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INSTITUTO DE AGROQUÍMICA Y
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ESTUDIO DE LA CONTRIBUCIÓN DE LA FRACCIÓN VOLÁTIL AL AROMA DE EMBUTIDOS CRUDOS CURADOS Y DEL EFECTO DE LA REDUCCIÓN DE GRASA SOBRE SU CALIDAD

TESIS DOCTORAL

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HACEN CONSTAR:

Que la memoria titulada “Estudio de la contribución de la fracción volátil al aroma de embutidos crudos curados y del efecto de la reducción de grasa sobre su calidad” presentada por Dña. Alicia Olivares Sevilla para optar al grado de Doctor por la Universidad Politécnica de Valencia, ha sido realizada bajo su dirección y supervisión en el Instituto de Agroquímica y Tecnología de Alimentos, reuniendo las condiciones exigidas para ser defendida por su autora.

Valencia, febrero de 2011

Fdo. Dra. Mónica Flores Llovera

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Resumen

El aroma es uno de los atributos que determinan en mayor medida la calidad de los embutidos crudos curados. Por este motivo, es de gran interés para la industria cárnica conocer cuáles son los principales compuestos aromáticos responsables de la aceptación del aroma de los embutidos crudos curados y cuál es su evolución a lo largo del proceso de elaboración para así establecer las estrategias tecnológicas que fomenten la generación de dichos compuestos. En la presente Tesis, el estudio de los compuestos volátiles con poder aromático se ha abordado desde dos perspectivas. Por un lado, se han aplicado técnicas de olfatometría para conocer el perfil aromático de embutidos tradicionales de gran calidad organoléptica. Por otro lado, se ha calculado el valor de la actividad aromática (OAV) de los compuestos volátiles presentes en los embutidos, tanto en la matriz como en el espacio de cabeza. Para conocer la concentración de compuestos volátiles en la matriz se ha hecho uso de la técnica de microextracción en fase sólida (SPME) múltiple. Además, esta técnica se ha aplicado para estudiar la participación de las fracciones magra y grasa en el desarrollo y liberación del aroma.

Por otro lado, teniendo en cuenta el alto porcentaje de grasa de los embutidos crudos curados y la creciente preocupación de los consumidores por la salud, se ha determinado el efecto de la reducción del contenido de grasa sobre la calidad de este producto cárnico. En este sentido, se han estudiado los parámetros físico-químicos y atributos sensoriales de embutidos de fermentación lenta con distintos porcentajes de grasa. Asimismo, se ha estudiado el efecto de la reducción de grasa sobre la generación de compuestos volátiles y se han empleado técnicas de olfatometría para conocer los compuestos aromáticos responsables de la aceptación sensorial.

Por último, el desarrollo de técnicas rápidas para el análisis de los compuestos volátiles de embutidos crudos curados puede ser de gran utilidad para el control de su calidad. A este respecto, en esta Tesis Doctoral se presenta por primera vez la aplicación de la técnica espectrométrica directa Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) para la determinación de compuestos volátiles en embutidos crudos curados.

Abstract

Aroma is one of the most important quality attributes of dry fermented sausages. Due to this fact, meat industry is interested in the elucidation of the aroma compounds responsible for the aroma acceptance of dry fermented sausages and its evolution during processing, in order to establish the technological strategies that enhance the generation of the mentioned compounds. In this Thesis, the study of aroma volatile compounds has been approached by two different perspectives. In the first place, olfactometric techniques have been applied to study the aroma profile of traditional fermented sausages with high organoleptic quality. Secondly, the odour activity values (OAV) of both matrix and headspace of dry fermented sausages have been determined. Multiple solid phase microextraction (SPME) technique has been applied to determine the total concentration of volatile compounds in the sausage matrix. Also, this technique has been used to study the contribution of lean and adipose tissues in the generation and release of volatile compounds.

On the other hand, considering the high proportion of fat in dry fermented sausages and the increased concerns of consumers about health, the effect of fat reduction on the quality of this meat product has been determined. In this sense, the chemical and sensory parameters of dry fermented sausages with different fat contents have been evaluated. Also, it has been studied the effect of fat reduction on aroma generation and olfactometric techniques have been employed to elucidate the aroma compounds responsible for sensory acceptance.

Finally, the development of rapid techniques for the analysis of volatile compounds of dry fermented sausages constitutes an interesting alternative for its quality control. In relation to this, in this Thesis, the direct spectrometric technique selected ion flow tube-mass spectrometry (SIFT-MS) has been applied for the first time for the analysis of volatile compounds of dry fermented sausages.

Resum

L'aroma és un dels atributs que determinen en major grau la qualitat del embotits crus curats. Per este motiu, és de gran interés per a la indústria càrnica conéixer quins són els principals compostos aromàtics responsables de l'acceptació de l'aroma dels embotits crus curats i quina és la seua evolució al llarg del procés d'elaboració per així establir les estratègies tecnològiques que fomenten la generació d'aquests compostos. En la present Tesi Doctoral, l'estudi dels compostos volàtils amb poder aromàtic s'ha abordat des de dos perspectives. D'una banda, s'han aplicat tècniques d'olfatometria per a conéixer el perfil aromàtic d'embotits tradicionals de gran qualitat organolèptica. D'altra banda, s'ha calculat el valor de l'activitat aromàtica (OAV) dels compostos volàtils presents en els embotits, tant en la matriu com en l'espai de capçalera. Per a conéixer la concentració de compostos volàtils en la matriu s'ha fet ús de la tècnica de microextracció en fase sòlida (SPME) múltiple. A més, esta tècnica s'ha aplicat per a estudiar la participació de les fraccions magre i greix en el desenvolupament i alliberament de l'aroma.

D'altra banda, tenint en compte l'alt porcentatge de greix dels embotits crus curats i la creixent preocupació dels consumidors per la salut, s'ha determinat l'efecte de la reducció del contingut de greix en aquest producte carnic. En este sentit, s'han estudiat els paràmetres físico-químics i atributs sensorials d'embotits amb distints percentatges de greix. Així mateix, s'ha estudiat l'efecte de la reducció de greix sobre la generació de compostos volàtils i s'han utilitzat tècniques d'olfatometria per a conéixer els compostos aromàtics responsables de l'acceptació sensorial.

Finalment, el desenvolupament de tècniques ràpides per a l'anàlisi dels compostos volàtils d'embotits crus curats pot ser de gran utilitat per al control de la seua qualitat. A este respecte, en aquesta Tesi Doctoral se presente per primera vegada l'aplicació de la tècnica espectromètrica directa Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) per a la determinació de compostos volàtils en embotits crus curats.

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ÍNDICE

I. Introducción

I. Introducción

La industria cárnica es uno de los cinco primeros sectores industriales de España y el primero en importancia económica de la industria española de alimentos y bebidas. Está formada básicamente por más de 3.000 pequeñas y medianas empresas repartidas por toda la geografía nacional (AICE, 2010). En 2009, su cifra de negocio supuso el 2% del producto interior bruto (PIB) total español, el 14% del PIB de la rama industrial y el 20% de todo el sector alimentario español, lo que se traduce en más de 19.000 millones de euros. Las carnes (incluidas las aves) frescas, congeladas y elaboradas son los productos más importantes en la cesta de la compra de alimentos de los españoles, representando el 21% del consumo en alimentación (MARM, 2010).

En la producción cárnica española destaca la carne de porcino, que en 2009 representó el 83% de las carnes de ungulados y el 62% de la carne si también se incluye la avicultura y la cunicultura. Con una producción cercana a los tres millones y medio de toneladas, España es el cuarto mayor productor mundial de carne de porcino, por detrás de China, EE.UU. y Alemania (AICE, 2010).

Dentro del sector cárnico español, la elaboración de productos cárnicos ocupa una gran parte de la actividad empresarial, siendo España el cuarto productor de la Unión Europea, por detrás de Alemania, Italia y Francia. En 2009, el 6% de la producción de carne de porcino se destinó a la producción de embutidos crudos curados, representando éstos el 15% del total de derivados cárnicos (AICE, 2010).

En España existe una amplia variedad de embutidos crudos curados con características muy diversas, siendo el chorizo y el salchichón los más importantes. Tradicionalmente, la elaboración de estos productos se ha llevado a cabo por empresas artesanales mediante tecnologías de curado lento que requieren largos periodos de maduración y son costosas en términos de tiempo y energía. Sin embargo, hoy en día, la industria cárnica emplea tecnologías de curado rápido que reducen el coste total de producción y permiten obtener productos seguros y de calidad normalizada. No obstante, la rapidez de algunos procesos de fabricación industrial impide una correcta maduración del embutido, con las consiguientes pérdidas de las características sensoriales típicas, principalmente debido a la

aparición de un intenso aroma y sabor ácidos que no es aceptado por los consumidores (Flores & Bermell, 1996).

Por otro lado, en los últimos años, la creciente preocupación de los consumidores por la salud ha tenido como consecuencia la demanda de alimentos con menor aporte energético y contenido en grasa. Por lo tanto, el alto porcentaje de grasa de los embutidos crudos curados, si bien es fundamental para el desarrollo de sus características sensoriales (Gandemer, 2002), puede ser un inconveniente a la hora de incluir estos productos en la dieta.

Estos hechos podrían explicar que, aunque la producción de embutidos crudos curados ha aumentado en los últimos diez años en un 125%, este aumento es menor que el producido en el resto de tecnologías del sector de la transformación del cerdo (147%) como son la producción de jamón curado, jamón cocido y platos preparados (AICE, 2010).

Por todo ello, es de gran interés para la industria cárnica conocer cuáles son los principales compuestos aromáticos responsables de la aceptación del aroma de los embutidos crudos curados y cuál es su evolución a lo largo del proceso de elaboración. A su vez, la determinación del papel de la grasa en el desarrollo y percepción del aroma puede ser útil para la fabricación de embutidos que se adapten a las preferencias de los consumidores. Por último, la aplicación de métodos rápidos para el análisis del aroma de los productos cárnicos es de gran importancia para controlar su proceso de elaboración.

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II. Antecedentes bibliográficos

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1. Embutidos crudos curados

Tanto la fermentación como el curado son métodos que tradicionalmente se han utilizado para prolongar la vida útil de los alimentos. Los alimentos fermentados se definen como aquellos que han sido sometidos a la acción de microorganismos o enzimas que producen cambios bioquímicos responsables de modificaciones significativas en el alimento (Lücke, 1994). La conservación de la carne mediante técnicas de fermentación se remonta a hace más de 2500 años (Zeuthen, 2007) ya que en China se elaboraban embutidos en estado crudo. Más tarde, la elaboración de embutidos crudos se extendió a Europa, donde la tradición cuenta con aproximadamente 2000 años. Por otro lado, el curado es una técnica de conservación de alimentos basada en la adición combinada de sal y agentes de curado (nitrato y/o nitrito) (Honikel, 2007). El término curado se aplica a numerosos productos cárnicos, aunque su significado varía según el país. En el norte de Europa se denomina "curado" a cualquier producto que ha sido tratado con sal y nitrito. En cambio, en el área mediterránea, los productos curados son aquellos a los que se les han adicionado agentes de curado y que han sido sometidos a un proceso de maduración y secado (Flores, 1997).

Las técnicas de fermentación y curado se utilizan de manera complementaria en la fabricación de los embutidos crudos curados, obteniéndose un producto estable y con escaso riesgo microbiológico (Ordoñez & de la Hoz, 2007). Así pues, los embutidos crudos curados pueden definirse como productos cárnicos obtenidos tras selección y picado de carnes magra y grasas, a las cuales se les han adicionado sales de curado (nitrito y/o nitrato sódico o potásico), ascorbato o isoascorbato sódico o potásico, azúcares como dextrosa o sacarosa, especias, y cultivos iniciadores o no, y que han sido embutidos y han sufrido un proceso de secado-maduración que puede estar precedido o no de una etapa de estufaje o de ahumado. En la actualidad, debido al desarrollo de otros métodos de conservación de la carne (refrigeración, congelación, tratamientos térmicos, etc.) la finalidad de la aplicación de técnicas de fermentación y curado no se limita a la preservación de la carne sino que constituye una forma de transformación y diversificación de productos.

1.1. Fabricación de embutidos crudos curados

El proceso de fabricación de los embutidos comprende una serie de pasos generales que se resumen en la **Figura 1**. Tal y como se indica, el proceso puede sufrir modificaciones que son determinantes sobre la calidad del embutido obtenido, como se explica con mayor detalle en el apartado 1.2.

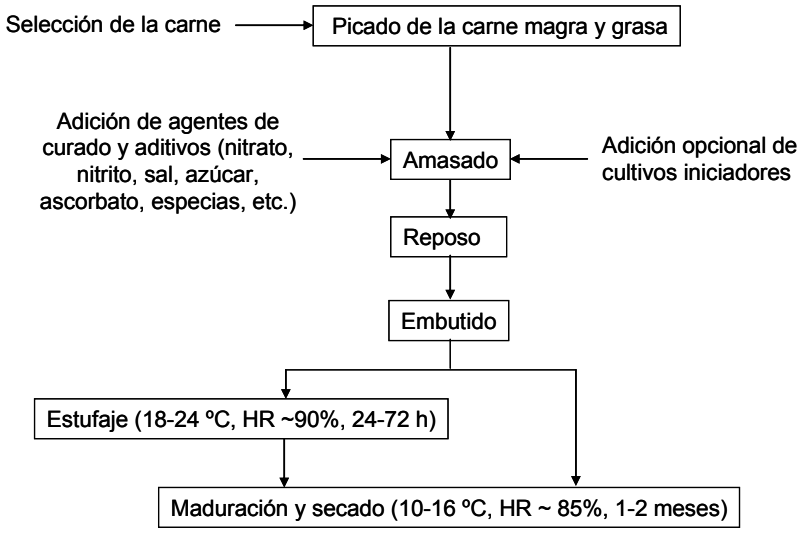

Figura 1. Diagrama de flujo de la elaboración de los embutidos crudos curados (adaptado de Toldrá & Flores, 2007).

En primer lugar, se selecciona la carne magra (generalmente de cerdo y/o vacuno) y la grasa (de cerdo debido a su punto de fusión). El control de la carga microbiana inicial de la carne es fundamental para evitar problemas tecnológicos (Solignat, 2002). La carne y la grasa se pican a baja temperatura (-2 ºC) para evitar la ruptura del glóbulo graso y que se forme una masa pegajosa (embarrado). A continuación, la carne picada se amasa a vacío junto al resto de ingredientes para evitar el contacto con el oxígeno. La masa cárnica así obtenida se mantiene en reposo a 0 ºC durante 24 h antes de embutir con el objetivo de que la flora responsable de la maduración se adapte al medio. Además, durante el reposo tiene lugar la ligazón de la masa cárnica debido a la solubilización de las proteínas por efecto de la adición de sal (Solignat,

2002). La siguiente etapa es el embutido de la masa, que puede realizarse en tripas, naturales o artificiales, permeables al agua. A continuación puede llevarse a cabo la etapa de estufaje (denominada fermentación en la industria cárnica) la cual tiene como objetivo estimular el proceso fermentativo, favoreciendo las condiciones de crecimiento de las bacterias ácido-lácticas (Flores, 1997). Normalmente se utilizan temperaturas entre 18 y 24 ºC durante 1-3 días y se controla la humedad relativa (HR < 90%) con el fin de evitar condensaciones sobre la superficie del embutido y deshidrataciones excesivas de la tripa. Finalmente, durante la etapa de maduración-secado se produce la deshidratación del embutido que es esencial para conseguir la textura de estos productos. Las condiciones de secado dependen de múltiples variables como el tipo y calibre de embutido. Generalmente el secado se realiza a temperaturas del orden 10-16 ºC y con humedades relativas decrecientes entre 90 y 75% durante 30-60 días.

Durante las etapas de estufaje y maduración-secado se producen reacciones químicas y enzimáticas, éstas últimas de origen endógeno y microbiano, que dan lugar al aroma y sabor característico de estos productos (Toldrá *et al.*, 2001). A su vez, se desarrolla una secuencia de barreras (conocido como efecto "hurdle") para el crecimiento bacteriano que asegura la estabilidad microbiológica de los embutidos crudos curados (Leistner, 1992). En primer lugar, el nitrito es determinante para la inhibición de microorganismos patógenos, especialmente *Clostridium botulinum*. En segundo lugar, el potencial de oxidorreducción disminuye rápidamente, lo que acentúa el efecto de los iones nitrito y favorece el desarrollo de las bacterias ácido-lácticas. Además, éstas últimas fermentan los azúcares generando ácido láctico y el pH disminuye hasta 4.5-5.0, valores muy cercanos al punto isoeléctrico de las proteínas. De esta forma, las proteínas pierden la capacidad de retención de agua y se favorece el secado del embutido. El resultado es un producto loncheable, microbiológicamente estable y que no necesita refrigeración para su conservación.

1.1.1. Efecto de los agentes de curado

Tal y como se ha mencionado anteriormente, los productos curados son aquellos a los que se han adicionado sal y nitrificantes (nitrato y/o nitrito).

El objetivo fundamental de la adición de nitrito es bacteriostático, esencialmente porque no existen buenas alternativas para inhibir la formación de la toxina botulínica. Además, el nitrito participa en el desarrollo del color característico de los embutidos debido a la formación del complejo nitrosomioglobina entre la mioglobina y el óxido nítrico derivado de la reducción del nitrito (Honikel, 2007). Asimismo, el nitrito contribuye a la generación del aroma típico a producto cárnico curado. Por último, el efecto antioxidante del nitrito retrasa el enranciamiento por oxidación. Por otro lado, en los embutidos de larga maduración, el nitrato se añade como reservorio de nitrito, ya que a pH superiores a 5.4 se produce la reducción del nitrato a nitrito por acción de las enzimas nitrato-reductasas bacterianas de los estafilococos coagulasa negativo (Toldrá *et al.*, 2001). Además, el uso de nitrato produce un aumento de la calidad sensorial de los embutidos no fermentados (apartado 1.2.), aunque también se ha observado que no hay diferencias entre el uso de nitrato o nitrito en embutidos fermentados (sometidos a estufaje) (Marco *et al.*, 2008).

El uso de nitratos y nitritos ha sido objeto de polémica en los últimos años debido al riesgo de formación de nitrosaminas, a las que se atribuyen efectos cancerígenos. Las nitrosaminas se forman por reacción de nitrito con aminas secundarias, favorablemente a altas temperaturas. Sin embargo, las dosis residuales de nitratos y nitritos en embutidos curados son extremadamente bajas en el producto final (aproximadamente un 5% de los contenidos iniciales) (Solignant, 2002) y el riesgo de la formación de nitrosaminas es pequeño comparado con los efectos positivos que ejercen las sales de curado (Honikel, 2007).

En cuanto a la sal, ésta ejerce distintos efectos en los embutidos crudos curados. Brevemente, es responsable del sabor salado, contribuye a la solubilización de las proteínas, a la capacidad de retención de agua y a la reducción de la actividad de agua (Ruiz, 2007).

1.1.2. Efecto de los microorganismos

Los microorganismos implicados en la fermentación y maduración de los embutidos pueden provenir de la propia carne o haber sido adicionados como cultivos iniciadores

o starters. En la **Tabla 1** se resumen los principales grupos microbianos que participan en el proceso y su efecto sobre los embutidos.

Tabla 1. Microbiota presente en la fermentación y secado-maduración de embutidos crudos curados.

Grupo microbiano	Especie	Actividad metabólica	Efecto sobre el embutido
Bacterias ácido-lácticas	*Lactobacillus sakei, L. curvatus, L. plantarum, L. pentosus, Pediococcus pentosaceus*	-Formación de ácido láctico -Producción de bacteriocinas	-Selección microbiana e inhibición de bacterias patógenas -Gelificación de las proteínas miofibrilares por acidificación y posterior pérdida de retención de agua
Estafilococos coagulasa negativa (CNS)	*Staphylococcus xylosus, S. carnosus, S. saprophyticus, S. equorum, Kocuria varians*	-Reducción del nitrato -Destrucción de peróxidos -Lipólisis, proteólisis y formación de ésteres	-Formación del color y generación del aroma
Levaduras	*Debaryomyces hansenii, Candida fumata*	-Destrucción de peróxidos -Lipólisis	-Estabilización del color y generación del aroma
Mohos	*Penicillium nalgiovense, P. chrysogenum*	-Actividad desaminasa -Consumo de ácidos orgánicos -Destrucción de peróxidos -Lipólisis	-Neutralización de la acidez -Estabilización del color y generación del aroma

En las primeras etapas de la fabricación tiene lugar una selección microbiana que se explica en gran medida por la teoría de las barreras de Leistner (1992) previamente mencionada. Las condiciones del medio (la presencia de nitrito, una menor actividad de agua debido a la disolución de la sal y los azúcares) favorecen la implantación de

bacterias de las familias *Lactobacillaceae* y *Micrococaceae*, mayoritariamente los géneros *Lactobacillus* y *Staphylococcus* respectivamente, y la inhibición del resto de la flora inicial de la carne, como *Pseudomonas, Enterobacteriaceae*, o *Enterococcus* (Ordoñéz & de la Hoz, 2007). Las bacterias ácido-lácticas (*Lactobacillus*) fermentan los azúcares y acidifican el medio y además producen bacteriocinas (Hugas, 1998) lo que a su vez contribuye a la selección microbiana (Garriga & Aymerich, 2007). El nivel de acidificación alcanzado depende de la cantidad y tipo de azúcares adicionados (simples o complejos).

Además de la selección microbiana, la acidificación ejerce otros efectos muy importantes en la fabricación de los embutidos crudos curados (Ordoñez *et al.*, 1999). Por un lado, la bajada del pH origina una desnaturalización de las proteínas cárnicas y éstas coagulan, lo que da lugar a la formación de un gel proteico que contiene los gránulos de grasa. Por otro lado, el valor de pH alcanzado es muy próximo al punto isoeléctrico de las proteínas (carga eléctrica neutra) por lo que éstas pierden su capacidad de retención de agua, favoreciéndose así el secado del producto. Estos procesos son los responsables de la textura y consistencia del producto final.

Con respecto a las bacterias del género *Staphylococcus*, en el embutido las especies presentes son coagulasa negativo (CNS) (Garriga & Aymerich, 2007). Estas bacterias además de reducir el nitrato a nitrito, contribuyen a la descomposición de peróxidos formados por las bacterias ácido-lácticas, previniendo así la actividad oxidativa y sus efectos negativos sobre el color y el aroma. Asimismo, los estafilococos contribuyen a la formación de los compuestos volátiles responsables del aroma a través de sus actividades lipolíticas, proteolíticas y esterasa (Toldrá *et al.*, 2001; Flores & Toldrá, 2010).

Por último, hongos y levaduras también forman parte de la población microbiana, aunque su crecimiento tiene lugar principalmente en el exterior del embutido sobre la tripa debido a su metabolismo aeróbico. Generalmente se trata de mohos del género *Penicillium* y levaduras del género *Debaryomyces* que contribuyen a la calidad sensorial de los embutidos como resultado de sus actividades metabólicas (Durá, 2003; Durá *et al.*, 2004). Los mohos presentan actividad desaminasa produciendo amoníaco y además consumen ácidos orgánicos como el ácido láctico, neutralizando

así la acidez del embutido (Garriga & Aymerich, 2007). Además hongos y levaduras presentan actividad catalasa y ejercen acciones lipolíticas que afectan a la calidad final del embutido (Flores & Toldrá, 2010).

1.1.3. Efecto de la grasa

El contenido inicial de la masa cárnica típicamente es del 30%, aunque este valor aumenta hasta aproximadamente 45% debido a la pérdida de agua durante el secado (Wirth, 1988). La grasa proviene mayoritariamente de la panceta de cerdo, constituida mayoritariamente por triglicéridos, aunque el magro también contiene grasa intramuscular. Concretamente, el magro contiene un 1.5-4% de grasa, de la cual un 60-80% son triglicéridos y un 16-34% son fosfolípidos (Schweigert, 1994). La grasa de los embutidos crudos curados, además de contribuir al valor nutricional debido al aporte de ácidos grasos esenciales y vitaminas liposolubles, cumple un papel fundamental desde el punto de vista tecnológico y sensorial. En primer lugar, los gránulos de grasa repartidos por la matriz cárnica ayudan a controlar el secado de los embutidos, disminuyendo el proceso de deshidratación (Wirth, 1988). Además, la grasa contribuye a la textura y jugosidad del producto. Por último, la grasa participa en el desarrollo del aroma característico, como se explica con mayor detalle en el apartado 2.

Sin embargo, un consumo excesivo de grasa, especialmente de grasa saturada, está relacionado con la prevalencia de enfermedades cardiovasculares, obesidad, etc. Por este motivo, se han llevado a cabo distintas estrategias para reducir el contenido de grasa de los embutidos y/o mejorar el perfil nutricional de la grasa (Mendoza *et al.*, 2001; Muguerza *et al.*, 2002; Muguerza *et al.*, 2003; Koutsopoulos *et al.*, 2008; Liaros *et al.*, 2009; Del Nobile *et al.*, 2009; Salazar *et al.*, 2009). Sin embargo, dada la importancia de la grasa en los embutidos crudos curados, la reducción de su contenido plantea problemas desde el punto de vista tecnológico, por ejemplo excesiva pérdida de humedad (Keeton, 1994). Generalmente, los embutidos con menores contenidos de grasa son peor valorados por los consumidores y frecuentemente se han descrito defectos en la calidad organoléptica (apariencia, textura, etc.) (Mendoza *et al.*, 2001; Muguerza *et al.*, 2002).

1.2. Tipos de embutidos crudos curados según la tecnología de fabricación

Los embutidos pueden ser clasificados atendiendo a distintos criterios, tales como la acidez, el grado de picado de los ingredientes, la adición o no de cultivos iniciadores, el diámetro y tipo de tripa empleada, etc. Flores (2001) propuso una clasificación de los embutidos basada en el tipo de fabricación desde el punto de vista tecnológico. En primer lugar, distingue entre embutidos industriales y embutidos tradicionales dependiendo de si se han adicionado o no cultivos iniciadores, respectivamente. Además, diferencia dos tipos de embutidos industriales según la realización o no de la etapa de estufaje; los embutidos fermentados y los no fermentados, tal y como se indica en la **Figura 2**.

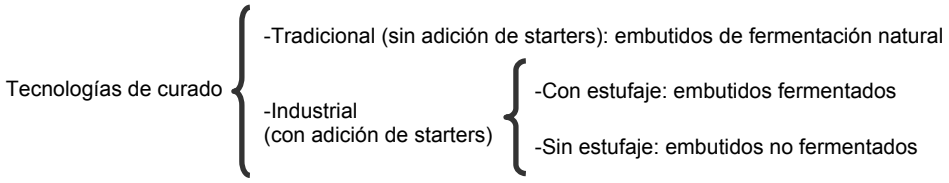

Figura 2. Tipos de embutidos según las de tecnologías de curado (basado en Flores, 2001).

1.2.1. Embutidos tradicionales o de fermentación natural

La fermentación natural es la tecnología que tradicionalmente se ha empleado para la fabricación de embutidos crudos curados aunque actualmente sólo es usada por empresas artesanales. Los métodos artesanales de elaboración están frecuentemente ligados a la historia y la cultura de la zona geográfica, presentado una gran variabilidad en cuanto a materias primas y parámetros de secado, lo que se traduce en una gran variedad de productos con unas características organolépticas singulares que son muy apreciados por los consumidores (Conter *et al.*, 2008). Estas tecnologías de curado lento o artesanal están basadas en la fermentación espontánea de la masa cárnica mediante la intervención de su flora microbiana autóctona, es decir, sin inoculación de cultivos iniciadores, denominándose el producto obtenido embutidos de fermentación natural (en inglés naturally fermented sausages) o embutidos tradicionales. Cuando las condiciones climáticas lo permiten, la fermentación y

maduración-secado se llevan a cabo en secaderos naturales, no siendo consideradas éstas dos etapas diferenciadas. Normalmente, la acidificación alcanza un valor de pH superior a 5 (Navarro *et al.*, 2001; Comi *et al.*, 2005; Aymerich *et al.*, 2003; Lebert *et al.*, 2007) por lo tanto se trata de embutidos crudos curados de baja acidez.

Diversos estudios se han llevado a cabo para identificar la flora endógena responsable de la fermentación espontánea de la carne (Talon *et al.*, 2007). Las bacterias ácido-lácticas constituyen la mayor parte de la flora, predominando las especies *L. sakei* y *L. curvatus* (Comi *et al.*, 2005; Aymerich *et al.*, 2003). Además, se han aislado bacterias del género *Staphylococcus*, principalmente *S. xylosus*, y en menor proporción mohos y levaduras.

Sin embargo, la fabricación mediante tecnologías de curado lento es extremadamente delicada y plantea serios problemas de control. En este sentido, Talon *et al.* (2008) desarrollaron un cultivo iniciador aislado de embutidos de fermentación natural con el objetivo de controlar la seguridad del producto a la vez que se preservan las características sensoriales típicas de los embutidos tradicionales.

1.2.2. Embutidos industriales

Los embutidos industriales se fabrican mediante tecnologías de curado rápido. Éstas se distinguen de las tecnologías de curado lento en la adición de cultivos microbianos iniciadores con el fin de asegurar la calidad y la homogeneidad del producto y acelerar el proceso de elaboración. Los cultivos iniciadores tienen que cumplir una serie de requisitos: deben ser no patógenos, no tóxicos o alergénicos, competitivos ante la flora autóctona bajo las condiciones típicas del proceso, tolerantes a la sal y a los nitrificantes, homofermentativos y no producir sabores anómalos (Toldrá *et al.*, 2001).

Dentro de las tecnologías de curado rápido que emplean cultivos iniciadores se distinguen dos tipos de embutidos en función de la realización o no de la etapa de estufaje, los fermentados y los no fermentados (Flores, 2001). En la literatura científica es común denominar embutidos de fermentación rápida a los embutidos que han sufrido etapa de estufaje, mientras que los embutidos no fermentados (sin estufaje) se corresponden con los embutidos de fermentación lenta.

1.2.1.1. Embutidos fermentados

Son aquellos embutidos a los que se les han adicionado cultivos iniciadores y que han sufrido una etapa de estufaje que acelera el inicio de la fermentación. Por esta razón, en la industria cárnica, la etapa de estufaje se denomina fermentación. Las condiciones de estufaje dependen de los microorganismos incorporados y de la velocidad e intensidad de acidificación que se desee alcanzar. En general, la industria cárnica distingue dos tipos de estufaje: estufaje largo (condiciones constantes durante un tiempo prolongado, por ejemplo 18 y 24 ºC durante 1-3 días) y estufaje corto (ciclos de estufaje seguidos de reposo, hasta un total de 72 h de fases calientes) (Solignant, 2002). Estas condiciones, junto a la adición de azúcares, favorecen el crecimiento de las bacterias ácido-lácticas. La bajada del pH alcanza valores inferiores a 5 y garantiza la seguridad del producto, siendo especialmente importante en embutidos de grueso calibre, los cuales son más difíciles de secar. Sin embargo una acidificación demasiado rápida puede tener efectos negativos. Por un lado, puede afectar a la formación del color al inhibir a las bacterias CNS (Toldrá *et al.*, 2001). Por otro, la acidificación intensa conduce a una pérdida de características sensoriales típicas debido al desarrollo de un excesivo sabor ácido que no es aceptado por los consumidores (Navarro *et al.*, 2001).

1.2.1.2. Embutidos no fermentados

El término embutidos no fermentados no es muy correcto desde el punto de vista de la ciencia de alimentos, puesto que los embutidos crudos curados siempre sufren un proceso de fermentación. Sin embargo, en este caso, el proceso de fermentación que llevan a cabo los cultivos iniciadores no está ligado a una operación de estufaje, razón por la cual la industria cárnica los considera como embutidos no fermentados. El objetivo de la supresión del estufaje es evitar una acidificación intensa que resulte en un excesivo sabor ácido (Navarro *et al.*, 2001). Así pues, el proceso de fabricación consta de una primera fase en la que se aplican bajas temperatura (10 ºC) y posteriormente un secado a temperaturas más elevadas (16-18 ºC) con el fin de acelerar la pérdida de agua y favorecer el desarrollo de las características sensoriales típicas.

Al igual que la mayoría de los embutidos tradicionales, los embutidos no fermentados son productos poco ácidos (pH superior a 5) lo cual puede suponer un riesgo a nivel microbiológico. Sin embargo, a diferencia de los embutidos tradicionales, los cultivos iniciadores que se adicionan en los embutidos no fermentados mejoran la seguridad microbiológica del producto (Garriga *et al.*, 2005).

2. El aroma de los embutidos crudos curados

El aroma es una característica muy importante en la calidad global de los embutidos crudos curados, siendo éste completamente diferente al de la carne procesada mediante tratamiento térmico. El aroma final es el resultado de las reacciones químicas y bioquímicas que ocurren simultáneamente durante los procesos de fermentación y maduración-secado (**Figura 3**).

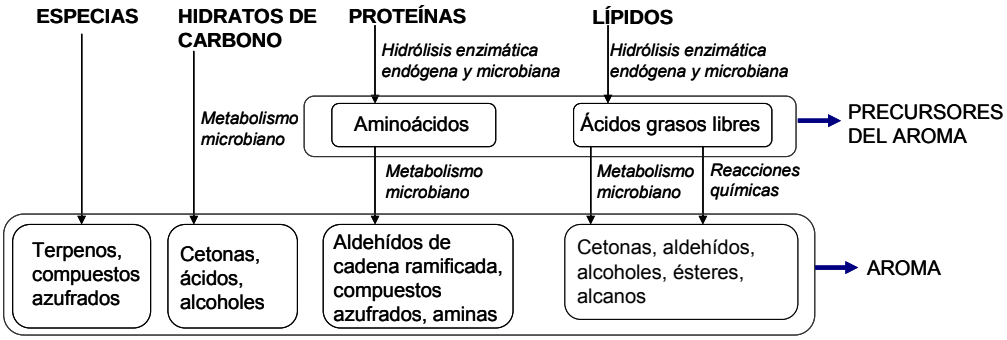

Figura 3. Esquema general de las principales vías de generación de compuestos volátiles durante la fermentación y maduración-secado de embutidos crudos curados (adaptado de Toldrá & Flores, 2007).

La fermentación de los hidratos de carbono, además de originar ácido láctico, conduce a la formación de otros compuestos que tienen impacto sobre el aroma. Por otro lado, los componentes del embutido (proteínas y lípidos) sufren reacciones de hidrólisis enzimática a partir de las cuales se generan los precursores de los compuestos volátiles. Posteriormente, estos precursores actúan como sustratos de reacciones, tanto químicas como del metabolismo microbiano, en las que se forman compuestos

volátiles responsables del aroma (Toldrá *et al.*, 2001). Por último, las especias añadidas también contribuyen al aroma de los embutidos.

2.1. Generación de precursores

Los procesos más importantes de generación de precursores de compuestos volátiles son la hidrólisis de lípidos y proteínas, conocidos como lipólisis y proteolisis respectivamente.

La lipólisis consiste en la hidrólisis enzimática de los lípidos presentes en el músculo y el tejido adiposo, siendo los triglicéridos (TG) la fracción lipídica más abundante en la grasa de ambos tejidos. La hidrólisis enzimática es llevada a cabo por lipasas que rompen los enlaces éster de los TG produciendo di- y monoglicéridos, ácidos grasos libres (AGL) y glicerol. Estas enzimas presentan especificidad por la posiciones 1 y 3 de los TG, donde suelen encontrarse ácidos grasos insaturados (Molly *et al.*, 1996). El origen de las lipasas es tanto endógeno (de la propia carne) como microbiano. Tradicionalmente, se consideraba que la hidrólisis de TG era principalmente debida a la acción de las lipasas microbianas, principalmente de los estafilococos coagulasa negativo (CNS), y en menor medida de mohos y levaduras (Montel *et al.*, 1998). Sin embargo, diferentes estudios llevados a cabo en embutidos no inoculados (Montel *et al.*, 1993) y embutidos elaborados asépticamente (Hierro *et al.*, 1997) concluyeron que entre el 60 y el 80% de la lipólisis se debe a lipasas musculares endógenas. Dado el pH ácido de los embutidos, dentro de las lipasas musculares la lipasa ácida tendría un papel destacado (Toldrá *et al.*, 2001). Por otro lado, los fosfolípidos representan entre el 16 y el 34% de la grasa muscular y son hidrolizados por fosfolipasas de origen muscular dando lugar a AGL poliinsaturados (Toldrá *et al.*, 2001). La liberación de ácidos grasos es más intensa en los fosfolípidos que en los TG si la liberación se expresa en porcentaje con respecto al contenido inicial de ácidos grasos de cada fracción. Sin embargo, en términos absolutos, la mayor parte de los AGL se genera a partir de los TG, ya que éstos representan la fracción grasa más abundante en los embutidos (Molly *et al.*, 1996; Marco *et al.*, 2006).

En embutidos, el contenido inicial de AGL en la masa cárnica es bajo (1-2% del contenido total de ácidos grasos) elevándose durante el procesado hasta valores del

5%, siendo especialmente importante el incremento de ácidos grasos libres insaturados (Molly *et al.*, 1996; Zanardi *et al.*, 2004) debido a la especificidad de la lipasa por las posiciones 1 y 3 de los TG y a la rotura de fosfolípidos, anteriormente mencionadas.

Diversos estudios se han llevado a cabo para determinar el efecto de las condiciones del proceso de fabricación en la intensidad de la lipólisis en embutidos crudos curados. Zanardi *et al.* (2004) concluyeron que la lipólisis se debe principalmente a la acción de las lipasas endógenas y que ésta depende en mayor medida de las variaciones en actividad lipolítica de la materia prima cárnica usada que de las condiciones del proceso de fabricación (agentes de curado, especias, cultivos iniciadores, pH, etc.). Sin embargo, otros autores han descrito efectos significativos de distintos parámetros del proceso (pH, contenido de sal, actividad de agua, temperatura, etc.) sobre la cantidad de ácidos grasos liberados (Toldrá, 2008). Por ejemplo, Marco *et al.* (2006) y Navarro *et al.* (2001) hallaron un mayor grado de lipólisis en embutidos con nitrito que en embutidos con nitrato. Además, Gandemer (2002) indicó que a mayor tiempo de proceso y mayor temperatura aplicada, más intenso es el proceso de lipólisis.

Por otro lado, la proteolisis consiste en la hidrólisis enzimática de proteínas sarcoplásmicas y miofibrilares por parte de proteasas (endopeptidasas y exopeptidasas). En una primera etapa, las endopeptidasas generan polipéptidos que posteriormente son degradados a péptidos y aminoácidos libres por acción de las exopeptidasas. El origen de las proteasas es tanto endógeno (de la propia carne) como microbiano. Sin embargo, al igual que ocurre con la lipólisis, la contribución de la actividad hidrolítica de las enzimas microbianas es menor que la de las enzimas musculares (Verplaetse, 1992). El 40% de la proteolisis se ha atribuido a los microorganismos, principalmente debida a las bacterias CNS, ya que la actividad proteolítica de las bacterias ácido-lácticas se inhibe a pH ácido (Montel *et al.*, 1998). De esta manera, la degradación de proteínas en péptidos es el resultado de la acción de endopeptidasas musculares con actividad óptima a pH ácido y de endopeptidasas extracelulares microbianas. Mientras que la degradación de péptidos en aminoácidos se debe a la acción conjunta de algunas exopeptidasas musculares, como las

aminopeptidasas y dipeptidilpeptidasas, y exopeptidasas bacterianas (Toldrá *et al.*, 2001; Ordoñez & de la Hoz, 2007; Flores & Toldrá, 2010).

2.2. Reacciones involucradas en la generación de compuestos volátiles

Las reacciones a partir de las cuales se generan los compuestos volátiles responsables del aroma de los embutidos crudos curados se pueden dividir en dos tipos: reacciones químicas y reacciones del metabolismo microbiano.

2.2.1. Reacciones químicas

La reacción química de mayor importancia en el procesado de embutidos crudos curados es el proceso de autooxidación de la grasa que afecta a los ácidos grasos liberados, especialmente a los poliinsaturados (Gandemer, 2002). Este proceso es una reacción en cadena que consta de tres etapas (Frankel, 1984) que se resumen en la **Figura 4**.

En la primera o iniciación tiene lugar la transferencia de un radical de hidrógeno adyacente a un doble enlace de un ácido graso (RH) debido a un agente iniciador (X), generándose un radical libre ($R^{\bullet}$). En la segunda etapa o propagación, el radical libre formado ($R^{\bullet}$) reacciona con el oxígeno para dar un radical peroxilo muy reactivo ($ROO^{\bullet}$), que a su vez puede actuar sobre otro ácido graso insaturado (RH), abstrayéndole un radical de hidrógeno e iniciando así de nuevo la cadena de propagación. Además, durante esta fase se forma una elevada cantidad de hidroperóxidos (ROOH) que se descomponen en radicales libres. En la tercera etapa o terminación, la reacción en cadena se interrumpe cuando los radicales libres se combinan y se forman productos inactivos y los hidroperóxidos se descomponen en productos no radicalarios.

La descomposición de los hidroperóxidos ha sido ampliamente estudiada debido a que se forman una gran variedad de compuestos volátiles como alcanos, aldehídos, alcoholes, ésteres y ácidos carboxílicos (Frankel, 1984). Algunos de ellos presentan bajos umbrales de percepción y por lo tanto tienen un gran impacto sobre el aroma (Shahidi *et al.*, 1986). Por esta razón, aunque la oxidación lipídica constituye una de las principales causas de deterioro de la calidad de los alimentos, es esencial para el

desarrollo del aroma de los embutidos crudos curados (Gandemer, 2002). La oxidación lipídica también es llevada a cabo por los microorganismos como se explicará más adelante en el apartado 2.2.2.3.

1) Iniciación

$RH + X^{\bullet} \rightarrow R^{\bullet} + XH$

2) Propagación

$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$

$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$

$ROOH \rightarrow RO^{\bullet} + HO^{\bullet}$

$2\ ROOH \rightarrow ROO^{\bullet} + RO^{\bullet} + H_2O$

3) Terminación

$ROO^{\bullet} + ROO^{\bullet} \rightarrow$ Productos inactivos

$ROOH \rightarrow$ Productos de descomposición

Figura 4. Etapas de la autooxidación lipídica.

Existen varios métodos para la evaluación del estado oxidativo de los productos cárnicos entre los que destacan el índice de peróxidos y el ensayo de las sustancias reactivas al ácido tiobarbitúrico (TBARS). El índice de peróxidos se basa en la medida de los hidroperóxidos formados durante las primeras etapas de la autooxidación lipídica. El carácter transitorio de estos productos es la principal limitación de este método, ya que los niveles de hidroperóxidos aumentan durante las primeras etapas de la fabricación de productos cárnicos y después disminuyen, por lo tanto es difícil determinar el momento en que éstos alcanzan el nivel máximo (Fernández *et al.*, 1997). Por otro lado, el TBARS se basa en la reacción colorimétrica del producto secundario de la oxidación lipídica malondialdehído (MDA) con el ácido tiobarbitúrico. Este método es el más utilizado para la evaluación del estado oxidativo de la carne y

los productos cárnicos (Shahidi, 1994) aunque también presenta desventajas, por ejemplo pueden producirse interferencias en la reacción colorimétrica por la presencia de otros componentes como azúcares o proteínas, o incluso nitritos. No obstante, el TBARS ha sido positivamente correlacionado con los análisis sensoriales de evaluación del enranciamiento (Shahidi, 1994; Campo *et al.*, 2006).

Por otro lado, durante el procesado de los embutidos crudos curados pueden desarrollarse otras reacciones químicas como la degradación de Strecker (Stanhke, 1994; Ordoñez & de la Hoz, 2007). Esta degradación es una de las vías de la reacción de Maillard, y consiste en la desaminación oxidativa y posterior descarboxilación de un aminoácido en presencia de un compuesto dicarbonilo (por ejemplo el diacetilo) generándose un aldehído. De esta manera, a partir de los aminoácidos ramificados valina, isoleucina y leucina se forman los compuestos volátiles 2-metil propanal, 2-metil butanal y 3-metil butanal, respectivamente (Ordoñez *et al.*, 1999). Las condiciones de pH y temperatura durante el procesado de embutidos limitan las posibilidades de que esta reacción química tenga lugar. Por el contrario, la baja actividad de agua, los tiempos largos de curado y la generación de aminoácidos libres y compuestos dicarbonilo la favorecen. En este sentido, la detección de productos secundarios de la reacción de Maillard como las pirazinas en embutidos parece indicar que esta reacción tiene lugar durante su procesado (Stahnke, 2002). No obstante, la degradación de los aminoácidos también se debe al metabolismo microbiano (apartado 2.2.2.2).

2.2.2. Reacciones del metabolismo microbiano

Las reacciones del metabolismo se pueden dividir en cuatro grupos: fermentación de los hidratos de carbono, degradación de los aminoácidos, β-oxidación lipídica y actividad esterasa de los estafilococos.

2.2.2.1. Fermentación de los hidratos de carbono

Los hidratos de carbono constituyen la fuente de energía básica que emplea la flora microbiana para crecer. El catabolismo de estos azúcares en la masa cárnica tiene lugar de manera anaerobia por las bacterias ácido-lácticas, por lo tanto se trata de un

proceso de fermentación. Fundamentalmente, la fermentación sigue la vía homofermentativa o vía de la glucólisis, de manera que el producto principal es el ácido láctico, el cual afecta al sabor de los embutidos. Sin embargo, también puede darse una fermentación heterofermentativa, ya sea por vía heterofermentativa tipo por acción de bacterias heterolácticas como *Lactobacillus fermentum* o *Leuconostoc lactis*, la vía alcohólica por acción de levaduras, o la vía de los ácidos mixtos por las enterobacterias (Solignat, 2002). En estos casos, además de ácido láctico, se producen otros compuestos que tienen impacto en el aroma, como diacetilo, acetaldehído, acetoína, etanol, o ácidos grasos de cadena corta como fórmico, acético, propiónico o butanoico (Toldrá, 2008).

2.2.2.2. Degradación de los aminoácidos

Los aminoácidos libres, además de ser degradados mediante la reacción química de Strecker, pueden ser catabolizados por la flora microbiana presente, principalmente por los estafilococos coagulasa negativo y *Debaryomyces hansenii,* generándose así compuestos volátiles de gran impacto sobre el aroma (Montel *et al.*, 1998). Por un lado, la degradación de la cadena lateral de los aminoácidos tirosina y triptófano da lugar a la formación de fenol e indol. En el caso de los aminoácidos azufrados cisteína y metionina, se generan compuestos volátiles azufrados con límites de detección muy bajos, que aportan notas desagradables en el aroma. Sin embargo, la degradación de los aminoácidos por parte de los microorganismos también conduce a la formación de compuestos que imparten notas positivas al aroma (**Figura 5**). Una de las numerosas vías metabólicas que pueden darse lugar es la degradación vía transaminación y descarboxilación de los aminoácidos ramificados valina, isoleucina y leucina, a partir de los cuales se generan aldehídos ramificados (Ordoñez & de la Hoz, 2007). Otros aminoácidos como la fenilalanina, cisteína, metionina y treonina también son transformados en aldehídos. A su vez estos aldehídos, tanto los ramificados como los lineales, pueden ser transformados en ácidos, alcoholes y ésteres.

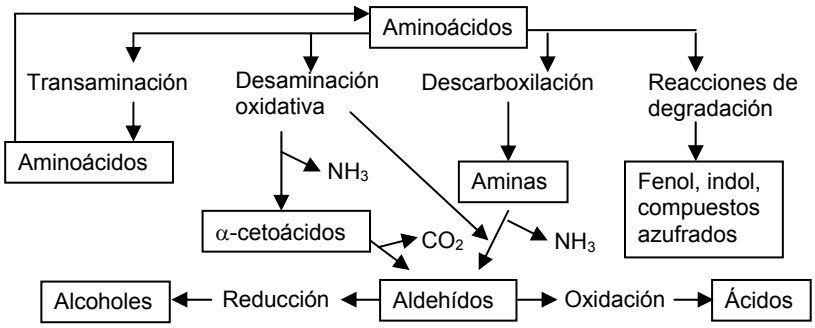

Figura 5. Generación de compuestos volátiles a partir de los aminoácidos (vías químicas y bioquímicas), adaptado de Toldrá *et al.* (2001).

2.2.2.3. β-oxidación lipídica

Los ácidos grasos además de ser degradados mediante autooxidación lipídica (reacción química) también pueden ser oxidados por los sistemas enzimáticos microbianos, dando lugar a ácidos grasos de cadena corta y β-cetoácidos. Los β-cetoácidos son transformados en metil-cetonas, como la 2-pentanona y la 2-heptanona, mediante descarboxilación, y a su vez éstas pueden ser reducidas a alcoholes secundarios como el 1-octen-3-ol (Montel *et al.*, 1998). La producción de metil-cetonas se ha atribuido a bacterias del género *Staphylococcus* y a levaduras del género *Penicillium* (Demeyer & Stahnke, 2002).

2.2.2.4. Actividad esterasa de los estafilococos

Los ésteres se generan por la reacción entre un ácido y un alcohol mediada por las esterasas de *Staphylococcus*. La intensidad de la actividad esterasa depende de la presencia de sustrato en el medio así como de la especie de estafilococo. En este sentido, en sistemas modelo, *S. carnosus* y *S. xylosus* mostraron mayor producción de ésteres que *S. warneri* (Montel *et al.*, 1998). Además, parece ser que las mismas esterasas que forman ésteres son responsables de la hidrólisis de éstos (Talon *et al.*, 1998). La mayoría de los ésteres identificados en embutidos son etil ésteres, es decir, son productos generados mediante la esterificación de un ácido con el etanol (Stahnke, 2002).

2.3. Especias y otros condimentos

Las especias son ingredientes vegetales que se utilizan en pequeñas cantidades (0.5-2%) para conferir determinados sabores, aromas y colores a los productos cárnicos. Las especias más comúnmente adicionadas a los embutidos son la pimienta negra, el pimentón, ajo, cebolla, mostaza, orégano, semillas de anís, etc. Los fabricantes de embutidos añaden las especias individualmente o bien usan preparados comerciales de especias con formulaciones específicas para sus propios productos (Chi & Wu, 2007). Cada especia imparte una nota característica al aroma de los embutidos, aunque en ocasiones un mismo aroma puede provenir de distintas especias. Por ejemplo, la adición de pimienta es responsable del contenido de monoterpenos, mientras que la adición de ajo proporciona un elevado contenido en compuestos azufrados (Ordoñez & de la Hoz, 2007). Por otro lado, se ha descrito el poder antioxidante de las especias en los embutidos (Mateo & Zumalacárregui, 1996), sin embargo Zanardi *et al.* (2004) indicaron que la acción antioxidante sólo es significativa si se emplea una cantidad de especias superior a la habitualmente adicionada (4%).

2.4. Factores que afectan a la generación de compuestos volátiles

Las reacciones responsables de la generación de compuestos volátiles pueden verse afectadas por un gran número de parámetros: condiciones del proceso, sales de curado, flora microbiana, materias primas, etc. (Demeyer & Stahnke, 2002).

2.4.1. Condiciones del proceso

Dentro de las condiciones del proceso, tanto la adición o no de cultivos iniciadores como la realización o no de la etapa de estufaje afectan tanto al tipo como a la cantidad de compuestos volátiles presentes. En relación a los cultivos iniciadores, se han llevado a cabo múltiples estudios para conocer el efecto de éstos en la generación de compuestos volátiles (Berdagué *et al.*, 1993; Ansorena *et al.*, 2000; Olesen *et al.*, 2004). Sin embargo, existe poca información acerca del perfil de compuestos volátiles de los embutidos a los que no se les han adicionado cultivos iniciadores. Además, en ocasiones no queda claramente reflejado si éstos han sido o no adicionados a los embutidos (Schmidt & Berger, 1998a; Bianchi *et al.*, 2007). En embutidos de

fermentación natural, Croizet *et al.* (1992) detectaron compuestos derivados tanto de las especias como de reacciones químicas y bioquímicas, mientras que Spaziani *et al.* (2009) observaron que los compuestos volátiles provenían fundamentalmente de las especias. Otros trabajos compararon el perfil de compuestos volátiles en embutidos con y sin adición de cultivos iniciadores y concluyeron que en general, los embutidos a los que se les han adicionado cultivos iniciadores presentaron mayores contenidos de alcoholes, ácidos, aldehídos, cetonas y ésteres (Schmidt & Berger, 1998b), mientras que los embutidos de fermentación espontánea se caracterizaron por una mayor proporción de terpenos (Di Cagno *et al.*, 2008) y compuestos azufrados (Mateo & Zumalacárregui, 1996).

Por otro lado, otros estudios se han centrado en determinar el efecto del estufaje sobre la fracción volátil. De esta forma, los embutidos que han sido sometidos a la etapa de estufaje (18-24ºC durante 1-3 días y HR <90%) presentan mayores niveles de ácidos y menores de ésteres, aldehídos de cadena ramificada y productos derivados de la oxidación lipídica (Stahnke, 1995), aunque Marco *et al.* (2008) observaron mayores niveles de productos de la oxidación lipídica en embutidos con estufaje. El empleo de altas temperaturas durante las primeras etapas del proceso afecta al metabolismo de los microorganismos. En general, puede concluirse que si se lleva a cabo la etapa de estufaje, la acidificación es más rápida e intensa y esto disminuye el crecimiento y metabolismo de las bacterias coagulasa negativo y por lo tanto dificulta su contribución al aroma (Ravyts *et al.*, 2010).

2.4.2. Agentes de curado

En relación al efecto de la adición de nitrato y/o nitrito en la generación de compuestos volátiles, el uso de nitrato origina mayores niveles de ésteres, productos de la degradación de aminoácidos y de la fermentación de carbohidratos (Stahnke, 1995; Olesen *et al.*, 2004; Marco *et al.*, 2006). Sin embargo, no parece claro el efecto de los agentes de curado en la generación de compuestos volátiles derivados de la oxidación lipídica. Por un lado, Stahnke (1995) observó mayores niveles de estos compuestos en los embutidos con nitrato mientras que Marco *et al.* (2006) en los embutidos con nitrito. Por otro lado, Marco *et al.* (2008) indicaron que el efecto de las sales de curado

depende en gran parte del tipo de proceso, siendo sólo apreciable en los procesos de fermentación lenta, es decir, cuando no se ha aplicado estufaje.

Con respecto a la sal, ésta actúa liberando los compuestos volátiles de la matriz, ya que modifica la fuerza iónica del medio y disminuye la solubilidad de los compuestos volátiles en la matriz. Este efecto es conocido como "salting out" (van Ruth & Roozen, 2010).

2.4.3. Contribución de las fracciones magra y grasa al aroma

Por último, los componentes de la carne ejercen un papel determinante en la generación del aroma. Como se ha explicado anteriormente, las vías de formación de compuestos volátiles a partir de la grasa, proteínas, e hidratos de carbono son conocidas (apartado 2.2). Sin embargo, la influencia de grasa y proteínas en el aroma no se limita a su papel como precursores de compuestos volátiles, sino que ambas fracciones también afectan a la liberación de los compuestos responsables del aroma, ya que la grasa actúa como solvente de los compuestos volátiles (Chevance & Farmer, 1998) y por otro existen interacciones entre los compuestos volátiles y la matriz proteica (Pérez-Juan *et al.*, 2008). No obstante, no se ha determinado claramente la contribución real de cada fracción (magra y grasa) ni el efecto de la reducción de la grasa en la generación del aroma. Los estudios sensoriales llevados a cabo en embutidos a los que se les ha reducido el contenido de grasa han aportado resultados contradictorios. Así, en los estudios de Muguerza *et al.* (2002) y Liaros *et al.* (2009) no se encontraron diferencias en el aroma de embutidos con distintos contenidos de grasa, mientras que Mendoza *et al.* (2001) obtuvieron mejores puntuaciones en la evaluación sensorial del aroma de los embutidos con 30% de grasa que en la de embutidos con 20 y 10% de grasa. Sin embargo, en estos estudios sensoriales no se determinaron los compuestos volátiles responsables de la aceptación sensorial. Con posterioridad, Muguerza *et al.* (2003) detectaron una mayor concentración de compuestos volátiles en embutidos con bajo contenido en grasa. En el caso de los compuestos derivados de la oxidación lipídica, la mayor abundancia fue atribuida al mayor porcentaje de grasa intramuscular, al ser ésta más insaturada, y por lo tanto más susceptible de sufrir procesos oxidativos que el tejido adiposo. El estudio

del efecto del contenido de grasa en los embutidos crudos curados es importante ya que ésta no sólo influye en la generación de compuestos volátiles, sino que los lípidos son excelentes disolventes para muchos de los compuestos volátiles, ya que la mayoría son lipofílicos. En general, los lípidos son responsables de una reducción de la presión de vapor (volatilidad) de los compuestos volátiles retrasando así su liberación y disminuyendo la intensidad del aroma percibido (Leland, 1997). En este sentido, Chevance *et al.* (2000) indicaron que la reducción del contenido de grasa en salami condujo a una mayor liberación de terpenos al espacio de cabeza.

3. Técnicas convencionales de análisis de la fracción volátil

Dada la importancia del aroma en la calidad global de los embutidos, diversas técnicas se pueden emplear para conocer los compuestos responsables del aroma característico y cómo los distintos parámetros del proceso de elaboración influyen en la generación y liberación de estos compuestos. La identificación y cuantificación de los compuestos volátiles mediante técnicas convencionales requiere una extracción previa de los mismos. A este respecto, el perfil de compuestos volátiles obtenido depende en gran medida de la técnica de extracción usada, de forma que técnicas diferentes aplicadas sobre una misma muestra conducen inevitablemente a la obtención de distintos resultados (Reineccius, 2010). A continuación se describen las principales técnicas de extracción de compuestos volátiles en alimentos, distinguiendo entre técnicas basadas en la extracción de los compuestos volátiles presentes en la matriz y las técnicas de extracción de los compuestos presentes en el espacio de cabeza (Flores, 2009). Las principales técnicas de extracción en alimentos se muestran en forma de esquema en la **Figura 6**. En este apartado también se describen las técnicas de análisis utilizadas para la determinación del poder aromático de los compuestos volátiles.

3.1. Técnicas de extracción de compuestos volátiles presentes en la matriz

Estas técnicas están basadas en separar de la matriz del alimento los compuestos volátiles de los que no lo son, de forma que se extraen los compuestos volátiles totales presentes en la matriz del alimento.

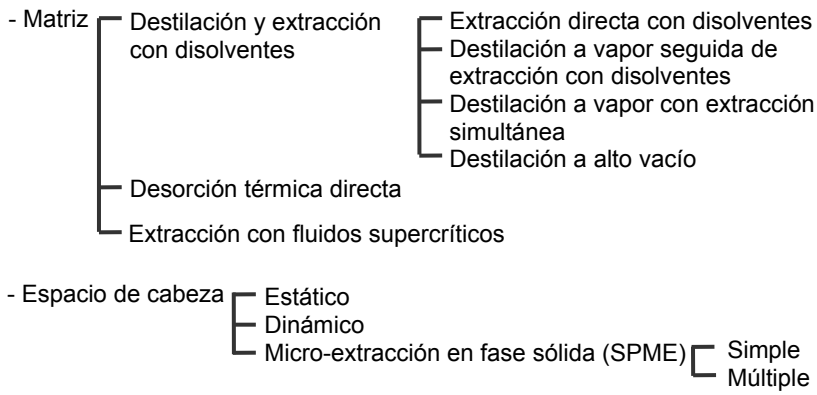

Figura 6. Principales métodos de extracción de compuestos volátiles en alimentos (adaptado de Flores, 2009).

3.1.1. Destilación y extracción con disolventes

La extracción con disolventes se basa en que el coeficiente de distribución entre el disolvente y la muestra es favorable para el primero (Parliment, 1997). La extracción puede llevarse a cabo mediante mezcla directa del disolvente con el alimento, sin embargo de esta forma también se arrastran los compuestos no volátiles como los lípidos. Una alternativa que evita la interferencia de los lípidos es la destilación a vapor de la muestra en suspensión acuosa y la extracción de los compuestos volátiles del condensado acuoso mediante disolventes. El disolvente puede extraer los compuestos volátiles una vez obtenido el condensado acuoso o bien extraerlos simultáneamente, como en el equipo de Lickens-Nickerson. Sin embargo, durante la destilación pueden formarse artefactos debido a las temperaturas aplicadas (Elmore, 2011). No obstante, si la destilación se realiza a alto vacío la formación de éstos disminuye. La evaporación de aromas asistida por disolventes (SAFE; solvent assisted flavour evaporation) es una técnica basada en la destilación a alto vacío y a baja temperatura (Engel *et al.*, 1999).

En embutidos, la técnica de destilación y extracción con disolventes más frecuentemente utilizada para la extracción de compuestos volátiles es la destilación con extracción simultánea Lickens-Nickerson (Mateo & Zumalacárregui, 1996;

Ansorena *et al.*, 2000). Más recientemente, Söllner & Schieberle (2009) emplearon la técnica SAFE para identificar los compuestos aromáticos del salami húngaro.

3.1.2. Desorción térmica directa

Es una técnica rápida y de fácil preparación para el análisis cualitativo de los compuestos volátiles presentes en matrices sólidas. Consiste en desorber térmicamente los compuestos volátiles de la muestra y transferirlos directamente a la cabeza de columna de un cromatógrafo (Grimm *et al.*, 1997). Los inconvenientes de esta técnica son la posible contaminación de un muestreo a otro y la pérdida de compuestos termolábiles.

3.1.3. Extracción con fluidos supercríticos

Esta técnica está basada en la capacidad que tienen determinados compuestos de modificar su poder disolvente en estado supercrítico. Por ejemplo, el dióxido de carbono, en condiciones de presión y temperatura superiores a su punto crítico, se comporta como un fluido supercrítico, de manera que difunde como un gas, y disuelve compuestos volátiles como lo haría un disolvente orgánico, aunque no disuelve los compuestos no volátiles (Elmore, 2011). Esta técnica es muy costosa ya que requiere un equipo de altas presiones y generalmente no se aplica en el estudio del aroma de los embutidos.

3.2. Técnicas de extracción de compuestos volátiles presentes en el espacio de cabeza

Estas técnicas han sido ampliamente utilizadas para estudiar el aroma de los embutidos y tienen como objetivo extraer los compuestos volátiles presentes en el espacio de cabeza de un alimento, es decir, en el aire que rodea al embutido cuando se encuentra confinado en un vial sellado. De esta forma, se aíslan los compuestos volátiles que son percibidos por la nariz antes de ingerir un alimento (aroma nasal). Además de evitar el uso de disolventes, estas técnicas permiten el análisis de compuestos de bajo peso molecular (Flores, 2011).

3.2.1. Espacio de cabeza estático

Es una técnica de extracción simple y rápida que consiste en, una vez alcanzado el equilibrio entre la matriz y el espacio de cabeza, retirar una porción del espacio de cabeza e inyectarla directamente en un cromatógrafo para su análisis (Wampler, 1997). El principal inconveniente de esta técnica es que no tiene la suficiente sensibilidad para la determinación de compuestos minoritarios debido a la ausencia de enriquecimiento previo del espacio de cabeza. Dado que el volumen de inyección normalmente oscila entre 0.1-2 mL, sólo una porción del espacio de cabeza es analizado y únicamente aquellos compuestos cuya concentración en el espacio de cabeza exceda 10^{-7} g/L y 10^{-5} g/L son detectados por cromatografía de gases acoplado a detector selectivo de masas (GC-MS) o acoplado a detector por ionización de llama (GC-FID), respectivamente (Reineccius, 2010). La sensibilidad de este método puede ser mejorada si se usan detectores más sensibles como el detector fotométrico de llama pulsada o en el caso de la cromatografía de quimioluminiscencia. Además, se puede enriquecer la concentración de volátiles del espacio de cabeza mediante la adición de sal o mediante el análisis del espacio de cabeza de un destilado de la muestra. Por otro lado, la cuantificación proporciona información acerca de la concentración de compuestos volátiles presente en el espacio de cabeza que depende del coeficiente de distribución de cada compuesto volátil entre el espacio de cabeza y la matriz (Wampler, 1997). Este coeficiente es muy difícil de determinar en el caso de matrices complejas como los embutidos, siendo por lo tanto complejo establecer la relación entre la concentración del espacio de cabeza y la presente en la matriz.

3.2.2. Espacio de cabeza dinámico

Esta técnica consiste en arrastrar los compuestos volátiles presentes en el espacio de cabeza mediante una corriente de gas inerte como nitrógeno o helio durante un tiempo suficiente para extraer la mayoría de los compuestos volátiles presentes en éste (Wampler, 1997). Posteriormente se hace pasar al gas portador a través de una serie de trampas que retienen los compuestos volátiles, por lo que esta técnica suele denominarse purga y trampa. Las trampas están constituidas por materiales porosos,

siendo el más usado el polímero Tenax®. Este material es capaz de concentrar una amplia variedad de compuestos volátiles, presentando alta afinidad por compuestos no polares (Reineccius, 2010). Una vez atrapados los compuestos, la desorción se lleva por calentamiento de la trampa. También pueden utilizarse trampas criogénicas o de carbón activado, aunque las primeras atrapan agua y en las segundas se generan artefactos en la etapa de desorción (Reineccius, 2010).

La principal ventaja de esta técnica es que presenta mayor sensibilidad que el espacio de cabeza estático, sin embargo el tiempo de preparación de la muestra es mayor y el perfil de volátiles obtenido depende del material que se use como adsorbente. La técnica de purga y trampa, generalmente empleando Tenax® como material adsorbente, ha sido ampliamente utilizada para el estudio de los compuestos volátiles de embutidos crudos curados (Croizet *et al.*, 1992; Berdagué *et al.*, 1993; Stahnke, 1995; Viallón *et al.*, 1996; Meynier *et al.*, 1999; Bruna *et al.*, 2001; Bianchi *et al.*, 2007).

3.2.3. Microextracción en fase sólida (SPME)

La microextracción en fase sólida es actualmente la técnica más utilizada para la extracción de compuestos volátiles en alimentos (Reineccius, 2010) ya que se trata de una técnica simple y rápida. Esta técnica fue desarrollada por Pawliszyn y colaboradores (Zhang & Pawliszyn, 1993) y tiene como objetivo concentrar los compuestos volátiles presentes en el espacio de cabeza. Consiste en exponer al espacio de cabeza una fibra SPME de sílice recubierta por una capa de fase estacionaria selectiva. Los compuestos volátiles presentes en el espacio de cabeza son adsorbidos por la fibra y posteriormente son desorbidos en el puerto de inyección de un cromatógrafo de gases para su separación y análisis.

En el análisis por SPME están implicadas tres fases, matriz, espacio de cabeza y fibra, entre las que existen procesos de partición. La concentración de compuestos volátiles en el espacio de cabeza depende del coeficiente de partición entre la matriz y el espacio de cabeza. A su vez, la concentración adsorbida por la fibra depende del coeficiente de partición entre el espacio de cabeza y la fibra. El equilibrio se alcanza cuando se satisfacen los valores de las constantes de partición entre las fases, siendo menor el tiempo necesario para ello si se aumenta la temperatura (Zhang *et al.*, 1994).

Por lo tanto, con esta técnica no se lleva a cabo una extracción absoluta de los compuestos volátiles presentes en la matriz, sino que se obtiene una proporción relativa de aquellos liberados al espacio de cabeza que depende en gran medida del tipo de matriz y de la fibra utilizada, así como de las condiciones de extracción. Aunque la cantidad de compuesto volátil adsorbido por la fibra en el equilibrio está directamente relacionada con la concentración total de compuesto volátil en la muestra (Zhang *et al.*, 1994), ésta última sólo podría calcularse si se conocieran los coeficientes de partición entre las fases para cada uno de los compuestos volátiles de los embutidos.

Además, hay que tener en cuenta que la adsorción de los compuestos volátiles por parte de la fibra depende en gran medida de la afinidad de ésta por los compuestos, por lo tanto las concentraciones obtenidas no son concentraciones "reales" en el espacio de cabeza, sino cantidad de compuesto volátil adsorbida por la fibra. No obstante, la obtención de proporciones relativas mediante la técnica SPME permite comparar entre muestras cuando se utilizan exactamente las mismas condiciones de extracción (Roberts *et al.*, 2000).

Existen varios tipos de fibras que se diferencian por el grosor y la polaridad de la fase estacionaria, presentando distinta afinidad por los compuestos volátiles (Roberts *et al.*, 2000). Por ejemplo, el polidimetilsiloxano (PDMS) tiene alta afinidad por compuestos no polares, pero no por los polares, mientras que el poliacrilato (PA) por los polares. El uso de fibras bipolares en general aumenta la sensibilidad de esta técnica.

Esta técnica fue aplicada por primera vez para el estudio de los compuestos volátiles de embutidos crudos curados por Marco *et al.* (2004). Posteriormente muchos otros autores la han aplicado (Di Cagno *et al.*, 2008; Spaziani *et al.*, 2009; Latorre-Moratalla *et al.*, 2011)

3.2.3.1. Microextracción en fase sólida múltiple (SPME múltiple)

La SPME múltiple es una variante del SPME desarrollada a partir del fundamento de la extracción del espacio de cabeza múltiple. Ésta última es un método de extracción por etapas que elimina la influencia de la matriz, lo que hace posible cuantificar de forma absoluta los compuestos volátiles de una muestra sólida o líquida (Kolb, 1982).

La SPME múltiple consiste en exponer la fibra SPME en el espacio de cabeza de la muestra repetidas veces en intervalos de tiempo idénticos (Tena & Carrillo, 2007) (**figura 7**). De esta manera, tras varias extracciones sucesivas de una misma muestra, la concentración del analito disminuye exponencialmente y se puede calcular la cantidad total de compuesto volátil en la matriz.

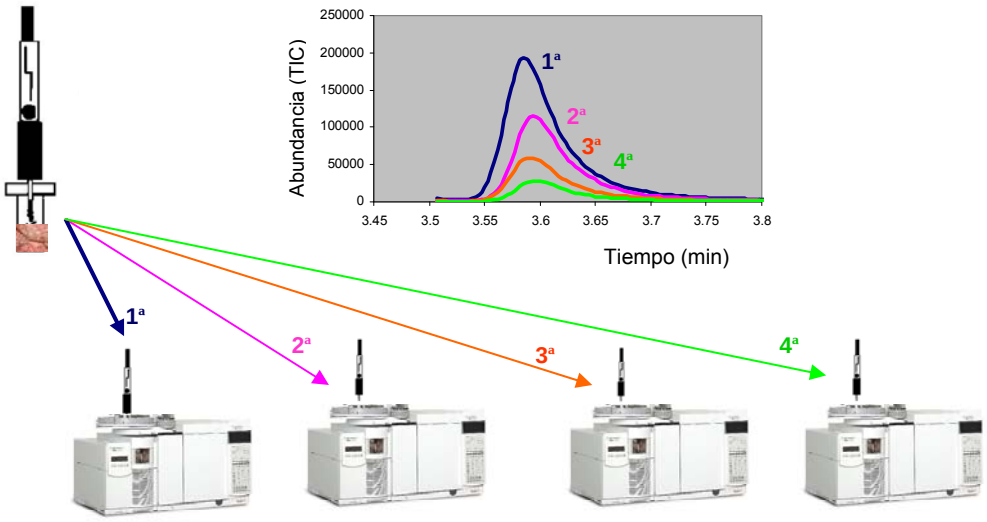

Figura 7. Esquema del procedimiento de la SPME múltiple. El gráfico representa los picos cromatográficos obtenidos tras 4 extracciones sucesivas.

La aplicación de la SPME múltiple debe cumplir 3 requisitos (Ezquerro *et al.*, 2003):

a) La relación entre el área del pico y la cantidad de compuesto volátil en la fibra debe ser lineal en el rango de estudio.
b) El volumen de cada fase (matriz, espacio de cabeza, y fibra) y los coeficientes de partición entre las fases deben ser constantes durante las extracciones sucesivas.
c) La concentración del analito debe estar en equilibrio con las tres fases para cada etapa de extracción.

La cantidad de compuesto volátil extraído por la fibra (m_f) está relacionada con el equilibrio de las tres fases del sistema y el volumen de cada fase. Una vez que se ha alcanzado dicho equilibrio m_f puede calcularse:

$$m_f = \frac{K_{fs}V_f}{K_{fs}V_f + K_{hs}V_h + V_s} * m_0 \quad (1)$$

donde:

m_0: cantidad inicial de compuesto volátil en el sistema de tres fases

K_{fs}: coeficiente de partición entre la fibra y la muestra

K_{hs}: coeficiente de partición entre el espacio de cabeza y la muestra

V_f: volumen de la fibra

V_h: volumen del espacio de cabeza

V_s: volumen de la muestra

Si los coeficientes de partición y el volumen de las tres fases son constantes, la cantidad de compuesto volátil adsorbida por la fibra es proporcional a la cantidad inicial, entonces:

$$m_f = \alpha m_0 \quad (2)$$

donde α ($0<\alpha\leq1$) es la constante que agrupa las anteriores constantes:

$$\alpha = \frac{K_{fs}V_f}{K_{fs}V_f + K_{hs}V_h + V_s} \quad (3)$$

La técnica SPME múltiple es un proceso que consta de extracciones sucesivas (N) sobre la misma muestra hasta que la totalidad de compuesto volátil es extraído, se considera que m_{fN} =0. Cuando se establezca el equilibrio al final de la primera etapa de extracción la cantidad de compuesto volátil adsorbida por la fibra será:

$$m_{f1} = \alpha m_0 \quad (4)$$

después de la segunda y la tercera extracción:

$$m_{f2} = m_{f1}(1-\alpha) \quad (5)$$

$$m_{f3} = m_{f1}(1-\alpha)^2 \quad (6)$$

y al final de la extracción *i*:

$$m_{fi} = m_{f1}(1-\alpha)^{i-1} \quad (7)$$

El área cromatográfica está relacionada con la cantidad de compuesto volátil inyectada, es decir, la adsorbida por la fibra, por lo tanto se puede considerar la cantidad de compuesto volátil adsorbido por la fibra de acuerdo a una constante instrumental *K*:

$$A_i = Km_{fi} \quad (8)$$

La ecuación (8) puede reescribirse como:

$$A_i = Km_{f1}(1-\alpha)^{i-1} = A_1(1-\alpha)^{i-1} = A_1\beta^{i-1} \quad (9)$$

donde β es $(1-\alpha)$ y $(0 \leq \beta < 1)$. La suma de las áreas obtenidas en cada extracción puede ser calculada:

$$A_T = A_1 + A_2 + A_3 + \bullet\bullet\bullet + A_N \quad (10)$$

$$A_T = A_1 (1 + \beta + \beta^2 + \bullet\bullet\bullet + \beta^{N-1}) \quad (11)$$

Siendo la ecuación (11) una progresión geométrica cuya suma es:

$$A_T = \sum_{i-1}^{N} A_i = \frac{A_1}{1-\beta} \quad (12)$$

Según la ecuación (12), el área total de compuesto volátil puede ser calculada a partir de dos valores: el área del pico de la primera extracción A_1, y la constante β que puede ser conocida a partir de la pendiente de la recta obtenida tras representar los valores del $Ln\ A_i$ frente a $(i-1)$, siendo i el número de extracciones realizadas con SPME. Si aplicamos logaritmos neperianos a la ecuación (9) se transforma en la ecuación de una recta:

$$ln\ A_1 = (i - 1)\ ln\ \beta + ln\ A_1 \qquad (13)$$

donde $ln\ \beta$ es la pendiente de la recta y $ln\ A_1$ la ordenada en el origen. Si despejamos $\beta = e^k$ y sustituimos en la ecuación (12) el área total de compuesto volátil (A_t) en la muestra se puede calcular mediante la siguiente ecuación:

$$A_t = \frac{A_1}{1 - e^k} \qquad (14)$$

El área total A_t se puede correlacionar con la cantidad total de compuesto volátil en la matriz interpolando su valor en una recta de calibrado obtenida con diluciones patrón de masa conocida y extraídas mediante SPME múltiple en idénticas condiciones a las de la muestra, es decir:

$$m_0 = \frac{A_t \times m_s}{A_{ts}} \qquad (15)$$

Donde m_s es la cantidad inicial de patrón y A_{ts} es el área total de éste.

La ventaja de esta técnica es que, de la misma forma que el resto de técnicas de espacio de cabeza, evita el uso de disolventes, pero en este caso es posible conocer la concentración total de compuestos volátiles sin interferencia de la matriz. Por otro lado, las principales limitaciones de la SPME múltiple son el incremento del tiempo de análisis, los menores rangos lineales y peor reproducibilidad debido a la pequeña

cantidad de muestra analizada, condición última necesaria para que tenga lugar la disminución en el área con las extracciones sucesivas (Tena & Carrillo, 2007).
Esta técnica fue optimizada para el estudio de compuestos volátiles de embutidos crudos curados por Flores & Hernández (2007) y fue aplicada por Marco *et al.* (2007) para la cuantificación de compuestos con poder aromático.

3.3. Determinación del poder aromático

En los últimos años, el interés se ha centrado en conocer cuáles de los compuestos volátiles presentes en los embutidos crudos curados son los responsables del aroma típico. Probablemente, aquellos cuya concentración sea superior a los umbrales de percepción son los que contribuyen en mayor medida al aroma. La determinación del poder aromático de los compuestos volátiles y la estimación de su importancia relativa puede ser abordada mediante el cálculo del valor de la actividad aromática (OAV) (Mistry *et al.*, 1997) o bien mediante técnicas olfatométricas (Delahunty *et al.*, 2006; Zellner *et al.*, 2008).

3.3.1. Cálculo del valor de la actividad aromática (OAV)

Consiste en calcular el ratio entre la concentración de un compuesto volátil (C) y su umbral de percepción (C_u), es decir, la concentración mínima necesaria para que un compuesto volátil sea percibido.

$$OAV = \frac{C}{C_u}$$

Hay que tener en cuenta el efecto del medio sobre los umbrales de percepción, por ejemplo, para un mismo compuesto los umbrales de percepción en aire, agua y en aceite son distintos (van Gemert & Nettenbreijer, 2004). En este sentido, Grosch (2001) recalcó la importancia de calcular los OAV usando los umbrales de percepción del medio que predomine en el alimento (agua, aceite, etc.).
Por otro lado, este parámetro no considera las interacciones de antagonismo y sinergismo que se establecen entre los compuestos volátiles de un alimento. De

hecho, Ryan *et al.* (2008) indicaron que algunos compuestos volátiles presentes a concentraciones inferiores a su umbral de percepción (OAV inferior a 1) pueden contribuir al aroma global de un alimento.

3.3.2. Técnicas olfatométricas

Las técnicas olfatométricas son aquellas en la que se combina la cromatografía de gases con detectores olfatométricos (GC-O), es decir, se emplea la nariz humana como detector (Zellner *et al.*, 2008). En la práctica, el eluyente de la columna se divide entre un detector convencional (detector selectivo de masas GC-MS, detector de ionización de llama GC-FID, detector de conductividad térmica GC-TCD, etc.) y un puerto de olfacción donde una persona evalúa el eluyente de la columna actuando así como detector (**Figura 8**).

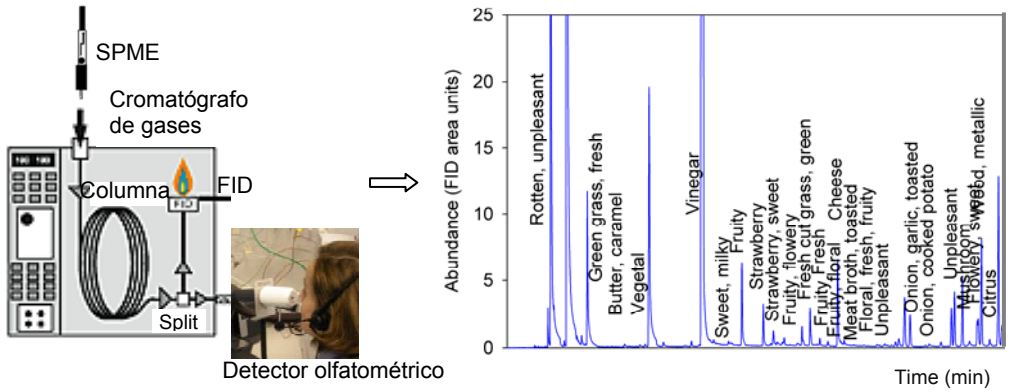

Figura 8. Esquema básico de GC-O y ejemplo de aromagrama obtenido.

De esta forma, el cromatograma obtenido recoge información adicional sobre los compuestos volátiles y es denominado aromagrama (**Figura 8**). Según la técnica olfatométrica empleada, el aromagrama contiene la descripción del aroma, intensidad, duración, etc. Las técnicas de GC-O se clasifican en tres categorías, tal y como se resume en la **Figura 9**.

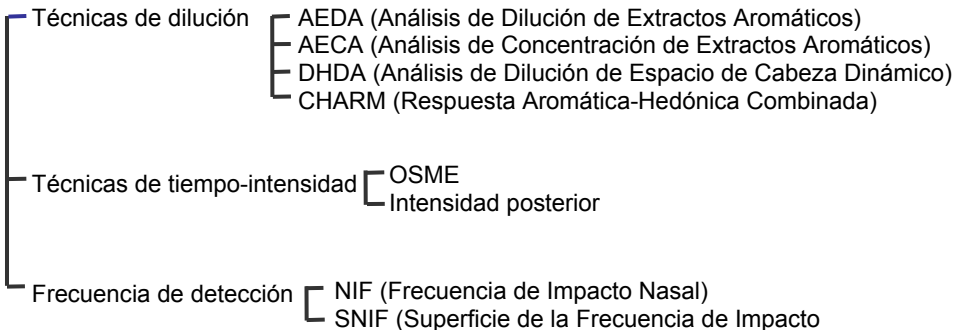

Figura 9. Principales técnicas de olfatometría (adaptado de Flores, 2009).

3.3.2.1. Técnicas de Dilución

Estas técnicas tienen como objetivo clasificar los compuestos volátiles en función de la potencia aromática de las diluciones progresivas de un extracto del alimento. Estas técnicas requieren un bajo número de panelistas (1-3), sin embargo, debido a que se analizan series diluidas de un mismo extracto (aproximadamente 10-12 diluciones) se emplea mucho tiempo para el análisis de una única muestra.

La técnica de Análisis de Dilución de Extractos Aromáticos (AEDA), desarrollada por Grosch y colaboradores (Ullrich & Grosch, 1987), consiste en evaluar diluciones sucesivas de un extracto aromático del alimento hasta que ningún olor es percibido por los panelistas. En base a esto, se define el factor de dilución como la relación existente entre la concentración del compuesto volátil en el extracto inicial y el extracto más diluido en el que ese compuesto fue detectado. Esta técnica ha sido empleada para cuantificar la intensidad y el carácter aromático de los compuestos volátiles de embutidos crudos curados (Schmidt & Berger, 1998a, 1998b; Blank *et al.*, 2001; Söllner & Schieberle, 2009) (ver **Tabla 2**).

La principal limitación de la técnica AEDA es la estimación inexacta de la importancia de los compuestos aromáticos, principalmente debido a que el extracto evaluado puede no ser representativo del aroma percibido (Reineccius, 2010) ya que pueden tener lugar pérdidas de compuestos aromáticos y/o formación de artefactos durante la obtención del extracto aromático inicial. Además, un extracto obtenido mediante disolventes contiene los compuestos volátiles totales de la matriz, mientras que el

aroma percibido de un alimento únicamente contiene los compuestos liberados al espacio de cabeza (Blank, 1997).

Con el objetivo de superar estos inconvenientes, se han desarrollado métodos basados en la técnica AEDA como el Análisis de Concentración de Extractos Aromáticos (AECA) y Análisis de Dilución de Espacio de Cabeza Dinámico (DHDA). En AECA se evalúan extractos cada vez más concentrados hasta llegar al umbral de detección. De esta forma, se previenen las pérdidas de compuestos aromáticos durante la evaporación del disolvente. Otra posibilidad es la técnica DHDA, que combina AEDA con la técnica de extracción del espacio de cabeza dinámico (Mistry *et al.*, 1997). De esta manera, es posible detectar compuestos muy volátiles que se pierden durante las etapas de concentración y que frecuentemente coeluyen con el disolvente en la separación cromatográfica. En el caso de DHDA, las diluciones se corresponden con menores tiempos de purga del espacio de cabeza.

Por último, la técnica Respuesta Aromática-Hedónica Combinada (CHARM), desarrollada por Acree *et al.* (1984), también tiene como objetivo evaluar la dilución máxima en la que un compuesto volátil es percibido, pero se diferencia de AEDA en que CHARM analiza de forma aleatoria una serie de extractos diluidos y además tiene en cuenta no sólo la intensidad sino la duración de la detección del aroma.

3.3.2.2. Técnicas de Tiempo-Intensidad

Estas técnicas determinan la intensidad del aroma percibido además de tener en cuenta el tiempo durante el que éste es percibido. Esto puede llevarse a cabo una vez el compuesto ha eluido (intensidad posterior) o de forma dinámica (OSME), es decir, mientras el compuesto volátil eluye de la columna cromatográfica (Delahunty *et al.*, 2006). A diferencia de las técnicas de dilución, un único extracto es evaluado, por lo que los resultados obtenidos no están basados en los umbrales de detección (Mistry *et al.*, 1997). Asimismo, el número de inyecciones es menor, pero requiere un mayor número de panelistas (3-10) y entrenamiento específico de éstos para evaluar la intensidad aromática de cada compuesto volátil. Hasta el momento, esta técnica no ha sido empleada en embutidos crudos curados.

3.3.2.3. Técnicas de frecuencia de detección (FD)

Esta técnica fue desarrollada por Pollien *et al.* (1997) con el objetivo de obviar el entrenamiento de los panelistas en cuanto a escalas de intensidad y obtener un compromiso aceptable entre la reproducibilidad y un moderado número de inyecciones. Consiste en la evaluación de un mismo extracto aromático por varios catadores (6-12). Las técnicas de frecuencia de detección asumen que la contribución de un compuesto volátil al aroma está relacionada con el número de veces que éste es detectado por los panelistas que analizan el extracto (frecuencia de impacto nasal: NIF). Si además se tiene en cuenta la duración se define la superficie de la frecuencia de impacto nasal (SNIF).

La principal limitación de este método es que no tiene escala, en otras palabras, aunque un compuesto sea percibido por todos los panelistas a una determinada concentración, el incremento de concentración no se verá reflejado en un incremento de la frecuencia de detección (Delahunty *et al.*, 2006). Por esta razón, esta técnica presenta menor poder discriminante entre muestras que las técnicas de intensidad. Sin embargo presenta mejor reproducibilidad que éstas últimas (van Ruth, 2004).

Esta técnica ha sido previamente aplicada en embutidos crudos curados (Chevance *et al.*, 2000; Marco *et al.*, 2007; Gianelli *et al.*, 2011) (**Tabla 2**).

3.3.3. Compuestos volátiles con poder aromático identificados en embutidos crudos curados

Distintos autores han tratado de identificar los compuestos volátiles con poder aromático de diferentes tipos de embutidos crudos curados mediante distintas técnicas de extracción de los compuestos volátiles y diferentes técnicas olfatométricas (**Tabla 2**). Los embutidos más ampliamente estudiados han sido los embutidos fermentados, aunque también se han analizado embutidos de fermentación natural y embutidos no fermentados. Para ello se han empleado tanto técnicas de destilación y extracción con disolventes como técnicas de análisis del espacio de cabeza, concretamente espacio de cabeza dinámico (purga y trampa) y SPME. En cuanto a las técnicas olfatométricas, los primeros trabajos que se llevaron a cabo consistieron en olfatear el

Tabla 2. Estudios de compuestos aromáticos realizados en embutidos crudos curados mediante GC-O.

Ref.*	Autores	Producto	Técnica de extracción	Técnica Olfatométrica
1	Croizet *et al.* (1992)	Embutido de fermentación natural	Espacio de cabeza dinámico (purga y trampa)	Sniffing GC effluent
2	Stahnke (1994)	Embutido fermentado	Espacio de cabeza dinámico (purga y trampa)	Sniffing GC effluent
3	Stahnke (1995)	Embutido fermentado	Espacio de cabeza dinámico (purga y trampa)	Sniffing GC effluent
4	Meynier *et al.* (1995)	Embutido fermentado (salami)	Espacio de cabeza dinámico (purga y trampa)	Sniffing GC effluent
5	Schmidt & Berger (1998a)	Embutidos fermentados (salami de Francia, Italia, España y Alemania)	Destilación a vapor, destilación a alto vacío, espacio de cabeza dinámico	AEDA
6	Schmidt & Berger (1998b)	Embutidos de fermentación natural y embutidos fermentados	Destilación a alto vacío	AEDA
7	Chevance *et al.* (2000)	Embutidos fermentados (salami) con distinto contenido de grasa	Espacio de cabeza dinámico	Frecuencia de detección
8	Blank *et al.* (2001)	Embutido fermentado (salami italiano)	Destilación a alto vacío	AEDA
9	Marco *et al.* (2007)	Embutidos no fermentados con adición de nitrato o nitrito	SPME múltiple	Frecuencia de detección
10	Söllner & Schieberle (2009)	Embutido fermentado (Salami húngaro ahumado)	Destilación a alto vacío (SAFE)	AEDA
11	Gianelli *et al.* (2011)	Embutido fermentado (sobrasada)	SPME	Frecuencia de detección

* Número de referencia empleado en la Tabla 3.

eluyente cromatográfico y describir el aroma percibido, pero no se determinó la importancia relativa de cada compuesto (Croizet *et al.*, 1992; Stahnke, 1994, 1995; Meynier *et al.*, 1999). Más tarde, la GC-O se ha llevado a cabo empleando las técnicas AEDA (Schmidt & Berger 1998a, b; Blank *et al.*, 2001; Söllner & Schieberle, 2009) y frecuencia de detección (Chevance, *et al.*, 2000; Marco *et al.*, 2007; Gianelli *et al.*,

2011). Mediante estas últimas técnicas, además de identificar los compuestos aromáticos, se han descrito como más potentes aquellos que presentaron los factores de dilución y las frecuencias de detección más altos, respectivamente. En global, más de 100 compuestos volátiles han sido descritos como "aroma activos" en embutidos crudos curados, incluyendo una gran variedad de aldehídos, cetonas, alcoholes, ácidos, ésteres, compuestos nitrogenados y azufrados, terpenos, hidrocarburos aromáticos, furanos y lactonas, que están recogidos en la **Tabla 3**.

Tabla 3. Compuestos volátiles con poder aromático detectados en embutidos crudos curados.

Grupo químico/compuesto	Descriptor olfatométrico*
Aldehídos	
Acetaldehído	verde[3]
Butanal	dulce, snacks[9]
2-Metil-propanal	fresco[11]
2-Metil-butanal	queso amargo, afrutado[2,3]; caramelo, rancio[4]; malta[10]
3-Metil-butanal	queso amargo, afrutado[2,3]; rancio, jamón curado[9]; malta[10]
Pentanal	sintético, hoja verde[2]; hierba recién cortada, rancio[9]
Hexanal	vegetales[2,3]; hierba recién cortada[4,9,11]; verde[5,6,8,10]
2-Hexenal	patata cocidas[3]; carne salada, jamón curado[9]
3-Metil-hexanal	amargo, verde, acetona[2]; hojas verdes[3]
Heptanal	verde, afrutado[2,3]; patatas[4]; jabón, jamón curado rancio[9]
2-Heptenal	rancio, sucio[9]
4-Heptenal	pan desagradable[3]
2,4-Heptadienal	tostado, mantequilla, jabón, carne cocinada, nueces[9]
Octanal	geranio, floral[2,9]; ortiga, seco[3]
2-Octenal	frito[3]; rancio[8]; jamón curado, salchichón[9]; floral, especias[11]
Nonanal	herbal, geranio[2]; ortiga, seco[3]; plástico, jabón, cítrico[9,10,11]
2-Nonenal	pepino, herbal, madera[2,3,9]; tostado, caramelo[11]
Decanal	pepino, hierba seca[2]
Benzaldehído	fresco, pino, herbal, especias[11]
Fenilacetaldeído	rosas[9]; floral, fresco[11]
2,4-Nonadienal	seboso, grasoso[8,10]
2,4-Decadienal	frito[2]; rancio[5]; seboso, grasoso[8]
4,5-Epoxi-2-decenal	metálico[10]
Cetonas	
Acetona	acetona, alcohol[9]

Diacetilo	mantequilla[2,3,5,6,11]; queso[9]
3-Hidroxi-2-butanona	ligero a mantequilla[5]
2-Pentanona	tostado, dulce[9]
2,3-Pentanodiona	mantequilla[2,3,9]
2-Heptanona	ortiga, frutal, medicinal[3,9]
1,5-Octanodiona	geranio[10]
1-Octen-3-ona	champiñón[3,8,10]
3-Octen-2-ona	champiñón, metálico[3]
2-Nonanona	herbal[2]; champiñón[3]; frutal ligero[5,6]; tostado, quemado[9]
6-Metil-5-hepten-2-ona	resina, pino, herbal, sintético[11]
2-Hidroxi-3,5-dimetil-2-ciclopenten-1-ona	caramelo[10]
2-Hidroxi-3,4-dimetil-2-ciclopenten-1-ona	condimento, aderezo[10]
Alcoholes	
Etanol	masa panaria, levadura[9]
1-Propanol	fresco, herbal[11]
1-Pentanol	tostado, carne tostada[9]; pino[11]
1-Hexanol	hierba verde, plástico[9]
2-Heptanol	plástico, cortezas de cerdo[9]
1-Octen-3-ol	champiñón[4,9]
Ácidos carboxílicos	
Ácido acético	vinagre[3,5,6,9,11]; punzante, agrio[10]
Ácido propanoico	agrio débil[3]; sudoroso[5,6,10]; queso[9]
Ácido 2-metil-propanoico	queso[3]; sudoroso[5,6]; grasoso, sabroso[9]
Ácido butanoico	queso intenso[3,9,10]; sudoroso[5,6,10]
Ácido 3-metil-butanoico	queso, pies, calcetines sucios[3,9,11]; sudoroso[5,6,10]
Ácido 2-metil-butanoico	pepino[3;] sudoroso[10]
Ácido pentanoico	sudoroso[5]
Ácido hexanoico	sudoroso, podrido[5]
Ácido heptanoico	rancio, jamón curado[9]; desagradable, medicinal[11]
Ácido octanoico	rancio, madera[9]
Ácido decanoico	punzante[5]
Ésteres	
Acetato de propilo	manzana, caramelo[2]; frutal, floral[6]
Acetato de etilo	sintético a fruta[5]; dulce, frutal[6,9]
Propanoato de etilo	frutal[5,6]
Butanoato de metilo	frutal, floral[5,6]
Butanoato de etilo	fruta, caramelo[2,3,10,11]; piña[5,6]; fresa[9,11]

3-Metil-butanoato de etilo	agrio, picante[2]; frutal, floral[3,5,6,8,10,11]
2-Metil-butanoato de etilo	dulce, piña[2]; fruta[3,10,11]; fresa[9]
3-Metil-butanoato de propilo	frutal[6]
2-Metil-propanoato de etilo	piña, fresa fruta, caramelo[2,3,8,9,10]
Acetato de 2-metil-propilo	frutal[3]
Pentanoato de etilo	frutal[2,3]; fresa[9]
Hexanoato de etilo	pera[6]; dulce, frutal, cereza[9]
Octanoato de etilo	frutal[6]; seboso, verde[8]; carne tostada[11]
Decanoato de etilo	frutal, cítrico[6]
Compuestos nitrogenados	
Dimetil pirazina	café tostado, pan tostado[3]
Tetrametil pirazina	azúcar tostado[11]
3-Isopropil-2-metoxipirazina	terroso, té[10]
3-Isobutil-2-metoxipirazina	terroso, té[10]
2-Etil-3,5(6)-dimetil-pirazina	terroso[10]
2,3-Dietil-5-metilpirazina	terroso[10]
3-Metil-indol (escatol)	bola antipolilla[6]; heces[10]
2-Acetil-pirrolina	tostado, palomitas[6,10]; fruto seco tostado[11]
1-Metil-pirrol	tostado, caldo[11]
Compuestos azufrados	
Metil-sulfuro de alilo	cebolla, desagradable[4]; ajo[5,6,8,10]
Metil-disulfuro de alilo	ajo[5]
Sulfuro de dialilo	ajo[5,6]
Disulfuro de dialilo	ajo[5,6]
Dimetil disulfuro	ajo[5]; caramelo, tostado[11]
Dimetil trisulfuro	col, azufrado, desagradable[7]
Metional (3-metiltiopropanal)	patata cocida[8,9,10]; caldo de carne, rancio[9]
Metanotiol	huevos podridos, coliflor[9]
2-Furil-metanotiol	tostado, azufrado[10]
Etil metil disulfuro	cebolla, punzante[2]
2-Metil-3-furil disulfuro	carne cocinada[10]
2-Acetil-2-tiazolina	tostado, palomitas[10]
Terpenos	
β-Mirceno	limón, frutal, verde[4]; herbal[5,11]; especia[6]
Limoneno	mentol[4]; herbal[5]; fresco[6]; cítrico, naranja[9]
Terpineno	herbal[5]
Terpinoleno	frutal, eucalipto[4]

α-Pineno	picante, resina[5,6]; pimienta[8]; pino[10]
β-Pineno	punzante[5]
Terpineol	verde[5]
1,8-Cineol	mentol[5]
3-Careno	desagradable[11]
Felandreno	limón, frutal, verde[4]
Linalol	floral intenso, rosa[5,6,7,8,10]
Cumarina	dulce[10]
Vanilina	vainilla, dulce[10]
Hidrocarburos aromáticos	
3-Metil fenol	humo[6]; fenólico[10]
4-Metil-fenol	establo, caballo[3,10,11]; mohoso, ahumado[5,6]
3-Etil fenol	fenólico[10]
4-Etil fenol	fenólico[10]
2-Metoxifenol (guiaicol)	salchicha cocida[3]; ahumado, dulce[10]
2,6-Dimetoxifenol	ahumado, dulce[10]
Eugenol	ahumado, especia, clavos[5,6,10]
Isoeugenol	clavos[10]
5-Metil-2-metoxifenol	ahumado, dulce[10]
4-Metil-2-metoxifenol	dulce[10]
4-Etil-2-metoxifenol	clavos[10]
Ácido fenilacético	miel[10]
2-Fenil etanol	floral, rosa[5,6]; miel[10]
Fenil etil alcohol	bodega[9]; floral, fresco, sintético[11]
Furanos	
Furfural	caldo, carne cocinada[11]
2-Etil-furano	tostado, ajo[9]
2-Pentil-furano	cebolla, sabroso, rancio[9]; establo, azufrado[11]
2-Metil-3-furanotiol	carne tostada[7]
2-Metil-3-metilditiofurano	tostado, carnoso[7]
3-Hidroxi-4,5-dimetil-2(5H)-furanona	condimento, aderezo[10]
Lactonas	
δ-Hexalactona	aceite esencial, piel de naranja, dulce[11]
d-Octolactona	frutal, floral[6]

* Los números [1-10] que aparecen en los descriptores olfatométricos se corresponden con el número de referencia de la Tabla 2.

Las diferencias en el perfil de compuestos aromáticos entre distintos tipos de embutidos sólo pueden compararse cuando se han empleado las mismas condiciones de extracción. En este sentido, Stanhke (1995) comparó embutidos fermentados y no fermentados, e indicó que las variaciones en el aroma global entre embutidos distintos se deben a diferencias en la proporción de los compuestos volátiles y no a la presencia de compuestos volátiles específicos en ciertos tipos de embutidos. Así, los embutidos no fermentados se caracterizaron por una mayor proporción de notas aromáticas a masa fermentada, grasa y disolvente que los embutidos fermentados, mientras que en éstos últimos se detectó una mayor proporción de notas ácidas y a queso. De la misma manera, Schmidt & Berger (1998b) compararon embutidos de fermentación natural y embutidos fermentados y observaron que las diferencias en el aroma también se debían a variaciones en la proporción de compuestos volátiles. Estos autores indicaron que el aroma típico de los embutidos fermentados provenía de la combinación de compuestos derivados de las especias (pimienta y ajo) y de otros compuestos derivados de reacciones bioquímicas, mientras que en el aroma de los embutidos de fermentación natural se caracterizaba por una mayor proporción de compuestos derivados de las especias y una menor proporción de los compuestos generados por los microorganismos. Por su parte, Marco *et al.* (2007) no hallaron diferencias significativas en relación a los compuestos con poder aromático de embutidos fabricados con nitrato o nitrito mediante la técnica de frecuencia de detección. Aunque distintos autores coinciden en el hecho de que las diferencias en el aroma entre embutidos distintos no se debe a la presencia de compuestos específicos sino a variaciones en la proporción de compuestos volátiles, cabe resaltar que en el caso de los embutidos ahumados y/o con especias la presencia de algunos compuestos volátiles, como compuestos fenólicos y terpenos, es característica del proceso de elaboración y los ingredientes empleados en estos embutidos, respectivamente (Schmidt & Berger, 1998a; Söllner & Schieberle, 2009).

Por otro lado, la técnica de extracción empleada influye en el perfil de compuestos volátiles obtenido. En general, mediante las técnicas de destilación y extracción con disolventes se han identificado compuestos minoritarios no detectados con las técnicas de análisis del espacio de cabeza, como las pirazinas (Söllner & Schieberle,

2009). Sin embargo, algunos compuestos de bajo peso molecular como el acetaldehído, butanal, 2-metil propanal, acetona o etanol, únicamente han sido detectados mediante técnicas del espacio de cabeza (Marco *et al.*, 2007).

En relación a los compuestos aromáticos más potentes, en primer lugar, los aldehídos constituyen una de las familias químicas más importantes en el aroma de los embutidos crudos curados, aportando numerosas notas verdes al aroma y en ocasiones a rancio. Entre ellos, el hexanal es el aldehído que más veces ha sido detectado en los embutidos analizados. Además, ha sido descrito como uno de los compuestos más potentes, tanto con la técnica de dilución AEDA (Blank *et al.*, 2001) como con la técnica de frecuencia de detección (Marco *et al.*, 2007). Otros aldehídos como el pentanal, octanal y 2-nonenal también presentaron altos valores de frecuencia de detección (Marco *et al.*, 2007).

Por otro lado, las cetonas aportan notas lácteas, principalmente olor a mantequilla por la presencia del diacetilo (2,3-butanodiona), y también notas de olor a champiñón, herbal, etc.

En general, los alcoholes no han sido descritos como importantes contribuyentes al aroma, la mayoría de ellos únicamente han sido descritos por Marco *et al.* (2007). Esto puede ser debido a que sus umbrales de percepción son elevados (van Gemert & Nettenbreijer, 2004).

Los ácidos carboxílicos tienen un aroma intenso principalmente a vinagre o a queso, siendo los más potentes los ácidos acético, butanoico y 3-metil butanoico (Schmidt & Berger, 1998a,b; Marco *et al.*, 2007; Söllner & Schieberle, 2009) al presentar altos valores de frecuencias de detección y altos factores de dilución por AEDA.

Por su parte, varios ésteres han sido identificados como responsables de notas afrutadas (piña, fresa) y a caramelo en el aroma de los embutidos. Entre ellos, los descritos como más potentes son el etil butanoato, etil 2-metil propanoato y etil pentanoato (Schmidt & Berger 1998b; Marco *et al.*, 2007).

Los compuestos nitrogenados han sido comúnmente descritos como compuestos clave en el aroma de la carne cocinada al impartir notas a fruto seco, sin embargo, han sido detectados en pocas ocasiones en embutidos crudos curados. La mayoría de ellos han sido únicamente descritos por Söllner & Schieberle (2009) probablemente

debido al tipo de extracción que estos autores emplearon (destilación a alto vacío SAFE). Además, la 2-acetil-pirrolina ha sido descrita como uno de los compuestos aromáticos más potentes de los embutidos (Blank *et al.*, 2008).

Los compuestos azufrados presentan muy bajos niveles de detección y su aroma ha sido descrito como a ajo, cebolla, patata cocida y en ocasiones a carne cocinada. Dentro de los compuestos azufrados con potencia aromática los compuestos disulfuro de dialilo, sulfuro de alilo, metil sulfuro de alilo, metanotiol y metional han sido descritos como los más potentes en el aroma de los embutidos (Schmidt & Berger 1998a,b; Blank *et al.*, 2001; Marco *et al.*, 2007; Söllner & Schieberle 2009).

Los terpenos imparten notas vegetales al aroma, tipo resina, pino o limón. Generalmente sólo han sido detectados en aquellos embutidos a los que se les ha adicionado especias, especialmente pimienta. En este grupo, el linalol ha sido identificado como uno de los compuestos aromáticos más potentes de los embutidos (Schmidt & Berger 1998a).

Por otro lado, los compuestos fenólicos son característicos de los productos cárnicos que han sido sometidos a una etapa de ahumado. Por esta razón, la mayoría de ellos sólo han sido detectados en el embutido húngaro ahumado (Söllner & Schieberle, 2009). En este producto, varios compuestos fenólicos (2-metoxifenol, eugenol, isoeugenol, 4-metil fenol) fueron identificados como los más potentes desde el punto de vista aromático.

Por último, los furanos contribuyen al olor a carne cocinada. El 2-pentil-furano ha sido descrito como uno de los compuestos aromáticos más potentes (Marco *et al.*, 2007) impartiendo notas a cebolla, sabroso y rancio.

4. Técnicas de espectrometría de masas directa

Las técnicas convencionales de análisis de compuestos volátiles requieren la extracción previa de los mismos (apartado 3.1. y 3.2.) y posteriormente su separación mediante cromatografía. Sin embargo, este procedimiento emplea mucho tiempo y otras técnicas se requieren cuando se desea analizar un gran número de muestras en poco tiempo. En este sentido, las técnicas de espectrometría de masas directa

desarrolladas recientemente constituyen una alternativa interesante debido a que analizan los compuestos volátiles a tiempo real (Taylor & Linforth, 2010).

Las técnicas de espectrometría de masas directa se basan en el análisis directo de los compuestos volátiles presentes en una mezcla gaseosa en un detector selectivo de masas sin extracción ni separación cromatográfica previa. Estos métodos requieren el uso de técnicas de ionización más suaves que la ionización por impacto de electrones (IE) comúnmente empleada en la CG acoplada a detector selectivo de masas (Dass, 2007). La ionización por IE generalmente usa energías de ionización de 70 eV que conducen a la fragmentación del compuesto volátil en varios iones (Dass, 2007). En cambio, la ionización química es un proceso más suave, ya que la energía de ionización equivaldría a 10 eV (Taylor & Linforth, 2010), lo que usualmente resulta en iones con una sola carga y fragmentación mínima de manera que, aunque no se realice una separación cromatográfica, los espectros de masas obtenidos son relativamente simples. De esta forma, al prescindir de las etapas de extracción y de la cromatografía, los tiempos requeridos para el análisis mediante las técnicas de espectrometría de masas directa son considerablemente menores que los de GC-MS. Esto permite el estudio de un gran número de muestras en muy poco tiempo, e incluso el análisis de los compuestos volátiles a tiempo real y análisis *in vivo*. Los análisis a tiempo real son útiles en aquellos casos en que los compuestos volátiles de los alimentos sufren cambios en poco tiempo, por ejemplo, en el espacio de cabeza de frutas y vegetales recién cortadas (Španěl & Smith, 1999). Por otro lado, los análisis *in vivo* permiten el estudio de la liberación del aroma durante el tiempo de consumo del alimento, siendo los compuestos volátiles muestreados directamente desde las fosas nasales al espectrómetro de masas (Taylor & Linforth, 2010).

4.1 Fundamento de la ionización química

La ionización química consiste en la formación de iones como resultado de reacciones ácido-base en fase gaseosa entre los compuestos de una muestra y un gas reactivo. La reacción predominante en la ionización química es la reacción de transferencia de protones. Tomando como ejemplo al ión H_3O^+ como gas reactivo, la reacción de transferencia de protones es la siguiente:

$$H_3O^+ + R \rightarrow RH^+ + H_2O$$

Donde *R* es compuesto volátil y RH^+ el ión producto.

Esta reacción tiene lugar si la afinidad protónica de los compuestos a ionizar es mayor que la afinidad protónica del gas reactivo. Por ejemplo, si se emplea H_3O^+ como gas reactivo los compuestos presentes en la muestra cuya afinidad protónica sea mayor que la del agua son ionizados (**Tabla 4**).

Tabla 4. Afinidad protónica de compuestos inorgánicos y orgánicos, adaptado de Blake *et al.* (2009).

Compuesto	Afinidad protónica (kJ/mol)
Gases inorgánicos	
O_2	421
N_2	494
CO_2	541
O_3	626
H_20	691
Compuestos orgánicos	
Acetaldehído	768
Etanol	776
Ácido acético	783
Ácido pentanoico	806
Acetona	812
Dimetil disulfuro	830
Butanoato de metilo	845

El espacio de cabeza de los alimentos, contiene los gases inorgánicos del aire (O_2, N_2, CO_2, etc.) y los compuestos volátiles liberados desde el alimento (compuestos orgánicos). Tal y como se puede observar en la **Tabla 4**, los compuestos orgánicos presentan mayor afinidad por los protones que los gases inorgánicos del aire (Blake *et al.*, 2009), por lo tanto cuando se analiza el espacio de cabeza de los alimentos la ionización es selectiva para los compuestos volátiles de la muestra.

Además de la ionización por transferencia de protones, la ionización química puede tener lugar, aunque en menor medida, mediante otras reacciones, como la ionización por formación de aductos o la ionización por sustracción del ión hidruro (Dass, 2007):

$$G^+ + R \rightarrow [R + G]^+$$

$$G^+ + R \rightarrow [R - H]^+ + G$$

En realidad, el desarrollo de las técnicas de espectrometría de masas directa tenía como objetivo el estudio de las cinéticas de las reacciones ión-molécula entre distintos gases ionizantes y los compuestos orgánicos (Španěl *et al.*, 1997; Španěl & Smith, 1997; Španěl & Smith 1998a; Španěl & Smith, 1998b). Inicialmente estas investigaciones fueron llevadas a cabo mediante espectrometría de masas por selección de iones en tubo de flujo (SIFT-MS) (apartado 4.1.3.). La **Tabla 5** recoge los iones producto característicos generados tras la ionización con diferentes gases precursores.

Tabla 5. Iones característicos formados tras las reacciones de distintos gases ionizantes (adaptado de Smith & Španěl, 2005).

Grupo químico	**H_3O^+**	**NO^+**	**O_2^+**
Alcoholes	RH^+; $[R\text{-}OH]^+$	$[R\text{-}H]^+$; $[R\text{-}OH]^+$	R^+; $[R\text{-}H]^+$
Aldehídos	RH^+; $[R\text{-}OH]^+$	$[R\text{-}H]^+$	R^+; $[R\text{-}H]^+$
Cetonas	RH^+	$NO^+.R$; R^+	R^+
Ésteres	RH^+	$[R\text{-}H]^+$	R^+
Ácidos carboxílicos	RH^+; $[R\text{-}OH]^+$	$NO^+.R$; $[R\text{-}OH]^+$	R^+; $[R\text{-}H]^+$
Compuestos azufrados	RH^+	R^+	R^+

Sin embargo, aunque el uso original de estas técnicas fue el estudio de las reacciones ión-molécula, una vez conocidos los iones producto característicos (RH^+) que se forman tras la ionización química de cada compuesto volátil (R) y los coeficientes de reacción (k) a un tiempo de reacción dado (t), mediante la señal $[RH^+]/[H_3O^+]$ detectada en el espectrómetro de masas, es posible llevar a cabo la cuantificación de los compuestos volátiles, tal y como se explica en la siguiente ecuación:

$$[RH^+]/[H_3O^+] = k\ t\ [R]$$

Donde $[RH^+]$ y $[H_3O^+]$ son las concentraciones de ión producto e ión precursor que no ha reaccionado (remanente) detectadas por el espectrómetro de masas, respectivamente, y *[R]* es la concentración inicial del compuesto volátil de interés.
Se distinguen 3 técnicas de espectrometría de masas directa que se diferencian entre ellas en el modo en que la ionización química se lleva a cabo; la espectrometría de masas por ionización química a presión atmosférica (APCI-MS), la espectrometría de masas por transferencia de protones (PTR-MS) y la espectrometría de masas por selección de iones en tubo de flujo (SIFT-MS).

4.1.1. Espectrometría de masas por ionización química a presión atmosférica (APCI-MS)

La técnica APCI-MS es una técnica de espectrometría de masas directa en la que la ionización se lleva a cabo a presión atmosférica (Taylor *et al.*, 2000). Los compuestos volátiles introducidos llegan a una corona de descarga y los iones formados son arrastrados mediante una corriente de nitrógeno al detector selectivo de masas (Dass, 2007) (**Figura 10**).

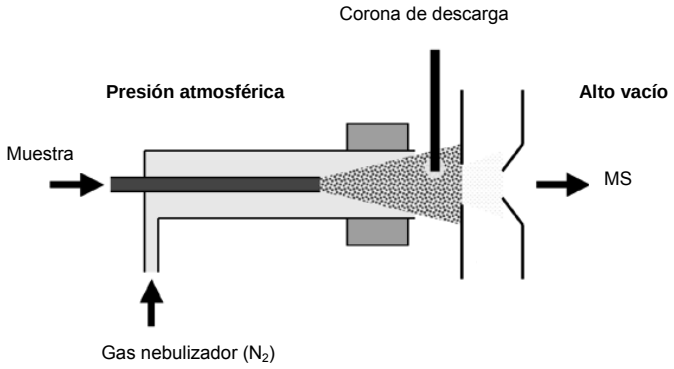

Figura 10. Esquema básico del equipo de ionización APCI-MS (adaptado de Taylor & Linforth, 2010).

La ionización a presión atmosférica conlleva dificultades en el control del proceso de ionización, por ejemplo, la presencia de agua en el ambiente puede modificar las

cinéticas de reacción de la transferencia de protones. Por lo tanto, es necesaria la calibración para las condiciones de cada experimento (Taylor & Linforth, 2010). Estos inconvenientes se evitan si se trabaja a vacío, como es el caso de las técnicas PTR-MS y SIFT-MS (apartados 4.1.2 y 4.1.3), donde las condiciones se mantienen constantes y no es necesario chequear las cinéticas de reacción para las condiciones de cada experimento (Taylor & Linforth, 2010).

La técnica APCI-MS ha sido previamente aplicada para el estudio de la fracción volátil de diversos alimentos (Taylor & Linforth, 2003; Jublot *et al.*, 2005), principalmente para determinar la liberación del aroma durante el consumo. Con respecto a los productos cárnicos ha sido utilizada para estudiar la liberación de compuestos aromáticos durante la masticación de salchichas cocidas con distintos contenidos de grasa (Carrapiso, 2007). En este estudio, se cuantificaron los iones (*m/z*) específicos de los compuestos volátiles que habían sido añadidos a la matriz cárnica y se encontraban de manera muy abundante, sin presentar interferencias de otros compuestos.

4.1.2. Espectrometría de masas por transferencia de protones (PTR-MS)

La técnica PTR-MS fue desarrollada por Lindinger *et al.* (1998). El instrumento PTR-MS está formado por una fuente de iones (comúnmente se trata de un cátodo hueco de descarga) seguido de un tubo de corriente ("drift tube") donde los gases ionizantes reaccionan con los compuestos volátiles (**Figura 11**).

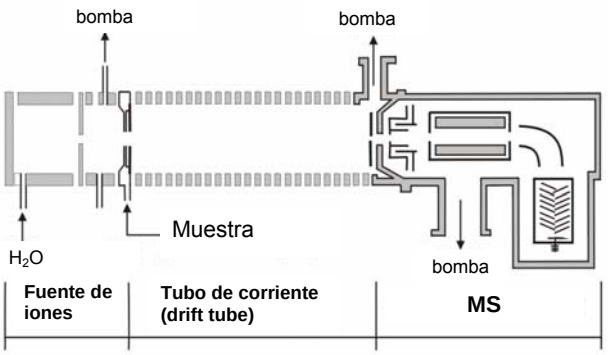

Figura 11. Esquema gráfico de un instrumento PTR-MS (adaptado de Blake *et al.*, 2009).

Generalmente se utiliza H_3O^+ como gas ionizante (Taylor & Linforth, 2010). Los iones producto generados son transportados al espectrómetro de masas mediante campos eléctricos (Blake *et al.*, 2009). Los detectores selectivos de masas generalmente acoplados son el cuadrupolo o el de tiempo de vuelo (TOF).

Esta técnica tiene una alta sensibilidad (1 ppbv) debido a que los iones son transportados mediante campos eléctricos, a diferencia de APCI-MS y SIFT-MS donde son arrastrados por una corriente de gas inerte que diluye la muestra. Sin embargo, este tipo de transporte también es responsable de la principal limitación de la técnica PTR-MS, ya que produce mayor fragmentación de los iones. Cuando se analizan muestras complejas como los alimentos, la excesiva fragmentación da lugar a superposiciones ("overlapping") de iones, es decir, un mismo ión producto procede de distintos compuestos volátiles, no siendo posible atribuir un valor *m/z* a un compuesto específico, lo cual dificulta la interpretación de los espectros (Blake *et al.*, 2009). En estos casos, el perfil de iones del espectro obtenido es considerado como una huella dactilar ("fingerprint") de la muestra, y la evaluación de distintas muestras generalmente se lleva a cabo mediante la comparación de sus espectros haciendo uso de herramientas estadísticas del tipo análisis de componentes principales (PCA) (Aprea *et al.*, 2007; Pozo-Bayón *et al.*, 2009).

Cuando se trata de muestras gaseosas cuyo contenido en compuestos volátiles es más simple o sistemas modelo en los que los compuestos de interés se encuentran en una proporción abundante (Roberts *et al.*, 2003), la cuantificación absoluta se lleva a cabo mediante los principios fundamentales de las reacciones ión-molécula (apartado 4.1.), sin necesidad de calibrado.

Esta técnica ha tenido dos aplicaciones principales en el estudio de los compuestos volátiles de los alimentos; en primer lugar, la evaluación de la calidad de los alimentos y en segundo lugar, la determinación de la liberación y percepción del aroma durante el consumo de los alimentos. A modo de ejemplo, la técnica PTR-MS se ha empleado para la evaluación del tiempo de maduración del queso y el estado oxidativo del aceite de oliva (Aprea *et al.*, 2006; Aprea *et al.* 2007). Además, ha sido utilizada para estudiar el deterioro de la carne, así como el efecto de las condiciones de envasado (Lindinger *et al.*, 1998; Mayr *et al.*, 2003). En cuanto a la medida del aroma durante el consumo,

se han determinado los compuestos volátiles liberados tras la masticación de cebolla, fruta, etc. (Blake *et al.*, 2009).

4.1.3. Espectrometría de masas por selección de iones en tubo de flujo (SIFT-MS)

La técnica SIFT fue desarrollada por Adams & Smith (1976) para el estudio de las cinéticas de reacción ión-molécula, aunque posteriormente el instrumento fue acoplado a un detector selectivo de masas (Smith & Španěl, 2005). El esquema del instrumento SIFT-MS se muestra en la **Figura 12**. El equipo SIFT-MS consta de una fuente de iones seguida de un cuadrupolo, un tubo de flujo (flow tube) y un segundo cuadrupolo. En primer lugar, en la fuente de iones se generan iones mediante descarga eléctrica en una mezcla de aire y agua. A continuación, el cuadrupolo selecciona los iones precursores en función de su relación masa/carga (*m/z*). A diferencia de APCI-MS y PTR-MS, esta técnica ofrece la posibilidad de elegir entre distintos iones precursores, en concreto H_3O^+, NO^+ y O_2^+. Una vez seleccionado el ión precursor, éste llega al tubo de flujo donde reacciona con los compuestos volátiles de la muestra. Los iones producto generados y el ión precursor excedente que no ha reaccionado, son arrastrados por una corriente de helio hacia el segundo cuadrupolo, donde son analizados.

El transporte de los iones mediante corriente de helio es responsable de la dilución de la muestra, y por lo tanto, de una menor sensibilidad (10 pbbv) que la técnica PTR-MS. Sin embargo, la posibilidad de usar tres iones diferentes como gases precursores supone una gran ventaja, dado que cada compuesto volátil resulta en diferentes iones producto según el ión precursor utilizado (**Tabla 5**), por lo tanto se obtienen tres espectros diferentes (Španěl & Smith, 1999). De esta forma, en el análisis de mezclas complejas, si tiene lugar la superposición de iones producto de distintos compuestos volátiles, se puede llevar a cabo la cuantificación de esos compuestos mediante el uso de otro ión precursor. Dado que se trabaja a vacío, las condiciones se mantienen constantes, es decir, para un tiempo de reacción dado (determinado por la velocidad de flujo del helio) el coeficiente de reacción se mantiene constante. Por lo tanto, midiendo el ratio ión producto/ión precursor $[RH^+]/[H_3O^+]$ se puede llevar a cabo la

cuantificación. De esta manera, la cuantificación se realiza mediante los principios fundamentales de las reacciones ión-molécula, sin necesidad de calibrado.

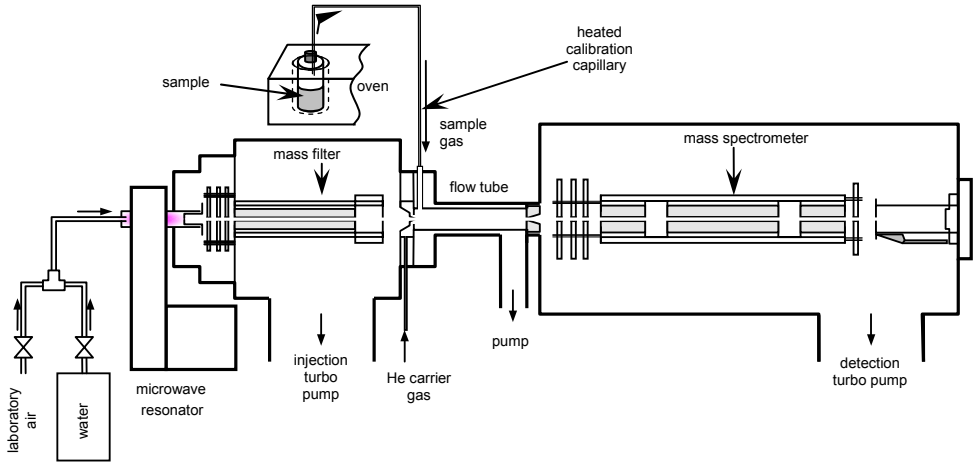

Figura 12. Esquema básico de un instrumento SIFT-MS (figura adaptada de Smith *et al.*, 2009).

Esta técnica ha sido ampliamente aplicada en el campo de la medicina (Smith & Španěl, 2005). Con respecto al área de ciencia de alimentos, Španěl & Smith (1999) estudiaron a tiempo real los compuestos liberados tras el picado de cebolla, ajo y plátano. Otros autores también han utilizado SIFT-MS para evaluar los compuestos volátiles del espacio de cabeza del aceite de oliva (Davis *et al.*, 2005), licores de cacao (Huang & Barringer, 2010), tomate (Xu & Barringer 2009, 2010a) y malta (De Clippeleer *et al.*, 2010). Además, también ha sido empleada para estudiar los compuestos volátiles liberados durante la masticación del tomate (Xu & Barringer, 2010b). Por último, SIFT-MS ha sido usada para evaluar otros parámetros de la calidad de los alimentos aparte del aroma. Por ejemplo, Davis & McEwan (2007) desarrollaron una técnica rápida para la evaluación del estado oxidativo del aceite de oliva y Noseda *et al.* (2010) para el control de la frescura del pescado. Sin embargo, no existen estudios sobre su aplicación en el estudio de compuestos volátiles de productos cárnicos.

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III. Objetivos

III. Objetivos

Teniendo en cuenta el interés de la industria cárnica por conocer los compuestos volátiles responsables de la aceptación de los embutidos crudos curados y el papel de la grasa en el desarrollo y percepción del aroma, y asimismo la demanda de métodos rápidos y sencillos para el análisis de compuestos volátiles, en la presente Tesis Doctoral se plantearon los siguientes objetivos:

1. Estudio de los compuestos con poder aromático responsables de la aceptación de los embutidos tradicionales.

2. Determinación de la contribución de la fracción volátil al aroma de embutidos crudos curados a lo largo del procesado.

3. Efecto de la reducción del contenido de grasa en la calidad de embutidos crudos curados.

4. Evaluación de una nueva técnica de espectrometría de masas directa para el estudio de la calidad de los embutidos crudos curados.

IV. Plan de trabajo

IV. Plan de trabajo

Con el fin de conseguir los objetivos planteados se planteó el siguiente plan de trabajo:

1. Determinación de los compuestos volátiles con poder aromático en embutidos tradicionales (IGP Embutido de Requena) mediante el uso de técnicas olfatométricas (GC-O).

2. Estudio de la contribución de los compuestos aromáticos durante el procesado de embutidos crudos curados mediante el cálculo del valor de la actividad aromática (OAV).

3. Estudio de la distribución de los compuestos aromáticos en las fracciones lipídica y proteica durante el curado de embutidos crudos curados mediante la técnica SPME múltiple.

4. Estudio del efecto de la reducción del contenido de grasa de embutidos crudos curados en los parámetros físico-químicos y en la aceptación por consumidores.

5. Efecto de la reducción del contenido de grasa de embutidos crudos curados en la generación del aroma mediante el uso de técnicas olfatométricas (GC-O).

6. Cuantificación de compuestos aromáticos en embutidos crudo curados mediante la técnica de espectrometría de masas directa Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS).

7. Comparación de la técnica de espectrometría de masas directa (SIFT-MS) frente a técnicas convencionales (GC-MS) en el análisis de compuestos volátiles.

V. Resultados

Capítulo 1

Aroma compounds responsible for the consumers' acceptability of naturally fermented sausages

Aroma compounds responsible for the consumers' acceptability of naturally fermented sausages

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Abstract

The objective of this study was to characterize naturally fermented dry sausages produced without the use of microbial starters and to determine which odour active compounds are responsible for their consumer acceptance. The naturally fermented sausages from different manufacturers were similar in sensory and chemical characteristics; however the traditional manufacture was responsible for different consumer's acceptance. The volatile compounds detected in the HS comprised a complex mixture of volatile compounds derived from bacterial metabolism (mainly esterase activity of Staphyloccoci), spices and lipid autooxidation. The odour-active volatile compounds were identified using gas-chromatography coupled to olfactometry (GC-O) using the detection frequency method. The aroma profile was characterized by the presence of several compounds such as acetic acid, ethyl butanoate, hexanal, methional, 1-octen-3-ol, benzeneacetaldehyde and 4-methyl-phenol. However, naturally fermented sausages were also characterized by numerous esters, both ethyl and methyl esters, which were responsible for the high acceptance of this product by consumers.

Keywords: dry sausage, naturally fermented, aroma, consumers' acceptance, odour-active volatile compounds.

INTRODUCTION

The traditional manufacture methods of artisan fermented sausages and their singular organoleptic attributes distinguish them from others of the same category and make them highly appreciated by consumers. A wide variety of traditional dry sausages are produced in the Mediterranean area, often at local or regional level (Talon, Leroy, Lebert, Giammarinaro, Chacornac, Latorre-Moratalla, Vidal-Carou, Zanardi, Conter & Lebecque, 2008). In the east of Spain, small factories manufacture artisan fermented sausages "Embutido de Requena" (Requena, Valencia, Spain) through a natural fermentation process by indigenous bacteria and using low temperatures during processing (10-12 ºC).

Previous research has been done to characterize the microorganisms involved in the fermentation on naturally fermented sausages (Aymerich, Martín, Garriga & Hugas, 2003; Talon *et al.*, 2008). Also, the changes of the lipid fraction that contribute to their final sensory quality have been studied (Navarro, Nadal, Nieto & Flores, 2001). Nevertheless, few works have studied the volatile compounds present in naturally fermented sausages, (Croizet, Denoyer, Tran, & Berdagué, 1992; Mateo and Zumalacárregui, 1996; Schmidt and Berger, 1998a; Di Cagno, Chaves López, Tofalo, Gallo, De Angelis, Paparella, Hammes, Gobbetti, 2008; Spaziani, Del Torre, & Stecchini, 2009). Although in some cases it is no clear if the sausages were naturally fermented or a starter was used (Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999; Schmidt and Berger, 1998b; Ansorena, Gimeno, Astiasarán & Bello, 2001; Blank, Devaud, Fay, Cerny, Steiner & Zurbriggen, 2001).

Among the hundreds of volatile compounds identified in dry-fermented sausages only a limited number are present at a concentration higher than its threshold value contributing to the aroma. A few works have studied the aroma active compounds in dry fermented sausages (Stahnke, 1994; Schmidt and Berger, 1998b; Meynier *et al.*, 1999; Marco, Navarro & Flores, 2007; Söllner and Schieberle, 2009; Olivares, Navarro & Flores, 2011). However, only Croizet *et al.* (1992) and Schmidt & Berger (1998a) have studied aroma compounds in naturally fermented sausages. Nevertheless, the

relationship between aroma active compounds of naturally fermented sausages and consumer acceptability has never been established.

The characterization of the aromatic profile of naturally fermented sausages can be useful for their authentication and differentiation based on the type and the relative amounts of volatile compounds. Therefore, the main objective of this study was to characterize naturally fermented Spanish dry sausages ("Embutido de Requena") and to determine which aroma compounds are responsible for their consumer acceptance.

MATERIALS AND METHODS

Dry fermented sausages

Naturally fermented dry sausages ("Embutido de Requena") from 10 different manufacturers (ER-1 to ER-10) were supplied by the "Consejo Regulador de la Indicación Geográfica Protegida Embutido de Requena" (Requena, Valencia, Spain). Artisan sausages were manufactured under the traditional specifications; no starter cultures were added and the drying process was carried out in room temperature chambers at low temperatures (approximately 10-12 ºC). The naturally fermented sausages were dried for approximately 1 month. The weight of naturally fermented sausages was 200-350 g and the diameter ranged from 30 to 40 mm. All the sausages were manufactured using lean pork meat, pork back fat, sodium chloride, nitrite, nitrate, sugars, dextrins, spices and different additives such as colorants (Ponceau 4R, carminic acid) and proteins (milk and soya). Three sausages from each "Salchichón de Requena" manufacturer (ER-1 to ER-10) were sliced, vacuum packed and frozen at -20 ºC for subsequent chemical, lipid and volatile analyses. The remaining sausages were immediately used for the sensory analysis. Results were expressed as the mean of three replicates in dry matter (dm).

Chemical analyses (moisture, total lipids, protein, pH, TBARS, nitrate, nitrite and free fatty acids)

Moisture content was determined according to the official method for analysis of meat products BOE (1979) by dehydration at 100 ºC until constant weight. Total lipids were extracted from 5 g of minced sausage according to the method of Folch, Lees and

Stanley (1957), using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1) as solvent due to its lower toxicity. The extracts were dried in a rotating vacuum evaporator and weighed to determine the total lipid quantity. Free fatty acids were analysed as described Olivares *et al.* (2011). Nitrogen content was determined by the Kjeldahl method. The pH was measured as described by ISO 2917:1974 by introducing a pH-meter (HI 99163, Hanna Intruments Inc., Hoonsocket, USA) into a sausage:water mixture (1:1). Thiobarbituric acid reactive substances (TBARS) were determined according to Bruna, Ordóñez, Fernández, Herranz and de la Hoz (2001), using tricloroacetic acid instead of perchloric acid as solvent. The nitrate and nitrite content was determined using an enzymatic kit (Cat. No. 09050658, Roche, Palo Alto, USA) according to Arneth and Herold (1988).

Analysis of headspace aroma compounds

Extraction of headspace (HS) volatile compounds was done using a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA) as described Olivares *et al.*, (2011). For the identification of the volatile compounds, a gas chromatograph HP 7890A equipped with an HP 5975C mass selective detector (Hewlett Packard, Palo Alto, CA) was used. The quantification of volatile compounds was done in a DB-624 capillary column (J&W Scientific; 60 m, 0.32 mm id, film thickness 1.8 μm) installed on an Agilent 6890 gas chromatograph (USA) equipped with a flame ionisation detector (FID). Helium was used as the carrier gas at a linear velocity of 37.0 cm/s. The GC oven programme began when the fibre was inserted in the injection port at 240 ºC during 15 min in splitless mode and using a 0.75 mm id liner. Then, the volatile compounds were separated using a temperature programme and after the injection of the fibre the oven was held at 38 ºC for 13 min, ramped at 100 ºC at 3 ºC/min and maintained at 100 ºC during 10 min, then to 150 ºC at 3 ºC/min, and to 210 ºC at 5 ºC/min, and finally held at 210 ºC for 20 min, the total run time was 82.3 min. Detector temperature was set at 240 ºC. Kovats indices (KI) of the volatile compounds were calculated. The identification of compounds was confirmed using authentic standards. The content of each volatile compound was calculated from the FID area and expressed as area units (AU 10^{-6}).

The identification of aroma-active compounds was done by SPME-GC-Olfactometry (SPME-GC-O) as described Olivares *et al.* (2011). The detection frequency method was used to estimate the aromatic impact of each volatile compound and two trained assessors evaluated the 10 naturally fermented sausages, therefore a total of 20 assessments were carried out.

Sensory analysis

The acceptability of naturally fermented sausages was evaluated by 78 consumer panellists. The analysis was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988) as described Olivares *et al.* (2011). The consumers were asked to evaluate each sausage based on appearance, aroma, hardness, juiciness, taste and overall quality using a 9-point hedonic scale. The analysis was done in two different sessions due to the high number of samples to avoid fatigue of the panellists, 5 samples in each session. Sensory evaluations were recorded by computer software using Compusense® five release 4.6 (Compusense Inc., Guelph, ON, Canada).

Statistical analysis

Data from chemical, free fatty acids, volatile and sensory analyses were analysed by one-way analysis of variance (ANOVA) in order to determine differences among manufacturers. Differences between particular sample means were analysed according to Fisher's least significant difference (LSD) test. Also, a Pearson correlation procedure was performed to evaluate any relationship between chemical and volatile compounds parameters. Furthermore, principal component analysis (PCA) was used to find the relationships among sausage manufacturers (ER-1 to ER-10) and chemical parameters. Statistical analysis was performed using the statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain).

RESULTS AND DISCUSSION

Chemical analyses (moisture, total lipids, pH, TBARS, nitrate and nitrite)

Table 1 shows the composition and chemical characteristics of Requena naturally fermented sausages produced by different manufacturers. The water content of naturally fermented sausages ranged from 24.6 to 35.6 % while fat and protein contents ranged from 28.7 to 55.2 % and 32.3 to 53.7 % in dry matter (dm), respectively. Moisture, lipid and protein content are within the range expected for dry fermented sausages (Wirth, 1988), although the variability among sausages can be due to the raw materials used and also to differences in the traditional manufacturing practices.

Table 1. Composition and chemical characteristics of naturally fermented sausages.

Samples	% water (g / 100g)	% fat (g / 100g DM)	% protein (g / 100g DM)	pH	TBARS (mg MDA/kg DM)	Nitrate (ppm DM)	Nitrite (ppm DM)
ER-1	29.40 c	39.94 bc	43.36 c	5.56 b	1.34 c	206.72 bcd	10.47 bc
ER-2	33.28 b	33.10 d	53.73 a	5.55 b	1.01 cd	499.94 a	11.06 b
ER-3	28.12 cd	40.99 bc	37.71 d	6.00 a	1.28 c	535.65 a	12.47 a
ER-4	31.80 b	55.18 a	32.27 e	5.08 d	0.76 d	336.18 b	9.78 c
ER-5	24.66 e	44.75 b	36.96 d	6.00 a	2.08 b	269.43 bc	9.98 c
ER-6	27.06 d	51.45 a	35.64 d	6.05 a	2.78 a	10.73 e	11.14 b
ER-7	33.77 ab	42.32 bc	45.33 bc	5.36 c	1.29 c	110.89 de	8.66 d
ER-8	27.91 cd	42.48 bc	45.19 bc	5.38 c	1.04 cd	175.90 cd	8.79 d
ER-9	35.67 a	28.69 d	46.15 b	5.47 d	0.63 d	11.96 e	8.97 d
ER-10	33.45 ab	38.89 c	44.65 bc	5.53 d	0.98 cd	14.74 e	8.53 d
SEM	0.784	1.697	0.927	0.032	0.173	52.280	0.251

[1]MDA: Malonaldehyde, [a-e]: Means that do not share any letter are significantly different (p <0.05). * SEM: Standard error of the mean

In relation to pH, all the sausages were above pH 5.0 and all of them were between 5.3 and 6.05, except ER-4 **(table 1).** Therefore “Salchichón de Requena” sausages can be considered as low-acid sausages (Aymerich *et al.*, 2003).

The TBARS ranged from 0.6 to 2.8 mg malonaldehyde (MDA) per Kg dm. The lipid oxidation process that takes place during the ripening of dry sausages is essential to develop their typical aroma (Gandemer, 2002). TBARS is used to evaluate the extent of the lipid oxidation process by measuring secondary lipid oxidation products. The level of TBARS found is within the range expected for ripened sausages (Marco, Navarro and Flores, 2006). A positive correlation Pearson coefficient was obtained

between the TBARS value and fat content (r = 0.865, p = 0.003) for all the manufacturers excluding ER-4. The highest lipid oxidation values were detected in the sausages that contained the highest amount of fat as also reported by Liaros, Katsanidis, & Bloukas (2009) and Olivares *et al.* (2011).

The nitrate content differed significantly between the sausages (**table 1**). The concentrations ranged from 10.7 to 535.6 ppm (dm). Five naturally fermented sausages (corresponding to manufacturers ER-1, ER-2, ER-3, ER-4 and ER-5) showed high nitrate residual levels. With respect to nitrite, only residual levels were found, as previously detected by Meynier *et al.* (1999).

In summary, the composition and chemical characteristics were similar among the naturally fermented sausages of different manufacturers, except for few differences in the composition and the high nitrate residual level of several of them.

Free fatty acid analysis

The total concentration of free fatty acids (FFA) in the naturally fermented sausages ranged from 1066.4 to 3211.1 mg/ 100 g dm (**table 2**) which represented 2.5-8.0 % of the total lipid content. A similar FFA concentration was observed by Zanardi, Ghidini, Battaglia & Chizzolini (2004) in dry fermented sausages. The FFA relationship detected in the sausages was MUFA>PUFA>SFA. This behaviour was previously observed by Navarro *et al.* (2001) in non-fermented sausages. However, Gandemer (2002) reported that the free fatty acid composition of back fat from industrial pigs was MUFA>SFA>PUFA and this relation was also found by Navarro *et al.* (2001) in the fresh meat paste. Therefore, it is supposed that the free fatty acid profile observed in this study was the consequence of the preferential release of the unsaturated fatty acids from the sausage lipids during ripening of sausages as previously reported (Navarro *et al.*, 2001; Zanardi *et al.*, 2004).

In summary, the FFA profile was similar among sausages, although several of them showed lower concentration. The differences in FFA among naturally fermented sausages are probably due to differences in the processing conditions among manufacturers.

Table 2. Free fatty acid (FFA) concentrations (mg/100 g dm) of naturally fermented sausages.

Free fatty acids	ER-1	ER-2	ER-3	ER-4	ER-5	ER-6	ER-7	ER-8	ER-9	ER-10	SEM
C14:0	34.2 a	16.5 c	19.3 c	26.9 b	15.0 c	13.5 c	20.0 c	15.5 c	18.4 c	39.3 a	2.34
C16:0	407.1 a	249.8 cd	218.8 cd	268.5 c	223.3 cd	201.3 de	224.0 cd	159.2 e	195.5 de	341.5 b	20.11
C18:0	212.6 a	132.2 bc	98.9 d	141.1 b	93.2 de	97.2 de	104.4 cd	69.8 e	86.8 de	145.4 b	9.70
SFA	653.9 a	398.5 cd	337.0 cde	436.5 bc	297.8 de	276.8 e	348.4 cde	244.5 e	300.6 de	526.2 b	36.64
C16:1	81.5 b	51.0 d	43.6 de	99.4 a	47.7 de	42.4 de	36.1 ef	36.8 ef	29.0 f	67.2 c	4.19
C18:1	1571.3 a	821.1 d	710.3 de	1347.0 b	646.6 def	688.9 de	571.5 efg	476.0 fg	387.5 g	1100.4 c	68.49
C20:1 n9	33.2 a	12.9 cd	12.1 cd	25.8 b	11.2 cd	17.0 c	12.9 cd	9.6 d	7.8 d	10.9 cd	2.28
MUFA	1685.9 a	885.0 c	766.0 cd	1472.1 a	698.7 cde	731.2 cde	620.6 def	522.4 ef	424.3 f	1178.4 b	74.31
C18:2 n6	708.7 a	346.8 cde	357.2 cde	428.9 c	319.9 def	307.7 def	367.3 cd	246.5 f	267.9 ef	587.1 b	32.46
C18:3 n3	45.3 a	17.3 cd	22.2 c	32.6 b	19.8 cd	19.1 cd	19.5 cd	14.9 d	15.3 d	32.8 b	2.10
C20:2 n6	25.6 a	10.4 ef	11.3 de	17.0 bc	11.7 de	13.8 cd	14.9 c	9.5 ef	7.7 f	19.0 b	1.07
C20:3 n6	10.6 a	8.2 bc	5.0 ef	6.2 de	4.8 ef	4.7 ef	6.9 cd	4.1 f	4.6 ef	8.8 b	0.55
C20:4 n6	55.0 a	43.1 b	30.4 c	29.1 cd	28.4 cd	29.6 cd	43.9 b	22.1 d	31.6 c	56.8 a	2.76
C22:4 n6	7.0 a	5.0 bc	4.3 cde	5.1 bc	3.9 de	4.4 cd	5.9 b	3.4 e	4.1 cde	7.4 a	0.34
C20:5 n3	1.5 a	1.1 bc	0.8 de	1.2 b	0.8 cde	0.6 e	1.1 bcd	0.6 e	0.7 e	1.2 b	0.11
C22:5 n3	14.1 a	8.0 bc	6.6 cd	12.7 a	6.2 cd	7.9 bc	9.8 b	5.9 d	7.7 cd	14.6 a	0.67
C22:6 n3	3.3 a	1.1 d	0.9 d	2.0 c	0.8 d	0.9 d	1.8 c	0.7 d	1.8 c	2.6 b	0.17
PUFA	871.3 a	441.0 cde	438.8 cde	534.7 c	396.3 def	388.8 def	471.2 cd	307.7 f	341.4 ef	730.3 b	39.38
Total	3211.1 a	1724.6 cd	1541.8 cd	2443.3 b	1848.7 c	1399.4 de	1440.1 cde	1074.6 e	1066.4 e	2434.9 b	151.39

[A] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. [a-g]: Means that do not share any letter are significantly different ($p < 0.05$). * SEM: Standard error of the mean.

Headspace volatile compounds

A total of 99 volatile compounds were identified by GC-MS in the HS of sausages and their structures were confirmed using authentic standards. However, several compounds could not be totally resolved and were quantified in the GC-FID as a mixture of volatile compounds (**table 3**). Moreover, 4 compounds were not quantified in the sausages HS due to its low concentration (methanethiol, butanoic acid, 1-octen-3-ol and phenylethyl alcohol). The compounds detected were 27 esters, 19 aldehydes, 14 hydrocarbons, 9 alcohols, 9 acids, 8 ketones, 7 terpenes, 3 sulphur compounds, 2 pyrazines and 1 lactone. All of them have been previously identified in dry fermented sausages (Meynier *et al.*, 1999; Schmidt and Berger, 1998; Ansorena *et al.*, 2001) except for methyl benzeneacetate and methyl nonanoate.

The abundance of the volatile compounds is shown in **table 3**, where the volatile compounds are grouped according to their possible origin: lipid autooxidation, bacterial metabolism (lipid β-oxidation, carbohydrate fermentation, amino acid degradation, and staphylococci esterase activity), spices and unknown origin (meat or food contaminants). However, several of the compounds listed can have more than one origin (Ordóñez, Hierro, Bruna & de la Hoz, 1999). With our extraction technique (SPME CAR/PDMS), the compounds extracted in higher amounts were those derived from lipid autooxidation, esterase activity of Staphylococci and spices (**figure 1**). However, the proportion of volatile compounds obtained depends on the stationary phase of the SPME fibre employed and the extraction conditions (time, temperature, etc). Therefore, the HS abundance and volatile compounds profile can not be compared with other studies which employed other extraction techniques such as dynamic headspace (Croizet *et al.*, 1992) and distillation techniques (Mateo & Zumalacárregui, 1996; Schmidt & Berger, 1998a) or other SPME extraction conditions (Di Cagno *et al.*, 2008; Spaziani *et al.*, 2009).

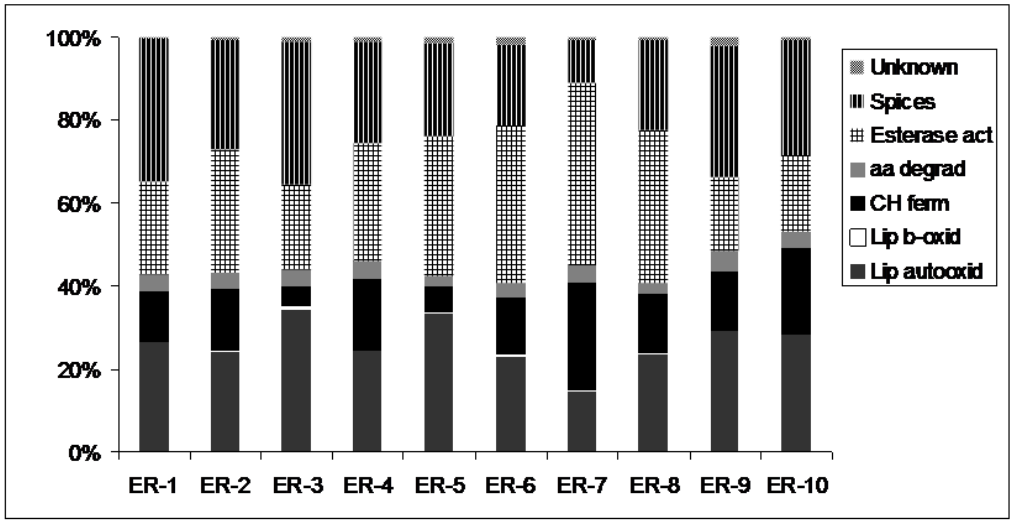

Figure 1. Percentage of volatile compounds present in naturally fermented sausages (ER-1 to ER-10) according to their origin lipid autooxidation (Lip autooxid), lipid, β-oxidation (Lip b-oxid), carbohydrate fermentation (CH ferm), amino acid degradation (aa degrad), esterase activity (Esterase act), spices, and unknown origin (Unknown).

Lipid oxidation was responsible for the generation of volatile compounds mainly by autooxidation process as lipid autooxidation products comprised 14.5-34-4% of the total extracted area whilst β-oxidation only 0.17-0.59% (**figure 1**). The manufacturers with the highest lipid derived compounds were ER-3, and ER-5 (**table 3**). In this group, the compounds that showed the highest abundance were hexanal, octanal, hexanoic acid, 2,4-heptadienal and nonanal (**table 3**). The total percentage of lipid autooxidation products is low compared to the observed using the same extraction technique in dry fermented sausages elaborated with starter culture addition, where these compounds represented up to 45% of the extracted area (Marco *et al.*, 2006) although these authors did not use spices. However, the relative abundance obtained is in agreement with other naturally fermented sausages where the percentage of lipid autooxidation volatile compounds was around 25% (Mateo and Zumalacárregui, 1996; Di Cagno *et al.*, 2008; Spaziani *et al.*, 2009), although different extraction techniques were used. In general, lipid derived volatile compounds represent one of the main groups in naturally fermented sausages although their relative importance is lower than in other fermented

sausages. This fact could be due to the low temperatures applied during naturally fermented sausage processing, since lipid autooxidation is increased by temperature (Stahnke, 1995).

The volatiles generated from the carbohydrate fermentation represented 4.9-25.8% of the total extracted area (**figure 1**), although only ER-7 and ER-10 showed percentages above 20%. In this group, the compound extracted in the highest proportion was acetic acid (**table 3**), although acetone was the most abundant compound in ER-3. It was observed an inversely relationship between acetic acid abundance and pH value, except for ER-4, since higher acetic acid abundance corresponded to lower pH (Pearson coefficient r=-0.7, p = 0.033). In general, the relative abundance of acetic acid in naturally fermented sausages is low compared to starters added sausages (Mateo & Zumalacárregui, 1996). This fact is related to the low-acid pH of these sausages (**table 1**). Other authors also reported a low proportion of acetic acid in naturally fermented sausages (Schmidt & Berger, 1998a; Di Cagno *et al.*, 2008).

The volatile compounds originated from the amino acid degradation comprised 2.5-5.2 % of the total extracted volatile compounds (**figure 1**). The most abundant compounds in this group were 2-methyl butanoic acid, benzaldehyde, phenol and benzeneacetaldehyde (**table 3**), which have been also detected in other naturally fermented sausages (Mateo and Zumalacárregui, 1996; Di Cagno *et al.*, 2008). The lowest abundance of this group was found in the sausages ER-5 and ER-8, mainly due to its lower abundance of benzaldehyde.

Esters were detected as the most abundant group of volatile compounds arising from microbial metabolism since they represented 17.6-44.1% of the total extracted area (**figure 1**). Within the sausages, ER-6, ER-7 and ER-8 showed the highest esters abundance.

Table 3. Volatile compounds (GC-FID area units; AU 10^{-6}) in the headspace of naturally fermented sausages.

KI	Compound/origin	ER-1	ER-2	ER-3	ER-4	ER-5	ER-6	ER-7	ER-8	ER-9	ER-10	SEM
	Lipid autooxidation											
500	Pentane	1.57 cd	1.16 d	2.35 cd	3.42 cd	17.66 a	9.54 b	3.06 cd	1.63 cd	6.26 b	2.17 cd	1.61
521	Propanal	1.10 cd	0.00 d	17.30 a	0.63 d	6.80 b	4.01 bc	0.00 d	0.00 d	1.66 cd	0.00 d	1.05
600	Hexane	11.19 f	30.88 abc	16.23 ef	21.03 cdef	30.12 abcd	25.46 bcde	19.77 def	26.14 bcde	33.86 ab	36.98 a	3.52
614	1-propanol	16.41 c	10.39 d	25.41 b	8.53 de	5.73 e	10.41 d	23.86 b	18.11 c	49.31 a	7.67 de	1.11
625	Butanal	0.00 c	0.00 c	1.01 a	0.00 c	0.80 ab	0.50 b	0.00 c	0.00 c	0.00 c	0.00 c	0.08
700	2-Methylbutanal / heptane	0.00 c	3.38 bc	8.32 b	2.05 bc	8.85 b	6.83 bc	3.04 bc	1.62 bc	25.61 a	2.77 bc	2.81
735	Pentanal	8.84 bc	3.53 c	30.58 a	7.89 bc	25.50 a	12.48 b	9.65 bc	6.50 bc	7.96 bc	3.20 c	2.40
739	Ethyl propionate / 2,3-Pentanedione	2.56 c	7.83 ab	9.33 a	7.27 b	2.62 c	7.87 ab	9.17 ab	9.50 a	2.60 c	3.25 c	0.70
795	Propanoic acid	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	18.58 b	0.00 d	30.48 a	0.00 d	4.85 c	0.52
800	Octane	2.10 c	3.29 bc	4.88 bc	4.80 bc	22.87 a	12.39 b	5.81 bc	3.50 bc	28.25 a	3.13 bc	3.21
836	Hexanal	82.20 c	47.65 c	323.38 a	95.04 bc	359.65 a	181.92 b	122.78 bc	96.14 bc	109.83 bc	62.48 c	31.93
904	2-Hexenal / Isopentyl acetate	0.00 h	1.77 g	3.73 e	2.50 efg	9.40 c	12.49 b	17.75 a	7.84 d	2.30 fg	3.30 ef	0.46
939	Heptanal	23.09 c	16.84 cd	40.62 b	40.38 b	67.90 a	38.77 b	55.65 a	4.54 d	38.54 b	16.09 cd	5.06
970	Methional / Pentanoic acid	10.85 a	1.60 d	5.20 c	0.88 d	5.91 c	5.53 c	9.11 b	1.75 d	9.63 ab	9.00 b	0.44
1011	2-Heptenal/ Butirolactone	5.67 bcd	0.00 d	13.30 b	3.19 cd	12.90 b	8.53 bc	7.70 bcd	3.66 cd	30.27 a	2.73 cd	2.85
1047	Octanal	3675.03 a	2552.22 bc	3705.44 a	1814.40 d	2061.78 cd	1565.85 d	672.33 e	1664.07 d	3174.85 ab	3796.34 a	212.11
1064	Hexanoic acid	219.36 a	139.44 c	177.86 b	72.49 e	96.80 d	59.43 e	76.55 de	139.00 c	173.64 b	193.64 b	8.09
1075	2,4-Heptadienal	113.92 a	49.39 c	110.44 ab	29.60 d	14.85 d	17.62 d	47.06 c	24.14 d	94.55 b	119.99 a	5.51
1116	2-Octenal	3.49 d	0.37 e	6.87 b	2.30 de	6.73 bc	4.07 cd	3.62 d	2.29 de	26.41 a	1.52 de	0.93
1151	Nonanal	81.38 bc	49.46 cd	86.66 bc	40.33 d	112.53 b	110.36 b	187.32 a	116.76 b	177.02 a	57.08 cd	12.97
1159	Heptanoic acid	20.37 d	24.62 cd	27.98 c	258.87 a	11.30 e	10.77 e	28.25 c	12.61 e	10.47 e	50.44 b	2.28
1200	Dodecane	5.09 cd	4.47 de	2.62 ef	5.57 bcd	1.11 fg	1.86 fg	10.77 a	0.00 g	7.13 bc	7.27 b	0.72
1221	2-Nonenal	2.67 c	4.26 bc	13.27 b	3.13 c	3.47 bc	2.65 c	2.87 c	2.10 c	81.15 a	1.52 c	3.34
1253	Octanoic acid/decanal	64.83 ab	45.66 c	49.91 bc	1.53 d	57.38 bc	57.54 bc	41.07 c	80.48 a	49.59 bc	76.53 a	5.69
1326	2-Decenal	3.66 bc	1.86 de	2.57 cde	1.70 de	3.95 bc	3.23 bcd	4.60 ab	5.89 a	3.92 bc	1.01 e	0.61
1344	2-Undecanone	5.58 bcd	5.87 bc	3.57 cde	7.93 b	2.69 e	2.18 e	3.29 de	28.41 a	6.80 b	7.17 b	1.02
1440	Decanoic acid	35.21 c	31.53 cd	49.24 b	35.90 c	28.24 de	21.21 f	54.36 a	32.54 cd	23.66 ef	33.68 c	1.71
	Lipid β-oxidation											
637	2-Butanone	13.24 b	17.24 a	18.23 a	8.39 c	5.97 cd	4.20 d	18.08 a	9.25 c	0.00 e	7.90 c	1.14
730	2-Pentanone	0.00 e	0.00 e	20.46 a	0.50 de	2.83 b	0.63 d	0.52 de	0.47 de	1.60 c	0.55 de	1.96
931	2-Heptanone	1.55 d	0.00 d	14.28 b	2.32 d	13.09 bc	1.03 d	1.10 d	10.27 c	25.27 a	0.00 d	0.92
1142	2-Nonanone	21.10 cd	31.20 a	28.50 ab	8.50 f	17.50 de	21.63 cd	24.50 bc	0.00 g	13.75 e	28.55 ab	1.93
	Carbohydrate fermentation											
506	Ethanol	6029.94 d	11581.87 ab	245.88 f	8005.49 c	3442.89 e	8094.45 c	10550.44 b	12197.66 a	5697.79 d	5353.21 d	425.81
528	Acetone	53.27 d	100.10 b	369.43 a	32.63 e	51.19 de	8.32 f	74.34 c	32.89 e	43.12 de	4.56 f	6.45
632	Diacetyl	0.00 e	2.33 cd	23.91 a	0.00 e	2.99 c	3.23 c	1.09 de	0.00 e	0.80 e	9.27 b	0.46
652	2-Butanol	3.52 f	7.05 cdef	7.44 cde	5.46 ef	9.34 bc	7.70 cde	6.02 def	8.71 bcd	11.44 ab	12.87 a	1.09
706	Acetic acid	1902.84 cd	1767.46 cd	273.76 f	1699.16 d	498.53 f	1309.27 e	2442.95 b	1396.48 e	1996.68 c	3292.36 a	98.41
	Amino acid catabolism											
590	2-Methylpropanal	1.42 cd	1.37 d	2.20 bc	0.81 d	3.64 a	1.29 d	1.24 d	0.86 d	2.68 b	0.84 d	0.27
680	Benzene	0.55 de	0.40 e	1.12 b	1.35 ab	1.61 a	1.35 ab	0.78 cd	0.45 e	1.07 bc	1.09 b	0.11
683	2-Methyl-1-propanol	0.00 f	9.52 c	0.00 f	7.79 c	2.79 de	18.43 b	16.53 b	28.52 a	4.93 d	1.40 ef	0.83
691	3-Methyl butanal	4.48 def	8.68 cde	10.54 bc	2.45 f	9.06 cd	5.44 cdef	3.90 ef	0.77 f	14.41 b	23.48 a	1.74
774	Dimethyl disulfide	2.72 cd	13.80 bcd	173.41 a	0.00 d	18.83 bc	11.43 cd	27.98 b	0.00 d	13.14 bcd	179.30 a	5.56
953	2-Methyl butanoic acid	98.06 a	76.96 b	64.63 b	30.65 c	17.49 cde	10.92 de	9.86 e	30.21 cd	73.75 b	19.36 cde	6.53
1021	Benzaldehyde	466.30 ab	287.05 de	248.06 e	329.40 cd	136.10 f	158.85 f	99.54 f	149.44 f	534.83 a	411.87 bc	24.97
1103	Phenol	52.13 a	19.69 e	54.81 a	28.65 bcd	22.64 de	25.36 cde	4.90 f	22.03 e	29.85 bc	33.50 b	2.06
1111	Benzeneacetaldehyde	69.76 c	55.64 c	18.76 efg	25.26 def	10.92 fg	39.90 d	238.20 a	5.23 g	30.20 de	114.75 b	5.27

	Staphylococci esterase activity											
549	Methyl acetate	513.20 b	345.55 d	185.61 e	432.85 c	218.83 e	654.42 a	635.17 a	314.88 d	326.88 d	287.86 d	22.97
642	Ethyl acetate	358.71 e	843.83 a	0.60 f	372.85 e	38.05 f	413.81 de	965.44 a	581.71 bc	513.90 cd	705.29 b	44.78
659	Methyl propanoate	26.84 cd	16.67 fg	41.53 b	30.31 c	44.80 ab	47.02 a	23.09 de	20.44 ef	22.54 de	14.88 g	1.71
744	Propyl acetate	8.84 a	0.52 cd	1.15 c	0.34 d	0.49 cd	0.64 cd	2.42 b	0.67 cd	2.59 b	0.72 cd	0.27
751	Methyl butanoate	994.51 b	618.56 cd	1024.70 ab	458.01 e	1127.54 a	711.16 c	538.49 de	470.45 e	324.12 f	522.46 de	42.91
789	Ethyl 2-methyl propanoate	10.67 b	2.95 f	0.00 g	4.15 ef	0.00 g	5.70 de	9.40 bc	13.50 a	7.30 cd	0.00 g	0.81
788	Toluene / Methyl 2-hydroxy-propanoate	131.50 cd	99.70 ef	79.08 f	166.55 b	153.70 bc	161.25 b	162.06 b	194.23 a	114.71 de	88.42 ef	9.16
803	Methyl 3-methyl butanoate	17.35 c	30.78 c	11.21 c	67.71 b	36.05 c	68.96 b	66.40 b	78.67 b	148.39 a	25.32 c	9.46
826	Ethyl butanoate	112.95 bc	211.14 a	1.30 f	90.16 cd	46.91 e	118.94 b	202.75 a	184.33 a	48.83 e	71.91 de	9.66
849	Methyl pentanoate	71.52 ab	39.21 d	77.41 a	43.25 d	76.73 a	61.59 bc	50.85 cd	16.80 e	44.96 d	63.32 abc	4.79
859	Ethyl 2-hydroxy-propanoate / methyl pirazine	22.83 c	44.37 b	0.00 e	46.68 b	4.32 e	20.04 cd	68.10 a	66.44 a	13.67 d	15.24 d	2.17
872	Ethyl 2-methyl butanoate	356.77 a	268.45 b	203.96 c	150.27 e	228.46 c	164.27 de	220.46 c	202.45 cd	161.59 e	281.67 b	13.29
876	Ethyl 3-methyl butanoate	0.00 e	19.68 c	0.00 e	22.21 c	3.75 de	18.91 c	34.30 b	44.57 a	4.73 de	8.53 d	2.75
924	Ethyl pentanoate	7.02 cd	12.23 b	0.96 f	6.30 d	4.02 e	8.62 c	15.13 a	0.00 f	11.23 b	7.07 cd	0.60
950	Methyl hexanoate	808.20 b	647.45 c	984.35 a	622.78 c	868.28 ab	656.59 c	668.40 c	849.79 b	548.38 c	577.06 c	42.80
1126	Ethyl heptanoate	3.04 bc	0.00 d	6.83 a	2.74 bc	7.73 a	3.72 b	3.83 b	2.10 bcd	1.90 bcd	1.13 cd	1.03
1156	Methyl octanoate	133.93 cd	92.78 de	132.78 cd	112.19 cde	104.67 cde	275.23 ab	293.81 a	233.49 b	146.16 c	77.06 e	14.16
1226	Ethyl octanoate	87.75 d	157.65 b	2.96 g	2.87 g	41.79 f	134.07 c	155.56 b	242.68 a	5.00 g	61.80 e	7.05
1237	Methyl benzeneacetate	0.00 d	202.73 a	1.93 cd	98.47 b	1.47 cd	0.36 d	3.16 cd	1.78 cd	5.60 c	2.13 cd	1.65
1255	Methyl nonanoate	9.60 e	34.93 d	45.39 c	76.40 a	12.48 e	3.67 f	34.94 d	13.34 e	38.37 d	58.03 b	1.45
1323	Ethyl nonanoate	2.95 cd	4.13 bc	0.25 d	6.70 ab	1.50 cd	1.96 cd	6.11 b	5.97 b	9.70 a	1.28 d	0.95
1354	Methyl decanoate	11.59 cd	10.79 cd	15.11 c	30.41 a	14.35 c	28.74 a	33.36 a	21.89 b	9.07 d	7.84 d	1.61
1421	Ethyl decanoate	28.18 d	87.02 b	8.85 f	59.57 c	9.58 ef	64.61 c	107.90 a	90.13 b	23.23 def	26.46 de	5.36
	Derived from spices											
945	α-Pinene	239.98 b	214.62 b	159.36 c	44.57 d	35.09 d	26.25 d	10.38 d	29.79 d	165.10 c	351.95 a	13.51
1003	β-Myrcene	2312.18 a	1183.03 d	1978.21 b	814.07 e	493.89 f	379.68 f	153.79 g	572.49 f	1635.03 c	2070.80 ab	74.14
1026	Ethyl hexanoate / 3-carene	1303.84 a	764.95 c	979.80 b	687.97 c	423.65 d	362.09 d	301.01 d	690.27 c	859.67 bc	772.38 c	66.70
1037	α-terpinene / trimethyl pyrazine / octanone	61.99 b	37.71 c	37.43 c	18.66 d	7.40 d	9.38 d	35.19 c	14.10 d	64.67 b	79.23 a	4.79
1052	p-Cymene	443.25 a	422.48 a	450.98 a	271.96 b	174.12 cd	107.64 e	130.12 de	199.28 c	428.40 a	328.60 b	19.72
1051	Limonene	468.55 a	361.33 b	301.88 b	177.32 c	180.54 c	178.40 c	98.25 c	85.02 c	520.54 a	295.87 b	33.64
1148	Linalol / Methyl benzoate	71.26 bc	77.71 ab	85.74 a	3.34 g	65.91 cd	52.87 ef	46.38 f	50.86 ef	50.21 ef	59.55 de	3.22
1255	Terpineol	13.09 ab	15.73 a	14.07 ab	13.80 ab	7.98 cd	5.81 d	5.88 d	6.52 cd	10.24 bc	15.13 a	1.40
1480	Caryophyllene	840.01 ab	214.21 e	710.74 bc	431.83 d	600.82 c	751.77 bc	213.05 e	429.92 d	820.18 ab	985.71 a	57.07
	Unknown origin, meat or food contaminants											
883	Ethyl benzene	2.40 c	9.47 b	3.23 c	2.38 c	1.72 c	2.71 c	2.03 c	0.74 c	32.58 a	0.65 c	1.41
893	p-xylene	14.04 cd	24.66 ab	15.67 c	24.84 a	12.82 cd	16.74 bc	13.63 cd	7.16 d	30.31 a	17.59 bc	2.44
918	o-xylene	4.06 d	9.17 cd	6.57 cd	11.66 c	22.01 b	43.90 a	6.02 cd	8.57 cd	26.30 b	3.41 d	2.15
920	Styrene	4.77 b	11.40 a	5.41 b	6.39 b	5.78 b	5.37 b	1.87 c	5.12 b	5.10 b	3.96 bc	0.97
1190	4-methyl phenol	3.67 c	3.47 c	3.73 c	13.70 a	3.20 c	3.03 c	3.23 c	4.23 c	8.37 b	7.66 b	0.48
130	Tridecane	3.23 d	2.10 e	2.13 e	7.74 a	1.70 e	2.17 e	3.08 d	6.52 b	4.73 c	3.32 d	0.29
1390	Caprolactame	1.07 bc	0.38 e	2.26 a	0.50 de	2.67 a	0.87 cde	1.04 cd	0.49 de	0.58 cde	1.62 b	0.19
1400	Tetradecane	13.30 g	10.87 g	114.67 b	41.57 efg	78.43 cd	91.24 bc	22.07 fg	52.63 def	195.37 a	64.83 cde	10.51

[A]: Kovats indices calculated for DB-624 capillary column (J&W Scientific: 60 m, 0.32 mm i.d., 1.8 μm film thickness) installed on a gas chromatograph equipped with FID detector. [a-h]: Means that do not share any letter are significantly different ($p < 0.05$). * SEM: Standard error of the mean

Two different types of esters were identified; methyl and ethyl esters (**table 3**). Methyl esters were more abundant than ethyl esters, as they accounted for 56.4-91.9 % of the total extracted area corresponding to esters. The methyl esters present in the highest abundance were methyl acetate, methyl butanoate and methyl hexanoate (**table 3**). Among the 12 methyl esters identified, 10 have been previously found in dry fermented sausages, except for methyl benzeneacetate and methyl nonanoate (Schmidt and Berger, 1998a; Ansorena, Hierro, de la Hoz, Mottram, Fernández & Ordóñez, 2000). The origin of methyl esters is not reported in the literature. Bruna Hierro, de la Hoz, Mottram, Fernández, Ordóñez (2001) remarked the ability of *Penicillium* strains to produce esters. However, Olivares *et al.* (2011) also detected the presence of methyl esters in dry fermented sausages in which natamycin and potassium sorbate were added to prevent moulds growth, therefore the generation of methyl esters could be attributed to Staphyloccoci. With respect to ethyl esters, with our extraction technique, the most abundant ethyl esters in naturally fermented sausages were ethyl acetate and ethyl 2-methyl butanoate (**table 3**).

The compounds derived from spices were mainly terpenes (**table 3**) and represented 10.2-34.6% of the total extracted area (**figure 1**) being ER-7 sausage the one with the lowest terpene abundance. In all the sausages, the most abundant compounds were β-myrcene, 3-carene and caryophyllene (**table 3**), which are derived from pepper (Croizet *et al.*, 1992). The variability observed in the content of terpenes can be ascribed to the different amount and kind of spices added (Bianchi *et al.*, 2007) as it was observed that some manufacturers used minced pepper while others used it as a whole grain. Spaziani *et al.* (2009) detected the terpenes as the most abundant class of aroma compounds (95% of the total extracted area) due to the spices added, mainly pepper and garlic, in naturally Italian fermented sausages, although this was probably due to the extraction conditions employed. However, the high proportion of terpenes has also been described in other naturally sausages in contrast to sausages with starter culture addition where other volatile compounds derived from biochemical reactions were dominant (Schmidt & Berger, 1998a; Di Cagno *et al.*, 2008). In Requena naturally fermented sausages, volatile compounds derived from spices

showed an important proportion of the extracted area, although they were not the main chemical class.

Aroma-active volatile compounds

The HS of the naturally fermented sausages comprised a complex mixture of volatile compounds as described above. In order to determine the impact odorants responsible for the singular aroma of "Salchichón de Requena" sausages, the HS was analyzed by GC-O using the detection frequency (DF) method (Zellner, Dugo, Dugo, & Mondello, 2008). A total of 42 odor-active regions were detected, although 11 of them could not be identified by GC (**table 4**), probably because these compounds were in very low concentration but have extremely low thresholds that can be detected by smell. All the aroma compounds identified have already been detected as odour active compounds in dry fermented sausages (Schmidt and Berger, 1998a; Meynier *et al.*, 1999; Marco, Navarro & Flores 2007; Söllner and Schieberle, 2009) except for several esters (ethyl 2-OH-propionate, methyl 3-methyl butanoate, methyl benzoate and methyl benzeneacetate).

The detection frequency (DF) method assumes that the compounds detected more frequently have a greater relative importance. In this sense, only 6 compounds presented DF values higher than 16: acetic acid (vinegar), ethyl butanoate (strawberry, fruity), 1-octen-3-ol (mushroom), benzeneacetaldehyde (roses), 4-methyl phenol (stable) and one unknown compound defined as meat broth and savoury. In addition, 16 had DF values higher than 10: ethyl 2-methyl propanoate (strawberry), methyl 3-methyl butanoate (fruity), hexanal (fresh cut grass), butanoic acid (cheese), methional (cooked potato), 2-heptenal (unpleasant), ethyl hexanoate (flowery, sweet), α-terpinene (wood, metallic), caryophyllene (spicy, cloves) and 7 unknown compounds which contributed to the aroma with roasted nuts, toasted, woody and dissolvent notes. Within the volatile compounds with DF values below 10, it is worthy of note the high proportion of ester compounds both ethyl and methyl esters which impart a wide variety of fruity notes. Although methyl esters were more abundant than ethyl esters (**table 3**), most of the odour active esters were ethyl esters (**table 4**) due to the higher thresholds of methyl esters than the ones of ethyl esters (Burdock, 2002).

Table 4. Odor-active compounds identified in the HS of dry fermented sausages.

IK*	Compound	GC-O descriptor	DF**
472	Methanethiol	Rotten	7
590	2-Methyl propanal	Fresh, cologne	5
630	Diacetyl	Butter, caramel	5
642	Ethyl acetate	Vegetal	4
702	Acetic acid	Vinegar	17
740	2,3-Pentanedione	Sweet, milky	5
753	Methyl butanoate	Fruity	4
784	Ethyl 2-methyl propanoate	Strawberry	12
802	Methyl 3-methylbutanoate	Fruity	11
824	Ethyl butanoate	Strawberry, fruity	17
835	Hexanal	Fresh cut grass	12
861	Ethyl 2-hydroxy-propanoate	Fresh	4
870	Ethyl 2-methyl butanoate	Fruity	9
874	Ethyl 3-methyl butanoate	Fruity, floral	8
872	Butanoic acid	Cheese	11
898	Unknown 1	Meat broth, savory	20
924	Ethyl pentanoate	Floral, fresh, fruity	4
937	Heptanal	Herbal	5
963	Unknown 2	Roasted nuts, toasted	15
966	Methional	Cooked potato	15
1007	2-Heptenal	Unpleasant, cabbage	12
1020	1-Octen-3-ol	Mushroom	20
1025	Ethyl hexanoate	Flowery, sweet	10
1031	α-Terpinene	Wood, metallic	14
1045	Octanal	Citric	4
1109	Benzeneacetaldehyde	Roses	16
1135	Unknown 3	Toasted, old wood, burnt hair	13
1147	Methyl benzoate	Fruity, sweet, waxy, metallic	9
1162	Heptanoic acid	Fresh, herbal	6
1172	Unknown 4	Woody, pine	12
1178	Unknown 5	Roasted nuts, toasted	14
1189	Unknown 6	Mustiness, woody	10
1190	4-Methyl phenol	Stable	16
1195	Phenylethyl alcohol	Roses	9
1201	Unknown 7	Mustiness	9
1222	Unknown 8	Wood, toasted, herbal	15
1236	Methyl benzeneacetate	Caramel, sweet, toffee	7
1245	Unknown 9	Toasted, coffee	9
1286	Unknown 10	Glue, dissolvent	14
1400	Unknown 11	Wood, pistachio, spicy	8
1424	Ethyl decanoate	Fruity	4
1443	Caryophyllene	Spicy, cloves	10

* Kovats index calculated for DB-624 capillary column (J&W Scientific 60m x 0.32 mm i.d., film thickness 1.8 µm) installed on a gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port. ** DF Detection frequency value.

However, 3 methyl esters, methyl butanoate, methyl benzoate and methyl benzeneacetate, have been identified as odour active compounds.

With respect to the origin of the aroma compounds identified, the main pathway of generation was staphylococci esterase activity, followed by lipid autooxidation, amino

acid degradation and to a lower extent spices and carbohydrate fermentation. Also, it is worthy of note, that many of the compounds which showed the highest HS abundance were detected as odour active. This is the case of hexanal, octanal, acetic acid, benzeneacetaldehyde, ethyl acetate, methyl butanoate and ethyl 2-methyl butanoate (**table 3**). However, as previously indicated, the contribution of volatile compound to the aroma depends not only in the HS abundance but in the perception threshold value. In this sense, some compounds were detected as important contributors because of their very low threshold values although they were extracted in low amounts (2-heptenal, methional, 4-methyl phenol) or even not quantified because of very low GC-FID signal (butanoic acid and 1-octen-3-ol) (**table 3**).

Among the volatile compounds identified as odour-active in dry fermented sausages in previous studies, some of them have been selected as the most potent because they presented the highest DF values using detection frequency method or the highest dilution factors in dilution techniques (Zellner *et al.*, 2008). In this sense, the most potent odorants of naturally fermented sausages were acetic acid, ethyl butanoate, 1-octen-3-ol, benzeneacetaldehyde and 4-methyl phenol. These compounds have already been described as essential contributors to the aroma of dry fermented sausages (Stahnke, 1994; Schmidt and Berger, 1998a; Meynier *et al.*, 1999; Marco *et al.*, 2007; Söllner and Schieberle, 2009).

In naturally fermented sausages, only two studies have analyzed the odour-active compounds present. In first place, Croizet *et al.* (1992) indicated that sulphur compounds, acetic acid, diacetyl, lineal and branched aldehydes were the main odorants in naturally fermented sausages, although these authors only described the aromas eluted from the GC but did not establish the most potent odorants. Secondly, Schmidt & Berger (1998a) reported that the aroma profile of naturally fermented sausages contained all the spicy odour-active compounds derived from pepper and garlic (methylallysulphide, diallylsulphide, diallyldisulphide and eugenol), but lacked of the aroma compounds produced by biochemical reactions the latter being described as typical of the starter culture sausages. However, Requena naturally fermented sausages aroma was characterized by a high proportion of volatile compounds coming from biochemical pathways and to a lesser extent by spice-derived compounds.

In conclusion, most of the odour-active compounds come from biochemical reactions that occur during processing and have been already identified in dry fermented sausages. Also, the most potent aroma compounds agree with previous studies in dry fermented sausages. However, Requena sausages were also characterized by other compounds coming from biochemical pathways such as esters.

Sensory analysis

In general, naturally fermented sausages did not differ too much among them (**table 4**). However, the ER-4 sausage was the less preferred by the consumers in terms of hardness, juiciness, taste and overall acceptability ($p < 0.05$) and ER-5 sausage in terms of aroma ($p < 0.05$).

Table 5. Sensory analysis (hedonic test) of naturally fermented (ER) sausages.

Samples	appearance	aroma	toughness/hardness	juiciness	taste	overall quality
ER-1	6.24 ab	6.35 a	6.38 ab	6.57 a	6.62 a	6.42 a
ER-2	6.11 ab	6.46 a	6.24 ab	6.24 a	5.91 c	6.1 a
ER-3	6.44 a	6.24 a	6.53 a	6.44 a	6.19 abc	6.16 a
ER-4	4.94 c	6.39 a	4.97 c	5.57 b	5.3 d	5.01 b
ER-5	5.84 b	5.67 b	6.44 ab	6.44 a	6.34 abc	6.25 a
ER-6	6.28 ab	6.48 a	5.97 b	6.38 a	6.08 bc	6.08 a
ER-7	6.42 a	6.23 a	6.23 ab	6.55 a	6.57 ab	6.37 a
ER-8	6.15 ab	6.29 a	6.34 ab	6.38 a	5.84 c	6.01 a
ER-9	6.2 ab	6.3 a	6.06 ab	6.33 a	6.26 abc	6.12 a
ER-10	5.23 c	6.14 a	6.53 a	6.61 a	6.24 abc	6.42 a
SEM	0.175	0.156	0.179	0.16	0.185	0.176

[a-d]: Means that do not share any letter are significantly different (p <0.05). * SEM: Standard error of the mean.

In order to establish which aroma compounds are responsible for the aroma acceptance of the naturally fermented sausages, a principal component analysis (PCA) was performed using the following parameters; lipolysis (FFA divided in SFA, MUFA and PUFA content), lipid oxidation (TBARS), aroma compounds abundance (only those compounds listed in **table 4**) and sensory attribute aroma. Results from PCA applied to mean scores of the parameters are summarized in **figure 2**. The PCA showed that about 52.15% of the variability was explained by the two first principal components. Principal component 1 (PC 1) was the most important variable in terms of differences among samples as it accounted for 30.22% of the total variability.

PC1 was positively related to the sensory attribute aroma and to several ester compounds such as ethyl acetate, ethyl butanoate, ethyl 2-methyl propanoate, ethyl 2-hydroxy-propanoate, ethyl decanoate and methyl 3-methyl butanoate. In addition, PC1 was inversely related to octanal and the aldehydes 2-heptenal and 2-methyl propanal. On the other hand, principal component 2 (21.93%) was positively related to free fatty acids (SFA, MUFA and PUFA) and the aroma compounds acetic acid, α-terpinene, ethyl 2-methyl butanoate and ethyl hexanoate, and inversely to lipid oxidation value (TBARS), heptanal and hexanal.

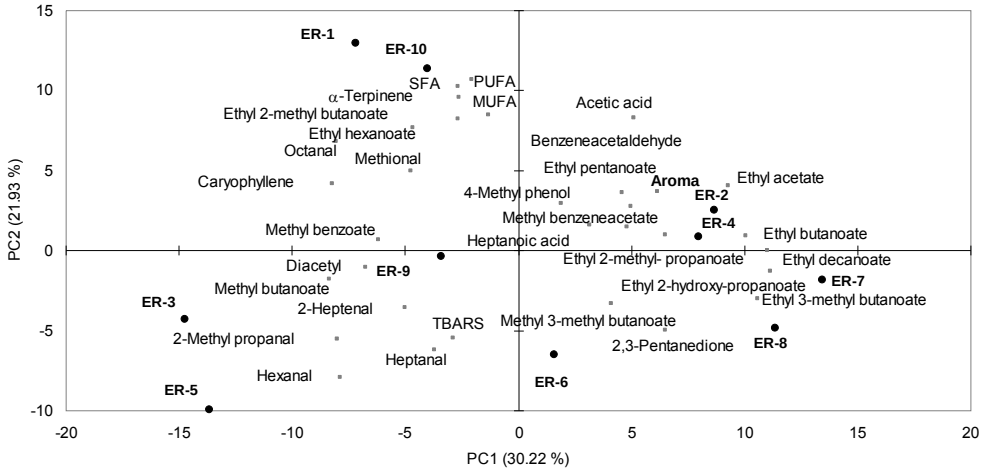

Figure 2. Loadings of the first two principal components (PC1-PC2) of the selected variables (■) and naturally fermented sausages (ER-1 to ER-10) (●). The selected variables were the aroma-active volatile compounds, TBARS, free fatty acids (SFA, MUFA, PUFA) and sensory attribute analysis (aroma).

The sausages ER-2, ER-4, ER-6, ER-7 and ER-8 had the greatest component 1 value therefore they were related to the consumers' acceptance as these sausages contained a high proportion of esters (**figure 1** and **table 3**). In contrast, the sausages ER-3, ER-5 and ER-9 were inversely correlated to the sensory aroma but were positively related to lipid derived volatile compounds as it was previously observed in the HS abundance (**figure 1**), while ER-1 and ER-10 sausages were in an intermediate position.

CONCLUSION

The naturally fermented sausages from different manufacturers (Requena, Valencia, Spain) studied in this manuscript were similar in sensory and chemical characteristics. However, there was a wide variability among them that produced different consumer's acceptance probably due to the traditional manufacture process. Naturally fermented sausages were characterized by a high free fatty acid content and a high proportion of volatile compounds derived from lipid autooxidation, Staphylococci esterase activity, and spices. Among the volatile compounds, 31 were identified as odour active. The aroma profile was characterized by the presence of acetic acid, ethyl butanoate, hexanal, methional, 1-octen-3-ol, benzeneacetaldehyde and 4-methyl-phenol. However, the HS of the naturally fermented sausages was also characterized by numerous esters, both ethyl and methyl esters, which were related to the high acceptance of this product by consumers.

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Capítulo 2

Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage

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Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage

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Abstract

The generation of aroma compounds through the curing process of dry fermented sausages was studied. The most important aroma compounds were determined using their odour-activity values (OAVs). The compound quantification in the headspace (HS) was carried out by solid phase microextraction (SPME) and the total concentration in the sausage by multiple headspace SPME (multiple HS-SPME) using gas chromatography and mass spectrometry. The main compounds that contributed to the aroma of dry fermented sausages were those with the highest oil OAVs such as 3-methyl butanal, 2-methyl butanal, octanal, diacetyl and ethyl 2-methyl butanoate that were important from the beginning of the process. Other compounds were important contributors as they were generated at the end of process, including propanal, pentanal, hexanal, ethyl 3-methyl butanoate, 1-octen-3-ol, 3-methyl butanoic acid, 2-methyl propanoic acid, ethyl hexanoate and nonanal. However, the aroma perceived in the HS was due to compounds with the highest air OAVs such as 3-methyl butanoic acid, ethyl 2-methyl butanoate, nonanal and octanal. In many cases, the percentage of the aroma compound released to the HS was around 10–20% of the total concentration in the sausage.

Keywords: Dry fermented sausage; Multiple headspace solid phase; microextraction; Cured aroma; Odour-activity value.

INTRODUCTION

Aroma is a very important characteristic for the overall quality of dry fermented sausages. It is mainly developed by bacterial metabolism and lipid oxidation reactions that occur in the sausage matrix during the manufacture process. The addition of spices also influences the content of volatile compounds, as well as several factors of the fabrication process, such as curing agents and fermentation stage (Marco, Navarro, & Flores, 2008).

The typical aroma of dry fermented sausage is not due to a single compound, but to the mixture of volatile compounds in the appropriate amounts (Marco, Navarro, & Flores, 2007). Hundreds of volatile compounds have been identified in fermented sausages. However, many of those volatiles do not contribute to the aroma because of their high sensory threshold values (Tjener & Stahnke, 2007). In previous work, Marco *et al.* (2007), using gas chromatography–olfactometry (GC–O) and detection frequency method, reported 55 compounds as odour-active constituents in dry fermented sausages.

Aroma perception in meat products depends on the interactions of volatile compounds with other meat components (fat and protein) (Grosch, 2001) that affect its gas phase concentration and also on the interaction among volatile compounds (Adhikari, Hein, Elmore, Heymann, & Willott, 2006). A criterion for the selection of the most important aroma compounds in a food is their odour activity value (OAV), which can be easily calculated if the concentration and the odour threshold of the aroma compounds are known (De Roos, 2007). However, Grosch (2001) indicated that the estimation of the odour threshold values of volatiles should be done in a medium that predominates in the food, e. g. water, oil. In the case of dry fermented sausages, the high proportion of fat (around 20%) produces a high effect on threshold calculations.

The composition of the odorants in the air above the food can be obtained by headspace (HS) analysis; however, it is necessary to estimate the proportion of volatile compounds in the whole product to predict their impact on the aroma. In this sense, the quantitative analysis of volatile compounds in a complex matrix was solved by Kolb (1982) with a procedure called multiple headspace extraction (MHE). The method is

based on a stepwise gas extraction at equal time intervals, allowing the total area for the compound to be calculated and eliminating the influence of the matrix. Multiple headspace solid phase microextraction (multiple HS-SPME) has the same aim as MHE. The amount of the analyte extracted by the fibre is proportional to the initial amount, and it is assumed that the analyte concentration will decay with the number of extractions. This method has already been applied to study the content of volatile compounds in dry fermented sausages (Flores & Hernández, 2007; Marco *et al.*, 2007).

In fermented sausages, many studies have been focused on protein and lipid degradation during processing (Lücke, 1985). However, the release of volatile compounds from the matrix could be also affected by the drying process because the content of fat, protein and salt continually rise during ripening as a result of moisture release.

However, nothing is known about the generation of aroma compounds through the different ripening stages and when those compounds are essential for the aroma. Therefore, the objective of this study was to determine which compounds and at which stage of the curing process are essential for the aroma, and also to determine if the use of different curing agents can affect their generation throughout the process.

MATERIALS AND METHODS

Reagents and standards

The chemical compounds used for the identification and to prepare the standard dilutions were all obtained from Fluka Chemie AG (Buchs, Switzerland) except diacetyl (97%) and 2-ethyl furan (97%), which were obtained from Aldrich (St. Louis, MO).

Dry fermented sausages

Two batches of fermented sausages, one containing sodium nitrite and another containing potassium nitrate were manufactured and submitted to a slow fermentation process. Dry fermented sausages were made with lean pork (80%) and pork back fat (20%). The following additives were added in gram per kilogram quantities to the meat mixture: sodium chloride (27), lactose (20), dextrin (20), sodium caseinate (20),

glucose (7), sodium ascorbate (0.5), and either sodium nitrite (0.15) or potassium nitrate (0.3). The meat was ground through a 10 mm diameter mincing plate, vacuum minced with the remaining ingredients and inoculated with a commercial starter culture (0.1) SP-318 (Danisco, Cultor, Madrid, España) containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus*, and *Staphylococcus carnosus*. The mixture was stuffed into collagen casings (Fibran, S.A., Girona, España, 75–80 mm diameter), with an approximate final sausage weight of 500 g. The sausages were kept in a chamber at 3–5 ºC for 24 h, and then dried during 10 d at 9 ºC, 10 d at 11 ºC and 21 d at 9 ºC and with 85–75% relative humidity. The total drying time was 42 d. At the end of the process, the sausages were vacuum packed and stored at 4 ºC for 50 d, giving a total processing time of 92 d.

The process was followed by performing microbial, moisture and pH analyses in the two batches (Marco *et al.*, 2008). In addition, at day 0, 200 g of the minced meat mixture were collected and at day 11, 21, 42 and 92, 3 sausages from each batch were randomly chosen. Each sausage was sliced, vacuum packed and stored at -20 ºC for chemical (TBARS, nitrite and nitrate) and volatile compound analyses. Results are expressed as the mean of the three replicates per kg of dry matter (dm) at each sampling time. Two sensory analyses were carried out at different times of processing, at the end of drying (42 d) and after vacuum storage (92 d).

Chemical analyses (TBARS, nitrite and nitrate)

Thiobarbituric reactive substances (TBARS) were determined according to Bruna, Ordoñez, Fernández, Herranz, and de la Hoz (2001), using trichloroacetic acid instead of perchloric acid as solvent.

The nitrite and nitrate content was determined using an enzymatic kit (Cat No. 10905658, 2r-Biopharm, Roche, Darmstadt, Germany) according to the procedure described by Arneth and Herold (1988) and as described Marco *et al.* (2008).

Sensory analysis

Sausages from both batches were submitted to sensory analysis at the end of the drying (42 d) and after vacuum storage (92 d). The casing was removed and the

sausage was cut into slices of approximately 5 mm thickness. Before serving, the slices were equilibrated at room temperature for 30 min. Samples were assessed by a consumer panel of 50 members. A preference test (ISO- 5495) was carried out to determine panellist's preference in terms of colour, aroma, taste and overall quality. Sensory evaluations were recorded by computer software using Compusense® five releases 4.6 (Compusense Inc., Guelph, ON, Canada).

Extraction of volatile compounds

Extraction of HS volatile compounds was done using a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA) with a 85 μm carboxen/polydimethylsiloxane StableFlex fibre (CAR/PDMS SF).

Gas chromatography–mass spectrometry (GC–MS)

The identification and quantification of volatile compounds was done in a gas chromatograph HP 5890 series II equipped with an HP 5972 mass-selective detector (Hewlett Packard, Palo Alto, CA). The compounds adsorbed by the fibre were desorbed in the injection port of the GC–MS for 15 min at 220 ºC with the purge valve off (splitless mode). The compounds were separated on a DB-624 capillary column J & W Scientific (Agilent Technologies, USA) and analysed as described Flores and Hernández (2007). The compounds were identified by comparison with mass spectra from the library database (NIST 98), Kovats retention index (Kovats, 1965), and by comparison with authentic standards. The quantification of the volatile compounds was done in selected ion monitoring (SIM) mode using the area of a specific ion for each compound (**Table 1**). The analysis was focused on those compounds previously identified as aroma active compounds in fermented sausages using olfactometry (Marco *et al.*, 2007) and the selection of the volatile compounds was done as described Flores and Hernández (2007) by using the detector in SCAN mode. The aroma properties of the compounds, odour description, air and oil thresholds and hydrophobicity values (Log K_{ow}) are indicated in **Table 1**.

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Table 1. Volatile compounds analysed by multiple HS-SPME.

Chemical group /compound	KI[a]	Ion (m/z)	Stock Solution (ng/mL)	Log Kow[b]	Threshold range (ng/g)[c]		Descriptor
					Air	Oils	
Aldehydes							
Propanal	522	58	24.24	0.33		9.4[2]	pungent[d]
Butanal	621	72	21.21	0.82	0.84-8800[1]	150[1]	sweet, snacks[e]
3-methyl-butanal	687	58	21.21	1.23	2-4[1]	13-13000[1]	rancid, dry cured ham[e]
2-methyl-butanal	698	58	21.21	1.23		2.21[2]	cacao, coffee[d]
Pentanal	733	58	27.27	1.31	120-17500[1]	240[3]	fresh cut grass, rancid[e]
Hexanal	839	58	27.27	1.80	20-330[1]	32-200[1]	fresh cut grass, rancid[e]
2-hexenal	905	41	21.21	1.58	50-1800[1]	850[1]	salty meat, dry cured ham[e]
Heptanal	941	70	12.12	2.29	60-260[1]	250[1]	citrus, soap, rancid cured ham[e]
2-heptenal	1011	41	21.21	2.07	34-2800[1]	1500[1]	rancid, dirty[e]
Octanal	1039	41	21.21	2.78	5-20[1]	56[2]	geranium, herbal, floral[e]
Nonanal	1148	98	21.21	3.27	5-230[1]	1000[1]	plastic, soap[e]
Ketones							
2-butanone	630	72	15.15	0.26			apricot[d]
Diacetyl (2,3-butanedione)	625	43	27.27	-1.34	5-20[1]	4.5-10[1]	cheese, snacks[e]
2-ethyl-furan	719	81	27.27	2.40		8000[1]	roasted, garlic[e]
2-pentanone	728	43	24.24	0.75	6700-30000[1]		roasted, sweet[e]
2,3-pentanedione	739	57	27.27	-0.85	10-60[1]		butter, cheese[e]
2-heptanone	934	43	18.18	1.73	45-330[1]	1500-392000[1]	medicinal, fruity[e]
2-nonanone	1140	58	21.21	2.71	75-5500[1]		roasted, burnt[e]
Esters							
Ethyl acetate	634	43	30.30	0.86	340-623000[1]	10000-100000[1]	fruity, toffees[e]
Ethyl butanoate	831	43	21.21	1.85	2.7-200[1]	28[1]	strawberry[e]
Ethyl 2-methyl-butanoate	877	57	21.21	2.26	0.06-10[1]	0.26[1]	strawberry[e]
Ethyl 3methyl-butanoate	881	88	27.27	2.26		0.62[2]	fruity[d]
Ethyl pentanoate	928	85	27.27	2.34	0.3-330[1]		strawberry[e]
Ethyl hexanoate	1023	88	24.24	2.83	3-90[1]	40[1]	sweet, fruity, cherry[e]
Ethyl octanoate	1156	88	24.24	3.81	5-92[4]		fruity[d]
Alcohols							
3-methyl-1-butanol	795	43	24.24	1.26			whiskey, malt[d]
1-octen-3-ol	1025	57	27.27	2.60	12-110[1]	34-900[1]	mushroom[e]
Acids							
2-methyl-propanoic acid	868	43	30.30	1.00	5-240[1]	755[1]	fatty, savoury snacks[e]
3-methyl-butanoic acid	950	60	18.18	1.49	0.22-14[1]	22-66[1]	cheese, feet, dirty stocks[e]
Hydrocarbons							
Limonene	1036	68	24.24	4.83	4-229[4]		citrus, orange[e]

[a]: Kovats index calculated for DB-624 capillary column (J & W Scientific: 30 m, 0.25 mm i.d., 1.4 μm film thickness) installed on a gas chromatograph equipped with a mass-selective detector. [b]: Log of octane/water partition coefficient. [c]: threshold values obtained from the following sources; [1] van Gembert (2004); [2] Reiners and Grosch (1998); [3] Meijboom (1964); [4] Burdock (2002). [d]: Odour description defined by Marco *et al.* (2007) using gas chromatography-olfactometry (GC-O). [e]: Odour description according to Burdock (2002).

Quantification of odour-active compounds of dry fermented sausages by multiple HS-SPME

The analysis was done as previously optimised by Flores and Hernández (2007). For the extraction procedure, a sausage slice was diced, 0.75 mg of butylated hydroxytoluene (BHT) were added, and minced with liquid nitrogen. One gram of the finely pulverised sausage was homogenised with 15 ml of double-distilled water. Then, 1 ml of the homogenised sample was added to a 10 ml HS vial, containing 0.5 g of NaCl and equilibrated at 30 ºC for 1 h. The sample was extracted for four consecutive times by exposing the SPME fibre for 90 min at 30 ºC each extraction. This procedure was done per triplicate on each batch and sampling time. After each extraction, the fibre was desorbed in the GC injection port of the GC–MS, using the conditions described previously.

The concentration of analyte decayed exponentially through the successive extractions and the total peak area (A_t) corresponding to an exhaustive extraction was calculated from Eq. (1):

$$A_t = \frac{A_1}{1 - e^k} \quad (1)$$

where A_1 is the peak area in the first extraction and k is the slope obtained when representing the natural logarithm of the peak area versus the number of extractions minus one. The initial mass of compound in the sample can be calculated with the value of A_t by using a calibration curve obtained using standard solutions (Tena & Carrillo, 2007).

The quantification of each volatile compound was done by the external standard method. Stock standard solutions of pure compounds were prepared in methanol (**Table 1**). The stock solution was diluted 1/5, 1/10, 1/20, 1/200, 1/500, and 1/800 in methanol, and all the dilutions were analysed by multiple HS-SPME using similar conditions as for the sausage. Each dilution was analysed in triplicate and data was acquired in SIM mode. Eq. (1) was used to calculate the total area of standard present in the vial. Then, a linear calibration was obtained by representing the total area against the standard concentration added in the vial. Limits of detection (LOD) and

quantification (LOQ) were calculated from the first extraction of a blank plus three and ten times the standard deviation of four blank replicates, respectively.

Quantification of odour-active compounds in the headspace (HS) of dry fermented sausages

At the end of the drying process, 3 g of minced sausage made with nitrite was weighed into a 10 ml HS vial sealed with a PTFE faced silicone septum and 0.75 mg of BHT were added. The vial was left in a thermoblock for 30 min at 30 ºC. The released compounds were extracted by exposing the SPME fibre to the HS while the sample was maintained at 30 ºC for different times (0.5, 1, 2 and 3 h). Each experiment was done in triplicate. The compounds adsorbed by the fibre were identified and quantified by GC–MS using SIM mode as described previously.

Calculation of odour-activity values (OAV)

The odour-activity values (OAVs) (De Roos, 2007) in air or oil of each compound present in the HS or in the matrix were calculated. For this purpose, the concentration of the compound in the HS or in the sausage (total concentration) was divided by the detection threshold in air or oil respectively, obtained from van Gemert and Nettenbreijer (2004), Burdock (2002), Reiners and Grosch (1998) and Meijboom (1964). The threshold value selected for OAV calculation was the minimum of the range (**Table 1**) because it indicates the minimum concentration necessary to detect the compound.

Statistical analysis

The effect the addition of curing agents (nitrite or nitrate) and ripening time on the volatile content and chemical parameters were tested by two-factor analysis of variance (ANOVA) using the statistic software Statgraphics plus (v. 5.1). Significant effects were compared using Fisher's least significant difference (LSD) test. The effect of nitrite and nitrate on the sensory preference was evaluated by ANOVA.

RESULTS

Dry fermented sausages

The growth of lactic acid bacteria (LAB) and Staphylococci was monitored during the manufacture process (data not shown). The growth was within the range expected for slow fermented sausages (Marco, Navarro, & Flores, 2006). Moisture content and pH decreased during the process without significant differences between batches. Moisture content decreased from 62% to 40% and the pH reached the lowest value 4.8 after 21 d of processing.

Nitrite content was higher in the samples with added nitrite than nitrate at day 0 (**Fig. 1a**). Then, the concentration decreased rapidly and only residual concentrations were found in both batches. The nitrate concentration decreased from 300 to 15 ppm from 0 d to 42 d, while a low concentration of nitrate was detected in nitrite added samples (**Fig. 1b**).

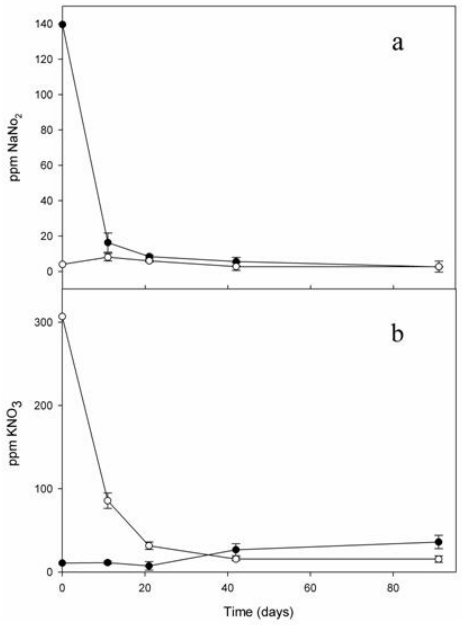

Fig. 1. Concentration (ppm in dm) of nitrite (a) and nitrate (b) during the ripening process of dry fermented sausages manufactured with added nitrite (●) or nitrate (○). Symbols represent the mean and standard error of the mean.

TBARS value increased throughout the drying process in both batches (**Fig. 2**), and it was significantly higher in the nitrate added batch. However, after vacuum storage the nitrate sausage showed a reduction in the TBARS value that resulted in the absence of differences between batches.

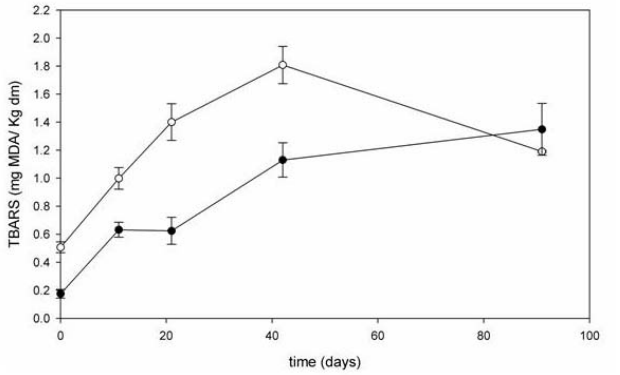

Fig. 2. Levels of TBARS (mg of manoladehyde (MDA) per kg of dm) during the ripening process of dry fermented sausages manufactured with added nitrite (●) or nitrate (○). Symbols represent the mean and standard error of the mean.

Results of the sensory analyses performed at 42 and 92 d are shown in **Table 2**. The preference tests did not show significant differences ($p < 0.05$) in aroma, taste and overall quality between batches except for a preference in the colour of nitrite samples at 42 d. However, the panel preferred the nitrate batch in aroma at 92 d although with a low significance value ($p < 0.12$).

Table 2. Sensory analysis (preference test) of the dry fermented sausages at the end of ripening (42 d) and storage (92 d).

	42 d			92 d		
	Preferred sample		P	Preferred sample		P
	N_2[a]	N_3[b]		N_2	N_3	
Colour	35	15	0.007	27	23	0.672
Aroma	21	29	0.322	19	31	0.119
Taste	23	27	0.672	22	28	0.480
Overall quality	24	26	0.888	24	26	0.888

P: significant value between both samples, tendency of the preference of one sample over the other.
[a] N_2: samples with added nitrite. [b] N_3: samples with added nitrate.

Determination of volatile compounds in fermented sausages by multiple HS-SPME

A total of 42 odour-active compounds were identified in fermented sausages. Of them, 30 presented exponential area decay when multiple HS-SPME was applied. The volatile compounds listed by their chemical classes were: 11 aldehydes, 7 ketones, 7 esters, 2 alcohols, 2 acids and 1 hydrocarbon (**Table 3**).

Table 3. Linearity, correlation coefficients, LODs and LOQs of the standard compounds analysed by multiple HS-SPME.

Chemical group/compound	Measurement range (ng)	Correlation coefficient (r^2)	m (At/ng x 10^{-3})*	LOD (ng)	LOQ (ng)
Aldehydes					
Propanal	6.06-121.21	0.999	16.65	0.26	0.57
Butanal	0.13-10.61	0.993	21.36	0.22	0.58
3-methyl-butanal	5.30-21.21	0.967	26.76	0.02	0.05
2-methyl-butanal	0.13-5.3	1.000	21.14	0.04	0.08
Pentanal	0.68-136.36	0.999	16.39	0.16	0.39
Hexanal	0.17-136.36	0.991	16.78	9.49	27.64
2-hexenal	0.21-106.06	0.977	0.80	5.64	11.91
Heptanal	0.30-60.61	0.991	30.71	0.21	0.56
2-heptenal	0.13-21.21	0.992	3.81	0.14	0.35
Octanal	0.53-106.06	0.990	3.15	1.79	4.81
Nonanal	0.53-106.06	0.990	1.42	4.01	11.01
Ketones					
2-butanone	3.79-15.15	0.924	74.24	0.47	1.18
Diacetyl (2,3-butanedione)	0.68-136.36	0.990	242.26	0.06	0.14
2-ethyl-furan	0.17-0.68	0.992	421.35	0.00	0.00
2-pentanone	0.15-0.61	0.999	284.42	0.00	0.00
2,3-pentanedione	0.68-13.64	0.996	9.39	0.13	0.32
2-heptanone	0.11-18.18	0.995	55.33	0.05	0.11
2-nonanone	0.13-21.21	0.977	9.85	0.17	0.45
Esters					
Ethyl acetate	0.19-30.30	0.883	118.19	0.53	1.19
Ethyl butanoate	0.13-0.53	0.999	181.78	0.01	0.02
Ethyl 2-methyl-butanoate	0.53-106.06	0.997	43.53	0.00	0.00
Ethyl 3methyl-butanoate	0.17-13.64	0.991	23.92	0.00	0.01
Ethyl pentanoate	0.17-6.82	0.999	12.76	0.07	0.15
Ethyl hexanoate	0.15-24.24	0.997	10.13	0.01	0.03
Ethyl octanoate	0.15-12.12	0.990	7.56	0.08	0.24
Alcohols					
3-methyl-1-butanol	0.61-121.21	1.000	0.80	0.21	0.46
1-octen-3-ol	0.27-27.27	0.962	2.97	0.07	0.16
Acids					
2-methyl-propanoic acid	7.58-151.52	0.996	51.29	0.00	0.01
3-methyl-butanoic acid	4.55-90.91	0.990	172.49	0.00	0.01
Hydrocarbons					
Limonene	0.24-6.06	1.000	5.96	2.32	6.23

* m: slope of the calibration curve, *At* is total area of compound calculated using Eq. (1) obtained in the multiple HS-SPME analysis.

The total area for each volatile compound in the dry fermented sausage analysed by multiple HS-SPME was calculated using Eq. (1). In order to calculate the concentration of the volatile compounds corresponding to the total area, the external standard calibrations were obtained. **Table 3** shows the measurement ranges of the volatile compounds, correlation coefficients and slopes of the standard compounds. The quantification of the 30 odour compounds during the ripening process and vacuum packaged is shown in **Table 4**. As can be observed, all the concentrations of the compounds were above LOQs and LODs.

Many compounds were absent in the mince meat mixture, although a few volatile compounds were detected such as 3-methyl butanal, diacetyl and others (**Table 4**). Throughout the drying process and vacuum packed storage, there was a significant increase in the concentration of almost all the compounds, aldehydes, ketones, esters and 3-methyl butanoic acid, except for 2-heptenal, 2-butanone, 2,3-pentanedione, ethyl pentanoate, 2-methyl propanoic acid, 3-methyl-1-butanol, 1-octen-3-ol and limonene (**Table 4**).

The addition of nitrate or nitrite affected the concentration of 10 volatile compounds (**Table 4**). Several volatile compounds were more concentrated in the nitrate added sausages such as those originated from amino acid degradation (3-methyl butanal, 2-methyl butanal and 3-methyl butanoic acid), staphylococci esterase activity (ethyl 2-methyl butanoate, ethyl 3-methyl butanoate and ethyl hexanoate) and lipid autooxidation products (propanal, hexanal and 2-heptenal). However, 2-pentanone, derived from lipid β-oxidation, was in high concentration in the nitrite added sausage.

With regards to the detection thresholds of the 30 odour-active compounds quantified (**Table 1**), all of them were above their air detection threshold, except for 2-pentanone and 2-heptanone. However, only 14 were found in a concentration higher than their oil thresholds values, although not all oil thresholds were available in the literature.

Odour-activity values (OAV) in oil of the compounds throughout the process were calculated and shown in **Table 5**. The oil OAV was 1 or higher when the oil threshold was achieved and the compound would contribute to the aroma of the sample. Since the beginning of the curing process, the compounds 3-methyl butanal, 2-methyl

Table 4. Volatile compound concentration (ng/g dm) obtained by multiple HS-SPME in dry fermented sausages during drying and storage.

Chemical group/compound	0d	11d		21d		42d		91d		SEM	S[g]	B	SxB
		N_2[e]	N_3[f]	N_2	N_3	N_2	N_3	N_2	N_3				
Aldehydes													
Propanal		463.7a	546.8a	404.9b	994.7b	792.0b	906.3b			5.4	**	**	*
Butanal	6.4a	14.8a	14.1a	20.4a	46.0a	32.1a	43.9a	108.0b	77.6b	16.6	**	ns	ns
3-methyl-butanal	120.1a	122.7a	220.8a	148.8ab	215.7ab	150.0bc	343.4bc	186.9c	379.8c	33.8	**	**	*
2-methyl-butanal	11.3a	12.5ab	35.6ab	30.6bc	42.6bc	39.7cd	77.0cd	41.1d	60.2d	6.8	**	**	ns
Pentanal		238.5a	291.8a	240.8a	500.2a	208.0ab	394.4ab	738.6b	462.0b	96.1	*	ns	ns
Hexanal		898.7a	984.5a	813.0bc	2799.7bc	803.6b	2307.4b	1911.0c	2620.3c	246.0	**	**	*
2-hexenal				43.4a	60.8a	179.0a	300.2a	794.3b	580.5b	117.0	**	ns	ns
Heptanal	126.7a	117.8a	128.5a	138.7a	142.8a	93.5a	167.3a	197.9b	179.0b	16.1	*	ns	ns
2-heptenal		41.4	53.1	60.8	156.4	55.7	291.0	92.7	256.1	51.9	ns	*	ns
Octanal	375.3a	300.2a	359.4a	776.6a	658.9a	316.8a	1120.4a	2329.9b	1044.0b	277.0	**	ns	*
Nonanal	266.5ab	275.9a	302.7a	579.0b	722.3b	263.5ab	833.0ab	1332.5c	701.3c	135.6	**	ns	**
Ketones													
2-butanone	175.0	85.1	195.9	142.8	119.4	152.3	222.2	167.4	269.0	48.3	ns	ns	ns
Diacetyl (2,3-butanedione)	750.5b	730.2c	1584.7c	772.3b	544.5b	279.5a	373.6a	345.3a	386.0a	72.0	**	**	**
2-ethyl furan		6.5d	6.8d	5.5c	5.7c	4.5a	4.7a	5.0b	5.0b	0.2	**	ns	ns
2-pentanone	0.4a	4.1ab	2.7ab	5.0ab	2.2ab	4.2b	2.4b	8.1c	5.0c	1.0	**	*	ns
2,3-pentanedione						30.1	36.2	25.8	29.1	3.9	ns	ns	ns
2-heptanone	2.5a	101.4b	58.3b	86.1b	52.1b	109.8c	166.7c	132.3c	185.0c	17.7	**	ns	*
2-nonanone		103.8a	8.5a	91.0a	20.1a	161.2b	248.5b	291.5b	198.5b	32.8	**	ns	ns
Esters													
Ethyl acetate						42.5a	17.8a	411.3b	257.0b	36.8	**	ns	ns
Ethyl butanoate		6.6b	7.1b	4.6a	4.8a	6.6b	7.8b	7.2b	7.8b	0.8	*	ns	ns
Ethyl 2-methyl-butanoate	90.5d	81.7d	95.7d	68.8c	73.1c	58.7a	66.4a	63.7b	69.2b	1.2	**	**	**
Ethyl 3-methyl-butanoate		22.6ab	86.5ab	25.3a	43.9a	44.1b	93.1b	86.2c	130.5c	12.1	**	**	ns
Ethyl pentanoate				0.8	1.4	0.1	2.6	2.2	8.6	2.1	ns	ns	ns
Ethyl hexanoate		18.9a	29.3a	19.1a	34.1a	29.6b	93.2b	101.5c	126.0c	12.1	**	**	ns
Ethyl octanoate							55.5	66.9	92.2				
Alcohols													
3-methyl-1-butanol						241.67	93.8	500.2	948.0	361.6	ns	ns	ns
1-octen-3-ol		69.4	70.6	62.2	62.7	63.1	62.7	61.4	67.4	3.0	ns	ns	ns
Acids													
2-methyl-propanoic acid				368.8	493.6	839.7	672.2	803.4	580.5	108.8	ns	ns	ns
3-methyl-butanoic acid				752.7a	996.9a	1194.6b	1830.9b	1138.6b	2009.6b	204.1	*	*	ns
Hydrocarbons													
Limonene	32.6	63.2	55.8	53.9	70.1	35.6	101.6	56.9	84.9	16.9	ns	ns	ns

B: batch. S x B: interaction between batches and stage. ns: no significant. a–d: means with different letters in the same batch indicate significant differences ($p < 0.05$) among processing times. [e] N_2: samples with added nitrite. [f] N_3: samples with added nitrate. [g] S: stage. * Significant $p < 0.05$. ** Significant $p < 0.01$.

butanal, octanal, diacetyl and ethyl 2-methyl butanoate showed oil OAVs above 1. All of them, with the exception of ethyl 2-methyl butanoate, also increased during the drying process. At the end of the fermentation stage (11 d), other compounds showed oil OAVs higher than 1 such as propanal, pentanal, hexanal, ethyl 3-methyl butanoate and 1-octen-3-ol.

Table 5. Odour-activity values (OAVs) in oil of volatile compounds detected in dry fermented sausages during ripening and storage.

Chemical	0d	11d		21d		42d		91d	
group/compound		N_2	N_3	N_2	N_3	N_2	N_3	N_2	N_3
Aldehydes									
Propanal		49.33	58.18	43.08	105.82	84.26	96.42		
Butanal	0.04	0.10	0.09	0.14	0.31	0.21	0.29	0.72	0.52
3-methyl-butanal	9.24	9.44	16.99	11.45	16.60	11.54	26.42	14.83	29.22
2-methyl-butanal	5.14	5.66	16.14	13.86	19.29	17.99	34.85	18.64	27.28
Pentanal		0.99	1.22	1.00	2.08	0.87	1.64	3.08	1.93
Hexanal		28.09	30.77	25.41	87.49	25.11	72.11	59.72	81.89
2-hexenal				0.05	0.07	0.21	0.35	0.93	0.68
Heptanal	0.51	0.47	0.51	0.55	0.57	0.37	0.67	0.79	0.72
2-heptenal		0.03	0.04	0.04	0.10	0.04	0.19	0.06	0.17
Octanal	6.70	5.36	6.42	13.87	11.77	5.66	20.01	41.61	18.64
Nonanal	0.27	0.28	0.30	0.58	0.72	0.26	0.83	1.33	0.70
Ketones									
2-butanone	-*	-	-	-	-	-	-	-	-
Diacetyl (2,3-butanedione)	166.79	162.27	352.17	171.63	121.02	62.12	83.04	76.74	85.79
2-ethyl furan		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2-pentanone	-	-	-	-	-	-	-	-	-
2,3-pentanedione	-	-	-	-	-	-	-	-	-
2-heptanone	0.002	0.07	0.04	0.06	0.03	0.07	0.11	0.09	0.12
2-nonanone	-	-	-	-	-	-	-	-	-
Esters									
Ethyl acetate						0.004	0.001	0.04	0.03
Ethyl butanoate		0.24	0.25	0.17	0.17	0.24	0.28	0.26	0.28
Ethyl 2-methyl-butanoate	348.30	314.52	368.44	264.68	281.49	225.85	255.76	245.20	266.17
Ethyl 3-methyl-butanoate		36.53	139.66	40.85	70.81	71.15	150.16	139.07	210.49
Ethyl pentanoate	-	-	-	-	-	-	-	-	-
Ethyl hexanoate		0.47	0.73	0.48	0.85	0.74	2.33	2.54	3.15
Ethyl octanoate	-	-	-	-	-	-	-	-	-
Alcohols									
3-methyl-1-butanol	-	-	-	-	-	-	-	-	-
1-octen-3-ol		2.04	2.08	1.83	1.84	1.86	1.85	1.81	1.98
Acids									
2-methyl-propanoic acid				0.49	0.65	1.11	0.89	1.06	0.77
3-methyl-butanoic acid				34.21	45.31	54.30	83.23	51.76	91.35
Hydrocarbons									
Limonene	-	-	-	-	-	-	-	-	-

[a] N_2: samples with added nitrite. [b] N_3: samples with added nitrate. * OAV not calculated due to absence of the oil threshold in literature.

Other compound that contributed to the aroma (oil OAVs higher than 1) in the middle of the process (21 d) was 3-methyl butanoic acid while 2-methyl propanoic acid and ethyl

hexanoate did it at the end of processing (42 d). However, nonanal contributed to the aroma after vacuum storage. In addition, compounds like 2-hexenal, heptanal and butanal showed oil OAVs close to 1.

Determination of volatile compounds in the HS of fermented sausages

The quantification by HS-SPME showed that the concentration of volatile compounds present in the HS is only a fraction of the total content of the sausage at any extraction time, although, as can be observed in **Fig. 3**, the content increases with the extraction time. **Table 6** shows the concentration found in the HS of a sausage (nitrite added sausage at 42 d of processing) by exposing the CAR/PDMS fibre during 3 h. In many of the compounds, the concentration in the HS was around 10% of the total obtained by multiple HS-SPME. However, there were compounds with a concentration in the HS that represented 20–40% of the total concentration, such as 2-heptenal, ethyl hexanoate, 2-nonanone and nonanal, or even above 70%, as in the case of ethyl acetate and ethyl pentanoate.

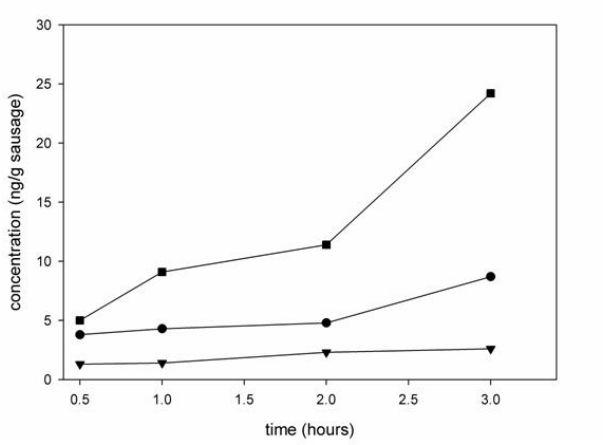

Fig. 3. Concentration (ng/g sausage) of ethyl hexanoate (●), 1-octen-3-ol (▼) and 2-nonanone (■) in the headspace of nitrite added dry fermented sausage (42 d of drying) at different extraction times.

Table 6. Odour-activity values in air of volatile compounds detected in nitrite added dry fermented sausage (42 d of drying) and percentage of volatile compounds in the HS respect to the total concentration.

Chemical group/compound	Concentration (ng/g)		%[c]	OAV in air[d]
	HS[a]	total[b]		
Aldehydes				
Propanal	2.94	488.1	0.6	*
Butanal	0.26	19.8	1.3	0.31
3-methyl-butanal	1.10	92.5	1.1	0.55
2-methyl-butanal	0.14	24.5	0.5	*
Pentanal	4.41	128.2	3.4	0.04
Hexanal	33.69	495.3	6.8	1.68
2-hexenal	2.60	110.3	2.3	0.05
Heptanal	3.80	57.7	6.5	0.06
2-heptenal	8.31	34.3	24.2	0.24
Octanal	34.28	195.3	17.5	6.86
Nonanal	57.60	162.4	35.4	11.52
Ketones				
2-butanone	tr	93.9	-	*
Diacetyl (2,3-butanedione)	2.36	172.3	1.3	0.47
2-ethyl furan	0.08	2.8	3.0	*
2-pentanone	0.35	2.6	13.2	0.00
2,3-pentanedione	1.38	19.1	7.2	0.14
2-heptanone	5.64	67.7	8.3	0.13
2-nonanone	24.21	99.4	24.3	0.32
Esters				
Ethyl acetate	24.4	26.2	93.1	0.07
Ethyl butanoate	0.35	4.1	8.8	0.13
Ethyl 2-methyl-butanoate	0.79	36.2	2.1	13.17
Ethyl 3-methyl-butanoate	0.53	27.2	1.9	*
Ethyl pentanoate	0.03	0.04	78.8	0.10
Ethyl hexanoate	8.69	18.3	47.6	2.90
Ethyl octanoate	16.44	n.q.	-	3.29
Alcohols				
3-methyl-1-butanol	23.07	148.9	15.4	*
1-octen-3-ol	2.63	38.9	6.7	0.22
Acids				
2-methyl-propanoic acid	3.76	517.5	0.7	0.75
3-methyl-butanoic acid	3.15	736.2	0.4	14.32
Hydrocarbons				
Limonene	2.67	22.0	12.2	0.67

tr: traces.
* odour threshold in air was not reported in literature. n.q.: not quantified.
[a] Concentration obtained after 3 h of CAR/PDMS fibre exposure in the HS of dry fermented sausage.
[b] Concentration obtained by multiple HS-SPME corresponding to the total content of volatile compound in the sausage.
[c] Percentage of volatile compound found in the HS of dry fermented sausages in relation to the total content obtained by multiple HS-SPME.
[d] Odour-activity values calculated using the concentration found in the HS and the air detection thresholds from Van Gemert and Nettenbreijer (2004) and Burdock (2002).

The air OAVs were also calculated (**Table 6**) and the volatile compounds that presented the highest air OAVs values in the HS of sausages were 3-methyl-butanoic acid, ethyl 2-methylbutanoate, nonanal, octanal, ethyl hexanoate, hexanal and ethyl octanoate (**Table 6**). In addition, limonene, 2-methyl-propanoic acid, 3-methyl butanal and diacetyl showed OAVs close to 1.

DISCUSSION

In this study, sausages made with nitrate or nitrite have been used. The results obtained were similar to those previously reported by Marco *et al.* (2006). Nitrite decreased very quickly due to its high reactivity (Cassens, Greaser, Ito, & Lee, 1979), while nitrite formation in the nitrate added samples was likely caused by the nitrate reductase activity of staphylococci (Talón, Walter, Chartier, Barriere, & Montel, 1999). The selection of a slow fermentation process was performed to allow the enzyme activity to convert nitrate to nitrite (Lücke, 1985).

In relation to TBARS values, the lower levels reported in the nitrite added batch during the drying process could be due to the potent antioxidant effect of nitrite. On the other hand, the TBARS decrease in the nitrate batch after vacuum storage could be explained, as Talon *et al.* (1999) observed, by the production and release of catalase in *S. xylosus* in presence of nitrate. The TBARS decrease during vacuum storage was previously observed by Marco *et al.* (2006) in nitrite and nitrate dry fermented sausages, although the levels of oxidation were higher in nitrite samples during drying and storage. In contrast, Navarro, Nadal, Nieto, and Flores (2001) reported that TBARS levels were higher in small diameter non-fermented sausages with added nitrate than with nitrite as also occurs in our study.

The effect of the addition of nitrate and nitrite on aroma generation was studied by Marco *et al.* (2006); however, the total amount of each volatile compound generated at the different stages of processing is not known. In the present study, multiple HS-SPME was applied throughout the drying process and after vacuum storage of dry fermented sausages. The compounds quantified were those previously reported as odour-active compounds in dry fermented sausages using gas chromatography–

olfactometry (Marco *et al.*, 2007). The concentrations observed after vacuum packed storage were within the range of concentrations reported by Marco *et al.* (2007) and Flores and Hernández (2007) using multiple HS-SPME in slow and fast fermented sausages, respectively. The low concentration of volatile compounds in the unfermented mince and the increase of most of them throughout the drying process and vacuum storage were previously observed by several authors using HS techniques, (Croizet, Denoyer, Tran, & Berdagué, 1992; Marco *et al.*, 2006; Olesen, Meyer, & Stahnke, 2004). As Olesen *et al.* (2004) concluded, volatile compound generation is linked to the fermentation and curing process.

The higher concentration of volatiles derived from amino acid catabolism, staphylococci esterase activity and lipid autooxidation in the nitrate added sausages was already reported by Marco *et al.* (2006) and Olesen *et al.* (2004) using HS techniques and this increment could be the reason for the aroma preference of nitrate sausages at 92 d. The previous application of multiple HS-SPME by Flores and Hernández (2007) also showed a higher proportion of almost all the volatile compounds in the nitrate fermented sausage. However, Marco *et al.* (2007) only reported few significant differences between batches.

The application of multiple HS-SPME allowed the selection of the main contributors to the aroma by comparing the total content with the oil thresholds values. In our study, 14 compounds were above their oil thresholds (**Table 5**). Five compounds showed concentrations above their oil thresholds since the beginning. Some of them, such as 3-methyl butanal, 2-methyl butanal, octanal and diacetyl also increased during the drying process, while ethyl 2-methyl butanoate was at a constant concentration. The other 9 compounds achieved the oil detection threshold throughout the drying process or even during the storage, these were propanal, pentanal, hexanal, 1-octen-3-ol, 3-methyl butanoic acid, 2-methyl propanoic acid, ethyl 3-methyl butanoate, ethyl hexanoate, and nonanal. These results confirmed that ripening time has a considerable effect on the aroma of dry fermented sausages and are partially in accordance with those from Marco *et al.* (2007) who only detected hexanal, heptanal and 1-octen-3-ol as the main contributors to the aroma (concentrations above their oil thresholds). In addition, Marco *et al.* (2007) indicated the presence of other compounds

(18 compounds) above their air threshold and therefore, they were also considered as odour-active compounds of fermented sausages. On the other hand, Schmidt and Berger (1998) applying olfactometry techniques to Spanish-fermented sausages, reported that, without taking into consideration the contribution of aroma compounds derived from spices, the most potent odorants were 2,3-butanedione, 3-methyl butanoic acid, ethyl propanoate, 2-phenylethanol and acetic acid. Only, two compounds, 2,3-butanedione (diacetyl) and 3-methyl butanoic acid, are in accordance with our results.

Nevertheless, aroma perception in meat products depends not only on the concentration and odour thresholds of volatile compounds, but also on their interactions with other food components and among volatile compounds (Adhikari *et al.*, 2006). In fact, the concentration found in the HS of nitrite added sausages was only a fraction, around 10–20%, of the total content determined by HS-SPME multiple (**Table 6**). However, other compounds can be in higher proportion (around 70%) such as esters. On the other hand, it was not possible to establish a significant relationship between the fraction released and the hydrophobicity of each compound. For this purpose, the parameter Log K_{ow} (**Table 1**) was used as it represents the partition coefficient in octanol/water and measures the hydrophobicity of each compound. Only the aldehydes showed a significant relationship ($p < 0.01$) between Log K_{ow} and release. In this case, the higher Log K_{ow} the higher percentage of aldehyde compound found in the HS in relation to the total content. The binding of aroma compounds by muscle proteins could be responsible for this behaviour (Chevance & Farmer, 1998; Pérez-Juan, Flores, & Toldrá, 2007). Other factors that have not been considered in the present study are water and sodium chloride content as they can affect the partition coefficients between matrix and vapour phase (Guichard, 2002).

In relation to air OAVs, the values obtained in the HS are partially in accordance with those calculated considering the total content and the oil OAVs. Reiners and Grosch (1998) indicated that the compounds with OAVs greater than five contribute strongly to the flavor of a sample. In the HS of fermented sausages, 3-methyl butanoic acid, ethyl 2-methyl butanoate, nonanal and octanal showed the highest air OAVs (**Table 6**). All of them also had high oil OAVs, except nonanal, whose oil OAV value increased after

vacuum storage although it was lower than 5 (**Table 5**). The most important compounds in relation to oil OAVs were those previously mentioned and also propanal, hexanal, diacetyl and ethyl 3-methyl butanoate. However, the air thresholds of propanal and ethyl 3-methyl butanoate were not available, and its air OAVs could not be calculated. With respect to diacetyl and hexanal, their air OAV were close to 1.

In summary, the positive effect of nitrate addition on the aroma of fermented sausages was confirmed. Also, the HS concentration of aroma compounds represents around 10–20% of the total concentration of the compounds in fermented sausages. Therefore, the release of compounds is highly dependent on matrix composition and compound concentration. In this study, the aroma perceived in the HS was mainly due to 3-methyl butanoic acid, ethyl 2-methyl butanoate, nonanal and octanal that showed the highest air OAVs. However, the main compounds that contributed to the aroma of dry fermented sausages during eating were those with the highest oil OAVs, such as 3-methyl butanal, 2-methyl butanal, octanal, diacetyl and ethyl 2-methyl butanoate that were important since the start of the ripening process. Other compounds, such as propanal, pentanal, hexanal, ethyl 3-methyl butanoate, 1-octen-3-ol, 3-methyl butanoic acid, 2-methyl propanoic acid, ethyl hexanoate and nonanal were also important contributors as they were generated at the end of process. Actually to confirm the results of the aroma analyses, further investigations are been carried out in our laboratory using aroma models in order to reproduce the fermented sausage aroma and the factors that affect their release.

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Capítulo 3.

Distribution of volatile compounds in lean and subcutaneous fat tissues during processing of dry fermented sausages

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Distribution of volatile compounds in lean and subcutaneous fat tissues during processing of dry fermented sausages

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Abstract

The distribution of volatile compound among lean and fat tissues during processing of dry fermented sausages manufactured with either nitrite or nitrate was studied. Twelve volatile compounds were quantified by multiple headspace solid-phase microextraction (multiple HS-SPME) in combination with gas chromatography and mass selective detector (GC–MS) in the lean and fat tissues. The lean tissue contained the highest amount of volatile compounds derived from the lipid oxidation process (heptanal, octanal, nonanal, 1-octen-3-ol, 2-pentanone and 2-heptanone), amino acid degradation reactions (2 and 3- methylbutanal) and esterase activity (ethyl 3-methylbutanoate and ethyl hexanoate). However, the compounds pentanal and hexanal showed similar concentration in both tissues. Few differences were observed in the concentration of volatile compounds due to curing agents throughout the ripening stages although they disappeared after vacuum storage. In conclusion, the main tissue contributing to the flavour development in sausages is the intramuscular fat and the protein fraction. However, the fat tissue contributes to flavour perception due to the solubilization of volatile compounds in it.

Keywords: Dry fermented sausage; Lean; Fat; Volatile compounds; Multiple SPME.

INTRODUCTION

The manufacture of dry fermented sausages is carried out by mincing lean and fat and mixing it with starter culture, additives, curing agents and spices. Then, the mince obtained is submitted to fermentation and drying process. During the fermentation, the pH decline produces the coagulation of myofibrillar proteins, and a protein gel is formed, which contains the granulated fat (Ordoñez & de la Hoz, 2007). Throughout the drying process, the protein gel suffers a continuous release of moisture and at the same time, flavour is developed as the result of biochemical changes derived from both the lean and the fat components (Mottram, 1991). Several factors of the manufacture process influence the generation of aroma compounds, such as fermentation and curing salts (Marco, Navarro, & Flores, 2008).

Subcutaneous fat is mainly composed by triglycerides (TG) while lean is composed by water (72%), protein (20%) and lipids (7%) (Schweigert, 1994). The lipids of the muscle tissue (intramuscular fat) are composed by TG (62–80%) and phospholipids (PL) (16–34%) (Ordóñez, Hierro, Bruna, & de la Hoz, 1999). Both subcutaneous and intramuscular fat contribute to aroma generation through lipolysis and lipid oxidation reactions (Gandemer, 2002). Even though phospholipids are present in minor amounts than triglycerides, they have a strong influence on flavour development and oxidation because they are relatively rich in polyunsaturated fatty acids (PUFA) in comparison to triacylglycerols (Toldrá, 2007).

The importance of fat on the sensory properties of meat products is due to its role as precursor of volatile compounds and also it acts as a solvent for aroma compounds, as most of them are lipophillic (Leland, 1997). Moreover, lipids influence flavour release (lowering the vapour pressure of lipophillic compounds) and stabilize flavour compounds by interfering with solubility of reactants (water and acids) (Leland, 1997).

On the other hand, the degradation of proteins also results in the formation of aromatic compounds (Ordóñez *et al.*, 1999). Also, proteins influence flavour release and perception because they interact with flavour components thus affect the volatile compounds headspace concentration (Pérez-Juan, Flores, & Toldrá, 2007).

Many authors have studied volatile compounds of several solid foods, however, little attention has been paid to the distribution of volatile compounds among the different fractions of the solid matrix. Gkatzionis, Linforth, and Dodd (2009) studied the aroma profile of three different sections of a variety of blue cheese. However, it is not clear which is the contribution of lean and fat tissues to flavour generation and retention during processing of dry fermented sausages. This information can be useful to select the technological strategies to enhance the generation of flavour compounds.

Therefore, our approach was to study the distribution of volatile compounds among the lean and fat tissues during processing of dry fermented sausages manufactured with either nitrite or nitrate. Multiple HS-SPME in combination with GC–MS was applied in order to measure the total content of volatile compound present in each fraction to determine their effect on flavour generation or retention.

MATERIALS AND METHODS

Reagents and standards

The volatile compounds used for identification and to prepare the standard dilutions were all obtained from Fluka Chemie AG (Buchs, Switzerland). The purity of the standard compounds was between 97% and 99.7% except a 90% and 95% purity for 2-methyl butanal and heptanal, respectively.

Dry fermented sausages

Two batches of dry slow fermented sausages, one containing sodium nitrite and another containing potassium nitrate, were manufactured with lean pork ham (80%), pork back fat (20%), sodium chloride, lactose, dextrin, sodium caseinate, glucose and sodium ascorbate, as explained in Olivares, Navarro, and Flores (2009). At day 0, 200 g of the minced meat mixture were collected and two sausages from each batch were randomly chosen throughout the drying process (11, 21 and 42 d) and after vacuum storage (91 d). Each sausage was sliced, vacuum packed and stored at -20 ºC for chemical analyses. For volatile analysis, from each sausage a slice (2 cm thickness) was taken, lean and subcutaneous fat were manually separated with a spatula. Each

fraction, lean and fat, was minced with liquid nitrogen in presence of 0.75 mg of butylated hydroxytoluene (BHT) to prevent oxidation. The homogenized sample was wrapped in aluminium foil, vacuum packaged and frozen at -20 ºC. One gram of the homogenized sample was used for GC–MS analysis.

Chemical analyses (moisture and total lipids)

Moisture content was determined according to the official method for analysis of meat products (BOE, 1979) by dehydration at 100 ºC until constant weight.

Total lipids were extracted from 5 g of minced sausage according to the method of Folch, Lees, and Sloane Stanley (1957), using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1) as solvent due to its lowest toxicity. The extracts were dried in a rotating vacuum evaporator and weighted to determine the total quantity of lipids.

Results of chemical analyses are expressed as the mean of three replicates at each sampling time (g/100 g dry fermented sausage).

Lean content was calculated from the total sausage weight minus fat and water content at each stage of the curing process.

Volatile compounds analysis

The total concentration of volatile compounds was determined by multiple HS-SPME using gas chromatography and mass spectrometry. Multiple HS-SPME is a stepwise extraction method for the quantitative analysis of volatile compounds in a complex matrix (Tena & Carrillo, 2007). The sample is extracted for consecutives times by exposing the SPME fiber to the HS at equal time intervals. It is assumed that the concentration of the analyte decays exponentially and the total peak area value can be calculated. This total peak area corresponds to the total amount of analyte in the sample. The optimized procedure extraction is described in Flores and Hernández (2007) for dry fermented sausages. One gram of sample (lean or fat) was homogenized in 15 mL of double-distilled water. Then, 1 mL of the homogenized sample was added to a 10 mL HS vial, containing 0.5 g of NaCl and equilibrated at 30 ºC for 1 h. The sample was extracted for four consecutives times using a solid-phase

microextraction fiber (85 μm carboxen/polydimethylsiloxane StableFlex, Supelco, Bellefonte, PA, USA) for 90 min at 30 ºC each extraction.

After each extraction, the fiber was desorbed in the GC injection port of the CG (HP 5890 series II, Hewlett–Packard, Palo Alto, CA) equipped with a HP 5972 mass selective detector (Hewlett–Packard) using the conditions described in Olivares *et al.* (2009). The compounds were identified by comparison with mass spectra from the library database (Nist' 98), kovats retention index (Kovats, 1965), and by comparison with authentic standards. The quantification of the volatile compounds was done in selected ion monitoring (SIM) mode. The analysis was focused on those compounds previously identified as aroma active compounds in fermented sausages using olfactometry (Marco, Navarro, & Flores, 2007) and the selection of the volatile compounds was done as described Flores and Hernández (2007) by using the detector in SCAN mode.

External standard method was used to calculate the mass of compound. Stock standard solutions were analysed by multiple HS-SPME using the same conditions as for the sausage and a total area value was calculated. Then, a linear calibration was obtained by representing the total area against the standard concentration added to the vial (Flores & Hernández, 2007). The linearity, limit of detection and quantification of the technique are reported in Olivares *et al.* (2009).

Results are expressed as volatile compound present in the lean or the fat per g of dry fermented sausage (dry matter) at each processing time and batch.

Statistical analysis

The effect of the addition of curing agents (nitrite or nitrate) and type of tissue (lean or fat) on the volatile content and chemical parameters were tested by two-factor analysis of variance (ANOVA) using the statistic software Statgraphics plus (v 5.1). Significant effects were compared using Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

As expected, the content of protein and fat rose in both batches during the drying process as a result of moisture release (**Table 1**) as indicated by Wirth (1988). There were not found differences neither in fat nor in lean content between batches. Moisture content was higher ($p < 0.05$) in the nitrate added sausage although this difference was only of 1% of relative humidity.

Table 1. Percentage of moisture, fat and lean content during the ripening of dry fermented sausages manufactured with added nitrate or nitrite.

Time (days)	Batch	Moisture	Fat	Lean
0	N_3	62.9^{a}	14.9^{a}	22.2^{a}
	N_2	61.5^{a}	14.1^{a}	24.7^{a}
11	N_3	58.9^{b}	14.8^{a}	26.2^{b}
	N_2	57.6^{b}	14.2^{a}	28.2^{b}
21	N_3	50.7^{c}	16.1^{a}	33.2^{c}
	N_2	49.1^{c}	16.4^{a}	34.5^{c}
42	N_3	40.2^{d}	22.5^{b}	37.6^{d}
	N_2	38.7^{d}	22.4^{b}	39.2^{d}
91	N_3	40.4^{d}	22.0^{b}	37.6^{d}
	N_2	39.1^{d}	23.3^{b}	37.6^{d}
SEM		0.80	1.08	1.4
S/B/SxB		**/*/ns	**/ns/ns	**/ns/ns

N_3: samples with added nitrate. N_2: samples with added nitrite. S: stage. B: Batch. S x B: interaction between batches and stage. ns: no significant.
$^{a–d}$ Means with different letter in the same batch indicate significant differences ($p < 0.05$) among processing times. SEM: Standard error of the mean. * Significant $p < 0.05$. ** Significant $p < 0.01$.

A total of 42 odour active compounds were identified in the fermented sausages (**Table 2**). However, only 12 of them were quantified in lean and fat tissues throughout the drying process (7 aldehydes, 2 ketones, 2 esters and 1 alcohol) because only these compounds showed the exponential decay in peak area with the successive extractions required in multiple HS-SPME. The compounds 3-methylbutanal and 2-

Table 2. Volatile compounds identified in the fermented sausages by GC–MS.

Compound	KI[A]	R[B]
Alcohols		
Ethanol	505	a
3-Methyl-1-butanol	795	a
1-Pentanol	827	a
1-Hexanol	925	a
1-Octen-3-ol	1025	a
Phenylethyl alcohol	1190	a
1-Octanol	1124	a
Aldehydes		
Propanal	522	a
Butanal	603	a
3-Methyl butanal	687	a
2-Methyl butanal	698	a
Pentanal	733	a
Hexanal	839	a
2-Hexenal	905	a
Heptanal	941	a
2-Heptenal	1011	a
Octanal	1039	a
Phenylacetaldehyde	1103	a
Nonanal	1148	a
Ketones		
Acetone	528	a
2-butanone	630	a
Diacetyl	625	a
2-Pentanone	728	a
2,3-Pentanedione	739	a
2-Heptanone	934	a
2-Nonanone	1140	a
Esters		
Ethyl acetate	634	a
Ethyl butanoate	831	a
Ethyl 2-methyl butanoate	877	a
Ethyl 3-methyl butanoate	881	a
Ethyl pentanoate	928	a
Ethyl hexanoate	1029	a
Ethyl octanoate	1226	a
Acids		
Acetic acid	717	a
Propanoic acid	815	a
2-Methyl propanoic acid	868	a
Butanoic acid	900	a
3-Methyl butanoic acid	930	a
Octanoic acid	1283	a
Furans		
2-Ethylfuran	719	a
2-Pentylfuran	1006	b
Sulfur compounds		
Methional	966	a
Terpenes		
Limonene	1048	a

[A] Kovats index for DB-624 capillary column (J&W Scientific: 30 m, 0.25 mm i.d., 1.4 μm film thickness) installed on a gas chromatograph equipped with a mass selective detector. [B] Reliability of identification: a, mass spectrum and retention time identical with an authentic standard; b, mass spectrum and KI from the literature in accordance.

methylbutanal could not been quantified at 42 d. In all cases, the concentration was expressed as ng of volatile compound present in the lean or the fat per 1 g of sausage. It should be taken into account that lean abundance is higher than fat and to a greater extent with processing time (**Table 1**).

In general, the concentration of all the compounds rose during the curing process in both tissues. As Olesen, Meyer, and Stahnke (2004) indicated volatile compounds generation is linked to the fermentation and curing process. In the case of linear aldehydes (**Fig. 1**), the concentration found in lean was significantly higher than in fat tissue, with the exception of pentanal and hexanal, which showed similar concentrations in both tissues during the process and also octanal at day 21. In relation to the evolution of 1-octen-3-ol, 2-pentanone and 2-heptanone (**Fig. 2**) it was observed that in all cases, the concentration found in the lean fraction was significantly higher than in fat, with the exception of 2-pentanone at day 91 and 2-heptanone at day 21, where there were not differences between tissues.

During dry fermented sausage processing, lipids in muscle and adipose tissues are subjected to degradation, mainly by lipolysis and oxidation reactions. Lipolysis of TG and PL leads to the formation of free fatty acids (FFA), which are substrates for oxidation reactions, particularly those unsaturated (oleic, linoleic and linolenic) (Gandemer, 2002). The main volatile compounds generated through lipid oxidation processes are aldehydes. Methylketones and alcohols can also be generated within lipid oxidation, although their main possible origin is the β-oxidation of FFA by Staphylococci (Ordóñez *et al.*, 1999). In this study, five linear aldehydes (pentanal, hexanal, heptanal, octanal and nonanal), two ketones (2-pentanone and 2-heptanone) and one unsaturated alcohol (1-octen-3-ol) were found in both subcutaneous fat and lean tissues. These findings could indicate that lipids from both fractions have acted as substrates for oxidation reactions to form volatile compounds with aroma properties. Moreover, the highest concentration observed in the lean tissue could be due to the oxidation of PL as they are relative rich in PUFA in comparison to TG (Marco, Navarro, & Flores, 2006). Therefore, this fact would confirm that PL have a strong influence in flavour development. In fact, Molly, Demeyer, Civera, and Verplaetse (1996) and Zanardi, Ghidini, Battaglia, and Chizzolini (2004) indicated that polyunsaturated fatty

acids are liberated mainly from the polar lipid fraction. However, until now it had not been confirmed the importance of volatile compounds derived from lipid oxidation in the lean tissue of dry fermented sausages and its effect on flavour generation.

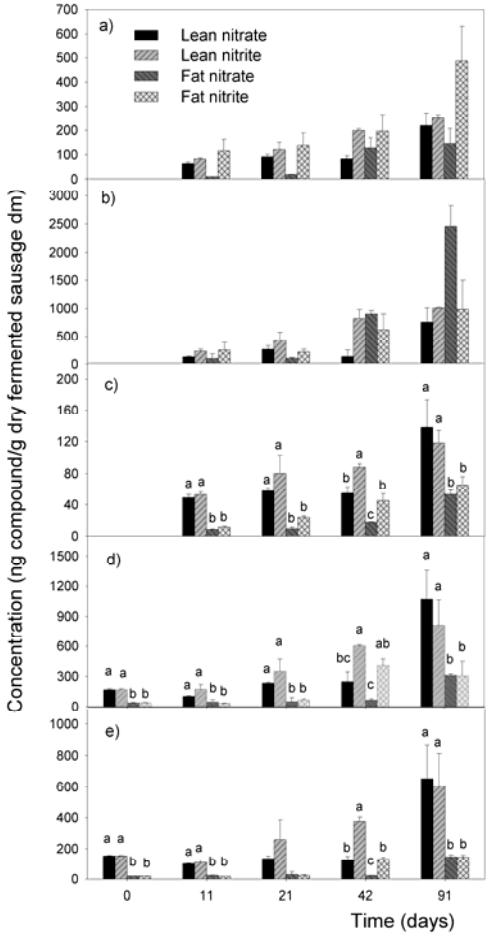

Fig. 1. Concentration of linear aldehydes: pentanal (a), hexanal (b), heptanal (c), octanal (d) and nonanal (e) in the lean and fat tissues during the ripening of dry fermented sausages manufactured with added nitrate or nitrite. Different letters in the same stage indicate significant differences ($p < 0.05$) among volatile content of lean and fat tissues.

With respect to the use of different curing salts, the only significant difference in the concentration of volatile compounds coming from lipid autooxidation was detected at

the end of the process (42 d), where the batch containing nitrite showed the highest abundance of heptanal, octanal and nonanal in both tissues, fat and lean.

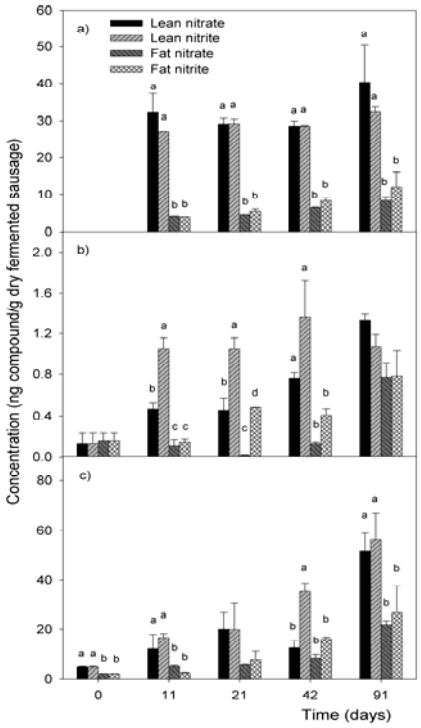

Fig. 2. Concentration of 1-octen-3-ol (a), 2-pentanone (b) and 2-heptanone (c) in the lean and fat tissues during the ripening of dry fermented sausages manufactured with added nitrate or nitrite. Different letters in the same stage indicate significant differences ($p < 0.05$) among volatile content of lean and fat tissues.

However, after the vacuum process (91 d) this difference disappeared. These results agree with Marco, Navarro, and Flores (2006, 2008) who reported few significant differences in lipid oxidation products among slow fermented sausages with added nitrite or nitrate, although Stahnke (1995) indicated that the addition of nitrate strongly increased the generation of straight aldehydes.

In the case of lipid β-oxidation products, the use of nitrite resulted in higher significant amounts of 2-pentanone (11 d) and 2-heptanone (42 d) in the lean tissue and 2-pentanone (21 d) in both tissues. Marco *et al.* (2006) found larger amounts of 2-

pentanone and 2-heptanone in nitrite added sausages throughout the manufacture process. In addition, Stahnke (1995) indicated that β-oxidation seems to increase with nitrite conditions, especially if the processing temperature is low, as in our study that the ripening was done at low temperature (slow fermented process).

In relation to the two branched aldehydes, their concentration is shown in **Fig. 3a** and **b** (3-methylbutanal and 2-methylbutanal respectively). Both of them are present at higher amounts in the lean tissue throughout the manufacture process. The main pathway by which 3-methylbutanal and 2-methylbutanal are generated is the catabolism of the branched amino acids leucine and isoleucine, respectively (Ordóñez *et al.*, 1999). As amino acids are components of protein, their presence in the lean fraction is explained. On the other hand, the amounts found in subcutaneous fat could be due to the role that fat plays as a solvent for aroma compounds. With respect to the type of curing salt used, there was a significant difference in the concentration of the lean tissue at day 11. In the case of 3-methylbutanal, it was more concentrated in the nitrate batch, while 2-methylbutanal was in the nitrite lean tissue. In addition, 2-methylbutanal was at significant higher amount in the fat of the nitrate batch at day 91. Several authors have reported the positive effect of nitrate in the generation of volatile compounds coming from the generation of amino acids (Marco *et al.*, 2006, 2008; Stahnke, 1995) and our results are partially in accordance.

Within the group of esters, the content of ethyl 3-methylbutanoate and ethyl hexanoate (**Fig. 3c** and **d**, respectively) was always higher in the lean than in the fat fraction. Talon, Chastagnac, Bergnais, Montel, and Berdagué (1998) indicated that the production of esters in sausages depends on the presence of ethanol and different acids, as well as on the esterase activity of Staphylococci. Alcohols and acids can be generated by the reduction or oxidation of the aldehydes formed with the lipid oxidation and protein degradation, therefore their presence in both tissues is explained. On the other hand, the use of nitrate or nitrite did not affect the amount of esters with the exception of ethyl 3-methylbutanoate at day 91, which showed the highest abundance in the fat tissue of the nitrite batch. Previous works (Marco *et al.*, 2006, 2008; Stahnke, 1995) indicated that the amount of esters was higher in sausages with added nitrate rather than those with nitrite. However, in our case few differences were observed.

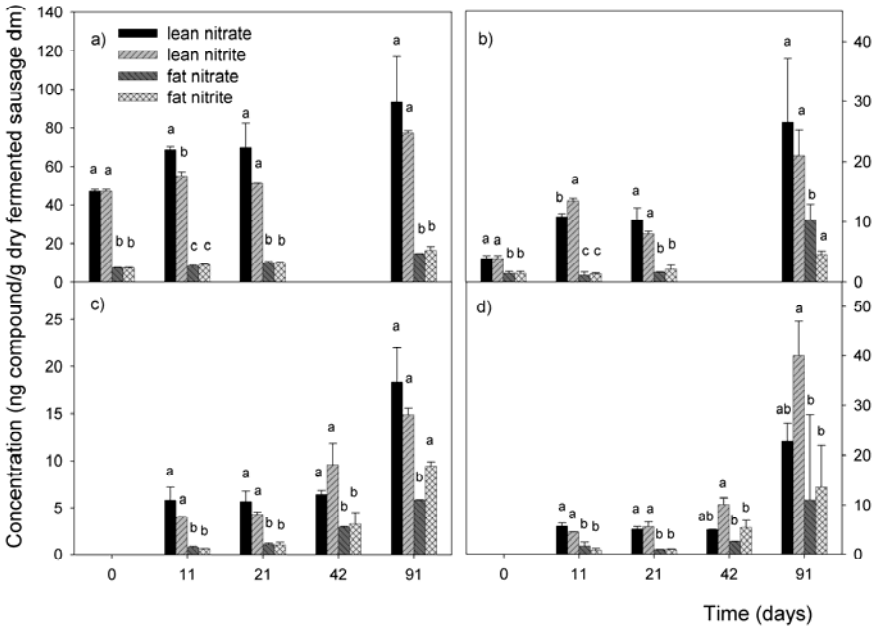

Fig. 3. Concentration of branched aldehydes: 3-methylbutanal (a) and 2-methylbutanal (b), and esters: ethyl 3-methyl butanoate (c) and ethyl hexanoate (d) in the lean and fat tissues during the ripening of dry fermented sausages manufactured with added nitrate or nitrite. Different letters in the same stage indicate significant differences ($p < 0.05$) among volatile content of lean and fat tissues.

Several works indicated that the addition of nitrate generates higher amounts of volatile compounds coming from lipid autooxidation reactions, amino acid degradation and esterase activity of Staphylococcus, while nitrite addition produces higher generation of β-oxidation products (Marco *et al.*, 2006; Stahnke, 1995). The increase of volatile compounds arising from amino acid degradation in nitrate added sausages was attributed to a higher microorganisms population and to the nitrate effect on their metabolism. However, this effect was not observed in the present study as the growth of lactic acid bacteria and Staphylococci was higher in the nitrite batch (data not shown), and this could be the reason for the absence of differences between batches.

Actually only one work has studied the release of aroma compounds from dry fermented sausage (Flores & Olivares, 2008) in presence of saliva and antioxidant. However, the study of the distribution of volatile compounds among the meat matrix is

important since it helps to understand the interactions between them and the components of the matrix that influence flavour release. As fat decreases the release of flavour compounds, reduces their headspace concentration and, therefore, their vapour pressure but also acts as a reservoir for volatile compounds since the losses are prevented (Leland, 1997).

In summary, both the type of tissue and the curing agents produced significant effect on the generation and distribution of volatile compounds. Generally, the lean tissue had a higher amount of volatile compounds derived from protein and lipid degradation than the fat tissue indicating its contribution to flavour development. Taken into account the low fat content in the lean, the highest abundance of compounds derived from lipid oxidation indicates that these compounds are mainly derived from the oxidation of PUFA contained in the PL of the intramuscular fat. In addition, the contribution of fat to flavour retention was confirmed by the presence of volatile compounds derived from amino acids (branched aldehydes) and esters in it. On the other hand, the use of different curing salts affected the concentration of some volatile compounds at several processing stages in both lean and subcutaneous tissues, producing an increase in the presence of nitrite. However, after vacuum storage the curing agents did not affect the volatile content in both tissues.

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Capítulo 4.

Sensory acceptability of slow fermented sausages based on fat content and ripening time

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Sensory acceptability of slow fermented sausages based on fat content and ripening time

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Abstract

Low fat dry fermented sausages were manufactured using controlled ripening conditions and a slow fermented process. The effect of fat content and ripening time on the chemical, colour, texture parameters and sensory acceptability was studied. The fat reduction in slow fermented sausages produced an increase in the pH decline during the first stage of the process that was favoured by the higher water content of the low fat sausages. Fat reduction did not affect the external appearance and there was an absence of defects but lower fat content resulted in lower sausage lightness. The sausage texture in low fat sausages caused an increase in chewiness and at longer ripening times, an increase in hardness. The sensory acceptability of the fermented sausages analyzed by internal preference mapping depended on the different preference patterns of consumers. A group of consumers preferred sausages with high and medium fat content and high ripening time. The second group of consumers preferred sausages with low ripening time regardless of fat content except for the appearance, for which these consumers preferred sausages of high ripening time. Finally, the limit to produce high acceptability low fat fermented sausages was 16% fat content in the raw mixture that is half the usual content of dry fermented sausages.

Keywords: Fermented sausages; Low fat; Sensory acceptability; Ripening.

INTRODUCTION

Dry fermented sausages are meat products with high fat content. Commercial sausages have fat contents around 32% directly after manufacture, but as a result of drying this rises to about 40–50% (Wirth, 1988). Fat is responsible for various properties of dry fermented sausages. Firstly, from a physiological point of view, fat acts as a source of essential fatty acids and fat soluble vitamins and constitutes the most concentrated source of energy in the diet (9 kcal/g) (Mela, 1990). Secondly, fat contributes to the flavour, texture, mouthfeel, juiciness and lubricity, which determine the quality and acceptability of dry sausages. Finally, the granulated fat has a technological function in the manufacture of dry fermented sausages as it helps to loosen up the sausage mixture to facilitate the continuous release of moisture from the inner layer of the sausage; a process necessary for undisturbed fermentation and flavour development (Wirth, 1988).

In recent years, increased concerns about the potential health risks associated with the consumption of high fat foods has led the food industry to develop new formulations or modify traditional food products to contain less fat (Mendoza, Garcia, Casas, & Selgas, 2001). One of the strategies for the development of low-fat fermented sausages was the reduction of fat content and the simultaneous addition of non-lipid fat replacers to minimize texture defects (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004). In this regard, the addition of inulin, cereal and fruit fibres, and short-chain fructooligosaccharides gave satisfactory results for the reduction of fat content in dry fermented sausages (Mendoza *et al.*, 2001; García, Dominguez, Galvez, Casas, & Selgas, 2002; Salazar, García, & Selgas,2009). Other strategies were focused on the replacement of pork back fat by olive oil in order to have a positive effect on consumer health (Bloukas, Paneras, & Fournitzis, 1997; Muguerza, Fista, Ansorena, Antiasarán, & Bloukas, 2002; Muguerza, Ansorena, Bloukas, & Antiasarán, 2003; Koutsopoulos, Koutsimanis, & Bloukas, 2008; del Nobile, Conte, Incoronato, Panza, Sevi, & Marino, 2009).

Dry fermented sausages are one of the most difficult meat products as far as fat reduction is concerned. Excessive fat reduction leads to harder or rubbery products

due to higher weight losses (Keeton, 1994) and also, unacceptable appearance produced by the presence of wrinkled surfaces and case hardening (Muguerza *et al.*, 2002). However, these defects can be avoided if appropriate processing or climatic conditions are applied as suggested Wirth (1988). Wirth suggested that fat reduced fermented sausages of acceptable standard can be made with fat contents in the raw material of about 15%, which rises to 20–30% in the finished product. Liaros, Katsanidis, and Bloukas (2009) have proposed the use of vacuum packaging during ripening as an effective technique to improve external appearance in low fat fermented sausages. However, high fat sausages still had the highest acceptability scores (Mendoza *et al.*, 2001), not only due to their appearance but also to other sensory characteristics such as texture and flavour. So, it is necessary to determine the effect of fat reduction on consumer acceptability in order to elucidate the limit of fat reduction. Moreover, processing conditions should be controlled to avoid the appearance of case hardening therefore; it is proposed to use a slow fermented process to obtain low fat fermented sausages of high organoleptic quality.

The aim of this study was to determine the limit of fat reduction based on consumer acceptability and taking into consideration the ripening process.

MATERIALS AND METHODS

Dry fermented sausages

Three batches of dry fermented sausages (20 kg meat batter for each batch) with different pork back fat contents (10%, 20% or 30%) were manufactured; low fat (LF), medium fat (MF) and high fat (HF) respectively. The lean pork and the pork back fat were ground through a 10 mm diameter mincing plate and vacuum minced with the following additives (g/kg): sodium chloride (27), lactose (20), dextrin (20), sodium caseinate (20), glucose (7), sodium ascorbate (0.5), sodium nitrite (0.15) and potassium nitrate (0.15). Also, a commercial starter culture (0.1) SP-318 was added (Danisco, Cultor, Madrid, España) containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus*, and *Staphylococcus carnosus*. The meat mixture was maintained at 3–5 °C for 24 h and then was stuffed into collagen casings

(Fibran, S.A., Girona, España, 75–80 mm diameter) the final weight of each sausage was 700 g. Approximately 30 sausages were made in each batch. The sausages were dried for 42 d at 10 °C and 80–90% relative humidity (RH). After 42 d of processing, the temperature was increased to 12 °C for 4 d and finally, was mantained for 17 d at 10 °C and 75–85% RH. The total drying time was 63 d (**Fig. 1**). Temperature and RH of the ripening chambers were continuously recorded. In order to control the ripening process, two sausages from each treatment were weighed almost every day to control weight losses that were expressed as a percentage of the initial weight. Also, two sausages from each batch were used to control the pH by introducing a pH meter HI 99163 (Hanna Instruments Inc., Hoonsocket, USA) into the centre of the sausage as described by ISO 2917 (1999).

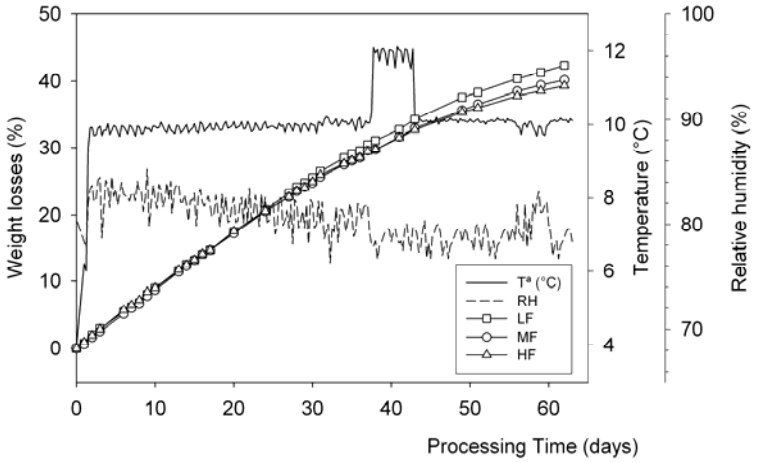

Fig. 1. Processing conditions (Tª and RH) applied in the manufacture of the slow fermented sausages. Weight losses of the different batches are shown as LF (□), MF (○) and HF (Δ).

From each batch (LF, MF and HF), 200 g of the minced meat mixture were collected and at days 9, 18, 42 and 63, four sausages from each batch were randomly chosen to study the effect of ripening time and fat content. In each sample colour analyses were done and then, 150 g of the sample were minced and used for moisture, water activity and pH analyses. The remaining minced sample was vacuum packed and frozen at −20 °C for subsequent analyses (protein and lipid contents). All the results were

expressed as the mean of four replicates at each sampling time. Finally, at 42 and 63 d of the drying process four sausages from each batch were taken for sensory and texture analyses.

Chemical analyses (pH, water activity, moisture, protein, and total lipids)

The pH was measured as described by ISO 2917 (1999) by introducing a portable pH meter (HI 99163, Hanna Instruments Inc., Hoonsocket, USA) into a mixture of sausage and water (1:1). Water activity was determined using a FAst-lab (Gbx, Romans sur Isère Cédex, France) water activity meter, previously calibrated with sodium chloride and potassium sulphate.

Moisture content was determined according to the official method for analysis of meat products BOE (1979) by drying at 100 °C to constant weight. Nitrogen content was determined by the Kjeldahl method and protein estimated by multiplying the nitrogen content by 6.25. Total lipids were extracted from 5 g of minced sausage according to Folch *et al.* (1957), using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1) as solvent. The extracts were dried in a rotating vacuum evaporator and weighed to determine the total lipid content.

Colour measurement

Colour measurements were carried out using a CR-410 colorimeter (Minolta Chroma Meter Measuring Head, Osaka, Japan) with D65 illuminant. Each sausage was cut and the colour of the slices was measured three times for each analytical point. L*, a*, and b* scale coordinates were obtained: L* (lightness), a* (redness) and b* (yellowness). Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Texture profile analysis (TPA)

Instrumental texture was measured with a TA-XT.plus Texture Analyzer using the Texture Exponent software (version 2.0.7.0. Stable Microsystems, Godalming, UK). Dry fermented sausage slices (4×1.5 cm) and cubes (2×2×1.5 cm) were evaluated. The speed was 1 mm s^{-1} with a strain of 50% of the original cube height for samples

stored 42 d and a strain of 25% of the original cube height for samples stored 63 d with a 5 s interval between compression cycles. A trigger force of 5 g was selected. The compression was performed using a 75 mm diameter aluminium plate (P/75). The samples were compressed twice to give a TPA from which the three primary textural parameters (Pons & Fiszman, 1996) were obtained: hardness (the peak force during the first compression cycle), springiness (the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite) and cohesiveness (the ratio of the positive force area during the second compression portion to the positive force area during the first compression), as well as the secondary parameter chewiness (the product of hardness, cohesiveness and springiness). Twelve samples per batch (LF, MF, and HF) and ripening time (42 and 63 d) were measured.

Sensory Analysis

Seventy-five consumers, 45 female and 30 male, who consumed dry fermented sausages on a regular basis, were used. Testing was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988). The casing was removed and the sausages were cut into slices of approximately 4 mm thickness and served at room temperature on white plastic dishes. Water and unsalted toasts were provided to cleanse the palate between samples. Consumers tasted, in two different sessions, three samples (HF, MF and LF) at each ripening time (42 and 63 d) identified with random, three-digit codes, following a balanced complete block experimental design. For each sample, consumers scored the overall acceptability as well as the acceptability of appearance, flavour, taste, hardness and juiciness using a 9-box scale labelled on the bottom with “dislike very much”, in the middle “neither like nor dislike” and on the top “like very much”. Data acquisition was performed using Compusense five release 5.0 software (Compusense Inc., Guelph, Ont., Canada).

Statistical Analysis

Two-way analysis of variance (ANOVA) (ripening time, fat level and interaction ripening time × fat level) was performed on the instrumental and sensory parameters to

evaluate differences among samples. The differences among batches in texture parameters were also analyzed by two-way analysis of variance (ANOVA) (fat level and sausage shape). Besides, Internal Preference Mapping applied to the individual hedonic rates on all samples was performed (van Kleef, van Trijp, & Luning, 2006). 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. After performing the internal preference mapping, cluster analysis was carried out to classify consumers according to their preference patterns. Agglomerative Hierarchical Clustering (AHC) was performed using Euclidian distance, with Ward's method as the aggregation criterion (XLStat 2006 Agglomerative hierarchical clustering). A dissimilarity plot was used to determine how many clusters were appropriate for each analysis. A dendrogram was then employed to determine the cluster structure of the data and support the decision that was made using the dissimilarity plot. Statistical analysis of instrumental parameters was performed using the SPSS 12 package program and statistical analysis of sensory parameters was performed using the statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain).

RESULTS AND DISCUSSION

Chemical analyses

The fat content of the slow fermented sausages was lower than formulated as the batches contained 13.2, 16.5 and 19.3% fat instead of the 10, 20 and 30% formulated (**Table 1**). The lower fat content was due to variations in the trimming of the pork meat. However, the batches had significant differences in fat contents that were useful. The protein content was similar among batches although at 9, 18 and 42 d, the protein content was significantly lower in the HF sausages than the LF sausages. The reduction in moisture during ripening caused the increase in protein and fat contents (**Table 1**). At the end of the process the sausages reached a fat content of 22.0, 24.1 and 28.4% fat in LF, MF and HF, respectively. The fat content of the sausage

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Table 1. Chemical composition of the dry sausages (g/100 g) manufactured with different pork back fat contents; low fat (LF), medium fat (MF) and high fat (HF).

	0 days			9 days			18 days			42 days			63 days			P_F*	P_R	P_{FxR}
	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF			
Fat	13.2^{g}	16.5^{efg}	19.3^{def}	13.5^{g}	16.1^{fg}	19.5^{de}	14.4^{g}	16.4^{fg}	19.4^{de}	18.2^{ef}	21.3^{cd}	25.8^{ab}	22.0^{cd}	24.1^{bc}	28.4^{a}	***	***	ns
	(1.18)	(1.03)	(0.50)	(0.66)	(1.31)	(1.23)	(0.97)	(0.99)	(1.13)	(1.15)	(1.19)	(1.27)	(0.86)	(1.78)	(1.15)			
Protein	19.1^{f}	18.5^{f}	19.1^{f}	22.0^{de}	19.4^{ef}	18.6^{f}	22.2^{d}	21.1^{def}	19.3^{f}	29.6^{b}	26.5^{c}	25.3^{c}	35.6^{a}	35.9^{a}	33.2^{a}	***	***	*
	(0.12)	(1.84)	(0.78)	(0.51)	(0.59)	(0.15)	(0.19)	(0.69)	(0.54)	(0.49)	(1.12)	(0.85)	(1.49)	(0.78)	(1.64)			
Moisture	65.3^{a}	61.5^{ab}	55.8^{cde}	60.2^{b}	59.5^{bc}	54.4^{de}	57.4^{bcd}	56.3^{cde}	53.1^{e}	45.0^{f}	44.7^{f}	42.6^{f}	36.9^{g}	35.2^{g}	33.6^{g}	***	***	*
	(4.63)	(0.09)	(2.65)	(0.69)	(0.75)	(1.89)	(0.47)	(0.96)	(1.89)	(0.67)	(0.72)	(0.64)	(0.91)	(1.92)	(0.81)			

* P_F: P value of fat content effect; P_R: P value of ripening effect; P_{FxR}: P value of interaction between fat content and ripening effects. ***: P < 0.001, **: P< 0.01, *: P<0.05, ns: P>0.05.
Identical letters in each parameter indicate that there is no significant difference at p>0.05 (Tukey's test). The values represent the mean and the standard deviation in parenthesis.

expressed on a dry matter basis (dm) were 34.8, 39.1 and 43.1% while the protein contents in dm were 54.6, 49.5 and 43.8% for LF, MF and HF, respectively.

Fig. 1 shows the weight losses during the ripening process as well as the temperature and relative humidity in the ripening chamber. No significant differences were detected in weight losses among batches until 50 d of ripening, meaning that the slow ripening conditions were controlled to minimize differences. Only at 63 d, did the LF batch show a higher significant loss than the MF and HF batches. This did not produce differences in the external appearance of the sausages. Many of the studies done to reduce fat content in fermented sausages reported higher weight losses in sausages with lower fat contents (Bloukas, *et al.*, 1997; Papadima & Bloukas, 1999; Muguerza *et al.*, 2002; Liaros *et al.*, 2009) probably due to the ripening conditions applied as all used higher temperatures (around 20 °C) during the first ripening days to produce fermented sausages in ripening times of around 30 d.

The pH was measured directly in the sausages to control the fermentation (**Fig. 2a**) however, a higher standard deviation was observed than when measuring the pH with an equal mixture of sausage and water (**Fig. 2b**). When measured in the lab, there was a significant effect of ripening time, fat level and the interaction of fat level and ripening time on pH ($p<0.001$). The pH decreased from 5.9 to 4.6 in 18 d (**Fig. 2b**). A similar trend has been reported for slow dry fermented sausages (Ordóñez, Hierro, Bruna, & de la Hoz, 1999; Marco, Navarro, & Flores, 2008; Olivares, Navarro, & Flores, 2009). With respect to fat content, the decrease in fat produced a faster pH decline that was significantly different at 9 d of processing ($p<0.001$) although on further ripening there were no differences among batches. This faster pH decrease in low fat sausages, was also seen by Soyer, Ertas, and Üzümcüoglu (2003) although other authors have not reported an effect of fat on pH decline, probably because it is highly dependent on the fermentation process (Bloukas *et al.*, 1997; Muguerza *et al.*, 2002; Salazar *et al.* 2009).

The moisture content of the sausages was different among batches (**Table 1**). As expected it was highest in LF sausages although at 42 and 63 d there were not differences among batches. The LF batch had a higher water content decline than the HF batch during the first 18 days. Water activity levels decreased from 0.96 to 0.87 during ripening in all batches (**Fig. 2c**) and were not affected by fat content ($p>0.05$) as

also reported by Mendoza *et al.* (2001) and García *et al.* (2002). The high water activity of LF sausages could be related to the high pH decline of this batch (**Fig. 2b**) as this difference was only detected at 9 d, when the highest significant differences ($p<0.001$) in pH were detected.

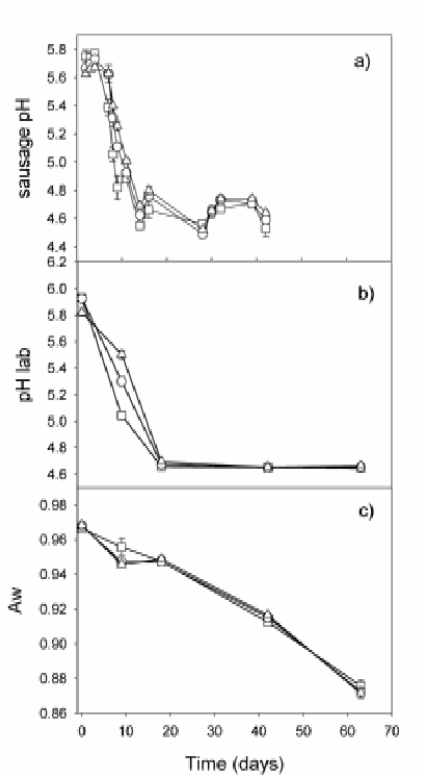

Fig. 2. Changes in pH (measured directly in the sausage (a) and in the lab (b)) and water activity (c) during the ripening of dry sausages manufactured with different pork back fat contents; low fat (LF, □), medium fat (MF, ○) and high fat (HF, ∆). Symbols represent the mean and standard error of the mean.

Fat level and ripening time affected the lightness (L*) and redness (a*) ($p<0.001$) of the sausages, while the yellowness (b*) was only affected by the ripening time ($p<0.001$) (**Fig. 3**). In relation to L* values, a decrease was observed during ripening, since sausages became darker due to weight loss. Higher fat contents resulted in lighter ($p<0.001$) sausages as also observed by Muguerza *et al.* (2002) and Soyer *et al.* (2003). With respect to a* values, an increase in redness was observed at day 9 due to

the formation of nitrosylmyoglobin, followed by a decrease during ripening ($p<0.001$) (**Fig. 3**). Moreover, HF sausages had the lowest redness ($p<0.001$) as observed Soyer *et al.* (2003). Finally, a decrease in yellowness ($p<0.001$) was detected in all batches as also reported Muguerza *et al.* (2002).

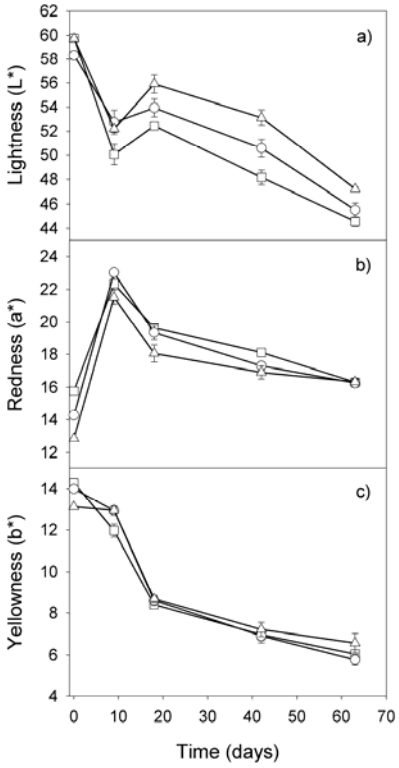

Fig. 3. Changes in L*, a* and b* values during the ripening of dry sausages manufactured with different pork back fat contents; low fat (LF, □), medium fat (MF, ○) and high fat (HF, ∆). Symbols represent the mean and standard error of the mean.

Texture profile analyses

TPA parameters of sausages analyzed at 42 and 63 d of ripening are summarized in **Tables 2** and **3**, respectively. The sausages were analyzed in two shapes, slices and cubes, due to the possible effect of the external layers on texture. Two factors were considered for statistical analysis, fat content and shape. No differences in hardness and springiness due to fat content were found at 42 d of ripening. However, the

sausages with the lower fat content showed a significantly higher value of cohesiveness and chewiness but only when they were analyzed in slice shape. At 63 d of ripening, the sausages showed significant differences due to fat content in hardness and chewiness and again, these differences were only detected in the slices. The low fat samples had the highest hardness and chewiness. An increase in hardness and chewiness has been reported by several authors in low fat dry fermented sausages (Salazar *et al.*, 2009; García *et al.*, 2002) while other authors only reported an increase in hardness (Mendoza *et al.*, 2001; Liaros *et al.*, 2009).

Table 2. Texture parameters of dry fermented sausages manufactured with different pork back fat contents; low fat (LF), medium fat (MF) and high fat (HF) and 42 days of ripening time.

		LF	MF	HF	P_S*	P_F	$P_{S \times F}$
Hardness (N)	Slices	293.7a (20.9)	281.2a (25.3)	282.9a (23.5)	***	ns	ns
	Cubes	71.1b (5.6)	68.6b (9.0)	67.4b (11.1)			
Springiness	Slices	0.730a (0.055)	0.696ab (0.041)	0.716a (0.043)	*	ns	*
	Cubes	0.699ab (0.025)	0.707ab (0.032)	0.667b (0.049)			
Cohesiveness	Slices	0.667ab (0.009)	0.668a (0.018)	0.649c (0.013)	***	***	ns
	Cubes	0.649abc (0.151)	0.660ab (0.149)	0.629c (0.025)			
Chewiness (N)	Slices	143.1a (14.7)	130.4b (13.2)	131.7ab (16.2)	***	*	ns
	Cubes	32.4c (3.5)	32.0c (4.5)	28.5c (6.7)			

* P_S: P value of shape effect; P_F: P value of fat content effect; P_{SxF}: P value of interaction between shape and fat content effects. ***: P < 0.001, **: P< 0.01, *: P<0.05, ns: P>0.05.
Identical letters in each parameter indicate that there is no significant difference at p>0.05 (Tukey's test). The values represent the mean and the standard deviation in parenthesis.

Sausage geometry also had a significant effect as both types of samples (slices and cubes) showed significant differences in hardness and chewiness at 42 d of ripening whereas at 63 d of ripening the differences were in hardness, chewiness and cohesiveness. At both ripening times, the cubes were less hard than the slices; as

expected since cubes were extracted from the inside of sausages where the moisture content was highest.

In summary, fat reduction in dry fermented sausages had a significant effect on the texture however, sample preparation and ripening time significantly affected the differences. The differences were more marked when the whole slice was analyzed rather than a portion of it. Moreover, the significant increase in hardness due to fat reduction was only detected at longer ripening times due to the loss of moisture. Fat reduction was responsible for an increase in chewiness regardless of ripening time.

Table 3. Texture parameters of dry fermented sausages manufactured with different pork back fat contents; low fat (LF), medium fat (MF) and high fat (HF) and 63 days of ripening time.

		LF	MF	HF	P_S*	P_F	$P_{S \times F}$
Hardness (N)	Slices	242.3a (17.7)	223.6b (17.2)	210.6b (31.3)	***	***	ns
	Cubes	56.8c (9.4)	48.0c (6.5)	39.4c (5.8)			
Springiness	Slices	0.783a (0.030)	0.800a (0.052)	0.764a (0.041)	ns	ns	ns
	Cubes	0.769a (0.053)	0.765a (0.051)	0.747a (0.093)			
Cohesiveness	Slices	0.778a (0.030)	0.768a (0.018)	0.773a (0.033)	***	ns	ns
	Cubes	0.704b (0.014)	0.712 b (0.017)	0.691b (0.032)			
Chewiness (N)	Slices	147.3a (15.8)	137.6a (14.8)	124.2b (19.0)	***	***	ns
	Cubes	30.9c (6.1)	26.1c (3.9)	20.4c (4.1)			

* P_S: P value of shape effect; P_F: P value of fat content effect; $P_{S \times F}$: P value of interaction between shape and fat content effects. ***: P < 0.001, **: P< 0.01, *: P<0.05, ns: P>0.05.
Identical letters in each parameter indicate that there is no significant difference at p>0.05 (Tukey's test). The values represent the mean and the standard deviation in parenthesis.

Sensory analyses

The fermented sausages were analyzed by consumers at two ripening times (42 and 63 d). The slow fermentation process prevented the appearance of external defects such as dry edges and shrunken diameter so, the external appearances of the batches were similar (**Fig. 4**). This is in accord with the weight losses as the LF batch showed

no differences during the process until 50 d when losses were around 2% higher than in the MF and HF batches (**Fig. 1**).

Fig. 4. Effect of fat content and ripening time on the external and cross sectional appearance of the fermented sausages.

The purpose of this study was to elucidate which instrumental measurements were related to consumer acceptance. Therefore, the information obtained from the internal "map" of consumers and products was related to selected instrumental parameters (colour, hardness and fat content).

An internal preference mapping of the sensory attribute "overall acceptability" was performed and the results were a sample map and a consumer map, corresponding to the scores and loadings of the Principal Components Analysis (PCA). Preference mapping examines individual consumers' acceptability instead of average hedonic ratings and takes into account heterogeneous acceptability degrees across consumers. Internal preference mapping informs about which products are preferred by consumers and allows visual identification of clusters of consumers with similar preference patterns (Guinard, Uotani, & Schlich, 2001; Jaeger, Andani, Wakeling, & MacFie, 1998).

For the purpose of understanding consumer responses, the preferences were also analyzed by cluster analysis using Euclidean distances. Internal preference mapping biplot representations of consumers' acceptability, samples and instrumental parameters on the basis of the first two components are shown in **Fig. 5**. The PCA of the preference scores showed that about 59% of the variation in the preferences was explained by the two first principal components. Preference component 1 was related

to MF63, HF63, HF42, MF42 and LF42 batches and to the instrumental parameters of "hardness" and colour (L*, a* and b*). Preference component 2 was related to HF63, LF63 and MF42 samples and to "fat content" and "L*" colour. Consumers were distributed into two clusters by the second component (**Fig. 5**). The largest group fell into the right quadrants with 50 consumers (cluster 1) and the other group was situated in the left quadrants with 25 consumers (cluster 2). Cluster 1 basically liked the MF63 sample which was related to hardness and fat content. Cluster 2 liked the HF42 sample followed by the MF42 sample. These samples were related to fat content, colour properties and lower hardness. Few consumers preferred the LF42 sample. Samples with high and low fat content at 63 d of ripening (HF63 and LF63) were not preferred by consumers.

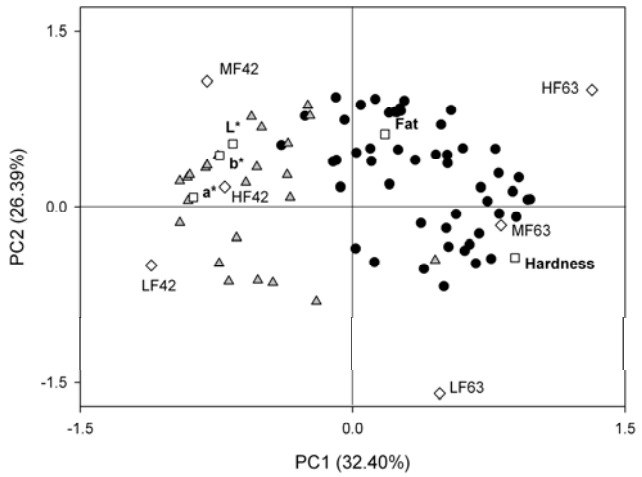

Fig. 5. Internal preference mapping biplot representation of consumers' acceptability (black circles: cluster 1; grey triangles: cluster 2), samples and instrumental parameters on the basis of the first two components.

Internal preference mapping of the other sensory attributes analyzed were similar to "overall acceptability" although the number of consumers in each cluster varied from one attribute to another. Forty-four and 30 consumers for "appearance", 31 and 43 consumers for "flavour", 46 and 29 consumers for "taste", 38 and 37 consumers for "hardness" and 37 and 38 consumers for "juiciness".

As can be seen in **Fig. 5**, sample preference was different for each cluster. For this reason the mean value of the different sensory attributes (overall acceptability, appearance, flavour, taste, hardness and juiciness) scored by each consumer cluster was studied by one-way ANOVA (**Fig. 6**). Cluster 1 preferred samples with high and medium fat content and high ripening time. However, cluster 2 preferred samples with low ripening time regardless of fat content except for "appearance", for which high ripening times samples were favoured (**Fig. 6**).

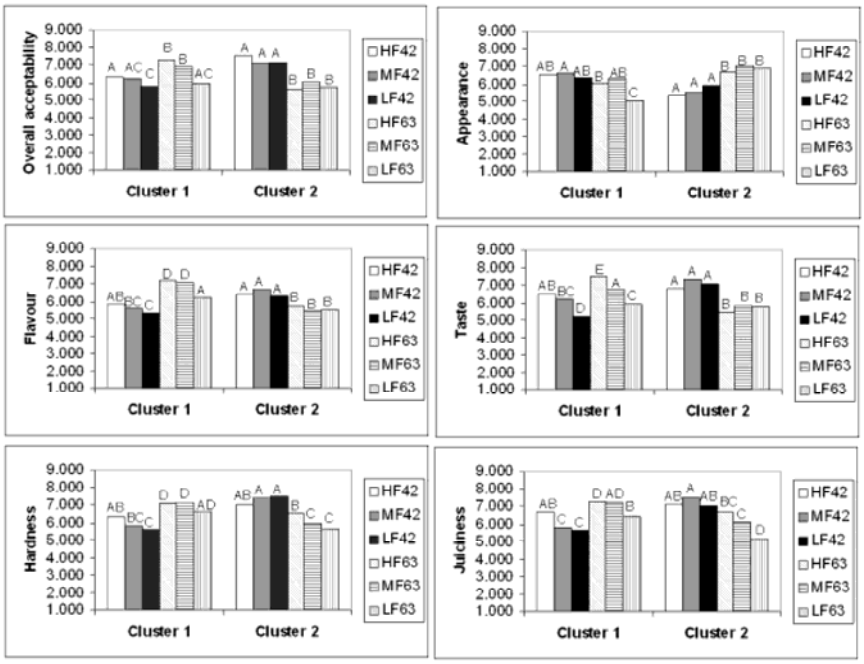

Fig. 6. Mean values of the different sensory attributes scored by each consumer cluster. Identical letters for each cluster indicate that there is no significant difference at p>0.05 (Tukey's test).

Previous sensory analyses performed on dry fermented sausages indicated that low fat fermented sausages had lower external and cross sectional appearance (Liaros *et al.*, 2009), higher hardness, lower juiciness, lower colour and higher saltiness and taste (Mendoza *et al.*, 2001), higher colour, lower odour, taste and appearance (Muguerza *et al.*, 2002) and lower hardness and higher smoke odour (Bovolenta, Boscolo, Dovier,

Morgante, Pallotti, & Piasentier, 2008) than high-fat fermented sausages. However, none of these studies were able to elucidate how these changes affect consumer acceptability to establish the minimum fat reduction.

CONCLUSIONS

The slow fermented process was able to produce low fat fermented sausages without negative effects on the external appearance, only a lower lightness of the cross section was seen. Fat reduction in fermented sausages affected the texture by causing an increase in chewiness and, at longer ripening times, an increase in hardness. Although the sensory acceptability of fermented sausages depended on the different preference patterns of consumers, it can be concluded that fermented sausages of low fat content (13% in the raw mixture, LF) were less appreciated by consumers. On the other hand, a fat content of 16% (MF) in the raw sausage mixture produced fermented sausages with high consumer acceptability, half the usual fat content of fermented sausages.

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Capítulo 5.

Effect of fat content on aroma generation during processing of dry fermented sausages

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Effect of fat content on aroma generation during processing of dry fermented sausages

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Abstract

Dry fermented sausages with different fat contents were produced (10%, 20% and 30%). The effect of fat content and ripening time on sensory characteristics, lipolysis, lipid oxidation and volatile compounds generation was studied. Also, the key aroma components were identified using gas chromatography (GC) and olfactometry. High fat sausages showed the highest lipolysis and lipid oxidation, determined by free fatty acid content and thiobarbituric acid reactive substances (TBARS), respectively. A total of 95 volatile compounds were identified using SPME, GC and mass spectrometry (MS). Fat reduction decreased the generation of lipid derived volatile compounds during processing while those generated from bacterial metabolism increased, although only at the first stages of processing. The consumers preference in aroma and overall quality of high and medium fat sausages was related to the aroma compounds hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol which contributed green, medicinal, tallowy, fruity and mushroom notes.

Keywords: Fermented sausages; Low fat; Lipolysis; Aroma compounds.

INTRODUCTION

The high fat content of dry fermented sausages (40–50%) is essential for sensory properties, such as hardness, juiciness and flavour, and for technological functions (Wirth, 1988). However, from a health point of view, excessive fat intake is not recommended. For this reason, several authors have focused on the reduction and partial substitution of fat in dry fermented sausages (Liaros, Katsanidis, & Bloukas, 2009; Mendoza, García, Casas, & Selgas, 2001; Muguerza, Ansorena, Bloukas, & Astiasarán, 2003; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002; Olivares, Navarro, Salvador, & Flores, 2010).

Low fat sausages become hard due to high weight losses and have an unacceptable appearance because of wrinkled surfaces and case hardening (Muguerza *et al.*, 2002). Nevertheless, Liaros *et al.* (2009) proposed the use of vacuum packaging during ripening as an effective strategy to produce low fat fermented sausages without negative effects on external appearance. However, high fat sausages still have the highest acceptability scores (Mendoza *et al.*, 2001; Olivares *et al.*, 2010) due to other sensory characteristics such as flavour.

Flavour formation in dry fermented sausages is mainly related to lipolysis (Gandemer, 2002) through the generation of free fatty acids (FFA) that are further subjected to lipid oxidation producing a large variety of volatile compounds (Zanardi, Ghidini, Battaglia, & Chizzolini, 2004). Although the role of fat as a precursor of aroma compounds is known, there is little information about the effect of fat content on dry fermented sausage flavour. Fat is important for dry fermented sausage flavour not only for the generation of flavour compounds but also as a solvent for aroma compounds (Leland, 1997).

However, the reduction of fat in fermented sausages has given controversial results in relation to flavour. While Mendoza *et al.* (2001) and Olivares *et al.* (2010) obtained higher aroma scores in high fat dry fermented sausages compared to low fat ones, other authors reported no differences in flavour (Liaros *et al.*, 2009; Muguerza *et al.*, 2002). Muguerza *et al.* (2003) reported an increase in oxidation and total volatile compounds in fat reduced sausages that was attributed to the higher intramuscular fat

content of reduced fat products. Also, Chevance, Farmer, Desmond, Novelli, Troy, and Chizzolini (2000) indicated that fat reduction in salami increased the release of odour compounds.

Nevertheless, it has never been shown how fat content, through the generation of volatile compounds affects consumer's acceptance. Therefore, the aim of this work was to study the effect of fat content on lipid changes and headspace volatile compounds during the processing of dry fermented sausages. Moreover, the ripened sausages were evaluated by consumers to determine which aroma compounds are responsible for acceptability.

MATERIALS AND METHODS

Dry fermented sausages

Three batches of dry fermented sausages with different pork back fat contents (10%, 20% or 30%) were manufactured; low fat (LF), medium fat (MF) and high fat (HF) respectively. The manufacturing process was as described by Olivares *et al.* (2010). Briefly, the lean pork and the pork back fat were minced and vacuum mixed with additives (sodium chloride, lactose, dextrin, sodium caseinate, glucose, sodium ascorbate, sodium nitrite and potassium nitrate) and commercial starter culture (*Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus* and *S. carnosus*). The meat mixture was stuffed into collagen casings (75–80 mm diameter) previously dipped in a solution of natamycin and potassium sorbate (Floracid N3, Ceylan, Spain) in order to prevent overgrowth of undesirable surface moulds.

From each batch (LF, MF and HF), 200 g of the meat mixture was collected at day 0. Also, at 9 and 18 d days of ripening and at two different final ripening times (42 and 63 d), four sausages from each batch were randomly removed from the storage chamber, sliced, vacuum packaged and frozen at −80 °C to await analysis.

Lipolysis and lipid oxidation analyses

Lipolysis was studied by the analysis of the free fatty acid (FFA) content. Total lipids were extracted from 5 g of minced sausage according to Folch, Lees, and Sloane

Stanley (1957) and the free fatty acids were separated from the lipid fraction using an ion exchange resin, as described by Needs, Ford, Owen, Tuckley, and Anderson (1983). Heneicosanoic acid (C21:0) was used as the internal standard. FFAs were converted into fatty acid methyl esters (FAME) using boron fluoride-methanol (Sigma-Aldrich, Chemical Co.,Milwaukee, WI) as the methylating reagent. Analysis of the FAME was carried out in a Fisons 8160 gas chromatograph (GC) equipped with a flame ionisation detector and a split injector (split ratio used 2:1). The capillary column was a CP-SIL 88 (Agilent, Las Rozas, Spain; 100 m, 0.25 mm i.d., 0.2 μm film thickness). The oven temperature program began at 140 °C for 10 min, ramped to 190 °C at 4 °C/min, held at 190 °C for 10 min, ramped to 220 °C at 2 °C/min, held at 220 for 5 min, ramped to 230 at 2 °C/min, and finally, held at 230 °C for 20 min. Helium was used as carrier gas at a flow rate of 17.7 cm/s. Detector and injector temperatures were 240 and 220 °C respectively. FAMES were identified by comparing their retention times with those of standard fatty acid methyl esters. For quantification, the response factors of the standard FAME with respect to the internal standard were used. FFA content was expressed as mg per 100 g dry matter (dm). Moisture content was determined according to the official method for analysis of meat products BOE (1979) by dehydration at 100 °C until constant weight. The results were expressed as the mean of four replicates at each batch and sampling time.

Lipid oxidation in the sausages was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Bruna, Ordoñez, Fernández, Herranz, and de la Hoz (2001), using tricloroacetic acid instead of perchloric acid as solvent. The results were expressed as mg malonaldehyde (MDA) per kg dm.

Analysis of headspace volatile compounds by SPME GC–MS

The analysis of volatile compounds in the headspace (HS) of the sausages was done as described Marco, Navarro, and Flores (2004). The extraction used a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA) with a 85 μm carboxen/polydimethylsiloxane StableFlex fibre (CAR/PDMS SF). 3 g of minced sausage was weighed into a 10 mL headspace vial, and 0.75 mg of BHT was added. The vial was left for 1 h in a thermoblock (J.P., Selecta, Barcelona, Spain) at 37 °C for

equilibration. The CAR/PDMS fibre was then exposed to the headspace for 3 h while maintaining the sample at 37 °C. The identification and quantification of volatile compounds was performed in a gas chromatograph HP 7890A equipped with a HP 5975C mass selective detector (Hewlett Packard, Palo Alto, CA). The compounds adsorbed by the fibre were desorbed in the injection port of the GC for 15 min at 220 °C with the purge in splitless mode. Then, the compounds were separated using a DB-624 capillary column J & W Scientific (Agilent Technologies, USA) (30 m, 0.25mm i.d., film thickness 1.4 μm). The GC oven temperature program began at 38 °C, held for 13 min, ramped to 110 °C at 3 °C/min, then to 150 °C at 4 °C/min and to 210 °C at 10 °C/min, and, finally, held at 210 °C for 5 min. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range 29–400 amu (scan mode). 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 standards used for the identification were obtained from Fluka Chemie AG (Buchs, Switzerland) except 2-methyl furan, 2-nonenal, diacetyl, methyl ethyl sulphide, 3-methyl thiophene, 3-methyl-2-buten-1-ol, 2-methylpyrazine, 2,6-dimethylpyrazine, dimethyl tripsulphide, 3-methylthio-propanol, benzeneacetaldehyde, methyl acetate, methyl 2-hydroxy-propanoate, methyl 3-hexenoate, methyl heptanoate, ethyl 2,4-hexadienoate, 2,4-hexadienoic acid and 4-methyl-phenol which were obtained from Aldrich (St. Louis, MO). Quantification was based on the total extracted area (TIC) or the area of a target ion when different compounds coeluted. The results were expressed as abundance units (AU) 10^{-6} per g dm and comprised the mean of three replicates at each batch and sampling time.

Gas chromatrography–olfactometry

The volatile compounds were adsorbed by the SPME fibre as described above but using 4 g of sausage from LF and HF batches at 63 d of ripening. Then, the fibre was desorbed in the gas chromatograph (Agilent 6890, USA) injection port for 15 min at 240 °C in splitless mode, the split valve was opened after 1 min. The compounds were separated using a DB-624 capillary column (60 m, 0.32 mm i.d., film thickness 1.8 μm). The capillary column was split (2:1) into deactivated and uncoated capillary tubing

connected with the sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) and flame ionization detector (FID), respectively. The sniffing port ODP3 was equipped with a humidified air make up and a computer voice recorder integrated in the Chemstation software (Agilent, USA). Helium was used as the carrier gas at a flow rate of 35.14 cm/s. The oven temperature program began at 38 °C for 13 min, ramped to 100 °C at 3 °C/min and maintained at 100 °C during 10 min, then ramped to 150 °C at 3 °C/min, ramped to 210 °C at 5 °C/min, and finally held at 210 °C for 20 min, the total run time was 82.3 min. Detector temperature was set at 240 °C.

The detection frequency method was used to estimate the aromatic impact of each volatile compound (Pollien, Ott, Montigon, Baumgartner, Muñoz-Box, & Chaintreau, 1997). Four trained assessors evaluated the odors from the GC-effluent. Each assessor evaluated two high fat and two low fat sausages (63 d of ripening), therefore a total of 16 assessments were carried out. The final detection frequency value (DF) for each compound was obtained by summation of the 16 sniffings. The detection of an odor by less than three assessors was considered to be noise, therefore the minimum DF value was 4 and the maximum was 16. For each assessment, evaluation of the odor took place over two different time intervals (0–35 and 35–70 min) in order to avoid olfactory fatigue of the assessors. Aroma compounds were identified by three different methods; comparison with mass spectra, comparison with the Kovats retention indices of authentic standards injected in the GC–MS and GC–O; and by coincidence of the assessors' descriptors with those in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).

Sensory analysis

At the end of the process (42 and 63 d), dry fermented sausages were tested by a panel of 75 consumers. The analysis was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988). The casing was removed and the sausages were cut in slices approximately 4 mm thick and served at room temperature on white plastic dishes. Water and unsalted toasts were provided to consumers to cleanse the palate between samples. Consumers tasted, in two different sessions, three samples (HF, MF and LF) of two different ripening times (42 and 63 d) identified with random,

three-digit codes, following a balanced complete block experimental design. For each sample, consumers scored the aroma and overall quality using a 9-box hedonic scale. Data acquisition was performed using Compusense five release 5.0 software (Compusense Inc., Guelph, Ont., Canada).

Statistical analysis

Two-way analysis of variance (ANOVA) (ripening time, fat content and interaction of ripening time and fat content) was performed on lipid, volatile compounds and sensory parameters to evaluate differences among samples. Differences between particular sample means were analysed according to Fisher's least significant difference (LSD) test. A correlation procedure was performed to evaluate any relationship among lipolysis, lipid oxidation and volatile compounds. Furthermore, principal component analysis (PCA) was used to find the relationships among sausages with different fat contents and ripening times (LF, MF and HF at 42 and 63 d of processing) and the parameters related to lipid changes (FFA and TBARS), aroma-active volatile compounds and sensory analysis (aroma and overall quality). Statistical analysis was performed using the statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain).

RESULTS

Lipolysis and lipid oxidation

The fat content of the sausages was different among batches and at 63 d of processing was 22.0±0.9 in LF, 24.1±1.8 in MF and 28.4±1.2 in HF. The chemical composition of the three batches during ripening was reported in Olivares *et al.* (2010). The levels of FFA in LF, MF and HF sausages during processing are shown in **Table 1**. At day 0, no differences in total FFA concentration were detected among batches and the total FFA concentration ranged from 162 to 220 mg/100 g dm, which represented 0.38–0.58 % of the total lipid content. The total FFA levels increased during processing as a result of lipolysis ($p<0.01$).

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Table 1. Free fatty acid (FFA) concentrations (mg/100 g dm) of dry fermented sausages with different fat contents.

	0 days			9 days			18 days			42 days			63 days						
FFA[A]	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF	SEM[B]	Ps[C]	Pb	Psxb
C14:0	2.6 ^{h}	1.9 ^{h}	2.8 ^{h}	6.4 ^{g}	7.5 ^{efg}	8.9 ^{def}	6.6 ^{g}	9.3 ^{cdef}	11.1 ^{bc}	7.2 ^{fg}	9.4 ^{cde}	12.5 ^{ab}	9.8 ^{cd}	11.4 ^{bc}	13.9 ^{a}	0.82	**	**	*
C16:0	48.7 ^{d}	34.7 ^{d}	44.2 ^{d}	94.5 ^{c}	101.0 ^{c}	111.4 ^{c}	101.5 ^{c}	107.1 ^{c}	112.3 ^{c}	102.2 ^{c}	115.4 ^{bc}	142.4 ^{ab}	141.7 ^{ab}	150.9 ^{a}	167.1 ^{a}	11.1	**	**	ns
C18:0	22.7 ^{g}	16.8 ^{g}	22.8 ^{g}	41.6 ^{f}	43.3 ^{f}	47.9 ^{ef}	57.2 ^{de}	51.5 ^{def}	53.2 ^{def}	53.7 ^{def}	62.6 ^{cd}	77.3 ^{ab}	73.4 ^{bc}	77.7 ^{ab}	87.6 ^{a}	4.7	**	**	ns
SFA	74.1 ^{f}	53.4 ^{f}	69.8 ^{f}	142.6 ^{e}	151.9 ^{de}	168.2 ^{de}	165.3 ^{de}	167.9 ^{de}	176.6 ^{de}	163.2 ^{de}	187.4 ^{cd}	232.2 ^{ab}	225.0 ^{bc}	240.0 ^{ab}	268.6 ^{a}	16.5	**	**	ns
C16:1	6.8 ^{h}	4.9 ^{h}	6.4 ^{h}	17.9 ^{g}	19.4 ^{g}	21.2 ^{fg}	21.0 ^{fg}	25.7 ^{def}	27.7 ^{de}	22.5 ^{efg}	28.0 ^{d}	36.6 ^{ab}	30.9 ^{cd}	35.1 ^{bc}	41.4 ^{a}	2.0	**	**	*
C18:1	89.3 ^{j}	65.8 ^{j}	87.3 ^{j}	205.4 ^{i}	229.1 ^{hi}	255.1 ^{ghi}	293.9 ^{fgh}	304.6 ^{fg}	324.6 ^{ef}	306.1 ^{fg}	377.8 ^{de}	502.7 ^{ab}	423.3 ^{cd}	479.5 ^{bc}	565.1 ^{a}	25.3	**	**	ns
C20:1 n9	1.6 ^{hi}	1.0 ^{i}	1.5 ^{hi}	3.2 ^{gh}	3.8 ^{h}	4.2 ^{fg}	6.7 ^{de}	5.6 ^{ef}	6.1 ^{e}	8.0 ^{d}	9.8 ^{c}	14.0 ^{b}	11.1 ^{c}	14.6 ^{b}	17.6 ^{a}	0.5	**	**	**
MUFA	97.7 ^{j}	71.7 ^{j}	95.1 ^{j}	226.5 ^{i}	252.4 ^{hi}	280.5 ^{ghi}	321.6 ^{fgh}	335.9 ^{fg}	358.4 ^{ef}	336.7 ^{fg}	415.6 ^{de}	553.3 ^{ab}	465.4 ^{cd}	526.0 ^{bc}	624.1 ^{a}	27.6	**	**	ns
C18:2 n6	36.7 ^{g}	27.3 ^{g}	40.2 ^{g}	99.4 ^{f}	100.9 ^{f}	110.8 ^{f}	148.4 ^{e}	157.8 ^{de}	165.2 ^{de}	156.9 ^{e}	186.3 ^{d}	250.3 ^{b}	216.3 ^{c}	237.9 ^{bc}	280.1 ^{a}	10.5	**	**	**
C18:3 n3	1.6 ^{g}	1.2 ^{g}	1.9 ^{g}	4.9 ^{f}	5.1 ^{f}	6.1 ^{f}	8.6 ^{e}	8.8 ^{e}	10.2 ^{de}	9.3 ^{e}	11.2 ^{d}	16.1 ^{b}	12.9 ^{c}	14.5 ^{bc}	18.0 ^{a}	0.7	**	**	**
C20:2 n6	1.0 ^{g}	0.7 ^{g}	1.0 ^{g}	2.6 ^{f}	2.8 ^{f}	3.2 ^{f}	5.3 ^{de}	5.0 ^{e}	5.1 ^{e}	6.3 ^{d}	8.0 ^{c}	11.4 ^{b}	9.0 ^{c}	10.5 ^{b}	12.9 ^{a}	0.4	**	**	**
C20:3 n6	0.8 ^{g}	0.8 ^{g}	1.0 ^{g}	2.1 ^{f}	1.9 ^{f}	2.0 ^{f}	3.4 ^{de}	3.0 ^{e}	2.9 ^{e}	4.0 ^{cd}	4.4 ^{c}	5.5 ^{b}	5.5 ^{b}	5.8 ^{ab}	6.2 ^{a}	0.2	**	**	*
C20:4 n6	6.0 ^{h}	4.4 ^{h}	6.3 ^{h}	12.8 ^{g}	12.4 ^{g}	12.1 ^{g}	20.7 ^{de}	18.2 ^{ef}	16.2 ^{f}	21.5 ^{de}	23.0 ^{cd}	25.5 ^{bc}	28.6 ^{ab}	30.4 ^{a}	30.6 ^{a}	1.1	**	ns	ns
C22:4 n6	0.7 ^{g}	0.6 ^{g}	0.8 ^{g}	1.5 ^{f}	1.5 ^{f}	1.4 ^{f}	2.4 ^{e}	2.2 ^{e}	1.7 ^{f}	2.9 ^{d}	3.4 ^{c}	3.6 ^{c}	3.8 ^{bc}	4.1 ^{ab}	4.4 ^{a}	0.1	**	ns	ns
C20:5 n3	0.2 ^{h}	0.1 ^{h}	0.2 ^{gh}	0.5 ^{fg}	0.5 ^{fg}	0.4 ^{fg}	0.6 ^{def}	0.7 ^{cde}	0.5 ^{ef}	0.8 ^{bc}	0.5 ^{def}	0.7 ^{cd}	0.9 ^{abc}	1.0 ^{ab}	1.1 ^{a}	0.1	**	ns	ns
C22:5 n3	1.5 ^{i}	1.3 ^{i}	2.0 ^{hi}	3.3 ^{fg}	2.7 ^{gh}	3.4 ^{fg}	6.9 ^{d}	4.7 ^{e}	4.0 ^{ef}	6.5 ^{d}	7.0 ^{d}	8.1 ^{c}	8.5 ^{bc}	9.2 ^{ab}	9.5 ^{a}	0.3	**	ns	**
C22:6 n3	0.4 ^{fg}	0.3 ^{g}	0.5 ^{fg}	0.7 ^{efg}	0.5 ^{fg}	0.8 ^{def}	1.4 ^{abc}	1.1 ^{cd}	0.9 ^{de}	1.3 ^{bc}	1.2 ^{cd}	1.4 ^{abc}	1.7 ^{a}	1.6 ^{ab}	1.7 ^{ab}	0.1	**	ns	ns
PUFA	48.9 ^{g}	36.7 ^{g}	53.8 ^{g}	127.9 ^{f}	128.2 ^{f}	140.3 ^{f}	197.8 ^{e}	201.4 ^{e}	206.7 ^{e}	205.3 ^{e}	245.0 ^{d}	322.5 ^{b}	287.2 ^{c}	315.0 ^{bc}	364.3 ^{a}	13.2	**	**	*
Total	220.7 ^{h}	161.9 ^{h}	218.7 ^{h}	497.0 ^{g}	532.5 ^{g}	589.0 ^{fg}	684.7 ^{ef}	705.2 ^{def}	741.8 ^{de}	705.1 ^{def}	848.0 ^{cd}	1108.1 ^{b}	977.6 ^{bc}	1081.0 ^{b}	1257.0 ^{a}	56.3	**	**	ns

[A] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
[a–j]: Identical letters in each parameter indicate no significant differences at p>0.05 (Fisher's test).
[B] SEM: Standard error of the mean.
[C] P_s: p value of ripening time effect; P_b: p value of fat content effect; P_{sxb}: p value of interaction between fat content and ripening time effects. **: p<0.01, *: p<0.05, ns: p>0.05.

At the end of processing, total FFA concentration was significantly higher ($p<0.01$) in high fat sausages (1257 mg/100 g dm) than in low and medium fat sausages (977 and 1081 mg/100 g dm, respectively) although total FFA content was similar (2.9% of the total lipid content) in the three batches. Therefore, total FFA levels were proportional to the amount of pork back fat used in the manufacture of the sausages. These results are in agreement with Molly, Demeyer, Civera, and Verplaetse (1996) and Marco, Navarro, and Flores (2006) who reported that the major proportion of FFA comes from triglycerides present in the subcutaneous fat.

At day 0, FFA in all batches maintained the relationship MUFA>SFA>PUFA as previously observed (Navarro, Nadal, Nieto, & Flores, 2001) in fresh meat paste. During ripening, all the main FFA's increased ($p<0.01$), however PUFA showed a greater release than SFA in all batches resulting in a free MUFA>PUFA>SFA profile as previously reported for dry fermented sausages (Marco *et al.*, 2006; Molly *et al.*, 1996; Navarro *et al.*, 2001; Zanardi *et al.*, 2004). With respect to fat content, differences among batches were detected at 42 and 63 d, since the release of all FFAs was significantly higher ($p<0.01$) in the HF sausage than in MF and LF sausages. Previously, Soyer and Ertas (2007) found that high fat fermented sausages showed greater lipolysis than reduced fat ones, as observed in the present study. In addition, Molly *et al.* (1996) and Marco *et al.* (2006) indicated that there was a very high specific fatty acid (FA) release from the polar fraction compared to the triglycerides (TG) when the release was expressed as a percentage of the initial amount of FA, however, the majority of FFA were derived from the TG fraction, the most abundant lipid fraction in sausages. Also, Molly *et al.* (1996) noted the specificity of lipases for the position 3 of the triglyceride molecules, where unsaturated FA are predominantly located.

The level of TBARS in the sausages was measured throughout processing as an index of lipid oxidation (**Fig. 1**). TBARS increased during fermentation and drying in all batches ($p<0.001$) from approximately 0.3 to 1.3–1.7 mg MDA/kg dm. However, no differences among batches were detected until the end of the process, when HF sausages had higher TBARS values than LF sausages ($p<0.05$). The highest lipid oxidation values were also detected in high fat sausages by Soyer and Ertas (2007) and Liaros *et al.* (2009). In contrast Muguerza *et al.* (2003) reported higher TBARS

values in low fat sausages that they attributed to the higher intramuscular fat content of reduced-fat products.

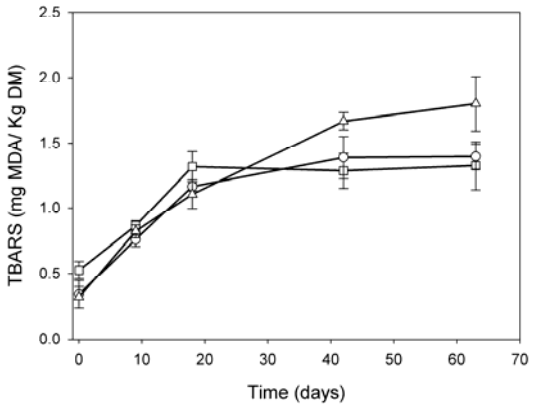

Fig. 1. Levels of TBARS (mg MDA/kg dm) during processing of dry fermented sausages manufactured with different pork back fat contents; low fat (LF,□), medium fat (MF,○) and high fat (HF, ∆). Symbols represent the mean and standard error of the mean.

In summary, the highest amount of pork back fat in HF sausages produced increased lipolysis and lipid oxidation. These reactions are related to flavour formation in dry sausages (Gandemer, 2002) due to the generation of flavour precursors, free fatty acids. However, until now it has not been shown how fat can act not only as a source but also as a solvent for flavour compounds in dry sausages and so, how both aspects affect consumer acceptance. Therefore, it is essential to study the volatile compounds present in the headspace of sausages to determine the reasons for consumer acceptance of high fat sausages.

Generation of volatile compound during processing

The proportion of volatile compounds analyzed depends on the stationary phase of the SPME fibre employed. The HS abundance and volatile compounds profile cannot be compared with other studies in which other SPME fibres or other extraction techniques have been used. However, the present extraction technique allows determination of

the effect of the studied factors (fat content and ripening time) on the HS volatiles of the sausages.

The analysis of the volatile compounds present in the HS of sausages gives an indication of the chemical and metabolic processes that occur during manufacture. In this sense, the volatile compounds listed in **Table 2** were grouped according to their possible origin (Ordóñez, Hierro, Bruna, & de la Hoz, 1999): lipid autooxidation, bacterial metabolism (lipid β-oxidation, carbohydrate fermentation, amino acid degradation, and Staphylococci esterase activity), and unknown origin (derived from meat or food contaminants). However, several of the compounds can have more than one origin.

A total of 95 volatile compounds were extracted by SPME and identified by GC–MS in the HS of the sausages and 90 of them were confirmed using authentic standards (**Table 2**). The mixture comprised 20 aldehydes, 17 esters, 15 hydrocarbons, 14 alcohols, 11 ketones, 7 sulphur compounds, 6 acids, 3 furans and 2 pyrazines. All the compounds have been previously identified in dry fermented sausages except for ethyl 2,4-hexadienoate. Lipid autooxidation was responsible for the generation of 36 volatile compounds comprising aldehydes, hydrocarbons, alcohols, acids and ketones (**Table 2**). Ripening time affected ($p<0.05$) the extracted area of all the compounds, except for nonane, 2-heptenal, 1-heptanol and 2,4-decadienal. Generally, the HS abundance of the volatile compounds increased until 42 d and then remained constant (**Fig. 2a**). At the end of the process (63 d), this group represented 20– 25% of the total extracted area. Similar evolution was seen in other dry fermented sausages using the same extraction technique (Marco *et al.*, 2006). Hexanal was the most abundant compound throughout processing, followed by hexanoic acid, octanoic acid, 4-hexen-1-ol, heptanal, pentanal, nonanal and decane. In relation to fat content, it affected the HS concentration of 21 out of 36 volatile compounds ($p<0.05$) and generally, these 21 compounds showed the highest extracted areas in the high fat sausage. In addition, a positive relationship was found between FFA and the total extracted area of volatile compounds derived from lipid autooxidation ($p<0.0001$, $r=0.926$). For instance, the compounds 2-hexenal, 2-heptenal and 2,4-decadienal were significantly higher in HF sausages than in low fat ones at 42 and 63 d.

Table 2. Volatile compounds quantified as AU x 10^{-6} per g dry matter in the headspace of dry fermented sausages during processing.

N[A]	Compound/origin	KI[B]	R[C]	0 d			9 d		
				LF	MF	HF	LF	MF	HF
	Lipid autooxidation								
3	Pentane	500	a				0.41 ^{f}	0.49 ^{ef}	
5	Propanal	523	a				0.06 ^{d}	0.04 ^{d}	0.78 ^{c}
8	Hexane	600	a	0.19 ^{fghi}	0.16 ^{ghi}	0.11 ^{i}	0.14 ^{hi}	0.90 ^{a}	0.66 ^{bc}
9	1-Propanol	611	a						
10	2-Methyl-furan	615	a				1.35 ^{abc}	1.26 ^{abcd}	0.80 ^{ef}
11	Butanal	621	a				0.14 ^{a}	0.18 ^{cd}	0.16 ^{d}
19	Heptane (71)	700	a	0.09 ^{b}	0.08 ^{b}		0.70 ^{b}	0.69 ^{b}	1.65 ^{a}
22	Pentanal	738	a				5.92 ^{c}	1.44 ^{e}	2.89 ^{cde}
30	Octane	800	a	2.88 ^{e}	1.62 ^{e}	1.73 ^{e}	8.34 ^{cd}	11.03 ^{abc}	3.58 ^{e}
32	1-Pentanol	826	a	1.42 ^{efg}	0.93 ^{fg}	0.96 ^{g}	2.60 ^{acb}	2.43 ^{bcd}	2.99 ^{ab}
35	Hexanal	840	a	4.03 ^{e}	3.83 ^{e}	2.97 ^{e}	15.48 ^{e}	13.20 ^{e}	54.14 ^{cde}
41	Nonane	900	a				0.35 ^{de}	0.56 ^{abc}	0.65 ^{ab}
42	2-Hexenal (Z)	905	a				0.00		
43	2-Butyl-furan	909	b				0.19 ^{bcd}	0.23 ^{bc}	0.18 ^{cd}
45	1-Hexanol	923	a	0.39 ^{g}	0.38 ^{g}	0.33 ^{g}	5.61 ^{ef}	2.29 ^{g}	1.41 ^{g}
46	4-Hexen-1-ol	927	b				3.21 ^{f}	1.16 ^{f}	2.36 ^{f}
48	Heptanal	940	a	1.33 ^{cd}	0.84 ^{d}	0.78 ^{d}	3.91 ^{cd}	2.54 ^{cd}	1.72 ^{cd}
53	Decane	1000	a	3.27 ^{c}	3.76 ^{c}	6.37 ^{c}	11.82 ^{b}	14.62 ^{ab}	11.12 ^{b}
55	2-Pentyl-furan (81)[E]	1009	a	0.31 ^{def}	0.15 ^{ef}	0.13 ^{f}	0.39 ^{de}	0.35 ^{def}	0.25 ^{def}
56	2-Heptenal (Z) (41)	1011	a						
58	1-Heptanol	1024	a				2.03 ^{ab}	1.83 ^{ab}	1.05 ^{b}
63	Octanal	1048	a	0.09 ^{b}	0.08 ^{b}	0.03 ^{b}	0.14 ^{b}	0.12 ^{b}	0.10 ^{b}
67	Hexanoic acid	1078	a	2.36 ^{f}	1.90 ^{f}	2.09 ^{f}	13.28 ^{d}	10.67 ^{d}	6.55 ^{e}
68	2-Ethyl-1-hexanol	1083	a				4.18 ^{a}	4.18 ^{a}	3.10 ^{bc}
69	Undecane	1100	a	0.36 ^{d}	0.43 ^{d}	0.48 ^{d}	1.07 ^{bc}	1.19 ^{b}	0.84 ^{c}
72	2-Octenal (E)	1116	a	0.22 ^{ef}	0.19 ^{f}	0.11 ^{f}	0.60 ^{de}	0.42 ^{ef}	0.36 ^{ef}
73	1-Octanol	1124	a	0.44 ^{g}	0.50 ^{g}	0.29 ^{g}	1.52 ^{de}	1.47 ^{de}	1.06 ^{f}
76	Nonanal	1149	a	3.48 ^{def}	3.30 ^{def}	1.88 ^{f}	5.15 ^{d}	4.21 ^{de}	2.54 ^{ef}
81	Dodecano	1200	a	0.43 ^{c}	0.53 ^{c}	0.57 ^{c}	1.15 ^{ab}	1.04 ^{ab}	1.01 ^{b}
82	2-Nonenal (Z)	1222	a	0.61 ^{de}	0.50 ^{e}	0.36 ^{e}	0.65 ^{cde}	0.65 ^{cde}	0.42 ^{e}
85	Octanoic acid	1267	a	1.89 ^{g}	1.58 ^{g}	1.25 ^{g}	18.25 ^{e}	14.36 ^{e}	10.00 ^{f}
86	2,4-Nonadienal (Z, Z)	1287	b				0.00		
87	Tridecane	1300	a	0.18 ^{cd}	0.18 ^{cd}	0.14 ^{d}	0.26 ^{ab}	0.23 ^{abc}	0.19 ^{cd}
98	2-Decenal (Z)	1327	a	0.25 ^{c}	0.27 ^{c}	0.16 ^{c}	0.34 ^{c}	0.26 ^{c}	0.26 ^{c}
92	2,4-Decadienal (Z, Z)	1392	a						
94	Decanoic acid	1451	a	0.22 ^{f}	0.20 ^{f}	0.19 ^{f}	1.03 ^{e}	0.83 ^{e}	0.86 ^{e}
	Lipid β-oxidation								
21	2-Pentanone	733	a	0.50 ^{d}	0.41 ^{d}	0.53 ^{d}	4.04 ^{a}	3.26 ^{b}	2.95 ^{bc}
23	2,3-Pentanedione	743	a				0.20 ^{cd}	0.40 ^{bc}	0.30 ^{bcd}
47	2-Heptanone	934	a				7.32 ^{bc}	4.30 ^{e}	2.22 ^{f}
59	2,3-Octanedione	1029	b				0.88 ^{de}		0.58 ^{e}
60	1-Octen-3-ol	1031	a	0.50 ^{de}	0.38 ^{e}	0.45 ^{e}	1.78 ^{de}	1.32 ^{de}	0.95 ^{de}
61	2-Octanone	1039	a				0.34 ^{ab}	0.29 ^{ab}	0.17 ^{c}
74	2-Nonanone	1141	a				3.79 ^{cd}	1.87 ^{e}	1.12 ^{e}
89	2-Undecanone	1349	a						
	Carbohydrate fermentation								
1	Acetaldehyde	466	a	18.06 ^{abc}	1.05 ^{g}	10.65 ^{f}	25.20 ^{a}	17.55 ^{bcd}	8.29 ^{f}
4	Ethanol	507	a		1.35 ^{e}	1.29 ^{e}	4.95 ^{c}	9.31 ^{b}	12.60 ^{a}
13	2,3-Butanedione	626	a	0.18 ^{cde}	0.22 ^{c}	0.14 ^{c}	4.74 ^{a}	1.24 ^{b}	1.72 ^{b}
14	2-Butanone	630	a	4.53 ^{efg}	3.67 ^{g}	4.21 ^{f}	12.01 ^{ab}	13.14 ^{a}	10.09 ^{bc}
20	Acetic acid	718	a				276.71 ^{f}	125.35 ^{g}	62.14 ^{g}
26	3-OH-2-butanone	781	a		5.49 ^{c}	4.34 ^{c}	47.32 ^{a}	45.00 ^{a}	28.60 ^{b}
40	Butanoic acid	895	a	6.28 ^{f}	6.90 ^{f}		51.64 ^{b}	43.07 ^{cd}	30.64 ^{e}

18 d			42 d			63 d			Ps^D	Pb	Psxb
LF	MF	HF	LF	MF	HF	LF	MF	HF			
0.50 ef	0.82 de		1.03 cd	2.34 b	2.55 ab	0.97 cd	1.37 c	3.41 a	**	**	**
0.07 d	0.39 d	0.87 c	1.03 bc	1.80 a	1.93 a	1.23 b	1.73 a	2.02 a	**	**	ns
0.50 cd	0.73 ab	0.52 cd	0.39 def	0.42 de	0.50 cd	0.29 efgh	0.37 defg	0.42 de	**	**	**
0.04 d	0.07 c	0.11 b	0.02 e	0.07 c	0.08 c	0.02 e	0.01 e	0.14 a	*	**	**
1.51 ab	1.56 a	1.35 abc	0.96 cdef	1.12 bcde	0.77 ef	0.90 def	0.72 f	0.84 ef	*	ns	ns
0.16 d	0.11 d	0.16 d	0.31 b	0.32 b	0.46 a	0.27 bc	0.28 bc	0.35 b	**	*	ns
0.42 b	0.54 b	0.44 b	0.25 b	0.50 b	0.33 b	0.24 b	0.44 b	0.42 b	*	ns	ns
5.09 cd	2.80 de	9.29 b	11.94 ab	11.54 ab	13.43 a	9.93 b	10.58 ab	11.33 ab	**	**	*
8.56 cd	9.36 bcd	9.56 bcd	7.94 d	11.60 ab	12.69 a	9.23 bcd	11.07 abc	11.08 abc	**	*	**
1.61 def	1.38 efg	2.81 ab	2.76 abc	1.89 cde	3.36 a	2.34 bcd	2.44 abcd	2.69 abc	**	**	ns
36.06 de	19.46 e	70.26 cd	161.72 ab	84.50 c	187.19 a	139.02 b	142.94 ab	169.75 ab	**	**	ns
0.26 e	0.48 bcd	0.66 ab	0.45 cd	0.64 ab	0.67 a	0.49 bcd	0.63 abc	0.63 ab	ns	**	ns
0.13 d	0.47 bc	0.39 bc	0.43 bc	0.31 c	0.89 a	0.42 bc	0.51 b	0.99 a	**	**	**
0.16 cd	0.14 d	0.18 cd	0.25 ab	0.13 d	0.20 bcd	0.26 ab	0.20 bcd	0.31 a	**	ns	*
8.78 de	3.04 fg	10.35 bcd	12.32 ab	14.88 a	11.29 b	7.65 cde	5.91 def	10.20 bc	**	ns	**
7.46 ef	7.56 ef	49.62 a	11.20 cde	17.51 c	26.78 b	10.06 de	14.22 cd	31.73 b	**	**	**
4.67 c	2.48 cd	3.84 cd	13.78 a	14.71 a	9.90 b	12.00 ab	12.16 ab	13.37 ab	**	ns	ns
11.22 b	13.00 ab	17.11 a	11.24 b	12.50 b	14.21 ab	12.24 b	13.18 b	12.00 ab	**	ns	ns
0.44 cd	0.29 def	0.38 de	0.89 b	0.86 b	1.22 a	0.75 b	0.71 bc	0.91 b	**	ns	ns
0.26 ab	0.20 bc	0.09 e	0.12 de	0.21 bc	0.32 a	0.13 de	0.18 cd	0.29 a	ns	**	**
2.06 ab	2.20 ab	2.33 ab	1.64 b	1.59 b	1.57 ab	1.34 b	1.11 a	1.32 b	ns	ns	ns
0.22 b	1.80 a	0.15 b	0.59 b	0.45 b	0.44 b	0.51 b	0.45 a	0.56 b	*	*	ns
22.07 ab	20.52 abc	21.56 abc	20.69 abc	22.88 a	20.02 abc	21.81 ab	19.46 bc	18.69 c	**	**	ns
4.03 ab	3.16 bc	3.19 abc	2.79 cd	3.10 bc	2.39 cde	2.35 cde	1.97 de	1.78 e	**	ns	ns
1.28 ab	1.08 bc	1.54 a	1.31 ab	1.18 b	1.36 ab	1.12 bc	1.06 bc	1.22 b	**	ns	ns
0.58 de	0.44 ef	0.93 d	1.37 bc	0.48 ef	1.81 b	1.31 c	1.53 bc	2.24 a	**	**	**
1.57 de	1.08 fg	1.52 de	1.82 cd	1.42 ef	2.39 b	1.92 cd	1.78 ef	2.97 a	**	**	**
7.71 c	3.81 def	4.36 de	13.50 a	7.62 c	8.46 c	11.10 b	12.11 ab	11.73 ab	**	**	**
1.25 ab	1.02 ab	1.10 ab	1.04 ab	1.17 ab	1.37 a	1.14 ab	1.05 ab	1.08 ab	**	ns	ns
0.60 de	0.45 e	0.63 de	1.03 ab	0.87 bcd	1.19 a	0.93 abc	0.87 bcd	1.12 ab	**	ns	ns
31.68 abc	25.75 d	26.03 d	34.40 a	28.33 bcd	27.42 cd	32.53 ab	28.33 bcd	26.07 d	**	**	ns
0.12 c		0.09 c	0.18 b	0.21 ab	0.24 ab	0.21 ab	0.18 b	0.25 a	**	ns	ns
0.23 abc	0.20 bc	0.26 a	0.20 c	0.19 cd	0.23 abc	0.21 ab	0.22 abc	0.21 abc	**	ns	ns
0.31 c	0.21 c	0.24 c	0.62 b	0.60 b	0.64 b	0.66 abc	0.59 b	0.96 a	**	ns	*
			0.20 bc	0.19 bc	0.29 a	0.18 c	0.19 bc	0.25 ab	ns	**	ns
1.87 cd	1.56 d	1.91 cd	2.86 a	2.15 bc	2.74 a	2.80 a	2.45 ab	2.51 ab	**	*	ns
2.89 bc	3.00 bc	2.72 c	0.51 d	0.75 d	0.60 d	0.57 d	0.70 d	0.54 d	**	ns	*
0.17 d	0.26 bcd	0.37 bcd	0.33 bcd	0.30 bcd	0.96 a	0.41 bcd	0.49 b	0.87 a	**	**	**
9.45 a	8.17 ab	7.58 b	5.68 de	6.10 cd	5.20 de	6.01 cd	5.40 de	5.16 de	**	**	**
1.09 cde	1.34 bcd	1.40 bcd	1.42 bcd	0.82 de	1.55 bc	1.91 ab	2.41 a	2.39 a	**	ns	ns
1.65 de	0.96 de	2.03 d	4.15 d	6.01 ab	6.46 a	3.63 c	4.53 bc	6.05 ab	**	*	ns
0.38 a	0.28 ab	0.28 ab	0.33 ab	0.28 ab	0.26 bc	0.34 ab	0.28 ab	0.30 ab	ns	ns	ns
5.96 a	4.60 bc	4.28 bcd	4.94 b	4.12 bcd	3.65 d	4.65 bc	3.74 cd	3.90 cd	**	**	ns
0.39 bcde	1.25 a	0.43 bcd	0.54 b	0.42 bcd	0.28 de	0.49 b	0.45 bcd	0.34 cde	**	**	**
18.64 bc	16.37 cd	23.34 ab	16.61 cd	15.76 cde	13.41 cdef	14.03 cdef	13.80 cdef	8.86 ef	**	**	**
4.41 cd	9.86 b	13.21 a	3.34 cde	5.83 c	12.64 a	2.08 de	4.31 cd	9.67 b	**	**	*
0.19 c	0.10 c	0.42 c	0.20 c	0.08 c	0.32 c	0.26 d	0.02 c	0.21 c	**	**	**
12.15 ab	10.83 abc	8.60 cd	6.35 def	7.11 de	4.35 fg	6.35 def	4.71 efg	3.83 fg	**	**	ns
651.23 a	594.19 ab	505.19 c	451.95 cd	535.04 bc	419.52 de	415.44 de	390.26 de	349.52 ef	**	**	*
23.10 b	24.33 b	23.54 b	3.86 c	6.13 c	5.42 c	3.43 c	1.92 c	2.17 c	**	ns	ns
69.38 a	72.73 a	64.95 a	36.81 cde	44.15 bc	35.97 de	33.52 e	33.72 e	31.44 e	**	**	*

Table 2. Continued.

N[A]	Compound/origin	KI[B]	R[C]	0 d			9 d		
				LF	MF	HF	LF	MF	HF
	Amino acid degradation								
7	2-Methyl-propanal	593	a				0.77 ^{ab}	0.69 ^{bcd}	0.50 ^{de}
12	Methyl ethyl sulphide	624	a						
17	3-Methyl-butanal	689	a				2.78 ^{c}	3.17 ^{bc}	2.45 ^{cd}
18	2-Methyl-butanal (58)	699	a				0.13 ^{bcd}	0.13 ^{bcd}	0.15 ^{abc}
25	Dimethyl disulphide	773	a						
27	Toluene	788	a	1.58 ^{gh}	1.43 ^{h}	1.04 ^{h}	2.92 ^{efg}	2.90 ^{efg}	1.80 ^{fgh}
29	3-Methyl-thiophene	794	a				11.75 ^{def}	10.72 ^{def}	8.11 ^{ef}
34	3-Methyl-2-buten-1-ol	834	a	0.82 ^{fg}	0.78 ^{g}	0.92 ^{efg}	3.01 ^{c}	1.38 ^{de}	1.59 ^{d}
37	2-Methylpyrazine	860	a				0.10 ^{de}	0.10 ^{e}	0.21 ^{bc}
38	Ethylbenzene/2,3-Butanediol	883	a	0.37 ^{e}	0.42 ^{e}	0.42 ^{e}	3.41 ^{a}	2.62 ^{cd}	3.33 ^{ab}
49	2,6-Dimethylpyrazine	944	a						
52	3-Methylthio-propanal	966	a	0.37 ^{fg}	0.22 ^{g}	0.31 ^{g}	0.90 ^{de}	0.40 ^{fg}	0.62 ^{efg}
54	Dimethyl trisulphide	1002	a						
57	Benzaldehyde	1018	a	1.31 ^{f}	1.04 ^{f}	0.94 ^{f}	6.58 ^{b}	6.05 ^{b}	3.54 ^{e}
66	3-Methylthio-propanol	1062	a						
70	Benzenacetaldehyde	1108	a						
71	Phenol	1111	a		0.14 ^{i}	0.23 ^{i}	2.38 ^{fg}	2.15 ^{g}	1.21 ^{h}
79	Phenyl ethyl alcohol	1193	a				0.20 ^{bc}	0.16 ^{cd}	0.13 ^{cd}
	Esterase activity								
6	Methyl acetate	551	a				25.19 ^{b}	25.79 ^{b}	13.26 ^{c}
15	Ethyl acetate	635	a				2.12 ^{cde}	3.55 ^{a}	1.42 ^{ef}
16	Methyl propanoate	650	a	0.41 ^{ef}	0.22 ^{f}	0.36 ^{ef}	1.47 ^{bc}	1.00 ^{cd}	1.46 ^{bc}
24	Methyl butanoate	755	a	16.88 ^{h}	11.91 ^{h}	15.45 ^{h}	87.50 ^{cd}	80.20 ^{cde}	71.33 ^{cde}
28	Methyl 2-OH-propanoate	793	a				7.54 ^{ef}	6.72 ^{ef}	3.34 ^{f}
31	Methyl 3-methyl-butanoate	805	a						
33	Ethyl butanoate	831	a						
36	Methyl pentanoate	855	a	0.83 ^{f}	0.59 ^{f}	0.67 ^{f}	4.02 ^{cd}	3.77 ^{cde}	2.82 ^{de}
50	Methyl hexanoate	951	a	13.94 ^{f}	9.89 ^{f}	11.45 ^{f}	49.30 ^{cd}	43.53 ^{de}	24.95 ^{ef}
51	Methyl 3-hexenoate (E)	963	a						
64	Methyl heptanoate	1057	a	0.14 ^{e}	0.12 ^{e}	0.11 ^{e}	0.48 ^{bc}	0.43 ^{cd}	0.30 ^{d}
77	Methyl octanoate	1156	a	8.47 ^{f}	6.99 ^{f}	7.84 ^{f}	30.29 ^{de}	25.80 ^{e}	21.50 ^{e}
83	Ethyl octanoate	1229	a				0.09 ^{f}	0.21 ^{de}	
84	Methyl nonanoate	1260	a	0.15 ^{h}	0.13 ^{h}	0.14 ^{h}	0.41 ^{bcd}	0.41 ^{bcd}	0.29 ^{efg}
90	Methyl decanoate	1358	a	0.74 ^{f}	0.76 ^{ef}	0.75 ^{f}	1.09 ^{def}	0.94 ^{ef}	0.67 ^{f}
	Unknown or contaminants								
2	Methanethiol	472	a	110.34 ^{cde}	155.31 ^{acb}	116.31 ^{bcde}	164.72 ^{a}	158.32 ^{ab}	113.30 ^{cde}
39	*p*-xylene	891	a	1.28 ^{f}	1.34 ^{f}	1.21 ^{f}	10.09 ^{bcd}	8.05 ^{e}	9.02 ^{bcde}
44	*o*-xylene	917	a	0.31 ^{g}	0.25 ^{gh}	0.35 ^{h}	4.06 ^{a}	3.33 ^{abcde}	2.38 ^{f}
62	Limonene	1045	a				1.83 ^{bcde}	1.76 ^{bcde}	3.11 ^{a}
65+67	Methyl 2,4-hexadienoate (E, E)	1059/1066	b	0.11 ^{e}	0.37 ^{e}	0.19 ^{e}	185.70 ^{b}	180.20 ^{b}	89.80 ^{d}
75	Ethyl 2,4-hexadienoate (E, E)	1144	a				0.22 ^{f}	0.44 ^{ef}	0.33 ^{ef}
78	2,4-Hexadienoic acid (E, E)	1180	a				104.73 ^{cd}	58.01 ^{ef}	42.95 ^{f}
80	4-Methyl-phenol	1196	a				0.56 ^{a}	0.48 ^{abc}	0.33 ^{cd}
91	Caprolactame	1387	a	0.36 ^{cde}	0.17 ^{g}	0.20 ^{fg}	0.72 ^{a}	0.39 ^{cde}	0.46 ^{cd}
93	Tetradecane	1400	a	0.11 ^{b}	0.18 ^{ab}	0.10 ^{b}	0.13 ^{a}	0.11 ^{b}	0.10 ^{b}

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound. [A] Number of the Peak in the chromatogram. [B] Kovats index calculated for DB-624 capillary column (J&W Scientific 30m×0.25mmi.d.×1.4 μmfilm thickness) installed on a gas chromatograph equipped with a mass selective detector. [C] Reliability of identification: a,mass spectrum and retention time identical with an authentic standard; b, tentative identification by mass spectrum. [a-i]: Identical letters in each parameter indicate that there is no significant difference at p>0.05 (Fisher's test). [D] P_b: p value of fat content effect; P_s: p value of ripening effect; P_{sxb}: p value of interaction between fat content and ripening effects. **: p<0.01, *: p<0.05, ns: p>0.05. [E] Target ion used to quantify the compound when the peak was not completely resolved.

18 d			42 d			63 d			Ps^D	Pb	Psxb
LF	MF	HF	LF	MF	HF	LF	MF	HF			
0.95 abcde	0.76 ab	0.64 bcd	0.53 cde	0.62 bcd	0.40 e	0.68 bcd	0.59 bcde	0.49 de	**	**	ns
0.49 b	0.23 cd	1.74 a	0.36 bc	0.25 cd	0.49 b	0.31 bcd	0.22 cd	0.16 d	**	**	**
2.50 cd	2.37 cd	1.82 d	2.42 cd	2.32 cd	1.76 d	4.86 a	3.95 b	2.92 c	**	**	ns
0.11 cde	0.11 cde	0.08 e	0.10 de	0.13 bcd	0.08 e	0.18 a	0.16 ab	0.13 bcd	**	*	ns
1.09 a	1.02 a	0.54 bcd	0.31 d	0.72 b	0.42 cd	0.53 bcd	0.65 bc	0.54 bcd	**	**	**
3.23 e	3.02 ef	3.26 e	4.19 de	4.84 d	4.04 de	13.11 a	9.90 b	8.04 c	**	**	**
28.35 a	20.74 b	15.23 cd	17.86 bc	13.40 cde	11.98 de	6.58 f	11.95 de	11.08 def	**	**	**
6.21 a	5.77 a	4.27 b	1.26 defg	1.35 def	0.93 efg	0.90 efg	0.79 g	0.71 g	**	**	**
0.11 de	0.18 c	0.23 b	0.23 b	0.21 bc	0.14 d	0.35 a	0.24 b	0.24 b	**	ns	**
3.49 a	3.00 abc	3.43 a	2.62 cd	2.69 cd	2.68 cd	2.82 bcd	2.35 d	2.68 cd	**	*	ns
0.15 cd	0.05 e	0.18 c	0.14 cd	0.09 de	0.12 cde	0.41 a	0.39 a	0.31 b	**	ns	**
0.78 def	0.47 efg	0.90 de	1.86 a	1.15 cd	1.39 bc	1.74 ab	1.40 bc	1.39 bc	**	**	ns
0.19 a	0.06 d	0.08 cd	0.09 cd	0.13 bc	0.15 ab	0.14 ab	0.15 ab	0.13 bc	ns	ns	**
8.27 a	6.39 b	6.28 b	6.41 b	5.41 bc	4.49 cde	6.26 b	4.81 cd	3.79 de	**	**	*
			0.09 c	0.19 a	0.10 c	0.19 a	0.16 ab	0.12 bc	ns	**	**
0.66 cd	0.44 d	0.49 d	1.37 b	0.93 c	1.33 b	1.83 a	1.79 a	1.53 ab	**	ns	ns
3.61 cd	2.95 ef	3.44 de	4.70 b	4.54 b	4.22 bc	5.38 a	4.75 ab	4.39 b	**	**	ns
0.10 d	0.13 cd	0.19 bc	0.26 ab	0.33 a	0.27 ab	0.19 bcd	0.16 cd	0.19 bc	**	ns	ns
28.73 bc	40.27 a	30.08 b	24.99 b	31.15 ab	30.33 b	22.86 b	21.98 bc	28.81 b	**	ns	*
2.40 bcd	3.15 ab	2.63 bc	1.69 def	2.16 cde	2.18 cde	1.27 bc	2.72 abc	2.76 abc	*	**	**
1.52 b	2.15 a	1.41 bc	0.78 de	1.00 cd	0.98 cd	0.68 def	0.70 def	0.78 de	*	ns	ns
115.05 ab	137.48 a	92.74 bc	58.62 efg	68.43 defg	47.93 g	55.22 fg	55.98 fg	52.39 fg	**	*	ns
12.87 de	26.07 ab	17.26 cd	13.53 de	19.98 abcd	19.16 bcd	22.24 abc	21.72 abc	27.46 a	**	ns	ns
0.70 bc	0.69 bc	0.55 c	0.69 bc	0.60 c	0.60 c	0.87 ab	1.04 a	0.64 c	**	*	ns
0.26 bc	0.27 bc	0.52 a	0.13 d	0.16 cd	0.34 b	0.18 cd	0.14 d	0.25 bc	**	**	ns
5.46 b	6.65 a	4.59 b	3.67 cde	3.96 cd	2.73 e	3.53 cde	3.41 de	3.20 de	**	**	ns
82.77 ab	87.85 a	73.61 ab	85.67 ab	74.76 ab	67.15 bc	81.08 ab	76.95 ab	76.61 ab	**	**	ns
			0.11 b	0.13 b	0.14 ab	0.14 ab	0.11 b	0.17 a	ns	*	ns
0.65 a	0.66 a	0.57 abc	0.62 ab	0.46 bc	0.43 cd	0.48 bc	0.44 cd	0.44 cd	**	*	ns
59.45 ab	52.78 abc	49.58 abc	64.75 a	43.99 bcd	45.65 bcd	46.22 bc	35.93 cde	41.39 cd	**	*	ns
0.20 de	0.27 d	0.42 c	0.18 de	0.26 d	0.67 b	0.13 ef	0.26 d	0.87 a	**	**	**
0.55 a	0.48 ab	0.43 bc	0.55 a	0.37 cde	0.30 efg	0.31 def	0.21 fgh	0.19 gh	**	**	ns
1.79 ab	1.64 abc	1.64 abc	2.03 a	1.43 bcd	1.68 abc	1.54 bcd	1.23 cde	1.41 bcd	**	ns	ns
133.78 abcde	167.75 a	141.63 abcd	117.46 bcde	99.55 de	93.48 e	114.02 cde	95.72 e	88.61 e	**	*	ns
12.14 a	9.85 bcde	11.27 ab	8.34 de	10.31 abcd	8.51 cde	10.54 abc	7.73 e	8.99 cde	**	ns	ns
3.89 abc	3.56 abcd	3.93 ab	2.97 cdef	2.83 def	3.06 bcdef	2.99 bcdef	2.52 ef	2.88 def	**	*	ns
1.95 bcd	1.59 cdef	3.02 a	1.12 f	1.37 ef	2.12 b	1.47 def	1.11 f	2.00 bc	**	**	ns
198.13 b	252.51 a	255.62 a	151.16 bc	114.94 cd	123.89 cd	102.74 cd	81.75 d	102.85 cd	**	ns	**
0.39 ef	0.60 de	1.33 a	0.60 de	0.52 def	0.94 bc	0.34 ef	0.73 cd	1.17 ab	**	**	**
146.79 ab	174.83 a	129.96 bc	109.89 bc	91.66 cde	87.72 cde	120.96 bc	69.84 def	92.08 cde	**	*	ns
0.54 a	0.32 d	0.56 a	0.63 a	0.60 a	0.61 a	0.63 a	0.49 ab	0.34 bcd	**	**	*
0.63 ab	0.35 de	0.50 bc	0.61 ab	0.38 cde	0.39 cde	0.63 cde	0.30 ef	0.62 ab	**	**	ns
0.15 b	0.13 b	0.09 b	0.12 b	0.15 b	0.10 b	0.14 b	0.14 b	0.12 b	ns	ns	ns

These compounds are generated from the degradation of linolenic (C18:3) and linoleic (C18:2) acids which also had the greatest concentration in high fat sausages at 42 and 63 d. Also, a significant correlation was detected between the TBARS values and the total extracted area of the volatile compounds derived from lipid autooxidation ($p<0.0001$, $r=0.928$). As indicated above, these results are in contrast to Muguerza *et al.* (2003) who reported higher oxidation values (TBARS) and an increase of lipid oxidation products, such as aldehydes, in low fat ripened sausages compared to high fat ones. Nevertheless, in the present study a significant relationship among lipolysis, lipid oxidation and fat content was found, and the greatest lipolysis and oxidation were observed in HF sausages.

The HS abundance of volatile compounds produced by β-oxidation of lipids was affected by processing time ($p<0.01$) except for 2-octanone. The HS abundance increased drastically until 18 d and then remained constant (**Fig. 2b**). Fat content affected the HS abundance of 2,3-pentanedione, 2-heptanone, 1-octen-3-ol, 2-nonanone and 2-undecanone ($p<0.05$). At 18 d, LF sausages showed significantly higher extracted area than high fat ones for 2-heptanone and 2-nonanone. Also, at the end of the process (42 and 63 d) LF sausages showed the greatest abundance of 2-nonanone and 2-undecanone, while HF sausages showed the highest abundance of 2,3-pentanedione and 1-octen-3-ol. The high content of fat in HF sausages, and therefore of free fatty acids, was the reason for the high proportion of lipid β-oxidation degradation products such as 1-octen-3-ol. Also Bovolenta *et al.* (2008) detected mould flavour which they attributed to 1-octen-3-ol in high fat sausages. However, other compounds derived from lipid β-oxidation had their highest abundance in LF sausages. These results probably mean that lipid β-oxidation depends not only on the amount of substrate but on the environmental conditions for bacterial growth, which were more favourable in LF sausages with their higher water content (Olivares *et al.*, 2010).

The area of volatile compounds coming from carbohydrate fermentation was affected by both processing time and fat reduction ($p<0.01$) (**Table 2**), except for 3-hydroxy-2-butanone that was not affected by fat content. The extracted area increased drastically after 9 d until 18 d, especially in LF sausages, and then it decreased until the end of

the process (**Fig. 2c**) when this group comprised 30–40% of the total extracted area. The most abundant compound was acetic acid, followed by butanoic acid, acetaldehyde and ethanol. These compounds showed greater abundance in LF sausages except for ethanol that had the greatest abundance in HF sausages. Carbohydrate fermentation by microorganisms takes place during the first days of processing causing the pH decline and generation of volatile compounds. Olivares *et al.* (2010) reported that fat reduction produced a faster pH decrease in low fat sausages at the beginning of the process, although no differences were observed in later stages. In the present study, volatile compounds derived from carbohydrate fermentation had greater abundance in LF sausages at the beginning of manufacture, in agreement with the higher lean and water content in LF sausages that would produce faster carbohydrate fermentation.

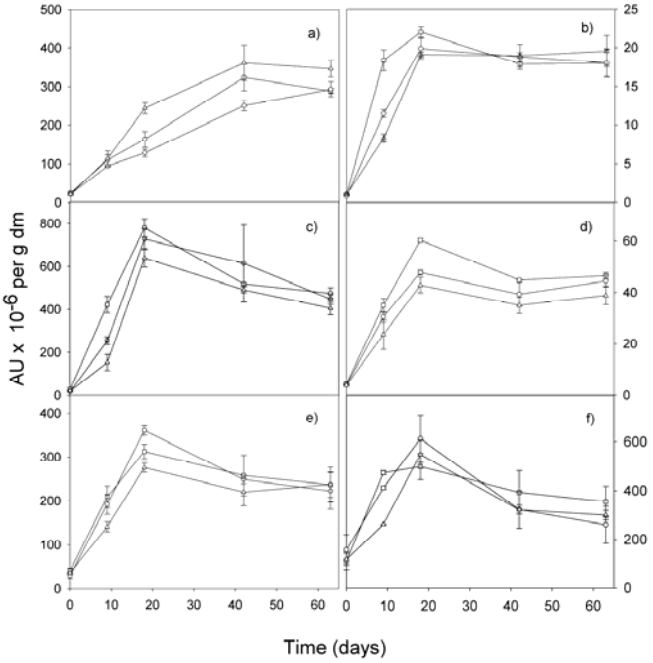

Fig. 2. Levels of total volatile compounds extracted from the HS of dry fermented sausages grouped according to their origin. Volatile compounds coming from: a) lipid autooxidation; b) lipid β-oxidation; c) carbohydrate fermentation; d) amino acid degradation; e) staphylococci esterase activity; f) unknown origin or contaminants. Low fat sausages (LF, □) medium fat sausages (MF, ○) and high fat sausages (HF, ∆). Symbols represent the mean and standard error of the mean.

The evolution of volatile compounds derived from amino acid degradation is shown in **Fig. 2d**. Ripening time ($p<0.01$) significantly affected the extracted area except for dimethyl trisulphide and 3-methylthio-propanol that were not detected in the earliest stages. The abundance increased until day 18 and thereafter it remained constant during the ripening process. Moreover, fat content affected the HS abundance of this group ($p<0.05$) except for 2-methylpyrazine, 2,6-dimethylpyrazine, dimethyl trisulphide, benzeneacetaldehyde and phenyl ethyl alcohol. During sausage ripening, proteins of the lean tissue are subjected to hydrolysis producing free amino acids which are transformed into different volatile compounds (Toldrá, Sanz, & Flores, 2001). A sharp increase was observed at 18 d mainly due to the generation of 3-methylthiophene, 3-methyl-2-buten-1-ol and benzaldehyde. However, the differences among batches for almost all compounds of this group were more marked at the longest ripening time (63 d) when LF sausages, which contained the largest proportion of lean meat, showed a higher abundance than MF and HF sausages (**Table 2**).

Esterase activity of Staphylococci produced 15 ester compounds, 12 methyl and 3 ethyl esters (**Table 2**). Ripening time affected the HS abundance of all esters except for methyl hexanoate. During processing, an increase in the HS abundance was seen which reached a maximum at 18 d, and then decreased slowly (**Fig. 2e**). The most abundant esters extracted by SPME were methyl acetate, methyl butanoate, methyl 2-hydroxy-propanoate, methyl hexanoate and methyl octanoate (**Table 2**). On the other hand, fat content significantly affected ($p<0.05$) the extracted area of all esters except for methyl acetate, methyl propanoate, methyl 2-hydroxy-propanoate and methyl decanoate. Generally, LF and MF sausages showed greater abundance of methyl esters in the first stages although few differences were detected among batches at the end of the process. In contrast, ethyl esters were extracted in higher amounts in the HF sausages at days 42 and 63. Esters have very low odour detection thresholds contributing to the aroma with fruity notes (Stahnke, 1994). Ethyl esters were in lower abundance than methyl esters, however, ethyl esters have lower air thresholds than methyl esters (Burdock, 2002) which results in a higher aroma impact, especially in HF sausages where the highest abundance was detected.

With respect to those volatiles of unknown origin (**Fig. 2f**) there was a significant effect of ripening time ($p<0.01$) in all cases expect for tetradecane since the extracted areas increased until 18 d and then decreased. Moreover, fat content also affected the extracted area of this group ($p<0.05$) that generally was the highest in LF sausages during the first stages of processing, with the exception of *p*-xylene, methyl 2,4-hexadienoate and tetradecane that were unaffected. The detection of 2,4-hexadienoic acid (sorbic acid) and also its ethyl and methyl esters came from the mixture of sorbic acid and natamycin applied to the sausage casing to prevent surface molds (Holley, 1981) as previously described. The compounds, 2,4-hexadienoic acid and methyl 2,4-hexadienoate, have already been reported in other dry fermented sausages (Mateo & Zumalacárregui, 1996; Muguerza *et al.*, 2003).

Generally, the volatile compounds coming from bacterial metabolism showed similar generation trends (**Fig. 2**). Volatile compounds derived from lipid β-oxidation (**Fig. 2b**), carbohydrate fermentation (**Fig. 2c**) and amino acid degradation (**Fig. 2d**) showed a higher abundance in LF sausages in the first stages of processing (9 and 18 d) than in HF sausages. This behaviour is related to the faster pH decrease and higher lean and moisture contents in LF sausages (Olivares *et al.*, 2010). The higher moisture content would favour fermentation producing a faster pH decline, however after 18 d no differences were observed among batches in the HS abundance of volatile compounds coming from bacterial metabolism.

With respect to the role of fat in fermented sausages, it is well known that it acts as a solvent for flavour compounds and thus delays their release, particularly for lipophilic compounds (Leland, 1997). When fat is reduced, different flavour profiles may result. Accordingly, it was reported that the release of spice- and smoke-derived volatile compounds such as terpenes and phenols was higher in low fat frankfurter and salami (Chevance & Farmer, 1998; Chevance *et al.*, 2000). The sausages used in the present study were not smoked and did not contain spices in order to avoid interferences in the volatiles analysis. However, dry fermented sausages are meat products subjected to ripening where numerous metabolic and chemical processes occur. Therefore, in dry fermented sausages with different fat contents, fat by itself affects flavour not only due to its role as a solvent but also as a flavour precursor.

In conclusion, the effect of fat reduction on volatile generation during processing was in two parts. First, lower generation of volatile compounds derived from lipid oxidation reactions during the whole ripening process and second, a higher generation of volatile compounds derived from lipid β-oxidation, carbohydrate fermentation and amino acid degradation during the fermentation stage, although at the end of the process fat reduction did not affect the abundance of volatile compounds produced by bacterial metabolism.

Volatile compound analysis by GC–O

To determine how fat reduction affects the aroma of sausages, GC–O analyses were applied to the sausages and 30 different aroma-active zones were detected (**Table 3**). The aroma compounds detected were present at concentrations higher than their threshold values and thus contributed to the aroma. The use of the detection frequency (DF) value gives information about the contribution of each compound to the aroma. Thus, those compounds showing the maximum DF value (16) were always detected by the panellist as being important for sausage aroma, such as acetic acid (vinegar), 1-octen-3-ol (mushroom) and 2-nonenal (medicinal). Also, ten of the 23 aroma compounds had DF values higher than 12. These compounds were hexanal (fresh cut grass), pentyl furan (meat broth, savory), 2-octanone (floral, geranium), butanoic acid (cheese), methional (cooked potato), methyl 3-methyl butanoate (fruity, strawberry, sweet) and 4-methyl-phenol (stable). Also, two unknown compounds showed DF equal to 15 and were described as onion, garlic-like (unknown 3) and roasted nuts (unknown 5). All the aroma compounds identified have been detected as odour active compounds in dry fermented sausages (Marco, Navarro, & Flores, 2007; Meynier, Novelli, Chizzolini, Zanardi, & Gandemer, 1999; Schmidt & Berger, 1998; Söllner & Schierberle, 2009), except for methyl ethyl sulphide, methyl 2-hydroxy-propionate, methyl 3-methyl-butanoate, 2-octanone and methyl octanoate.

Table 3. Odor-active compounds identified in the HS of dry fermented sausages.

KI[a]	Compound	GC-O descriptor	DF[b]	previously reported in dry sausages
472	Methanethiol	Rotten, unpleasant	9	1
589	2-Methyl-propanal	Green grass, fresh	5	5
631	Methyl ethyl sulfide	Rotten onion, unpleasant	4	-
633	2,3-Butanedione	Butter	7	1,4,6
691	3-Methyl butanal	Green, herbal	10	1,2,6
702	Acetic acid	Vinegar	16	1,2,4,6
790	Methyl 2-hydroxy-propionate	Green grass, fresh	4	-
803	Methyl 3-methyl butanoate	Strawberry, sweet	12	-
824	Ethyl butanoate	Fruity, flowery	5	1,2,4,5,6
837	Hexanal	Fresh cut grass, green	14	1,2,3,45,6
873	Butanoic acid	Cheese	15	1,2
904	Unknown 1	Meat broth, snacks, roasted	11	-
926	Unknown 2	Cheese, feet	5	-
939	Heptanal	Unpleasant	7	1,3,6
964	Unknown 3	Onion, garlic	15	-
969	Methional (3-methyl-thiopropanal)	Onion, cooked-potato	12	1,5
1010	2-Pentyl furan	Meat broth, savory, metallic	14	1,5
1023	1-Octen-3-ol	Mushroom	16	1,3
1035	2-Octanone	Floral, geranium	13	-
1046	Octanal	Citrus	11	1
1111	Benzeneacetaldehyde	Roses	8	1,2,5
1148	Unknown 4	Roasted, toasted	7	-
1159	Methyl octanoate	Fruity	7	-
1180	Unknown 5	Roasted nuts, snacks	15	-
1992	4-Methyl phenol	Stable, horse	12	5
1203	Unknown 6	Mustiness, fruity	9	-
1220	2-Nonenal	Medicinal	16	1,2,5,6
1226	Unknown 7	Roasted nuts	8	-
1236	Octanoic acid	Toasted, coffee	5	1
1288	2,4-Nonadienal (E, E)	Tallowy	5	2

[a] Kovats index calculated for DB-624 capillary column (J&W Scientific 60 m×0.32 mm i.d., film thickness 1.8 µm) installed on a gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port.
[b] DF Detection frequency value. Previously identified in dry sausages by: 1 Marco *et al.* (2007), 2 Söllner and Schieberle (2009), 3 Meynier *et al.* (1999), 4 Schmidt and Berger (1998), 5 Gianelli *et al.* (in press); 6 Stahnke (1994).

Sensory analysis

The consumer sensory analyses were carried out at 42 and 63 d and results are shown in **Table 4**. There were no differences in aroma and overall quality due to ripening time. Fat content did not affect aroma and overall quality at 42 d, although significant differences were detected at 63 d as MF and HF sausages had the highest

acceptability for aroma and overall quality. The highest acceptability of high fat sausages has been reported by Mendoza *et al.* (2001) although Liaros *et al.* (2009) found no marked differences.

Table 4. Sensory analysis (hedonic test) of dry fermented sausages with different fat contents at 42 and 63 days of ripening.

	42 d			63 d			S	B	S x B
	LF	MF	HF	LF	MF	HF			
aroma	5.88 ^{b}	6.21 ^{ab}	6.20 ^{ab}	5.83 ^{b}	6.15 ^{ab}	6.35 ^{a}	0.942	0.014	0.730
overall quality	6.23 ^{bc}	6.49 ^{ab}	6.68 ^{ab}	5.91 ^{c}	6.60 ^{ab}	6.69 ^{a}	0.605	0.000	0.366

Identical letters for each parameter indicate no significant differences at p>0.05 (Fisher's test). * S: *p* value of ripening time effect; B: *p* value of fat content effect; SxB: *p* value of interaction between ripening time and fat content effects.

Principal component analysis (PCA)

To establish which aroma compounds were responsible for the high acceptability of MF and HF sausages, a principal component analysis (PCA) was performed using the following parameters; lipolysis (FFA), lipid oxidation (TBARS), aroma compounds abundance and sensory attributes (aroma and overall quality) at the two ripening times (42 and 63 d). Only the aroma active volatile compounds reported in **Table 3** were selected for analysis. Results for PCA applied to mean scores of the parameters are summarized in **Fig. 3**. The PCA showed that about 68.9% of the variability was explained by two first principal components. Principal component 1 (PC 1) was the most important variable in terms of differences among samples as it accounted for 44.2% of the total variability. PC1 was positively related with TBARS value, free fatty acids (SFA, MUFA and PUFA), lipid content, aroma compounds; hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol and the sensory parameters; aroma and overall quality. In addition, PC 1 was inversely related to protein content and the aroma compounds methanethiol, methional, 2-methyl-propanal, 2-octanone and octanoic acid. High fat sausages at both ripening times (HF-42 and HF-63) had the greatest component 1 value therefore, they were related to the consumer acceptance. In contrast, LF sausages at both ripening times (LF-42 and LF-63) were inversely correlated to aroma and overall quality while MF (MF-42 and MF-63) sausages were

intermediate. These differences were also observed in the sensory analysis (**Table 4**). On the other hand, principal component 2 (24.73%) was positively related to the aroma compounds; 3-methyl-butanal, methyl 3-methyl-butanoate, octanal, benzeneacetaldehyde and methyl 2-hydroxy-propanoate and inversely to 4-methyl-phenol, methyl octanoate, acetic acid, methyl ethyl sulphide and 2-pentyl-furan. Sausages at 63 d of ripening were on the positive PC 2 axis while those at 42 d were on the negative PC 2 axis.

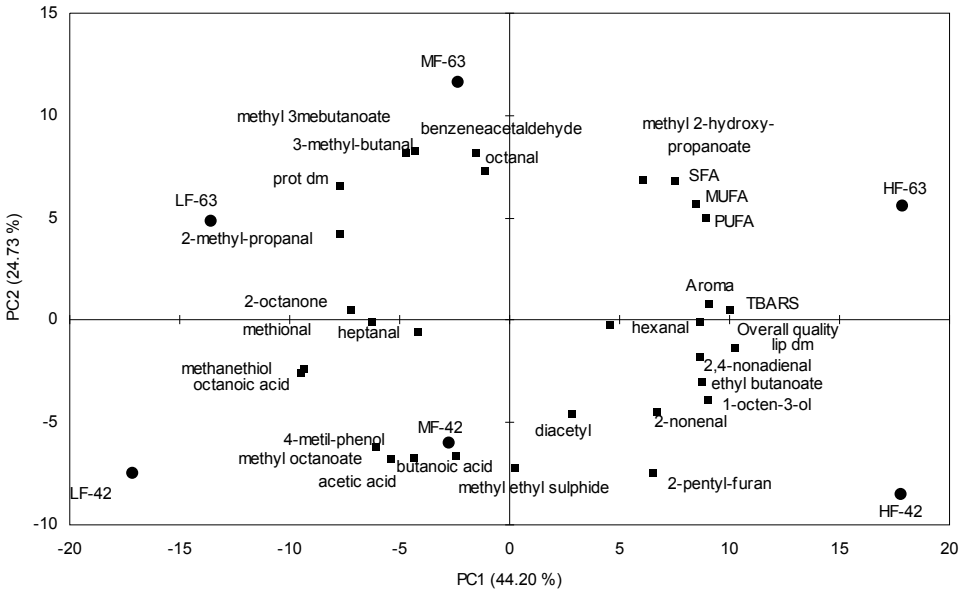

Fig. 3. Loadings of the first two principal components (PC1–PC2) of the selected variables for dry fermented sausages with different fat contents (low fat LF; medium fat MF; high fat HF) at two ripening times (42 and 63 d). The selected variables were the aroma active compounds, TBARS values (TBARS), protein content (prot/dm), lipid content (lip/dm), saturated free fatty acids (SFA), monounsaturated free fatty acids (MUFA), polyunsaturated free fatty acids (PUFA), aroma and overall quality scored by consumers.

In conclusion, PC1 differentiated the sausages on fat content while PC2 on ripening time. Fat content was related to the aroma compounds hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol which were more abundant in the HS sausages followed by MF sausages and they contributed to the aroma with green, medicinal, tallowy, fruity and mushroom notes. On the other hand, ripening time was related to the aroma compounds 3-methylbutanal, methyl 3-methyl-butanoate, octanal,

benzeneacetaldehyde and methyl 2-hydroxy-propanoate which were more abundant in the sausages with the longest ripening times (63 d) and contributed to the aroma with green, strawberry, citrus, roses and green-fresh notes.

CONCLUSIONS

Fat reduction in dry fermented sausages decreased lipolysis, lipid oxidation and lipid derived volatile compounds during processing while the volatile compounds generated from bacterial metabolism increased, although only in the first stages of processing. Sensory analysis revealed that consumers prefer the aroma and overall quality of high and medium fat sausages at longer ripening times (63 d). The aroma compounds associated with highest consumer acceptability of high fat sausages were hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol which contributed to the aroma with green, medicinal, tallowy, fruity and mushroom notes. The results indicate that consumer acceptance is strongly linked to high fat content and ripening time, although fat content reduced to 20%, medium fat sausages (MF) were not differentiated by consumers from HF sausages.

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Capítulo 6.

Selected Ion Flow Tube-Mass Spectrometry for absolute quantification of Aroma Compounds in the Headspace of Dry Fermented Sausages

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Selected Ion Flow Tube-Mass Spectrometry for Absolute Quantification of Aroma Compounds in the Headspace of Dry Fermented Sausages

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Abstract

The meat industry is interested in rapid control of the sensory quality of meat products. Selected ion flow tube mass spectrometry (SIFT-MS) has been applied to the rapid, real-time quantification of 31 volatile aroma compounds present in the headspace (HS) of dry fermented sausages. Three batches of fermented sausages with different fat contents (10, 20, and 30%) were monitored throughout the processing time. SIFT-MS revealed significant changes in the concentration of all the aroma compounds during the processing time. Moreover, among the various batches of the sausages, significant differences were revealed in their HS concentration of butanal, 2-pentenal, hexanal, 2-butanone, 2,3-butanedione, ethanol, ethyl formate, hexanoic acid, and dimethyl disulfide. In addition, highly volatile compounds were detected and quantified using our real-time SIFT-MS technique that are apparently not generally seen by trace gas extraction and GC techniques.

INTRODUCTION

The consumption of dry fermented sausage is widespread throughout Europe, and a great variety of dry sausage exists due to different manufacturing processes and consumer preferences.[1] Despite these differences, all dry fermented sausages have a high fat content (typically 40-50%)[2] that contributes to aroma development through lipolysis and lipid oxidation reactions.[3] The fat content also affects aroma perception, because fat acts as a solvent for aroma compounds, most of which are lipophilic.[4]

A demand of the meat industry, driven by consumer nutritional requirements, is for the production of highly aromatic, quality meat products that have a low fat content. However, reduction of the fat content affects the aroma acceptance and other sensory characteristics.[5] Many studies have characterized the aroma of dry fermented sausage,[6-11] although only one study has indicated how fat reduction affects the generation of volatile compounds.[12] More than 400 volatile compounds released by fermented sausages have been identified[13] using gas chromatography mass spectrometry (GC/MS) and olfactometry analyses (GC-O).[14] However, not all of these compounds contribute to the aroma; clearly, only those whose concentrations are higher than a threshold value can contribute to the aroma.[14,15]

The demands of the food industry for rapid analysis mean that GC/MS is not so convenient because, although it is a highly sensitive and reliable technique, analysis can take a considerable amount of time and operator training is essential. Most desirable are real time techniques to monitor manufacturing processes when the mixture of the emitted volatile compounds are sampled directly into the MS and are immediately detected and quantified.[16,17] Direct analyses of volatile compounds in air have been demonstrated using different ionization techniques, including atmospheric pressure chemical ionization (APCI),[18] proton transfer reaction mass spectrometry (PTR-MS),[19] and selected ion flow tube mass spectrometry (SIFT-MS).[20]

SIFT-MS is a direct mass spectrometric technique based on the chemical ionization of a gas sample (to the exclusion of the major air gases) using specific, selected precursor (reagent) positive ions. Using SIFT-MS, real time quantification of volatile compounds in humid air can be achieved without external calibration.[16] This technique

has been widely used for online trace gas analysis in biology and medicine.[20] With respect to flavor analysis, it has been successfully demonstrated by a case study of the real-time release of volatile compounds from newly cut onion, crushed garlic, and ripe banana.[16] In this same study, the kinetics of the reactions of the precursor ions used in SIFT-MS with some food flavor compounds were described, thus exemplifying the use of SIFT-MS in flavor research.

As is now appreciated, aldehydes are almost always components of the aroma mixtures emitted by foods. A detailed study of the reactions of the precursor ions used for analysis in SIFTMS, viz., H_3O^+, NO^+, and O_2^+, with several aldehydes was carried out some years ago using the SIFT technique,[21] and the kinetics data obtained have allowed studies of aldehydes present in and emitted from some fruits and vegetables[16] and other media, including exhaled human breath, to be carried out.[22,23] Subsequently, a similar study was made of the reactions of several saturated and unsaturated aldehydes in support of food flavor and other humid media.[24] Later, Davis, McEwan, and co-workers[25,26] used SIFT-MS to monitor the major volatile compounds emitted by olive oil in order to establish differences in their quality and oxidative status. Recently, Xu and Barringer[27] measured the release of lipid-related volatiles in tomato puree using SIFT-MS. These few examples indicate that SIFT-MS is a fast reliable technique that is ideal for use in the food manufacturing/ monitoring industry.

SIFT-MS has not yet been used to study meat products, but it clearly has great potential for the rapid and reliable monitoring of volatile aromatic compounds and the control of sensory qualities. Therefore, the aim of the present study was to apply SIFT-MS to the measurement of volatile compounds produced during the processing of fermented sausages and to use this technique to study the effect of sausage fat content on the generation of volatile compounds.

MATERIALS AND METHODS

Dry fermented sausages

Three batches of dry fermented sausages with different pork back fat content were processed; 10%, low fat (LF); 20%, medium fat (MF); and 30%, high fat (HF). The

processing and fermentation followed the same procedure as described in recent papers.[28,29] Briefly, the lean pork and the pork back fat were ground and then minced together with the following additives: sodium chloride, lactose, dextrin, sodium caseinate, glucose, sodium ascorbate, sodium nitrite, and potassium nitrate. Also, a commercial starter culture containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus*, and *S. carnosus* was added. The meat mixture was maintained at 3-5 °C for 24 h and then extruded into collagen casings (Fibran, S.A., Girona, España, 75-80 mm diameter). The sausages so formed were dried for a period of 42 days at 10 °C and 80-90% relative humidity (RH) and then for 21 days at 10-12 °C and 75-85% RH. The total drying time was 63 days.

Three sausages from each batch type (LF, MF, and HF) were randomly chosen at day 0 (immediately following production) and then at days 9, 18, 42, and 63 of processing after which they were sliced, vacuum packaged, and frozen at -80 °C to await analysis.

Selection of Volatile Compounds by GC/MS and GC-Olfactometry

The identification of the headspace (HS) volatile compounds was carried out using GC/MS and solid phase microextraction (SPME), as described by Marco *et al.*[11] A SPME device (Supelco, Bellefonte, PA) with a 85 μm carboxen/polydimethylsiloxane StableFlex fiber (CAR/PDMS SF) was used to extract the volatile compounds from a known volume of headspace. The compounds were separated in a DB-624 capillary column (J&W Scientific, Agilent Technologies, USA) and then identified in the usual way by comparison with the mass spectra from the library database (NIST 2005) and by the Kovats linear retention index, LRI,[30] using authentic reference standards. (See the list in **Table 1**.)

Among all the volatile compounds identified in the HS of the sausages, a selection was made focusing on the main odorants responsible for the typical dry-cured aroma in fermented sausages. GC-olfactometry (GC-O) employs human assessors to detect and evaluate volatile compounds eluting from the GC column with the aim to determine the potent aroma-active compounds responsible for the aroma.[31] Our detailed data for sausage emissions is not yet fully assessed and published, and so at this time, we adopt the list of compounds obtained in our previous studies[32] and also presented in

Table 1. Volatile Compounds Identified by GC/MS in the Headspace of Dry Fermented Sausages in the Present Study.

Compound	Mw[A]	Sample LRI[B]	Standard LRI[C]	Compound	Mw	Sample LRI	Standard LRI
Aldehydes				***Esters***			
Acetaldehyde	44	466	466	Methyl acetate	74	551	551
Propanal	58	523	521	Ethyl acetate	88	635	635
2-Methyl-propanal	72	593	590	Methyl propanoate	88	650	650
Butanal	72	621	620	Methyl butanoate	102	755	754
3-Methyl-butanal	86	689	693	Methyl 2-OH-propanoate	104	793	791
2-Methyl-butanal	86	699	702	Methyl 3-methyl-butanoate	116	805	804
Pentanal	86	738	737	Ethyl butanoate	116	831	829
Hexanal	100	840	840	Methyl pentanoate	116	855	854
2-Hexenal (Z)	98	905	903	Methyl hexanoate	130	951	952
Heptanal	114	940	942	Methyl 3-hexenoate (E)	128	963	964
2-Heptenal (Z)	112	1011	1011	Methyl heptanoate	144	1057	1057
Benzaldehyde	106	1018	1014	*Methyl 2,4-hexadienoate (E, E)*	*126*	*1059/1066*	
Octanal	128	1048	1044	Ethyl 2,4-hexadienoate (E, E)	140	1144	1144
Benzenacetaldehyde	120	1108	1103	Methyl octanoate	158	1156	1155
2-Octenal (E)	126	1116	1113	Ethyl octanoate	172	1229	1226
Nonanal	142	1149	1149	Methyl nonanoate	172	1260	1258
2-Nonenal (Z)	140	1222	1218	Methyl decanoate	186	1358	1358
2,4-Nonadienal (Z, Z)	*138*	*1287*		***Ketones***			
2-Decenal (Z)	154	1327	1324	2,3-Butanedione	86	625	625
2,4-Decadienal (Z, Z)	152	1392	1392	2-Butanone	72	630	630
Hydrocarbons				2-Pentanone	86	733	728
Pentane	72	500	500	2,3-Pentanedione	100	743	742
Hexane	86	600	600	3-OH-2-butanone	88	781	778
Heptane	100	700	700	2-Heptanone	114	934	933
Toluene	92	788	790	*2,3-Octanedione*	*142*	*1029*	
Octane	114	800	800	2-Octanone	128	1039	1034
Ethylbenzene	106	883	881	2-Nonanone	142	1141	1139
p -xylene	106	891	890	2-Undecanone	170	1347	1349
Nonane	128	900	900	Caprolactam	113	1387	1383
o -xylene	106	917	915	***Sulphur compounds***			
Decane	142	1000	1000	Methanethiol	48	472	472
Limonene	136	1045	1041	Methyl ethyl sulphide	76	624	624
Undecane	156	1100	1100	Dimethyl disulphide	94	773	768
Dodecano	170	1200	1200	3-Methyl-thiophene	98	794	799
Tridecane	184	1300	1300	3-Methylthio-propanal	104	966	964
Tetradecane	198	1400	1400	Dimethyl trisulphide	126	1002	1002
Alcohols				3-Methylthio-propanol	106	1062	1062
Ethanol	46	507	509	***Acids***			
1-Propanol	60	611	611	Acetic acid	60	718	720
1-Pentanol	88	826	823	Butanoic acid	88	895	899
3-Methyl-2-buten-1-ol	86	834	834	Hexanoic acid	116	1078	1080
2,3-Butanediol	90	883	883	2,4-Hexadienoic acid (E, E)	112	1180	1182
1-Hexanol	102	923	921	Octanoic acid	144	1267	1258
4-Hexen-1-ol	*100*	*927*		Decanoic acid	172	1451	1447
1-Heptanol	116	1024	1021	***Furans***			
1-Octen-3-ol	128	1031	1028	2-Methyl-furan	82	615	615
2-Ethyl-1-hexanol	130	1083	1081	*2-N-butyl-furan*	*124*	*909*	
Phenol	94	1111	1109	2-Pentyl-furan	138	1009	1010
1-Octanol	130	1124	1123	***Pyrazines***			
Phenyl ethyl alcohol	122	1193	1191	2-Methylpyrazine	94	860	859
4-Methyl-phenol	108	1196	1196	2,6-Dimethylpyrazine	108	944	942

[A] Mw is the molecular weight of the compound. [B] The linear retention indices (LRI) calculated for a DB-624 capillary column (J&W Scientific 30 m × 0.25 mm i.d. × 1.4 μm film thickness). [C] The reliability of identification was confirmed by the mass spectrum of the sample peak being identical to a mass spectrum of an authentic standard. The several compounds given in italics were only tentatively identified by a library match of the sample mass spectrum, and no standard LRI was obtained.

the available literature[10,14,33,34] to select the key aroma compounds of dry fermented sausages.

SIFT-MS analysis.

The SIFT-MS method for absolute quantification of volatile compounds in humid air (including human breath) has been described in detail previously.[16,20] It exploits chemical ionization using selected precursor ions, which are injected into helium carrier gas in a flow tube. The flow speed of the ion swarm/helium carrier gas defines the reaction time of the precursor ions with the trace compounds present in an analyte mixture (sample) that is introduced at a known flow rate into the helium. Precursor ions H_3O^+, NO^+, and O_2^+ are used, because they do not react with the major components of air but selectively ionize only the trace gases and volatile compounds present in the air sample being analyzed producing characteristic product ions that identify the neutral trace gas reactant molecules. Quantitative analysis is achieved by measuring the count rates of both the precursor ions and the characteristic product ions using a downstream mass spectrometer.[35] For the present study, a SIFT-MS Profile 3 instrument[36] was used to directly measure the volatile compounds released into the headspace (HS) above the dry fermented sausages. First, in order to confirm that SIFT-MS was able to detect the presence of the volatile compounds of interest, as indicated by the GC-O studies, several consecutive full spectral scans (FS) of the SIFT-MS instrument, ranging from mass-to-charge ratios, *m/z*, of 10-200, were each performed for 1 min using all three precursor ions (H_3O^+, NO^+, and O_2^+) for up to a total time of 1 h to produce clearly defined ion peaks in the FS spectra. Different characteristic product ions resulted for the different precursor ions, so three mass spectra were obtained for each sausage sample analyzed. The characteristic product ions were assigned to specific volatile compounds on the basis of the kinetic library compiled from the results of the previous ion chemistry studies, as mentioned above. (The results will be given in the Confirmation by SIFT-MS of the Presence of the Selected Volatile Compounds in the HS of Fermented Sausages section.)

For accurate quantification, the multiple ion monitoring (MIM) mode was used to target specific volatile compounds; in this mode, the analytical mass spectrometer is rapidly

switched between selected *m/z* values of both the precursor ions and the characteristic product ions. MIM achieves higher sensitivity and reproducibility than do analyses using full scan (FS) mass spectra. The selected *m/z* values were carefully chosen (as will be discussed in the Confirmation by SIFT-MS of the Presence of the Selected Volatile Compounds in the HS of Fermented Sausages section) to avoid overlap between the characteristic product ions of the different volatile compounds, and this allowed unambiguous identification and quantification of the compounds. The known rate coefficients for the analytical reactions were then used to quantify the absolute HS concentrations of the aroma compounds using the standard SIFT-MS data analysis software and the general method of quantification[35].

For each measurement, a frozen sample slice of the sausage was diced and introduced in a mortar together with 0.75 mg of antioxidant (butylated hydroxytoluene) and liquid nitrogen. Then, using the pestle, the sausage was finely pulverized. Five grams of the finely pulverized sausage were then transferred to a 15 mL volume vial which was sealed with a silicone septum cap. The emitted volatiles were allowed to develop in the HS air at 37 °C for 1 h. Then, the HS air/volatile compounds were sampled directly by piercing the septum with a stainless steel needle connected directly to the SIFT-MS sampling line. Thus, the sample entered the helium carrier gas via a heated (70 °C) capillary line at a measured rate of 0.45 Torr L s^{-1}. A second syringe needle pierced the septum to maintain the pressure in the vial at atmospheric pressure by introducing laboratory air at a rate that balances the small loss rate due to the sampling into the SIFT-MS instrument. (See further comments on this procedure in the Reproducibility of SIFT-MS Quantification and Validity of the Sampling Procedure section.) Background (laboratory air) concentrations of all the volatile compounds included in the analysis were routinely recorded before and after the analysis of each sample. As indicated above, selected volatile compounds were analyzed using the MIM mode and the H_3O^+, NO^+, and O_2^+ precursor ions. Data for each precursor ion were collected and integrated for a period of 200 s, and the mean values over this sampling time were recorded.

The HS of three sausages were analyzed in duplicate measurements for each fat batch and for each processing time. Thus, in total, six measurements were obtained for

each combination of fat content and processing time. The results are then expressed in parts-per-billion by volume of the headspace, ppbv (nanoliters of volatile compound per liter of air). The measuring order of the samples was randomized. The reproducibility of the sampling procedure and the validity of the choice of the amount of sample (5 g) were explored by dividing the same pulverized sample into six separate 15 mL vials in amounts of 0.5, 1, 2, 3, 4, and 5 g.

Statistical Analysis

The effect of processing time and fat content on the concentrations of the HS volatile compounds was tested by two-factor analysis of variance (ANOVA) using the statistic software Statgraphics plus (v 5.1). The statistical significance was assessed using the Fisher's least significant difference (LSD) test.

RESULTS AND DICUSSION

Volatile Compounds in HS of Fermented Sausages As Indicated by GC/MS

In the present study, a total of 95 volatile compounds were identified by GC/MS in the HS of the pulverized sausages, and 90 of them were confirmed using authentic standards. (See **Table 1a**.) The mixture comprised 20 aldehydes, 17 esters, 15 hydrocarbons, 14 alcohols, 11 ketones, 7 sulfur compounds, 6 acids, 3 furans, and 2 pyrazines. Out of these 95 volatile compounds, 32 represent known potent contributors to the aroma of dry-cured fermented sausages. The aroma descriptors for each of these volatile compounds are summarized in **Table 2**. As explained in the Materials and Methods section, we were guided in our selection of these 32 aroma (olfactory-active) compounds by the previously reported GC-O analytical data on dry fermented sausages[10,14,32-34].

Confirmation by SIFT-MS of the Presence of the Selected Volatile Compounds in the HS of Fermented Sausages.

Full scan (FS) SIFT-MS spectra were obtained using the three precursor ions H_3O^+, NO^+, and O_2^+, in turn, as the HS of the fermented sausage was introduced into the

Table 2. Aroma compounds released by dry fermented sausages as compiled from previous work. Those compounds given in italics were quantified by SIFT-MS in the present study.

Compound	GC-O descriptor[a]
Aldehydes	
Butanal	sweet, snacks[32]
3-Methyl butanal	malty[14]; rancid, dry cured ham[32]; sour, cheese, fruity[7]
2-Methyl butanal	malty[14]; butter, caramel[33]; sour, cheese, fruity[7]
Pentanal	fresh cut grass, rancid[32]
Hexanal	green[14,10,33]; fresh cut grass, rancid[32]; green leaves[7]
Heptanal	citrus, soap, rancid dry cured ham[32]; potatoes[33]; green leaves[7]
2-heptenal	rancid, dirty[32]
Octanal	geranium, herbal, floral[32]; nettle[7]
Benzeneacetaldehyde	roses[10]
2-octenal	dry cured ham, dry cured sausage[32]; deep fried[7]
Nonanal	plastic, soap[32,10]; nettle[7]
2-nonenal	cucumbers, herbal, woody[32,7]; fatty, tallowy[10]
Decanal	potatoes, butter[33]
2,4-decadienal	rancid[10]
Ketones	
Acetone	acetone, alcohol[32]
2,3-butanedione	cheese, snacks[32]; butter[10,7]
2-pentanone	roasted, sweet[32]
2-heptanone	medicinal, fruity[32]
2-pentyl furan	onions, savoury, rancid[32]
2-nonanone	roasted, burnt[32]
Alcohols	
Ethanol	bread dough, yeast[32]
Esters	
Ethyl acetate	fruity, toffees[32]
Acids	
Acetic acid	pungent, sour[14]; vinegar[32,10,7]
Propanoic acid	sweaty[14,10]; cheese[32]; sour[7]
Butanoic acid	sweaty[14,10]; cheese[32,7];
Pentanoid acid	sweaty[10]
Hexanoic acid	sweaty, putrid[10]
Octanoic acid	rancid, woody[32]
Sulfur compounds	
Methanethiol	rotten, eggs, cauliflower[32]
Dimethyl disulfide	garlic[10]
Methional (3-methyl-thiopropanal)	cooked potato-like[14]; meat broth, rancid, savoury snack[32]
Others	
2,6-Dimethyl pyrazine	mashed potatoes[33]; toasted[7]

[a] Those compounds given in italics were quantified by SIFT-MS in the present study. [b] Odor description defined using gas chromatography-olfactometry (GC-O) analyses reported in the references quoted in the table.

helium carrier gas. Sample spectra obtained using H_3O^+ and NO^+ precursor ions are presented in **Figure 1**. The spectra obtained using the more energetic O_2^+ precursor ions are much more complicated, and so they are not given.

Most of the 32 aroma compounds of interest were identified in the FS spectra from their characteristic product ions, with the exception of 2- and 3-methyl butanal, phenyl acetaldehyde, 2-pentyl furan, octanoic acid, methional, and 2,6-dimethyl pyrazine, simply because their kinetic parameters were not available.

Twenty-two (shown in italics in **Table 2**) of the 25 aroma compounds of the 25 aroma compounds were unambiguously identified in the full scan spectra, because the *m/z* values of their characteristic product ions did not overlap with those of other compounds in the sample. However, overlap of the product ions for propanoic, butanoic, and pentanoic acids did occur with those of other volatile compounds in the HS sample for all three precursors, and therefore, it was not possible to accurately quantify these three carboxylic acids.

In addition to those shown in **Table 2**, other compounds were identified in the HS of the sausages by SIFT-MS, viz., propanal, 2-butanone, 1-propanol, 2-pentenal, ethyl formate, methanol, hydrogen sulfide, dimethyl sulfide, and carbon disulfide. (Note that propanal, 2-butanone, and 1-propanol were also identified by GC/MS without olfactory detection and are listed in **Table 1**.) The common feature of these volatile compounds is their relatively small molecular weight and, therefore, their likely high evaporation rates. Although these compounds have not been reported as potent odorants by GC-O, some of them have previously been seen to be released by dry fermented sausages using other chromatographic techniques,[11,13] with the exception of ethyl formate, methanol, and hydrogen sulfide. As Davis *et al.*[25] indicated, the type of column used in GC analysis is normally chosen to separate larger, more hydrophobic volatiles, such as long chain aldehydes and alcohols, at the expense of smaller, more hydrophilic molecules that are reported as being at low concentrations or not reported at all. Therefore, even though these low molecular weight compounds are not generally potent odorants, it was considered to be important to include these highly volatile molecules in this SIFT-MS study.

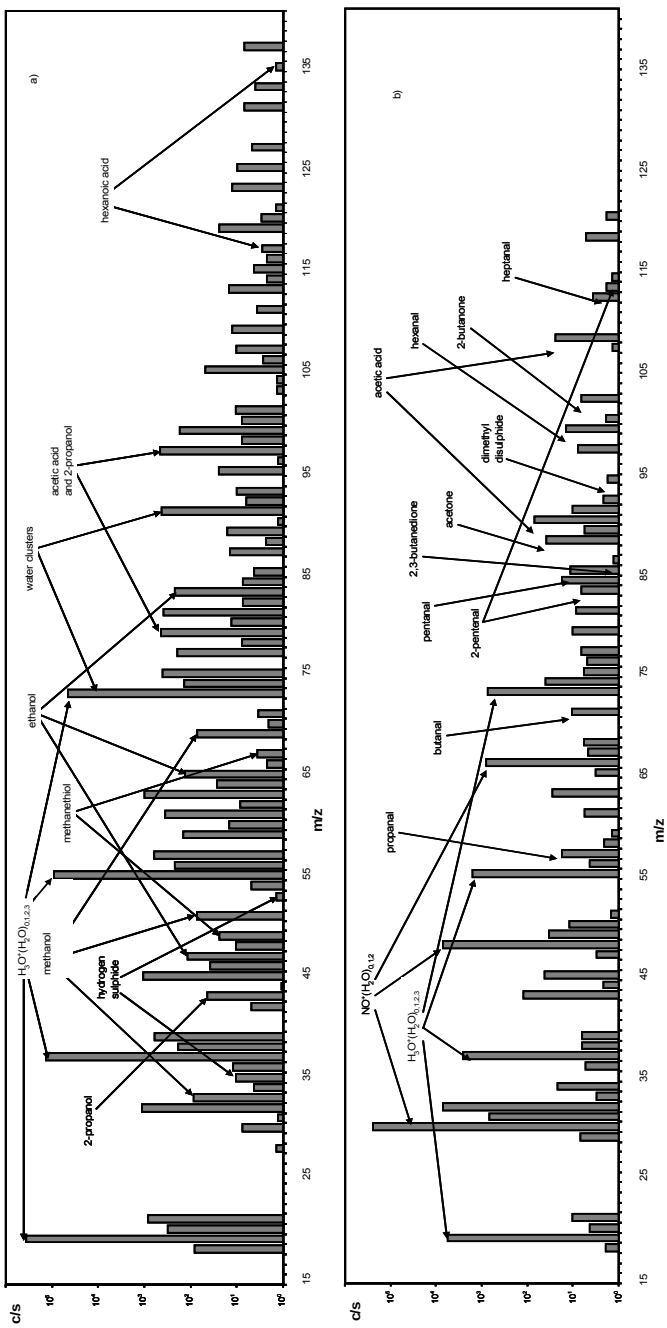

Figure 1. SIFT-MS full scan (FS) mass spectra obtained as the HS above a dry fermented sausage is introduced into the helium carrier gas using (a) H_3O^+ and (b) NO^+ precursor ions. The individual characteristic product ions that appear, including their hydrates, are assigned to the analyte molecules indicated.

Hence a total of 31 volatile compounds (22 shown in italics in **Table 2** and the nine additional compounds given above) were quantified by SIFT-MS throughout the processing of the fermented sausages. These compounds are included in **Table 3**, together with the precursor ion used for their quantification and the characteristic product ions generated. As can be seen, NO^+ was the most suitable precursor ion for most of these volatile compounds. However, the use of the three available precursor ions can greatly assist identification and quantification in SIFT-MS analyses, especially when potential overlaps of characteristic ions are possible using a particular precursor ion, as can often occur when H_3O^+ is used. Background (laboratory air) concentrations of all compounds measured were much less than 10% of the levels observed in the HS of the sausage samples. Actually, the only two notable impurities in the laboratory air during the course of this study were acetone and methanol (typically 20 ppb), while the background concentrations of the other compounds were much lower. This applied also to the specific case of acetic acid, which we focus on below. Thus, the introduction of laboratory air into the sampling vials to stabilize the sampled gas pressure has no significant affect on the measured compound levels. It is worthy of note that the number of compounds monitored by SIFT-MS in this work is much greater than those reported in previous work using this technique.[25-27].

Reproducibility of SIFT-MS Quantification and Validity of the Sampling Procedure

Measured amounts (0.5, 1, 2, 3, 4, and 5 g) of a frozen pulverized sausage sample were introduced into the 15 mL vials, and the HS of each was analyzed twice according to the protocol described in the Selection of Volatile Compounds by GC/MS and GC-Olfactometry section. Sample data obtained for the quantification of acetic acid are shown in **Figure 2**. Note that the HS acetic acid concentration for the smallest amount of sample (0.5 g) is significantly lower than for the larger amounts of 1-5 g.

Table 3. The molecular formula and the mass-to-charge ratios, *m/z* values of the characteristic product ions of the aroma compounds shown analyzed by SIFT-MS using H_3O^+ and NO^+ precursor ions.

Compound	molecular formula	precursor ion	*m/z*	Characteristic product ions	reference
Aldehydes					
Propanal	C_3H_6O	NO^+	57	$C_3H_5O^+$	21
Butanal	C_4H_8O	NO^+	71	$C_4H_7O^+$	21
Pentanal and methylbutanal	$C_5H_{10}O$	NO^+	85	$C_5H_9O^+$	21
2-Pentenal	C_5H_8O	NO^+	83, 114	$C_5H_7O^+$, $C_5H_8O{\cdot}NO^+$	24
Hexanal	$C_6H_{12}O$	NO^+	99	$C_6H_{11}O^+$	21
Heptanal	$C_7H_{14}O$	NO^+	113	$C_7H_{13}O^+$	24
2-Heptenal	$C_7H_{12}O$	H_3O^+	111, 142	$C_7H_{11}O^+$, $C_7H_{12}O{\cdot}NO^+$	24
Octanal	$C_8H_{16}O$	NO^+	127	$C_8H_{15}O^+$	24
2-Octenal	$C_8H_{14}O$	NO^+	125, 156	$C_8H_{13}O^+$, $C_8H_{14}O{\cdot}NO^+$	24
Nonanal	$C_9H_{18}O$	NO^+	141	$C_9H_{17}O^+$	-
2-Nonenal	$C_9H_{16}O$	NO^+	139, 170	$C_9H_{15}O^+$, $C_9H_{15}O{\cdot}NO^+$	24
Decanal	$C_{10}H_{20}O$	NO^+	155	$C_{10}H_{19}O^+$	24
2,4-Decadienal	$C_{10}H_{16}O$	NO^+	151	$C_{10}H_{15}O^+$	24
Ketones					
Acetone	C_3H_6O	NO^+	88	$NO^+{\cdot}C_3H_6O$	21
2-Butanone	C_4H_8O	NO^+	102	$NO^+{\cdot}C_4H_8O$	36
2,3-Butanedione	$C_4H_6O_2$	NO^+	86	$C_4H_6O_2^+$	21
2 and 3-Pentanone	$C_5H_{10}O$	NO^+	116	$NO^+{\cdot}C_5H_{10}O$	21
2-Heptanone	$C_7H_{14}O$	NO^+	144	$NO^+{\cdot}C_7H_{14}O$	39
2-Nonanone	$C_9H_{18}O$	NO^+	172	$NO^+{\cdot}C_9H_{18}O$	39
Alcohols					
Methanol	CH_4O	H_3O^+	33, 51, 69	CH_5O^+ , $CH_5O^+(H_2O)$, $CH_5O^+(H_2O)_2$	40
Ethanol	C_2H_6O	H_3O^+	47, 65, 83	$C_2H_7O^+$, $C_2H_7O^+(H_2O)$, $C_2H_7O^+(H_2O)_2$	40
2-Propanol	C_3H_8O	H_3O^+	43	$C_3H_7^+$	40
Esters					
Ethyl formate	$C_3H_6O_2$	NO^+	104	$NO^+{\cdot}HCOOC_2H_5$	41
Ethyl acetate	$C_4H_8O_2$	NO^+	118	$NO^+{\cdot}CH_3COOC_2H_5$	41
Acids					
Acetic acid	$C_2H_4O_2$	NO^+	90, 108	$NO^+{\cdot}CH_3COOH$, $NO^+{\cdot}H_2OCH_3COOH$	39
Hexanoic acid	$C_6H_{12}O_2$	H_3O^+	117, 135	$C_6H_{12}O_2H^+$, $C_6H_{12}O_2H^+(H_2O)$	40
Sulphur compounds					
Hydrogen sulphide	H_2S	H_3O^+	35, 53	H_3S^+ , $H_3S^+(H_20)$	41
Dimethyl sulphide	C_2H_6S	O_2^+	62, 80	$C_2H_6S^+$, $C_2H_6S^+(H_2O)$	42
Dimethyl disulphide	$C_2H_6S_2$	NO^+	94	$C_2H_6S_2^+$	41
Carbon disulphide	CS_2	O_2^+	76	CS_2^+	42
Methanethiol	CH_4S	H_3O^+	49, 67	$CH_3SH_2^+$, $CH_3SH_2^+(H_2O)$	43

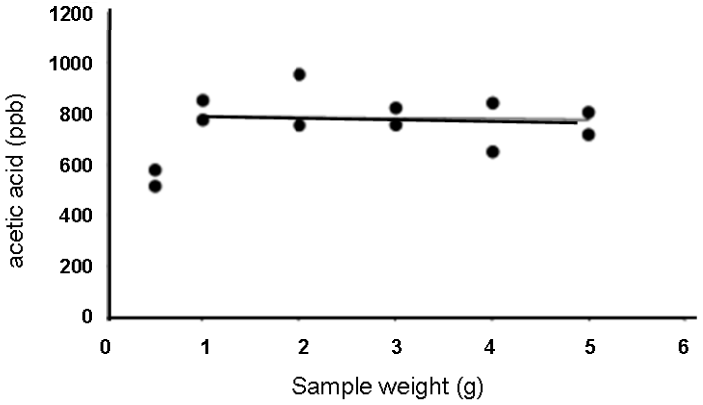

Figure 2. Concentration (in parts-per-billion, ppb) of acetic acid measured in the ambient air headspace above various sample weights (i.e., 0.5, 1, 2, 3, 4, and 5 g) of pulverized sausage contained in glass vials; see the text for further information.

However, in common with the acetic acid, the concentrations of all compounds recorded during these tests, including water vapor, acetone, propanal, hexanal, heptanal, methyl formate, ethyl formate, methanol, and ethanol, measured above 1-5 g samples, did not vary significantly with the amount of the sample, indicating that, even though there was a small flow of ambient air into the vials and, hence, a slow exchange of the air above the sausage samples, this exchange did not significantly disturb the dynamic equilibrium between the solid phase (ground sausage) and the vapor phase (headspace) for these volatile compounds. Hence, these headspace volatiles are equilibrated with the sample, and their content in the sausage sample is not significantly depleted by evaporation into the HS. These results indicate that the HS concentrations of the several compounds above 5 g samples, as reported below, are essentially representative of the equilibrated HS concentrations irrespective of the amount of sample.

The coefficients of variation (cv) were calculated for the measured concentration of several compounds in the HS as follows: water vapor, 4%; acetic acid, 10%; acetone, 11%; propanal, 17%; hexanal, 20%; heptanal, 11%; methyl formate, 11%; ethyl formate, 10%; methanol, 8%; ethanol, 12%. These percentages describe the reproducibility of single MIM quantifications; therefore, the mean values of the six measurements (duplicate measurements for three independent samples) will have

better reproducibility. Although such detailed assessments have not been performed for all the compounds listed in **Table 3**, similar results to the above "test" compounds are expected for all compounds.

HS Levels of Aldehydes.

The 13 aldehydes listed in **Table 3** were quantified by SIFT-MS in the HS of the pulverized sausages. However, 7 of them were present at concentrations close to their quantification limit (10 ppb), and so, the data for these were not analyzed further. The HS concentrations of propanal, butanal, pentanal, 2-pentenal, hexanal, and heptanal at the different processing times are shown in **Figure 3**. In all cases, significant changes are seen in the HS concentration of these aldehydes during processing ($p < 0.01$, with the possible exception of heptanal, $p < 0.05$). The most abundant volatile compound is propanal, although it has not been reported as the major aldehyde released by dry fermented sausages when extraction and GC techniques were used.[11,13] Propanal has the smallest molecular weight and is the most hydrophilic aldehyde among those monitored in this study. This can be explained by the fact that while the sensitivity of previously used GC techniques relying on extraction (solvent, SPME, etc.) is reduced for smaller (less than four C atoms) compounds, the sensitivity of SIFT-MS does not change greatly with the molecular (ionic) size. A similar observation was reported previously by Davis *et al.*[25] in their studies of olive oil emissions using SIFT-MS.

In general, these data reveal an increase in the HS concentrations of these aldehydes with processing time. The fat content of the sausages apparently increases the production of 2-pentenal and hexanal (**Figure 3d,e**, respectively) ($p < 0.01$). During dry fermented sausage processing, aldehydes are the main volatile compounds, as generated by the oxidation of lipid components such as free fatty acids (FFA).[3] The majority of the FFA is derived from the tryglicerides present in the adipose tissue (pork back fat),[11] and so, the fat appears to be the major source of 2-pentenal and hexanal.

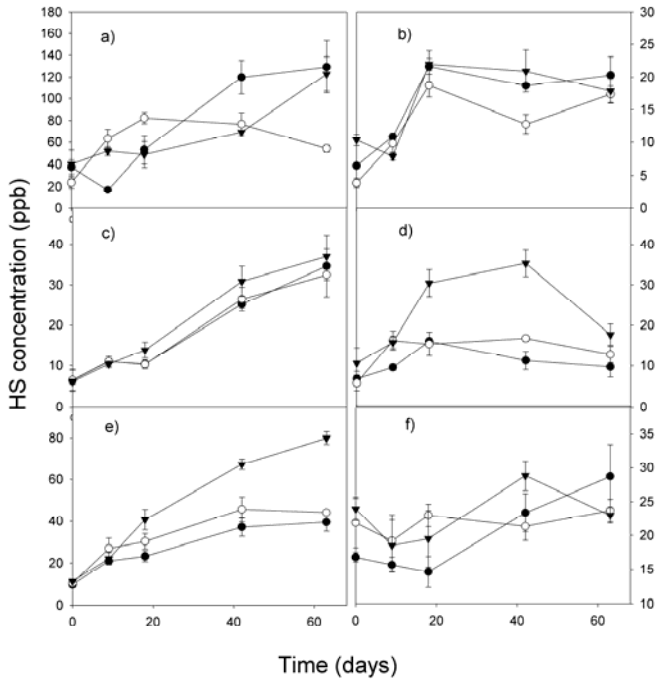

Figure 3. Headspace (HS) concentrations (in parts-per-billion, ppb) of (a) propanal, (b) butanal, (c) pentanal, (d) 2-pentenal, (e) hexanal, and (f) heptanal at the times (days) after the initial processing of dry sausages containing different pork back fat content; low fat (LF, ●), medium fat (MF, ○), and high fat (HF, ▼). Each data point is the mean of six measurements, and the bars represent the standard error of the mean; see the text for further explanation.

HS Levels of Ketones and Alcohols.

A total of six ketones were quantified in the HS of the fermented sausages by SIFTMS (see **Table 3**), although the levels of three of them were close to their detection limits (1 ppb). The HS concentrations of acetone, 2-butanone, and 2,3-butanedione (**Figure 4a,b,c**, respectively) were significantly altered by the processing time ($p < 0.05$). Acetone, a very volatile compound, was the most abundant ketone in the HS, although its concentration was apparently not affected by fat content (**Figure 4a**). In contrast, 2-butanone and 2,3-butanedione (see **Figure 4b,c**, respectively) were influenced by the fat content ($p < 0.05$) although differences in their concentrations were observed in the batches only at the latest processing times. The median value of the total concentration of both pentanone isomers was estimated as 8 ppb, and the median

concentrations of both heptanone and nonanone were 4 ppb; no statistically significant relation of their concentrations to fat content or processing time was evident.

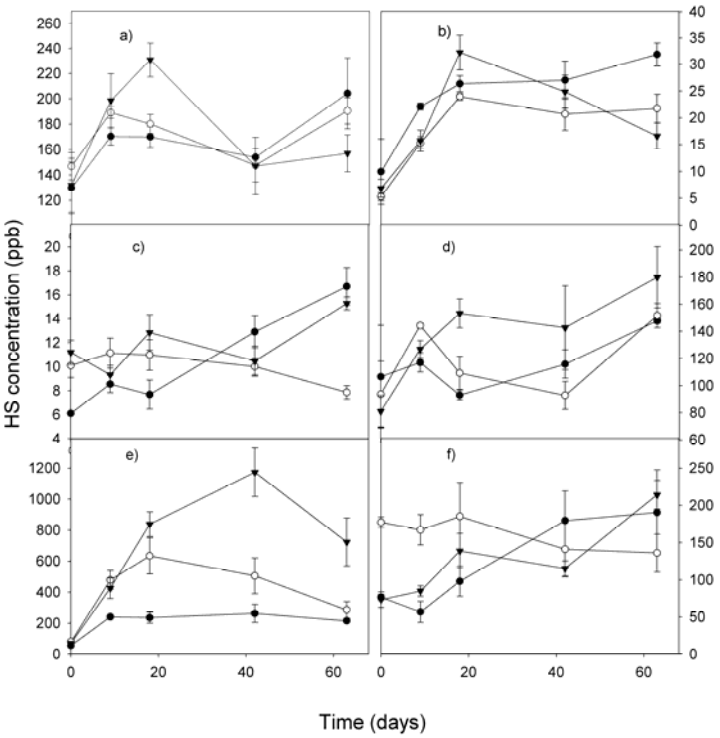

Figure 4. Headspace (HS) concentrations (in parts-per-billion, ppb) of (a) acetone, (b) 2-butanone, (c) 2,3-butanedione, (d) methanol, (e) ethanol, and (f) 2-propanol at the times (days) after the initial processing of dry sausages containing different pork back fat contents; low fat (LF, ●), medium fat (MF, ○), and high fat (HF, ▼). Each data point is the mean of six measurements, and the bars represent the standard error of the mean; see the text for further explanation.

There is a general increase in the HS alcohol concentrations with increasing processing time ($p < 0.01$) (see **Figure 4d,e,f**, respectively) and a very clear increase in the ethanol content with increasing fat content ($p < 0.05$) (**Figure 4e**). Ethanol was the most abundant alcohol detected, which is in accordance with previous studies using conventional GC analysis that describe ethanol as one of the most abundant products of bacterial metabolism in dry fermented sausages.[11] However, the present studies using SIFT-MS are the first to recognize methanol in the HS of fermented

sausages (**Figure 4d**). Nevertheless, its presence is to be expected following the identification of a wide variety of methyl esters in naturally fermented sausages.[28]

HS Levels of Esters and Acids.

Two esters, ethyl formate and ethyl acetate, and two acids, acetic and hexanoic acids, were monitored by SIFT-MS in the HS of the sausages throughout the processing period (**Figure 5**). Significant changes in the concentration of both the esters and the acetic acid were detected with the processing time ($p < 0.01$). The fat content of the sausage resulted in a clear increase in the HS concentration of both ethyl formate and hexanoic acid ($p < 0.05$).

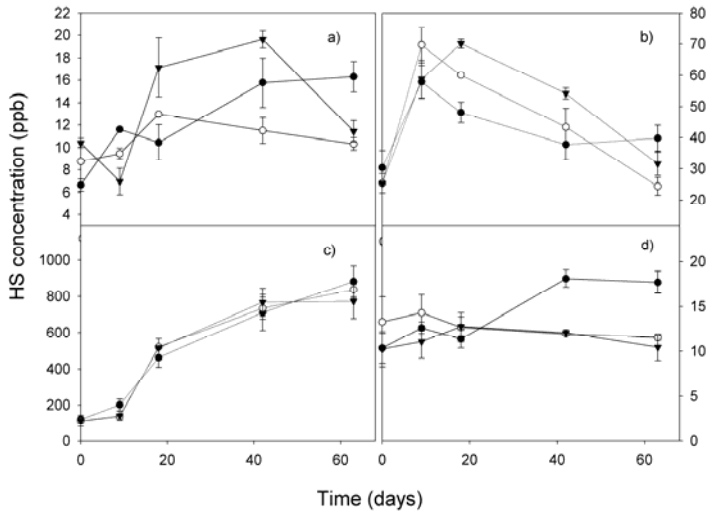

Figure 5. Headspace (HS) concentration (in parts-per-billion, ppb) of (a) ethyl formate, (b) ethyl acetate, (c) acetic acid, and (d) hexanoic acid at the times (days) after the initial processing of dry sausages containing different pork back fat contents; low fat (LF, ●), medium fat (MF, ○), and high fat (HF, ▼). Each data point is the mean of six measurements, and the bars represent the standard error of the mean; see the text for further explanation.

As is expected, the generation of esters and acetic acid is linked to the fermentation and curing process. Ethyl acetate has been reported as the most abundant ester in the HS of dry fermented sausages analyzed by GC/MS.[11] However, the generation of ethyl formate is reported here for the first time. Ethyl formate, like methanol, is a highly volatile compound that is difficult to detect by GC. Acetic acid was detected at a

relatively high level (approaching 1000 ppb); it was readily detected by Marco *et al.*[11] using SPME with GC/MS.

HS Levels of Sulfur Compounds.

Five sulfur-containing compounds were monitored by SIFT-MS in the HS of the sausages. (See **Table 3** and **Figure 6**.) Significant changes occurred ($p < 0.01$) in the HS concentration of all five compounds with increasing processing time. The concentration of dimethyl sulfide, dimethyl disulfide, carbon disulfide, and methanethiol increased with time, whereas hydrogen sulfide greatly decreased after 9 days of processing. The fat content affected only the concentration of dimethyl disulfide, as can be seen in **Figure 6c**.

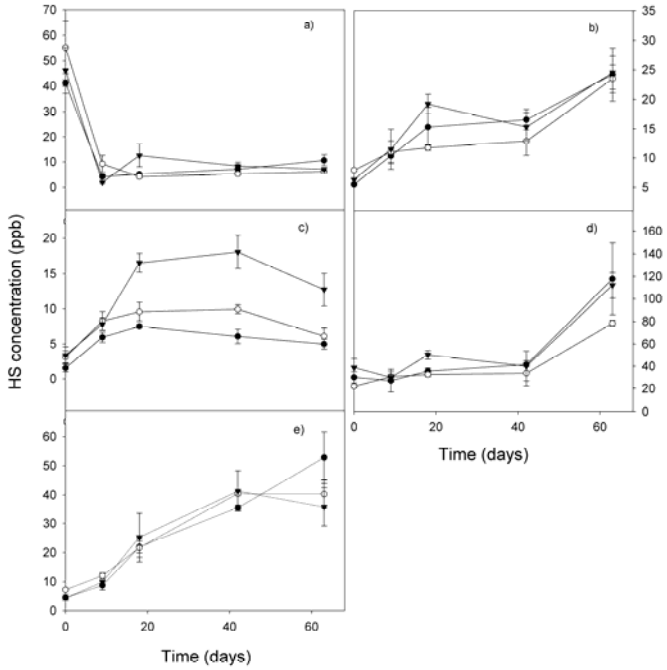

Figure 6. Headspace (HS) concentration (in parts-per-billion, ppb) of (a) hydrogen sulfide, (b) dimethyl sulfide, (c) dimethyl disulfide, (d) carbon disulfide, and (e) methanethiol at the times (days) after the initial processing of dry sausages containing different pork back fat contents; low fat (LF, ●), medium fat (MF, ○), and high fat (HF, ▼). Each data point is the mean of six measurements, and the bars represent the standard error of the mean; see the text for further explanation.

These sulfur compounds are highly volatile and are difficult to identify and quantify by conventional GC techniques. However, they are potent odorants with very low odor detection thresholds within the parts-per-trillion range.[37] It has been suggested that the relatively rapid decrease in the observed level of hydrogen sulfide (**Figure 6a**) is due to its reaction with other compounds present in the meat that results in the formation of other volatile compounds,[38] although this suggestion requires further investigation.

CONCLUSIONS

This study has demonstrated the value of SIFT-MS for the real time monitoring and quantification of the volatile compounds released by food products. This particular SIFT-MS investigation has focused on the aroma compounds present in the headspace of dry fermented sausages.

A sensory study had already been carried out on the same sausages prior to this SIFT-MS study, and the results have been published in a recent paper.[28] The sensory analysis performed by 75 consumers of the sausages with different fat contents has revealed significant differences in all the attributes evaluated, which were flavor, taste, juiciness, hardness, appearance, and overall acceptability. The low fat content fermented sausages were less appreciated by consumers and resulted in lower flavor acceptance. The SIFT-MS results presented here for the selection of volatile organic carbons (VOCs) recognized as the aroma active compounds indicate that the subjective sensory indicators can be explained by the quantitative changes in the HS concentrations of specific compounds, as shown in **Figures 3**, **4**, **5**, and **6**. In summary, those aldehydes that are derived from lipid oxidation that occurs during the drying process and those compounds that are generated by bacterial metabolism, including ketones, alcohols, esters, and acids, are readily detected by SIFT-MS, and the changes in their concentration/generation rate during processing can be quantified by real time analysis. In addition, highly volatile compounds, which are not generally recognized by extraction and GC techniques, can be detected and quantified by SIFT-MS. Thus, SIFT-MS could be used for a great effect for the fast control of the sensory quality of meat products.

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Capítulo 7

SPME-GC-MS versus Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) analyses for the study of volatile compounds generation and oxidation status during dry fermented sausage processing

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SPME-GC-MS versus Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) analyses for the study of volatile compounds generation and oxidation status during dry fermented sausage processing

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Abstract

The use of selected ion flow tube mass spectrometry (SIFT-MS) and gas chromatography-mass spectrometry together with solid phase micro-extraction (GC-MS-SPME) have been compared in the analysis of volatile compounds during dry fermented sausage processing. Thus, the headspace (HS) of samples of dry fermented sausages with different fat contents was analysed during their manufacture using both techniques and significant and positive correlations were found between SIFT-MS and SPME-GC-MS measurements for the compounds pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanone, 2-pentanone, ethanol, acetic acid and hexanoic acid. The oxidative status of fermented sausages during processing was also evaluated and a significant correlation was obtained between the HS concentration of lipid auto-oxidation volatile compounds measured by SIFT-MS and SPME-GC-MS and the level of thiobarbituric acid reactive substances (TBARS) in the sausage. The hexanal measured by SIFT-MS resulted in a higher correlation coefficient (r = 0.936) than that obtained using SPME-GC-MS (r = 0.927). SIFT-MS is shown to be a fast, real time analytical technique for monitoring changes in the profile of volatile compounds in dry fermented sausages during processing and a useful tool to evaluate the oxidative status of meat products.

Keywords: SIFT-MS, dry fermented sausages, oxidation, volatile organic compounds.

INTRODUCTION

The typical aroma of dry-fermented sausages is due to a mixture of volatile compounds generated by bacterial metabolism and lipid oxidation during processing. However, among the hundreds of volatile compounds identified, only a limited number are the odorants responsible for the dry-cured aroma (1, 2, 3). Gas chromatography/mass spectrometry (GC-MS) is the most widely used technique for identification and quantification of flavour compounds. This technique requires pre-concentration of the volatiles in the gas (headspace, HS) sample using methods such us vacuum distillation or solid phase micro-extraction (SPME). Using the latter method, the volatile compounds are released from the adsorbent thermally and injected into the column in order to separate them, however the chromatographic separation takes time. Although GC-MS is a highly sensitive and reliable technique, the demands of the food industry for rapid analysis indicate that GC-MS is not so convenient and faster analytical techniques are required. This has lead to the development of direct mass spectrometric techniques without chromatographic separation in which the mixture of the emitted volatile compounds from a food matrix is sampled directly into a mass spectrometer and the compounds are immediately detected and quantified. Direct analyses of volatile compounds in air have been demonstrated using different ionization techniques, including atmospheric pressure chemical ionization (APCI; 4), proton transfer reaction mass spectrometry (PTR-MS; 5), and selected ion flow tube mass spectrometry (SIFT-MS; 6,7).

SIFT-MS is a direct mass spectrometric technique based on the chemical ionization of a gas (analyte) sample (to the exclusion of the major air gases N_2, O_2, H_2O and CO_2) using specific, selected precursor (reagent) positive ions. Using SIFT-MS, real time quantification of volatile compounds in humid air can be achieved without external calibration. This is because the absolute concentrations are calculated from the ratios of the count rates of the product analyte-derived ions to those of the precursor whilst taking into account known values of the reaction rate coefficients, reaction time and the influence of diffusion and mass discrimination (8). Even so, SIFT-MS analyses have been validated using standard mixtures (9) and it has been widely applied in biology

and medicine (7, 10, 11). With respect to flavour analysis, Španěl and Smith (6) described the kinetics of the reactions of the precursor ions used in SIFT-MS with some food flavour compounds and studied the real-time release of volatile compounds from freshly cut onion, crushed garlic, and ripe banana. Recently, several papers have demonstrated the value of SIFT-MS for flavour research; the applications continue to broaden as this article goes to press. Xu and Barringer used SIFT-MS to study headspace tomato volatiles and their release during chewing (12, 13). Davis *et al.* (14) and Davis and McEwan (15) monitored the major volatile compounds emitted by olive oil in order to establish differences in their quality. SIFT-MS has also been employed to quantify volatile basic nitrogen compounds released from cod fillets (16), the release of alkylpyrazines and other volatiles by cocoa liquors (17), and aldehydes from malt (18). Most recently, we have successfully exploited SIFT-MS to quantify the volatile aroma compounds released by dry fermented sausages (19).

SIFT-MS has also been described as a tool for the evaluation of other quality parameters in food products apart from flavour. For instance, Noseda *et al.* (16) compared SIFT-MS with the traditional methods for the quantification of amines (steam distillation of an alkalized sample) and proposed SIFT-MS as a fast non-destructive technique for the evaluation of raw fish freshness. Davis and McEwan (15) have positively correlated SIFT-MS measurements with oxidative status as determined by the peroxide value (PV) and developed a fast reliable method for PV prediction by SIFT-MS in olive oil. To date, the results of only one study have been reported in which both SIFT-MS and GC-MS have been used in tandem, this study involving the differentiation of malt varieties (18).

Therefore, the aim of the present work was to evaluate the potential of SIFT-MS to monitor the generation of volatile compounds in fermented sausages during ripening by comparing conventional SPME-GC-MS analyses with real-time SIFT-MS analyses. Additionally, the capability of SIFT-MS to determine the oxidative status of dry fermented sausages during processing was investigated. The results reveal the usefulness of SIFT-MS in quality control applications.

MATERIALS AND METHODS

Reagents and standards.

The chemical compounds used for volatile identification were all obtained from Fluka Chemie AG (Buchs, Switzerland) except 2-octenal and 2,3-butanedione which were obtained from Aldrich (St. Louis, MO).

Dry fermented sausages

Three batches of dry fermented sausages with different pork-back fat percentage were selected for analysis; 10% - low fat (LF); 20% - medium fat (MF) and 30% - high fat (HF). The processing conditions of the sausages are described in Olivares *et al.* (20). From each batch, three sausages (LF, MF and HF) were chosen at 0, 9, 18, 42 and 63 days of processing after which they were sliced, vacuum packaged and frozen at –80 ºC to await analysis. The lipid oxidation in the sausages was determined using the thiobarbituric acid reactive substances (TBARS) method, as described by Bruna *et al.* (21), using tricloroacetic acid instead of perchloric acid as solvent. The results are expressed as mg malonaldehyde (MDA) per kg. The lipid oxidation determinations were replicated three times and the results expressed as the mean of the three values.

SPME GC-MS analyses

The analysis of volatile compounds present in the headspace (HS) of the sausages was carried out as described by Marco *et al.* (22). The compounds shown in **Table 1** were quantified due to their aroma properties described previously in dry fermented sausages (1, 2, 3). For each measurement, 3 g of minced sausage was weighted into a 10 mL headspace vial, and 0.75 mg of antioxidant (butylated hydroxytoluene, BHT) was added. The vial was left for 1 h in a thermoblock (J.P., Selecta, Barcelona, Spain) at 37 ºC to equilibrate. The volatile compounds were extracted by solid phase micro-extraction (SPME) using a 85 µm carboxen/polydimethylsiloxane StableFlex fibre (CAR/PDMS SF, Supelco, Bellefonte, Pennsylvania, USA) that was exposed to the headspace for 3 h while maintaining the sample at 37 ºC. The fibre was then placed in the injection port of a gas chromatograph (HP 7890A) equipped with a HP 5975C mass selective detector (Hewlett Packard, Palo Alto, CA). The compounds adsorbed onto

the fibre were desorbed in the injection port of the GC with the purge in the split-less mode. The released compounds were separated using a DB-624 capillary column (J & W Scientific, Agilent Technologies, USA) and identified by comparison with the mass spectra constructed from the (NIST 05) library database and by the Kovats linear retention index (23) using authentic standards. The volatile compounds were analyzed by the SCAN mode and the total ion current (TIC) across the *m/z* range 29–400 was acquired. Quantification was based on total extracted area (TIC). The headspace of the sausages was analyzed in triplicate for each fat batch and each processing time. The measuring order of the samples was randomised.

SIFT-MS analyses

The optimization of SIFT-MS for quantification of the volatile compounds in the headspace of sausages was achieved as described in Olivares *et al.* (19). For each measurement, 5 g of crushed sausage was weighted into a 15 mL headspace vial, together with 0.75 mg of butylated hydroxytoluene (BHT) used as antioxidant. The emitted volatiles were allowed to develop in the HS of the sealed vial (initially purged with laboratory air) at 37 ºC for 1 hour. A SIFT-MS *Profile 3* instrument manufactured by Instrument Science Limited (Crewe, UK) was used to measure the volatile compounds. The air/volatile compounds of the sealed vial were sampled directly by piercing the septum with a stainless steel needle connected directly to the SIFT-MS sampling line. The sample entered the helium carrier gas via a heated (70 °C) capillary tube at a measured rate of 0.45 Torr L/s. A second syringe needle pierced the septum to maintain the pressure in the vial at atmospheric pressure by introducing laboratory air at a rate that balances the small loss rate due to the sampling into the SIFT-MS instrument. Background (laboratory air) concentrations of all the volatile compounds included in the analysis were routinely recorded before and after the analysis of each sample. H_3O^+, NO^+ and O_2^+ were used as precursor ions. Flow tube temperature was 26 ºC, flow tube pressure was 1.0 Torr, flow tube diameter was 1 cm and reaction length was 4 cm. For accurate quantification, the multiple ion monitoring (MIM) mode was used to target specific volatile compounds (11). In this mode, the analytical mass

spectrometer is rapidly switched between selected *m/z* values of both the precursor ions and the characteristic product ions. Precursor ion count rates were in the range from 10000 to 1000000 counts/second. The known rate coefficients for the analytical reactions were then used to quantify the absolute HS concentrations of the compounds using the standard SIFT-MS data analysis software and the general method of quantification (24). Ionic diffusion and mass discrimination was corrected by the SIFT-MS software according to procedure described in Smith *et al.* (8). The absolute quantification was continuously verified by analyses of absolute humidity (25). In **Table 1** is shown the volatile compounds quantified together with the precursor ion and product ions used for each compound. Data for each precursor ion were collected and integrated for a period of 200 seconds and the mean values over this sampling time were recorded. The results were then expressed in parts-per-billion by volume of the headspace, ppbv (nL of volatile compound per L of air). The headspace of the sausages was analyzed in duplicate for each fat batch and each processing time. The measuring order of the samples was randomised.

Statistical analysis

The effect of ripening time on the HS volatile compounds concentration obtained by both techniques was assessed using analysis of variance (ANOVA). Pearson correlation analysis was performed to correlate the results obtained by SIFT-MS and SPME-GC-MS analyses. In addition, Pearson correlation analysis was performed between the analysis of volatile compounds (SIFT-MS and GC-MS) and the oxidative status of the sausages (TBARS values). The statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain) package was used for these analyses.

RESULTS

Comparison of SIFT-MS and SPME-GC-MS throughout sausage processing

Quantification of volatile compounds (shown in **Table 1**) was achieved by GC-MS for the three batches of sausages throughout the processing period (0, 9, 18, 42 and 63 days). Each SPME-GC-MS analysis required a total time of 5 hours (1 h equilibration, 3 h CAR/PDMS fibre extraction, 1 h GC-MS run). A single fibre was used so only 2

analyses per day were possible. However, the number of analyses per day can be improved if an automatic device is used. The volatile compounds were selected based on their aroma properties described for dry fermented sausages by previous GC-olfactometric data (1-3, 26-29). The mixture comprised 11 aldehydes, 6 ketones, 2 alcohols, 2 acids, 2 organosulphur compounds and 1 ester (**Table 1**).

Table 1. Molecular weight, precursor ion, mass-to-charge ratio (*m/z*) of the characteristic product ions and GC-O descriptors of the aroma compounds analyzed by SIFT-MS.

Compound	R[1]	M_W[2]	Precursor ion[3]	Product ion (m/z)[4]
Aldehydes				
Propanal	a	58	NO^+	57
Butanal	a	72	NO^+	71
Pentanal	a	86	NO^+	85
Hexanal	a	100	NO^+	99
Heptanal	a	114	NO^+	113
2-Heptenal	a	112	H_3O^+	111 + 142
Octanal	a	128	NO^+	127
2-Octenal	a	126	NO^+	125 + 156
Nonanal	a	142	NO^+	141
2-Nonenal	a	140	NO^+	139 + 170
2,4-Decadienal	a	152	NO^+	151
Ketones				
2-Butanone	a	72	NO^+	102
2,3-Butanedione	a	86	NO^+	86
2-Pentanone	a	86	NO^+	116
2-Heptanone	a	114	NO^+	144
2-Octanone	a	128	NO^+	158
2-Nonanone	a	142	NO^+	172
Esters				
Ethyl acetate	a	88	NO^+	118
Alcohols				
Ethanol	a	46	H_3O^+	47 + 65 + 83
1-Propanol	a	60	H_3O^+	43
Acids				
Acetic acid	a	60	NO^+	90 + 108
Hexanoic acid	a	116	H_3O^+	117 + 135
Sulphur compounds				
Dimethyl disulphide	a	94	NO^+	94
Methanethiol	a	48	H_3O^+	49 + 67

[1] R: Reliability of identification: a, mass spectrum and retention time identical with an authentic standard [2]: MW: Molecular weight. [3]: Precursor ion used for the quantification. [4]: Product ion generated after ionization as described in Olivares et al., (2010a).

The volatile compounds were also quantified during ripening using SIFT-MS in order to determine the potential of this real time analytical method. The sample preparation conditions were exactly the same as those used in SPME/GC-MS analyses (crushing the sample, antioxidant addition, ratio sample/HS volume in the vial, temperature and equilibration time). For each sample little more than 1 h was needed (1 h equilibration, 200 seconds for SIFT-MS data acquisition with each precursor ion species). Even though preparation of the sample was identical for both techniques, SIFT-MS analysis required a much shorter time because it does not involve SPME and GC separation, even though an automatic device is used. Several samples were crushed, and after equilibration were measured consecutively. For instance, **Figure 1** shows SIFT-MS real-time monitoring data obtained using the MIM mode analysis for the compounds ethanol, acetic acid, butyric acid, propanol and water vapour in the HS of four high fat sausages (HF) at different ripening times (9, 18, 42 and 63 d). Although **Figure 1** represents only 1 measurement of 1 sample at each processing time, a change with ripening time of the HS concentration of ethanol, acetic acid, butyric acid and propanol can be seen and only 15 minutes were necessary for SIFT-MS data acquisition, in this case using H_3O^+ precursor ion.

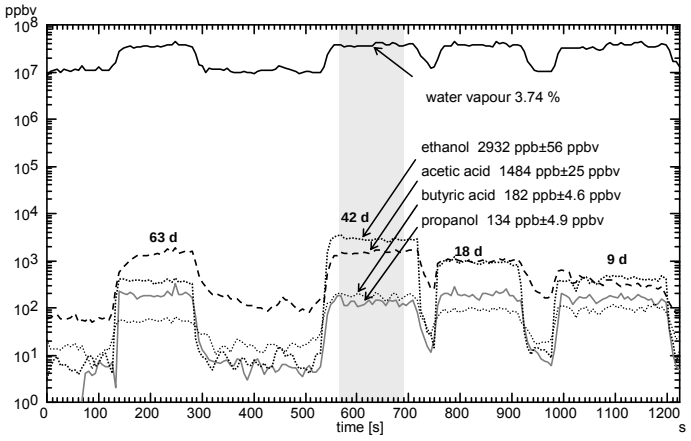

Figure 1. Real time monitoring of the HS of sausages by SIFT-MS. Four high fat sausages (HF) at different ripening times 9, 18, 42 and 63 days (indicated as d in the figure) were analyzed. The levels of several compounds indicated in parts-per-billion by volume (ppbv) are given together with their estimated uncertainties for the 42 d sample, the vertical shading indicating the integration interval.

For the quantification of the volatile compounds the parameters described in **Table 1** were used. In general NO^+ was used as the precursor ion, but H_3O^+ was used for 2-heptenal, ethanol, 1-propanol, hexanoic acid and methanethiol. This selection was made based on previous work that indicated the *m/z* values of characteristic products ions (shown in **Table 1**) that did not overlap with those characteristic ions produced by other compounds in the sausage HS (19). Thus, the MIM mode was used to quantify the volatile compounds, as described in materials and methods section. All the analyses were carried out in duplicate for all the samples and the mean values obtained. The results of quantification realized by both techniques are shown in **Table 2** where the values represent the mean of the three fat batches analyzed. The values of the SPME-GC-MS analysis were expressed in abundance ($TICx10^{-6}$) as their values depend on the affinity of the compounds to the fibre and the concentration will not be the real concentration present in the sausage HS. These data indicate significant increases in almost all the volatile compounds during ripening, except for octanal, 2,4-decadienal, ethanol, 1-propanol, hexanoic acid and methanethiol. The latter is probably due to the high standard error obtained in the analyses of these compounds due to the different fat batches employed. Nevertheless, SPME-GC-MS can be used to monitor changes during ripening in most all the emitted volatile compounds. Therefore, the results obtained can be an index of the ripening process and can also relate to sensory properties (30, 31).

On the other hand, the SIFT-MS analyses express the concentrations of the volatile compounds in ppbv in the HS. Many volatile compounds increased significantly during ripening except for heptanal, 2-heptenal, nonanal, 2,3-butanedione, 2-octanone, 2-nonanone, ethanol, 1-propanol, hexanoic acid and dimethyl disulphide. Previously, it was mentioned that the absence of observable differences can be due to the high standard error that can result when collectively analysing the three fat batches but, in addition, several of these compounds were at very low concentration in the HS close to the quantification limits of the SIFT-MS (limit of quantification is 10 ppbv) (24). This is the case for nonanal, 2,3-butanedione, 2-octanone, 2-nonanone that were present at concentrations lower than 10 ppbv (**Table 2**).

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Table 2. Quantification of volatile compounds in dry fermented sausages during ripening (values represents the mean of the three fat batches analyzed) by SMPE-GC-MS and SIFT-MS after 0 (initial), 9, 18, 42, and 63 days.

	SPME GC-MS							SIFT-MS						
Compound	**0d**	**9d**	**18d**	**42d**	**63d**	**SEM***	***p*****	**0d**	**9d**	**18d**	**42d**	**63d**	**SEM**	***p***
Aldehydes														
Propanal	0.00 ^{b}	0.40 ^{b}	0.61 ^{b}	2.68 ^{a}	3.24 ^{a}	0.38	0.000	33.80 ^{c}	44.03 ^{bc}	61.38 ^{abc}	88.61 ^{ab}	94.04 ^{a}	14.18	0.049
Butanal	0.00 ^{c}	0.20 ^{b}	0.19 ^{b}	0.61 ^{a}	0.59 ^{a}	0.05	< 0.0001	6.90 ^{b}	9.50 ^{b}	20.77 ^{a}	17.45 ^{a}	20.10 ^{a}	1.88	0.001
Pentanal	0.00 ^{c}	4.25 ^{bc}	7.76 ^{b}	20.67 ^{a}	20.61 ^{a}	1.61	< 0.0001	6.32 ^{c}	10.76 ^{c}	11.46 ^{c}	27.47 ^{b}	38.54 ^{a}	2.05	< 0.0001
Hexanal	4.18 ^{b}	36.20 ^{b}	56.82 ^{b}	243.30 ^{a}	293.38 ^{a}	29.28	< 0.0001	10.32 ^{c}	23.30 ^{c}	31.38 ^{bc}	50.00 ^{ab}	57.16 ^{a}	6.93	0.004
Heptanal	1.13 ^{b}	3.37 ^{b}	4.87 ^{b}	21.40 ^{a}	24.24 ^{a}	1.23	< 0.0001	20.87	17.81	19.04	24.50	24.97	1.96	0.094
2-Heptenal	0.00 ^{b}	0.00 ^{b}	0.24 ^{a}	0.36 ^{a}	0.39 ^{a}	0.07	0.004	31.38	25.27	26.99	34.73	31.94	2.75	0.176
Octanal	0.07	0.15	0.95	0.82	1.91	0.50	0.144	3.37 ^{b}	3.54 ^{b}	3.69 ^{b}	5.49 ^{a}	4.70 ^{ab}	0.47	0.040
2-Octenal	0.20 ^{c}	0.57 ^{c}	0.87 ^{bc}	2.06 ^{ab}	3.30 ^{a}	0.42	0.002	1.55 ^{b}	3.50 ^{ab}	4.88 ^{a}	4.04 ^{a}	3.87 ^{a}	0.63	0.039
Nonanal	3.31 ^{c}	4.91 ^{c}	6.99 ^{c}	16.50 ^{b}	22.60 ^{a}	1.56	< 0.0001	4.22	4.28	4.78	4.16	4.51	0.46	0.870
2-Nonenal	0.56 ^{b}	0.71 ^{b}	0.74 ^{b}	1.73 ^{a}	1.88 ^{a}	0.12	< 0.0001	0.78 ^{b}	2.80 ^{a}	3.69 ^{a}	3.73 ^{a}	3.73 ^{a}	0.56	0.016
2,4-Decadienal	0.00 ^{b}	0.00 ^{b}	0.37 ^{a}	0.38 ^{a}	0.40 ^{a}	0.11	0.059	0.25 ^{c}	1.09 ^{bc}	1.87 ^{ab}	2.03 ^{a}	1.33 ^{ab}	0.28	0.008
Ketones														
2-Butanone	5.05 ^{c}	14.72 ^{a}	13.93 ^{a}	9.92 ^{b}	9.60 ^{b}	0.99	0.000	7.37 ^{c}	17.71 ^{b}	27.51 ^{a}	24.19 ^{ab}	23.39 ^{ab}	2.70	0.003
2,3-Butanedione	0.21 ^{b}	3.18 ^{a}	0.32 ^{b}	0.34 ^{b}	0.31 ^{b}	0.58	0.018	9.13	9.67	10.51	11.13	12.31	1.50	0.613
2-Pentanone	0.56 ^{c}	4.27 ^{a}	3.82 ^{a}	1.04 ^{b}	1.17 ^{b}	0.15	< 0.0001	3.85 ^{b}	8.34 ^{a}	9.01 ^{a}	7.34 ^{a}	8.87 ^{a}	1.00	0.024
2-Heptanone	0.00 ^{c}	5.68 ^{b}	11.14 ^{a}	9.49 ^{a}	10.77 ^{a}	0.80	< 0.0001	1.37 ^{c}	4.63 ^{b}	5.11 ^{b}	8.28 ^{a}	5.28 ^{b}	0.93	0.006
2-Octanone	0.00 ^{d}	0.33 ^{c}	0.41 ^{bc}	0.49 ^{ab}	0.59 ^{a}	0.03	< 0.0001	1.22	3.96	3.61	5.58	3.77	0.94	0.087
2-Nonanone	0.00 ^{c}	2.78 ^{b}	6.56 ^{a}	7.09 ^{a}	7.95 ^{a}	0.56	< 0.0001	0.40	3.69	3.35	5.39	2.86	1.00	0.058
Esters														
Ethyl acetate	0.00 ^{b}	2.94 ^{a}	3.63 ^{a}	3.34 ^{a}	4.38 ^{a}	0.58	0.003	27.05 ^{c}	62.12 ^{a}	59.41 ^{a}	45.13 ^{b}	31.98 ^{bc}	4.49	0.001
Alcohols														
Ethanol	1.64	11.50	12.38	12.36	10.53	3.92	0.466	69.57	382.11	570.00	647.70	408.64	164.80	0.200
1-Propanol	0.00	0.00	0.10	0.10	0.11	0.04	0.238	109.05	102.92	140.63	145.25	180.24	27.46	0.338
Acids														
Acetic acid	0.00 ^{c}	189.48 ^{b}	773.71 ^{a}	785.62 ^{a}	747.03 ^{a}	44.47	< 0.0001	115.77 ^{d}	160.04 ^{d}	498.49 ^{c}	737.93 ^{b}	831.37 ^{a}	20.30	< 0.0001
Hexanoic acid	2.47	12.61	28.47	35.54	38.77	9.30	0.060	11.30	12.67	12.29	14.06	13.23	1.51	0.759
Sulphur compounds														
Dimethyl disulphide	0.00 ^{b}	0.00 ^{b}	1.16 ^{a}	0.80 ^{a}	1.11 ^{a}	0.14	0.000	2.70	7.37	11.23	11.37	8.38	2.26	0.113
Methanethiol	149.33	181.60	196.66	173.39	192.78	13.98	0.202	5.40 ^{c}	11.24 ^{c}	22.91 ^{b}	39.12 ^{a}	42.97 ^{a}	2.52	< 0.0001

$^{a.-d}$: Means with different letters indicate significant differences ($p<0.05$) among ripening times.*: SEM: standard error of the mean, **: *p* value for the statistical analysis.

Generally, both the GC-MS and SIFT-MS techniques detected differences in volatile compound concentrations and increases with ripening time. However, in order to determine if both techniques were able to see the same differences a Pearson correlation analysis was done using the data obtained in the three batches during the different ripening times. **Figure 2** shows the correlation between the measurement of hexanal by both techniques (GC-MS and SIFT-MS) for the three batches and ripening times. The symbol grey intensity decreases with ripening time (**Figure 2**), so it can be seen that both techniques showed an increase in the hexanal concentration during ripening with a positive and significant correlation coefficient (r = 0.948).

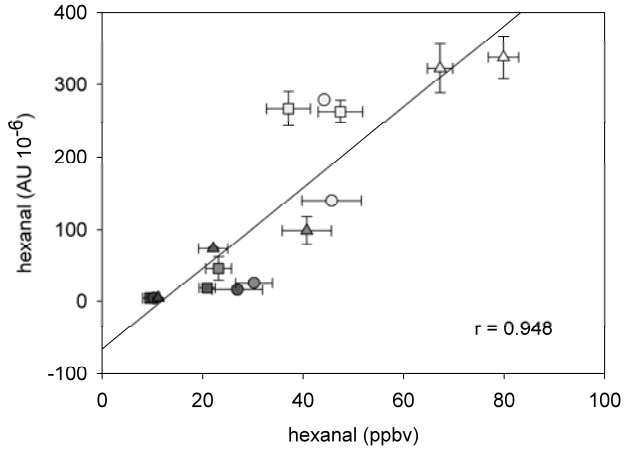

Figure 2. Pearson correlation coefficient (r) of the hexanal levels measured by SIFT-MS (ppbv) and GC-MS (Abundance units; AU 10^{-6}) during sausage processing. Low fat (LF, □), medium fat (MF, ○) and high fat (HF, Δ) sausages. The plotted values are the mean values for the three sausages and the bars represent the standard errors for both techniques. Grey intensity in symbols decreases with ripening time.

The same analysis was carried out for all the volatile compounds analysed and the correlation coefficients for each fat batch and the whole set of batches are shown in **Table 3**. When the three batches were analyzed together, significant and positive correlations were obtained for 13 of the 24 volatile compounds analyzed such as for the linear aldehydes C5 to C8 and 2-heptenal, 2-octenal and 2-nonenal, ketones: 2-butanone, 2,3-butanedione and 2-pentanone, the acids acetic and hexanoic and

ethanol. The best correlations (r > 0.8) were obtained for hexanal, pentanal, acetic acid, ethanol and 2-pentanone. However, when the correlation analysis was carried out for the different fat samples of each batch, the LF batch was the one where a higher number of significant correlations were obtained. Probably the presence of a higher fat content of the MF and HF samples can contribute to interfere in the analysis of volatile compounds. Fat can not only act as a solvent of the volatile compounds thus decreasing its concentration in the HS, but also it is one of the main precursors of volatile compounds during ripening (30).

Table 3. Pearson correlation coefficients of volatile compounds obtained by SIFT-MS and SPME-GC-MS in dry fermented sausages during processing with different pork back fat contents; low fat (LF), medium fat (MF) and high fat (HF).

Compound	LF		MF		HF		all batches	
	r	p	r	p	r	p	r	p
Aldehydes								
Propanal	0.920	**0.001**	0.430	0.249	0.751	**0.012**	0.517	0.085
Butanal	0.378	0.225	0.045	0.895	0.909	**0.001**	0.337	0.283
Pentanal	0.840	**0.001**	0.957	**<0.0001**	0.892	**<0.0001**	0.861	**<0.0001**
Hexanal	0.857	**0.002**	0.869	**0.020**	0.960	**<0.0001**	0.948	**0.001**
Heptanal	0.804	**0.002**	0.510	0.197	0.239	0.454	0.548	**0.034**
2-Heptenal	0.719	**0.044**	0.747	**0.033**	0.871	**0.019**	0.761	**0.029**
Octanal	0.892	**<0.0001**	0.701	**0.050**	0.841	**0.001**	0.751	**0.006**
2-Octenal	0.846	**0.001**	0.500	0.207	0.597	0.090	0.549	**0.042**
Nonanal	0.704	**0.016**	0.148	0.609	0.285	0.425	0.271	0.395
2-Nonenal	0.875	**0.001**	0.652	**0.030**	0.769	**0.006**	0.774	**0.010**
2,4-Decadienal	0.298	0.474	0.598	0.117	0.531	0.092	0.316	0.374
Ketones								
2-Butanone	0.718	**0.019**	0.754	**0.005**	0.858	**0.0004**	0.763	**0.003**
2,3-Butanedione	0.495	0.212	0.598	**0.040**	0.583	**0.047**	0.560	**0.049**
2-Pentanone	0.848	**0.002**	0.918	**0.0002**	0.671	**0.017**	0.803	**0.001**
2-Heptanone	0.205	0.522	0.190	0.553	0.285	0.369	0.254	0.433
2-Octanone	0.255	0.448	0.539	0.169	0.032	0.929	0.189	0.557
2-Nonanone	0.766	**0.016**	0.435	0.182	0.084	0.812	0.211	0.678
Esters								
Ethyl acetate	0.673	**0.047**	0.344	0.406	0.724	0.066	0.474	0.166
Alcohols								
Ethanol	0.679	**0.031**	0.843	**0.001**	0.796	**0.003**	0.822	**0.002**
1-Propanol	0.423	0.297	0.164	0.729	0.777	**0.014**	0.299	0.435
Acids								
Acetic acid	0.872	**0.001**	0.934	**<0.0001**	0.786	**0.004**	0.843	**0.001**
Hexanoic acid	0.797	**0.003**	0.805	**0.005**	0.752	**0.012**	0.780	**0.002**
Sulphur compounds								
Dimethyl disulphide	0.871	**0.001**	0.571	0.108	0.527	0.095	0.599	0.081
Methanethiol	0.696	**0.050**	0.277	0.470	0.775	**0.041**	0.406	0.133

Nevertheless, these results indicated that in spite of the different fat content present in the sausages both the GC-MS and SIFT-MS analyses were correlated and were able to detect the same changes with processing time for 13 volatile compounds.

However, several compounds did not show such correlations in any batch; one reason can be because the HS concentrations are too low for accurate SIFT-MS analysis. This was so for 2-heptanone and 2-octanone the concentrations of which increased significantly during ripening when measured by SPME-GC-MS (**Table 2**) but their concentrations were close to the quantification limits for SIFT-MS (24). Another reason could be the fact that SPME-GC-MS concentrates the volatile compounds from the HS while SIFT-MS measures the concentration directly without pre-concentration. Similar results were obtained by Pozo-Bayón *et al.* (32) when comparing GC-MS and PTR-MS. In any case, the positive and significant correlations obtained for many of the compounds analysed indicate that SIFT-MS is a useful tool for monitoring changes in the concentrations of HS volatile compounds above fermented sausages during processing much faster than SPME-GC-MS.

SIFT-MS as a tool for the evaluation of oxidative status

Lipid oxidation is one of the major causes of deterioration in meat quality, but it is also essential for the development of the characteristic dry cured aroma (33). In meat products, lipid oxidation is currently evaluated using the peroxide value and TBA-reactive substance measurements, and more recently by volatile compound quantification (33). Thiobarbituric acid reactive substances (TBARS) measures secondary lipid oxidation products and has been correlated with consumers' perception of lipid oxidation (34). However, the TBARS method requires sample preparation and is not convenient for the real-time monitoring of the oxidative status of dry fermented sausages.

In order to determine if SIFT-MS and SPME-GC-MS can be used to evaluate the oxidative status of the sausages throughout the processing period a Pearson correlation procedure between the measurements of aldehydes, produced during lipid oxidation reaction in the sausage, and the measurement of TBARS was performed.

Figure 3a, **c**, **e**, **g** and **i** shows the correlation between TBARS and the HS concentration of the aldehydes C3-C7 obtained by SPME-GC-MS, while **Figure 3b**, **d**, **f**, **h** and **j** includes the correlation between TBARS and SIFT-MS measurement of aldehydes C3-C7. The selection of the aldehydes as markers of the lipid oxidation process in meat has been widely studied (34). For this study, the linear aldehydes (C3 to C7) were chosen as possible markers. The TBARS values increased during processing time (from 0.2 to 1.2 mg MDA/kg, as shown in **Figure 3**, in the three fat batches as it has been reported in other dry fermented sausages (29, 30, 35).

The TBARS data were well correlated with the HS concentration of the linear aldehydes (C3- to C7) as detected by both SPME-GC-MS and SIFT-MS techniques (**Figure 3**). Using the SPME-GC-MS data, high positive correlation coefficients ($r > 0.9$, $p < 0.0001$) were obtained between the TBARS levels and the HS concentration for all the aldehydes C3-C7 (**Figures 3 a**, **c**, **e**, **g** and **i**). However, the results obtained by SIFT-MS resulted in good correlation for the aldehydes C3-C6 (**fig 3b**, **d**, **f**, **h**) but a poorer for heptanal (**Fig 3 j**). The hexanal measured by SIFT-MS resulted in a higher correlation coefficient ($r = 0.936$) than the one obtained using SPME-GC-MS ($r = 0.927$). The best correlation coefficients obtained by SIFT-MS were for pentanal and hexanal. Nevertheless, the correlation coefficients for the other aldehydes using SIFT-MS were lower than those obtained using GC-MS. These results indicate that both the SPME-GC-MS and SIFT-MS analytical techniques can be used to evaluate the oxidation process during dry fermented sausage ripening. Although, it would be necessary to analyze a higher number of meat samples to confirm these results. SIFT-MS measurements are obtained more rapidly than both TBARS and SPME-GC-MS measurements. SIFT-MS provided similar information about the hexanal levels, as has been reported as the most abundant volatile compound derived from lipid oxidation in meat and has often been chosen as an index of lipid oxidation level (29).

In summary, significant and positive correlations are seen between SIFT-MS and SPME-GC-MS measurements for the volatile compounds pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanone, 2-pentanone, ethanol, acetic acid and hexanoic acid generated during the processing of dry fermented sausages. So this study demonstrates that SIFT-MS is a reliable technique for monitoring changes in the

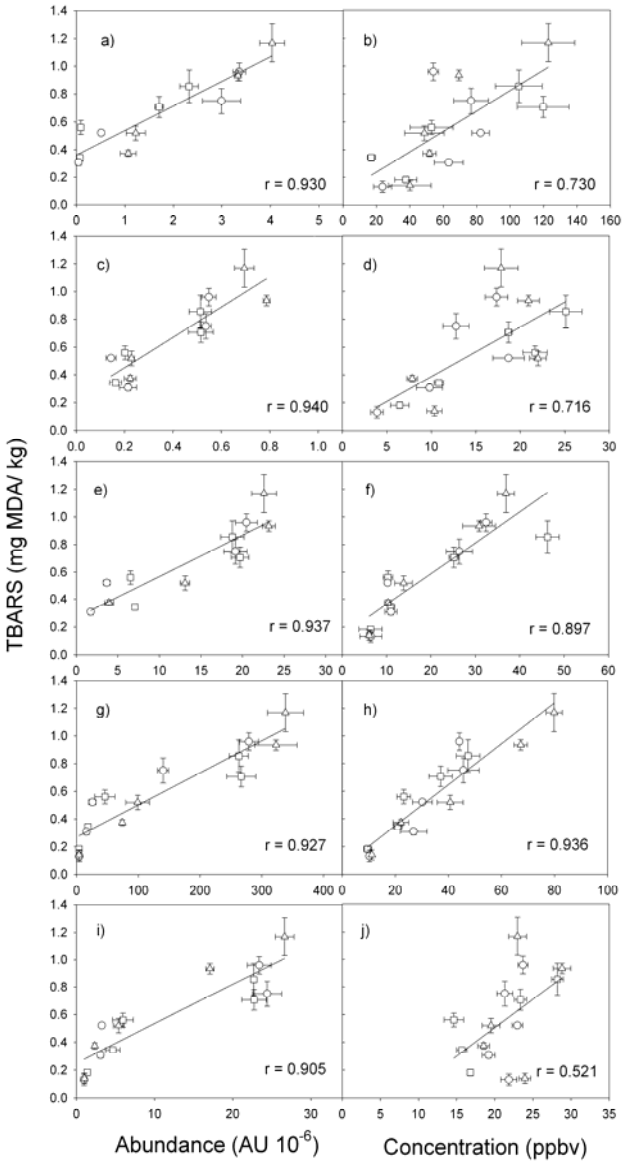

Figure 3. Correlation between lipid oxidation value (TBARS) and volatile compounds monitored by GC-MS and SIFT-MS during the manufacture of dry fermented sausages with different fat contents. Plots in the left column represent abundance obtained by GC-MS (AU 10^{-6}) for a) propanal, c) butanal, e) pentanal, g) hexanal and i) heptanal. Plots b), d), f), h) and j) in the right column represent concentration obtained by SIFT-MS (ppbv) for the same sequence of compounds. The symbols correspond to low fat (LF, □), medium fat (MF, ○) and high fat (HF, Δ) sausages. The plotted values are the mean values for the three sausages and the standard errors.

volatile compounds, providing similar information to the traditional SPME-GC-MS analyses, but more rapidly and, if desired, in real time. Finally, this study reveals that SIFT-MS can be used to evaluate the oxidative status of meat products by measuring the hexanal content of the sausages much more rapidly than conventional techniques.
In summary, significant and positive correlations are seen between SIFT-MS and SPME-GC-MS measurements for the volatile compounds pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanone, 2-pentanone, ethanol, acetic acid and hexanoic acid generated during the processing of dry fermented sausages. So this study demonstrates that SIFT-MS is a reliable technique for monitoring changes in the volatile compounds, providing similar information to the traditional SPME-GC-MS analyses, but more rapidly and, if desired, in real time. Finally, this study reveals that SIFT-MS can be used to evaluate the oxidative status of meat products by measuring the hexanal content of the sausages much more rapidly than conventional techniques.

ABBREVIATIONS

BHT: butylated hydroxytoluene; CAR/PDMS SF: Carboxen/polydimethylxylosane StableFlex; GC: Gas chromatograph; HS: Headspace; MS: Mass spectrometry; MW: molecular weight; SIFT-MS: selected ion flow tube mass spectrometry; SPME: Solid phase micro-extraction; TBARS: thiobarbituric acid reactive substances.

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VI. Discusión general

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En esta tesis doctoral se plantearon cuatro objetivos y los resultados obtenidos han dado lugar a siete capítulos incluidos en el apartado V. No obstante, en esta discusión general se ponen de manifiesto los resultados más relevantes.

La calidad global de los embutidos crudos curados está determinada en gran parte por su aroma, el cual está formado por una mezcla compleja de compuestos volátiles. Estos compuestos son el resultado de múltiples reacciones bioquímicas que tienen lugar durante el proceso de fabricación, donde las fracciones lipídicas y proteicas juegan un papel destacado. Sin embargo, aunque los compuestos volátiles presentes en embutidos crudos curados se han estudiado ampliamente (Stahnke, 2002), únicamente aquellos cuya concentración sea superior a los umbrales de percepción son importantes desde el punto de vista aromático (Grosch, 2001).

Dentro de los embutidos crudos curados, los fabricados mediante técnicas tradicionales presentan un aroma característico que es muy apreciado por los consumidores. Por ello, la primera parte de este estudio se ha centrado en la determinación de los compuestos responsables del aroma de embutidos de alta calidad organoléptica fabricados mediante técnicas tradicionales (capítulo 1). La determinación de la contribución de los compuestos volátiles al aroma se puede abordar desde dos perspectivas diferentes; el uso de técnicas olfatométricas y el cálculo de la actividad aromática. En este caso, los compuestos volátiles responsables del aroma característico de embutidos tradicionales se determinaron mediante olfatometría acoplada a cromatografía de gases. Esta técnica permitió la identificación de compuestos con potencia aromática previamente detectados como compuestos esenciales para el aroma de embutidos crudos curados, como los compuestos etil butanoato, ácido acético, 1-octen-3-ol, bencenoacetaldehído, 4-metil fenol, hexanal, etil hexanoato y metional entre otros (Blank *et al.*, 2001; Marco *et al.*, 2007). Además, se observó que los embutidos tradicionales presentaban otras notas aromáticas características, impartidas por otros compuestos derivados de distintas reacciones bioquímicas. Entre ellos destacan una gran variedad de ésteres, que fueron relacionados con la apreciación de este producto por parte de los consumidores. Cabe destacar que algunos de los compuestos identificados como aromáticos fueron

detectados en baja proporción en el espacio de cabeza (a modo de ejemplo, los compuestos metional, 4-metil fenol y 1-octen-3-ol). Así pues, estos resultados confirman que la importancia relativa de cada compuesto no sólo depende de su concentración en el espacio de cabeza sino de su umbral de percepción, y que la combinación de técnicas cromatográficas y olfatométricas es de gran utilidad para determinar el impacto real de cada compuesto y de este modo conocer qué compuestos están relacionados con la aceptación por parte de los consumidores.

Con el fin de ampliar el conocimiento de los compuestos volátiles de mayor impacto aromático, se estudió su evolución en la matriz cárnica a lo largo del proceso de secado de los embutidos (capítulo 2). Para ello, se empleó la técnica SPME múltiple ya que, a diferencia de la SPME convencional, permite conocer la concentración total presente en el embutido (Tena & Carrillo, 2007), y no sólo la liberada al espacio de cabeza. Los embutidos analizados, si bien contenían cultivos iniciadores, fueron fabricados mediante técnicas de fermentación lenta similares a las empleadas en los procesos de fabricación tradicionales. Dado que las concentraciones de los compuestos volátiles eran conocidas, en este caso la estimación de su contribución al aroma se llevó a cabo mediante el cálculo de la actividad aromática (OAV), relacionando la concentración de los compuestos volátiles en la matriz con los umbrales de percepción (Mistry *et al.*, 1997). Debido al alto contenido de grasa de los embutidos (aproximadamente 25%) se tomaron como referencia los umbrales de percepción en aceite (Grosch, 2001). Este estudio concluyó que algunos compuestos volátiles presentan OAV positivos (concentraciones superiores a los umbrales) desde las primeras etapas del proceso de curado (2-metil butanal, 3-metil butanal, octanal, 2,3-butanodiona y etil 2-metil butanoato). Además, a lo largo del proceso otros compuestos aromáticos aumentan su concentración y presentan OAV positivos (propanal, pentanal, hexanal, etil 3-metil butanoato, 1-octen-3-ol, 3-metil butanoico, 2-metil propanoico, etil hexanoato y nonanal). No obstante, hay que tener en cuenta que este cálculo fue obtenido a partir de la concentración total de compuesto volátil en la matriz, la cual no está necesariamente relacionada con la concentración liberada al espacio de cabeza. Por este motivo, también se calculó la actividad aromática en función de la concentración presente en el espacio de cabeza y los umbrales de

percepción en aire. En este caso, tal y como se observa en la **figura 1**, sólo algunos de los compuestos con altos valores de actividad aromática en la matriz, mostraron altos valores de actividad aromática en el aire (3-metil butanoico, etil 2-metil butanoato, etil hexanoato, hexanal, octanal y nonanal) aunque la mayor parte de ellos fueron siempre menores que los obtenidos en la matriz excepto octanal y nonanal. Por lo tanto, estos resultados indican que la matriz del embutido retiene una gran proporción de los compuestos volátiles presentes en ella.

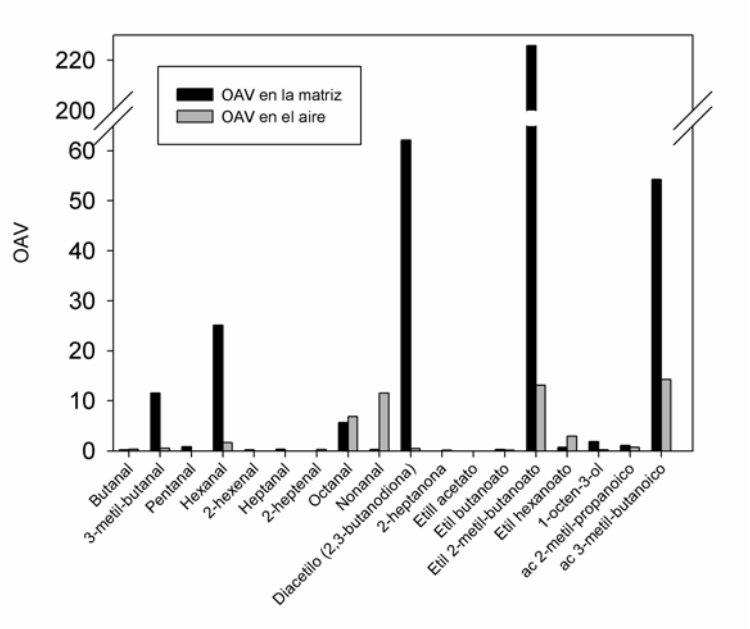

Figura 1. Actividad aromática de los compuestos volátiles de embutidos en la matriz y en el espacio de cabeza.

Los procedimientos de estudio de la determinación del poder aromático de los compuestos volátiles (técnica olfatométrica y el parámetro OAV) empleados en los capítulos 1 y 2 proporcionaron información que puede ser útil para establecer las estrategias tecnológicas que fomenten la generación de los compuestos de interés, como en el caso del capítulo 2 donde se demostró el efecto positivo de la adición de nitrato en el aroma de los embutidos. Sin embargo, cabe destacar que los resultados obtenidos mediante ambos procedimientos (olfatometría y OAV) difieren en parte en

cuanto a la estimación de la importancia de los compuestos volátiles en el aroma. Por un lado, las diferencias detectadas eran esperables debido a que en cada trabajo se estudiaron embutidos distintos (capítulos 1 y 2). No obstante, pudo observarse que el cálculo de OAV subestima el poder aromático de los compuestos en comparación con la técnica olfatométrica, ya que aunque los compuestos analizados habían sido previamente identificados como aromático activos en embutidos mediante GC-O, sólo algunos de ellos presentaron altos OAV. Esto es probablemente debido a que el parámetro OAV depende de los valores umbral empleados para su cálculo, los cuales varían enormemente según la fuente bibliográfica consultada (van Gemert & Nettenbreijer, 2004).

Una vez conocida la evolución de los compuestos volátiles con potencia aromática durante el proceso de secado de los embutidos, se determinó la distribución de los compuestos volátiles entre las fracciones grasa y magra para así conocer la contribución de ambos tejidos en la generación del aroma y además establecer su importancia en la retención de los compuestos volátiles (capítulo 3). Los resultados obtenidos indicaron que ambas fracciones actúan como precursores de los compuestos volátiles, ya que los compuestos derivados de los aminoácidos se hallaron en el magro, y los generados a partir de la grasa se detectaron tanto en el tejido adiposo como en el músculo debido al contenido de grasa intramuscular de éste último. Además, se observó que el tejido adiposo actúa como disolvente de los compuestos volátiles, lo cual afecta a su liberación y por lo tanto a la percepción del aroma.

Estos resultados pusieron de manifiesto el papel destacado de la fracción lipídica en relación al aroma. No obstante, el alto contenido de grasa de los embutidos crudos curados supone un inconveniente para su consumo debido a la relación existente entre las altas ingestas de grasa y la prevalencia de enfermedades cardiovasculares, obesidad, etc. En este sentido, se han adoptado distintas estrategias para mejorar el perfil nutricional de los embutidos. Sin embargo, los trabajos previos han puesto de manifiesto distintos problemas desde el punto de vista tecnológico, ya que la grasa ayuda a controlar el proceso de deshidratación que tiene lugar durante el secado de

los embutidos (Keeton, 1994). Además, es responsable de distintas características organolépticas además del aroma, como la dureza y jugosidad.

Por todo ello, se evaluó el efecto de la reducción del contenido de grasa de los embutidos en su calidad sensorial (capítulo 4), y más concretamente en la generación de compuestos volátiles (capítulo 5), con el objetivo de evitar que la reducción de la grasa produzca una pérdida de las características sensoriales mediante el control de factores tecnológicos.

Mediante el proceso de fabricación de fermentación lenta fue posible controlar las pérdidas de humedad y evitar la deshidratación excesiva de los embutidos. Se observó que la disminución del pH fue más rápida en los embutidos con menores contenidos de grasa, probablemente debido al mayor contenido de agua disponible para el crecimiento microbiano. Por otro lado, tanto la lipólisis como la oxidación lipídica tuvieron lugar de manera más intensa en los embutidos más grasos. En cuanto a los compuestos volátiles presentes en el espacio de cabeza, su abundancia se vio afectada de distinta manera según la vía de generación de éstos. Los compuestos derivados del metabolismo microbiano aumentaron en los embutidos con reducción de grasa, aunque únicamente en las etapas iniciales del proceso de fabricación. En cambio, los compuestos volátiles derivados de la oxidación de la grasa fueron más abundantes en los embutidos con mayores porcentajes de grasa (tejido adiposo), en concordancia con la mayor lipólisis y oxidación lipídica hallada en estos embutidos. Estos resultados indican que el papel de la grasa en el aroma de los embutidos, no se limita a la retención y liberación de los compuestos volátiles debido a su carácter disolvente, tal y como se observó en el capítulo 3, sino que, al tratarse de un producto sometido a un proceso de maduración, la grasa también contribuye al aroma porque actúa como precursora de compuestos volátiles.

Además, en esta experiencia se emplearon técnicas de olfatometría para dilucidar los compuestos con potencia aromática en estos embutidos. Se comprobó que algunos de los compuestos detectados como aromáticos presentaron mayor abundancia en los embutidos con mayores porcentajes de grasa (hexanal, 2-nonenal, 2,4-nonadienal, etil butanoato y 1-octen-3-ol) siendo éstos relacionados con la mayor aceptación sensorial de los mencionados embutidos. No obstante, cabe destacar que, si bien los análisis

sensoriales pusieron de manifiesto que la mayor parte de los consumidores prefiere los embutidos con mayores contenidos de grasa, especialmente cuando éstos han sido sometidos a largos tiempos de curado, es posible reducir el porcentaje de grasa, que normalmente supone un 30% de la materia prima, hasta un 20% sin detrimento de la aceptación sensorial. Esta disminución de grasa supone una reducción del 15% del contenido calórico.

Por otro lado, aunque los embutidos fabricados con distintos porcentajes de grasa habían sido producidos mediante un proceso de fermentación lenta similar al que se aplica a los embutidos de fermentación natural, se observaron ciertas diferencias en el perfil aromático de los embutidos tradicionales (capítulo 1) y el de los fabricados mediante cultivos iniciadores (capítulo 5), aunque se emplearon las mismas técnicas de extracción y de olfatometría en ambos estudios. Los compuestos identificados mediante los análisis olfatométricos de ambos embutidos (tradicionales y fabricados con adición de cultivos iniciadores) se resumen en la **tabla 1**. No obstante, en esta tabla no se incluyen aquellos aromas a los que no se pudo atribuir el compuesto responsable del aroma percibido.

En primer lugar, un mayor número de compuestos aromáticos fueron detectados en los embutidos tradicionales que en los embutidos con cultivos iniciadores, principalmente debido al alto número de ésteres detectados en los primeros, responsables de notas aromáticas descritas como frutal, floral, fresa, fresco, caramelo, etc. Sin embargo, otros grupos químicos como los aldehídos y cetonas fueron detectados en un mayor número de ocasiones en los embutidos con cultivos iniciadores.

Además, de los 40 compuestos mostrados en la **tabla 1**, sólo 15 fueron detectados como aroma activos en ambos embutidos. Varios de estos compuestos comunes mostraron altos valores de frecuencia de detección (por encima de 10) en ambos embutidos, como es el caso de hexanal, bencenoacetaldehído, ácido acético, ácido butanoico, 1-octen-3-ol, 4-metil fenol, metil 3-metil butanoato, etil butanoato y metional. Sin embargo, otros compuestos potentes desde el punto de vista aromático sólo fueron detectados en uno de los embutidos. Por ejemplo, los compuestos 3-metil butanal, 2-nonenal y 2-pentil furano que mostraron altos valores DF en los embutidos

con cultivos iniciadores, mientras que 2-heptenal, etil 2-metil propanoato, α-terpineno y cariofileno únicamente en los embutidos tradicionales. Lógicamente, estos dos últimos compuestos se detectaron en los embutidos fabricados con especias (capítulo 5).

Tabla 1. Compuestos volátiles identificados como aroma-activos en embutidos tradicionales y embutidos con cultivos iniciadores.

Compuestos	Descriptor olfatométrico	LRI*	DF**	
			Embutido tradicional	Embutido con cultivos iniciadores
Aldehídos				
2-Metil propanal	Hierba verde, fresco	589	5	6
3-Metil butanal	Verde, herbal	691		10
Hexanal	Hierba recién cortada, verde	837	12	17
Heptanal	Desagradable	939	5	8
2-Heptenal	Desagradable, col	1007	12	
Octanal	Cítrico	1046	4	13
Bencenoacetaldehído	Rosas	1111	16	10
2-Nonenal	Medicinal	1220		19
2,4-Nonadienal	Seboso	1288		7
Ácidos				
Ácido acético	Vinagre	702	17	20
Ácido butanoico	Queso	873	11	18
Ácido heptanoico	Fresco, herbal	1162	6	
Ácido octanoico	Tostado, café	1236	9	5
Cetonas				
2,3-Butanodiona	Mantequilla, caramelo	633	5	10
2,3-Pentanodiona	Dulce, lácteo	740	5	
2-Octanona	Floral, geranio	1035		16
Alcoholes				
1-Octen-3-ol	Champiñón	1023	20	20
4-Metil fenol	Establo, caballo	1192	16	15
Fenil etil alcohol	Rosas	1195	9	
Ésteres				
Etil acetato	Vegetal	642	4	
Metil butanoato	Frutal	753	4	
Etil 2-metil propanoato	Fresa	784	12	
Metil 2-hidroxi-propanoato	Hierba, verde, fresco	790		4
Metil 3-metil butanoato	Fresa, dulce	802	11	14
Etil butanoato	Frutal, floral	824	17	5
Etil 2-hidroxi-propanoato	Fresco	861	4	
Etil 2-metil butanoato	Frutal	870	9	
Etil 3-metil butanoato	Frutal, floral	874	8	
Etil pentanoato	Floral, fresco, frutal	924	4	
Etil hexanoato	Floral, dulce	1025	10	
Metil benzoato	Frutal, dulce	1147	9	
Metil octanoato	Frutal	1159		10
Metil bencenoacetato	Caramelo	1236	7	
Etil decanoato	Frutal	1424	4	
Compuestos azufrados				
Metanotiol	Podrido, desagradable	472	7	10
Sulfuro de metil etil	Cebolla podrida, desagradable	631		4
Metional	Cebolla, patata cocinada	969	15	15
Terpenos				
α-Terpineno	Madera, metálico	1031	14	
Cariofinelo	Especias, clavos	1443	10	
Furanos				
2-Pentil furano	Caldo de carne, sabroso, metálico	1010		18

*LRI: Índice de retención lineal (columna capilar DB-624; J&W Scientific 60 m x 0.32mm x 1.8 μm).
**DF: valor de frecuencia de detección.

En resumen, de los estudios olfatométricos puede concluirse que el perfil aromático de los embutidos de fermentación natural no sólo se debe a las especias añadidas, tal y como indican otros autores (Di Cagno *et al.*, 2008; Spaziani *et al.*, 2009) sino que es el resultado de la combinación de compuestos volátiles generados mediante procesos bioquímicos durante su elaboración y en menor medida de las especias añadidas. Por último, aunque ambos embutidos analizados fueron producidos mediante un proceso de fermentación lenta, otros factores del proceso de fabricación, como la adición de especias y el tipo de microorganismo presente, influyen en gran medida en el perfil aromático de los embutidos.

Los compuestos detectados como aroma-activos son los compuestos responsables del aroma percibido, y por tanto de la calidad de los embutidos y su aceptación sensorial por parte de los consumidores. Sin embargo, las técnicas convencionales empleadas para su análisis consumen mucho tiempo, ya que requieren una extracción previa y posterior separación cromatográfica. Con el fin de desarrollar un nuevo método rápido para el análisis de los compuestos volátiles, en este trabajo se ha aplicado por primera vez la técnica espectrométrica directa SIFT-MS (Smith & Španěl, 2005) para la medida a tiempo real de compuestos con potencia aromática de embutidos crudos curados (capítulo 6). Esta técnica fue capaz de detectar los cambios producidos en la concentración de compuestos volátiles aroma activos a lo largo del proceso de fabricación, y en algunos casos según el contenido de grasa (butanal, 2-pentenal, hexanal, 2-butanona, 2,3-butanodiona, etil formato, etanol, ácido hexanoico y dimetil disulfuro).

Además, para confirmar la efectividad de la técnica, los resultados obtenidos mediante SIFT-MS fueron comparados con la técnica convencional de análisis GC-MS (capítulo 7). Así, se obtuvieron correlaciones positivas en el caso de los compuestos pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanona, 2-pentanona, etanol, ácido acético y ácido hexanoico. Asimismo, las medidas obtenidas en relación a la concentración de compuestos derivados de la oxidación lipídica (propanal, butanal, pentanal, hexanal y heptanal) fueron positivamente correlacionadas con la determinación del grado de oxidación en los embutidos mediante TBARS ($p < 0.0001$).

Por lo tanto, estos resultados demuestran que SIFT-MS puede emplearse como técnica de control rápido de la calidad de los embutidos.

En resumen, los resultados obtenidos demuestran la contribución específica de determinados compuestos volátiles al aroma de los embutidos crudos curados sometidos a proceso de fermentación lenta, y el efecto que determinados factores tecnológicos tienen sobre ellos. Este es el caso del efecto positivo sobre el aroma de la adición de nitrato. En cuanto a la reducción de grasa, se demuestra que es posible obtener embutidos crudos curados con menos contenido de grasa (20%) que el habitual (30%) con un aroma y calidad sensorial aceptable, siempre y cuando se controlen los parámetros tecnológicos. Por último, se ha confirmado que la tecnología de fabricación y el tipo de microorganismo presente influyen en mayor medida que las especias en el perfil aromático de los embutidos crudos curados.

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VII. Conclusiones

VII. CONCLUSIONES

I. El aroma de los embutidos crudos curados tradicionales (Salchichón de Requena) se caracteriza por la presencia de 31 compuestos aroma-activos, que han sido detectados mediante técnicas olfatométricas. De entre ellos, el ácido acético, etil butanoato, hexanal, metional, 1-octen-3-ol, bencenoacetaldehído y 4-metil-fenol son los más potentes. Estos resultados indican que el perfil aromático de los embutidos se debe principalmente a los compuestos volátiles derivados de reacciones bioquímicas y en menor medida a las especias añadidas. Además, se ha puesto de manifiesto que los ésteres imparten notas aromáticas relacionadas con la aceptación sensorial de dichos embutidos.

II. El valor de la actividad aromática (OAV) calculado en la matriz de los embutidos crudos curados pone de manifiesto la contribución al aroma de los compuestos 2-metil butanal, 3-metil butanal, propanal, pentanal, hexanal, octanal, nonanal, 2,3-butanodiona, etil 2-metil butanoato, etil 3-metil butanoato, 3-metil butanoico, 2-metil propanoico, etil hexanoato y 1-octen-3-ol. En cambio, los OAV calculados en el espacio de cabeza de los embutidos indican que los compuestos volátiles que más contribuyen al aroma son hexanal, octanal, nonanal, 3-metil butanoico, etil 2-metil butanoato y etil hexanoato.

III. Se ha confirmado el efecto positivo de la adición de nitrato en el aroma de embutidos crudos curados. También se ha demostrado la diferente contribución de los tejidos magro y graso a la generación de compuestos aromáticos en embutidos crudos curados. Así, los compuestos volátiles derivados de los aminoácidos están presentes en el magro mientras que los generados a partir de la grasa están tanto en el tejido adiposo como en el tejido magro. Además, el tejido adiposo actúa como disolvente de los compuestos volátiles, lo cual afecta a su liberación y por lo tanto a la percepción del aroma.

IV. El proceso de fermentación lenta permite la obtención de embutidos crudos curados con menores contenidos de grasa sin afectar a la apariencia. La mayoría de los consumidores prefiere los embutidos con mayor contenido de grasa y largos tiempos de curado, aunque es posible reducir el porcentaje de grasa hasta un 20% sin detrimento de la aceptación sensorial. Esta reducción de grasa produce una menor lipólisis, oxidación lipídica y generación de compuestos volátiles derivados de los lípidos, y en las primeras etapas del proceso, una mayor generación de los compuestos volátiles derivados del metabolismo microbiano. Los análisis olfatométricos evidencian que los compuestos volátiles asociados con la aceptación sensorial de los embutidos con mayor contenido de grasa son hexanal, 2-nonenal, 2,4-nonadienal, etil butanoato y 1-octen-3-ol.

V. El análisis de la fracción volátil mediante técnicas olfatométricas de embutidos tradicionales y embutidos elaborados con adición de cultivos iniciadores, ambos de fermentación lenta, revela diferencias en el perfil aromático. Un mayor número de compuestos aromáticos están presentes en los embutidos tradicionales, entre los que cabe destacar los ésteres y los terpenos. En cambio, los aldehídos y las cetonas tienen mayor importancia aromática en los embutidos con cultivos iniciadores.

VI. La técnica de espectrometría de masas directa SIFT-MS se ha revelado como un método rápido aplicable para la cuantificación de compuestos volátiles de embutidos. Está técnica presenta una alta correlación con la técnica convencional GC-MS. Asimismo se ha demostrado su utilidad en la evaluación del estado oxidativo de los embutidos crudos curados.