EFECTO DE LA REDUCCIÓN DEL CONTENIDO DE NaCl Y/O GRASA EN LA CALIDAD DE EMBUTIDOS CURADO-MADURADOS Y ESTUDIO DE NUEVAS ESTRATEGIAS PARA LA POTENCIACIÓN DEL AROMA.

TESIS DOCTORAL

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Hace constar:

Que el trabajo de investigación titulado “Efecto de la reducción del contenido de NaCl y/o grasa en la calidad de embutidos curado-madurados y estudio de nuevas estrategias para la potenciación del aroma” presentado por Dña. Sara Corral Silvestre para optar al grado de Doctor por la Universidad Politécnica de Valencia, ha sido realizado en el Instituto de Agroquímica Tecnología de los Alimentos (IATA-CSIC) bajo mi dirección y supervisión reuniendo las condiciones exigidas para ser defendida por su autora.

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Resumen

El proceso de fabricación de embutidos curado-madurados es crucial para el desarrollo de unas características sensoriales óptimas siendo el aroma y sabor uno de los parámetros de calidad más importantes para los consumidores. Por ello, es de interés conocer los compuestos de gran potencia aromática que determinan la aceptación de dicho producto para así, diseñar nuevas estrategias tecnológicas que potencien el aroma de los embutidos curado-madurados. En vista de ello, la presente Tesis ha abordado el estudio de los compuestos volátiles con poder aromático mediante dos pasos críticos en la identificación de los compuestos como son la técnica de extracción y detección. Desde el punto de vista de la extracción de compuestos volátiles, se ha aplicado una técnica de extracción con disolventes (SAFE) y una de espacio de cabeza (SPME) a embutidos curado-madurados tradicionales. Además, se aplicaron técnicas olfatométricas (cromatografía de gases-olfatometría y "odour activity value") con el fin de conocer qué técnica genera un extracto representativo del aroma del embutido. Desde el punto de vista de la detección de los compuestos volátiles, se emplearon diferentes detectores específicos cromatográficos (fotométrico de llama, nitrógeno-fósforo, olfatómetro) con el objetivo de facilitar la identificación, mediante cromatografía gases acoplada a espectrometría de masas y cromatografía de gases multidimensional, de compuestos volátiles minoritarios generados a lo largo del proceso de maduración.

Por otro lado, es de gran interés para la industria cárnica satisfacer los intereses cardiosaludables de los consumidores. A este efecto, se ha determinado el efecto de la reducción de sal sobre la calidad de embutidos curado-madurados, asi como, la reducción de grasa y sal/grasa. Además, se ha empleado una cepa de *Debaryomyces hansenii* como alternativa para potenciar el aroma de dichos embutidos reformulados. De tal forma que se han estudiado los parámetros físico-químicos, microbiológicos y sensoriales de embutidos reducidos en sal y/o grasa inoculados con *D. hansenii*. Al igual que se ha estudiado la generación de los compuestos volátiles y su potencial aromático en dichos embutidos reformulados.

Abstract

Dry fermented sausages process is crucial to develop suitable sensory characterics, being aroma and taste the most important quality parameters for consumers. Therefore, the knowledge of aroma active compounds is of great interest to find out which of them define the sensory acceptability, in order to design new technological strategies to enhance the aroma of dry fermented sausages. In view of this, the present Thesis deals with the study of aroma volatile compounds by means of two decisive steps in compounds identification; the extraction and detection technique employed. From standpoint of the volatile compound extraction, solvent extraction (SAFE) and headspace (SPME) techniques were applied to traditional dry fermented sausages. Furthermore, olfactometry techniques (gas chromatography-olfatometry and odour activity value) were applied to know which technique obtains a representative aroma extract of the dry fermented sausages. From the point of view of the volatile compounds detection, different specific chromatographic detectors (flame photometric, nitrogen-phosphorus, olfatometry) were used to facilitate the identification, using gas chromatography-mass spectrometry and multidimensional gas chromatography, of minor volatile compounds generated throughout the dry curing process.

On the other hand, consumer health interests are of great importance to meat industry. To this end, the effect of salt reduction on sausage quality has been determined, as well as, the effect of fat and salt/fat reduction. In addition, a *Debaryomyces hansenii* strain was used as alternative to enhance the aroma of reformulated sausages. In this sense, the physic-chemical, microbiological and sensory parameters of reduced salt and/or fat sausages inoculated with *D. hansenii* were studied. Also, the generation of volatiles compounds and their aroma potency in these reformulated sausages were studied.

Resum

El procés de fabricació d'embotits curat-madurats és crucial per al desenvolupament d'unes característiques sensorials òptimes, sent l'aroma i sabor un dels paràmetres de qualitat més importants per als consumidors. Per això, és de interés conéixer els compostos de gran potència aromàtica que determinen l'acceptació d'aquest producte per tal d'així, dissenyar noves estratègies tecnològiques que potencien l'aroma dels embotits curat-madurats. En vista d'això, la present Tesi ha abordat l'estudi dels compostos volàtils de poder aromàtic per mitjà de dos passos crítics en la identificació dels compostos com són la tècnica d'extracció i de detecció. Des del punt de vista de l'extracció de compostos volàtils, s'ha aplicat una tècnica d'extracció amb dissolvents (SAFE) i una d'espai de cap (SPME) a embotits curat-madurats tradicionals. A més, es varen aplicar tècniques olfatomètriques (cromatografia de gasos-olfatometría i "odour activity value") a fi de conéixer quina tècnica genera un extracte representatiu de l'aroma de l'embotit. Des del punt de vista de la detecció dels compostos volàtils, es van emprar diferents detectors cromatogràfics específics (fotomètric de flama, nitrogen-fòsfor, olfatometre) amb l'objectiu de facilitar la identificació, per mitjà de cromatografia de gasos acoblada a espectrometria de masses i cromatografia de gasos multidimensional, de compostos volàtils minoritaris generats al llarg del procés de maduració.

D'altra banda, és de gran interés per a la indústria càrnica satisfer els interessos cardiosaludables dels consumidors. A este efecte, s'ha determinat l'efecte de la reducció de sal sobre la qualitat d'embotits curat-madurats, així com, la reducció de greix i sal/greix. A més, s'ha utilitzat un cep de *Debaryomyces hansenii* com a alternativa per a potenciar l'aroma dels embotits reformulats. De tal forma que s'han estudiat els paràmetres fisicoquímics, microbiològics i sensorials d'embotits reduïts en sal y/o greix inoculats amb *D. hansenii*. Al igual que s'ha estudiat la generació dels compostos volàtils i el seu potencial aromatic en dites embotits reformulats.

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Abreviaturas

AECA: Análisis de concentración de extracto aromático
AECOSAN: Agencia Española de Consumo, Seguridad Alimentaria y Nutrición
AED: Detector de emisión atómica
AEDA: Análisis de dilución de extracto aromático
AESAN: Agencia Española de Seguridad Alimentaria y Nutrición
BAL: Bacterias ácido lácticas
CAR: Carboxen
CHARM: Método de respuesta aromática-hedónica combinada
CNS: Estafilococos coagulasa negativa
CW: Carbowax
DHDA: Análisis de dilución de espacio de cabeza dinámico
DVB: Divinilbenceno
ECD: Detector de captura de electrones
FAO: Organización de las Naciones Unidas para la Alimentación y la Agricultura
FID: Detector de ionización de llama
FPD: Detector fotométrico de llama
GC: Cromatografía de gases
HID: Detector de ionización de helio
HSSE: Extracción absorbente de espacio de cabeza
IR: Infrarrojo
LRI: Índice de retención lineal
MCSS: Cromatografía correlativa espectral de componentes múltiples
MDGC: Cromatografía de gases multidimensional
MS: Espectrometría de masas
NCD: Detector de quimiluminiscencia de nitrógeno
N-FID: Detector de nitrógeno de ionización de llama
NIF: Frecuencia de impacto nasal
NPD: Detector de nitrógeno-fósforo
OAV: Valor de actividad aromática
OMS: Organización Mundial de la Salud
OSME: derivado de la palabra griega oler
PA: Poliacrilato
PDHID: Detector de ionización de helio de descarga pulsada
PDMS: Polidimetilsiloxano
PEG: Polietilenglicol

PFPD: Detector fotométrico de llama pulsado
PID: Detector de fotoionización
SAFE: Evaporación del aroma asistida con disolvente
SBSE: Extracción absorbente de barra agitadora
SCD: Detector de quimioluminiscencia de azufre
SNIF: Superficie de la frecuencia de impacto
SPME: Microextracción en fase sólida
TCD: Detector de conductividad térmica
TID: Detector termoiónico

ÍNDICE

I. Introducción

I. INTRODUCCIÓN

La industria cárnica tiene como principal actividad la obtención, preparación y conservación de la carne, así como la elaboración de productos a base de ésta. Es el quinto sector industrial de España y el primero dentro de la industria agroalimentaria. El sector cárnico se caracteriza por su elevada atomización, es decir, por la existencia de un gran número de pequeñas y medianas empresas. Pese a la crisis económica, la industria cárnica ha mantenido su nivel de actividad reduciéndose sólo en un 2,6 % el número de empresas respecto a 2012 mientras que la media nacional de desaparición de empresas industriales fue entorno al -9,2 % (MAGRAMA, 2013). En 2012 generó unas ventas netas de 19.499 millones de euros, es decir, el 21,6 % de la industria alimentaria y el 4,4 % del total de la industria representando el 2 % del producto interior bruto (PIB) nacional.

El gasto total en alimentación en España, incluyendo la alimentación en el hogar y extradoméstica, en 2013 aumentó en un 0,6 % rompiendo la tendencia al descenso de los dos años anteriores. En los hogares, el gasto en carne fue entorno al 22,1 % en 2013. Y supuso una evolución respecto al 2012 de un descenso de un 0,1 %, debiéndose principalmente a la disminución del consumo de carne congelada y de carne de vacuno, mientras que la carne de cerdo aumentó en un 0,8 % (MAGRAMA, 2014).

Dentro de la industria cárnica, el sector porcino es el predominante en nuestro país y el cuarto productor mundial tras China, EEUU y Alemania. 3,43 millones de toneladas de carne de cerdo fueron procesadas en los mataderos en 2013, seguido por el sector avícola y vacuno (Cruz, 2014). Además, la Comisión Europea estima un aumento de la producción de carne de porcino de un 0,8 % para 2015. Dentro del sector porcino, alrededor de la mitad (48 %) de la carne de cerdo sacrificada en España se destina a la elaboración de productos cárnicos a base de ésta. Además, la producción de los productos cárnicos se ha visto incrementada en los últimos años igualándose a los niveles registrados en 2008, previo a la crisis económica.

En España hay una gran tradición chacinera vinculada a la zona geográfica donde se produce, por ello, encontramos una amplia gama de productos cárnicos, siendo el chorizo y el salchichón los más importantes. La elaboración de estos productos de forma artesanal da lugar a productos muy apreciados por su gran calidad organoléptica. Sin embargo, la sociedad actualmente demanda alimentos con una calidad definida y constante, lo que ha

llevado a la industria cárnica a desarrollar tecnologías de procesado en los que determinados parámetros de interés puedan ser controlados y regulados. Además, el incremento de consumo de estos productos ha llevado a la sustitución de los métodos tradicionales de maduración lenta por nuevos métodos de maduración rápida para responder a la fuerte demanda. Sin embargo, este tipo de maduración rápida produce una ligera pérdida de las características organolépticas de los embutidos ya que, impide una correcta maduración del embutido resultando en productos más ácidos y menos aromáticos.

Por otra parte, el gran interés y preocupación de los consumidores por la salud se ha visto reflejado en una tendencia de consumo de alimentos cardiosaludables con menor contenido en sodio y grasa. Sin embargo, dichos ingredientes tienen un papel relevante en el desarrollo de las características tecnológicas y sensoriales de los embutidos curado-madurados.

Por todo ello, es de gran interés para la industria cárnica satisfacer los intereses de los consumidores. Para ello, es necesario conocer el efecto de la reducción de NaCl y grasa en el aroma de los embutidos curado-madurados y conocer los compuestos de gran potencia aromática para diseñar nuevas estrategias que potencien el aroma de los embutidos.

Referencias bibliográficas

MAGRAMA (2013). Ministerio de Agricultura, Alimentación y Medio Ambiente. Informe Industria alimentaria en 2012-2013.

MAGRAMA (2014). Ministerio de Agricultura, Alimentación y Medio Ambiente. Disponible en http://www.magrama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/panel-de-consumo-alimentario/ultimos-datos/default.aspx

Cruz J. (2014). Las industrias alimentarias y cárnicas se muestran positivas ante la evolución de su facturación. Eurocarne, Nº 224, Marzo.

II. Antecedentes bibliográficos

II. ANTECEDENTES BIBLIOGRÁFICOS

1. Embutidos curado-madurados

En la reciente norma de calidad de derivados cárnicos (Real Decreto 474/2014, BOE, 18 Junio 2014) se define como embutido curado-madurado a los derivados cárnicos constituidos por trozos de carne o carne y grasa no identificables anatómicamente que, con carácter general y no limitativo, se han sometido a un proceso de picado más o menos intenso, mezclados con especias, ingredientes, condimentos y aditivos, embutidos en tripas naturales o envolturas artificiales, y sometidos a un proceso de salazón seguido de curado-maduración, acompañado o no de fermentación, suficiente para conferirles las características organolépticas propias y su estabilidad a temperatura ambiente. Las carnes podrán ser todas del mismo tipo o ser una mezcla de carnes de distinta procedencia, naturaleza, parte anatómica y especie animal (BOE, 18 Junio 2014).

Para ir desmenuzando esta definición, sería recomendable clarificar el término curado-madurado. Se entiende como tal, al tratamiento de curado con posterior desecación en condiciones ambientales adecuadas para provocar, en el transcurso de una lenta y gradual reducción de la humedad, la evolución de los procesos naturales de fermentación o enzimáticos necesarios para aportar al producto cualidades organolépticas características y que garantice su estabilidad durante el proceso de comercialización, dando lugar a lo que tradicionalmente se conoce como derivado cárnico curado (BOE, 18 Junio 2014). Y como curado se denomina al tratamiento con sal, que puede ir acompañada del uso de nitritos, nitratos y otros componentes o una combinación de ellos, que debe responder a una necesidad tecnológica, dando lugar a compuestos procedentes de la combinación de estos conservantes con las proteínas de la carne. El tratamiento se puede realizar mediante la aplicación en seco, a la superficie de la carne, de la mezcla de curado, mediante inmersión de la misma en la solución de curado o mediante inyección de la solución de curado en la pieza cárnica (BOE, 18 junio 2014).

Durante la etapa de desecación se aplican las condiciones ambientales para que se vea favorecido el desarrollo de la flora microbiana natural teniendo lugar la fermentación del producto. Tradicionalmente, tanto el secado como la fermentación, eran dos tecnologías que se utilizaban para prolongar la vida útil de los alimentos. Sin embargo, hoy en día, dichas técnicas han perdido importancia como métodos de conservación debido a la aparición de nuevas tecnologías de conservación de la carne (refrigeración, congelación, tratamientos

térmicos, envasado…). Sin embargo, han adquirido una nueva perspectiva, persiguiendo la diversificación de la oferta de productos de mayor valor añadido.

1.1. Tecnología de fabricación de los embutidos curado-madurados

La Figura 1 muestra un diagrama del proceso de elaboración de embutidos curado-madurados. Sin embargo, existen amplias variaciones en el proceso tecnológico, siempre y cuando se mantengan las reducciones adecuadas de pH y a_w, de ahí el motivo de la amplia variedad de embutidos curado-madurados (Leistner, 1992).

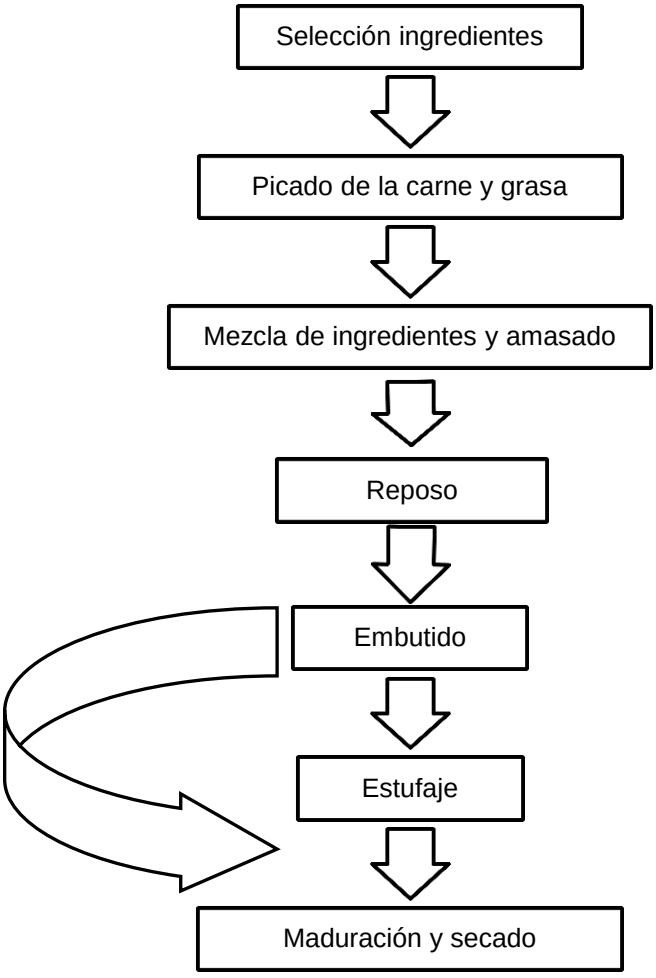

Figura 1. Diagrama de fabricación de embutido curado-madurado.

En primer lugar, se selecciona la carne magra y grasa de animales saludables, generalmente de cerdo y/ vacuno. A continuación, la carne y la grasa se pican parcialmente congeladas, para evitar su calentamiento y la rotura de los adipocitos. Una vez picada la carne y grasa, se procede a amasar a vacío todos los ingredientes para evitar el contacto con el oxígeno y su influencia en el desarrollo del color. Además de la carne magra y grasa, comúnmente, se añaden los siguientes ingredientes:

- Nitritos y nitratos: su función es la inhibición de microorganismos patógenos como *Clostridium botulinum*. Además, contribuyen en el desarrollo del color y aroma típicos de los embutidos curado-madurados.
- Ascorbato: es un coadyuvante del proceso de curado que ayuda a reducir el nitrito a óxido nítrico por lo tanto, mejora la formación y estabilización del color.
- Sal común: conocida popularmente como “sal”, corresponde a la sal denominada cloruro sódico (NaCl). Es un potenciador del sabor entre otras funciones (apartado 1.2.1.).
- Azúcares: la fermentación se lleva a cabo por acción de los microorganismos presentes en la masa cárnica al metabolizar los azúcares a ácidos. El tamaño y cantidad de azúcar añadido condicionará la velocidad de la acidificación.
- Especias: son ingredientes vegetales con carácter aromático que se utilizan habitualmente en pequeñas cantidades para conferir determinados sabores, aromas y colores a los productos cárnicos.
- Cultivos iniciadores o starters: son microorganismos seleccionados (apartado 1.1.1.).

Tras un reposo de 24 horas, para favorecer la adaptación de la flora microbiana al medio y la ligazón de la masa, la masa cárnica es embutida en tripas naturales o artificiales permeables al agua. A continuación, se lleva a cabo de forma opcional la etapa del estufaje, consiste en dar un golpe de calor a los embutidos (18-24 ºC y < 90 % de humedad relativa (HR) durante 1-3 días en Europa y 30-45 ºC en EE.UU.) (Ordóñez *et al*., 1999) tras haberlos embutido en la tripa, para asegurar la proliferación del cultivo iniciador, el cual inhibirá la flora autóctona de la masa. Consecuentemente, se produce una bajada rápida del pH por la fermentación llevada a cabo por las bacterias ácido lácticas (BAL). Según el tipo de embutido a procesar, se lleva a cabo o no dicho estufaje dependiendo

de la adicción o no de azúcares y starters. Sin embargo, los productores de embutidos tradicionales obvian dicha etapa para evitar el riesgo que puede suponer. Por último, se lleva a cabo la etapa de maduración y simultáneamente el secado del producto. Los embutidos se mantienen durante periodos variables de tiempo en condiciones de temperatura y humedad relativa, controladas. La duración de este periodo es variable en función del tipo de producto y su diámetro, pero suele oscilar entre 1-3 meses (Flores, 1997).

1.1.1. Cultivos iniciadores o starters en embutidos curado-madurados

En numerosas ocasiones, para corregir posibles defectos en la maduración del embutido u homogeneizar su calidad, se opta por utilizar cultivos de microorganismos seleccionados o starters. Las bacterias inoculadas como starters se encuentran en un nivel de 10^6 colonias viables/g de masa (Talon *et al.*, 2002). En Europa, las bacterias que se adicionan como starters son ácido lácticas y estafilococos coagulasa negativa (CNS), y en el área mediterránea también se inoculan mohos y levaduras creciendo en la superficie del embutido y dando lugar a un velo blanco superficial. En la Tabla 1 se muestran los microorganismos utilizados comúnmente como starter en embutidos curado-madurados.

Las BAL tienen un papel importante tecnológica y sensorialmente, ya que consumen los azúcares presentes y/o adicionados a la masa generando ácido láctico (metabolismo homofermentativo) que proporciona un sabor ácido al producto. Como consecuencia, se produce el descenso de pH (5,2-4,6) alcanzando el punto isoeléctrico de las proteínas. De esta forma, se favorece el secado del producto ya que, se ve disminuida la capacidad de retención de agua de las proteínas miofibrilares y tiene lugar la coagulación de éstas favoreciendo su gelificación. Además, el descenso de pH favorece el desarrollo del color, al tener lugar la reacción del óxido nítrico con la mioglobina ya que, el nitrito es espontáneamente reducido a pH ácido (Talon *et al*., 2002). De forma directa, contribuyen levemente a la proteólisis ya que, reducen el pH favoreciendo la actividad de las catepsinas musculares a pH ácido (Talon *et al*., 2002). También, las BAL contribuyen a la conservación del producto por la generación de bacteriocinas.

Por otra parte, los CNS tienen mayor influencia sobre las características sensoriales. Estos microorganismos favorecen el desarrollo del color reduciendo el nitrato a nitrito y, éste es reducido a óxido nítrico que reacciona con la mioglobina formando nitrosomioglobina (color rojo característico de los embutidos). Además, favorece el desarrollo del aroma del producto a través de la

lipolisis, proteólisis y, en menor medida, a través del metabolismo de los hidratos de carbono (Talon *et al*., 2002). En condiciones anaerobias, las BAL y CNS metabolizan los azúcares a ácido láctico, acético y etanol y, éste último reacciona con alcoholes generando ésteres metílicos a través de la actividad esterasa de los *Staphylococci*. Además por su actividad catalasa, retrasa la rancidez, estabiliza el color, el aroma y sabor del producto eliminando el peróxido de hidrógeno. La fermentación debe de ser lenta para permitir su crecimiento y actuación ya que, las CNS son sensibles a pH ácidos.

Tabla 1. Microorganismos utilizados como starters en embutidos curado-madurado.

Microorganismos	**Especies**	**Modo de acción**
Bacterias acido lácticas	*L. sakei, L. curvatus,*	Producción de bateriocinas
	L. plantarum, L. pentosus	Producción de ácido láctico
	P. pentosaceus,	Proteólisis
	P. acidilactici	Eliminación del nitrito
Estafilococos coagulasa negativa	*K. varians*	Reducción del nitrato
	S. xylosus, S. carnosus	Consumo de oxígeno
		Eliminación de peróxidos
		Catabolismo hidratos de carbono, proteínas, lípidos
		Producción de ésteres
		Eliminación del nitrito
Levaduras	*D. hansenii*	Consumo de oxígeno
	C. fumata	Eliminación de peróxidos
		Lipolisis, proteolisis
		Protección frente al oxígeno y la luz
Mohos	*P. nalgiovense,*	Consumo de oxígeno
	P. chrysogenum	Eliminación de peróxidos
		Actividad desaminasa
		Oxidación de lactato
		Lipolisis, proteolisis
		Inhibición de la microorganismos indeseables
		Protección frente al oxígeno y la luz
		Protección frente a secado excesivo

Adaptada de Talon *et al*. (2002) y Lücke (1994).

Dado el carácter aerobio de mohos y aerobio facultativo de las levaduras, crecen principalmente en la superficie de los embutidos. Sin embargo, las levaduras poseen un metabolismo fermentativo débil que le permite crecer en el interior del producto (Toldrá *et al*., 2001). Estos microorganismos pueden producir un aumento del pH final del embutido por el consumo de ácidos orgánicos y utilización de proteínas, que producen compuestos moduladores de la acidez. Asimismo, participan principalmente en el desarrollo del aroma y sabor del producto ya que, participan en la lipolisis y proteólisis formando productos metabólicos que mejoran el aroma y sabor (Toldrá, 2008). Al igual que los CNS, pueden retrasar la rancidez del embutido por su actividad catalasa y, dado que crecen en la superficie del embutido, evitan la penetración del oxígeno y la luz. También, pueden reducir los nitratos a nitritos mejorando el color superficial del embutido, además de producir sustancias antibióticas capaces de inhibir el crecimiento de microorganismos patógenos. Además, los mohos y levaduras regulan la salida de agua al crecer en la superficie del embutido (Talon *et al*., 2002).

Levaduras como starter en embutidos curado-madurados

La alta concentración de sal, la baja actividad de agua (a_w) junto con la acidez del embutido hacen un medio hostil para el crecimiento de determinadas especies de levaduras. La especie predominante en este medio cárnico es *Debaryomyces hansenii* por excelencia (Ravyts *et al*., 2012; Encinas *et al*., 2000). De ahí, el hecho de aislar una cepa idónea que potencie las características organolépticas del producto inoculado. Por un lado, Bolumar *et al*. (2006) adicionaron un extracto de la cepa *D. hansenii* CECT 12487, como starter al proceso de fabricación de embutidos, mejorando sensorialmente el producto al afectar especialmente a la generación de compuestos volátiles. Por otra lado, Olesen *et al.* (2000), Flores *et al.* (2004), Iucci *et al.* (2007) y Cano-García *et al*. (2014) inocularon diferentes cepas mostrando diferentes resultados sensoriales. Olesen *et al.* (2000) y Cano-García *et al.* (2014) observaron un impacto de *D. hansenii* sobre los compuestos volátiles pero, en cambio, ese impacto no se apreció sensorialmente, probablemente debido al efecto bacteriostático del ajo y del starter bacteriano utilizado, respectivamente. Por el contrario, Flores *et al.* (2004) y Iucci *et al.* (2007) apreciaron sensorialmente un efecto positivo sobre el aroma de los embutidos. Por lo tanto, es importante la selección de una cepa apropiada junto con la formulación, starter y condiciones del proceso de fabricación.

1.2. Composición nutricional de los embutidos curado-madurados e impacto sobre la salud

En la Tabla 2 se muestra la composición nutricional de diferentes tipos de carne y embutido curado-madurado. La carne se caracteriza principalmente por su alto contenido proteico y en menor medida lipídico. Nutricionalmente, la principal diferencia entre los diferentes tipos de carne es el contenido en lípidos siendo la carne de cerdo la más grasa. Por lo tanto, la composición nutricional de los embutidos varía dependiendo de la naturaleza de la carne e ingredientes adicionados. No obstante, los embutidos curado-madurados se caracterizan principalmente por su alto contenido en proteínas de alto valor biológico mientras el contenido de lípidos es muy variable y su aporte en hidratos de carbono es escaso (Tabla 2). Sus proteínas de alto valor biológico aportan todos los aminoácidos esenciales, es decir, aquellos que deben ser aportados por los alimentos ya que, no pueden ser sintetizados por el organismo o no en cantidad suficiente. Además, dada la proteólisis, que tiene lugar en este tipo de productos cárnicos, aumenta la digestibilidad proteica de estos productos (Varman & Sutherland, 1995).

Tabla 2. Composición nutricional de diferentes tipos de carne y embutidos curado-madurados.

(g/100g)	Vacuno	Pollo	Cerdo	Embutido curado-madurado
Energía (Kcal/100g)	130	135	135	350
Agua	71-75	71-75	72-76	40-45
Proteínas	20-22	18-20	18-20	22-24
Lípidos	3-5	3-6	3-6	26-28
Carbohidratos	1	1	1	2-3
Minerales	1	1	1	5-6

Adaptada de ANICE, 2014

También cabe destacar el alto contenido en minerales y vitaminas de la carne de cerdo y de los embutidos curado-madurados, principalmente vitaminas hidrosolubles del complejo B, especialmente tiamina (Tabla 3). Asimismo, la carne de cerdo es más rica en riboflavina y piridoxina que otras carnes, y en ella también está presente la vitamina B12 (Tabla 3). Los embutidos curado-madurados tienen cantidades significativas de ácido ascórbico ya que, se adiciona como coadyuvante. La carne de cerdo, y por tanto los embutidos a

partir de ésta, son buena fuente dietética de hierro fácilmente asimilable, fósforo y de otros minerales como zinc, magnesio, etc (Tabla 3).

Tabla 3. Contenido (mg/100g) en vitaminas y minerales en distintos tipos de carne y en embutidos curado-madurados.

(mg/100g)	Vacuno	Pollo	Cerdo	Embutido curado-madurado
Minerales				
Sodio	90	81	70	1100
Potasio	160	350	300	160
Calcio	11	10	10	11
Magnesio	16	25	20	9
Hierro	3	1	2,5	2
Zinc	3,1	1,1	1,8	1,7
Fósforo	206	200	170	116
Iodo (µg)	-	-	-	-
Flúor (µg)	-	140	66	-
Cobre (µg)	-	70	160	50
Selenio (µg)	-	14	31	35
Vitaminas				
Vitamina A (µgeq)	-	-	-	-
Vitamina D (µg)	-	-	-	-
Vitamina E (µgeq)	-	0,2	-	-
Vitamina C	-	4	-	-
Tiamina (B1)	0,17	0,1	1	0,1
Riboflavina (B2)	0,27	0,2	0,2	0,2
Niacina (B3)	6	7	4,5	6
Piridoxina (B6)	0,23	0,42	0,45	0,07
Cianocobalamina (B12) (µg)	2	0	2	1
Ácido fólico (µg)	9	12	3	1

Adaptado de Jiménez *et al.* (2005).

En resumen, los embutidos juegan un papel importante en la dieta por su elevado contenido en proteínas y micronutrientes. Sin embargo, su elevado contenido en grasa y NaCl hace que se consideren alimentos no saludables relacionándose con enfermedades cardiovasculares, pudiendo ser consumidos de forma ocasional dentro de una dieta variada. Además, hoy en día existe una preocupación de los consumidores por su salud que repercute en los planteamientos de la industria alimentaria, interesada en atender las demandas y preferencias de los consumidores, respondiendo con ofertas de productos alimentarios capaces de aportar algún beneficio saludable. Considerando estas

necesidades y las recomendaciones de la Organización Mundial de la Salud (OMS/FAO, 2003), la industria cárnica está desarrollando productos reducidos en NaCl y grasa. No obstante, dichas reducciones pueden conllevar problemas tecnológicos, sensoriales y de seguridad en el producto.

1.2.1. Reducción de cloruro de sodio

1.2.1.1. Problemática de terminología

El ión sodio (Na^+) tiene un impacto sobre la salud como veremos en el siguiente apartado (1.2.1.2.). El sodio está presente de forma natural en los alimentos a muy bajos niveles. Sin embargo, la principal fuente de sodio en la dieta es el cloruro sódico, es decir, la sal común popularmente conocida. Por tanto, existe una problemática en la terminología utilizada en el etiquetado de los alimentos procesados, por el uso de "sodio" o "sal".

En el comité de expertos del Codex Alimentarius sobre etiquetado de alimentos (2011), hubo unanimidad en que el término "sodio" es técnica y científicamente correcto para el nutriente que causa problemas en la salud pública. También hubo acuerdo en que los consumidores deben entender la declaración (sea sal o sodio) ya que, el etiquetado de los alimentos va dirigido a los mismos. Sin embargo, no hubo consenso sobre si la declaración en la lista de nutrientes debería ser como "sal" o como "sodio" y por tanto, el uso de ambos términos sería según el dictamen de las autoridades nacionales. También remarcaron la importancia de la educación de los consumidores para cualquiera de los dos enfoques.

Centrándonos en el ámbito de la Unión Europea, de acuerdo al Reglamento nº1169/2011, el término a utilizar en el etiquetado nutricional debe ser "sal", en vez de la correspondiente denominación del nutriente "sodio", para facilitar el entendimiento de la información del etiquetado a los consumidores a los que va dirigido el etiquetado nutricional y pudiéndose calcular mediante la fórmula: sal = sodio x 2,5 (Anexo I, punto 11, del Reglamento). Además, la sal debe ser incluida en la declaración sobre las propiedades nutrititivas en el etiquetado de todos los alimentos excepto en aquellos sin transformar que incluyan un solo ingrediente, productos transformados cuya única transformación haya consistido en ser curados y que incluyan un solo ingrediente, agua destinada al consumo humano, especias, sal y sucedáneos de la sal, edulcorantes de mesa, extractos de café o achicoria, infusiones, vinagres fermentados y sus sucedáneos, aromas, aditivos alimentarios, coadyuvantes tecnológicos, enzimas alimentarias, gelatina, espesantes para mermeladas, levadura, gomas de mascar, alimentos en envases cuya superficie sea inferior a

25 cm^2 y alimentos artesanales suministrados por el fabricante en pequeñas cantidades. Sin embargo, dada la diferencia de opiniones entre países (Codex Alimentarius, 2011) que existe sobre el uso del término de "sal" o "sodio", la Unión Europea, para solventar el problema que se presenta en el etiquetado de alimentos que no contengan sal (NaCl) adicionada pero, en cambio, sean fuente natural de sodio, añade que cuando proceda, se podrá incluir una indicación señalando que el contenido de sal obedece exclusivamente al sodio presente de forma natural en el alimento.

Por tanto, dado el ámbito cientifíco en el que se enmarca este documento, el término utilizado a lo largo de él será sodio o NaCl cuando proceda.

1.2.1.2. Impacto sanitario y tecnológico del NaCl en embutidos curado-madurado

La sal (NaCl) como fuente de sodio es un nutriente esencial en el organismo ya que, actúa en el correcto funcionamiento del metabolismo, regula el balance hídrico corporal, el transporte de nutrientes o la eliminación de sustancias a través de los fluidos de agua y sal (Doyle & Glass, 2010). Además, la sal común es un vehículo para el aporte de yodo y flúor previniendo la deficiencia de estos minerales. Sin embargo, un alto consumo de sodio produce la retención de líquidos en el organismo aumentando la presión arterial. Además, esta asociación es más evidente cuando aumenta la edad y el peso del individuo, y sus valores iniciales de presión arterial (AESAN, 2005). Por tanto, la OMS/FAO recomienda reducir el consumo de NaCl (de toda procedencia) a menos de 5 g/día (OMS/FAO, 2003), lo que llevó a la Unión Europea a desarrollar una iniciativa para reducir el consumo de sodio en un 4% a lo largo de 4 años haciendo la disminución de forma progresiva permitiendo a los consumidores adaptarse al menor sabor salado de los alimentos (Comisión Europea, 2008).

En España, el consumo actual de NaCl es de 9,7 g/día persona procediendo el 70-75 % de alimentos procesados y comidas fuera del hogar. La principal fuente de sodio en la dieta española es el pan, aportando el 19 % del total ingerido, seguido por los productos cárnicos como el jamón, embutidos y fiambres (OMS, 2013). El contenido de NaCl en los embutidos es variable según el área geográfica y de cada productor pero, su contenido es alrededor de 3-5 % (Rust, 1987). Sin embargo, la reducción del contenido de sodio es un reto para la industria cárnica, dadas las funciones que desarrolla el NaCl en los embutidos curado-madurados.

El NaCl, no sólo mejora el sabor sino que ejerce diferentes funciones tecnológicas y microbiológicas. Por un lado, reduce la a_w por lo tanto, tiene un efecto bacteriostático retardando el crecimiento microbiano. Por otro lado, en

particular los iones cloro se unen a las proteínas aumentando su carga negativa causando la repulsión entre las proteínas miofibrilares y solubilizándolas proporcionando una película adhesiva que sirve para envolver las partículas de grasa y agua formando una emulsión estable (Rust, 1987). Además, condiciona las reacciones químicas y bioquímicas que se llevan a cabo durante la maduración de los embutidos desarrollando el aroma del producto final. A pesar de los efectos beneficiosos que puede tener, también puede afectar negativamente al producto provocando la rancidez de la grasa debido a la acción de metales pesados contaminantes en la sal común además de su propio efecto prooxidante (Rust, 1987).

1.2.2. Reducción de grasa: impacto sanitario y tecnológico de la grasa en embutidos curado-madurados

La grasa actúa como reserva energética en el cuerpo, como precursor de otras sustancias como las hormonas, forma parte de la membrana celular y vehiculiza las vitaminas liposolubles. Sin embargo, un consumo excesivo de grasa, especialmente saturadas y ácidos grasos trans, está relacionado con enfermedades cardiovasculares como la obesidad, un aumento de la presión arterial, colesterol y diabetes mellitus 2 (OMS/FAO, 2003). Por tanto, se recomienda que el consumo de grasa no supere el 30 % de la ingesta diaria reduciéndose el consumo de grasas saturadas y ácidos grasos trans (AESAN, 2005).

El alto consumo de grasas se debe principalmente a una dieta caracterizada por un exceso de carnes, embutidos, lácteos y alimentos con alta densidad energética, como productos de bollería. En el caso de los embutidos curado-madurados, el contenido de grasa es alrededor de 25-55 % (Ordóñez, 1999). Por ello, la Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN) ha desarrollado un plan estratégico, “Plan Cuídate+2012” (AECOSAN, 2012) en el que se acuerda con los proveedores reducir el contenido de grasa un 5 % y de NaCl un 10 % en productos cárnicos en un periodo de 2 años.

El contenido y calidad de la grasa de cerdo varía, no sólo de una especie animal a otra, sino también, según la región de la canal dentro de una misma especie, de la alimentación del animal, sexo y edad. Los lípidos del tejido adiposo del cerdo contienen un 99 % de triglicéridos y pequeñas cantidades de colesterol y productos derivados de la degradación de los triglicéridos (di y monoacilglicéridos, ácidos grasos libres) mientras que, los lípidos del tejido muscular se componen principalmente por triglicéridos (62-80 %) y fosfolípidos

(16-34 %) (Gandemer, 2002). En la Tabla 4 podemos observar el perfil lipídico de la grasa de cerdo. Es rica en ácidos grasos monoinsaturados (50 %), seguido por saturados (40 %) y poliinsaturados (9 %) con los siguientes ácidos grasos principales: miristíco, palmítico, esteárico, palmitoleico, oleico, linoleico y linolenico (Tabla 4). Los ácidos grasos poliinsaturados mayoritariamente presentes en la grasa animal son el linoleico y araquidónico, los cuales son esenciales y poseen propiedades nutricionales especiales. Por tanto, éstos deben ser aportados por la dieta ya que, no pueden ser sintetizados por el organismo.

Sin embargo, una reducción del contenido de grasa en los embutidos curado-madurados puede conllevar un detrimento en su calidad, dado el papel que desarrolla en dichos productos. No sólo contribuye al aporte energético sino que desempeña diferentes funciones tecnológicas y sensoriales. Por un lado, es imprescindible para que se consiga una ligazón adecuada en el amasado de la masa cárnica; además, los gránulos de grasa permiten y controlan la continua liberación de agua favoreciendo el proceso de deshidratación (Wirth, 1988). Asimismo, proporciona jugosidad, untuosidad o suavidad al producto facilitando el corte o masticación del embutido (Roncales, 1994). Por último, participa en diferentes reacciones químicas o bioquímicas que determinarán el aroma y sabor del producto final (Toldrá *et al*., 2001).

Tabla 4. Composición de ácidos grasos de diferentes tipos de carne y embutidos curado-madurados.

Ácidos grasos (g/100g)	**Vacuno**	**Pollo**	**Cerdo**	**Embutido curado-madurado**
Saturados	0,8	1,42	11,45	9,8
Mirístico	0,03	0,05	0,35	0,4
Palmítico	0.35	1,08	7,3	6,15
Esteárico	0,4	0,29	3,65	3,2
Monoinsaturados	0,5	1,93	12,9	12,3
Palmitoleico	0,07	0,29	0,9	0,95
Oleico	0,45	1,62	11,75	11,3
Poliinsaturados	0,2	0,65	2,2	2,25
Linoleico	0,09	0,55	2	1,8
Linolenico	-	0,03	0,2	0,15
Colesterol (mg)	70	87	72	100

Adaptado de Jiménez *et al*. (2005)

2. El aroma de los embutidos curado-madurados

El aroma de los embutidos curado-madurados depende de la generación de compuestos volátiles a lo largo del proceso de maduración, a través de varias reacciones químicas o bioquímicas. Tanto un tipo como otro de reacción ocurren de forma simultánea y se ven reguladas por las condiciones del proceso generando un aroma y sabor característico de los embutidos.

2.1. Reacciones bioquímicas implicadas en el aroma de los embutidos curado-madurado

Las reacciones bioquímicas que tienen lugar en los embutidos pueden llevarse a cabo por las enzimas musculares o por los microorganismos presentes en la masa cárnica.

2.1.1. Degradación de los carbohidratos

Los hidratos de carbono son el sustrato para el crecimiento y desarrollo de la flora microbiana presente y adicionada como starter al embutido. En general, la fermentación de los azúcares se lleva a acabo anaeróbicamente por las BAL vía homofermentativa generando ácido láctico, el cual contribuye al sabor del embutido. En menor medida, *Staphylococci* también contribuye a la generación de ácido láctico (Montel *et al*., 1993). Sin embargo, si los starters utilizados son heterofermentatitivos pueden producir compuestos volátiles como ácido acético, etanol, diacetilo, acetoína, butanediol, acetaldehído (Talon *et al*., 2002).

Además, dependiendo de los starters y las condiciones aplicadas, la degradación de los azúcares genera di- y tri-carbonilos que pueden ser sustrato para subsecuentes reacciones (Toldrá & Flores, 2007).

2.1.2. Degradación de las proteínas

Dado el gran porcentaje que supone el tejido muscular (50-70 %) en los embutidos curado-madurado, éste tiene un papel primordial en la generación de compuestos volátiles. Existen tres tipos de proteínas musculares: miofibrilares, sarcoplásmicas y del tejido conjuntivo, y éstas son degradadas principalmente por las proteasas endógenas (enzimas del tejido muscular) y en menor medida por exógenas (microbianas). La degradación de las proteínas incluye dos etapas, en primer lugar, ocurre la proteólisis, la cual genera péptidos y éstos son degradados a aminoácidos, y en segundo lugar, el catabolismo de los aminoácidos (Toldrá & Flores, 2007).

2.1.2.1. Proteólisis

Las proteínas mayoritariamente degradadas son las miofibrilares seguidas por las sarcoplásmicas, principalmente, por acción de las enzimas musculares mientras que las enzimas microbianas tienen un papel más limitado (Hierro *et al*., 1999, Verplaeste *et al*., 1992). Sin embargo, las BAL indirectamente contribuyen a la proteólisis ya que, al reducir el pH se favorece la actividad de las catepsinas. En primer lugar, las proteínas son degradadas a polipéptidos y péptidos por acción de endoproteasas (calpaínas, catepsinas y proteasoma) (Figura 2). Y en segundo lugar, actúan las exoproteasas (tripeptidilpeptidasas, dipeptidilpeptidasas y aminopeptidasas) sobre los polipéptidos y péptidos generando gran cantidad de aminoácidos libres (Flores & Toldrá, 2011). El papel de las enzimas microbianas cobra más sentido en la degradación de péptidos a aminoácidos contabilizándose alrededor del 40 % su contribución frente a las enzimas musculares (Molly *et al*., 1997).

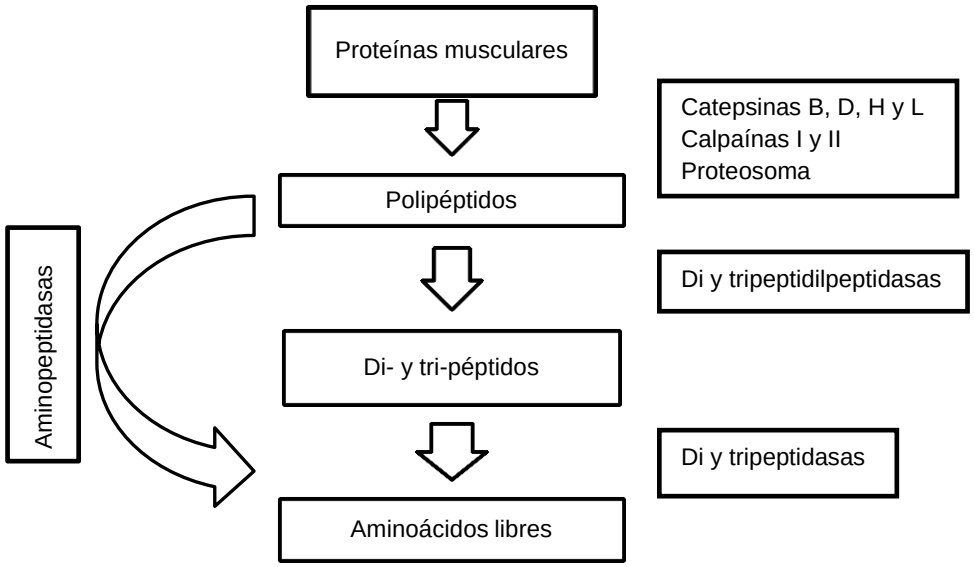

Figura 2. Diagrama de la proteólisis en embutidos curado-madurados (Adaptado de Toldrá, 1998)

2.1.2.2. Catabolismo de los aminoácidos

Los aminoácidos generados contribuyen directamente al flavor del embutido o indirectamente como precursores de otros compuestos volátiles a

través de otras reacciones enzimáticas, como la degradación de aminoácidos, o químicas, como la degradación de Strecker (Toldrá & Flores, 2007). Los aminoácidos pueden seguir los siguientes tipos de reacciones enzimáticas:

Reacciones de eliminación: son catalizadas por enzimas liasas musculares o microbianas generando fenol, indol, metanotiol, amoníaco y piruvato (Toldrá *et al*., 2001).

Descarboxilación: por descarboxilación microbiana, principalmente de microorganismos contaminantes. De los aminoácidos se producen aminas biógenas como tiramina, triptamina, feniletilamina, cadaverina, histamina y putrescina, las cuales afectan al aroma de los embutidos y pueden conllevar un riesgo para salud de los consumidores (Toldrá *et al*., 2001).

Desaminación: puede ser oxidativa o no oxidativa. La desaminación oxidativa se lleva a cabo principalmente por enzimas microbianas generando oxoácidos y amoníaco de los aminoácidos. La desaminación no oxidativa se lleva a cabo por sustituciones en el carbono en posición β del aminoácido, y el oxoácido puede ser transformado a un aldehído por descarboxilación y para más tarde, ser reducido a su correspondiente alcohol u oxidado a su ácido (Toldrá *et al.*, 2001).

Transaminación: el grupo α-amino es transferido al α-carbono de un α-cetoácido generando un nuevo aminoácido (Toldrá *et al.,* 2001).

Además la combinación de estas reacciones bioquímicas puede generar aldehídos, alcoholes, ácidos y ésteres. Por ejemplo, la transaminación-descarboxilación de la leucina da lugar a 3-metilbutanal (Ordóñez *et al*., 1999). En particular, los aminoácidos con cadena lateral ramificada (leucina, isoleucina, valina) pueden ser catabolizados a aldehídos (2 ó 3-metilbutanal o 2-metilpropanal), alcoholes (2 ó 3-metilbutanol o 2-metilpropanol), ácidos (2 ó 3-metilpropanoico). Además, los aminoácidos generados pueden reaccionar con compuestos carbonilo procedentes de la degradación de los lípidos produciendo compuestos volátiles que contribuirán al aroma del embutido. Los *Micrococcaceae* y *D. hansenii* juegan un papel importante en el catabolismo de los aminoácidos (Toldrá, 2008).

2.1.3. Degradación de los lípidos

La lipolisis consiste en la hidrólisis enzimática de los tri-, di- y monoglicéridos y fosfolípidos, presentes en el tejido muscular y adiposo, liberando ácidos grasos libres. Las enzimas involucradas son lipasas endógenas y exógenas, siendo un 60-80 % el porcentaje de contribución por parte de las lipasas musculares (Molly *et al*., 1997), en particular la lipasa ácida (Toldrá *et al*.,

2001). El porcentaje restante es principalmente atribuido a la actividad lipolítica de CNS, mohos y levaduras y en menor medida a las BAL (Toldrá, 2008). En el caso de los fosfolípidos, sólamente las fosfolipasas musculares son responsables de su hidrólisis siendo más importante la fosfolipasa ácida (Toldrá *et al*., 2001). Los lípidos del tejido muscular y adiposo son principalmente triglicéridos mientras que los fosfolípidos representan alrededor del 16-34 % de la grasa muscular. Los ácidos grasos liberados son generalmente ácidos grasos poliinsaturados. Por tanto, los fosfoslípidos son fácilmente hidrolizados, ya que tienen una mayor proporción de ácidos grasos poliinsaturados, mientras que los triglicéridos son hidrolizados preferiblemente en la posición 1 y 3, ocupada por ácidos grasos insaturados (Ordóñez *et al*., 1999).

Los ácidos grasos libres generados contribuyen directamente al sabor e indirectamente al aroma a través de reacciones posteriores de oxidación microbiana (β-oxidación) o química (autoxidación lipídica).

2.1.3.1. β-oxidación

En general, mediante la β-oxidación, los ácidos grasos saturados generan grupos acetilCoA mediante cuatro reacciones (oxidación por FAD, hidratación, oxidación por NAD^+ y tiólisis). Sin embargo, los intermediarios de esterCoA pueden ser liberados y convertidos a α-cetoácido mediante la actividad tioesterasa y luego a metilcetona generando alcoholes como 1-octen-3-ol vía decarboxilasa y dehidrogenasa (Montel *et al*., 1998).

2.1.4. Producción de ésteres

Los ésteres se producen mediante la esterificación de un ácido con un alcohol. Los ésteres son compuestos aromáticos, que tienen un bajo umbral de detección e imparten notas afrutadas enmascarando el aroma rancio de los embutidos (Stahnke, 1994). En general, los ésteres generados en los embutidos son los ésteres etílicos (Stahnke, 2002). La actividad esterasa de los microorganismos depende del pH, temperatura, disponibilidad de sustrato y de la cepa (Talon *et al*., 1998). Los microorganismos involucrados en la formación de ésteres son principalmente *Staphylococci*, levaduras y mohos (Stahnke, 2002).

2.2. Reacciones químicas implicadas en el aroma de los embutidos curado-madurados

2.2.1. Oxidación de los lípidos

Dado el gran porcentaje de grasa que contienen los embutidos, la oxidación lipídica juega un papel importante en la generación de compuestos volátiles. Los ácidos grasos insaturados son los más susceptibles a esta

oxidación. Este proceso consta de tres etapas: iniciación, propagación y terminación (Figura 3). La oxidación se inicia por la presencia de catalizadores como luz, calor, humedad, cationes metálicos o enzimas oxidativas musculares (peroxidasas o ciclooxigenasas) provocando la pérdida de un átomo de hidrógeno de un ácido graso formando un radical libre. En la segunda etapa o propagación, el radical libre formado reacciona con el oxígeno para formar un radical peroxilo, el cual sustrae un hidrógeno de otro ácido graso para formar un hidroperóxido y un radical libre, iniciando así la reacción de propagación de nuevo. Por último, en la etapa de terminación, los radicales peroxilo reaccionan entre sí formando productos no radicalarios. Y los hidroperóxidos reaccionan con las proteínas, péptidos, aminoácidos. A continuación, se dan a cabo una serie de reacciones secundarias como polimerizaciones o descomposiciones generando compuestos de bajo peso molecular. De los productos producidos en estas reacciones (furanos, ácidos, alcoholes, aldehídos, cetonas), los aldehídos son los compuestos aromáticos más importantes en embutidos curado-madurados (Stahnke, 1995).

Iniciación: $RH + In\ (iniciador) \rightarrow R^{\bullet} + InH$
Propagación: $R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$
$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$
Terminación: $ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$
$ROOH \rightarrow$ Productos de descomposición

Figura 3. Etapas de la autooxidación de los lípidos. (Adaptado de Kołakowska, 2003).

2.2.2. Degradación de los ribonucleótidos

La degradación de los ribonucleótidos contribuye indirectamente a la generación de compuestos volátiles. A partir de inosinfosfato u otros ribonucleótidos se libera ribosa la cual participa en la reacción de Maillard generando compuestos aromáticos activos (Toldrá & Flores, 2007).

2.2.3. Degradación de la tiamina

La degradación de la tiamina puede ser térmica o ácida, pero dado las condiciones aplicadas a este tipo de producto será la degradación ácida la que probablemente tenga lugar (Güntert *et al*., 1990). A partir de esta vitamina se generan compuestos volátiles azufrados de gran potencia aromática, dado su bajo umbral de detección (Mottram, 1998), como son furanos, furantioles, tiofenos, tiazoles y compuestos azufrados alifáticos.

2.2.4. Reacción de Maillard

Esta reacción se lleva a cabo entre un azúcar reductor con un grupo amino libre, procedente de un aminoácido o un péptido, generando una gran variedad de compuestos aromáticos. La reacción de Maillard comprende numerosas reacciones complejas de condensación, deshidratación, reordenación y degradación para formar furanos, furfural, aldehídos, dicarbonilos, cetonas, pirazinas, compuestos azufrados (sulfuros, tiazoles, tioles y tiofenos) (Mottram, 1991). Además, los productos de esta reacción pueden reaccionar de nuevo con compuestos carbonilo de origen lipídico generando una gran variedad de compuestos volátiles. Esta reacción requiere un aporte de calor, que en los embutidos es suave pero, la baja a_w, el largo proceso de maduración y la gran cantidad de aminoácidos libres facilitan su transcurso (Ordóñez *et al*., 1999).

2.2.5. Degradación de Strecker

La reacción de Strecker consiste en una desaminación oxidativa y una descarboxilación de un α-aminoácido en presencia de un dicarbonilo. Los dicarbonilos provienen de la degradación de azúcares o reacción de Maillard. Los productos de la reacción son un aldehído, con un átomo de carbono menos que su aminoácido original, y una α-aminocetona. Las aminocetonas son intermediarios en la formación de compuestos heterocíclicos, como pirazinas, oxazoles y tiazoles (Mottram, 1991). Algunos ejemplos son acetaldehído, propanal, 3-metilbutanal, 2-metilbutanal, 2-metilpropanal, fenilacetaldehído y metional. Es importante mencionar, el papel de los aminoácidos azufrados (cisteína y metionina) en esta reacción por la generación de compuestos volátiles azufrados muy reactivos (sulfuro de hidrógeno) que serán precursores para la formación de otros compuestos volátiles azufrados con bajo umbral de detección (Toldrá & Flores, 2007).

2.3. Especias y condimentos

Tradicionalmente, especias y condimentos se han añadido en la elaboración de embutidos curado-madurados dependiendo del área geográfica, generalmente pimienta, ajo, mostaza, romero, orégano, cebolla. Por lo tanto, los compuestos volátiles presentes en los embutidos dependerán de la especia añadida. A modo de ejemplo, la pimienta negra proporciona terpenos, el ajo o pimentón compuestos azufrados y 3-hexenol, respectivamente (Toldrá & Flores, 2007).

2.4. Influencia de NaCl y grasa en el aroma de embutidos curado-madurados

2.4.1. Cloruro de sodio

El NaCl tiene una gran influencia en la percepción y generación de los compuestos volátiles. La percepción de los aromas no sólo depende de su volatilidad sino también, de la interacción con la matriz que facilitará o impedirá su liberación. El NaCl facilita la liberación de los compuestos volátiles desde la matriz ya que, cambia la presión osmótica y hace que los compuestos volátiles sean menos solubles dentro de la matriz alimentaria (Guichard, 2002). Sin embargo, este efecto es más pronunciado para los alcoholes que para los aldehídos y menos significativo para los ésteres (Poll & Flink, 1984).

Por otra parte, el NaCl afecta a la generación de los compuestos volátiles ya que, tiene un papel regulador de la actividad enzimática tanto muscular como microbiana. Por lo tanto, las reacciones principales para el desarrollo del aroma (lipólisis y proteólisis) se van a ver afectadas por la concentración de NaCl presente en el producto. La actividad de catepsinas y aminopeptidasas, excepto la aminopeptidasa B y m-calpaína (Flores *et al*., 1993; Rosell & Toldrá, 1996), se ve inhibida por concentraciones altas de NaCl (Rico *et al*., 1990, 1991; Toldrá *et al*., 1992, 1993). En el caso de las enzimas proteolíticas micobianas, éstas se ven afectadas o no por el contenido de sal dependiendo de la enzima y del microorganismo (Sanz & Toldrá, 1997; Khalid & Marth, 1990).

Respecto a la actividad lipolítica endógena, la actividad de la lipasa ácida se ve activada cuando aumenta la concentración de sal y disminuye la a_w mientras que, las lipasas básicas y neutras son inactivas a valores de a_w reducidos y, ésta última se ve inhibida por un incremento de la concentración de sal (Motilva & Toldrá, 1993). Y la actividad lipolítica microbiana de *Staphylococci xylosus* se ve inhibida a altas concentraciones de sal (Kenneally *et al*., 1998).

Además de su contribución en la lipólisis, también puede contribuir en la oxidación lipídica generando compuestos volátiles. Sin embargo, este efecto sobre la oxidación lipídica no está claro. Algunos autores han encontrado un efecto prooxidante del NaCl en carne y productos cárnicos (Kanner *et al*. 1991; Shahidi *et al*., 1988) mientras que, otros no han observado dicho efecto (Sárraga & García-Regueiro, 1998; Zanardi *et al*., 2010). El efecto prooxidante del NaCl se ha atribuido principalmente a su capacidad para desplazar los iones hierro quedando éstos libres y pudiendo participar en las reacciones de oxidación (Ordóñez *et al*., 1999).

No obstante, apenas se ha determinado el efecto del contenido de NaCl sobre la generación del aroma en embutidos curados-madurados. Sólo Campagnol *et al*., (2011) estudiaron la generación de los compuestos volátiles en embutidos reducidos en NaCl sustituidos con KCl y con extracto de levadura. En dicho estudio, no se observaron grandes diferencias al reducir un 25 % el contenido de NaCl mientras que una reducción del 50 % conllevó diferencias apreciables en la fracción volátil. A pesar de ello, sensorialmente los consumidores apreciaron diferencias en el aroma tanto con un 25 como con un 50 % de reducción del contenido NaCl. Olesen *et al*. (2004) también indicaron un efecto considerable en la producción de compuestos volátiles cuando llevaron a cabo una reducción de NaCl del 50 %, sin embargo, este efecto fue fuertemente relacionado con el tipo de starter utilizado y con el proceso de secado. Mientras que Ravyts *et al*. (2010) no encontraron diferencias significativas de la reducción de NaCl. No obstante, diferentes características sensoriales se han visto afectadas por la reducción de NaCl en embutidos curado-madurados como el sabor (Gelabert *et al*., 2003), la textura (Gou *et al*., 1996) o el aroma (Campagnol *et al*., 2011).

2.4.2. Grasa

Como hemos visto anteriormente, los lípidos actúan como precursores de la generación de compuestos volátiles por medio de la lipolisis seguida por la oxidación lipídica. Además, los ácidos grasos liberados pueden estar involucrados en otras reacciones como reacciones de Maillard, Strecker. Sin embargo, no sólo actúan como precursores sino también, como solvente de los compuestos volátiles ya que, la mayoría de ellos presentan naturaleza hidrofóbica (Van Ruth & Roozen, 2010), por lo tanto, afectan a la liberación y percepción del aroma del embutido. En este aspecto, Chevance & Farmer (1998) señalaron que el menor contenido de grasa produjo una mayor liberación de terpenos en salami.

En la generación de los compuestos volátiles, existe controversia sobre el efecto del contenido de grasa. Por un lado, Muguerza *et al*. (2003) indicaron una mayor oxidación lipídica y abundancia de compuestos volátiles en embutidos reducidos en grasa mientras que, Olivares *et al.* (2011) observaron una menor oxidación y aceptabilidad del aroma. A pesar de ello, las reducciones llevadas a cabo han producido diferentes defectos en la apariencia, dureza, intensidad del sabor y aroma percibida por los consumidores (Liaros *et al*., 2009; Olivares *et al*., 2010).

2.5. Compuestos aromáticos en embutidos curado-madurados

A lo largo del proceso de maduración y secado de los embutidos se generan un gran número de compuestos volátiles. No obstante, no todos ellos contribuyen activamente al aroma del embutido, es decir, sólo aquellos que se encuentran en concentraciones superiores a su umbral de detección de olfacción (Grosch & Schieberle, 2009). Los compuestos aromáticos comprenden un gran número de clases químicas, algunos de ellos siendo muy reactivos y presentes en concentraciones extremadamente bajas (Grosch & Schieberle, 2009). Por lo tanto, los análisis cualitativos o cuantitativos de los compuestos aromáticos se basan en estas características. La elucidación de los compuestos aromáticos lleva los siguientes pasos: extracción de los compuestos volátiles, separación cromatográfica, detección e identificación.

2.5.1. Técnicas de extracción de compuestos aromáticos

En primer lugar, se debe determinar la cantidad de muestra de la que se partirá para detectar incluso aquellos compuestos que se encuentran en concentraciones del rango de ppb o ppt pero que contribuyen al aroma del embutido. La muestra seleccionada debe ser representativa del producto con un aroma adecuado y sin "off-flavor" ni artefactos (Flores, 2011). Dada la gran diversidad de estructuras químicas que comprenden los compuestos aromáticos, y por lo tanto sus propiedades físicas y químicas, es una tarea crítica seleccionar una técnica u otra para la extracción de los compuestos. Por ello, según la propiedad física o química en la que se base la técnica de extracción seleccionada se obtendrá un perfil aromático determinado (Reineccius, 2010).

Los métodos de extracción de compuestos volátiles se dividen principalmente en extracción con disolvente o extracción a partir del espacio de cabeza.

2.5.1.1. Extracción con disolventes

Extracción directa con disolventes

Una de las técnicas más simples y eficaces es la extracción directa con disolventes. Los disolventes más utilizados son éter dietílico, mezcla de éter dietílico/pentano, hidrocarbonos, freons y cloruro de metileno (Parliment, 1997). También es utilizado el CO_2 supercrítico como disolvente. En este caso, se basa en alterar la capacidad solvente del disolvente mediante cambios de presión y temperatura a la cual tiene lugar la extracción (Reineccius, 2010). Bajo condiciones supercríticas del CO_2, éste presenta una gran afinidad por un gran número de compuestos mientras que, los compuestos no volátiles son insolubles.

Así, tras la extracción el disolvente se elimina fácilmente dando un extracto aromático concentrado.

A pesar de ser una técnica atractiva, su coste es muy alto debido al gran consumo de CO_2 y a la necesidad de un equipo de altas presiones. Además, las presiones y temperaturas altas pueden modificar la matriz de la muestra o los compuestos volátiles (Timón *et al*., 1998). En embutidos, no se ha aplicado previamente esta técnica.

Destilación a vapor seguida de extracción con disolventes

La limitación de la extracción directa con disolvente es el arrastre de compuestos no volátiles como los lípidos. Por lo tanto, está ampliamente extendida la combinación de destilación y extracción con disolventes. Los compuestos volátiles se obtienen por destilación de una suspensión acuosa y son condensados mediante trampas refrigeradas para luego extraerlos con disolventes orgánicos. Las principales desventajas son los grandes volúmenes de disolvente utilizados y el paso de concentración requerido que puede conllevar la formación de artefactos.

Destilación a vapor con extracción simultánea

El equipo Lickens-Nickerson (1964) (Figura 4) fue ampliamente utilizado para llevar a cabo la destilación y extracción de forma simultánea. Como consecuencia del calentamiento los compuestos volátiles en el vapor se transfieren al disolvente y ambos son condensados. El extracto que se obtiene, se seca con sulfato de sodio anhidro y concentra antes del análisis. Las principales desventajas son la pérdida de compuestos de bajo punto de fusión en la concentración del extracto y formación de artefactos como consecuencia del calentamiento. En embutidos, fue una técnica comúnmente utilizada en el siglo XX (Mateo & Zumalacárregui, 1996).

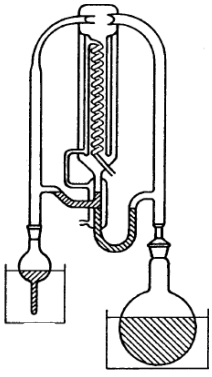

Figura 4. Equipo Lickens-Nickerson (1964).

Destilación a alto vacío

Una mejora de la última técnica fue introducida por Engel & Schieberle (1999), al combinarla con alto vacío diseñando el aparato "solvent-assisted flavour evaporation" (SAFE) (Figure 5), aumentando así la eficacia de extracción y reduciendo el tiempo de análisis ya que, se pueden introducir directamente alimentos acuosos sin previa extracción con disolventes (Reineccius, 2007). Además, el extracto obtenido puede ser fraccionado dando como resultado un incremento de la separación de los compuestos facilitando la identificación de compuestos minoritarios.

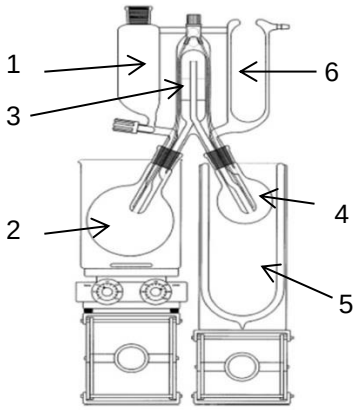

Figura 5. Equipo solvent assisted flavour evaportation (SAFE). (Adaptado de Engel & Schieberle, 1999).

Brevemente, el procedimiento a seguir con este equipo es el siguiente. Después de aplicar alto vacío (5 mPa), la destilación comienza al dejar caer la muestra líquida o extracto desde el embudo (Figura 5, nº 1) hasta el matraz de fondo redondo (Figura 5, nº 2), el cual es calentado a 35-40 ºC en un baño de agua. Los compuestos volátiles junto con el disolvente son transferidos al cuerpo de destilación del equipo (Figura 5, nº 3), donde posibles impurezas se retienen en la espiral. A continuación, el destilado se condensa en el matraz (Figura 5, nº 4) que se encuentra sumergido en nitrógeno líquido (Figura 5, nº 5). También se adiciona nitrógeno líquido en el embudo superior (Figura 5, nº 6) para proteger a la bomba de alto vacío. Söllner & Schieberle (2009) aplicaron esta técnica identificando un gran número de compuestos aromáticos en un embutido de "salami tipo húngaro".

2.5.1.2. Desorción térmica directa

Se basa en transferir los compuestos desde la matriz, generalmente por calentamiento, directamente a la cabeza de columna del cromatografo (Grimm *et al*., 1997). Es una técnica sencilla y rápida que permite el análisis cualitativo de la muestra mientras que, el análisis cuantitativo es menos reproducible por variaciones en la purga, pérdidas de compuestos termolábiles en el puerto de inyección, contaminantes y contaminación en el cromatógrafo de gases. En embutidos, no se ha aplicado previamente esta técnica.

2.5.1.3. Técnicas de extracción del espacio de cabeza

Estas técnicas se basan en la volatilidad de los compuestos por lo tanto, se analiza la atmosfera adyacente a la muestra introducida en un vial sellado. De esta forma se analizan los compuestos volátiles que están en la fase gaseosa a una concentración detectable por la nariz humana. Son técnicas rápidas, sencillas, de fácil preparación, libre de disolventes y escasa probabilidad de formación de artefactos.

La cuantificación de los compuestos volátiles mediante las técnicas de extracción del espacio de cabeza cuantifican la concentración presente en el espacio de cabeza, por tanto, no cuantifican la concentración total presente en la muestra. Además, la concentración en el espacio de cabeza depende del coeficiente de distribución de cada compuesto volátil entre el espacio de cabeza y la matriz (Wampler, 1997). Dicho coeficiente resulta complicado de determinar dada la complejidad de determinadas matrices, como los embutidos, siendo por lo tanto, difícil establecer una relación entre la concentración del espacio de cabeza y la presente en la matriz de la muestra.

Las técnicas de espacio de cabeza se dividen principalmente en: espacio de cabeza estático, dinámico o purga y trampa, microextracción en fase sólida (Solid-Phase Microextraction, SPME) y extracción por absorción del espacio de cabeza (Headspace Sorptive Extraction, HSSE).

Espacio de cabeza estático

En este caso, un pequeño volumen (1 ml) de la atmosfera alrededor de la muestra se inyecta directamente en la columna del cromatógrafo de gases (GC). La concentración mínima que detecta GC-MS (cromatografía de gases acoplada a espectrometría de masas) en 1 ml es 10^{-4} g/l, y generalmente la concentración de los compuestos volátiles en el espacio de cabeza de los alimentos se encuentran en el rango de 10^{-4}-10^{-10} g/l (Reineccius, 2010), por lo tanto, esta técnica presenta problemas de sensibilidad.

Espacio de cabeza dinámico

Consiste en arrastrar los compuestos volátiles presentes en el espacio de cabeza con una corriente de gas inerte (nitrógeno o helio). Seguidamente, los volátiles quedan atrapados en trampas criogénicas, Tenax®, carbón activado u otros, de ahí el nombre de la técnica de purga y trampa. Más tarde la desorción de los compuestos tiene lugar por calentamiento de la trampa. Esta técnica presenta mayor sensibilidad que la técnica de espacio de cabeza estático pero, conlleva mayor complejidad y coste. Además, el tipo de trampa utilizado determinará el perfil de compuestos volátiles. En embutidos, esta técnica empleando Tenax® como material adsorbente se ha utilizado ampliamente (Viallon *et al*., 1996; Berdagué *et al*., 1993; Meynier *et al*., 1999).

Microextracctión en fase sólida (SPME)

Es una técnica rápida, simple y libre de disolventes, basándose en la previa concentración de los volátiles en el espacio de cabeza para más tarde exponer una fibra de sílice recubierta por una fase estacionaria (Arthur & Pawliszyn, 1990). Los compuestos volátiles se van extrayendo de la muestra hacia la fibra hasta que se alcanza el equilibrio y se desorben en el puerto de inyección de un cromatógrafo de gases (Figura 6). Las fases estacionarias disponibles comercialmente son: polidimetilsiloxano (PDMS), carboxen (CAR), divinilbenceno (DVB), poliacrilato (PA), carbowax (CW) y polietilenglicol (PEG). En general, se utilizan mezclas de fases estacionarias para conseguir mayor eficacia en la extracción, tanto de compuestos apolares como polares. Sin embargo, los compuestos extraídos dependen de los siguientes factores: su polaridad, volatilidad, coeficientes partición aceite/agua, el volumen de muestra, el volumen de espacio de cabeza, el ratio de agitación, el pH de la disolución, de la temperatura, fase estacionaria. Actualmente, existen muestreadores del espacio de cabeza automáticos así, permiten controlar la temperatura e incluir agitación de la muestra. En embutidos, su uso se encuentra ampliamente extendido (Marco *et al*., 2007; Andrade *et al*., 2010; Olivares *et al.*, 2011).

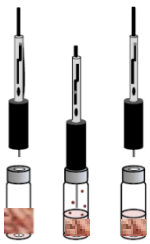

Figura 6. Procedimiento de extracción por SPME.

Una variante de esta técnica es SPME múltiple, se basa en hacer extracciones consecutivas de los compuestos volátiles del espacio de cabeza, es decir, exponer la fibra SPME en el espacio de cabeza de la muestra repetidas veces en intervalos de tiempo idénticos. De esta forma se elimina el efecto matriz de la muestra permitiendo la cuantificación de los compuestos volátiles presentes en la muestra (Kolb *et al*., 1982). Esta variante de SPME se ha utilizado en la extracción y cuantificación de compuestos volátiles en embutidos (Olivares *et al*., 2009).

Extracción con barra magnética (SBSE o HSSE)

Es una nueva versión de SPME por lo tanto, se basa en el mismo fundamento. En este caso se coloca una barra agitadora con PDMS como material absorbente, llamada comercialmente Twister®, en el espacio de cabeza (Headspace sorptive extraction, HSSE) o sumergida en la muestra (stir bar sorptive extraction, SBSE) (Baltussen *et al*., 1999). La principal diferencia es la gran cantidad de material absorbente (PDMS) que contiene la barra (25-125 µl) en contraste con las fibras de PDMS de SPME (0,6 µl). Del mismo modo que SPME, la barra se desorbe automáticamente en el puerto de inyección. Hoy en día, comercialmente sólo hay disponibles Twister® de PDMS o PEG aunque, en el futuro se esperan nuevos materiales (Bicchi *et al*., 2005). En embutidos no se ha aplicado previamente esta técnica.

2.5.2. Técnicas de separación de compuestos aromáticos

Para determinar los compuestos aromáticos de una matriz compleja, como es el embutido, es necesaria una separación eficaz de los compuestos. La eficacia en la separación y detección facilitará la identificación de los compuestos. Generalmente, para el análisis de compuestos volátiles en embutidos se utiliza la cromatografía de gases con columnas capilares recubiertas de una película de fase estacionaria. Es importante seleccionar un tipo u otro de fase estacionaria según la muestra a analizar. Los criterios principales para la selección de la columna son la selectividad y la polaridad. La selectividad vendrá ligada a la capacidad de la fase estacionaria de diferenciar entre dos moléculas por sus propiedades físicas y químicas. Mientras que la polaridad viene determinada por la estructura de la fase estacionaria y ejercerá el efecto de separación. Existen tres tipos de columnas según su polaridad: apolares, de polaridad intermedia y polares.

Sin embargo, la coelución, las bajas concentraciones de los compuestos aromáticos, la similitud de estructuras químicas o la preseparación de la muestra a menudo no permiten elucidar completamente el aroma de los embutidos

mediante GC-MS (Olivares *et al*., 2011). En este sentido, la cromatografía multidimensional (MDCG) es una alternativa y una técnica muy potente para conseguir una separación más completa de los compuestos volátiles debido a su alta resolución por el uso de dos columnas cromatográficas. El concepto de MDGC es prácticamente similar a las operaciones realizadas para GC, para el cual se utiliza una columna para obtener una fracción separada parcialmente, la cual se reinyecta en una segunda columna, normalmente de una fase estacionaria opuesta para lograr una mayor separación. La gran diferencia es que no se requiere la recolección del efluente obtenido de la primera columna ya que, las dos columnas están interconectadas y la transferencia es directa mediante válvulas o conmutadores neumáticos (Herrero *et al*., 2009). Se debe diferenciar entre MDCG convencional (CG-CG) y la MDCG comprensiva (CGxCG), no obstante, existen diferentes diseños de cada una de ellas según los detectores y la interfase utilizada.

2.5.2.1. Cromatografia de gases multidimensional convencional ("Heart-cut") (GC-GC)

La MDCG convencional o "heart-cut" consiste en hacer pasar una porción o efluente de la primera columna a una segunda columna. Existen diferentes configuraciones (Herrero *et al*., 2009) pero, la configuración básica consiste en dos hornos independientes con dos columnas analíticas y varios detectores. Comúnmente, el efluente de la primera columna se conecta a un detector universal, como un detector de ionización de llama, y la segunda columna a un espectrómetro de masas (Figura 7). De esta forma, podemos reanalizar una zona concreta del cromatograma resultando en un cromatograma más sencillo.

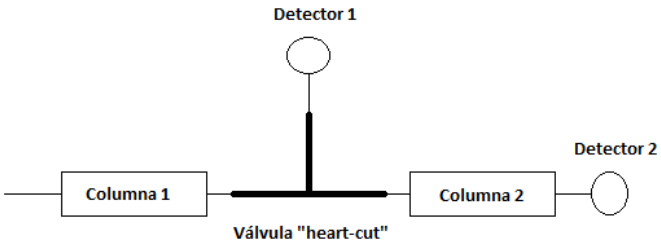

Figura 7. Esquema básico de la MDCG convencional (Adaptado de Herrero *et al*., 2009)

Los inconvenientes que puede presentar son el uso de más detectores, el tiempo de análisis es mayor que con una sola columna, tampoco permite hacer un corte ilimitado antes de la segunda columna sino que, varios cortes son

necesarios para obtener una separación completa. Por ello, el tiempo de análisis para cada columna es significativamente mayor con respecto a la GCxGC (Lewis, 2002) (como se describe en el apartado 2.5.2.2.).

2.5.2.2. Cromatografia de gases multidimensional comprensiva (GCxGC)

En los años 90 se introdujo esta técnica y supuso un gran avance científicamente (Marriott, 2002). Básicamente, consiste en reanalizar el efluente de la primera columna en una segunda columna de diferente selectividad de la fase estacionaria. La gran diferencia frente a la MDCG convencional es la transferencia total de todo el efluente de la primera columna a la segunda columna mediante un modulador, dentro de un tiempo fijado (4-8 segundos), cuyo objetivo es retener, enfocar y transferir todas las fracciones eluidas de la primera columna a la segunda columna a intervalos de tiempo previamente determinados. Las columnas utilizadas son de diferente polaridad, generalmente, la fase estacionaria de la primera columna es menos polar que la de la segunda por lo tanto, la separación en la primera columna se basa en las propiedades del punto de ebullición de los compuestos mientras en la segunda columna se debe a su polaridad. La primera columna generalmente es de 15-30 m x 0,25-0,32 mm de diámetro interno y grosor de la película de 0,1-1 µm. El modulador normalmente separa el eluyente de la primera columna en una gran número de pequeñas fracciones adyacentes, las cuales son redirigidas e inyectadas en la segunda columna que es más corta y estrecha (1-2 m x 0,1 mm x 0,1 µm). La separación en la segunda columna es rápida de 1-10 segundos en contra de 45-120 min de la separación de la primera columna. Las columnas pueden estar en el mismo horno estando sometidas así a las mismas temperaturas mientras que, en otros diseños la segunda columna está colocada en un segundo horno permitiendo más flexibilidad y temperaturas independientes.

Una pieza crítica es el dispositivo que permite la conexión de las dos columnas. Diversas revisiones han tratado este ítem en profundidad (Herrero *et al*., 2009). Hoy en día, los modulares disponibles en el mercado son más robustos y la mayoría se basan en sistemas de criofocalización doble (Marriott, 2002). La válvula tipo Deans (Deans, 1968) permite la transferencia selectiva de fracciones del efluente de la primera a la segunda columna mediante el control de flujos y presiones del sistema. Además, puede ser necesario un punto de criofocalización al inicio de la segunda columna para favorecer el estrechamiento de las bandas cromatográficas en el segundo horno.

Algunos inconvenientes son su alto coste y mantenimiento, la dificultad de su optimización (frecuencia de modulación, temperatura del modulador), la

necesidad de detectores rápidos debido a la gran velocidad y generación de datos, un software sofisticado y potente. Además, de la difícil interpretación de los cromatogramas, dada su complejidad.

En el caso de los embutidos, la cromatografía multidimensional no se ha utilizado previamente para la identificación de compuestos aromáticos. Sin embargo, Söllner & Schieberle (2009) utilizaron la cromatografía multidimensional (sistema de cromatografía correlativa espectral de componentes múltiples (MCSS)) para la cuantificación de compuestos aromáticos en embutidos tipo húngaro. Este sistema es un método de algoritmo quimiométrico que consiste en la construcción de una matriz ortogonal utilizando espectros obtenidos de la muestra y por comparación de los espectros de compuestos puros y mixtos (muestras) que se proyectan en la matriz y se observa la presencia o ausencia de correlación cromatografíca espectral. En general, MCSS se ha utilizado en el campo de la medicina (Hu *et al*., 2004).

2.5.3. Detectores cromatográficos de gases

Una vez los compuestos se han separado cromatográficamente deben de ser detectados y posteriormente identificados. Los detectores son dispositivos que miden los solutos en la corriente del gas portador, convirtiendo la señal en una señal eléctrica que es amplificada y registrada en el momento de salida de la columna. De forma genérica, los detectores pueden clasificarse en detectores universales, que responden a cualquier compuesto que eluye de la columna, y en detectores específicos o selectivos que responden solamente a un grupo limitado de compuestos o a una determinada propiedad fisicoquímica dando cromatogramas más simplificados. En los últimos años, las investigaciones se han centrado en determinar los compuestos volátiles con capacidad aromática en los embutidos curado-madurados. Para ello, dichos compuestos deben estar presentes en concentraciones superiores a su umbral de percepción. No obstante, muchos de ellos se encuentran a nivel traza y es una labor tediosa identificarlos, de ahí el uso de detectores específicos. En la Figura 8 se muestra la sensibilidad de los detectores más utilizados.

En embutidos curado-madurados, tan sólo se ha utilizado el detector FID (Schmidt & Berger, 1998 a, b), como detector universal, y el olfatometro para determinar los compuestos aromáticos (Marco *et al*., 2007), como detector específico. En la mayoría de los casos, el olfatometro se ha acoplado a un CG-FID (Stahnke, 1994, 1995; Meynier *et al*, 1999; Chevance *et al.*, 2000; Söllner & Schieberle, 2009; Olivares *et al*., 2011). A continuación se describen los diferentes tipos de detectores específicos y universales.

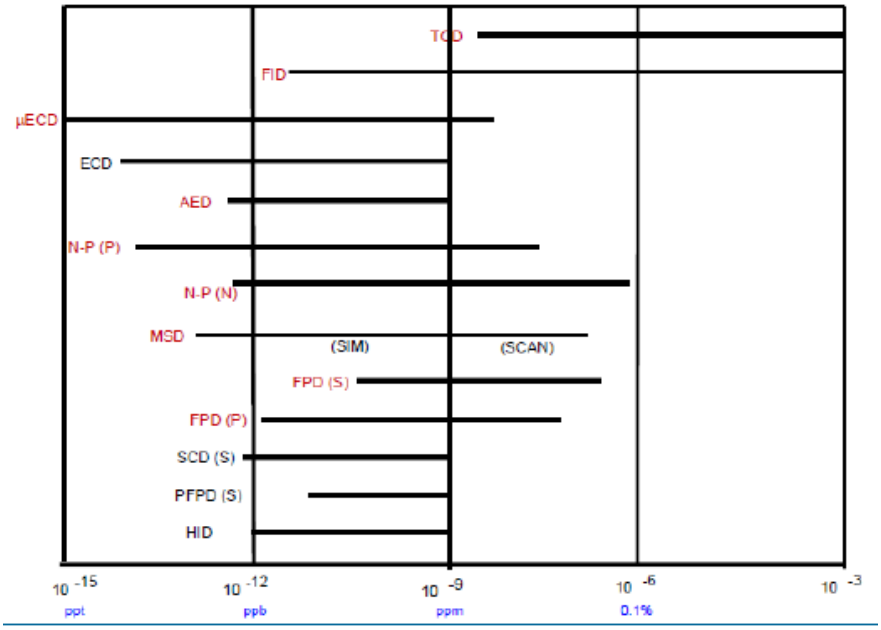

Figura 8. Sensibilidad de los detectores de CG más utilizados (siglas definidas en listado) (Agilent Technologies, 2011).

2.5.3.1. Detectores específicos

Detector fotométrico de llama (FPD)

Básicamente consiste en una llama de hidrógeno reductora que produce especies quimioluminiscentes que emiten una luz característica filtrada por la longitud de onda deseada, la cual determinará que componentes son detectados. Los filtros más comunes son para la detección de compuestos azufrados y fosforados en mezclas complejas y su límite de detección 10-10^{12} pg/s (Wardencki & Zygmunt, 1991).

Detector fotométrico de llama pulsado (PFPD)

Su configuración se basa en una fuente de llama y un flujo de gases combustibles pero, la llama no es continua como en el FPD. Tiene una cámara de combustión donde por su parte inferior entran los dos flujos de gas combustibles (hidrógeno y aire). Los gases se queman en la llama, se propagan, y la llama se apaga. El flujo continuo de gases elimina productos de la combustión y facilita el reencendido de la llama. La emisión de carbonos se completa en unos pocos milisegundos mientras que la emisión de compuestos

azufrados empieza después de la combustión, por tanto, es capaz de detectar compuestos azufrados a menor concentración. La emisión se filtra y se detecta en un tubo fotomultiplicador. La sensibilidad y selectividad de hidrocarbonos con azufre, fósforo y nitrógeno de PFPD (límite de detección de 0,01-2 pg/s) es mayor que FPD trabajando con llama continúa (Jing, 1998).

Detector de quimioluminiscencia de azufre (SCD)

Se basa en la formación de monóxido de azufre procedente de compuestos que contienen azufre por combustión en una llama reductora de hidrógeno/oxígeno. Los productos de la combustión se transfieren a una célula de reacción a vacío donde reaccionan con ozono resultando en una reacción de quimioluminiscencia. La luz que emiten los compuestos se monitoriza en un tubo fotomultiplicador. La sensibilidad de este equipo es del orden de 1 pg/s (Yan, 2006).

Detector de nitrógeno-fósforo (NPD)

También se denomina detector termoiónico (TID) o detector de nitrógeno de ionización de llama (N-FID). El efluente de la columna se mezcla con hidrógeno, pasa a través de una llama y se combustiona. El gas fluye alrededor de una esfera cerámica de silicato de rubidio calentada eléctricamente, la cual se mantiene a unos 180 V con respecto al colector. La esfera caliente forma un plasma que alcanza una temperatura de 600 a 800 ºC. Lo que ocurre exactamente en el plasma, que hace que se produzcan una gran cantidad de iones a partir de las moléculas que contienen fósforo o nitrógeno, realmente no está bien establecido, pero el resultado es una gran corriente de iones, la cual se utiliza para la determinación de compuestos que contienen P y N. El límite de detección de este detector es de 10^{-2}-10^{4}pg/s (Rubinson & Rubinson, 2001).

Detector de quimioluminiscencia de nitrógeno (NCD)

Es un detector específico para nitrógeno que genera una respuesta equimolar y lineal a los compuestos de nitrógeno basada en una reacción quimioluminiscente del NO con el ozono. Su fundamento es similar al SCD y su sensibilidad está en el rango de pg/s (Yan, 2006).

Detector de captura de electrones (ECD)

El efluente de la columna pasa sobre un emisor β, como niquel-63 o tritio (adsorbido sobre lámina de platino o titanio). Un electrón (e-) del emisor provoca la ionización del gas portador (con frecuencia N_2) y la producción de una ráfaga de e-. De este proceso de ionización, en ausencia de especies orgánicas, resulta una corriente constante entre un par de electrodos. Sin embargo, la corriente disminuye en presencia de moléculas orgánicas que tienden a capturar

electrones. Este detector es sensible a grupos funcionales electronegativos: halógenos, peróxidos, quinonas y grupos nitro. En cambio, no es sensible a aminas, alcoholes e hidrocarburos. Presenta un límite de detección alrededor de 10^{-3} pg/s. (Rubinson & Rubinson, 2001)

Detector de fotoionización (PID)

Utiliza luz ultravioleta (106-150 nm) para ionizar los componentes que eluyen de la columna. Los iones producidos son recogidos por electrodos siendo la corriente generada la medida de la concentración del analito. Es un método no destructivo ya que, los iones se recombinan al descargarse en los electrodos para volver a la forma de la molécula original. Es selectivo para compuestos alifáticos, aromáticos, hetrocíclicos, organosulfurados y algunos organometálicos, cetonas, ésteres, aldehídos y aminas con una sensibilidad de $1\text{-}10^{4}$ pg/s (Rubinson & Rubinson, 2001).

Olfatómetro

Emplea la nariz humana como detector, es decir, una persona entrenada puede oler el efluente de la columna y describir el olor percibido, su intensidad, duración, etc. La información proporcionada por el detector se registra y el cromatograma obtenido se denomina aromagrama. Existen diversas técnicas olfatométricas que se clasifican en: análisis de dilución, de tiempo-intensidad y frecuencia de detección.

Las técnicas de dilución son las más empleadas y se basan en realizar diluciones sucesivas del extracto aromático hasta que los panelistas no detectan ningún aroma. Se requiere un bajo número de panelistas y se representa generalmente como CHARM (Método de Respuesta Aromática-Hedónica Combinada) (Acree *et al*., 1984), AEDA (Análisis de Dilución de Extracto Aromático) (Ullrich & Grosch, 1987), AECA (Análisis de Concentración de Extractos Aromáticos) y DHDA (Análisis de Dilución de Espacio de Cabeza Dinámico) (Mistry *et al*., 1997).

Las técnicas de tiempo-intensidad se basan en clasificar los compuestos aromáticos según su intensidad y la duración de tiempo en el que es percibido. El número de inyecciones es menor pero se requiere mayor número de personas y requieren un mayor entrenamiento. Las técnicas más comunes son OSME (viene de la palabra griega aroma) e intensidad posterior (Delahunty *et al*., 2006).

Las técnicas de frecuencia de detección se basan en oler un mismo extracto por varios panelistas en sesiones diferentes, posteriormente se relaciona la contribución de un compuesto al aroma con el número de veces detectado, por lo tanto en este caso, no se requieren personas entrenadas en

escalas de intensidad. Se distinguen NIF (Frecuencia de Impacto Nasal) y SNIF (Superficie de la Frecuencia de Impacto) (d'Acampora Zellner *et al*., 2008).

De estas técnicas, sólo AEDA (Schmidt & Berger, 1998 a, b; Blank *et al*., 2001; Söllner & Schieberle, 2009) y NIF (Chevance *et al*., 2000; Marco *et al*., 2007; Olivares *et al*., 2011) se han utilizado en embutidos curado-madurados para determinar la potencia aromática de sus compuestos volátiles. Y en el caso de los estudios de Croizet *et al*., (1992), Stahnke (1994, 1995) y Meynier *et al*., (1999) tan sólo describieron los aromas presentes en el efluente de la GC.

2.5.3.2. Detectores universales

A menudo, los detectores específicos se encuentran acoplados en un CG con otro detector universal para facilitar la identificación de los compuestos detectados. Los detectores universales más comunes son:

Detector de conductividad térmica (TCD)

Es un detector simple, no destructivo, con una sensibilidad baja (alrededor de 10^{-5}-10^{-6} g/s de gas portador). Se basa en los cambios de la conductividad térmica del gas portador ocasionados por la presencia de las moléculas del componente a analizar. El sensor consiste en un elemento calentado eléctricamente cuya temperatura, a una potencia eléctrica constante, depende de la conductividad térmica del gas circundante. El elemento calentado puede ser un hilo fino de platino, oro, wolframio o un termistor semiconductor. La resistencia del hilo o del termistor da una medida de la conductividad térmica del gas (Ševčik, 1976).

Detector de ionización de llama (FID)

Es uno de los detectores más utilizados para desarrollar nuevos métodos de análisis, dado que es un detector casi universal, ya que responde en base al número de carbonos y otros elementos como halógenos y oxígenos presentes que reducen la combustión (Holm, 1999). Consiste en una llama de hidrógeno/aire y una placa colectora. Las moléculas que salen de la columna pasan a través de la llama, la cual rompe las moléculas orgánicas y produce iones. Los iones son colectados en un electrodo parcial y produce una señal eléctrica. Es muy sensible a un amplio rango de concentraciones (límite de detección 100 pg/seg) pero su principal inconveniente es la destrucción de la muestra.

Detector de ionización de helio (HID)

Utiliza una fuente radiactiva, generalmente β-emisor, para generar especies de helio metaestables. Éstas tienen una energía hasta 19,8 eV y pueden ionizar todos los compuestos excepto el neón, el cual tiene un potencial

de 21,56 eV. Los compuestos eluyen de la columna y colisionan con las especies de helio metaestables, las cuales luego ionizan los componentes. Su principal ventaja es el uso de helio tanto como gas acarreador como gas ionizante (Ševčik, 1976).

Detector de ionización de helio de descarga pulsada (PDHID)

Se basa en un pulso de voltaje entre dos electrodos en una corriente de helio circulando por encima de la presión atmosférica. La emisión de luz del helio excitado ioniza los analitos presentes. Si se mezcla un dopante como el CH_4 con el helio o con el efluente de la columna, el detector funciona como un ECD. El dopado con otros gases inertes provoca un comportamiento similar al de un PID. El PDHID es un excelente sustituto a los FID en ambientes peligrosos donde el uso de nitrógeno o de llamas puede ser un problema, como refinerías de petróleo o petroquímicas. Su sensibilidad está en el rango de pg/s (Rubinson & Rubinson, 2001).

Detector de emisión atómica (AED)

El gas de la columna se introduce en un plasma de helio obtenido por inducción de microondas, el cual se acopla a un espectrómetro óptico de emisión de diodos en serie. El plasma atomiza los elementos de la muestra, los excita y se obtienen espectros de emisión atómica. La radiación emitida se colima y se refleja en una red de difracción, descomponiéndose en sus longitudes de onda individuales, y se recoge en una fila de diodos. Otras fuentes de plasma son la corriente directa de argón (DCP) y el plasma de argón acoplado por inducción (ICP). La sensibilidad de este detector es del orden de 10^{-11} g/s (Wardencki & Zygmunt, 1991).

2.5.4. Identificación de compuestos aromáticos

La identificación y confirmación de los compuestos aromáticos se lleva a cabo mediante los siguientes métodos:

Tiempo de retención/índice de retención

El tiempo de retención, en términos absolutos, no es un parámetro adecuado para llevar a cabo la identificación de los compuestos. Por tanto, para evitar variaciones en el tiempo de retención originadas por la técnica o hardware, se calcula el tiempo de retención relativo de cada compuesto en base a los tiempos de retención de una serie de alcanos (Kovats, 1965). Estos índices pueden compararse con los de su estándar puro o publicado en la bibliografía para el mismo tipo de columna utilizada (bases de datos: Flavornet, Nist, Pherobase, Odour).

Espectrometría infrarrojo de transformada de Fourier (IR)

Esta técnica proporciona información sobre los grupos funcionales presentes en las moléculas. Se basa en las formas vibracionales de una molécula, la cual viene determinada por su estructura y grupos funcionales (Reineccius, 2010). Las moléculas gaseosas de la muestra se ionizan en el centro de una celda por electrones acelerados. Los iones generados se almacenan en la celda de atrapar iones durante un cierto tiempo, para más tarde someterse a un impulso de radiofrecuencia que aumenta linealmente en frecuencia durante su tiempo de vida. Una vez finaliza el barrido de frecuencias, la placa receptora amplifica la corriente imagen (Rubinson & Rubinson, 2001).

Previamente fue una técnica muy útil para la identificación de los compuestos volátiles. Sin embargo, dado el gran muestreo requerido y la facilidad de acoplamiento de GC con un espectrómetro de masas, actualmente esta técnica se ha visto desplazada (Reineccius, 2010).

Espectrometría de masas (MS)

Los compuestos eluyen de la columna del cromatografo de gases, entran en la fuente de iones del MS donde se ionizan y se generan sus fragmentos. Los fragmentos se separan por su relación masa/carga (m/z) generando un espectro y proporcionando información sobre su estructura. La identificación se lleva a cabo por comparación de los espectros obtenidos con librerías bibliográficas comerciales (Nist, Wiley, Mainlib, Replib). Por una parte, es un detector universal trabajando en modo SCAN, es decir, realizando barridos completos. Por otra parte, puede clasificarse como un detector específico y muy sensible trabajando en modo SIM, realizando barridos de iones seleccionados previamente. A menudo el uso de detectores específicos proporciona información que facilita la interpretación de los espectros obtenidos y por tanto su identificación (Reineccius, 2010).

Confirmación del poder aromático

Una vez confirmada la estructura química de los compuestos volátiles mediante las técnicas anteriores, se debe determinar el poder aromático de los compuestos volátiles. Para ello, existen dos métodos: el cálculo del valor de la actividad aromática (OAV) o análisis olfatométrico (d'Acampora Zellner *et al*., 2008). El OAV se calcula mediante la concentración de un compuesto volátil (C) y su umbral de detección (C_u) utilizando los umbrales de percepción predominantes en el alimento determinado (aire, agua, aceite) (ecuación 1) (Grosch, 2001). Si el OAV de un compuesto volátil es mayor a la unidad, éste contribuirá al aroma del producto ya que, se encuentra presente en

concentraciones superiores a las de su umbral de detección. Sin embargo, esta técnica no tiene en cuenta las interacciones que pueden existir en la matriz del alimento que podrían facilitar o impedir la liberación del mismo.

$$OAV = C/ C_u \quad \textbf{(1)}$$

Tras aplicar las técnicas olfatométricas comentadas anteriormente (apartado 2.5.3.1.), se debe contrastar con la bibliografía si el compuesto químicamente confirmado imparte el olor detectado mediante las técnicas olfatométricas. Como bases de datos se pueden utilizar las siguientes: "Flavornet", "Fenaroli's Handbook of Flavor Ingredients" (Burdock, 2002) o "The Good Scents Company".

2.5.5. Compuestos nitrogenados y azufrados en embutidos curado-madurados

Diversos estudios han enfocado sus investigaciones en elucidar los compuestos aromáticamente activos en los embutidos curado-madurados. Los primeros estudios se centraron en identificar los compuestos aromáticos (Croizet *et al*., 1992; Stahnke, 1994, 1995; Meynier *et al*., 1999) mientras que los más recientes en determinar la potencia aromática mediante diferentes técnicas olfatométricas AEDA (Schmidt & Berger, 1998 a, b; Blank *et al*., 2001; Söllner & Schieberle, 2009) o frecuencia de detección (Chevance *et al*., 2000; Marco *et al*., 2007; Gianelli *et al*., 2011; Olivares *et al*., 2011). No obstante, todavía existen aromas detectados olfatométricamente sin poder identificar su estructura química (Olivares *et al.*, 2011, Blank *et al.*, 2001). En general, dichos aromas recuerdan a frutos secos tostados, snacks, alimento descompuesto, azufrado, ajo, carnoso, caramelo, café, caldo (Stahnke, 1995; Meynier *et al*., 1999; Chevance *et al*., 2000; Blank *et al*., 2001; Marco *et al*., 2007; Söllner & Schieberle, 2009; Gianelli *et al*., 2011; Olivares *et al*., 2011) y probablemente procedan de compuestos volátiles azufrados y nitrogenados ya que, este tipo de "notas" aromáticas recuerdan a los aromas producidos por estos compuestos (Burdock, 2002).

Algunos autores observaron que aquellos aromas que recordaban a ajo o azufrado procedían de compuestos alifáticos azufrados pertenecientes al ajo adicionado como ingrediente en la fabricación del embutido (Meynier *et al*., 1999). Sin embargo, otros autores identificaron compuestos similares en embutidos sin ajo, por tanto, confirmaron su procedencia de la actividad proteólitica de las enzimas musculares o microbianas sobre los aminoácidos azufrados (Stahnke, 1995, Bianchi *et al.*, 2007). El compuesto azufrado con mayor poder aromático encontrado en embutidos es el metional, seguido entre otros por metil-sulfuro de

alilo, bis(2-metil-3-furil)disulfuro, metanotiol, disulfuro de dialilo (Schmidt & Berger, 1998 a; Blank *et al*., 2001, Marco *et al*., 2007; Söllner & Schieberle, 2009). Por otra parte, los compuestos nitrogenados pueden proceder de cualquier fuente nitrogenada como los aminoácidos (Meynier & Mottram, 1995). Estos compuestos no se han elucidado en los embutidos a pesar del potente aroma que evocan. Tan sólo se han encontrado con poder aromático 2-acetil-1-pirrolina, 1-metil-1H-pirrol, 2-acetilpirrol, 2-acetiltiazolina, 3-metilindol y algunas pirazinas (Blank *et al*., 2001; Gianelli *et al*., 2011; Schmidt & Berger, 1998 b; Söllner & Schieberle, 2009).

En la Tabla 5 se recogen todos los compuestos volátiles azufrados y nitrogenados identificados como aromáticamente activos en embutidos curado-madurados. Sin embargo, en otros estudios donde se ha estudiado la fracción volátil del embutido se han identificado diferentes compuestos azufrados y nitrogenados (Tabla 6), pero, en dichos estudios no se describió el carácter aromático de dichos compuestos volátiles.

Como se ha comentado anteriormente, dependiendo de la técnica de extracción utilizada se obtendrá diferente perfil aromático. En embutidos, los primeros estudios de la fracción volátil se determinaron mediante la técnica de espacio de cabeza dinámico (purga & trampa) (Berdagué *et al*., 1993) y en menor medida mediante la técnica de destilación como Likens-Nickerson y destilación a alto vacío o destilación molecular (Schmidt & Berger, 1998 a). Mientras que recientemente la técnica más ampliamente aplicada es la técnica de SPME (Marco *et al*., 2007; Olivares *et al*., 2011). En general, mediante las técnicas de extracción de espacio de cabeza se han detectado compuestos volátiles azufrados de menor peso molecular como metanotiol, metionol, dimetiltrisulfuro, etil metil disulfuro, metilpropilsulfuro, 1-metiltio-1-propeno, 1-metilpirrol (Marco *et al*., 2007, Olivares *et al*., 2011; Meynier *et al*., 1999); mientras que, los compuestos de mayor peso molecular se han identificado mediante técnicas de destilación a alto vacío como 2-metil-3-furil disulfuro, 2-furilmetanotiol (Söllner & Schieberle, 2009). Además mediante las técnicas de destilación a alto vacío únicamente se detectaron compuestos minoritarios como 2-acetil-1-pirrolina o pirazinas (Söllner & Schieberle, 2009; Blank *et al*., 2001) los cuales imparten notas a frutos secos clave para el aroma final del producto.

Por lo tanto, resulta de gran interés conocer los compuestos azufrados y nitrogenados presentes en los embutidos curado-madurados dado su gran potencial aromático. Hoy en día, la disposición de técnicas de separación cromatográfica, como GC-GC o GCxGC, y detectores específicos con una mayor

sensibilidad, pueden ser de gran utilidad para aportar información adicional en la identificación de la estructura química de los compuestos volátiles detectados olfatométricamente.

Tabla 5. Compuestos aromáticos azufrados y nitrogenados detectados en embutidos curado-madurados.

Grupo químico	Descriptor olfatométrico	Autores[a]
Sulfuros		
Dimetildisulfuro	Ajo, caramelo, caldo, tostado	4,10
Dimetiltrisulfuro	Col, azufrado, desagradable	6
Sulfuro de dialilo	Ajo	4,5
Disulfuro de dialilo	Ajo	4,5
Etil metil sulfuro	Cebolla en descomposición, desagradable	11
Etil metil disulfuro	Cebolla, pungente	1
Metil-sulfuro de alilo	Ajo	1,3,4,5,9
Metil-disulfuro de alilo	Ajo	4
Dipropiltrisulfuro	Carnoso, tostado	3
2-Metil-3-furil disulfuro	Carne cocida	9
Tioles		
Metanotiol	Huevo podrido, coliflor	8,11
2-Furilmetanotiol	Asado, azufrado	9
2-Metil-3-furantiol	Carne asada	6,9
Otros compuestos azufrados		
Metional	Cebolla, patata cocida, carne, caldo, rancio, sabroso	7,8,9,10,11
2-Metil-3-metilditiofurano	Carne asada	6
Tiazolinas		
2-Acetil-2-tiazolina	Asado, palomitas	9
Derivados pirroles		
1-Metilpirrol	Tostado, caldo	10
2-Acetilpirrol	Frutos secos tostados, snack fritos	10
2-Acetil-1-pirrolina	Asado, palomitas	7,9
Pirazinas		
Dimetilpirazina	Café, tostado	2

Tabla 5. Continuación.

Grupo químico	Descriptor olfatométrico	Autores[a]
2-Etil-3,5(6)-dimetilpirazina	Tierra	9
Tetrametilpirazina	Azúcar caramelizado	10
2,3-Dietil-5-metilpirazina	Tierra	9
3-Isopropil-2-metoxipirazina	Tierra, guisante	9
3-Isobutil-2-metoxipirazina	Tierra, guisante	9
Indoles		
3-metilindol	Naftalina	5

[a] 1 Stahnke (1994); 2 Stahnke (1995); 3 Meynier *et al.* (1999); 4 Schmidt & Berger (1998a); 5 Schmidt & Berger (1998b); 6 Chevance *et al*. (2000); 7 Blank *et al*. (2001); 8 Marco *et al*. (2007); 9 Söllner & Schieberle (2009); Gianelli *et al*. (2011); Olivares *et al*. (2011).

Tabla 6. Compuestos volátiles azufrados y nitrogenados detectados en embutidos curado-madurados.

Grupo químico	Autores[a]
Sulfuros	
Dióxido de azufre	11
Disulfuro de carbono	1,7,14-22, 24,28,29,32
Dimetilsulfuro	22
Trisulfuro de dialilo	9
Metil-trisulfuro de alilo	2,33,34
Tetrasulfuro de alilo	2
Isopropilsulfuro de alilo	3
Metilpropilsulfuro	3,6,10
Metil propenil disulfuro	2
Metilpropildisulfuro	3
Disulfuro de 1-propenilalilo (*E)*	2
Disulfuro de 1-propenilalilo *(Z)*	2
Fenil sulfuro	13

Tabla 6. Continuación.

Grupo químico	Autores[a]
Tiofenos	
2,3-Dihidrotiofeno	4
Metiltiofeno	12
3-Metiltiofeno	8,17,23,27
2-Metiltiofeno	11
Tioles	
Tiol alilo	2,33,34
1-Propanotiol	11
Etil metanotiol	28
Otros compuestos azufrados	
1-Metiltio-1-propeno	3,6,10,19
Dimetil sulfona	26
Metionol	23,27,32
3-Metiltiopropanoato de metilo	30,31
2-Vinil-4H-1,3-ditin	9
Derivados pirroles	
1-Metil-2-pirrolidona	5
Pirazinas	
2-Metilpirazina	3,25,27
2,6-Dimetilpirazina	3,4,6,25,27
2,5-Dimetiliprazina	4,26
Indoles	
Indol	4,9
Otros compuestos nitrogenados	
Dimetil nitrosamina	3
Piridina	16

Tabla 6. Continuación.

Grupo químico	Autores[a]
5-Methyl-2,4-imidazolidinodiona	4
1-Acetilpiperidina	5
N-(2-metilbutil)-acetamida	5

[a]1 Berdagué *et al*. (1993); 2 Mateo & Zumalacárregui (1996); 3 Viallon *et al.* (1996); 4 Schmidt & Berger (1998 a); 5 Schmidt & Berger (1998 b); 6 Meynier *et al*. (1999); 7 Bruna *et al*. (2000a); 8 Bruna *et al*. (2000b); 9 Ansorena *et al.* (2001); 10 Sunesen *et al.* (2001); 11 Bruna *et al.* (2003); 12 Muguerza *et al*. (2003); 13 Benito *et al.* (2004); 14 Flores *et al.* (2004); 15 Marco *et al.* (2004); 16 Flores *et al.* (2005); 17 Herranz *et al*. (2005); 18 Marco *et al.* (2006); 19 Bianchi *et al*. (2007); 20 Marco *et al.* (2008); 21 Rivas-Cañedo *et al*. (2009); 22 Andrade *et al.* (2010); 23 Olivares *et al.* (2010); 24 Casquete *et al.* (2011); 25 Gianelli *et al.* (2011); 26 Latorre-Moratalla *et al*. (2011); 27 Olivares *et al*. (2011); 28 Partidário *et al.* (2011); 29 Hospital *et al.* (2012); 30 Fonseca *et al.* (2013); 31 Gómez & Lorenzo (2013); 32 Cano-García *et al*. (2014); 33 Kargozari *et al.* (2014a); 34 Kargozari *et al.* (2014b).

3. Referencias bibliográficas

Acree, T. E., Barnard, J., & Cunningham, D. G. (1984). A procedure for the sensory analysis of gas chromatographic effluents. *Food Chemistry*, 14, 273-286.

Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) (2005). Estrategia NAOS (para la Nutrición, Actividad Física y prevención de la Obesidad). Madrid, Ministerio de Sanidad, Asusntos Sociales e Igualdad. Disponible en: http://www.naos.aesan.msssi.gob.es/home.html

Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN) (2012). PlanCuídate+2012. Madrid, Ministerio de Sanidad, Asuntos Sociales e Igualdad. Disponible en: http://www.plancuidatemas.aesan.msssi.gob.es/

Agilent Technologies (2011). Seminario Fundamentos de cromatografía y técnicas de espectrometría de masas, Madrid (España).

Andrade, M. J., Córdoba, J. J., Casado, E. M., Córdoba, M. G., & Rodríguez, M. (2010). Effect of selected strains of *Debaryomyces hansenii* on the volatile compound production of dry fermented sausage "salchichón". *Meat Science*, 85, 256-264.

ANICE (2014). Asociación Nacional de Industrias de la Carne de España. Disponible en http://www.anice.es

Ansorena, D., Gimeno, O., Astiasarán, I., & Bello, J. (2001). Analysis of volatile compounds by GC–MS of a dry fermented sausage: chorizo de Pamplona. *Food Research International*, 34, 67-75.

Arthur, C. L., & Pawliszyn, J. (1990). Solid-Phase Microextraction with Thermal-Desorption Using Fused-Silica Optical Fibers. *Analytical Chemistry*, 62, 2145-2148.

Baltussen, E., Sandra, P., David, F., & Cramers, C. (1999). Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *Journal of Microcolumn Separations*, 11, 737-747.

Benito, M. a. J., Rodríguez, M., Martín, A., Aranda, E., & Córdoba, J. J. (2004). Effect of the fungal protease EPg222 on the sensory characteristics of dry fermented sausage “salchichón” ripened with commercial starter cultures. *Meat Science*, 67, 497-505.

Berdagué, J. L., Monteil, P., Montel, M. C., & Talon, R. (1993). Effects of starter cultures on the formation of flavour compounds in dry sausage. *Meat Science*, 35, 275-287.

Bianchi, F., Cantoni, C., Careri, M., Chiesa, L., Musci, M., & Pinna, A. (2007). Characterization of the aromatic profile for the authentication and differentiation of typical Italian dry-sausages. *Talanta*, 72, 1552-1563.

Bicchi, C., Cordero, C., Liberto, E., Rubiolo, P., Sgorbini, B., David, F., & Sandra, P. (2005). Dual-phase twisters: A new approach to headspace sorptive extraction and stir bar sorptive extraction. *Journal of Chromatography A*, 1094, 9-16.

Blank, I., Devaud, S., Fay, L. B., Cerny, C., Steiner, M., & Zurbriggen, B. (2001). Odor-active compounds of dry-cured meat: Italian-type salami and Parma ham. En: *Aroma-Active Compounds in Foods*. Chemistry and Sensory Properties. Ed. G.R.

Takeoka, M. Güntert & K. H. Engel. American Chemical Society: Washington, D.C., 9-20.

BOE (2014). Norma de calidad de derivados cárnicos. *Boletín Oficial del Estado*, de 18 de junio de 2014, Madrid (España), 46058-46078.

Bolumar, T., Sanz, Y., Flores, M., Aristoy, M. C., Toldrá, F., & Flores, J. (2006). Sensory improvement of dry-fermented sausages by the addition of cell-free extracts from *Debaryomyces hansenii* and *Lactobacillus sakei*. *Meat Science*, 72, 457-466.

Bruna, J. M., Fernández, M., Hierro, E. M., Ordóñez, J. A., & de la Hoz, L. (2000 a). Combined use of Pronase E and a fungal extract (*Penicillium aurantiogriseum*) to potentiate the sensory characteristics of dry fermented sausages. *Meat Science*, 54, 135-145.

Bruna, J. M., Fernández, M., Hierro, E. M., Ordóñez, J. A., & De La Hoz, L. (2000 b). Improvement of the sensory properties of dry fermented sausages by the superficial inoculation and/or the addition of intracellular extracts of *Mucor racemosus*. *Journal of Food Science*, 65, 731-738.

Bruna, J. M., Hierro, E. M., de la Hoz, L., Mottram, D. S., Fernández, M., & Ordóñez, J. A. (2003). Changes in selected biochemical and sensory parameters as affected by the superficial inoculation of *Penicillium camemberti* on dry fermented sausages. *International Journal of Food Microbiology*, 85, 111-125.

Burdock, G. A. (2002). *Fenaroli's handbook of flavor ingredients (4th ed*.). CRC Press Inc., Boca Raton, Florida.

Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R. (2011). The effect of yeast extract addition on quality of fermented sausages at low NaCl content. *Meat Science*, 87, 290-298.

Cano-García, L., Belloch, C., & Flores, M. (2014). Impact of *Debaryomyces hansenii* strains inoculation on the quality of slow dry-cured fermented sausages. *Meat Science,* 96, 1469-1477.

Casquete, R., Martín, A., Benito, M. J., Ruiz-Moyano, S., Nevado, F. P., & Córdoba, M. D. G. (2011). Impact of Pre-Selected Autochthonous Starter Cultures on the Flavor Quality of Iberian Dry-Fermented "Salchichón" Sausage with Different Ripening *Processes. Journal of Food Science*, 76, S535-S544.

Chevance, F. F. V., & Farmer, L. J. 1998. Influence of fat on the flavour of an emulsified meat product. En: *Food Flavors: Formation, analysis and packaging influences*. Ed. E.T. Contis, C. T. Ho, C. J. Mussinan, T. H. Parliament, F. Shahidi & A. M. Spanier. Elsevier: Amsterdam, 255-270.

Chevance, F. F. V., Farmer, L. J., Desmond, E. M., Novelli, E., Troy, D. J., & Chizzolini, R. (2000). Effect of some fat replacers on the release of volatile aroma compound from low-fat meat products. *Journal of Agricultural and Food Chemistry*, 48, 3476-3484.

Codex Alimentarius (2011). Recomendaciones sobre la declaración del sodio (sal). Comité del Codex Alimentarius sobre etiquetado de alimentos, del 9 al 13 de mayo de 2011 (Quebec, Canadá).

Comisión Europea (2008). Salt reduction. Disponibles en http://ec.europa.eu/health/ph_determinants/life_style/nutrition_salt_en.htm

Croizet, F. Denoyer, C., Tran, N. & Berdagué, J. L. (1992). Les composes volatils du saucisson sec. Évolution au cours de la maduration. *Viandes Produits Carnés*, 13, 167-170.

d'Acampora Zellner, B., Dugo, P., Dugo, G. & Mondello, L. (2008). Gas chromatography-olfactometry in food flavour analysis. *Journal of Chromatography A*, 1186, 123-143.

Deans, R. R. (1968). A new technique for heart cutting in gas chromatography. *Chromatographia*, 1, 18-22.

Delahunty, C. M., Eyres, G., & Dufour, J. P. (2006). Gas chromatography-olfactometry. *Journal of Separation Science*, 29, 2107-2125.

Doyle, M. E., & Glass, K. A. (2010). Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety,* 9, 44-56.

Encinas, J. P., López-Díaz, T. M., García-López, M. L., Otero, A., & Moreno, B. (2000). Yeast populations on Spanish fermented sausages. *Meat Science*, 54, 203-208.

Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation - A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *European Food Research and Technology*, 209, 237-241.

Flavornet, http://www.flavornet.org/flavornet.html

Flores, M., Aristoy, M. C., & Toldrá, F. (1993). HPLC purification and characterization of porcine muscle aminopeptidase B. *Biochimie*, 75, 861-867.

Flores, J. (1997). Mediterranean vs northern European meat products. Processing technologies and main differences. *Food Chemistry*, 59, 505-510.

Flores, M., Durá, M. A., Marco, A., & Toldrá, F. (2004). Effect of *Debaryomyces spp*. on aroma formation and sensory quality of dry-fermented sausages. *Meat Science*, 68, 439-446.

Flores, M., Nieto, P., Ferrer, J. M., & Flores, J. (2005). Effect of calcium chloride on the volatile pattern and sensory acceptance of dry-fermented sausages. *European Food Research and Technology,* 221, 624-630.

Flores, M. (2011). Flavor of meat products. En: *Sensory Analysis of Foods of Animal Origin*. Ed. Leo M.L. Nollet & F. Toldrá. CRC Press, Boca Ratón, Florida, EEUU, 131-145.

Flores, M. & Toldrá, F. (2011). Microbial enzymatic activities for improved fermented meats. *Trends in Food Science & Technology*, 22, 81-90.

Fonseca, S., Cachaldora, A., Gómez, M., Franco, I., & Carballo, J. (2013). Effect of different autochthonous starter cultures on the volatile compounds profile and sensory properties of Galician chorizo, a traditional Spanish dry fermented sausage. *Food Control*, 33, 6-14.

Gandemer, G. (2002). Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. *Meat Science*, 62, 309-321.

Gelabert, J., Gou, P., Guerrero, L., & Arnau, J. (2003). Effect of sodium chloride replacement on some characteristics of fermented sausages. *Meat Science*, 65, 833-839.

Gianelli, M. P., Olivares, A., & Flores, M. (2011). Key aroma components of a dry-cured sausage with high fat content (Sobrassada). *Food Science and Technology International,* 17, 63-71.

Gómez, M., & Lorenzo, J. M. (2013). Effect of fat level on physicochemical, volatile compounds and sensory characteristics of dry-ripened "chorizo" from Celta pig breed. *Meat Science*, 95, 658-666.

Gou, P., Guerrero, L., Gelabert, J., & Arnau, J. (1996). Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. *Meat Science*, 42, 37-48.

Grimm, C. C.; Lloyd, S. W.; Miller, J. A., & Spanier, A. M. (1997). The Analysis of Food Volatiles Using Direct Thermal Desorption. En: *Techniques for Analyzing Food Aroma.* Ed. R. Marsili. Marcel Dekker Inc., New York, EEUU, 59-80.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chemical Senses*, 26, 533-545

Grosch, W. & Schieberle, P. (2009). Aroma compounds. En: *Food Chemistry*. Ed. H—D. Belitz, W. Grosch & P. Schieberle. Springer-Verlag, Berlin Heidelberg, 340-402.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International*, 18, 49-70.

Güntert, M., Brüning, J., Emberger, R., Köpsel, M., Kuhn, W., Thielmann, T., & Werkhoff, P. (1990). Identification and formation of some selected sulphur-containing flavor compounds in various meat model systems. *Journal of Agricultural and Food Chemistry,* 38, 2027-2041.

Herranz, B., de la Hoz, L., Hierro, E., Fernández, M., & Ordóñez, J. A. (2005). Improvement of the sensory properties of dry-fermented sausages by the addition of free amino acids. *Food Chemistry*, 91, 673-682.

Herrero, M., Ibáñez, E., Cifuentes, A., & Bernal, J. (2009). Multidimensional chromatography in food analysis. *Journal of Chromatography A*, 1216, 7110-7129.

Hierro, E., De La Hoz, L., & Ordóñez, J. A. (1999). Contribution of the microbial and meat endogenous enzymes to the free amino acid and amine contents of dry fermented sausages. *Journal of Agricultural and Food Chemistry*, 47, 1156-1161.

Holm, T. (1999). Aspects of the mechanism of the flame ionization detector. *Journal of Chromatography A,* 842, 221-227.

Hospital, X. F., Hierro, E., & Fernández, M. (2012). Survival of *Listeria innocua* in dry fermented sausages and changes in the typical microbiota and volatile profile as affected by the concentration of nitrate and nitrite. *International Journal of Food Microbiology,* 153, 395-401.

Hu, Y., Liang, Y.-Z., Li, B.-Y.,Li, X.-N., & Du, Y.-P. (2004). Multicomponent spectral correlative chromatography applied to complex herbal medicines. *Journal of Agricultural and Food Chemistry*, 52, 7771-7776.

Iucci, L., Patrignani, F., Belletti, N., Ndagijimana, M., Elisabetta Guerzoni, M., Gardini, F., & Lanciotti, R. (2007). Role of surface-inoculated *Debaryomyces hansenii* and *Yarrowia lipolytica* strains in dried fermented sausage manufacture. Part 2: Evaluation of their effects on sensory quality and biogenic amine content. *Meat Science,* 75, 669-675.

Jiménez, A., Cervera P. & Bacardí M. (2005). *Tabla de composición de alimentos*. Ed. Novartis Medical Nutrition, 9ª edición, Barcelona (España).

Jing, H., & Amirav, A. (1998). Pulsed flame photometric detector – a step forward towards universal heteroatom selective detection. *Journal of Chromatography A*, 805, 177-215.

Kanner, J., Harel, S., & Jaffe, R. (1991). Lipid peroxidation of muscle food as affected by NaCl. *Journal of Agricultural and Food Chemistry*, 39, 1917-1921.

Kargozari, M., Moini, S., Akhondzadeh Basti, A., Emam-Djomeh, Z., Gandomi, H., Revilla Martin, I., Ghasemlou, M., & Carbonell-Barrachina, Á. A. (2014a). Effect of autochthonous starter cultures isolated from Siahmazgi cheese on physicochemical, microbiological and volatile compound profiles and sensorial attributes of sucuk, a Turkish dry-fermented sausage. *Meat Science*, 97, 104-114.

Kargozari, M., Moini, S., Akhondzadeh Basti, A., Emam-Djomeh, Z., Ghasemlou, M., Revilla Martin, I., Gandomi, H., Carbonell-Barrachina, Á. A., & Szumny, A. (2014b). Development of Turkish dry-fermented sausage (sucuk) reformulated with camel meat and hump fat and evaluation of physicochemical, textural, fatty acid and volatile compound profiles during ripening. *LWT - Food Science and Technology*, 59, 849-858.

Kenneally, P. M., Leuschner, R. G., & Arendt, E. K. (1998). Evaluation of the lipolytic activity of starter cultures for meat fermentation purposes. *Journal of Applied Microbiology,* 84, 839-846.

Khalid, N. M., & Marth, E. H. (1990). Purification and partial characterization of a prolyl-dipeptidyl aminopeptidase from *Lactobacillus helveticus* CNRZ 32. *Applied and Environmental Microbiology,* 56, 381-388.

Kołakowska, A. (2003). Lipid oxidation in food systems. En: *Chemical and Functional Properties of Food Lipids*. Ed. Z.E. Sikorski & A. Kołakowska. CRC Press, Boca Raton, USA, 133-166

Kolb, B. (1982). Multiple headspace extraction-A procedure for eliminating the influence of the sample matrix in quantitative headspace, gas chromatography. *Chromatographia*, 15, 587-594.

Kovats, E.S. (1965).Gas chromatographic characterization of organic substances in the retention index system. En: *Advances in chromatography*. Ed. J. C. Giddings, R. A. Keller. Marcel Dekker, Inc., New York, 229-247.

Latorre-Moratalla, M. L., Bosch-Fusté, J., Bover-Cid, S., Aymerich, T., & Vidal-Carou, M. C. (2011). Contribution of enterococci to the volatile profile of slightly-fermented sausages. *LWT - Food Science and Technology*, 44, 145-152.

Leistner, F. (1992). The essentials of producing stable and safe raw fermented sausages. En: *New technologies for meat and meat products*. Ed. F. J. M. Smulders, F. Toldrá, J. Flores & M. Prieto. Utrecht: ECCEAMST, Nijmegen: Audet Tijdschriften, Holanda, 1-19.

Lewis, A.C. (2002). Multidemensional High Resolution Gas Chromatography. En: *Multidimensional Chromatography*. Ed. L. Mondello, A. C. Lewis & K. D. Bartle. John Wiley & Sons Ltd, New York, USA, 47-76.

Liaros, N. G., Katsanidis, E., & Bloukas, J. G. (2009). Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages*. Meat Science*, 83, 589-598.

Lickens, S.T. & Nickerson, G.B. (1964). Detection of certain hop oil constituents in brewing products, *Proceedings of American Society of Brewing Chemists*, 5.

Lücke, F. K. (1994). Fermented meat products. *Food Research International*, 27, 299-307.

Marco, A., Navarro, J. L., & Flores, M. (2004). Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). *Food Chemistry*, 84, 633-641.

Marco, A., Navarro, J. L., & Flores, M. (2006). The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage. *Meat Science,* 73, 660-673.

Marco, A., Navarro, J. L., & Flores, M. (2007). Quantitation of selected odor-active constituents in dry fermented sausages prepared with different curing salts. *Journal of Agricultural and Food Chemistry*, 55, 3058-3065.

Marco, A., Navarro, J. L., & Flores, M. (2008). The sensory quality of dry fermented sausages as affected by fermentation stage and curing agents. *European Food Research and Technology,* 226, 449-458

Marriott, P. (2002). Orthogonal GC-GC. En: *Multidimensional Chromatography*. Ed. L. Mondello, A. C. Lewis & K. D. Bartle. John Wiley & Sons Ltd, Nueva York, USA, 77-108.

Mateo, J., & Zumalacárregui, J. (1996). Volatile compounds in chorizo and their changes during ripening. *Meat Science*, 44, 255-273.

Meynier, A., & Mottram, D. S. (1995). The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chemistry*, 52, 361-366.

Meynier, A., Novelli, E., Chizzolini, R., Zanardi, E., & Gandemer, G. (1999). Volatile compounds of commercial Milano salami. *Meat Science*, 51, 175-183.

Mistry, B. S., Reinecius, T., & Olson, L. K. (1997). Gas-Chromatography-Olfactometry for the Determination of Dey Odorants in Foods. En: *Techniques for Analyzing Food Aroma.* Ed. R. Marsili. Marcel Dekker Inc., New York, EEUU, 265-292.

Molly, K., Demeyer, D., Johansson, G., Raemaekers, M., Ghistelinck, M., & Geenen, I. (1997). The importance of meat enzymes in ripening and flavour generation in dry fermented sausages. First results of a European project. *Food Chemistry*, 59, 539-545.

Montel, M. C., Talon, R., Berdagué, J. L., & Cantonnet, M. (1993). Effects of starter cultures on the biochemical characteristics of French dry sausages. *Meat Science*, 35, 229-240.

Montel, M. C., Masson, F., & Talon, R. (1998). Bacterial role in flavour development. *Meat Science*, 49(Suppl. 1), S111-S123.

Motilva M. J., Toldrá, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity*. Zeitschrift für Lebensmittel-Untersuchung und Forschung,* 196, 228-232.

Mottram, D.S. (1991). Meat. En: *Volatile compounds in foods and beverages*. Ed. H. Maarse. Marcel Dekker Inc, New York, USA, 107-177.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, 62, 415-424.

Muguerza, E., Ansorena, D., Bloukas, J. G., & Astiasarán, I. (2003). Effect of fat level and partial replacement of pork backfat with olive oil on the lipid oxidation and volatile compounds of Greek dry fermented sausages. *Journal of Food Science*, 68, 1531-1536.

Nist, http://webbook.nist.gov/chemistry/

Odour*,* http://www.odour.org.uk/

Olesen, P. T., & Stahnke, L. H. (2000). The influence of *Debaryomyces hansenii* and *Candida utilis* on the aroma formation in garlic spiced fermented sausages and model minces. *Meat Science*, 56, 357-368.

Olesen, P. T., Meyer, A. S., & Stahnke, L. H. (2004). Generation of flavour compounds in fermented sausages - The influence of curing ingredients, *Staphylococcus* starter culture and ripening time. *Meat Science*, 66, 675-687.

Olivares, A., Navarro, J. L., & Flores, M. (2009). Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage. *Food Chemistry*, 115, 1464-1472.

Olivares, A., Navarro, J. L., Salvador, A., & Flores, M. (2010). Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Science*, 86, 251-257.

Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science*, 87, 264-273.

OMS/FAO Expert Consultation (2003). Diet, nutrition and the prevention of chronic diseases. *OMS technical report series* 916, Geneva.

OMS (2013). Mapping salt reduction initiatives in the WHO European Region. Regional Office for Europe, Dinamarca.

Ordóñez, J. A., Hierro, E. M., Bruna, J. M., & De La Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition,* 39, 329-367.

Parliment, T.H. (1997). Solvent Extraction and Distallition Techniques. En: *Techniques for Analyzing Food Aroma*. Ed. R. Marsili. Marcel Dekker Inc., New York, EEUU, 27-58.

Partidário, A. M., Roseiro, C., & Santos, C. (2011). Effect of processing in volatiles from a Portuguese traditional dry-fermented ripened sausage "Chourico Grosso Borba-Estremoz PGI". *Food Science and Technology International*, 17, 15-22.

Pherobase, http://pherobase.com/

Poll, L., & Flink, J. M. (1984). Aroma analysis of apple juice: Influence of salt addition on headspace volatile composition as measured by gas chromatography and corresponding sensory evaluations. *Food Chemistry*, 13, 193-207.

Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L., & Leroy, F. (2010). The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages. *Food Microbiology*, 27, 945-954.

Ravyts, F., Vuyst, L. D., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. Engineering in Life Sciences, 12, 356-367.

Reglamento nº1169/2011. Parlamento Europeo y el Consejo de la Unión Europea, 6 de julio de 2011.

Reineccius, G.A. (2007). Flavour-Isolation Techniques. En: Flavour and Fragrances: chemistry, bioprocessing and sustainability. Ed. R.G. Berger. Springer- Verlag Berlin Heidelberg (Alemania), 409-426

Reineccius, G. (2010). Instrumental methods of analysis. En: *Food Flavour Technology second edition.* Ed. A. J. Taylor & R. S.T. Linforth. Willey-Blackwell, Oxford, Reino Unido, 229-265.

Rico, E., Toldrá, F., & Flores, J. (1990). Activity of cathepsin D as affected by chemical and physical dry-curing parameters. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung,* 191, 20-23.

Rico, E., Toldrá, F., & Flores, J. (1991). Effect of dry-curing process parameters on pork muscle cathepsin B, H and L activity. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 193, 541-544.

Rivas-Cañedo, A., Nuñez, M., & Fernández-García, E. (2009). Volatile compounds in Spanish dry-fermented sausage 'salchichón' subjected to high pressure processing. Effect of the packaging material. *Meat Science*, 83, 620-626.

Roncales P. (1994). Tecnología, cambios bioquímicos y calidad sensorial de los embutidos curados. En Tendencias en el curado de productos cárnicos. Ed. J. Flores, S. Bermell & F. Toldrá. *Jornada técnica Expocarne*, Valencia, España.

Rosell, C. M., & Toldrá, F. (1996). Effect of curing agents on m-calpain activity throughout the curing process. *European Food Research and Technology*, 203, 320-325.

Rubinson, K. A. & Rubinson, J. F. (2001). *Análisis instrumental*. Pearson, Madrid, España.

Rust, R. E. (1987). Sausage Products. En: *The Science of meat and meat products third edition*. Ed. J. F. Price & B. S. Schwigert. Food & Nutrition Press, Inc, Westport, Connecticut USA, 457-485.

Sanz, Y., & Toldrá, F. (1997). Aminopeptidase activities from *Lactobacillus sake* in models of curing ingredients and processing conditions for dry sausage. *Journal of Food Science*, 62, 1211-1213+1234.

Sárraga, C., & García-Regueiro, J. A. (1998). Oxidation of microsomal fraction in the processing conditions of dry-cured ham. The in-vitro effect of brine. *Meat Science*, 50, 295-302.

Schmidt, S., & Berger, R. G. (1998a). Aroma Compounds in Fermented Sausages of Different Origins. *LWT - Food Science and Technology*, 31, 559-567.

Schmidt S. & Berger R.G (1998b). Microbially formed aroma compunds during the maturation of dry fermented sausage (Salami). *Advances in Food Sciences*, 20, 144-152.

Ševčik, J. (1976). Detectors in Gas Chromatography. En: *Journal of Chromatography Library-volume 4*. Elsevier Scientific Publishing Company, Amsterdam, Holanda.

Shahidi, F., Rubin, L. J., & Wood, D. F. (1988). Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Science*, 22, 73-80.

Söllner, K., & Schieberle, P. (2009). Decoding the key aroma compounds of a Hungarian-type salami by molecular sensory science approaches. Journal of Agricultural and *Food Chemistry*, 57, 4319-4327.

Stahnke, L. H. (1994). Aroma components from dried sausages fermented with Staphylococcus xylosus. *Meat Science*, 38, 39-53.

Stahnke, L. H. (1995). Dried sausages fermented with Staphylococcus xylosus at different temperatures and with different ingredient levels - Part III. Sensory evaluation. *Meat Science*, 41, 211-223.

Stahnke, L. (2002). Flavour formation in fermented sausages. En: *Research Advances in the quality of meat and meat products*. Ed. F. Toldrá. Research Signpost, India. 193-223.

Sunesen, L. O., Dorigoni, V., Zanardi, E., & Stahnke, L. (2001). Volatile compounds released during ripening in Italian dried sausage. *Meat Science*, 58, 93-97.

Talon, R., Chastagnac, C., Vergnais, L., Montel, M. C., & Berdagué, J. L. (1998). Production of esters by staphylococci. *International Journal of Food Microbiology*, 45, 143-150.

Talon, R., Leroy-Sétrin, S. & Fadda, S. (2002). Bacterial starters involved in the quality of fermented meat products. En: *Research Advances in the quality of meat and meat products*. Ed. F. Toldrá. Research Signpost, India. 175-191.

The good scents company, http://www.thegoodscentscompany.com/index.html.

Timón, M. L., Ventanas, J., Martín, L., Tejeda, J. F., & García, C. (1998). Volatile Compounds in Supercritical Carbon Dioxide Extracts of Iberian Ham. *Journal of Agricultural and Food Chemistry*, 46, 5143-5150.

Toldrá, F., Rico, E., & Flores, J. (1992). Activities of pork muscle proteases in model cured meat systems. *Biochimie*, 74, 291-296.

Toldrá, F., Cerveró, M. C., & Part, C. (1993). Porcine aminopeptidase activity as affected by curing agents. *Journal of Food Science*, 58, 724-726.

Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science*, 49, Suppl 1, S101-S110.

Toldrá, F., Sanz, Y. & Flores, M. (2001). Meat fermentation technology. En: *Meat Science and Aplications.* Ed. Y.H. Hui, W.K. Nip, R.W. Rogers & A.Y. Owen. Marcel Dekker, New York, USA, 537-561.

Toldrá, F. & Flores, M. (2007). Processed pork meat flavors. En: *Handbook of Food Products Manufacturing*. Ed. Y. H. Hui, R. Chadan, S. Clark, N. Cross, J. Dobbs, W. J. Hurst, L. M. L. Nollet, E. Shimoni, N. Sinha, E. B. Smith, S. Surapat, A. Titchenal & F. Toldrá. John Wiley & Sons, New York, EEUU, 281-301.

Toldrá F. (2008) Biotechnology of flavor generation in fermented meats. En: *Meat Biotechnology.* Ed. F. Toldrá. Springer, New York, USA, 199-215.

Ullrich, F., & Grosch, W. (1987). Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung,* 184, 277-282.

Van Ruth, S.M. & Roozen, J.P. (2010). Delivery of flavours from food matrices. En: *Food Flavour Technology second edition*. Ed. A. J. Taylor & R. S.T. Linforth. Willey-Blackwell , A John Wiley & Sons, Ltd., Publication, UK, 190-206.

Varnam A. H. & Sutherland J. P. (1995). Embutidos fermentados. En: *Carne y productos cárnicos, Tecnología, química y microbiología*. Acribia S.A. Zaragoza (España).307-346.

Verplaeste, A., Demeyer, D., Gerard, S & Buys, E. (1992).*Proceedings of the 38th International Congress of Meat Science and Technology*, Clermont-Ferrand, Francia, 851.

Viallon, C., Berdagué, J. L., Montel, M. C., Talon, R., Martin, J. F., Kondjoyan, N., & Denoyer, C. (1996). The effect of stage of ripening and packaging on volatile content and flavour of dry sausage. *Food Research International*, 29, 667-674.

Wampler, T.P. (1997). Analysis of Food Volatiles Using Headspace-Gas Chromatographic Techniques. En: *Techniques for Analyzing Food Aroma*. Ed. R. Marsili. Marcel Dekker Inc., New York, EEUU, 27-58.

Wardencki, W., & Zygmunt, B. (1991). Gas chromatographic sulphur-sensitive detectors in environmental analysis*. Analytica Chimica Acta*, 255, 1-13.

Wirth, F. (1988). Technologies for making fat-reduced meat products. *Fleischeirtsch*, 509 68, 1153-1156

Yan, X. (2006). Unique selective detectors for gas chromatography: Nitrogen and sulfur chemiluminescence detectors. *Journal Separation Science*, 29, 193-1945.

Zanardi, E., Ghidini, S., Conter, M., & Ianieri, A. (2010). Mineral composition of Italian salami and effect of NaCl partial replacement on compositional, physico-chemical and sensory parameters*. Meat Science*, 86, 742-747.

III. Objetivos

III. OBJETIVOS

Considerando la problemática que plantea la ingesta de elevadas cantidades de sal y grasa en la dieta sobre la salud cardiovascular así como, la importancia de conocer el efecto de dicha reducción en el aroma de los embutidos curado-madurados y diseñar nuevas estrategias que lo potencien, se plantearon dos objetivos principales:

1. Estudio de los compuestos volátiles minoritarios de gran potencia aromática en embutidos curado-madurados.
2. Efecto de la reducción del contenido de sal y/o grasa en la calidad de embutidos curado-madurados y estudio del efecto del empleo de levaduras en la potenciación del aroma de dichos embutidos.

Con el fin de alcanzar los objetivos principales se plantearon los siguientes objetivos parciales:

- Identificación y cuantificación de los compuestos aromáticos en embutidos curado-madurados mediante diferentes técnicas de extracción.

- Identificación de compuestos aromáticos azufrados y nitrogenados en embutidos curado-madurados mediante diferentes métodos de separación y detección cromatográfica, así como el estudio de su generación a lo largo del procesado.

- Estudio de la reducción del contenido de sal en la calidad de embutidos curado-madurados.

- Estudio de la reducción del contenido de grasa y/o sal en la calidad sensorial de embutidos curado-madurados inoculados con *Debaryomyces hansenii*.

- Efecto de la inoculación de *Debaryomyces hansenii* en el aroma de embutidos curado-madurado con contenido reducido de grasa y/o sal.

IV. Resultados

Capítulo 1

Elucidation of key aroma compounds in traditional dry fermented sausages using different extraction techniques

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Elucidation of key aroma compounds in traditional dry fermented sausages using different extraction techniques

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Runing tittle: key aroma compounds in dry fermented sausages

Abstract

BACKGROUND: The use of different extraction techniques – solid phase microextraction (SPME) and solvent assisted flavour evaporation (SAFE) – can deliver different aroma profiles and it is essential to determine which is most suitable to extract the aroma compounds from dry fermented sausages.

RESULTS: Forty-five aroma-active compounds were detected by SPME and SAFE, with 11 of them reported for the first time as aroma compounds in dry fermented sausages: ethyl 3-hydroxy butanoate, trimethyl pyrazine, D pantolactone, isobutyl hexanoate, ethyl benzoate, α-terpineol, ethyl 3 pyridinecarboxylate, benzothiazole, 2,3-dihydrothiophene, methyl eugenol, γ-nonalactone. The aromaconcentration and odour activity values (OAVs) were calculated. Flavour reconstitution analyses were performed using 20 odorants with OAVs above 1 obtained from the SAFE and SPME extracts to prepare the aroma model.

CONCLUSION: SPME and SAFE techniques were complementary and necessary to reproduce the overall dry fermented sausage aroma. The final aroma model included the odorants from both extraction techniques (SPME and SAFE) but it was necessary to incorporate the compounds 2,4-decadienal (E,E), benzothiazole, methyl eugenol, α-terpineol, and eugenol to the final aroma model to evoked the fresh sausage aroma although a lowest cured meat aroma note was perceived.

Keywords: solid phase microextraction (SPME), solvent assisted flavour evaporation (SAFE), volatile compound, aroma, fermented sausage.

1. INTRODUCTION

Traditional fermented sausages are meat products highly appreciated by consumers due to their characteristics developed throughout the ripening process (Conter *et al*., 2008). Their flavour depends on many factors such as the raw meat and additives (curing agents, spices, etc), fermentation stage and finally, the ripening conditions (Toldrá & Flores, 2007). In particular, traditional (slow) fermented sausages are highly appreciated by consumers due to the lower oxidation and the higher proportion of volatile compounds derived from carbohydrate fermentation and amino acid catabolism (Toldrá & Flores, 2007).

Aroma research has mainly been focused on aroma composition using gas chromatography-mass spectrometry (GC-MS) techniques to identify the compounds and previously extracting the volatile compounds from the sample (Toldrá & Flores, 2007). Nevertheless, the aroma profile obtained for fermented sausages depends on the volatile extraction technique used (Flores, 2011). The different extraction techniques are applied directly to extract the compounds present in the food matrix such as simultaneous distillation extraction, solvent-assisted flavour evaporation (SAFE) or present in the headspace (HS) such as static, dynamic headspace, solid phase microextraction (SPME) and headspace sorptive extraction (HSSE) (Elmore, 2011). However, not all of them have been applied to study fermented sausage aroma. The HS techniques analyse the vapour phase above a food minimising solvent consumption and enabling the extraction of low weight compounds and in addition, these techniques are simple, efficient and inexpensive. SPME has been widely applied to dry fermented sausages (Marco *et al.*, 2007; Olivares *et al.*, 2011); however, the results obtained depend on the extraction conditions applied, such as extraction temperature, time and fibre phase.

On the other hand, the techniques based on the extraction of volatile compounds present in the food matrix apply high temperatures during distillation. However, Schmidt & Berger (1998a) and Blank *et al.* (2001) reported the presence of aroma compounds in high vacuum distillates from salami and recently, in 2009 Söllner & Schieberle, undertook the extraction of volatile compounds from the matrix of Hungarian Salami using solvent-assisted flavour evaporation (SAFE) with high vacuum distillation at low temperature minimising the artefact formation (Engel & Schieberle, 1999). They reported a great variety of odorants extracted from Hungarian Salami.

Nevertheless, research on the aroma of dry fermented sausages has mainly been done using dynamic HS techniques (Schmidt & Berger, 1998a;

Stahnke, 1994; Meynier *et al.*, 1999; Chevance *et al.*, 2000), SPME using CAR/PDMS fibre (Marco *et al.*, 2007; Olivares *et al.*, 2011; Gianelli *et al.*, 2011; Corral *et al.*, 2013) or molecular distillation techniques (Schmidt & Berger, 1998b). All of these studies reported olfactometry analyses but until now, only Söllner & Schieberle (2009) have studied salami aroma using the SAFE technique and confirmed the fermented aroma by GC-olfactometry, odour activity values and flavour reconstitution. However, there are no available studies about which technique is most suitable to extract the aroma compounds from traditional dry fermented sausages.

The aim of this work was to compare different extraction techniques (SPME and SAFE) to show which would be the most suitable to elucidate the potent odorants of dry fermented sausages. For this purpose, the results obtained were confirmed by sensory profile analysis.

2. MATERIALS AND METHODS

2.1. Dry sausage samples

Three batches, each composed of three traditional fermented dry sausages, were purchased from the PGI "Embutido de Requena" (Requena, Valencia, Spain) (Olivares *et al.*, 2010). Traditional sausages were manufactured under traditional specifications; no addition of starter cultures. The ingredients were 80 g kg^{-1} lean pork meat, 20 g kg^{-1} pork back fat, sodium chloride, nitrite, nitrate, sugars, dextrin, spices, additives such as colorants (Ponceau 4R, carminic acid) and proteins (milk and soya) and the ripening process was around 2 month at 10-12 ºC at atmospheric conditions from these geographic area. Aroma profile analyses was performed directly after sausage purchase and the remaining sausages were sliced and wrapped in aluminium foil, vacuum packaged and stored at -20 ºC for the extraction of volatile compounds.

2.2. Chemicals

The aroma compounds were obtained from Aldrich (Germany) except the following aroma compound which were purchased from the commercial sources given in parentheses: (2Z)-2-phenyl-2-butenal, 2,4-decadienal (E,E), tetramethylpyrazine, butyl acetate, isobutyl isobutanoate, ethyl 3-hydroxybutanoate, diethyl succinate, bornyl acetate, 2-pentylfuran and caprolactam (Safc, St. Louis, Missouri, USA); 1-butanol (Merck, Darmstadt, Germany); pyridine, acetic acid, toluene (Panreac, Spain). Dichloromethane

(99.8%) was also obtained from Aldrich (Seelze, Germany); methanol (99.9%) was from Scharlau (Barcelona, Spain) and anhydrous sodium sulfate (99%), sodium carbonate (99.5%), hydrochloric acid (37%) and propylene glycol (99.5%) were from Panreac.

2.3. Isolation methods to extract volatile compounds

2.3.1. Solid phase micro extraction (SPME)

The extraction was performed as already reported (Olivares *et al.*, 2011). Three types of fibres were used to analyse the HS: PDMS (1cm length, 100 µm thickness), CAR/PDMS (1 cm length, 85 µm thickness) and DVB/CAR/PDMS (1cm lenght, 50/30 µm thickness) (Supelco, Bellefonte, PA, USA). All were purchased from Supelco (Supelco) and preconditioned according to manufacturer instructions. Dry fermented sausages (25 g) were cut into pieces and minced in a blender (Waring Laboratory, Torrington, Connecticut, USA). Five grams sample was introduced into a 20 ml HS vial sealed with a PTFE faced silicone septum. The vial was maintained at 37 ºC during 30 min to equilibrate its HS and then, extracted using a SPME fibre for 3 h at 37 ºC. This procedure was done in triplicate using each fibre.

2.3.2. Solvent assisted flavour evaporation (SAFE)

Dry fermented sausages (50 g) were cut into pieces, frozen with liquid nitrogen and powdered in a blender (Waring Laboratory). 2-methyl-3-heptanone (5.1 µg) was used as internal standard and directly added to sausage powder. The powder was extracted with dichloromethane (1:2, w:v) three times (total volume 250 ml). After drying over anhydrous sodium sulfate, the volatiles were isolated using the SAFE technique (Engel & Schieberle, 1999). A blank was also run with dichloromethane without a sausage sample. The obtained extract was dried over anhydrous sodium sulfate and concentrated at 45 ºC to a final volume of 500 µl by distilling of the solvent by means of a Vigreux column and under a stream of nitrogen. This procedure was performed in triplicate.

2.4. Identification of volatile compounds by GC-MS

The identification of the volatile compounds extracted from the sausages using the different isolation processes were analysed in an Agilent 7890 GC with a 5975 MS system (Hewlett Packard, Palo Alto, CA, USA). The instrument was equipped with a Gerstel MPS2 autosampler (Gerstel, Mülheim an der Ruhr, Germany). The compounds were separated on the following capillary columns: DB-624 column (30 m, 0.25 mm i.d., film thickness 1.4 µm) (Agilent Technologies,

Santa Clara, California, USA) and HP-INNOWAX column (30 m, 0.25 mm i.d., film thickness 0.25 µm) (Agilent). The chromatographic conditions, oven temperature and flow were as described by Olivares *et al.* (2011). The GC-mass spectrometer interface was maintained at 240 ºC. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range 29-400 amu. Retention indices of the volatile compounds were calculated using the series of *n*-alkanes (Aldrich).

For SPME extraction, the fibres (SPME) were desorbed in the injection port of the GC-MS for 5 min at 240 ºC with purge valve off (splitless mode). Before each injection, the fibre was baked at 250 ºC for 15 min to avoid carry-over effects. For SAFE extract, 1µl was introduced in the injection port of the GC-MS at 240°C with 10:1 split. The compounds extracted were analysed in both columns (DB-624 and HP-INNOWAX capillary columns; Agilent).

The compounds were identified by comparison with mass spectra from the library database (Nist'05), linear retention index (Kovats, 1965) and by comparison with authentic standards. The relative quantification of volatiles compounds was done in SCAN mode using the total ion count (TIC) or the area of a target ion when different compounds co-eluted. The results comprised the mean of three replicates. In SAFE extract the results were expressed as normalised area of volatile compound (AU) versus the area of the internal standard. However, when a target compound co-eluted, the area of a target ion was used.

2.5. Gas-Chromatrography-olfactometry

The SAFE and SPME extracts were subjected to olfactometry analyses in a gas chromatograph (Agilent 6890) equipped with a flame ionization detector (FID) and sniffing port (ODP3, Gerstel, Germany) and a capillary column (DB-624, 60 m, 0.32 mm i.d., film thickness 1.8 µm) (Olivares *et al.*, 2011). The aroma-active compounds extracted by SPME were identified by the detection frequency (DF) method (Pollien *et al.*, 1997). DVB/CAR/PDMS fibre of 2 cm was used to extract the volatile compound. The compounds adsorbed by the fibre were desorbed in the GC injection port for 15 min at 240 ºC in splitless mode, and the split valve was opened after 1 min. Each assessment was carried out using 4 trained assessors (Olivares *et al.,* 2011). Each assessor evaluated two sausages, therefore a total of eight assessments were carried out. The detection of an odour by less than three assessors was considered to be noise; therefore the minimum and the maximum DF value were 3 and 8, respectively.

The aroma impact of volatile compound extracted by SAFE was determined by aroma extract dilution analysis (AEDA) (Ullrich & Grosch, 1987). The SAFE extract was diluted 1:1, 1:2, 1:10, 1:50, 1:100 and 1:500 with dichloromethane. One microliter of each dilution was injected into the capillary column and analysed by four experienced assessors until no odours were detected. A flavour dilution (FD) factor was assigned to the highest dilution at which and odour active compound was detected.

Aroma compounds were identified using the following techniques: comparison with mass spectra, comparison with linear retention indices (LRI) of authentic standards injected in the GC-MS in both used columns and GC-O, and by coincidence of the assessors's descriptors with those described in the *Fenaroli's Handbook of Flavour Ingredients* (Burdock, 2002).

2.6. Quantification of aroma compounds and calculation of odour activity values

The quantification of selected aroma compounds in the SPME and SAFE extracts was done using the GC-MS described above. The external standard method was used to quantify the aroma compounds extracted by SPME (Olivares *et al.*, 2009). Five-point calibration curves for each standard compound were obtained ($R^2 > 0.94$). The results were expressed as ng of compound extracted in the HS g^{-1} of fermented sausages. All calibration curves were done in triplicate (Table S1 in the supporting information).

For the quantification of selected aroma compounds in the SAFE extract, the curves (five-point calibration curves, $R^2 > 0.98$) were constructed by the direct addition of known amounts of standards (as shown in section "Chemicals") and a fixed concentration of internal standard (2-methyl-3-heptanone). The results were expressed as ng g^{-1} of fermented sausages (Table S2 in the supporting information).

The odour-activity values (OAVs) in air or oil of each aroma compound extracted by SPME or by SAFE were calculated. The OAVs were calculated as the ratio of concentration in HS (SPME extract) or in the matrix (SAFE extract) to odour thresholds in air or oil, respectively (Söllner & Schieberle, 2009; Burdock, 2002; Van Gemert & Nettenbreijer, 2004).

2.7. Sensory analysis

2.7.1. Aroma profile analysis

The sensory panel was composed of nine trained expert judges. A total of 18 sessions (1 h per session) were carried out at room temperature to evaluate ortho-nasal aroma (smell) of dry fermented sausages in two types of assessment: training and formal assessment. In the training assessment, thin slices of different types of dry fermented sausages were presented to panellists in a Petri dish. In the initial training sessions, the aroma descriptive terms were discussed and defined in an open session with the panel leader. The selected aroma descriptors were pepper, rancid, sour, cheesy, fruity, cured meat and stable. Following sessions different aroma references were presented for each aroma descriptors; pepper (black pepper), rancid (pork-fat kept at 30º for 15 days), sour (acetic acid), cheesy (butyric acid), fruity (ethyl 2-methylbutanoate), cured meat (dry-cured loin) and stable (4-methylphenol) and discussed by the panel to achieve a better finding of descriptors in dry fermented sausages. The panellists used a 10-cm intensity scale (1 = not perceived; 10 = very intense) to score the final aroma descriptors. During each session, panellists evaluated the intensity of each aroma descriptor in dry fermented sausages until consensual judgments were achieved. Panel members were then individually trained in the use of an intensity scale to check their reproducibility.

The evaluation of dry fermented sausages aroma was done using fresh sliced fermented sausages (4 mm thickness). The formal assessment was carried out in duplicate (two different sessions) using Compusense *five* release 5.0 (Compusense Inc., Guelph, Ontario, Canada). The intensity of the aroma descriptors was scored on a 10-cm unstructured line scale. To avoid aroma carryover, coffee beans were provided inside a vial to allow panellists to smell them between samples. The presentation order of the samples was randomised and served with a random three-digit code.

2.7.2. Flavour reconstitution

The odorants with OAV above 1 identified in the SPME and SAFE extracts were prepared in a stock solution in propylene glycol. This stock was diluted to an aroma model (40:60, sunflower oil:1 mol L^{-1} phosphate buffer pH 4.82) to obtain the concentrations determined by each technique: SPME (CAR/PDMS), SPME (DVB/CAR/PDMS) and SAFE. However, SPME concentrations were increased by 100-fold since the headspace concentration represents around 10% of the total concentration present in the matrix (Olivares *et al.*, 2009). Then, the aroma model (50 mL) was presented in an amber bottle

(100 mL) to the panel and evaluated through aroma profile analysis as explained above. Different sessions were performed with mixtures of the previous aroma models and including selected aroma compounds. The aroma models were evaluated according to the same scale and procedure used for aroma profile analysis. In the formal assessment, a balanced complete block experimental design was carried out in duplicate (in two different sessions).

2.8. Statistical analysis

The effect of the different coating fibres used in SPME technique on the volatile compounds was done by one-factor ANOVA analysis ($\alpha = 0.05$) using the statistic software XLSTAT (v 2009.4.03, Addinsoft, Barcelona, Spain). The Fisher test was used to evaluate differences among conditions. In order to check panel performance for each aroma descriptor, a two-factor analysis of variance (ANOVA) was done (assessors and samples and their interaction as factors).To study the similarity between dry fermented sausage aroma and the aroma model for each descriptor, one-factor ANOVA analysis was applied.

3. RESULTS

3.1. Extraction of volatile compounds using different isolation techniques

With regards to SPME technique three fibre types were used (PDMS, CAR/PDMS and DVB/CAR/PDMS) to extract the volatile compounds from dry fermented sausages. A total of 22 volatile compounds were identified by GC-MS in the HS of sausages using PDMS fibre while 115 and 106 volatile compounds using CAR/PDMS and DVB/CAR/PDMS fibres, respectively. The compounds identified were classified by chemical class and their abundance is shown in Table 1. However, the data for the PDMS fibre was not shown in Table 1 because the fibre was less effective. As observed in Table 1, the CAR/PDMS fibre significantly extracted the highest abundance of aldehydes, alkanes, ketones, nitrogen compounds, acids, alcohols, esters, furans, lactones and aromatic hydrocarbons, followed by DVB/CAR/PDMS fibre (Table 1). However, the sulfur compounds were highly extracted by DVB/CAR/PDMS, followed by CAR/PDMS fibre. It was observed that high molecular weight volatile compounds significantly had higher affinity for DVB/CAR/PDMS than CAR/PDMS fibre (tetradecane, dimethyl trisulfide, benzothiazole, nonanoic acid, decanoic acid, benzyl alcohol, ethyl octanoate, and others). Also several compounds were only extracted by DVB/CAR/PDMS (3-(methylthio)propanal and dimethyl disulfide). By

contrast, several low molecular weight compounds (propyl acetate, methyl butanoate, 2-methylfuran, 2-butylfuran etc) were only extracted by the CAR/PDMS fibre.

In relation to SAFE technique, a total of 101 volatile compounds were isolated and identified in the SAFE extract from dry fermented sausage (Table 1). All the volatile compounds in SAFE fraction were confirmed using authentic standards except 2,3-dihydrothiophene and ethyl 9-decenoate which were tentatively identified (Table 1).

3.2. Aroma analysis

To determine the aroma active compounds present in traditional fermented sausages an olfactometry analysis was carried out. Thirty-five different aroma-active zones were detected by SPME when using the DVB/CAR/PDMS fibre (Table 2). From these compounds, 27 were identified by mass spectra, liner retention indices and by comparison with the odour description in literature (Burdock, 2002). However, eight compounds could not be identified and were numbered as unknown from 1 to 8. The detection frequency (DF) method was applied to select the aroma compounds. The most important aroma compounds with highest DF values were 1-octen-3-ol, benzothiazole and the following esters contributing to fruity notes: ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2 and 3-methylbutanoate, ethyl 3-hydroxybutanoate, ethyl hexanoate and ethyl benzoate. All of them were found at low concentration (ng g^{-1}) (Table 2). In addition, there were differences in the quantity of aroma compounds extracted by each SPME fibre.

In the SAFE extract 28 aroma-active zones were detected. All of them were identified except one aroma zone (unknown 6 in Table 2) and several of them were already detected in the SPME extract (Table 2). AEDA was applied and their flavour dilution factors calculated (FD factor in Table 2). The most important aroma compounds with the highest FD factor (500) were ethyl 2-methyl propanoate, ethyl 3-methylbutanoate and methyl eugenol followed by those showing 100 FD factor ethyl 2-methylbutanoate, 4-methylphenol, ethyl benzoate, benzothiazole, 2,4-decadienal (*E,E*), 2,3-dihydrothiophene and *γ*-nonalactone.

Table 1. Volatile compounds identified in dry fermented sausages using different extraction techniques (SPME and SAFE).

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
Aldehydes									
Acetaldehyde	466	709	a	2.50	0.80	0.15	***		
Propanal	521	763	a	9.73	2.32	0.21	***		
2-Methylpropanal	591	813	a	0.62	0.32	0.03	**	9.00	0.028
Butanal	621	880	a	1.19	0.26	0.03	***		
3-Methylbutanal	689	918	a	5.51	3.29	0.37	*	14.00	0.037
2-Methybutanal (58)[f]	699	914	a	0.26	0.14	0.02	**	0.73	0.003
Pentanal	737	979	a	25.92	6.65	0.60	***	24.19	0.077
2-Pentenal (*E*)	804	1121	a	1.61	0.22	0.07	***		
Hexanal	840	1074	a	314.05	102.76	9.96	***	301.02	0.593
2-Hexenal (*E*)	903	1210	a	1.62	0.57	0.14	**	2.31	0.004
Heptanal (44)[f]	939	1182	a	2.05	0.97	0.11	**	2.56	0.004
2,4-Hexadienal (*E,E*)	967	1399	a	0.58	0.39	0.03	*		
2-Heptenal (*Z*)(41)[f]	1009	1317	a	1.10	0.74	0.09	*	2.47	0.004
Benzaldehyde (106)[f]	1014	1516	a	1.55	0.99	0.09	*	1.77	0.001
Octanal (43)[f]	1045	1287	a	1.65	1.02	0.21	ns	2.29	0.002
2,4-Heptadienal (*E,E*) (81)[f]	1075	1491	a	0.78	0.64	0.06	ns		
Benzeneacetaldehyde	1107	1647	a	1.55	1.68	0.20	ns	19.82	0.096
2-Octenal (*E*)	1113	1424	a	3.00	2.99	0.26	ns	7.74	0.011
Nonanal	1149	1391	a	15.84	18.82	0.54	*	88.51	0.194
2-Nonenal (*Z*) (43)[f]	1219	1527	a	0.08	0.10	0.01	ns	0.23	0.0001
2,4-Nonadienal (*E,E*)	1285	1697	a	1.17	1.56	0.17	ns		
(2Z)-2-phenyl-2-butenal (117)[f]	1358	1932	a	0.02	0.020	0.00	ns	1.18	0.004

Table 1. Continued.

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
2,4-Decadienal (*E,E*) (81)[f]	1389	1807	a	0.12	0.22	0.04	ns	2.46	0.005
Total				392.49	147.47	11.24	***	480.31	0.899
Alkanes									
Pentane	499	518	a	5.35	1.79	0.23	***		
Hexane	600	608	a	0.64	0.21	0.03	***	1472.8	3.181
Heptane (71)[f]	700	701	a	0.36	0.13	0.01	***	1.53	0.005
Octane	800	800	a	5.40	1.81	0.28	***	29.68	0.067
2-Octene	810	836	a	2.48	0.61	0.10	***		
Decane	1000	1000	a	1.15	0.47	0.12	*	4.48	0.002
Dodecane	1198	1200	a	0.48	0.53	0.17	ns		
Tridecane	1297	1300	a	0.40	0.34	0.03	ns		
Tetradecane (57)[f]	1398	1403	a	0.13	0.20	0.01	**		
Total				16.40	6.09	0.86	***	1508.5	3.248
Ketones									
Acetone	527	818	a	3.24	0.82	0.23	**		
2,3-Butanedione	625	1001	a	3.39	0.70	0.06	***		
2-Butanone	629	906	a	2.04	0.44	0.03	***	8.58	0.035
3-methyl-2-butanone	711		a					19.36	0.059
2-Pentanone	732	976	a	0.16		0.06			
2,3-Pentanedione	742	1055	a	17.07	4.78	1.63	**		
3-Hydroxy-2-butanone	779	1285	a	8.71	2.60	0.43	***	62.15	0.063
2-Heptanone (43)[f]	932	1177	a	0.88	0.46	0.05	**		
3-Octen-2-one	1093	1402	a	1.47	1.46	0.31	ns		
Total				36.96	11.26	2.25	***	90.09	0.149

Table 1. Continued.

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
Nitrogen compounds									
Pyridine	783	1176	a	0.36		0.10			
2-Ethylpyridine (106)[f]	933	1277	a	0.07	0.06	0.00	ns	0.15	0.0004
2,5-Dimethylpyrazine	943	1320	a	4.68	2.82	0.34	*		
Trimethylpirazine (42)[f]	1037	1395	a	1.72	1.36	0.08	***	1.23	0.001
Tetramethylpyrazine (54)[f]	1115	1467	a	0.11	0.11	0.01	ns	0.33	0.001
1-Piperidinecarboxaldehyde	1239	1756	a					4.24	0.008
Ethyl 3-pyridinecarboxylate	1280	1810	a					5.98	0.004
Total				6.94	4.35	0.41	***	11.92	0.017
Sulfur compounds									
3-(Methylthio)propanal	966	1457	a		0.58	0.07		4.75	0.002
Dimethyl disulfide (94)[f]	771	1059	a		0.04	0.002			
Dimethyl trisulfide (126)[f]	1001	1368	a	0.01	0.03	0.003	*		
Dimethyl sulfone	1060	1909	a					50.59	0.046
3-(Methylthio)propanol (106)[f]	1073	1724	a	0.08	0.08	0.01	ns		
Benzothiazol (135)[f]	1292	1950	a	0.04	0.08	0.01	*	0.35	0.003
2,3-Dihydrothiophene (85)[f]	1389	2232	b					4.49	0.016
Total				0.12	0.81	0.06	**	60.18	0.060
Acids									
Acetic acid	713	1473	a	229.51	40.37	15.80	***		
Propanoic acid	807	1559	a	0.17		0.01			
Butanoic acid (60)[f]	888	1655	a	8.37	2.48	0.28	***		
3-Methylbutanoic acid	937	1693	a	1.23	0.97	0.12	ns		
Pentanoic acid	974	1763	a	0.47	0.17	0.03	**		

Table 1. Continued.

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
4-Methylpentanoic acid (74)[f]	1044	1826	a	0.34	0.19	0.04	ns		
Hexanoic acid (60)[f]	1047	1867	a	2.68	1.74	0.19	*		
Octanoic acid	1262	2040	a	1.66	3.42	0.11	***		
Benzoic acid (105)[f]	1285	2264	a	0.02	0.01	0.002	ns		
Nonanoic acid (60)[f]	1354	2083	a	0.03	0.06	0.001	***		
Decanoic acid	1447	2117	a	1.44	2.48	0.05	***		
Total				245.91	51.89	11.89	***		
Alcohols									
Ethanol	505	939	a	166.61	45.40	9.78	***		
1-Propanol	611	1034	a	0.60	0.18	0.05	**	29.06	0.115
2-Methyl-1-propanol	681	1103	a					1240.6	2.906
1-Butanol	725	1151	a					30.55	0.069
1-Penten-3-ol	740	1168	a	3.67	0.91	0.32	**	16.47	0.027
3-Methyl-1-butanol	793	1214	a					455.68	0.178
2-Methyl-1-butanol	796	1213	a					95.21	0.105
1-Pentanol	825	1258	a	1.45	0.52	0.52	ns	18.07	0.024
2,3-Butanediol (45)[f]	889	1589	a					66.77	0.184
3-Ethoxy-1-propanol	895	1590	a					15.45	0.013
1-Hexanol (56)[f]	922	1361	a	0.02	0.01	0.003	ns	25.40	0.033
1-Heptanol (70)[f]	1020	1463	a	0.33	0.291	0.02	ns		
1-Octen-3-ol (57)[f]	1030	1460	a	0.62	1.59	0.23	ns	10.47	0.023
2-Ethyl-1-hexanol (57)[f]	1082	1498	a	0.29	0.23	0.02	ns	1.55	0.001
Phenol	1111	2012	a	0.71	0.57	0.01	***	17.65	0.004
Benzyl alcohol (79)[f]	1119	1883	a	0.19	0.43	0.05	*	13.88	0.015

Table 1. Continued.

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
1-Octanol (56)[f]	1125	1560	a	0.01	0.004	0.001	ns	3.83	0.010
Phenylethylalcohol (91)[f]	1194	1917	a	0.08	0.03	0.01	*	105.93	0.105
4-Methylphenol (107)[f]	1195	2048	a	0.49	0.31	0.03	**	7.21	0.008
2-Ethylphenol (107)[f]	1252	2098	a	0.04	0.05	0.005	ns	0.37	0.001
2-Phenoxyethanol (94)[f]	1310	2071	a					0.60	0.0004
Total				175.11	50.52	9.42	***	2155.3	2.831
Esters									
Methyl acetate	549	830	a	0.76	0.17	0.02	***		
Ethyl acetate	634	897	a	73.53	18.75	4.59	***	820.48	2.335
Ethyl propanoate (102)[f]	743	957	a	0.05	0.01	0.002	***	1.15	0.002
Propyl acetate	749	1001	a	0.11		0.01		4.02	0.010
Methyl butanoate	754	1012	a	0.18		0.04			
Ethyl 2-methylpropanoate (43)[f]	787	965	a	0.09	0.06	0.01	ns	76.57	0.034
Isobutyl acetate	805	1012	a					61.33	0.095
Ethyl butanoate (71)[f]	830	1036	a	2.72	0.89	0.05	***	41.14	0.053
Butyl acetate	847	1061	a					4.16	0.022
Ethyl 2-hydroxypropanoate	865	1351	a	2.59	1.20	0.21	*	882.07	0.647
Ethyl 2-methybutanoate	877	1044	a	0.57	0.55	0.06	ns	45.07	0.033
Ethyl 3-methylbutanoate (88)[f]	881	1061	a	0.27	0.21	0.02	ns	21.14	0.020
3-Methyl-1-butanol acetate	906	1116	a	0.35	0.25	0.03	ns	14.09	0.009
Ethyl pentanoate	926	1130	a	1.21	0.58	0.05	***	16.10	0.020
Isobutyl isobutanoate (71)[f]	940	1086	a					5.28	0.007
Isobutyl butanoate	981	1162	a					9.31	0.009
Ethyl 4-methylpentanoate	991	1190	a					2.36	0.005

Table 1. Continued.

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
Ethyl 3-hydroxybutanoate (45)[f]	994	1520	a	0.06	0.04	0.002	***	0.36	0.001
Methyl hexanoate	949	1183	a	0.67	0.39	0.10	*		
Ethyl hexanoate (88)[f]	1028	1234	a	1.95	1.52	0.11	*	53.14	0.042
Ethyl heptanoate	1125	1334	a	0.60	0.89	0.07	*	32.30	0.071
Isobutyl hexanoate	1180	1354	a					7.62	0.018
Ethyl benzoate (105)[f]	1222	1665	a	0.06	0.07	0.003	ns	3.59	0.011
Ethyl octanoate (88)[f]	1227	1436	a	0.81	1.17	0.03	***	828.60	2.673
Diethyl succinate (101)[f]	1235	1686	a					0.72	0.001
Ethyl benzeneacetate	1299	1788	a					6.08	0.023
Ethyl nonanoate (88)[f]	1325	1533	a	0.025	0.046	0.002	**	6.65	0.013
Bornyl acetate (95)[f]	1336	1567	a	0.122	0.167	0.01	***	0.61	0.001
Methyl decanoate (74)[f]	1356	1596	a					0.37	0.001
Isobutyl octanoate	1378	1543	a					8.79	0.015
Ethyl benzenepropanoate (104)[f]	1406	1885	a					1.96	0.009
Ethyl 9-decenoate	1421	1694	b					53.36	0.239
Ethyl decanoate	1425	1645	a	3.26	5.66	0.22	***	1140.7	3.331
Total				90.00	32.64	3.76	***	3504.3	2.785
Furans									
2-Methylfuran	615		a	0.44		0.05			
2-Ethylfuran	719	953	a	5.73	1.94	0.64	*		
2-Butyl furan	907	1134	a	0.60		0.03			
2-Pentylfuran (81)[f]	1007	1232	a	2.02	1.58	0.30	ns	5.32	0.003
Total				8.79	3.51	0.78	**	5.32	0.003

Table 1. Continued.

Compound	LR[lc] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
Lactones									
Butyrolactone (42)[f]	1019	1625	a	2.05		0.08	***	58.01	0.088
γ-Pentalactone	1062	1604	a	3.89	2.20	0.21	*	51.93	0.049
D-Pantolactone	1168	2045	a					6.31	0.012
γ-Nonalactone	1481	2014	a					9.23	0.029
Total				5.94	2.20	0.11	***	125.48	0.158
Aromatic hydrocarbons									
Benzene	674		a	0.31		0.03			
Tolueno (91)[f]	788	1031	a	1.57	0.51	0.03	***	5.86	0.018
Ethylbenzene (91)[f]	883	1112	a	0.09	0.03	0.01	*	0.94	0.003
p-Xylene (91)[f]	891	1111	a	0.29	0.14	0.01	***	2.65	0.005
o-Xylene	915	1172	a	0.21		0.01		2.28	0.006
Styrene	917	1252	a	0.29		0.03			
α-Pinene (93)[f]	940	1013	a	8.00	9.86	0.86	***	16.10	0.021
β-Myrcene (41)[f]	1001	1160	a	20.75	13.02	2.60	**	2.88	0.007
3-Carene (93)[f]	1022	1134	a	12.15	21.49	1.45	***	83.73	0.544
α-Terpinene	1034	1174	a	9.59	7.24	0.51	*	20.53	0.031
D-Limonene (68)[f]	1045	1182	a	79.33	48.23	7.67	***	83.24	0.474
p-Cymene	1050	1264	a	139.88	86.54	6.06	***	110.55	0.240
4-Terpineol (71)[f]	1227	1600	a	5.86	6.05	0.23	***	23.57	0.108
α-Terpineol	1250	1467	a	3.71	4.27	0.34	ns	28.45	0.028
Caprolactam	1382	2183	a					17.82	0.002
Eugenol	1435	2081	a					27.63	0.141
Methyleugenol	1461	2021	a	7.14	10.26	0.31	***	3.70	0.006

Table 1. Continued.

Compound	LRI[c] DB624	LRI HP-INNOWAX	RI[d]	SPME[a] CAR/PDMS fiber	SPME[a] DVB/CAR/PDMS fiber	SPME[a] SEM	SPME[a] P_{SPME}[e]	SAFE[b] SAFE	SAFE[b] SEM
Caryophyllene	1469	1575	a	104.37	165.79	15.42	**	413.52	0.110
Total				393.55	373.44	37.44	***	860.46	2.893

[a] Abundance expressed as AU x 10^{-6} (AU: abundance units, expressed as total ion chromatogram (TIC) or area of the target ion.

[b] Normalised area (10^{-2}) using as internal standard 2-methyl-3-heptanone.

[c] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a DB-624 capillary column and HP-INNOWAX.

[d] Reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard in both columns; b, tentatively identification by mass spectrum.

[e] P_{SPME} value of fibre effect, *** P<0.001, ** P<0.01, * P<0.05, ns, P>0.05.

[f] Target ion used to quantify the compound when the peak was not completely resolved.

Eighteen of the aroma-active compounds were found in both extraction techniques; SPME and SAFE (Table 2). However, SAFE elucidated two unknown compounds detected by SPME being *α*-terpineol (unknown 7) and 2,3-dihydrothiophene (unknown 8). Furthermore, SAFE extracted other compounds which were not detected by SPME such as 1-pentanol, 2-octenal (E), D-pantolactone, isobutyl hexanoate, 2-nonenal (*Z*), ethyl 3-piridinecarboxylate, 2,4-decadienal (*E,E*), eugenol, methyl eugenol and *γ*-nonalactone, all being high molecular weight compounds. However, SPME mainly extracted low molecular weight compounds such as ethyl acetate, acetic acid, 2,3-pentanedione, 3-methylbutanoic acid, heptanal, 2-heptenal (*Z*), trimethylpyrazine, octanal, 2,4-heptadienal (*E,E*), benzeneacetaldehyde, tetramethylpyrazine and phenylethylalcohol which were not extracted by SAFE.

3.3. Aroma active compound quantification and OAVs

The volatile concentrations calculated in SPME and SAFE extracts are shown in Table 2. The compounds present in SPME extract at highest concentration were acetic acid, hexanal, 2-heptenal (*Z*), octanal and nonanal when using both fibres. On the other hand, the most abundant compounds in the SAFE extract were hexanal, ethyl 2-methylpropanoate, ethyl hexanoate, ethyl butanoate and ethyl 3-methylbutanoate. The concentration calculated in the SAFE extract represented the total concentration present in the sausage matrix whilst SPME concentrations only represented the HS concentration. Therefore, the SPME concentrations only represented 0.006-18.4 % of the total concentration quantified in the SAFE extract (sausage matrix). The majority of aroma compounds in the HS were around 1-7 % of the total concentration except hexanal, *α*-terpinene and nonanal which represented 18.4 - 6.03%, 16.6 - 14.1% and 7.5 - 9.1%, respectively, for CAR/PDMS and DVB/CAR/PDMS fibres.

The contribution of the quantified aroma compounds to the overall aroma of dry fermented sausages was calculated by the OAVs (Table 3). In general, in SPME and SAFE extracts, the same compounds showed OAVs higher than 1 except for ethyl 2-methylpropanoate, ethyl hexanoate and 1-octen-3-ol which showed high OAVs in the SAFE extract although lower than 1 in SPME extract. On the contrary, nonanal and 4-methylphenol were extracted by SPME above their air detection threshold, and not by SAFE (Table 3).

3.4. Flavour reconstitution and sensory analysis

The olfactometry technique and OAVs revealed which aroma compounds contribute to dry fermented sausage aroma, but these techniques do not predict the aroma quality of the complex mixture of fermented sausage aroma. For this reason, the aroma-active compounds identified by GC-O and with OAVs above 1 were reconstituted in an aroma model and evaluated by sensory profile analysis and compared to fresh fermented sausage aroma. The chosen compounds were the 20 first compounds (Table 3) with OAVs above 1.

The first evaluated models were SPME (CAR/PDMS), SPME (DVB/CAR/PDMS) and SAFE. The results obtained in the aroma profile analysis for these models were compared to the fresh fermented sausage aroma and are shown in Figure 1A. The aroma profile analysis showed that dry fermented sausage aroma, SAFE and SPME aroma models evoked the same intensities for the rancid, cheesy and stable aroma descriptors (Fig. 1A). However, the cured meat and pepper aroma descriptors were significantly lowest in all aroma models while sour and fruity aroma descriptors were significantly different for SAFE and SPME (DVB/CAR/PDMS) aroma models. SAFE aroma model had a significant lower sour and higher fruity aroma than dry fermented sausage.

Due to the differences found between aroma models and sausage aroma, it was decided to prepare mixtures of SAFE with each SPME aroma models and these mixtures were compared to fresh fermented sausage by the trained panel (Fig. 1B). Significant differences were only detected for pepper, sour and cured meat aromas (Fig. 1B) which were scored lower than sausage aroma. The SAFE plus SPME (CAR/PDMS) aroma model showed the best similarity to the intensity of the attributes evaluated therefore, it was used as the aroma base for the following analysis. Finally, the following compounds: 2,4-decadienal (*E,E*), benzothiazole, methyl eugenol, *α*-terpineol, and eugenol were incorporated to the SAFE plus SPME (CAR/PDMS) model because they were responsible for pepper, sour and cured notes as evaluated by GC-O (Table 2). The results obtained in the aroma profile analysis compared to fresh sausage aroma are showed in Fig. 1C. The aroma intensities of pepper, rancid, sour, cheesy, fruity and stable aroma notes in the model were similar to sausage aroma except for a lower significant cured meat aroma.

Table 2. Odour active compounds identified and quantified in dry fermented sausages by SAFE and in the headspace of dry fermented sausages by SPME.

Compound	LRI[b] GC-O	LRI standard	RI[c]	Descriptor
Ethyl acetate	638	643	a	Peach, spicy, yeast
Acetic acid	701	700	a	Vinegar
2,3-Pentanedione	736	739	a	Sweet, vanilla, roasted nuts
Ethyl 2-methylpropanoate	787	789	a	Strawberry, pineapple, red apple
1-Pentanol	821	820	a	Sweet
Ethyl butanoate	825	825	a	Strawberry, fruity
Hexanal	836	836	a	Fresh cut grass
Ethyl 2-methylbutanoate	871	872	a	Sweet, strawberry, pineapple
Ethyl 3-methybutanoate	875	876	a	Fruity, floral
Ethyl pentanoate	922	924	a	Fruity
3-Methylbutanoic acid	930	930	a	Cheese, unpleasant
Heptanal	939	937	a	Unpleasant, cabbage
Unknown 1	963		c	Roasted nuts, toasted
3-(Methylthio)propanal	968	969	a	Cooked potato, asparagus
Ethyl 3-hydroxybutanoate	990	994	a	Sweet, fruity, pineapple
2- Heptenal (Z)	1009	1011	a	Unpleasant, toasted, vegetables
1-Octen-3-ol	1024	1028	a	Mushroom
Ethyl hexanoate	1026	1027	a	Sweet, acid strawberry
α-Terpinene	1035	1037	a	Mustiness, floral, green pepper
2-Octenal (E)	1111	1103	a	Glue, talcum powder, fruity
Trimethylpyrazine	1038	1038	a	Coffee, hot chocolate
Octanal	1046	1048	a	Citric
2,4-Heptadienal (E,E)	1071	1077	a	Glue, herbal, fresh
Unknown 2	1077		c	Toasted, spicy
Benzeneacetaldehyde	1111	1112	a	Roses, pollen
Tetramethylpyrazine	1117	1118	a	Roasted nuts, toasted, varnished wood
Nonanal	1150	1151	a	Fresh, orange, spicy
Unknown 3	1159		c	Floral, toasted
D-Pantolactone	1164	1170	a	Cooked vegetables, unpleasant
Unknown 4	1173		c	Cherry, strawberry, varnished wood
Isobutyl hexanoate	1177	1180	a	Sweet, fruity, floral
Unknown 5	1179		c	Roasted nuts, toasted
Phenylethylalcohol	1192	1195	a	Roses
4-Methylphenol	1193	1190	a	Stable, leather, horse
Unknown 6	1202		c	Grease, wood, floral, savoury
2-Nonenal (Z)	1216	1222	a	Toast, fruity
Ethyl benzoate	1225	1226	a	Fruity, herbal, mustiness
Unknown 7/α-Terpineol[h]	1250	1255	a	Freshness, soap, herbal
Ethyl 3-pyridinecarboxylate	1284	1284	a	Mustiness
Benzothiazole	1293	1292	a	Barbecue sauce, onion, herbal
2,4-Decadienal (E,E)	1395	1393	a	Herbal, roasted, glue
Unknown 8/ 2,3-Dihydrothiophene[h]	1401		b	Savoury, herbal, wood
Eugenol	1442	1439	a	Tallowy, spicy
Methyleugenol	1455	1459	a	Spicy, clove, floral
γ-Nonalactone	1488	1488	a	Fruity, roasted, metallic

[a]Concentration expressed as ng compound in the HS/ g fermented sausage. [b]Linear retention indices (LRI) of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column.[c] Reliability of identification: a, identification by mass spectrum, coincidence with the LRI of an authentic standard in both columns and by coincidence of the assessors's descriptors with those described in the Fenaroli's handbook of flavour ingredients (Burdock, 2002); b, tentatively identification by mass spectrum; c, unknown compounds.

SPME			SAFE		
	Concentration (ng g^{-1})[a]				
DF[d]	CAR/PDMS	DVB/CAR/PDMS	FD[e] factor	Concentration[f] range(ng g^{-1})	Concentration[f] mean (ng g^{-1})
4	51.16	15.02	-[g]	- -	-
5	254.62	60.47	-	- -	-
4	24.05	7.97	-	- -	-
8	0.04	0.02	500	531.68 - 617.09	575.16
-	-	-	2	27.17 - 40.56	33.32
8	3.432	1.01	10	293.89 - 427.59	346.12
5	148.70	48.76	1	650.84 - 967.19	808.35
8	0.27	0.27	100	52.66 - 66.52	61.11
8	0.75	0.67	500	194.10 - 259.37	230.19
3	0.55	0.23	50	17.17 - 24.69	20.42
4	4.48	4.33	-	- -	-
3	7.74	4.21	-	- -	-
6	-	-	-	- -	-
6	3.18	2.05	10	61.87 - 71.97	67.21
8	0.34	0.24	50	3.29 - 8.54	5.92
4	13.33	9.82	-	- -	-
8	2.55	3.86	50	43.56 - 75.54	55.86
8	1.94	1.02	10	453.74 - 581.40	506.96
5	3.24	2.76	50	14.28 - 22.48	19.54
-	-	-	1	9.62 - 15.49	13.27
4	2.80	2.44	-	- -	-
5	10.93	7.12	-	- -	-
3	1.58	1.46	-	- -	-
3	-	-	-	- -	-
6	1.34	1.44	-	- -	-
6	0.24	0.25	-	- -	-
4	10.40	12.61	2	82.63 - 175.91	138.52
3	-	-	-	- -	-
-	-	-	10	13.72 - 25.91	19.53
7	-	-	-	- -	-
-	-	-	50	8.88 - 15.00	12.71
6	-	-	-	- -	-
6	0.74	0.67	-	- -	-
4	0.96	0.66	100	18.20 - 39.23	36.57
5	-	-	2		
-	-	-	1	11.99 13.17	12.52
8	0.04	0.04	100	7.34 - 16.30	11.88
5	-	-	50	15.80 - 18.34	17.08
-	-	-	1	13.39 - 15.11	14.40
8	0.07	0.10	100	3.13 - 4.74	3.94
-	-	-	100	21.24 - 28.32	25.76
4	-	-	100	- -	-
-	-	-	1	34.76 - 82.42	58.64
-	-	-	500	8.68 - 10.31	9.50
-	-	-	100	12.98 - 26.24	17.71

[d]DF Detection frequency value of the extracted compounds from HS with DVB/CAR/PDMS fiber.[e]FD flavour dilution factor.[f]Concentration expressed as ng compound/g fermented sausage.[g]Not detected in the HS of SPME extract or in SAFE extract.[h]The compound was identified exclusively by SAFE while was not identified in the HS by SPME.

Table 3. Odour Activity Values of aroma active compounds extracted by SPME and SAFE.

Compound	Threshold air (ng g^{-1})[b]	Threshold oil (ng g^{-1})[b]	OAV[a]		
			CAR/PDMS	DVB/CAR/PDMS	SAFE
Ethyl 2-methylpropanoate	0.3[1]	1.2[1]	<1	<1	479
Ethyl 3-methylbutanoate	0.07[1]	0.62[1]	11	10	371
3-(Methylthio)propanal	0.063[1]	0.2[1]	51	33	336
Ethyl 2-methylbutanoate	0.06[1]	0.26[1]	4	4	235
Nonanal	0.3[1]	1000[1]	35	42	<1
3-Methylbutanoic acid	0.22[1]	22[1]	20	20	-
4-Methylphenol	0.05[1]	68[3]	19	13	<1
Ethyl pentanoate	0.3[1]	1.5[2]	2	<1	14
Ethyl hexanoate	3[1]	40[1]	<1	<1	13
Ethyl butanoate	2.7[1]	28[1]	1	<1	12
Acetic acid	25[1]	124[1]	10	2	-
Hexanal	20[1]	120[1]	7	2	7
2-Nonenal (Z)	0.1[1]	4.5[1]	-	-	3
γ-Nonalactone	7[2]	7[2]	-	-	3
2,3-Pentanedione	10[1]	-	2	<1	-
Benzeneacetaldehyde	0.61[1]	22000[1]	2	2	-
Octanal	5[1]	320[1]	2	1	-
Phenylethylalcohol	0.35[1]	211[1]	2	2	-
1-Octen-3-ol	12[1]	34[1]	<1	<1	2
Heptanal	6[1]	250[1]	1	<1	-
2- Heptenal (Z)	34[1]	1500[1]	<1	<1	-
α-Terpinene	2350[1]	85[1c]	<1	<1	<1
Ethyl acetate	340[1]	10000[1]	<1	<1	-
2,4-Decadienal (E,E)	0.04[1]	180[1]	-	-	<1
Methyl-eugenol	68[1]	68[2]	-	-	<1
Eugenol	0.022[1]	512[3]	-	-	<1
Ethyl benzoate	0.6[1]	100[2]	<1	<1	<1
α-Terpineol	10[1]	280[2]	-	-	<1
Trimethylpyrazine	50[1]	27000[1]	<1	<1	-
Benzothiazole	80[1]	80[2]	<1	<1	<1
1-Pentanol	20[1]	850[1]	-	-	<1
2,4-Heptadienal (E,E)	57[1]	10000[1]	<1	<1	-
Ethyl 3-hydroxybutanoate	1000[1d]	1000[1d]	<1	<1	<1
D-Pantolactone	2200[1d]	2200[1d]	-	-	<1
2-Octenal (E)	9[1]	7000[1]	-	-	<1
Tetramethylpyrazine	690[1]	38000[1]	<1	<1	-
Isobutyl hexanoate	-	-	-	-	-
Ethyl 3-pyridinecarboxylate	-	-	-	-	-
2,3-Dihydrothiophene	-	-	-	-	-

[a]Odour activity values for SPME and SAFE extractions were calculated using the concentration found in the HS or sausages respectively.

[b]Threshold values obtained from the following sources: (1) Van Gemert and Nettenbreijer (2004), (*2*) Burdock (2002) and (*3*) Söllner and Schieberle (2009).

[c]Threshold value from *α*- Terpinene was only reported in water.

[d]Threshold value from ethyl 3-hydroxybutanoate and D-Pantolactone were only reported in 14 % (v/v) ethanol/ tartaric acid to pH 3.5.

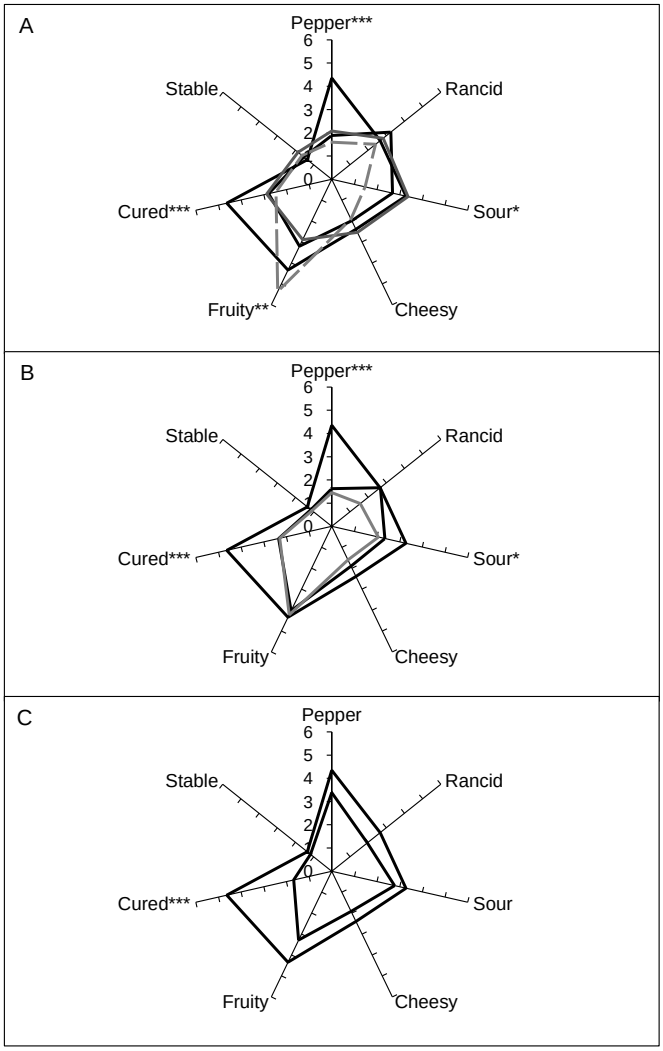

Figure 1. Aroma profile analyses of dry fermented sausage (black line) compared to: (A) model mixture containing the volatile compounds extracted by SPME (CAR/PDMS) (grey stippling), SPME (DVB/CAR/PDMS) (grey line) and SAFE (grey broken line); (B) model mixture containing the volatile compounds extracted by SAFE plus SPME (CAR/PDMS (grey stippling) or DVB/CAR/PDMS (grey line); (C) model mixture containing the volatile compounds extracted by SPME (CAR/PDMS) plus SAFE plus 2,4-decadienal (*E,E*), methyl eugenol, eugenol, *α*-terpineol and benzothiazole (black stippling). Asterisks in each aroma descriptor indicate significant differences (****P*<0.001, ***P*<0.01, **P*<0.05).

4. DISCUSSION

The first screening for the identification of key aroma compounds was to detect all the volatile compounds present in the sausage sample by both techniques (SPME and SAFE results shown in Table 1), and further reduce the number of volatiles by olfactometry techniques (Table 2). However, the use of different extraction techniques (SPME and SAFE) in dry fermented sausages produced qualitative and quantitative different aroma extracts. CAR/PDMS fibre significantly extracted the highest amount of volatile compounds (Table 1) as already shown in other meat products (Gianelli *et al.*, 2002; Marco *et al.*, 2004). The DVB/CAR/PDMS fibre showed a highest affinity to high molecular weight compounds while CAR/PDMS fibre to low molecular weight compounds (Gianelli *et al.*, 2002). This fact can be explained by the larger pores present in the DVB solid polymer than in CAR polymer. In addition, the highest sulphur extraction capacity of the DVB/CAR/PDMS fibre was also indicated by Marco *et al.* (2004) in dry fermented sausages (Table 1).

In contrast to HS technique, the distillation techniques allow to extract the volatile compounds present in the matrix. It was observed that several of the volatile compounds present in the HS by SPME were not present in the SAFE extract (Table 1) probably due to their high volatility and therefore, they were lost during the distillation and concentration steps (Söllner & Schieberle, 2009). Some of them such as acetaldehyde, pentane, ethanol, propanal, acetone and methyl acetate were not detected due to solvent delay applied in the GC-MS. However, 27 new compounds were identified by SAFE and they were not detected by SPME probably due to a matrix effect that did not allow the release of these compounds to the HS (Majcher & Jelen, 2009). In summary, SAFE allowed mainly the isolation of compounds presents in low concentration in dry fermented sausages whilst SPME of highly volatile compounds.

However, not all the identified compounds contribute to dry fermented sausages aroma as it was shown by the olfactometry analysis (Table 2). All of the aroma-active compounds detected by SPME and SAFE GC-O have been previously reported as aroma compounds in dry fermented sausages (Marco *et al.*, 2007; Olivares *et al.*, 2011; Schmidt & Berger, 1998a, b; Blank *et al.*, 2001; Söllner & Schieberle, 2009; Stahnke, 1994; Gianelli *et al.*, 2011; Corral *et al.*, 2013) except ethyl 3-hydroxybutanoate, trimethylpyrazine, D-pantolactone, isobutyl hexanoate, ethyl benzoate, *α*-terpineol, ethyl 3-pyridinecarboxylate, benzothiazole, 2,3-dihydrothiophene, methyl eugenol, *γ*-nonalactone. Furthermore, the SAFE technique was able to elucidate two unknown aroma

compounds (*α*-terpineol and 2,3-dihydrothiophene, Table 2) that were detected by olfactometry analysis on the SPME extract but due to their low concentration they were not identified. Therefore, the SPME and SAFE techniques are complementary for providing a more complete aroma profile of dry fermented sausages.

The quantification of compounds extracted by SPME in the HS of the sausage does not directly represent the real amount present in the sample as it depends on the kinetic absortion to the fibre (Arthur & Pawliszyn, 1990) and interactions with other food components (Guichard, 2002). By contrast, SAFE is a quantitative extraction technique and represents the concentration present in the food matrix. Similar HS percentages in dry fermented sausages were also observed by Olivares *et al.* (2009) when they compared the HS extraction to the total concentration calculated using multiple-HS-SPME analysis.

On the other hand, aroma perception not only depends on concentration and odour threshold, but different interactions take place also between aroma compounds that can affect the aroma perception of single compounds (Guichard, 2002). Therefore, flavour reconstitution analyses were performed using the mixture of the aroma compounds described by SPME and SAFE (Table 2, Fig. 1). The fruity character of the fermented sausages was due to the high OAVs of ester compounds (ethyl 2-methylpropanoate, ethyl 2-methyl and 3-methylbutanoate, ethyl butanoate, ethyl pentanoate and ethyl hexanoate). Previous studies by AEDA had demonstrated their contribution to fermented sausage aroma (Schmidt & Berger, 1998a, b) but these authors were not able to select which of them either their concentrations, were the contributors to the final aroma. Finally, the aroma profile analysis revealed that dry fermented sausages aroma compounds were properly identified and quantified except those aroma compounds that evoked cured meat aroma (Fig. 1C). However, all panellists agreed that the aroma model reminded them of the sausage aroma. The lower cured meat aroma score could be attributed to 2,3-dihydrothiophene which was not included in the aroma model or to other unknown compounds that were not identified, as unknown 6 that contributes to greasy, wood, savoury notes (Table 2).

In previous studies (Marco *et al.*, 2007; Olivares *et al.*, 2011; Gianelli *et al.*, 2011; Corral *et al.*, 2013), several compounds were described as meaty odorants in dry fermented sausages such as 2-ethylfuran, 2-pentylfuran, dimethyl disulphide, 2,4-heptadienal (*E,E*). Although they were also detected in the present study, they were not included in the aroma models assayed due to their

low OAVs (<1). Moreover, several meaty odorants identified in Mediterranean fermented sausages such as 2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran and dipropyl trisulphide (Meynier *et al.*, 199; Chevance *et al.*, 2000) were not found in the dry fermented sausages used. However, the contribution of these compounds to fermented sausage aroma was not studied by sensory analysis.

A previous study in a fermented sausage, Hungarian-type salami, reported different aroma notes such as sweaty, roasty,-popcorn, potato, meat, honey, smoky, spicy and garlic (Söllner & Schieberle, 2009). The presence of smoky notes is due to the smoking process applied in these northern European sausages that provided a high concentration of phenolic compounds. Also, different ingredients such as spices provided characteristics notes (garlic notes). However, these authors did not report any characteristic cured meat note. Also, our study did not find any compound responsible for a cured meat aroma note (Table 2) only few compounds contributed to savoury or meaty notes (unknown 6, benzothiazole and 2,3-dihydrothiophene). In addition, in meat products the effect of nitrite as curing agent has a crucial influence on flavour, especially on cured meat aroma due to the balance of oxidation and sulfur compounds more than on a specific aroma compound (Marco *et al.*, 2007; Thomas *et al.*, 2013).

In summary, SPME and SAFE techniques were complementary and necessary to reproduce the overall dry fermented sausage aroma as it has been demonstrated by aroma profile analysis. However, the final aroma model including odorants with OAV>1 from SPME and SAFE extracts, was incorporated with compounds 2,4-decadienal (*E,E*), benzothiazole, methyl eugenol, *α*-terpineol, and eugenol to achieve the fatty, cured meat, pepper and sour aroma notes. Nevertheless, further studies should be performed to elucidate those aroma compounds that evoke the cured meat note characteristic of dry fermented sausages from southern Europe.

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REFERENCES

Arthur, C. L. & Pawliszyn, J. (1990). Solid-phase microextraction with thermal-desorption using fused-silica optical fibers. *Analytical Chemistry,* 62, 2145-2148.

Blank, I., Devaud, S., Fay, L.B., Cerny, C., Steiner, M. & Zurbriggen, B. (2001).Odor-active compounds of dry-cured meat: Italian-type salami and Parma ham. In *Aroma-Active Compounds in Foods. Chemistry and Sensory Properties, ed. by* G. R. Takeoka, M. Güntert & K. H. Engel, American Chemical Society: Washington, D.C., pp. 9-20.

Burdock, G. A. (2002).*Fenaroli's handbook of flavor ingredients* (4th ed.). Boca Raton, Florida: CRC Press Inc.

Chevance, F. F. V., Farmer, L. J., Desmond, E. M., Novelli, E., Troy, D. J. & Chizzolini, R. (2000). Effect of some fat replacers on the release of volatile aroma compound from low-fat meat products. *Journal of Agricultural and Food Chemistry,* 48, 3476-3484.

Conter, M., Zanardi, E., Ghidini, S., Pennisi, L., Vergara, A., Campanini, G., Ianieri, A. (2008). Consumers' behaviour toward typical Italian dry sausages. *Food Control,* 19, 609–615.

Corral, S., Salvador, A. & Flores, M. (2013). Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science,* 93, 776-785.

Elmore, J. S. (2011). Aroma. In*. Sensory analysis of foods of animal originm,* ed. by L. M. L. Nollet, F. Toldrá, Boca Raton , Florida: CRC Press Taylor & Francis Group, pp. 243-265.

Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation, a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *European Food Research and Technology,* 209, 237-241.

Flores, M. (2011). Flavour of meat products. In *Sensory analysis of foods of animal origin,* ed. by L. M. L. Nollet & Toldrá, F, Boca Raton, Florida: CRC Press Taylor & Francis Group., pp. 131-145.

Gianelli, M. P., Flores, M. & Toldrá, F. (2002). Optimisation of solid phase microextraction (SPME) for the analysis of volatile compounds in dry-cured ham. *Journal of the Science of Food and Agriculture,* 82, 1703-1709.

Gianelli, M. P., Olivares, A. & Flores, M. (2011). Key aroma components of a dry-cured sausage with high fat content (Sobrassada). *Food Science and Technology International,* 17, 63-71.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International,* 18, 49-70.

Kovats, E.S. (1965).Gas chromatographic characterization of organic substances in the retention index system. In *Advances in chromatography,* ed. by J. C. Giddings, R. A. Keller, New York: Marcel Dekker, Inc., pp. 229-247.

Majcher, M. & Jeleń, H. H. (2009).Comparison of suitability of SPME, SAFE and SDE methods for isolation of flavor compounds from extruded potato snacks. *Journal of Food Composition and Analysis,* 22, 606-612.

Marco, A., Navarro, J. L. & Flores, M. (2004).Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). *Food Chemistry,* 84, 633-641.

Marco, A., Navarro, J. L. & Flores, M. (2007). Quantitation of selected odor-active constituents in dry fermented sausages prepared with different curing salts. *Journal of Agricultural and Food Chemistry,* 55, 3058-3065.

Meynier, A., Novelli, E., Chizzolini, R., Zanardi, E. & Gandemer, G. (1999). Volatile compounds of commercial Milano salami. *Meat Science,* 51, 175-183.

Olivares, A., Navarro, J. L. & Flores, M. (2009). Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage. *Food Chemistry,* 115, 1464-1472.

Olivares, A., Navarro, J. L. & Flores, M. (2010). Comparison of flavor components in dry sausages obtained from commercial and non fermented sausages from protected origin. In *Recent Advances in Food and Flavour Chemistry. Food Flavors and encapsulation, health benefits, analytical methods, and molecular biology of functional foods*, ed. by C.T. Ho, C.J. Mussinan, F. Shahidi, E. Tratas Contis, RSC Publishing, The Royal Society of Chemistry, pp 150-156.

Olivares, A., Navarro, J. L., Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science,* 87, 264-273.

Pollien, P., Ott, A., Montigon, F., Baumgartner, M., Muñoz-Box, R. & Chaintreau, A. (1997). Hyphenated headspace-gas chromatography-sniffing technique: screening of impact odorants and quantitative aromagram comparisons. *Journal of Agricultural and Food Chemistry,* 45, 2630-2637.

Schmidt, S., & Berger, R. G. (1998a). Aroma compounds in fermented sausages of different origins. *LWT - Food Science and Technology,* 31, 559-567.

Schmidt, S., & Berger, R. G. (1998b). Microbially formed aroma compounds during the maturation of dry fermented sausage (salami). *Advances in Food Sciences,* 20, 144-152.

Söllner, K. & Schieberle, P. (2009). Decoding the key aroma compounds of a Hungarian-type salami by molecular sensory science approaches. *Journal of Agricultural and Food Chemistry,* 57, 4319-4327.

Stahnke, L. H. (1994). Aroma components from dried sausages fermented with *Staphylococcus xylosus*. *Meat Science,* 38, 39-53.

Thomas, C., Maercier, F, Tournayre, P., Martin, J. L. & Berdagué, J. L. (2013). Effect of nitrite on the odourant volatile fraction of cooked ham. *Food Chemistry,* 139, 432-438.

Toldrá F. & Flores, M., (2007). Sensory quality of meat products. In *Handbook of Food Products Manufacturing,* ed. by Y. H. Hui, John Willey & Sons, U.S.A, pp. 303-328.

Ullrich, F. & Grosch, W. Z. (1987). Identification of the most intense odor compound formed during the autoxidation of linoleic acid. *Zitschrift für Lebensmittel-Untersuchung und-Forschung* 184: 277-282.

Van Gemert, L. & Nettenbreijer, A. (2004). *Compilation of odour threshold values in air, water and other media*. The Netherlands: BACIS: Zeist.

SUPPORTING INFORMATION

Table 1. Linearity and correlation coefficients of the standard compounds analyzed by HS-SPME

Compound	Measure range (ng)	Correlation coefficient (R^2)	Slope (A/ng x 10^{-4})[a]	Intercept (10^{-4})
Ethyl acetate	11.76 - 188.13	0.9992	28.99	-325.03
Acetic acid	13.75 - 440.00	0.9988	18.68	-1671.2
2,3-Pentanedione	1.48 - 47.50	0.9920	14.58	-108.57
Ethyl 2-methylpropanoate	0.05 - 3.33	0.9892	22.28	4.38
Ethyl butanoate	0.15 - 19.44	0.9857	14.42	15.66
Hexanal	20.94 - 335.00	0.9977	40.37	329.54
Ethyl 2-methylbutanoate	0.18 - 2.81	0.9974	49.21	-11.28
Ethyl 3-methylbutanoate	0.29 - 18.75	0.9984	12.39	-21.13
Ethyl pentanoate	0.14 - 2.22	0.9819	37.56	14.38
3-Methylbutanoic acid	2.81 - 22.50	0.9637	20.11	-343.14
Heptanal	3.05 - 48.75	0.9993	5.76	-25.81
3-(Methyl)thiopropanal	0.63 - 5.00	0.9854	7.33	-18.03
Ethyl 3-hydroxybutanoate	0.10 - 3.33	0.9885	3.66	-0.57
2- Heptenal	1.70 - 54.38	0.9719	1.85	-17.93
1-Octen-3-ol	1.18 - 75.56	0.9992	15.47	-142.35
Ethyl hexanoate	3.44 - 110.00	0.9766	8.78	106.89
α-Terpinene	1.56 - 50.00	0.9989	82.49	-424.98
Trimethylpyrazine	1.95 - 15.63	0.9946	16.37	-65.29
Octanal	0.25 - 16.25	0.9976	3.05	-8.05
2,4-Heptadienal (E,E)	1.41 - 11.25	0.9941	18.29	-71.49
Benzeneacetaldehyde	0.27 - 17.50	0.9940	43.75	-149.61
Tetramethylpyrazine	0.02 - 1.53	0.9908	8.75	-0.02
Nonanal	1.82 - 116.67	0.9985	30.17	-40.42
Phenylethyl alcohol	0.26 - 4.22	0.9427	11.3	-35.67
4-Methylphenol	0.09 - 11.73	0.9931	11.1	-6.32
Ethyl benzoate	0.01 - 1.11	0.9922	32.55	0.04
Benzothiazole	0.04 - 5.33	0.9931	22.37	-3.86

[a] A represents the area of compound

Table 2. Linearity and correlaciton coefficients of the standard compounds analyzed by SAFE.

Compound	Measure range (ng)	Correlation coefficient (R^2)	Slope (A/ng x 10^{-4})[a]	Intercept (10^{-4})
Ethyl 2-methylpropanoate	22.23 - 711.47	0.9944	14.22	-3.88
1-Pentanol	5.10 - 326.09	0.9997	63.36	-2.69
Ethyl butanoate	21.54 - 689.23	0.9959	13.03	-3.22
Hexanal	25.94 - 830.04	0.9997	38.21	-2.78
Ethyl 2-methylbutanoate	10.42 - 667.00	0.9981	77.52	-1.52
Ethyl 3-methylbutanoate	22.93 - 733.70	0.9974	10.41	-2.43
Ethyl pentanoate	6.72 - 429.84	0.9997	92.8	-2.54
3-(Methyl)thiopropanal	2.78 - 177.87	0.9962	7.84	-0.43
Ethyl 3-hydroxybutanoate	0.35 - 22.23	0.9980	7.29	-0.06
1-Octen-3-ol	7.87 - 503.96	0.9992	23.34	-2.35
Ethyl hexanoate	22.93 - 733.70	0.9994	11.06	-2.01
α-Terpinene	6.02 - 385.38	0.9998	118.59	-2.26
2-Octenal	2.26 - 144.52	0.9962	61.78	-3.24
Nonanal	12.16 - 778.17	1.0000	68.95	-5.43
DL-Pantolactone	6.14 - 49.10	0.9871	33.45	-0.11
Isobutyl hexanoate	7.18 - 459.49	0.9998	94.08	-4.14
4-Methylphenol	11.12 - 711.47	0.9988	28.7	-3.11
2-Nonenal	2.61 - 166.75	0.9958	4.18	-0.28
Ethyl benzoate	7.87 - 503.96	0.9999	42.43	-1.37
α-Terpineol	5.56 - 355.73	0.9993	216.72	-7.96
Ethyl 3-pyridinecarboxylate	2.26 - 144.52	0.9957	68.43	-3.71
Benzothiazole	1.51 - 96.34	0.9984	32.1	-0.89
2,4-Decadienal (E,E)	11.46 - 733.70	0.9986	22.38	-3.21
Eugenol	8.57 - 548.42	0.9994	60.1	-7.04
Methyleugenol	1.97 - 125.99	0.9967	79.28	-3.71
γ -Nonalactone	2.03 - 129.69	0.9977	68.77	-2.75

[a] A represents the area of compound

Capítulo 2

Determination of sulphur and nitrogen compounds during dry fermented sausages processing and their relationship with amino acid generation

In preparation

Determination of sulphur and nitrogen compounds during dry fermented sausages processing and their relationship with amino acid generation

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In preparation

Abstract

The identification of potent odorants sulphur and nitrogen compounds was investigated during the processing of dry fermented sausages. Also, the generation of free amino acids was followed in order to elucidate their contribution to the production of these potent odorants. The volatile compounds present in the dry sausages were extracted using Solvent Assisted Flavour Evaporation (SAFE) and monitored by one and two-dimensional gas chromatography with different detectors; mass spectrometry (MS), nitrogen phosphorous (NPD), flame photometric (FPD) and olfactometry (O) detectors. A total of seventeen sulphur and nitrogen compounds were identified and quantified and their content increased throughout the ripening process. Amongst them, 2-acetyl-1-pyrroline, methional, 2-ethylpyrazine and 2,3-dihydrothiophene were the most potent odorants characterized by toasted, cooked potato, and nutty notes. The free amino acids generated during the first stages of processing acted as a source of potent odorants, together with sausage environment conditions, acidic pH and low a_w values obtained during the long ripening time. The degradation of free amino acids was related to the production of volatile compounds such as methionine with methional, thiazole, 2,4-dimethylthiazole and benzothiazole while ornithine produced 2-acetyl-1-pyrroline and glycine 2,6-dimethlypyrazine and 2-ethyl pyrazine.

Keywords: Nitrogen, Sulphur, Volatile compounds, Amino acid, Dry fermented sausage

1. INTRODUCTION

Traditional fermented sausages are dried for long ripening times where a high number of microbial, biochemical and chemical reactions take place. These reactions are related to flavour development (Toldrá, Sanz & Flores, 2001). Although the sausage flavour is caused by a high number of volatile compounds, volatile sulphur and nitrogen compounds play a crucial role on the formation of sausage aroma due to their low odour threshold values and their characteristic olfactive notes (Mottram, 1998). Several odour notes like onion, roasted nuts, meat broth, rotten egg, cabbage, popcorn, fried snacks, chocolate have been reported in dry fermented sausages although few of them were identified (Corral, Salvador & Flores, 2013; Gianelli, Olivares & Flores, 2011; Olivares, Navarro & Flores, 2011). Therefore, the further identification of these compounds remains a challenge for the dry fermented process industry to determine the mechanism related to their generation and their effect on final sensory quality.

The use of one dimensional gas chromatography mass spectrometry (GC-MS) is widely used to elucidate sausage aroma although the low concentration, in the ppb to ppt range, of volatile sulphur and nitrogen compounds in meat products (Thomas, Mercier, Tournayre, Martin & Berdagué, 2014) requires the use of highly sensitive and selective detectors such as flame photometric, pulsed flame photometric, sulphur chemiluminescence, nitrogen-phosphorus or olfactometry detectors. In addition, volatile identification can be a laborious task since coelution of compounds of interest is common in complex food matrices. A strong tool to achieve a high separation and identification of compounds is two dimensional GC with mass spectrometric detector (heart-cutting 2DGC, GC-GC; or comprehensive 2D-GC, GCxGC). Anyway, the selection of a suitable volatile extraction technique is a crucial point, as the aroma profile obtained will depend on this technique. In this sense, the use of solvent assisted flavour evaporation (SAFE) can be a good selection to enrich the compounds at trace level such as volatile sulphur and nitrogen compounds as it is a solvent extraction at vacuum which avoids artefact formation by thermal degradation (Engel, Bahr & Schieberle, 1999).

Several mechanisms are involved in the generation of sulphur compounds in dry fermented sausages. However, it is well known that sulphur containing amino acids (cysteine and methionine) together with thiamine are precursor of these volatile sulphur compounds (Mottram, 1998). While pork contains the highest thiamine content of any meat (Mottram, 1991), during dry fermented sausage production a great generation of free amino acids is produced mainly

due to endogenous proteolytic activities (Toldrá, *et al.*, 2001). The degradation of methionine and cysteine produces methanethiol and hydrogen sulfide, respectively, and owing to their high reactivity generate other volatile sulphur compounds (Mottram, 1991). On the other hand, volatile nitrogen compounds can be formed from a nitrogen source such as any amino acid produced in the Strecker degradation (Meynier & Mottram, 1995). Whilst enzymatic endogenous activity is predominant at the beginning of the ripening process, the microorganism metabolism plays a crucial role during the amino acid degradation to volatile compounds (Molly, Demeyer, Raemaekers, Ghistelinck & Geenen, 1997). Thus, the relationship between the generation of free amino acids and volatile sulphur and nitrogen compounds can be useful to understand the mechanism of production of these compounds in dry fermented sausages.

Therefore, the aim of the present work was to identify the odour potent volatile sulphur and nitrogen compounds produced during the ripening of dry fermented sausages. Furthermore, the generation of free amino acids was followed during processing in order to elucidate their contribution to the production of sulphur and nitrogen odour active compounds.

2. MATERIAL AND METHODS

2.1. Dry fermented sausage processing

Dry fermented sausages were manufactured with the following ingredients and additives (g/Kg): lean pork (75 %), pork back fat (25 %) and lactose (20); dextrin (20); sodium caseinate (20); glucose (7); sodium ascorbate (0.5); sodium nitrite (0.15); potassium nitrate (0.15) and starter culture (0.1) C-P-77-S bactoferm (Chr. Inc. Hansen, Denmark) containing *Lactobacillus pentosus and Staphylococcus carnosus*. The manufacturing process was described by Corral *et al.* (2013). The meat mixture was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) and the sausages were subjected to drying at initial stage of 15-20 ºC and 75-85 % for 24 h, followed by ripening at 9 ºC and 75-85 % HR for 66 days. In order to control the ripening process, weight losses, and pH were measured during processing (Corral *et al.*, 2013).

Three sausages were randomly collected at 0, 15, 36 and 66 days to study the effect of ripening time on volatile sulphur and nitrogen compounds. Several sausage slices (1 cm thickness) were wrapped in aluminum foil, vacuum packaged and stored at -80 ºC for volatile compound and free amino acids

analyses. In addition, sausage moisture and water activity was measured from the minced sausage as described by Corral *et al.* (2013). All results were expressed as the mean of three replicates at each sampling time.

2.2. Isolation of volatiles by solvent assisted flavour evaporation (SAFE)

Dry fermented sausages (100 g) were cut into pieces, frozen with liquid nitrogen and powdered in a blender (Waring Laboratory, USA). 2-methyl-3-heptanone (5.1 µg) was used as internal standard and directly added to sausage powder. The powder was extracted with dichloromethane (1:2, w:v) three times (total volume 500 ml). After drying over anhydrous sodium sulphate, the volatiles were isolated using the solvent-assisted flavour evaporation (SAFE) technique (Engel et al, 1999). The obtained extract was dried over anhydrous sodium sulphate and concentrated at 45 ºC to a final volume of 500 µl by distilling off the solvent by means of a Vigreux column and under a stream of nitrogen. This procedure was performed in triplicate at each sampling time.

2.3. Volatile sulphur and nitrogen compounds analyses

After the isolation of sulphur and nitrogen compounds from fermented sausages, the SAFE extract was analyzed by gas chromatography (GC) and multidimensional GC (MDGC) analyses and using different detectors; mass spectrometer, flame photometric and nitrogen-phosphorous detectors. Different GC equipments were used and all of them were equipped with an autosampler CTC Combi Pal (CTC Analytics AG, Zwingen, Switzerland).

2.3.1. Separation of volatiles by GC-MS analysis

An Agilent 7890 GC coupled with a 5975 MS detector (Hewlett Packard, Palo Alto, CA) was used. The SAFE extract was injected in the injection port at 240 ºC with 10:1 split. For separation, two different columns were used; a capillary column HP-5 (30 m length, 0.25 mm i.d., 1 µm film thickness) (Agilent Technologies, USA) used with helium as carrier gas at a 33 cm/sec. The GC oven temperature was -10 ºC for 1 min (cooling with liquid nitrogen), ramped to 240 ºC at 8 ºC/min and then to 280 ºC at 40 ºC/min. The MS interface temperature was set at 280 ºC. The second column used was a capillary column DB-624 (30 m length, 0.25 mm i.d., 1.4 µm film thickness) used with helium as carrier gas at a 34 cm/sec. The GC oven temperature was 38 ºC for 13 min, ramped to 100 ºC at 3 ºC/min and held at 100 ºC for 5 min, then to 150 ºC at 4 ºC/min and to 210 ºC at 5 ºC/min and finally, held at 210 ºC for 10 min. The MS interface temperature was set at 240 ºC. Mass spectra were obtained by electron

impact at 70 eV, and data were acquired across the range 29-400 amu. Retention indices of the volatile compounds were calculated using the series of n-alkanes (Aldrich, Germany) for both columns. Compounds were identified by comparison with mass spectra from the library database (Nist'05 and '08), linear retention indices (Kovats, 1965) from literature data and from authentic standards.

2.3.2. Separation of sulphur volatiles by GC-FID-FPD analysis

Volatile sulphur compounds in the SAFE extract were detected using a flame photometric detector (FPD) installed on an Agilent 6890 GC also equipped with a FID detector (Hewlett Packard, Palo Alto, CA). The SAFE extract was injected in the injection port at 220 ºC with 15:1 split and helium was used as carrier gas at constant flow rate of 26.3 cm/sec. The compounds were separated on a capillary column ZB-5 (30 m length, 0.25 mm i.d., 1 µm film thickness) (Phenomenex, Inc). The GC oven temperature was -10 ºC for 1 min (cooling the oven with liquid nitrogen), ramped to 280 ºC at 12 ºC/min and kept at 280 ºC for 3 minutes. FID and FPD temperature were 280 ºC and 240 ºC, respectively. Linear retention index of the eluted volatile compounds were calculated as indicated above.

2.3.3. Separation of sulphur volatiles by multidimensional GC-MS (MDGC) analysis

The MDGC system from Shimazdu (Japan) QP-2010 consisted of two independent gas chromatographs interconnected by means of a Deans switch device (Valco Instruments, Houston, TX, USA). Chromatograph 1 (GC1) was equipped with SPL-2010 (Plus) injection port, Deans switching device and FID detector. The SAFE extract was injected at 220 ºC with 5:1 split and helium gas as carrier gas at a linear velocity of 16.8 cm/sec. The compounds were separated on a capillary column Optima 5 Accent (30 m length, 0.25 mm i.d., 0.25 µm film thickness) (Macherey-Nagel GmbH & Co. KG, Germany). The GC oven temperature was -10 ºC for 1 min (cooling with liquid nitrogen), ramped to 260 ºC at 7 ºC/min and held at 280 ºC for 7 min. The FID temperature was kept at 300 ºC. Chromatograph 2 (GC2) was coupled to an MS detector. The column was connected to the Deans switch placed in the first chromatograph via the thermostated transfer line at 250ºC. The compounds were separated on fused silica capillary column ZB-WAX plus (30 m length, 0.25 mm i.d., 0.25 µm thickness). The oven temperature program was 40 ºC for 1 min, ramped to 240 ºC at 6ºC/min and held at 240 ºC for 2 min. The ion source and interface temperature was 200 and 220 ºC, respectively. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range 46-200 amu

in SCAN mode and SIM mode using specific m/z ratio of ions: 97 and 98 for 3-methylthiophene, 76, 80, 104, 122 for methional and 61 and 106 for methionol. The identification of sulphur compounds was performed by comparing the mass spectra to those from the library database (NIST) and confirmed by injection of the pure standard analysed under the same experimental conditions.

2.3.4. Separation of nitrogen volatiles by GC-NPD-PID analysis

Volatile nitrogen compounds from SAFE extract were detected using a nitrogen-phosphorous detector (NPD) installed on an Agilent 7890 GC also equipped with a photo ionization detector (PID) (SRI Instruments, California, USA). The SAFE extract was injected into the injection port at 250 ºC with 8:1 split and helium was used as carrier gas at 17.69 cm/sec. The compounds were separated on a fused silica capillary column ZB-5 (30 m length, 0.25 mm i.d., 1 µm film thickness) (Phenomenex, Inc). The GC oven temperature was -10 ºC for 1 min (cooling the oven with liquid nitrogen), ramped to 240 ºC at 8 ºC/min and then to 280 ºC at 40 ºC/min. NPD and PID temperature was 200 ºC. Auxiliary temperature was 280 ºC. Linear retention index of eluted volatile compounds was calculated as indicated above.

2.3.5. Quantification of volatile nitrogen and sulphur volatile compounds

The relative quantification of volatile compounds identified was done in the GC-MS (section 2.3.1.) in SCAN mode using extracted ion chromatogram (TIC or EIC) except for 2-acetyl-1-pyrroline and 2-methyl-3-furanthiol which were relatively quantified in SIM mode using the mass-to-charge ratio (m/z) area of the characteristic ions 43 and 111, respectively. The volatiles compounds were quantified by comparison to the internal standard (2-methyl-3-heptanone) and expressed as ng/g dry matter.

2.4. Sensory evaluation of sulphur and nitrogen compounds by olfactometry analysis (GC-O)

The SAFE extract were subjected to olfactometry analyses in a gas chromatograph (Agilent 6890, USA) equipped with a flame ionization detector (FID) and a sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) and a capillary column DB-624 (60 m, 0.32 mm i.d., film thickness 1.8 µm) (Olivares *et al.*, 2011). The aroma impact of volatile compounds was determined by aroma extract dilution analysis (AEDA) (Ullrich & Grosch, 1987) by dilution of the SAFE extract with dichloromethane. One microliter of each dilution was injected into the GC and analysed by four experienced assessors until no odours were detected. Flavour dilution factor (FD factor) was assigned to the highest dilution at which

and odour active compound was detected. Aroma compounds were identified using the following techniques: comparison with mass spectra, comparison with linear retention indices (LRI) of authentic standards injected in the GC-MS and GC-FID-O, and by coincidence of the assessors's descriptors with those described in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).

2.5. Free amino acids analysis

Free amino acids content in fermented sausages was determined according to method described by Aristoy & Toldrá (1991) using norleucine (65.6 µg) as internal standard. Phenylthiocarbamyl amino acids derivatives were analysed by reversed-phase HPLC in a 1200 Series Agilent chromatograph (Agilent, Palo Alto, CA, USA) using a Waters Nova Pack C18 column (3.9 x 300mm) (Waters Corporation, Milford, USA) and ultraviolet detection (254nm) as described by Flores, Aristoy, Spanier & Toldrá (1997). The concentration of reduced glutathione, cysteine, glutathione and cystine was determined as described by Marušić, Aristoy & Toldrá (2013) using an Agilent 1100 Series HPLC with fluorescence detector (λ_{ex} = 330 nm, λ_{em} = 376 nm). The analysis was performed in three sausages at each sampling time and each sausage sample was derivatized in triplicate. The results were expressed as mg/100 g sausage in dry matter.

2.6. Statistical analysis.

The effect of ripening time on the sulphur and nitrogen compounds and free amino acids content was done by one factor ANOVA analysis using the statistic software XLSTAT (v 2009.4.03, Addinsoft, Barcelona, Spain). Fisher test was used to evaluate differences among ripening times. A correlation procedure was performed to evaluate any relationship between sulphur and nitrogen volatile compounds and free amino acids.

3. RESULTS AND DISCUSSION

The use of conventional 1-D GC-MS allowed the identification of only four volatile sulphur and nitrogen compounds in the SAFE extract of dry fermented sausages (Table 1). However, the use of specific detectors, FPD and NPD, increased the sensitivity for sulphur and nitrogen detection together with the use of two dimensional GC with mass spectrometric detection (Figure 1). The SAFE sausage chromatograms using FPD and MDGC are shown in Figure 1. Three signals were selected at 776, 908 and 981 LRI (HP-5 column) according to FPD

signal. Therefore, three heart-cuts were transferred onto a second column (Figure 1B) and the corresponding chromatograms obtained from the second dimension are shown in Figure 1C, 1D, 1E. The sulphur compounds identified in each heart-cut were 3-methylthiophene, methional and methionol, respectively. On the other hand, the identification of volatile nitrogen compounds was performed using NPD detector and 1-D GC-MS identifying ten volatile nitrogen compounds (Table 2).

Table 1. First screening by GC-MS of sulfur and nitrogen compounds in dry fermented sausages at the end of process (66 days).

	LRI[a] compound	LRI[b] standard
2-Methyl-3-furanthiol	868	868
Dimethyl sulfone	923	925
Benzothiazole	1233	1232
2,3-Dihydrothiophene	1219	na[c]

[a] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a HP-5 capillary column (J&W Scientific 30 m x 0.25 mm i.d. x 0.25 µm film thickness).
[b] Linear retention indices from pure standard, database (http://www.flavornet.org/flavornet.html or http://webbook.nist.gov/chemistry)
[c]Na: not available.

A total of seventeen sulphur and nitrogen compounds previously identified (tables 1, 2 and figure 1) was semi-quantified in course of the sausage ripening process at 0, 15, 36 and 66 days (Table 3). Different chemical structures were identified: thiazoles (4), pyrroles (3), thiophenes (2), pyrazines (3), methionine derived compounds (2), thiols (1), pyridines (1), and others (1). Thiazole, 2,4-dimethylthiazole, 2-acethylthiazole, 2-ethylpyrazine and 2-ethylpyridine have not been previously reported in dry fermented sausages (Corral *et al.*, 2013; Gianelli *et al.*, 2011; Söllner & Schieberle, 2009). In general, all sulphur and nitrogen compounds showed an increase in concentration throughout the ripening process. At the same time, the measurement of free amino acids in the analysed sausages also showed an increase in concentration (Table 4). However, the highest increase was observed during the first stages of processing until 36 days, even though a few of them (Thr, Ile, Lys, Asn) continued their concentration increase until 66 days. This fact indicates further degradation of the released amino acids by the microbial fermented activity present in the sausages to produce volatile compounds (Ravyts, Vuyst & Leroy, 2012).

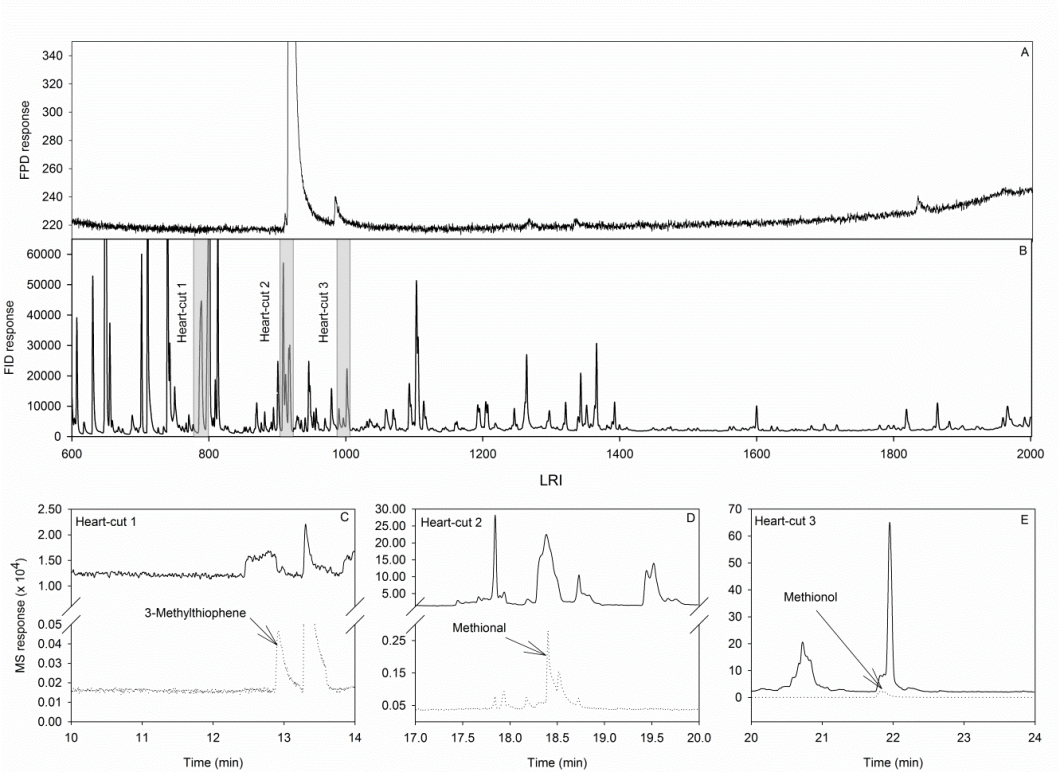

Figure 1. Sulfur compounds detected by FPD and separated by MDGC (heart-cut technique) in dry fermented sausages at the end of process (66 days). A) FPD chromatogram, B) First dimension chromatogram of MDGC system. C, D, E) Second dimension chromatogram of each heart-cut shown in graph B: dotted line (SIM response) and straight line (SCAN response) for each compound.

Due to the odour contribution of volatile sulphur and nitrogen compounds, an olfactometry analysis was performed and revealed nine aroma active compounds from the seventeen compounds identified (Table 3). Pyrrole, 2-ethylpyridine and 2-ethylpyrazine, have not been reported before as aroma active compounds in dry fermented sausages (Corral *et al.*, 2013, 2014; Gianelli *et al.*, 2011; Olivares *et al.*, 2011; Söllner & Schierberle, 2009; Schmidt & Berger, 1998). On the other hand, 3-methylthiophene and 2-methyl-3-furanthiol reported as aroma active compounds in dry fermented sausages (Corral *et al.*, 2013; Söllner & Schieberle, 2009), did not show influence on the aroma indicating that in these product their concentrations were below their odour thresholds. In our experiments, 2-acetyl-1-pyrroline was the most potent odorant followed by

methional, 2-ethylpyrazine and 2,3-dihydrothiophene and then pyrrole, 2-ethylpyridine, 2,6-dimethlpyrazine, 2-acetylpyrrole and benzothiazole (Table 3).

Table 2. Nitrogen compounds identified by GC-MS in dry fermented sausages at the end of the process (66 days).

	LRI [a] compound	LRI[b] standard
Thiazole	745	742
Pyrrole	767	762
2-Methylpyrazine	839	839
2,4-Dimethylthiazole	894	892
2-Ethylpyridine	908	906
2,6-Dimethylpyrazine	919	919
2-Ethylpyrazine	918	918
2-Acetyl-1-pyrroline	930	931
2-Acetylthiazole	1018	1020
2-Acetylpyrrole	1060	1060

[a] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a HP-5 capillary column (J&W Scientific 30 m x 0.25 mm i.d. x 0.25 µm film thickness).
[b] Linear retention indices from pure standard or database (http://www.flavornet.org/flavornet.html or http://webbook.nist.gov/chemistry)

In relation to the generation of these compounds during dry sausage processing, four thiazoles compounds, thiazole, 2,4-dimethylthiazole, 2-acetylthiazole and benzothiazole, were quantified. Their concentration increased (p<0.05) until 36 days of process and then it was maintained being thiazole the most abundant compound. This group of compounds is mainly formed by two pathways; thiamine acid degradation or by non-enzymatic browning reactions between reducing sugars and amino acids in the presence of hydrogen sulphide (H_2S) originated from the degradation of sulphur containing amino acids (Güntert, Brüning, Emberger, Köpsel, Kuhn, Thielmann & Werkhoff, 1990). This is the first time that these identified thiazole compounds are reported in dry fermented sausages, although they have been found in other meat products such as cooked ham probably as result of the thermal degradation of thiamine (Thomas *et* al., 2014). However, the ripening process in dry fermented sausages does not reach high temperatures to favour the generation of these thiazole compounds although, the generation of 2-acetylthiazole was reported in model systems under mild conditions, low temperatures and low pH values (Pripis-Nicolau, De Revel, Bertrand & Maujean (2000). About their relationship with the amino acid concentration, the high increase of methionine and in low proportion of cysteine

(table 4) may favour their generation. In this sense, a positive correlation between thiazole, 2,4-dimethylthiazole and benzothiazole with the amino acid methionine (0.99, 0.99 and 0.88, respectively) were observed. These findings confirmed the origin from sulphur containing amino acid as reported by Meynier & Mottram (1995) who detected thiazole and 2-acetylthiazole in heated model systems containing cysteine and ribose and favoured at a pH range of 5.5 to 6.5.

Regarding pyrrol compounds, pyrrole, 2-acetyl-1-pyrroline and 2-acetylpyrrole were quantified. Their relative concentration increased throughout the process being significantly high at the end of the process for pyrrole and 2-acetyl-1-pyrroline. These compounds have been described to be derived from amino acid pyrolysis (proline), reaction of ammonia with dicarbonyls derived from the breakdown of Amadori products or interaction of furfurals and ammonia (Mottram, 1991). In the dry fermented process, they can be generated from microbial amino acid degradation (Stahnke, 2002). 2-Acetyl-1-pyrroline is a potent odorant in meat products (Söllner & Schieberle, 2009) which has been mainly found in Mediterranean sausages covered with yeast (Demeyer *et al.*, 2000) and its formation was attributed to yeast degradation of proline and ornithine amino acids (Schieberle, 1990). The results of our studies confirmed these findings, as a positive correlation between 2-acetyl-1-pyrroline concentration and ornithine (r 0.97) was obtained in the dry fermented sausages.

Concerning thiophene compounds, 3-methylthiophene and 2,3-dihidrothiophene were quantified. 3-Methylthiophene was only detected at 66 days as well as the 2,3-dihydrothiophene concentration which increased significantly in course of the fermentation until 66 days. In dry fermented sausages, they can be originated by yeast metabolism such as from *Debaryomyces hansenii*; though their metabolic activity is strain dependent and therefore, sulphur production (López Del Castillo-Lozano, Delile, Spinnler, Bonnarme & Landaud, 2007). However, their formation have also been reported by means of the reaction of hydrogen sulfide or other volatile sulphur compounds derived from sulphur containing amino acids, with sugar degradation products from Maillard reaction, the heating of furans with hydrogen sulfide or the thermal degradation of thiamin (Mottram, 1991).

Table 3. Sulphur and nitrogen compounds generation (ng/g dm) during the ripening of dry fermented sausages.

	HP-5		DB-624		Time					
	LRI[a]	LRI[b]	LRI[a]	LRI[b]						
Compound	**comp**	**std**	**comp**	**std**	**0 days**		**15 days**		**36 days**	
Thiazoles					**19.226**	**c**[f]	**41.64**	**b**	**52.233**	**a**
Thiazole	745	742	776	776	1.94	b	10.90	a	11.65	a
2,4-Dimethylthiazole	894	892	910	913			0.27		0.58	
2-Acetylthiazole	1018	1020	1074	1076			0.63	b	1.65	a
Benzothiazole	1233	1232	1292	1292	17.29	b	29.84	ab	38.36	a
Pyrroles					**29.29**	**c**	**69.20**	**bc**	**147.40**	**b**
Pyrrole	767	762	840	842			1.26	b	2.69	b
2-Acetyl-1-pyrroline	930	931	958	960	28.83	c	66.06	bc	142.71	b
2-Acetylpyrrole	1060	1060	1158	1155	0.46		1.88		2.00	
Thiophenes					**34.88**	**b**	**34.95**	**b**	**60.00**	**b**
3-Methylthiophene	776	776		803						
2,3-Dihydrothiophene[g]	1219	na[h]	1388	Na	34.88	b	34.95	b	60.00	b
Pyrazines					**7.83**	**ab**	**3.32**	**c**	**5.71**	**b**
2-Methylpyrazine	839	839	865	860	1.52		2.25		2.75	
2,6-Dimethylpyrazine	919	914	943	943	5.57	a	0.53	b	1.25	b
2-Ethylpyrazine	918	918	947	946	0.74	b	0.53	b	1.70	b
Methionine derived					**1.02**	**c**	**56.66**	**b**	**109.74**	**a**
Methional	908	908	964	964	0.72	b	3.49	ab	5.57	a
Methionol	981	981	1062	1062	0.30	c	53.17	b	104.17	a
Thiols										
2-Methyl-3-furanthiol	868	868	888	894	31.95		37.98		34.75	
Pyridines										
2-Ethylpyridine	908	906	935	934	0.54		0.83		1.07	
Others										
Dimethyl-sulfone	923	925	1060	1060	1769.35		1287.34		1512.44	

[a] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a HP-5 capillary column (J&W Scientific 30 m x 0.25 mm i.d. x 0.25 µm film thickness) or a DB-624 capillary column (J&W Scientific 30 m x 0.25 mm i.d. x 1.4 µm film thickness).

[b] Linear retention indices from pure standard or database (http://www.flavornet.org/flavornet.html or http://webbook.nist.gov/chemistry)

[c] Standard error of the mean.

Compound	Time 66 days		SEM[c]	LRI[d] GC-O	Descriptor	FD[e] factor
Thiazoles	**51.165**	**a**	**4.171**			
Thiazole	18.11	a	2.18			
2,4-Dimethylthiazole	1.50		0.53			
2-Acetylthiazole			0.11			
Benzothiazole	31.55	a	4.36	1298	Green, strange, damp, acid	1
Pyrroles	**279.37**	**a**	**26.76**			
Pyrrole	17.71	a	1.53	848	Coffe, sweet	1
2-Acetyl-1-pyrroline	260.05	a	26.40	962	Fried corn, toasted, roasted meat, crust	50
2-Acetylpyrrole	1.62		0.65	1149	Toasted, strong, bitter	1
Thiophenes	**135.58**	**a**	**11.05**			
3-Methylthiophene	1.92		0.50			
2,3-Dihydrothiophene[g]	133.65	a	11.08	1386	Walnut or hazelnut, acid	2
Pyrazines	**10.50**	**a**	**0.91**			
2-Methylpyrazine	2.60		0.68			
2,6-Dimethylpyrazine	0.92	b	0.64	945	Toasted, spicy, acid	1
2-Ethylpyrazine	6.98	a	0.30	949	Toasted, spicy, acid	2
Methionine derived	**13.79**	**c**	**10.97**			
Methional	6.70	a	1.23	968	Cooked potate, cauliflower	10
Methionol	7.09	c	9.59			
Thiols						
2-Methyl-3-furanthiol	24.91		12.98			
Pyridines						
2-Ethylpyridine	0.60		0.15	936	Rotten mushroom, detergent	1
Others						
Dimethyl-sulfone	1503.86		606.00			

[d] Linear retention indices (LRI) of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (J&W Scientific 60 m x 0.32 mm i.d. x 1.8 µm film thickness).

[e] FD Flavor dilution factor.

[f] Different letters in each volatile compound indicate significant differences during the ripening process at $p<0.05$ (Fisher's test).

[g] Tentative identification by mass spectrum.

[h] No available.

Table 4. Free amino acids generation during the ripening of dry fermented sausages (mg/100 g dry matter).

	Time				
Amino acid	**0 days**	**15 days**	**36 days**	**66 days**	**SEM[a]**
Essential					
His	6.89 c[b]	25.19 b	49.70 a	59.19 a	4.89
Thr	11.37 d	28.96 c	63.88 b	84.58 a	5.11
Val	15.02 c	65.74 b	131.17 a	138.52 a	5.26
Met	5.01 d	25.83 c	61.77 a	55.79 b	1.36
Ile	10.43 d	37.55 c	82.66 b	95.45 a	1.90
Leu	14.89 c	141.14 b	275.73 a	271.52 a	3.85
Phe	9.56 c	64.12 b	129.05 a	128.97 a	2.82
Trp	3.35 d	16.70 c	30.70 a	26.20 b	0.84
Lys	17.06 c	62.96 c	141.00 b	185.50 a	15.39
Nonessential					
Asp	41.67 d	99.17 c	193.88 a	153.83 b	9.46
Glu	26.32 c	77.87 b	135.33 a	112.65 a	8.10
Ser	11.81 c	35.71 b	58.12 a	29.57 bc	6.99
Asn	3.50 d	13.89 c	26.79 b	33.44 a	2.04
Gly	27.35 b	35.41 b	60.12 a	65.50 a	4.02
Gln	91.17	99.89	117.73	100.00	10.67
Ala	74.77 b	108.69 b	166.77 a	172.42 a	11.57
Arg	20.76 a	20.96 a	9.61 c	14.56 b	1.37
Pro	10.65 c	86.46 b	151.82 a	137.16 a	5.84
Tyr	11.17 c	40.29 b	53.05 a	41.46 b	2.43
Orn	0.58 c	18.18 b	41.99 a	47.66 a	4.78
Cys	10.08 c	14.32 bc	18.90 a	17.11 ab	1.39
Others and di- or tripeptides					
β-ala	8.70	7.07	7.91	6.99	0.85
Tau	138.49	116.62	152.38	151.25	15.08
Ans	28.24 a	17.53 c	20.94 bc	25.79 ab	1.95
Car	702.78	489.62	466.11	537.71	66.44
GSH	21.13 b	34.72 b	51.71 a	22.39 b	4.94
GSSG	223.89 a	121.65 b	124.19 b	82.31 c	9.36
Cys-cys	6.32	5.32	5.86	8.25	1.60

[a] Standard error of the mean.

[b] Different letters in each amino acid indicate significant differences at $p<0.05$ (Fisher's test).

With regard to pyrazines, 2-methylpyrazine, 2,6-dimethylpyrazine and 2-ethylpyrazine were quantified. 2-Ethylpyrazine significantly increased in course of the process while the 2,6-dimethylpyrazine content decreased after 15 days. Generally, alkyl pyrazine formation is derived from carbohydrate/amine system being the carbohydrates the carbon source and amino acids the nitrogen source, therefore, all amino acids can generate them. The results obtained showed two positive correlations for 2,6-dimethlypyrazne and 2-ethyl pyrazine with glycine

(1.0 and 0.97, respectively) (Meynier & Mottram, 1995). Their formation is favoured by high temperatures, basic pH, carnosine presence or low moisture (Jayasena, Ahn, Nam & Jo, 2013). Among dry sausage factors, the low a_w values (Figure 3) and high amino acid concentration including the presence of carnosine (Car, Table 4) will favour their formation, even though the temperatures and pH are low.

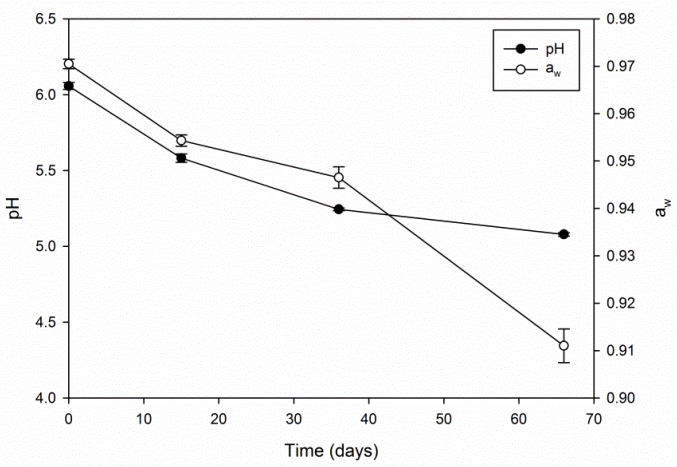

Figure 3. Evolution of pH and a_w throughout the ripening of dry fermented sausages.

In relation to methionine derived compounds, methional and methionol were quantified while methanethiol was not detected probably due to its high volatility and could has been lost during concentration steps (Söllner & Schieberle, 2009). These methionine derived compounds can be formed by Strecker degradation or microbial enzyme activity (Martínez-Cuesta, Peláez & Requena, 2013). The dry sausage conditions and the fermentation process favour the microbial activity (lactic acid bacteria and yeast) to produce them. Methionine degradation by bacteria can be done by three pathways: S-adenosylmethionine; degradation pathway initiated by an aminotransferase; and the simultaneous deamination and demethiolation by action of C-S lyases which lead to thiol precursor methanethiol and further to methional, methionol, dimethylsulfide and dimethyltrisulfide among other sulphur volatiles (Martínez-Cuesta *et al.*, 2013). During sausage processing, methional concentration significantly increased at 36 days keeping its increase until 66 days. However, methionol concentration increased until 36 days followed by a significant decrease at 66 days. A positive correlation was observed for methional and methionine (0.999) however, methionol was not correlated.

About thiols, only 2-methyl-3-furanthiol was quantified and no significant differences were observed throughout the ripening process, probably due to its instability and susceptibility to oxidation (Hofmann & Schieberle, 1995). 2-Methyl-3-furanthiol has been described to be formed from the reaction between cysteine and sugar whereas in this context, pentoses are more effective than hexoses (Hofmann & Schieberle, 1998) or by thiamine degradation (Münch & Schieberle, 1998). In contrast to this chemical pathway, in dry fermented sausages, an alternative way for 2-methylfuranthiol can be yeast metabolism (Münch & Schieberle, 1998). On the other hand, in meat products such as cooked ham, thiamine has been shown to be more important as a source for thiol formation than cysteine probably due to the thermal treatment (Thomas *et al.* 2014).

In relation to pyridines, only 2-ethylpyridine was quantified and no significant concentrations changes were found throughout the fermentation process. 2-Ethylpyridine could have been formed from aspartic acid and isoleucine amino acids (Hwang, Hartman & Ho, 1995) and 2,4-heptadienal (E,E) (Elmore, Campo, Enser & Mottram, 2002).

On the other hand, dimethylsulfone did not show significant changes throughout the ripening process. Silva Ferreira, Rodrigues, Hogg & De Pinho (2003) suggested that dimethyl-sulfone could be formed from dimethyl sulfoxide by dimethyl sulfoxide reductase activity of yeast which comes from methionine or cysteine.

In summary, different factors can be involved in the formation of aroma active sulphur and nitrogen compounds in dry fermented sausages. The main pathways for their formation are chemical, such as Maillard, or microbial reactions. In general, Maillard reaction requires high temperatures which do not take place in dry fermented sausage processing. However, this study clearly demonstrated how the long ripening time and the presence of high amounts of free amino acids, obviously favour these reactions even at the temperatures of the fermentation process (Ventanas, Estévez, Delgado & Ruiz, 2007). In fact, the production of free amino acid as a result of endogenous meat proteases (Durá *et al.*, 2004) and the amino acids catabolism by the microbial activity of *LAB, Staphylococci* or yeast is a source for the generation of volatile compounds (Toldrá *et al.*, 2001). As observed in our study, sulphur volatile compounds arose mainly from methionine as it was present at higher concentrations than cysteine, cystine (cys-cys) and tripeptide GSH (Glu-Cys-Gly) (Marušić *et al.*, 2013). Even though, the hydrolysis of GSH and cystine is a source of the constituent's amino acids and thereby, cysteine will be available to be microbially metabolized during

sausage processing. This microbial activity has been confirmed by a high abundance of volatile sulphur compounds in dry sausages inoculated with *Staphylococcus carnosus* (Tjener, Stahnke, Andersen & Martinussen 2004) and *D. hansenii* strains (Cano-García, Rivera-Jiménez, Belloch & Flores, 2014). Nevertheless, scarce microbial mechanisms have been proposed before for nitrogen containing compounds like pyrazines and pyridines (Larroche, Besson & Gros, 1999; Cheng, Reineccius, Bjorklund & Leete, 1991). Even though, a study done in a model system under mild conditions using low temperatures and low pH indicated the formation of these compounds especially in the presence of cysteine (Pripis-Nicolau *et al.*, 2000). Due to the impact of these sulphur and nitrogen compounds on sausage aroma, further studies should be carried out to control the processing factors that affect their generation and therefore, the final sausage aroma.

4. CONCLUSION

The analytical methods used revealed the generation of volatile sulphur and nitrogen compounds throughout dry sausage ripening process. Among the seventeen volatile compounds quantified, 2-acetyl-1-pyrroline, methional, 2-ethylpyrazine and 2,3-dihydrothiophene were the most potent odorants characterized by toasted, cooked potato, and nutty notes. The sausage environment, acid pH, low a_w, high concentration of free amino acids and especially methionine, cysteine and GSH as a source of cysteine, favoured the formation of sulphur compounds (methional, thiazole, 2,4-dimethylthiazole and benzothiazole). In addition, the microbial degradation of ornithine produced 2-acetyl-1-pyrroline while glycine degradation generated 2,6-dimethlypyrazine and 2-ethyl pyrazine.

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REFERENCES

Aristoy, M. C., & Toldra, F. (1991). Deproteinization techniques for HPLC ammo acid analysis in fresh pork muscle and dry-cured ham. *Journal of Agricultural and Food Chemistry, 39*(10), 1792-1795.

Burdock, G. A., (2002). *Fenaroli's handbook of flavor ingredients* (4th ed.). Boca Raton, Florida: CRC Press Inc..

Cano-García, L., Rivera-Jiménez, S., Belloch, C., & Flores, M. (2014). Generation of aroma compounds in a fermented sausage meat model system by Debaryomyces hansenii strains. *Food Chemistry, 151*(0), 364-373.

Cheng, T.B., Reineccius, G.A., Bjorklun J.A. & Leete, E. (1991). Biosysthesis of 2-methoxy-3-isopropylpyrazine in *Pseudomonas perolens*. *Journal of Agricultural and Food Chemistry*, 39, 1009-1012.

Corral, S., Salvador, A., & Flores, M. (2013). Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science, 93*(3), 776-785.

Corral, S., Salvador, A., & Flores, M. (2014). Elucidation of key aroma compounds in traditional dry fermented sausages using different extraction techniques. Journal of the Science of Food and Agriculture. http://dx.doi.org/10.1002/jsfa.6830.

Demeyer, D., Raemaekers, M., Rizzo, A., Holck, A., De Smedt, A., ten Brink, B., Hagen, B., Montel, C., Zanardi, E., Murbrekk, E., Leroy, F., Vandendriessche, F., Lorentsen, K., Venema, K., Sunesen, L., Stahnke, L., De Vuyst, L., Talon, R., Chizzolini, R., & Eerola, S. (2000). Control of bioflavour and safety in fermented sausages: first results of a European project. *Food Research International, 33*(3–4), 171-180.

Durá, MA, Flores M., Toldrá, F. (2004). Effect of *Debaryomyces spp*. on the proteolysis of dry-fermented sausages. *Meat Science* 68, 319-328.

Elmore, J. S., Campo, M. M., Enser, M., & Mottram, D. S. (2002). Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids. *Journal of Agricultural and Food Chemistry, 50*(5), 1126-1132.

Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation - A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *European Food Research and Technology, 209*(3-4), 237-241.

Flores, M., Aristoy, M. C., Spanier, A. M., & Toldrá, F. (1997). Non-volatile components effects on quality of 'Serrano' dry-cured ham as related to processing time. *Journal of Food Science, 62*(6), 1235-1239.

Gianelli, M. P., Olivares, A., & Flores, M. (2011). Key aroma components of a dry-cured sausage with high fat content (Sobrassada). *Food Science and Technology International,* 17, 63-71.

Güntert, M., Brüning, J., Emberger, R., Köpsel, M., Kuhn, W., Thielmann, T., & Werkhoff, P. (1990). Identification and formation of some selected sulphur-containing flavor compounds in various meat model systems. *Journal of Agricultural and Food Chemistry, 38*(11), 2027-2041.

Hofmann, T., & Schieberle, P. (1995). Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *Journal of Agricultural and Food Chemistry, 43*(8), 2187-2194.

Hofmann, T., & Schieberle, P. (1998). Quantitative Model Studies on the Effectiveness of Different Precursor Systems in the Formation of the Intense Food Odorants 2-Furfurylthiol and 2-Methyl-3-furanthiol. *Journal of Agricultural and Food Chemistry, 46*(1), 235-241.

Hwang, H. I., Hartman, T. G., & Ho, C. T. (1995). Relative reactivities of amino acids in the formation of pyridines, pyrroles, and oxazoles. *Journal of Agricultural and Food Chemistry, 43*(11), 2917-2921.

Jayasena, D. D., Ahn, D. U., Nam, K. C., & Jo, C. (2013). Flavour chemistry of chicken meat: A review. *Asian-Australasian Journal of Animal Sciences, 26*(5), 732-742.

Kovats, E.S. (1965). Gas chromatographic characterization of organic substances in the retention index system. In J. C. Giddings, & R. A. Keller (Eds.), *Advances in chromatography* (pp. 229-247). New York: Marcel Dekker, Inc..

Larroche, C., Besson, I., & Gros, J.-B. (1999). High pyrazine production by Bacillus subtilis in solid substrate fermentation on ground soybeans. *Process Biochemistry*, 34(6–7), 667-674.

López Del Castillo-Lozano, M., Delile, A., Spinnler, H. E., Bonnarme, P., & Landaud, S. (2007). Comparison of volatile sulphur compound production by cheese-ripening yeasts from methionine and methionine-cysteine mixtures. *Applied Microbiology and Biotechnology*, 75(6), 1447-1454.

Martínez-Cuesta, M. D. C., Peláez, C., & Requena, T. (2013). Methionine Metabolism: Major Pathways and Enzymes Involved and Strategies for Control and Diversification of Volatile Sulphur Compounds in Cheese. *Critical Reviews in Food Science and Nutrition*, 53(4), 366-385.

Marušić, N., Aristoy, M. C., & Toldrá, F. (2013). Nutritional pork meat compounds as affected by ham dry-curing. *Meat Science, 93*(1), 53-60.

Meynier, A., & Mottram, D. S. (1995). The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chemistry, 52*(4), 361-366.

Molly, K., Demeyer, D., Johansson, G., Raemaekers, M., Ghistelinck, M., & Geenen, I. (1997). The importance of meat enzymes in ripening and flavour generation in dry fermented sausages. First results of a European project. *Food Chemistry, 59*(4), 539-545.

Mottram, D.S. (1991). Meat. In: Volatile compounds in foods and beverages; Maarse H. (ed.). Marcel Dekker Inc, New York, pp 107-177.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry, 62*(4), 415-424.

Münch, P., & Schieberle, P. (1998). Quantitative Studies on the Formation of Key Odorants in Thermally Treated Yeast Extracts Using Stable Isotope Dilution Assays. *Journal of Agricultural and Food Chemistry, 46*(11), 4695-4701.

Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science,* 87, 264-273.

Pripis-Nicolau, L., De Revel, G., Bertrand, A., & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *Journal of Agricultural and Food Chemistry*, 48(9), 3761-3766.

Ravyts, F., Vuyst, L. D., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. *Engineering in Life Sciences*, 12(4), 356-367.

Schieberle, P. (1990). The role of free amino acids present in yeast as precursors of the odorants 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine in wheat bread crust. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 191*(3), 206-209.

Schmidt, S., & Berger, R. G. (1998). Aroma Compounds in Fermented Sausages of Different Origins. *LWT - Food Science and Technology,* 31, 559-567.

Silva Ferreira, A. C., Rodrigues, P., Hogg, T., & De Pinho, P. G. (2003). Influence of some technological parameters on the formation of dimethyl sulfide, 2-mercaptoethanol, methionol, and dimethyl sulfone in port wines. *Journal of Agricultural and Food Chemistry, 51*(3), 727-732.

Söllner, K., & Schieberle, P. (2009). Decoding the key aroma compounds of a Hungarian-type salami by molecular sensory science approaches. *Journal of Agricultural and Food Chemistry,* 57, 4319-4327.

Stahnke, L. H. (2002). Flavour formation in fermented sausage. In Research Advances in the Quality of Meat and Meat Products. Eds Toldrá, F., Research Signpost, India.

Thomas, C., Mercier, F., Tournayre, P., Martin, J. L., & Berdagué, J. L. (2014). Identification and origin of odorous sulphur compounds in cooked ham. *Food Chemistry, 155*, 207-213.

Tjener, K., Stahnke, L. H., Andersen, L., & Martinussen, J. (2004). Growth and production of volatiles by Staphylococcus carnosus in dry sausages: Influence of inoculation level and ripening time. *Meat Science, 67*(3), 447-452.

Toldrá, F. Sanz, Y. & Flores, M. (2001). Meat Fermentation Technology. In: Meat Science and Applications. Eds Hui, Y.H., Nip, W.K., Rogers, R. W. and Young W. A., Marcel Dekker, Inc.New York,Basel.

Ullrich, F. & Grosch, W. Z. (1987).Identification of the most intense odor compound formed during the autoxidation of linoleic acid. *Zitschrift für Lebensmittel-Untersuchung und-Forschung* 184: 277-282.

Ventanas, S., Estévez, M., Delgado, C. L., & Ruiz, J. (2007). Phospholipid oxidation, non-enzymatic browning development and volatile compounds generation in model

systems containing liposomes from porcine Longissimus dorsi and selected amino acids. *European Food Research and Technology*, 225(5-6), 665-675.

Capítulo 3

Salt reduction in slow fermented sausages affects the generation of aroma active compounds

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Salt reduction in slow fermented sausages affects the generation of aroma active compounds

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Abstract

Slow fermented sausages with different salt content were manufactured: control (2.7% NaCl, S), 16% salt reduced (2.26% NaCl, RS) and 16% replaced by KCl (2.26% NaCl and 0.43% KCl, RSK). The effect of salt reduction on microbiology and chemical parameters, sensory characteristics, texture and volatile compounds was studied. The aroma compounds were identified by GC-MS and olfactometry analyses. Small salt reduction (16%) (RS) affected sausage quality producing a reduction in the acceptance of aroma, taste, juiciness and overall quality. The substitution by KCl (RSK) produced the same acceptability by consumers as for high salt (S) treatment except for the aroma that was not improved by KCl addition. The aroma was affected due to the reduction in sulfur and acids and the increase of aldehyde compounds. Aroma compounds that characterized the high salt treatment (S) were dimethyl trisulfide, 3-methyl thiophene, 2,3-butanedione, 2-nonanone and acetic acid.

Keywords: Fermented sausages, Salt reduction, Volatile compounds, Aroma, Flavour.

1. INTRODUCTION

The relation between high salt intake and incidence/prevalence of hypertension has led the European Union (EU) to implement salt reduction initiatives in the EU framework (Commission European, 2008). EU proposed salt reduction of 16% in 4 years, decreasing 4% per year in order to allow consumers to adapt to the slightly decreasing salty taste. In some products, salt reduction means lower salty taste, but others products, such as dry curing and processed meats, can lead to safety and technological problem.

Salt is an essential ingredient in dry fermented sausages; it is involved in myofibrillar protein solubilization, improves texture, decreases water activity (a_w) controlling the growth of pathogens microorganism and, finally, controls the biochemical and enzymatic reactions during ripening, affecting the final flavour (Ruusunen & Puolanne, 2005). The reduction of salt in fermented meat products has been studied through different strategies such as the use of KCl alone (Gou, Guerrero, Gelabert, & Arnau, 1996) or together with other chloride salts ($CaCl_2$, $MgCl_2$) (Gimeno, Astiasarán & Bello, 1998; Zanardi, Ghidini, Conter & Ianieri, 2010) and also different flavour enhancers have been used (lactate, amino acids and yeast extracts) (Gou *et al.*, 1996; Guàrdia, Guerrero, Gelabert, Gou & Arnau, 2008; Campagnol, dos Santos, Wagner, Terra & Pollonio, 2011). However, KCl provides metallic or bitter tastes when it is used at concentrations equal or higher than 40% (Gou *et al.*, 1996, Gelabert, Gou, Guerrero & Arnau, 2003). Furthermore, sausage texture is affected depending on the type of salts used in the substitution. When KCl is used alone it produced an increase in sausage hardness (Gou *et al.*, 1996; Guàrdia *et al.*, 2008) while the use of KCl in combination with other divalent salts resulted in a decrease in hardness (Gimeno, Astiasarán & Bello, 1999).

Moreover, other sensory characteristics are affected by salt substitution such as a decrease in aroma and taste when KCl is used at high percentages (>40%) (Campagnol *et al.*, 2011; Guàrdia *et al.*, 2008). Nevertheless, the effect of salt substitution on aroma has been poorly studied; only Campagnol *et al.* (2011) studied the volatile compounds generated in fermented sausages when NaCl was substituted by KCl and yeast extracts. These authors reported few differences in aroma among sausages when NaCl was reduced in a 25% but after 50% substitution, the decrease in aroma and taste was evident. In addition, other studies performed on sausage models indicated that salt modifications affected volatile compounds but it depended on ripening time and the type of starter culture used (Olesen, Meyer & Stahnke, 2004; Tjener, Stahnke, Andersen

& Martinussen, 2004). In contrast, Ravyts, Steen, Goemaere, Paelinck, De Vuyst & Leroy (2010) indicated that modifications of salt concentrations in sausages produced a very limited impact on the growth and composition of the microbiota without detecting an effect on volatile composition. However, all these previous studies did not evaluate the effect of salt reduction on aroma active compounds as they mainly focused on several volatile compounds.

Furthermore, these studies were done in fermented sausages but there are no reports about the reduction of NaCl content in slow fermented sausages (i.e. Chorizo de Cantimpalos, Cacciatore salami, Hungarian type salami, and others). These slow fermented sausages are typically produced in Southern European countries by using low temperatures during ripening (Flores, 1997) and the rate of acidification is low allowing the activity of acid-sensitive bacteria (*micrococcaceae* and *staphylococci*). The flavour of these sausages is mostly formed by endogenous or bacterial enzymatic activities and the oxidation of the lipid fraction (Ravyst *et al.*, 2010). Nothing is known about the effect of salt reduction on volatile aroma compounds in slow fermented sausages. Therefore, it is necessary to evaluate if salt reduction may affect flavour quality because the acceptability of slow fermented sausages is largely dependent upon its flavour (Flores, 1997). NaCl plays an important role in flavour development, since it provides the salty taste, enhances savory and meaty flavours and improves the release of volatile aroma compound from the food matrix (Ruusunen & Puolanne, 2005). However, the adaptation to less salty taste by consumers is important as it can be a way to reduce salt content in meat products. For all these reasons, the aim of this work was to study the effect of a 16 % salt reduction in the production of aroma active compounds in slow fermented sausages and to determine the effects produced by KCl used during salt reduction.

2. MATERIALS AND METHODS

2.1 Dry fermented sausages preparation

Three treatments of dry fermented sausages were manufactured with different salt contents: control treatment (S) with 2.7% NaCl, low salt treatment (RS) with 2.26% NaCl and a third treatment (RSK) with 2.26% NaCl and 0.43% KCl.

Sausages was prepared with lean pork (75%) and pork back fat (25%) and the following additives (g/kg): lactose (30); dextrin (10); sodium caseinate (20); glucose (7); sodium ascorbate (0.5); sodium nitrite (0.15); potassium nitrate

(0.15) and starter culture (0.1) SP318 TEXEL SA-301 (Danisco, Cultor, Madrid, Spain) containing *Lactobacillus sakei, Pediococcus pentosaceus, Staphylococcus xylosus and Staphylococcus carnosus*. The manufacturing process was the same as described (Olivares, Navarro & Flores, 2010). The meat mixture was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) and the sausages were subjected to drying at 10-12 ºC and 70-85% HR for 57 days. In order to control the ripening process, weight losses and pH were measured during processing (Olivares *et al.*, 2010).

From each treatment, a 200 g portion of the meat mixture (0 days) and three sausages at 9, 29 and 57 days were randomly collected to study the effect of ripening time and formulation. A 150 g portion of the sample was minced and used for moisture, water activity and pH tests. In addition, sausage color was measured and a 10 g portion was taken for microbiological analysis. The remaining minced sample was vacuum packed and frozen at -20 ºC for subsequent analyses (TBARS, lipid, protein and ions content). At 57 days, several slices (1 cm thickness) were wrapped in aluminum foil, vacuum packaged and stored at -80 ºC for volatile and aroma analyses. All results were expressed as the mean of three replicates at each sampling time. Finally, the texture and sensory analysis were carried out at the end of the drying process (57 days).

2.2 Chemical analysis

pH was measured by introducing a pH meter HI 99163 (Hanna Instruments Inc., Hoonsocket, USA) into a mixture of sausage and distilled water (1:1) (ISO 2917:1999). Water activity was determined using a Fast-lab water activity meter (Gbx, Romans sur Isère Cédex, France) as described Olivares *et al.* (2010). Color evaluation was made through the CIE L*, a*, b* space. The color of the sausages was measured using a colorimeter CR-400/410 (Konica Minolta Sensing Inc., Japan) with D65 illuminant (Olivares *et al.*, 2010).

Moisture content was determined after dehydration at 100 ºC to a constant weight, according to the official method of analysis of meat products (BOE, 1979). Total lipids were extracted from 5 g of minced sausage according to the method of Folch, Lees & Sloane Stanley (1957), using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1) as solvent. The extract obtained was evaporated in a rotating vacuum evaporator and weighed to determine the total lipid content. Nitrogen content was determined by the Kjeldahl method and protein was estimated by multiplying the nitrogen content by a factor of 6.25.

Thiobarbituric acid reactive substances (TBARS) were quantified to determine the degree of lipid oxidation, as described Olivares, Navarro & Flores (2011) using trichloroacetic acid as solvent instead of perchloric acid. The results were expressed as mg malonaldehyde (MDA)/ kg in dry matter.

Cations (sodium, potassium) were analyzed by ion chromatography as described (Armenteros, Aristoy, Barat, & Toldrá, 2009). Chloride anion in sample solutions was determined by using Metrohm 761 Compact IC with Metrohm 833 IC Liquid Handling Suppressor unit to improve chromatographic signal. Guard column A-Supp 4/5 (5.0 x 4.0 mm) and analytical column Supp 5-250 (4.0 × 250 mm) were used to analyze chloride anion. The mobile phase consisted of 1 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 with 30 ml/l acetone. The concentration of each ion was determined from respective calibration curves, using a set of standard solutions of Na^+, K^+ and Cl^- (Fluka, Switzerland, Sigma, St. Louis, MO). The results (means of three determinations) were expressed as mg/100 g of sample in dry matter.

2.3 Microbiological analysis

Minced sausage sample was aseptically homogenized with peptone water (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min and decimal dilutions were prepared. Lactic acid bacteria population was determined by the overlay technique to promote anaerobic growth using MRS agar (Scharlau Chemie SA, Barcelona, Spain). *Staphylococci* counts were obtained on mannitol salt agar (Scharlau Chemie SA, Barcelona, Spain). Both mediums were incubated at 30 ºC for 3 days.

2.4 Analysis of volatile compounds

2.4.1 Gas chromatography-mass spectrometry (GC-MS)

An Agilent HP 7890 series II GC (Hewlett- Packard, Palo Alto, CA) with an HP 5975C mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2 multipurpose sampler (Gerstel, Germany) was used in all experiments. Extraction of headspace volatile compounds was performed using a solid-phase microextraction (SPME) with an 85 µm Carboxen/ Polydimethylsiloxane (CAR/PDMS) fibre for automatic holder (Supelco, Bellefonte, PA). Before the analysis, the fibre was preconditioned as indicated by the manufacturer.

For each experiment, 5 g of dry fermented sausages was minced and weighted into a 20 ml headspace vial sealed with a PTFE faced silicone septum and 0.75 mg of BHT was added. The vial was maintained at 37 ºC during 30 min

to equilibrate its headspace. Then, the SPME fibre was exposed to the headspace while maintaining the sample at 37 ºC during 3 h. Before each injection, the fiber was baked at 250 ºC for 15 min. The compounds adsorbed by the fibre were desorbed in the injection port of the GC-MS for 5 min at 240 ºC with purge valve off (splitless mode). The analysis of volatile compounds in the GC-MS was done as described Olivares *et al.* (2011). The compounds were identified by comparison with mass spectra from the library database (Nist'05), kovats retention index (Kovats, 1965) and by comparison with authentic standards. The quantification of volatile compounds was done in SCAN mode using either total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale.

2.4.2 Gas-chromatrography-olfactometry

A gas chromatograph (Agilent 6890, USA) equipped with a FID detector and sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) was used to analyze aroma compounds as described Olivares *et al.* (2011) using SPME technique. The detection frequency method was used to estimate the aromatic impact of each volatile (Pollien *et al.*, 1997). Each assessment was carried out according to Olivares *et al.* (2011). Four trained panellists evaluated the odours from the GC-effluent. Each assessor evaluated 3 sausages per treatment (57 days of ripening); therefore, a total of 12 assessments were carried out. The final detection frequency value (DF) for each compound was obtained by summation of the 12 sniffings. The detection of an odor by less than three assessors was considered to be noise.

Compounds were identified using the following techniques: comparison with mass spectra, comparison with kovats retention indices of authentic standards injected in the GC-MS and GC-O, and by coincidence of the assessors's descriptors with those in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).

2.5 Texture profile analysis

Texture profile analysis (TPA) was performed using TA-XT.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0. Stable Microsystems, Godalming, UK). Four dry fermented sausage slices (diameter 3.5 cm and thick 1.5 cm) of three sausages per treatment were compressed twice to 50% of their original height as described Olivares *et al.* (2010). TPA curves were obtained and the main parameters of texture were calculated: hardness, springiness, cohesiveness and as secondary parameter chewiness.

2.6 Sensory analysis

Testing was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988). A panel of 85 untrained consumers was used. The casing was removed and the sausages were cut into slices of 4 mm thickness. Samples from each treatment (S, RS, RSK) were labeled with random, three-digit codes and presented on a plate at room temperature with water and bread without salt to cleanse the palate between samples. An acceptability test was carried out using 9-box hedonic scale (1extremely dislike – 9 extremely like). The attributes evaluated were appearance, flavour, taste, hardness, juiciness and overall quality. One slice of each treatment was placed inside a camera with D65 illuminant to evaluate the appearance. Data acquisition was performed using Compusense® five release 5.0 (Compusense Inc., Guelph, Ontario, Canada).

2.7 Statistical analysis

Effect of reduction/replacement of NaCl and processing time on the variables studied (chemical and microbial) was done by a two-factor analysis of variance (ANOVA) using the statistic software XLSTAT 2009.4.03 (Addinsoft, Barcelona, Spain). Fisher test was used to evaluate differences among treatments. The effect of reduction/replacement of NaCl on texture, sensory parameters and volatile compounds at the end of the process was done by one factor ANOVA analysis. Furthermore, principal component analysis (PCA) was done to evaluate the relationships among sausages and different parameters (pH, TBARS, ions, texture parameters, moisture, lipids and protein content and aroma active compounds).

3. RESULTS

3.1. Chemical and microbiology analyses

At the end of the ripening process, the three treatments showed weight losses of 38.9-39.2% (data not shown), that are suitable values for this kind of sausage. Salt reduction did not produce differences in weight losses among treatments at the end of process, as also observed other authors (Campagnol *et al.*, 2011).

Two essential factors such as a_w and pH guarantee the stability and safety of the sausage. The pH and a_w values are shown in Fig. 1A. The pH dropped to 4.5 due to the LAB growth in the treatments and then, pH experienced a slight increase due to ammonia formation. No differences in pH

were observed among treatments as also was observed in salt reduced small caliber fermented sausages (Gelabert *et al.*, 2003; Gou *et al.*, 1996). Concerning a_w, it decreased throughout the processing to 0.92 value in all treatments. At 9 days, differences were found in a_w as seen by a highest a_w in the RS treatment; nevertheless, the final sausages did not show differences (p>0.05) as also has been reported by other authors (Campagnol *et al.*, 2011). However, Olesen *et al.* (2004) also observed a lowest a_w in highly salted sausages as we detected, although they performed a higher salt reduction in their assays (50%) than the reduction done in our study (16%).

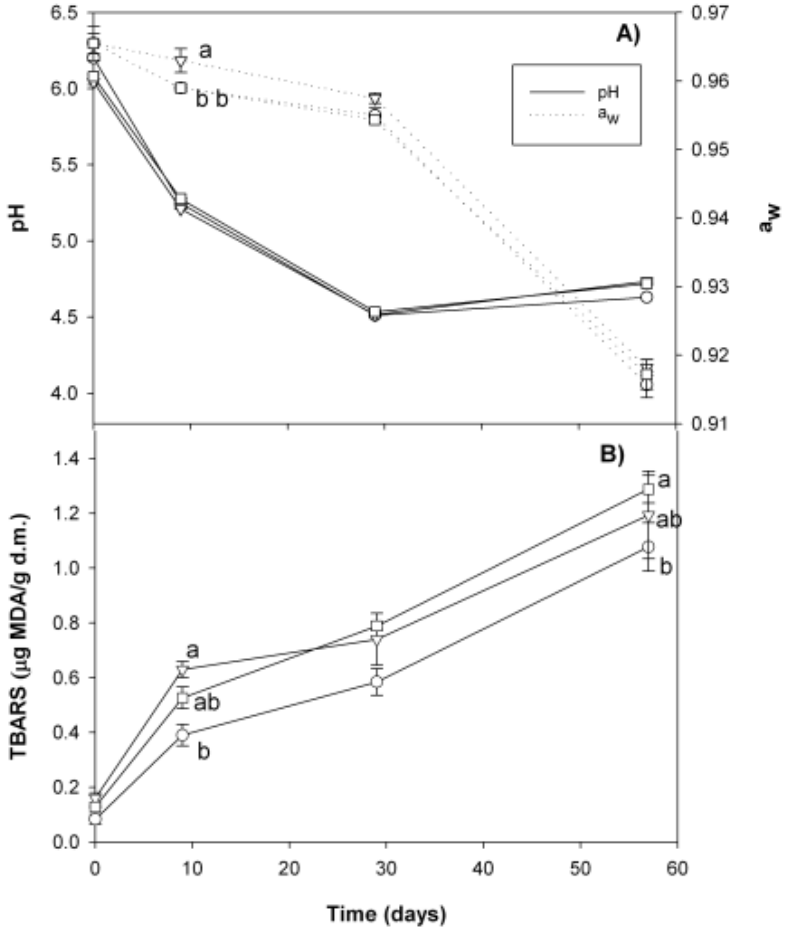

Figure 1. Changes in pH, a_w (A) and TBARS (B) during the ripening of dry fermented sausages: S (control, ○), RS (16% reduced salt, ∇) and RSK (16% KCl to replace NaCl, □).

The number of LAB and Staphylococci was within the range of what could be expected in dry fermented sausages and no differences were observed (p>0.05) among treatments throughout the process (data not shown). The population of LAB experienced a growth of 3 logarithmic cycles during the first 9 days and it was maintained stable until the end of the process. The number of *Staphylococci* suffered a slight decrease of 2 logarithmic cycles during processing.

In relation to fat and protein content, an increase in both contents was observed as a result of the reduction in moisture content during ripening. Salt content did not cause significant differences (p>0.05) in chemical composition among treatments at the end of the process (Table 1).

Table 1. Chemical composition and ion contents in dry fermented sausages with different salt content: S (control), RS (16% reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process (57 days).

		S		RS (red16%)		RSK(red16% +KCl)		*P*
Moisture	(%)	49.51		48.06		49.23		ns
Fat	(%)	10.75		12.73		12.32		ns
Protein	(%)	35.32ab		37.00		34.88b		ns
Na^+	(mg/100 g d.m.)	3435.38	a	3074.55	ab	2748.67	b	***
K^+	(mg/100 g d.m.)	952.26	b	996.35	b	1458.14	a	***
Cl^-	(mg/100 g d.m.)	3257.52		2946.78		2992.77		ns

P: *P* value of salt content effect. *** p<0.001, ** p<0.01, * p<0.05, ns: p>0.05. Identical letters in each row indicate the absence of differences at p>0.05 (Fisher test).

The color of sausages was also measured along the process, obtaining L*, a* and b* coordinates (data not shown). The trend in the three color coordinates throughout the process was similar to that observed by Olivares *et al.* (2010). No differences were detected in the final product among treatments as also has been observed in similar fermented sausages (Campagnol *et al.*, 2011).

TBARS values increased during the drying process in the three treatments (Fig. 1B) as it has been reported in similar sausages (Olivares *et al.*, 2011). A highest oxidation was observed in the reduced salt treatments at 9 days. TBARS values were significantly higher in the RS treatment than S treatment, but at the end of the process only RSK was significantly higher than S treatment. The effect of NaCl on lipid oxidation is not clear. Several authors have reported a pro-oxidant effect of NaCl in meat and meat products (Kanner, Harel & Jaffe, 1991; Shahidi, Rubin & Wood, 1988) while other authors have not observed this effect

in model system (Sárraga & García-Regueiro, 1998). Nevertheless, Zanardi *et al.* (2010) also observed a highest oxidation in reduced salt sausages and they attributed this highest oxidation to the use of $CaCl_2$ that can favored the lipid oxidation. In our study, the highest oxidation observed in RSK treatment could be due to a slightly highest fat content observed in this treatment (RSK).

As expected, a significant reduction (p<0.05) of the Na^+ ion content was achieved in RS and RSK treatments throughout the process (data at the end of the process 57days are shown in Table 1); however, at the end of the process only significant differences were found between S and RSK. The content of K^+ ion was increased (p<0.05) in RSK treatment, since this treatment was the one containing KCl (Table 1). Finally, significant differences were detected in Cl^- ion content throughout the process among treatments although at the end of the process there were not significant. The salt reduction detected in the treatments (RS and RSK) could be considered as a healthy benefit, following EU indications.

3.2. Texture profile analysis

Sausage TPA parameters were analyzed at the end of ripening and are shown in Table 2. Salt content did not produce differences in hardness, adhesiveness and springiness. However, reduced/replaced treatments presented lower significant values of cohesiveness and consequently chewiness, since this second parameter is the product of hardness, cohesiveness and springiness. It is well known that salt favors the gel formation in fermented sausages and leads to the desirable texture (Ruusunen, M., & Puolanne, E. 2005). However, we have obtained that in slow fermented sausages the reduction/substitution of low salt percentages can affect the cohesiveness and chewiness, although the hardness is not affected. Therefore, it is necessary to determine if these changes can be detected by consumers. Only few studies have detected differences in texture parameters by TPA analyses when the level of salt substitution was 40% or higher but not in lower percentages of substitution as we have performed. In this sense, Gou *et al.* (1996) did not detect differences in texture parameters when KCl was used as unique salt substitute while only Gimeno *et al.* (1999) reported a decrease in sausage hardness when KCl was used in combination with other divalent salts.

Table 2. Texture parameters of dry fermented sausages with different salt content: S (control), RS (16% reduced salt) and RSK (16% KCl to replace NaCl).

	S		RS (red16%)		RSK (red16% +KCl)		*P*
Hardness (N)	257.85 (22,60)		245.43 (17.22)		246.23 (14.64)		ns
Adhesiveness (N·s)	-3.34 (0.54)		-3.26 (0.47)		-3.68 (0.58)		ns
Springiness	0.63 (0.02)		0.61 (0.03)		0.61 (0.03)		ns
Cohesiveness	0.64 (0.02)	a	0.62 (0.02)	b	0.62 (0.01)	b	*
Chewiness	103.49 (9.21)	a	93.11 (8.96)	b	93.24 (7.94)	b	**

P: *P* value of salt content effect. *** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns: $p>0.05$. Identical letters in each row indicate the absence of differences at $p>0.05$ (Fisher test).The values represent the mean and (standard deviation).

3.3. Sensory analysis

The results of sensory analysis are shown in Table 3. The sensory panel did not detect significant differences among treatments in appearance and tenderness acceptability; however, S treatment had the highest acceptability in aroma, taste, juiciness and overall quality.

Previous studies detected differences in sensory texture parameters when the level of salt substitution was 40% or higher but not in lower percentages of substitution as we have performed. In this sense, Gou *et al.* (1996), Gelabert *et al.* (2003) and Guàrdia *et al.* (2008) reported an increase in hardness when KCl was used as unique salt substitute. However, the use of KCl in combination with other divalent salts or lactate resulted in a decrease in sausage hardness (Gimeno *et al.*, 1999; Gelabert *et al.*, 2003). Also, it is important to remark that these previous studies were performed mainly in small diameter fermented sausages and there are no reports in slow fermented sausages. Nevertheless, only the juiciness acceptability was the texture parameter that the consumers detected as lowest in reduced salt treatment (RS). The lowest juiciness acceptability detected in RS treatment could be due to the lower cohesiveness and chewiness (Table 2) observed in this treatment.

Table 3. Sensory acceptability of dry fermented sausages with different salt content: S (control), RS (16% reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process.

	S	RS (red16%)	RSK (red16% + KCl)	*P*
Appearance	6.14 (1.57)	5.86 (1.41)	5.96 (1.48)	ns
Aroma	6.33 a (1.46)	5.93 b (1.37)	5.89 b (1.44)	*
Taste	5.96 a (1.89)	5.34 b (1.80)	5.84 a (1.94)	**
Tenderness	6.13 (1.64)	6.00 (1.69)	6.18 (1.61)	ns
Juiciness	6.22 a (1.63)	5.75 b (1.60)	5.99 ab (1.58)	*
Overall quality	5.92 a (1.72)	5.54 b (1.61)	5.92 a (1.78)	*

P: *P* value of salt content effect. *** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns: $p>0.05$. Identical letters in each row indicate the absence of differences at $p>0.05$ (Fisher test). The values represent the mean and (standard deviation).

It was remarkable to observe that the addition of KCl removed the differences observed in taste, juiciness and overall quality and the consumers showed the same acceptance between S and RSK treatments in these parameters. Therefore a 16% substitution by KCl can be carried out although the aroma was the unique parameter that was not improved by KCl addition. Therefore, it is necessary to understand which aroma compounds are affected by the salt reduction and substitution.

3.4. Aroma compound analyses

In order to study how salt reduction and substitution affects aroma development in slow fermented sausages, the volatile compounds were extracted by SPME and analyzed by GC-MS and olfactometry analysis (Table 4 and 5, respectively). It is necessary to take into account that the proportion of volatile compounds depends on the extraction method used. In the present study, SPME technique with CAR/PDMS fiber was used. A total of 96 compounds were identified at the end of the process (Table 4) being 20 aldehydes, 11 alkanes, 13 ketones, 1 pyrazynes, 8 sulfur compounds, 8 acids, 17 alcohol, 10 esters, 6 aromatic hydrocarbons and 1 terpene. The volatile compounds present in the

sausage treatments classified by chemical groups are shown in Fig. 2. The reduction and substitution of salt produced an increase in aldehyde compounds but also a reduction in the abundance of sulphur and acid compounds (Fig. 2). One of the chemical groups that was present in highest abundance in the three treatments were the acids, representing 61-72% of the total extracted area, followed by aldehydes (8-16%) and alcohols (5-9%). Acetic acid was the most abundant compound in headspace (HS) (Table 4). Other abundant compounds were hexanal, 3-methyl-thiophene, octanoic acid, phenol, octane, heptanal, 1-hexanol, hexanoic acid, nonanal, octanal, pentanal, 2-butanone and 3-methyl-2-butenal (Table 4). All the identified compounds have been previously reported in fermented sausages (Marco, Navarro & Flores, 2004, 2006, 2008; Olivares *et al.*, 2011) using the same extraction technique except 3-methyl-2-butenal, 2-hydroxy benzaldehyde and butyl acetate. The identification of compounds was confirmed with authentic standards except benzyl alcohol and methyl 2,4-hexadienoate which were tentatively identified.

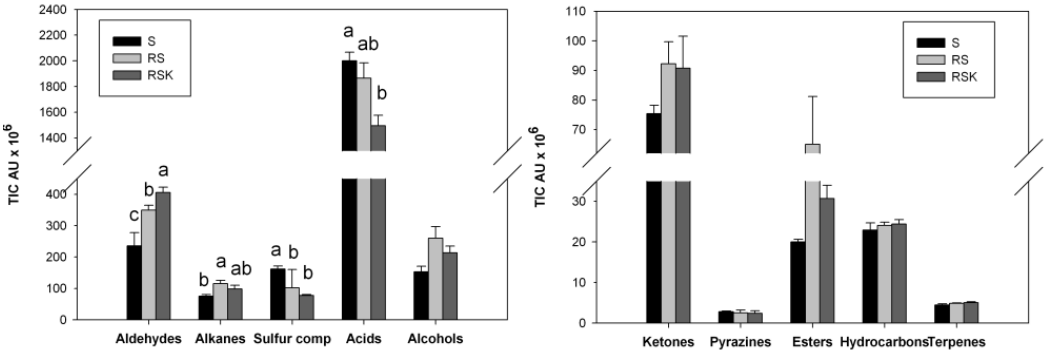

Figure 2. Total volatile compounds abundance expressed as AU x 106 in the headspace of dry fermented sausages with different salt content: S (control), RS (16% reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process. Different letters in the same chemical group indicate significant differences (p<0.05) among treatments.

In order to study the effect of salt reduction/substitution on the processes involved in the generation of aroma compounds it is better to classify the volatile compounds according to their possible origin: lipid autooxidation, bacterial metabolism (lipid β-oxidation, carbohydrate fermentation, amino acid degradation and Staphylococci esterase activity) and unknown or contaminant compounds (Table 4) as indicated Ordóñez, Hierro, Bruna and De La Hoz (1999).

Table 4. Volatile compounds (expressed as AU x 10^6 extracted by HS-SPME) identified in the headspace of dry fermented sausages with different salt content: S (control), RS (16% reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process.

			Sausage batches							
Compound	**LRI** [b]	**RI**[c]	**S**		**RS (red16%)**		**RSK (red16% +KCl)**		**SEM**	***P***[d]
Lipid autooxidation										
Pentane	500	a	8.25	c	19.98	b	32.45	a	1.66	***
Propanal	524	a	4.78		11.00		9.78		2.62	ns
Isopropyl alcohol	542	a	3.26		6.58		10.41		1.92	ns
Hexane	600	a	12.05	a	5.81	b	5.88	b	1.32	*
1-Propanol (31)[a]	613	a	0.06	b	0.11	a	0.09	ab	0.01	*
2-Methylfuran (82)[a]	615	a	1.23		1.21		0.69		0.15	ns
Butanal	622	a	0.56	b	1.41	b	3.04	a	0.27	**
1-Heptene (55)[a]	693	a	0.03	b	0.25	a	0.31	a	0.03	**
Heptane (71)[a]	700	a	1.84		5.55		5.31		1.19	ns
2-Ethylfuran (81)[a]	720	a	1.55	b	1.13	b	3.11	a	0.32	*
Pentanal (44)[a]	737	a	18.82		33.63		33.67		5.19	ns
Octane	800	a	25.95	b	60.93	a	37.00	b	5.21	*
2-Octene	810	a	13.15		8.91		6.13		1.66	ns
1-Pentanol	826	a	9.35	b	12.22	b	17.79	a	1.11	**
Hexanal (44)[a]	840	a	72.90	b	92.41	b	137.28	a	10.45	*
Nonane	900	a	3.14		2.56		2.36		0.28	ns
2-Hexenal (Z)	904	a	1.84		1.47		2.56		0.39	ns
2-Butylfuran	908	a	1.71		2.06		1.88		0.25	ns
1-Hexanol	922	a	28.19		45.87		37.14		5.18	ns
Heptanal	940	a	19.06		54.79		55.22		9.72	ns
Decane	1000	a	1.81		1.84		1.85		0.20	ns
2-Penthylfuran	1009	a	3.61	b	8.73	ab	11.78	a	1.51	*
Octanal	1047	a	19.79		31.80		33.30		3.36	ns
Hexanoic acid	1075	a	37.54		42.70		43.92		2.15	ns
2-Ethyl 1-hexanol	1082	a	6.89		7.54		8.00		0.60	ns
Undecane (57)[a]	1100	a	0.16		0.15		0.18		0.02	ns
2-Octenal (Z)	1115	a	1.03	b	1.88	b	4.24	a	0.64	*
1-Octanol	1123	a	1.02	b	2.84	a	2.74	a	0.40	*
Nonanal	1149	a	28.86		37.94		37.95		3.62	ns
Heptanoic acid	1165	a	2.03	b	3.23	a	2.16	b	0.29	*
Dodecane	1200	a	6.32		7.19		5.36		0.62	ns
2-Nonenal (Z)	1221	a	2.12		3.09		2.90		0.39	ns
Decanal	1256	a	1.99		2.36		1.73		0.35	ns
Octanoic acid	1266	a	87.82	a	35.80	b	36.79	b	5.78	***
2,4-Nonadienal (E, E)	1287	a	tr.		tr.		tr.			
Tridecane	1300	a	2.83	a	2.05	ab	1.39	b	0.24	*
Nonanoic acid	1357	a	3.20		2.83		2.85		0.16	ns
Decanoic acid	1449	a	7.98		6.80		6.97		0.90	ns
Total			442.70	b	566.66	a	606.23	a	36.77	*

Table 4. Continued.

Compound	LRI [b]	RI[c]	Sausage batches						SEM	P[d]
			S		RS (red16%)		RSK (red16% +KCl)			
Bacterial metabolism										
Lipid β oxidation										
2,3-Pentanedione (85)[a]	744	a	0.17		0.14		0.08		0.03	ns
2-Heptanone	933	a	4.64		7.88		7.70		1.10	ns
2-Heptanol	946	a	5.92		4.32		4.01		0.67	ns
1-Octen-3-ol (57)[a]	1030	a	2.13		2.01		3.80		0.56	ns
2-Octanone	1039	a	0.62		0.61		0.90		0.09	ns
2-Nonanone	1140	a	5.60	a	3.01	b	3.78	b	0.29	**
2-Undecanone	1306	a	1.27		1.25		1.11		0.16	ns
Total			20.36		19.22		21.38		2.20	ns
Carbohydrate fermentation										
Acetaldehyde	466	a	9.94		12.44		10.67		0.67	ns
Ethanol	508	a	13.11	c	96.86	a	49.08	b	12.11	**
Acetone	530	a	20.48		27.79		24.48		4.18	ns
2,3-Butanedione (43)[a]	626	a	0.52	a	0.34	b	0.30	b	0.04	*
2-Butanone	631	a	28.96		30.95		28.48		2.41	ns
Acetic acid	737	a	1447.65		1295.19		1228.50		59.09	ns
2,3-Butanediol	887	a	1.63	a	0.26	b	0.33	b	0.25	*
Butanoic acid	892	a	72.74		80.23		82.45		4.31	ns
Total			1595.03		1544.06		1424.30		75.20	ns
Amino acid degradation										
2-Methyl propanal	594	a	7.42		7.38		5.59		1.06	ns
Ethyl methyl sulfide (61)[a]	624	a	0.98	a	0.79	a	0.41	b	0.10	*
Benzene	675	a	0.95		1.00		1.20		0.13	ns
2-Methyl 1-propanol	682	a	0.36		0.20		0.31		0.08	ns
3-Methyl butanal (44)[a]	689	a	8.89		9.32		8.36		0.96	ns
2-Methyl butanal (58)[a]	700	a	2.50		2.30		2.11		0.21	ns
Dimethyl disulfide	772	a	4.40	a	3.11	ab	2.06	b	0.51	*
Toluene	788	a	12.83		14.11		13.63		0.76	ns
3-Methyl-3-buten-1-ol (41)[a]	789	a	0.30		0.24		0.29		0.05	ns
3-Methyl thiophene	794	a	125.16	a	68.25	b	42.68	b	6.88	***
3-Methyl 2-butenal (55)[a]	840	a	14.73	b	17.74	b	28.74	a	2.61	*
Ethyl benzene	883	a	2.46		2.74		2.90		0.20	ns
2,5-Dimethyl pyrazine	944	a	2.81		2.53		2.42		0.52	ns
3-Methyl thiopropanal	966	a	6.24	b	9.89	a	11.66	a	0.75	**
Dimethyl trisulfide	1002	a	1.80		1.25		1.01		0.20	ns
Benzaldehyde	1017	a	16.33	b	21.55	a	22.29	a	1.40	*
2-Hydroxy benzaldehyde (122)[a]	1100	a	0.27		0.27		0.28		0.01	ns
Benzeneacetaldehyde	1107	a	3.69	c	6.82	a	5.65	b	0.30	***
Phenol	1111	a	75.55		74.23		73.78		2.99	ns
Benzyl alcohol	1120	b	1.63		1.66		1.53		0.05	ns
Phenylethyl alcohol (91)[a]	1194	a	0.39	b	2.32	a	0.92	b	0.16	***
Total			289.69	a	247.69	b	227.82	ab	16.78	ns

Table 4. Continued.

Compound	LRI [b]	RI[c]	Sausages batches						SEM	P[d]
			S		RS (red16%)		RSK (red16% +KCl)			
Staphylococci esterasa activity										
Methyl acetate	551	a	2.70		1.98		1.62		0.27	ns
Ethyl acetate	635	a	6.76	b	26.99	a	13.21	b	2.58	**
Ethyl propanoate (57)[a]	744	a	0.27		0.62		0.47		0.12	ns
Ethyl butanoate (71)[a]	831	a	0.28	c	2.90	a	1.29	b	0.30	**
Butyl acetate	847	a	2.05		2.55		4.69		0.64	ns
Ethyl 2-hydroxy-propanoate	869	a	1.78	b	16.45	a	1.97	b	0.44	***
Ethyl hexanoate (88)[a]	1028	a	0.10	c	1.22	a	0.71	b	0.09	***
Ethyl octanoate	1229	a	2.73		4.42		3.14		0.83	ns
Total			16.68		57.12		27.09		9.78	ns
Unknown or contaminants compound										
Methanethiol	472	a	7.83		9.17		9.84		0.47	ns
Carbon disulfide	537	a	13.78		7.75		8.53		1.59	ns
o-Xylene	916	a	3.24		3.23		3.33		0.23	ns
Styrene	918	a	1.61		1.27		1.36		0.15	ns
2-Butoxyetanol	952	a	2.35		2.74		2.87		0.31	ns
Butyrolactone	1023	a	5.07		7.15		6.49		0.82	ns
p-Cymene	1050	a	1.81		1.72		2.00		0.13	ns
D-Limonene	1045	a	4.48		4.85		5.03		0.20	ns
Dimethyl sulfone	1061	a	1.27	b	1.37	b	1.61	a	0.06	*
Methyl 2,4-hexadienoate (67)[a]	1065	b	0.19	a	0.22	a	0.06	b	0.01	***
Ethyl 2,4-hexadienoate	1144	a	3.13	b	7.72	a	3.57	b	0.75	*
Sorbic acid	1179	a	342.07	a	397.89	a	91.74	b	42.89	**
4-Methyl-phenol (107)[a]	1195	a	0.46		0.58		0.43		0.04	ns
Total			387.29		445.65		136.87		82.35	ns

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound. Means followed by different letters in the same compound indicate significant differences (p<0.05) among batches.

[a] Target ion used to quantify the compound when the peak was not completely resolved.

[b] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a DB-624 capillary column (J&W Scientific 30 m x 0.25 mm i.d. x 1.4 µm film thickness).

[c] Reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentatively identification by mass spectrum.

[d] P value of salt content effect. ***: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05.

The carbohydrate fermentation volatile compounds were the most abundant compounds, representing 53-58% of the total extracted area, since acetic acid just represented a 44-52%. Then, lipid autooxidation volatile compounds represented 16-24%, amino acid degradation products 8-10%, volatile compounds derived from staphylococci esterase activity 0.6-1% and lipid β-oxidation products 0.6-0.8%.

Volatile compounds derived from lipid autooxidation have an important role in the odor of dry fermented sausages due to their low olfactory threshold (Marco *et al.*, 2007). Predominantly, the lipid oxidation originates aldehydes among other products such as alkanes, ketones, and alcohols. Salt content affected (p<0.05) the HS abundance of volatile compounds as observed by a highest abundance in RS and RSK treatments (Table 4). Several compounds have a significant higher abundance in RSK treatment than S and RS treatments such as hexanal, butanal, 2-ethylfuran, 1-pentanol and 2-octenal (Table 4). However, only 2-pentylfuran was more abundant in the HS of RSK treatment than in S treatment while tridecane showed opposite effect. Only two compounds, 1-octanol and 1-heptene, showed more abundance in the HS of RS and RSK than S treatment while hexane and octanoic acid displayed the opposite effect. Finally, octane and heptanoic acid and 1-propanol showed a greater abundance in HS of RS than S and RSK treatments. The higher abundance of compounds derived from lipid oxidation in RS and RSK treatments is in accordance to the TBARS values obtained as they were significantly higher in RS and RSK treatments, probably due to their highest fat content.

On the other hand, salt content did not produce significant differences on volatile compound derived from lipid β-oxidation reactions except for 2-nonanone which was more abundant in the HS of S treatment than in reduced/replaced treatments (RS, RSK). However, several compounds derived from carbohydrate fermentation were affected by salt reduction. The carbohydrate fermentation reactions mainly generate acids, followed by alcohols and ketones. Only ethanol, 2,3-butanediol and 2,3-butanedione showed significant differences among treatments (Table 4). 2,3-Butanediol and 2,3-butanedione showed a greater HS abundance (p<0.05) in the S treatment while ethanol had the lowest abundance in S treatment. The reduction in 2,3-butanediol abundance was also observed by Olesen *et al.* (2004); however, they detected the opposite effect for 2,3-butanedione. These authors related the 2,3-butanedione concentration to the activity of *Staphylococcus* starter, although in the present study we did not detect differences in S*taphylococci* grow among the treatments.

Volatile compounds derived from amino acid degradation depend on free amino acid concentration present in sausages. Branched chain amino acid produces branched aldehydes, alcohols and acids; in addition sulfur amino acids generate sulfur volatile compounds as well as aromatic amino acids produce aromatic compounds. Salt affected the total abundance of this group of compounds as a highest HS abundance (p<0.05) was detected in S than RS

treatment. The most abundant compound in HS of sausages within this chemical family was 3-methyl thiophene (Table 4). This compound had a higher abundance in S treatment than RS and RSK treatments, and dimethyl disulfide was also more abundant in S treatment, but it was only significantly different from RSK. Nevertheless, ethyl methyl sulfide showed the lowest abundance in RSK treatment while 3-methyl 2-butenal showed the opposite effect. On the other hand, several compounds, 3-methyl thiopropanal and benzaldehyde, showed a greater abundance in the HS of reduced treatments (RS, RSK) than in S treatment. However, phenylethyl alcohol and benzeneacetaldehyde were only more abundant in the HS of RS treatment (Table 4).

The compounds derived from the *Staphylococci* activity were also affected by the salt reduction. A higher abundance of total ester compounds was found in the HS of RS treatment, but due to variability among sausages, the differences were not significant. However, several compounds were significantly different among treatments: ethyl acetate, ethyl butanoate, ethyl 2-hydroxy propanoate and ethyl hexanoate were significantly higher in RS treatment than in S and RSK treatments (Table 4). These ester compounds provide fruity notes and have been widely detected in slow fermented sausages (Olivares *et al.*, 2011). Talon, Chastagnac, Vergnais, Montel & Berdagué (1998) indicated that the production of esters compounds depended on the presence of the substrates (ethanol and acids) and on the *Staphylococci* esterase activity. In the present study we detected highest ethanol abundance in RS treatment followed by RSK treatment and S treatment that would explain the highest ester production found at the same proportion in the treatments.

About unknown or contaminants compounds, few differences were found among treatments. The presence of sorbic acid and its ethyl and methyl esters came from the potassium sorbate applied to the sausage casing to avoid mold growth as also reported Olivares *et al.* (2011). Sorbic acid and their esters showed highest abundance in RS treatment while dimethyl sulfone had a highest abundance RSK treatment.

Generally, all the studies performed on volatile compounds in reduced fermented sausages studied a percentage reduction of 25% or higher (Olesen *et al.*, 2004; Ravyts *et al.*, 2010; Campagnol *et al.*, 2011). While Campagnol *et al.* (2011) reported few changes in the profile of volatile compounds when salt was substituted in 25 and 50% by KCl, other authors such as Olesen *et al.* (2004) indicated a considerable impact on the volatile profile when NaCl was reduced in a 50% in fermented sausages. In addition, Olesen *et al.* (2004) indicated that a

half percent salt reduction produced an activation of lactic acid bacteria growth giving a higher pH drop affecting negatively the growth of *Staphylococci*. This effect produced a decrease in the generation of branched derived volatile compounds in low salted sausages. However these authors reported that the differences observed among high and low salted sausages were narrowed as the ripening process continued. In the present study, we only analyzed a reduction of the 16% of the total salt content without observing any effect on the growth of lactic acid bacteria and *Staphylococci*; also no differences in pH were detected along the process among treatments. Therefore, the differences that we have observed in volatile compounds derived from amino acid degradation cannot be attributed to a higher *Staphyloccoci* activity. This fact is also in accordance to Ravyts *et al.* (2010) who indicated that modifications of salt concentrations had a limited impact on the growth of sausage microbiota and they did not find a significant effect on volatile production. Probably these authors did not find an effect on volatile compound production because they only extracted few volatile compounds as they used static headspace gas chromatography analysis. However, it is necessary to remember that the flavour of fermented sausages is affected by recipe and the type of starter culture used (Leroy, Verluyten & De Vuyst, 2006).

In order to reveal the aroma contribution of the volatile compounds present in the slow fermented sausages an olfactometry analysis was performed showing the presence of 31 different aroma active zones. Twenty-six of them were identified by mass spectra, linear retention indices and odor description, while 5 of them could not be identified (Table 5). All of identified compounds have been previously detected as aroma impact compounds in fermented sausages (Chevance, Farmer, Desmond, Novelli, Troy & Chizzolini, 2000; Gianelli, Olivares & Flores, 2011; Marco *et al.*, 2007; Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999; Olivares *et al.*, 2011; Schmidt & Berger, 1998a, 1998b; Söllner & Schierberle, 2009; Stahnke, 1995a, 1995b) except 2-hydroxy benzaldehyde (herbal, stable, roasted bread), butyl acetate (spice, rancid, wood, boiled vegetables) and 3-methyl thiophene (cooked potato, green, wood). The detection frequency (DF) method was applied to determine the contribution of the different volatile compounds to the aroma of slow fermented sausages. The highest DF values mean a highest aroma impact. The most potent odorants detected were 2-hexenal (roasted, meat broth), 1-octen-3-ol (mushroom), acetic and butanoic acids (vinegar and cheese odors, respectively), dimethyl trisulfide (onion, cabbage), 2-nonanone (plastic, wood), 3-methyl thiopropanal (cooked potato,

savory) and D-limonene (citrus). Four of these aroma compounds (acetic and butanoic acids, 1-octen-3-ol and 3-methyl thiopropanal) were also detected as potent odorants in fermented sausages (Olivares *et al.*, 2011). However other potent aroma compounds, contributing with roasted nuts odors, were detected although they were not identified (unknown compounds with LRI 1179 and 1223). These last unknown aroma compounds were also detected in similar fermented sausages by Olivares *et al.* (2011) using the same extraction technique.

In order to study which aroma compounds were responsible for the highest acceptability of the salted treatment (S), a principal component analysis was done using the following parameters: chemical composition (fat, protein and moisture content), pH, ions (Na^+, K^+, Cl^-), aroma active volatile compounds (those shown in Table 5) and texture parameters. Fig. 3 illustrates the results of the PCA analysis. Two principal components were able to explain the 57.88% of the total variance observed. PC1 is the most important variable because it accounted for 39.83% of the variance while PC2 accounted for 18.05% of the variance. PC1 differentiated the sausages by their salt content. S treatment, with the highest salt content, appeared separately in the positive part of PC1, associated with higher texture parameters, higher Na^+ and Cl^- ions content and the presence of the volatile compounds such as 2-nonanone, dimethyl trisulfide, 3-methyl thiophene, 2,3 butanedione and acetic acid. However, PC2 differentiated samples with reduced salt content (RS) to S and RSK treatments showing a negative correlation with RS treatment. Therefore, sausage samples with KCl as substitute (RSK treatments) were differentiated but more similar to the S treatment than the reduced salt treatment (RS).

Table 5. Odor active compounds identified in the HS of dry fermented sausages.

Compound	LRI[a] GC-O	LRI standard	Descriptor	DF[b]	Previously reported in dry sausages[c]
Lipid autooxidation					
2-Ethylfuran	725	718	Tallowy, savory, sweet	4	8
Pentanal	734	735	Green, nut, meat broth	7	1,8
1-Pentanol	815	820	Floral, butter, roasted nuts	5	8,10
Hexanal	835	836	Fresh cut grass	8	1,2,5,7-9,11
2-Hexenal (Z)	903	904	Sweet, roasted, meat broth	12	8
Nonanal	1148	1151	Citrus, laurel, carnation	8	1,2,7-10
Heptanoic acid	1163	1162	Herbal, rancid	6	8,10
2-Nonenal (Z)	1219	1222	Herbal, strawberry	4	1,2,8-11
2,4-Nonadienal (E, E)	1290	1288	Herbal, unpleasant, roasted	6	7,9,11
Bacterial metabolism					
Lipid β oxidation					
1-Octen-3-ol	1024	1028	Mushroom	12	5,8,11
2-Octanone	1031	1037	Green, garlic	10	11
2-Nonanone	1137	1142	Plastic, wood, pop-corn, roasted	11	1,3,4,8
Carbohydrate fermentation					
2,3-Butanedione	632	632	Butter	4	1-4,6,8,10,11
Acetic acid	705	700	Vinegar	11	1-3,8-11
Butanoic acid	871	876	Cheese	12	1-4,8,9,11
Amino acid degradation					
3-Methyl butanal	691	691	Green	5	1,2,8,9,11
3-Methyl thiophene	795	796	Cooked potato, green, wood	4	11
3-Methyl thiopropanal	967	969	Cooked potato, savory	11	7-11

Table 5. Odor active compounds identified in the HS of dry fermented sausages.

Compound	LRI[a] GC-O	LRI standard	Descriptor	DF[b]	Previously eported in dry sausages[c]
Dimethyl trisulfide	1007	1009	Onion, rotten, cabbage	12	6
2-Hydroxy benzaldehyde	1105	1107	Herbal, stable, roasted bread	4	-
Benzeneacetaldehyde	1110	1112	Rancid, musk, jasmine	6	8-11
Staphylococci esterase					
Ethyl butanoate	825	825	Pineapple, strawberry	6	1-5,8-11
Butyl acetate	843	840	Spice, rancid, wood, boiled vegetables	9	-
Unknown or contaminants compound					
Methanethiol	472	471	Rotten, stable	8	8,11
D-Limonene	1046	1048	Citrus	11	3-5,8
4-Methyl-phenol	1194	1190	Plastic, stable, rancid,	7	3,4,9-11
Unknown 1	922		Green, rancid, manure, cheese	6	
Unknown 2	962		Onion, Swiss chard	7	
Unknown 3	1001		Herbal, roasted, damp, vanilla	6	
Unknown 4	1179		Roasted nuts	10	
Unknown 5	1223		Roasted nuts, unpleasant, cardboard	11	

[a] Linear retention indices (LRI) of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (J&W Scientific 60 m x 0.32 mm i.d. x 1.8 µm film thickness).

[b] DF Detection frequency value

[c] Previously reported in dry fermented sausages by: 1 Stahnke (1994), 2 Stahnke (1995b), 3 Schmidt and Berger(1998a), 4 Schmidt and Berger (1998b), 5 Meynier *et al.* (1999), 6 Chevance *et al.* (2000), 7 Blank *et al.* (2001), 8 Marco *et al.* (2007), 9 Söllner & Schieberle (2009), 10 Gianelli *et al.* (2011), 11 Olivares *et al.* (2011).

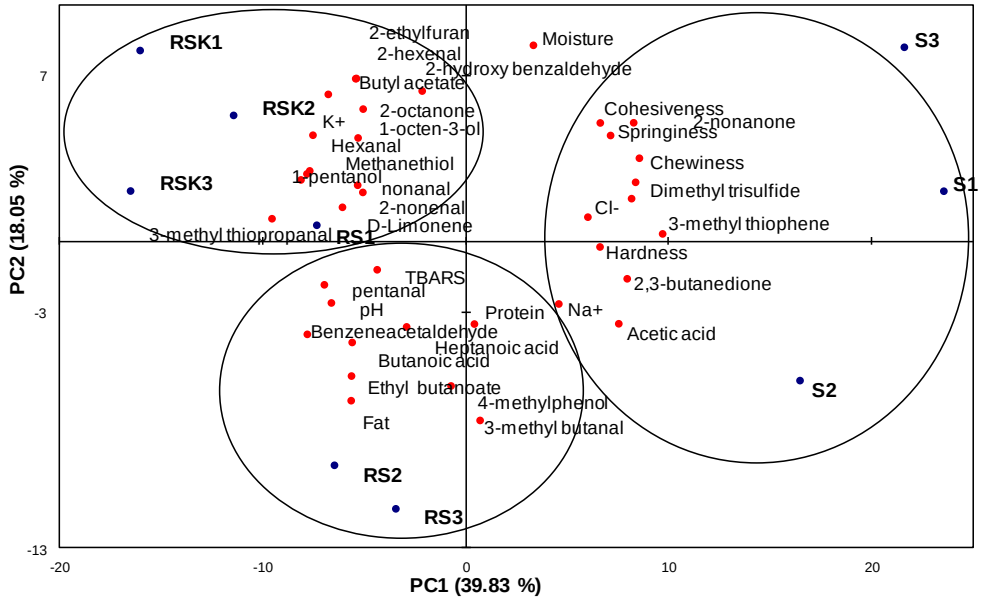

Figure 3. Loadings of the first two principal components (PC1-PC2) of the analyzed parameters (pH, TBARS, ions, texture parameters and aromatic active compounds) of fermented sausages with different salt content: S (control), RS (16% reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process.

4. CONCLUSION

In summary, small salt reduction (16%) affected the quality of slow fermented sausages producing a reduction in the acceptance of aroma, taste, juiciness and overall quality. However, the substitution by KCl removed the differences observed in taste, juiciness and overall quality and the consumers showed the same acceptance for high salt (S) and substituted (RSK) treatments. Therefore a 16% substitution by KCl can be carried out; however, the aroma was the unique parameter that was not improved by KCl addition. The aroma perceived by consumers was affected due to the reduction detected in sulfur and acid compounds and the increase in aldehyde compounds. Moreover, the aroma compounds that characterized the high salt treatment (S) were dimethyl trisulfide, 3-methyl thiophene, 2,3-butanedione, 2-nonanone and acetic acid. In addition, the decrease in chewiness and cohesiveness detected in reduced and substituted treatments (RS and RSK) could affect the perception of the aroma

compounds. To improve the aroma of reduced salt slow fermented sausages is necessary to look for other alternatives to KCl addition to improve the aroma perception. Further studies about the use of salt-associated odours which can induce a saltiness enhancement should be performed.

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REFERENCES

Armenteros, M., Aristoy, M. C., Barat, J. M., & Toldrá, F. (2009). Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. *Food Chemistry,* 117, 627-633.

BOE (1979). Métodos oficiales de análisis de productos cárnicos. *Boletín Oficial del Estado, de 28 de agosto de 1979, Anexo II* (pp. 20233-20240). (Madrid, Spain).

Burdock, G. A., (2002). *Fenaroli's handbook of flavor ingredients* (4th ed.). Boca Raton, Florida: CRC Press Inc..

Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R. (2011). The effect of yeast extract addition on quality of fermented sausages at low NaCl content. *Meat Science,* 87, 290-298.

Chevance, F. F. V., Farmer, L. J., Desmond, E. M., Novelli, E., Troy, D. J., & Chizzolini, R. (2000). Effect of some fat replacers on the release of volatile aroma compound from low-fat meat products. Journal of Agricultural and Food Chemistry, 48, 3476-3484.

Commission, European (2008). Salt reduction. Avaliable at. http://ec.europa.eu/health/ph_determinants/life_style/nutrition_salt_en.htm

Flores, J. (1997). Mediterranean vs northern European meat products. Processing technologies and main differences. *Food Chemistry,* 59, 505-510.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of biological chemistry,* 226, 497-509.

Gelabert, J., Gou, P., Guerrero, L., & Arnau, J. (2003). Effect of sodium chloride replacement on some characteristics of fermented sausages. *Meat Science,* 65, 833-839.

Gianelli, M. P., Olivares, A., & Flores, M. (2011). Key aroma components of a dry-cured sausage with high fat content (Sobrassada). *Food Science and Technology International,* 17, 63-71.

Gimeno, O., Astiasarán, I., & Bello, J. (1998). A Mixture of Potassium, Magnesium, and Calcium Chlorides as a Partial Replacement of Sodium Chloride in Dry Fermented Sausages. *Journal of Agricultural and Food Chemistry,* 46, 4372-4375.

Gimeno, O., Astiasarán, I., & Bello, J. (1999). Influence of partial replacement of NaCl with KCl and $CaCl_2$ on texture and color of dry fermented sausages. *Journal of Agricultural and Food Chemistry,* 47, 873-877.

Gou, P., Guerrero, L., Gelabert, J., & Arnau, J. (1996). Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. *Meat Science,* 42, 37-48.

Guàrdia, M. D., Guerrero, L., Gelabert, J., Gou, P., & Arnau, J. (2008). Sensory characterisation and consumer acceptability of small calibre fermented sausages with 50% substitution of NaCl by mixtures of KCl and potassium lactate. *Meat Science,* 80, 1225-1230.

ISO 2917 (1999). Meat and meat products. Determination of the pH (Reference method).

ISO 8589 (1988). Sensory analysis. General guidance for design of test rooms. Standard no.8589 (Geneva, Switzerland).

Kanner, J., Harel, S., & Jaffe, R. (1991). Lipid peroxidation of muscle food as affected by NaCl. *Journal of Agricultural and Food Chemistry,* 39, 1917-1921.

Kovats, E.S. (1965). Gas chromatographic characterization of organic substances in the retention index system. In J. C. Giddings, & R. A. Keller (Eds.), *Advances in chromatography* (pp. 229-247). New York: Marcel Dekker, Inc..

Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology,* 106, 270-285.

Marco, A., Navarro, J. L., & Flores, M. (2004). Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). *Food Chemistry,* 84, 633-641.

Marco, A., Navarro, J. L., & Flores, M. (2006). The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage. *Meat Science,* 73, 660-673.

Marco, A., Navarro, J. L., & Flores, M. (2007). Quantitation of selected odor-active constituents in dry fermented sausages prepared with different curing salts. *Journal of Agricultural and Food Chemistry,* 55, 3058-3065.

Marco, A., Navarro, J. L., & Flores, M. (2008). The sensory quality of dry fermented sausages as affected by fermentation stage and curing agents. *European Food Research and Technology,* 226, 449-458.

Meynier, A., Novelli, E., Chizzolini, R., Zanardi, E., & Gandemer, G. (1999). Volatile compounds of commercial Milano salami. *Meat Science,* 51, 175-183.

Olesen, P. T., Meyer, A. S., & Stahnke, L. H. (2004). Generation of flavour compounds in fermented sausages - The influence of curing ingredients, Staphylococcus starter culture and ripening time. *Meat Science,* 66, 675-687.

Olivares, A., Navarro, J. L., Salvador, A., & Flores, M. (2010). Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Science,* 86, 251-257.

Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science,* 87, 264-273.

Ordóñez, J.A., Hierro, E.M., Bruna, J.M., De La Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition*, 39, 329-367.

Pollien, P., Ott, A., Montigon, F., Baumgartner, M., Muñoz-Box, R., & Chaintreau, A. (1997). Hyphenated Headspace-Gas Chromatography-Sniffing Technique: Screening of Impact Odorants and Quantitative Aromagram Comparisons. *Journal of Agricultural and Food Chemistry,* 45, 2630-2637.

Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L., & Leroy, F. (2010). The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages. *Food Microbiology,* 27, 945-954.

Ruusunen, M.M & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science,* 70, 531-541.

Sárraga, C., & García-Regueiro, J. A. (1998). Oxidation of microsomal fraction in the processing conditions of dry-cured ham. The in-vitro effect of brine. *Meat Science,* 50, 295-302.

Schmidt, S., & Berger, R. G. (1998a). Aroma Compounds in Fermented Sausages of Different Origins. *LWT - Food Science and Technology,* 31, 559-567.

Schmidt, S., & Berger, R. G. (1998b). Microbially formed aroma compounds during the maturation of dry fermented sausage (salami). *Advances in Food Sciences,* 20, 144-152.

Shahidi, F., Rubin, L. J., & Wood, D. F. (1988). Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Science,* 22, 73-80.

Söllner, K., & Schieberle, P. (2009). Decoding the key aroma compounds of a Hungarian-type salami by molecular sensory science approaches. *Journal of Agricultural and Food Chemistry,* 57, 4319-4327.

Stahnke, L. H. (1995a). Dried sausages fermented with Staphylococcus xylosus at different temperatures and with different ingredient levels - Part II. Volatile components. *Meat Science,* 41, 193-209.

Stahnke, L. H. (1995b). Dried sausages fermented with Staphylococcus xylosus at different temperatures and with different ingredient levels - Part III. Sensory evaluation. *Meat Science,* 41, 211-223.

Talon, R., Chastagnac, C., Vergnais, L., Montel, M. C., & Berdagué, J. L. (1998). Production of esters by staphylococci. *International Journal of Food Microbiology,* 45, 143-150.

Tjener, K., Stahnke, L. H., Andersen, L., & Martinussen, J. (2004). The pH-unrelated influence of salt, temperature and manganese on aroma formation by Staphylococcus xylosus and Staphylococcus carnosus in a fermented meat model system. *International Journal of Food Microbiology,* 97, 31-42.

Zanardi, E., Ghidini, S., Conter, M., & Ianieri, A. (2010). Mineral composition of Italian salami and effect of NaCl partial replacement on compositional, physico-chemical and sensory parameters. *Meat Science,* 86, 742-747.

Capítulo 4

Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii yeast*

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Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii yeast*

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Abstract

The inoculation of a *Debaryomyces hansenii* strain in dry fermented sausages with reduced fat and salt contents was evaluated in terms of chemical, microbial and consumer acceptability. The implantation of the inoculated yeast strain was confirmed by RAPDs of M13 minisatellite. A reduction of 17-20 % salt and 10-16% fat content was achieved. These reductions affected the sausage quality by producing an increase in a_w, hardness and chewiness values and a decrease of staphylococci growth. However, *D. hansenii* inoculation compensated these changes although it was not able to modify neither the hardness of reduced fat batches nor the staphylococci growth decrease. In terms of sensory acceptability, different preferences patterns of consumers were found. Yeast inoculation improved the aroma and taste quality when fat or salt reductions were carried out in dry fermented sausages.

Keywords: Fat, Salt, sensory, *Debaryomyces hansenii*, Dry fermented sausage, Yeast.

1. INTRODUCTION

The intake of meat products has grown around 10% in industrialized countries in spite of their fat and salt content (WHO/FAO, 2003). However, during the last years a demand for low fat and salt food has grown (Ruusunen & Puolanne, 2005). Therefore, meat products such as dry fermented sausages are being reformulated to adjust their fat and salt content (Aaslying, Vestergaard & Koch, 2014; Beriain, Gómez, Petri, Insausti & Sarriés, 2011). However, these ingredients cannot be reduced without affecting organoleptic and technologic characteristics. On the one hand, fat contributes to nutritional (source of essential fatty acids, liposoluble vitamins and energy), organoleptic (flavour, texture, mouthfeel) and technological properties (release of moisture) (Olivares, Navarro, Salvador & Flores, 2010). On the other hand, salt is also involved in organoleptic (flavour, texture) and technologies properties (myofibrillar protein solubilization, a_w decrease) (Corral, Salvador & Flores, 2013).

Different strategies have been studied to reduce fat and salt content in dry fermented sausages, since preservation of product acceptability is a decisive criterion when developing this kind of products (Wirth, 1988). Fat content has been replaced by soy oil (Muguerza, Ansorena & Astiasarán, 2003), olive oil (Bloukas, Paneras & Fournitzis, 1997), konjac gel (Ruíz-Capillas, Triki, Herrero, Rodriguez-Salas & Jiménez-Colmenero, 2012), inulin (Mendoza, García, Casas & Selgas, 2001), and fibres (Salazar, García, & Selgas, 2009). However, few studies have dealt with fat reduction without replacers and its effect on sensory characteristics. Generally, fat reduction (10 %) affected the external appearance and flavour intensity of fermented sausages (Liaros, Katsanidis & Bloukas, 2009) while higher reduction percentages (20 %) produced a suitable acceptability (Papadima & Bloukas, 1999 and Olivares *et al.* 2010); in spite of a lowest taste, aroma and hardness (Olivares *et al.*, 2010 and 2011) and appearance (Papadima & Bloukas, 1999) perceived by consumers.

Regarding salt reduction, different salts have been used as NaCl substitute (Corral *et al.*, 2013; Gimeno, Astiasarán & Bello, 1999) although they did not achieve sensory acceptable products (Gimeno *et al.*, 1999). Major salt reductions (40-50%) had a negative effect on many sensory characteristics such as hardness, bitterness, aroma and taste acceptability (Gelabert, Gou, Guerrero & Arnau, 2003; Campagnol, dos Santos Wagner, Terra & Pollonio, 2011). With small salt reductions (16 %), the best results were found when KCl alone was used even though the aroma acceptability was still affected (Corral *et al.*, 2013).

The effect of fat and salt reductions together in dry fermented sausages has been scarcely studied. A strategy of combining a reduction in the salt content and a simultaneous modification in the lipid fraction using olive or linseed oils resulted in products with lowest sodium content, highest calcium content and a significant supply of omega-3 fatty acids (García-Iñiguez de Ciriano, Berasategi, Navarro-Blasco, Astiasarán & Ansorena, 2013) and highest MUFA content (Beriain *et al.*, 2011) producing an improved nutritional sausage profile. Both studies indicated a sensory acceptable characteristic of the new formulations although differences in texture and taste were reported by the trained panel.

The use of starter yeasts can be an alternative to improve the sensory characteristics of the dry fermented sausages. *Debaryomyces hansenii* is the predominant yeast which proliferates in dry fermented sausage environment (Cocolin, Urso, Rantsiou, Cantoni & Comi, 2006). The growth of *D.hansenii* in the sausage surface can control water release in low fat sausages thus improving sausage aroma lost by salt reduction as reported by Campagnol *et al.* (2011) who tried to improve the sensory characteristic by the addition of a yeast extract (*Saccharomyces cerevisiae*). The mechanisms behind aroma loss due to salt reduction are probably due to the salting out effect that salt produces on volatile compounds (Desmond, 2006) in addition to the effect of salt on the biochemical reactions involved in aroma generation.

Nevertheless, important differences have been observed when different yeasts strains are inoculated in fermented sausages (Olesen, & Stahnke, 2000; Andrade, Córdoba, Sánchez, Casado & Rodríguez, 2009). Recently, Cano-García, Flores & Belloch (2013) isolated *D. hansenii* strains from traditional fermented sausages and reported their aroma potential using a meat model system (Cano-García, Rivera-Jiménez, Belloch & Flores, 2014). Therefore, the aim of this work was to elucidate the effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *D. hansenii*, since this yeast could offset the quality defects produced by fat and/or salt reductions.

2. MATERIALS AND METHODS

2.1. Preparation of yeast inoculum

D. hansenii P2 previously isolated from naturally fermented sausages (Cano-García *et al.*, 2013) was used as starter in the production of dry fermented sausages. Yeast was cultivated on GPY medium (2% glucose, 0.5% peptone, 0.5% yeast extract, pH 6.0) and the grown cells washed with sterile saline

solution (0.9% NaCl) and centrifuged (7000 rpm for 10 minutes at 4ºC) to remove the culture medium. The collected cells were prepared to a concentration of 10^8 c.f.u./ml using dilution plates. The concentrated yeast cells were directly stored at -80°C until their inoculation in dry fermented sausage batches.

2.2. Dry fermented sausages and sampling

Seven batches of dry fermented sausages were manufactured: a control batch (C) was prepared using 70% pork lean meat and 30% pork back fat and 27g/kg NaCl content while six batches were manufactured varying salt and/or pork back fat content with or without yeast inoculation (*D. hansenii*). The reformulated batches were: reduced fat (RF); reduced salt (RS); reduced fat and salt (RF+RS); and the same three batches but inoculated with *D. hansenii* yeast (RF+Y, RS+Y, RF+RS+Y). Reduced salt batches were 25 % salt reduced adding 20.25 g/kg NaCl and 6.75 g/Kg KCl. Fat reduced batches were 50 % fat reduced adding 85% lean pork meat and 15% back fat. Appropriate volumes of yeast strain *D. hansenii* P2 suspension were added to the inoculated batches at final concentration of 5 10^6 c.f.u./g of yeast strain. All fermented sausage batches were produced using the following ingredients: lactose (20 g/kg); dextrin (20g/Kg); sodium caseinate (20 g/kg); glucose (7 g/kg); sodium ascorbate (0.5 g/kg); sodium nitrite (0.15 g/kg); potassium nitrate (0.15 g/kg) and starter culture (0.1 g/kg) SP318 TEXEL SA-301 (Danisco, Cultor, Madrid, Spain) containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus carnosus*. The batches were manufactured under the conditions described by Olivares *et al.* (2010). The meat mixture was kept at 3-5°C for 24h and then was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) being the final weight of each sausage approximately 700 g. The sausages were subjected to drying in a controlled drying chamber at 10-14°C and 70-85% relativity humidity (RH) for 61 days. The weight losses and pH were measured during ripening to control the drying process.

From each batch, 300 g of the meat mixture at 0 days and three sausages at 61 days were randomly chosen for microbial and chemical analysis. From each sample, sausage colour was measured, 20 g were taken for microbial analysis and 150 g were minced and used to measure moisture, water activity and pH. The remaining minced sausages from each batch were vacuum packed and frozen at -20°C till subsequent analysis (fat, protein and ions content). In addition, at 61 days the remaining sausages of each batch were vacuum packaged and stored at 4°C for sensory and texture analysis. All the results were expressed as

means of the three replicates per 100 g of dry matter at each processing time and batch.

2.3. Microbial analysis

Sausages samples (20 g) were aseptically homogenized with sterile saline solution (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min and decimal dilutions were prepared. Lactic acid bacteria population was determined by spread plating on MRS Agar anaerobically (Scharlau Chemie SA, Barcelona, Spain) and staphylococci population by using Mannitol Salt Agar (Scharlau Chemie SA, Barcelona, Spain) both medium were incubated at 37°C for 2 days. Yeast count was determined in Rose Bengal Agar with chloramphenicol (RBA) (Conda SA, Madrid, Spain) at 28°C for 3 days.

Ten yeast strains isolated from each batch at the initial and final time of the ripening process were subjected to molecular characterization by minisatellite PCR amplification using the M13 primer as described in Cano-García et al (2013). The M13 Minisatellite PCR patterns obtained were compared with the originals previously obtained by Cano-García et al (2013).

2.4. Chemical analysis

The measurement of pH, water activity, colour evaluation (CIELab L*, a*, b*), moisture and fat content was performed as described by Olivares *et al.* (2010). Nitrogen content was determined by the Kjeldhal method and protein was estimated multiplying the nitrogen content by a factor of 6.25.

Cations (sodium and potassium) and chloride anion were analysed by ion chromatography as described by Corral *et al.* (2013). The concentration of each ion was determined by calibration curves using a set of standard solutions of Na^+, K^+ and Cl^- (Fluka, Switzerland, Sigma, St. Louis, MO). All results were expressed as mg/100g of sample in dry matter.

2.5. Texture profile analysis

Texture profile analysis (TPA) was carried out using TA-XT.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0 Stable Microsystems, Godalming, UK). At the end of the process, three different slices (3.5 cm diameter and 1.5 cm thick) of three sausages from each batch were compressed twice to 50 % of their original height as described by Olivares *et al.* (2010). The main texture parameters (hardness, springiness and cohesiveness) and the secondary parameter chewiness were obtained from the deformation curves.

2.6. Sensory analysis

Sensory analysis of fermented sausages (61 days) was carried out by 81 untrained assessors. The analysis was made in a sensory laboratory equipped with individual booths (ISO 8589, 1988). The casing was removed and each sausage was cut into slices of 4mm thickness. One slice of each sausage batch was randomly labelled with three digit codes and presented on a small white plate at room temperature. Water and unsalted bread was provided to clean the palate between samples. A hedonic test was carried out using a 9-hedonic scale labelled on the bottom with “dislike extremely” and on the top “like extremely”. The assessors evaluated their liking of appearance, flavour, taste, hardness, juiciness and overall acceptability. Data acquisition and analysis was performed by Compusense *five* release 5.0 (Compusense Inc., Guelph, ON, Canada).

2.7. Statistical analysis

Analyses of variance (ANOVA) were performed for the chemical and texture parameters to evaluate the differences among samples. A Fisher’s test was used to identify significant ($p<0.5$) differences between types of fermented sausages evaluated. Internal Preference Mapping applied to the mean individual hedonic rates of overall acceptability on all samples was performed. For each product, the coordinates on the preference space determined by the first two components were kept. Then, consumers' hedonic ratings were regressed onto these coordinates, and plotted into the map. Mean values of instrumental parameters were considered as supplementary variables. Clusters analysis was performed to classify consumers according to their preference about dry fermented sausages aroma, taste and overall acceptability. Agglomerative Hierarchical Clustering (AHC) was carried out using Euclidian distance with Ward’s method as the aggregation criterion (XLSTAT 2011 Agglomerative hierarchical clustering). A dissimilarity plot was performed to determine how many clusters were suitable for each analysis. A dendrogram was used to define the cluster structure of the data and support the decision that was made using the dissimilarity plot. All statistical analyses were performed using the statistic software XLSTAT 2011 v5.01 (Addinsoft, Barcelona, Spain)

3. RESULTS AND DISCUSSION

3.1. Chemical analysis

The pH, a_w and weight losses are shown in table 1. The pH showed a reduction in all batches from an initial value of 5.9-6.1 to 4.9-5.3 considered enough to ensure the safety of meat products together with drying and low a_w values (Papadima *et al.*, 1999). In uninoculated batches, RS and RF+RS showed significantly higher pH values than control batch and RF (Table 1) although this has not been previously reported (Liaros *et al.*, 2009; Olivares *et al.*, 2010; Corral *et al.* 2013). However, the greatest significant differences were found in yeast inoculated batches as all of them presented the highest significant pH probably due to the ability of yeasts to consume organic acids such as lactic acid (Durá, Flores & Toldrá, 2004).

Water activity (a_w) also controlled through the ripening process reached values of 0.90-0.91 thus securing product stability. Few significant differences were observed among batches because the control batch had the lowest a_w (Table 1). The effect of salt reduction in a_w values of dry fermented sausages produced different results, no effect was reported in 16 % reduced salt sausages (Corral *et al.*, 2013) while highest a_w values were reported in 50 % reduced salt sausages (Olesen, Meyer & Stahnke, 2004). Moreover fat reduction produced an increase in a_w values (Gómez & Lorenzo, 2013).

Weight losses mainly depend on climatic conditions applied for product ripening (Bloukas *et al.*, 1997). The weight losses in all batches were 39.8-41.6 % at the end the process (Table 1). The slow ripening conditions applied during process prevented the effect of fat or/and salt reduction on weight loss (Olivares *et al.* 2010). However, these processing conditions were not able to avoid the differences (p<0.05) found when both reductions were applied together in the inoculated *D. hansenii* batch (RF+RS+Y). The highest weight losses produced by fat reduction have been also reported by other authors (Bloukas *et al.*, 1997; Papadima & Bloukas, 1999; Liaros *et al.*, 2009).

Table 2 shows the chemical composition of dry fermented sausages. A fat reduction of 10-16% was achieved. The moisture content was the highest (p<0.05) in fat reduced batches (Gómez & Lorenzo 2013). However, this effect was not seen (p>0.05) in inoculated batches (RF+Y, RS+Y and RF+RS+Y) being in agreement with a_w values obtained. As expected, the highest (p<0.05) protein content was found in fat reduced batches due to the highest lean content present as also observed Olivares *et al.* (2010).

A total sodium reduction of 17-20% was achieved (Table 3). Salt reduced batches both uninoculated and yeast inoculated (RS, RF+RS, RS+Y and RF+RS+Y batches) presented significantly lower Na^+ content and higher K^+ content than no salt reduced batches (C, RF, RF+Y) (Table 3). By contrast, no significant differences were found for Cl^- content among batches since, salt reduced batches were substituted by KCl. Overall, fat or/and salt reduction and *D. hansenii* inoculation did not produce significant differences in colour parameters (L*, a* and b*) (Corral *et al.*, 2013) (data not shown).

3.2. Microbial analysis

LAB and staphylococci are essential for the ripening process and play an important role in the safety and organoleptic characteristics of dry fermented sausages (Ravyts, Vuyst & Leroy, 2012). The levels of LAB, staphylococci and yeast population were analysed at the beginning and end of the ripening process. At the beginning of the process the mean counts of LAB and staphylococci were 10^6 c.f.u./ g d.m. in all batches while for yeasts 10^7 u.f.c/ g d.m. were found in the inoculated batches. At the end of the process, the levels of LAB and staphylococci were within the range of what could be expected in this product (Table 4) (Durá *et al.*, 2004). The population of LAB experienced a growth of 3-4 logarithmic cycles whilst the population of staphylococci was within the same logarithmic units (Andrade, Córdoba, Casado, Córdoba & Rodríguez, 2010). No significant differences were found among batches for LAB level except the RS+Y batch which presented a significant higher LAB level than inoculated C and RF+RS+Y. However, these results were not correlated to the pH values obtained. Concerning staphylococci growth, all batches with fat and/or salt reduction had lower staphylococci growth than the control batch. Moreover, *D. hansenii* inoculation did not affect the growth of LAB and neither Staphylococcus (Table 4). Acidification carried out by LAB causes the inhibition of staphylococci growth (Leroy, Verluyten & De Vuyst, 2006), but in this study the batches which presented the highest pH values were those where the lowest staphylococci counts were found. Therefore, the staphylococci growth could have been affected by fat or/and salt reduction. However, controversial results about their effect on staphylococci have been reported. Several authors reported an absence of effect on staphylococci growth by salt reduction (Corral *et al.*, 2013, Campagnol et al, 2011) while others attributed to KCl the capacity to increase staphylococci growth (Gelabert *et al.*, 2003). With respect to the effect of fat reduction, Del Nobile, Conte, Incoronato, Panza, Sevi & Mariano (2009) reported no effect on

staphylococci while Liaros *et al.* (2009) found a decrease in staphylococci growth in fat reduced sausages attributing it to the lower counts presents in beef and pork meat than in pork back fat. However, Ravyts, Steen, Goemaere, Paelinck, Vuyst & Leroy (2010) reported a limited effect of fat and salt reduction on microbiota growth. In summary, in the present study fat and salt reduction produced a significant decrease on staphylococci growth.

Regarding yeast level at the beginning of the process, all the inoculated batches (RF+Y, RS+Y, RF+RS+Y) showed 10^7 c.f.u./g. yeast while no yeast growth was detected in the uninoculated batches (C, RF, RS, RF+RS). At the end of the process, a low yeast growth was detected in the uninoculated batches (10^4 c.f.u./g) although no differences were detected among batches (Table 4). However, the inoculated batches showed significant higher levels of yeast (10^7 c.f.u./g) than control batches (10^4 c.f.u./g) and this fact was also observed in each respective inoculated versus uninoculated batch.

At the beginning of the process, 100 % of the isolated yeasts from the inoculated batches displayed the original M13 minisatellite pattern of P2 strain indicating the correct inoculation of the batches (Fig. 1A Supplementary Material). Similarly, at the end of the process, all isolated yeasts (100%) showed the same pattern as the strain P2 originally inoculated (Fig. 1B, Supplementary Material). These results demonstrate that *D. hansenii* P2 was able to survive and replicate in the sausage environment even with fat and/or salt reduction, confirming the dominance of the inoculated P2 *D. hansenii* along the ripening process. In the case of the control batch, solely 20 % of the M13 patterns corresponded to P2 (Fig. 1C, Panel C, Supplementary Material). However, the percentage increased between 60% and 70% in the uninoculated batches with salt and fat reduction (Fig. 1C, Panels RS and RF, Supplementary Material) and reached 90 % in the uninoculated batch with both salt and fat reduction (Fig. 1C, Panel RS+RF, Supplementary Material). The presence of *D. hansenii* strains in the uninoculated sausage batches was probably due to the dispersion of the inoculated yeasts along the 61 days of ripening favoured by air circulation in the drying chamber. Nevertheless, the non-inoculated batches presented lower *D. hansenii* P2 counts than the inoculated ones.

Table 1. Effect of salt and fat reduction on pH, a_w and weight losses of dry fermented sausages inoculated with *D. hansenii* yeast.

	pH				a_w				Weight losses (%)			
	Uninoculated		Inoculated		Uninoculated		Inoculated		Uninoculated		Inoculated	
C	5	Ab	5	Ab	0.906	Ab	0.906	Aa	39.8	Aa	39.8	Ab
RF	4.9	Bb	5.2	Aa	0.914	Ba	0.916	Aa	40.6	Aa	39.8	Ab
RS	5.1	Ba	5.2	Aa	0.912	Aa	0.912	Aa	41.2	Aa	40.6	Ab
RF+RS	5.1	Ba	5.3	Aa	0.916	Aa	0.913	Aa	40	Ba	41.6	Aa

Different small letters in the same column indicate significant differences at $p<0.05$. Different capital letters in each row for each parameter indicate significant differences at $p<0.05$.

Table 2. Effect of salt and fat reduction on chemical composition of dry fermented sausages inoculated with *D. hansenii* yeast.

	Moisture (%)				Fat (%)				Protein (%)			
	Uninoculated		Inoculated		Uninoculated		Inoculated		Uninoculated		Inoculated	
C	46.0	Ac	46.0	Aa	17.5	Aa	17.5	Aa	33.3	Ac	33.3	Ab
RF	46.9	Ab	47.3	Aa	15.3	Abc	15.7	Aab	34.5	Ab	35.4	Aa
RS	46.2	Ac	46.3	Aa	16.7	Aab	16.6	Aa	34.7	Ab	34.9	Aab
RF+RS	47.6	Aa	46.4	Aa	15	Ac	14.7	Ab	35.2	Aa	35.9	Aa

Different small letters in the same column indicate significant differences at $p<0.05$. Different capital letters in each row for each parameter indicate significant differences at $p<0.05$.

Table 3. Effect of salt and fat reduction on ions content in dry fermented sausages inoculated with *D. hansenii* yeast.

	Na^+ (mg/100g d.m.)		K^+ (mg/100g d.m.)		Cl^- (mg/100g d.m.)	
	Uninoculated	**Inoculated**	**Uninoculated**	**Inoculated**	**Uninoculated**	**Inoculated**
C	2925.6 Ab	2925.6 Aa	968.2 Ab	968.2 Ac	4261.8 Aa	4261.8 Aa
RF	3078.1 Aa	3032.7 Aa	1024.8 Ab	1064.7 Ab	4127.2 Aa	4207.4 Aa
RS	2527.5 Ac	2473.6 Ab	1937.6 Aa	1910.7 Aa	4176.7 Aa	4054.9 Aa
RF+RS	2553.9 Ac	2495.0 Ab	1980.9 Aa	1969.0 Aa	4176.8 Aa	4181.3 Aa

Different small letters in the same column indicate significant differences at $p<0.05$. Different capital letters in each row for each parameter indicate significant differences at $p<0.05$.

Table 4. Effect of salt and fat reduction on Lactic acid bacteria, Staphylococci and yeast counts in dry fermented sausages inoculated with *D. hansenii* yeast.

	LAB (cfu/g dm)		Staphylococci (cfu/g dm)		Yeast (cfu/g dm)	
	Uninoculated	**Inoculated**	**Uninoculated**	**Inocualated**	**Uninoculated**	**Inoculated**
C	2.7E+09 Aa	2.7E+09 Ab	1.7E+07 Aa	1.7E+07 Aa	3.2E+04 Aa	3.2E+04 Ab
RF	2.6E+09 Aa	7.4E+09 Aab	2.3E+06 Ab	1.7E+06 Ab	1.8E+04 Ba	5.5E+07 Aa
RS	3.7E+09 Aa	12.1E+09 Aa	3.1E+06 Ab	1.6E+06 Ab	1.3E+04 Ba	2.7E+07 Aa
RF+RS	2.8E+09 Aa	4.3E+09 Ab	2.0E+06 Ab	1.9E+06 Ab	1.6E+04 Ba	4.1E+07 Aa

Different small letters in the same column indicate significant differences at $p<0.05$. Different capital letters in each row for each parameter indicate significant differences at $p<0.05$.

3.3. Texture profile analysis

TPA parameters were analysed in the final product (Table 5). Hardness and consequently chewiness were affected by the different formulations whilst no effect was observed on springiness and cohesiveness. In uninoculated batches, RF, RS and RF+RS showed significant higher hardness and chewiness than control batch. Moreover, RS batch showed the highest hardness and chewiness. On the contrary, in inoculated batches, only RF+Y and RF+RS+Y batches showed significant higher hardness than the control batch. The effect of the inoculated *D. hansenii* yeast was significant in salt reduced batches (RS and RS+Y) as it produced a decrease in the hardness and chewiness. However, springiness and cohesiveness were not affected by neither formulation nor *D. hansenii* inoculation. This increase in hardness and chewiness has been already reported in dry fermented sausage when fat was reduced (Olivares *et al.*, 2010). However, salt effect on fermented sausage texture is contradictory as generally low changes in texture have been reported when KCl was used as unique salt substitute (Gou, Guerrero, Gelabert & Arnau, 1996) or a decrease in sausage hardness (Gimeno *et al.* 1999); although any reference has indicated an increase in hardness as observed in our uninoculated sausages.

3.4. Sensory analysis

An Internal Preference Mapping was done with mean scores of overall acceptability and the following supplementary parameters which showed significant differences between batches: pH, a_w, weight losses, ions content (Na^+, K^+, Cl^-), fat, protein and moisture content, microbiological analysis (LAB, Staphylococcus and yeast counts), texture analysis (hardness, chewiness) and consumer liking (appearance, aroma and taste) (Fig. 1). Two principal components were able to explain the 42.8% of the total variance. PC1 accounted for 22.9% of the variance and distinguished samples according to consumer preferences placing C, RF+RS and inoculated samples on the right quadrants and salt (RS) or fat (RF) reduced samples on the left quadrants. However, PC2 accounted for 19.9% of the variance and distinguished samples by the presence of yeast (in the positive part of the axe are placed samples without yeast inoculation and in the negative part of the axe inoculated samples). Taking into account supplementary parameters plotted, C, RF+RS and RF+Y samples were related with moisture, fat and Na^+ and Cl^- content, staphylococci counts and taste liking; RS and RF samples were related with texture parameters and RS+Y, RF+RS+Y samples were related with pH, protein and potassium content and yeast.

Table 5. Effect of salt and fat reduction on texture parameters in dry fermented sausages inoculated with *D. hansenii* yeast.

	Hardness				Springiness				Cohesiveness				Chewiness			
	Uninoculated		Inoculated		Uninoculatd		Inoculated		Uninoculated		Inoculated		Uninoculated		Inoculated	
C	172.7	Ac	172.7	Ac	0.69	Aa	0.69	Aa	0.66	Aa	0.66	Aa	77.4	Ac	77.4	Aa
RF	203.9	Aab	200.7	Aa	0.66	Aa	0.67	Aa	0.65	Aa	0.65	Aa	88.5	Aab	87.3	Aa
RS	217.2	Aa	178.4	Bbc	0.67	Aa	0.71	Aa	0.65	Aa	0.64	Aa	95.4	Aa	80.9	Ba
RF+RS	201.8	Ab	192.7	Aab	0.66	Aa	0.68	Aa	0.65	Aa	0.65	Aa	86.6	Ab	85.2	Aa

Different small letters in the same column indicate significant differences at $p<0.05$. Different capital letters in each row for each parameter indicate significant differences at $p<0.05$.

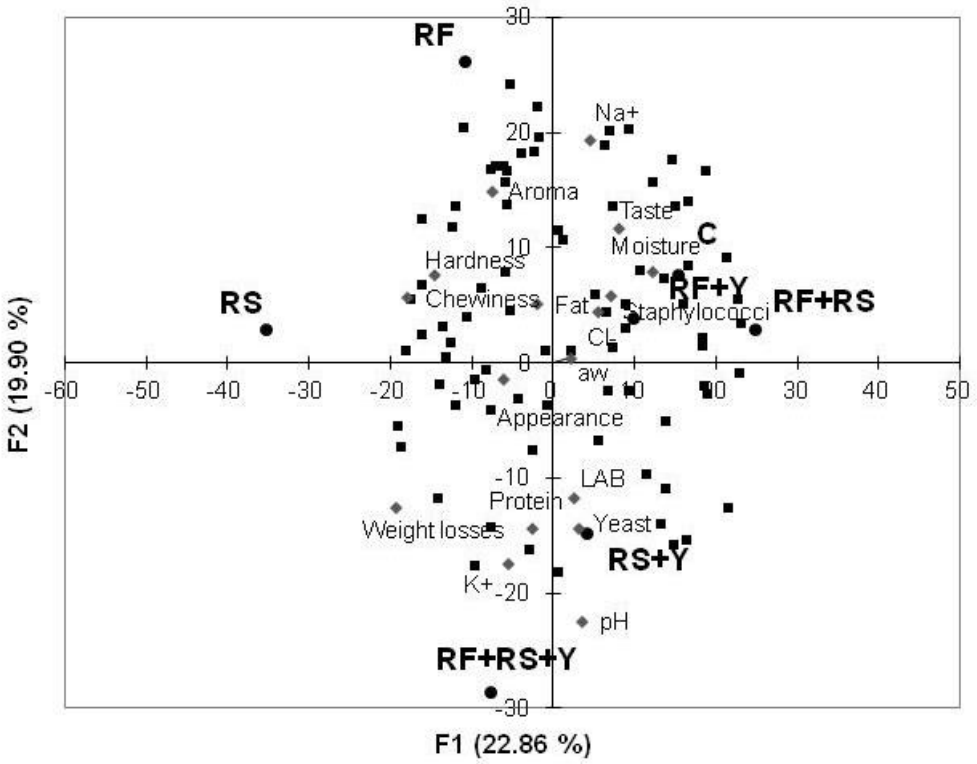

Figure 1. Loadings of the first two principal components (PC1-PC2) of consumer acceptability (black square), samples (black circles) and instrumental parameters (grey rhombus).

For a better understanding of consumer responses, the preferences for attributes that showed significant differences (taste and overall acceptability) plus the attribute aroma were also analysed by cluster analysis using Euclidean distances (Fig. 2). The attribute aroma was analysed due to the effect of yeast on sausage aroma (Cano-García *et al.*, 2013). The number of consumers in each cluster was different and depended on the analysed attribute, thirty-nine and forty-two consumers for aroma, thirty-four and twenty-seven for taste and fifty and thirty-one for overall acceptability, in cluster 1 and 2 respectively. The sausage preference of each cluster was elucidated by one-way ANOVA. Based on aroma, taste and overall acceptability, cluster 1 preferred fat reduced sausages without

yeast inoculation while cluster 2 preferred inoculated and fat reduced sausages (Fig. 2).

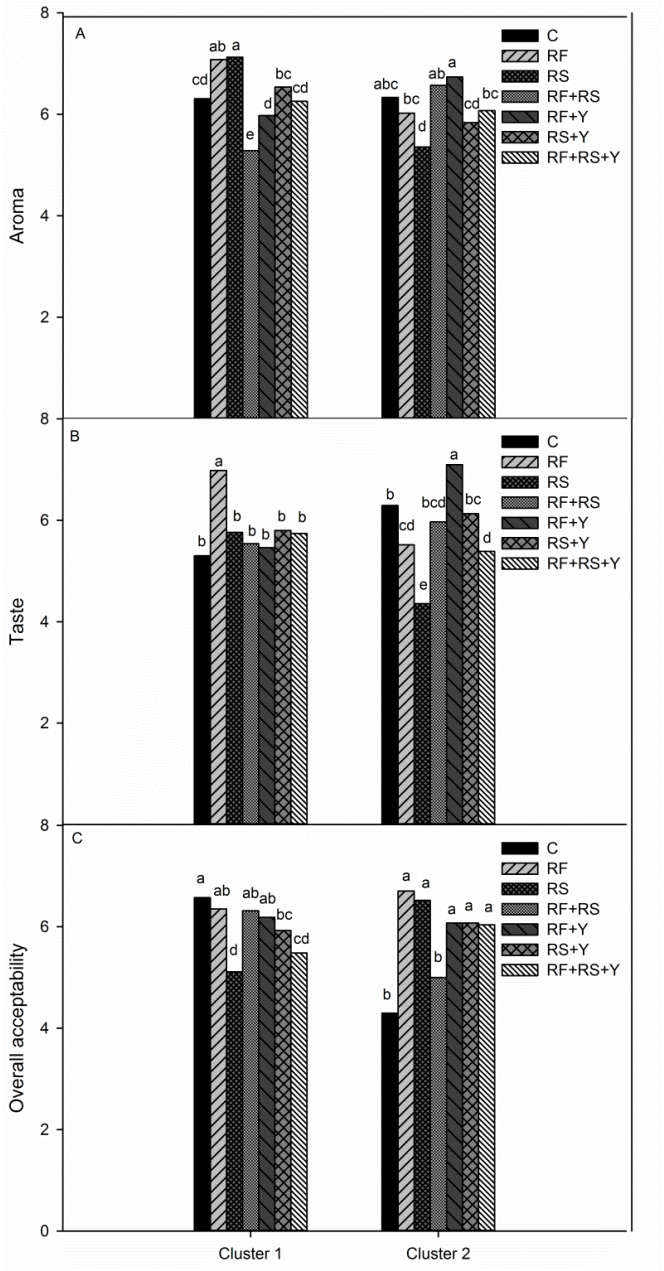

Figure 2. Mean values of the A) aroma; B) taste; C) overall acceptability by consumer cluster. Different letters in each cluster indicate significant differences at $p<0.05$.

Salt reduced sausage (RS) was perceived less tasty and the cluster 2 also perceived it less aromatic. Although RS was overall accepted by cluster 2, cluster 1 did not sensory accepted it. Moreover, the yeast inoculation on salt reduced sausages (RS+Y) was accepted better than uninoculated salt reduced sausages by both clusters, even though the sausage aroma and taste did not significantly improve by yeast inoculation. This fact agree with those reported by Corral *et al.* (2013) and Aaslying *et al.* (2014) who pointed out that consumers consider reduced sausages an acceptable product although the sensory characteristic are affected. Also, other studies have reported an improvement of sausage aroma and taste using yeast extracts (Campagnol *et al.*, 2011).

Fat reduced sausage was sensory accepted by the two consumer clusters; although, cluster 2 perceived it less tasty and aromatic. However, the yeast inoculation on fat reduction sausage was perceived with more sausage taste and aroma by cluster 2. This result agrees with Olivares *et al.* (2011) who reported a lower aroma in low fat sausages although Liaros *et al.* (2009) and Papadima *et al.* (1999) reported no effect of fat reduction on sausage odour or taste.

When the salt and fat reduction was carried out together, the two clusters perceived this sausage the less tasty than the other formulations, and cluster 1 even perceived it less aromatic. In this case, the yeast inoculation did not improve the sausage taste or aroma.

Nevertheless, different preferences patterns of consumers were found; the yeast inoculation improved the aroma and taste quality when the fat or salt reductions were carried out in dry fermented sausages. Several authors have studied the effect of *D. hansenii* on sausage aroma reporting an increase in some volatile compounds (Andrade *et al.*, 2010) while Olesen & Stahnke (2000) reported few differences between control and yeast inoculated sausages. However, there are not reports about the effect *D. hansenii* inoculation on consumer acceptability and when fat or salt is reduced. In this sense, the addition of yeast extracts produced an increase in the aroma acceptability of sausages (Bolumar, Sanz, Flores, Aristoy, Toldrá & Flores 2006, and Campagnol *et al.*, 2011) while only Flores, Durá, Marco & Toldrá (2004) confirmed the beneficial effect of the inoculation of *D. hansenii* in fermented sausages on consumer aroma acceptability. However, they did not confirm the implantation of the inoculated yeast strain. In addition, further studies are necessary to elucidate the biochemical process involved in the improvement of quality and aroma in low salt and fat dry fermented sausages by the inoculation of *D. hansenii* yeast.

4. CONCLUSION

The inoculation of *D. hansenii* yeast on salt and fat reduced sausages was able to compensate the changes in a_w and texture although it was not able to modify neither the hardness of reduced fat batches nor the decrease in staphylococci growth. In terms of sensory analysis, yeast inoculation improved the aroma and taste quality when fat or salt reductions were done. However, when salt and fat reduction was carried out together, yeast inoculation did not improve sausage taste or aroma. Further studies are necessary to elucidate the biochemical process involved in aroma generation and the interactions with salt and fat reductions.

ACKNOWLEDGEMENTS

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REFERENCES

Aaslyng, M. D., Vestergaard, C., & Koch, A. G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science, 96*, 47-55.

Andrade, M. J., Córdoba, J. J., Casado, E. M., Córdoba, M. G., & Rodríguez, M. (2010). Effect of selected strains of *Debaryomyces hansenii* on the volatile compound production of dry fermented sausage "salchichón". *Meat Science, 85*, 256-264.

Andrade, M.J., Córdoba, J.J., Sánchez, B., Casado, E.M.,& Rodríguez, M. (2009). Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. *Food Chemistry*, *113*,457-463.

Beriain, M. J., Gómez, I., Petri, E., Insausti, K., & Sarriés, M. V. (2011). The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. *Meat Science, 88*, 189-197.

Bloukas, J. G., Paneras, E. D., & Fournitzis, G. C. (1997). Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science, 45*, 133-144.

Bolumar, T., Sanz, Y., Flores, M., Aristoy, M. C., Toldrá, F., & Flores, J. (2006). Sensory improvement of dry-fermented sausages by the addition of cell-free extracts from *Debaryomyces hansenii* and *Lactobacillus sakei*. *Meat Science, 72*, 457-466.

Cano-García, L., Flores, M., & Belloch, C. (2013). Molecular characterization and aromatic potential of *Debaryomyces hansenii* strains isolated from naturally fermented sausages. *Food Research International, 52*, 42-49.

Cano-García L, Rivera-Jiménez S, Belloch C and Flores M. Generation of aroma compounds in a fermented sausage meat model system by *D. hansenii* strains. (2014) *Food Chemistry, 151*, 364-373.

Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R. (2011). The effect of yeast extract addition on quality of fermented sausages at low NaCl content. *Meat Science, 87*, 290-298.

Corral, S., Salvador, A., & Flores, M. (2013). Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science, 93*, 776-785.

Cocolin, L., Urso, R., Rantsiou, K., Cantoni, C. & Comi, G. (2006). Dynamics and characterization of yeasts during natural fermentation of Italian sausages. *FEMS Yeast Research*, *6*, 692–701.

Del Nobile, M.A., Conte, A., Incoronato, A.L., Panza, O, Sevi, A.,& Mariano, R. (2009). New strategies for reducing the pork back-fat in typical Italian salami. *Meat Science*, *81*, 263-269.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*, 74, 188-196.

Durá, M. A., Flores, M., & Toldrá, F. (2004). Effect of *Debaryomyces* spp. on the proteolysis of dry-fermented sausages. *Meat Science, 68*(2), 319-328.

Flores, M., Durá, M. A., Marco, A., & Toldrá, F. (2004). Effect of *Debaryomyces* spp. on aroma formation and sensory quality of dry-fermented sausages. *Meat Science, 68*, 439-446.

García-Íñiguez de Ciriano, M., Berasategi, I., Navarro-Blasco, I., Astiasarán, I., & Ansorena, D. (2013). Reduction of sodium and increment of calcium and ω-3 polyunsaturated fatty acids in dry fermented sausages: Effects on the mineral content, lipid profile and sensory quality. *Journal of the Science of Food and Agriculture, 93*, 876-881.

Gelabert, J., Gou, P., Guerrero, L., & Arnau, J. (2003). Effect of sodium chloride replacement on some characteristics of fermented sausages. *Meat Science, 65*, 833-839.

Gimeno, O., Astiasarán, I., & Bello, J. (1999). Influence of partial replacement of NaCl with KCl and $CaCl_2$ on texture and color of dry fermented sausages. *Journal of Agricultural and Food Chemistry, 47*, 873-877.

Gómez, M., & Lorenzo, J. M. (2013). Effect of fat level on physicochemical, volatile compounds and sensory characteristics of dry-ripened “chorizo” from Celta pig breed. *Meat Science, 95*, 658-666.

Gou, P., Guerrero, L., Gelabert, J., & Arnau, J. (1996). Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. *Meat Science, 42*, 37-48.

ISO 8589 (1988). Sensory analysis. *General guidance for design of test rooms*. Standard no.8589 (Geneva, Switzerland).

Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology, 106*, 270-285.

Liaros, N. G., Katsanidis, E., & Bloukas, J. G. (2009). Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages. *Meat Science, 83*, 589-598.

Mendoza, E., García, M. L., Casas, C., & Selgas, M. D. (2001). Inulin as fat substitute in low fat dry fermented sausages. *Meat Science, 57*, 387-393.

Muguerza, E., Ansorena, D., & Astiasarán, I. (2003). Improvement of nutritional properties of Chorizo de Pamplona by replacement of pork backfat with soy oil. *Meat Science, 65*, 1361-1367.

Olesen, P. T., Meyer, A. S., & Stahnke, L. H. (2004). Generation of flavour compounds in fermented sausages - The influence of curing ingredients, *Staphylococcus* starter culture and ripening time. *Meat Science, 66*, 675-687.

Olesen, P. T., & Stahnke, L. H. (2000). The influence of *Debaryomyces hansenii* and *Candida utilis* on the aroma formation in garlic spiced fermented sausages and model minces. *Meat Science, 56*, 357-368.

Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science, 87*, 264-273.

Olivares, A., Navarro, J. L., Salvador, A., & Flores, M. (2010). Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Science, 86*, 251-257.

Papadima, S. N., & Bloukas, J. G. (1999). Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science, 51*, 103-113.

Ravyts, F., Vuyst, L. D., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. *Engineering in Life Sciences, 12*, 356-367.

Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L., & Leroy, F. (2010). The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages. *Food Microbiology, 27*, 945-954.

Ruiz-Capillas, C., Triki, M., Herrero, A. M., Rodriguez-Salas, L., & Jiménez-Colmenero, F. (2012). Konjac gel as pork backfat replacer in dry fermented sausages: Processing and quality characteristics. *Meat Science, 92*, 144-150.

Ruusunen, M. & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science*, 70, 531-541.

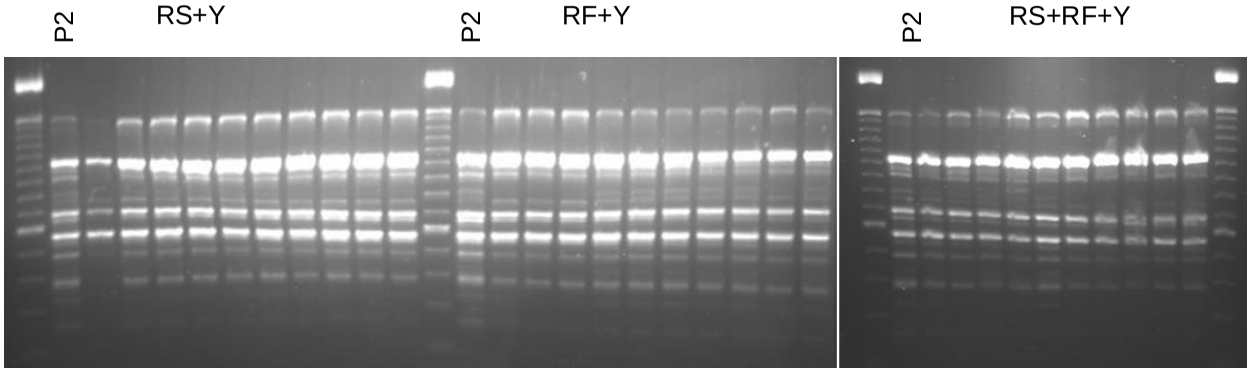
Salazar, P., García, M. L., & Selgas, M. D. (2009). Short-chain fructooligosaccharides as potential functional ingredient in dry fermented sausages with different fat levels. *International Journal of Food Science and Technology, 44*, 1100-1107.

WHO/FAO Expert Consultation (2003). *Diet, nutrition and the prevention of chronic diseases*. WHO technical report series 916, Geneva.

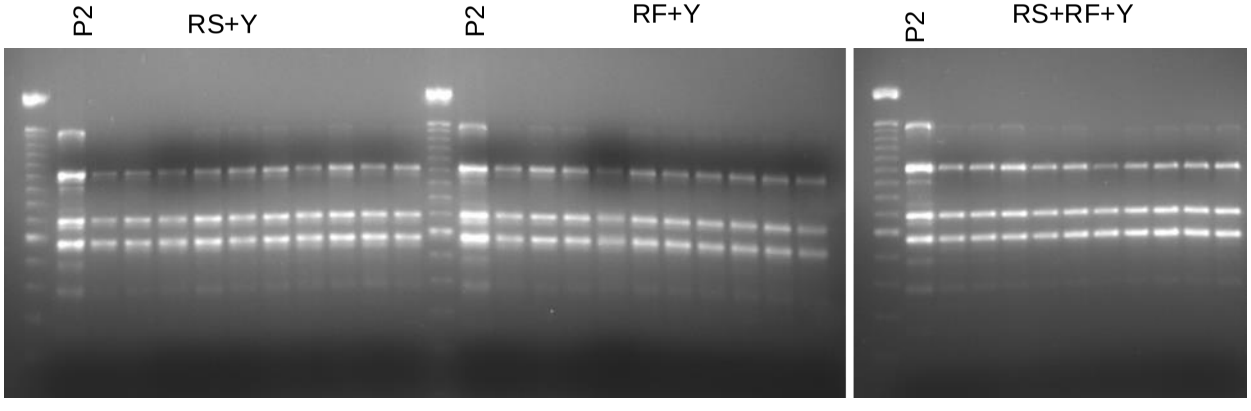
Wirth, F. (1988). Technologies for making fat-reduced meat products. *Fleischeirtsch*, *68*, 1153-1156.

SUPLEMENTARY DATA

A) Time 0 days



B) Time 61 days



C) Time 61 days

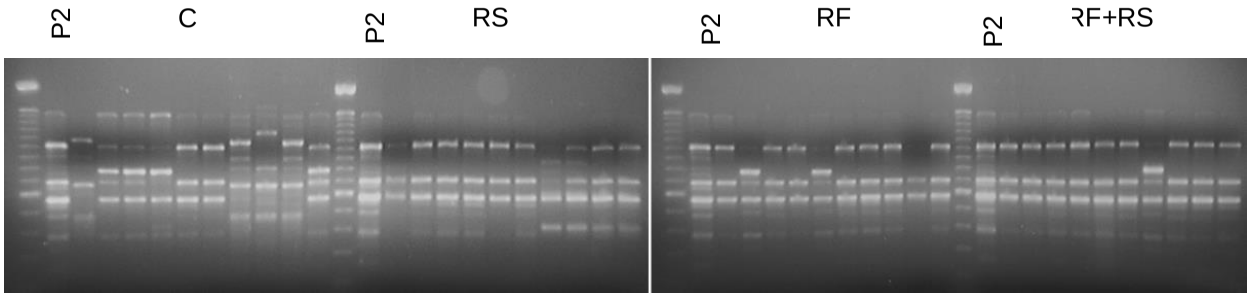


Fig. 1 Supplementary data. Electrophoretic patterns of minisatellite M13 PCR amplification of strains isolated at 0 d (A) and 61 days (B, C). In each photograph: first lane represents “M” 100 pb ladder (Invitrogen, Carlsbad, CA, USA) followed by the original inoculated P2 yeast (P2) and the 10 isolated strains in the sausage batches. Sausage batches; Control (C), reduced fat (RF), reduced salt (RS), reduced salt and fat (RS+RF) and inoculated reduced fat (RF+Y), inoculated reduced salt (RS+Y) and inoculated reduced salt and fat (RS+RF+Y).

Capítulo 5

Improvement the aroma of reduced fat and salt fermented sausages by *Debaryomyces hansenii* inoculation

Food Control, 2015, 47, 526-535

Improvement the aroma of reduced fat and salt fermented sausages by *Debaryomyces hansenii* inoculation

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Abstract

The effect of *Debaryomyces hansenii* inoculation in dry fermented sausages with fat and/or salt reductions was studied in terms of lipolysis, lipid oxidation, volatile compounds production and sensory analysis. The aroma of the identified volatile compounds was evaluated by olfactometry analysis while overall aroma perception was evaluated by sensory descriptive profiling. Salt reduction in dry sausages increased lipolysis and contributed to a high oxidation rate and rancid aroma generation while fat reduction resulted in sausages with a high content of aroma compounds from carbohydrate fermentation. The inoculation of *D. hansenii* yeast in the reformulated dry sausages produced an increase in lipolysis and, at the same time, an antioxidant effect. The most important contribution of *D. hansenii* yeast was the increase in aroma compounds derived from amino acid degradation (3-methylbutanoic acid and benzothiazole) and ester activities increasing the perception of fruity and cured aroma notes (2-methylpropanoate, 2-methylbutanoate and 3-methylbutanoate). However, when both reductions were carried out together, *D. hansenii* inoculation did not show a clear effect.

Keywords: Fat reduction, Salt reduction, *D. hansenii*, Volatile compounds, aroma, Fermented sausages

1. INTRODUCTION

The reduction of salt and saturated fat in meat products is one key point for the meat industry due to dietetic recommendations from the World Health Organization (WHO/FAO, 2003) as they may be associated with cardiovascular diseases. In dry fermented sausages, fat and salt are two essential ingredients in the development of aroma, a crucial sensory attribute. Fat plays an important role in aroma release and generates lipid derived compounds such as aldehydes, ketones, alcohols (Olivares, Navarro & Flores, 2011) while salt controls biochemical and enzymatic key reactions necessary for flavour development in addition to its contribution to the salting out effect (Corral, Salvador & Flores, 2013).

Several authors have studied the effect of fat reduction on volatile compounds and aroma although inconsistent findings have been reported. While, Muguerza, Fista, Ansorena, Astiasarán & Bloukas,*et al.* (2003) observed a high oxidation level and amount of total volatile compounds in low fat sausages, Olivares *et al.* (2011) reported a low oxidation and aroma acceptability. On the other hand, low fat sausages are generally perceived too salty due to the relatively large amount of moisture release; therefore, a salt reduction of 20-25 % is recommended in low fat dry sausages (Wirth, 1988). In addition, salt reduction at 16% (Corral *et al.*, 2013) or 25% (Campagnol, Santos, Wagner, Terra & Pollonio, 2011b) reduction and substitution with KCl produced a decrease in aroma perception even though the sausages were acceptable to consumers.

Nevertheless, few studies have been conducted examining the effects of fat plus salt reductions in dry fermented sausages. García-Íñiguez de Ciriano, Berasategi, Navarro-Blasco, Astiasarán & Ansorena (2013) improved the fat profile by using linseed oil (unsaturated fat) and replaced salt by calcium ascorbate, obtaining differences in colour intensity and juiciness. Whilst Beriain, Gómez, Petri, Insausti & Sarriés (2011) used an alginate emulsion and KCl with CaCl2 as fat and salt substitutes, respectively, reporting the lowest score for taste and texture by a trained panel.

Flavour enhancers such as amino acids, glutamate, ribonucleotides, yeast extract and lactate have been used to improve aroma in fat or salt reduced dry sausages (Campagnol, Santos, Morgano, Terra & Pollonio, 2011a and b; Gelabert, Gou, Guerrero & Arnau, 2003; Ruusunen, Simolin & Puolanne, 2001). However, the use of yeast strains can be an alternative as they affect flavour development (Leroy, Verluyten & De Vuyst, 2006). *Debaryomyces hansenii* is resistant to the low aw and high salt concentration typically found in dry

fermented sausages. Its effect on the aroma of fermented sausages has been reported although it depends on the *D. hansenii* strain inoculated (Andrade, Córdoba, Casado, Córdoba & Rodríguez, 2010; Cano-García, Belloch, Flores, 2014a; Olesen & Stahnke, 2000). Moreover, the lipolytic and ester activities reported in *D. hansenii* yeasts (Cano-García *et al.*, 2014a, b) seem to be related to the production of lipid derived and fruity aroma compounds with high aroma impact.

Accordingly, *D. hansenii* inoculation in low fat and salt dry fermented sausages may provide a strategy to improve the aroma lost produced by these reductions. In a previous study, Corral, Salvador, Belloch & Flores (2014) reported the positive impact on the sensory quality of fat and/or salt reduced dry sausages inoculated with *D. hansenii*, although its biochemical origin was not elucidated. Therefore, the aim of the present work is to evaluate the *D. hansenii* ability to improve aroma in fat and/or salt reduced dry sausages. Moreover, in order to get a better knowledge of the relationship between aroma perception and yeast inoculation, the production of volatile compounds, free fatty acids and lipid oxidation markers were assessed.

2. MATERIALS AND METHODS

2.1. Dry fermented sausages and sampling

Seven batches of dry fermented sausages were manufactured as described Corral, Salvador, Belloch, *et al.* (2014) with a total ripening time of 61 days at 10-14 ºC and 70-85 %. The seven dry fermented sausage batches were: control batch (C); reduced fat (RF); reduced salt (RS); reduced fat and salt (RF+RS) and the same three reformulated batches but inoculated with the yeast *D. hansenii* (RF+Y, RS+Y and RF+RS+Y). The control batch was manufactured with 70% pork lean meat and 30% pork back fat and 27g/kg NaCl content while reduced salt batches were 25 % salt reduced adding 20.25 g/kg NaCl and 6.75 g/Kg KCl. Fat reduced batches were 50 % fat reduced adding 85% lean pork meat and 15% back fat. Appropriate volumes of yeast strain *D. hansenii* P2 suspension (Cano-García *et al.*, 2014a) were added to the inoculated batches at final concentration of 5 x 106 c.f.u./g of meat batter.

From each batch, 300 g of the meat mixture at 0 days and three sausages at 61 days were randomly collected from each batch. The sausage sample was minced, vacuum packed and frozen at -20ºC for moisture, lipid, free fatty acids and TBARS analyses. At 61 days, three sausages per batch were taken wrapped

in aluminum foil, vacuum packed and stored at -80ºC for volatile and aroma analysis. In addition, three additional sausages were directly used for sensory analyses. All the results were expressed as the mean of three replicates per batch.

2.2. Lipolysis and lipid autooxidation analysis

Lipolysis was determined by analysis of free fatty acids (FFA). Total lipids were extracted from 5 g of minced sausages as described by Corral *et al.* (2013). FFA were determined in total lipids and methylated as described by Olivares *et al.* (2011) using heneicosanoic acid (C21:0) as internal standard.

The analysis was performed with an Agilent HP 7890A gas chromatograph (GC) equipped with a flame ionisation detector (FID) set at 240°C and autosampler (Agilent Technologies 7683B). 1 µl was injected in split injector (split ratio 100:1) set at 220°C.The fatty acid methyl esters were separated in HP-88 capillary column (Agilent, Las Rozas, Spain, 100m, 0.25 mm i.d, 0.25µm film thickness) using helium at a flow rate of 26.03 cm/s. The oven temperature began at 140ºC for 10 min, ramped to 190ºC at 4ºC/min, held at 190ºC for 15 min, ramped to 220ºC at 2ºC/min and held for 10 min. FAMEs were identified by comparing their retention times with those of standard fatty acid methyl esters. For quantification, response factors of the standards respect to the internal standard (C21:0) were used. The results were expressed as mg of fatty acid/100g of dry fermented sausages in dry matter. Moisture content was determined as described by Corral, Salvador, Belloch, *et al.* (2014).

Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Corral *et al.* (2013). Results were expressed as mg malonaldehyde (MDA)/kg in dry matter.

2.3. Extraction of volatile compounds

Extraction of headspace (HS) volatiles compounds was performed using solid phase microextraction (SPME) with an 85 µm Carboxen/ Polydimethylsiloxane (CAR/PDMS) fibre as described by Corral *et al.* (2013). Five grams of minced sausage were weighted into a 20 ml HS vial sealed with a PTFE faced silicone septum and 0.75 mg of BHT was added. Before extraction, the vial was equilibrated at 37 ºC for 30 min and then, SPME fibre was exposed to the headspace during 3h at 37 ºC.

2.4. Gas chromatography-mass spectrometry (GC-MS)

The identification and quantification of HS volatile compounds was performed using an Agilent HP 7890 series II GC (Hewlett-Packard, Palo Alto, CA) with an HP 5975C mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2 multipurpose sampler (Gerstel, Germany). Extraction of HS volatile compounds was performed using SPME as indicated above.

The compounds extracted by the fibre were desorbed in the injection port of the GC-MS for 5 min at 240 ºC with purge valve off (splitless). The analysis of volatile compounds in the GC-MS was performed as described Olivares *et al.* (2011). The compounds were identified by comparison with mass spectra from the library database (Nist'05), kovats retention index (Kovats, 1965) and by comparison with authentic standards. The quantification of volatile compounds was done in SCAN mode using either total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale. The results were expressed as abundance units (AU) 10^{-6}.

2.5. Gas-chromatrography-olfactometry

The analysis of aroma compounds extracted by SPME was performed using a gas chromatograph (Agilent 6890, USA) equipped with a FID and sniffing port detectors (ODP3, Gerstel, Mülheim an der Ruhr, Germany) as described by Olivares *et al.* (2011). Each assessment was carried out according to Olivares *et al.* (2011). Four trained panellists evaluated the odours from the GC-effluent of the sausages (61 days). The detection of an odour by less than three assessors was considered to be noise.

Compounds were identified using the following techniques: comparison with mass spectra, comparison with kovats retention indices of authentic standards injected in the GC-MS and GC-O, and by coincidence of the assessors's descriptors with those in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).

2.6. Sensory analysis

An Aroma profile analysis was performed by a panel of 8 trained judges with previous experience in quantitative descriptive analysis (QDA). The training of the panel was described previously (Corral, Salvador, & Flores, 2014). The selected aroma descriptor during training were pepper, rancid, sour, cheesy, fruity, cured and stable and they were scored using a 10-cm intensity scale (1 = no perceived; 10 = very intense).

The evaluation of dry fermented sausage aroma was done using fresh sliced sausages (4 mm thickness). The sample evaluation sessions were carried out in duplicate with a balanced complete block experimental design (Williams design for 7 samples and 8 assessors) using Compusense five release 5.0 (Compusense Inc., Guelph, Ontario, Canada). Each assessor evaluated the aroma intensity of the selected aroma descriptors at individual booths in a standardized test room (ISO 8589). Samples were presented in petri dish coded with a random three digit numbers. To avoid aroma carryover, coffee beans were provided inside a vial to allow assessors to smell them between samples.

2.7. Statistical analysis

Analyses of variance (ANOVA) were performed for FFAs, TBARS and volatile compounds to elucidate the differences among samples. Differences between particular sample means were analysed according to Fisher's least significant difference (LSD) test. To check panel performance for each aroma descriptor, a two factor analysis of variance (ANOVA) was done (assessors and samples and their interaction as factors). In addition, principal component analysis (PCA) was performed to evaluate the relationships among aroma descriptors and different parameters (FFAs, TBARS and volatile compounds) among sausages. The sensory aroma intensities were used as parameters and aroma compounds abundance, FFA and TBARS value as supplementary variables. All statistical analyses were performed using the statistic software XLSTAT 2011 (v5.01) (Addinsolft, Barcelona, Spain).

3. RESULTS

The chemical composition and microbiology analysis of the seven sausage batches previously reported (Corral, Salvador, Belloch, *et al.*, 2014) confirmed the yeast inoculation and fat (10-16%) and salt (17-20%) reduction in the manufactured batches. Presence of the yeast *D. hansenii* was checked by microbial and molecular analyses and the dominance of the yeast inoculated in the sausages was confirmed (Corral, Salvador, Belloch, *et al.*, 2014).

3.1. Lipolysis and lipid oxidation

Table 1 shows the levels of FFA at the end of the ripening process. Initially, no differences in total FFA concentration were observed among batches and levels of 144-191 mg/100g dm were detected (data not shown). During the ripening process, FFA concentrations increased in all batches to around 2000

mg/100g dm. MUFA showed a greater increase than PUFA and SFA, in particular C18:1.

At the end of processing, no significant differences were detected in total FFA concentration in uninoculated batches. The fat reduction scarcely showed significant differences for SFA, MUFA and PUFA. Only C18:0 concentration was significantly higher in the fat reduced batches than in C batch. On the other hand, salt reduced batch (RS) had a higher (p<0.05) SFA level than control batch (C) as also was observed in fat and salt reduced batch (RF+RS). In inoculated batches, RS+Y and RF+Y, a higher release of total FFA than in the C batch was observed mainly due to the increase in SFA and MUFA. However, this effect was not detected when both reductions (RS+RF+Y) were carried out together. In summary, the effect of *D. hansenii* yeast inoculation produced a significant increase of the lipolysis in RF+Y batch in contrast to the FFA detected in the RF batch.

Regarding lipid oxidation, TBARS values increased throughout the ripening process from an initial value of 0.4 to 1.7-2.5 mg MDA/kg dm (Table 1). No differences in TBARS values among batches were observed at the beginning of process (data not shown). At the end of the process, salt reduced batches (RS, RF+RS) showed the highest (p<0.05) lipid oxidation values. In contrast, the presence of the yeast produced a decrease of the TBARS values in RS+Y and RF+Y batches in comparison to their respective uninoculated batches although it was only significant in RF+Y batch.

3.2. Volatile compounds

The SPME headspace composition cannot be compared with other studies in which other SPME fibres or other extraction techniques have been used as it depends on many factors (fibre, extraction conditions, etc.). However, the present extraction technique allows determination of the effect of the studied factors (fat and salt reduction and yeast inoculation) on the volatiles of the sausages.

A total of 95 volatiles compounds were identified in the sausage headspace; although two of them were tentatively identified (Table 2). The identified volatile compounds were classified according to the most likely origin (Ordóñez, Hierro, Bruna & De La Hoz, 1999): from lipid autooxidation reactions (27), or from bacterial metabolism by lipid β-oxidation (7), carbohydrate fermentation (9), amino acid degradation (29) and esterase activity (15) reactions and, finally, a group of unknown or contaminant compounds (8) (Table 2). The

most abundant volatile compounds were those originated from carbohydrate fermentation and amino acid degradation reactions which represented 15-55 % and 11-43% of the total extracted area, respectively.

In general, volatile compounds originated from lipid autooxidation reactions were mainly affected by salt reduction and *D. hansenii* inoculation (Table 2). Among the uninoculated batches, salt reduced batch (RS) showed the highest HS abundance of the main lipid autoxidation volatile compounds, especially the significant increase of hexanal. Other linear aldehydes such as pentanal and nonanal also showed a highest HS abundance. On the other hand, fat reduction affected few lipid autooxidation volatile compounds. Only 1-propanol, octanoic and decanoic acid showed a higher HS abundance in the RF batch while propanoic acid had a lower HS abundance than in the C batch. When both reductions were carried out together, several volatile compounds were also generated in higher abundance than in the C batch. Among inoculated batches, a significant increase in some volatile compounds was observed in all batches, such as propanal, 1-propanol, 2-methylfuran, 2-pentylfuran, hexanoic acid, 2-ethyl-1-hexanol and decanoic acid. However, the effect of *D. hansenii* inoculation was significant in the RS+Y batch as it partly inhibited the lipid oxidation by decreasing the concentration of many of the aldehydes observed in the RS batch. In addition, the reduction observed in hexanal abundance was significant.

Volatile compounds coming from lipid β-oxidation were also affected by salt reduction and *D. hansenii* inoculation in salt reduced batches. Salt reduced uninoculated batches (RS and RF+RS) were characterized by a high abundance of 2-nonanone, 1-octen-3-ol, 3-pentanone and 2-undecanone. The effect of *D. hansenii* was significant in the RS+Y batch as reduced the abundance of 3-pentanone, 4-heptanone, 2-heptanone and 1-octen-3-ol indicating a decrease in the lipid β-oxidation reactions.

Regarding the volatiles originated from carbohydrate fermentation reactions, they were affected by the different formulations. Uninoculated salt reduced batches (RS and RF+RS) had the lowest abundance of acetic acid, 3-hydroxy-2-butanone, 2,3-butanediol and butanoic acid (Table 2). In contrast, the RF batch showed the highest abundance of acetic acid, 2,3-butanediol and butanoic acid. The effect of the inoculated *D. hansenii* was significant as it produced the highest ethanol and acetaldehyde abundance whilst a significant reduction of 2,3-butanedione, acetic acid, 3-hydroxy-2-butanone, 2,3-butanediol and butanoic acid was observed in all inoculated batches.

Table 1. Effect of fat and salt reduction on free fatty acid content (FFA, mg/100g dm) and TBARS value (mg MDA/kg dm) of dry fermented sausages (61days) inoculated with *D.hansenii* yeast.

Free fatty	Uninoculatetd					Inoculated				
acids	C	RF	RS	RF+RS	SEM	C	RF+Y	RS+Y	RF+RS+Y	SEM
C12:0	2.3	2.2 [2]	2.4	2.7	0.2	2.3	2.6 [1]	2.8	2.5	0.1
C14:0	20.4	20.9 [2]	22.5	22.2	1.9	20.4 b	23.9 ab[1]	25.9 a	21.1 b	1.2
C15:0	1.6	1.6 [2]	1.7	1.7	0.1	1.6 c	1.8 ab[1]	2.0 a	1.7 bc	0.1
C16:0	310.5 c	358.7 bc[2]	398.3 ab	420.2 a	14.6	310.5 c	395.3 b[1]	448.6 a	350.2 bc	15.9
C17:0	5.9	6.6	6.6	6.4	0.5	5.9 b	7 ab	7.9 a	6.6 b	0.4
C18:0	138.7 c	161.5 b[2]	181.0 ab	186.1 a	6.8	138.7 d	180.5 b[1]	200.4 a	162.9 c	6.4
C20:0	1.2	1.2 [2]	1.2	1.3	0.1	1.2	1.3 [1]	1.4	1.2	0.1
SFA	480.6 c	552.8 bc[2]	613.7 ab	640.6 a	22.9	480.6 c	612.5 b[1]	689.1 a	546.1 bc	23.1
C14:1	0.7	0.7	0.7	0.7	0.1	0.7	0.7	0.8	0.7	0.1
C16:1	47.0	49.9 [2]	48.3	47.0	3.9	47.0 bc	55.2 ab[1]	60.0 a	43.9 c	3.2
C17:1	11.3	13.6	12.3	14.2	2.1	11.3	12.2	12.4	13.3	2.3
C18:1	701.9	704 [2]	776.2	791.5	37.5	701.9 b	789.5 ab[1]	898.7 a	670.1 b	36.3
C20:1 n9	19.9	19.8	21.3	21.1	1.7	19.9	21.7	22.5	18.4	1.1
MUFA	780.1	788.0 [2]	858.2	873.4	45.1	780.1 b	879.3 a[1]	993.3 a	745.7 b	45.0
C18:2n6	455.2	434.2	478.6	499.6 [1]	17.6	455.2 b	451.2 b[1]	507.3 a	410.6 b[2]	12.5
C18:3 n3	18.3	18.0	19.0	18.6	1.5	18.3	20.0	21.1	17.8	1.1
C18:3 n6	0.6	0.6	0.7	0.6	0.1	0.6	0.7	0.7	0.6	0.0
C20:2 n6	18.1	16.8	18.4	17.5	1.4	18.1	18.0	19.9	16.1	0.9
C20:3 n6	2.8	3.0 [2]	3.0	3.4	0.2	2.8	3.3 [1]	3.4	3.1	0.2
C20:4 n6	51.0 b	55.9 b[2]	52.8 b	69.5 a	3.4	51.0	61.9 [1]	63.1	58.9	3.7
C22:4 n6	8.9	10.1 [2]	10.2	11.7	0.8	8.9 b	11.2 a[1]	11.6 a	11.2 a	0.7
C20:5 n3	5.3	5.1 [2]	4.7	5.6	0.4	5.3	5.6 [1]	5.1	4.9	0.4
C22:5 n3	18.7	19.8 [2]	18.7	21.1	1.6	18.7	21.4 [1]	22.4	19.0	1.1
C22:6 n3	1.8	2.0	1.9	1.9	0.2	1.8 b	2.1 ab	2.4 a	1.7 b	0.1
PUFA	580.5	565.5	608.9	649.3 [1]	35.0	580.5	595.2	657.3	543.7 [2]	29.8
TOTAL FFA	1775.8	1885.6 [2]	1963.7	1951.1	151.3	1775.8 b	2087.0 ab[1]	2198.8 a	1829.1 b	106.0
TBARS	1.7 b	1.7 b[1]	2.0 ab	2.5 a	0.2	1.7 b	0.9 c[2]	1.7 b	2.4 a	0.2

Different letters in the same row of each group (uninoculated and inoculated) indicate significant differences at $p<0.05$ among batches. Different number superscript indicate significant differences at $p<0.05$ between uninoculated and inoculated samples within the same reformulation.

Table 2. Effect of fat and salt reduction on volatile compounds (expressed as AU x 10^{-6}) of dry fermented sausages (61 days) inoculated with *D.hasenii* yeast.

		Uninoculated								
Compound	**LRI[a]**	**C**		**RF**		**RS**		**RF+RS**		**SEM**
Lipid autooxidation		**96.24**	**b[b]**	**118.49**	**b**	**1420.93**	**a[1]**	**246.74**	**b**	85.59
Propanal	523	0.78	b	0.83	b[2]	7.74	a[1]	1.36	b[2]	0.28
1-Propanol	611	2.01	c	4.99	b	6.77	a	4.49	b	0.48
2-Methylfuran	615	0.78	b	0.83	b[2]	10.70	a	1.36	b[2]	1.50
Butanal (44)[c]	622	0.05	b	0.04	b	0.30	a[1]	0.05	b	0.01
Tetrahydrofuran (42)[c]	643	0.13		0.06		0.39		0.43		0.14
2-Ethylfuran (81)[c]	720	0.07	b	0.15	b[1]	2.95	a[1]	0.32	b	0.47
2,5-Dimethylfuran (96)[cd]	726	0.21		0.22		0.24	[1]	0.18		0.03
Pentanal	737	1.76	b	2.36	b[1]	66.68	a[1]	4.66	b	3.80
Propanoic acid	807	6.28	a	4.18	b	2.89	b[2]	6.25	a	0.54
1-Pentanol	826	8.78	b	7.50	b	56.49	a[1]	14.14	b	8.58
Hexanal	840	15.18	b	14.26	b	991.73	a[1]	35.49	b	26.61
2-Butylfuran (81)[c]	909	0.07	b	0.07	b	0.66	a[1]	0.08	b	0.01
1-Hexanol	922	15.93	b	19.44	b	128.98	a[1]	23.56	b	8.74
Heptanal (44)[c]	940	0.88	b	0.29	b	3.37	a[1]	1.00	b	0.23
2-Pentylfuran	1009	1.56	c	2.29	c[2]	17.22	a[1]	13.71	b	0.69
1-Heptanol (70)[c]	1023	0.14	b	0.11	b	0.60	a[1]	0.12	b	0.05
Octanal (43)[c]	1047	0.51	b	0.41	b	1.52	a[1]	0.49	b	0.05
Hexanoic acid	1074	10.91	b	16.31	b	29.25	a	28.90	a	1.89
2-Ethyl-1-hexanol	1082	3.66	c	4.83	c[2]	8.92	b	18.84	a	1.20
1-Octanol (56)[c]	1123	0.14	b	0.13	b	0.31	a[1]	0.11	b	0.01
Nonanal	1149	9.93	c	8.33	c	28.66	a	17.89	b	1.68
Heptanoic acid (60)[c]	1167	0.08	b	0.06	b[1]	0.14	a	0.05	b[1]	0.01
Decanal	1256	1.15	b	2.65	b[1]	1.94	b[2]	11.66	a	0.52
Octanoic acid	1264	7.80	c	13.69	b	18.81	b[2]	26.61	a	1.57
Nonanoic acid	1357	1.41	c	3.16	bc[1]	5.62	b	8.68	a	0.89
Decanoic acid	1450	2.47	c	5.88	b[1]	7.63	b[2]	12.89	a	0.61
1-Dodecanol[cd]	1523	3.57	b	5.41	b	20.38	a	13.40	a[2]	2.60
Bacterial metabolism										
Lipid β oxidation		**38.67**	**b**	**44.83**	**b**	**86.23**	**a[1]**	**61.67**	**ab**	7.83
2-Pentanone	733	7.24		8.13		7.06		7.94		2.10
3-Pentanone(57)[c]	740	1.52	b	1.92	b[1]	9.93	a[1]	2.30	b	0.45
4-Heptanone[d]	911	8.07		4.75		10.50	[1]	8.79	[1]	1.22
2-Heptanone	933	14.70		21.44		27.54	[1]	18.01	[1]	4.49
1-Octen-3-ol (57)[c]	1030	0.82	b	1.00	b	13.18	a[1]	2.40	b	2.36
2-Nonanone	1140	5.67	b	6.01	b	16.35	a	17.42	a	1.25
2-Undecanone	1346	0.64	c	1.58	b[1]	1.68	b[2]	4.82	a	0.24
Carbohydrate fermentation		**626.27**	**b**	**776.57**	**a[1]**	**334.02**	**c**	**553.45**	**b**	54.56
Acetaldehyde	466	8.28	ab	10.73	a	5.72	b[2]	11.30	a	1.23
Ethanol	507	95.39		146.86		112.71	[2]	244.50		38.44
Acetone (43)[c]	529	47.39		49.21		31.93	[2]	45.73		5.65
2,3-Butanedione	626	9.11	ab	12.06	a[1]	2.85	c	6.51	bc	1.75
2-Butanone	630	27.32	a	22.95	ab[2]	19.03	b[2]	26.07	a[2]	1.74
Acetic acid	717	234.68	b	326.72	a[1]	137.81	c	171.96	c	16.91
3-Hydroxy-2-butanone (45)[c]	782	143.01	a	115.46	a	12.75	b[2]	39.20	b	23.41
2,3-Butanediol (45)[c]	891	51.82	b	80.24	a[1]	4.46	c	2.77	c[1]	5.94
Butanoic acid (60)[c]	889	9.28	b	12.34	a[1]	6.77	c[1]	5.41	c	0.43
Amino acid degradation		**294.48**	**b**	**366.86**	**a[2]**	**225.60**	**b[2]**	**466.59**	**a[2]**	27.05
Methanethiol	472	1.42		1.28		1.75		1.28	[2]	0.22
Dimethyl sulfide (62)[c]	532	0.06	ab	0.04	b[2]	0.09	a	0.06	b[2]	0.01

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound. The main groups according to the most likely origin in bold. [a] Linear retention index (LRI) of the compounds eluted from

Inoculated								
C		**RF+Y**		**RS+Y**		**RF+RS+Y**		**SEM**
96.24	**c**	**112.19**	**c**	**229.10**	**b**[2]	**278.55**	**a**	18.39
0.78	b	2.28	a[1]	3.08	a[2]	2.42	a[1]	0.26
2.01	b	9.74	a	10.96	a	10.09	a	2.03
0.78	b	2.28	a[1]	3.08	a	2.42	a[1]	0.26
0.05		0.03		0.03	[2]	0.03		0.01
0.13	a	0.05	bc	0.06	b	0.03	c	0.01
0.07	b	0.04	b[2]	0.05	b[2]	0.17	a	0.03
0.21	a	0.09	bc	0.03	c[2]	0.11	b	0.02
1.76	b	0.96	b[2]	2.34	b[2]	8.86	a	1.18
6.28	a	3.65	b	3.74	b[1]	4.50	ab	0.56
8.78	b	5.00	c	8.78	b[2]	12.01	a	0.71
15.18		6.48		20.04	[2]	36.39		7.88
0.07	b	0.04	c	0.06	bc[2]	0.09	a	0.01
15.93	bc	7.45	c	16.69	b[2]	29.18	a	2.54
0.88		0.81		1.12	[2]	1.23		0.18
1.56	b	5.59	a[1]	8.02	a[2]	9.24	a	1.25
0.14		0.10		0.12	[2]	0.12		0.03
0.51		0.54		0.55	[2]	0.60		0.10
10.91	c	20.42	b	24.32	ab	26.60	a	1.98
3.66	d	10.15	c[1]	12.77	b	15.67	a	0.73
0.14		0.12		0.12	[2]	0.13		0.01
9.93	b	13.36	b	23.88	a	22.29	a	1.69
0.08	a	0.04	b[2]	0.03	b	0.03	b[2]	0.01
1.15	b	1.38	b[2]	7.19	a[1]	9.86	a	0.91
7.80	b	11.37	b	27.46	a[1]	31.23	a	1.42
1.41	b	1.38	b[2]	8.70	a	10.25	a	1.07
2.47	c	4.40	b[2]	12.73	a[1]	14.09	a	0.53
3.57	b	4.44	b	24.95	a	30.91	a[1]	2.45
38.67	**ab**	**30.28**	**b**	**47.53**	**ab**[2]	**51.24**	**a**	5.16
7.24		5.50		5.12		5.62		1.21
1.52	ab	0.75	c[2]	1.30	bc[2]	1.94	a	0.20
8.07	a	2.76	c	3.76	bc[2]	5.27	b[2]	0.76
14.70		11.90		13.48	[2]	10.64	[2]	2.13
0.82	b	2.02	a	1.91	a[2]	2.29	a	0.23
5.67	b	6.48	b	17.78	a	19.32	a	0.87
0.64	b	0.87	b[2]	4.17	a[1]	6.16	a	0.66
626.27		**450.50**	**[2]**	**524.12**		**473.48**		55.14
8.28	b	14.44	a	17.85	a[1]	12.34	ab	1.84
95.39	b	212.70	a	219.62	a[1]	221.72	a	24.07
47.39		52.14		50.60	[1]	49.98		4.58
9.11	a	3.02	b[2]	3.74	b	2.07	b	1.19
27.32		32.13	[1]	37.60	[1]	36.48	[1]	3.62
234.68	a	113.40	b[2]	123.74	b	133.54	b	16.23
143.01	a	16.36	b	65.07	ab[1]	12.51	b	21.35
51.82	a	2.34	b[2]	1.85	b	0.68	b[2]	4.24
9.28	a	3.97	b[2]	4.05	b[2]	4.16	b	0.37
294.48	**b**	**688.45**	**a**[1]	**675.12**	**a**[1]	**725.91**	**a**[1]	53.35
1.42		1.43		1.64		1.84	[1]	0.13
0.06	b	0.31	a[1]	0.11	b	0.10	b[1]	0.03

the GC-MS using a DB-624 column capillary column (30m x 0.25mm i.d. x 1.4µm film thickness) .[b] Different letters in the same row of each group (uninoculated and inoculated) indicate significant differences at $p<0.05$

Tabla 2. Continued

Compound	LRI[A]	Uninoculated C		RF		RS		RF+RS		SEM
2-Methylpropanal	593	3.25		4.99	[2]	2.75	[2]	2.22	[2]	0.79
2-Methyl-3-buten-2-ol	653	1.23	a	0.83	b	0.60	b	0.83	b[1]	0.13
2-Methyl-1-propanol	680	24.88	ab	25.83	a	10.45	c[2]	18.21	b	2.19
3-Methylbutanal	689	21.92	ab	28.71	a[2]	16.76	b[2]	29.03	a[2]	3.02
2-Methylbutanal	700	14.51		18.64	[2]	26.39	[2]	19.15	[2]	3.58
Dimethyl disulfide	772	2.72	ab	1.81	b	2.16	b	3.74	a	0.32
3-Methyl-3-buten-1-ol (56)[c]	789	1.34	b	1.34	b	0.84	c[2]	1.50	a	0.05
3-Methyl-1-butanol	794	147.41	b	200.56	a	104.79	c[2]	174.13	ab	13.06
2-Methyl-1-butanol	796	35.59	bc	51.71	a	27.51	c[2]	41.87	ab	3.85
3-Methyl-2-buten-1-ol	833	12.24		11.88		13.67		15.55		1.07
3-Methyl-2-butenal (84)[c]	842	0.25		0.24		0.28		0.33		0.04
Methylpyrazine (94)[c]	859	0.52	a	0.43	ab	0.31	b	0.43	ab	0.04
2-Methylpropanoic acid (43)[c]	861	6.44	b	2.65	b[2]	1.19	b[2]	26.66	a[2]	5.17
Ethyl benzene (91)[c]	883	0.34		0.36	[1]	0.44	[1]	0.33		0.03
3-Methylbutanoic acid (60)[c]	939	11.97	b	5.24	b[2]	2.77	b[2]	85.39	a[2]	5.27
2,6-Dimethylpyrazine (108)[c]	943	0.86	b	1.48	a[1]	0.37	c[1]	0.41	c	0.09
2-Methylbutanoic acid (74)[c]	945	1.60	b	1.83	b[2]	1.03	b[2]	23.31	a[2]	3.51
2-Acetyl-1-pyrroline (43)[c]	960	0.32	c	0.08	d	0.48	a	0.39	b[2]	0.02
3-Methylthiopropanal (48)[c]	966	0.06	b	0.08	b[2]	0.19	a	0.07	b[2]	0.02
Benzaldehyde	1016	1.76	c	2.13	c	5.31	b[2]	9.33	a	0.43
3-Thiophenethiol (116)[c]	1047	0.06	b	0.04	b[2]	0.05	b[2]	0.17	a[2]	0.02
3-Methylthiopropanol (106)[c]	1061	0.02	b	0.08	a	0.08	ab	0.09	a	0.02
Benzene acetaldehyde (91)[c]	1107	0.16	b	0.29	b[2]	0.87	a	0.21	b[2]	0.08
Phenol (94)[c]	1111	0.96	a	1.29	a[1]	0.88	a[1]	0.34	b[1]	0.14
Benzyl alcohol (79)[c]	1119	0.06	a	0.06	a[1]	0.02	b	0.03	b	0.01
Phenylethylalcohol (91)[c]	1192	1.40	b	1.28	b[2]	1.79	b	4.83	a[2]	0.55
Benzothiazole	1294	1.12	b	1.68	b	1.78	b[2]	6.69	a	0.55
Esterase activity		**41.96**	**b**	**67.75**	**b[2]**	**61.81**	**b[2]**	**121.65**	**a[2]**	17.93
Methyl acetate (43)[c]	552	0.17		0.17		0.30		0.18		0.04
Ethyl acetate	635	6.87	c	28.94	a[1]	7.59	c[2]	19.19	b	2.64
Ethyl propanoate (102)[c]	744									
Ethyl 2-methylpropanoate (43)[c]	788	1.86	b	0.54	b[2]	0.28	b[2]	6.44	a	0.95
Ethyl butanoate (71)[c]	830	0.55		0.98	[1]	1.00		0.63		0.15
Isobutyl acetate	805	2.68	b	2.09	b[2]	2.42	b[2]	4.09	a	0.40
Butyl acetate (43)[c]	846	0.12	bc	0.27	a	0.23	ab	0.04	c	0.04
Ethyl 2-hydroxypropanoate	866	12.19	bc	14.78	b[2]	8.20	c[2]	22.64	a[2]	1.47
Ethyl 2-methylbutanoate	877	6.11	b	2.43	b[2]	2.12	b[2]	26.42	a[2]	3.15
Ethyl 3-methylbutanoate (88)[c]	881	0.53	b	0.38	b[2]	0.26	b[2]	7.74	a[2]	0.93
3-Methyl 1-butanol acetate	906	5.36	a	6.93	a	2.64	b	2.17	b	0.65
2-Methyl-1-butanol acetate (43)[c]	909	0.21		0.27	[1]	0.19		0.13	[2]	0.03
Ethyl hexanoate	1028	3.06	b	3.96	b[2]	30.70	a[1]	12.27	b	3.56
Ethyl octanoate	1229	1.09	c	3.63	b[1]	4.79	b[2]	12.01	a	0.82
Bornyl acetate	1343	1.16	b	2.39	b	1.09	b[2]	7.68	a	0.52
Unknown compounds or contaminants		41.02	ab	30.36	bc[2]	23.24	c[2]	57.72	a	6.08
Carbon disulfide	538	38.04	ab	26.86	bc	19.57	c[2]	46.82	a	6.28
Pyridine (79)[c]	785	0.57	a	0.61	a[1]	0.42	b[2]	0.37	b	0.03
3-Carene (93)[c]	1021	0.07		0.06	[2]	0.09		0.08		0.01
Butyrolactone (42)[c]	1020	0.88	b	0.66	bc[2]	0.46	c[2]	1.97	a[2]	0.09
D-Limonene (68)[c]	1045	0.28		0.24	[2]	0.35		0.37		0.04
p-Cymene	1050	0.65	c	1.40	b[2]	1.83	b	7.54	a[1]	0.20
Dimethylsulfone (79)[c]	1060	0.29		0.34		0.26		0.26		0.02
4-Methylphenol (107)[c]	1196	0.24		0.19	[2]	0.28		0.31	[2]	0.04

among batches. Different numbers superscript indicate significant differences at $p<0.05$ between uninoculated and inoculated samples within the same reformulation. [c] Target ion used to quantify the compound when the

Inoculated								
C		**RF+Y**		**RS+Y**		**RF+RS+Y**		**SEM**
3.25	b	11.31	a[1]	11.07	a[1]	9.65	a[1]	1.07
1.23	a	0.57	b	0.51	b	0.45	b[2]	0.11
24.88	a	21.52	ab	15.36	bc[1]	14.52	c	2.47
21.92	b	73.60	a[1]	81.54	a[1]	74.02	a[1]	6.14
14.51	b	48.02	a[1]	44.05	a[1]	42.12	a[1]	4.63
2.72		1.45		1.97		2.51		0.31
1.34		1.26		1.29	[1]	1.37		0.04
147.41		196.37		160.06	[1]	160.90		18.96
35.59		51.86		40.05	[1]	42.44		4.33
12.24		13.49		13.52		14.50		1.03
0.25		0.26		0.30		0.28		0.03
0.52		0.30		0.43		0.35		0.06
6.44	b	61.10	a[1]	52.46	a[1]	65.04	a[1]	10.33
0.34		0.26	[2]	0.29	[2]	0.34		0.02
11.97	b	130.36	a[1]	156.60	a[1]	176.80	a[1]	21.31
0.86	a	0.26	b[2]	0.23	b[2]	0.25	b	0.05
1.60	b	58.44	a[1]	63.06	a[1]	84.40	a[1]	10.73
0.32		0.65		0.44		0.59	[1]	0.10
0.06	b	0.12	b[1]	0.27	a	0.30	a[1]	0.03
1.76	b	2.54	b	8.46	a[1]	7.66	a	0.90
0.06	c	0.21	b[1]	0.24	ab[1]	0.27	a[1]	0.01
0.02	b	0.20	a	0.13	ab	0.17	a	0.03
0.16	c	0.59	b[1]	0.83	a	0.87	a[1]	0.06
0.96	a	0.20	b[2]	0.16	b[2]	0.18	b[2]	0.11
0.06	a	0.02	b[2]	0.03	b	0.03	b	0.00
1.40	b	10.29	a[1]	10.57	a	13.33	a[1]	2.08
1.12	b	1.46	b	9.45	a[1]	10.63	a	1.39
41.96	**b**	**276.60**	**a[1]**	**328.66**	**a[1]**	**342.24**	**a[1]**	36.46
0.17		0.13		0.13		0.19		0.02
6.87	b	11.67	ab[2]	17.86	a[1]	16.07	a	2.37
				0.02		0.02		0.00
1.86		7.59	[1]	9.60	[1]	10.26		2.03
0.55		0.50	[2]	0.52		0.51		0.08
2.68	b	4.15	ab[1]	3.80	ab[1]	4.95	a	0.56
0.12		0.15		0.16		0.11		0.05
12.19	b	196.01	a[1]	203.86	a[1]	213.72	a[1]	25.22
6.11	c	29.00	b[1]	50.05	a[1]	48.78	a[1]	5.88
0.53	b	10.61	a[1]	14.62	a[1]	14.19	a[1]	2.93
5.36	a	4.09	a	1.73	b	5.91	a	0.63
0.21	a	0.10	b[2]	0.10	b	0.15	b[1]	0.02
3.06	b	9.18	a[1]	10.23	a[2]	9.81	a	0.59
1.09	b	1.98	b[2]	9.89	a[1]	11.02	a	0.37
1.16	b	1.45	b	6.09	a[1]	6.56	a	0.22
41.02	**b**	**54.56**	**ab[1]**	**72.85**	**ab[1]**	**79.46**	a	9.98
38.04		46.35		63.85	[1]	67.97		10.27
0.57	b	0.45	c[2]	0.64	a[1]	0.35	d	0.02
0.07		0.08	[1]	0.08		0.08		0.00
0.88	b	2.74	a[1]	3.74	a[1]	3.44	a[1]	0.37
0.28	b	0.35	b[1]	0.38	ab	0.50	a	0.05
0.65	c	3.96	b[1]	3.53	b	6.50	a[2]	0.48
0.29	a	0.28	ab	0.23	bc	0.21	c	0.02
0.24	b	0.34	a[1]	0.40	a	0.42	a[1]	0.03

peak was not completely resolved. [d] Tentatively identification by mass spectrum.

With respect to volatile compounds originated from amino acid degradation reactions, the most abundant compound was 3-methyl-1-butanol. In general, these compounds were mainly affected by fat reduction and *D. hansenii* inoculation (Table 2). Uninoculated fat reduced batches (RF and RF+RS) were characterized by the highest abundance of branched alcohols (2 and 3-methyl-1-butanol), in addition to a high abundance of branched acids (2-methylpropanoic and 2- and 3-methylbutanoic acids) and sulphur compounds (3-thiophenethiol, 3-methylthiopropanol, and benzothizole) in RF+RS batch. However, salt reduction (RS batch) produced a decrease in the abundance of several compounds (2-methyl-3-buten-2-ol, 2-methyl-1-propanol, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol, methylpyrazine, 2,6-dimethylpyrazine and benzyl alcohol) while other compounds were increased (2-acetyl-1-pyrroline, 3-methlythiopropanal, benzaldehyde and benzene acetaldehyde). The effect of *D. hansenii* was significant as observed by the highest HS abundance of methyl branched aldehydes, acids and sulphur compounds in all inoculated batches.

Ester compounds originated from microbial activity were affected by reformulation and *D. hansenii* inoculation. When fat and salt reductions were carried out together (RF+RS) the highest significant abundance of many ester compounds was observed, while salt reduction (RS) only increased ethyl hexanoate and fat reduction (RF) augmented ethyl acetate, butyl acetate and ethyl octanoate abundance (Table 2). In contrast, inoculation of *D. hansenii* produced a significant increase of many ethyl ester compounds in all the inoculated batches being characterized by the high increase of ethyl 2-hydroxypropanoate and in lowest proportion of ethyl 2-methyl and 3-methylbutanoate.

Several compounds from unknown or contaminant origin were identified, being the most abundant carbon disulphide. While the highest abundance of this compound was observed in the RF+RS batch, no effect due to yeast inoculation was detected.

An olfactometry analysis performed to determine which volatile compounds contributed to sausage aroma detected twenty-seven aroma active zones although five of them were not identified (Table 3). In addition to the green notes produced by lipid oxidation derived compounds, it was important the contribution of compounds derived from amino acid degradation and ester activity that contributed to toasted-savoury and fruity notes respectively. The abundance of these aroma compounds in the dry sausage batches was represented in Fig. 1. The reformulation produced a significant effect. Salt reduction (RS) produced the

Table 3. Odour active compounds identified in the HS of dry fermented sausages.

Compound	LRI[a] compound	LRI[a] standard	Descriptor
Lipid autooxidation			
Propanoic acid	813	802	Glue, wax
Hexanal	835	836	Fresh cut grass
2-Pentylfuran	1011	1011	Metallic, green, unpleasant, cabbage
Octanal	1046	1047	Lemon, floral
Decanal	1245	1254	Green wood
Nonanoic acid	1357	1347	Spicy, hazelnut, walnut
Bacterial metabolism			
Lipid β-oxidation			
3-Pentanone	739	739	Butter, damp
1-Octen-3-ol	1025	1028	Mushroom
2-Nonanone	1139	1142	Toasted
Carbohdrate fermentation			
2,3-Butanedione	632	632	Butter, cooked ham
Acetic acid	695	700	Vinegar
Amino acid degradation			
Methylpyrazine	852	858	Green, cooked potato
3-Methylbutanoic acid	924	926	Strong cheese
2-Acetyl-1-pyrroline	962	960	Savoury, snacks
3-Methylthiopropanal	968	969	Cooked potato or vegetables
Benzaldehyde	1016	1021	Toasted, tobacco
Phenol	1113	1112	Wet dog hair
Benzothiazole	1293	1305	Toasted, savoury, sulfurous, pepper
Esterase activity			
Ethyl 2-methylpropanoate	786	789	Sweet, strawberry
Ethyl 2-methylbutanoate	871	872	Sweet, strawberry
Ethyl 3-methylbutanoate	875	876	Fruity, floral, sweety
Unknown compounds			
Unknown 1	1032		Fish, amines, metallic, herbal
Unknown 2	1178		Savoury, snacks, toasted almond
Unknown 3	1186		Sulfurous, cooked vegetable, burnt
4-Methylphenol	1193	1190	Burnt plastic, dung
Unknown 4	1203		Unpleasant, plastic, rubber
Unknown 5	1218		Green, freshness

[a] Linear retention index (LRI) of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (60m x 0.32mm x 1.8µm).

highest abundance of aroma compounds derived from lipid autooxidation and β-oxidation and the lowest from carbohydrate fermentation reactions. Fat reduction (RF) increased the aroma compounds derived from carbohydrate fermentation reactions. In contrast, when both reductions (RF+RS) were carried out together, an increase in all the aroma compounds from the different origins was observed except from carbohydrate fermentation and unknown origins. The effect of *D. hansenii* was significant as produced the highest abundance of aroma compounds derived from amino acid degradation, ester activity and unknown compounds and the lowest of carbohydrate fermentation aroma compounds (Fig. 1). In addition, yeast inoculation reduced significantly the aroma compounds

derived from lipid autooxidation and β-oxidation reactions in the salt reduced batch (RS+Y) that were highly increased in the uninoculated batch (RS).

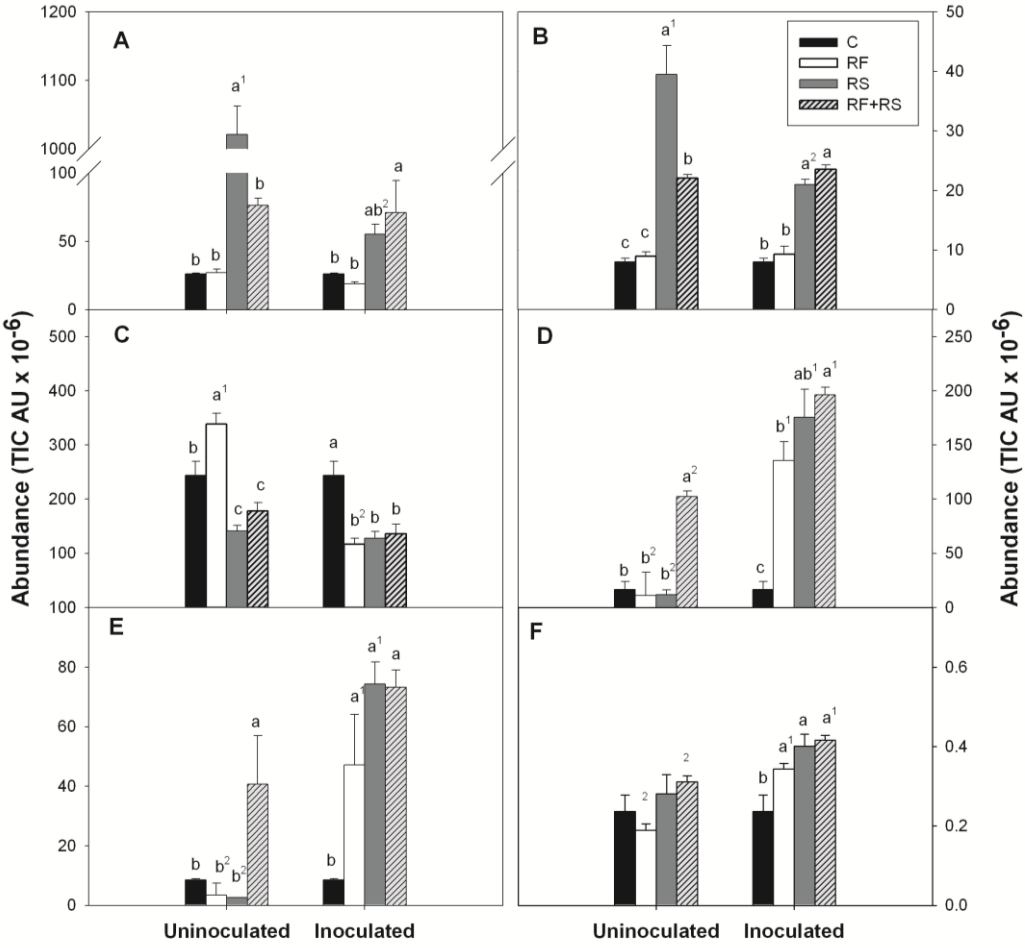

Figure 1. Effect of fat and salt reduction on total aroma compounds (expressed as total ion current abundance units TIC AU) of dry fermented sausages (61 days) inoculated with *D. hansenii*. Aroma compounds derived from: A) lipid autooxidation; B) Lipid β-oxidation; C) Carbohydrate fermentation; D) Amino acid degradation; E) Esterase activity; F) Unknown or contaminant compounds. Different letters in the same group (uninoculated and inoculated) indicate significant differences (p<0.05) among batches. Different number superscripts indicate significant differences (p<0.05) between uninoculated and inoculated samples within the same reformulation.

3.3. Sensory analysis

In order to study the effect of reformulation and yeast inoculation on the perception of sausage aroma an aroma profile analysis was carried out (Fig. 2). The effect of yeast inoculation was compared against the control sausage for each reformulation; RF (Fig. 2A), RS (Fig. 2B) and RF+RS (Fig. 2C). Fat reduction did not affect the aroma perception but the inoculation of *D. hansenii* significantly increased the perception of fruity and cured aromas (Fig. 2A). Moreover, salt reduction did not affect the aroma perception but the inoculation of *D. hansenii* significantly increased the perception of stable and cured aromas and although rancid aroma was increased it was not significant (Fig. 2B). Finally, when both reductions were done together a higher intensity of cured and fruity aroma was perceived in inoculated and uninoculated batches than in the control sausages (Fig. 2C).

4. DISCUSSION

The reformulation of fermented sausages in terms of fat and salt reduction produced important changes in aroma compounds and aroma perception. First, salt reduction produced an increment in lipolysis, TBARS and aroma compounds derived from lipid autooxidation and β-oxidation reactions (Corral *et al.*, 2013). This fact indicated that lipolysis was affected by salt content (Molly, Demeyer, Civera & Verplaaetse 1996; Toldrá, 1992) whereas the highest generation of SFA and MUFA pointed out an activation of the lipase activities in the salt reduced sausages (Stahnke, 1995). An opposite effect, activation of muscle acid lipase activity by salt, had been previously reported (Motilva & Toldrá, 1993). In addition to this, a positive correlation between the lipid oxidation value (TBARS) and hexanal abundance in salt reduced sausages was found ($r = 0.82$) (Olivares *et al.* 2011). Therefore, salt reduction in dry sausages increased lipolysis and contributed to a high oxidation rate and rancid aroma generation.

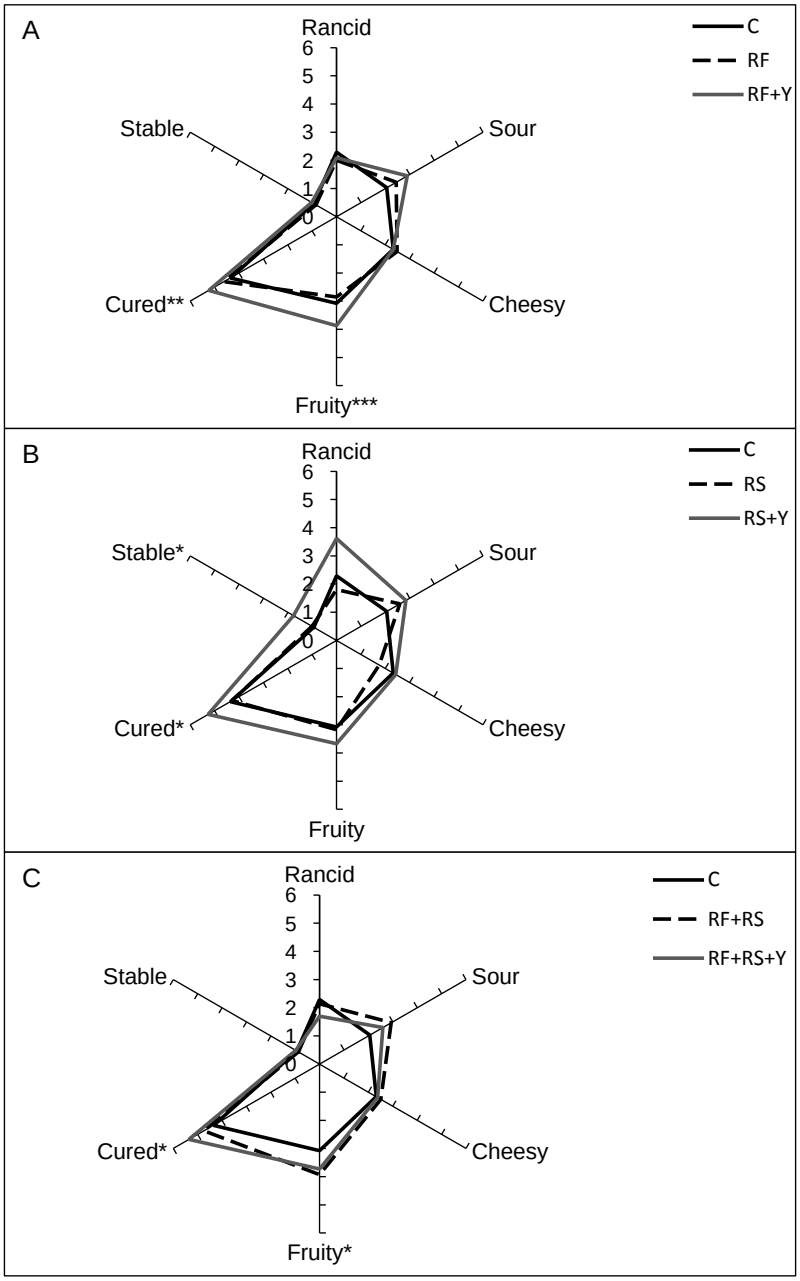

Figure 2. Aroma profile analysis of control sausage (solid line) compared to: A) Fat reduced sausages (RF and RF+Y), B) Salt reduced sausages (RS and RS+Y), C) Fat plus salt reduced sausages (RF+RS and RF+RS+Y). Asterisks in each aroma descriptor indicate significant differences at *p<0.05, **p<0.01, ***p<0.001.

On the other hand, the small fat reduction achieved (10-16%) had a limited impact on lipolysis, TBARS values and aroma compounds derived from lipid oxidation reactions. Even though an increase in aroma compounds derived from carbohydrate fermentation was detected (Olivares *et al.*, 2011). In contrast, when salt and fat reductions were carried out together not only lipolysis and lipid oxidation values were increased but also many of the aroma compounds. The highest abundance of volatile compounds coming from amino acid degradation reactions could be related to the largest proportion of lean meat in these sausages (Olivares *et al.*, 2011). In addition, the highest abundance of ester compounds could be due to the interaction of the fat and salt reduction effects, on microbial activity. Previously, a weak effect of fat or salt content on sausage microbiota was reported (Ravyts, Steen, Goemaere, Paelinck, De Vuyst & Leroy, 2010) probably due to the limitation of the analysis performed using static headspace that did not allowed the detection of ester compounds in the sausages.

In relation to the effect of *D. hansenii* on dry sausages, a significant increase in lipolysis was detected (Table 1) (Bolumar, Sanz, Flores, Aristoy, Toldrá & Flores, 2006) which can be related to the lipolytic activity found in *D. hansenii* (Cano-García *et al.*, 2014b). In contrast, the antioxidant effect produced by *D. hansenii* in salt reduced and partially in fat reduced sausages, can be due to the decomposition of hydrogen peroxide by the catalase activity of *D. hansenii* (Segal-Kischinevzky, Rodarte-Murguía, Valdés-López, Mendoza-Hernández, González & Alba-Lois., 2011). This antioxidant effect was also confirmed in dry sausages (Cano-García *et al.* 2014a, Flores, Durá, Marco & Toldrá 2004) although not observed by Andrade *et al.* (2010). In addition, the inhibition of Staphylococci growth by *D. hansenii* (Corral, Salvador, Belloch, *et al.*, 2014; Durá, Flores & Toldrá, 2004a) resulted in a decrease in the abundance of compounds from the β-oxidation reactions as they are responsible for their generation. On the other hand, the observed decrease in aroma compounds derived from carbohydrate fermentation in yeast inoculated sausages was related to the lowest abundance of acetic acid although opposite results had been found by Cano-García *et al.* (2014a).

The most notable effect of *D. hansenii* was observed in the generation of volatile compounds derived from amino acid degradation and ester activities. In inoculated sausages, a clear catabolism of branched amino acids yielded α-keto acids (2-methylpropanoic, 2 and 3-methlbutanoic) which were descarboxylated to branched aldehydes (2-methylpropanal, 2 and 3-methylbutanal, 3-methyl-2-

butenal) and reduced to corresponding branched alcohol (2-methyl-1-propanol, 2 and 3-methyl-1-butanol and 3-methyl-2-buten-1-ol) by means of Ehrlich pathway (Durá, Flores & Toldrá, 2004b). This fact agrees with other authors studies (Andrade *et al.*, 2010; Flores *et al.*, 2004; Bolumar *et al.*, 2006) although it was not clearly appreciated in a previous study (Cano-García *et al.*, 2014a). Moreover, sulphur compounds, derived from amino acids degradation reactions, were detected in a high proportion in the yeast inoculated sausages contributing actively to sausage aroma due to their low odour thresholds (Mottram, 1998). The ability of *D. hansenii* to produce volatile sulphur compounds has been previously reported (Olesen & Stahnke, 2000, Cano-García *et al.* 2014b) although it depends on media composition and on the other starter cultures present (Cano-García *et al.*, 2014a). The inoculated *D. hansenii* also enhanced the production of ester compounds in dry sausages (Flores *et al.* 2004; Bolumar *et al.*, 2006; Cano-García *et al.*, 2014a; Andrade *et al.*, 2010) by means of the esterification of carboxylic acids and alcohols such as ethanol which was found in the highest abundance.

In order to understand the interrelationships between aroma perception and the chemical parameters analysed (aroma active compounds, FFA and oxidation index TBARS) in the dry sausages, a principal component analysis was performed (Fig. 3). The PCA biplot described 75.3% of the variability by the two first principal components. PC1 is the most important variable in terms of differences among sausages because it accounts for 51.3% of the total variability while PC2 accounts for 24.0% of the variability. PC1 was strongly related to yeast inoculation placing the inoculated batches on the positive part of PC1. While PC2 distinguished the samples according to fat or salt reduction. The distribution of the variables according to PC1 suggested that fruity, cured and sour aromas of inoculated batches were associated to aroma compounds such as ethyl esters, acids, sulphur and nitrogen compounds which produced fruity and savoury odour notes in olfactometry (GC-O) analysis (Table 3). PC2 was related to rancid, cheesy and stable aromas of salt reduced batches mainly dominated by FFA concentration (MUFA and PUFA) and hexanal abundance. These results confirm not only the negative effect of salt reduction on aroma generation and perception and the contribution of specific aroma compounds but also the aroma enhancement produced by *D. hansenii* P2 yeast. Moreover, it is essential to select the appropriate yeast strain for aroma enhancement as wide differences among yeast strains in ester and sulphur production had been detected (Cano-García *et al.*, 2014a, b). Nevertheless, the limited impact of *D. hansenii* on

sausage sensory characteristics reported by other authors (Cano-García *et al.*, 2014a; Olesen & Stahnke, 2000) may be due to the metabolic activity of these yeasts as it is affected by many factors such as; meat ingredients, technological parameters and also other microorganism present throughout the ripening process (Ravyts, De Vuyst, L. & Leroy, 2012) which affect the final sensory characteristics.

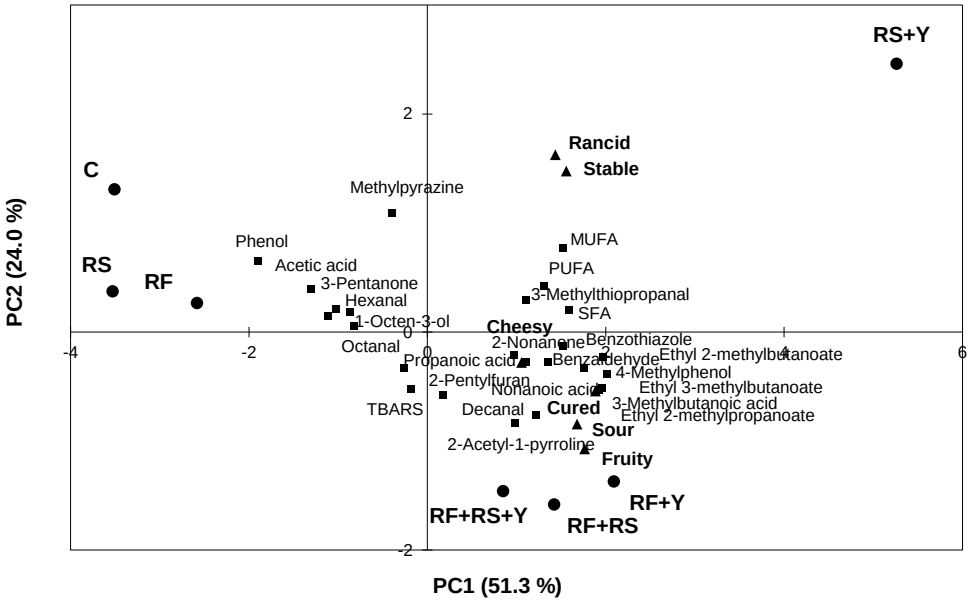

Figure 3. Loadings of the first two principal components (PC1-PC2) of trained panel, samples (●), aroma descriptors (▲) and aroma active compounds, lipolysis and lipid oxidation (■).

5. CONCLUSION

In summary, the reformulation of sausages by the decrease in fat and salt content produced important changes in the generation of aroma compounds and aroma perception but the inoculation of *D. hansenii* was shown to compensate for these effects and improve the sensory characteristics of the dry sausages. The most important contribution of *D. hansenii*, in addition to the lipolysis increase and antioxidant effect, was the enhance in aroma compounds derived from amino acid degradation and ester activity increasing the perception of fruity and cured aroma notes. However, when both reductions were carried out together, *D. hansenii* inoculation did not show a clear effect.

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REFERENCES

Andrade, M. J., Córdoba, J. J., Casado, E. M., Córdoba, M. G., & Rodríguez, M. (2010). Effect of selected strains of *Debaryomyces hansenii* on the volatile compound production of dry fermented sausage "salchichón". *Meat Science*, 85, 256-264.

Beriain, M. J., Gómez, I., Petri, E., Insausti, K., & Sarriés, M. V. (2011). The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. *Meat Science*, 88, 189-197.

Bolumar, T., Sanz, Y., Flores, M., Aristoy, M. C., Toldrá, F., & Flores, J. (2006). Sensory improvement of dry-fermented sausages by the addition of cell-free extracts from *Debaryomyces hansenii* and Lactobacillus sakei. *Meat Science*, 72, 457-466.

Burdock, G. A., (2002). Fenaroli's handbook of flavor ingredients (4th ed.). Boca Raton, Florida: CRC Press Inc..

Campagnol, P. C. B., Santos, B. A. d., Morgano, M. A., Terra, N. N., & Pollonio, M. A. R. (2011a). Application of lysine, taurine, disodium inosinate and disodium guanylate in fermented cooked sausages with 50% replacement of NaCl by KCl. *Meat Science*, 87, 239-243.

Campagnol, P. C. B., Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R. (2011b). The effect of yeast extract addition on quality of fermented sausages at low NaCl content. *Meat Science*, 87, 290-298.

Cano-García, L., Belloch, C., & Flores, M. (2014a). Impact of *Debaryomyces hansenii* strains inoculation on the quality of slow dry-cured fermented sausages. *Meat Science*, 96, 1469-1477.

Cano-García, L., Rivera-Jiménez, S., Belloch, C., & Flores, M. (2014b). Generation of aroma compounds in a fermented sausage meat model system by *Debaryomyces hansenii* strains. *Food Chemistry*, 151, 364-373.

Corral, S., Salvador, A., & Flores, M. (2013). Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science*, 93, 776-785.

Corral, Salvador, Belloch & Flores (2014). Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii* yeast. *Food Control*, 45, 1-7.

Corral, S., Salvador, A. & Flores, M. (2014) Elucidation of key aroma compounds in traditional dry fermented sausages using different extraction techniques. *Journal of the Science of Food and Agriculture*. https://dx.doi.org/10.1002/

Durá, M. A., Flores, M., & Toldrá, F. (2004a). Effect of *Debaryomyces spp*. on the proteolysis of dry-fermented sausages. *Meat Science*, 68, 319-328.

Durá, M. A., Flores, M., & Toldrá, F. (2004b). Effect of growth phase and dry-cured sausage processing conditions on *Debaryomyces spp*. generation of volatile compounds from branched-chain amino acids. *Food Chemistry*, 86, 391-399.

Flores, M., Durá, M. A., Marco, A., & Toldrá, F. (2004). Effect of *Debaryomyces spp*. on aroma formation and sensory quality of dry-fermented sausages. *Meat Science*, 68(3), 439-446.

García-Íñiguez de Ciriano, M., Berasategi, I., Navarro-Blasco, I., Astiasarán, I., & Ansorena, D. (2013). Reduction of sodium and increment of calcium and ω-3 polyunsaturated fatty acids in dry fermented sausages: Effects on the mineral content, lipid profile and sensory quality. *Journal of the Science of Food and Agriculture*, 93, 876-881.

Gelabert, J., Gou, P., Guerrero, L., & Arnau, J. (2003). Effect of sodium chloride replacement on some characteristics of fermented sausages. *Meat Science*, 65, 833-839.

ISO 8589 (2007). *Sensory analysis. General guidance for design of test rooms*. Standard no.8589 (Geneva, Switzerland).

Kovats, E.S. (1965). Gas chromatographic characterization of organic substances in the retention index system. In J. C. Giddings, & R. A. Keller (Eds.), *Advances in chromatography.* New York: Marcel Dekker, Inc., pp. 229-247.

Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology*, 106, 270-285.

Molly, K., Demeyer, D., Civera, T., & Verplaetse, A. (1996). Lipolysis in a Belgian sausage: Relative importance of endogenous and bacterial enzymes. *Meat Science*, 43, 235-244.

Motilva M. J. & Toldrá, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 196, 228-232.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, 62, 415-424.

Muguerza, E., Ansorena, D., Bloukas, J. G., & Astiasarán, I. (2003). Effect of fat level and partial replacement of pork backfat with olive oil on the lipid oxidation and volatile compounds of Greek dry fermented sausages. *Journal of Food Science*, 68, 1531-1536.

Olesen, P. T., & Stahnke, L. H. (2000). The influence of *Debaryomyces hansenii* and Candida utilis on the aroma formation in garlic spiced fermented sausages and model minces. *Meat Science*, 56, 357-368.

Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science*, 87, 264-273.

Ordóñez, J. A., Hierro, E. M., Bruna, J. M., & De La Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition*, 39, 329-367.

Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L., & Leroy, F. (2010). The application of *staphylococci* with flavour-generating potential is affected by acidification in fermented dry sausages. *Food Microbiology*, 27, 945-954.

Ravyts, F., De Vuyst, L., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. *Engineering in Life Sciences*, 12, 356–367.

Ruusunen, M., Simolin, M., & Puolanne, E. (2001). The effect of fat content and flavor enhancers on the perceived saltiness of cooked 'bologna-type' sausages. *Journal of Muscle Foods*, 12, 107-120.

Segal-Kischinevzky, C., Rodarte-Murguía, B., Valdés-López, V., Mendoza-Hernández, G., González, A., & Alba-Lois L. (2011). The euryhaline yeast *Debaryomyces hansenii* has two catalase genes encoding enzymes with differential activity profile. *Current Microbiology*, 62, 933-943.

Stahnke, L. H. (1995). Dried sausages fermented with Staphylococcus xylosus at different temperatures and with different ingredient levels - Part I. Chemical and bacteriological data. *Meat Science*, 41, 179-191.

Toldrá, F. (1992). In J. M. Smulders, F. Toldra, J. Flores & M. Prieto (Eds.). *New technologies for meat and meat products* (pp. 209).

WHO/FAO Expert Consultation (2003). *Diet, nutrition and the prevention of chronic 507 diseases*. WHO technical report series 916, Geneva.

Wirth, F. (1988). Technologies for making fat-reduced meat products. *Fleischeirtsch*, 509 68, 1153-1156.

V. Discusión general

V. DISCUSIÓN GENERAL

El proceso de fabricación de embutidos curado-madurado es crucial para el desarrollo de unas características sensoriales óptimas. Estas características se ven influenciadas en gran parte por el aroma del producto, el cual es completamente diferente al de la carne procesada mediante tratamiento térmico. Aunque se conocen una gran cantidad de compuestos volátiles originados como resultado de reacciones químicas y bioquímicas durante el proceso de fabricación (Ordóñez *et al*., 1999), sólo algunos de ellos contribuyen al aroma. Para ello, dichos compuestos deben estar presentes en concentraciones superiores a su umbral de detección de olfacción (Grosch & Schieberle, 2009). Como se ha visto en los antecedentes bibliográficos, las técnicas de análisis de compuestos volátiles son muy variadas y el poder determinar su contribución al aroma es esencial para diseñar estrategias de potenciación. Por ello, se planteó el primer objetivo que consistió en determinar los compuestos volátiles minoritarios de gran potencia aromática en embutidos curado-madurados.

La primera parte de este estudio (capítulo 1) se centró en el uso de diferentes técnicas de extracción de compuestos volátiles con el fin de elucidar los compuestos aromáticos de embutidos de alta calidad organoléptica, como son los embutidos tradicionales. El estudio se abordó con la utilización de una técnica de extracción de espacio de cabeza (SPME) y otra de extracción con disolventes (SAFE). En vista de los resultados obtenidos en el capítulo 1, en general, la técnica de SAFE frente a SPME fue capaz de extraer un mayor número de compuestos volátiles de mayor peso molecular, los cuales, además, se presentaban a baja concentración. Mientrás que la técnica de SPME extrajo un mayor número de compuestos de menor peso molecular y, en concreto, se observaron diferencias según el tipo de fibra empleada. La fibra DVB/CAR/PDMS extrajo mayor número de compuestos volátiles de mayor peso molecular que la fibra CAR/PDMS.

Dicha selectividad en la extracción permitió la identificación de compuestos aromáticos minoritarios ya que, se observó que la técnica de SAFE extrajo un mayor porcentaje de ésteres y compuestos azufrados (Figura 1), los cuales imparten un aroma “afrutado” y “curado-sabroso” relacionado con la apreciación de este producto por los consumidores (Olivares *et al*., 2010). Además, la técnica de SAFE esclareció la identificación de dos compuestos (α-terpineol y 2,3-dihidrotiofeno) que con la técnica de SPME se detectaron olfatométricamente pero, en cambio, no permitió su identificación. Con el fin de

determinar cual de dichas técnicas, SAFE vs SPME, permitía elucidar el aroma de los embutidos curado-madurados se cuantificaron los compuestos aromáticos identificados por ambas técnicas para calcular su OAV, y de dicha forma poder seleccionar los compuestos de mayor potencia aromática. Aquellos compuestos que mostraron valores de OAV superiores a 1 se seleccionaron para llevar a cabo la reconstitución del aroma del embutido en una matriz similar (compuesta por aceite de girasol/tampón fosfato). El empleo de un panel entrenado permitió confirmar que, mediante análisis cuantitativo descriptivo del aroma, las fórmulas reconstituidas impartieron el aroma a pimienta, rancio, ácido, queso, afrutado y establo que se habían descrito en el embutido control (embutido de fermentación natural). Este análisis permitió la selección de los compuestos aromáticos en la concentración adecuada aunque, los compuestos que impartieron el aroma a "curado" no se pudieron identificar (Capítulo 1).

Debido a que las notas aromáticas que no se pudieron identificar en el capítulo 1 estaban relacionadas con aromas a "tostado" y éstas con compuestos azufrados y nitrogenados, generalmente presentes a nivel traza, ppb o ppt (Burdock, 2002), se propuso centrarse en la identificación de estos compuestos aromáticos minoritarios. Por lo tanto, el siguiente trabajo (Capítulo 2) se centró en elucidar los compuestos azufrados y nitrogenados generados a lo largo del proceso de maduración de embutidos curado-madurados.

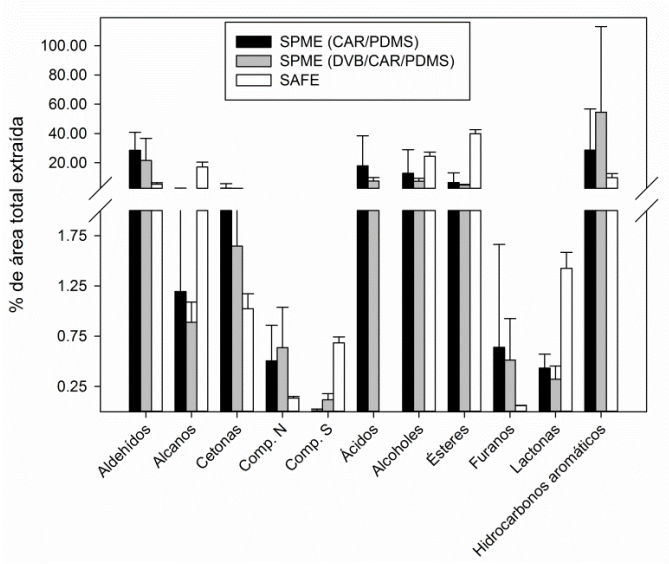

Figura 1. Porcentaje de extracción por grupo químico de la técnica de SPME y SAFE aplicada a embutidos curado-madurados.

Para ello, se decidió emplear la técnica de extracción de SAFE, dada su capacidad de extraer un mayor porcentaje de compuestos traza, como compuestos azufrados (Figura 1). En este caso, se utilizaron detectores específicos para facilitar la identificación de los compuestos azufrados y nitrogenados, como son los detectores FPD, NPD y Olfatómetro. Con ayuda de la cromatografía multidimensional convencional (GC-GC) y GC-MS se identificaron 17 compuestos volátiles azufrados y nitrogenados, de los cuales, 9 de ellos presentaron actividad aromática. Los siguientes compuestos, 2-acetil-1-pirrolina, metional, 2-etilpirazina y 2,3-dihidrotiofeno, fueron los de mayor impacto aromático. Además, este estudio permitió identificar los compuestos aromáticos desconocidos 1, 2, 3 del capítulo 1 ("unknown 1, 2, 3"), siendo éstos 2-acetil-1-pirrolina, 2-acetiltiazol y 2-acetilpirrol. Sin embargo, en este estudio (Capítulo 2) 2-acetiltiazol no impartió aroma, probablemente debido a que se encontraba presente en una concentración inferior a la de su umbral de detección.

El origen de los compuestos aromáticos minoritarios durante la maduración de los embutidos puede ser principalmente de dos fuentes, los aminoácidos azufrados (cisteína y metionina) o de la tiamina. Por ello, se evaluó la generación de aminoácidos libres a lo largo del proceso de maduración, con el fin de establer estrategias tecnológicas que promuevan la generación de los compuestos azufrados y nitrogenados. Dada la intensa proteólisis que tiene lugar en el embutido liberando aminoácidos libres, los cuales son posteriormente degradados química o bioquímicamente (apartados 2.1. y 2.2. de antecedentes bibliográficos), éstos pueden ser la fuente principal de generación de compuestos volátiles azufrados a partir de los aminoácidos azufrados. Mientras que los compuestos nitrogenados proceden de cualquier fuente nitrogenada, principalmente de los aminoácidos, dado el gran porcentaje que suponen las proteínas en la composición del embutido. Los resultados obtenidos demostraron la relación positiva entre metional, tiazol, 2,4-dimetiltiazol y benzotiazol con la generación de metionina, 2-acetil-1-pirrolina con ornitina y dos pirazinas (2-etilpirazina y 2,6-dimetilpirazina) con la glicina lo cual demostró la contribución de los aminoácidos a la generación de compuestos aromáticos minoritarios.

Por otro lado, el segundo objetivo de esta Tesis Doctoral se centró en la reformulación de embutidos curado-madurados con un perfil saludable (reducción de grasa y/o sal) manteniendo el aroma característico y, para ello, se seleccionó una estrategia de potenciación basada en la inoculación de levaduras. En primer lugar se estudió el efecto de la reducción de NaCl en la calidad aromática de embutidos curado-madurados.

La actual demanda de productos cardiosaludables ha provocado que los organismos gubernamentales (AECOSAN) promuevan la reducción del contenido de NaCl y por ello, la industria cárnica se ha centrado en reducir el contenido de NaCl en estos productos, dado su gran aporte de sodio, el cual se encuentra relacionado con el aumento de la presión arterial (Doyle & Glass, 2010). En el ámbito europeo, la Comisión Europea (2008) recomendó la reducción del 16 % del contenido de sodio de todos los productos alimentarios como de los consumidos en restauración. Además, señalaron que dicha reducción debía hacerse con reducciones progresivas de un 4 % cada año para que los consumidores se adaptaran al menor sabor salado. Sin embargo, no existen evidencias científicas sobre si pequeñas reducciones de NaCl (16 %) producen un impacto negativo en la calidad sensorial de los embutidos y si es necesario el empleo de KCl como sustito al NaCl.

En este estudio (Capítulo 3) se consiguió una reducción del 16 % del contenido de sodio, propocionando así un producto más cardiosaludable a los consumidores. Sin embargo, los parámetros de textura, cohesividad y masticabilidad se vieron disminuidos por la reducción del contenido de NaCl, puesto que éste afecta a la solubilización de las proteínas y así a la textura del producto. Además, este estudió demostró claramente que el aroma del embutido se vio afectado por la reducción del contenido de NaCl, principalmente los compuestos aromáticos presentes en el espacio de cabeza procedentes de la autooxidación lipídica, degradación de aminoácidos, actividad esterasa de *Staphylococci* y compuestos de origen desconocido (Figura 2). Probablemente el contenido NaCl tuvo un efecto tanto en la actividad enzimática muscular como microbiana implicada en la generación de compuestos aromáticos (Rosell & Toldrá, 1996; Sanz & Toldrá, 1997). Sin embargo, el uso de KCl, como sustituto de NaCl, demostró ser esencial para mantener la textura y el sabor de los embutidos y, por tanto, conseguir un producto aceptable por los consumidores. No obstante, el KCl no fue capaz de potenciar el aroma del embutido. Por ello, es necesaria la búsqueda de estrategias que potencien el aroma de los embutidos reducidos en sal con el fin de conseguir productos de alta calidad sensorial.

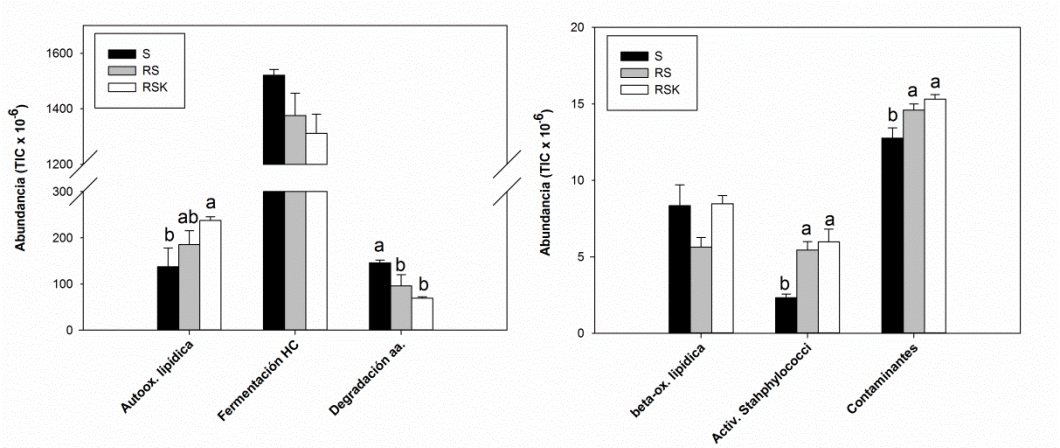

Figura 2. Efecto de la reducción y sustitución del contenido de sal sobre los compuestos aromáticamente activos.

En base de los resultados obtenidos en el Capítulo 3, se planteó como alternativa para la mejora de la calidad organoléptica de embutidos con contenido reducido en NaCl, la combinación de la sustitución de KCl junto con la inoculación de una cepa de *D. hansenii*. Dicha cepa de *D. hansenii* fue aislada de embutidos tradicionales (Cano-García *et al*., 2013) y previamente se demostró su potencial aromático en diversos medios, desde sistemas modelo hasta embutidos curado-madurados (Cano-García et al., 2014 a, b). Asimismo, se estudió el efecto de la reducción de grasa junto con la reducción de NaCl e inoculación de *D. hansenii* ya que, en estudios anteriores la reducción de grasa dio lugar a embutidos curado-madurados menos aromáticos (Olivares *et al*., 2011), consiguiendo así un embutido más cardiosaludable. Así pues, se evaluó la calidad sensorial (Capítulo 4) y en detalle el aroma del embutido con contenido reducido en grasa y NaCl inoculado con *D. hansenii* (Capítulo 5).

Los resultados obtenidos demostraron que la inoculación de *D. hansenii* compensó los cambios en la a_w y textura en los embutidos reducidos en grasa y NaCl pero, por otra parte, no fue capaz de modificar la dureza en los embutidos reducidos en grasa ni tampoco el descenso del crecimiento de *Staphylococci* (Capítulo 4). Respecto al aroma, por una parte, el aroma de los embutidos reducidos en NaCl se vio afectado principalmente por un incremento de los compuestos aromáticos generados a través de la oxidación lipídica y β-oxidación, dado el incremento de la lipolisis y oxidación lipídica que tuvo lugar en estos embutidos. Estos resultados confirmaron los resultados obtenidos en el Captítulo

3 demostrando que la oxidación lipídica se vio favorecida por la reducción del contenido de NaCl (Stahnke, 1995). Por otro lado, el aroma de los embutidos reducidos en grasa se vio afectado en menor medida mientras que la combinación de ambas reducciones (NaCl y grasa), del mismo modo que anteriormente, dio lugar a una mayor lipolisis y oxidación lipídica. Además, ambas reducciones dieron lugar a una mayor generación de compuestos volátiles procedentes de la generación de aminoácidos, puesto que existe un mayor contenido proteico en estos embutidos, y de compuestos éster, probablemente debido a una interacción entre ambas reducciones y a la actividad microbiana. No obstante, a pesar de los efectos producidos por las modificaciones en la formulación, la inoculación de *D. hansenii* compensó algunos de ellos y mejoró otros. Tal es el caso del efecto antioxidante debido a la actividad catalasa de *D. hansenii* (Aquilanti *et al*., 2007) pese a su efecto lipolítico (Cano-García *et al*., 2014 b) o su intensa actividad esterasa y sobre la degradación de aminoácidos, dando lugar a una gran abundancia de compuestos azufrados y ésteres aromáticos en los embutidos inoculados, demostrando así el potencial aromático de la levadura.

A lo largo de esta Tesis Doctoral se han identificado compuestos volátiles con capacidad aromatica en los distintos embutidos curado-madurados estudiados. En la Figura 3 se describen las distintas experiencias realizadas junto con las técnicas de extracción empleadas para el análisis.

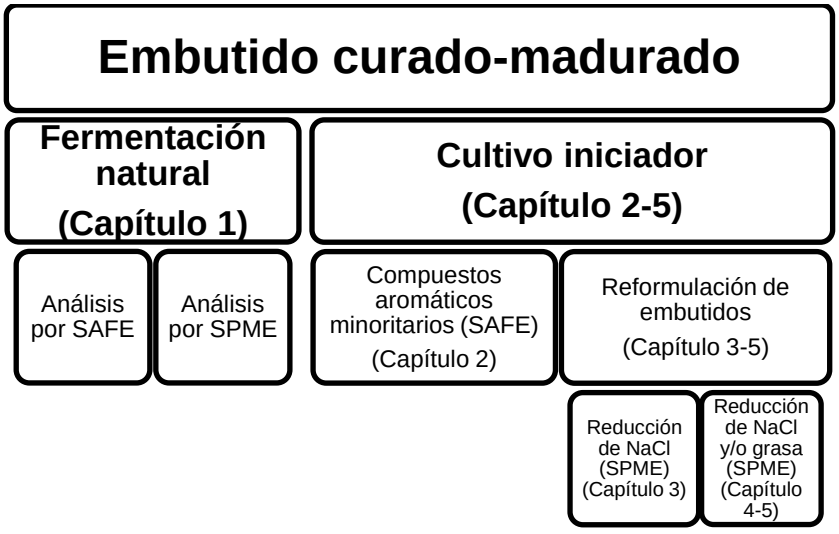

Figura 3. Descripción de las experiencias realizadas.

A la hora de comparar los compuestos aromáticos identificados se muestra en la Tabla 1 los compuestos ordenados por grupo químico y su presencia en los distintos embutidos estudiados.

Tabla 1. Compuestos volátiles aromáticos identificados en los embutidos crudo-madurados y reformulados.

			Embutido tradicinal		Embutido con cultivo iniciador		
			SPME	SAFE	SAFE (C)	SPME (RS)	SPME (*D. hansenii*)
Compuesto	**LRI[a]**	**Descriptor aromático**	**Cap. 1**	**Cap. 1**	**Cap. 2**	**Cap. 3**	**Cap. 4-5**
Aldehídos							
3-Metilbutanal	691	Verde				+	
Pentanal	734	Verde, fruto seco, caldo carne				+	
Hexanal	836	Cesped recién cortado	+	+		+	+
2-Hexenal (Z)	903	Dulce, asado, caldo de carne				+	
Heptanal	939	Desagradable, col	+				
2- Heptenal (Z)	1009	Vegetales, desagradable	+				
Benzaldehído	1016	Tostado, tabaco					+
Octanal	1046	Floral, cítrico	+				+
2,4-Heptadienal (E,E)	1071	Herbal, fresco, pegamento	+				
2-Hidroxibenzaldehído	1105	Herbal, pan tostado, establo				+	
2-Octenal	1111	Pegamento, polvos de talco, afrutado		+			
Benceneacetaldehído	1111	Rosas, polen, jazmín, almizcle	+			+	
Nonanal	1150	Cítrico, fresco, laurel, especia	+	+		+	
2-Nonenal	1216	Afrutado, herbal, fresco		+		+	
Decanal	1245	Madera verde					+
2,4-Nonadienal (E,E)	1290	Herbal, desagradable				+	
2,4-Decadienal (E,E)	1395	Pegamento, herbal		+			
Alcoholes							
1-Pentanol	821	Dulce, floral, mantequilla		+		+	
1-Octen-3-ol	1024	Champiñón	+	+		+	+
Fenol	1113	Pelo de perro mojado					+
Feniletil alcohol	1192	Rosas	+				
4-Metilfenol	1193	Establo, cuero, caballo	+	+		+	+
Ácidos							
Ácido acético	701	Vinagre	+			+	+
Ácido propanoico	813	Pegamento, cera					+
Ácido butanoico	871	Queso				+	
Ácido 3-metilbutanoico	930	Queso, desagradable	+				+
Ácido heptanoico	1163	Herbal, rancio				+	
Ácido nonanoico	1357	Especia, avellana, nuez					+
Cetonas							
2,3-Butanodiona	632	Mantequilla				+	+
2,3-Pentanodiona	736	Dulce, vainilla, fruto seco tostado	+				

Tabla 1. Continuación.

			Embutido tradicional		Embutido con cultivo iniciador		
			SPME	SAFE	SAFE (C)	SPME (RS)	SPME (*D. hansenii*)
Compuesto	**LRI[a]**	**Descriptor aromático**	**Cap. 1**	**Cap. 1**	**Cap. 2**	**Cap. 3**	**Cap. 4-5**
3-Pentanona	793	Mantequilla, humedad					+
2-Octanona	1031	Herbal, ajo				+	
2-Nonanona	1137	Madera, plástico, asado				+	+
Compuestos azufrados							
Metanotiol	472	Podrido, establo				+	
3-Metiltiofeno	795	Patata cocida, verde, madera				+	
3-Metiltiopropanal	968	Patata o espárragos cocidos	+	+	+	+	+
Dimetil trisulfuro	1007	Cebolla, podrido, col				+	
2-Acetiltiazol	1077	Tostado, espcia	D[b]			+	
Benzotiazol	1293	Salsa barbacoa, cebolla, herbal, sabroso	+	+	+		+
Desconocido/2,3-Dihidro tiofeno	1401	Sabroso, herbal, madera, avellana, nuez	+	+	+		
Compuestos nitrogenados							
Pirrol	848	Café, dulce			+		
Metilpirazina	852	Patata cocida, verde					+
2-Etilpiridina	936	Setas, detergente			+		
2,6-Dimetilpirazina	945	Tostado, especia			+		
2-Etilpirazina	949	Tostado, especia			+		
2-Acetil-1-pirrolina	963	Fruto seco tostado, palomitas, snacks, caldo carne	D		+	D	+
Trimetilpirazina	1038	Café, chocolate caliente	+				
Tetrametilpirazina	1117	Fruto seco tostado, madera barnizada	+				
2-Acetilpirrol	1159	Floral, tostado	D		+		
3-Piridinecarboxilato de etilo	1284	Humedad		+			
Ésteres							
Acetato de etilo	638	Melocotón, especia, levadura	+				
2-Metilpropanoato de etilo	787	Fresa, piña, manzana roja	+	+			+
Butanoato de etilo	825	Afrutado	+	+		+	
Acetato de butilo	843	Especia, rancio, madera, vegetales cocidos				+	
2-Metilbutanoato de etilo	871	Dulce, fresa, piña	+	+			+
3-Metilbutanoato de etilo	875	Afrutado, floral	+	+			+
Pentanoato de etilo	922	Afrutado, verde, rancio	+	+		D	
3-Hidroxibutanoato de etilo	990	Dulce, afrutado	+	+			
Hexanoato de etilo	1026	Dulce, fresa ácida	+	+			
Hexanoato de isobutilo	1177	Dulce, floral		+			
Benzoato de etilo	1225	Afrutado, herbal, humedad, armario cerrado	+	+		D	D
Furanos							
2-Etillfuran	725	Grasa, sabroso, dulce				+	
2-Pentilfuran	1011	Metálico, herbal, col, desagradable					+

Tabla 1. Continuación.

			Embutido tradicional		Embutido con cultivo iniciador		
			SPME	SAFE	SAFE (C)	SPME (RS)	SPME (*D. hansenii*)
Compuesto	**LRI[a]**	**Descriptor aromático**	**Cap. 1**	**Cap. 1**	**Cap. 2**	**Cap. 3**	**Cap. 4-5**
Lactonas							
D-Pantolactona	1164	Vegetales cocidos, desagradable		+			
Ɣ-Nonalactona	1488	Afrutado, metálico		+			
Hidrocarburos aromáticos							
α-Terpineno	1035	Humedad, floral, herbal, pimiendo verde, metálico	+	+			+
D-Limoneno	1046	Cítrico				+	
Desconocido/α-Terpineol	1250	Fresco, jabón, herbal	+	+			
Eugenol	1442	Especia, grasa		+			
Metileugenol	1455	Especia, clavo, floral		+			
Compuestos desconocidos							
Desconocido	1001	Herbal, tostado, humedad, vainilla				D	
Desconocido	1173	Cereza, fresa, madera barnizada	D				
Desconocido	1179	Frutos secos tostados	D			D	D
Desconocido	1186	Vegetales cocidos, azufrado					D
Desconocido	1202	Floral, madera, grasa, plástico, desagradable	D	D			D

[a]LRI: Índice de retención lineal (columna capilar DB-624; J&W Scientifici 60 m x 0,32 mm x 1,8 µm).
[b]D: compuesto desconocido.

Cuantitativamente no se pueden comparar los estudios ya que se utilizaron diferentes metodologías para la extracción de los compuestos volátiles y se realizaron en diferentes tipos de embutidos. También debe tenerse en cuenta que, en el caso del capítulo 3, dado su objetivo, sólo se centró en el estudio de los compuestos volátiles azufrados y nitrogenados sin determinar todos los compuestos aromáticos presentes. A lo largo de la presente Tesis, en algunos casos, el aroma de los compuestos se detectó pero no se pudo identificar su estructura química ("unknown" en cada uno de los capítulos) y en la Tabla 1 se indican como D (compuesto desconocido). De la mayoría de ellos se pudo determinar su identificación puesto que presentaron índices de retención lineal (LRI) similares además de coincidir el descriptor aromático. Los compuestos identificados fueron pentanoato de etilo, 2-acetil-1-pirrolina, 3-hidroxibutanoato de etilo, α-terpineno, 2-acetiltiazol, 2-acetilpirrol y benzoato de etilo. En cambio, los únicos aromas que no se consiguieron dilucidar son los que

corresponden a los LRI 1001, 1173, 1179, 1186, 1202. Como se observa en la Tabla 1, la técnica de SPME junto con la de SAFE consiguió extraer un mayor número de compuestos aromáticos. Además, la reducción de sal (Capítulo 3) produjo un mayor número de aldehídos aromáticos, debido a la mayor oxidación lipídica observada, mientras que la inoculación de *D. hansenii* (Capítulo 5) disminuyó el número de dichos aldehídos en los embutidos reformulados. Asimismo, la inoculación de la levadura produjo de forma más intensa el compuesto nitrogenado 2-acetil-1-pirrolina, el cual se ha identificado como uno de los compuestos más aromáticos del embutido junto con metional y hexanal (Blank *et al*., 2001) permitiendo identificarlo aun cuando se empleó la técnica de SPME, siendo que tan sólo se había identificado por técnicas de mayor rendimiento como SAFE (Blank *et al*., 2001; Söllner & Schieberle, 2009).

Concluyendo así, se ha desmostrado el poder aromático de la levadura *D. hansenii* (P2) en la producción de compuestos altamente aromáticos mejorando la calidad organoléptica disminuida en los embutidos con contenido reducido en NaCl y grasa.

Referencias bibliográficas

Aquilanti, L., Santarelli, S., Silvestri, G., Osimani, A., Petruzzelli, A., & Clementi, F. (2007). The microbial ecology of a typical Italian salami during its natural fermentation. *International Journal of Food Microbiology*, 120, 136–145.

Blank, I., Devaud, S., Fay, L.B., Cerny, C., Steiner, M., & Zurbriggen, B. (2001). Odor-active compounds of dry-cured meat: Italian-type salami and Parma ham. En: *Aroma-Active Compounds in Foods. Chemistry and Sensory Properties*. Ed. G.R. Takeoka, M. Güntert, K. H. Engel. American Chemical Society, Washington, D.C., 9-20.

Burdock, G. A. (2002). *Fenaroli's handbook of flavor ingredients (4th ed.).* CRC Press Inc., Boca Raton, Florida.

Cano-García, L., Flores, M., & Belloch, C. (2013). Molecular characterization and aromatic potential of *Debaryomyces hansenii* strains isolated from naturally fermented sausages*. Food Research International*, 52, 42-49.

Cano-García, L., Belloch, C., & Flores, M. (2014 a). Impact of *Debaryomyces hansenii* strains inoculation on the quality of slow dry-cured fermented sausages. *Meat Science*, 96, 1469-1477.

Cano-García, L., Rivera-Jiménez, S., Belloch, C., & Flores, M. (2014 b). Generation of aroma compounds in a fermented sausage meat model system by *Debaryomyces hansenii* strains. *Food Chemistry*, 151, 364-373.

Comisión Europea (2008). Salt reduction. Disponible en http://ec.europa.eu/health/ph_determinants/life_style/nutrition_salt_en.htm

Doyle, M. E., & Glass, K. A. (2010). Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety,* 9, 44-56.

Grosch, W. & Schieberle, P. (2009). Aroma compounds. En: *Food Chemistry*. Ed. H—D. Belitz, W. Grosch & P. Schieberle. Springer-Verlag, Berlin Heidelberg, 340-402.

Olivares A., Navarro J. L. & Flores M. (2010). Comparison of flavor components in dry sausages obtained from commercial and non fermented sausages from protected origin. En: *Recent Advances in Food and Flavour Chemistry*. Food Flavors and Encapsulation, Health Benefits, Analytical Methods, and Molecular Biology of Functional Foods. Ed. C. T. Ho, C.J. Mussinan, F. Shahidi & E. Tratas Contis. Royal Society of Chemistry, Cambridge, UK, 150–156.

Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science*, 87, 264-273.

Ordóñez, J. A., Hierro, E. M., Bruna, J. M., & De La Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition,* 39, 329-367.

Rosell, C. M., & Toldrá, F. (1996). Effect of curing agents on m-calpain activity throughout the curing process*. European Food Research and Technology*, 203, 320-325.

Sanz, Y., & Toldrá, F. (1997). Aminopeptidase activities from *Lactobacillus sake* in models of curing ingredients and processing conditions for dry sausage. *Journal of Food Science*, 62, 1211-1213+1234.

Söllner, K., & Schieberle, P. (2009). Decoding the key aroma compounds of a Hungarian-type salami by molecular sensory science approaches. *Journal of Agricultural and Food Chemistry*, 57, 4319-4327.

Stahnke, L. H. (1995). Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels - Part I. Chemical and bacteriological data. *Meat Science*, 41, 179-191.

VI. Conclusiones

VI. CONCLUSIONES

- Las técnicas de SPME y SAFE fueron complementarias para reproducir el aroma de embutidos curado-madurados tradicionales. El aroma de los embutidos se compuso de 45 compuestos aromáticos, de los cuales los más potentes aromáticamente fueron 1-octen-3-ol, benzotiazol, metil eugenol y algunos ésteres etílicos. Para los análisis de reconstitución se emplearon 20 compuestos con OAV superiores a 1 e identificados por ambas técnicas pero fue necesario incorporar los compuestos 2,4-decadienal (E,E), benzotiazol, metil eugenol, α-terpineol y eugenol para evocar el aroma de los embutidos a pesar de no conseguir impartir totalmente el aroma a curado.

- En la identificación de compuestos aromáticos minoritarios, como compuestos volátiles azufrados y nitrogenados, fue necesario el uso de detectores cromatográficos específicos (FPD, NPD y O) junto con GC-GC. Estos compuestos se generaron principalmente de los aminoácidos liberados a través de la intensa proteólisis que tuvo lugar durante la maduración del producto. De los compuestos azufrados y nitrogenados, 2-acetil-1-pirrolina, metional, 2-etilpirazina y 2,3-dihidrotiofeno fueron los de mayor potencia aromática siendo su origen más probable la degradación de los aminoácidos ornitina, metionina y glicina.

- La reducción de un 16 % de NaCl y sustitución de NaCl por KCl dio lugar a un embutido sensorialmente aceptado por los consumidores excepto en el aroma. El detrimento en el aroma se debió principalmente a una reducción de los compuestos azufrados y ácidos y a un incremento de los aldehídos. Los análisis olfatométricos revelaron que los compuestos aromáticos asociados a la aceptación del producto fueron dimetil trisulfuro, 3-metiltiofeno, 2,3-butanodiona, 2-nonanona y ácido acético.

- Se confirmó la mejora de las características organolépticas de embutidos reducidos en sal y/o grasa mediante la inoculación de la levadura *D. hansenii* (P2) a pesar de que no se consiguió evitar el incremento de la dureza, así como el descenso del crecimiento de *Staphylococci* de dichos embutidos. Los cambios producidos por la inoculación de la levadura se debieron al incremento de la lipólisis y a su efecto antioxidante. Además, la

levadura aumentó la generación de compuestos aromáticos derivados de la degradación de aminoácidos (ácido 3-metilbutanoico y benzotiazol) y de la actividad esterasa (ésteres de etilo 2-metilpropanoato, 2 y 3-metilbutanoato) intensificando la percepción de las notas aromáticas a “fruta” y “curado”. No obstante, cuando ambas reducciones (NaCl y grasa) se realizaron de manera conjunta se observó un efecto significativamente mayor que el producido por la inoculación de *D. hansenii.*