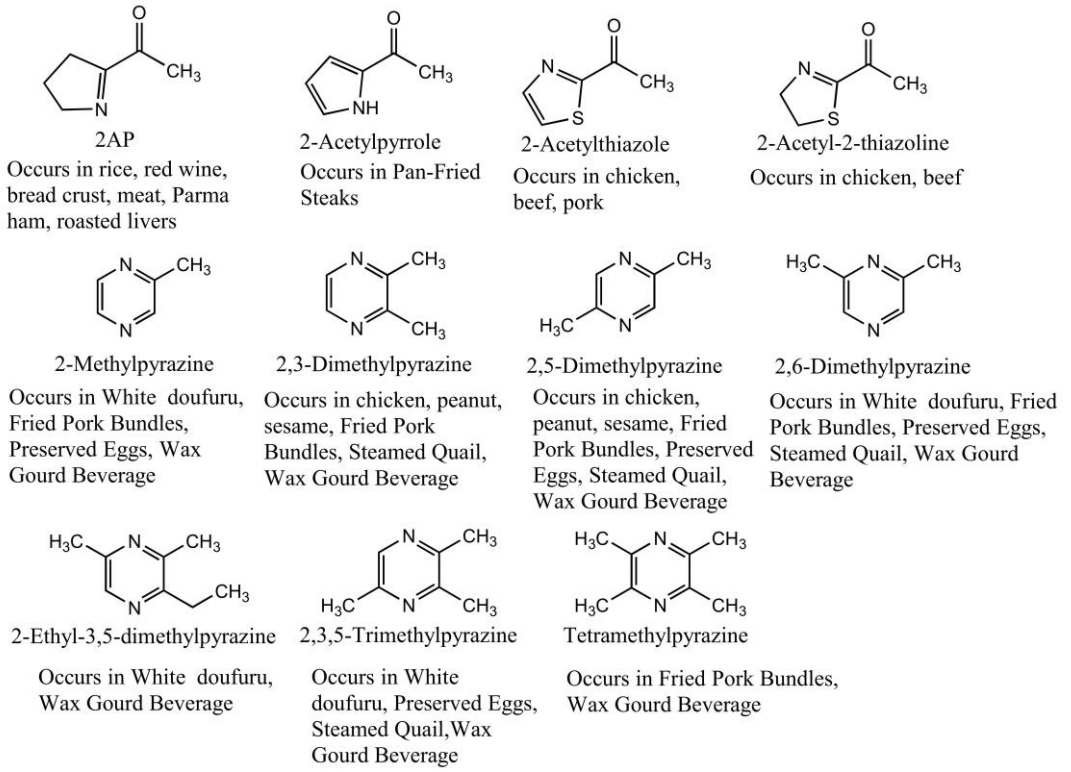

Fig. 13. Chemical structure and distribution of toasted aromas in food products.

Table 7. Aroma properties and production of toasted compounds.

Compounds	Descriptor[a]	Precursor or/and microflora	Reaction type	Reference[b]
2AP	Nutty, roasted, sweet	*Acremonium nigricans*, *Aspergillus awamori*, *Aspergillus oryzae* and *Kluyveromyces marxianus*	Biosynthesis	1
		Pro and Orn, *B. cereus*	Biosynthesis	2,3
		Orn, *Dekkera/Brettanomyces*	Biosynthesis	4
		Lactic acid bacteria	Biosynthesis	5
		Pro, Orn, putrescine rhizobacterial isolates	Biosynthesis	6
		Pro, *Penicillium nalgiovense*	Biosynthesis	7
		Pro, Orn	Maillard	8-11
		Pro and Orn (BAD2, BADH2, OsBADH2, GmAMADH2, CnAMADH2 is Inactive)	Biosynthesis	12-16
2-Acetylpyrrole	Musty, nut, walnut	Ala, Gly, Leu, Val	Maillard	17,18
2-Acetylthiazole	Nutty, popcorn, peanut, roasted, peanut	Cys, Met, Pro, Thr, Pro-Cys	Maillard	19,20,21,22,23
2-Acetyl-2-thiazoline	Corn chip, popcorn, roasted	Cys	Maillard	24
2-Methylpyrazine	Nutty, cocoa, roasted, chocolate, peanut, green	Ala, Cys, Gln, Glu, Gly, Leu, Lys, Met, Phe, Pro, Ser, Thr, Ala-Gln, Ala-Leu, Cys-Met-Thr, Lys-Ala, Lys-Cys, Lys-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Phe, Lys-Ser, Pro-Cys,	Maillard and Biosynthesis	3,21,23,25,26,27,28,29

Table 7 (continued).

2,3-Dimethylpyrazine	Nutty, coffee, peanut, butter, caramel	Ala, Cys, Glu, Gln, Gly, Leu, Lys, Phe, Ser, Ala-Leu, Lys-Ala, Lys-Cys, Lys-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Phe, Lys-Ser	Maillard	23,25,27,29,30
2,5-Dimethylpyrazine	Cocoa, roasted, nutty, beefy	Ala, Cys, Glu, Gln, Gly, Leu, Lys, Met, Phe, Ser, Thr, Ala-Leu, Ala-Gln, Lys-Ala, Lys-Cys, Lys-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Phe, Lys-Ser	Maillard and Biosynthesis	3,23,25,26,27,28,29,31
2,6-Dimethylpyrazine	Cocoa, nutty, roasted, meaty	Ala, Cys, Glu, Gln, Gly, Leu, Lys, Phe, Ser, Ala-Leu, Ala-Gln, Lys-Ala, Lys-Cys, Lys-Leu, Lys-Lys, Lys-Glu, Lys-Gly, Lys-Phe, Lys-Ser	Maillard	21,23,27,28,29
2-Ethyl-3,5-dimethylpyrazine	Burnt, almond, roasted, nutty, coffee	Ala, Leu, Lys, Glu, Gly, Ala-Leu, Lys-Gly, Lys-Leu, Lys-Ser	Maillard	27,28,29
2,3,5-Trimethylpyrazine	Nutty, earthy, roasted, peanut, musty	Ala, Cys, Glu, Gly, Leu, Lys, Phe, Ser, Thr, Ala-Leu, Lys-Ala, Lys-Cys, Lys-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Phe, Lys-Ser	Maillard and Biosynthesis	3,25,27,28,29,30,31
Tetramethylpyrazine	Nutty, musty, chocolate, coffee, cocoa	Leu, Lys, Gly, Thr	Maillard and Biosynthesis	29,30,31

[a] Odor descriptors from The Good Scents Company database (www.thegoodscentscompany.com).

[b] Reported in: 1. Rungsardthong & Noomhoom (2005); 2. Romanczyk et al. (1995); 3. Adams & De Kimpe (2007); 4. Grbin (1998); 5. Costello et al. (2001); 6. Deshmukh et al. (2015); 7. Stahnke (2000); 8. Hofmann & Schieberle (1998a); 9. Blank et al. (2003); 10. Schieberle (1990); 11. Rückriemen et al. (2015); 12. Bradbury et al. (2008); 13. He et al. (2015); 14. Niu et al. (2008); 15. Arikit et al.

(2011); 16. Dumhai et al. (2019); 17. Cho et al. (2010); 18.Yaylayan & Keyhani (2000); 19. Liu et al., (2022); 20. Wang et al., (2021); 21. Yu & Zhang (2010); 22. Yu et al. (2012); 23. Xu et al. (2021); 24. Hofmann & Schieberle (1995); 25. Yu et al. (2017); 26. Cerny & Guntz-Dubini (2006); 27. Zou et al. (2018); 28. Amrani-Hemaimi et al. (1995); 29. Van Lancker et al. (2010); 30. Guerra & Yaylayan (2012); 31. Zhang et al. (2019).

4.3.1 Formation of pyrrole and pyrroline compounds

Proline and ornithine have been proposed as the amino acids that interact with 2-oxopropanal (intermediate of the Maillard reaction) to create 2AP (Fig. 14), being 4-aminobutyraldehyde and 1-pyrroline the intermediates (Schieberle, 1990). The compound 2AP can quickly oxidize to 2-acetylpyrrole at room temperature (Daygon et al., 2017).

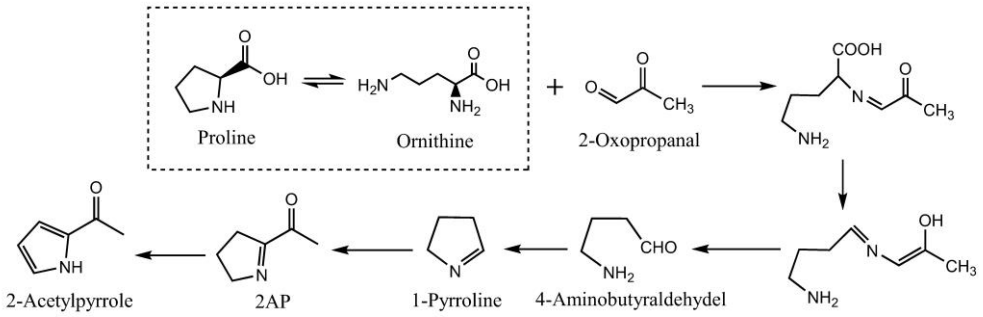

Fig. 14. Formation of 2AP and 2-acetylpyrrole by chemical degradation. Adapted from Schieberle (1990) with the publisher's permission.

Biochemical degradation pathways mediated by molds have also been proposed for the production of 2AP. *Penicillium nalgiovense*, growing on the surface of Mediterranean sausages, has been identified as the producer of 2AP (Stahnke, 2000). In wine, *Dekkeral/Brettanomyces* produced 2AP when ornithine was the sole nitrogen source. In rice, the absence of enzymatic activity betaine aldehyde dehydrogenase 2 (BAD2) plays a key role in 2AP synthesis (Bradbury et al., 2008) produced by proline catabolism (Fig. 15).

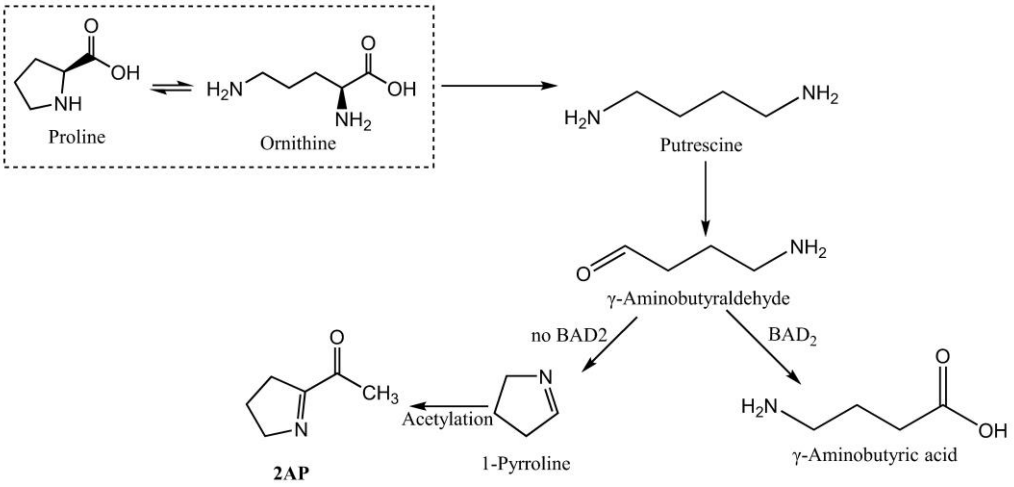

Fig. 15. Pathway from proline to 2AP by biotransformation. Adapted from Bradbury et al (2008) with the publisher's permission.

4.3.2 Formation of thiazole and thiazoline compounds

Formation of these compounds has been commonly studied under high temperature conditions. Liu et al. (2022) found that 2-acetylthiazole was the dominant product from the reaction of cysteine with 2-oxopropanal (intermediates of the Maillard reaction) (Chansataporn et al., 2019) (Fig. 16).

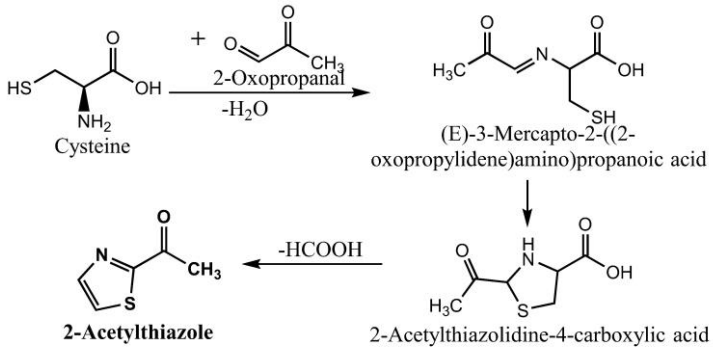

Fig. 16. Pathway converting cysteine into 2-acetylthiazole. Adapted from Liu et al. (2022) with the publisher's permission.

Hofmann & Schieberle (1995) established that 2-(l-Hydroxyethyl)-4,5-dihydrothiazole (HDT) is an important intermediate in the formation of 2-acetyl-2-thiazoline (Fig. 17), which is produced from cysteamine at 6 °C previously decarboxylated from cysteine (Pinto et al., 2009).

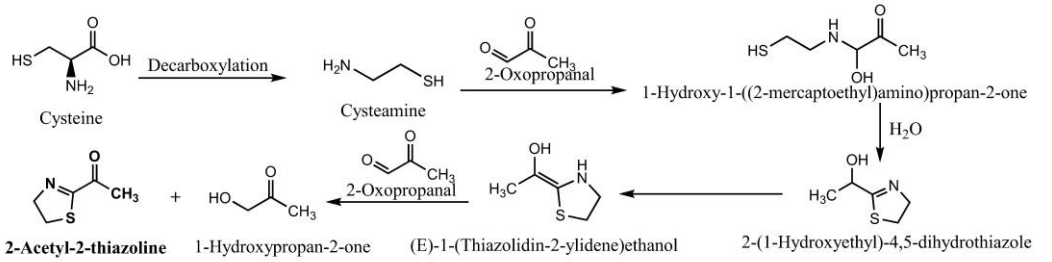

Fig. 17. Pathway from cysteine to 2-acetyl-2-thiazoline. Adapted from Hofmann & Schieberle, (1995) and Pinto et al. (2009) with the publisher's permission.

4.3.3 Formation of pyrazine compounds

Pyrazines are a family of nitrogen-containing heterocyclic compounds (Fig. 13), which are recognized as crucial aromas in raw, high temperature treated, or fermented foods (Zhang et al., 2019). Pyrazines are formed from the carbonyl-amine condensation of two amino ketones, throughout Strecker degradation of amino acids (Gly, Ala, Cys, Thr and so on) with α-dicarbonyl compounds (from Maillard reaction) to form a dihydropyrazine (Parker, 2015), which is subsequently oxidized (Fig. 18).

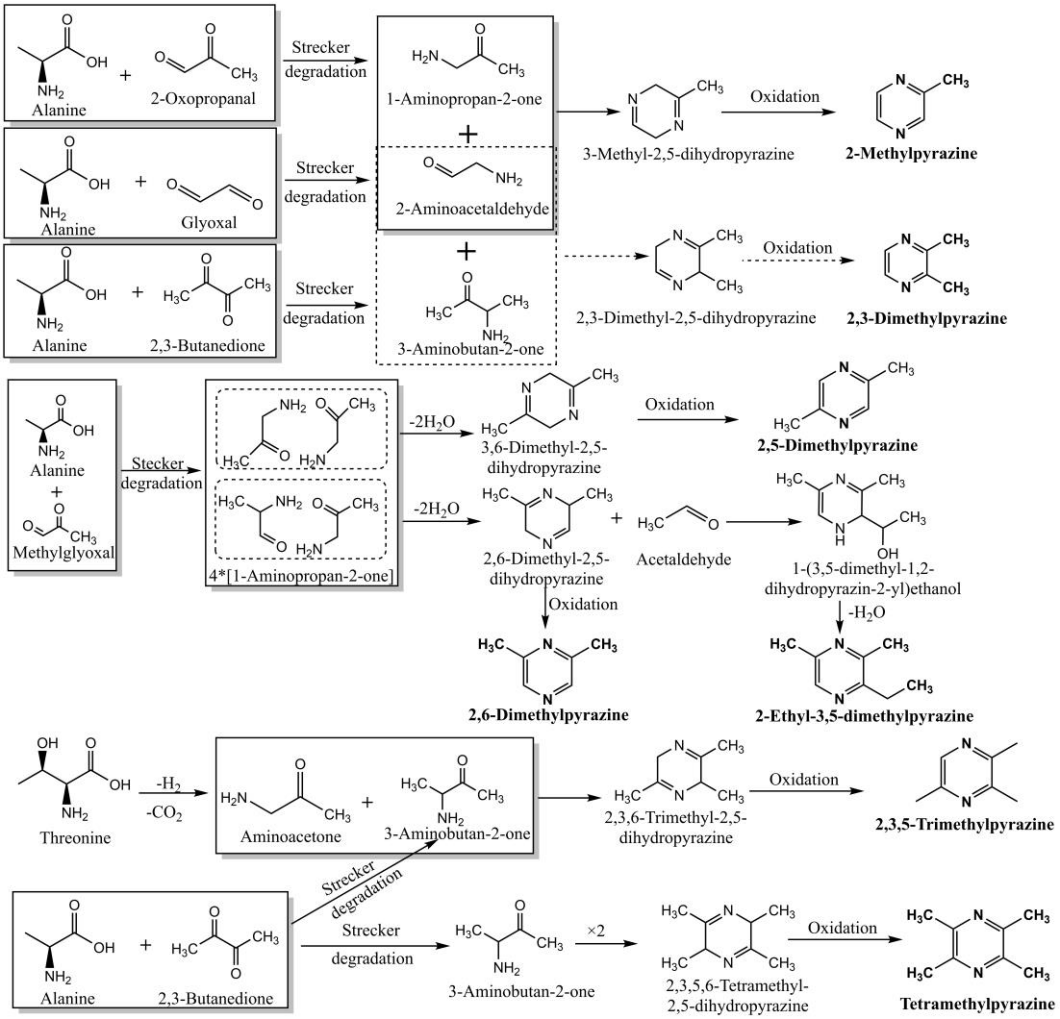

Fig. 18. Formation of pyrazines. Adapted from Glomb (2017), Parker (2015), Zhang et al. (2019) and Divine et al. (2012) with the publisher's permission.

4.4 The role of yeast in the production of savory and toasted aromas

Different yeast genera have been found in dry cured meat products, among these we can find *Debaryomyces*, *Candida*, *Yarrowia*, *Pichia*, *Rhodotorula*, *Cryptococcus, Hansenula, Torulopsis,* and *Trichosporon* (Gardini et al., 2001; Garcia-Cano et al., 2013). The yeast population in these products varies depending on the stage of processing; however, the most frequent and dominant yeast throughout the fermentation and maturation

process of dry cured meat products, such as dry cured loins and dry-fermented sausages, is *Debaryomyces hansenii*. Several studies in dry-cured ham (Andrade et al., 2009), dry cured loins (Martín, Córdoba, Benito, Aranda, & Asensio, 2003), dry-cured "lacón" (Purriños, Carballo, & Lorenzo, 2013), and dry-fermented sausages (Andrade et al., 2010; Bolumar et al., 2006; Corral, Salvador, Belloch, et al., 2015; Flores et al., 2004; Patrignani et al., 2007; Perea-Sanz et al., 2020) have demonstrated their influence on the development of characteristic flavor.

Among *D. hansenii* strains isolated from different foods sources, the production of savory aromas (sulfur compounds) from sulfur amino acids, and toasted aromas varied substantially (Table 8) and, at least some of these compounds were produced by yeast metabolism (Perea-Sanz, Peris, Belloch, & Flores, 2019). Taking into account that many studies about the mechanisms involved in the production of savory and toasted aromas have been done in cooked meat products, it is necessary to understand the contribution of yeasts to their formation in dry cured meat products.

Studies performed to determine the contribution of yeast to the formation of savory compounds have been done on different types of media and meat products (Table 8). The particular interest is the presence of methanethiol and DMDS, which were mainly promoted by inoculated *D. hansenii* on culture medium (Casado, Córdoba, Andrade, & Rodríguez, 2011) or meat model systems (Cano-García, Rivera-Jiménez, et al., 2014). Yeast inoculation promoted the generation of more savory flavors and also more toasted flavors in inoculated dry cured meat products (Table 8). The compound 2-acetylthiazole was found more frequently in fermented sausages.

Table 8. Savory and toasted compounds detected in model systems and dry cured products inoculated with *D. hansenii*.

	Sample	Model minces	Culture medium	Culture media	Meat model systems	Meat model medium	Dry-cured ham
	Reference[a]	1	2	3	4	5	6
Savory compounds	Methional					+	+
	Methionol				+	+	
	Methanethiol		+	+	+		+
	DMS						+
	DMDS	+	+	+	+		+
	DMTS	+		+	+		
	MFT						
	MMFDS						
Toasted compounds	2AP						
	2-Acetylthiazole						
	2-Methylpyrazine						
	2,5-Dimethylpyrazine					+	
	2,6-Dimethylpyrazine						
	2-Ethyl-3,5-dimethylpyrazine						+
	2,3,5-Trimethyl-pyrazine						
	Tetramethylpyrazine						

Table 8 (continued).

	Sample	Dry-cured Iberian ham	Pork loins	Iberian cured pork loin	Slow dry-cured fermented sausages	Dry fermented sausages	Dry fermented sausages
	Reference[a]	7	8	9	10	11	12
Savory compounds	Methional	+		+		+	+
	Methionol	+			+	+	
	Methanethiol	+	+	+	+	+	+
	DMS		+			+	
	DMDS	+	+		+	+	+
	DMTS	+			+		+
	MFT						+
	MMFDS						
Toasted compounds	2AP						+
	2-Acetylthiazole					+	+
	2-Acetyl-2-thiazoline						
	2-Methylpyrazine						
	2,5-Dimethylpyrazine						+
	2,6-Dimethylpyrazine		+			+	
	2-Ethyl-3,5-dimethylpyrazine						
	2,3,5-Trimethyl-pyrazine						
	Tetramethylpyrazine		+				

[a] Reported in:1. Olesen & Stahnke (2000); 2. Casado et al. (2011); 3. Cano-García et al. (2013); 4. Cano-García, Rivera-Jiménez, et al. (2014); 5. Gong et al. (2023); 6. Martín et al., (2006); 7. Andrade et al. (2009); 8. Martín et al. (2003); 9. Ramos-Moreno et al. (2019); 10. Cano-García, Belloch, et al. (2014); 11. Corral, Salvador, Belloch, et al. (2015); 12. Perea-Sanz et al. (2020).

4.5 Savory and toasted odors in dry cured loins and dry fermented sausages

In summary, savory and toasted aromas detected in dry cured loins and dry fermented sausages can be originated from a variety of precursors as shown in sections 4.2 (Table 6) and 4.3 (Table 7). Up to now, 12 compounds have been detected in both loins and sausages, and 9 compounds have been exclusively found in sausages (Table 9) indicating a contribution of the microbiota metabolism (Table 4).

As observed in Table 9, MFT, MMTF, and MMFDS have been only detected in sausages. Several pathways for MFT generation from methionine, cysteine and thiamine have been found (section 4.2.1, 4.2.2 and 4.2.3). Moreover, MFT can act as precursor of MMTF and MMFDS (Kim, Cadwallader, Kido, & Watanabe, 2013). However, improved analytical techniques and methodologies are required to determine the presence of MFT in dry cured meat products like loins.

Regarding the presence of toasted compounds, 2AP, 2-acetylpyrrole, 2-acetylthiazole and 2-acetyl-2-thiazoline have been exclusively found in sausages (Table 9). The synthesis of these compounds (section 4.3.1 and 4.3.2) may be produced by chemical or microbial origins, although it is necessary to improve the analytical methodologies to determine their presence in dry cured meat products like loins.

Regarding the contribution of these savory and toasted odors to the aroma of dry loins and fermented sausages, Table 9 shows a variety of descriptors for these compounds. The reasons for this variety may be due to the sensory technique used and the panel training, as well as the effect of the meat product matrix on aroma perception.

In summary, it is necessary to unveil the mechanisms involved in the production of savory and toasted aromas in dry cured meat products, and the factors affecting their production, as well as the contribution of yeasts.

Table 9. Savory and toasted odors in loins and sausages.

Compounds	Loins		Sausages	
	Reference[a]	Odor descriptor[b]	Reference[a]	Odor descriptor[a]
Savory compounds				
Methional	1, 4	Savory, meaty, brothy	12-17, 21-26, 28, 30, 31	Meat broth[13], cooked potato[15-17,21-23,25,26], meaty[30]
Methionol	2, 3	Savory, soup, cooked	19-23, 31	
Methanethiol	1, 2, 3, 9	Savory, meaty	12, 13, 16, 18-20, 22, 24, 26, 28, 30	Rotten[13,16,18,26,30], cauliflower[13]
DMS	1, 2, 3, 7	Sulfurous, fishy	22, 24	
DMDS	1, 2, 3, 8, 9	Sulfurous, cabbage	10-12, 14, 16, 17, 19-22, 24-28, 30-31	Garlic[10], toasted[17,26], rotten[25]
DMTS	2, 3	Sulfurous, savory, meaty	16, 18-22, 24, 25, 30, 31, 33	Onion[18,25], sulfur[25,30]
MFT			23, 30, 31	Fatty[31], sulfur[31]
MMTF			31	
MMFDS			30, 31	Meaty[30], rotten[30]
Toasted compounds				
2AP			15, 22-26, 28, 30, 31, 33	Roasty[15,30], savoury[22,25], toasted[23,26]
2-Acetylpyrrole			17, 23, 31, 32	Roasted nuts[17], toasted[23]
2-Acetylthiazole			34	
2-Acetyl-2-thiazoline			30	
2-Methylpyrazine	1,6	Popcorn, roasted, grain,	22-24, 32	Cooked potato[22], toasted[23]
2,3-Diethyl-5-methylpyrazine			15	Earthy[15]
2.3-Dimethylpyrazine			32	

Table 9 (continued).

2,5-Dimethylpyrazine	1	Cocoa, roasted, nutty	10, 18, 32, 34	
2,6-Dimethylpyrazine	2-4,6	Cocoa, nutty, roasted	10,11,17,21-24,26-28,32,33	Toasted[23], acid[23]
2-Ethyl-3,5-dimethylpyrazine	1	Burnt, roasted, nutty	15, 34	Earthy[15]
2,3,5-Trimethylpyrazine	1-6	Nutty, roasted, peanut	21, 27, 29, 34	Coffee[21], chocolate[21]
Tetramethylpyrazine	1-3,6,9	Nutty, musty	17, 21, 29	Toasted[17,21], roasted nuts[21]

[a] Reported in:1. Martín et al. (2003); 2. Muriel, Antequera, Petrón, Martin, et al. (2004); 3. Muriel, Antequera, Petrón, Andrés, et al. (2004); 4. Ramírez & Cava (2007); 5. Campus et al. (2008); 6. Soto et al. (2008); 7. Ventanas et al. (2008); 8. Ventanas et al. (2010); 9. Belloch et al. (2021); 10. Schmidt & Berger (1998); 11. Meynier et al. (1999); 12. Marco et al. (2006);13. Marco et al. (2007); 14. Marco et al. (2008); 15. Söllner & Schieberle (2009); 16. Olivares et al. (2011); 17. Gianelli et al. (2011); 18. Corral et al. (2013); 19. Cano-García, Rivera-Jiménez, et al. (2014); 20. Cano-García, Belloch, et al. (2014); 21. Corral, Salvador, & Flores (2015); 22. Corral, Salvador, Belloch, et al. (2015); 23. Corral, Leitner, et al. (2016); 24. Corral, Salvador, et al. (2016); 25. Corral et al. (2018); 26. Perea-Sanz et al. (2018); 27. Bis-Souza et al. (2019); 28. Perea-Sanz et al. (2019); 29. Xiao et al. (2020); 30. Perea-Sanz et al. (2020); 31. Flores et al. (2021); 32. Belleggia et al. (2022); 33. Liu et al. (2021); 34. Mi et al. (2021).

[b] Odor descriptors from The Good Scents Company database (www.thegoodscentscompany.com).

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JUSTIFICATION AND OBJECTIVES

JUSTIFICATION AND OBJECTIVES

The meaty (savory) flavor is composed of hundreds of volatile compounds, but only a minor part of which are responsible for the characteristic odor. In contrast to cooked meat products, savory and toasted odor compounds in dry cured meat products are not completely characterized. In addition, the presence of volatile compounds (nitrogen- and sulfur- containing compounds) at low levels but characterized by low odor thresholds make them as essential contributors to the cured flavor as they may complement or mask the flavor of other compounds. However, until now scarce studies have demonstrated the contribution of nitrogen and sulfur precursors to the formation of toasted and savory odour notes in dry cured products. Therefore, the main hypothesis is that the degradation and transformation pathways of nitrogenous amino acids (Pro and Orn) and amino acids containing sulfur atoms like cysteine, cystine and methionine, as well as the vitamin thiamine may impact the toasted and savory odour notes in dry cured products like fermented sausages and dry cured loin. Therefore, this project proposes the exploration of the effect of meat products ingredients and the study of the reaction mechanisms involved in the production of toasted and savoury aromas in dry cured meat products.

The objectives of the Ph.D. project are:

1.Investigation of the mechanisms involved in the chemical degradation and biotransformation of amino acids (Pro and Orn) and thiamine during the processing of dry-cured meat products (dry cured loins versus dry fermented sausages) and their transformation into nitrogen and sulfur compounds contributing the toasted and savory aromas. The results are presented in **chapter 1**, **chapter 2** and **chapter 3**.

2. Study the role of the Maillard reaction for aroma generation in model systems under mild conditions simulating the dry cured meat process, and also the biotransformation of amino acids in model systems as affected by fat and yeast inoculation for aroma enhancement. The results are presented in **chapter 4** and **chapter 5**.

RESULTS

Chapter 1

Aroma enhancement in dry cured loins by the addition of nitrogen and sulfur precursors

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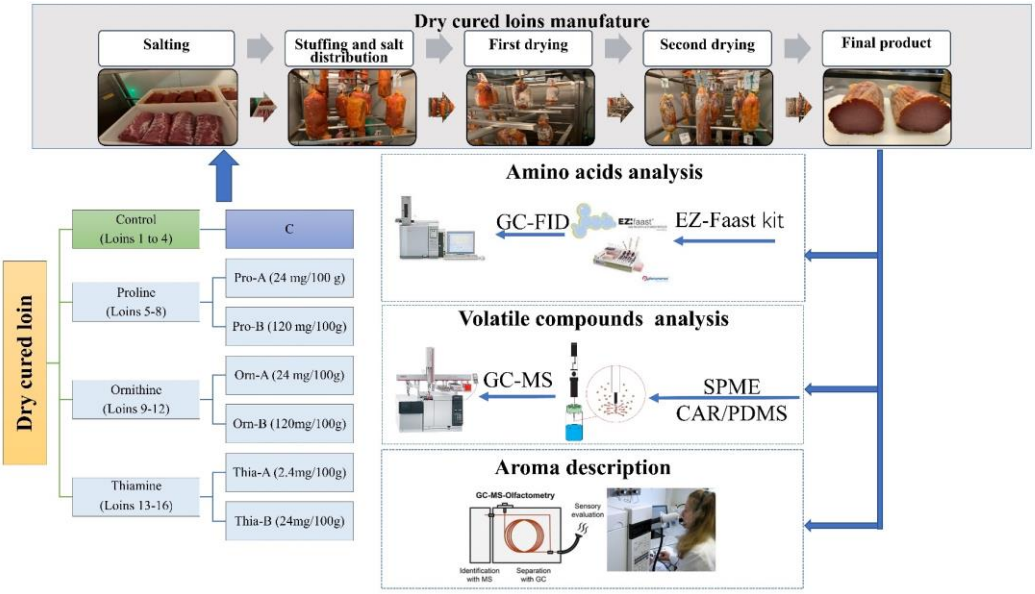

Aroma enhancement in dry cured loins by the addition of nitrogen and sulfur precursors

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ABSTRACT

Dry cured loins containing nitrogen (proline and ornithine) and sulfur (thiamine) compounds as precursors of aroma compounds at two concentration levels were manufactured. The effect of precursor addition on the microbiology and chemical parameters of loins was studied together with the aroma study performed by olfactometry and Free Choice Profile sensory analyses. Addition of precursors did not affect the microbial and chemical parameters, while aroma was affected when precursors were added at the highest level. The dry loin aroma profile was mainly composed by compounds 3-methylbutanal, methional, ethyl 3-methylbutanoate, 3-methylbutanoic acid, 1-octen-3-ol, 2-acetyl-1-pyrroline and 2-acetylpyrrole that contribute to musty, cooked potatoes, fruity, cheesy, mushroom, roasted and meaty odor notes. Proline and ornithine supplementation modified the loins aroma profile producing toasted odors, while the effect of thiamine supplementation on the aroma was revealed by the presence of sulfur derived compounds (methional and 2-methyl-3-(methylthio)furan) that contribute to the “cured meat odor”.

Keywords: proline; ornithine; thiamine; aroma; dry cured loins

1. Introduction

Dry cured loins are made using the whole or caudal half of the *Longissimus thoracis et lumborum* muscle of all animals. Pork loins are rubbed by a mixture of curing agents (salt, nitrite and nitrate) and several spices (paprika and garlic) onto the surface of the loin pieces, subsequently stuffed into natural casings and ripened-dried for several months until the product achieves a specific aroma, texture and taste (Muriel, Ruiz, Martin, Petron, & Antequera, 2004; Ventanas, Estevez, Tejeda, & Ruiz, 2006). Their sensory properties are highly affected by genetic factors, feeding practices and ingredients used during product manufacture (Ventanas, Ventanas, Estévez, & Ruiz, 2010) while the length of the dry cured process is essential for aroma development (Ventanas, Estevez, Andrés, & Ruiz, 2008).

The reduction of the use of curing agents in meat product formulations as well as the purpose to reduce the time for processing has directed to the search for alternatives to enhance the dry cured aroma. However, aroma development in dry cured products is based on complex biochemical reactions occurring during ripening that result in a large number of volatile compounds which contribute to their characteristic flavor (Flores, 2018). Studies in dry cured loin flavor have elucidated the volatile profile, but little information about its aroma contribution is available (Belloch, Neef, Salafia, López-Diez, & Flores, 2021; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, & Ruiz, 2004; Ventanas et al., 2010). Most of the identified volatile compounds are derived from garlic and paprika together with other compounds derived from microbial activity such as alcohols and ethyl esters (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004). However, differences in volatile compounds between the surface and within the loin revealed the presence of oxidation and Strecker degradation volatile compounds on the loin surface together with volatile compounds derived from yeasts and moulds metabolism (Martín, Córdoba, Benito, Aranda, & Asensio, 2003; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, & Ruiz, 2004). Although dry cured process is performed at mild temperatures, Maillard reactions between nitrogen compounds from proteolysis and lipid oxidation products have been attributed as one of the main chemical

reactions contributing to the aroma profile of dry cured meats (Li, Belloch, & Flores, 2021; Ventanas et al., 1992) .

The presence of nitrogen- and sulfur-containing compounds in dry cured meat products point to their important role in meat product flavor because of their olfactory properties and low odor thresholds (Flores, 2018; Flores, Perea-Sanz, López-Díez, & Belloch, 2021). Nitrogen volatile compounds with nut and toasted odor notes such as 2-acetyl-1-pyrroline, 2,6-dimethyl pyrazine and tetramethyl pyrazine have been detected in dry cured ham (Liu et al., 2014; Théron et al., 2010) and dry cured loins (Martín et al., 2003; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, & Ruiz, 2004; Soto et al., 2008). Noteworthy is the contribution of the compound 2-acetyl-1-pyrroline to nuts and toasted corn (popcorn) odor notes in dry cured products (Söllner & Schieberle, 2009), although their formation may have several origins, by degradation of amino acids such as proline (Pro) and ornithine (Orn), by microbial metabolism or through the Maillard reactions (Blank, Devaud, Matthey-Doret, & Robert, 2003; Schieberle, 1990). On the other hand, sulfur volatile compounds are mainly produced from sulfur amino acids (Corral, Leitner, Siegmund, & Flores, 2016; Perea-Sanz, Peris, Belloch, & Flores, 2019) and thiamine in meat products (Flores, 2018). Some volatile compounds derived from thiamine like 2-methyl-3-furanthiol, 2-methyl-3-(methyldithio)furan and 2-acetyl-2-thiazoline have already been identified in dry cured ham (Carrapiso, Ventanas, & García, 2002; Song & Cadwallader, 2008) but nothing has been reported in dry cured loins.

Taking into account all this information, determination of the factors that affect the degradation and transformation of nitrogen and sulfur volatile precursors such as Pro, Orn and thiamine, in dry cured products is essential for its ability to generate key aroma compounds. Therefore, the aim of this work was to elucidate the role of nitrogen and sulfur precursors added during the salting stage of the dry cured loin process, and its effect on aroma development. In addition, the possible production of amines from the decarboxylation of amino acids was determined in dry cured loins as well as the content of the precursors added.

2. Materials and methods

2.1. Manufacture of dry cured loins

Seven different dry cured loin formulations differing in their nitrogen (Pro and Orn) and sulfur (thiamine) precursors concentrations were prepared using four loins as replicates for each formulation. A total of twenty-eight loins (*Longissimus thoracis et lumborum*) were used in the experiments. The seven formulations were: control treatment without precursors (C), two formulations with Pro at 24 mg/100 g meat (Pro-A) and 120 mg/100 g meat (Pro-B), two containing Orn at 24 mg/100 g meat (Orn-A) or 120 mg/100 g meat (Orn-B) and two formulations with thiamine at 2.4 mg/100 g meat (Thia-A) and 24 mg/100 g meat (Thia-B).

The processing of dry cured loins started with the salting stage by rubbing the loin's surface with the mixture of salt (NaCl 2.4%), additives (sodium nitrite 150 mg/kg, potassium nitrate 150 mg/kg, and sodium ascorbate 0.5 g/kg), paprika (*Capsicum anuun*) (9 g/kg) and the amount of precursor indicated for each formulation. Then the loins were kept at refrigeration temperature for 6 days, stuffed into collagen casings (10 cm diameter) and kept 7 additional days at 4 °C. Afterwards, the loins were dried at 11 °C temperature and relative humidity 70-80%. Weight losses were controlled during drying for 68 days until a final mean weight loss of 39% was reached. A slice from the fresh loins (50 g) was taken for the analysis of the thiamine before the salting stage. At the end of drying (68 d), samples were taken aseptically as follows: a slice (25 g) from the central part of each loin for microbial analysis, 2 or 3 slices up to 100 g were minced and directly used for moisture, water activity (a_w) and pH analysis. The remaining minced loin was vacuum packed and frozen at −20 °C for physicochemical analyses (TBARS, thiamine, protein, free amino acids (FAA) and biogenic amines). Colour was measured directly on a slice and two additional slices were wrapped in aluminium foil, vacuum packed and stored at −80 °C for volatile and aroma analyses. Finally, several dry cured loins from each formulation were stored at 4 °C until sensory analysis.

2.2. Measurement of physicochemical parameters

Measurements of pH, a_w, colour (CIE L*, a*, b*), moisture, and protein were done as described in (Perea-Sanz, Montero, Belloch, & Flores, 2018). Lipid autoxidation was measured by the thiobarbituric acid reactive substances (TBARS) method and results expressed as mg malonaldehyde (MDA)/kg (Perea-Sanz et al., 2018).

2.3. Analysis of thiamine

Analysis of thiamine in loins was carried out as described in Flores et al. (2021). Minced loins (1.5 g) were used and extracted with 15 ml HCl 0.1 N and then heated at 90 °C for 30 min to extract the thiamine. The sample was centrifuged at 10000 g for 20 min and the supernatant filtrated through a 0.45 μm nylon filter. Only fresh loin meat samples were previously treated with 0.1 g of clara-diastase (Sigma, Missouri, USA) added to 5 ml of supernatant, incubated for 18 h at 37 °C with agitation, and afterwards centrifuged at 10000g for 20 min and filtered. An aliquot of the filtrate was used for the derivatization of thiamine. For this, potassium ferrocianure 1% was added to filtrates to achieve the oxidation of thiamine to its fluorescent derivative thiochrome. The analysis of thiamine fluorescence derivative was done as described in Flores et al. (2021) using an Agilent 1100 HPLC series chromatograph with fluorescence detector (Agilent, Palo Alto, CA, USA) and a reverse phase column XDB-C18 column. Thiamine derivative was detected at excitation and emission wavelengths of 360 and 430 nm, respectively. A calibration curve was used, and results were expressed as mg thiamine/100 g of loin.

2.4. Microbial analysis

Viable counts of mesophilic bacteria (TMB), lactic acid bacteria (LAB), Gram positive cocci (GC+), enterobacteriaceae (EB), yeasts and moulds (YM) were carried out as described in Perea-Sanz et al. (2018).

2.5. Biogenic amines analysis

Biogenic amines were analysed by ion-pair reverse-phase high performance liquid chromatography, as described in Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou (1996). Briefly, this method is based on the formation of ion-

pairs between biogenic amines, previously extracted with 0.6 M perchloric acid from 5 g of loins, and octanesulfonic acid present in the mobile phase. Amine separation was performed using a Agilent 1100 HPLC series chromatograph with fluorescence detector (Agilent, Palo Alto, CA, USA) and a C-18 reverse phase column, followed by a post-column derivatization with o-phthalaldehyde and spectrofluorimetric detection (ex: 340 nm and em: 445 nm). Calibration curves for each amine were used and results expressed as mg/kg.

2.6. Analysis of Free amino acids (FAA)

Analysis of FAA was carried out as described in Flores et al. (2021) using the Phenomenex kit (Phenomenex, Torrance, CA, USA). Five grams of minced loins were extracted by homogenisation with 20 ml of 0.1 M HCl. After centrifugation at 10000 g for 20 min, the supernatant was diluted 1/7 (v/v) and analysed following the manufacturer instructions using norvaline as internal standard. The derivatized amino acids were analysed in a GC Agilent Technologies 7890B equipped with an automatic liquid sampler 7693A and a flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). The retention times and peak area of the reference compounds (Phenomenex standard solutions and Sigma, St. Louis, MO) were used for the identification and quantification. Calibration curves for each amino acid were obtained and results expressed in mg/100g of loin.

2.7. Analysis of volatile compounds

Analysis of volatile compounds was carried out by headspace (HS) solid-phase microextraction (SPME) with an 85 μm carboxen/poly-dimethylsiloxane (CAR/PDMS) fibre (Supelco, Bellefonte, USA) as described in Corral, Salvador, & Flores (2013) with slight modifications. The volatile compounds were analysed using a gas chromatograph with a quadrupole mass detector (Agilent HP 7890 series II, HP 5975C, Hewlett- Packard, Palo Alto, USA) and equipped with an autosampler (MPS2 multipurpose sampler, Gerstel, Mülheim an der Ruhr, Germany). Four grams of loins supplemented with 0.75 mg BHT to avoid oxidation, were placed into a headspace vial and incubated at 37 °C for 30 min. The extracted volatile compounds were adsorbed into the fibre for 2 h at 37 °C and desorbed

in the injection port of the GC–MS for 5 min at 240 °C in splitless mode. The volatile compounds were separated using a DB-624 capillary column (30 m, 0.25 mm i.d., film thickness 1.4 μm, J&W Scientific, Agilent Technologies, USA) using the conditions described by (Corral, Leitner, et al., 2016) with slight modifications. The GC oven temperature was 40 °C for 13 min, ramped to 100 °C at 3 °C/min and held at 100 °C for 5 min, then to 150 °C at 4 °C/min and to 210 °C at 5 °C/min and finally, held at 210 °C for 10 min. The MS interface temperature was set to 240 °C. The compounds were identified in full scan mode and by comparison with mass spectra from the library database (NIST'05), with linear retention indices calculated using the series of C5-C22 n-alkanes (Van Den Dool & Dec.Kratz, 1963) and with authentic standards. The quantitation was done in SCAN mode using either total or extracted ion current (TIC or EIC) on an arbitrary scale and data was expressed as abundance units (AU x 10^6).

In addition, compounds identification was confirmed by analysed the extracted volatile compounds using a DB-5 capillary column (30 m, 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific, Agilent Technologies, USA) installed in an Agilent 8890 gas chromatograph with a 5977 mass selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA). The SPME extraction conditions and desorption into GC-MS were those indicated for the analysis of compounds using the column DB-624. Volatile analysis was done using helium with a constant flow rate of 1 mL/min. The GC oven temperature was held at 15 °C for 5 min, ramped to 20 °C at 2 °C/ min, held at 20 °C for 5 min, ramped to 100 °C at 3 °C/min and to 170 °C at 8 °C/min and to 230 °C at 10 °C/min, and held at 230 °C for 5 min. Electron-impact mass spectra were generated at 70 eV. The analysis was done in full scan mode and linear retention indices were calculated using the series of C5-C22 n-alkanes for the DB-5 column. Compounds were identified by matching with the reference mass spectra in the NIST'17 mass spectral library, and by comparison of their LRI with the NIST Chemistry WebBook database (Linstrom, 2003) and with authentic standards.

2.8. Olfactometry analysis

The analysis of aroma compounds extracted by SPME using 4 g of loins was carried out in a gas chromatograph (Agilent 6890, USA) equipped with an FID detector and a sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) as described in Olivares, Navarro, & Flores (2011). The aromatic impact of each volatile compound was carried out using the detection frequency method (Pollien et al., 1997). Five trained panellists evaluated the odors from the GC-effluent and a total of 14 assessments were undertaken. Aroma compounds were identified by comparison with mass spectra, using linear retention indices of authentic standards injected in GC–MS and GC-O and by coincidence of the assessor's descriptors with those in the Fenaroli's handbook of flavour ingredients (Burdock, 2010).

2.9. Sensory analysis

A group of 21 consumers, 16 women and 5 men with ages ranging from 21 to 55 years old, were chosen based on its ability to describe the presence of odors in dry cured meat products and took part in a Free Choice Profile (FCP) analysis as reported by Corral, Salvador, et al. (2016). The first session consisted on the generation of the odor terms used by each consumer describing the differences among dry cured loins aroma by Repertory Grid Method. The samples were presented in plastic petri dishes and each consumer described the similarities and differences among the aroma of samples within each dish in their own terms. This method was repeated until all samples were tried. In the second session, sensory differences among dry cured loin formulations (Control, Pro-B, Orn-B and Thia-B loins) were analysed by FCP (Williams & Langron, 1984). Each consumer evaluated his/her own list of odor terms by rating the intensity for each sample using 10 cm unstructured scale with the extremes “Not perceived” and “Intense”. A quarter of loin slice (5 mm thickness) was randomly labelled with three-digit codes and presented on a closed petri-dish at room temperature and panellists were instructed to smell the aroma. The presentation order was balanced to avoid a serving order effect.

2.10. Statistical analysis

Data were analysed using the Generalized Linear Model (GLM) procedure of statistical software (XLSTAT 2018, Addinsoft, Barcelona, Spain). The model included the effect of

loin formulations as fixed effect and replicates were considered as random effect. When a significant effect of the treatment group was detected ($P<0.05$), least squares means (LSM) were compared using Tukey test. Principal components analysis (PCA) was plotted to evaluate the relationships among loin formulations, physicochemical and microbiological parameters and aroma compounds at the end of the ripening process. A Generalized Procrustes Analysis (GPA) was applied to the Free Choice Profile data.

3. Results

3.1. Physicochemical characteristics of dry cured loins

The physicochemical properties of the manufactured dry cured loins analysed at the end of process (68 d) are shown in Table 1. No differences in composition, pH, aw and colour parameters among loins formulations were detected except for the oxidation value (TBARS). The control loins showed higher significant TBARS values than loins manufactured with the highest content of precursors Pro-B and Orn-B. Regarding thiamine concentration, no differences were observed among the fresh loins. A high thiamine content was detected in dry loins supplemented with thiamine, although the differences between loin formulations were only significant at the highest thiamine content (Thia-B).

3.2. Microbiology of dry cured loins

The results of microbiological counts are shown in Supplementary Table 1. The microbiological counts of dry cured loins were not affected by the addition of nitrogen (Pro and Orn) and sulfur (thiamine) precursors.

3.3. FAA content of dry cured loins

The concentration of FAA in the dry cured loins is presented in Table 2. As expected, significant differences were detected in the concentration of Pro and Orn in loins supplemented with these precursors. In addition, the highest supplementation of Pro and Orn added during salting resulted in the highest FAA concentration at the end of drying (Pro-B and Orn-B). Regarding the other FAA, proteolysis was similar among loins except for a highest proteolysis activity observed in Orn-A loins. This formulation showed a highest significant content of several amino acids (Ala, Ile, Asn, and Glu).

Table 1. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the physicochemical parameters of dry cured loins.

	C[1]		Pro-A		Pro-B		Orn-A		Orn-B		Thia-A		Thia-B		RMSE[2]	*P*[3]
Moisture (%)	56.68		55.94		56.86		56.46		54.59		53.90		54.91		2.11	ns
Protein (%)	36.98		37.77		37.71		37.70		36.88		39.08		38.90		1.45	ns
pH	5.88		5.82		5.79		5.72		5.76		5.69		5.70		0.08	ns
a_w	0.942		0.943		0.943		0.940		0.938		0.940		0.941		0.005	ns
TBARS (mg DA/kg)	0.38	a[4]	0.25	ab	0.21	b	0.36	a	0.21	b	0.27	ab	0.25	ab	0.06	**
L*	44.58		46.36		42.06		44.19		44.40		45.71		44.58		2.32	ns
a*	17.19		16.92		17.96		17.77		17.25		16.80		16.64		0.69	ns
b*	8.94		9.03		8.24		8.96		9.78		9.28		9.26		0.82	ns
Thiamine (fresh)[5](mg/100g)	0.68		0.54		0.54		0.70		0.70		0.65		0.65		0.10	ns
Thiamine (mg/100g)	0.95	b	0.77	b	0.75	b	0.96	b	0.94	b	3.50	b	29.00	a	2.39	***

[1] C: Control loins without precursors, addition of precursors: proline and ornithine at 24 mg/100 g (Pro-A and Orn-A) and 120 mg/100 g (Pro-B and Orn-B), and thiamine at 2.4 mg/100 g (Thia-A) and 24 mg/100 g (Thia-B). [2] RMSE: root mean square error. [3] *P* value of formulation effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] Different letters in the same row indicate significant differences at $P < 0.05$ among formulations. [5] Thiamine content measured in the fresh loins.

Table 2. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the concentration of free amino acids (mg/100g) in dry cured loins.

FAA	C[1]		Pro-A		Pro-B		Orn-A		Orn B		Thia-A		Thia-B		RMSE[2]	*P*[3]
Ala	152.29	ab[4]	149.81	b	162.90	ab	185.93	a	159.65	ab	165.69	ab	149.57	b	14.69	*
Gly	79.50		78.65		85.09		94.71		79.56		85.34		79.02		8.08	ns
Val	99.53		92.06		93.26		102.88		91.30		101.67		94.13		8.15	ns
β-Ala	65.32		66.06		49.00		53.69		49.16		57.39		49.99		9.81	ns
Leu	115.12		107.38		121.00		134.20		110.81		130.39		115.88		13.59	ns
Ile	65.05	ab	61.51	b	68.18	ab	76.43	a	65.10	ab	73.56	ab	67.94	ab	5.46	*
Thr	71.98		65.54		70.61		82.22		68.31		73.22		67.36		7.64	ns
Ser	114.81	ab	100.86	b	119.12	ab	142.07	a	114.65	ab	125.06	ab	119.77	ab	14.96	*
Pro	82.10	bc	98.85	b	178.99	a	87.03	bc	77.76	bc	73.99	bc	68.75	c	11.99	***
Asn	32.53	ab	26.44	b	31.94	ab	42.85	a	29.34	b	31.77	ab	29.17	b	5.60	*
Asp	56.62		55.24		46.39		45.99		44.50		51.44		47.23		7.13	ns
Met	31.61	ab	27.14	b	29.10	b	32.35	ab	34.15	ab	38.36	a	28.86	b	3.93	*
Hyp	7.13		6.12		6.11		8.13		6.28		5.86		5.94		1.37	ns
Glu	119.20	ab	128.23	ab	131.14	ab	137.50	a	115.75	ab	129.89	ab	106.09	b	11.35	*
Phe	62.35		56.54		64.97		71.31		58.24		68.16		63.25		6.89	ns
Gln	89.12		93.51		96.95		102.85		94.99		85.50		84.37		9.96	ns
Orn	7.26	c	13.05	bc	7.86	c	21.40	b	74.27	a	7.15	c	5.43	c	5.59	***
Lys	146.51		141.22		158.87		173.76		148.39		155.59		145.79		17.60	ns
His	203.80		189.94		141.04		161.40		154.92		177.30		141.71		33.19	ns
Tyr	63.90		56.31		60.27		65.91		55.62		64.14		58.09		6.83	ns
Trp	20.51		19.25		20.46		21.89		19.83		20.79		19.33		1.72	ns
Car	444.32	bc	382.07	c	449.72	bc	532.84	abc	588.42	ab	629.31	a	555.88	abc	75.11	**
Total Aac	1700.57		1652.18		1759.63		1861.58		1668.96		1738.61		1573.24		120.27	ns

[1] C: Control loins without precursors, addition of precursors: proline and ornithine at 24 mg/100 g (Pro-A and Orn-A) and 120 mg/100 g (Pro-B and Orn-B), and thiamine at 2.4 mg/100 g (Thia-A) and 24 mg/100 g (Thia-B). [2] RMSE: root mean square error. [3] *P* value of formulation effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] Different letters in the same row indicate significant differences at $P < 0.05$ among formulations.

3.4. Biogenic amines in dry cured loins

As Supplementary Table 2 shows, histamine and phenethylamine were present in dry cured loins at very low levels, while high levels of polyamines (spermidine and spermine) were detected. No differences in histamine and phenethylamine were observed among loin formulations. However, Orn-B loins showed the highest significant content of putrescine, spermidine and spermine and Pro-A loins in cadaverine.

3.5. Volatile compounds in dry cured loins

A total of seventy-seven volatile compounds were identified in dry cured loins and two of them (2-methoxyphenol and 2-methylphenol) were tentatively identified (Table 3). Volatile compounds were grouped in chemical families including aldehydes (13), alcohols (15), alkanes (10), ketones (10), esters (9), acids (8), nitrogen compounds (6) and sulfur compounds (6). The most abundant groups of compounds extracted using SPME fibre were alcohols (60.75–70.84%) and acids (9.74–14.12%) since aldehydes and ketones represented 5.05–14.99% and 3.36–7.22% of the total chromatographic area, respectively. Individually, the major compounds were ethanol, 3-methyl-1-butanol, acetic acid and 2-methyl-1-butanol.

In the aldehydes group, acetaldehyde, 3-methylbutanal, 2-methylbutanal and hexanal were the most abundant. The main differences among loin formulations were detected in hexanal, heptanal, octanal and nonanal compounds which were significantly detected in the largest abundance in Pro-B and Orn-A loins. In addition, 2-methylbutanal was particularly abundant in Orn-A loins, whereas 3-methylbutanal was clearly abundant in Thia-B loins although no significant due to the high standard error of the mean.

Concerning the alcohols, ethanol had the uppermost abundance in all loins and only two compounds (1-hexanol and 1-octen-3-ol) were in high significant abundance in Pro-B loins. Among alkanes, toluene was the most abundant specially in Thia-B loins even though there were no significant differences among loin formulations. Concerning ketones, the abundance of butyrolactone was highest in Thia-B loins while Pro-A loins contained a significant abundance of 2,3-butanedione and 3-hydroxy-2-butanone. An assortment of

ester compounds, mainly ethyl esters, were found; however, no differences among loin formulations were detected. In contrast, a high number of acids were detected, although only three of them (propanoic, butanoic and octanoic acids) showed differences among loin formulations. The control loins had the lowest content of propanoic and octanoic acids in comparison to the large abundance of these compounds in Orn-B loins. In addition, Thia-B loins contained a significant amount of octanoic acid in comparison with the control loins.

Relating to nitrogen compounds, which represented a low percentage of the total chromatographic area (0.40-0.77%), the most abundant were 1-methyl-1H-pyrrole, pyridine and tetramethyl pyrazine. No differences among loin formulations were detected, except in the case of Orn-B loins containing a significant abundance of pyridine.

Sulfur compounds represented 1.94-3.95% of the total chromatographic area. Among these compounds, carbon disulfide showed the highest levels. Only methional and 2-methyl-3-(methylthio)furan appeared significantly different among loin formulations, and the highest levels of abundance appeared in Thia-B loins.

3.6. Aroma characteristics of dry cured loins

Gas chromatography-olfactometry (Table 4) of the extracted volatile compounds using SPME revealed the presence of 24 aroma active zones in dry cured loins which were identified except for 2 odor zones. Most of the aromas detected were sweet, fruity and vegetal odors except for the roasted odors produced by 2-acetyl-1-pyrroline, tetramethyl pyrazine and 2-acetyl-pyrrole. In addition, savoury odors produced by methionol, grass odors produced from aldehydes, and cheesy odors produced by acids were described. The odor described as fried corn was only tentatively identified as a 2-methylphenol. Nevertheless, the most important aroma compounds detected were those showing the highest DF (detection frequency) values (DF>10). They were 2,3-butanedione, 3-hydroxy-2-butanone, 3-methylbutanal, methional, ethyl 3-methylbutanoate, 3-methylbutanoic acid, 1-octen-3-ol, 2-acetyl-1-pyrroline and 2-acetylpyrrole.

Table 3. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the volatile compounds (expressed as AU × 10^6) detected in the headspace of dry cured loins.

Compound	LRI[1]	RI[2]	C[3]		Pro-A		Pro-B		Orn-A		Orn-B		Thia-A		Thia-B		RMSE[4]	*P*[5]
Acetaldehyde	467	a	8.25		4.18		6.56		9.55		7.10		10.36		9.60		3.13	ns
2-Methylpropanal	598	a	1.46	ab[6]	1.05	b	1.62	ab	2.43	a	1.59	ab	1.82	ab	1.61	ab	0.48	*
Butanal	624	a	0.15	ab	0.13	b	0.23	a	0.19	ab	0.22	ab	0.16	ab	0.16	ab	0.04	*
3-Methylbutanal	693	a	24.30		16.79		42.18		27.36		22.33		37.55		97.26		33.35	ns
2-Methylbutanal	702	a	8.78	ab	7.13	b	10.55	ab	15.01	a	10.00	ab	9.56	ab	10.45	ab	2.94	*
Pentanal (44)[7]	739	a	0.26	abc	0.18	c	0.42	a	0.39	ab	0.35	abc	0.21	bc	0.28	abc	0.08	**
Hexanal	842	a	8.63	b	7.37	b	15.12	a	15.15	a	12.08	ab	7.05	b	9.48	ab	2.46	***
Heptanal (70)	942	a	0.24	bc	0.28	bc	0.57	a	0.58	a	0.44	ab	0.20	c	0.33	bc	0.08	***
Benzaldehyde	1019	a	1.92		2.03		1.86		1.62		1.35		1.75		1.86		0.44	ns
Octanal	1049	a	2.57	b	2.98	ab	4.69	a	4.57	a	3.55	ab	2.02	b	3.02	ab	0.81	***
Benzeneacetaldehyde (91)	1109	a	0.22		0.21		0.25		0.39		0.22		0.84		0.79		0.37	ns
Nonanal	1150	a	4.50	c	5.37	bc	9.13	a	8.90	a	7.77	ab	4.35	c	7.23	abc	1.32	***
Decanal	1258	a	0.71		0.73		0.54		0.49		0.53		0.70		0.67		0.23	ns
Aldehydes			***62.33***	***ab***	***48.92***	***b***	***93.65***	***ab***	***86.07***	***ab***	***67.90***	***ab***	***76.23***	***ab***	***141.48***	***a***	***37.75***	*
Ethanol	511	a	483.60		430.16		473.83		475.10		460.37		458.26		464.77		47.22	ns
1-Propanol	613	a	4.09		4.10		4.98		3.30		7.22		5.55		2.70		3.25	ns
2-Butanol (59)	645	a	0.16		0.14		0.14		0.17		0.13		0.12		0.13		0.03	ns
2-Methyl-1-propanol	683	a	15.26		17.83		11.89		16.29		19.69		11.05		10.73		5.69	ns
2-Pentanol	756	a	2.74		2.49		2.27		1.90		2.48		1.56		1.52		0.59	ns
3-Methyl-1-butanol	796	a	76.29		110.27		87.27		103.06		95.26		59.71		57.63		24.23	ns
2-Methyl-1-butanol	798	a	39.28		55.14		44.53		53.00		52.14		30.37		24.38		14.52	ns
1-Hexanol	924	a	1.93	ab	2.24	a	2.12	a	1.66	ab	1.64	ab	1.38	b	1.70	ab	0.29	**
1-Octen-3-ol	1032	a	1.84	ab	1.63	ab	2.42	a	1.66	ab	1.41	b	1.33	b	1.59	ab	0.40	*
2-Ethyl-1-hexanol	1083	a	2.89		2.95		2.56		2.13		1.93		2.17		2.00		0.63	ns
Phenol	1113	a	2.30		2.87		2.72		2.86		2.24		2.16		2.55		0.44	ns
2-Methoxyphenol	1160	b	1.39		1.82		1.24		1.44		0.94		1.24		1.10		0.40	ns
2-Methylphenol	1171	b	0.87		1.02		0.89		0.87		0.62		0.74		0.72		0.26	ns
Phenylethylalcohol	1195	a	1.78		2.69		2.70		1.56		0.92		1.17		1.27		0.99	ns

Table 3 (continued).

4-Methylphenol (107)	1199	a	0.32		0.47		0.46		0.41		0.36		0.40		0.41		0.07	ns
Alcohols			***634.93***		***634.32***		***600.23***		***664.36***		***646.66***		***577.55***		***573.42***		***78.00***	***ns***
Pentane	501	a	0.88		0.48		2.99		1.78		0.66		0.78		0.72		1.09	ns
Hexane	601	a	3.54		1.48		1.95		2.42		2.09		2.01		3.63		1.16	ns
Toluene	790	a	15.50		16.85		14.18		17.74		28.18		24.97		35.52		15.14	ns
Octane	801	a	2.89	b	2.79	b	4.53	ab	6.56	a	3.08	b	4.88	ab	4.16	ab	1.19	**
Ethylbenzene (91)	885	a	0.12		0.12		0.16		0.13		0.14		0.15		0.16		0.04	ns
o-Xylene	918	a	0.57		0.66		0.70		0.64		0.63		0.60		0.70		0.19	ns
Styrene	920	a	0.89		0.84		1.74		0.88		0.53		0.55		2.32		1.43	ns
Decane	1000	a	0.47		0.42		0.37		0.30		0.27		0.41		0.42		0.14	ns
Limonene	1045	a	0.98	ab	1.09	ab	1.14	ab	1.21	ab	0.94	b	1.60	a	1.55	ab	0.27	*
p-Cymene (119)	1051	a	0.06	b	0.08	ab	0.10	ab	0.10	ab	0.09	ab	0.10	ab	0.13	a	0.03	ns
Alkanes			***22.55***		***24.23***		***28.02***		***31.75***		***36.53***		***34.33***		***48.85***		***15.91***	***ns***
Acetone	532	a	11.95		12.53		12.78		10.00		15.94		19.19		9.84		4.07	ns
2,3-Butanedione	628	a	1.46	b	3.44	a	1.30	b	1.04	b	1.70	ab	2.08	ab	1.29	b	0.81	**
2-Butanone	633	a	4.31		5.13		4.33		4.61		4.54		5.98		3.70		0.96	ns
2-Pentanone	735	a	4.75		5.69		4.93		4.69		4.56		6.62		3.65		1.21	ns
3-Hydroxy-2-butanone	784	a	13.11	ab	37.51	a	9.28	b	11.11	b	12.66	ab	19.42	ab	11.12	b	9.84	*
2-Heptanone	935	a	4.16		2.92		2.40		2.53		2.00		3.06		2.53		1.10	ns
6-Methyl-5-hepten-2-one	1037	a	0.91		1.12		0.94		0.92		0.78		0.93		0.96		0.21	ns
Butyrolactone	1025	a	2.46	ab	2.14	ab	3.00	a	1.79	ab	1.31	b	1.65	ab	3.06	a	0.66	**
Acetophenone	1134	a	0.63		0.78		0.60		0.54		0.40		0.58		0.55		0.23	ns
2-Nonanone (58)	1142	a	0.27		0.10		0.07		0.05		0.06		0.14		0.13		0.11	ns
Ketones			34.14	b	69.93	a	40.14	ab	37.71	ab	44.15	ab	57.77	ab	31.69	b	14.17	*
Ethyl acetate	637	a	14.03		16.41		12.85		15.22		17.13		10.27		12.66		7.44	ns
Ethyl propanoate	745	a	1.17		1.17		1.31		1.31		1.27		1.10		1.36		0.33	ns
Ethyl butanoate	833	a	3.16		3.93		4.19		3.17		3.49		4.39		3.68		0.94	ns
Ethyl 2-hydroxypropanoate	868	a	3.09		3.73		3.52		3.08		3.11		3.24		3.20		0.58	ns
Ethyl 2-methylbutanoate	879	a	2.01		2.62		2.25		1.92		1.83		1.77		1.83		0.87	ns
Ethyl 3-methylbutanoate (88)	883	a	0.31		0.43		0.38		0.33		0.41		0.34		0.38		0.23	ns
2-Methyl-1-butanol acetate	908	a	0.47		0.82		0.43		0.56		0.58		0.31		0.45		0.23	ns

Table 3 (continued).

Ethyl hexanoate (88)	1030	a	0.10		0.09		0.10		0.11		0.08		0.09		0.09		0.02	ns
Ethyl octanoate	1230	a	0.72		0.69		0.51		0.61		0.39		0.44		0.36		0.24	ns
Esters			***25.05***		***29.86***		***25.54***		***26.14***		***28.17***		***21.97***		***23.80***		***8.69***	***ns***
Acetic acid	717	a	75.53		117.34		74.90		81.00		97.39		70.10		82.93		28.11	ns
Propanoic acid	812	a	1.80	b	2.27	ab	2.34	ab	2.17	ab	2.64	a	2.11	ab	2.09	ab	0.31	*
2-Methylpropanoic acid	865	a	2.02		3.54		3.88		2.57		4.91		3.66		2.95		1.94	ns
Butanoic acid (60)	892	a	2.37	ab	4.01	ab	4.01	ab	2.19	b	2.88	ab	4.44	a	3.43	ab	0.95	*
3-Methylbutanoic acid (60)	941	a	1.14		2.28		1.69		1.36		2.00		1.93		1.72		1.07	ns
2-Methylbutanoic acid	946	a	3.09		5.58		4.54		3.63		4.05		3.89		3.79		1.80	ns
Hexanoic acid	1077	a	1.33		1.64		1.59		1.51		1.50		1.53		1.79		0.46	ns
Octanoic acid (60)	1266	a	0.04	b	0.05	ab	0.05	ab	0.08	a	0.07	a	0.07	ab	0.08	a	0.01	**
Acids			***87.30***		***136.71***		***92.99***		***94.50***		***115.44***		***87.72***		***98.79***		***27.74***	***ns***
1-Methyl-1H-pyrrole (81)	785	a	1.72		1.57		1.31		3.00		1.46		1.48		4.43		2.39	ns
Pyridine (79)	787	a	1.88	ab	1.04	ab	0.72	b	0.73	b	2.09	a	1.15	ab	1.76	ab	0.47	*
2,6-Dimethyl pyrazine (108)	945	a	0.07		0.09		0.13		0.13		0.22		0.21		0.24		0.14	ns
2-Acetyl-1-pyrroline (83)	962	a	0.01		0.01		0.01		0.01		0.01		0.01		0.01		0.01	ns
Tetramethyl pyrazine	1117	a	1.18		1.26		1.34		1.26		1.09		1.16		1.30		0.25	ns
2-Acetylpyrrole (94)	1156	a	0.03		0.04		0.04		0.04		0.03		0.03		0.04		0.01	ns
Nitrogen compounds			***4.82***		***4.05***		***3.64***		***4.43***		***4.45***		***3.96***		***7.23***		***2.44***	***ns***
Methanethiol (47)	473	a	0.02		0.01		0.01		0.02		0.02		0.01		0.02		0.01	ns
Dimethyl sulfide (62)	534	a	0.19		0.14		0.08		0.18		0.08		0.16		c		0.07	ns
Carbon disulfide	541	a	24.75		26.60		36.36		27.88		26.53		16.40		18.08		9.09	ns
Methional (104)	968	a	0.05	ab	0.03	b	0.05	ab	0.06	a	0.03	b	0.04	ab	0.08	a	0.02	*
2-Methyl-3-(methylthio)furan (128)	978	a	0.04	b	0.05	b	0.04	b	0.04	b	0.03	b	0.07	b	0.22	a	0.04	***
Methionol (106)	1064	a	0.05		0.10		0.06		0.07		0.03		0.04		0.04		0.03	ns
Sulfur compounds			***25.11***		***20.16***		***36.35***		***28.16***		***26.69***		***17.04***		***18.71***		***10.05***	***ns***
Total compounds			***896.24***		***968.19***		***920.57***		***973.12***		***969.98***		***876.58***		***943.95***		***119.83***	***ns***

[1] Linear retention indices (LRI) of the compounds eluted from the GC–MS using a DB-624 capillary column (J&W Scientific 30 m×0.25 mm i.d. ×1.4 μm film thickness). [2] RI: reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentatively identification by mass spectrum. [3] C: Control loins without precursors, addition of precursors: proline and ornithine at 24 mg/100 g (Pro-A and Orn-A) and 120 mg/100 g (Pro-B and Orn-B), and thiamine at 2.4 mg/100 g (Thia-A) and 24 mg/100 g (Thia-B). [4] RMSE: root mean square of errors. [5] *P* value of formulation effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P <$

0.05, ns: $P > 0.05$. [6] Different letters in the same row indicate significant differences at $P < 0.05$ among formulations. [7] Target ion used to quantify the compound when the peak was not completely resolved.

Table 4. Odor-active compounds identified in the headspace of dry cured loins.

Compound	DB-624		DB-5		GC-O description	DF[4]	Previously found in dry loins[5]
	LRI GC-O[1]	LRI GC-O std	LRI GC-MS[2]	LRI GC-MS std[3]			
Ketones							
2,3-Butanedione	629	626	-	-	Sweet, lactic	10	1,2
3-Hydroxy-2-butanone	777	777	730	721	Sweet, strawberry, fruity	10	2-7
6-Methyl-5-hepten-2-one	1031	1024	990	989	Earthy, vegetable, spices	7	6
Aldehydes							
3-Methylbutanal	693	692	650	652	Sour, grass, musty	11	1-9
Hexanal	835	836	808	807	Fresh cut grass	8	1-9
Sulfur compounds							
Methional	970	971	-	-	Cooked potatoes	12	1,9
2-Methyl-3-(methylthio)furan	976	981	951	952	Meaty, sour	4	
Methionol	1060	1054	985	980	Potato, savoury	4	3,4
Esters							
Ethyl butanoate	818	825	814	808	Sweet, fruity	8	3-5,7
Ethyl 3-methylbutanoate	871	871	866	859	Sweet, fruity, strawberry	11	1,3,4,7,9
Ethyl octanoate	1230	1226	1198	1196	Sweet, cooked ham, savoury	8	3,4,9
Acids							
Butanoic acid	855	859	840	844	Cheesy, pungent	8	1-6,8,9
3-Methylbutanoic acid	916	915	880	877	Cheesy, sour, pungent	12	1-4,6,9
Alcohols							
2-Methyl-1-propanol	676	676	629	621	Vinegar, sweet, fresh	5	1-4,6

Table 4 (continued).

1-Octen-3-ol	1019	1018	985	985	Mushrooms	11	1-4,8
2-Methylphenol	1178	-			Toasted bread, fried corn	8	1
4-Methylphenol	1199	1196	1083	1084	Unpleasant, sweaty	8	5
Phenylethyl alcohol	1208	1208	1111	1110	Vegetal, floral	5	
Alkanes							
Limonene	1043	1048	1025	1025	Grass, green, fresh, flowers	6	2-4,6,9
Nitrogen compounds							
2-Acetyl-1-pyrroline	964	963	-	-	Roasted, toasted corn	12	
Tetramethyl pyrazine	1118	1122	-	-	Toasted, roasted	5	1,3,4,5
2-Acetylpyrrole	1168	1156	1065	1064	Roasted, toasted corn, nuts, meaty	13	
Unknown							
unknown	1279	-	-	-	Toasted, unpleaseant grass	7	
unknown	1281	-	-	-	Toasted, meaty	7	

[1] LRI: Linear retention indices of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (J&W Scientific 60 m×0.32 mm i.d.×1.8 μm film thickness). [2] LRI: Linear retention indices of the compounds eluted from the GC–MS using a DB-5 capillary column (J&W Scientific 30 m× 0.25 mm i.d. × 0.25 μm film thickness). [3] Linear retention indices from standard compounds and database (http://webbook.nist.gov/chemistry). [4] DF: Detection frequency value. [5] Previously reported in dry cured loins by GC-MS: 1. Martín et al., 2003, 2. Ventanas et al., 2008, 3. Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004, 4. Muriel, Antequera, Petrón, Martin, et al., 2004, 5. Soto et al., 2008, 6. Ventanas et al., 2010, 7.Gamero-Negrón, García, Reina, & Sánchez del Pulgar, 2018, 8. Campus, Flores, Martinez, & Toldrá, 2008, 9. Ramírez & Cava, 2007.

3.7. Sensory analysis of dry cured loins

A Free Choice Profile analysis (FCP) was performed on dry cured loins formulated with the highest level of precursors (Orn-B, Pro-B and Thia-B loins) and control loins (Fig. 1). Fig. 1A shows the odor descriptors used by each assessor in different colours. In addition, in Fig. 1B the two dimensions obtained by Generalized Procrustes Analysis (GPA) indicates the main mentioned odor descriptors explained by each dimension listed next to them. The total variance attributed to the two first dimensions was 75.62% related to odor terms. Dimension 1 accounted for 40.06% of the variance. In the negative side of X axis, odor terms like cured, acid, vegetal and meaty characterized the formulations of Thia-B and Orn-B loins. While in the positive side of the X axis, odor terms like peppery, smoked, toasted, rancid and moldy appeared characterizing the formulation of Pro-B loins. On the other hand, dimension 2 accounted for 35.56% of the variance. In the positive side of Y axis, odor terms like acid, savory, cured, nutty and cheesy characterized the control loins while in the negative side of Y axis, terms like cooked meat, paprika, leather and brine, characterized the formulation of Orn-B loins.

3.8. Principal component analysis

To determine the relationship between dry cured loin formulations and aroma compounds, a principal component analysis was made (Fig. 2). Two principal components were able to explain 56.94% of the total variability observed. PC1 accounted for 32.85% of the variance and distinguished formulations placing Pro-A and Pro-B on the right quadrant and C, Orn-A, Orn-B, Thia-A and Thia-B on the left quadrants. PC2 accounted for 24.09% of the variance. Taking into account the volatile compounds detected as aroma impact compounds, most of them were related to the right quadrant. Pro-B loins were related to nitrogen compounds 2-acetylpyrrole and tetramethyl pyrazine, while Pro-A loins to 2,3-butanedione and 3-hydroxy-2-butanone. In contrast, control loins, Orn-A and Orn-B loins were related to hexanal and 2-acetyl-1-pyrroline, whereas Thia-A and Thia-B loins were related to 3-methylbutanal, limonene and sulfur compounds (methional and 2-methyl-3-methylthiofuran).

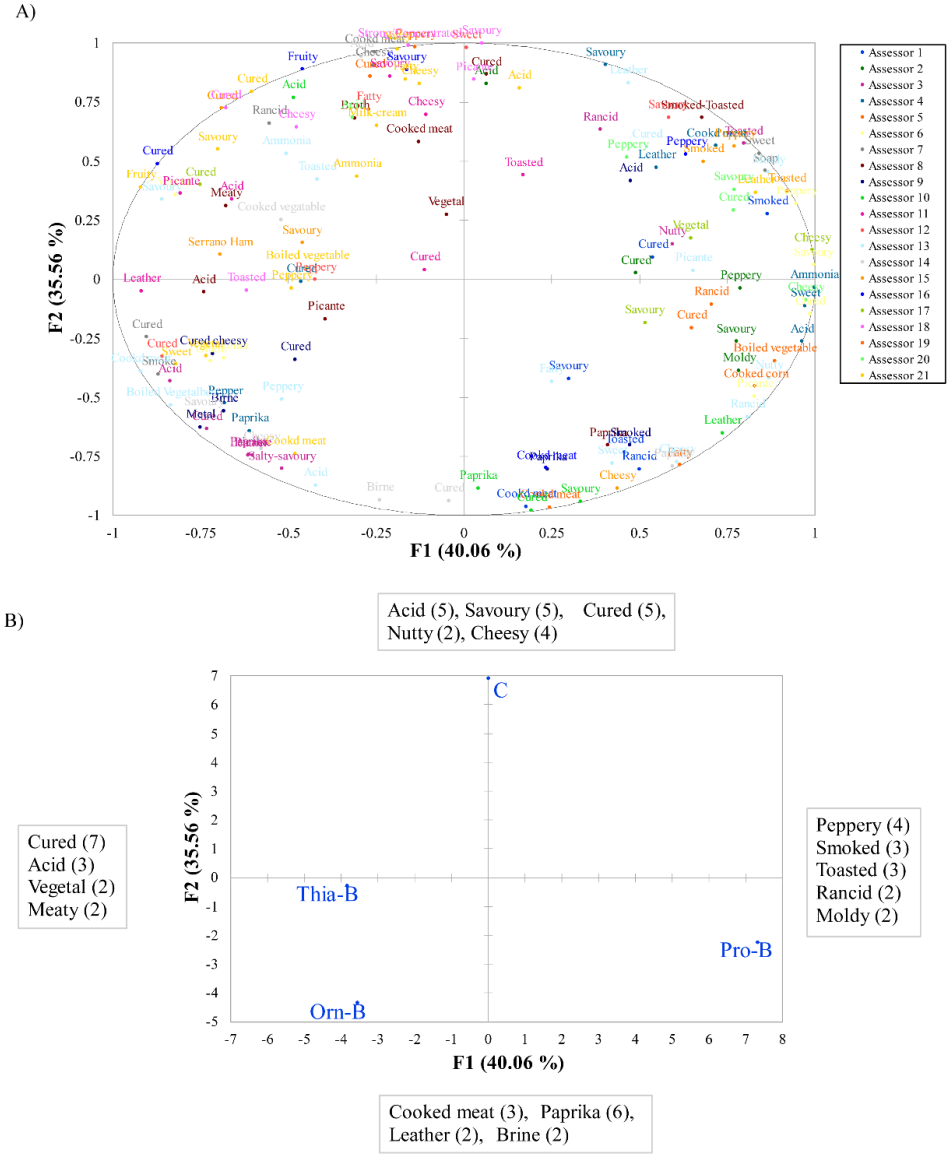

Fig. 1. A) Correlation of the GPA analysis of the free choice profile data. B) Two dimension GPA plot of the differences among dry cured loins manufactured with the precursors (C: Control loins, Pro-B and Orn-B: dry loins with proline and ornithine at 120 mg/100 g respectively, Thia-B: loins with thiamine at 24 mg/100 g). The main descriptors correlated with the first two dimensions of the average space are listed on the boxes with the number of times that the descriptor was mentioned.

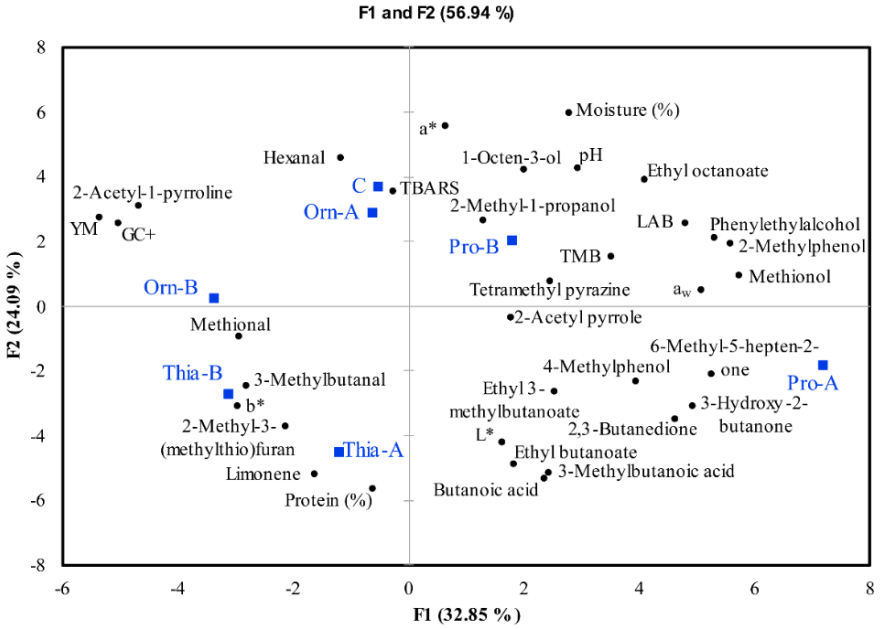

Fig. 2. Loadings of the first two principal components (PC1-PC2) of physic-chemical variables, aroma compounds and microbiology results in the different formulations of dry cured loins. C: Control loins without precursors, addition of precursors: proline and ornithine at 24 mg/100 g (Pro-A and Orn-A) and 120 mg/100 g (Pro-B and Orn-B), and thiamine at 2.4 mg/100 g (Thia-A) and 24 mg/100 g (Thia-B). The abbreviations used for variables are indicated in Tables 1 and Table 1S.

4. Discussion

The addition of aroma precursors (Pro, Orn, and thiamine) in the manufacture of dry cured loins may be an alternative to enhance the sensory characteristics and specially the aroma of these meat products. The absorption of the precursors into the loin muscle was confirmed by the presence of Pro, Orn, and thiamine at the end of the drying process in similar proportional quantities as added (Table 1 and Table 2), despite their different penetration rates (Pro, Orn and thiamine) as reported for sodium or potassium ions (Aliño et al., 2009). The addition of these precursors did not affect the drying and maturation process, as indicated by the small differences in the physic-chemical (Table 1) and microbial characteristics observed (Supplementary Table 1). The oxidation of loins was low and differences in oxidation levels were detected in Control and Orn-A dry loins,

although only Orn-A loins showed a large abundance of lipid oxidation volatile compounds like linear aldehydes (Table 3). Nevertheless, the reported TBARS values (Table 1) in the dry cured loins were low, in agreement with the low fat content of loins and, in contrast to other manufactured minced meat products (dry fermented sausages), where the high fat content increases the TBARS values (Olivares et al., 2011). In addition, the proteolytic process of the manufactured dry cured loins was measured by the content of FAA (Table 2), which showed few proteolytic activity differences among formulations, and confirmed the supplementation of Pro and Orn.

Dry cured loins are not fermented products and microorganisms are mainly present on the surface (Campus et al., 2008; Martín et al., 2003; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, et al., 2004). This fact explains the low levels of biogenic amines detected in dry cured products like dry hams and dry loins (Supplementary Table 2) in comparison to the high levels reported in fermented dry cured products, except for the endogenous amines spermine and spermidine (Vidal-Carou, Veciana-Nogués, Latorre-Moratalla, & Bover-Cid, 2014). The descarboxilation process of amino acids into amines (Suzzi & Gardini, 2003) explains that the supplementation of Orn, precursor of putrescine (Paulsen, Nauman, Bauer, Avagnina, & Smulders, 2017) resulted in a highest significant concentration of putrescine at the highest supplementation level (Orn-B loins) (Supplementary Table 2). On the other hand, the high cadaverine concentration detected in Pro-A loins maybe related to the high lactic acid bacteria (LAB) detected in these loins although it was not significant.

The addition of precursors of nitrogen and sulfur volatile compounds had a significant impact on the volatile profile of the manufactured dry cured loins (Table 3). The majority of volatile compounds detected had been previously reported in dry cured loins (Campus et al., 2008; Gamero-Negrón et al. 2018; Martín et al., 2003; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, et al., 2004; Ramírez & Cava, 2007; Soto et al., 2008; Ventanas et al., 2008; Ventanas et al., 2010) except for nine compounds (2-pentanol, phenylethyl alcohol, styrene, p-cymene, acetone, 2-methyl-1-

butanol acetate, 2-acetyl-1-pyrroline, 2-acetylpyrrole and 2-methyl-3-(methylthio)furan) that were detected for the first time being several of them important aroma impact compounds (Table 4). The aroma profile of dry cured loins was the result of the combination of several compounds, mainly 3-methylbutanal, methional, ethyl 3-methylbutanoate, 3-methylbutanoic acid, 1-octen-3-ol, 2-acetyl-1-pyrroline and 2-acetylpyrrole that contribute to musty, cooked potatoes, fruity, cheesy, mushroom, roasted and meaty odor notes, respectively. Nevertheless, other compounds contributed to the overall profile like 2-methyl-3-(methylthio)furan, that impart a meaty character.

Few differences among dry cured loins formulation were detected in volatile compounds, although the presence of volatile compounds derived from amino acid degradation showed a correlation with the highest content of Ile and Val in Orn-A loins (Table 2). This result may be directly related to the abundance of 2-methylbutanal and 2-methylpropanal in these loins, respectively (Table 3). However, only 3-methylbutanal was detected as an aroma impact compound in dry loins (Table 4), as it has been reported for other dry cured meat products (Théron et al., 2010). On the other hand, the high LAB counts in Pro-A loins may explain the higher abundance of 2,3-butanedione and 3-hydroxy-2-butanone in these loins (Liu, 2003). This would explain the completely different aroma profile of Pro-A loins in comparison to the other loin formulations (Fig. 2).

The presence of nitrogen compounds in dry cured meat products has been associated with Maillard reactions taking place between lipid oxidation products and certain amino acids (Ventanas et al., 1992). In our study, the amount of precursors added did not produce differences in the concentration of these volatile compounds among loins, including the two expected degradation products of the precursors added (2-acetylpyrrole and 2-acetyl-1-pyrroline). Their mechanism of formation in dry cured loins has not been elucidated yet, although the formation of 2-acetyl-1-pyrroline has been attributed to yeasts degradation of Pro and Orn (Schieberle, 1990; Stahnke, 2000). Meanwhile formation of 2-acetylpyrrole has been demonstrated through the Strecker degradation of Pro and hydroxyproline (Cho, Lee, Jun, Roh, & Kim, 2010). These results would indicate that other factors are necessary

to increase the degradation of precursors into nitrogen aroma compounds when added at the salting stage. Nevertheless, nitrogen compounds are very potent aroma compounds due to their low odor thresholds values (Buttery, Turnbaugh, & Ling, 1988； Koehler, Mason, & Odell, 1971), as shown by the contribution of 2-acetyl-1-pyrroline, and tetramethyl pyrazine to toasted nutty odors in dry cured loin aroma (Table 4).

Among the sulfur compounds, methional and 2-methyl-3-(methylthio)furan were particularly abundant in dry loins supplemented with the highest concentration of thiamine (Table 3). Methional is derived from the Strecker reaction of methionine (Met) and it breaks down rapidly to 2-propenal and methanethiol (Resconi, Escudero, & Campo, 2013). The low content of Met in Thia-B, Pro-A, and Pro-B loins may indicate a higher degradation rate of this amino acid into the aldehyde, although this was only confirmed in Thia-B loins. The highest 2-methyl-3-(methylthio)furan content in Thia-B loins may be related to the degradation of thiamine (Baek, 2007); however, the low temperatures used during loin processing did not favour its formation. Another thiol compound derived from sulfur amino acids and thiamine found in dry fermented sausages (Flores et al., 2021), as well as in dry cured ham (Del Pulgar, García, Reina, & Carrapiso, 2013), is 2-methyl-3-furanthiol. In contrast, this compound was not detected in the dry loins, although its detection possibly needs an optimized extraction and detection process due to its low content reported in meat products (Flores et al., 2021). In addition, this thiol compound is very unstable, and it reacts quickly with the decomposition products of methionine to form thiophenes due to aldol condensation and the condensation between the two SH groups (Tang, Jiang, Yuan, & Ho, 2013). These mechanisms would explain that only one compound derived from thiamine, 2-methyl-3-(methylthio)furan, was detected in dry loins and confirmed its formation at the highest supplemented level of thiamine in loins (Thia-B loins) (Table 3). In addition, its low odor threshold value (0.05 μg/kg) and its meaty aroma at levels below 1 μg/kg (Mottram, 1998), make it an important aroma compound contributing to the meaty odor in dry cured loins (Table 4). Regarding other sulfur compounds, none of the garlic derived sulfur compounds, especially methylallyl sulphide, were detected (Ventanas et al., 2008) as

garlic was not added.

The FCP sensory analysis performed on the loins supplemented with the highest concentrations of supplements (B formulations), in comparison with the control loins, confirmed the impact on the aroma of the precursors added. The aroma of Thia-B loins was perceived as cured aroma (Fig. 1), and the highest abundance of methional and 2-methyl-3-(methylthio)furan, which have meaty and vegetable odors (Table 4), is consistent with results in Fig. 1 and Fig. 2. These results demonstrate that olfactometry and FCP can explain the aroma enhancement generated by the addition of precursors. Moreover, Pro-B loins had a different aroma profile (Fig.1) described as toasted and mostly related to the presence of tetramethyl pyrazine and 2-acetylpyrrole as shown in the PCA (Fig. 2). Although the content of these compounds did not show significant differences among loins (Table 3), their quantitation needs to be optimized due to their low abundance. In addition, the Orn-B loins were mostly related to 2-acetyl-1-pyrroline, which has roasted odors and has been attributed to yeasts degradation (Sunesen & Stahnke, 2003). Moreover, a similar relation between this compound and Orn degradation has been observed in dry fermented sausages by Corral, Leitner, et al. (2016).

5. Conclusions

The addition of nitrogen (Pro and Orn) and sulfur (thiamine) compounds during the manufacture of dry cured loins produced a modification of the aroma profile of loins, which was confirmed by olfactometry and FCP sensory analyses. The impact on the aroma of loins produced by the addition of these precursors depends on their concentration, as higher amounts are needed to modify the aroma profile. The contribution of nitrogen precursors into the generation of toasted odours in the aroma profile of loins may be probably related to the presence of tetramethyl pyrazine, 2-acetylpyrrole and 2-acetyl-1-pyrroline, although further studies may be necessary to confirm this conclusion. Moreover, the effect of thiamine supplementation on the aroma was confirmed by the presence of sulfur derived compounds (methional and 2-methyl-3-(methylthio)furan), which contribute to the “cured

meat odor". The mechanism of formation of these aroma compounds in dry cured loins is mainly the result of lipid oxidation, Maillard and thiamine degradation reactions, although microbial metabolism of moulds and yeasts on loin's surface should be considered.

Credit author statement

Lei Li: Formal analysis, Methodology, Investigation, Writing - original draft. **Laura Perea-Sanz**: Formal analysis, Methodology, Investigation. **José Javier López-Díez**: Formal analysis, Methodology, Investigation. **Ana Salvador**: Formal analysis, Methodology, Investigation. **Carmela Belloch**: Funding acquisition, Conceptualization, Writing - review & editing. **Monica Flores**; Funding acquisition, Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

None.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on microbial counts (log cfu/g) of dry cured loins.

	Culture Medium	C[1]	Pro-A	Pro-B	Orn-A	Orn-B	Thia-A	Thia-B	RMSE[2]	*P*[3]
Total mesophilic bacteria (TMB)	PCA[4]	5.94	6.43	5.92	6.10	6.02	5.54	6.05	0.36	ns
Lactobacillus (LAB)	MRS	5.61	6.39	5.48	5.84	5.25	4.83	5.31	0.65	ns
Gram positive cocci (GC+)	MSA	5.33	4.78	5.26	5.47	5.68	4.98	5.61	0.42	ns
Enterobacteriaceae（EB）	VRBGA	1.20	0.50	0.00	0.68	0.00	0.00	0.73	0.96	ns
Yeasts and moulds (YM)	RB	5.58	4.91	5.25	5.50	5.46	5.26	5.57	0.44	ns

[1] C: Control loins without precursors, addition of precursors: proline and ornithine at 24 mg/100 g (Pro-A and Orn-A) and 120 mg/100 g (Pro-B and Orn-B), and thiamine at 2.4 mg/100 g (Thia-A) and 24 mg/100 g (Thia-B). [2] RMSE: root mean square error. [3] *P* value of formulation effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] PCA: Plate Count Agar. MRS: Man Rogosa Sharpe agar. MSA: Mannitol Salt Agar. VRBGA: Violet Red Bile Glucose Agar. RB: Rose Bengal Chloramphenicol Agar.

Supplementary Table 2. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the biogenic amine content in dry cured loins (mg/Kg).

	C[1]		Pro-A		Pro-B		Orn-A		Orn-B		Thia-A		Thia-B		RMSE[2]	*P*[3]
Tyramine	1.30		3.52		0.68		0.29		0.68		13.65		0.65		10.44	ns
Histamine	0.00		0.00		0.00		0.00		0.00		0.18		0.16		0.16	ns
Putrescine	0.33	c[4]	3.47	ab	0.66	c	0.34	c	5.13	a	1.12	bc	1.16	bc	1.12	***
Cadaverine	13.81	ab	25.36	a	1.04	b	0.00	b	2.97	b	4.97	ab	0.90	b	9.37	**
Phenethylamine	0.00		0.00		0.16		0.18		0.00		1.01		0.41		0.43	ns
Spermidine	6.34	ab	8.06	ab	8.03	ab	8.90	ab	9.56	a	5.81	b	6.94	ab	1.49	*
Spermine	40.10	b	62.59	ab	62.97	ab	71.32	a	70.34	a	55.36	ab	64.64	ab	12.62	*
BA[5]	15.45		32.35		2.54		0.81		8.77		20.94		3.28		17.70	ns
PA[5]	46.45	b	70.65	ab	71.00	ab	80.21	a	79.91	a	61.17	ab	71.59	ab	13.66	*
Total	55.55		94.94		65.51		72.13		79.11		76.30		67.92		19.60	ns

[1] C: Control loins without precursors, addition of precursors: proline and ornithine at 24 mg/100 g (Pro-A and Orn-A) and 120 mg/100 g (Pro-B and Orn-B), and thiamine at 2.4 mg/100 g (Thia-A) and 24 mg/100 g (Thia-B). [2] RMSE: root mean square error. [3] *P* value of formulation effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] Different letters in the same row indicate significant differences at $P < 0.05$ among batches. [5] BA = biogenic amines (tyramine +histamine + putrescin+cadaverine+phenethylamine); PA = polyamines (spermine + spermidine).

Chapter 2

Understanding the impact of nitrogen and sulfur precursors on the aroma of dry fermented sausages

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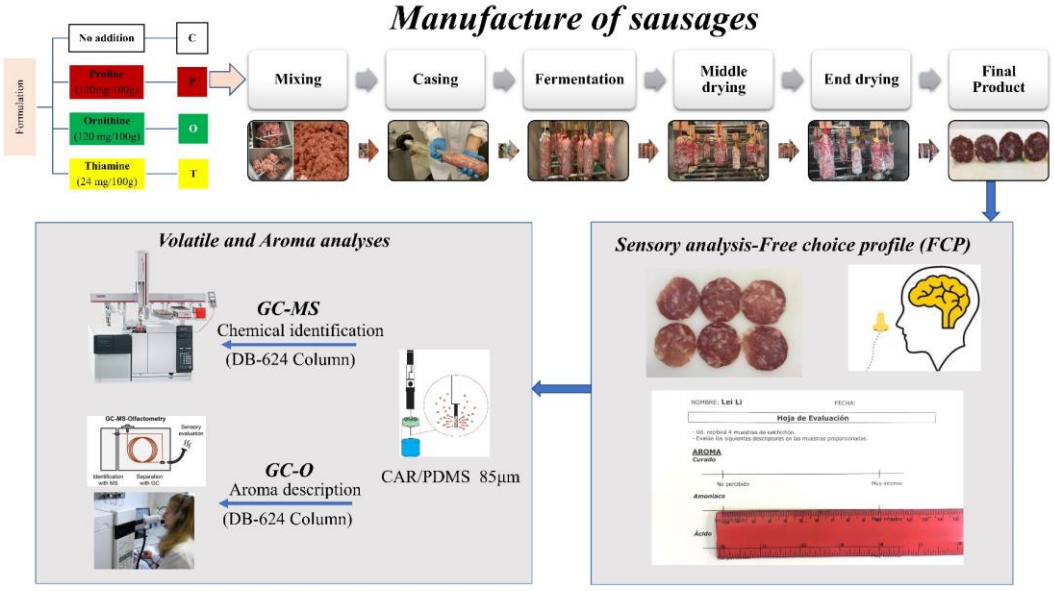

Understanding the impact of nitrogen and sulfur precursors on the aroma of dry fermented sausages

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ABSTRACT

The goal of this study was to confirm and acquire more information about the nitrogen and sulfur compounds existing in the volatile profile of dry fermented sausages from the addition of precursors (proline, ornithine and thiamine), and their role in sausage aroma. To this end, the precursors were added to the formulation of sausages, which were submitted to a fermentation and drying process. The sausage aroma was analyzed by olfactometry technique and Free Choice Profile sensory analysis. The results showed that the addition of precursors impacted the aroma, and reduced the level of oxidation in the final sausages while microbial differences were mainly observed in Orn-sausages. Among the aroma compounds detected only 2-methyl-3-(methylthio)furan verified the effect of thiamine supplementation and the impact on the cured and savoury odours detected in Thia-sausages by Free Choice profile sensory analysis, while no clear effect could be attributed to specific volatile compounds in the nitrogen supplemented sausages.

Keywords: aroma; savoury; proline; ornithine; thiamine; dry fermented sausages

1. Introduction

The current challenges of the meat industry are aimed at the development of healthier meat products that maintain the desired sensory characteristics. Among these, "savoury" (meaty) flavours are highly demanded in meat products, in which the generation of aroma and flavour occurs naturally during processing (Flores, Perea-Sanz, López-Díez, & Belloch, 2021). Moreover, savoury aromas are constituted by a complex mixture of compounds producing a specific character and present at part per billion levels and due to their low sensory thresholds they affect meat perception (Cook, Linforth, & Taylor, 2003). Consequently, the control of the factors that favour the development of specific savoury aroma compounds is considered of great interest for the meat industry.

The flavour of dry fermented sausages is due to a large number of microbiological and chemical reactions (Flores & Olivares, 2014; Toldra, Flores, & Sanz, 2001). The formation of aroma in dry fermented sausages occurs mainly throughout the ripening process and the aroma impact of the biochemical reactions depends on the microbial diversity, which is highly affected by the manufacturing conditions. Latest studies trying to correlate bacterial diversity with the development of volatile compounds in dry fermented sausages in Europe and Asia revealed the differences in microbiota and explained their differences in the volatile profile (Flores & Piornos, 2021; Yang et al., 2022). However, many differences in recipes and manufacturing processes produce a wide variety of aroma compounds that may be related to the presence of precursors and bacteria diversity. In contrast, the formation of compounds producing savoury and toasted odours in dry fermented sausages is scarce. Flores (2018) summarized the impact of important aroma compounds in dry fermented sausages, such as sulfur, thiols, thiazoline, thiophene and pyrazine compounds and revealed that they are mainly originated from sulfur amino acids, thiamine and 5´ribonucleotides as well as some of the sulfide compounds that are derived from the spices used as ingredients.

The main contributors to the savoury and toasted odours are compounds present at very low concentrations like sulfur and nitrogen compounds. While the contribution of the microbial degradation of sulfur amino acids in fermented sausages produces sulfur aroma

compounds (Perea-Sanz, Peris, Belloch, & Flores, 2019), the contribution of proline and ornithine released during the process (Flores, Sanz, Spanier, Aristoy, & Toldrá, 1998) is important as both amino acids are precursors of 2-acetyl-1-pyrroline, a potent toasted odour compound identified in Hungarian salami (Söllner & Schieberle, 2009). A previous study in dry cured loin related proline and ornithine concentration to toasted odours (Li et al., 2022) and revealed that in addition to the effect of precursor concentration, other compounds like pyrazines may be related to the toasted odour perception. However, the microbial metabolism presents in dry fermented sausages (Schieberle, 1990) may contribute as another degradation route to 2-acetyl-1-pyrroline formation instead of the Maillard reaction (Blank, Devaud, Matthey-Doret, & Robert, 2003).

Regarding other savoury precursors, thiamine is recognized to be a crucial precursor of meaty aroma in thermal degradation processes (Cerny, 2007). The addition of thiamine to cooked ham can increase the content of sulfur-containing compounds (2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran, and bis(2-methyl-3-furyl)disulphide) and it is considered a better aroma precursor than sulfur amino acids like cysteine (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2014; Thomas, Mercier, Tournayre, Martin, & Berdagué, 2015). On the contrary, the role of thiamine in the generation of important aroma compounds in other meat products like Beijing roasted duck or fried pork chops was not clearly elucidated (Zou, Kang, Yang, Song, & Liu, 2019). In products like dry fermented sausages, little research has been done about the contribution of the precursor thiamine to the production of savoury odour. Previous studies have revealed the presence of two compounds (2-methyl-3-furanthiol and methyl-2-methyl-3-furyl disulphide), derived from thiamine degradation, in dry fermented sausages. Moreover, these compounds were confirmed as impact aroma compounds as their concentrations were above their odour thresholds (Flores et al., 2021). However, sausages showed low thiamine content, which was not affected during the drying period, therefore it was not possible to relate the formation of these savoury odour compounds to its degradation. Other factors have been related to the formation of these savoury odours in addition to thiamine degradation, like

the presence in the sausages of compounds derived from amino acid methionine degradation, methanethiol and methional. These compounds react either with themselves or with the degradation compounds of thiamine during the long ripening times favoured by the low water activity and acid pH of the fermented sausages (Flores et al., 2021).

In this study, precursors were added to the formulation of fermented sausages for the elucidation of the factors affecting the production of savoury and toasted odours during the ripening process. Thus, the objective of the study was to reveal the role of nitrogen and sulfur precursors in the aroma of dry fermented sausages by the addition of these precursors into the meat batter.

2. Materials and methods

2.1. Dry sausage manufacture and sampling

The sausage manufacture consisted of four different formulations. Three formulations were prepared adding the following precursors into the meat batter: proline (1.2 g/kg, Pro), ornithine (1.2 g/kg, Orn) and thiamine (0.24 g/kg, Thia) (Li et al., 2022), and a fourth formulation control (C) without precursors was also prepared.

The four formulations consisted of a meat batter with pork lean meat (50%) and pork back fat (50%) purchased in a local company (Cárnicas La Cope, Torrente, Spain) that were ground via a minced plate of 10 mm diameter. The ingredients added into the meat batter were: 20 g/kg lactose, 20 g/kg dextrin, 6 g/kg glucose, 27 g/kg sodium chloride, 0.150 g/kg sodium nitrite, 0.178 g/kg potassium nitrate, 0.5 g/kg sodium ascorbate and spices (1.5 g/kg black pepper). For fermentation, a commercial starter culture TRADI-302 (Chr. Hansen, Hørsholm, Denmark) was included into the meat batter at 0.125 g/kg. After resting for 24 h at 3-5 °C, the formulations were stuffed into collagen casings of 95 mm diameter (Fibran, S.A., Girona, Spain), up to approximately 500 g weight per sausage. The sausages were then surface inoculated with a yeast strain (*D. hansenii* L1, Perea-Sanz et al., 2019) by introducing them for a few seconds in a suspension of 2 log cells/ml.

Fermentation and drying of all sausages were done in climatic chambers by a slow

ripening process at 10 °C and 75–90% relative humidity (RH). The temperature and RH of the chambers were continuously monitored. The control of weight losses was performed by weighting regularly one sausage from each formulation until reaching 45-46% weight loss. Fermentation was controlled by pH measurement using a pH meter HI 99163 (Hanna Instruments Inc., Hoonsocket, USA) introduced in one sausage per formulation as indicated Olivares, Navarro, Salvador, & Flores (2010).

Three independent replicates of each formulation were done; therefore, a total of 12 batches (4 formulations ×3 replicates) were manufactured. Sampling consisted on taking approximately 200 g of the minced batter at 0 d for analyses while two sausages from each formulation and replicate were randomly taken at 8, 34, and 62 d. From each sample, 25 g were taken for microbial analyses. The colour of the sausage was evaluated on the slice surface before the sample was chopped and analyzed for moisture, water activity (a_w) and pH. For physicochemical analysis, thiamine, free amino acids and biogenic amines, the remaining minced sample was vacuum packaged and kept frozen at -20 °C. In addition, for volatile and aroma analyses several slices of each selected sausage were wrapped in aluminium foil, packaged at vacuum and stored at -80 °C. At 62 d of ripening, 2 sausages were taken from each formulation and replicate, vacuum packaged and stored at 4 °C until sensory analysis.

2.2. Physicochemical parameters measurement

The pH, a_w, colour (CIE L*, a*, b*), moisture and protein measurements were done as described (Perea-Sanz, Montero, Belloch, & Flores, 2018). Lipid oxidation was evaluated by the TBARS method, using tricloroacetic acid instead of perchloric acid as solvent, and the results were represented in mg malonaldehyde (MDA) per kg dry matter (Olivares, Navarro, & Flores, 2011).

2.3. Analysis of thiamine

Thiamine analysis was performed based on the methodology of Tang, Cronin, & Brunton (2006). Thiamine was extracted from minced sausage in a proportion of 1:10 w/v with a solution of HCl 0.1 N. Then, the mixture was heated for 30 min in a water bath at

90 °C followed by centrifugation at 10000 g for 20 min to obtain the supernatant for the thiamine analysis after filtration through a 0.45 µm nylon filter (Flores et al. 2021). The thiamine fluorescence derivative was analyzed using a HPLC system with fluorescence detector (Agilent series 1100, Agilent, Palo Alto, CA, USA) and a column XDB-C18. Excitation at 360 nm and emission at 430 nm wavelengths were used to detect thiamine derivatives. The results were expressed as mg thiamine/100 g dry matter using a calibration curve.

2.4. Analysis of free amino acids (FAA)

The minced sausages (5 g) were homogenized with HCl (0.1 M) at 1:4 (w/v) and then, centrifuged at 10000 g for 20 min. Free amino acid were analyzed in the supernatant using a commercial kit (Phenomenex, Torrance, CA, USA) (Flores et al. 2021). Before the derivatization process, supernatant was diluted with distilled water 1/2 (v/v) or 1/5 (v/v) to adjust the concentration into the calibration curves and norvaline was added as internal standard. A gas chromatograph with FID detector and including a liquid automatic injector (Agilent 7890B GC/FID, 7693A Liquid Autosampler, Palo Alto, CA, USA) was used for the separation of derivatized amino acids. For identification and quantification, the retention times and calibration curves of the standard compounds from the manufacturer (Phenomenex) and Sigma (St. Louis, MO, USA) were employed. Each amino acid's calibration curve was obtained and adjusted to levels found in sausages, and the results were expressed in mg/100g dry matter.

2.5. Microbial analysis

Twenty-five grams of sausage samples were used after aseptically removing the collagen casings, mixed with 225 ml of buffered peptone water (Pronadisa, Madrid, Spain) in a filtered bag (Scharlau, Barcelona, Spain). The samples were homogenized in a Pulsifier II (Microgen Biotech, Carlow, Ireland) for three times 30 s and several decimal dilutions were prepared with the filtrate, which were spread in triplicates on appropriated media plates for microbial counts (Belloch, Neef, Salafia, López-Diez, & Flores, 2021). Total Mesophilic bacteria (TMB) were counted on Plate Count Agar (Pronadisa, Madrid,

Spain), lactic acid bacteria (LAB) on MRS Agar (Scharlau, Barcelona, Spain), Gram positive cocci (GC+) on Mannitol Salt Agar (Scharlau, Barcelona, Spain), Enterobacteriaceae (EB) on Violet Red Bile Glucose Agar (Pronadisa, Madrid, Spain), and yeasts and moulds (YM) on Rose Bengal Agar with chloramphenicol (Scharlau, Barcelona, Spain).

2.6. Biogenic amines analysis

Ion-pair reverse-phase high-performance liquid chromatography was used to examine biogenic amines, as described by Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou (1996). In summary, amines from minced sausages (5 g) were extracted with perchloric acid (0.6 M). The separation was done using a HPLC system with fluorescence detector (Agilent series 1100, Palo Alto, CA, USA) with a C-18 column and post-column derivatization using o-phthalaldehyde. Then, the detection was performed at excitation 340 nm and emmission 445 nm and the results were reported as mg/kg dry matter using calibration curves for each amine (Li et al., 2022).

2.7. Analysis of volatile compounds

Four grams of sausages previously minced using liquid nitrogen, were added with BHT (0.75 mg) to avoid oxidation and introduced into a 20 ml headspace vial (Gerstel, Mülheim an der Ruhr, Germany) for 30 min incubation before volatile extraction using a solid-phase microextraction (SPME) device (including a 85 µm carboxen/poly-dimethylsiloxane CAR/PDMS fibre, Supelco, Bellefonte, USA) (Li et al., 2022). A gas chromatograph (GC) with a quadrupole mass spectrometer detector (Agilent GC 7890 series II, MS 5975C, Agilent, Palo Alto, USA) and an autosampler (MPS2, Gerstel, Mülheim an der Ruhr, Germany) were used. The adsorption of volatile compounds into the fibre was done at 37 °C for 2 h and then they were desorbed in splitless mode of the GC injection port for 5 min at 240 °C. Separation of volatile compounds was done as described by Corral, Leitner, Siegmund, & Flores (2016) in a DB-624 capillary column (30 m × 0.25 mm I.D. × 1.4 µm thickness, J&W Scientific, Agilent Technologies, USA). Full scan

mode and mass spectra from the library database (NIST'17) were used to identify the compounds together with the calculated linear retention indices (LRI) for the DB-624 column, using a n-alkanes series (C5-C22) (Van Den Dool & Dec.Kratz, 1963), and by comparison with those included in the NIST Chemistry WebBook database (Linstrom, 2003) and calculated with authentic standards. A relative quantitation based on the abundance of area units ($AUx10^6$) allows the comparison among sausage formulations analyzed under the same sampling time and temperature conditions. The quantitation was done in SCAN mode using either total ion (TIC) or extracted ion (EIC) current on an arbitrary scale.

Furthermore, confirmation of identified compounds was done by the analysis of the extracted compounds in a GC Agilent 8890 equipped with a 5977 mass selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA) using a DB-5 capillary column (30 m × 0.25 mm ID × 0.25 μm thickness, J&W Scientific, Agilent Technologies, USA). In this case, a manual SPME holder with the same conditions indicated above was used for the extraction of volatile compounds. Separation of volatile compounds was done as reported by Li et al. (2022). The analysis was carried out in full scan mode, and the identification of volatile compounds was done as indicated above but using linear retention indices for the DB-5 column.

2.8. Olfactometry analysis

The olfactometry analysis was done by the detection frequency method as described Li et al. (2022) in a GC equipped with an FID detector (Agilent GC 6890, USA) that included a sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany). Four g of sausage were extracted by using a manual SPME holder with the same conditions indicated above. Odours were evaluated by four trained panellists from the GC-olfactometry port and performed a total of 14 assessments to reveal the aromatic impact of compounds. The panellists evaluated the odours by smelling and recording the retention time and odour descriptors of every perceived odour. The detection of an odour in the sniffing port by less than three assessors was considered to be noise while the final Detection Frequency (DF)

value for each compound was obtained by summation of the 14 sniffings. The aroma components were identified by comparing mass spectra, linear retention indices for DB-624 and DB-5 columns of authentic standards in GC-MS and GC-O, and then, assessor's descriptors were matched to those reported in the Fenaroli's handbook of flavour ingredients (Burdock, 2010).

2.9. Sensory analysis

The Free Choice Profile (FCP) sensory analysis (Williams & Langron, 1984) was employed as reported Li et al. (2022) with slight modifications. Based on the earlier selection done by Li et al. (2022), a set of 20 consumers (15 women and 5 men) between 21 to 55 years old, were employed. The evaluation consisted on using a 10-cm unstructured scale ranging from “not perceived” to “intense”, and each consumer rated his or her own list of aroma and appearance words by scoring the strength in the sample. Panelists were directed to sniff the aroma and judge the appearance of a sausage slice (5 mm thickness) displayed on a closed petri-dish, labelled with three-digit code and maintained at room temperature. The sausage presentation order was balanced.

2.10. Statistical analysis

The Generalized Linear Model (GLM) procedure was used to analyze the data by using statistical software (XLSTAT 2018, Addinsoft, Barcelona, Spain). The effect of sausage formulation and ripening time were included in the model as fixed effect and replicates were considered as random effect. In the case of volatile compounds, the analysis only included one factor, sausage formulation, as fixed effect and the replicates as random effect. Tukey test was used to compare means when there was a significant effect of the treatment group ($P<0.05$). A Generalized Procrustes Analysis (GPA) was applied to the sensory data obtained by Free Choice Profile. Heatmaps of FAA and volatile compounds were calculated based on the relative concentration obtained at 62 d of processing using XLSTAT 2018.

3. Results

3.1. Physicochemical characteristics

The evolution of physico-chemical parameters during the fermentation and drying process of the four sausage formulations is shown in Table 1. The effect of the processing time (stages) was significant in all parameters but the addition of precursors only affected pH, colour parameter a* and TBARS values. Regarding pH and a* no significant differences among formulations were observed at the end of process (62 d). In the case of pH, the evolution of the four sausage formulations was similar, while a* values of Thia- and Pro- sausages were the highest in the meat batter (0 d). TBARS values increased significantly during the drying process, and at the end of process, the C-sausages showed a higher oxidation value than the Thia-sausages.

3.2. Thiamine and FAA content

Fig. 1 shows the content of the precursors added (thiamine, proline and ornithine) analyzed during the process. The content of Thia, Pro and Orn was the highest in the supplemented formulations confirming the addition of precursors. The content of precursors showed different trends in the supplemented sausages during the drying process. The content of thiamine in Thia-sausages showed a slight reduction during drying as observed for proline in Pro-sausages. In contrast, the content of ornithine in Orn-sausages increased indicating the contribution of proteolysis activity. Regarding other FAA, proteolysis was similar among formulations and stages (Supplementary Table 1) and only a few FAA (Ala and Gly) showed significant differences at the end of process with the lowest concentration detected in Orn-sausages. Furthermore, the total FAA content was significantly lowest in C-sausages and Thia-sausages (Supplementary Table 1). In addition, the heatmap (Fig. 2) with hierarchical clustering based on the relative content of amino acids obtained at 62 d of drying showed similar FAA profiles in Orn and Pro-sausages, whereas Thia-sausages clustered in another group. The absence of differences between proline and ornithine sausages may be due to the variability observed between replicates.

Table 1. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the physicochemical parameters of dry fermented sausages.

	Time (d)	C[1]		Pro		Orn		Thia		RMSE[2]	P_f[3]	P_s	P_{f*s}
Moisture (%)	0	64.28	a[4]	63.75	a	64.21	a	64.27	a	1.43	ns	***	*
	8	62.50	a	61.53	a	62.30	a	62.88	a				
	34	49.73	b	49.31	b	51.35	b	51.97	b				
	62	34.53	c	36.48	c	35.29	c	34.78	c				
Protein (%)	0	17.02	e	17.20	e	17.42	d	17.53	de	0.89	ns	***	ns
	8	19.31	cd	19.41	c	19.38	c	19.27	cd				
	34	25.43	b	25.54	b	26.43	b	24.82	b				
	62	32.06	a	32.39	a	32.47	a	31.58	a				
pH	0	5.76	a	5.77	a	5.78	a	5.78	a	0.03	***	***	**
	8	4.92	b	4.94	b	4.88	b	4.88	b				
	34	4.75	cd	4.79	c	4.77	cd	4.75	cd				
	62	4.71	d	4.77	cd	4.78	cd	4.71	d				
a_w	0	0.976	a	0.973	a	0.972	a	0.973	a	0.01	ns	***	ns
	8	0.966	a	0.966	a	0.968	a	0.970	a				
	34	0.932	b	0.928	b	0.933	b	0.934	b				
	62	0.851	c	0.843	c	0.845	c	0.841	c				
L*	0	57.72	a	55.96	abcd	57.47	ab	57.22	abc	1.18	ns	***	ns
	8	54.78	de	55.42	abcde	55.12	bcde	55.01	cde				
	34	54.17	de	53.24	e	54.03	de	53.81	de				
	62	46.55	f	46.31	f	46.81	f	47.17	f				

Table 1 (continued).

a*	0	15.01	e	16.73	bcd	15.20	e	16.78	bc	0.70	**	***	**
	8	18.41	a	18.07	abcde	18.55	a	18.49	a				
	34	16.42	cde	16.77	bc	16.74	bcd	17.16	abc				
	62	15.05	e	15.15	e	15.29	e	15.32	de				
b*	0	11.14	a	11.07	a	10.86	a	10.94	a	0.24	ns	***	ns
	8	8.98	b	8.96	b	8.76	b	8.73	b				
	34	6.80	c	6.66	c	6.54	c	6.63	c				
	62	5.04	d	5.03	d	5.18	d	5.04	d				
TBARS (μg MDA/g DM)	0	0.38	f	0.51	ef	0.74	cdef	0.50	ef	0.25	***	***	**
	8	0.49	ef	0.63	cdef	0.74	cdef	0.44	f				
	34	1.15	abcd	1.28	ab	0.81	bcdef	0.62	def				
	62	1.50	a	1.15	abc	1.08	abcd	0.98	bcde				

[1] C: Control sausages without precursors, addition of precursors: proline and ornithine at 120 mg/100 g (Pro and Orn), and thiamine at 24 mg/100 g (Thia). [2] RMSE: root mean square error. [3] *P* value of formulation (f), stage time (s) and formulation and stage (f*s) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] Different letters in the same row and column of each parameter indicate significant differences at $P < 0.05$.

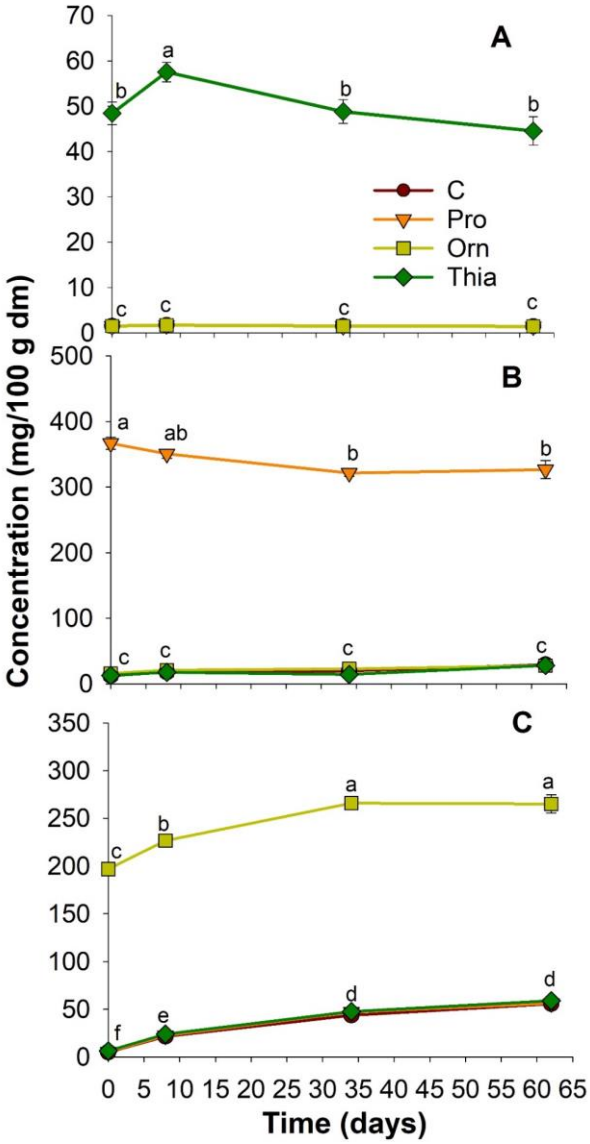

Fig. 1. Evolution of thiamine (A), Pro (B) and Orn (C) concentration (mg/100g DM) throughout the manufacture of dry fermented sausages. C: Control sausages without addition (●, dark red), addition of proline (Pro) (▼, orange) and ornithine (Orn) (■, light green), and thiamine (◆, dark green) (Thia). Each number represents the average of three individual manufacturing trials. Data are expressed as means ± SE and different letters indicate significant differences at $P < 0.05$. (For interpretation of the references to colour in this fig ure legend, the reader is referred to the web version of this article.)

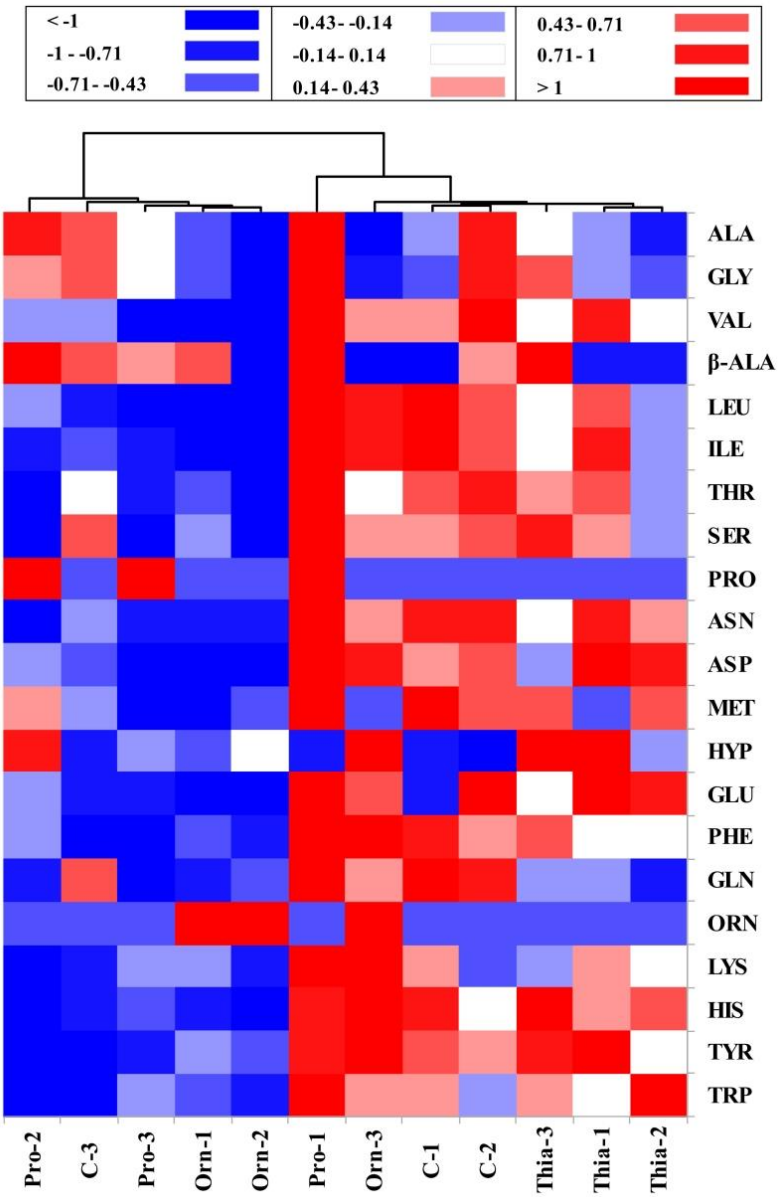

Fig. 2. Heatmap visualizing differences in the concentration of free amino acids (mg/100 g dry matter) at the end of process (62 d) in the different dry fermented sausages. C: Control sausages without addition, and formulations with proline and ornithine (Pro and Orn), and thiamine (Thia). The map's colours indicate the following: red represents a comparatively high abundance, blue represents a relatively low abundance of the compound, and white indicates no difference.

3.3. Microbiology

The results of microbiological counts are presented in Fig .3. Enterobacteriaceae were not detected in any sample. The added precursors of Pro, Orn and thiamine affected only slightly LAB, staphylococci and yeast and molds (YM) counts in the sausages. The largest differences were found at 62 d of drying, when Orn-sausages showed the highest counts of LAB and GC+, and Thia-sausages showed a significant lower YM counts.

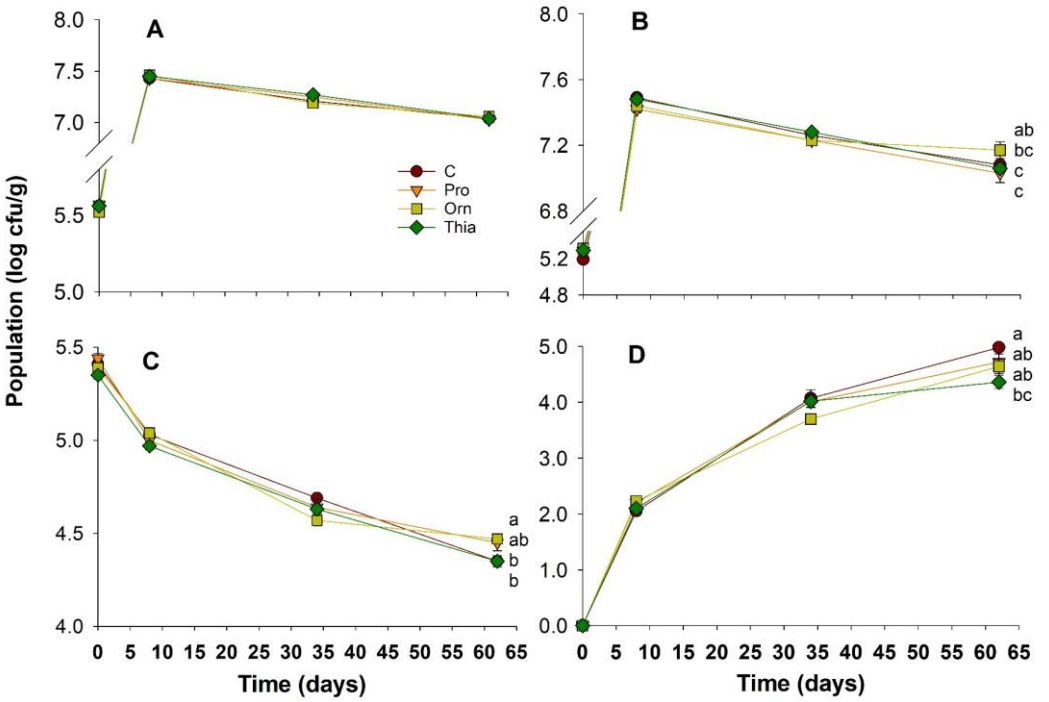

Fig. 3. Evolution of microbial counts (total mesophilic bacteria (A), Lactobacillus (B), Gram positive cocci (C) and yeast and moulds (D)) during the processing of dry fermented sausage formulations. C: Control sausages without addition (●, dark red), addition of proline (▼, orange) and ornithine (■, light green) (Pro and Orn), and thiamine (◆, dark green) (Thia). Each number represents the average of three individual manufacturing trials. Data are expressed as means ± SE and different letters at the end of process indicate significant differences at $P < 0.05$.

3.4. Biogenic amines

The results of biogenic amines are shown in Supplementary Table 2. Biogenic amines were not found while only the polyamines, spermine and spermidine were detected. Only Pro-sausages had a significantly higher content of spermidine than the C-sausages.

3.5. Volatile compounds

Eighty-five volatile compounds were identified in sausages dried for 62 d (Fig. 5 and Supplementary Table 3). The volatiles identified were categorized into 9 aldehydes, 7 alkanes, 10 acids, 6 ketones, 14 alcohol, 10 esters, 21 terpenes, 6 sulfur compounds and 2 nitrogen compounds. Fourteen additional volatiles were only tentatively identified.

Volatile compounds were classified according to their probable origins (Fig. 4 and Supplementary Table 3) into those originated from microbial activity due to the degradation of carbohydrates, degradation of amino acids, esterase activity and lipid β-oxidation, and also those derived from lipid autooxidation, spices, thiamine degradation and those of unknown origin (Ordóñez, Hierro, Bruna, & De La Hoz, 1999). Compounds derived from carbohydrate fermentation were the most abundant compounds, accounting for 43.4-53.1% of the total extracted area, with acetic acid and ethanol as the most abundant compounds of this group (Supplementary Table 3). The percentage of amino acid degradation products was 2.4-4.7% with 3-methylbutanal, 2-methylpropanoic and 2-methylbutanoic acids as high abundant compounds. Compounds generated from esterase activity represented 2.2-3.8% with ethyl acetate, ethyl butanoate and ethyl pentanoate as the most abundant compounds. Lipid autooxidation volatile compounds were 17.2-26.1%, with hexanal and nonanal as high abundant compounds. The group of spice-derived compounds accounted for 21.3-27.2% and contained terpenes compounds. The groups represented by very low percentages were lipid β-oxidation compounds (0.1-0.3%), unknown origin compounds (0.3-1.1%) and those derived from thiamine degradation (0.01%) that contained only one compound 2-methyl-3-(methylthio)furan. Differences among formulations regarding total volatile compound groups were observed in the Thia-sausages, which had a significantly higher content of compounds generated from carbohydrate fermentation, esterase activity

and thiamine degradation. The Orn-sausages had also a high content of compounds generated from lipid β-oxidation and those from unknown origin (Fig .4).

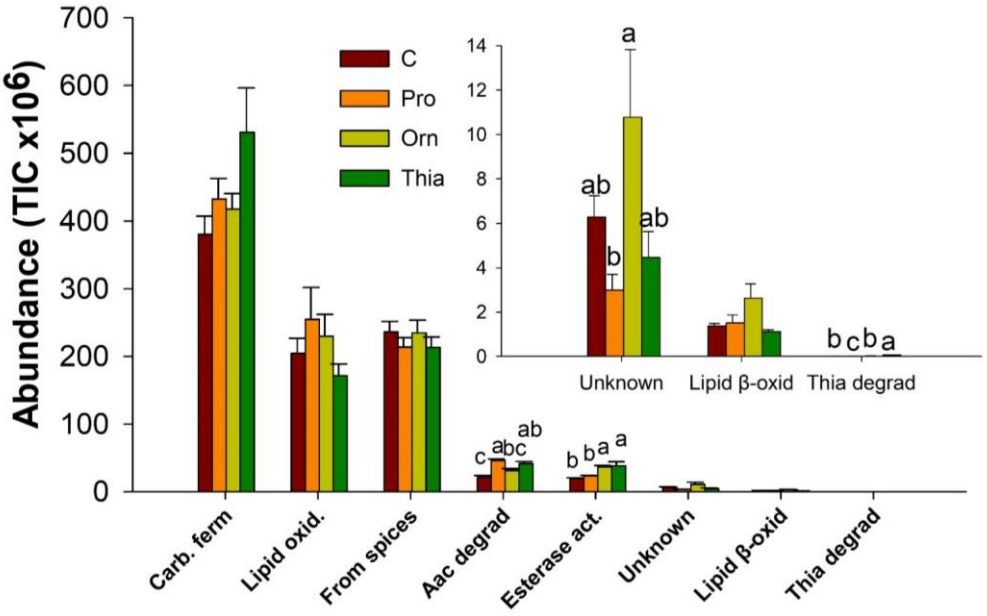

Fig. 4. Effect of precursors addition on the total content of volatile compounds (classified by the group of origin as reported in Supplementary Table 3) in the different dry fermented sausages; C: dark red bar, Pro: orange bar, Orn: light green bar and Thia: dark green bar. Data are expressed as means ± SE and different letters in each group indicate significant ($P < 0.05$) differences among formulations.

Several volatile compounds were significantly higher in the supplemented sausages (Supplementary Table 3). The Pro-sausages had a significant high abundance of propanal, hexanal, methanethiol, 3-methylbutanal, methional and benzeneacetaldehyde. The Orn-sausages had a significant higher abundance of acetone, 2,3-butanodione, 3-hydroxy-2-butanone, 1-propanol, heptane, octane, octene, propanoic acid, 3-methyl-2-buten-1-ol, decane and 2-ethyl-1-hexanol. The Thia-sausages were distinguished by the highest abundance of ethanol, ethyl acetate, ethyl 2-hydroxy-propanoate, ethyl hexanoate and 2-methyl-3-(methylthio)furan.

These differences among formulations can be seen in Fig. 5, which represents the

heatmap with hierarchical clustering based on the relative abundance of volatiles obtained at the end of drying, taking into account sausage formulations and replicates. The heatmap clearly distinguished the three replicates of the Pro-sausages, mainly due to the high relative abundance of linear aldehydes, branched aldehydes and linear acid compounds and the low abundance of branched acids and their respective ethyl ester compounds. Moreover, high variability among replicates was observed in Orn-sausages as two of the replicates (Orn-2 and Orn-3 sausages) were in a separated cluster. Furthermore, Thia- and C-sausages clustered in the same group, although Thia-2 and Thia-3 were distinguished by the high relative abundance of ethyl ester compounds and two sulfur compounds (methanethiol and 2-methyl-3-(methylthio)furan).

3.6. Aroma characteristics

Gas chromatography-olfactometry (Table 2) confirmed the presence of 29 aroma notes in dry fermented sausages, except for 5 unidentified notes. Sweet, green, herbal and vegetal notes were the main notes detected. Also, several savoury (meaty) odours were detected (LRI 907 and 1231) although not identified as well as several toasted odour notes (LRI 966, 1185 and 1217). In addition, some unidentified terpenes producing herbal notes were detected. However, those compounds showing high DF (detection frequency) values (DF>10) such as butanoic acid, 2-methyl-1-propanol, 3-methylbutanoic acid, methional, ethyl 2-methylbutanoate, 1-octen-3-ol, hexanal, α-pinene and an unknown compound (LRI 966) with a toasted odour would be the most important compounds for the aroma of dry fermented sausages.

3.7. Sensory analysis

Fig. 6A shows the odour and appearance descriptors defined by each assessor per colour in the free choice profile analysis (FCP). Fig. 6B shows the two GPA dimensions where the main descriptors explained by each dimension are shown next to them. The total variance explained by the two dimensions was 74.86%. Dimension 1 explained 40.50% of the variance. On the left side, aroma terms like “fatty” and appearance terms “white fat amount”, “bright”, “dark colour” and “oily appearance” characterized the dry fermented

sausages containing ornithine (Orn). On the right side of the plot, aroma terms “pepper”, “sour”, “boiled meat”, “toasted”, “mould” and “cheese” appeared characterizing the dry fermented Pro-sausages. Dimension 2 explained 34.36% of the variance and differentiated C from Thia-sausages. On the top of the plot, aroma terms “cured”, “savoury” “onion”, “pepper”, “fruity”, “spicy” and “toasted” and appearance terms “dark colour”, “brilliant” and “oily appearance” characterized the Thia-sausages. At the bottom of the plot, aroma terms like “fatty” characterized the C-sausages.

4. Discussion

Aroma formation in dry fermented sausages is a complex process, and many microbiological and biochemical reactions are involved (Flores, 2018). The impact of precursors like Thia, Pro and Orn on the aroma of dry fermented sausages has been studied based on the relation of thiamine to savoury (Flores et al., 2021) and proline and ornithine to toasted odours (Söllner & Schieberle, 2009).

Precursors supplementation as well as the changes in their content during the manufacture of the sausages was confirmed (Fig.1). The addition of these precursors had little effect on the fermentation and drying processes, except for several differences in TBARS values and microbiological characteristics (Table 1 and Figure 3). The lower oxidation values (TBARS) observed in the formulations containing precursors is in agreement with a recently reported study on dry cured loins (Li et al., 2022) that confirms an effect of precursors on the oxidation process. However, the antioxidant activity of yeasts cannot justify the lowest oxidation as all formulations had similar yeast counts except for the lower YM counts in the Thia-sausages.

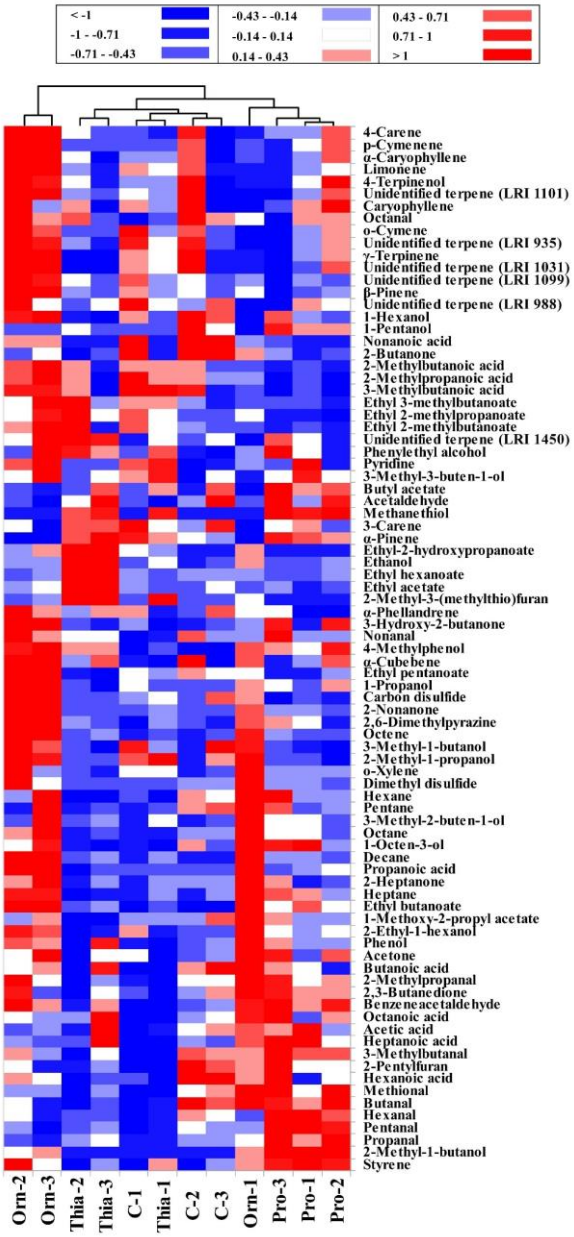

Fig. 5. Heatmap visualizing differences in VOCs formation (abundance area TIC × 10^6 in the headspace) at the end of process (62 d) in the different dry fermented sausages: C: Control without addition, and formulations with proline and ornithine (Pro and Orn), and thiamine (Thia). The map's colours indicate the following: red represents a comparatively high abundance, blue represents a relatively low abundance of the compound, and white indicates no difference.

Table 2. Odor-active compounds identified in the headspace of dry fermented sausages.

Compounds	Column DB-624		Column DB-5		GC-O description	DF[4]
	LRI GC-O[1]	LRI GC-O std	LRI GC-MS[2]	LRI GC-MS std[3]		
Carbohydrate fermentation						
2,3-Butanedione	628	626	598	593	Sweet, pungent, malty	9
3-Hydroxy-2-butanone	776	777	720	721	Sweet, lactic	9
Butanoic acid	858	859	848	844	Cheesy, buttery	11
Amino acid degradation						
Methanethiol	457	471	465	464	Sulfurous, cooked vegetable	8
2-Methyl-1-propanol	673	676	626	621	Vinegar, cologne	12
3-Methylbutanal	690	691	651	652	Sweet, herbal	8
3-Methylbutanoic acid	918	915	882	877	Cheesy	12
2,6-Dimethylpyrazine	941	945	914	914	Toasted, sour	5
Methional	970	971	907	908	Cooked potato	11
Staphylococci esterase activity						
Ethyl butanoate	824	825	811	808	Sweet, fresh	5
Ethyl 2-methylbutanoate	871	872	859	859	Sweet, fruity	11
Ethyl 3-methylbutanoate	875	876	861	858	Sweet, strawberry, green	6
Lipid β-oxidation						
1-Octen-3-ol	1020	1018	983	980	Mushroom	14
Lipid autooxidation						
Pentanal	730	735	701	699	Acrid, green, floral	8
Propanoic acid	811	802	731	728	Green, fresh, dairy	4
Hexanal	836	836	803	800	Fresh green, herbal	13
2-Pentylfuran	1008	1011	990	991	Floral, caramel	5
Nonanal	1151	1151	1099	1098	Cooked food and sweet	8

Table 2 (continued).

Derived from spices						
α-Pinene	943	945	932	935	Herbal, vegetal	·12
3-Carene	1020	1026	1011	1010	Pungent, spicy	8
γ-Terpinene	1077	-	1066	1063	Earthy, herbal	4
Unidentified terpene	1031	-	-	-	Green, herbal, metallic	9
Limonene	1045	1047	1027	1033	Fresh, citrus, herbal	6
Unidentified terpene	1097	-	-	-	Herbal, sweet	4
Unknown origin						
Unknown	907	-	-	-	Roasted meat, spicy	4
Unknown	966	-	-	-	Toasted	12
Unknown	1185	-	-	-	Roasted, toasted	7
Unknown	1217	-	-	-	Bread	6
Unknown	1231	-	-	-	Roasted meat, herbal	8

[1] LRI: Linear retention indices of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (J&W Scientific 60 m×0.32 mm i.d.×1.8 μm film thickness). [2] LRI: Linear retention indices of the compounds eluted from the GC–MS using a DB-5 capillary column (J&W Scientific 30 m× 0.25 mm i.d. × 0.25 μm film thickness). [3] Linear retention indices from standard compounds and database (http://webbook.nist.gov/chemistry). [4] DF: Detection frequency value.

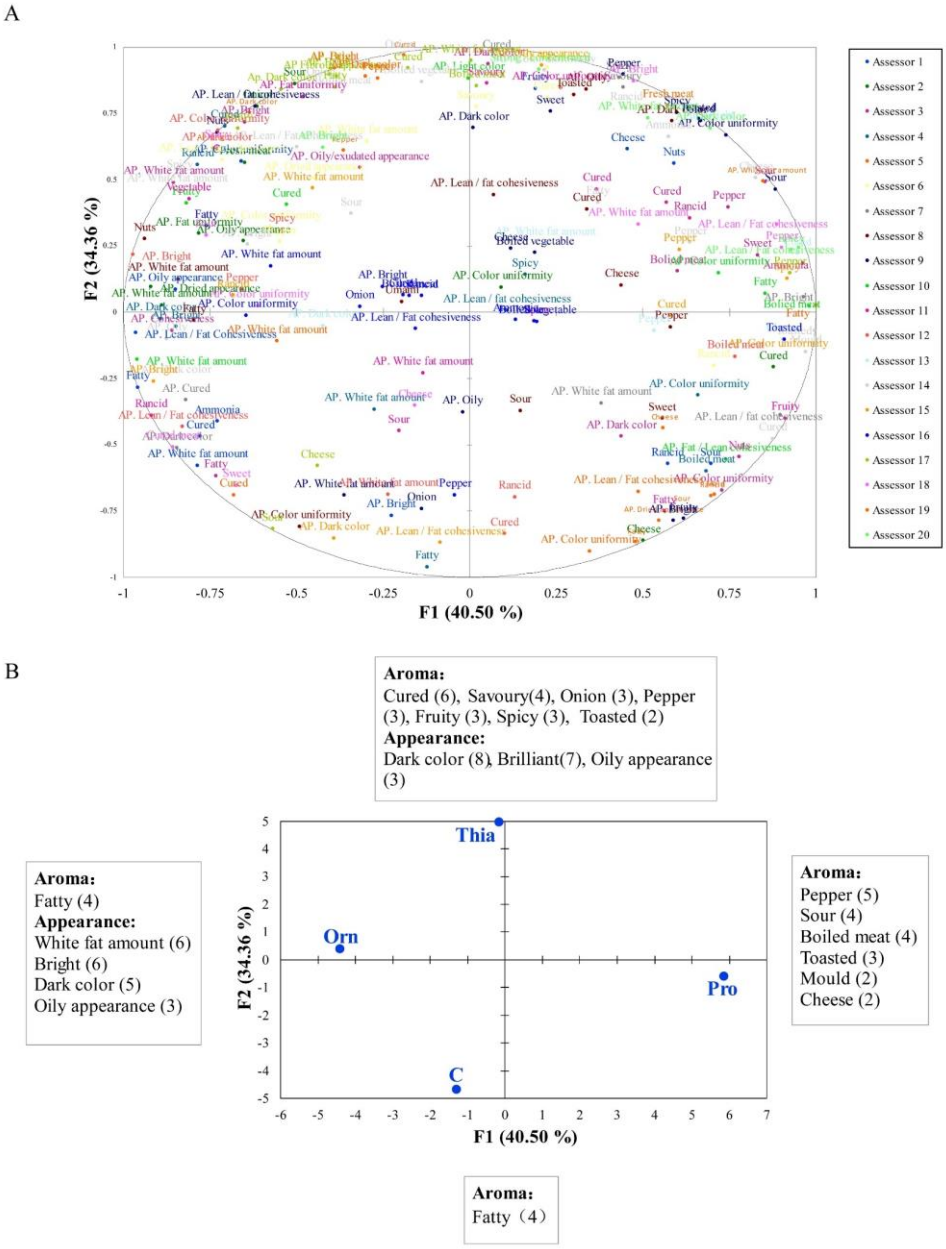

Fig. 6. The relationship of the GPA analysis from the free choice profile data (A) GPA plot of the differences between dry fermented sausages in two dimensions (B). The key descriptors associated with the first two dimensions are listed in the boxes, along with the number of times they were mentioned. C: Control without precursors, and formulations with precursors: proline and ornithine (Pro and Orn), and thiamine (Thia).

The supplementation of Pro and Orn in high quantities at the beginning of the sausage manufacture process may be a source of undesirable compounds like biogenic amines. These are generated by microbial decarboxylation of amino acids in fermented sausages (Suzzi & Gardini, 2003). Generally, starter cultures are selected to avoid or minimize the generation of biogenic amines (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000). In this case, the use of the starter culture (TRADI-302), with negative amino acid-decarboxylase activity, ensured the absence of biogenic amines in the sausages, and especially in those supplemented with Pro and Orn. Moreover, only the polyamines spermidine and spermine were detected and showed changes in concentration during the ripening process (Supplementary Table 2). However, these polyamines are amines found naturally in fresh meat (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997) and are not associated with microbial activity.

In order to study the flavour of the precursors supplemented dry fermented sausages at the end of process, the volatile profile in the headspace was analyzed by SPME extraction (Fig. 5 and Supplementary Table 3). Most of the volatile compounds identified have been found in dry fermented sausages when using SPME extraction (Corral, Salvador, et al., 2016; Olivares et al., 2011; Perea-Sanz et al., 2020) except for the presence of several compounds (*o*-cymene, *p*-cymenene, 4-terpinenol, 4-carene, α-cubebene and α-caryophyllene) mainly derived from the use of spices like black pepper (Aquilani et al., 2018). Among the most abundant compounds extracted by SPME, limonene, 3-carene and caryophyllene which are typical main black pepper aroma compounds were detected (Liu et al., 2013). The effect of spices in dry sausages depends on their use in the formulation (Aquilani et al., 2018) and they mainly contribute to the group of terpene compounds. The high variability of Orn-2 and Orn-3 sausages is probably related to a higher relative abundance of spices derived compounds than in Orn-1 sausages (Fig .5). Moreover, the role of black pepper microbiota metabolism in the volatile profile of the sausages has not been determined, but it should not be underestimated as it has been reported in the case of paprika added to dry cured loins (Belloch et al., 2021). Differences in abundance of volatile

compounds derived from spices between the four formulations could be attributed to a variety of external factors like uneven distribution and heterogeneity (Aquilani et al., 2018), as all of them were manufactured with the same content of spices.

The fermentation process in the precursor supplemented dry sausages was similar. However, differences between sausages, such as higher content of ethanol and ethyl esters in Thia-sausages, could possibly be linked to the activity of the microbial groups especially their esterase activity (Perea-Sanz et al., 2020), as yeast counts were the lowest in these sausages. Similarly, the higher abundance of acetone, 2,3-butanedione and 3-hydroxy-2-butanone in Orn-sausages may be directly related to the highest counts of LAB and GC+ (Montel, Masson, & Talon, 1998).

The microbial degradation of amino acids produced from the proteolysis process acted as a source of volatile compounds (Flores, 2018). The total content of amino acids was the lowest in C and Thia-sausages due to the absence of Pro and Orn supplementation; therefore, proteolysis should be considered excluding Pro and Orn amino acids. Moreover, a clear relationship between amino acid production and volatile compounds generation was not observed. Branched aldehydes, alcohols, and acids produced by branched-chain amino acids (Val, Leu, and Ile) (Flores, 2018) were at their highest abundance in Pro- and Orn-sausages, although no differences regarding amino acid content were detected among formulations. Despite the variability within each group of sausages (Fig .2), the concentration of FAA in Thia-sausages seemed to be different and suggested a different level of proteolytic activity.

The aroma of the supplemented dry fermented sausages was similar to those studies using the same volatile extraction conditions, and confirms the absence of off-odours due to the precursor supplementation (Aquilani et al., 2018; Corral et al., 2018; Olivares et al., 2011; Perea-Sanz et al., 2020). The aroma of the sausages was defined by the combination of several odour active compounds, mainly butanoic acid, 2-methyl-1-propanol, 3-methylbutanoic acid, ethyl 2-methylbutanoate, 1-octen-3-ol, hexanal and α-pinene that contribute to cheesy, vinegar, cheesy, cooked potato, sweet, mushroom, herbal and vegetal,

respectively. Even though, other compounds played a role in the overall profile like methional, 2,6-dimethylpyrazine and the unknown compounds, that imparted the meaty (savoury) and toasted character. However, none of the expected compounds derived from Pro and Orn, 2-acetylpyrrole and 2-acetl-1-pyrroline (Söllner & Schieberle, 2009), and 2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran, and bis(2-methyl-3-furyl)disulphide) derived from thiamine (Thomas et al., 2014; Thomas et al., 2015) were detected in the GC-MS, and only 2-methyl-3-(methylthio)furan was found by GC-MS. The main reason may be the presence of these compounds in traces in dry fermented sausages, thus making their detection and quantitation a difficult task in this complex matrix (Flores et al., 2021). Furthermore, the presence of terpenes derived from spices may have interfered in the identification of precursor-derived compounds. Therefore, the volatile profile determined by SPME does not represent in full the savoury and toasted odours because of the presence of unknown compounds detected by GCO (Table 2). These unknown compounds would have explained the differences in the sensory properties observed by the panel during the FCP analysis (Fig .6) of the dry sausages.

Previous studies regarding the effect of these precursors in another meat product, dry cured loin, revealed the existence of 2-acetyl-1-pyrroline in addition to tetramethyl pyrazine, and 2-acetylpyrrole derived from the nitrogen precursor addition (Pro and Orn) and responsible for the toasted odours (Li et al., 2022). However, the mechanism of formation can be different in dry loins and dry fermented sausages, as microbial fermentation in sausages is mainly produced by the starter culture added, mainly LAB in combination with coagulase cocci and yeast, while the natural microbiota in dry loins is highly affected by the spices used and processing factors like nitrite addition (Belloch et al., 2021). Moreover, the appropriate selection of a yeast culture needs to be done to promote the generation of 2-acetyl-1-pyrroline, which has been linked to the degradation of proline and ornithine by yeast (Schieberle, 1990).

Amongst the sulfur compounds, only 2-methyl-3-(methylthio)furan was identified and especially abundant in Thia-sausages. This compound was initially identified in cooked

beef with a low odour threshold value of 0.05 μg/kg while it produces a meaty aroma at levels below 1 μg/kg (Macleod & Ames, 1986). However, the low content of this compound precluded its identification as an impact aroma compound by GCO in sausages (Table 2), although it contributes to the "cured" meat odour in dry cured loins where the same amount of thiamine was used (Li et al., 2022). However, the ripening and drying conditions of sausages might have affected the formation of 2-methyl-3-(methylthio)furan as it is highly dependent on pH and a_w. Previous studies have found that its formation is favored at pH 5.5 in the presence of myofibrillar proteins, and at a_w 0.9 in cooked systems (Modi, Linforth, & Taylor, 2008).

The FCP sensory analysis confirmed the aroma impact caused by the addition of precursors as stated previously in dry cured loins (Li et al., 2022). The term "cured odour" described in the FCP methodology has been described in several dry cured meat products, like fermented sausages (Corral, Salvador, et al., 2016) and dry cured ham (Guàrdia, Aguiar, Claret, Arnau, & Guerrero, 2010). The definition of cured odour is not clear, buot cannot be based on one unique compound, because it is the balance among a variety of compounds (Flores, 2018; Thomas et al., 2015). The Thia-sausages were clearly distinguished by the terms "cured" and "savoury". These sausages exhibited the greatest abundance of ethanol, ethyl acetate, ethyl 2-hydroxy-propanoate, ethyl hexanoate and 2-methyl-3-(methylthio)furan, although none of them were detected as aroma active compounds by GCO. On the contrary, the "peppery", "sour" and "boiled meat" terms in Pro-sausages may be related to the highly significant abundance of the aroma compounds hexanal and methional (Table 2) producing green and cooked potato odour notes in the GCO. Similarly, the "fatty" term described in Orn-sausages may be due to the significant highest abundance of 3-hydroxy-2-butanone, 2,3-butanedione, and propanoic acid described as aroma impact compounds producing lactic, pungent, and green odours.

In summary, the differences in aromas produced by the addition of precursors into dry sausages formulations might be explained in part by the identified volatile compounds. However, the identification of unknown meaty and toasted odours may be essential for the

evaluation of their contribution to dry sausage aroma by optimized analytical techniques. The low concentrations of sulfur and nitrogen compounds together with their high reactivity, as well as, the prone to oxidation of sulfur compounds (Wei, Handoko, Pather, Methven, & Elmore, 2017; Tang, Jiang, Yuan, & Ho, 2013) requires a deep investigation to discover the impact of these minor compounds on dry meat products aroma.

5. Conclusions

In conclusion, the impact of precursors addition in the manufacture of dry fermented sausages produced an impact on the final sausage aroma. However, only the detected sulfur-derived compound 2-methyl-3-(methylthio)furan verified the effect of thiamine supplementation and the impact on the cured and savoury odours detected in Thia-sausages, while no clear effect could be attributed to specific volatile compounds in the nitrogen supplemented sausages (Orn- and Pro-sausages). Moreover, the differences in aroma produced by the precursors added might be explained in part by the identified volatile compounds, however it is essential to evaluate the contribution of unknown meaty and toasted odours to dry sausages aroma by optimized analytical techniques.

Credit author statement

Lei Li: Formal analysis, Investigation, Writing - original draft, **Laura Perea-Sanz**: Formal analysis, Methodology, **A. Salvador**: Formal analysis, Methodology, Investigation, **Carmela Belloch and Monica Flores**: Funding acquisition, Conceptualization, supervision, Writing - review & editing.

Declaration of Competing Interest

None

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the concentration of free amino acids (mg/100g DM) in dry fermented sausages.

FAA	Time(d)	C[1]		Pro		Orn		Thia		RMSE[2]	P_f[3]	P_s	P_{f*s}
Ala	0	108.12	defg[4]	105.38	efg	97.56	g	99.62	fg	13.53	***	***	**
	8	131.67	de	134.31	d	125.99	def	122.63	defg				
	34	191.23	bc	207.07	abc	199.39	bc	179.65	c				
	62	216.29	ab	229.97	a	186.31	c	203.35	abc				
Gly	0	47.01	e	46.53	e	44.04	e	43.91	e	8.15	*	***	*
	8	55.89	e	57.50	e	56.00	e	53.58	e				
	34	81.61	d	88.39	cd	87.97	cd	77.53	d				
	62	105.24	ab	111.45	a	90.90	bcd	101.40	abc				
Val	0	12.64	d	13.99	d	13.61	d	13.06	d	6.23	ns	***	ns
	8	32.06	c	32.43	c	32.58	c	32.11	c				
	34	77.98	b	81.46	b	84.36	b	81.72	b				
	62	105.27	a	103.26	a	97.28	a	103.98	a				
β-Ala	0	44.40	ab	44.80	ab	41.89	ab	42.43	ab	2.95	**	ns	ns
	8	42.95	ab	43.22	ab	43.55	ab	43.82	ab				
	34	42.50	ab	47.42	a	44.42	ab	43.02	ab				
	62	42.41	ab	46.01	ab	41.19	b	42.58	ab				

Supplementary Table 1 (continued).

Leu	0	15.27	e	16.09	e	14.60	e	14.94	e	10.92	ns	***	ns
	8	58.98	d	57.21	d	58.14	d	57.85	d				
	34	136.53	c	142.20	c	149.51	bc	144.37	c				
	62	178.68	a	177.16	a	171.45	ab	176.53	a				
Ile	0	14.90	e	14.95	e	14.65	e	14.32	e	4.85	ns	***	ns
	8	27.58	d	26.71	d	27.23	d	26.58	d				
	34	63.54	c	64.23	c	67.74	bc	67.04	c				
	62	81.87	a	80.02	a	76.95	ab	80.74	a				
Thr	0	8.41	f	8.26	f	8.09	f	8.44	f	4.32	ns	***	ns
	8	22.90	e	22.60	e	23.07	e	21.68	e				
	34	53.24	d	56.69	cd	59.58	bcd	52.01	d				
	62	70.28	a	67.73	ab	64.33	abc	68.87	a				
Ser	0	24.96	e	20.95	e	26.22	e	25.67	e	9.54	ns	***	*
	8	56.74	d	50.54	d	58.11	d	54.47	d				
	34	108.24	bc	115.74	abc	122.32	ab	102.04	c				
	62	130.24	a	121.25	abc	121.07	abc	126.80	ab				
Pro	0	12.57	c	366.86	a	15.95	c	13.17	c	16.13	***	ns	***
	8	18.04	c	350.92	ab	21.16	c	17.88	c				
	34	20.02	c	321.49	b	23.06	c	14.44	c				
	62	29.48	c	326.88	b	27.86	c	28.10	c				

Supplementary Table 1 (continued).

Asn	0	5.36	d	5.00	d	4.40	d	4.66	d	3.06	ns	***	ns
	8	8.87	d	8.99	d	8.78	d	7.99	d				
	34	23.57	c	24.74	c	26.31	bc	21.72	c				
	62	35.60	a	32.60	a	32.32	ab	35.41	a				
Asp	0	3.27	c	2.64	c	2.67	c	2.09	c	4.49	ns	***	ns
	8	3.42	c	3.53	c	4.08	c	3.31	c				
	34	31.10	b	29.40	b	36.72	b	36.04	b				
	62	58.35	a	53.94	a	55.39	a	60.92	a				
Met	0	8.79	e	8.74	e	7.59	e	8.41	e	4.27	ns	***	ns
	8	22.37	d	21.81	d	21.56	d	21.86	d				
	34	51.47	c	51.72	c	54.65	bc	54.70	bc				
	62	63.97	a	62.51	ab	58.51	abc	62.45	ab				
Hyp	0	4.44	def	4.39	def	4.38	def	4.19	ef	1.76	ns	***	***
	8	4.26	ef	4.38	def	4.07	f	4.99	cdef				
	34	10.79	a	7.94	abcd	9.36	a	8.92	ab				
	62	5.47	bcdef	7.76	abcde	8.25	abc	10.93	a				
Glu	0	23.51	e	27.15	e	23.89	e	21.62	e	12.50	ns	***	ns
	8	74.16	d	80.82	d	73.59	d	76.97	d				
	34	144.07	c	146.11	c	155.34	bc	163.12	abc				
	62	174.90	ab	178.12	ab	166.35	abc	186.37	a				

Supplementary Table 1 (continued).

Phe	0	12.41	d	17.15	d	12.77	d	12.34	d	6.23	ns	***	ns
	8	33.23	c	32.32	c	33.66	c	33.48	c				
	34	78.19	b	78.10	b	83.31	ab	84.10	ab				
	62	92.16	a	92.97	a	92.05	a	94.06	a				
Gln	0	214.36	a	184.45	ab	208.60	a	201.15	a	20.53	ns	***	ns
	8	137.60	cd	149.67	bcd	156.42	bc	141.89	cd				
	34	134.10	cd	145.57	bcd	151.18	bcd	118.57	cd				
	62	130.16	cd	116.94	cd	112.26	d	111.74	d				
Orn	0	5.01	g	5.28	g	196.88	c	6.37	fg	8.10	***	***	**
	8	21.43	efg	22.41	ef	226.74	b	23.87	e				
	34	43.68	d	47.02	d	266.05	a	47.60	d				
	62	55.66	d	56.28	d	265.31	a	58.95	d				
Lys	0	13.26	f	13.49	f	14.35	f	13.93	f	10.80	**	***	*
	8	36.55	e	40.05	e	39.73	e	37.17	e				
	34	116.94	d	131.45	cd	146.40	bc	118.29	d				
	62	166.36	ab	175.95	a	171.74	a	172.25	a				
His	0	33.04	ef	30.53	f	32.80	ef	33.75	ef	7.54	*	***	ns
	8	40.17	ef	44.23	ef	47.04	e	47.07	e				
	34	87.32	d	92.17	cd	98.23	bcd	99.26	bcd				
	62	109.12	ab	105.19	abc	106.30	abc	115.66	a				

Supplementary Table 1 (continued).

Tyr	0	8.02	f	9.74	f	9.37	f	10.24	f	4.76	***	***	ns
	8	23.35	e	24.90	e	26.11	e	26.99	e				
	34	42.11	d	49.42	cd	52.36	bc	55.32	abc				
	62	58.63	abc	57.37	abc	60.73	ab	63.69	a				
Trp	0	8.04	ef	7.31	f	7.20	f	7.37	f	1.14	ns	***	ns
	8	10.25	de	10.68	d	10.44	d	10.33	de				
	34	17.85	c	18.40	bc	19.21	abc	18.99	abc				
	62	19.62	abc	20.49	ab	19.55	abc	20.75	a				
Total	0	625.53	gh	949.90	ef	801.94	fgh	602.09	h	119.99	***	***	ns
	8	862.20	efg	1221.45	d	1098.51	de	860.11	efg				
	34	1557.23	c	1909.68	b	1939.56	b	1584.48	c				
	62	1903.28	b	2218.31	a	2018.92	ab	1925.65	b				

[1] C: Control sausages without precursors, addition of precursors: proline and ornithine at 120 mg/100 g (Pro and Orn), and thiamine at 24 mg/100 g (Thia). [2] RMSE: root mean square error. [3] *P* value of formulation (f), stage (s) and formulation and stage (f*s) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] Different letters in the same row and column of each amino acid indicate significant differences at $P < 0.05$.

Supplementary Table 2. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the biogenic amine content in dry fermented sausages (mg/Kg DM).

	Time(d)	C[1]	Pro	Orn	Thia	RMSE[2]	P_f[3]	P_s	P_{f*s}
Tyramine	0	ND[4]	ND	ND	ND	0.00	ns	ns	ns
	8	ND	ND	ND	ND				
	34	ND	ND	ND	ND				
	62	ND	ND	ND	ND				
Histamine	0	ND	ND	ND	ND	0.00	ns	ns	ns
	8	ND	ND	ND	ND				
	34	ND	ND	ND	ND				
	62	ND	ND	ND	ND				
Putrescine	0	ND	ND	ND	ND	0.00	ns	ns	ns
	8	ND	ND	ND	ND				
	34	ND	ND	ND	ND				
	62	ND	ND	ND	ND				
Cadaverine	0	ND	ND	ND	ND	0.00	ns	ns	ns
	8	ND	ND	ND	ND				
	34	ND	ND	ND	ND				
	62	ND	ND	ND	ND				
Phenethylamine	0	ND	ND	ND	ND	0.00	ns	ns	ns
	8	ND	ND	ND	ND				
	34	ND	ND	ND	ND				
	62	ND	ND	ND	ND				

Supplementary Table 2 (continued).

Spermidine	0	12.64	abc[5]	11.93	abcd	11.13	abcde	12.22	abc	1.38	***	***	***
	8	9.96	bcdef	8.93	ef	11.64	abcde	10.91	abcde				
	34	7.77	f	11.45	abcde	10.63	abcde	9.90	cdef				
	62	9.14	def	13.31	a	11.83	abcd	12.76	ab				
Spermine	0	29.72	efg	26.42	fg	24.81	g	27.29	fg	3.83	ns	***	***
	8	38.41	cd	32.81	def	38.17	cd	33.79	def				
	34	35.35	de	43.36	bc	38.81	cd	38.22	cd				
	62	43.80	bc	54.11	a	49.80	ab	54.08	a				
BA[6]	0	0.00	ND	0.00	ND	0.00	ND	0.00	ND	0.00	ns	ns	ns
	8	0.00	ND	0.00	ND	0.00	ND	0.00	ND				
	34	0.00	ND	0.00	ND	0.00	ND	0.00	ND				
	62	0.00	ND	0.00	ND	0.00	ND	0.00	ND				
PA	0	42.36	efgh	38.35	gh	35.94	h	39.51	fgh	5.02	ns	***	***
	8	48.37	cdefg	41.74	efgh	49.81	cde	44.70	cdefgh				
	34	43.12	defgh	54.81	bc	49.44	cdef	48.13	cdefg				
	62	52.94	bcd	67.42	a	61.62	ab	66.83	a				
Total	0	42.36	efgh	38.35	gh	35.94	h	39.51	fgh	5.02	ns	***	***
	8	48.37	cdefg	41.74	efgh	49.81	cde	44.70	cdefgh				
	34	43.12	defgh	54.81	bc	49.44	cdef	48.13	cdefg				
	62	52.94	bcd	67.42	a	61.62	ab	66.83	a				

[1] C: Control sausages without precursors, addition of precursors: proline and ornithine at 120 mg/100 g (Pro and Orn), and thiamine at 24 mg/100 g (Thia). [2] RMSE: root mean square error. [3] *P* value of formulation (f), stage (s) and formulation and stage (f*s) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] ND: not detected. [5] Different letters in the same row and column of each biogenic amine indicate significant differences at $P < 0.05$. [6] BA = biogenic amines (tyramine +histamine + putrescine+ cadaverine+ phenethylamine); PA = polyamines (spermine + spermidine).

Supplementary Table 3. Effect of the addition of nitrogen (proline and ornithine) and sulfur (thiamine) precursors on the volatile compounds (expressed as AU × 10^6) detected in the headspace of dry fermented sausages.

Compounds	LRI[1]	RI[2]	C[3]		P		O		T		RSME[4]	*P*[5]
Carbohydrate fermentation			380.22		432.73		417.70		530.67		91.97	ns
Acetaldehyde	467	a	2.47		2.80		1.82		2.47		0.65	ns
Ethanol	509	a	68.48	b[6]	68.65	b	97.41	b	216.71	a	40.85	***
Acetone	530	a	6.90	ab	8.32	ab	9.78	a	5.63	b	1.69	**
2,3-Butanedione (86)[7]	627	a	0.04	ab	0.05	ab	0.05	a	0.03	b	0.01	*
2-Butanone	631	a	7.47	a	3.02	c	4.30	b	2.25	d	0.41	***
Acetic acid	718	a	300.42		334.55		313.90		326.39		65.85	ns
3-Hydroxy-2-butanone (45)	782	a	0.26	b	0.34	ab	0.44	a	0.22	b	0.08	**
Butanoic acid	893	a	11.74		11.22		12.07		10.75		1.69	ns
Amino acid degradation			20.90	c	45.93	a	31.28	bc	41.12	ab	7.12	***
Methanethiol	473	a	2.20	b	26.31	a	2.17	b	23.05	a	3.87	***
2-Methylpropanal	594	a	0.80	b	1.03	ab	1.28	a	0.64	b	0.24	**
2-Methyl-1-propanol	682	a	0.55	ab	0.39	b	0.87	a	0.62	ab	0.20	**
3-Methylbutanal	690	a	7.24	b	9.99	a	8.26	ab	5.59	b	1.55	**
Dimethyl disulfide (94)	773	a	0.04	b	0.03	b	0.20	a	0.02	b	0.08	*
3-Methyl-1-butanol (55)	795	a	0.73		0.34		0.94		0.42		0.34	ns
2-Methyl-1-butanol	798	a	0.26	a	1.73	b	1.04	ab	0.30	b	0.57	**
2-Methylpropanoic acid (73)	866	a	4.78	ab	1.12	b	6.28	a	2.98	ab	2.21	*
3-Methylbutanoic acid (60)	943	a	3.83		0.91		4.57		4.04		2.21	ns
2,6-Dimethylpyrazine	946	a	1.00	b	1.15	b	1.58	a	1.06	b	0.19	**
2-Methylbutanoic acid (74)	948	a	1.63	ab	0.40	b	4.74	a	1.47	ab	1.67	*

Supplementary Table 3 (continued).

Methional (104)	969	a	0.04	b	0.08	a	0.07	ab	0.04	b	0.02	*
Benzeneacetaldehyde	1109	a	0.83	b	1.16	a	1.18	a	0.83	b	0.18	**
Phenol	1115	a	1.67	b	1.88	ab	2.31	a	1.75	ab	0.35	*
Phenylethyl alcohol	1194	a	0.49		0.88		0.88		0.97		0.33	ns
Staphylococci esterase activity			19.28	b	23.28	b	36.44	a	38.03	a	7.25	***
Ethyl acetate (56)	635	a	10.05	b	6.58	b	9.05	b	21.24	a	6.23	**
Ethyl 2-methylpropanoate (71)	788	a	0.37		0.05		0.53		0.51		0.26	ns
Ethyl butanoate	832	a	1.93	c	6.83	b	12.00	a	4.34	bc	1.53	***
Butyl acetate (56)	848	a	0.09	ab	0.15	a	0.07	b	0.10	ab	0.03	*
Ethyl-2-hydroxypropanoate (45)	867	a	2.27	b	1.68	b	3.51	ab	4.73	a	1.42	**
Ethyl 2-methylbutanoate (57)	878	a	0.17		0.04		0.32		0.27		0.14	ns
Ethyl 3-methylbutanoate (88)	882	a	0.14	ab	0.09	b	0.31	ab	0.47	a	0.20	*
1-Methoxy-2-propyl acetate	910	b	0.41	ab	0.40	ab	0.56	a	0.32	b	0.11	*
Ethyl pentanoate	929	a	8.61	ab	7.33	b	10.58	a	6.98	b	1.64	**
Ethyl hexanoate (88)	1028	a	0.06	b	0.06	b	0.06	b	0.19	a	0.07	*
Lipid β-oxidation			1.39		1.51		2.63		1.13		0.95	ns
2-Heptanone (58)	936	a	0.28	b	0.27	b	0.67	a	0.27	b	0.19	**
1-Octen-3-ol (57)	1030	a	0.90	ab	1.24	a	1.24	a	0.56	b	0.35	*
2-Nonanone	1142	a	0.16	b	0.27	b	1.18	a	0.25	b	0.27	***
Lipid autooxidation			204.42		254.49		229.78		171.70		79.64	ns
Pentane	500	a	12.14		11.45		19.51		9.87		9.81	ns
Propanal	524	a	1.14	b	2.50	a	1.19	b	1.17	b	0.47	***
Hexane	600	a	1.92		2.29		2.93		1.03		1.18	ns
1-Propanol	612	a	0.52	b	0.59	b	0.97	a	0.42	b	0.22	**
Butanal	622	a	0.70		0.82		0.72		0.58		0.22	ns

Supplementary Table 3 (continued).

Heptane	700	a	10.26	b	16.47	ab	20.89	a	9.99	b	5.19	*
Pentanal	738	a	12.79	b	23.86	b	12.18	b	10.93	b	5.29	**
3-Methyl-3-buten-1-ol (56)	790	a	0.11		0.12		0.12		0.12		0.02	ns
Octane	800	a	12.87	b	15.51	ab	25.03	a	8.82	b	5.84	**
Octene	810	b	0.23	b	0.31	b	0.84	a	0.29	b	0.15	***
Propanoic acid	812	a	1.99	b	2.16	b	3.30	a	1.86	b	0.36	***
1-Pentanol（55）	828	a	1.05		0.96		0.53		0.63		0.44	ns
3-Methyl-2-buten-1-ol	836	a	2.03	b	2.38	ab	3.18	a	2.33	b	0.49	**
Hexanal	841	a	105.26	ab	166.27	a	96.77	b	87.37	b	38.90	*
Decane	1000	a	0.23	b	0.28	b	0.52	a	0.28	b	0.07	***
2-Pentylfuran	1009	a	1.41		1.66		1.30		1.04		0.53	ns
Octanal (56)	1047	a	1.22		1.14		1.40		1.11		0.34	ns
1-Hexanol	925	a	7.61	a	5.27	ab	6.43	ab	2.88	b	1.86	*
Hexanoic acid	1078	a	4.97		4.45		4.48		3.32		0.99	ns
2-Ethyl-1-hexanol	1083	a	1.77	b	1.77	b	3.04	a	1.14	b	0.52	***
Nonanal	1151	a	27.77		33.76		38.07		29.42		6.86	ns
Heptanoic acid	1170	a	0.44	b	0.81	b	0.56	ab	0.42	b	0.18	*
Octanoic acid	1267	a	1.65		2.18		2.14		1.90		0.45	ns
Nonanoic aicd (60)	1360	a	0.18	a	0.05	c	0.11	b	0.04	c	0.02	***
Derived from spices			236.07		213.63		234.50		213.21		38.55	ns
o-Xylene	917	a	0.42	b	0.48	ab	0.75	a	0.43	b	0.17	*
Styrene	919	a	0.28	b	0.56	ab	0.49	a	0.39	ab	0.11	**
Unidentified terpene (93)	935	b	1.97		1.60		2.01		1.65		0.40	ns
α-Pinene (93)	941	a	1.37	ab	1.47	ab	0.91	b	1.56	a	0.36	*

Supplementary Table 3 (continued).

Unidentified terpene	988	b	15.49		14.08		14.25		14.13		2.98	ns
β-Pinene	1003	a	5.85	b	4.61	b	8.48	a	5.35	b	1.59	**
α-Phellandrene	1017	a	7.14		6.11		8.36		6.73		1.50	ns
3-Carene	1020	a	63.69		57.51		50.36		62.25		10.94	ns
Unidentified terpene (93)	1031	b	0.46		0.35		0.47		0.30		0.14	ns
Limonene	1043	a	62.36		55.75		78.52		55.47		14.63	ns
o-Cymene	1049	b	25.85		18.87		24.54		18.24		7.25	ns
γ-Terpinene	1074	b	7.42		4.81		7.36		4.49		2.05	ns
Unidentified terpene (93)	1099	b	0.07	ab	0.06	b	0.10	a	0.07	ab	0.02	*
Unidentified terpene (121)	1101	b	0.29		0.26		0.31		0.27		0.08	ns
p-Cymenene	1120	b	0.89	ab	0.93	ab	1.10	a	0.85	b	0.14	*
4-Terpinenol	1229	a	7.67		8.51		9.83		7.36		1.91	ns
4-Carene	1365	b	2.07		2.18		2.52		2.00		0.47	ns
α-Cubebene	1405	b	2.04		2.15		2.51		2.12		0.56	ns
Unindentified terpene	1450	b	2.43		2.64		2.68		3.02		0.61	ns
Caryophyllene	1469	a	28.43		29.49		27.41		26.10		5.28	ns
α-Caryophyllene	1505	b	1.79		1.84		2.19		1.73		11.54	ns
Thiamine degradation			0.02	b	0.01	c	0.03	b	0.07	a	0.00	***
2-Methyl-3-(methylthio)furan (128)	978	a	0.02	b	0.01	c	0.03	b	0.07	a	0.00	***
Unknown origin			6.28	ab	2.99	b	10.77	a	4.46	ab	4.41	*
Carbon disulfide (76)	538	a	5.94	b	2.88	b	12.45	a	4.57	b	3.20	**
Pyridine(79)	786	a	0.26		0.31		0.42		0.30		0.13	ns
4-Methylphenol (108)	1198	a	0.08	c	0.10	ab	0.11	a	0.10	b	0.01	***
Total			868.58		974.58		963.13		1000.38		138.93	ns

The main groups according to the most likely origin in bold.

[1]Linear retention indices (LRI) of the compounds eluted from the GC–MS using a DB-624 capillary column (J&W Scientific 30 m×0.25 mm i.d. ×1.4 μm film thickness). [2]RI: reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentatively identification by mass spectrum. [3]C: Control loins without precursors, addition of precursors: proline and ornithine at 120 mg/100 g (Pro and Orn), and thiamine at 24 mg/100 g (Thia). [4]RMSE: root mean square of errors. [5]*P* value of formulation effect. ***: $P < 0.001$,**: $P < 0.01$,*: $P < 0.05$,ns: $P > 0.05$. [6]Different letters in the same row indicate significant differences at $P < 0.05$ among formulations. [7]Target ion used to quantify the compound when the peak was not completely resolved.

Chapter 3

A comparative study of savory and toasted aromas in dry cured loins versus dry fermented sausages

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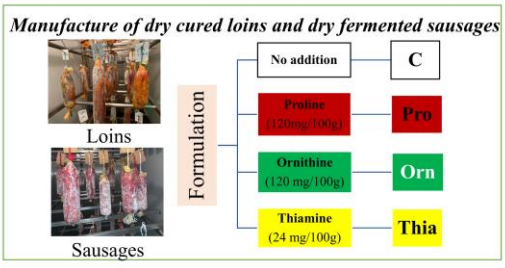

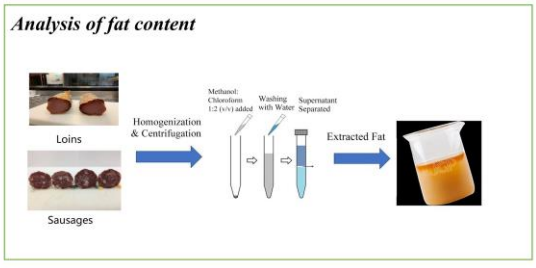

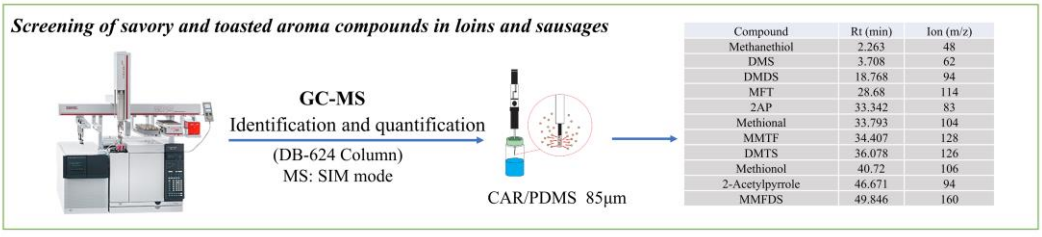

Compound	Rt (min)	Ion (m/z)
Methanethiol	2.263	48
DMS	3.708	62
DMDS	18.768	94
MFT	28.68	114
2AP	33.342	83
Methional	33.793	104
MMTF	34.407	128
DMTS	36.078	126
Methionol	40.72	106
2-Acetylpyrrole	46.671	94
MMFDS	49.846	160

A comparative study of savory and toasted aromas in dry cured loins versus dry fermented sausages

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ABSTRACT

Dry cured meat products, loins and sausages, have a different cured aroma due to differences in their formulation and manufacture. In these products, savory and toasted notes produced by sulfur and nitrogenated volatile compounds are essential for cured aroma. The interest of the meat industry to modulate the aroma profile and the creation of specific flavourings recognizes the necessity of gaining knowledge about the pathways involved into aroma generation. For this, a comparative study was done in dry cured loins and sausages. The two products were supplemented with either proline, ornithine or thiamine and compared to a control without supplement. The concentration of these volatile compounds was analysed by GC-MS in SIM mode. Eleven volatile compounds were identified, nine in loins and eight in sausages. Loin aroma was impacted by methional and 2-acetyl-1-pyrroline, contributing to cooked potato and toasted notes; meanwhile, sausage aroma was modulated by 2-methyl-3-furanthiol, methyl 2-methyl-3-furyl disulfide, producing fatty and meaty odors, and methional. The contribution of the microbial starters to the development of sausage aroma seems to have a higher effect than the addition of precursors. In loins, thiamine addition produced the highest concentration of 2-methyl-3-(methylthio)furan, while 2-acetyl-1-pyrroline and 2-acetylpyrrole increased in the proline supplemented loins.

Keywords: savory; toasted; aroma; dry loins; fermented sausages

1. Introduction

Traditionally cured meat products are highly appreciated due to their distinct aroma and flavor caused by variations in their manufacture and formulation (Flores, 2017). In this sense, two of the most representative dry cured meat products in the Mediterranean region, dry cured loins and dry fermented sausages, are characterized by rather different cured aroma profile. Despite the interest in these "cured" odor notes, little research has been paid regarding the factors influencing the formation of meaty (savory) and toasted aromas in dry cured meat products.

The toasted and savory aromas are thought to be mostly caused by nitrogen and sulfur molecules, which produce a significant impact on the global meat aroma in dry cured meat products (Flores, Perea-Sanz, López-Díez, & Belloch, 2021). The identification and quantification of these compounds in meat products at trace levels remains a challenge, and it is crucial for the development of "savory" aromas. Among the precursors of these odors, amino acids containing sulfur atoms like cysteine, cystine and methionine, as well as the vitamin thiamine, have been found crucial precursors of odor notes "roasted" or "cooked meaty" in cooked meat products (Güntert et al., 1990; Meynier & Mottram, 1995;Thomas, Mercier, Tournayre, Martin, & Berdagué, 2015). Previous studies have indicated that cysteine was the precursor of 2-methyl-3-furanthiol (Meynier & Mottram, 1995; Tang, Jiang, Yuan, & Ho, 2013)，while methionine was the origin of methional, methionol, methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide. These compounds are generated via the Maillard reaction, Strecker degradation and Ehrlich pathway (Perpète, Gijs, & Collin, 2008). Thiamine has a significant impact on the formation of 2-methyl-3-furanthiol, 2-methyl-3-(methylthio)furan and bis(2-methyl-3-furyl) disulfide in cooked ham (Thomas et al., 2015). On the other hand, toasted odor note produced by 2-acetyl-1-pyrroline has been found in cooked lean beef (Specht & Baltes, 1994) and fermented sausages (Stahnke, 2000), although the origin of this compound in meat products is not clear as it may be originated from Maillard or biological reactions (Wei, Handoko,

Pather, Methven, & Elmore, 2017).

The impact of these precursors in the generation of aroma in dry cured meat products is not entirely clear. Dry fermented sausages savory aroma is based on the crucial role of free amino acids (Corral, Leitner, Siegmund, & Flores, 2016; Li, Perea-Sanz, Salvador, Belloch, & Flores, 2022), although yeast inoculation seems to have an important effect on the aroma profile in terms of savory aroma generation. The mild temperatures (10 to 20 °C) used during their manufacture do not favor the transformation of thiamine into cured odors (Flores et al., 2021). Furthermore, the supplementation of thiamine in dry fermented sausages confirmed its degradation and formation of 2-methyl-3-(methylthio)furan (Li, Perea-Sanz, Salvador, et al., 2022). A similar effect was seen in dry cured loins (Li, Perea-Sanz, López-Díez, et al., 2022).

Nitrogen-compounds like 2-acetyl-1-pyrroline, producing toasted odors, have been detected in meat products manufactured under mild temperature conditions such as dry fermented sausages (Corral, Leitner, et al., 2016; Li, Perea-Sanz, Salvador, et al., 2022). Moreover, this compound has been also found in other dry cured products such as Iberian Hams (Carrapiso, Jurado, Timón, & García, 2002) and Jinhua ham (Song, Cadwallader, & Singh, 2008). Proline and ornithine, produced during the dry curing process, were identified as the precursors of 2-acetyl-1-pyrroline (Adams & De Kimpe, 2007).

The elucidation of the mechanisms, as well as the factors that affect the formation of savory and toasted aromas in dry cured meat products, will allow to establish strategies to enhance the aroma of dry meat products. Therefore, the goal of this study was to investigate the differences in savory and toasted aroma compounds between dry-cured loins and dry-fermented sausages supplemented with sources of nitrogen and sulfur compounds (proline, ornithine and thiamine) into their formulation. Moreover, owing to the low concentration of nitrogen and sulfur aroma compounds and the interference of other compounds, optimized analytic techniques were applied.

2. Materials and methods

2.1. Reagents and Standards

Chemical compounds dimethyl sulfide (DMS), dimethyl disulfide (DMDS), 2-methyl-3-furanthiol (MFT), methional, 2-methyl-3-(methylthio)furan (MMTF), dimethyl trisulfide (DMTS), methionol, 2-acetylpyrrole and methyl 2-methyl-3-furyl disulfide (MMFDS) were purchased from Sigma-Aldrich (St Louis, MO), and 2-acetyl-1-pyrroline (2AP) from Toronto Research Chemicals Inc (North York, Canada).

2.2. Manufacture of dry cured loins and dry fermented sausages

Two different dry cured meat products, dry cured loins and dry fermented sausages, produced at the pilot plant were analysed. Sausages were composed of a mixed batter (pork lean meat (50%) and pork back fat (50%)) fermented by the addition of carbohydrates and starter culture. Dry cured loins were manufactured as an entire muscle using *Longissimus thoracis et lumborum* which was mostly composed of lean meat. Among each type of product different samples taken at the end of the drying process were used corresponding to three treatments manufactured with a supplementation containing proline (1.2 g/kg, Pro), ornithine (1.2 g/kg, Orn) or thiamine (0.24 g/kg, Thia) and a fourth treatment without supplementation which was used as control (Li, Perea-Sanz, López-Díez, et al., 2022; Li, Perea-Sanz, Salvador, et al., 2022). Analysis was performed on 24 sausages dried for 68 d (six sausage replicates per treatment group) and 16 dry cured loins dried for 62 d (four loin replicates per treatment group). From each product, sausages and loins, several slices were taken, minced and wrapped in aluminium foil, vacuum sealed and stored at -80 °C until analysis.

2.3. Analysis of fat content

The total fat content was analysed by the method of (Folch, Lees, & Sloane Stanley, 1957). Total fat was extracted using chloroform: methanol (2:1) from 10 g of loins and 5 g of sausages minced samples. BHT (0.05%) was added beforehand to prevent lipid oxidation during extraction.

2.4. Screening of nitrogen and sulfur aroma compounds in meat products

The analysis of aroma compounds was carried out in an Agilent 7890/5975C GC-MS equipped with an autosampler (MPS2 multipurpose sampler from Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) employing a 85 μm Carboxen/polydimethylsiloxane CAR/PDMS SPME fiber (Supelco, Bellefonte, PA). Four grams of minced loins or sausages along with 0.75 mg BHT were introduced in a 20 ml headspace vial (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany), which was purged with nitrogen gas for 10 seconds. An internal standard, 2-methyl-3-heptanone (256 ng), was added to the vials. The vial was beforehand equilibrated at 37 °C for 30 min and then submitted to fiber extraction at 37 °C.

The screening of nitrogen and sulfur volatile compounds was fine-tuned by extending the extraction time of the fiber in the headspace until their presence was confirmed. Different extraction times were assayed at 2, 7, and 20 h. The extracted volatiles were analysed by desorbing the fiber for 5 min in splitless mode at 220 °C at the GC-MS injection port. The separation of volatile compounds was done using a 30 m × 0.25 mm × 1.4 μm DB-624 capillary column (J&W Scientific, Agilent Technologies, Santa Clara, CA, USA) (Flores et al., 2021). The GC oven temperature began at 38 °C, was held for 13 min, and then it was ramped to 100 °C at 3 °C/min, held for 5 min, and followed by a second ramp at 4 °C/min to 150 °C and a final ramp at 10 °C/min up to 210 °C with a final holding time of 5 min, giving a total run time of 62.2 min. The MS interface's temperature was set at 240 °C. Mass spectra in the full-scan mode were obtained at 70 eV electron energy in the range 31–350 amu. The identification was based on mass spectra using the NIST 17 library and comparison of the retention index (LRI) of each compound calculated according to authentic standards based on Van Den Dool and Kratz (1963) and using a alkane standard C8-C22 solution (Sigma-Aldrich, Switzerland).

Quantitation of nitrogen and sulfur compounds was done by means of the internal standard method. A stock solution containing the compounds (DMS 46 ppm, DMDS 0.22 ppm, MFT 300 ppm, methional 200058 ppm, MMTF 0.6 ppm, DMTS 436 ppm,

methionol 1603 ppm, 2-acetylpyrrole 59 ppm, MMFDS 111ppm was prepared in ethanol and diluted to different concentrations using ethanol. Also a solution of 2-methyl-3-heptanone (internal standard) at 8 ppm was prepared and diluted with ethanol. Four grams of fresh loins or sausage batter were minced and used as matrix and introduced in the 20 ml headspace vials. For calibration, several dilutions (1/10, 1/20, 1/40, 1/80, 1/120, 1/160, 1/200, 1/240, 1/320) of the compounds stock solution were prepared and then, 10 µl of the dilutions were added to the vials. Different amounts of internal standard (2-methyl-3-heptanone) were added into the vials, 4 ng to loins and 8 ng to sausages by adding 20µl of 0.2 ppm and 0.4 ppm solutions, respectively, thus taking into account the matrix effect. The vials were analyzed by GC-MS using detector in SIM mode by the previous selection of specific ions (m/z), indicated in Tables 2 and 3, and obtained by the GC-MS SCAN mode (Flores et al., 2021). Aroma compounds in the meat product samples were quantified after fiber extraction for 2 h at 37 °C, as indicated above, and calibration curves constructed by desorbing the SPME fiber into the GC-MS. Calibration curves for each compound were obtained by plotting the area ratio of the specific ion (m/z) of each compound to the internal standard against the ng of compounds added in each matrix, except for methanethiol and 2AP. For the quantitation of methanethiol the calibration curve of dimethyl disulfide was used, while the quantitation of 2AP was based on the calibration curve reported in sausages (Flores et al., 2021). The compounds were expressed in nanograms per gram of loins or sausages expressed in dry matter (DM), correcting the data by the sample humidity content calculated by drying the sample at 100 °C until constant weight was reached (BOE, 1979).

The analysis of the compounds was validated in terms of linearity and sensitivity. Linearity was checked using standard solutions at five concentration levels which were repeated three times. The sensitivity was determined by the limits of detection (LOD) and quantification (LOQ) that were calculated from the extraction of a blank sample, plus three times and ten times the standard deviation respectively, of five blank replicates for fresh loin and sausage batter (Olivares, Navarro, & Flores, 2009).

The estimation of odor-activity values (OAVs) of the nitrogen and sulfur compounds was done by calculation of the ratio of the concentration in loin or sausages by their respective odor threshold reported in oil or water (Van Gemert, 2011).

2.5. Statistical analysis

Data were analysed using the statistical software (XLSTAT 2018, Addinsoft, Barcelona, Spain) using a linear mixed model including meat product type (loin or sausage) and manufacture treatment as fixed effects, and replicates (six for sausages and four for loins) as a random effect. Tukey test was employed to compare means when a significant effect was detected ($P < 0.05$).

3. Results and discussion

The main difference in composition between dry loins and fermented sausages after drying was observed in their fat content due to the manufacturing process (Supplementary Fig. 1). As expected, fat content in dry-cured loins ranged from 5.3 to 7.0% without differences among replicates, while fat content in fermented sausages ranged from 28.1 and 30.3% (Supplementary Fig. 1). Similar results have been reported for sausages (Olivares, Navarro, & Flores, 2011), whereas in loins the content of fat may vary depending on pork breed and humidity content, as reported in Iberian dry cured loins ranging between 2.3 to 6.8% (Ventanas, Estevez, Andrés, & Ruiz, 2008). In this last case, sausages with proline (S-Pro) contained significantly more fat than the control sausages (S-C), although these differences were estimated at less than 2.3%. Differences in fat content between loins and sausages were investigated because they may affect not only the generation, but also the perception of flavor compounds due to the physical differences of volatile compounds in volatility and hydrophobicity values (calculated from the boiling point and the octanol-water partition coefficient Log $P_{o/w}$, respectively) (Table 1).

The analysis of nitrogen and sulfur compounds in loins and sausages revealed the presence of 9 compounds in loins and 8 in sausages (Fig. 1), being 6 of them detected in both products (methanethiol, DMS, DMDS, methional, MMTF, and DMTS) (Table 1).

Additionally, 3 compounds were exclusively detected in dry loins (2-acetyl-pyrroline, methionol and 2-acetyl pyrrole) and 2 in fermented sausages (MFT and MMFDS). The confirmation of the identity of these compounds was achieved using longer fiber extraction times (Table 1). Seven hours of extraction time were needed to confirm the presence of DMDS and MFT in sausages and DMDS in loins, while 20 h were needed for DMTS in both products and MMFDS in sausages.

Several of these compounds have been detected previously in dry cured loins, (methanethiol, DMS, DMDS, DMTS, 2AP, methional, MMTF, methionol and 2-acetylpyrrole) (Li, Perea-Sanz, López-Díez, et al., 2022; Martín, Córdoba, Benito, Aranda, & Asensio, 2003; Muriel, Antequera, Petrón, Andrés, et al., 2004; Muriel, Antequera, Petrón, Martin, et al., 2004; Ventanas et al., 2008) and in dry fermented sausages (methanethiol, DMDS, methional, 2AP, MMTF, MFT, DMTS and MMFDS) (Corral et al., 2016; Li, Perea-Sanz, López-Díez, et al., 2022; Olivares et al., 2011; Perea-Sanz, López-Díez, Belloch, & Flores, 2020). In these studies, the content of the volatile compounds was expressed as area units or equivalent to internal standard, therefore their aroma impact could not be estimated due to the absence of concentration units.

The quantitation of the identified nitrogen and sulfur compounds was optimized taking into account the different matrix (loin muscle and sausage batter), and calibration curves were prepared in each matrix (Tables 2 and 3), except for the compounds indicated. Among the sulfur and nitrogen compounds quantified in loins, five of them are key aroma contributors as detected previously by GC olfactometry (Li, Perea-Sanz, López-Díez, et al., 2022) (Table 2) and six in fermented sausages (Li, Perea-Sanz, López-Díez, et al., 2022; Perea-Sanz, Montero, Belloch, & Flores, 2018; Perea-Sanz et al., 2020) (Table 3). The concentration of nitrogen and sulfur compounds showed noticeable differences between meat products (loins and sausages) as well as significant differences among treatments (Fig. 2). It is remarkable the high content of methionol, methional, DMS, DMTS, 2AP, and 2-acetylpyrrole in dry loins, while fermented sausages are characterized by the high content of MFT and MMFDS. Regarding the fermented sausages, a previous study showed a

similar profile and content of these compounds in products ripened for longer times and inoculated with yeast (Flores et al., 2021); although methionol was not found in the present study. In case of the loins, these compounds have been detected, but no information about their concentration and their impact in aroma and perception has been provided (Li, Perea-Sanz, López-Díez, et al., 2022).

The impact of these compounds on the aroma of the dry cured products was explained by the calculation of odor-activity values (OAVs) (Table 4), considering that an odor-activity value (OAV) of 1 or higher indicates that the compound contributes to the aroma of the sample (Grosch, 2001). Among the compounds with the highest OAVs in loins, methional and 2AP were found. They have been identified as aroma impact compounds with high detection frequency values by GCO analysis, contributing to cooked potatoes and roasted, toasted corn aromas (Li, Perea-Sanz, López-Díez, et al., 2022). In dry fermented sausages, the compounds impacting the aroma were those with the highest OAVs, like MFT, methional and MMFDS, contributing to fatty, cooked potato and meaty odors (Table 3). However, the contribution of methanethiol to the aroma was not clear as shown by an OAV slightly higher than 1.

The differences observed between dry loins and fermented sausages aroma may be highly influenced by the ingredients and manufacture process applied. In addition to the different fat content of the products, another important difference was the fermentation process and the surface inoculation of a yeast strain (*D. hansenii* L1) on the dry fermented sausages. This yeast has the ability to reduce the content of methional, MMTF, DMTS and MMFDS in fermented sausages (Flores et al., 2021) based on the yeast antioxidant activity (Perea-Sanz et al., 2020). Therefore, the inoculated yeast may be responsible for the lower content of methional, methionol, methanethiol, DMS and DMTS of sausages in comparison to loins (Fig. 2).

Table 1. Sulfur and Nitrogen volatile compounds identified in the headspace of dry cured loins and dry fermented sausages at different SPME extraction times (2, 7 or 20 h) and their physical parameters.

N[a]	Compound	LRI[b]	LRI-std[c]	SPME extraction time (h)		Log $P_{o/w}$[d]	Boiling Point (°C) [e]	Threshold		
				Loin	Sausage			Air[f] (mg/m^3)	Water (mg/kg)	Oil (mg/kg)
1	Methanethiol	472	472	2	2	0.932	5.9-6.0	0.0000003	0.00002	0.00006
2	Dimethyl sulfide (DMS)	531	531	2	7	0.977	37.3	0.027	0.01	0.0012
3	Dimethyl disulfide (DMDS)	773	773	7	2	1.77	109-110	0.050-0.078	0.0003	0.012
4	2-Methyl-3-furanthiol (MFT)	895	894	-	7	1.941	57-60	0.000001-0.000002	0.000005	
5	2-Acetyl-1-pyrroline (2AP)	963	961	2	-	-0.019	182-183	0.00002 - 0.00004	0.00012	0.0001
6	Methional	969	969	2	2	0.436	165-166	0.000063	0.0002	0.0002
7	2-Methyl-3-(methylthio)furan (MMTF)	978	979	2	2	2.324	164.22	0.0076-0.0304	0.025	
8	Dimethyl trisulfide (DMTS)	1002	1003	20	20	1.926	165-170	0.014	0.00001	0.0025
9	Methionol	1064	1064	2	-	0.417	194-195	1.71	0.25	
10	2-Acetylpyrrole	1157	1158	2	-	0.93	220	>2	170	
11	Methyl 2-methyl-3-furyl disulfide (MMFDS)	1219	1220	-	20	2.278	213-217	0.00002-0.00008	0.000004	

[a] Number of compounds as shown in Fig. 1.
[b] LRI, Linear retention indices of the compounds calculated using a DB-624 capillary column (J&W Scientific 30 m×0.25 mm i.d. ×1.4 µm film thickness).
[c] LRI-std: Linear retention indices of the standard compounds.
[d] Octanol-water partition coefficient Log $P_{o/w}$ from The Good Scents Company, http://www.thegoodscentscompany.com.
[e] Boiling point at 760 mm Hg except for 2-methyl-3-furanthiol obtained at 40 mmHg.
[f] Thresholds in oil or other media reported by Van Gemert, 2011.

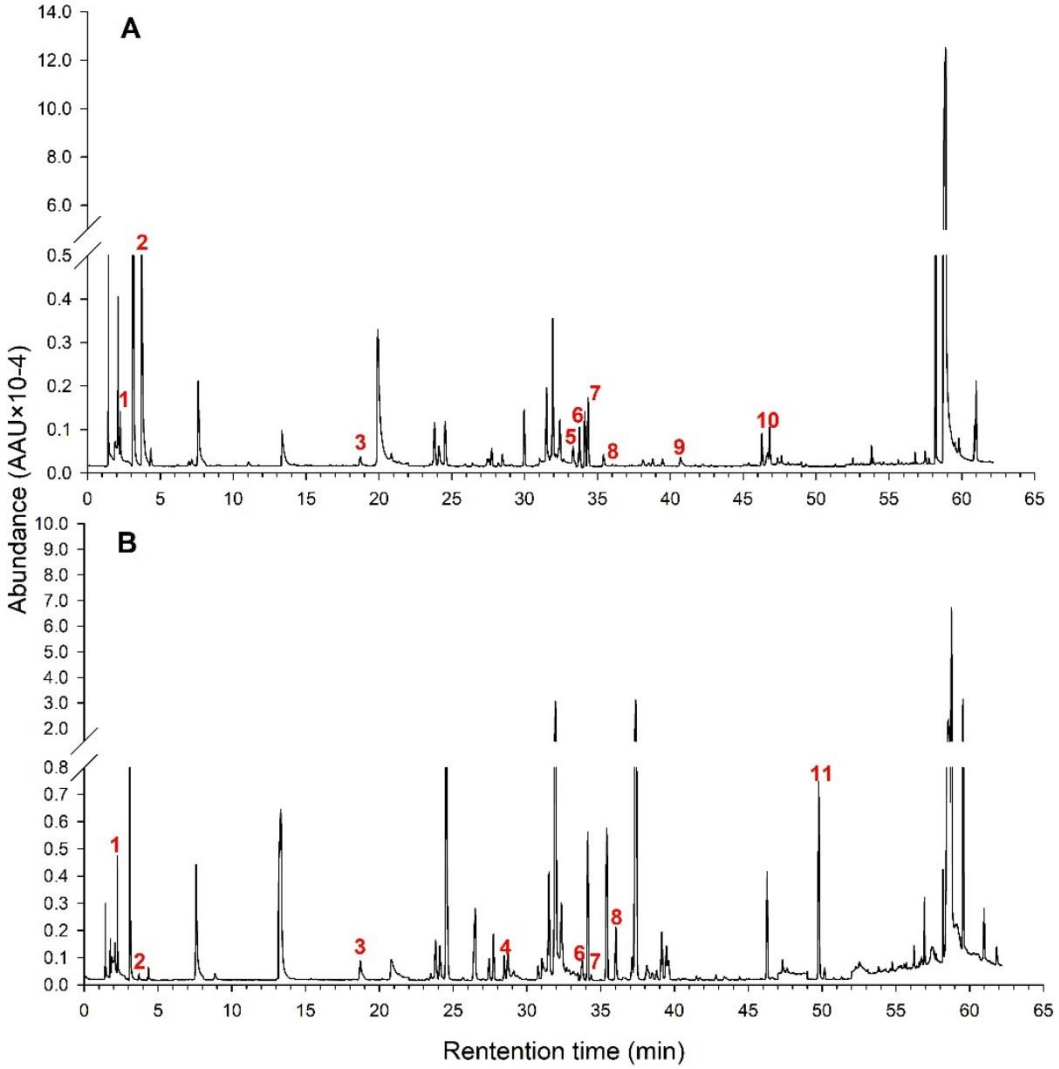

Fig. 1. Chromatograms obtained by SPME GC–MS in SIM mode in the headspace of dry cured loins (A) and dry fermented sausages (B). The savory and toasted aroma compounds identified are represented by the numbers as listed in Table 1.

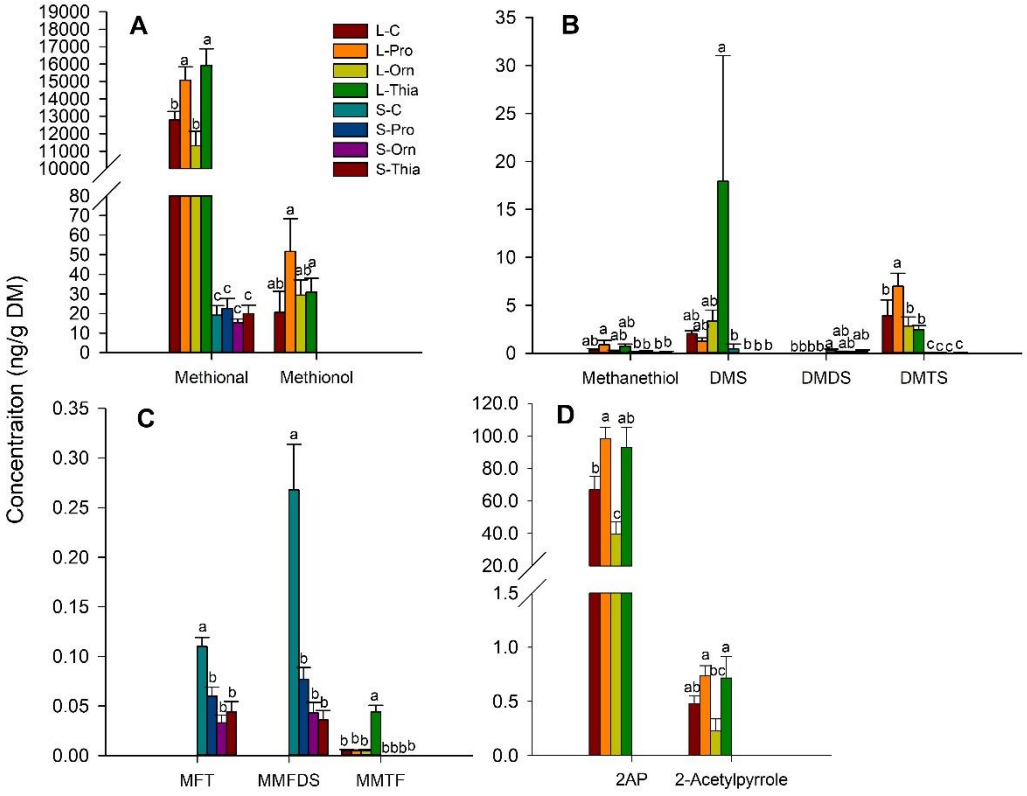

Fig. 2. Content of savory and toasted aroma compounds (ng of volatile compounds /g of meat products in dry matter) in dry cured loins (L) and dry fermented sausages (S) manufactured with different supplemented aroma precursors (C: control, Pro and Orn: proline and ornithine at 120 mg/100 g, and Thia: thiamine at 24 mg/100 g). Data are expressed as means ± SE and the different letters indicate significant differences at $P < 0.05$.

Table 2. Parameters of the optimized SPME-GC-MS method for the analysis of nitrogen and sulfur compounds in dry cured loins and their aroma description.

Compound	LRI[a]	LRIstd	Ion (m/z)[b]	Range (ng)	LOD (ng)	LOQ (ng)	Calibration equation[c]	R[d]	Aroma description[e]
Methanethiol	473	472	48	1.44 - 11.50	0.13	0.26	y=0.16x+0.50[f]	0.9753	-
DMS	532	531	62	1.44 - 11.50	0.17	0.32	y=0.16x+0.50	0.9753	-
DMDS	773	773	94	0.01- 0.02	0.00001	0.00003	y=2768.84x-10.46	0.9241	-
2AP	963	961	83	3-12	0.00	0.00	y = 0.0019x+ 0.011[g]	0.9752	Roasted, toasted corn
Methional	969	969	104	6251-50014	9767.45	27143.69	y=0.000027x+0.33	0.979	Cooked potatoes
MMTF	978	979	128	0.02- 0.61	0.03	0.07	y=8.86x-0.02	0.9025	Meaty, sour
DMTS	1002	1003	126	13.65- 54.58	1.05	2.46	y=0.010x+0.093	0.8371	-
Methionol	1063	1064	106	50.1-801.58	0.00	0.00	y=0.0024x+0.34	0.9484	Potato, savory
2-Acetylpyrrole	1158	1158	94	1.85-14.83	0.04	0.09	y=0.17x+0.33	0.9026	Roasted, toasted corn, nut, meaty

[a] LRI: Linear retention indices of the compounds eluted from the GC-MS using a DB-624 capillary column (J & W Scientific 30 m × 0.25 mm i.d. × 1.4 µm film thickness) and LRI-std of the standard compound.
[b] Quantifier ion (m/z).
[c] Variables: y (peak area relative to that of the internal standard 2-methyl-3-heptanone), and x (ng of compound in the sample).
[d] R: correlation coefficient.
[e] Aroma description by GC-olfactometry analysis reported in Li, Perea-Sanz, López-Díez, et al. (2022).
[f] Standard curve reported for dimethyl sulfide in this study.
[g] Standard curve reported in Flores et al., 2021.

Table 3. Parameters of the optimized SPME-GC-MS method for the analysis of nitrogen and sulfur compounds in dry fermented sausages and their aroma description.

Compound	LRI[a]	LRIstd	Ion (m/z)[b]	Range (ng)	LOD (ng)	LOQ (ng)	Calibration equation[c]	R[d]	Aroma description[e]
Methanethiol	473	472	48	1.44-46	1.25	3.14	y=0.02x+0.15[f]	0.9821	Sulfurous, cooked vegetable
DMS	532	531	62	1.44-46	1.90	3.99	y=0.02x+0.15	0.9821	-
DMDS	773	773	94	9.38-300	1.36	3.30	y=0.014x+0.078	0.9987	Toasted, garlic
MFT	894	895	114	1.36-43.67	0.00	0.00	y=0.012x-0.012	0.9889	Fatty, medicinal, sulfur
Methional	969	969	104	625.18-10002.90	2587.04	6039.01	y=0.000093x+0.03	0.9837	Cooked potato
MMTF	978	979	128	0.0019-0.0077	0.0002	0.0004	y=285.39x-0.13	0.9474	-
DMTS	1002	1003	126	2.73-43.67	0.45	1.02	y=0.019x-0.027	0.9518	Spicy, pungent, sulfur, rotten, vegetable
MMFDS	1219	1220	160	4.64-111.33	0.60	1.35	y=0.011x-0.002	0.9971	Meaty, unpleasant, wet wood, fermented, rotten

[a] LRI: Linear retention indices of the compounds eluted from the GC-MS using a DB-624 capillary column (J & W Scientific 30 m × 0.25 mm i.d. × 1.4 µm film thickness) and LRI-std of the standard compound.
[b] Quantifier ion (m/z).
[c] Variables: y (peak area relative to that of the internal standard 2-methyl-3-heptanone), and x (ng of compound in the sample).
[d] R: correlation coefficient.
[e] Aroma description determined by GC-olfactometry analysis in dry fermented sausages (Li, Perea-Sanz, Salvador, et al., 2022; Perea-Sanz et al., 2018; Perea-Sanz et al., 2020).
[f] Standard curve reported for dimethyl sulfide in this study.

Table 4. Odor activity values (OAVs) of the nitrogen and sulfur compounds in control (C) formulations of dry cured loin (L-C) and dry fermented sausages (S-C).

Compound[a]	L-C	S-C
Methanethiol	6.1	2.3
DMS	1.7	<1
DMDS	<1	<1
MFT[b]	-	22.0
2AP	669.2	-
Methional	63992.3	95.9
MMTF[b]	<1	<1
DMTS	1.56	<1
Methionol[b]	<1	-
2-Acetylpyrrole[b]	<1	-
MMFDS[b]	-	67.1

[a] OAV calculated based on the odor threshold in oil except those marked with
[b] which were calculated in water (Van Gemert, 2011).

Flavour physical properties and food matrix can also explain differences in the key sulfur and nitrogen aroma compounds between the meat products. The octanol-water partition coefficient (Log $P_{o/w}$) is an index of hydrophobicity (Kellogg & Abraham, 2000) that could explain which compounds are highly retained in the fat phase of the meat product. Methional and methionol are the sulfur compounds with the lowest hydrophobicity values (Table 1), which may explain the high content of methional (13774 ng/g DM, mean value) and methionol (33.10 ng/DM, mean value) in loins (Fig. 2A), as this meat product has a lower fat content than sausages (Supplementary Fig. 1). Methional is an aldehyde formed when α-dicarbonyl reacts with the amino acid methionine in a Strecker reaction (Pripi-Nicolau, De Revel, Bertrand, & Maujean, 2000). Moreover, methional can be subsequently reduced to methionol (Labuda, 2009) (Fig. 3). However, the mean content of methionine in loins (70.16 mg/100 g DM) and sausages (61.63 mg/100 g DM) was very similar (Li, Perea-Sanz, López-Díez, et al., 2022; Li, Perea-Sanz, Salvador, et al., 2022), therefore, the matrix composition and hydrophobicity are essential factors affecting the presence of methional and methionol in the two meat products.

The increase of Log $P_{o/w}$ value of methanethiol, DMS, DMDS and DMTS (Table 1) corresponds with the higher content of these compounds in loins except for DMDS (Fig. 2B). There are other factors besides hydrophobicity and meat matrix that could influence the content of these sulfur compounds. DMS, DMDS and DMTS are generated by methanethiol (Fig. 3), as the result of methional degradation due to its high reactivity and sensitivity to oxygen (Weimer, Seefeldt, & Dias, 1999). Differences in the content of these compounds between the different loins formulations were very similar except for the L-thia loins (Fig. 2B). The high DMS content (56.65 ng/g DM) detected in this loin would account also for the high standard error. The formation and content of DMS in loins could be also related to the disparity in redox balance due to different surface microbiota (Belloch, Neef, Salafia, López-Diez, & Flores, 2021). Therefore, the measurement of the redox value in meat products could be key to the elucidation of methanethiol, DMS, DMDS and DMTS generation.

The most hydrophobic compounds, MFT and MMFDS, were only detected in fermented sausages (Table 1; Fig. 2C). This would correlate with the high fat content in sausages (Supplementary Fig. 1), which may have retained the compounds in the fat phase of the product. Other hydrophobic compound, MMTF, was also detected but at low content in fermented sausages (Fig. 2C). Generation of MFT can be achieved by the Maillard reaction between carbohydrates and cysteine as well as by thiamine degradation (Tang et al., 2013) (Fig.3). Taking into account that the content of thiamine in loins and sausages was similar (mean value calculated without thiamine supplementation was 2.00 mg/100 g DM in loins and 1.48 mg/100 g DM in sausages as in Li, Perea-Sanz, López-Díez, et al. (2022) and Li, Perea-Sanz, Salvador, et al. (2022)), MFT formation may be affected by other factors such as pH. Low pH values have been seen to favor MFT formation in heated products (140 °C meat-related model systems, Meynier & Mottram (1995)). In dry cured products, the low pH values in sausages (pH 4.7-4.8 in Li, Perea-Sanz, Salvador, et al. (2022)) may favor MFT formation, in contrast to the high pH values and absence of MFT in loins (pH 5.7-5.9 in Li, Perea-Sanz, López-Díez, et al. (2022)). Generation of MFT in sausages may be also

associated with the lipase-assisted enzymatic reaction (Bel Rhlid, Matthey-Doret, Blank, Fay, & Juillerat, 2002) due to the high lipase activity naturally present in the meat and in the starter culture (Flores & Toldrá, 2011). Furthermore, the interaction of MFT with methanethiol (Fig. 3) (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2014) may be the source of MMFDS in sausages S-C (Fig. 2C). Lastly, MMTF derives from MFT (Fig. 3) (Tang et al., 2013), and its high content in loin L-thia (Fig. 2C) (0.044 ng/g DM) confirmed the role of thiamine as its precursor (Cerny, 2007; Li, Perea-Sanz, López-Díez, et al., 2022). However, the role of thiamine in sausages couldn't be confirmed, although it was previously observed (Li, Perea-Sanz, Salvador, et al., 2022). The reason may be the low content of MMTF detected (4.5×10^{-6} to 8.6×10^{-6} ng/g DM) that was not significantly different among treatments.

The roasted toasted notes produced by compounds with the lowest hydrophobicity values, 2AP and 2-acetylpyrrole, were only detected in loins which suggests a relationship with the higher moisture content found in these meat products (55.76%, Li, Perea-Sanz, López-Díez, et al. (2022)) than sausages (35.27%, Li, Perea-Sanz, Salvador, et al. (2022)). The absence of 2AP in the analysed sausages, may be due to a low content as it was previously detected but at very low concentrations (1 to 2.7 ng / g DM, Flores et al. (2021)) around 100 times lower than the amount found in loins (Fig. 2D). The compound 2AP is derived from the conversion of proline and ornithine (Wei et al., 2017), whereas 2-acetylpyrrole is a further oxidation product of 2AP (Daygon et al., 2017) (Fig. 3). Proline addition produced a significant highest concentration of 2-acetylpyrrole and 2AP in L-Pro (Fig. 2D), but this effect was not detected when ornithine was added (Fig. 2D). Probably, the high concentration of proline maybe the key factor together with the humidity content in promoting the production of 2AP and 2-acetylpyrrole in loins. Nevertheless, fungi and yeast strains participating in the surface microbiota may be involved as they have been reported as dominating the 2AP production (Wei et al., 2017). In Mediterranean fermented sausages, the first source of 2AP detected was *Penicillium nalgiovense* (Stahnke, 2000). In addition, 2AP has been found in several types of fermented sausages (Corral et al., 2016; Perea-Sanz et al., 2018; Perea-Sanz et al., 2020) but its exact origin has not been

elucidated yet. The presence of yeasts and molds may modulate the content of 2AP and 2-acetylpyrrole, although further research on the different species of fungi responsible for their generation seems necessary. Microbial counts in both products, sausages and loins, indicated similar yeast and molds numbers (around 5 Log ufc/g) (Li, Perea-Sanz, López-Díez, et al., 2022; Li, Perea-Sanz, Salvador, et al., 2022); therefore, pending further investigation on the type of fungal microbiota in both meat products, the presence of precursors and matrix composition may be the key elements for 2AP and 2-acetylpyrrole formation.

4. Conclusions

In conclusion, the physical properties of the volatile compounds and the meat matrix, together with the oxidation reactions modulated by the microbiota, could explain the differences in the key sulfur and nitrogen aroma compounds found in loins and sausages. The aroma of dry cured loins was greatly impacted by the presence of methional and 2AP, while sausages aroma was essentially modulated by MFT, methional and MMFDS. Methional, showing the highest OAV in loins and sausages, contributed to the cooked potato aroma, whereas 2AP added the toasted notes in loins and MFT and MMFDS contributed to fatty and meaty odors in sausages. The positive effect on the aroma of the precursors, added into the meat before processing, was only confirmed in dry cured loin. In this meat product, the presence of sulfur-derived compound MMTF in L-Thia confirmed the positive effect of thiamine supplementation, while the presence of 2AP and 2-acetylpyrrole confirmed the effect of proline addition. Moreover, essential factors such as humidity and pH regulate the kinetics of the aroma formation reactions in meat products, although the role of yeast and moulds in the formation of the potent 2AP compound in dry cured meat products needs to be elucidated. Also, the oxidation processes regulated by the yeasts and by the matrix chemistry affected the stability of sulfur derived compounds modulating the final volatile profile.

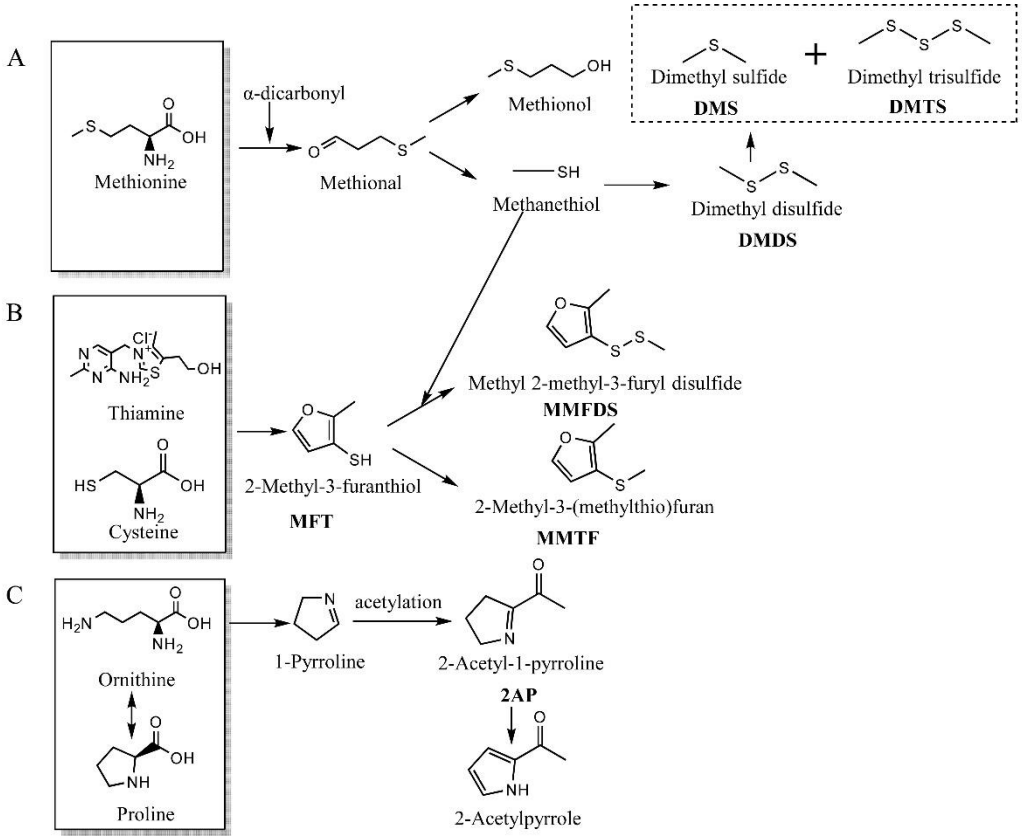

Fig. 3. Mechanisms of generation of savory (A and B, adapted from Wolle et al. (2006), Tang et al. (2013), Thomas et al. (2015) and Parker (2017)) and toasted aroma compounds (C, adapted from Wei et al. (2017) and Daygon et al. (2017)).

Credit author statement

Lei Li: Formal analysis, Methodology, Investigation, Writing - original draft, **Carmela Belloch and Monica Flores**: Funding acquisition, Conceptualization, supervision, Writing - review & editing.

Declaration of Competing Interest

None

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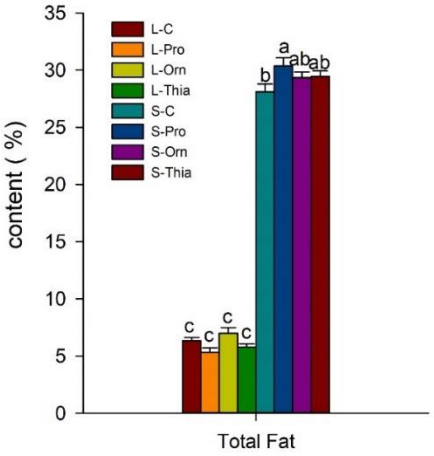
SUPPLEMENTARY MATERIAL



Supplementary Fig. 1. Total fat content in dry cured loins (L) and dry fermented sausages (S) manufactured with different supplemented aroma precursors (C: control, Pro and Orn: proline and ornithine at 120 mg/100 g, Thia: thiamine at 24 mg/100 g). Data are expressed as means ± SE and the different letters indicate significant differences at $P < 0.05$.

Chapter 4

The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions

Molecules 2021, 26, 223.

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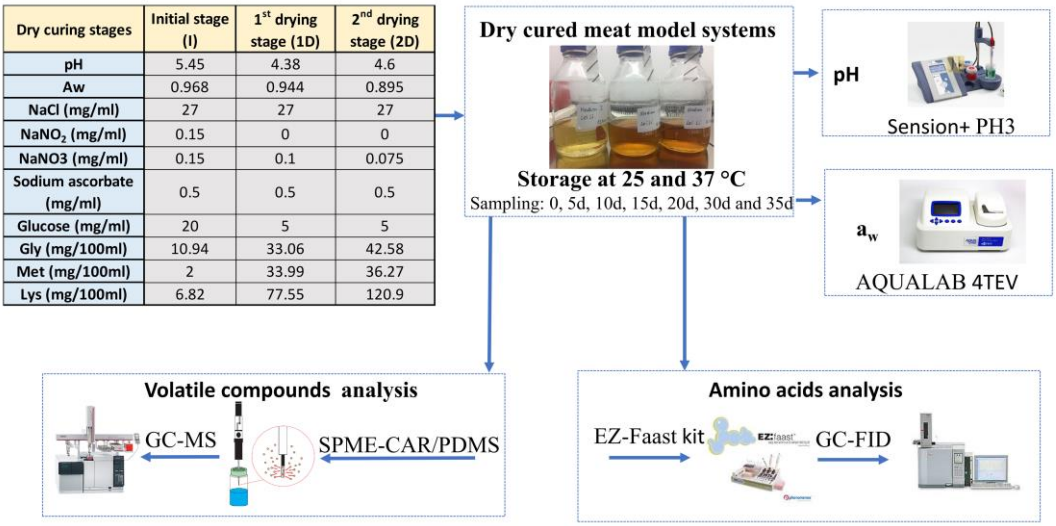

The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions

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Abstract

Flavor is amongst the major personal satisfaction indicators for meat products. The aroma of dry cured meat products is generated under specific conditions such as long ripening periods and mild temperatures. In these conditions, the contribution of Maillard reactions to the generation of the dry cured flavor is unknown. The main purpose of this study was to examine mild curing conditions such as temperature, pH and a_w for the generation of volatile compounds responsible for the cured meat aroma in model systems simulating dry fermented sausages. The different conditions were tested in model systems resembling dry fermented sausages at different stages of production. Three conditions of model system, labeled initial (I), 1st drying (1D) and 2nd drying (2D) and containing different concentrations of amino acid and curing additives, as well as different pH and a_w values, were incubated at different temperatures. Changes in the profile of the volatile compounds were investigated by solid phase microextraction and gas chromatography mass spectrometry (SPME-GS-MS) as well as the amino acid content. Seventeen volatile compounds were identified and quantified in the model systems. A significant production of branched chain volatile compounds, sulfur, furans, pyrazines and heterocyclic volatile compounds were detected in the model systems. At the drying stages, temperature was the main factor affecting volatile production, followed by amino acid concentration and a_w. This research demonstrates that at the mild curing conditions used to produce dry cured meat product volatile compounds are generated via the Maillard reaction from free amino acids. Moreover, in these conditions a_w plays an important role promoting formation of flavor compounds.

Keywords: meat flavor; dry cured; mild conditions

1. Introduction

Flavor is among the most important quality indicators of meat products. Flavor compounds produced during the processing of meat products are generated through different mechanisms including the Maillard reaction, Strecker degradation, lipid oxidative reactions, degradation of thiamine, ribonucleotides and carbohydrates, as well as microbial metabolism [1,2]. The contribution of these reactions to meat flavor depends on the manufacture conditions applied during processing.

Flavor compounds present in cooked cured meat products have been studied qualitatively and quantitatively [3], the Maillard reaction being the most important reaction for the production of meaty aroma compounds [4]. The Maillard reaction between an amino group and a reducing sugar is commonly divided into three stages: Amadori/Heyns rearrangement, sugar fragmentation and retro-aldolization. In these reactions the free amino group of amino acids peptides or proteins participates in dehydration, fragmentation, cyclization and polymerization reactions. The different paths involved in the Maillard reaction depend on conditions such as temperature, pH and water content that regulate the reaction kinetics, and type of sugar and amino acids that modulate the flavor compounds formed [5]. Extensive investigation of flavor formation in cooked cured meat products [6] has revealed the effect of cooking temperature, amino acid and sugar types and lipid composition [7]. Meaty aroma seems to be constituted by different volatile compounds, although the contribution of sulfur-containing compounds is essential [1]. Among these, thiophenes are derived mainly from the Maillard reaction between cysteine and ribose [5]. Amino acids such as cysteine and glycine are of utmost importance as precursors of sulfur-containing meaty compounds [8,9]. Furthermore, meat post-mortem conditioning increases the formation of Maillard reaction-derived meaty flavor compounds by the presence of ribose, methionine and cysteine [10].

Few studies have focused on the role of Maillard kinetics for flavor generation during dry cured meat processing. In these types of products, low temperatures are applied during

long processing times depending on products, ranging between 2 to 3 months in dry fermented sausages, and up to 2–3 years in dry cured hams [11]. Flavor production during dry cured meat processing is influenced by the use salt and nitrate and/or nitrite, which are rubbed on the surface of meat or mixed with the minced meat. As a result of the proteolysis occurring during dry curing, there is an increase in free amino acids which are flavor precursors in dry cured meat products [12]. Both pH and a_w exert a profound influence on the abundance of meaty flavor compounds in thermal treatments [13]. However, flavor composition of dry cured meat products and cooked cured meat products is significantly different due to the different processing methods [1]. The meaty flavor compounds of dry cured meat products are produced under long ripening periods where mild temperatures are applied.

The mechanisms involved in the generation of meaty flavor compounds in dry fermented sausages and the influence of the physicochemical conditions such as pH and low temperature are poorly understood, whereas many studies have focused on the microbial contribution during fermentation [14,15]. Additionally, several studies have focused on the lipid oxidation effect on dry sausage flavor [16]. However, the role of Maillard reactions in dry sausage flavor generation has not been elucidated. The control of flavor formation in meat products from Maillard reactions is essential for flavor quality [17]. Therefore, new studies on Maillard reactions are necessary to identify the influence of critical process parameters (pH, temperature, a_w) as well as determine the effect of reactant concentrations on the formation of flavor compounds in dry cured meat products.

The main purpose of this study was to examine mild curing conditions as far as temperature, pH and a_w for the generation of volatile compounds responsible for the cured meat aroma in model systems simulating dry fermented sausages. Model systems characterized by different amino acid and additive composition were incubated at 25 and 37 °C and samples taken at different times for profiling of generated volatile compounds and amino acid content.

2. Results

2.1. Analysis of Physicochemical Changes in the Model Systems

Water activity (a_w) and pH (Figures S1 and S2 Supplementary material, respectively) were measured during the incubation of sausage model systems (media I, 1D and 2D) at mild conditions (25 and 37 °C), and both parameters remained stable from the first day of incubation until 35 d.

2.2. Identification of Volatile Compounds Generated in the Model Systems

The identification of volatile compounds generated in the model systems is listed in Table 1. Seventeen volatile compounds were identified, and 1 of them (furan) was only tentatively identified. The compounds identified belonged to different chemical classes: 2 ketones, 2 furans, 6 aldehydes, 1 sulfur compounds, 1 pyrazine, 1 alcohol, 1 acid and 3 hydrocarbons. Not all compounds were detected in the three models. Furan, dimethyl disulfide, thiazole and methyl-pyrazine were absent in model I at both temperature conditions. Additionally, furfural was only detected when the three models were incubated at 37 °C. The volatile compounds detected were mainly derived from branched chain amino acids (Val, Leu and Ile); sulfur amino acids (Met and Cys); and Phe, Ala, Thr and Ser.

2.3. Quantitation of Volatile Compounds Generated in the Model Systems

The quantitation of all the volatile compounds generated in the model systems incubated at 25 and 37 °C is shown in Tables S1 and S2 of Supplementary material. Figures 1–3 show the changes in abundance of the most variable volatile compounds derived from branched-chain amino acids Val, Leu and Ile (Figure 1); sulfur amino acids (Met and Cys) and Phe (Figure 2); and heterocyclic compounds (Figure 3).

Table 1. Volatile compounds identified in the model systems.

Compound	LRI [1]	LRI std [2]	RI [3]	Initial Stage		1st Drying Stage		2nd Drying Stage		Aac Precursor [5]	Odor [6]	Threshold in Oil (mg/kg) [7]
				I-25°	I-37°	1D-25°	1D-37°	2D-25°	2D-37°			
Furan	514		b			+[4]	+	+	+	Ala, Thr, Ser [18]	ethereal	4.5–6 [a]
Acetone	530	527	a	+	+	+	+	+	+		ethereal	100–1000
2-Methylpropanal	594	590	a	+	+	+	+	+	+	Val[19]	pungent, floral	0.043
2-Butanone	632	629	a		+	+	+	+	+		woody, yogurt	10–100
3-Methylbutanal	692	687	a	+	+	+	+	+	+	Leu [19]	fruity green cocoa	0.0054–0.013
Heptane	700	700	a	+	+	+	+	+	+		-	
2-Methylbutanal	702	698	a	+	+	+	+	+	+	Ile [19]	musty chocolate, malty	0.0022–0.152
Acetic acid	715	714	a	+	+	+	+	+	+		pungent vinegar	0.12–0.75
Dimethyl disulfide	773	774	a			+	+	+	+	Met [20]	onion, cabbage	0.012
Thiazole	776	776	a			+	+	+	+	Cys [21]	nutty, meaty	0.038–3.1 [a]
Methylpyrazine	860	860	a			+	+	+	+	Gly,Lys,Cys [22,23]	nutty, cocoa, roasted,	27
p-xylene	892	893	a	+	+	+	+	+	+		-	
Furfural	898	898	a		+		+		+		brown, bready, nutty, caramel	155 [b]
3-(Methylthio)propanal	968	968	a	+	+	+	+	+	+	Met [24]	cooked potato, onion,	0.0002
Benzaldehyde	1020	1013	a	+	+	+	+	+	+	Phe [25]	bitter almond, cherry	0.06
2-Ethyl-1-hexanol	1083	1083	a	+	+	+	+	+	+		sweet, fatty, fruity	0.27–25 [a]
Benzeneacetaldehyde	1110	1104	a	+	+	+	+	+	+	Phe [26]	floral, chocolate	0.002–0.03 [a]

[1] LRI: Linear retention indices of the compounds eluted from the GC-MS using a DB-624 capillary column. [2] LRI std: Linear Retention Indices of authentic standard compounds. [3] RI: Reliability of identification: a: identification by mass spectrum and coincident with LRI of an authentic standard; b: tentative identification by mass spectrum. [4] Blank space means not generated; + indicates the compound was detected. [5] Bibliographic references indicating the amino acid precursor of the volatile compound are between brackets. [6] Burdok, G. A. (2010). Fenaroli's handbook of flavor ingredients (6th ed.). Florida: Boca Raton. CRC Press Inc. [7] Van Gemert, L., and Nettenbreijer, A. (2011). Compilation of odor threshold values in air, water and other media. The Netherlands: BACIS: Zeist. [a] Threshold reported in water; [b] threshold reported in propylenglycol.

Figure 1 shows that model system I (Figure 1a, d) was very poor in volatile compounds derived from Val, Ile and Leu at both incubation temperatures and any time. Model 1D (Figure 1b, e) showed a significant ($p < 0.001$) increase in the content of 3-methylbutanal during the incubation time. This increase was even higher when the model system was incubated at 37 °C. A similar trend was also observed in the case of 2-methylbutanal, although this compound was produced in lower amounts. In model 2D (Figure 1c, f), the evolution of 3-methylbutanal and 2-methylbutanal was similar than in model 1D, but the changes were significantly higher ($p < 0.001$) when the model system was incubated at 37 °C.

In Figure 2, the changes observed in the volatile compounds in model system I (Figure 2a, d) were not significant at any incubation temperature or time. On the contrary, model systems 1D and 2D showed a significant ($p < 0.001$) increase in benzaldehyde and benzeneacetaldehyde during the incubation time and, as in Figure 1, this increase was significantly higher at 37 °C (Figure 2b and 2c vs. 2e and 2f). Dimethyl disulfide was detected depending on the temperature and time of incubation. At 25 °C, it was detected at the end of incubation time (35 d) (Figure 2b, c), whilst at 37 °C, it was detected at 15 (2D) or 20 d (1D) of incubation time (Figure 2e, f). The abundance of this compound increased with the incubation time. Moreover, 3-(methythio)propanal was detected in higher abundance at 37° (Figure 2,f) than at 25 °C (Figure 2b,c), although in both conditions it increased until 10–15 d but showed a small decrease afterwards.

In Figure 3, we can observe an increase in furfural in model system I at 37 °C (Figure 3a, d). In model systems 1D and 2D (Figure 3b, e; Figure 3c, f), methylpyrazine, thiazole, furan and furfural increased significantly ($p < 0.001$) during the incubation time, although this increase was significantly higher when the models were incubated at 37 °C.

In order to examine the relationship between the different volatile profiles in the model systems under mild conditions (25 and 37 °C) and the flavor compounds produced, a principal component analysis (PCA) was performed (Figure 4). Two principal components were able to explain the 75.93% of the total variability. PC1 accounts for 62.25% of the variability and was strongly related with the incubation time. Model I, characterized by

short incubation times, was located on the left quadrant, while model systems 1D and 2D, incubated for longer times, were on the right. This would indicate that PC1 takes into account model system composition and incubation time. PC2 accounts for 13.88% of the variability and distinguishes samples by different groups of flavor compounds. Most of the aldehydes and ketones were on the upper quadrants, whereas on the lower quadrants, furans (furan and furfural), cyclic compounds (methylpyrazine and thiazole) and phenyl compounds (benzaldehyde) were related to models 1D and 2D incubated at 37 °C during longer times.

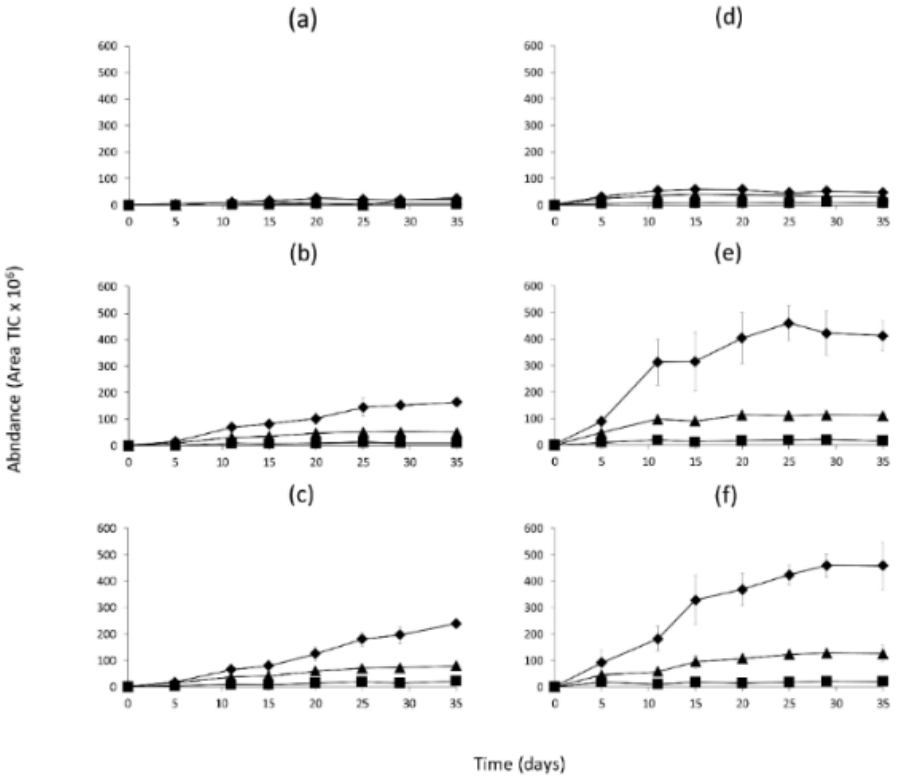

Figure 1. Abundance of volatile compounds derived from Val, Ile and Leu in the model systems during incubation at 25 or 37 °C and classified according to the dry curing stages (I-Initial stage, 1D-1st drying stage, 2D-2nd drying stage). (**a**) I-25 °C, (**b**) 1D-25 °C, (**c**) 2D-25 °C, (**d**) I-37 °C, (**e**) 1D-37 °C, (**f**) 2D-37 °C. Symbols represent the different compounds: 2-Methylpropanal (■); 3-Methylbutanal (◆); 2-Methylbutanal (▲).

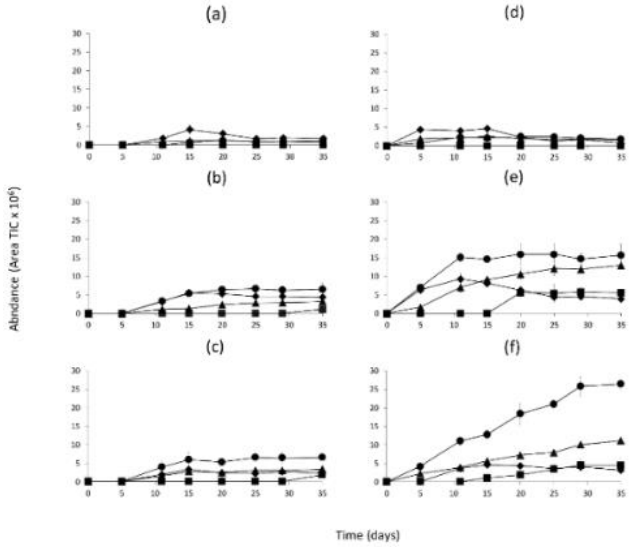

Figure 2. Abundance of volatile compounds derived from sulfur amino acids and Phe in the model systems during incubation at 25 or 37 °C and classified according to the dry curing stages (I-Initial stage, 1D-1st drying stage, 2D-2nd drying stage). (**a**) I-25 °C, (**b**) 1D-25 °C, (**c**) 2D-25 °C, (**d**) I-37 °C, (**e**) 1D-37 °C, (**f**) 2D-37 °C. Symbols represent the different compounds: Dimethyl disulfide (■); 3-(Methylthio)propanal (◆); Benzaldehyde (▲); Benzeneacetaldehyde (●).

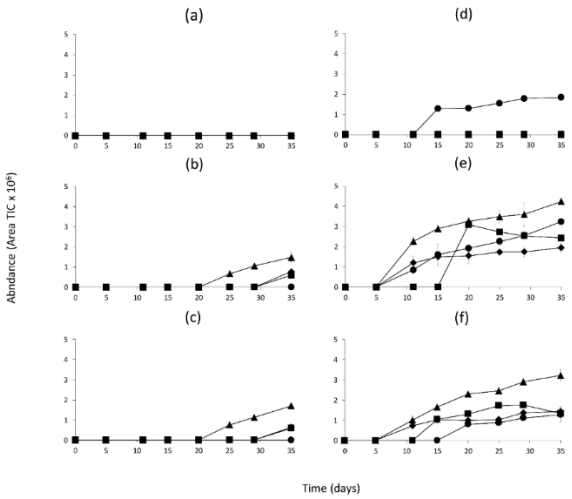

Figure 3. Abundance of heterocyclic volatile compounds derived from other amino acids in the model systems during incubation at 25 or 37 °C and classified according to the dry curing stages (I-Initial stage, 1D-1st drying stage, 2D-2nd drying stage). (**a**) I-25 °C, (**b**) 1D-25 °C, (**c**) 2D-25 °C, (**d**) I-37 °C, (**e**) 1D-37 °C, (**f**) 2D-37 °C. Symbols represent the different compound: Furan (■); Thiazole (◆); Methylpyrazine (▲); Furfural (●).

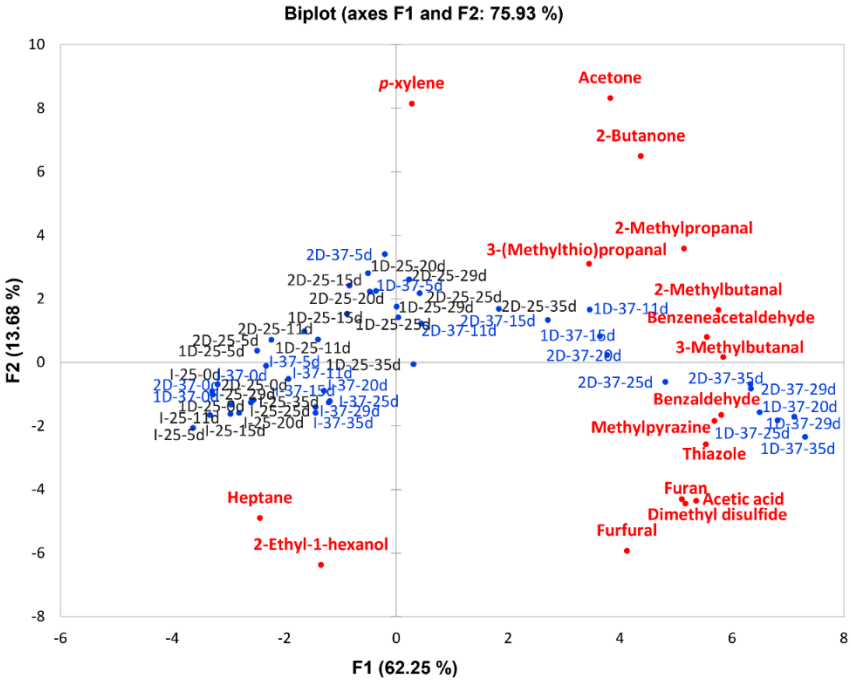

Figure 4. Loadings of the first two principal components (PC1-PC2) representing the variability (volatile compounds) in the model systems simulating the dry curing stages (I-Initial stage, 1D-1st drying stage, 2D-2nd drying stage). Sample loadings in black and blue represent models incubated at 25 and 37 °C, respectively.

2.4. Amino Acid Concentration in the Model Systems

The concentration of amino acids was compared among the model systems during the incubation time (Table S3 Supplementary material). Due to limitations of the analysis method, two amino acids, Arg and Cys, were not analyzed. The concentrations of the amino acids (Val, Leu, Ile, Pro, Met, Phe and orn) during the incubation times (0 to 35 d) and temperatures (25 and 37°) in the different models (I, 1D and 2D) are shown in Figure 5. Amino acid concentration in model system I did not show significant differences due to incubation times at any temperatures 25 or 37 °C (Figure 5a, d). On the other hand, a significant ($p < 0.001$) decrease in met during the incubation time was observed in models 1D and 2 D (Figure 5b, c and Figure 5e, f) at both temperatures.

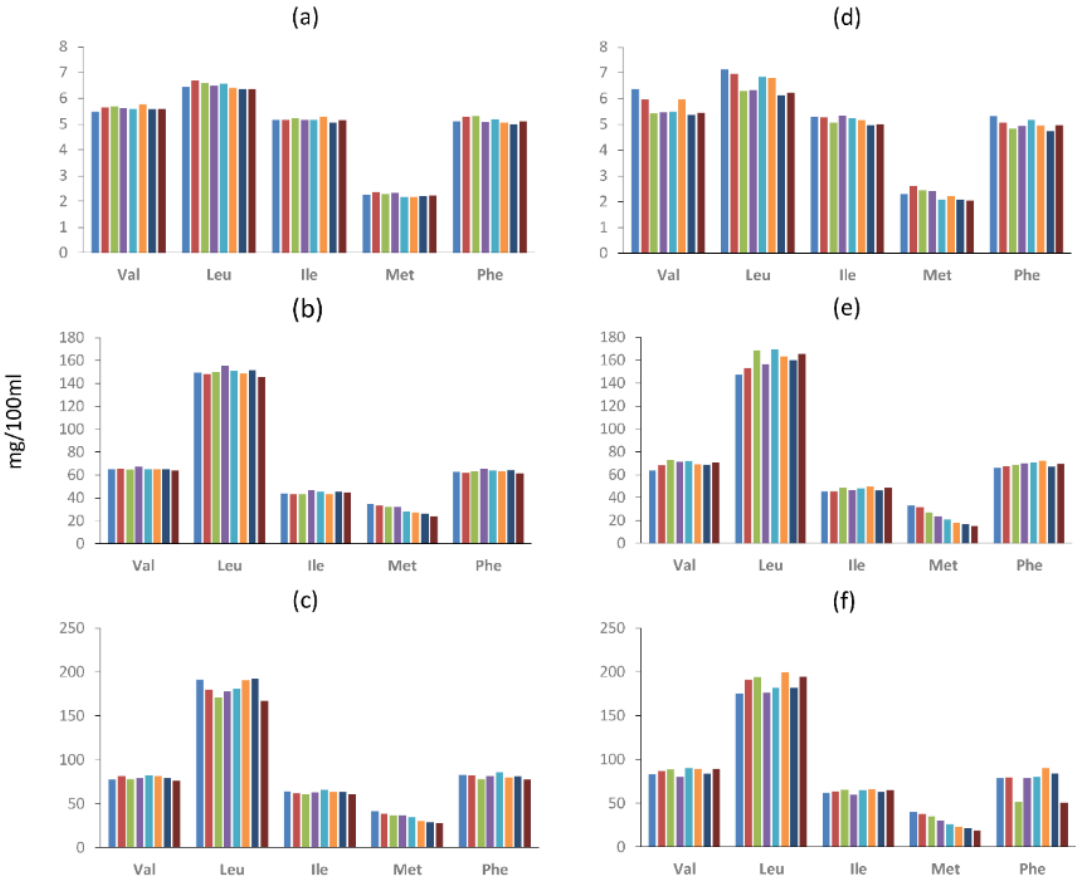

Figure 5. Amino acid concentration in the model systems (mg/100 mL) during incubation at 25 or 37 °C and classified according to the dry curing stages (I-Initial stage, 1D-1st drying stage, 2D-2nd drying stage). (**a**) I-25 °C, (**b**) 1D-25 °C, (**c**) 2D-25 °C, (**d**) I-37 °C, (**e**) 1D-37 °C, (**f**) 2D-37 °C. Bars from right to left in each amino acid represent concentrations at 0, 5, 9, 11 until 35 d.

3. Discussion

Flavor formation in dry cured meat products is very complex because many processing conditions affect its generation [1]. For example, Zamora and Hidalgo [27] indicated the importance of lipid oxidation and Maillard reactions in food processing, and indicated that both reactions are interrelated and the products of each reaction can modify the other.

The results from our experiments demonstrated that model systems that simulate the intermediate processing stages of dry fermented sausages, 1D and 2D, have different volatile profiles than the model simulating the initial conditions (model I) (Table 2). The conditions in model I were not sufficient to promote Maillard reactions and formation of volatile compounds, because only furfural was detected at 37 °C (Figure 3). This would indicate that whilst amino acids were present in model I, higher concentrations of amino acids (10 times more) are necessary for volatile generation, as occurs in models 1D and 2 D (Figure 5 and Table S3). Therefore, the proteolytic activity occurring during the drying process is essential to the release of free amino acids [12,14] and generation of Maillard reaction products under mild conditions. Moreover, the results showed that formation of Maillard volatile compounds in models 1D and 2D was compound-specific and dependent on the physicochemical conditions (pH, a_w, temperature).

The compounds derived from Val, Leu and Ile were produced in 1D and 2D models and dependent on the concentration of these amino acids in the models. The compound produced in the highest quantity 3-methylbutanal was derived from Leu, which was at the highest concentration (Figure 5). Compound 2-methylpropanal appeared always in low amounts (Figure 1) and was not affected by the mild conditions (pH, a_w and temperature) or concentration of precursor Val (Figure 5). The most probable explanation for the generation of this compound in fermented sausages is its production by the activity of Micrococcaceae such as *Staphylococcus* [28] and not from Maillard reactions. The increase in 3-methylbutanal with the incubation time in models 1D and 2D, which could be related to the amino acid content and favored by the lowest a_w in 2D (Figure S1). Previous studies have pointed out that Strecker aldehydes reach a maximum at high temperatures because they are not end products and, therefore, can react further to form other compounds [29]. Similarly, 3-methylbutanal in model 1D at 37 °C reached its highest value at 25 days and afterwards decreased. Moreover, a_w seems to be an essential parameter in Maillard reactions [30], as maximum reaction rates were found at 0.6–0.8 a_w values [31]. These branched aldehydes have been identified as key aroma compounds in dry-cured fermented sausages

due to their aroma notes and low thresholds (Table 1) [32]. Similar dynamics were found for 2-methylbutanal generation in models 1D and 2D; however, the low Ile concentration (Figure 5) would have prevented higher production of this compound. In summary, a high amino acid content favored the production of branched aldehydes, the production of which further increased under low a_w. On the other hand, pH was revealed as an important factor in Maillard reactions [17], affecting the volatile compounds generated [22] even at the small differences observed between 1D and 2D models (0.2 units).

Dimethyl disulfide and 3-(methylthio)propanal generation was related to the Strecker degradation of the amino acid Met (Table 1). The dynamic of 3-(methylthio)propanal generation was different from other volatile compounds produced in the three models. The concentration of this compound increased with incubation time until 10 to 15 d and then decreased specially in 1D followed by 2D and I model systems. This decrease could be related to an oxidative chemical reaction from L-methionine as observed in other studies [33]. Met in models 1D and 2D favored the generation of 3-(methylthio)propanal versus dimethyl disulfide (Figure 2e,f). Met content was very similar in 1D and 2D (Figure 5), showing a significant decrease during incubation which was more pronounced at 37 °C, in agreement with the higher generation of volatiles at this temperature (Figure 5). This demonstrated that temperature was a key factor in the formation of volatile compounds from Met, but the low temperature applied during dry cured meat processing (ranging from 10 to 25 °C depending on the ripening stage [11]) does not favor their generation. Regarding pH and a_w conditions, model 1D at lower pH and higher a_w than 2D favored the formation of dimethyl disulfide and 3-(methylthio)propanal. Therefore, the lowest pH values in 1D seemed to favor formation of these compounds, as observed in Maillard models submitted to high cooking temperatures [22]. Additionally, Phe concentration in models 1D and 2D together with 37 °C temperature seemed to be the most important factor for volatile production, although the lower a_w in 2D (Figure S1) produced a significant effect.

Furan is generated by two major formation pathways: (1) the intact sugar skeleton and (2) recombination of reactive C2 and/or C3 fragments which come from the amino acids

Ala, Thr or Ser [18]. In the model systems, furan formation was specially favored by high temperature as observed in models incubated at 37 °C. Concentration of Ala and Thr was higher in 2D than 1D, although the higher Ser content in 1D may have favored furan production in this model (Table S3 Supplementary material). Furfural appeared to be an intermediate of the Maillard reaction, and its concentration increased with temperature and time (Figure 3). Furfural was the only compound detected in I model, which contained the highest glucose concentration (Table 3). Furfural has been detected as a major product in model systems related to sugar degradation in Maillard reactions and its formation has been related with low pH values (pH < 7.0) [31]. In models 1D and 2 D, where glucose was low in comparison to I model, furfural formation was favored by the lowest pH (4.3) value in 1D model.

Generation of methylpyrazine exclusively in 2D and 1D at 25 and 37 °C was favored by the presence of Lys and high pH values [22,23]. Methylpyrazine content was the highest in model 1D, although Lys was in higher quantity in model 2D. This would indicate that in addition to Lys other factors affect the generation of this compound.

Other volatile compounds produced in the model systems such as acetic acid and thiazole were also produced in higher quantities in models incubated at 37° than at 25 °C (Table S3 Supplementary material). Acetic acid (Table 1), an indicator of the progress of Maillard reaction [34], was identified as byproduct of sugar degradation [18]. Thiazole, a reaction product between cysteine and carbonyls [21], was only generated in models 2D and 1D at 37 °C; therefore, temperature seems to be a key factor in the formation of this compound.

This study demonstrates that mild processing conditions such as low temperatures (25 and 37°), pH and a_w can be responsible for the different volatile profiles found in the model systems. These mild conditions applied during dry curing show that different aroma compounds can be generated by α-dicarbonyl compounds and amino acids as reported in wine aging [35]. In dry meat products, Zhu [36] evaluated the reactions between glucose and acetaldehyde with histidine and lysine in the generation of meat flavor compounds under

mild conditions. These authors observed that histidine and lysine were key precursors of Maillard reaction products that constitute the specific flavor of Jinhua ham. The results obtained with the model systems revealed a higher number of volatile compounds such as pyrazines, furan and sulfur compounds, not found in previous experiments [36] due to the limited number of amino acids and carbonyl compounds.

Another important result of our study is that high glucose content seems not essential for the generation of Maillard reaction volatile compounds, because the four times higher concentration of this compound in model I with respect to models 1D and 2D only resulted in the generation of furfural. Sugar content in dry fermented products decreases during the fermentation stage as it is used by the microorganisms to produce a decline in pH that favors sausage drying. However, although the nature and content of saccharides has been described as essential in Maillard reactions [31], in dry meat processes, it seems that the limiting factor to produce volatile compounds from Maillard reactions is the amino acid content as well as temperature and a_w conditions.

The contribution of the volatile compounds to the aroma of the dry cured products is a balance among all the volatile compounds identified in the product [1]. Nevertheless, taking into consideration that the compounds contributing to the aroma, such as 2-methylpropanal, 2 and 3-methylbutanal, 3-(methylthio)propanal, benzaldehyde, benzeneacetaldehyde, dimethyl disulfide and thiazole (Table 1), have the lowest threshold values in oil, they can be considered important contributors to the aroma in dry sausages [37,38,39]. Moreover, generation of these aroma compounds was mainly observed at long incubation times at mild temperatures in models 1D and 2D indicating the key impact of temperature on aroma formation in dry meat processes.

4. Materials and Methods

The different dry cured meat model systems were prepared according to the dry fermented sausages composition [40,41] regarding the concentration of additives and free amino acids at the initial (I), 1st drying (1D) and 2nd drying (2D) stages of the process

(Table 2). Model systems were prepared in 0.2 mM phosphate buffer, and their composition included nitrate, nitrite, NaCl, sodium ascorbate and glucose at the concentrations included in Table 3. a_w of the model systems at the different processing stages was adjusted with glycerol. Sterilization was performed by filtration using a 0.2 μm filter (Sartorius, Göttingen, Germany), and incubation of the three model systems was performed at 25 and 37 °C. The experiments were performed in triplicate. Samples from each media and temperature were taken at 0, 5, 11, 15, 20, 25, 29 and 35 days for physicochemical analysis, volatiles and amino acid composition.

Table 2. Composition in amino acids (mg/100 mL) of each model system according to the concentration of amino acids reported in different ripening stages of dry fermented sausages [40,41].

Amino Acids	Initial Stage	1st Drying Stage	2nd Drying Stage
Ala	29.91	91.85	111.80
Gly	10.94	33.06	42.58
Val	6.01	72.05	90.35
β-Ala	3.48	4.35	4.54
Leu	5.96	151.80	176.80
Ile	4.17	45.49	62.08
Thr	4.55	35.15	54.99
Ser	4.72	31.96	19.24
Pro	4.26	83.60	89.05
Asn	1.40	14.74	21.71
Asp	16.67	106.70	100.10
Met	2.00	33.99	36.27
Glu	10.53	74.25	73.45
Phe	3.82	70.95	83.85
Gln	36.47	64.90	65.00
Orn	0.23	23.10	31.01
Lys	6.82	77.55	120.90
His	2.76	27.34	38.48
Tyr	4.47	29.21	26.98
Trp	1.34	16.89	17.03
C-C	2.53	3.22	5.36
Arg	8.30	5.29	9.49
Cys	4.03	10.40	11.12

Table 3. Physicochemical conditions of the model systems simulating the ripening stages in the production of dry fermented sausages.

Dry Curing Stages	Initial Stage	1st Drying Stage	2nd Drying Stage
pH	5.45	4.38	4.60
Glycerol (ml/100 mL)	0	9	23
a_w	0.968	0.944	0.895
NaCl (mg/mL)	27	27	27
$NaNO_2$ (mg/mL)	0.15	0	0
$NaNO_3$ (mg/mL)	0.15	0.10	0.075
Sodium ascorbate (mg/mL)	0.5	0.5	0.5
Glucose (mg/mL)	20	5	5
Amino acids (mg/100 mL)	Table 2 (Initial stage)	Table 2 (1st drying stage)	Table 2 (2nd drying stage)

4.1. Analysis of Water Activity and pH

Water activity (a_w) was measured using an AQUALAB® 4 Water Activity Meter (METER Group, München, Germany), and pH was measured using a pH-meter (Orion EA 920, Boston, MA, USA).

4.2. Analysis of Volatile Compounds

Analysis of volatile compounds was carried out by headspace (HS) solid-phase microextraction (SPME) with an 85 μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) using a gas chromatograph (Agilent HP 7890 series II, Hewlett-Packard, Palo Alto, CA, USA) with a quadrupole mass detector (HP 5975C, Hewlett-Packard, Palo Alto, CA, USA). In summary, 5 mL of the model system was placed into a headspace vial using an automatic injector Gerstel MPS2 (Gerstel, Germany) [15] and incubated at 37 °C for 30 min. The extracted volatile compounds were adsorbed in the fiber for 60 min at 30 °C and desorbed in the injection port of the GC–MS for 5 min at 240 °C in splitless mode. The volatile compounds were separated using a DB-624 capillary column (30 m, 0.25 mm i.d., film thickness 1.4 μm (J&W Scientific, Agilent Technologies, Palo Alto, CA, USA)) using the conditions described by Corral et al. [42]. The MS interface temperature was set to 240 °C. The compounds were identified in full scan mode and by

comparison with mass spectra from the library database (Nist'05), with linear retention indices [43] and using authentic standards. The quantitation was performed in SCAN mode using either total ion current (TIC) on an arbitrary scale and expressed as abundance units (AU) × 10^6.

4.3. Analysis of Amino Acids

The analysis of free amino acids was performed using the EZ-Faast kit from Phenomenex (Torrance, CA, USA). Model system samples were diluted at ratio 1:5 (v/v) for I stage, and 1:25 (v/v) for 1D and 2D stages with distilled water previously to derivatization. The derived amino acids were analyzed using GC-FID. A gas chromatograph (Agilent Technologies 7890B) with a flame ionization detector (FID) equipped with an autosampler G4513A and a ZB-AAA 10 m× 0.25mm GC column (Phenomenex) was used. The injection volume was 2.5 µL at 250 °C in split mode (15:1). Helium was used as a carrier gas at a constant flow of 27 mL/min during the run, and the column head pressure was 8.78 psi. The GC oven temperature was initially held at 110 °C and then raised to 320 °C at 32 °C/min; the inlet temperature was 250 °C, and the detector was set at 320 °C. Identification and quantification were based on retention time and peak area integration of the reference amino acids. Norleucine was used as the internal standard. Calibration curves for each amino acid were obtained with the standard amino acids solutions (Phenomenex). In addition, several amino acids were prepared at high concentrations to adjust the calibration curves to the levels present in media samples. Results were expressed in milligrams per 100 mL of model system.

4.4. Statistical Analyses

The effect of different model system composition and incubation time on the generation of volatile compounds were tested by two-factor analysis of variance (ANOVA) at each temperature (25 and 37 °C) using the statistic software XLSTAT2018 (Addinsoft, Barcelona, Spain). The effect of temperature and time on the amino acid content was studied in each media by a two-factor analysis of variance (ANOVA). Significant differences

between samples means were analyzed according to Tukey test ($p < 0.05$). Principal component analysis (PCA) was plotted to evaluate the relationships among the different ripening stages, incubation temperature and volatile compounds in model systems measured at each sampling time.

5. Conclusions

This study has shown high yields of volatile compounds produced in model systems resembling dry fermented meat products at different curing stages in terms of amino acid content, pH, lower a_w and temperature (37 vs. 25 °C). In order to improve the formation of aroma compounds during the ripening process, the increase in temperature during dry curing process would favor their generation once the a_w value is low and amino acid concentration increases from the proteolytic activity. This research demonstrates that in dry fermented meat products, free amino acids participate in the generation of volatile compounds via the Maillard reaction in addition to the microbial activity. Moreover, a_w and temperature play an important role in these processes promoting the formation of flavor compounds in dry cured meat products.

Author Contributions: L.L. carried out the experiments and wrote the manuscript with support from C.B. and M.F.; C.B. and M.F. supervised the project and contributed to the design and analysis of the results. All authors have read and agreed to the published version of the manuscript.

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Sample Availability: Samples of the compounds are available from the authors.

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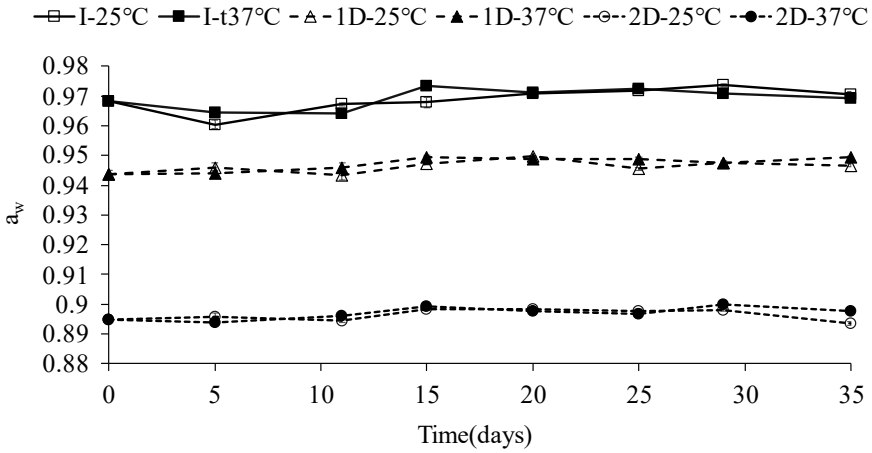
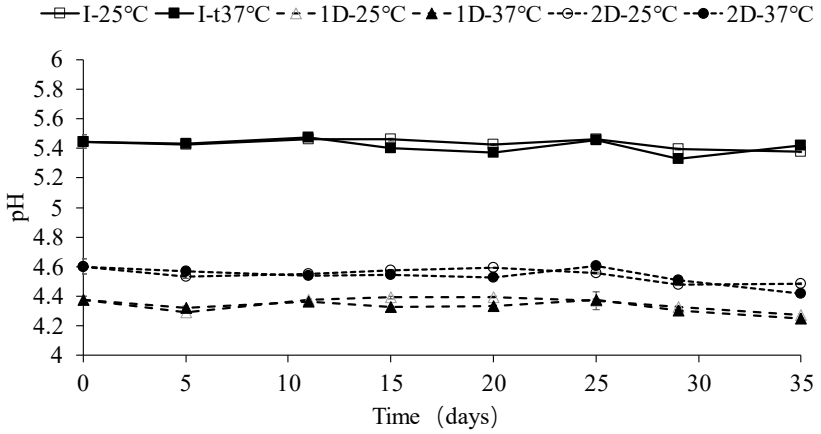
SUPPLEMENTARY MATERIAL

Supplementary Figure 1. The evolution of Water activity (a_w) in the model systems.



Supplementary Figure 2. The evolution of pH in the model systems.



Supplementary Table 1. Volatile compounds produced at 25 °C in the model systems simulating the dry curing stages (expressed as abundance (AU) of total ion current (TIC) as AU×10^6).

	Compound	Furan	Acetone	2-Methylpropanal	2-Butanone	3-Methylbutanal	Heptane	2-Methylbutanal	Acetic acid	Dimethyl disulfide
	LRI sample[2]	514	530	594	632	692	700	702	715	773
	LRI stand[3]	-	527	590	629	687	700	698	714	774
	RI[4]	b	a	a	a	a	a	a	a	a
I[1]	I-0d	nd[7]	0.411 g[8]	nd	nd	1.614 h	8.029 cd	nd	nd	nd
	I-5d	nd	3.887 fg	nd	nd	4.370 h	24.159 ab	nd	nd	nd
	I-11d	nd	nd	1.885 de	nd	11.082 h	nd	11.047 hi	nd	nd
	I-15d	nd	1.832 fg	5.072 cde	nd	22.200 gh	13.581 bc	15.493 ghi	0.542 abc	nd
	I-20d	nd	2.130 fg	5.663 cde	nd	26.736 gh	24.530 ab	7.538 hi	1.194 abc	nd
	I-25d	nd	1.776 fg	3.841 cde	nd	21.840 gh	29.720 a	0.000 i	1.165 abc	nd
	I-29d	nd	1.626 g	5.147 cde	nd	21.657 gh	nd	20.300 fgh	1.739 ab	nd
	I-35d	nd	1.644 g	4.235 cde	nd	26.257 gh	nd	21.787 fgh	1.232 abc	nd
1D	1D-0d	nd	3.633 fg	nd	nd	1.760 h	8.418 cd	nd	nd	nd
	1D-5d	nd	9.380 ef	nd	2.826 ab	16.307 h	nd	10.012 hi	nd	nd
	1D-11d	nd	12.172 de	8.328 bcde	2.295 bc	65.101 fg	nd	32.070 efg	nd	nd
	1D-15d	nd	14.391 cde	8.189 bcde	3.533 ab	85.630 ef	nd	36.157 def	0.560 abc	nd
	1D-20d	nd	14.768 cde	9.020 bcde	3.538 ab	102.697 def	nd	47.631 cde	1.211 abc	nd
	1D-25d	nd	17.284 bcd	13.665 abc	4.191 a	144.442 bcd	nd	51.795 cd	1.383 abc	nd
	1D-29d	nd	15.413 cde	10.768 abcd	3.748 ab	152.277 bc	nd	52.603 bcd	1.530 ab	nd
	1D-35d	0.599 a	16.180 cde	10.241 bcde	nd	164.551 bc	nd	51.156 cd	1.909 a	1.103 b
2D	2D-0d	nd	4.422 fg	nd	nd	1.665 h	7.145 cd	nd	nd	nd
	2D-5d	nd	14.092 cde	2.310 de	3.012 ab	16.606 h	nd	13.879 hi	nd	nd

	2D-11d	nd	17.334 bcd	8.266 bcde	0.722 cd	65.400 fg	nd	35.918 def	0.312 bc	nd
	2D-15d	nd	16.034 cde	7.098 cde	2.808 ab	78.952 ef	nd	39.695 de	1.389 abc	nd
	2D-20d	nd	18.923 abcd	13.025 abc	3.066 ab	124.558 cde	nd	58.556 bc	1.226 abc	nd
	2D-25d	nd	24.433 ab	18.394 ab	4.016 ab	180.210 b	nd	70.345 ab	1.094 abc	nd
	2D-29d	nd	20.210 abc	12.936 abc	3.335 ab	188.617 b	nd	70.037 ab	1.413 abc	nd
	2D-35d	0.587 a	25.833 a	20.946 a	4.329 a	238.605 a	nd	78.853 a	1.856 a	1.637 a
	RMSE[5]	0.02	2.42	3.27	0.55	15.20	4.03	5.68	0.47	0.14
	*P*t[6]	***	***	***	***	***	***	***	***	***
	*P*m[7]	***	***	***	***	***	***	***	ns	***
	*P*t*m[8]	***	***	**	***	***	***	***	ns	***

Supplementary Table 1 (continued).

	Compound	Thiazole	Methylpyrazine	*p*-xylene	Furfural	3-(Methylthio)propanal	Benzaldehyde	2-Ethyl-1-hexanol	Benzeneacetaldehyde
	LRI sample[2]	776	860	892	898	968	1020	1083	1110
	LRI stand[3]	776	860	893	893	968	1013	1083	1104
	RI[4]	a	a	a	a	a	a	a	a
	I-0d	nd	nd	nd	nd	nd	nd	nd	nd
	I-5d	nd	nd	nd	nd	nd	nd	8.341 bc	nd
	I-11d	nd	nd	nd	nd	1.785 g	0.897 h	8.896 bc	nd
I[1]	I-15d	nd	nd	nd	nd	3.875 bcd	1.174 gh	10.574 ab	1.011 de
	I-20d	nd	nd	1.324 abc	nd	3.053 def	1.262 gh	12.215 a	1.198 de
	I-25d	nd	nd	0.979 abc	nd	1.640 g	0.872 h	6.564 cd	0.906 de
	I-29d	nd	nd	nd	nd	1.898 fg	0.896 h	8.725 bc	0.945 de
	I-35d	nd	nd	nd	nd	1.764 g	0.845 h	9.355 abc	1.152 de

1D	1D-0d	nd	nd	nd	nd	nd	nd	2.416 ef	nd
	1D-5d	nd	nd	nd	nd	nd	nd	nd	nd
	1D-11d	nd	nd	nd	nd	3.134 de	1.126 gh	2.324 ef	3.274 cd
	1D-15d	nd	nd	0.506 bc	nd	5.592 a	1.592 fg	2.526 ef	5.390 abc
	1D-20d	nd	nd	3.307 a	nd	5.292 a	2.331 de	2.954 ef	6.403 ab
	1D-25d	nd	0.792 cd	nd	nd	4.571 ab	2.810 abcd	2.721 ef	6.632 a
	1D-29d	nd	1.048 bc	0.605 bc	nd	4.580 ab	2.884 abcd	nd	6.273 ab
	1D-35d	0.793 a	1.476 a	0.204 bc	nd	4.390 abc	3.313 ab	3.324 e	6.500 ab
2D	2D-0d	nd	nd	nd	nd	nd	nd	nd	nd
	2D-5d	nd	nd	nd	nd	nd	nd	nd	nd
	2D-11d	nd	nd	1.469 abc	nd	1.904 fg	1.862 ef	3.684 de	3.937 bc
	2D-15d	nd	nd	2.051 abc	nd	3.298 cde	2.741 bcd	0.922 ef	5.924 ab
	2D-20d	nd	nd	1.330 abc	nd	2.312 efg	2.501 cd	2.334 ef	5.281 abc
	2D-25d	nd	0.754 d	0.683 bc	nd	2.202 efg	2.988 abc	2.664 ef	6.509 ab
	2D-29d	nd	1.128 b	2.564 ab	nd	2.637 efg	3.015 abc	2.582 ef	6.395 ab
	2D-35d	0.627 b	1.700 a	0.590 bc	nd	2.303 efg	3.340 a	2.759 ef	6.519 ab
	RMSE[5]	0.02	0.08	0.76		0.96	0.83	0.96	0.83
	*P*t[6]	***	***	***		***	***	***	***
	*P*m	***	***	**		***	***	***	***
	*P*t*m	***	***	**		***	***	***	***

[1] Media models: Initial stage (I), 1st drying stage (1D), 2nd drying stage (2D). [2] LRI: Linear retention Indices of the compounds eluted from the GC-MS using a DB-624 capillry column. [3] LRI standard: Linear Retention Indices of an authentic standard compounds from internal laboratory database. [4] RI: Reliability of identification: a: identification by mass spectrum and by coincidence with LRI of an authentic standard, b: tentative identification by mass spectrum. [5] RMSE: Roost mean square error. [6] *P* value of time (t), media (m) and time and media (t*m) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [7] nd: not detected. [8] Different letters in the same columns means significant differences at $P < 0.05$.

Supplementary Table 2. Volatile compounds produced at 37 °C in the model systems simulating the dry curing stages (expressed as abundance (AU) of total ion current (TIC) as AU×10^6).

	Compound	Furan	Acetone	2-Methylpropanal	2-Butanone	3-Methylbutanal	Heptane	2-Methylbutanal	Acetic acid	Dimethyl disulfide
	LRI sample[2]	514	530	594	632	692	700	702	715	773
	LRI stand[3]	-	527	590	629	687	700	698	714	774
	RI[4]	b	a	a	a	a	a	a	a	a
I[1]	I-0d	nd[7]	4.205 bcdefg[8]	nd	nd	1.614 d	7.067 b	nd	nd	nd
	I-5d	nd	nd	4.855 bc	nd	31.604 cd	nd	24.398 cd	nd	nd
	I-11d	nd	nd	7.151 abc	0.000 d	54.922 cd	nd	35.478 cd	2.893 fgh	nd
	I-15d	nd	2.338 efg	8.183 abc	1.564 cd	59.784 cd	nd	40.876 c	3.012 fgh	nd
	I-20d	nd	nd	10.799 abc	1.781 bcd	58.157 cd	nd	39.082 cd	3.424 fgh	nd
	I-25d	nd	2.936 defg	8.058 abc	1.531 cd	46.038 cd	nd	35.874 cd	3.408 fgh	nd
	I-29d	nd	1.829 fg	10.047 abc	1.742 bcd	52.875 cd	nd	34.847 cd	4.275 efg	nd
	I-35d	nd	2.646 defg	7.835 abc	1.511 cd	47.972 cd	nd	33.525 cd	3.750 efgh	nd
1D	1D-0d	nd	3.633 cdefg	nd	nd	1.760 d	nd	nd	nd	nd
	1D-5d	nd	17.684 abcd	10.215 abc	4.365 ab	89.001 cd	nd	45.461 c	nd	nd
	1D-11d	nd	18.481 abc	18.905 a	4.923 a	313.634 ab	nd	88.920 ab	4.108 efg	0.371 d
	1D-15d	nd	14.955 abcdefg	13.136 abc	3.688 abc	315.535 ab	nd	90.752 ab	6.604 def	0.000 d
	1D-20d	2.645 a	17.185 abcde	16.869 ab	4.354 ab	403.442 a	nd	110.854 a	9.655 cd	5.226 a
	1D-25d	2.592 a	17.084 abcdef	18.737 a	4.591 a	469.147 a	nd	113.810 a	12.190 abc	5.406 a
	1D-29d	2.536 a	17.847 abcd	19.268 a	4.577 a	422.233 a	nd	109.071 a	13.365 abc	5.712 a
	1D-35d	2.505 a	15.627 abcdef	16.410 ab	3.832 abc	412.190 a	nd	111.220 a	15.838 a	5.586 a
2D	2D-0d	nd	4.422 bcdefg	nd	nd	1.665 d	7.145 b	nd	nd	nd
	2D-5d	nd	23.201 a	14.715 ab	4.957 a	91.357 cd	nd	43.823 c	nd	nd

2D-11d	nd	19.400 ab	9.129 abc	2.337 abcd	181.429 bc	nd	56.993 bc	1.991 gh	0.000 d
2D-15d	0.899 bc	21.549 a	16.307 ab	3.630 abc	328.012 ab	nd	94.623 ab	3.905 efgh	1.056 cd
2D-20d	1.249 b	18.356 abc	13.779 abc	3.082 abc	368.152 a	nd	107.100 a	7.761 de	1.832 bcd
2D-25d	1.587 ab	19.134 ab	16.906 ab	3.151 abc	422.772 a	nd	123.104 a	10.067 bcd	3.358 abc
2D-29d	1.747 ab	21.707 a	20.892 a	3.639 abc	458.502 a	nd	127.312 a	13.883 ab	4.456 a
2D-35d	1.331 b	20.946 a	19.545 a	3.198 abc	457.705 a	nd	125.737 a	14.944 a	4.400 ab
RMSE[5]	0.354	4.850	4.402	0.837	54.533	0.274	12.722	1.301	0.817
*P*t[6]	***	**	***	***	***	***	***	***	***
*P*m[7]	***	***	***	***	***	ns	***	***	***
*P*t*m[8]	***	*	ns	***	***	**	***	***	***

Supplementary Table 2(continued).

	Compound	Thiazole	Methylpyrazine	*p*-xylene	Furfural	3-(Methylthio)propanal	Benzaldehyde	2-Ethyl-1-hexanol	Benzeneacetaldehyde
	LRI sample[2]	776	860	892	898	968	1020	1083	1110
	LRI stand[3]	776	860	893	893	968	1013	1083	1104
	RI[4]	a	a	a	a	a	a	a	a
	I-0d	nd	nd	nd	nd	nd	nd	nd	nd
	I-5d	nd	nd	1.322 a	nd	4.326 c	1.834 hij	7.980 b	0.708 h
	I-11d	nd	nd	1.119 a	0.000 g	4.010 c	2.082 hij	8.975 ab	2.271 h
I[1]	I-15d	nd	nd	0.000 b	1.284 def	4.574 c	2.450 hi	9.999 a	2.052 h
	I-20d	nd	nd	0.537 ab	1.297 def	2.340 def	1.913 hij	8.072 b	2.404 gh
	I-25d	nd	nd	0.000 b	1.521 de	1.226 fg	1.599 hij	8.344 ab	2.348 gh
	I-29d	nd	nd	0.473 ab	1.802 cde	1.582 efg	1.828 hij	9.329 ab	2.003 h
	I-35d	nd	nd	0.000 b	1.840 cde	0.684 g	1.486 ij	8.043 b	1.710 h

1D	1D-0d	nd	nd	nd	nd	nd	nd	2.416 c	nd
	1D-5d	nd	nd	0.873 ab	nd	6.340 b	1.692 hij	2.162 c	7.032 fg
	1D-11d	1.201 cde	2.252 e	0.535 ab	1.194 ef	9.260 a	6.998 ef	2.823 c	15.109 cde
	1D-15d	1.499 bc	2.904 cd	1.055 a	1.600 de	8.105 a	9.139 cde	3.471 c	14.585 cde
	1D-20d	1.541 abc	3.255 bc	nd	1.923 bcd	6.280 b	10.565 bc	2.946 c	15.916 cd
	1D-25d	1.735 ab	3.483 b	nd	2.390 bc	4.397 c	12.051 ab	3.188 c	15.905 cd
	1D-29d	1.739 ab	3.485 b	0.141 b	2.546 b	4.532 c	11.851 ab	2.687 c	14.652 cde
	1D-35d	1.947 a	4.232 a	0.800 ab	3.233 a	3.921 cd	12.940 a	3.322 c	15.651 cde
2D	2D-0d	nd	nd	nd	nd	nd	nd	nd	nd
	2D-5d	nd	nd	1.328 a	nd	nd	2.177 hij	2.607 c	4.163 gh
	2D-11d	0.732 f	1.006 g	0.717 ab	0.000 g	3.623 cd	3.820 gh	2.963 c	10.994 ef
	2D-15d	1.020 def	1.648 f	0.812 ab	0.000 g	4.537 c	5.611 fg	2.800 c	12.742 de
	2D-20d	0.988 ef	2.293 e	0.749 ab	0.816 f	4.287 c	7.202 ef	2.980 c	18.344 bc
	2D-25d	1.029 def	2.456 de	0.000 b	0.871 f	3.560 cd	7.868 de	3.202 c	20.986 b
	2D-29d	1.369 bcde	2.900 cd	0.000 b	1.251 ef	4.018 c	10.019 bcd	3.442 c	25.794 a
	2D-35d	1.436 bcd	3.214 bc	0.457 ab	1.363 def	3.108 cde	11.099 abc	3.807 c	26.402 a
	RMSE[5]	0.142	0.152	0.282	0.206	0.521	0.710	0.596	1.496
	*P*t[6]	***	***	***	***	***	***	***	***
	*P*m	***	***	ns	***	***	***	***	***
	*P*t*m	***	***	***	***	***	***	***	***

[1] Media models: Initial stage (I), 1st drying stage (1D), 2nd drying stage (2D). [2] LRI: Linear retention Indices of the compounds eluted from the GC-MS using a DB-624 capillry column. [3] LRI standard: Linear Retention Indices of an authentic standard compounds from internal laboratory database. [4] RI: Reliability of identification: a: identification by mass spectrum and by coincidence with LRI of an authentic standard, b: tentative identification by mass spectrum. [5] RMSE: Roost mean square error. [6] *P* value of time (t), media (m) and time and media (t*m) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [7] nd: not detected. [8] Different letters in the same columns means significant differences at $P < 0.05$.

Supplementary Table 3. Concentration of amino acids (mg/100mL) in the model systems simulating the dry curing stages.

		Amino acids	ALA	GLY	VAL	β-ALA	LEU	ILE	THR	SER	PRO	ASN
I[1]	25 °C	0d	3.276	10.039	5.531	27.286	6.492	5.172	1.804 ab[4]	nd[5]	3.731	0.494
		5d	3.322	10.181	5.660	27.289	6.712	5.171	1.820 ab	nd	3.847	0.517
		11d	3.243	10.148	5.664	26.970	6.569	5.220	1.345 b	nd	3.923	0.513
		15d	3.353	10.067	5.595	27.331	6.516	5.166	1.375 b	nd	3.860	0.503
		20d	3.195	9.699	5.592	27.033	6.481	5.181	1.236 b	nd	3.800	0.465
		25d	3.228	9.741	5.758	26.487	6.444	5.291	1.536 ab	nd	3.811	0.501
		29d	3.287	9.837	5.584	26.895	6.435	5.064	1.641 ab	nd	3.770	0.501
		35d	3.227	9.779	5.614	26.646	6.405	5.137	1.493 ab	nd	3.821	0.499
	37 °C	0d	3.351	10.684	6.508	31.887	7.316	5.341	2.207 ab	nd	4.083	0.480
		5d	3.186	10.294	6.077	26.518	7.005	5.306	2.897 a	nd	3.966	0.446
		11d	3.191	9.577	5.466	26.153	6.326	5.082	1.882 ab	nd	3.680	0.443
		15d	3.263	9.740	5.490	26.499	6.362	5.324	1.759 ab	nd	3.774	0.434
		20d	3.236	10.379	5.520	26.049	6.970	5.261	1.970 ab	nd	4.124	0.417
		25d	3.259	10.101	6.048	26.907	6.820	5.143	1.748 ab	nd	3.923	0.446
		29d	3.166	9.436	5.403	25.809	6.147	4.981	1.616 ab	nd	3.636	0.404
		35d	3.172	9.701	5.544	26.106	6.377	5.087	1.648 ab	nd	3.929	0.445
		RMSE[2]	0.081	0.662	0.460	1.985	0.600	0.209	0.378		0.320	0.045
		*P*T[3]	ns	ns	ns	ns	ns	ns	**		ns	**
		*P*t	ns	ns	ns	ns	ns	ns	ns		ns	ns
		*P*T*t	ns	ns	ns	ns	ns	ns	ns		ns	ns

Supplementary Table 3 (continued).

		Amino acids	ASP	MET	GLU	PHE	GLN	ORN	LYS	HIS	TYR	TRP
		0d	11.491	2.265	7.270	5.103	35.891 a	1.074	2.770	7.848 a	4.296 a	2.400 a
		5d	12.139	2.380	7.412	5.289	31.865 ab	1.094	2.772	7.568 abc	4.689 a	2.364 ab
		11d	11.962	2.289	7.427	5.306	22.115 bc	1.095	2.629	7.009 abc	4.603 a	2.158 abc
	25 °C	15d	12.139	2.314	7.708	5.079	18.625 cd	1.097	2.710	7.256 abc	4.439 a	2.027 abcd
		20d	11.354	2.175	7.020	5.190	16.290 cd	1.093	2.645	6.990 abc	4.547 a	1.984 bcde
		25d	11.514	2.175	7.004	5.068	13.507 cd	1.167	2.749	7.199 abc	4.319 a	1.934 cdef
		29d	11.703	2.205	7.440	5.004	11.586 cd	1.111	2.755	7.119 abc	4.297 a	1.893 cdefg
		35d	11.566	2.218	7.348	5.077	10.259 d	1.111	2.627	7.188 abc	4.387 a	1.852 cdefg
		0d	12.048	2.273	7.125	5.372	32.809 ab	1.047	3.151	7.774 ab	4.276 a	2.361 ab
I[1]		5d	11.933	2.552	7.406	5.067	15.023 cd	1.077	3.048	6.809 abcd	4.006 a	1.892 cdefg
		11d	11.911	2.416	7.531	4.846	14.134 cd	1.065	1.926	5.673 cd	3.757 a	1.677 defg
	37 °C	15d	11.936	2.406	7.655	4.956	14.226 cd	1.123	2.087	6.253 abcd	4.056 a	1.782 cdefg
		20d	13.676	2.119	9.328	5.213	16.040 cd	1.101	1.514	5.673 cd	3.858 a	1.486 g
		25d	13.532	2.224	7.725	4.927	14.497 cd	1.121	2.140	6.462 abcd	3.802 a	1.501 g
		29d	11.436	2.104	7.317	4.779	14.444 cd	1.102	1.984	5.887 bcd	3.875 a	1.610 efg
		35d	11.833	2.092	7.607	5.041	15.410 cd	1.093	1.840	4.907 d	4.013 a	1.540 fg
		RMSE[2]	1.253	0.121	0.824	0.202	2.737	0.052	0.500	0.595	0.288	0.113
		*P*T[3]	ns	ns	ns	ns	**	ns	ns	***	***	***
		*P*t	ns	**	ns	ns	***	ns	ns	**	ns	***
		*P*T*t	ns	ns	ns	ns	***	ns	ns	ns	ns	ns

Supplementary Table 3 (continued).

		Amino acids	ALA	GLY	VAL	β-ALA	LEU	ILE	THR	SER	PRO	ASN
1D	25 °C	0d	5.297	30.266	65.485	82.570	149.058	43.902	22.789 ab	0.762 a	70.964	8.886
		5d	5.225	29.452	65.471	81.537	147.414	43.126	22.361 ab	1.098 a	71.229	8.958
		11d	5.115	29.137	64.720	82.169	150.012	43.318	20.558 b	8.051 a	69.723	8.231
		15d	5.116	30.384	67.815	85.231	155.724	47.060	22.040 b	8.947 a	72.515	8.760
		20d	4.996	28.975	65.106	83.139	151.094	45.070	20.027 b	9.014 a	69.541	8.120
		25d	5.008	28.698	64.683	80.966	147.498	43.207	20.843 ab	9.730 a	69.650	8.098
		29d	4.827	27.515	64.700	81.879	150.294	45.265	17.335 ab	12.631 a	68.279	7.597
		35d	4.941	28.061	63.787	80.123	145.485	44.794	18.420 ab	7.100 a	68.035	7.960
	37 °C	0d	4.787	26.617	64.703	77.655	149.641	45.617	25.090 ab	20.810 a	70.029	7.046
		5d	5.012	27.667	68.249	80.206	154.269	45.260	26.073 a	20.742 a	73.711	7.789
		11d	4.983	28.188	72.926	81.318	168.725	48.884	28.324 ab	21.811 a	79.272	8.019
		15d	4.998	28.312	72.499	81.337	157.372	47.056	28.515 ab	23.370 a	78.439	7.924
		20d	4.836	27.300	71.841	80.247	168.691	48.324	27.316 ab	25.525 a	77.188	7.196
		25d	4.941	28.230	68.862	84.057	162.285	48.421	25.369 ab	26.757 a	73.581	7.686
		29d	4.794	26.178	68.363	81.096	159.822	46.386	23.087 ab	24.257 a	68.658	6.917
		35d	4.767	25.896	70.433	80.424	165.770	48.906	24.718 ab	26.837 a	75.129	6.996
		RMSE[2]	0.179	2.265	5.171	3.270	11.359	2.815	2.361	4.5475908	6.317	0.739
		*P*T[3]	**	**	**	ns	**	**	***	***	**	***
		*P*t	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
		*P*T*t	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Supplementary Table 3 (continued).

		Amino acids	ASP	MET	GLU	PHE	GLN	ORN	LYS	HIS	TYR	TRP
1D	25 °C	0d	92.346	35.309	56.402	62.490	80.267	18.721	34.260 ab	40.256 a	19.372	18.096
		5d	94.567	33.518	59.517	61.129	64.913	18.742	34.597 ab	36.773 a	18.997	17.652
		11d	87.768	32.267	53.640	62.890	49.625	18.345	33.412 b	39.029 a	19.406	17.595
		15d	93.191	31.912	57.430	65.080	51.070	19.320	35.229 b	40.228 a	20.328	17.690
		20d	88.069	28.072	54.621	63.418	42.055	18.506	33.391 b	38.865 a	19.743	16.460
		25d	90.342	27.232	56.111	62.683	38.230	17.941	31.837 ab	39.221 a	19.655	16.132
		29d	90.750	26.278	56.509	64.174	33.307	17.255	29.869 ab	41.672 a	19.842	15.021
		35d	88.598	23.531	58.376	59.874	33.573	18.360	34.014 ab	40.090 a	18.778	14.818
	37 °C	0d	83.610	31.401	47.440	65.583	61.475	16.902	29.164 ab	39.457 a	20.507	17.869
		5d	91.064	30.205	51.132	67.001	36.733	17.612	30.376 a	42.981 a	20.945	17.558
		11d	89.842	26.799	49.881	67.883	18.661	16.685	29.411 ab	41.084 a	21.570	14.870
		15d	90.966	23.694	49.406	69.309	19.519	15.954	27.166 ab	46.743 a	22.029	13.914
		20d	85.598	20.757	46.574	69.793	24.267	16.278	27.133 ab	42.979 a	22.253	13.319
		25d	87.766	19.539	48.899	70.569	23.294	17.993	31.886 ab	42.703 a	22.597	13.694
		29d	85.250	16.188	47.884	66.903	23.191	16.906	29.445 ab	39.408 a	21.125	11.774
		35d	83.245	15.505	45.175	69.219	22.246	18.060	32.926 ab	36.129 a	21.733	11.712
		RMSE[2]	6.435	1.994	6.519	4.616	4.660	1.335	3.264	5.909	1.816	1.387
		*P*T[3]	ns	***	***	***	***	**	***	ns	***	***
		*P*t	ns	***	ns	ns	***	ns	ns	ns	ns	***
		*P*T*t	ns	ns	ns	ns	**	ns	ns	ns	ns	ns

Supplementary Table 3 (continued).

		Amino acids	ALA	GLY	VAL	β-ALA	LEU	ILE	THR	SER	PRO	ASN
2D[1]	25 °C	0d	5.072	42.663	77.880	95.589	189.115	63.790	39.085 ab	22.269 a	74.080	11.831
		5d	4.896	41.374	81.308	99.591	181.143	62.063	41.767 ab	19.120 a	75.590	12.911
		11d	4.738	39.252	78.083	93.396	173.127	61.064	37.471 b	16.755 a	70.058	12.402
		15d	4.776	42.152	79.675	98.099	177.026	62.913	39.090 b	20.574 a	79.274	12.539
		20d	4.728	43.949	82.463	97.823	181.915	65.586	39.429 b	19.515 a	83.438	12.406
		25d	5.080	41.569	82.909	99.301	191.940	63.883	43.824 ab	18.850 a	79.649	14.860
		29d	4.676	40.254	79.115	96.656	190.414	62.879	37.346 ab	19.169 a	73.193	12.702
		35d	5.608	42.082	78.791	94.371	173.340	61.903	38.089 ab	17.379 a	75.291	12.707
	37 °C	0d	5.119	43.022	83.389	97.663	179.236	62.143	42.587 ab	16.304 a	76.649	13.569
		5d	5.297	42.630	87.104	110.588	193.847	63.966	40.952 a	20.184 a	78.673	12.828
		11d	5.383	43.776	89.188	100.203	195.061	65.251	41.790 ab	17.908 a	77.585	12.739
		15d	4.956	41.987	80.571	98.812	177.537	60.347	40.965 ab	20.834 a	73.837	12.644
		20d	5.502	43.879	90.825	113.832	183.092	65.047	43.417 ab	18.676 a	77.177	13.617
		25d	4.913	46.208	88.884	110.173	200.519	65.604	41.396 ab	20.642 a	77.342	12.922
		29d	5.161	43.989	83.987	100.026	183.567	62.948	42.945 ab	20.095 a	79.720	13.516
		35d	5.284	44.682	90.022	109.453	197.373	64.785	45.052 ab	18.759 a	85.182	15.427
		RMSE[2]	0.501	3.410	7.593	10.485	20.581	4.425	3.607	2.9260663	8.140	1.558
		*P*T[3]	ns	ns	*	*	ns	ns	*	ns	ns	ns
		*P*t	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		*P*T*t	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Supplementary Table 3 (continued).

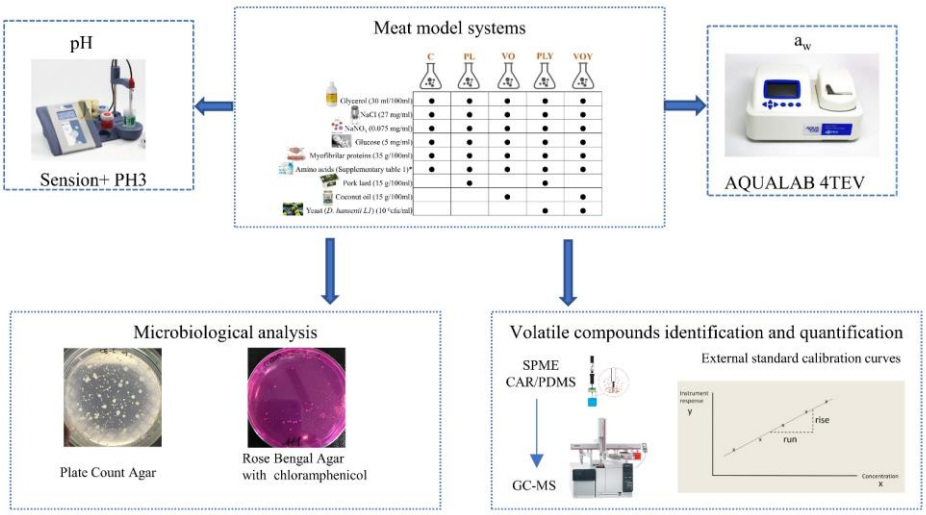
		Amino acids	ASP	MET	GLU	PHE	GLN	ORN	LYS	HIS	TYR	TRP
2D	25 °C	0d	74.266	42.036	45.727	83.342	69.111	21.645	46.404 ab	49.868 a	20.923	20.014
		5d	81.658	37.480	51.919	81.864	68.818	22.833	51.377 ab	60.979 a	19.941	19.903
		11d	77.232	35.954	48.115	78.131	56.052	21.315	47.501 b	63.625 a	19.509	19.805
		15d	81.028	36.920	59.234	81.534	50.016	21.446	47.037 b	52.343 a	19.540	18.695
		20d	78.763	34.205	48.600	84.722	47.020	23.793	52.934 b	59.907 a	21.573	19.755
		25d	91.494	29.583	54.171	78.027	44.850	24.419	55.369 ab	62.198 a	20.050	18.531
		29d	78.095	28.770	48.052	81.837	39.994	21.875	49.569 ab	65.506 a	20.895	19.164
		35d	81.513	27.133	52.464	74.491	30.508	22.589	49.279 ab	65.336 a	19.719	18.104
	37 °C	0d	82.687	38.454	49.520	76.718	58.572	21.193	44.719 ab	53.068 a	18.826	18.508
		5d	87.970	37.662	49.253	78.654	35.539	23.509	52.529 a	49.028 a	19.330	17.737
		11d	80.192	34.747	47.251	50.114	23.116	23.347	52.747 ab	60.945 a	19.613	16.954
		15d	76.395	30.363	46.533	78.149	24.026	22.333	49.901 ab	58.687 a	19.302	17.182
		20d	95.646	25.702	63.086	80.119	24.227	23.952	54.611 ab	53.265 a	19.055	15.288
		25d	81.764	22.607	45.695	94.685	23.775	22.003	49.208 ab	50.288 a	19.971	16.480
		29d	83.825	22.558	48.718	81.938	24.258	21.531	47.250 ab	65.961 a	20.194	16.333
		35d	90.089	17.903	51.368	49.760	18.576	21.184	52.507 ab	65.868 a	18.743	13.411
		RMSE[2]	7.931	1.134	6.082	19.595	6.294	2.547	6.405	10.552	1.281	1.461
		*P*T[3]	ns	***	ns	ns	***	ns	ns	ns	*	***
		*P*t	ns	***	ns	ns	***	ns	ns	ns	ns	*
		*P*T*t	ns	***	ns	ns	ns	ns	ns	ns	ns	ns

[1] Media models: Initial stage (I), 1st drying stage (1D), 2nd drying stage (2D). [2] RMSE: Roost mean square error. [3] P value of temperate (T), time (m) and temperate and time (T*t) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$. [4] nd: not detected. [5] Different letters in the same columns means significant differences at $P < 0.05$.

Chapter 5

Biotransformation of amino acids into aroma compounds in meat model systems as affected by fat replacement and yeast inoculation

This chapter has been sent submitted for publication.



Biotransformation of amino acids into aroma compounds in meat model systems affected by fat replacement and yeast inoculation

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ABSTRACT

The purpose of this study was to assess, in a meat model system, the effect of the replacement of pork lard with coconut oil and inoculation of *Debaryomyces hansenii* on the biotransformation of amino acids into volatile compounds. Yeast counts, Solid Phase Micro-Extraction and gas chromatography/mass spectrometry were used to assess yeast growth and volatile production. Yeast growth was confirmed until 28 d, although the volatile profile changed until 39 d. Forty-three volatiles were quantified and their odor activity values (OAVs) calculated. Differences in volatiles were related to the presence of fat and yeast. Production of branched-chain aldehydes, benzaldehyde, and methional was enhanced by pork lard's high concentration of carbonyl compounds at the initial incubation stages. The antioxidant action of *D. hansenii* was supported by the delay in hexanal formation in models containing coconut oil, and in formation of heptanal, octanal, and nonanal in those containing pork lard. The aroma profile in coconut models was influenced by hexanal, acid compounds, and their respective esters, whereas in pork lard models aroma was impacted by methional (musty, potato) and 3-methylbutanal (green, cocoa). Yeast inoculation contributed to 3-methylbutanoic (cheesy) and phenylethyl alcohol (floral). Fat type and yeast produced a differential effect on aroma.

Keywords: amino acids; yeast; aroma; biotransformation; *Debaryomyce hansenii*

1. Introduction

Amino acids are important precursors for the production of the characteristic flavor in dry cured meat products. Manufacturing practices submit these products to mild temperature during dry-curing and key aroma compounds can be produced from different reactions. Some aroma compounds are generated via Maillard reactions, as detected in model systems containing free amino acids and glucose resembling the conditions along the dry curing process (Li et al. 2021). Amino acids catabolism produced by microorganisms in dry cured fermented meats is also responsible for the production of aroma compounds. *Debaryomyces hansenii*, found at all stages of Spanish fermented sausage manufacture (Encinas, López-Díaz, García-López, Otero, & Moreno, 2000), can produce sulfur compounds (such as dimethyl disulfide, dimethyl trisulfide, and methional) via biochemical reactions from sulfur amino acids (Perea-Sanz, Peris, Belloch, & Flores, 2019). In addition, Strecker aldehydes can be produced when reactive carbonyls derived from lipid oxidation reactions react with amino acids (Hidalgo & Zamora, 2016; Zamora & Hidalgo, 2011). The combined action of phenylalanine with carbonyl and free radicals derived from lipid oxidation generated benzaldehyde (Hidalgo & Zamora, 2019), thus the formation of free radicals from different fatty acid compositions also affects the formation of flavor compounds derived from amino acids.

Among the starter cultures in fermented sausages *D. hansenii* and *Y. lipolytica* have been used for aroma production together with different bacteria (Flores et al. 2004; Iucci et al. 2007; Lorenzo et al. 2016). Moreover, the antioxidant effect of *D. hansenii* may impact flavor generation (Corral, Salvador, Belloch, & Flores, 2015). This effect has been seen in the reduction of oxidation values (TBARS) in fermented sausages (Corral et al., 2015); however, there is no information about the formation of carbonyl compounds from fats with different fatty acid compositions, and their effect on the formation of flavor compounds derived from amino acids in the presence of *D. hansenii* strains.

The use of model systems can simulate the conditions of processing imitating the complex matrix found in meat products where proteins and lipids make up the majority of

its composition. Myofibrillar proteins can bind key aroma compounds in dry cured meat (3-methyl butanal, 2-methyl butanal, hexanal, and methional) affecting aroma perception (Pérez-Juan, Flores, & Toldrá, 2008). Proteins are not the only molecules affecting the binding process, but also the fat of products like dry fermented sausages (nearly 30% fat content) has an impact on the aroma formation and release by acting as solvents of the generated compounds (Flores & Olivares, 2008).

Understanding the reactions involved and the production of key aroma compounds in dry cured meat products, such as fermented sausages, requires taking into account both Maillard reactions and lipid oxidation in complex systems, as well as the role of the starters in their formation. Accordingly, the objective of this study was to unveil the biochemical mechanisms involved in the production of key aroma compounds in a meat model system similar in composition to a dry fermented sausage, containing myofibrillar proteins, free amino acids, and fat from different origins, and inoculated with a starter culture of *D. hansenii*.

2. Materials and methods

2.1. Reagents and standards

All volatile compounds used for identification and quantitation were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of the yeast starter

D. hansenii strain L1 isolated from fermented sausage (Bolumar et al., 2006) was selected for the study. Yeasts were grown in GPY medium (glucose 2%, yeast extract 0.5%, peptone 0.5%) at 25 °C overnight. Cells were collected using saline solution (0.9% salt), and the cell suspensions were adjusted using a spectrophotometer V-1200 (VWR, Radnor, PA, USA) at 600 nm to reach a concentration of 10^6 cells ml^{-1} in the model systems.

2.3. Preparation of the meat model systems

A meat model system similar in composition to a dry fermented sausage containing myofibrillar proteins, free amino acids, and fat from different origins was prepared. Myofibrillar proteins were obtained from fresh minced meat using the muscle *Longissimus dorsi* without apparent fat or connective tissue. Myofibrillar proteins were isolated from meat and homogenized with 0.1 M Tris-HCl and 20 mM EDTA at pH 7.0 (1:2 w/v) using a mixer Krups 577 (Solingen, Germany). The homogenate was centrifuged for 30 min at 10,000 rpm at 4 °C and the supernatant was discarded. This process was repeated three times to eliminate the supernatant containing sarcoplasmic proteins. Then, the pellet containing myofibrillar proteins was stored at -20 °C until use for preparation of the meat models. The meat models were prepared with a similar composition in terms of additives and free amino acids as in fermented sausages (Corral et al., 2016; Olivares et al., 2010) mixed with the extracted myofibrillar proteins, and different fat types (pork lard (El Pozo, Murcia, Spain) or coconut oil (La Masía, Sevilla, Spain)).

Five meat model systems containing myofibrillar proteins (35%) and fat (15%) were prepared as follows: Control (C), pork lard (PL), pork lard with yeast (PLY), coconut oil (VO), and coconut oil with yeast (VOY) (Table 1). The meat model systems were prepared homogenizing the extracted myofibrillar proteins with a solution adjusted to pH 5 and a_w 0.895 containing the additives, sodium chloride, nitrate, glucose (Table 1) and free amino acids (Supplementary Table 1) previously sterilized by a vacuum-driven filtration system (0.22 μm), using a mixer Krups 577 (Solingen, Germany). Pork lard or coconut oil were then added and mixed until an emulsion was obtained. The PLY and VOY models were inoculated with *D. hansenii* L1 (10^6 ml^{-1}). Each meat model system, 255 g, was prepared and distributed in sterile Erlenmeyer flasks under sterile conditions (MSC-Advantage, Thermo-Fisher, Waltham, MA, USA). The model systems were incubated at 30 °C and the experiment was performed in three independent replicates. Samples of 35 g were taken for analyses at 0, 7, 14, 28, and 39 days from each model and replicate. Five grams of sample were used for microbial analysis and 30 g were centrifuged at 16,000 rpm for 30 min at 4

°C to remove protein, fat, and yeast cells, and the supernatant was used first for pH measurement (pH meter 50 VioLab, LabProcess, Barcelona, Spain) and then stored at -20 °C for volatile compounds analysis.

Table 1. Physical-chemical parameters and composition of the model systems.

Meat model systems[a]	C	PL	VO	PLY	VOY
pH	5	5	5	5	5
a_w[b]	0.895	0.895	0.895	0.895	0.895
NaCl (g/100 mL)	2.7	2.7	2.7	2.7	2.7
$NaNO_3$ (mg/100 mL)	7.5	7.5	7.5	7.5	7.5
Glucose (g/100 mL)	0.5	0.5	0.5	0.5	0.5
Amino acids[c]	+	+	+	+	+
Myofibrilar proteins (g/100 mL)	35	35	35	35	35
Pork lard (g/100 mL)		15		15	
Coconut oil (g/100 mL)			15		15
Yeast (*D. hansenii* L1) (cells ml^{-1})				10^6	10^6

[a] C: Control meat model system; PL and VO, pork lard and coconut oil model systems, respectively; PLY and VOY, pork lard and coconut oil model systems inoculated with yeast.
[b] The a_w was adjusted using glycerol (30 ml/100 mL).
[c] The concentration of amino acids is shown in Supplementary Table 1.

2.4. Microbial analysis

Samples, 5 g, were mixed with 45 ml of buffered peptone water (Pronadisa, Madrid, Spain) in a filtered bag (Scharlau, Barcelona, Spain) and homogenized using a Pulsifier II (Microgen Bioproducts Ltd., Camberley, Surrey, UK). Decimal dilutions of the filtrate were prepared and spread in triplicate on media plates for microbial counts (Belloch, Neef, Salafia, López-Diez, & Flores, 2021). Plate Count Agar (Pronadisa, Madrid, Spain) was used to count total mesophilic bacteria (TMB) and Rose Bengal Agar with chloramphenicol (Scharlau, Barcelona, Spain) was used to count yeasts.

2.5. Analysis of volatile compounds

The volatile compounds present in the headspace of the sample were analysed using a GC-MS 7890-5975 system (Agilent Technologies, Hewlett-Packard, Palo Alto, USA). The device was equipped with autosampler (MPS2 multipurpose sampler, Gerstel,

Mülheim an der Ruhr, Germany) and a DB-624 capillary column (30 m × 0.25 × 1.4 µm, J&W Scientific, Agilent Technologies, USA). The samples, 5 g of the supernatant from the models, were introduced into 20 ml headspace vials (Gerstel, Mülheim an der Ruhr, Germany) with a PTFE-faced silicon septum and incubated at 37 °C for 15 min for equilibration (250 rpm speed and 10 s time interval). The compounds were adsorbed onto an 85 µm CAR/PDMS fiber (Supelco, Bellefonte, USA) for 60 min at 37 °C by Headspace Solid-phase microextraction (HS-SPME) and then, desorbed for 5 min at 240 °C in splitless mode in the injection port of the GC-MS. Helium was employed as carrier gas at a constant flow rate of 0.897 mL/min and a constant average velocity of 34.34 cm/sec. The GC oven temperature was set at 40 °C for 10 min, then ramped to 100 °C at 3 °C/min for 5 min, then raised to 150 °C at 4 °C/min, then to 210 °C at 5 °C/min, and finally to 210 °C for 5 min. The temperature of the MS interface was fixed at 240 °C.

The identification of volatile compounds was done in full scan mode and by comparing mass spectra from the library database (NIST17). The identity of compounds was confirmed by comparing retention time and spectra with those of authentic standard compounds. The retention time index (RI) was calculated using an n-alkane series (C6–C20) under the same analysis conditions (Van Den Dool & Dec.Kratz, 1963).

The selected volatile compounds were quantified using external standard calibration curves (Jeleń & Wieczorek, 2023). Stock standard solutions of pure compounds were prepared in methanol except for those eluting in the initial 5 min were prepared in propylene glycol. Then, serial dilutions (1/2, 1/5, 1/10, 1/25, 1/50, 1/100, 1/125, 1/250) were prepared and analysed by GC-MS under the same chromatographic conditions. Calibration curves were obtained by plotting the total ion current (TIC) area against the ng of each compound except for 2-methyl butanal, which required the selection of a specific ion (m/z). The quantification was expressed in ng of the volatile compound extracted by the SPME fibre per g of meat model. The sensitivity and linearity of the volatile compound analysis were calculated. Standard solutions at six different concentration levels, each repeated three times, were used to test the linearity. For the

determination of sensitivity, limits of detection (LOD) and quantification (LOQ) were calculated from a blank sample (n=5) plus three and ten times the standard deviation respectively.

The estimation of odor activity values (OAVs) of volatile compounds extracted was done by calculation of the ratio of the concentration in the meat model by their respective odor threshold reported in air (Van Gemert, 2011).

2.6. Statistical analysis

The Generalized Linear Model (GLM) method was used to analyse the data by statistical software (XLSTAT 2018, Addinsoft, Barcelona, Spain). The influence of formulations and incubation time was treated as a fixed effect, and replicates as a random effect. The Tukey test was used to compare means when a significant effect ($P < 0.05$) was detected.

3. Results and discussion

The results of microbial counts in the models are shown in Fig. 1. Total mesophilic bacteria, present in the C, PL, and VO models, disappeared after 28 d, 14 d, and 7 d respectively. Total mesophilic bacteria were not detected in the PLY and VOY models, although the inhibitory effect of *D. hansenii* on the growth of total mesophilic bacteria had not been previously observed and requires further research. The absence of yeast after 28 d in PLY and VOY models indicates inhibition of yeast growth, most probably due to a decrease in pH of the meat model system at 28 d (Supplementary Fig. 1), because low pH can inhibit growth of *D. hansenii* (Belloch, Perea-Sanz, Gamero, & Flores, 2022). Moreover, the increase in pH followed by a sustained decrease in the model may be due to an increase in alkaline amino acids followed by an increase in acidic amino acids and fatty acids (Supplementary Fig. 1).

The volatile compounds identified in the five meat model systems are shown in Table 2. The compounds were classified according to their most probable origin into carbohydrate fermentation, lipid oxidation, amino acid degradation, esterase activity, lipid β-oxidation, and unknown origins. A total of 63 compounds were identified, and the volatile

composition differed among model systems. Two compounds were only present in the C model, 1-octanol, and dimethyl disulfide, while 2-methylbutanoic acid was only detected in the PLY model. The VO model was characterized by the presence of 3 compounds: dodecane, hexadecane, and heptadecane. In addition, 5 compounds were only present in VOY and PLY models: butanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, phenylethyl alcohol, and 2-pentanone.

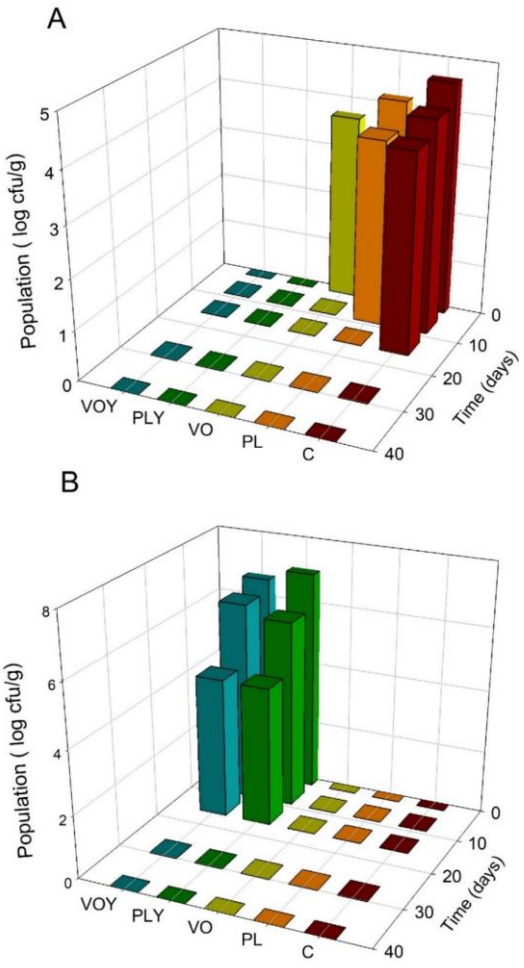

Fig. 1. Evolution of the microbial counts (log cfu/g) in the model systems (A) Total mesophilic bacteria, (B) Yeast. C, Control model system; PL and VO, pork lard and coconut oil model systems, respectively; PLY and VOY, pork lard and coconut oil model systems inoculated with *D. hansenii*.

Table 2. Volatile compounds detected in the model systems.

N[a]	Compounds	LRI[b]	LRI[c]	RI[d]	Meat model systems[e]				
					C	PL	VO	PLY	VOY
	Carbohydrate fermentation								
	Acetaldehyde	467	466	a	+[f]	+	+	+	+
1	Ethanol	508	507	a	+	+	+	+	+
2	Acetone	530	527	a	+	+	+	+	+
3	2-Butanone	635	629	a	-	+	+	+	+
4	Acetic acid	713	714	a	+	+	+	+	+
5	3-Hydroxy-2-butanone	783	783	a	-	-	+	+	+
6	Butanoic acid	888	891	a	-	-	-	+	+
	Lipid oxidation								
	Pentane	500	500	a	+	+	+	+	+
	Hexane	600	600	a	+	+	+	+	+
	Heptane	700	700	a	+	+	+	+	+
	Pentanal	737	736	a	+	+	+	+	+
	2-Methylheptane	767		b	+	+	+	+	+
	4-Methylheptane	768		b	+	+	+	+	+
	3-Methylheptane	775		b	+	+	+	+	+
	Octane	800	800	a	+	+	+	+	+
	2,4-Dimethylheptane	823		b	-	+	-	-	+
7	1-Pentanol	826	823	a	+	-	+	-	+
8	Hexanal	841	839	a	+	+	+	+	+
9	1-Hexanol	924	921	a	+	+	+	+	+
10	Heptanal	942	941	a	+	+	+	+	+
11	2-Pentylfuran	1009	1009	a	+	+	+	+	+
12	Octanal	1048	1044	a	+	+	+	+	+
13	Hexanoic acid	1080	1075	a	+	+	+	+	+
14	2-Ethyl-1-hexanol	1084	1083	a	+	+	+	-	+
15	1-Octanol	1126	1123	a	+	-	-	-	-
16	Nonanal	1151	1148	a	+	+	+	+	+
	Dodecane	1200	1200	a	-	-	+	-	-
17	Nonanoic acid	1360	1350	a	-	-	+	-	+

Table 2 (continued).

18	Octanoic acid	1269	1264	a	+	+	+	+	+
19	n-Decanoic acid	1455	1451	a	+	+	+	+	+
	Tetradecane	1399	1400	a	-	-	+	-	+
	Pentadecane	1500	1500	a	-	-	+	-	+
	Hexadecane	1600	1600	a	-	-	+	-	-
20	Dodecanoic acid	1645	1645	a	-	-	+	-	+
	Heptadecane	1700	1700	a	-	-	+	-	-
	Amino acid degradation								
21	2-Methylpropanal	594	590	a	+	+	-	+	+
22	3-Methylbutanal	692	687	a	+	+	+	+	+
23	2-Methylbutanal (58)[g]	701	698	a	+	+	+	+	+
24	Dimethyl disulfide	771	774	a	+	-	-	-	-
25	3-Methyl-1-butanol	795	795	a	+	+	+	+	+
26	2-Methylbutanol	798	795	a	-	+	-	+	+
27	2-Methylpropanoic acid	860	864	a	-	-	-	+	+
28	3-Methylbutanoic acid	939	933	a	-	-	-	+	+
29	2-Methylbutanoic acid	945	947	a	-	-	-	+	-
30	Methional	969	968	a	+	+	-	+	+
31	Benzaldehyde	1018	1013	a	+	+	+	+	+
	Benzeneacetaldehyde	1110	1104	a	+	+	+	+	+
32	Phenylethyl alcohol	1195	1191	a	-	-	-	+	+
	Esterase activity								
33	Ethyl acetate	638	636	a	+	+	+	+	+
34	Methyl hexanoate	953	952	a	-	-	+	-	+
35	Ethyl hexanoate	1028	1026	a	-	-	+	-	+
36	Ethyl octanoate	1229	1226	a	+	+	+	+	+
37	Ethyl decanoate	1427	1421	a	+	+	+	+	+
38	Ethyl dodecanoate	1623	1623	a	-	-	+	-	+
	Lipid β-oxidation								
39	2-Pentanone	733	731	a	-	-	-	+	+
40	2-Heptanone	935	933	a	+	+	+	+	+
	2,3-Octanedione	1028	1029	a	+	-	-	-	+
41	1-Octen-3-ol	1030	1028	a	+	+	+	+	+

Table 2 (continued).

	Unknown origin								
	Carbon disulfide	538	532	a	+	+	+	+	+
42	*p*-Xylene	892	892	a	+	+	+	+	+
43	Styrene	920	921	a	+	+	+	+	+
	3-Carene	1021	1026	a	+	+	+	+	+
	D-Limonene	1044	1046	a	+	-	+	+	+

[a] Compounds quantified in the meat model systems.
[b] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a DB-624 capillary column (J & W Scientific 30 m × 0.25 mm i.d. × 1.4 μ m film thickness).
[c] Linear retention indices (LRI) of the authentic standard compounds.
[d] RI: reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, identification by mass spectrum.
[e] C: Control meat model system; PL and VO, pork lard and coconut oil model systems, respectively; PLY and VOY, pork lard and coconut oil model systems inoculated with yeast.
[f] +: detected; -: not detected.
[g] Target ion used to quantify the compound when the peak was not completely resolved.

Considering the odor threshold of the compounds and their importance in the flavor of meat products (Perea-Sanz et al., 2020; Li et al., 2022), 43 compounds were selected for quantification (Table 1). These volatile compounds were quantified by using the external standard method (Supplementary table 2). The quantification showed significant differences among models and along the incubation time (Supplementary table 3). The evolution of the volatile compounds content grouped according to their probable origin is shown in Fig. 2. Ethanol, was excluded from the group of compounds derived from carbohydrate degradation due to its significant abundance concerning the rest of compounds in this group. The content of ethanol was significantly higher in C, PL and VO models than in the yeast inoculated models (PLY and VOY) (Fig. 2A). This may be related with the highest mesophilic bacteria counted in these models. Also, the PLY and VOY models showed higher levels of compounds derived from carbohydrate fermentation (Fig. 2B) and amino acid degradation (Fig. 2D) during the first 28 d of incubation. In contrast, the models with coconut oil (VO and VOY) showed higher content of compounds derived from lipid oxidation (Fig. 2C) and esterase activity reactions (Fig. 2E). The concentration

of compounds derived from lipid β-oxidation reactions was only different in the VOY model and mainly at 7 d (Fig. 2F).

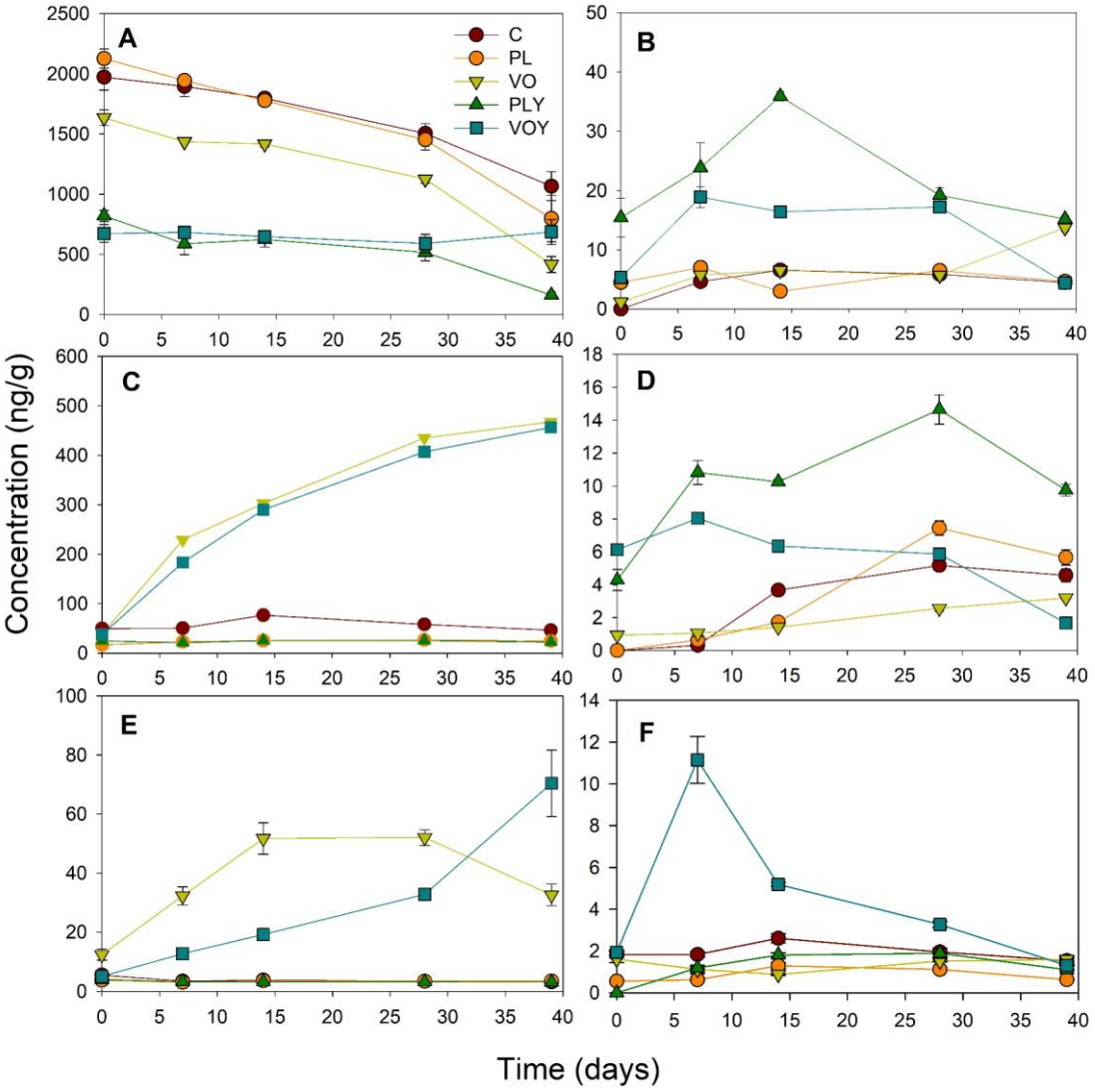

Fig. 2. Quantification (ng/g) of volatile compounds in the headspace of model systems classified according to the most likely origin (A, ethanol; B, carbohydrate fermentation (excluding ethanol); C, lipid oxidation; D: amino acid degradation; E, esterase activity; F, lipid β-oxidation reactions). Data are expressed as means ± SE. C, Control model system; PL and VO, pork lard and coconut oil model systems, respectively; PLY and VOY, pork lard and coconut oil model systems inoculated with *D. hansenii*.

The profile of volatile compounds produced in each model system was summarized in a heatmap with hierarchical clustering based on the concentration of the volatile compounds quantified in the headspace of the models (Fig. 3). Compounds derived from lipid oxidation reactions were clustered in the same group on the top of the heatmap, except for some acid compounds. Ester compounds were in the middle of the heatmap while those derived from amino acid catabolism (branched chain aldehydes, alcohols, and acids) were clustered at the bottom of the heatmap, together with the compounds derived from carbohydrate degradation reactions.

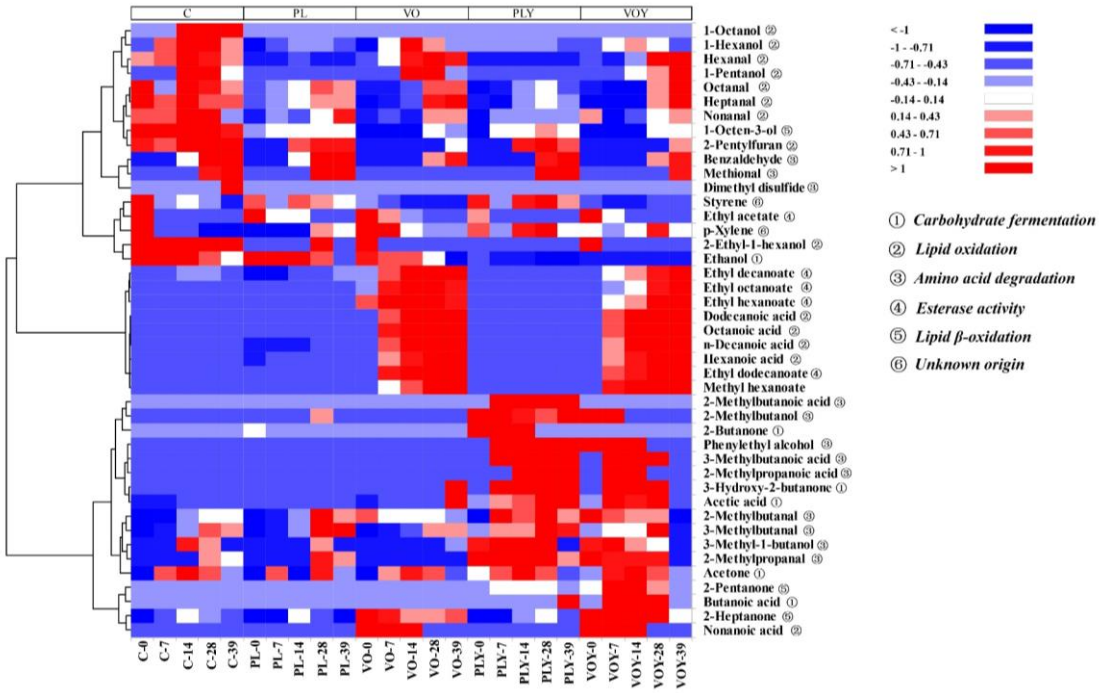

Fig. 3. Heatmap with hierarchical clustering based on volatile content in the headspace visualizing differences among model systems. The map's colors use red to represent relatively high concentrations, blue represents a relatively low concentration, and white indicates no difference. The numbers (0, 7, 14, 28, and 39) represent the sampling time. C, Control model system; PL and VO, pork lard and coconut oil model systems, respectively; PLY and VOY, pork lard and coconut oil model systems inoculated with *D. hansenii*. Compounds were asigned to the most probable origin as shown in Table 2, and indicated with numbers (①, ②, ③, ④, ⑤, and ⑥).

The addition of pork lard or coconut oil had different effects on the flavor compounds present in the meat model systems. Four acid compounds (hexanoic, octanoic, decanoic, and dodecanoic acids) were detected in the volatile profile of the VO and VOY models, being octanoic acid at the highest content in the models. Acids, hexanoic, octanoic, n-decanoic, and dodecanoic have been found in coconut oil in amounts of 0.52%, 7.6%, 5.5%, and 47.7% of total fatty acid methyl esters (FAMEs), respectively (Orsavova, Misurcova, Vavra Ambrozova, Vicha, & Mlcek, 2015). The highest amount of octanoic acid found in our analysis may be due to the different adsorption kinetics of the SPME fiber (Pinho, Ferreira, & Ferreira, 2002). The absence of these compounds in C, PL, and PLY models is due to the fatty acid composition of subcutaneous pork lard, which is characterized by mono and unsaturated fatty acids like palmitic acid 19.99%, oleic acid 46.31% and linoleic acid 12.96% acids (Daza, Menoyo, Olivares, Cordero, & López-Bote, 2007). A similar trend in the concentration of the four acids (hexanoic acid, octanoic acid, n-decanoic acid, and dodecanoic acid) was confirmed in the VO and VOY models, as well as their corresponding ethyl esters (Fig. 3 and Supplementary table 3). The origin of these ethyl esters (ethyl hexanoate and ethyl octanoate) in dry fermented sausages has been attributed to the inoculation of *D. hansenii* (Perea-Sanz et al., 2020). However, in the PLY model ethyl hexanoate was not detected and the content of ethyl octanoate was not significantly different during the incubation time. On the contrary, in the VOY model *D. hansenii* esterase activity increased the amount of these esters compounds along the incubation time (Supplementary table 3); however, an increase in these compounds was detected from days 7 to 28 in the VO model. This may suggest that the most probable origin of these esters compounds in the models is a chemical reaction (Park, Shaffer, & Bennett, 2009).

Yeast inoculation significantly affected the production of compounds derived from carbohydrate fermentation, amino acid degradation, and lipid β-oxidation reactions in both models, although this happened mainly during the first 14 d of incubation (Fig. 2). Compounds derived from carbohydrate degradation reactions were produced by yeast, as observed in compound 2-butanone in PLY, and acetic acid and 3-hydroxy-2-butanone in

PLY and VOY models (Fig. 3 and Supplementary table 3). This was previously observed in a model system inoculated with different strains of *D. hansenii* (Cano-García, Rivera-Jiménez, Belloch, & Flores, 2014). Furthermore, the abundance of products from the microbial metabolism of valine, leucine, and isoleucine, branched-chain aldehydes (2-methylpropanal, 3-methylbutanal and 2-methylbutanal, respectively) (Durá, Flores, & Toldrá, 2004) characterised the inoculated models VOY and PLY up to 28 d, while the corresponding acids compounds were also present but in lower amounts (Fig. 3 and Supplementary table 3). These branched acids have been reported in meat model medium inoculated with *D. hansenii* (Gong et al., 2022), as well as, in minced meat model (Olesen & Stahnke, 2000). In addition, phenylethyl alcohol was also majorly produced by *D. hansenii* in the VOY and PLY models as derivative from phenylalanine (Zhang, Ma, Meng, Li, & Ding, 2021). In the case of compounds derived from β-oxidation of fatty acids, only 2-pentanone was produced in PLY and VOY (Fig. 3 and Supplementary table 3).

The antioxidant effect of *D. hansenii* has been reported in fermented sausages by the reduction in heptanal, octanal, and nonanal contents (Flores et al. 2004). In the assayed model systems, the different fat employed affected the oxidation process. The presence of the yeast in VOY delayed the production of hexanal up to 28 d (Fig. 3, Supplementary table 3). In the PLY model, heptanal, octanal, and nonanal were reduced by the yeast inoculation, confirming the antioxidant effect of *D. hansenii* yeast; however, no differences in hexanal content were found.

The different oxidation processes in the models (pork lard and coconut oil) may have affected the formation of reactive carbonyls derived from lipid oxidation reactions, as well as, further reactions with the amino acids present in the models (Hidalgo & Zamora, 2016; Zamora & Hidalgo, 2011). The formation of branched-chain aldehydes is favored by the amino acid converting enzymes from yeast (Smit, Engels, & Smit, 2009). A similar role has been attributed to *D. hansenii* yeast strain D18335 inoculated on a cheese-surface model (Sørensen, Gori, Petersen, Jespersen, & Arneborg, 2011).

Regarding the concentration of amino acids, these as well as myofibrillar protein were in the same concentration in the models (Table 1). However, the content of branched-chain aldehydes in the PLY model was higher than in the VOY model (Supplementary table 3). This can be explained by the highest content of carbonyl compounds derived from the lipid peroxidation, which may have promoted branched-chain aldehyde production (Zamora & Hidalgo, 2011), which is in agreement with the higher carbonyl compounds (heptanal, octanal, and nonanal) content in the PL and PLY models detected up to 28 d derived from the unsaturated fatty acids present in pork lard (Wang & Cui, 2015). This was also confirmed by the highest content in PL and PLY of benzaldehyde (Supplementary table 3), which is derived from benzeneacetaldehyde produced from phenylalanine degradation by the action of reactive carbonyls (Hidalgo & Zamora, 2019) and benzeneacetaldehyde also can be reduced to phenylethyl alcohol (Lapadatescu, Giniès, Le Quéré, & Bonnarme, 2000). The same effect was observed in methional derived from the degradation of methionine (Corral et al., 2016) in PL and PLY models. Other compounds derived from methionine, like dimethyl disulfide, were only found in the C model, as already reported in a model containing exclusively free amino acids (Li et al., 2021). The absence of dimethyl disulfide in models VOY and PLY could be attributed to the antioxidant effect of *D. hansenii* (Corral et al., 2015), although a very low content of this compounds would make it undetectable in our analysis.

Taking into account that not all the identified compounds may contribute to the aroma of the model a calculation of their odor activity values (OAV) was done to explain the effect of fat and yeast inoculation on the headspace aroma of models (Supplementary table 4). However, it should be taken into account that the OAVs were calculated based on SPME extraction and using CAR/PDMS fiber, therefore the OAVs profile might change if other extraction techniques are used. Nevertheless, compounds showing OAV > 1 (Fig. 4) indicate that they impact the aroma, as their content is above its threshold (Olivares, Navarro, & Flores, 2009). Among the compounds quantified, aldehydes (hexanal, octanal, and nonanal), and acids (hexanoic, dodecanoic and octanoic acids) showed the highest

OAVs indicating the impact of the oxidation reaction on the aroma of the models (Flores, 2018) (Fig. 4). Moreover, hexanal and the acids octanoic, dodecanoic and hexanoic, had a large impact on the aroma of the models containing vegetable oil (VO and VOY). Moreover, their derived ester compounds, ethyl octanoate, ethyl hexanoate, ethyl decanoate and ethyl dodecanoate, which contribute to rancid, fatty, green, fruity, and cheesy odors were also very abundant in the VO and VOY models. In contrast, the aroma of the models containing pork lard (PL) was largely impacted by octanoic acid (rancid), although additional compounds derived from amino acids had also a high impact, like methional (musty, potato) and 3-methylbutanal (fruity, green, cocoa). Furthermore, yeast inoculation in pork lard models, PLY, enhanced the presence of compounds, 3-methylbutanoic and butanoic acids, and phenylethyl alcohol that contributed to cheesy and floral odor notes.

4. Conclusions

In summary, both factors fat type and yeast strain had a significant effect on the generation of volatile compounds in complex model systems by producing a differential effect on the aroma of the models. The antioxidant and amino acid converting effects of *D. hansenii* inoculation were confirmed in both models, PLY and VOY. Moreover, the different oxidation processes in the models affected the formation of reactive carbonyls derived from lipid oxidation reactions. The highest carbonyl content in pork lard models (PL and PLY), detected during the initial incubation period (up to 28 d) and derived from unsaturated fatty acids, favored the formation of branched-chain aldehydes in the models. This process was favored by amino acid converting enzymes from *D. hansenii*. The production of branched-chain aldehydes, benzaldehyde and methional, in pork lard models inoculated with *D. hansenii* confirms the action of reactive carbonyls on amino acids. The detected differences among models affected the aroma profile.

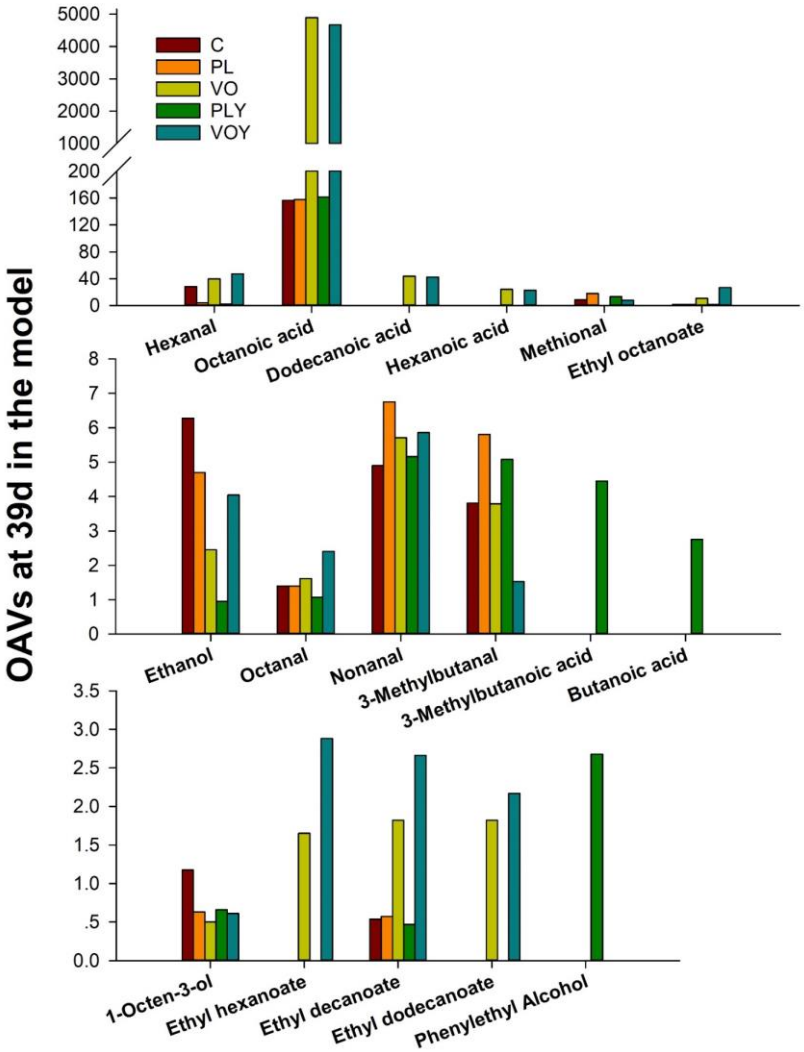

Fig. 4. Odour activity values (OAVs > 1) of volatile compounds in the headspace of model systems at 39 d. C, Control model system; PL and VO, pork lard and coconut oil model systems, respectively; PLY and VOY, pork lard and coconut oil model systems inoculated with *D. hansenii*.

Credit author statement

Lei Li: Formal analysis, Methodology, Investigation, Writing - original draft. **Carmela**

Belloch: Funding acquisition, Conceptualization, Writing - review & editing. **Monica Flores**; Funding acquisition, Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

None.

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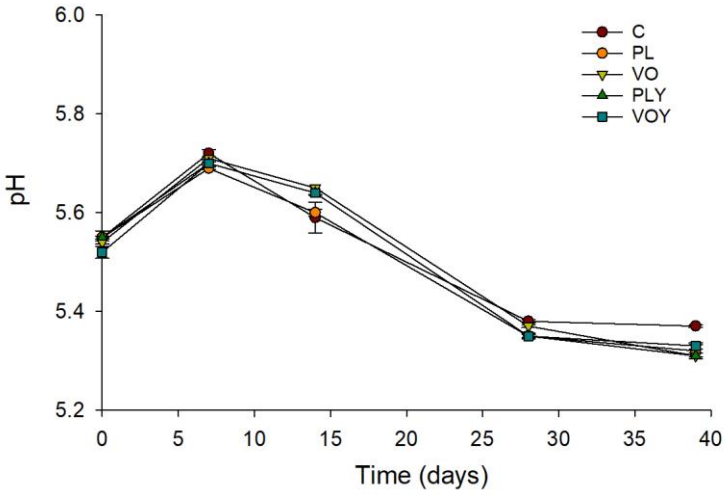
SUPPLEMENTARY MATERIAL



Supplementary Fig. 1. Evolution of pH in the model systems. Data expressed as means ± SE.

Supplementary Table 1. The concentration of amino acids (mg/100 mL) in the model systems.

Amino Acids	Concentration (mg/100 mL)	Amino Acids	Concentration (mg/100 mL)	Amino Acids	Concentration (mg/100 mL)	Amino Acids	Concentration (mg/100 mL)
Ala	111.8	Thr	54.99	Glu	73.45	Tyr	26.98
Gly	42.58	Ser	19.24	Phe	83.85	Trp	17.03
Val	90.35	Pro	89.05	Gln	65	C-C	5.36
β-Ala	4.54	Asn	21.71	Orn	31.01	Arg	9.49
Leu	176.8	Asp	100.1	Lys	120.9	Cys	11.12
Ile	62.08	Met	36.27	His	38.48		

Supplementary Table 2. Calibration curves, linearity and correlation coefficients of volatile compounds quantified in the model systems by HS-SPME.

Compounds	**Measure range (ng)**	**Slope (A/ng × 10^6)**	**Intercept (10^6)**	**Correlation coefficient (r^2)**	**LOD (ng)**	**LOQ (ng)**
Carbohydrate fermentation						
Ethanol	304.40-15220	0.04	28.49	0.9967	0.00	0.00
Acetone	2.96-74	0.10	-0.02	0.9991	0.00	0.00
2-Butanone	0.78-195	0.26	0.60	0.9998	0.00	0.00
Acetic acid	3.90-78	0.43	-1.97	0.9879	5.87	7.16
3-Hydroxy-2-butanone	9.60-240	0.16	-2.55	0.9968	11.72	12.75
Butanoic acid	6.32-31.60	0.38	-1.41	0.9981	4.98	7.03
Lipid oxidation						
1-Pentanol	1.18-11.8	0.61	-0.20	0.996	0.00	0.00
Hexanal	1.88-470	0.39	0.12	0.9994	1.23	2.90
1-Hexanol	1.32-16.5	1.13	-1.19	0.9967	1.56	2.35

Supplementary Table 2 (continued).

Heptanal	0.8-20	0.74	-0.56	0.9991	1.07	1.53
2-Pentylfuran	2.3-57.5	0.21	-0.80	0.9915	0.00	0.00
Octanal	1.72-17.2	0.22	-0.32	0.9941	0.00	0.00
Hexanoic acid	10.24-512	0.65	-9.98	0.9968	16.09	16.39
2-Ethyl-1-hexanol	1.32-6.6	0.87	-0.78	0.9951	0.00	0.00
1-Octanol	4.1-41	1.47	-5.60	0.9954	0.00	0.00
Nonanal	4.3-43	0.81	-2.05	0.9994	2.81	3.14
Nonanoic acid	2.64-13.2	1.36	-3.69	0.982	0.00	0.00
Octanoic acid	34.36-1718	0.53	-25.60	0.9971	0.00	0.00
n-Decanoic acid	9.84-123	0.78	-7.19	0.9984	0.00	0.00
Dodecanoic acid	21.6-216	0.51	-14.98	0.982	32.13	34.41
Amino acid degradation						
2-Methylpropanal	1.56-39	0.13	-0.14	0.9933	2.45	3.97
3-Methylbutanal	0.88-22	0.41	0.25	0.9992	0.00	0.00
2-Methylbutanal (58)[a]	0.84-21	0.04	-0.01	0.9997	0.00	0.00
Dimethyl disulfide	1.56-15.6	0.10	-0.04	0.9958	1.71	2.59
3-Methyl-1-butanol	1.84-46	0.54	-1.11	0.9942	2.42	2.81
2-Methylbutanol	1.11-11.41	1.06	-0.05	0.9989	0.20	0.32
2-Methylpropanoic acid	6.48-32	0.33	-0.89	0.9891	4.05	5.76
3-Methylbutanoic acid	2.69-13.4	0.41	-1.07	0.9868	0.00	0.00
2-Methylbutanoic acid	5.32-26.6	0.51	-2.37	0.9924	5.88	7.89
Methional	2.04-25.5	0.37	-0.75	0.9917	2.41	2.76
Benzaldehyde	0.94-9.4	0.82	-0.74	0.9053	1.97	3.86
Phenylethyl alcohol	2.65-26.5	0.80	-2.97	0.9892	4.06	4.43

Supplementary Table 2 (continued).

Esterase activity						
Ethyl acetate	1.9-19	0.37	0.02	0.997	0.00	0.00
Methyl hexanoate	2.08-10.4	0.26	-0.16	0.9929	0.00	0.00
Ethyl hexanoate	4-100	0.36	-1.90	0.9948	0.00	0.00
Ethyl octanoate	6.64-415	0.44	-5.10	0.9985	12.50	13.21
Ethyl decanoate	2.4-48	0.70	-1.84	0.9968	0.00	0.00
Ethyl dodecanoate	5.3-106	0.24	-1.44	0.9976	0.00	0.00
Lipid β-oxidation						
2-Pentanone	1.32-66	0.48	-0.24	0.9978	0.00	0.00
2-Heptanone	1.2-30	0.49	-0.68	0.9986	2.36	3.78
1-Octen-3-ol	2.48-31	0.85	-1.84	0.9965	0.00	0.00
Unknown origin						
p-Xylene	0.82-8.2	0.32	-0.04	0.9884	0.00	0.00
Styrene	0.52-5.2	0.58	-0.29	0.9938	0.87	1.35

[a] Target ion used to quantify the compound.

Supplementary Table 3. Quantification (ng/g) of volatile compounds in the headspace of model systems.

Group	Times (days)	***Carbohydrate fermentation***											
		Ethanol		Acetone		2-Butanone	Acetic acid		3-Hydroxy-2-butanone	Butanoic acid	***Total Carbohydrate fermentation (excluding ethanol)***	
C[a]	0	1972.84	ab[d]	-[e]		-	-		-	-	-	
	7	1896.25	abc	4.64	ab	-	-		-	-	4.64	de
	14	1797.97	abcd	5.44	a	-	1.13	fg	-	-	6.57	d
	28	1504.81	cde	4.68	ab	-	1.17	fg	-	-	5.85	de
	39	1067.24	fgh	3.35	de	-	1.15	fg	-	-	4.50	de

	0	2127.34	a	-		2.28	c	1.16	fg	-		-		3.44	de
	7	1944.64	ab	4.78	ab	1.08	c	1.17	fg	-		-		7.02	d
PL	14	1777.99	abcd	1.30	f	0.59	c	1.13	fg	-		-		3.02	de
	28	1452.55	def	5.30	ab	-		1.18	fg	-		-		6.49	de
	39	798.75	ghi	3.29	de	-		1.37	fg	-		-		4.66	de
	0	1635.58	bcd	-		1.17	c	-		-		-		1.17	de
	7	1436.30	def	4.26	bcd	0.30	c	1.18	fg	-		-		5.75	de
VO	14	1417.96	def	5.23	ab	0.13	c	1.14	fg	-		-		6.51	d
	28	1125.76	efg	4.50	abc	-		1.22	fg	-		-		5.71	de
	39	416.63	ij	2.82	e	-		6.58	abcd	4.32	c	-		13.72	c
	0	820.99	ghi	3.57	cde	10.27	b	1.58	fg	-		-		15.42	c
	7	589.19	i	4.51	abc	11.79	b	3.16	ef	4.36	bc	-		23.83	b
PLY	14	625.11	i	5.47	a	21.26	a	4.39	de	4.78	abc	-		35.89	a
	28	517.15	ij	4.56	abc	0.49	c	9.00	a	5.15	ab	-		19.20	bc
	39	161.43	j	2.88	e	-		6.05	bcd	5.28	a	0.96	a	15.17	c
	0	673.61	hi	3.03	e	0.71	c	1.57	fg	-		-		5.31	de
	7	683.71	hi	4.99	ab	0.29	c	7.88	ab	4.69	abc	1.05	a	18.90	bc
VOY	14	647.55	i	5.47	a	0.09	c	5.25	cde	4.67	abc	0.93	a	16.41	c
	28	590.91	i	4.84	ab	-		7.12	abc	4.25	c	1.02	a	17.22	c
	39	687.08	hi	2.95	e	-		1.46	fg	-		-		4.41	de
RMSE[b]		132.35		0.33		1.88		0.78		0.26		0.05		2.05	
*P*g[c]		***		***		***		***		***		***		***	
*P*t[c]		***		***		***		***		***		***		***	
*P*g*t[c]		***		***		***		***		***		***		***	

Supplementary Table 3 (continued).

Group	Times (days)	*Lipid oxidation*													
		1-Pentanol		Hexanal		1-Hexanol		Heptanal		2-Pentylfuran		Octanal		Hexanoic acid	
	0	-		24.75	def	0.28	de	0.87	abc	1.75	bcd	1.72	ab	3.57	d
	7	-		28.45	cde	0.65	bcd	0.72	bcdef	1.47	bcd	1.06	cdefghi	3.43	d
C[a]	14	1.54	a	46.68	ab	1.61	a	1.19	a	2.62	a	2.17	a	3.43	d
	28	1.62	a	31.70	cd	1.03	bc	0.72	bcdef	2.25	ab	1.53	bc	3.50	d
	39	0.37	cd	23.06	defg	0.56	cd	0.74	bcdef	2.21	ab	1.23	bcdefg	3.42	d
	0	-		4.05	hi	-		0.55	bcdef	-		0.86	defghi	-	
	7	-		6.16	hi	0.30	de	0.56	bcdef	-		1.03	cdefghi	3.31	d
PL	14	-		7.06	hi	0.39	de	0.66	bcdef	1.46	bcd	1.17	bcdefgh	3.32	d
	28	-		5.54	hi	0.40	de	0.68	bcdef	1.62	bcd	1.33	bcde	3.41	d
	39	-		3.36	i	0.25	de	0.67	bcdef	1.61	bcd	1.23	bcdefg	3.45	d
	0	-		9.66	ghi	-		0.42	ef	-		0.82	efghi	4.78	d
	7	-		16.95	efgh	0.44	de	0.48	cdef	-		0.79	efghi	24.88	c
VO	14	0.89	b	34.36	bcd	1.07	b	0.51	bcdef	-		0.88	defghi	36.97	b
	28	1.42	a	57.78	a	0.56	cd	0.79	abcde	-		1.40	bcd	60.89	a
	39	0.15	de	32.53	cd	0.34	de	0.84	abcd	0.98	d	1.42	bcd	69.75	b
	0	-		2.63	i	0.34	de	0.47	cdef	-		0.65	hi	3.59	d
	7	-		1.83	i	0.40	de	0.41	ef	-		0.76	fghi	3.35	d
PLY	14	-		3.97	hi	0.40	de	0.57	bcdef	1.73	bcd	0.96	defghi	3.37	d
	28	-		3.50	i	0.36	de	0.62	bcdef	1.97	abc	1.10	cdefghi	3.50	d
	39	-		1.72	i	0.28	de	0.60	bcdef	1.55	bcd	0.94	defghi	3.46	d
	0	-		8.18	hi	0.31	de	0.43	ef	-		0.78	efghi	4.25	d
	7	-		5.25	hi	0.50	de	0.33	f	-		0.58	i	23.02	c
VOY	14	0.24	de	12.35	fghi	0.52	d	0.44	def	-		0.71	ghi	37.31	b
	28	0.51	c	39.04	bc	0.42	de	0.71	bcdef	-		1.31	bcdef	63.58	a
	39	0.90	b	38.75	bc	0.27	de	0.89	ab	1.25	cd	2.12	a	66.43	a

RMSE[b]	0.08	4.24	0.16	0.13	0.26	0.18	3.28
Pg[c]	***	***	***	***	***	***	***
Pt[c]	***	***	***	***	***	***	***
*Pg***t*[c]	***	***	***	***	***	***	***

Supplementary Table 3 (continued).

Group	Times (days)	***Lipid oxidation***																	
		2-Ethyl-1-hexanol		1-Octanol		Nonanal		Nonanoic acid		Octanoic acid		n-Decanoic acid		Dodecanoic acid		***Total Lipid oxidation***	
C[a]	0	0.57	a	-		1.92	bcd	-		12.27	d	2.13	f	-		49.83	d
	7	0.39	ab	-		1.89	bcd	-		10.72	d	1.94	f	-		50.72	d
	14	0.48	ab	0.98	c	3.24	a	-		10.87	d	1.97	f	-		76.79	d
	28	0.41	ab	0.92	b	2.32	b	-		10.37	d	1.96	f	-		58.35	d
	39	0.38	ab	0.91	b	1.47	cde	-		10.18	d	1.95	f	-		46.47	d
PL	0	-		-		1.31	cde	-		9.97	d	-		-		16.74	d
	7	-		-		1.57	bcde	-		10.04	d	-		-		22.97	d
	14	-		-		1.43	cde	-		10.07	d	-		-		25.57	d
	28	0.42	ab	-		1.70	bcde	-		10.09	d	1.90	f	-		27.09	d
	39	-		-		2.02	bc	-		10.25	d	1.97	f	-		24.82	d
VO	0	0.35	b	-		1.29	cde	0.60	d	18.84	d	2.06	f	-		38.80	d
	7	-		-		1.34	cde	0.64	abc	163.55	bc	11.70	de	8.38	d	229.15	bc
	14	-		-		1.31	cde	0.64	abc	198.24	b	15.90	cd	11.78	c	302.55	d
	28	-		-		1.70	bcde	-		277.00	a	19.21	bc	14.18	bc	434.92	a
	39	-		-		1.71	bcde	-		318.05	a	24.39	a	17.43	a	467.62	a
PLY	0	-		-		0.95	e	-		13.94	d	2.15	f	-		24.71	d
	7	-		-		1.51	bcde	-		11.22	d	1.94	f	-		21.43	d
	14	-		-		1.53	bcde	-		11.06	d	1.95	f	-		25.54	d
	28	-		-		1.45	cde	-		10.90	d	2.17	f	-		25.59	d

	39	-		-	1.55	bcde	-		10.50	d	1.95	f	-		22.56	d
	0	0.34	b	-	1.74	bcde	0.63	bc	19.04	d	2.15	f	-		37.84	d
	7	-		-	1.15	de	0.67	d	134.38	c	9.29	e	8.18	d	183.35	c
VOY	14	-		-	1.42	cde	0.67	ab	208.18	d	15.97	cd	12.23	c	290.03	bc
	28	-		-	1.58	bcde	-		265.60	a	19.90	abc	14.43	bc	407.09	a
	39	-		-	1.76	bcd	-		303.38	a	23.68	ab	16.95	ab	456.37	a
RMSE[b]		0.06		0.02	0.25		0.01		17.61		1.47		0.88		23.70	
*P*g[c]		***		***	***		***		***		***		***		***	
*P*t[c]		***		***	***		***		***		***		***		***	
*P*g*t[c]		***		***	***		***		***		***		***		***	

Supplementary Table 3 (continued).

Group	Times (days)	***Amino acid degradation***															
		2-Methylpropanal		3-Methylbutanal		2-Methylbutanal		Dimethyl disulfide		3-Methyl-1-butanol		2-Methylbutanol		2-Methylpropanoic acid	
	0	-		-		-		-		-		-		-	
	7	-		0.32	kl	-		-		-		-		-	
C[a]	14	-		0.80	hijk	0.59	efg	-		1.96	cde	-		-	
	28	0.63	de	1.51	cdef	0.73	bcdef	-		1.11	fg	-		-	
	39	0.47	ef	1.33	defghi	0.63	efg	0.47	a	-		-		-	
	0	-		-		-		-		-		-		-	
	7	-		0.37	kl	0.27	fg	-		-		-		-	
PL	14	-		0.90	fghijk	0.53	efg	-		-		-		-	
	28	1.00	bcd	2.27	b	1.37	abc	-		1.28	efg	0.15	d	-	
	39	0.71	cde	2.03	bc	0.87	bcdef	-		-		-		-	
	0	-		-		0.94	bcde	-		-		-		-	
VO	7	-		0.42	jkl	0.63	efg	-		-		-		-	
	14	-		0.75	ijk	0.67	def	-		-		-		-	
	28	-		1.44	cdefg	0.72	cdef	-		-		-		-	

	39	-		1.33	defghi	0.63	efg	-		0.69	gh	-		-	
	0	1.17	abc	0.88	ghijk	-		-		1.92	cde	0.31	b	-	
	7	1.20	ab	1.31	defghi	1.38	ab	-		3.59	a	0.49	a	-	
PLY	14	1.14	abc	1.40	defghi	0.94	bcde	-		2.88	ab	0.24	bcd	0.87	bc
	28	1.59	a	3.38	a	1.71	a	-		2.61	bc	0.18	cd	1.01	ab
	39	0.63	de	1.78	bcde	0.81	bcdef	-		-		0.49	a	1.08	a
	0	1.12	abc	0.79	hijk	1.30	abcd	-		1.79	def	0.29	bc	-	
	7	1.05	bcd	1.18	efghi	0.96	bcde	-		2.24	bcd	0.27	bcd	0.81	cd
VOY	14	1.02	bcd	1.03	fghij	0.87	bcdef	-		1.28	efg	-		0.68	d
	28	1.05	bcd	1.91	bcd	0.89	bcdef	-		1.03	fg	-		-	
	39	-		0.53	jkl	-		-		-		-		-	
RMSE[b]		0.15		0.20		0.21		0.03		0.25		0.04		0.06	
*P*g[c]		***		***		***		***		***		***		***	
*P*t[c]		***		***		***		***		***		***		***	
*P*g*t[c]		***		***		***		***		***		***		***	

Supplementary Table 3 (continued).

Group	Times (days)	***Amino acid degradation***											
		3-Methylbutanoic acid		2-Methylbutanoic acid		Methional		Benzaldehyde		Phenylethyl Alcohol		***Total Amino acid degradation***	
	0	-		-		-		-		-		-	
	7	-		-		-		-		-		0.32	m
C[a]	14	-		-		-		0.32	e	-		3.67	hij
	28	-		-		0.48	e	0.72	bc	-		5.17	fgh
	39	-		-		0.56	de	1.11	a	-		4.57	fghi
	0	-		-		-		-		-		0.00	m
PL	7	-		-		-		-		-		0.64	m
	14	-		-		-		0.31	e	-		1.73	klm

	28	-		-		0.70	bc	0.69	bc	-		7.45	de
	39	-		-		1.13	a	0.91	ab	-		5.65	efg
	0	-		-		-		-		-		0.94	lm
	7	-		-		-		-		-		1.05	lm
VO	14	-		-		-		-		-		1.42	klm
	28	-		-		-		0.41	de	-		2.57	jkl
	39	-		-		-		0.55	cde	-		3.19	ijk
	0	-		-		-		-		-		4.29	ghij
	7	0.77	ab	1.14	b	-		-		0.94	a	10.82	b
PLY	14	0.75	ab	1.16	ab	-		-		0.87	bc	10.25	b
	28	0.83	ab	1.20	ab	0.64	cd	0.61	cd	0.90	ab	14.65	a
	39	0.98	a	1.49	a	0.83	b	0.75	bc	0.94	d	9.76	bc
	0	-		-		-		-		0.83	c	6.13	ef
	7	0.62	b	-		-		-		0.91	ab	8.04	cd
VOY	14	0.61	b	-		-		-		0.87	bc	6.35	def
	28	0.58	b	-		-		0.40	de	-		5.87	efg
	39	-		-		0.50	e	0.64	cd	-		1.67	klm
RMSE[b]		0.08		0.11		0.04		0.08		0.02		0.58	
*P*g[c]		***		***		***		***		***		***	
*P*t[c]		***		***		***		***		***		***	
*P*g*t[c]		***		***		***		***		***		***	

Supplementary Table 3 (continued).

Group	Times (days)	***Esterase activity***											
		Ethyl Acetate		Methyl hexanoate	Ethyl hexanoate	Ethyl octanoate		Ethyl decanoate		Ethyl dodecanoate	***Esterase activity***		
C[a]	0	1.93	a	-	-	2.96	e	0.63	g	-	5.52	de	
	7	-		-	-	2.78	e	0.64	g	-	3.43	de	
	14	-		-	-	2.92	e	0.87	fg	-	3.79	de	
	28	-		-	-	2.68	e	0.72	g	-	3.40	e	

	39	-		-		-		2.53	e	0.65	g	-		3.18	e
	0	1.32	abc	-		-		2.44	e	-		-		3.76	de
	7	0.39	de	-		-		2.64	e	-		-		3.03	e
PL	14	0.27	de	-		-		2.72	e	0.60	g	-		3.59	de
	28	-		-		-		2.79	e	0.64	g	-		3.43	e
	39	-		-		-		2.77	e	0.69	g	-		3.45	e
	0	1.65	ab	-		3.79	cde	6.11	e	0.84	fg	-		12.39	de
	7	0.46	cde	0.18	bc	7.44	ab	20.47	cd	1.35	de	2.37	cd	32.27	c
VO	14	0.21	de	0.27	bc	7.93	ab	37.85	b	2.09	bc	3.38	b	51.73	b
	28	-		0.50	ab	7.96	a	36.70	b	2.47	g	4.41	a	52.04	b
	39	-		0.61	ab	4.95	cd	21.21	c	2.18	g	3.64	b	32.59	c
	0	0.54	cde	-		-		2.87	e	0.58	g	-		3.99	de
	7	-		-		-		2.68	e	0.57	g	-		3.25	e
PLY	14	-		-		-		2.64	e	0.57	g	-		3.21	e
	28	-		-		-		2.58	e	0.64	g	-		3.21	e
	39	-		-		-		2.55	e	0.56	g	-		3.11	e
	0	1.01	bcd	-		-		3.37	e	0.66	g	-		5.04	de
	7	0.39	de	0.37	abc	1.98	ef	7.10	de	0.97	efg	1.92	d	12.73	de
VOY	14	-		0.51	ab	2.84	de	12.16	cde	1.29	def	2.43	de	19.23	cd
	28	-		0.52	ab	5.50	bc	22.42	c	1.68	cd	2.70	c	32.83	c
	39	-		0.78	a	8.64	a	53.48	a	3.19	a	4.34	a	70.44	a
RMSE[b]		0.29		0.14		0.78		4.34		0.14		0.20		4.96	
*P*g[c]		*		***		***		***		***		***		***	
*P*t[c]		***		***		***		***		***		***		***	
*P*g*t[c]		***		***		***		***		***		***		***	

Supplementary Table 3 (continued).

Group	Times (days)	*Lipid β-oxidation*								*Unknown*					
		2-Pentanone		2-Heptanone		1-Octen-3-ol		***Total Lipid β-oxidation***		*p*-Xylene		Styrene		***Total Unknown***	
	0	-		-		1.83	a	1.83	def	0.45	ab	1.57	a	2.02	a
	7	-		0.45	efg	1.39	b	1.84	def	0.14	fgh	0.29	bcdef	0.43	efg
C[a]	14	-		0.71	def	1.90	a	2.61	cd	0.13	gh	0.41	bcdef	0.54	cdefg
	28	-		0.52	ef	1.43	b	1.95	cde	-		0.29	cdef	0.29	fg
	39	-		0.37	fg	1.17	b	1.54	def	-		0.15	f	0.15	g
	0	-		-		0.56	c	0.56	fg	-		0.52	bcdef	0.52	defg
	7	-		-		0.63	c	0.63	efg	-		0.33	bcdef	0.33	fg
PL	14	-		0.64	def	0.65	c	1.30	defg	-		0.57	bcdef	0.57	cdefg
	28	-		0.44	efg	0.68	c	1.12	efg	0.26	cdefg	0.44	bcdef	0.70	bcdef
	39	-		-		0.63	c	0.63	efg	0.20	efg	0.36	bcdef	0.56	cdefg
	0	-		1.61	ab	-		1.61	def	0.54	a	0.32	bcdef	0.86	bcde
	7	-		1.12	bcd	-		1.12	efg	0.39	abc	0.22	def	0.61	bcdef
VO	14	-		0.88	de	-		0.88	efg	0.19	efg	0.14	f	0.34	fg
	28	-		0.91	de	0.61	c	1.52	def	0.15	fgh	0.14	f	0.30	fg
	39	-		1.10	cd	0.50	c	1.60	def	0.15	fgh	0.13	f	0.28	fg
	0	-		-		-		-		0.31	bcdef	0.67	bc	0.98	bc
	7	0.52	cd	-		0.67	c	1.19	efg	0.16	fgh	0.27	def	0.43	efg
PLY	14	0.55	cd	0.57	ef	0.69	c	1.81	def	0.33	bcde	0.62	bcd	0.95	bcd
	28	0.42	cd	0.74	def	0.73	c	1.90	def	0.37	bcd	0.69	b	1.06	b
	39	-		0.45	efg	0.66	c	1.11	efg	0.22	defg	0.43	bcdef	0.65	bcdef
	0	0.43	cd	1.50	abc	-		1.94	cde	0.20	efg	0.17	ef	0.37	fg
	7	9.23	a	1.91	a	-		11.15	a	0.15	fgh	0.15	f	0.30	fg
VOY	14	3.69	b	1.50	abc	-		5.19	b	0.19	efg	0.14	f	0.33	fg
	28	1.18	c	1.48	abc	0.61	c	3.27	c	0.35	bcde	0.18	ef	0.53	defg
	39	-		0.69	def	0.61	c	1.30	defg	0.20	efg	0.16	f	0.36	fg

RMSE[b]	0.30	0.16	0.09	0.43	0.05	0.12	0.14
*P*g[c]	***	***	***	***	***	***	***
*P*t[c]	***	***	***	***	***	***	***
*P*g*t[c]	***	***	***	***	***	***	***

[a] C: Control meat model system; PL and VO: pork lard and coconut oil meat model systems, respectively; PLY and VOY: pork lard and coconut oil meat model systems with inoculated yeast.
[b] RMSE: root mean square error.
[c] *P* value of group (g), ripening time (t) and group and ripening time (g*t) effect at ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: $P > 0.05$.
[d] Different letters in columns indicate significant differences at $P < 0.05$.
[e] -: not detected

Supplementary Table 4. Odor descriptor and OAVs of volatile compounds in the model systems at 39 days.

Compounds	Odor descriptor[a]	Threshold in air (ng/g)[b]	Meat model systems[d]				
			C	PL	VO	PLY	VOY
Carbohydrate fermentation							
Ethanol	Alcoholic	170–76000000	6.3	4.7	2.5	<1	4.0
Acetone	Solvent	1100-27900000	<1	<1	<1	<1	<1
2-Butanone	Fruity, green	750-1000000	<1	<1	<1	<1	<1
Acetic acid	Pungent, sour, fruit	10-500000	<1	<1	<1	<1	<1
3-Hydroxy-2-butanone	Creamy, dairy, sweet	14-10000[c]	<1	<1	<1	<1	<1
Butanoic acid	Acidic, sour, cheesy	0.35-9000	<1	<1	<1	2.8	<1
Lipid oxidation							
1-Pentanol	Pungent, fermented, bready	20-1100000	<1	<1	<1	<1	<1
Hexanal	Green, woody, vegetable	0.82-84500	28.1	4.1	39.7	2.1	47.3
1-Hexanol	Green, fruity, apple	10-65000	<1	<1	<1	<1	<1

Supplementary Table 4 (continued).

Heptanal	Green, aldehydic, oily	0.85-9500	<1	<1	1.0	<1	1.0
2-Pentylfuran	Green, waxy, musty	19-270	<1	<1	<1	<1	<1
Octanal	Aldehydic, green, peely	0.88-5000	1.4	1.4	1.6	1.1	2.4
Hexanoic acid	Cheesy, fruity, phenolic	2.9-3500	1.2	1.2	24.1	1.2	22.9
2-Ethyl-1-hexanol	Sweet, fatty, fruity	198-25482.2	<1	<1	<1	<1	<1
1-Octanol	Green, citrus, orange	5-9000	<1	<1	<1	<1	<1
Nonanal	Aldehydic, citrus, cucumber	0.3-230	4.9	6.7	5.7	5.2	5.9
Nonanoic acid	Cheesy, sweet, creamy	1.6-120	<1	<1	<1	<1	<1
Octanoic acid	Rancid, soapy, cheesy	0.065-3200	156.6	157.7	4893.2	161.5	4667.4
n-Decanoic acid	Soapy, waxy, fruity	50-90	<1	<1	<1	<1	<1
Dodecanoic acid	Fatty, coconut, bay	0.4-100	<1	<1	43.6	<1	42.4
Amino acid degradation							
2-Methylpropanal	Fresh, aldehydic, floral, pungent	1-410	<1	<1	<1	<1	<1
3-Methylbutanal	Fruity, dry, green, cocoa	0.35-6	3.8	5.8	3.8	5.1	1.5
2-Methylbutanal	Musty, rummy, nutty	100	<1	<1	<1	<1	<1
Dimethyl disulfide	Sulfurous, cabbage, malty, creamy	1.1-5600	<1	<1	<1	<1	<1
3-Methyl-1-butanol	Fusel, fermented, fruity	19–6300	<1	<1	<1	<1	<1
2-Methylbutanol	Ethereal fresh	140-329000	<1	<1	<1	<1	<1
2-Methylpropanoic acid	Acidic, sour, cheesy	5-240	<1	<1	<1	<1	<1
3-Methylbutanoic acid	Cheesy, dairy, acidic, sour	0.22-3000	<1	<1	<1	4.4	<1
2-Methylbutanoic acid	Fruity, dirty, acidic	20	<1	<1	<1	<1	<1
Methional	Musty, tomato, potato	0.063-60	8.9	18.0	<1	13.2	7.9
Benzaldehyde	Sharp, sweet, bitter	10-3400000	<1	<1	<1	<1	<1
Phenylethyl alcohol	Sweet, floral, fresh	0.35-3800	<1	<1	<1	2.7	<1
Esterase activity							
Ethyl acetate	Ethereal, fruity, sweet	340-1120000	<1	<1	<1	<1	<1

Supplementary Table 4 (continued).

Methyl hexanoate	Ethereal, fruity, pineapple	39-87[c]	<1	<1	<1	<1	<1
Ethyl hexanoate	Sweet, fruity, pineapple	3-18100	<1	<1	1.7	<1	2.9
Ethyl octanoate	Fruity, winey, waxy, sweet	2-40	1.3	1.4	10.6	1.3	26.7
Ethyl decanoate	Sweet, waxy, fruity, apple	1.2-530	<1	<1	1.8	<1	2.7
Ethyl dodecanoate	Sweet, waxy, floral, soapy	2	<1	<1	1.8	<1	2.2
Lipid β-oxidation							
2-Pentanone	Sweet, fruity, ethereal, winey	98-230000	<1	<1	<1	<1	<1
2-Heptanone	Cheesy, fruity, ketonic, green	3.5-330	<1	<1	<1	<1	<1
1-Octen-3-ol	Mushroom, earthy, green	1-110	1.2	<1	<1	<1	<1
Unknown origin							
p-Xylene		250-9100	<1	<1	<1	<1	<1
Styrene	Sweet, balsamic, floral	12-258000	<1	<1	<1	<1	<1

[a] Odor descriptors from The Good Scents Company database (www.thegoodscentscompany.com).
[b] Threshold values in air obtained from Van Gemert (2011).
[c] Threshold values in water obtained from Van Gemert (2011).
[d] C: Control meat model system; PL and VO: pork lard and coconut oil meat model systems, respectively; PLY and VOY: pork lard and coconut oil meat model systems with inoculated yeast.

GENERAL DISCUSSION

GENERAL DISCUSSION

Many different dry cured meat products are manufactured in the Mediterranean region. Formulation and processing of these meat products can vary between locations depending on local gastronomic traditions (Patarata, Carvalho, & Fraqueza, 2022). The organoleptic characteristics of meat and meat products influence customer choice and their purchase intention (Ventanas, González-Mohino, Estévez, & Carvalho, 2020). Flavor is thought to have the biggest impact on whether customers will accept meat products. The aroma development in dry cured products is based on complex chemical (Maillard reaction, Strecker degradation, and lipid autooxidation) and biochemical (carbohydrate fermentation, amino acid degradation, lipid β-oxidation, and esterase activity) reactions. These reactions occur mainly during ripening, and result in a large number of volatile compounds, which contribute to the characteristic flavor of dry cured meat products (Flores, 2018). Volatile compounds derived from amino acid degradation reactions are considered as the main source of "dry cured" flavor compounds, such as 3-methylbutanal which derives from Leu (Flores & Olivares, 2014). Moreover, nitrogen- and sulfur-containing volatile compounds derived from nitrogen and sulfur amino acids present specific olfactory characteristics and low odor thresholds in dry-cured meat products, indicating an important role in meat product flavor (Flores, 2018).

Since no specific compound is responsible for the distinctive odor of "cured" meat, the presence of compounds producing savory (meaty) and toasted aromas in meat products may produce an aroma balance that results in the characteristic "cured odor". The actual knowledge indicates that the toasted (2AP and 2-acetylpyrrole) and savory (MFT, methional, methionol, DMS, DMDS, DMTS, and MMFT) smell notes in cooked meat products may be influenced by the degradation and transformation pathways of nitrogenous amino acids (Pro and Orn) and amino acids containing sulfur atoms like cysteine, cystine, and methionine, as well as the vitamin thiamine (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2015). Though the contribution of these compounds to the meaty (savory) odors in cooked meat is well known (Parker, 2017), there is a lack of knowledge regarding the

mechanisms for the formation of savory and toasted aroma compounds in dry cured meat products.

Among the different types of dry cured meat products manufactured in the Mediterranean area (Flores, 1997), we can find those submitted to a fermentation process like dry fermented sausages, and those salted and ripened like dry cured loins and ham. The mechanisms for generation of savory and toasted aroma compounds can be different depending on the chemical and biochemical reactions involved in the process. Therefore, the study of the mechanisms involved in the generation of sulfur and nitrogen compounds in two different dry meat products (dry fermented sausages and dry cured loins) can help to understand the presence of savory and toasted aromas in these meat products. Accordingly, both dry fermented sausages and dry cured loins were used as representatives of meat products in the Ph.D. project (Chapters 1, 2 and 3). In addition, two different model systems were employed to study the role of the Maillard reaction for aroma generation (Chapter 4), and the effect of fat oxidation and *Debaryomyces hansenii* inoculation on the biotransformation of amino acids (Chapter 5).

In order to investigate the mechanisms involved in the chemical degradation and biotransformation of amino acids (Pro and Orn) and thiamine during the processing of dry-cured meat products, dry cured loins (Chapter 1) and dry fermented sausages (Chapter 2) were manufactured. Both meat products were supplemented with aroma precursors (Pro, Orn and thiamine) and submitted to a drying process. Loins were taken for study at the end of the process (68 d), while sausages were analysed at different days of the processing (8, 34, and 62 d).

The aromagram of dry cured loins and dry fermented sausages obtained by GC-FID-O, using the DF method, is shown in Fig. 1 and Fig. 2. The FID signal is shown at the top of the figure and the odor intensity (DF value) appears at the bottom. The numbers included in the graphs indicate the odor description produced by the identified or unknown chemical compound in loin and sausages (Chapters 1 and 2). The comparison between both figures (1 and 2) shows that, using the same volatile extraction technique, the aroma profile of the

products is different, although some of the aroma compounds are present in both products. The target of this study was the identification of savory and toasted aromas among all those detected. In this sense, the compounds detected by GC-O in loins were savory odors (methional (N.11, cooked potatoes), MMTF (N.12, meaty, sour) and methionol (N.16, potato, savory) in Fig. 1) and toasted odors (2AP (N.12, roasted, toasted corn), tetramethyl pyrazine (N.17, toasted, roasted), 2-acetylpyrrole (N.18, roasted, toasted corn, nuts, meaty) and 2 unknown compounds producing toasted notes at Linear retention index (LRI) DB624: 1279 (N.23) and 1281(N.24) in Fig. 1). In sausages the odors detected were savory odors (methanethiol (N.1, sulfurous, cooked vegetable), and methional (N.18, cooked potato) in Fig. 2) and toasted odors (2,6-dimethylpyrazine (N.15, toasted, sour), and five roasted and toasted meaty notes produced by unknown compounds at LRI DB-624: 907 (N.13), 966 (N.17), 1185 (N.27), 1217 (N.28), 1231 (N.29) in Fig. 2). "Unknown toasted" scents in loins and sausages require careful examination using advanced analytical techniques. Furthermore, these results confirm the low threshold odors of the toasted and savory aromas in the two meat products.

Several factors affect the analysis of the savory and toasted odors, the SPME extraction technique, their low concentration, and the matrix composition, which may affect the concentration of compounds in the headspace, etc. (Jeleń & Wieczorek, 2023). The abundance of sulfur and nitrogen compounds, primarily responsible for the savory and toasted odors, was measured in the headspace of dry sausages and loins (Chapters 1 and 2). In some cases, a specific ion (m/z) was selected to quantify the compound due to coelution with other compounds, and the results were expressed as abundance units ($AU \times 10^6$). A comparison of the abundance of these compounds in dry fermented sausages and dry cured loins headspace from the data obtained in Chapters 1 and 2 is represented in Fig. 3. The yield of savory and toasted aroma compounds extracted by SPME fiber was very low due to the low abundance of these compounds present in the headspace of loins and sausages. Not all the compounds were detected in the headspace of both products and, furthermore, their abundance was different between products. For example, the abundance of

methanethiol and 2,6-dimethylpyrazine was significantly higher in sausages than in loins (Fig. 3). Compounds 2AP and 2-acetylpyrrole were, for the first time, detected in the headspace of dry cured loins. However, the abundance of these two compounds did not differ significantly between loins, and the effect of the addition of precursors was not clear. In both products, loins and sausages, MMTF was at the highest abundance when thiamine was added, confirming the origin of MMTF from thiamine degradation.

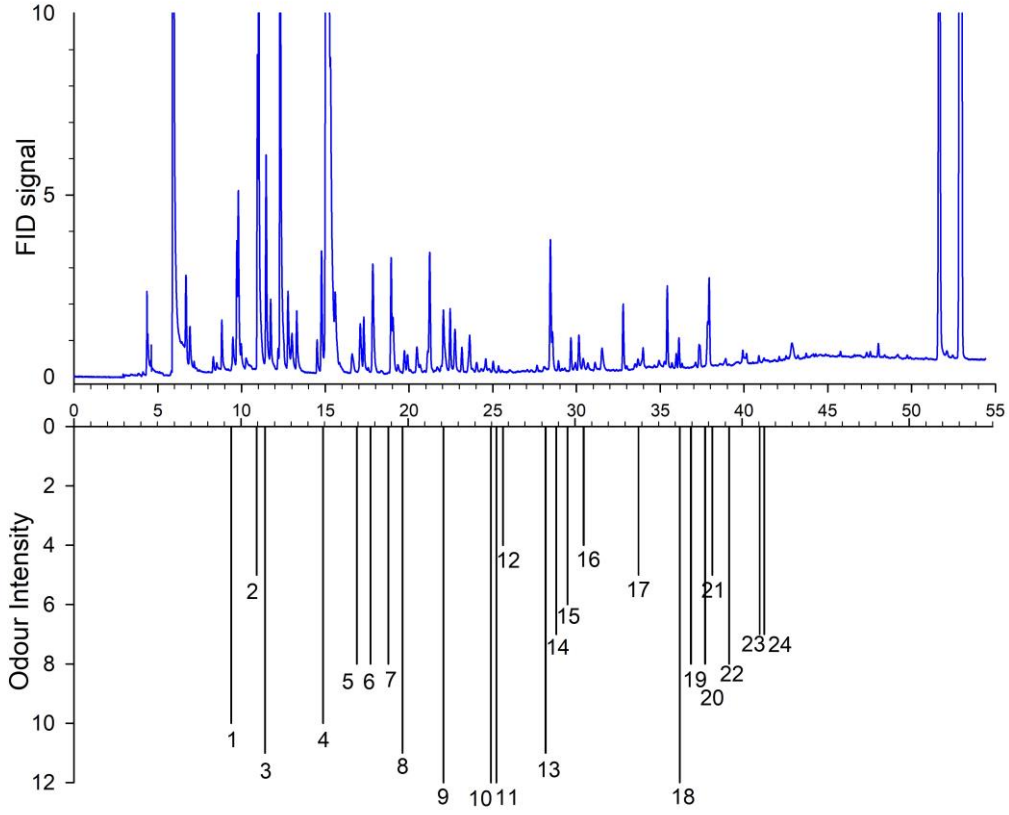


Fig. 1. Aromagram of dry cured loins obtained by GC-FID-O using the detection frequency method (Data from Chapter 1).

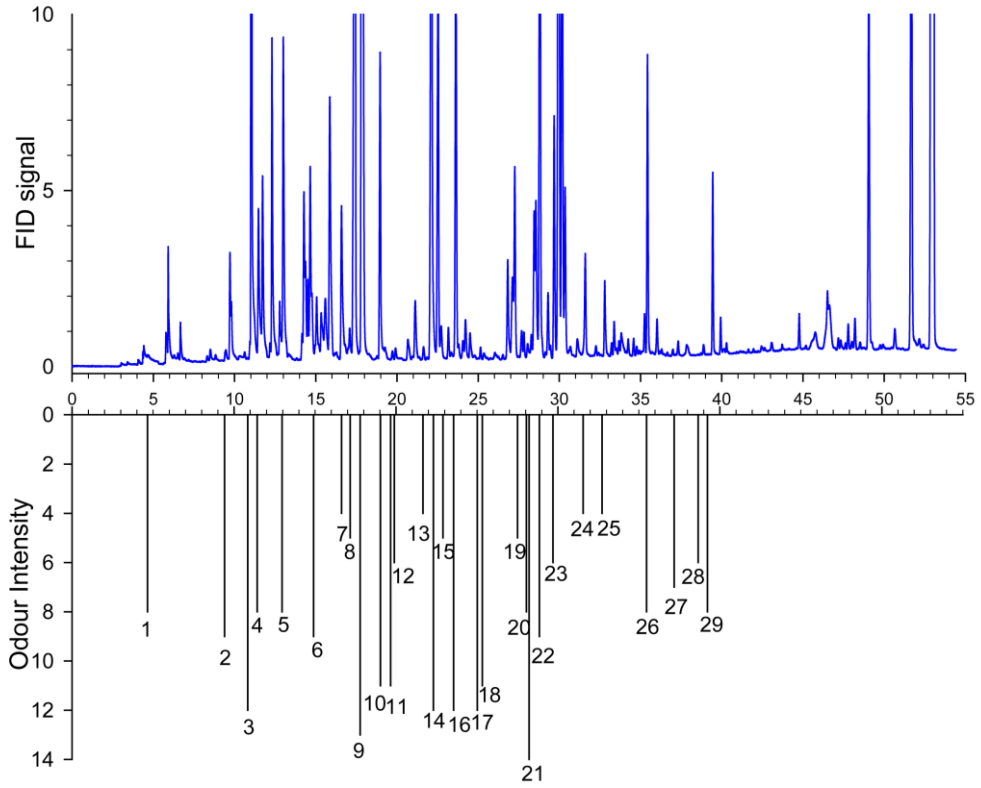


Fig. 2. Aromagram of dry fermented sausages obtained by GC-FID-O using the detection frequency method (Data from Chapter 2).

The abundance of compounds in the headspace of the two products does not provide an accurate quantitation, which is highly affected by matrix composition (Muriel, Antequera, Petrón, Martin, & Ruiz, 2004). However, this measurement technique allowed to determine the effect of precursor addition. Moreover, the impossibility to identify some of the toasted and meaty aromas detected in the products (unknown compounds, Table 4 in Chapter 1 and Table 2 in Chapter 2, respectively) directed this study to the development of an accurate quantification method using SPME-GC-MS and selected ion monitoring (SIM) mode (Chapter 3). The quantification of the compounds was expressed as ng of volatile compounds/g of meat products in dry matter (Chapter 3). In order to determine the effect

of matrix composition, the quantification was performed using a calibration curve obtained in each matrix (fresh loin and fresh sausage batter). A comparison of savory and toasted aroma compounds content in both products is shown in Chapter 3. The content of some compounds ranged from ppb to ppm levels, as in case of methional. This optimized quantitation process allowed the quantitation of DMDS and DMTS in loins, and MFT, DMS, DMTS, and MMFDS in sausages. In this comparison, the matrix effect was taken into account, thus allowing the measurement of the real compound content in the products. The greater concentration of MMTF in loins when thiamine was added, confirmed the origin of the compound and the effect of thiamine supplementation. Furthermore, the higher content of 2AP and 2-acetylpyrrole in loins after Pro addition confirmed its effect on the production of toasted volatile compounds as reported in Chapter 3.

In order to link the results from Chapters 1, 2 and 3, a Principal Components Analysis (PCA) was plotted to evaluate the relationships among formulations, physicochemical and microbiological parameters, and the concentration of savory and toasted aromas (Fig. 4) in dry cured loins and dry fermented sausages, at the end of the ripening process. Two principal components were able to explain 89.44% of the total variability observed. PC1 accounted for 81.3% of the variance and distinguished formulations placing loins on the right quadrant and sausages on the left quadrant, whereas PC2 accounted for 8.13% of the variance. The results of the PCA are consistent with the hydrophobicity values (Log $P_{o/w}$) of the compounds (Table 1 in Chapter 3). Compounds MFT, DMDS, and MMFDS, showing the higher Log $P_{o/w}$ values (1.941, 1.77, and 2.278 respectively), grouped with the variable fat, which was highest in sausages. In contrast, pH, water activity (a_w), moisture content, and protein content grouped with the loins (Fig. 4). Taking into account that sausages are fermented after inoculation of a commercial starter culture (TRADI-302) composed by *Lactobacillus sakei*, *Staphylococcus carnosus,* and *Staphylococcus xlyosus*, higher counts of TMB and LAB were detected in sausages than in loins (Fig. 4). On the contrary, the low GC+ counts in sausages may be related to the acid pH, whereas GC+ counts were higher in loins with higher pH (Danilović & Savić, 2017). Recent studies have shown that the

presence of "popcorn" aroma, identified as 2AP, can be related to *D. hansenii* inoculation in fermented sausages (Corral, Leitner, Siegmund, & Flores, 2016). However, inoculation of yeast strain *D. hansenii* L1 on the surface of sausages (Chapter 2), did not result in the formation 2AP, which was not found in sausages (Chapters 2 and 3). This result can be explained taking into account that 2AP formation in fermented sausages can have different origins, such degradation of Pro or Orn by microbial metabolism, or throughout a Maillard reaction (Schieberle, 1990). Moreover, the microbial origin of 2AP in fermented sausages has been related to the presence of *Penicillium nalgiovense* (Stahnke, 2000). The relationship between inoculation of yeasts and molds and formation of 2AP in sausages needs to be further investigated. Additionally, Jost et al. (2019) found that 2AP can easily be produced at higher pH and temperature through chemical reaction, which may be the cause of 2AP presence in loins showing higher pH and cured at mild temperature conditions (Fig. 2 in Chapter 3).

The main conclusion from the results obtained in Chapters 1, 2 and 3 is that the concentration of precursors in meat products is a key factor in promoting the formation of savory and toasted aroma compounds. However, the concentration of the precursors in the final product varied considerably (Fig. 5), although each precursor was added at the same concentration in the fresh meat (loin) or meat batter (sausage). The meat matrix of loins and sausages is essentially different due to the fat content (5.3 to 7.0% in loins and 28.1-30.3% in sausages). However, at similar concentrations of methionine in loins and sausages (Fig. 5), the content of the savory aromas resulting from methionine degradation differed significantly in loins and sausages (Chapter 3). The reason for this differences might be the different matrix composition of loins and sausages, which promotes different stability of these savory compounds (Cerny, 2015) due to the disparity in redox balance. Regarding toasted aromas, the concentration of Pro and Orn in the supplemented loins was already significantly different from the other loins (Fig. 5), but the abundance (AU × 10^6) of 2AP and 2-acetylpyrrole was no significantly different (Chapter 1). However, using the optimized quantitation technique (Chapter 3) a significant difference in concentration (ng/g

of meat products in dry matter) of 2AP and 2-acetylpyrrole was detected among loins. Therefore, the amount of precursors added affects the formation of toasted compounds. Nevertheless, the addition of Pro and Orn into the sausage batter deserves further study to confirm their mechanism of origin as their presence in loins mainly suggests a chemical origin.

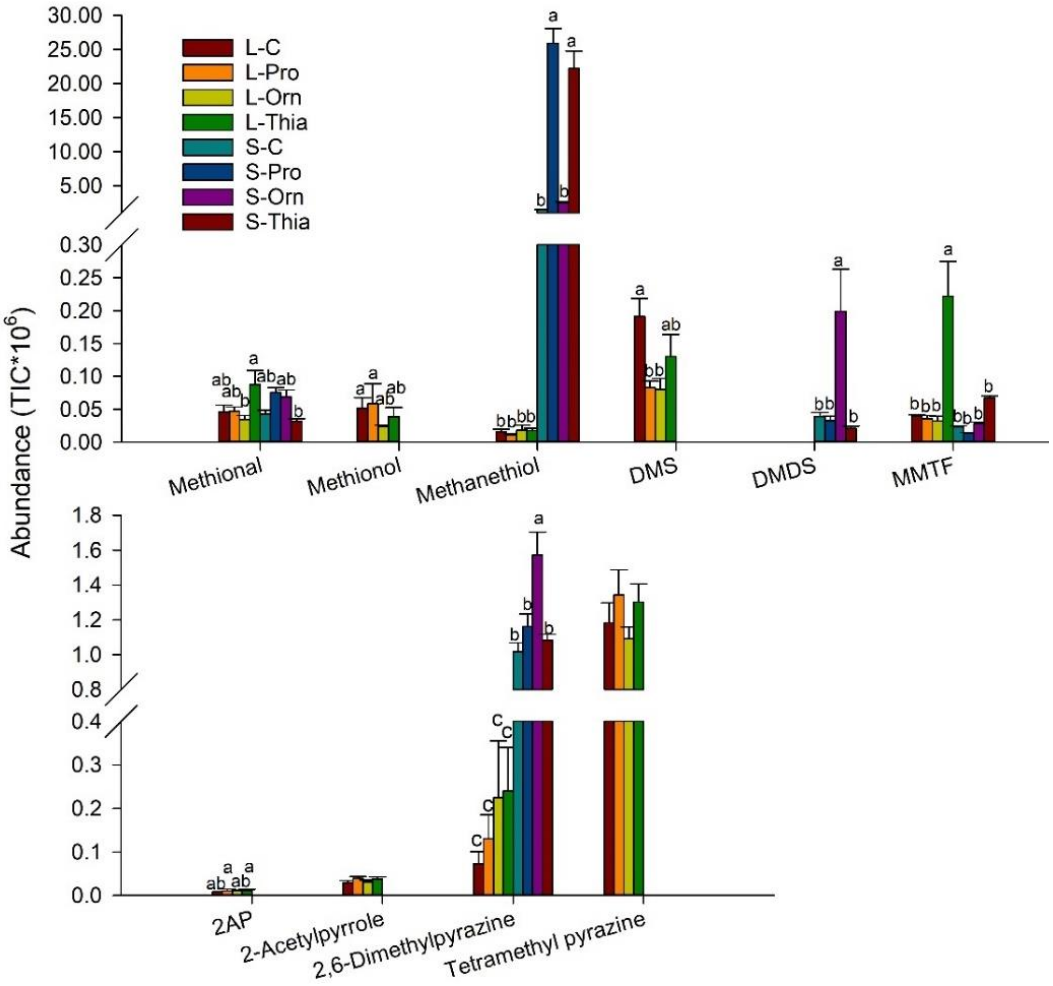


Fig. 3. Abundance of savory and toasted aroma compounds (TIC× 10^6) in the headspace of dry cured loins (68 d product L-Chapter 1) and dry fermented sausages (62 d product S-Chapter 2) manufactured with different aroma precursors (C: control, Pro and Orn: proline and ornithine at 120 mg/100 g, and Thia: thiamine at 24 mg/100 g). Data are expressed as means ± SE and the different letters indicate significant differences at $P < 0.05$.

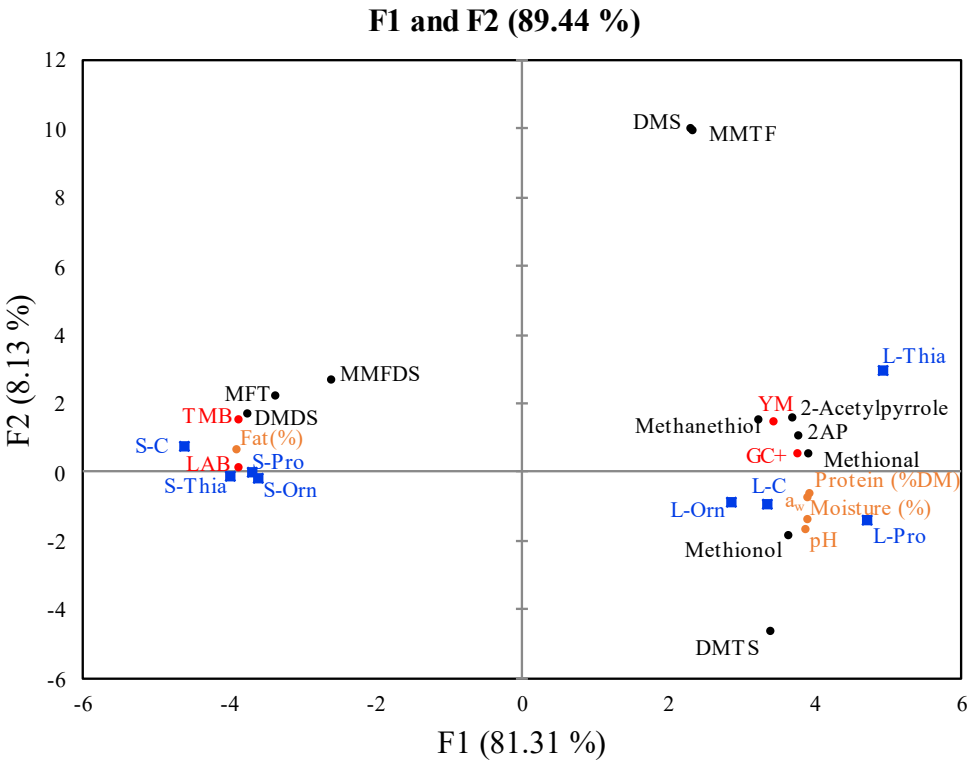


Fig. 4. Loadings of the first two principal components (PC1 and PC2) grouping physic-chemical and microbiology variables, as well as savory and toasted aroma compounds (Chapter 3) in dry cured loins (at 68 d L-Chapter 1) and dry fermented sausages (at 62 d S-Chapter 2) manufactured with different aroma precursors (C: Control, Pro and Orn: proline and ornithine at 120 mg/100 g, and Thia: thiamine at 24 mg/100 g). TMB (Total mesophilic bacteria), LAB (lactic acid bacteria), GC+ (Gram positive cocci), YM (yeasts and molds).

Thiamine content in the loins and sausages supplemented with this vitamin was also significantly different from the no supplemented products (Fig. 5). MMTF originating from thiamine decomposition (Cerny, 2007) was detected in very low abundance (Chapter 1) and concentration (Chapter 3) in loins and sausages. This could be due to the low amount of thiamine added, but also could be related to the chemical reactivity of MMTF (Mottram, 1998). It has been reported that the concentration of MMTF increased gradually with the addition of thiamine in cooked hams (Thomas et al., 2015), and the same was observed for MFT (Thomas et al., 2014; Thomas et al., 2015). In this Ph.D. project, thiamine at a

concentration of 24 mg thiamine/100 g supplemented to both products, confirmed the formation of MMTF in loins and sausages, although in the later the quantitation of MMTF did not confirm the effect (Chapter 3). Furthermore, MMTF was detected by GC-O as responsible for the meaty odor in loins (Chapter 1); however, no correlation was found between the concentration of thiamine addition and MFT, which was only quantified in sausages (Fig. 2 in Chapter 3). These results indicate that increasing thiamine supplementation in dry cured meat products to increase the concentration of MFT deserves further investigation, although MFT has been identified as responsible for the fatty aroma in these products (Perea-Sanz, López-Díez, Belloch, & Flores, 2020).

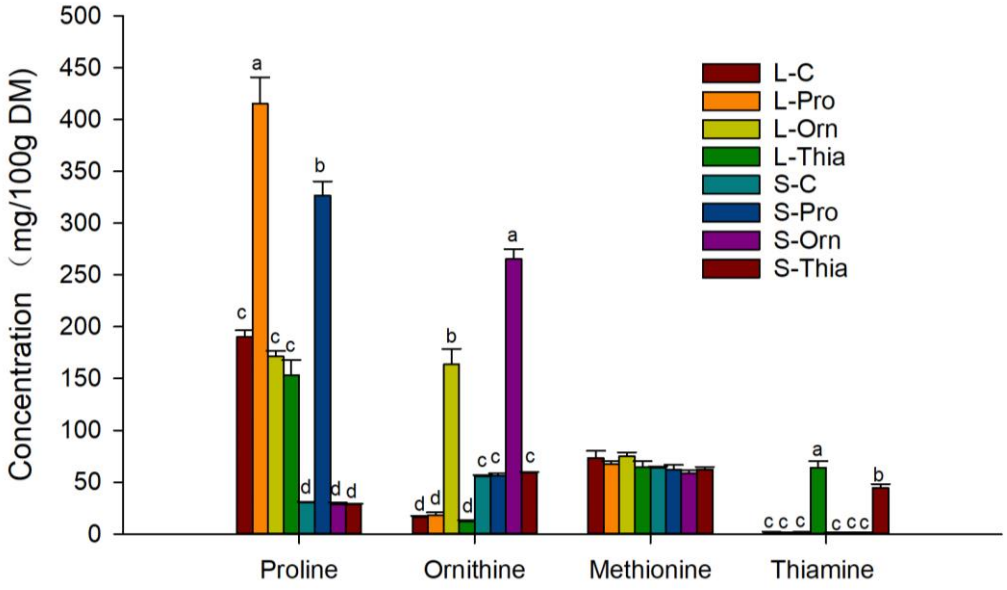


Fig. 5. Content of savory and toasted aroma precursors measured in dry cured loins (at 68 d L-Chapter 1) and dry fermented sausages (at 62 d S-Chapter 2) at the end of process in the supplemented products. (C: control, supplemented with Pro and Orn: proline and ornithine at 120 mg/100 g, and Thia: thiamine at 24 mg/100 g). Data are expressed as means ± SE and the different letters indicate significant differences at $P < 0.05$.

The sensory analysis applied (FCP) was able to distinguish between samples (Corral, Salvador, & Flores, 2016) and confirmed the effect of the supplementation in loin and sausages. Supplementation with Pro in loins was related to pepper, toasted, and mold

descriptors, while supplementation with thiamine imparted cured and savory descriptors in both products, loins (Chapter 1) and sausages (Chapter 2). The aroma contribution of savory and toasted compounds was evaluated by the OAV calculated. This calculation obtained from the concentration of compounds (Chapter 3) revealed that the aroma of dry cured loins was greatly impacted by the presence of methional and 2AP, while the aroma of sausages was essentially modulated by MFT, methional, and MMFDS (Fig. 6). These results confirm at least partially the results obtained by the sensory panel using the FCP methodology (Chapter 1 and 2). The aroma profile of the loins supplemented with Pro was probably related to the presence and concentration of 2AP, although 2AP was not detected in sausages probably due to their extremely low levels. Moreover, the presence of MMTF verified the effect of thiamine supplementation in loins, but its impact on the cured and savory attributes was not confirmed by OAV.

The complexity of the meat products and the reactions involved in the generation of savory and toasted odors made difficult reaching clear conclusions. Therefore, different model systems simulating the meat products processing were used for this purpose. The first models simulated different stages in the processing of fermented sausages in terms of free amino acid composition, pH and a_w conditions employed. The models were used to investigate the mechanisms of amino acid conversion to savory and toasted compounds in dry cured meat products (Chapter 4) via the Maillard reaction in presence of glucose at the mild temperatures used during processing (25 °C and 37 °C). Three models were designed to mimic the stages of the process in terms of amino acid composition (initial (I), 1st drying (1D) and 2nd drying (2D). The second model system (Chapter 5) was designed to investigate the biotransformation of amino acids and the influence of the oxidation process and yeast metabolism in the presence of pork myofibrillar proteins. In this case, models formulated with different fat types (PL-pork lard and VO-coconut oil) and inoculated with *D. hansenii*, but containing the amino acid composition and additives simulating the final drying stage of fermented sausages, were assayed. The models studied in Chapter 4 and Chapter 5, were different based on their composition but the results can be compared among models.

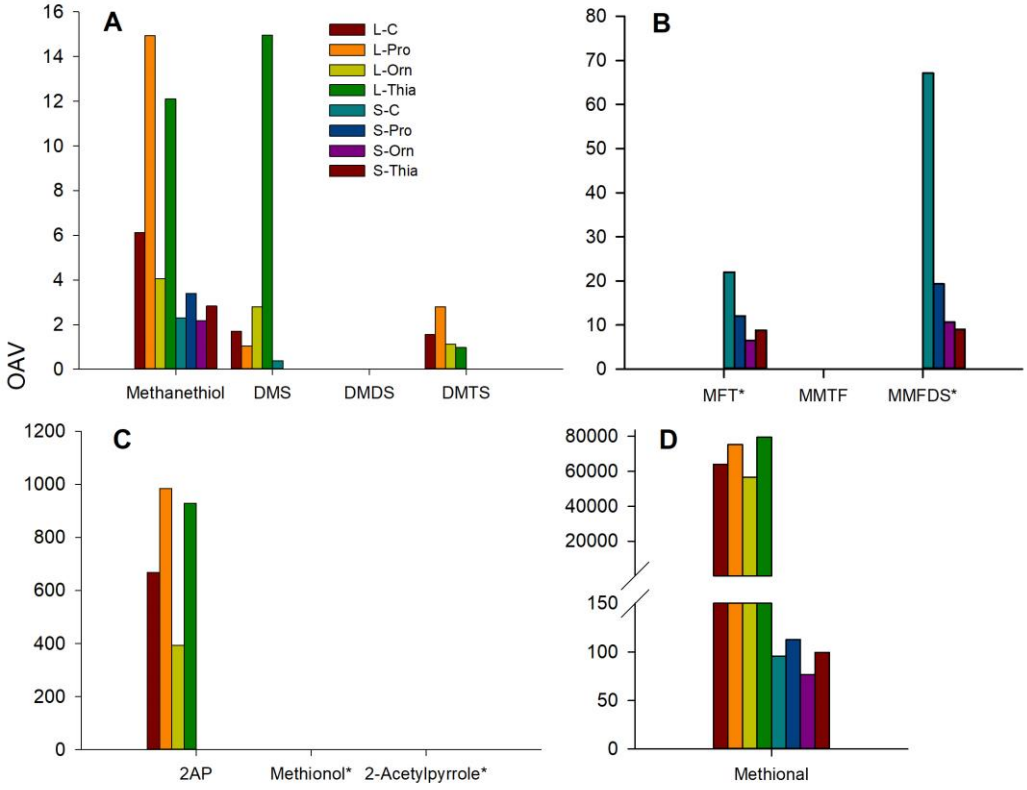


Fig. 6. Odor activity values (OAV) of nitrogen and sulfur compounds quantified (Chapter 3) in L-dry cured loins at 62 d and S-dry fermented sausages at 62 d manufactured with different aroma precursors (C: control, Pro and Orn: proline and ornithine at 120 mg/100 g, and Thia: thiamine at 24 mg/100 g). Values were calculated based on odor thresholds in oil, except for MMFDS, methionol and 2-acetylpyrrole where values in water were used (Van Gemert, 2011).

A Venn diagram was generated with the web tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) using the data from Chapters 4 and 5. The Venn diagram identified similarities and differences between samples (Feng et al., 2021). The Venn diagram in Fig. 7A shows the similarities and differences regarding the volatile compounds found in models simulating the final drying stage (2D-37 °C, Chapter 4) and the Control model (C, Chapter 5). Additionally, Fig. 7B shows the volatile profile of the models in Chapter 5: C: Control meat model system; PL and VO: Pork lard and Coconut oil meat

model systems; PLY and VOY: Pork lard and Coconut oil meat model systems inoculated with yeast. Different colors represent different groups, while the numbers in them represent the number of volatile compounds common or different between the models. Five compounds (furfural, methylpyrazine, thiazole, furan, and 2-butanone) were only detected in model system 2D-37 °C (Chapter 4), while 13 compounds were present in models 2D-37 °C (Chapter 4) and Control (C, Chapter 5) (Fig. 7A). This indicates that many compounds (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional, benzaldehyde and benzeneacetaldehyde) derived from amino acid degradation reactions are present in both models. This would indicate that the Maillard reaction is the principal cause of volatile generation, and the main factors affecting volatile production from free amino acids are the temperature conditions, followed by amino acid concentration and a_w, as was concluded from results in Chapter 4. In addition to these factors, the effect of the oxidation process and biotransformation of amino acids by yeasts should be also considered, as reported in Chapter 5.

The comparison of the five models in Chapter 5 (Fig. 7B) showed that many of the volatile compounds were present in all models (36 compounds). However, differences observed in the number of volatile compounds may explain the effect of the oxidation process (fat type) or *D. hansenii* activity. In Chapter 5, branched-chain aldehydes, benzaldehyde and methional content were enhanced by pork lard's high concentration due to the carbonyl compounds derived from lipid peroxidation. Consequently, in pork lard models (PL and PLY) aroma was impacted by methional and 3-methylbutanal, as both compounds derive from methionine and leucine degradation, respectively (Corral, Leitner, et al., 2016). In *D. hansenii* inoculated models, a delay in the formation of hexanal was observed in models containing coconut oil, whereas a delay in heptanal, octanal, and nonanal formation was observed in models containing pork lard, which confirmed the antioxidant action of *D. hansenii*. The presence of compounds such as 3-methylbutanoic and butanoic acids, as well as phenylethyl alcohol, which contributed to the cheesy and flowery odor notes, was enhanced by yeast inoculation in pork lard models (PLY).

However, in models inoculated with *D. hansenii* only methional as savory aroma compound was detected. Different reasons may have affected the low generation of savory compounds in the models, such as the low content of precursors like thiamine (not in the formulation of the models), and the absence of other starter cultures (LAB and CN-Stp), which modulate amino acid degradation reactions. On the contrary, in similar models containing high amounts of methionine and cysteine and inoculated with different *D. hansenii* strains (Perea-Sanz, Peris, Belloch, & Flores, 2019), savory (sulfur compound) aromas have been found. Moreover, 2AP responsible for the toasted aroma was not detected in models inoculated with *D. hansenii*. This result was probably due to the low precursor (Pro and Orn) content, and lack of chemical and biochemical mechanisms responsible for 2AP formation (Schieberle, 1990). Therefore, the inoculation of *D. hansenii* to promote the formation of savory and toasted aromas requires further research; although, the antioxidant and amino acid converting effects of *D. hansenii* was revealed in the model systems.

In summary, the Ph.D. project has shown that addition of aroma precursors (Pro, Orn, and thiamine) in the manufacture of dry cured loins and sausages may be an alternative to enhance the sensory characteristics and, especially, the savory and toasted aroma of these meat products. Moreover, the mechanisms of formation of these compounds depend highly on the chemical and biochemical reactions that take part in the meat matrix during the processing.

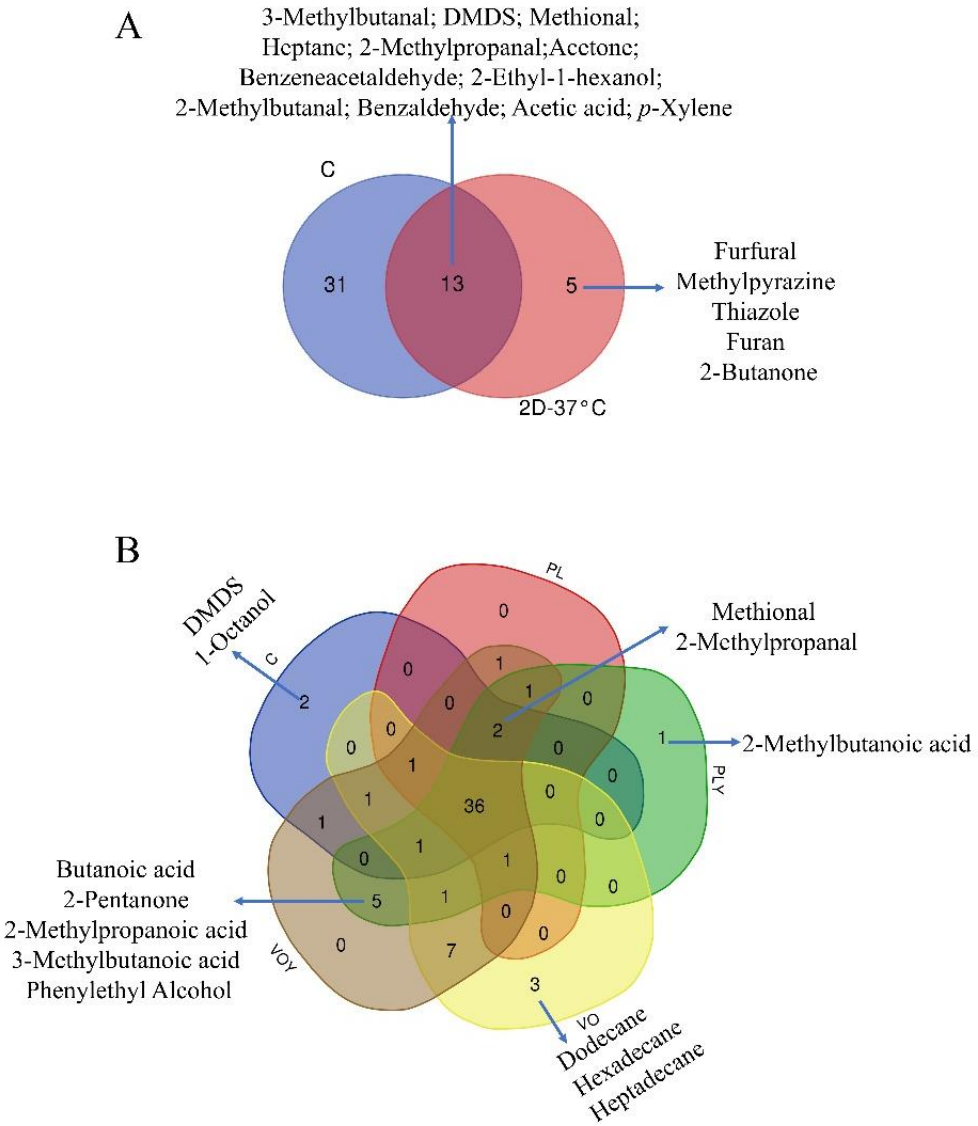


Fig. 7. Venn diagram showing the similarities and differences of volatile compounds in the different meat model systems. (A) Similarities and differences of volatile compounds between meat model systems in Chapter 5 (C: Control meat model system without inoculated yeast) and Chapter 4 (2D-2nd drying stage at 37 °C). (B) Similarities and differences of volatile compounds between meat model systems in Chapter 5 (C: Control meat model system; PL and VO: Pork lard and Coconut oil meat model systems; PLY and VOY: Pork lard and Coconut oil meat model systems with inoculated yeast)

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CONCLUSIONS

CONCLUSIONS

1. The contribution of nitrogen precursors (Pro and Orn) to the generation of toasted odors was confirmed in dry cured loins by the presence of 2-acetylpyrrole and 2-acetyl-1-pyrroline. In addition, other compounds contributing to toasted scents like tetramethyl pyrazine were found in loins, as well as 2,6-dimethyl pyrazine in both, loins and sausages.
2. The effect of thiamine supplementation on the aroma of dry cured loins and dry fermented sausages was confirmed by the presence of sulfur derived compounds 2-methyl-3-(methylthio)furan producing savory odors. In addition, other sulfur compounds, 2-methyl-3-furanthiol and methyl 2-methyl-3-furyl disulfide, were detected in sausages.
3. Methionine degradation contributed to the generation of savory scents derived from sulfur compounds, methional, methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide in loins and sausages, whereas methionol was exclusively found in loins.
4. The content of savory and toasted compounds at ppb levels except for methional, which was found at ppm levels, together with their physical properties and the meat matrix composition, explain the differences in savory and toasted aroma perception in dry meat products (loins and sausages).
5. Meat model systems, used to simulate the conditions of dry cured processing, show that at mild temperatures free amino acids are substrates of the Maillard reactions. However, oxidation reactions (fat type) and *D. hansenii* inoculation modify the volatile profile and consequently model aroma. Moreover, water activity (a_w) plays an important role promoting the formation of savory aroma compounds.
6. The mechanisms of formation of savory and toasted aroma compounds in dry cured loins and fermented sausages are related to reactions such as lipid oxidation, Maillard reactions and thiamine degradation, although microbial metabolism of molds and yeasts

in fermented sausages and on the loin's surface should be considered. In fermented sausages, the contribution of the microbial starters to the development of savory and toasted aromas seems to have a higher effect than the addition of precursors.

ANNEX 1. PUBLICATIONS

Authorisation for publication in the Doctoral Thesis.

Aroma enhancement in dry cured loins by the addition of nitrogen and sulfur precursors

Author: Lei Li,Laura Perea-Sanz,José Javier López-Díez,Ana Salvador,Carmela Belloch,Mónica Flores

Publication: Meat Science

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Aroma enhancement in dry cured loins by the addition of nitrogen and sulfur precursors

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ARTICLE INFO

Keywords:
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ABSTRACT

Dry cured loins containing nitrogen (proline and ornithine) and sulfur (thiamine) compounds as precursors of aroma compounds at two concentration levels were manufactured. The effect of precursor addition on the microbiology and chemical parameters of loins was studied together with the aroma study performed by olfactometry and Free Choice Profile sensory analyses. Addition of precursors did not affect the microbial and chemical parameters, while aroma was affected when precursors were added at the highest level. The dry loin aroma profile was mainly composed by compounds 3-methylbutanal, methional, ethyl 3-methylbutanoate, 3-methylbutanoic acid, 1-octen-3-ol, 2-acetyl-1-pyrroline and 2-acetylpyrrole that contribute to musty, cooked potatoes, fruity, cheesy, mushroom, roasted and meaty odor notes. Proline and ornithine supplementation modified the loins aroma profile producing toasted odors, while the effect of thiamine supplementation on the aroma was revealed by the presence of sulfur derived compounds (methional and 2-methyl-3-(methylthio)furan) that contribute to the "cured meat odor".

1. Introduction

Dry cured loins are made using the whole or caudal half of the *Longissimus thoracis et lumborum* muscle of all animals. Pork loins are rubbed by a mixture of curing agents (salt, nitrite and nitrate) and several spices (paprika and garlic) onto the surface of the loin pieces, subsequently stuffed into natural casings and ripened-dried for several months until the product achieves a specific aroma, texture and taste (Muriel, Ruiz, Martin, Petron, & Antequera, 2004; Ventanas, Estevez, Tejeda, & Ruiz, 2006). Their sensory properties are highly affected by genetic factors, feeding practices and ingredients used during product manufacture (Ventanas, Ventanas, Estévez, & Ruiz, 2010) while the length of the dry cured process is essential for aroma development (Ventanas, Estevez, Andrés, & Ruiz, 2008).

The reduction of the use of curing agents in meat product formulations as well as the purpose to reduce the time for processing has directed to the search for alternatives to enhance the dry cured aroma. However, aroma development in dry cured products is based on complex biochemical reactions occurring during ripening that result in a large number of volatile compounds which contribute to their characteristic flavor (Flores, 2018). Studies in dry cured loin flavor have elucidated the volatile profile, but little information about its aroma contribution is available (Belloch, Neef, Salafia, López-Díez, & Flores, 2021; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, & Ruiz, 2004; Ventanas et al., 2010). Most of the identified volatile compounds are derived from garlic and paprika together with other compounds derived from microbial activity such as alcohols and ethyl esters (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004). However, differences in volatile compounds between the surface and within the loin revealed the presence of oxidation and Strecker degradation volatile compounds on the loin surface together with volatile compounds derived from yeasts and moulds metabolism (Martín, Córdoba, Benito, Aranda, & Asensio, 2003; Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004; Muriel, Antequera, Petrón, Martin, & Ruiz, 2004). Although dry cured process is performed at mild temperatures, Maillard reactions between nitrogen compounds from proteolysis and lipid oxidation products have been attributed as one of the main chemical reactions contributing to the aroma profile of dry cured meats (Li, Belloch, & Flores, 2021; Ventanas et al., 1992).

The presence of nitrogen- and sulfur-containing compounds in dry cured meat products point to their important role in meat product flavor because of their olfactory properties and low odor thresholds (Flores,

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Understanding the impact of nitrogen and sulfur precursors on the aroma of dry fermented sausages

Author: Lei Li,Laura Perea-Sanz,Ana Salvador,Carmela Belloch,Mónica Flores

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Understanding the impact of nitrogen and sulfur precursors on the aroma of dry fermented sausages

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ARTICLE INFO

Keywords:
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ABSTRACT

The goal of this study was to confirm and acquire more information about the nitrogen and sulfur compounds existing in the volatile profile of dry fermented sausages from the addition of precursors (proline, ornithine and thiamine), and their role in sausage aroma. To this end, the precursors were added to the formulation of sausages, which were submitted to a fermentation and drying process. The sausage aroma was analyzed by olfactometry technique and Free Choice Profile sensory analysis. The results showed that the addition of precursors impacted the aroma, and reduced the level of oxidation in the final sausages while microbial differences were mainly observed in Orn-sausages. Among the aroma compounds detected only 2-methyl-3-(methylthio)furan verified the effect of thiamine supplementation and the impact on the cured and savoury odours detected in Thia-sausages by Free Choice profile sensory analysis, while no clear effect could be attributed to specific volatile compounds in the nitrogen supplemented sausages.

1. Introduction

The current challenges of the meat industry are aimed at the development of healthier meat products that maintain the desired sensory characteristics. Among these, "savoury" (meaty) flavours are highly demanded in meat products, in which the generation of aroma and flavour occurs naturally during processing (Flores, Perea-Sanz, López-Díez, & Belloch, 2021). Moreover, savoury aromas are constituted by a complex mixture of compounds producing a specific character and present at part per billion levels and due to their low sensory thresholds they affect meat perception (Cook, Linforth, & Taylor, 2003). Consequently, the control of the factors that favour the development of specific savoury aroma compounds is considered of great interest for the meat industry.

The flavour of dry fermented sausages is due to a large number of microbiological and chemical reactions (Flores & Olivares, 2014; Toldra, Flores, & Sanz, 2001). The formation of aroma in dry fermented sausages occurs mainly throughout the ripening process and the aroma impact of the biochemical reactions depends on the microbial diversity, which is highly affected by the manufacturing conditions. Latest studies trying to correlate bacterial diversity with the development of volatile compounds in dry fermented sausages in Europe and Asia revealed the differences in microbiota and explained their differences in the volatile profile (Flores & Piornos, 2021; Yang et al., 2022). However, many differences in recipes and manufacturing processes produce a wide variety of aroma compounds that may be related to the presence of precursors and bacteria diversity. In contrast, the formation of compounds producing savoury and toasted odours in dry fermented sausages is scarce. Flores (2018) summarized the impact of important aroma compounds in dry fermented sausages, such as sulfur, thiols, thiazoline, thiophene and pyrazine compounds and revealed that they are mainly originated from sulfur amino acids, thiamine and 5ŕibonucleotides as well as some of the sulfide compounds that are derived from the spices used as ingredients.

The main contributors to the savoury and toasted odours are compounds present at very low concentrations like sulfur and nitrogen compounds. While the contribution of the microbial degradation of sulfur amino acids in fermented sausages produces sulfur aroma compounds (Perea-Sanz, Peris, Belloch, & Flores, 2019), the contribution of proline and ornithine released during the process (Flores, Sanz, Spanier, Aristoy, & Toldrá, 1998) is important as both amino acids are precursors of 2-acetyl-1-pyrroline, a potent toasted odour compound identified in Hungarian salami (Söllner & Schieberle, 2009). A previous study in dry cured loin related proline and ornithine concentration to toasted odours (Li et al., 2022) and revealed that in addition to the effect of precursor concentration, other compounds like pyrazines may be related to the

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A comparative study of savory and toasted aromas in dry cured loins versus dry fermented sausages

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A comparative study of savory and toasted aromas in dry cured loins versus dry fermented sausages

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Institute of Agrochemistry and Food Technology (IATA-CSIC), Agustín Escardino Avenue 7, 46980, Paterna (Valencia), Spain

ARTICLE INFO

Keywords:
Savory
Toasted
Aroma
Dry loins
Fermented sausages

ABSTRACT

Dry cured meat products, loins and sausages, have a different cured aroma due to differences in their formulation and manufacture. In these products, savory and toasted notes produced by sulfur and nitrogenated volatile compounds are essential for cured aroma. The interest of the meat industry to modulate the aroma profile and the creation of specific flavourings recognizes the necessity of gaining knowledge about the pathways involved into aroma generation. For this, a comparative study was done in dry cured loins and sausages. The two products were supplemented with either proline, ornithine or thiamine and compared to a control without supplement. The concentration of these volatile compounds was analysed by GC-MS in SIM mode. Eleven volatile compounds were identified, nine in loins and eight in sausages. Loin aroma was impacted by methional and 2-acetyl-1-pyrroline, contributing to cooked potato and toasted notes; meanwhile, sausage aroma was modulated by 2-methyl-3-furanthiol, methyl 2-methyl-3-furyl disulfide, producing fatty and meaty odors, and methional. The contribution of the microbial starters to the development of sausage aroma seems to have a higher effect than the addition of precursors. In loins, thiamine addition produced the highest concentration of 2-methyl-3-(methylthio)furan, while 2-acetyl-1-pyrroline and 2-acetylpyrrole increased in the proline supplemented loins.

1. Introduction

Traditionally cured meat products are highly appreciated due to their distinct aroma and flavor caused by variations in their manufacture and formulation (Flores, 2017). In this sense, two of the most representative dry cured meat products in the Mediterranean region, dry cured loins and dry fermented sausages, are characterized by rather different cured aroma profile. Despite the interest in these "cured" odor notes, little research has been paid regarding the factors influencing the formation of meaty (savory) and toasted aromas in dry cured meat products.

The toasted and savory aromas are thought to be mostly caused by nitrogen and sulfur molecules, which produce a significant impact on the global meat aroma in dry cured meat products (Flores, Perea-Sanz, López-Díez, & Belloch, 2021). The identification and quantification of these compounds in meat products at trace levels remains a challenge, and it is crucial for the development of "savory" aromas. Among the precursors of these odors, amino acids containing sulfur atoms like cysteine, cystine and methionine, as well as the vitamin thiamine, have been found crucial precursors of odor notes "roasted" or "cooked meaty" in cooked meat products (Güntert et al., 1990; Meynier & Mottram, 1995; Thomas, Mercier, Tournayre, Martin, & Berdagué, 2015). Previous studies have indicated that cysteine was the precursor of 2-methyl-3-furanthiol (Meynier & Mottram, 1995; Tang, Jiang, Yuan, & Ho, 2013) , while methionine was the origin of methional, methionol, methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide. These compounds are generated via the Maillard reaction, Strecker degradation and Ehrlich pathway (Perpète, Gijs, & Collin, 2008). Thiamine has a significant impact on the formation of 2-methyl-3-furanthiol, 2-methyl-3-(methylthio)furan and bis(2-methyl-3-furyl) disulfide in cooked ham (Thomas et al., 2015). On the other hand, toasted odor note produced by 2-acetyl-1-pyrroline has been found in cooked lean beef (Specht & Baltes, 1994) and fermented sausages (Stahnke, 2000), although the origin of this compound in meat products is not clear as it may be originated from Maillard or biological reactions (Wei, Handoko, Pather, Methven, & Elmore, 2017).

The impact of these precursors in the generation of aroma in dry cured meat products is not entirely clear. Dry fermented sausages savory aroma is based on the crucial role of free amino acids (Corral, Leitner, Siegmund, & Flores, 2016; Li, Perea-Sanz, Salvador, Belloch, & Flores,

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Open Access Article

The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions

by Lei Li, Carmela Belloch and Mónica Flores *

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The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions

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Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Avda. Agustín Escardino 7, 46980 Paterna, Valencia, Spain; leili@iata.csic.es (L.L.); belloch@iata.csic.es (C.B.)

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Abstract: Flavor is amongst the major personal satisfaction indicators for meat products. The aroma of dry cured meat products is generated under specific conditions such as long ripening periods and mild temperatures. In these conditions, the contribution of Maillard reactions to the generation of the dry cured flavor is unknown. The main purpose of this study was to examine mild curing conditions such as temperature, pH and a_w for the generation of volatile compounds responsible for the cured meat aroma in model systems simulating dry fermented sausages. The different conditions were tested in model systems resembling dry fermented sausages at different stages of production. Three conditions of model system, labeled initial (I), 1st drying (1D) and 2nd drying (2D) and containing different concentrations of amino acid and curing additives, as well as different pH and a_w values, were incubated at different temperatures. Changes in the profile of the volatile compounds were investigated by solid phase microextraction and gas chromatography mass spectrometry (SPME-GS-MS) as well as the amino acid content. Seventeen volatile compounds were identified and quantified in the model systems. A significant production of branched chain volatile compounds, sulfur, furans, pyrazines and heterocyclic volatile compounds were detected in the model systems. At the drying stages, temperature was the main factor affecting volatile production, followed by amino acid concentration and a_w. This research demonstrates that at the mild curing conditions used to produce dry cured meat product volatile compounds are generated via the Maillard reaction from free amino acids. Moreover, in these conditions a_w plays an important role promoting formation of flavor compounds.

Keywords: meat flavor; dry cured; mild conditions

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1. Introduction

Flavor is among the most important quality indicators of meat products. Flavor compounds produced during the processing of meat products are generated through different mechanisms including the Maillard reaction, Strecker degradation, lipid oxidative reactions, degradation of thiamine, ribonucleotides and carbohydrates, as well as microbial metabolism [1,2]. The contribution of these reactions to meat flavor depends on the manufacture conditions applied during processing.

Flavor compounds present in cooked cured meat products have been studied qualitatively and quantitatively [3], the Maillard reaction being the most important reaction for the production of meaty aroma compounds [4]. The Maillard reaction between an amino group and a reducing sugar is commonly divided into three stages: Amadori/Heyns rearrangement, sugar fragmentation and retro-aldolization. In these reactions the free amino group of amino acids peptides or proteins participates in dehydration, fragmentation, cyclization and polymerization reactions. The different paths involved in the Maillard reaction depend on conditions such as temperature, pH and water content that regulate the

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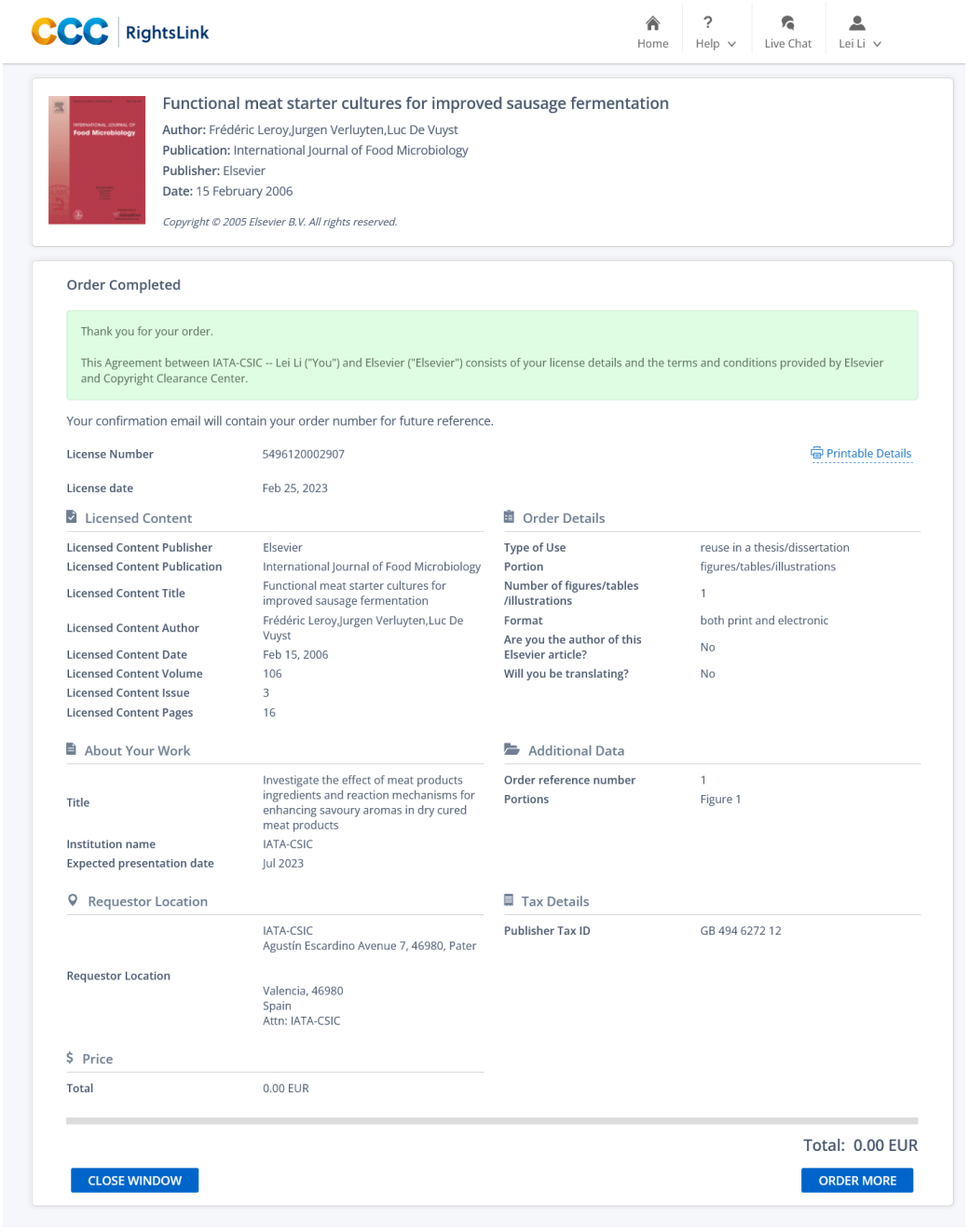

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Functional meat starter cultures for improved sausage fermentation

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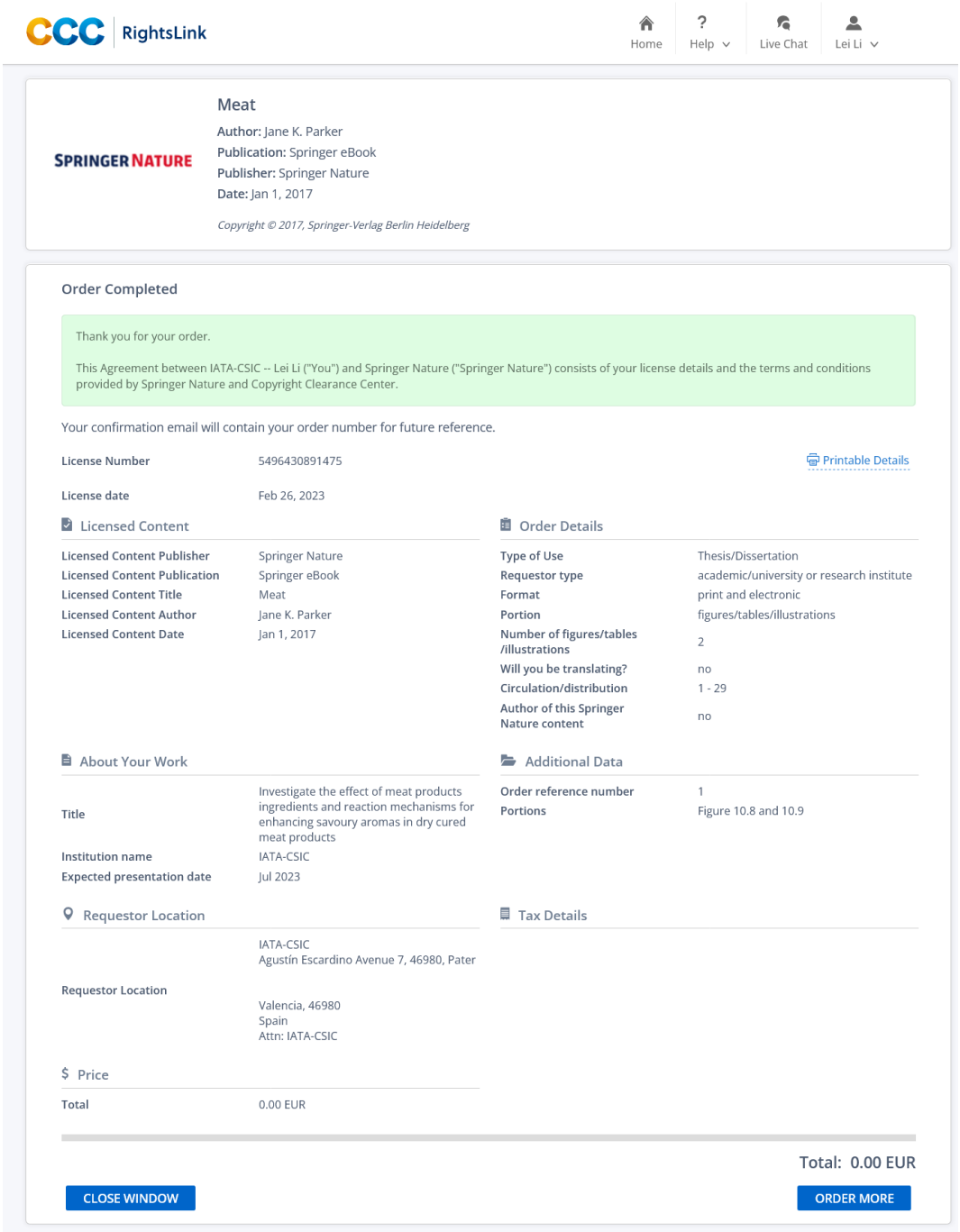

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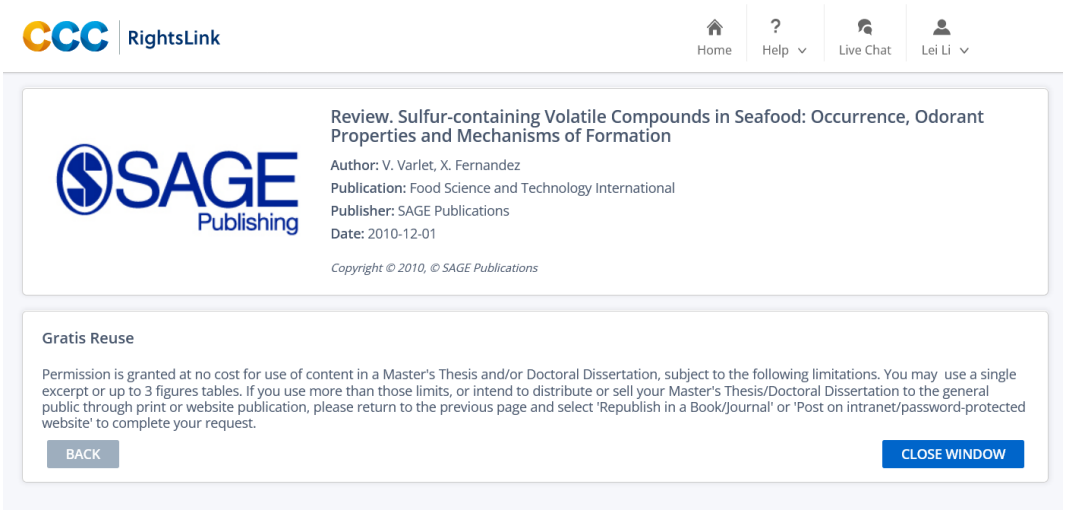

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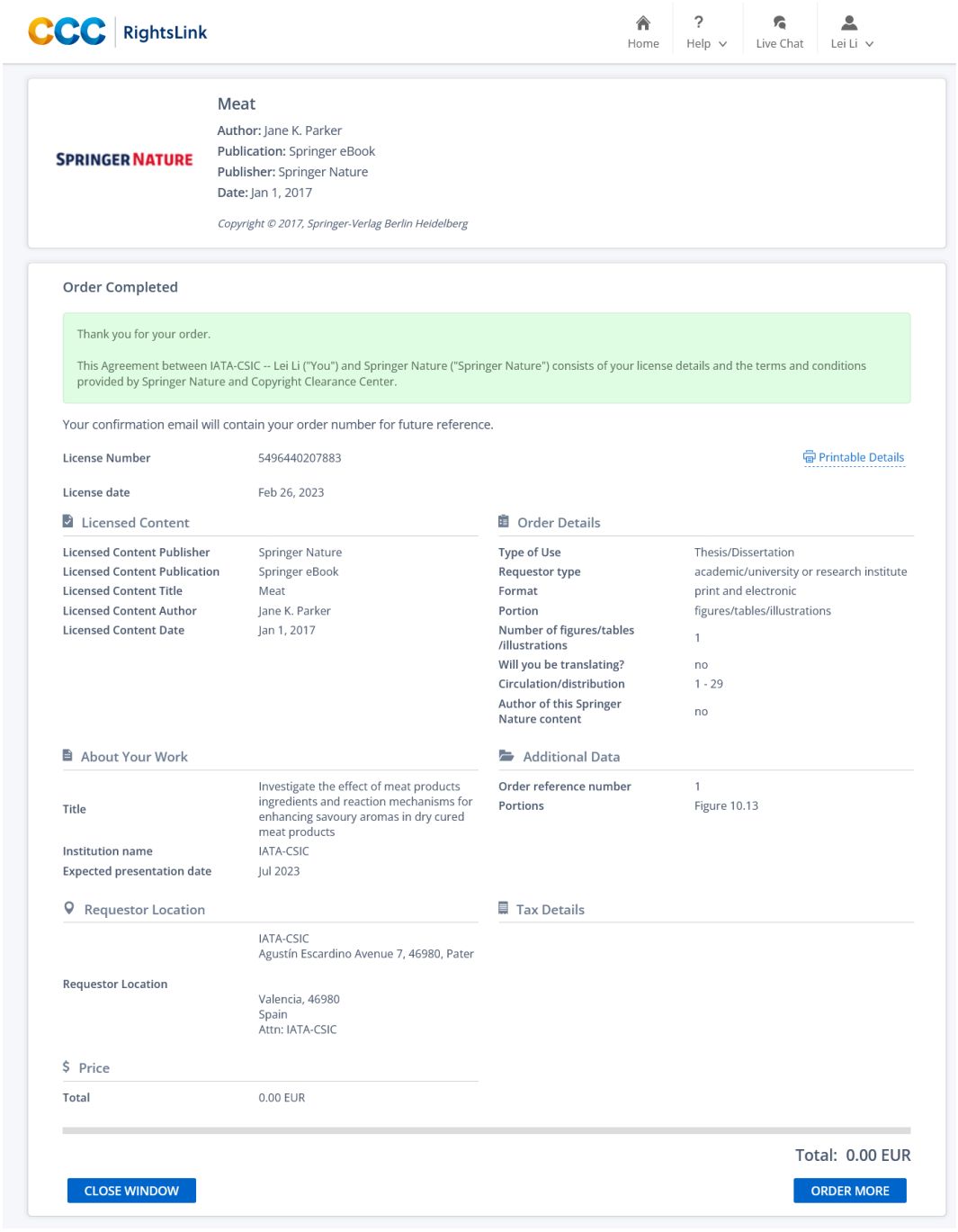

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Meat

Author: Jane K. Parker
Publication: Springer eBook
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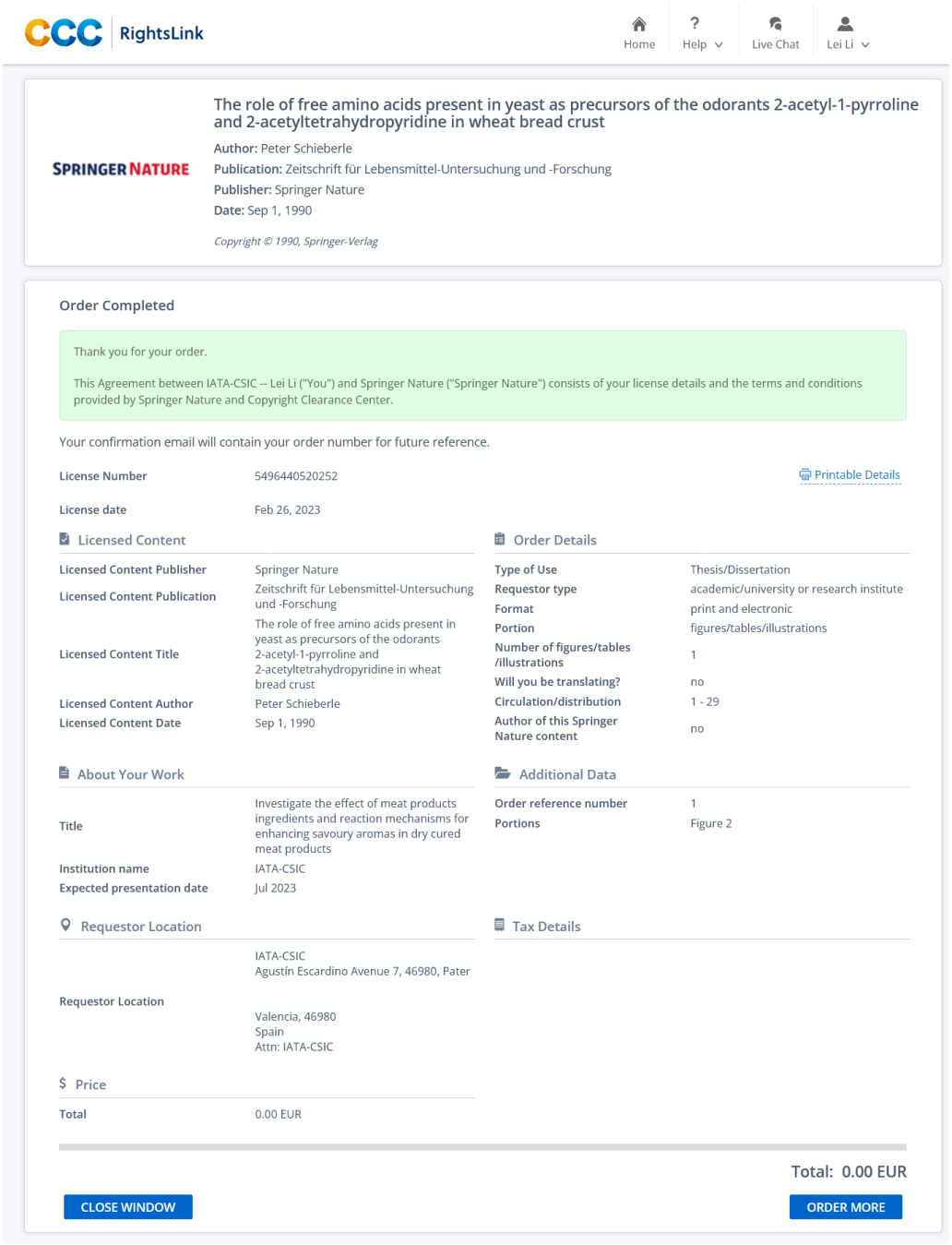

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Author: Peter Schieberle
Publication: Zeitschrift für Lebensmittel-Untersuchung und -Forschung
Publisher: Springer Nature
Date: Sep 1, 1990

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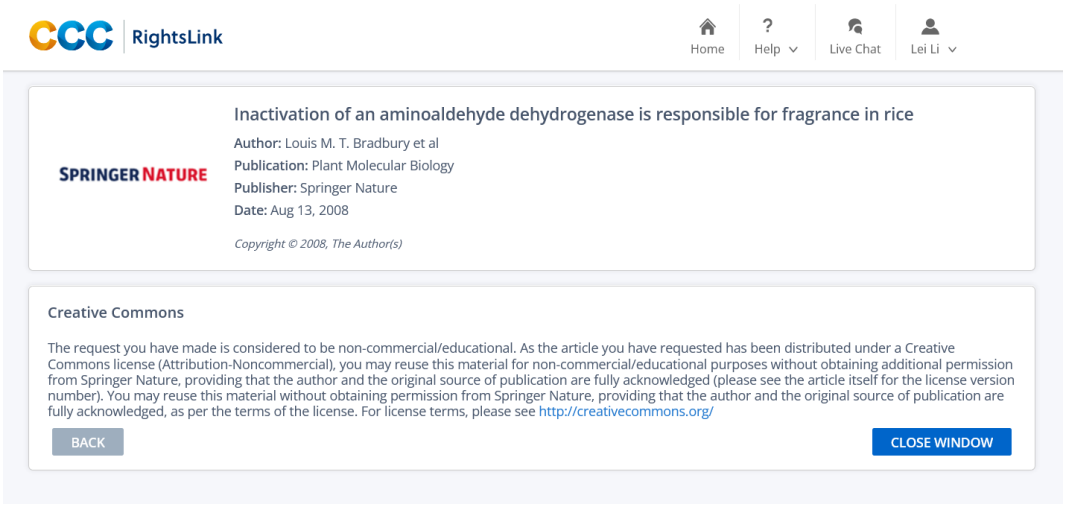

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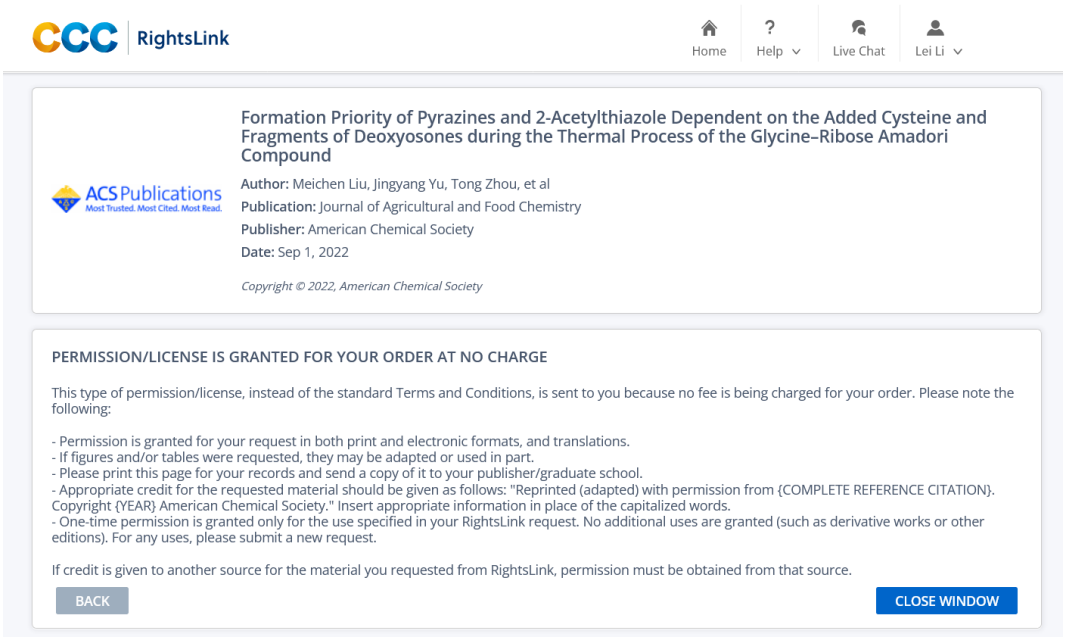

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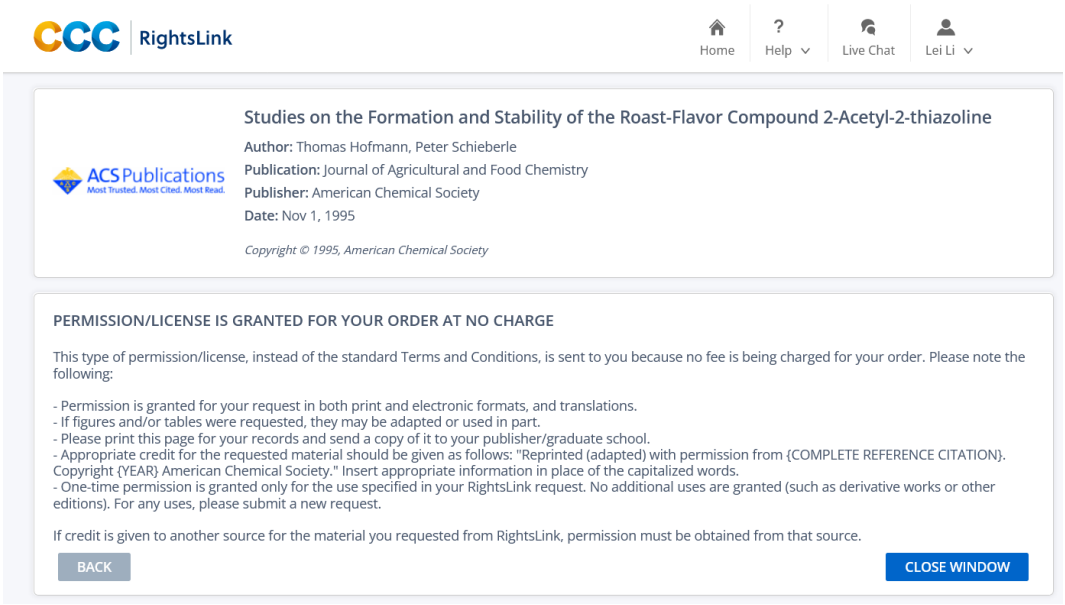

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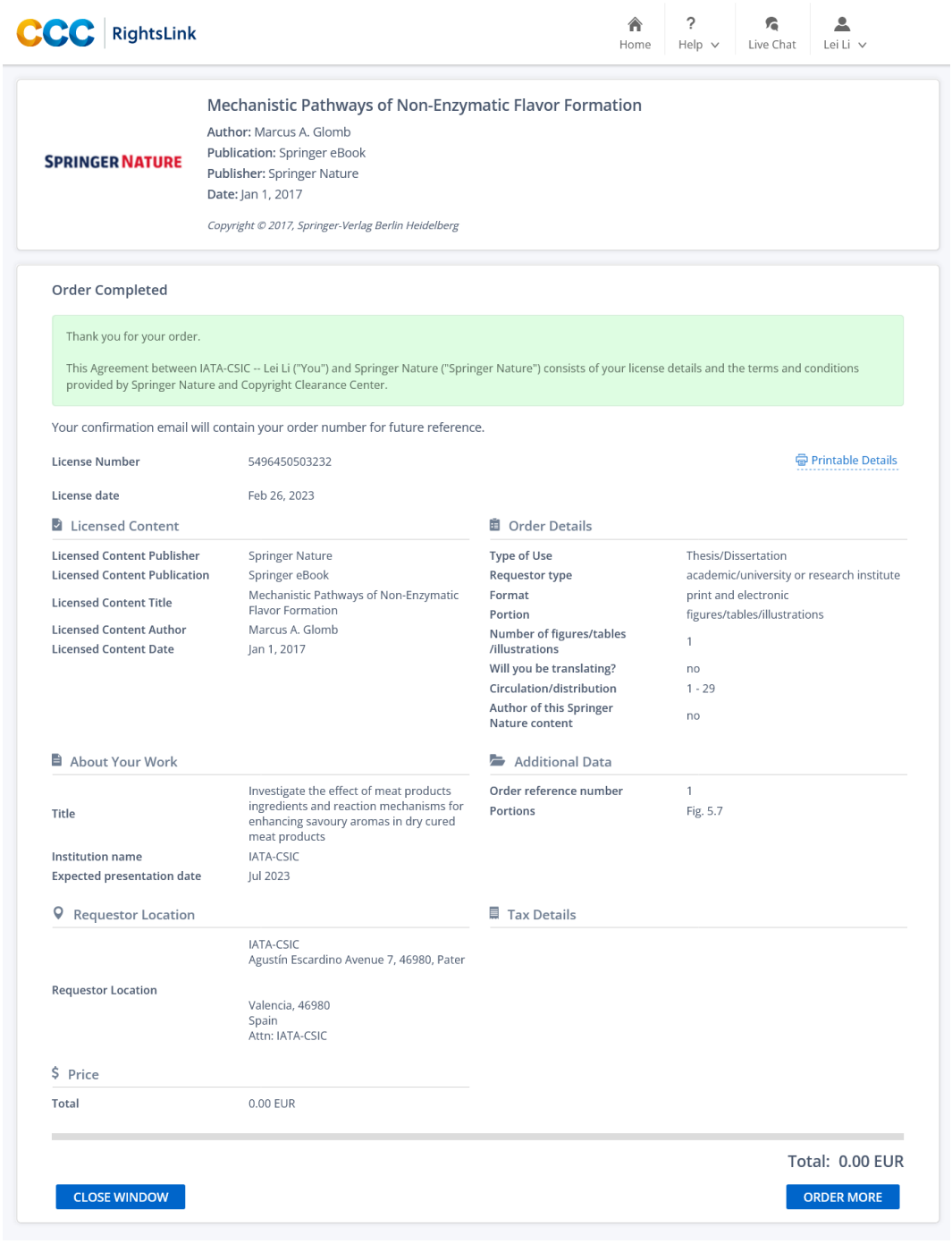

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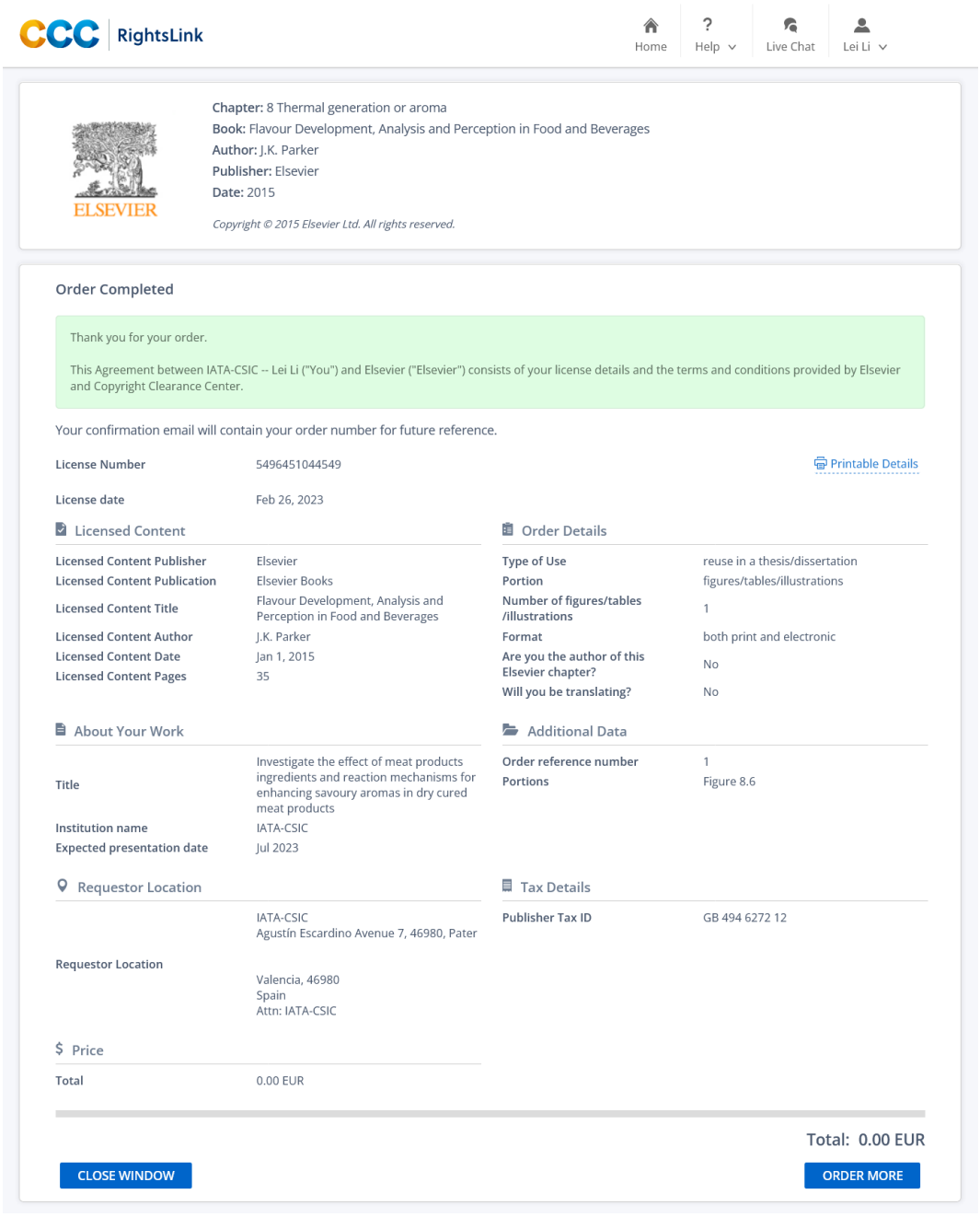

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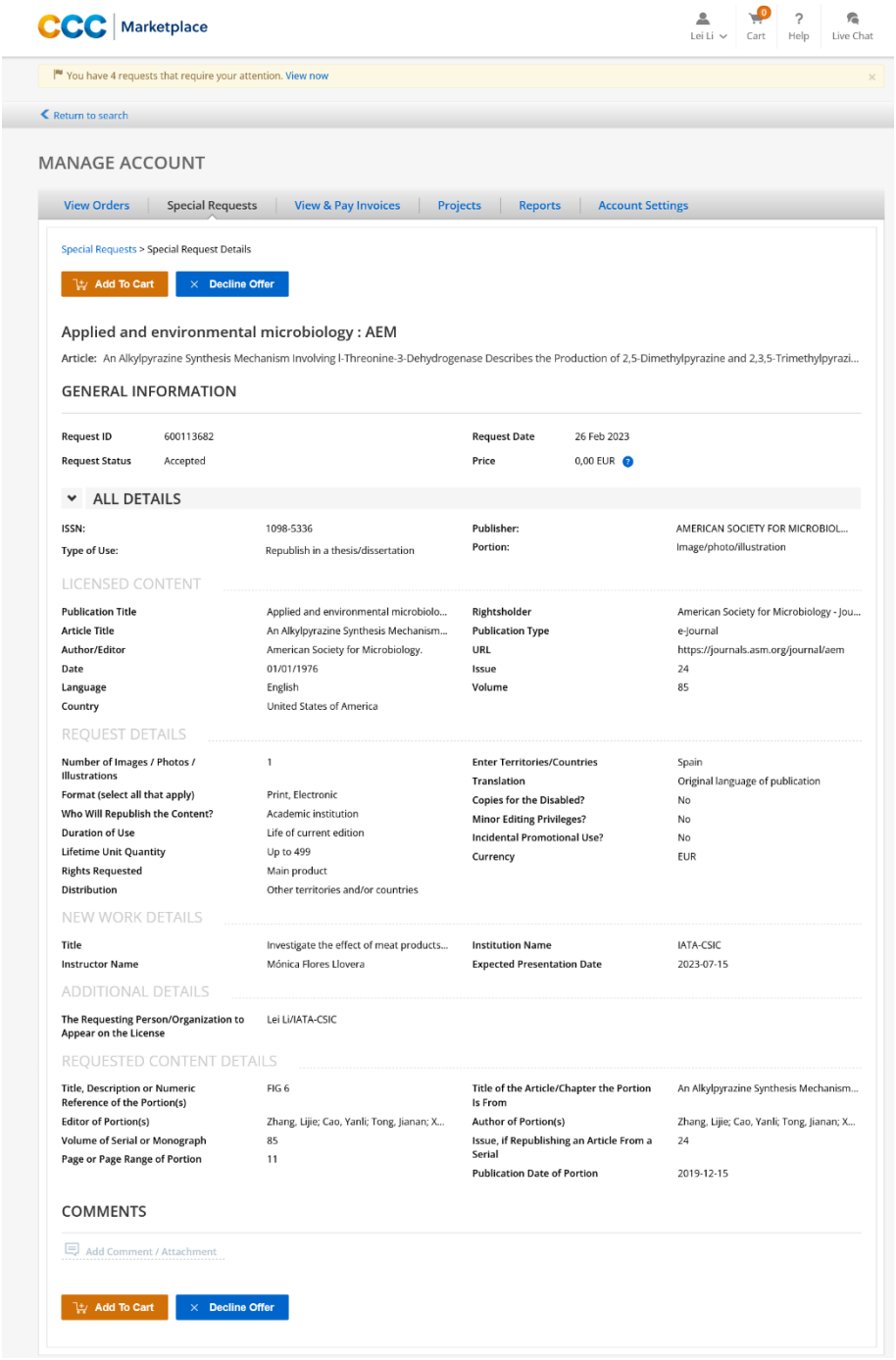

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