UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

Avances en la microencapsulación y biodisponibilidad de ácidos grasos omega-3 en derivados cárnicos

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Programa de doctorado en Ciencia de los Alimentos

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ÍNDICE

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Resumen

Los objetivos de esta tesis doctoral fueron: i) optimizar el proceso de elaboración de microcápsulas de aceite de pescado mediante spray-drying a partir de emulsiones monocapa y multicapa para obtener una fuente estable de ácidos grasos poliinsaturados Omega-3 (AGPI ω-3), ii) evaluar la viabilidad de la adición de estas microcápsulas a derivados cárnicos para etiquetarlos como "fuente de ácidos grasos omega-3" sin perjudicar sus características de calidad y iii) evaluar la bioaccesibilidad de AGPI ω-3 en microcápsulas y productos cárnicos enriquecidos.

El proceso de elaboración de emulsiones monocapa y multicapa se optimizó mediante el uso de altas presiones de homogeneización, obteniéndose microcápsulas estables de AGPI ω-3 que se probaron en sistemas modelo y derivados cárnicos (salchichas cocidas y fuet). La adición de las microcápsulas consiguió enriquecer los productos en AGPI ω-3 sin afectar a sus principales características de calidad, aunque disminuyó la intensidad de algunos atributos de flavor y aumentó la percepción del sabor salado. La aceptabilidad e intención de compra de los productos enriquecidos aumentaron cuando se acompañaron de la información sobre la composición y la declaración nutricional "fuente de ácidos grasos omega-3". Los derivados cárnicos enriquecidos con microcápsulas multicapa obtuvieron una mayor bioaccesibilidad intestinal de AGPI ω-3 y protección frente a la formación de compuestos volátiles de oxidación lipídica. El uso de microcápsulas de aceite de pescado monocapa y multicapa es viable para enriquecer derivados cárnicos en AGPI ω-3, recomendándose ajustar el contenido en sal, aromatizantes y mostrar una información precisa en el etiquetado.

Palabras clave: acido eicosapentaenoico y docosahexaenoico, microcápsulas, derivado cárnico

Abstract

The objectives of the present doctoral thesis were: i) to optimize the conditions for the production of fish oil microcapsules by spray-drying from monolayer and multilayer emulsions to obtain a stable source of Omega-3 polyunsaturated fatty acids (PUFA ω-3), ii) to evaluate the possibility of adding these microcapsules to meat derivatives to be labeled as a source of omega-3 fatty acids with appropriate quality characteristics and iii) to evaluate the bioaccessibility of PUFA ω-3 in microcapsules and enriched meat products.

The production process of monolayer and multilayer emulsions was optimized using high pressure homogenization, obtaining a stable source of PUFA ω-3 microcapsules that were tested firstly in model systems and then in meat derivatives (cooked sausages and fuet). The microcapsules addition enriched meat derivatives in PUFA ω-3 without affect their main quality characteristics, however, it decreased the intensity of some flavor attributes and increased the salty taste perception. The acceptability and purchase intent of the fortified products increased when the information on the composition and the nutritional claim "source of omega-3 fatty acids" were included. Meat derivatives enriched with multilayer microcapsules showed greater intestinal bioaccessibility and protection against the formation of volatile lipid oxidation compounds. The use of monolayer and multilayer fish oil microcapsules is suitable for enriching meat derivatives with PUFA ω-3, being necessary to adjust the content of salt, flavorings and showing a precise information on the label.

Keywords: eicosapentaenoic and docosahexaenoic acids, microcapsules, meat derivative

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1. Introducción

El estado del arte del tema desarrollado en la presente tesis se ha estructurado en cinco apartados, en el primero se describen las generalidades de los ácidos grasos omega-3, sus beneficios y declaraciones nutricionales, y las estrategias para enriquecer los alimentos con dichos ácidos grasos. Los apartados segundo y tercero se centran en la búsqueda de trabajos sobre microencapsulación de ácidos grasos omega-3 mediante secado por atomización y su aplicación a alimentos; en el cuarto apartado se trata del desarrollo de derivados cárnicos más saludables y finalmente, en el quinto apartado, se revisan los mecanismos de liberación de ácidos grasos omega-3 durante el proceso de digestión.

1.1. Ácidos grasos omega-3

1.1.1. Características generales

Los componentes mayoritarios de los lípidos son los triacilglicéridos y fosfolípidos que están constituidos por ácidos grasos, que son biomoléculas formadas por una cadena alifática de átomos de carbono con un grupo carboxilo terminal. Los ácidos grasos se pueden clasificar en función de la longitud de su cadena (de cadena corta: de 4 a 6 átomos de carbono; media: con 8 a 12 átomos de carbono; y larga: de 14 a 24 átomos de carbono), y el número y localización de sus dobles enlaces. Esto permite agruparlos en ácidos grasos saturados (AGS), aquellos que no presentan ningún doble enlace, monoinsaturados (AGMI), los que tienen un solo enlace doble, y poliinsaturados (AGPI), con dos o más enlaces dobles. A su vez, los AGPI se agrupan en familias según el carbono en el que se sitúa el primer doble enlace a partir del grupo metilo terminal, encontrándose así las familias omega-9, omega-6 y omega-3. La familia omega-3 (ω-3 o n-3), está constituida por los ácidos grasos cuyo primer doble enlace se encuentra en el carbono 3 (C-3), omega-6 (ω-6 o n-6), cuando el primer doble enlace se sitúa en el carbono 6 (C-6) y omega-9 (ω-9 o n-9), cuando el primer doble enlace se encuentra en el carbono 9 (C-9). Dentro de la familia de los AGPI ω-3 destacan el ácido α-linolénico (ALA, C18:3 ω-3), el ácido eicosapentaenoico (EPA, C20:5 ω-3) y el ácido docosahexaenoico (DHA, C22:6 ω-3). La familia de los AGPI ω-6 está principalmente representada por el ácido α-linoleico (LA, C18:2 ω-6), ácido gamma linolénico (GLA, C18:3 ω-6) y ácido araquidónico (ARA, C20:4 ω-6).

ALA junto con LA son ácidos grasos esenciales en la dieta, ya que no pueden ser sintetizados por el organismo, y por ello es necesario ingerirlos con la dieta. Las principales fuentes de ALA son las nueces, y especialmente los aceites vegetales de linaza, colza, cártamo, soja, onagra y lino. No obstante, la mayoría de estas semillas y sus correspondientes aceites también contienen elevadas concentraciones de ácido linoleico (LA, C18:2 ω-6) y, por lo tanto, la ratio ω-6/ω-3 alcanza valores por encima de los niveles recomendados (<4) en estos derivados [1]. A partir de LA y ALA, pueden sintetizarse otros AGPI ω-6 y ω-3 cadena larga como GLA-ARA y EPA-DHA, respectivamente, como se muestra en la **figura 1**. No obstante la biosíntesis de EPA y DHA a partir de ALA es un proceso de baja eficiencia metabólica en el adulto, siendo la conversión de ALA a EPA inferior a

5% y a DHA inferior a 1% [2]. Esto es debido principalmente a las altas cantidades de LA procedentes de la dieta y a la competencia entre ALA y LA por las mismas enzimas y sistemas de transporte (Δ4, Δ5 y Δ6 desaturasas y elongasas, que son los reguladores más importantes de estas dos rutas metabólicas) [3]. Incluso un incremento de la ingesta de ALA en la dieta es insuficiente para lograr unos niveles adecuados de AGPI ω-3. En consecuencia, la alimentación es la principal fuente de AGPI ω-3 en el ser humano [4], siendo los aceites de pescado y el pescado azul las fuentes más ricas de estos ácidos grasos, [5]. No obstante, la cantidad de AGPI ω-3 puede variar en función de la especie de pescado, su localización, así como la estación del año y la disponibilidad de fitoplancton.

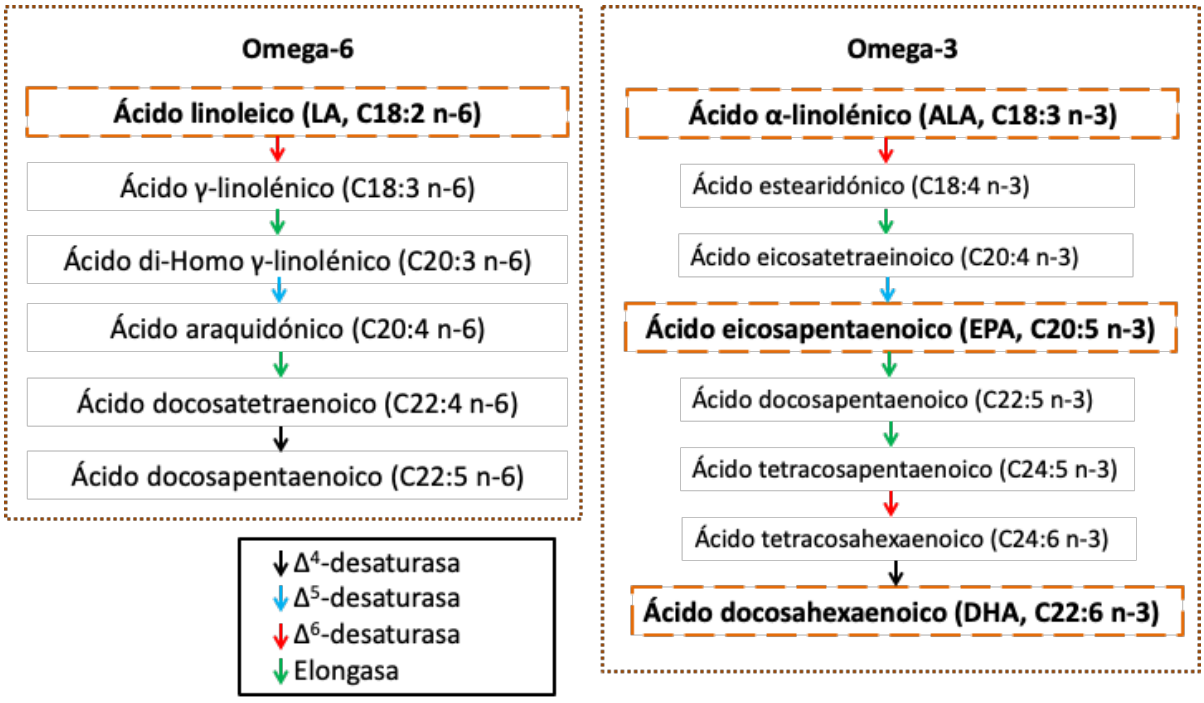


Figura 1. Rutas metabólicas de los ácidos grasos poliinsaturados.

1.1.2. Efectos beneficiosos para la salud y declaraciones nutricionales

Los AGPI ω-3 han demostrado tener una amplia variedad de efectos beneficiosos para la salud de los consumidores ejerciendo dicho efecto a través de varios mecanismos de actuación:

- Intervienen en la regulación de la glucosa en sangre, disminuyendo el riesgo de padecer diabetes tipo 2 mediante su incorporación en los fosfolípidos de la membrana celular. Esta unión aumenta la sensibilidad de proteínas de la membrana celular, como es el caso del receptor de la insulina, que, al estar más expuesto al medio aumenta el paso de la glucosa al interior de la célula [6].
- Previene o mejora algunas enfermedades inflamatorias como artritis reumatoide, colitis ulcerosa o asma. Todas estas patologías cursan con una elevada concentración de

derivados lipídicos proinflamatorios como prostaglandinas, leucotrienos y citoquinas. Los AGPI ω-3 ejercen su función antiinflamatoria a partir de la regulación de genes blanco al ligarse a los receptores (kB, PPAR o GPR 120) o mediante la producción de sustancias como protectinas y resolvinas generadas por una serie de reacciones de elongación y desaturación por parte de las enzimas Δ^6 y Δ^5 desaturasas, provocando una reducción en el número de citoquinas proinflamatorias [7].

- Intervienen en el correcto funcionamiento de los tejidos neuronales como el cerebro, la retina y las membranas sinápticas que en condiciones fisiológicas contienen elevadas cantidades de DHA. Sin embargo, deficiencias en el consumo de AGPI ω-3 pueden ocasionar alteraciones en la transmisión dopaminérgica en la corteza frontal y en la actividad celular de la bomba y los canales de sodio. Incluso la composición lipídica de las membranas celulares afecta a la estructura terciaria y cuaternaria de los receptores colinérgicos, dopaminérgicos y adrenérgicos y por consiguiente a la correcta transmisión del impulso nervioso [8].
- Disminuye la incidencia de padecer enfermedades cardiovasculares (ECV) como la cardiopatía isquémica y el infarto de miocardio al actuar sobre los lípidos plasmáticos, disminuyendo las concentraciones de triglicéridos y aumentando el transporte de colesterol por las lipoproteínas de alta densidad (HDL), además de presentar un efecto antitrombótico por su acción sobre la agregación plaquetaria, previniendo la formación de coágulos sanguíneos y depósitos grasos [9].

Debido a los beneficios del consumo de AGPI ω-3 para la salud, la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) y la Organización Mundial de la Salud (OMS) en un informe emitido en el año 2003 sobre dieta, nutrición y prevención de enfermedades cardiovasculares, recomendaban un porcentaje de AGPI totales en torno al 6-10% de la energía total, y de estos, los AGPI ω-3 deberían suponer el 1-2% [10]. Con relación a los niveles establecidos por la FAO y OMS, la fundación británica de nutrición (BNF) y la Asociación Americana del Corazón (AHA) en 2007 y 2009 respectivamente, han hecho recomendaciones específicas sobre la ingesta mínima de AGPI ω-3, la cual está en adultos sobre 500 mg/día de EPA + DHA. También la Autoridad Europea de Seguridad Alimentaria (EFSA) estableció en 2010 una Ingesta Adecuada (AI) de 0.5 % de la energía total diaria para ALA y 0.25 g/día para la suma de EPA más DHA [11]. La recomendación de la Sociedad Internacional para el Estudio de Ácidos Grasos y Lípidos (ISSFAL) en 2004 estableció un consumo diario de la suma de EPA y DHA de 0.65 g y un mínimo de 0.22 g de cada uno de estos ácidos grasos [12]. Sin embargo, aunque las pautas de consumo de estos ácidos grasos son claras, las sociedades occidentales modernas tienden a incluir poco pescado en la dieta, siendo evidenciada una ingesta por debajo de las

recomendaciones internacionales para la población adulta [13]. Los efectos beneficiosos de los AGPI ω-3 junto con la ingesta insuficiente de éstos, han propiciado, que en los últimos años haya un creciente interés en el desarrollo de suplementos dietéticos y alimentos enriquecidos en estos ácidos grasos.

En la actualidad el Reglamento (UE) nº 116/2010 de 9 de febrero de 2010 [14] en lo relativo a la lista de declaraciones nutricionales indica que para que un alimento pueda declararse y etiquetarse como "fuente de ácidos grasos omega-3" debe contener al menos 0.3 g de ALA o 40 mg de la suma de EPA y DHA por 100 g y por 100 Kcal. En el caso de declarar un alimento con "alto contenido en ácidos grasos ω-3", el derivado deberá de contener el doble de las cantidades mencionadas anteriormente por 100 g y por 100 Kcal.

1.1.3. Estrategias para enriquecer alimentos en ácidos grasos omega-3

La preocupación por la salud se está convirtiendo en un factor determinante a la hora del consumo de alimentos [15], observándose un incremento en la intención de compra de derivados que contienen información relativa a propiedades saludables [16,17] y relacionados con una disminución del riesgo de sufrir determinadas enfermedades [18].

Con relación a los derivados cárnicos, se han investigado distintos procedimientos para incorporar AGPI ω-3, principalmente debido a los efectos beneficiosos que estos compuestos bioactivos tienen sobre la salud como se ha comentado anteriormente, y también por las oportunidades de comercialización por parte de las industrias del sector cárnico como potencial nicho de mercado. En la **tabla 1** se recogen las principales estrategias llevadas a cabo para incorporar AGPI ω-3 a diferentes derivados cárnicos, así como los principales efectos observados, llevándose a cabo la suplementación bien a través de la alimentación animal, mediante la adición directa de vegetales y aceites a distintos alimentos o la incorporación de estos últimos en forma de pre-emulsión o microencapsulados.

Tabla 1. Estrategias para incrementar el contenido de AGPI ω-3 en carne y derivados cárnicos. NI: no indicado; ↑, ↓, = incremento, disminución o sin cambios en comparación con el lote control.

Estrategia de incorporación	Fuente de ω-3	Especie animal	Derivado cárnico	Ácidos grasos			Oxidación	Calidad sensorial	Referencia
				ALA	EPA	DHA			
Alimentación animal	Aceite de linaza	Cerdo	-	↑	NI	NI	↑	↓	[19–22]
		Pollo	-	↑	↑	↑	NI	=	[23,24]
		Pavo	-	↑	↑	↑	↑	↓	[25]
	Aceite de pescado	Cerdo	-	NI	↑	↑	↑	↓	[26,27]
		Pollo	-	NI	↑	↑	↑	↓	[28–30]
	Alga	Cerdo	-	NI	↑	↑	↑	↓	[27]
		Pollo	-	NI	↑	↑	NI	NI	[31]
Adición directa	Aceite de pescado	Vacuno	Empanadas de carne	NI	NI	NI	↑	↓	[32]
	Nuez	Vacuno	Filete de Vacuno reestructurado	↑	↑	↑	↑	↓	[33]
	Aceite de pescado	Cerdo	Chorizo	Ni	↑	↑	↑	↓	[34]
	Aceite de oliva, linaza y pescado	Cerdo	Salchichas Frankfurt	NI	NI	NI	↑	↓	[35]
Pre-emulsión	Aceite de alga	Cerdo	Pechuga de pavo, salchicha y jamón reestructurado	NI	NI	NI	↑	↓	[36]
	Aceite de linaza	Cerdo	Salchicha fresca	↑	=	=	↑	=	[37]
		Cerdo	Mortadela	↑	NI	NI	↑	=	[38]
		Pollo	Salchichas frankfurt con antioxidante	↑	NI	NI	↓	↓	[39]

Estrategia de incorporación	Fuente de ω-3	Especie animal	Derivado cárnico	Ácidos grasos			Oxidación	Calidad sensorial	Referencia
				ALA	EPA	DHA			
Microcápsula	Aceite de pescado	Cerdo	Mortadela con antioxidante	NI	↑	↑	=	=	[40]
		Cerdo	Salchichón con antioxidante	NI	↑	↑	=	=	[41]
		Cerdo	Salchichas frescas con antioxidante	NI	↑	↑	=	=	[37]
		Cerdo	Salchichón	NI	↑	↑	=	=	[42]
		Cerdo	Mortadela	NI	↑	↑	↑	=	[38]
	Aceites de oliva, linaza y pescado	Cerdo	Salchichas Frankfurt	↑	↑	↑	↑	=	[43]
	Aceites de linaza y alga	Cerdo	Mortadela con antioxidante	↑	NI	↑	=	=	[44]
	Aceite de pescado	Pollo	Nuggets	NI	NI	NI	=	=	[45]
		Cerdo	Salchichón	=	↑	↑	↑	↑	[46]
		Cerdo	Hamburguesa	NI	↑	↑	=	=	[47]
		Cerdo	Salchichón	=	↑	↑	↑	↓	[47]
	Aceite de linaza	Cerdo	Salchichón	↑	↑	↑	↑	↓	[46]

En relación con la suplementación a través de la alimentación animal, se han usado distintas combinaciones de semillas oleaginosas, forrajes y aceites ricos tanto en ALA como en EPA y DHA [49]. La mayoría de los estudios se han centrado en animales monocavitarios, en su mayor medida aves de corral y cerdos, ya que en animales policavitarios, tras la lipólisis, los ácidos grasos insaturados son biohidrogenados por los microorganismos del rumen, convirtiéndolos en su mayoría en AGS [50] lo que hace que esta estrategia no sea eficiente. En el caso de animales monocavitarios, fundamentalmente se ha empleado aceite de linaza como fuente de ALA con el fin de incrementar la biosíntesis de EPA y DHA. Sin embargo, aunque en la mayoría de los casos se ha observado un incremento en el contenido de EPA y DHA, acompañado de la reducción de la ratio ω-6 / ω-3, la cantidad de dichos ácidos ha sido baja, lo cual está relacionado con la baja tasa de conversión de ALA no solo en el organismo humano, como ya se ha mencionado anteriormente, sino también en la mayoría de las especies animales utilizadas en la industria alimentaria. Por otro lado, los derivados cárnicos obtenidos de animales sometidos a este tipo de dietas experimentales exhibieron mayores valores de oxidación lipídica y peores puntuaciones en determinadas características sensoriales, relacionadas fundamentalmente con un flavor desfavorable a rancio [51]. Otros estudios se han centrado en la adición directa de aceites ricos en EPA y DHA en los piensos, fundamentalmente aceites de pescado y alga [26–28,31]. Sin embargo, el porcentaje de inclusión de fuentes de AGPI ω-3 es muy limitado (0.2-0.4 %) debido al aumento en los valores de oxidación lipídica, aparición de atributos sensoriales indeseables como olores y flavores “a pescado” y “a rancio” y modificaciones de las propiedades texturales relacionadas con un incremento de la oleosidad, siendo fácilmente detectadas por los consumidores [52] y provocando un rechazo de los derivados cárnicos elaborados con este tipo de materias primas. También se han llevado a cabo estudios que han incluido distintos antioxidantes como tocoferol en estas dietas enriquecidas con aceite de pescado, obteniéndose derivados cárnicos con mayor estabilidad oxidativa, pero persistiendo los problemas asociados con la calidad sensorial [53].

Por ello, con la finalidad de aumentar la estabilidad oxidativa de estos aceites, en lugar de adicionarlos de forma directa, en los últimos años se ha investigado sobre la emulsificación como estrategia para vehicular biocompuestos lipídicos altamente insaturados. Relacionado con esto, diferentes tipos de emulsiones han sido evaluadas, variando fundamentalmente el tipo de aceite empleado: pescado [37,38,40–42], alga [36] o linaza [37–39] fundamentalmente, solos o combinados; el tipo de emulsionante (caseinato de sodio, aislado de proteína de soja o aislado de proteína de suero) y su concentración, ya que en la mayoría de los casos suele oscilar en torno al 10 % con respecto a la cantidad de aceite, además de los procedimientos para la estabilización de las emulsión (tipo de homogeneización, tiempo, y temperatura de la emulsión) y la adición o no de antioxidantes naturales como las vitaminas E y C, carotenoides y compuestos fenólicos o

sintéticos como el Butilhidroxianisol (BHA), Butilhidroxitolueno (BHT) y Butilhidroquinona Terciaria (TBHQ) [54].

En general, los derivados cárnicos enriquecidos con fuentes de AGPI ω-3 emulsificados y no adicionados con antioxidantes muestran mayores valores de oxidación lipídica y peores puntuaciones en las características sensoriales, en contraste con los derivados cárnicos enriquecidos con fuentes de AGPI ω-3 emulsificados y adicionados con antioxidantes, con valores similares de oxidación lipídica pero iguales o peores puntuaciones en las características sensoriales al compararlos con sus análogos no enriquecidos [55]. Por lo tanto, aunque la adición de emulsiones de AGPI ω-3 logra un perfil de ácidos grasos más saludable en los derivados cárnicos fortificados, la oxidación lipídica y la disminución de la calidad sensorial constituyen importantes problemas cuando se emplea esta estrategia.

Por ello, en los últimos años ha cobrado importancia la microencapsulación como estrategia para proteger los AGPI ω-3 frente a la oxidación, minimizando el contacto y reactividad del sustrato con el agua, oxígeno, hierro y otros promotores oxidantes. De forma muy simple esta técnica se fundamenta en "envolver" a los ingredientes funcionales en una matriz que actúa de estructura protectora [56]. Como se muestra en la **tabla 1**, el enriquecimiento con EPA y DHA ya se ha logrado con éxito en salchichón, hamburguesas de cerdo y nuggets de pollo [57–60], mostrando los derivados enriquecidos con microcápsulas de aceite de pescado valores de oxidación y características sensoriales similares a los no adicionadas con fuentes de AGPI ω-3 microencapsulados y muy inferiores a las registradas en alimentos enriquecidos con aceites de pescado adicionados de forma directa o emulsificada, lo cual indicaría, que el uso de microcápsulas podría ser la estrategia más adecuada para enriquecer derivados cárnicos. No obstante, resulta necesario llevar cabo más investigaciones que centren sus esfuerzos en elaborar y caracterizar distintas microcápsulas que se adecúen a los diferentes derivados cárnicos, los cuales difieren en ingredientes, procesado, estructura, tratamiento culinario, etc.

1.2. Microencapsulación

1.2.1. Generalidades, objetivos y aplicaciones de la técnica.

La microencapsulación se define como la técnica o proceso para recubrir una sustancia en estado sólido, líquido o gaseoso (sustancia activa o núcleo) a través de la aplicación de una cubierta delgada denominada membrana, matriz o pared constituida por otro u otros materiales de distinta naturaleza (generalmente poliméricos), conformando una estructura de membrana sólida, esférica y semipermeable que recubre la sustancia encapsulada. El producto resultante muestra un aspecto macroscópico en forma de polvo y un diámetro interno inferior a 1 μm

(micrómetro) y usualmente es denominado como "microcápsula", "microesfera" o "micropartícula" [61,62].

El origen de esta técnica se remonta al año 1931, siendo sometida a diversas variaciones durante la década de los cuarenta, estando su aplicación prácticamente limitada a la industria gráfica, que empleaba dicha técnica para encapsular colorantes en la elaboración de papel de calco sin carbón. Sobre la década de los setenta se hallaron interesantes aplicaciones de esta técnica en diversos sectores industriales como la industria textil, metalúrgica, química, cosmética, alimentaria, farmacéutica y médica. Respecto a su empleo en la industria alimentaria, puede afirmarse que su uso se ha incrementado ampliamente con el fin de convertir líquidos en sólidos o para enmascarar el sabor desagradable de determinados ingredientes alimentarios. En la actualidad, se encuentra en plena expansión ya que la pared de la microcápsula protege a los compuestos encapsulados de factores como el calor, la humedad, el oxígeno o la luz, otorgándoles mayor estabilidad y durabilidad. La microencapsulación ayuda a que determinados compuestos, que forman parte de las diferentes formulaciones alimentarias resistan condiciones de procesamiento, almacenamiento, transporte y comercialización, mejorando de esta forma el sabor, aroma, estabilidad, valor nutritivo y apariencia general del alimento adicionado [63].

Como ya se ha mencionado, esta técnica puede utilizarse con diferentes propósitos en el área de la industria alimentaria y que van a depender fundamentalmente del tipo de alimento al que se aplica y de la sustancia o principio activo que se pretende microencapsular. Así, entre las distintas aplicaciones en la industria alimentaria destacan:

- ✓ Disminuir la volatilidad de la sustancia encapsulada hacia la matriz alimentaria, limitando las posibles modificaciones del perfil odorífero del producto original [64].
- ✓ Enmascarar el mal olor y sabor de algunas sustancias empleadas como aditivos en el procesado de alimentos, evitando los posibles efectos negativos sobre el perfil del producto [65].
- ✓ Liberar de forma controlada la sustancia recubierta en un determinado momento y lugar concreto, bajo unas condiciones específicas de humedad, pH, tensión superficial, acción enzimática, etc., mejorando la absorción de los compuestos microencapsulados debido a su liberación específica y controlada en el tracto gastrointestinal de los consumidores [66].
- ✓ Limitar la exposición del material encapsulado a factores ambientales promotores de la oxidación como la luz, calor, humedad, iones metálicos y oxígeno, otorgándoles mayor estabilidad y durabilidad al proporcionar una barrera física entre el compuesto

microencapsulado y el ambiente que retrasa la auto-oxidación y limita la movilidad de los radicales libres por efecto protector de los materiales de pared [67].

- ✓ Mejorar la manipulación de las sustancias microencapsuladas, ya que con esta técnica se puede transformar un material en estado líquido a sólido, dando lugar a un producto en polvo, más fácil de manejar y almacenar que el producto sin encapsular y facilitando su dosificación, así como la distribución uniforme a lo largo de toda la matriz alimentaria [68].
- ✓ Introducir en el interior de la matriz alimentaria compuestos bioactivos protegidos, capaces de resistir las condiciones de procesamiento, almacenamiento, transporte y comercialización [69].

En el caso concreto de la microencapsulación de AGPI ω-3, se ha estudiado sobre la producción de partículas sólidas mediante esta técnica, como estrategia para limitar la degradación del aceite de pescado por exposición a promotores oxidantes y mitigar los posibles efectos negativos sobre el perfil sensorial de los derivados enriquecidos [47,48,70]. Así tras la microencapsulación, se obtiene un producto en forma de polvo donde el aceite de pescado queda recubierto por uno o varios materiales de pared, evitando la transferencia de olores y sabores desagradables a la matriz alimentaria e incrementando la estabilidad oxidativa y durabilidad del compuesto encapsulado.

La viabilidad de esta técnica viene determinada por la suma de varios factores como la eficiencia del proceso de microencapsulación y la estabilidad oxidativa de los AGPI ω-3 durante el almacenamiento, que están directamente correlacionado con la ratio núcleo/material de recubrimiento, y materiales de pared empleados en la elaboración de las emulsiones, entre otros factores [71].

1.2.2. Materiales de pared

Antes de llevar a cabo la microencapsulación de cualquier compuesto, el primer paso es la elección apropiada del material o materiales de recubrimiento, ya que las propiedades fisicoquímicas de los microencapsulados dependen en gran medida de los constituyentes de la cubierta. La elección de un material u otro depende de su aplicación final en la matriz alimentaria, el momento deseado de liberación de la sustancia, el método de microencapsulación seleccionado y las condiciones ambientales a las que las microcápsulas serán sometidas. A continuación, se mencionan algunas de las características que debería de tener un buen material de pared para su uso como componente de recubrimiento en microencapsulados de destino alimentario:

- ✓ Ser capaz de emulsionar y estabilizar el material activo sin reaccionar con él.

- ✓ Sellar y mantener la sustancia o principio activo en el interior de su estructura durante todo el proceso.
- ✓ Mostrar baja viscosidad a altas concentraciones.
- ✓ Baja capacidad de absorción de humedad atmosférica a fin de evitar su aglomeración y facilitar su dosificación.
- ✓ Ser estable en la matriz alimentaria donde será adicionado.
- ✓ Permitir la liberación completa del compuesto bioactivo en el lugar deseado del tracto gastrointestinal.
- ✓ Capacidad para tener un cambio de fase como fusión o gelificación.
- ✓ No mostrar olor ni sabor que puedan influir en el perfil sensorial del producto.

Muchos materiales de pared disponibles poseen estas propiedades, pero el número de ellos aprobados para uso alimentario es limitado [72]. Se han empleado biopolímeros de diversas fuentes en la encapsulación de ingredientes alimentarios como lípidos (lecitinas, ceras, parafinas, aceites hidrogenados o ésteres de ácidos grasos), carbohidratos (almidones, almidones modificados, β-ciclodextrinas, maltodextrinas con diferentes equivalentes de dextrosa, gomas y quitosanos) y proteínas (caseinatos, gelatinas o ceras).

Los lípidos son excelentes formadores de películas, capaces de cubrir las partículas individuales, proporcionando una encapsulación uniforme alrededor del compuesto bioactivo a microencapsular [73], normalmente actúan como emulsificantes en el transcurso de elaboración de las emulsiones como paso previo a la microencapsulación.

Los carbohidratos son extensivamente utilizados en la encapsulación. Dentro de este amplio grupo se encuentran los almidones, considerados buenos agentes de recubrimiento al mostrar bajas viscosidades a altas concentraciones y buena solubilidad [74], pero la mayoría de ellos carecen de las propiedades interfaciales necesarias para lograr una alta eficiencia en la microencapsulación. Un enfoque novedoso para mejorar las propiedades de encapsulación de estos materiales de pared consiste en modificaciones químicas de los carbohidratos, dando lugar a almidones modificados con propiedades de superficie activa [75]. La maltodextrina es considerada como un material de pared adecuado para la encapsulación de ingredientes alimentarios lipofílicos al proporcionar una buena estabilidad oxidativa, ser económica, con elevada solubilidad en agua y con capacidad para la formación de emulsiones estables [76]. Otro material de pared comúnmente utilizado en encapsulación es el quitosano, un polisacárido catiónico soluble en medio ácido y obtenido por desacetilación de la quitina, con una amplia gama

de bioactividades como antioxidante, antimicrobiano o antifúngico, con alta capacidad para la formación de emulsiones y películas y una adecuada estabilidad oxidativa [77].

Las proteínas se utilizan habitualmente para encapsular materiales de núcleo lipídico debido a su carácter anfifílico, alta actividad emulsionante y estabilizadora, solubilidad en agua y tendencia a formar una red fina y densa [78], se suelen emplear en combinación con carbohidratos, con el fin de suplir sus pobres propiedades interfaciales, necesitando la ayuda de un agente tensioactivo para encapsular materiales a base de aceite. Entre las más empleadas en la microencapsulación se encuentra el caseinato de sodio, proteínas del lactosuero, aislados de proteínas vegetales procedentes de la soja o trigo o gelatinas, pero sin lugar a duda, la más utilizada por su alta solubilidad en agua, facilidad de rehidratación y estabilidad térmica es el caseinato de sodio [79].

1.2.3. Emulsiones de ácidos grasos omega-3 para microencapsulación

La microencapsulación de AGPI ω-3 mediante la técnica de secado por atomización comienza con la producción de una emulsión simple de aceite-en-agua (O/W, *oil-in-water*) donde la fase oleosa está constituida por pequeñas gotas de naturaleza lipídica (aceite rico en AGPI ω-3), o emulsiones dobles (O/W/O, *oil-in-water-in-oil*), que son sistemas multicompartimentados caracterizados por la coexistencia de emulsiones de aceite-en-agua, en las que los glóbulos de la fase dispersa contienen dentro de ellos gotas igualmente dispersas más pequeñas de grasa, requiriendo este último tipo de emulsión un combinación de dos tipos de emulsionantes (uno de naturaleza hidrofílica y otro hidrofóbico) para la estabilización de la interfaz final de la emulsión O/W/O que contendrá el aceite rico en AGPI ω-3, como es el caso del aceite de pescado, y el material que se empleará como pared aparece disuelto en una fase continua de carácter acuoso.

La emulsión resultante constituye un sistema termodinámicamente inestable debido a la tensión superficial existente entre ambas fases monocapa [80]. Sin embargo, estos fenómenos pueden reducirse con el uso de surfactantes, que estabilizan cinéticamente la muestra, al tratarse de compuestos orgánicos anfifílicos que disminuyen la tensión interfacial e impiden la formación de espuma durante el proceso de homogeneización. De esta forma se obtienen emulsiones con rangos de tamaños de partícula extremadamente variables que oscilan entre 80 nm y 100 μm [81,82], por lo cual, se hace necesario prolongar la estabilidad de estas emulsiones a través de una reducción mecánica del tamaño de los glóbulos. La homogeneización a alta presión es una técnica muy utilizada en la elaboración de emulsiones con la finalidad de prevenir la floculación o separación de fases y reducir el tamaño del glóbulo hasta alcanzar valores en torno a 6-12 μm [83,84]. El proceso general de homogeneización a alta presión se encuentra esquematizado en la **figura 2**. En esta operación, la premezcla de fluidos que formará la emulsión es depositada en una tolva de alimentación y a través de la válvula de entrada pasa a una cámara de homogeneización, donde bajo el efecto de una presión elevada en el rango de 6.8-34 MPa (1000-5000 psi) y previo

ajuste de las válvulas homogeneizantes hasta la presión deseada indicada en el manómetro, los fluidos son sometidos a una combinación de fuerzas de elongación, cizallamiento, impactos y cavitación, recogiendo el resultado del homogeneizado a través de un tubo de recirculación [85].

Figura 2. homogeneizador a alta presión (SPX, modelo APV-200a).

Además de emulsiones simples [86] o dobles [87] se han desarrollado técnicas basadas en la superposición de materiales de pared con el fin de incrementar la protección de los AGPI ω-3, dando lugar a las emulsiones multicapa [67], donde las gotas de aceite se rodean por múltiples capas de material de cobertura, constituidas por una combinación de emulsionantes y uno o varios polielectrolitos de cargas opuesta, unidos mediante la técnica de deposición electrolítica capa por capa, del inglés "Layer-by-layer," como aparece representado en la **figura 3**.

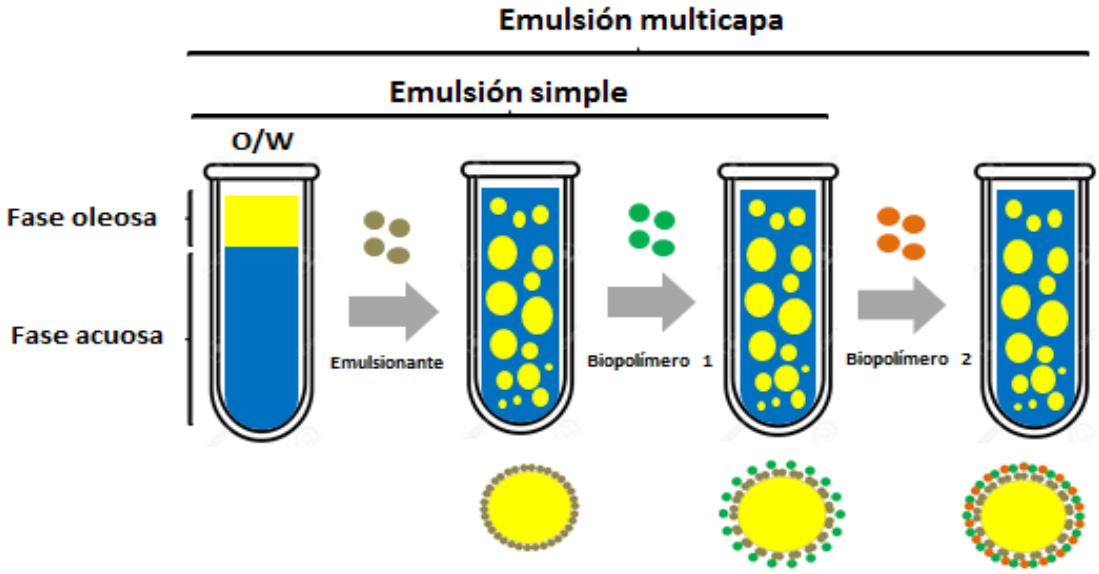


Figura 3. Producción de emulsión simple y multicapa mediante superposición polielectrolítica (elaboración propia).

Esta técnica consiste en la formación de una emulsión primaria, tras la integración del emulsionante iónico en las gotas de aceite de aceite previa homogeneización. El proceso continúa con la adición de un biopolímero (biopolímero 1) de carga opuesta al emulsionante, el cual queda adherido por interacción electrostática a la superficie de las gotas de grasa, originándose así la emulsión secundaria, adicionando a continuación otro biopolímero (biopolímero 2) de igual carga al 1, cuya función no es otra que la de reforzar la protección de la emulsión secundaria, al mejorar la estabilidad física y química de los componentes microencapsulados además de permitir una liberación específica y controlada de los compuestos bioactivos, pudiendo repetirse este proceso tres o más veces [88].

1.2.4. Microencapsulación mediante secado por atomización (spray drying)

El secado por atomización de emulsiones es una de las tecnologías de microencapsulación más utilizadas en la industria alimentaria porque el proceso es flexible, económico, eficiente, fácil de ampliar y produce un polvo de buena calidad [89], siendo aplicado a un amplio rango de materiales como grasas, aceites, sabores e ingredientes solubles en aceite

Dicho proceso se encuentra esquematizado en la **figura 4** e implica la dispersión de un material central en una solución de uno o varios biopolímeros, formando un líquido o emulsión que es conducido por una bomba peristáltica hacia una tobera de pulverización, donde una corriente concéntrica de aire caliente es mezclada con las gotas dispersas de la emulsión, evaporando instantáneamente el agua a través del cilindro de pulverización y produciendo un polvo (microcápsula) que tras pasar por un ciclón de separación de partículas es almacenado en un

recipiente de recogida de producto, obteniendo finalmente microcápsulas con un rango de tamaños que oscilan entre los 10-50 µm a 2-3 nm [61,90].

La evaporación de la fase acuosa de la emulsión se alcanza por una transferencia de calor entre el aire seco y el líquido a través del aporte de una corriente concéntrica de temperatura de entrada que oscila ente los 150-220 °C, ocurriendo una evaporación instantánea y exponiendo el polvo seco a una temperatura de salida moderada de 50-60 °C. A pesar de la elevada temperatura de contacto gota-aire caliente, el impacto en la estabilidad oxidativa de los ácidos grasos encapsulados mediante esta técnica es bajo, ya que la emulsión solo queda expuesta durante fracciones de segundo a dichas temperaturas y durante este periodo de tiempo, se establece un balance de transferencia de agua que ocasiona una refrigeración mientras se produce la evaporación del medio líquido [91,92].

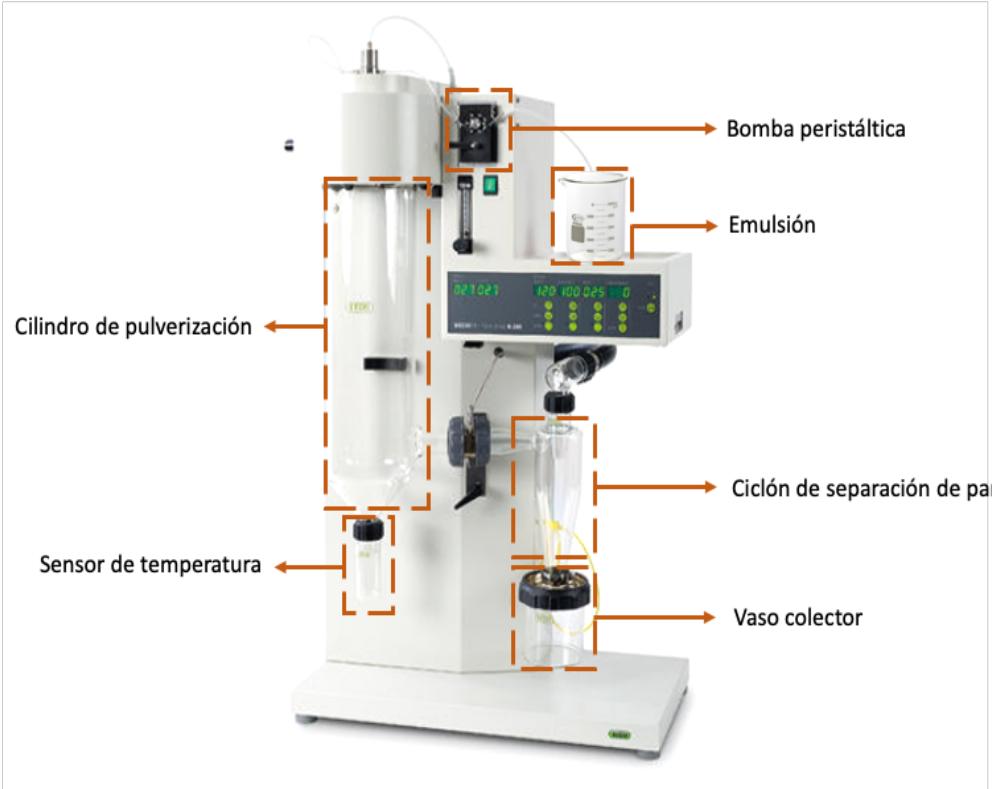


Figura 4. Secado por atomización "spray-drying" (Spray Dryer Buchi B-290).

1.3. Aplicación de la técnica de secado por atomización para microencapsular ácidos grasos omega-3

Con el objetivo de proteger de la oxidación a los aceites ricos en AGPI ω-3, se han venido realizando en las últimas décadas estudios en los que se evalúa la técnica de microencapsulación mediante spray-drying con el fin de producir microcápsulas estables [88, 89]. Para ello se han aplicado distintos aceites y materiales de recubrimiento que dependen del momento deseado de liberación del compuesto bioactivo, de las condiciones ambientales a las que serán sometidos

dichos microencapsulados y de su aplicación de destino en función de la matriz alimentaria susceptible de ser fortificada [93]. Tras evaluar todos estos factores, algunos investigadores sugieren la combinación de distintos materiales para lograr una encapsulación uniforme alrededor del compuesto bioactivo que incluyen en la mayoría de los casos un biopolímero con carácter emulsionante como la lecitina o ésteres de mono y diglicéridos de ácidos grasos y una combinación de uno o varios materiales de pared que aseguren el sellado y el mantenimiento del principio activo en el interior de la matriz durante todo el proceso [71,91,92,94,95].

Varios estudios han aplicado la microencapsulación de aceite de pescado como fuente de AGPI ω-3 a partir de emulsiones monocapa y multicapa mediante la técnica de deposición electrostática, empleando una combinación de lecitina-quitosano junto con distintos tipos de carbohidratos como material de recubrimiento [58,60,86,96]. En la mayoría de estos estudios se observó que las emulsiones multicapa de aceite de pescado eran más estables que el aceite de pescado solo o emulsionado junto con la lecitina y que sus correspondientes microcápsulas, obtenidas mediante la técnica de spray-drying, mostraron una elevada estabilidad oxidativa, muy por encima del propio aceite de pescado y de las emulsiones de partida [57,97–100]. Estos resultados ponen de manifiesto que la combinación de varios materiales de pared mediante la técnica de deposición electrostática junto con el secado por atomización de las emulsiones resultantes dan lugar a un producto con una elevada estabilidad oxidativa, capaz de soportar las distintas condiciones que tienen lugar durante el procesado de alimentos como son los tratamientos térmicos de cocción, escaldado, pasteurización, esterilización, calentamiento culinario, congelación, descongelación, liofilización y altas concentraciones de sodio [57–60,101], convirtiéndose en una opción prometedora a la hora de enriquecer alimentos con fuentes de AGPI ω-3.

1.4. Empleo de microcápsulas de ácidos grasos omega-3 en alimentos para el desarrollo de derivados cárnicos más saludables

El enriquecimiento de alimentos con AGPI ω-3 a través de la alimentación animal o la adición directa o en forma de emulsiones de aceite de pescado ha sido ampliamente estudiado [102]. Sin embargo, en relación con la microencapsulación de AGPI ω-3, la mayoría de las investigaciones se han centrado en el estudio de las características de calidad de las microcápsulas, en términos de rendimiento, eficiencia de microencapsulación, estabilidad oxidativa, tamaño de partícula y morfología. Sin embargo, son escasas las investigaciones que evalúan la viabilidad de su uso para enriquecer derivados cárnicos con AGPI ω-3. La **tabla 2** muestra una revisión bibliográfica de los artículos científicos que han empleado microcápsulas de AGPI ω-3para enriquecer derivados cárnicos hasta el momento de comenzar la presente tesis doctoral.

Todos estos estudios tienen un denominador común, el empleo de aceite de pescado como fuente de EPA y DHA, existiendo diferencias importantes en el tipo de microcápsulas empleadas para el enriquecimiento, así como la variedad de derivados cárnicos enriquecidos. Pelser et al., [46] y Josquin et al., [103] añadieron microcápsulas comerciales de aceite de pescado en salchichón de cerdo. Jiménez-Martín et al., [45] y Aquilani et al., [47] utilizaron microcápsulas de aceite de pescado elaboradas con la técnica de secado por atomización "Spray-drying" a partir de emulsiones multicapa para enriquecer nuggets de pollo y hamburguesas de cerdo, respectivamente, y Lorenzo et al., [48] prepararon microcápsulas de aceite de pescado mediante secado por atomización a partir de emulsiones monocapa en una matriz de konjac para enriquecer salchichones de cerdo. En todos estos estudios los derivados cárnicos enriquecidos fueron comparados con un lote control sin enriquecer a través de distintos parámetros de calidad, como contenido en humedad, proteína, ceniza, carbohidratos, valor energético, color, textura, contenido de AGPI ω-3, oxidación lipídica y características sensoriales. A nivel general, el enriquecimiento con microcápsulas de aceite de pescado fue llevado a cabo de forma exitosa en todos los derivados cárnicos fortificados, sin embargo, los resultados relacionados con la oxidación lipídica de los derivados enriquecidos mostraron ciertas variaciones. El enriquecimiento con microcápsulas de aceite de pescado comerciales [46,103] y microcápsulas de aceite de pescado elaboradas a partir de emulsiones multicapa [47,104] dieron lugar a derivados cárnicos con valores de oxidación lipídica inferiores en comparación con los lotes enriquecidos con aceite de pescado mediante adición directa o preemulsificada. En el extremo opuesto encontramos el trabajo de Lorenzo et al., [48] donde se registraron valores de oxidación lipídica superiores en los derivados cárnicos enriquecidos con microcápsulas de aceite de pescado en una matriz de konjac. Los resultados de los análisis sensoriales también mostraron diferencias en los lotes enriquecidos con microcápsulas de aceite de pescado en un matriz de konjac, influyendo negativamente en varios atributos sensoriales, aumentando la dureza, gomosidad y masticabilidad de los derivados cárnicos enriquecidos [48]. Por otro lado, no se encontraron efectos sensoriales negativos al usar microcápsulas de aceite de pescado elaboradas a partir de emulsiones multicapa [45,47] y microcápsulas comerciales de aceite de pescado [46,103].

Tabla 2. Resumen de la revisión bibliográfica realizada en derivados cárnicos enriquecidos con microcápsulas de AGPI ω-3.

Derivado enriquecido	Fuente AGPI ω-3	Tipo de emulsión	Técnica de microencapsulación	EPA	DHA	Oxidación lipídica	Análisis sensorial	Referencia
Salchichón de cerdo	Aceite de pescado	NI	NI	↑	↑	↑	↑	[101]
Salchichón de cerdo	Aceite de pescado	NI	NI	↑	↑	=	↑	[57]
Nuggets de pollo	Aceite de pescado	Multicapa	Spray-drying	NI	NI	=	=	[58]
Hamburguesas de cerdo	Aceite de pescado	Multicapa	Spray-drying	↑	↑	=	=	[59]
Salchichón de cerdo	Aceite de pescado	Monocapa	Spray-drying	↑	↑	↑	↓	[60]

Estos resultados indican la posibilidad de enriquecer distintos tipos de derivados cárnicos con microcápsulas de aceite de pescado. En relación con esto, merece la pena indicar que, a la hora de elegir un determinado alimento susceptible de ser fortificado debemos de tener en cuenta varios factores como son: el consumo promedio de este alimento en la población, su composición nutricional y las demandas y requerimientos actuales del mercado. En este sentido los derivados cárnicos pueden considerarse una buena opción, debido a su consumo habitual, que según las estadísticas recogidas en un informe de la Organización Mundial de la Salud se sitúan en torno a 3-4 veces por semana [105]. La carne y derivados cárnicos contienen proteínas de alto valor biológico con un alto contenido en aminoácidos esenciales como valina, leucina, isoleucina, metionina, triptófano, fenilalanina y lisina, además de histidina y arginina, consideradas esenciales en niños, de vitaminas fundamentalmente del complejo B (tiamina, riboflavina, niacina, ácido pantoténico, piridoxidina y cobalamina (B1,2,3,5,6,12, respectivamente)), aportando además ciertos cortes grasos y vísceras algunas vitaminas liposolubles, especialmente la vitamina A o retinol y minerales entre los que destacan el hierro y el fósforo por su relevancia nutricional y selenio, zinc, potasio y magnesio, que variarán dependiendo de la raza y pieza cárnica empleada. Por el aporte de todos los macro y micronutrientes citados anteriormente, el consumo de carne y derivados cárnicos está contemplado en el conjunto de una dieta saludable [21]. Sin embargo, su perfil lipídico en ocasiones es cuestionado debido fundamentalmente a su alta o moderada cantidad de AGS, bajo contenido de AGPI y mayor contenido en AGPI ω-6 que ω-3 lo que hace que la ratio ω-6/ω-3 sea más alta que la recomendada [22]. Por esto, en los últimos años las industrias cárnicas están mostrando un creciente interés en la producción de derivados cárnicos enriquecidos en AGPI ω-3 [106].

1.5. Digestibilidad de matrices alimentarias enriquecidas

La ingesta de alimentos enriquecidos en AGPI ω-3 es una opción disponible que puede ser eficaz en la reducción de factores de riesgo de enfermedades cardiovasculares, principal causa de mortalidad en los países occidentales, por lo que está recibiendo mucha atención en las últimas décadas por parte de la comunidad científica y, aunque el mercado de alimentos funcionales está en constante expansión, se necesitan más estudios que evalúen en qué medida el consumo de alimentos a los que se les añaden compuestos bioactivos produce efectos beneficiosos para la salud, ya que las propiedades bioactivas de la ingesta de estos alimentos pueden verse mermadas por diversas circunstancias como: interacciones con la matriz alimentaria donde se vehicula, su comportamiento durante el proceso de digestión, saber si serán reconocidos por las enzimas digestivas, si los derivados de hidrólisis resultantes serán absorbibles por el organismo o si estos derivados liberados a nivel intestinal serán capaces de llevar a cabo la función bioactiva deseada. Cualquier inconveniente en alguno de estos aspectos daría lugar a una pérdida de funcionalidad,

al no llegar a alcanzarse concentraciones bioactivas en el torrente circulatorio suficientes para ejercer su función [107].

Por todo ello, tras la ingesta de alimentos enriquecidos en AGPI ω-3 resulta imprescindible conocer su comportamiento durante el proceso de digestión como paso previo a la indagación del potencial bioactivo de estos AGPI ω-3, más allá del tracto gastrointestinal. En definitiva, resulta fundamental conocer cuál será su bioaccesibilidad, entendida esta como la fracción de compuesto ingerido que estaría disponible para ser absorbida [108] para lo cual, en el caso concreto de los lípidos, es necesario que se desarrollen tanto fenómenos de hidrólisis a nivel gastrointestinal, como de dispersión de estos productos de la hidrólisis en formas físicas concretas en el medio acuoso intestinal y facilitar así su acceso a las células del epitelio intestinal [109].

La hidrólisis lipídica comienza en el estómago mediante la acción de la lipasa gástrica, enzima lipolítica secretada por las células principales de la mucosa gástrica, donde se han observado niveles de hidrólisis entre 10-30% de los componentes lipídicos [110]. En esta misma etapa, las fuerzas de cizallamiento producidas por las contracciones de los músculos del estómago junto con los diferentes productos que se han liberado de la hidrólisis gástrica, fundamentalmente triglicéridos sin digerir y pequeñas proporciones de diglicéridos, monoglicéridos y ácidos grasos libres ayudarán a la formación de una emulsión [111].

Esta emulsión pasará al intestino delgado, donde el hígado a través del conducto biliar vierte su secreción compuesta fundamentalmente por sales biliares, fosfolípidos y colesterol y a su vez se estimula la secreción del jugo pancreático, que será secretado en la primera porción del intestino delgado. Dicha secreción contiene todas las enzimas digestivas, incluidas las lipolíticas, como son las enzimas lipasa pancreática, fosfolipasa o esterol esterasa que modificarán sustancialmente la emulsión lipídica que se había formado inicialmente a nivel gástrico, dando lugar a una compleja estructura coloidal formada por una mezcla de micelas en forma de discos y vesículas líquidas cristalinas [112]. Debido a la acción conjunta de todas estas enzimas, a nivel intestinal es donde tiene lugar la mayor parte de la hidrólisis de los componentes lipídicos, observándose niveles de hidrólisis en torno al 50-90% dependiendo de la naturaleza del compuesto lipídico [111].

Debido al carácter hidrofóbico de los lípidos, resulta fundamental la formación de las distintas formas de dispersión (micelas y vesículas) para asegurar una correcta distribución de los productos lipídicos en el medio acuoso intestinal y así facilitar su acceso a través de las células del epitelio intestinal, que es donde tendrá lugar la absorción de los productos liberados de la hidrólisis gastrointestinal [113].

Como ya se ha comentado anteriormente, uno de los apartados imprescindibles cuando se quiere evaluar el potencial bioactivo de los AGPI ω-3 añadidos a un derivado cárnico es saber su comportamiento durante el proceso de digestión. Una forma de llevar a cabo esto sería mediante un estudio de digestibilidad *in vivo*, ya sea mediante estudios de intervención en humanos o bien mediante modelos animales, los cuales proporcionan resultados fisiológicos y reales. Sin embargo, los estudios *in vivo* tienen una serie de limitaciones como son el elevado coste, la gran variabilidad de los resultados, el consumo de una gran cantidad de tiempo para desarrollarlos, así como determinados conflictos éticos para su aplicación. Por ello, se han buscado alternativas más sencillas como son los modelos de simulación *in vitro*. Dichos modelos nos permiten conocer cómo sería el comportamiento gastrointestinal del compuesto bioactivo de interés, al imitar de la forma más parecida posible a la realidad las condiciones fisiológicas que se desarrollan durante el proceso de digestión [114].

Los modelos de digestión *in vitro* pueden ser clasificados atendiendo a distintos criterios:

- Capacidad de simulación de fenómenos físicos, tales como movimientos peristálticos, reducción del tamaño de partícula o cambios en la viscosidad del medio, existiendo modelos dinámicos, que intentan reproducir los fenómenos físicos que suceden durante la digestión fisiológica [115,116] y modelos estáticos, que no reproducen dichas condiciones, siendo estos últimos por su fácil aplicación los más empleados [117].
- Tipo de transformación que sufre el compuesto, existiendo modelos fermentativos, basados en las reacciones de fermentación llevadas a cabo por la flora bacteriana del colon [118] y modelos hidrolíticos, basados en las reacciones enzimáticas de hidrólisis a nivel oral, gástrico e intestinal, siendo estos últimos los más habituales en las digestiones simuladas [116,119].
- Número de etapas de digestión, existiendo modelos policompartimentales, que simulan cada una de las etapas del proceso de digestión [120] y modelos monocompartimentales donde las etapas de la digestión se desarrollan en un solo compartimento, siendo estos últimos los más empleados [121].

Según lo dicho anteriormente, los modelos de digestión intestinal *in vitro* más utilizados son del tipo estático, hidrolítico y monocompartimental. Ahora bien, para desarrollar dicho modelo se deben de tener en cuenta una serie de parámetros como:

- Relación enzima/sustrato lo más parecida posible a la utilizada en los estudios *in vivo* [122].

- Cantidad de sustrato a digerir, simulando las cantidades habituales de consumo, con el objetivo de obtener un resultado representativo de lo que podría llegar a suceder a nivel de ingesta real.
- Tiempo de digestión, el cual vendrá determinado por el tramo del tracto gastrointestinal que se pretenda simular, siendo necesario mantener el sustrato en la digestión *in vitro* el tiempo necesario para alcanzar valores de hidrólisis comparables con los observados en estudios *in vivo* [123].
- pH del medio de digestión, al tratarse de un parámetro muy variable según el tramo del tracto gastrointestinal simulado y de especial importancia ya que las enzimas llevarán o no a cabo su función hidrolítica en función del pH del medio, para lo cual es necesario mantenerlo estable dentro de los rangos óptimos de actividad de las enzimas empleadas [124].
- Composición mineral de los fluidos digestivos, resaltando el papel del calcio en el grado y velocidad de la hidrólisis en la digestión al actuar como co-factor sobre la actividad de la lipasa pancreática, sin embargo, un exceso de este mineral en el medio de digestión podría llevar a una disminución de la digestibilidad de los lípidos al favorecer la floculación de las gotas lipídicas, siendo fundamental mantener la concentración de calcio en el medio de digestión en un rango fisiológico [125].
- Simulación de los movimientos peristálticos, al romper las uniones existentes y facilitar el mezclado de los alimentos con los fluidos gastrointestinales, facilitando la acción de las enzimas digestivas, lo que dará lugar a que se produzca con mayor eficacia y eficiencia las reacciones de hidrólisis [122].

Considerando la gran variedad de parámetros a tener en cuenta para la aplicación correcta de un modelo de digestión *in vitro* y la diversidad en los parámetros de digestión descritos en la bibliografía, se hace necesario unificar las condiciones de digestión *in vitro,* con el fin de poder comparar resultados entre estudios. En este sentido, Minekus et al., [126] proponen aplicar un método de digestión estandarizado apto para matrices alimentarias de forma que se siga la metodología que proponen estos investigadores siempre que se realicen estudios de digestibilidad estática *in vitro*, facilitando así la comparación entre resultados de estudios similares.

2. Planteamiento y objetivos

Como paso previo al desarrollo de los objetivos de la presente tesis doctoral, es necesario poner en contexto los antecedentes y estado actual del tema a tratar, con el fin de identificar la hipótesis de partida y los objetivos tanto generales como específicos que se pretenden conseguir con este trabajo. Dicho esto, esta tesis doctoral se centra en investigar el desarrollo de derivados cárnicos enriquecidos en AGPI ω-3.

Entre las estrategias investigadas para conseguir la mejora del perfil lipídico de los derivados cárnicos se encuentra la microencapsulación, centrándose las investigaciones de los últimos años en el desarrollo de tecnologías de encapsulación eficientes con el fin de aumentar la estabilidad de los AGPI y mejorar su biodisponibilidad en el organismo. Sin embargo, existen muy pocos trabajos en los que se haya evaluado la encapsulación como técnica de protección de este tipo de compuestos tanto en sistemas modelos como en alimentos elaborados y la información es aún mucho más limitada si se trata de derivados cárnicos. Existen algunas evidencias en derivados cocidos y curados de que las muestras enriquecidas con aceites insaturados encapsulados muestran una estabilidad oxidativa similar al control y mucho mayor a las enriquecidas con los mismos aceites sin encapsular, sin embargo, se han encontrado efectos negativos importantes a nivel sensorial, relacionados fundamentalmente con un sabor y olor desagradables, que podrían dar lugar a rechazo por parte de los consumidores. Además, las determinaciones llevadas a cabo en estos estudios se han centrado en analizar el porcentaje de EPA y DHA, la estabilidad oxidativa y la aceptabilidad de los derivados enriquecidos, siendo necesario una evaluación más amplia de la calidad de los derivados, atendiendo especialmente a la formación de compuestos de oxidación lipídica, perfil sensorial e intención de compra por parte de los consumidores, así como un estudio de la bioaccesibilidad de los AGPI ω-3

En relación con estas nuevas posibilidades de incorporación de compuestos bioactivos a alimentos, los investigadores del grupo TECAL de la Universidad de Extremadura iniciaron en 2011 una línea de investigación para desarrollar en primer lugar microcápsulas de aceite de pescado, con el fin de ser añadidas a derivados cárnicos, mejorando así el contenido de estos productos en ácidos grasos ω-3. Hasta ahora en el grupo TECAL se han desarrollado distintos tipos de microcápsulas elaboradas a partir de emulsiones de aceite de pescado mediante la técnica de spray-drying y se ha evaluado el efecto de su adición a hamburguesas de cerdo y nuggets de pollo. Las principales conclusiones obtenidas en los primeros estudios realizados han indicado la necesidad de mejorar algunas de las características de las microcápsulas desarrolladas, así como la evaluación de las características de calidad de los derivados enriquecidos a lo largo de su procesado y/o cocinado.

Teniendo en cuenta los planteamientos mencionados anteriormente, la hipótesis de partida de la presente tesis doctoral fue: optimizar tanto el proceso de elaboración como las características de distintas microcápsulas de aceite de pescado como técnica de protección de AGPI ω-3 con el fin de vehicular compuestos bioactivos estables a diferentes derivados cárnicos y obtener productos más

saludables, tecnológicamente viables y con características de calidad semejantes a los derivados no enriquecidos.

Para desarrollar esta hipótesis se propusieron tres objetivos generales:

1. Optimizar el proceso de elaboración de microcápsulas de aceite de pescado mediante spray-drying para garantizar su estabilidad en diferentes derivados cárnicos.
2. Evaluar la viabilidad de adición de microcápsulas de aceite de pescado a distintos derivados cárnicos con el fin de ser declarados fuente de ácidos grasos omega-3, sin que ello perjudique a sus características de calidad.
3. Evaluar la bioaccesibilidad de los AGPI ω-3 contenidos en las microcápsulas de aceite de pescado.

Para desarrollar estos objetivos generales, se propusieron los siguientes objetivos parciales:

1. Optimizar el proceso de homogeneización de emulsiones de aceite de pescado.
2. Caracterizar los distintos tipos de emulsiones de aceite de pescado obtenidas, así como sus correspondientes microcápsulas.
3. Estudiar las características de los distintos tipos de microcápsulas de aceite de pescado en sistemas modelo.
4. Evaluar el efecto de la adición de distintas microcápsulas de aceite de pescado como fuente de ácidos grasos omega-3 sobre la calidad sensorial de distintos derivados cárnicos.
5. Analizar el efecto de la adición de microcápsulas de aceite de pescado como fuente de ácidos grasos omega-3 sobre el perfil lipídico de distintos derivados cárnicos.
6. Determinar el efecto de la adición de microcápsulas de aceite de pescado como fuente de ácidos grasos omega-3 sobre el desarrollo de procesos oxidativos en distintos derivados cárnicos.
7. Analizar el efecto de la adición de microcápsulas de aceite de pescado como fuente de ácidos grasos omega-3 sobre el desarrollo del aroma en distintos derivados cárnicos.
8. Evaluar la adición de microcápsulas de aceite de pescado como fuente de ácidos grasos omega-3 sobre la liberación de estos compuestos bioactivos a nivel gastrointestinal.

3. Diseño experimental

La presente tesis doctoral está dividida en tres capítulos bien diferenciados (**figura 5**). En el primer capítulo se ha llevado a cabo una revisión bibliográfica sobre las estrategias más relevantes de enriquecimiento de derivados cárnicos con AGPI ω-3, que varían desde la suplementación a través de la alimentación animal hasta la adición directa de vegetales y aceites líquidos a distintos alimentos o la incorporación de estos últimos en forma de pre-emulsión o microencapsulados. En el segundo capítulo se ha evaluado el efecto de la homogeneización a alta presión de emulsiones monocapa y multicapa de aceite de pescado sobre las características de calidad de éstas y de las correspondientes microcápsulas obtenidas, con el fin de optimizar su proceso de elaboración. Para ello, en primer lugar, se evaluó el efecto de la homogenización a alta presión sobre las características de calidad de las emulsiones (creaming index, pH, microscopía óptica y tamaño de partícula) de aceite de pescado (monocapa y multicapa) y de sus correspondientes microcápsulas (humedad, rendimiento y eficiencia de la microencapsulación, estabilidad oxidativa, microestructura y tamaño de partícula). A continuación, se aplicó la metodología de superficie de respuesta para optimizar las condiciones de homogenización de las emulsiones; los parámetros experimentales fueron la presión y el número de ciclos de homogeneización a alta presión, y como respuestas evaluadas se consideraron el rendimiento, la eficiencia de la microencapsulación y la estabilidad oxidativa de las microcápsulas. El aceite de pescado que se utilizó para los ensayos realizados fue suministrado por Biomega Natural Nutrients S.L. (Galicia, España), con un contenido aproximado del 4,6 % de EPA y 8,9 % de DHA. Los materiales de pared empleados para la microencapsulación fueron: lecitina de soja, suministrada por Across Organics, (Madrid, España), maltodextrina con un 12 % de equivalente de dextrosa, suministrado por Roquette S.L. (Lestrem, Francia) y quitosano con un 95% de deacetilación, suministrado por Trade S.L. (Murcia, España). Las emulsiones monocapa y multicapa se prepararon con lecitina-maltodextrina y lecitina-maltodextrina-quitosano, respectivamente, que fueron sometidas a un proceso de secado mediante spray-drying para obtener las microcápsulas siguiendo la metodología de [127].

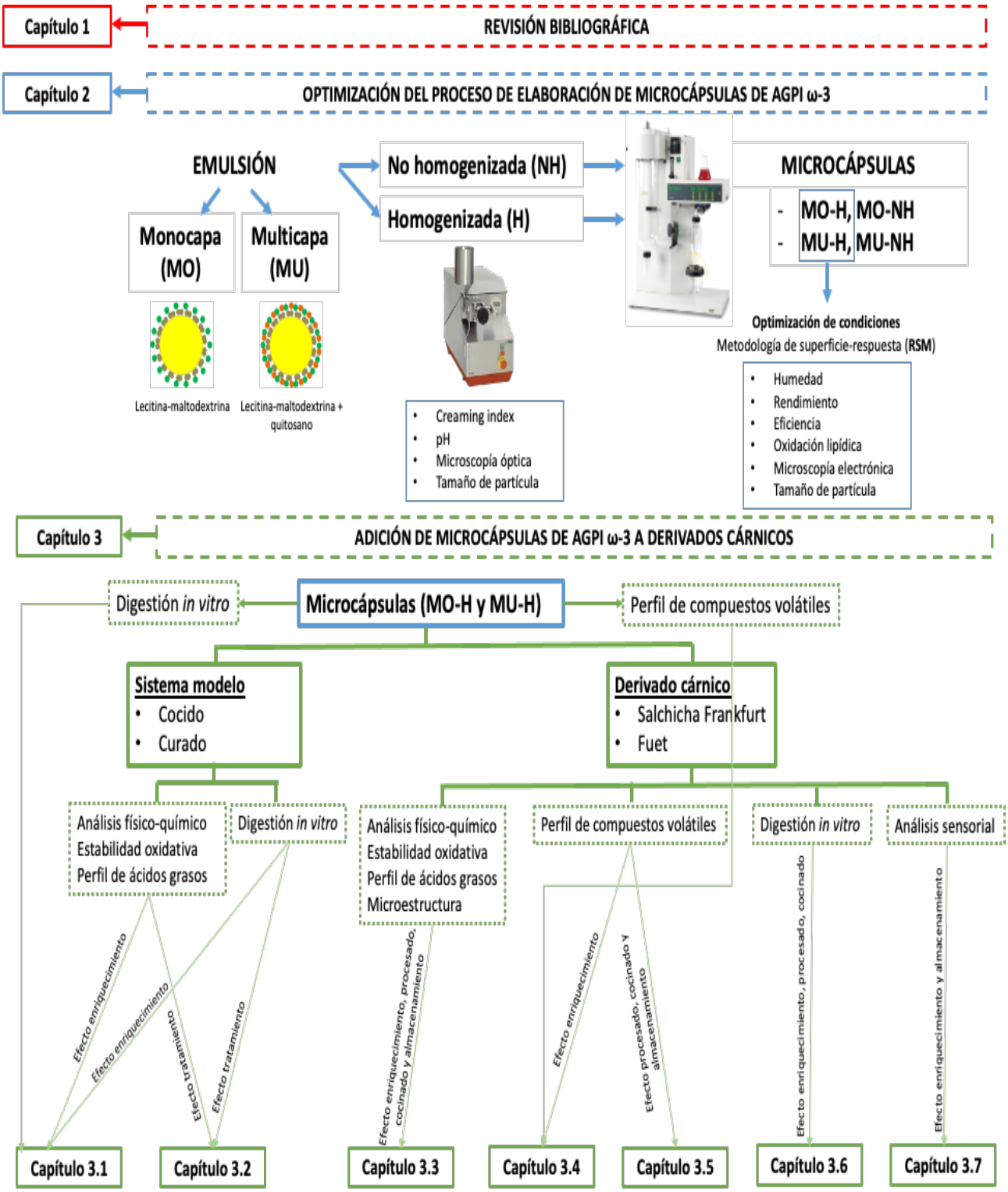
En el tercer capítulo se adicionaron microcápsulas de aceite de pescado (procedentes de emulsiones monocapa y multicapa homogeneizadas con alta presión) a diferentes sistemas modelos y derivados cárnicos. La cantidad de microcápsulas monocapa y multicapa añadida fue de 3 y 5 gramos respectivamente, por cada 100 gramos de derivado. Estas cantidades se estimaron para obtener la cantidad requerida de la suma de EPA y DHA para etiquetar los alimentos como “fuente de ácidos grasos omega-3” (40 mg por 100 g de muestra), considerando el contenido de EPA y DHA, el cual fue similar en ambos tipos de microcápsulas y la eficiencia de la microencapsulación, la cual fue mayor en las microcápsulas monocapa en comparación con la multicapa.

Se elaboraron dos sistemas modelos cárnicos distintos, uno cocido y otro curado, adicionados con microcápsulas monocapa y multicapa a partir de emulsiones homogeneizadas a alta presión, además de un lote control (sin enriquecimiento) para cada uno de los derivados cárnicos. El sistema modelo cocido

se elaboró con carne (65%) y grasa (15%) de cerdo picadas, agua (20%) y sal (20g/kg). Una vez elaborada la masa, se embutió en tubos Falcon de 50 mL y se cocinó a 80 °C durante 15 minutos y posteriormente se enfrió a temperatura ambiente. El sistema modelo curado se elaboró con carne (87%) y grasa (13%) de cerdo picadas y sal (20g/kg). Una vez elaborada la masa, se introdujo en tubos Falcon perforados y se sometió a un proceso de curado a 15 °C y 80 % de humedad relativa durante 12 días. Los sistemas modelos cárnicos se analizaron por triplicado (n = 3), evaluándose el efecto del enriquecimiento con las distintas microcápsulas (Capítulo 3.1) y del tipo de tratamiento de las muestras (cocido vs. curado) (Capítulo 3.2) sobre las características físico-químicas, perfil de ácidos grasos, estabilidad frente a la oxidación, y también se determinó la bioaccesibilidad de AGPI ω-3 en las microcápsulas y sistemas modelo enriquecidos.

Los dos tipos de microcápsulas de aceite de pescado (monocapa y multicapa) elaboradas a partir de emulsiones homogeneizadas a alta presión, también se adicionaron a diferentes derivados cárnicos (salchichas cocidas tipo Viena y fuet), los cuales fueron elaborados en una industria cárnica. Además de los lotes enriquecidos, también se elaboró un lote control (sin enriquecimiento) para cada uno de los derivados cárnicos. Las salchichas fueron elaboradas con carne de pollo separada mecánicamente, agua, grasa de cerdo, sal, plasma de cerdo, estabilizante (E-450), aromas, fibra vegetal, especias, extractos de especias, humo líquido, antioxidante (E-316) y conservante (E-250). Una vez elaborada la masa, se embutió y las salchichas obtenidas se cocieron a 85 °C durante 30 minutos, se envasaron al vacío y se almacenaron a temperatura de refrigeración (0-5 °C). Los fuets fueron elaborados con carne y grasa picada de cerdo ibérico, sal, dextrosa, proteína de soja, especias, estabilizantes (E-450 y E-451), antioxidante (E-301), conservantes (E-250 y E-252), potenciador del sabor (E-621) y colorante (E-120). Una vez elaborada la masa, se embutió en tripas de colágeno con una longitud de 40 cm y un diámetro de 60 mm; los fuets se sometieron a un proceso de curado a 4 °C y 82% de humedad relativa durante 3 días, y después se mantuvieron durante 21 días en una cámara de madurado a 8 °C y 80% de humedad relativa, pasando finalmente a 5 °C y 85% de humedad, hasta alcanzar un porcentaje de pérdida de peso en torno a 38-40% (aproximadamente 14 días). Los derivados cárnicos se analizaron por triplicado (n = 3) y además del efecto del enriquecimiento, también se evaluó la influencia del procesado (cocido o curado), del almacenamiento durante 4 meses (a temperatura de refrigeración para las salchichas y a temperatura ambiente para los fuets) y también, en el caso de las salchichas, del calentamiento culinario (90 °C durante 3 min) sobre las características físico-químicas, perfil de ácidos grasos, estabilidad frente a la oxidación, microestructura, perfil de compuestos volátiles, calidad sensorial y bioaccesibilidad de los AGPI ω-3, dando lugar a los capítulos 3.3 a 3.7 de la presente Tesis Doctoral, tal y como se especifica en la **figura 5.**

Figura 5. Diseño experimental.



4. Resultados

Capítulo 1

Strategies for Enrichment in ω-3 fatty acids aiming for Healthier meat products

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Strategies for Enrichment in ω-3 Fatty Acids Aiming for Healthier Meat Products

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ABSTRACT

Meat and meat products are important sources of high-quality proteins, some vitamins, and minerals, but their lipid profile is sometimes put in question. Some approaches have been suggested to improve the lipid profile. This review deals with some of the most relevant strategies aiming for the ω-3 fatty acids enrichment of meat products, given the beneficial health effects and recommended daily intakes of these bioactive compounds. Relevant information about the procedure and the achieved results are reported, with a special emphasis to the eicosapentaenoic (EPA, C20:5 ω-3) and docosahexaenoic (DHA, C22:6 ω-3) acids, the development of lipid oxidation phenomena and the changes in sensory attributes in the ω-3 enriched products. The main features of the different vehicles (animal feeding, emulsions, microcapsules) used for ω-3 addition to meat products are also reviewed in detail. The use of microcapsules seems to be the most appropriate strategy, but future researches are needed.

KEYWORDS

Animal feeding; eicosapentaenoic and docosahexaenoic fatty acids; emulsion; lipid oxidation; microencapsulation

Introduction

ω−3 fatty acids (FA) (also named as ω−3 or *n*−3 FA) are polyunsaturated FA (PUFA) in which the first double bond is between the third and fourth carbon atoms from the methyl end. This structural feature accounts for their benefits to human health.[1] The most significant ω-3 FA are α-linolenic acid (ALA, C18:3 ω-3), eicosapentaenoic acid (EPA, C20:5 ω-3) and docosahexaenoic acid (DHA, C22:6 ω-3). Beneficial effects of EPA and DHA intake are well known, such as contribution to the development of infant brain and liver[2] and decrease in the risk of tumors[3], cardiovascular diseases[4], and inflammatory disorders.[5]

ALA is one of the two essential FA in the human diet, together with linoleic acid (LA, C18:2 ω-6). ALA can be desaturated and elongated in the human body to its longer-chain relatives, ω-3 PUFA, but this conversion is very limited, and its efficiency is even further reduced by high intake levels of LA, which competes more effectively than ALA for desaturation and elongation enzymes. There is evidence suggesting that the conversion rate of dietary ALA to ω-3 PUFA is insufficient to achieve adequate levels of the latter, even when the ALA intake is enhanced.[6] Consequently, diet is the main source of ω-3 PUFA for humans.

Different health organizations have established dietary recommendations for the daily intakes of ω-3 PUFA. The European Food Safety Authority (EFSA)[7] and The

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Food and Agricultural Organization (FAO)[8] have established an Adequate Intake (AI) of 0.25 g for EPA plus DHA. The European Academy of Nutritional Sciences (EANS), as well as the UK dietary guidelines, recommend the intake of at least an average of 0.2 g of EPA plus DHA per person per day.[1,9] Recommendations of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) suggests the AI of ω-3 PUFA to be 0.5 g of DHA plus EPA per person per day.[10]

The highest quantities of ALA are found in oilseeds, principally chia, flax, and canola, while the main sources of EPA and DHA are fish and seafood. According to the study of Ackman[11], the concentrations of these FA can vary by region and species. For example, Mediterranean marine fish such as squid, mullet, hake, and sepia contain comparably higher amounts of EPA and DHA (5–30%), and Australian and tropical fish such as perch and tropical halibut contain 20–30% of EPA and DHA. In crustaceans, such as mussels, oysters, shrimp, scallop, crabs and clams, the content of EPA and DHA is around 0.1–2.5%. However, the consumption of these foodstuffs in current Western diets is not enough to reach ω-3 PUFA recommended intake.[6] In many Western countries, the average fish intake is presently below the recommended two to three fish servings per week[12], which leads to an average intake level of ω-3 PUFA of 0.15 g per person per day. These figures are quite far from the recommended ones. This is the result of a prominent consumption of vegetable fats and oils rich in ω-6 PUFA.[13,14] This fact, together with the cultural awareness of the benefits of ω-3 PUFA and the high percentage of population with the intention of incorporating these FA in their diet[15], has led to numerous investigations on the possibility of increasing the content of ω-3 PUFA in different foodstuffs.

Meat and meat products are highly valuable for consumers, mainly due to their sensory properties. On top of this positive aspect, these products are also relevant due to their nutritional impact. They are important sources of high-quality proteins and some vitamins (especially vitamin B6 and B12) and minerals (iron, selenium, and zinc).[16] However, the lipid profile of meat and meat products is nutritionally not so desirable in most cases, due to their high to moderate amount of saturated FA (SFA) and low PUFA contents and to the higher content in ω-6 PUFA than in ω-3.[17] In order to counteract these facts, strategies aiming to improve the lipid profile of meat and meat products have been tested and implemented, the main ones being enrichment of animal feeding with high ω-3 PUFA oils and direct addition of ω-3 PUFA oils to foods as pre-emulsions or microcapsules.

The present paper aims to review these strategies aiming to increase the content of ω-3 PUFA in meat and meat products, with a focus on the developed procedures and the most remarkable outcomes (Fig. 1).

Meat enrichment trough animal feeding

Addition of high ω-3 PUFA oils to animal feeding has been mostly conducted on non-ruminant animals, mainly poultry and pigs, since rumen digestion leads to the hydrogenation of most unsaturated fatty acids, making this strategy mostly inefficient.[18] Sources of both ALA and ω-3 PUFA have been tested. Table 1 shows a summary of the studies following this strategy, highlighting their most remarkable results. In the case of adding ALA sources to feeds, the aim is increasing EPA and DHA biosynthesis in livestock. For example, diverse meat products (dry-cured loin, dry-fermented sausage, cooked ham, and dry-cured ham) from pigs fed different oil-enriched diets have been studied.

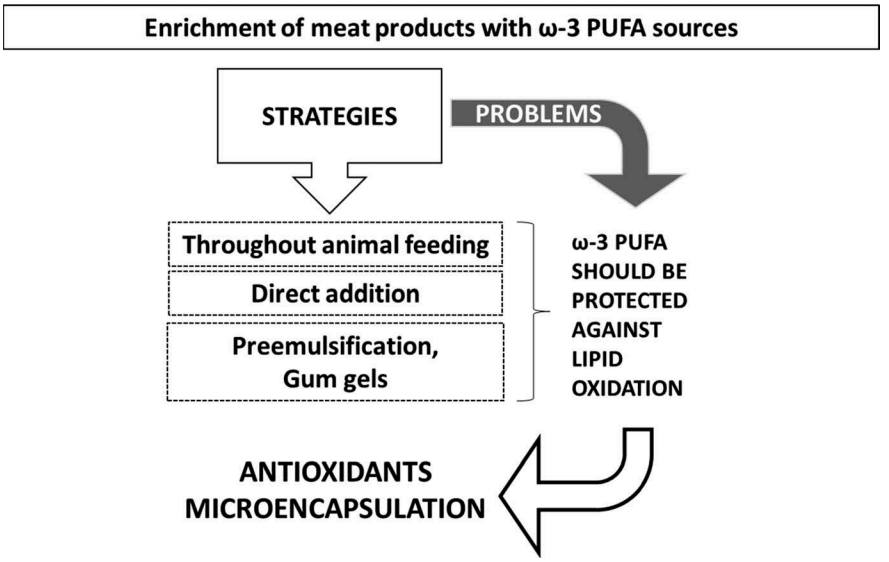

Figure 1. Strategies for enrichment meat products with ω-3 PUFA sources.

Table 1. Animal feeding strategies to increase ω-3 content: effect on meat quality characteristics.

			MEAT QUALITY CHARACTERISTICS					
ω-3 source	ANIMAL	ANTIOXIDANT	C18:3 ω-3	C20:5 ω-3	C22:5 ω-3	Oxidation values	Sensory quality	REFERENCE
Linseed oil	Pig	**NO**	↑	**NI**	**NI**	↑	↓	[19,20]
	Pig	**YES**	↑	**NI**	**NI**	=	=	[19,20]
	Pig	**NO**	↑	**NI**	**NI**	↑	↓	[21]
	Pig	**YES**	↑	**NI**	**NI**	=	↓	[21]
	Pig	**NO**	↑	**NI**	**NI**	↑	↓	[22]
	Chicken	**NO**	↑	↑	↑	**NI**	=	[23]
	Rooster	**YES**	↑	↑	↑	**NI**	**NI**	[24]
	Turkey	**NO**	↑	↑	↑	↑	↓	[25]
Fish oil	Pig	**NO**	**NI**	↑	↑	↑	↓	[26]
	Pig	**NO**	**NI**	↑	↑	↑	↓	[27]
	Pig	**YES**	=	↑	↑	↑	**NI**	[28]
	Chicken	**NO**	**NI**	↑	↑	↑	↓	[23]
	Chicken	**NO**	**NI**	↑	↑	**NI**	**NI**	[29]
	Chicken	**NO**	**NI**	↑	↑	**NI**	↓	[30]
	Chicken	**NO**	↑	↑	↑	**NI**	=	[31]
Microalgae	Pig	**NO**	**NI**	↑	↑	↑	↓	[28]
	Chicken	**NO**	**NI**	↑	↑	**NI**	**NI**	[32]

NI: not indicated
↑, ↓,= : increase, decrease or not changed, respectively, in comparison to a control batch.

Hoz et al.[19, 20] compared diets containing sunflower oil (batch control) with others enriched in linseed oil or linseed plus olive oil. Santos et al.[21] and Nuernberg et al.[22] studied the effect of feeding pigs with a basal concentrate diet supplemented with 5% olive oil or 5% linseed oil. The inclusion of linseed oil in feeds for chicken[23], rooster[24] and turkey[25] has also been studied. Overall, in these studies, meat products from pigs fed the experimental enriched diets showed a high enhancement in ALA, and consequently in total ω-3 PUFA, as well as a significant reduction in the ω-6/ω-3 ratio, whereas the content of EPA did not notably increase and DHA levels remained constant. This fact is connected

to the low conversion rate of dietary ALA to ω-3 PUFA.[6] As it happens in humans, biosynthesis of EPA and DHA from ALA is also limited in most livestock species.[23] In the case of poultry, ALA, EPA, and DHA significantly increased in meat from linoleic oil fed animals, which indicates a significant conversion of ALA to EPA and DHA. In fact, Lopez-Ferrer et al.[23] have reported that chicken could convert ALA to ω-PUFA in the liver at 24 d of age. Besides, the amount of added linseed oil as well as that of ω-3 PUFA content, could enhance or diminish, respectively, this conversion.[23]

Dry-cured meat products from pigs fed on linseed oil-enriched diets exhibited the highest lipid oxidation values and the worst scores for sensory characteristics, with an unfavorable degree of rancidity.[19–21] Similarly, in the research of Nuernberg et al.[22], the overall flavor of samples, which were models of a meat/backfat mixture, was negatively influenced by linseed oil supplementation compared to supplementation with olive oil, and the oxidative stability of muscle lipids was lower in linseed oil-fed pigs compared to those fed on olive oil enriched feeding. Similar results have been reported in turkey meat, with dietary linseed oil having a negative effect on the sensory properties and susceptibility towards oxidation.[25]

An alternative strategy is that based on adding oils rich in EPA and DHA to animal feeding. In pigs, the effects of dietary fish silage and fish oil on back-fat lipid composition and sensory quality of loin, flank, and bacon were evaluated by Kjos et al.[26] These authors observed that inclusion of fish silage in the diet increased the levels of DHA and eicosaenoic acid (C20:1 ω-9) in pig tissues to a greater extent than enrichment with fish oil, while EPA levels were not affected by diet. Authors associated these differences to the higher proportion of DHA in fish silage. As side consequences, high levels of fish oil in the feeding (>2.5 g/kg feed) reduced sensory quality (off-flavor) of bacon and loin, but this could be avoided by reducing the fish oil level in the diet.

Feeds with moderate and high PUFA levels (31 and 50%, respectively) and feeds added with different concentrations of fish oil (0.2 and 0.4%) were also evaluated on pigs[27], by means of fatty acid profile and sensory analysis. Back-fat from pigs fed with fish oil showed more EPA than their counterparts fed without fish oil, while the level of DHA did not show differences as a consequence of diets, which was ascribed to the low concentration of fish oil (0.2 and 0.4%) in the experimental diets. Results on sensory analysis showed higher rancidity scores in loins and sausages from pigs fed with high PUFA levels, whereas addition of small amounts of fish oil (0.4%) to the diet did not affect the sensory features of pork.

In chicken, Lopez-Ferrer et al.[33] assessed the effects of feeding regimens based on fish oil alone or with added linseed oil, and analyzed the fatty acid composition and sensory attributes of thighs. Feeding regimens based on fish oil led to an increase of EPA and DHA in the thighs, while LA content slightly varied. However, feeds added with fish and linseed oils resulted in the increase of ALA and LA. Sensory panelists described unacceptable odors in carcasses from broilers fed fish oil but they could not distinguish meat from broilers fed the different diets.

More recently, also on poultry, Konieczka et al.[29] replaced lard by fish oil in combination to lard, flaxseed or rapeseed oils, Martínez et al.[30] substituted soybean oil with tuna fish oil, and Panda et al.[31] supplemented fish oil to broiler chicks during the finisher phase. These studies were more focused on productive parameters, such as performance or body weight gain, paying less attention to meat quality characteristics. Nevertheless, the fatty acid composition showed higher levels of EPA and DHA on animals as a consequence of fish oil inclusion in the diets, but also a negative influence on the acceptability of the meat.

A different approach to increase the levels of EPA and DHA in meat is adding microalgae, as ω-3 PUFA source, to the animal feeding. Vossen et al.[28] fed pigs on a diet with added microalgae at different concentrations and addressed the effects on dry-cured hams and loins from these experimental animals. Gatrell et al.[32] conducted a similar study on chicken. As expected, in both studies the level of EPA and DHA increased in the meat from animals fed on microalgae added diets. However, dry-cured hams from algae fed animals had higher lipid oxidation values compared to the control treatment, with detrimental color stability and instrumental texture parameters. Unfortunately, quality parameters of chickens were not addressed.

A potential tool aiming to control abnormally high levels of lipid oxidation in meat enriched in ω-3 PUFA through animal feeding is the addition of tocopherol in animal diets.[19–21] In pigs, diets enriched in linseed oil and with added tocopherol led to dry-cured meat products with a higher lipid oxidative stability and better hedonic sensory scores than those without tocopherol supplementation. Nevertheless, despite the added tocopherol, dry-cured hams from animals fed on diets enriched in linseed oil were rejected by consumers, because of rancid flavor notes and off-odors.[21] The inclusion of supra-nutritional levels of α-tocopherol in fish oil added concentrates for pigs did not allow to maintain the oxidative stability of ω −3 PUFA in dry fermented sausages.[28]

Overall, most approaches focused on improving the lipid profile of meat products by means of animal feeding have found sensory and lipid oxidation problems. Still, some of those products are available in the market, such as ω-3 enriched poultry, but their actual presence is rather low. As a consequence of this lack of success, other strategies for increasing the content of ω-3 PUFA in meat products have been developed in the last two decades, such as the direct addition of ω-3 PUFA sources to the formula, either as bulk oil or as emulsions or microcapsules, as reviewed below.

Meat enrichment by PUFA direct addition

ω-3 rich plant foods (mainly walnuts) and oils (i.e., linseed, chia, and fish) have been used to fortify food in ω-3 PUFA by direct addition. The use of ALA sources allows an increase of this FA in different added meat products (frankfurters, patties, dry-sausages) and a subsequent decrease in the ω-6/ω-3 ratio, whereas the differences in EPA or DHA are not so notable.[34–36] What is even more interesting is that these strategies have shown to have a limited influence on sensory attributes of some of these products. However, high oxidation levels in products enriched with linseed oil was observed. More recently, other authors[37] have reformulated chicken nuggets by replacing chicken skin by chia flour up to 10%, allowing the increase of ALA without compromising the acceptability of this meat product. Regarding enriching products with fish oil, as a direct consequence, EPA and DHA are significantly enhanced and the ω-6/ω-3 ratio is reduced[38,39], but a great increase of lipid oxidation values and products negatively influencing odor, taste, and flavor are strongly limiting their potential use.

A straightforward approach for counteracting the oxidative susceptibility of meat products added with ω-3 PUFA sources would be to use antioxidants. For example, butylhydroxyanisole (BHA), a mixture of tocopherols and rosemary extract (0.01. 0.03, and 0.2%, respectively, based on fat content) were evaluated in ground beef patties fortified with fish oil (500 mg/110 g meat).[40] Results from this work showed the effectiveness of rosemary extract and BHA for minimizing lipid oxidation while

tocopherols were not able to decrease lipid oxidation. In other study, frankfurters, cooked ham, and cooked turkey breast were enriched with deodorized salmon oil (0.16% w/w), with rosemary extract (0.02% w/w) and vitamin E (0.001% w/w). The functional ingredients were incorporated into the meat products through a brine with a concentration of 16.6%[39]; these authors found that the lipid oxidation values of the meat products with rosemary extract and vitamin E were significantly lower than the products without these antioxidants. Chicken sausages formulated with vitamin E (12 mg per 100 g) and flaxseed oil (2 g per 100 g) had a negative effect on oxidation stability and consumer acceptance in comparison to a control batch (not enriched).[41] In algal oil (1%) enriched chevon patties, the use of bioactive peptide fractions of papain hydrolysates (0.09%) from goat blood, with antioxidant properties, seemed to improve the oxidative stability without influencing sensory acceptability.[42]

The main drawback of adding antioxidants to the oil enriched products is the different behavior of the antioxidant depending on the food matrix. The same antioxidant could show different efficiencies in different products or even be antioxidant in some products and prooxidant in others.[43]

Meat enrichment by PUFA emulsification

So far, the addition of linseed, fish, or algae oil emulsions to meat products has been mainly used as a fat replacer, although several applications of this type of emulsions as a way to fortify food in ω-3 PUFA have been described in the scientific literature. For both objectives, many different types of oil emulsions have been reported (Table 2), differing in several aspects: the oily phase, which can be constituted by only one oil, as occurred in most cases, or by different kinds of oil; constituents of the emulsion, especially the emulsifier, and their concentration; and the procedure to stabilize the emulsion.

These studies have been focused on emulsified fish oil[45–47], algae[44,50] and linseed oils[41,47,51]. In this sense, it is also worth noting the use of a mixture of emulsified olive, linseed and fish oils[48,52] and of algae and linseed oils.[49,53]

Among the constituents, the oil is usually added as it is.[45–49] However, it can also be previously stabilized by adding, for example, α-tocopherol, ascorbyl palmitate.[44] The most used emulsifiers are sodium caseinate and soy protein isolate[45–49], whereas the use of whey protein isolate is not so common.[44] As for the water phase, most studies use warm water (50–65°C). The proportion of each emulsion constituent is also a key factor for the stability and the efficiency of the method. In most cases, the proportion of oil and water is similar, and the emulsifier makes up normally around 10% of the oil. For example, Valencia et al.[47] used 245 g of oil and water and 49 g of sodium caseinate, and Delgado-Pando et al.[48] and Berasategui et al.[49] made the emulsions with 10 parts of oil, 8 parts of water, and 1 part of soy protein isolate.

Most studies do not pay much attention to the procedure for making the emulsion. The common method involves firstly homogenizing or mixing the water with the emulsifier, and thereafter, adding this mixture to the oil. Usually, these steps are carried out by stirring or by using a Stephan type machine. Generally, the emulsifying time is around 2–3 mins. Some studies consider additional final steps, such as pasteurization of the emulsion at 75°C for 30 min[50] or keeping the emulsion at refrigeration (4°C) prior to use.[47]

Table 2. Data on oil emulsions used to enrich meat products in ω-3.

ω-3 source	EMULSION CONSTITUENTS	CONSTITUENT CONCENTRATION	PROCEDURE	REFERENCE
Algae oil	– Oil – Aqueous phase Whey protein isolated Sodium citrate buffer pH = 3 Potassium sorbate EDTA	25% 75% 2.5% 10 mM 0.2% 100 μM	Homogenize oil with the aqueous phase Emulsion pasteurization (75°C, 30 min)	[44]
Fish oil	– Oil (stabilized with α-tocopherol, ascorbil palmitate and soy protein) – Water (60°C) – Sodium caseinate	4.5 (v) 4.5 (w) 1 (v)	Mix all constituents at the same time by stirring	[45]
Fish oil	– Oil – Hot water – Soy protein isolated	10 parts 8 parts 1 part	Mix hot water with soy protein isolated for 2 min Add the oil and then mix for 3 min	[46]
Fish oil Linseed oil	– Oil – Water (50°C) – Soy protein isolated	245 g 245 g 49 g	Mixing vigorously for 2 min Stored at 4°C prior to use	[47]
Mixture of olive, linseed and fish oils	– Oil – Water – Sodium caseinate	10 parts 8 parts 1 part	Homogenize water and sodium caseinate for 2 min Add the oil and then homogenize for 3 min	[48]
Mixture of linseed and algae oils (1:1)	– Oil – Hot water – Soy protein isolated	10 parts 8 parts 1 part	Mix hot water with soy protein isolated for 2 min Add the oil and mix for 3 min	[49]

There are a number of studies focused on increasing the ω-3 PUFA in the meat products by using emulsified oil, mainly differing on the type of oil emulsified and on the inclusion, or not, of antioxidants (Table 3). Thus, linseed oil emulsions have been added to fresh pork sausages (15% replacement of back-fat)[47] and to bologna-type sausage (8.75% replacement of back-fat)[51], with the addition of different antioxidants: green tea catechin and green coffee extract in the former, and an extract of *Melissa officinalis* and BHA in the latter. The addition of linseed oil increased ALA levels in the sausage and also lipid oxidation values, but it did not influence sensory scores of any of these two products. Enriched ω-3 products with green tea catechin, BHA and *Melissa officinalis* (lemon balm) extract showed a decrease in lipid oxidation values, while the effect of the green coffee extract was not so notable.

Caceres et al.[45] studied the effects of adding a fish oil emulsion to *mortadella*, a large Italian emulsified sausage, and found a significant enhancement in the content of ω-3 PUFA in enriched samples. It is remarkable that no significant differences in lipid oxidation nor in the sensory analysis were found between enriched and control batches. The source of fish oil used by these authors was Ropufa '30' *n-3* Food Oil (F. Hoffman-La Roche, Switzerland) with an ω-3 PUFA minimum content of 30%, stabilized with tocopherol, ascorbyl palmitate, and lecithin. The inclusion of the antioxidants could be the reason behind the absence of increased levels of

Table 3. Addition of oil emulsion strategies to increase ω-3 content: effect on meat products quality characteristics.

			MEAT PRODUCT QUALITY CHARACTERISTICS					
ω-3 SOURCE	ANTIOXIDANT	ENRICHED PRODUCT	C18:3 ω-3	C20:5 ω-3	C22:5 ω-3	Oxidation values	Sensory quality	REFERENCE
Algae oil	NO	Ground turkey	**NI**	**NI**	**NI**	=	↓	[44]
Algae oil	NO	Pork sausages	**NI**	**NI**	**NI**	↑	↓	[44]
Algae oil	NO	Restructured hams	**NI**	**NI**	**NI**	↑	↓	[44]
Algae oil	YES (in the product)	Dry-fermented sausages	**NI**	↑	↑	=	↓	[50]
Linseed oil	NO	Fresh pork sausage	↑	=	=	↑	=	[47]
Linseed oil	YES (in the product)	Fresh pork sausage	↑	=	=	=	=	[47]
Linseed oil	NO	Bologne-type sausage	↑	**NI**	**NI**	↑	=	[51]
Linseed oil	YES (in the product)	Bologne-type sausage	↑	**NI**	**NI**	=	=	[51]
Linseed oil	YES (in the product)	Chicken sausage	↑	**NI**	**NI**	↓	↓	[41]
Fish oil	YES (in the product)	Dry-fermented sausages	**NI**	↑	↑	=	=	[46]
Fish oil	YES (in the oil)	Mortadella	**NI**	↑	↑	=	=	[45]
Fish oil	NO	Fresh sausages	**NI**	↑	↑	↑	↓	[47]
Fish oil	YES (in the product)	Fresh sausages	**NI**	↑	↑	=	=	[47]
Linseed + algae oil	YES (in the product)	Bologne-type sausage	↑	=	↑	=	=	[49]
Linseed + algae oil	YES (in the product)	Dry-fermented sausages	↑	=	↑	=	=	[53]
Olive + linseed + fish oil	NO	Frankfurters	↑	↑	↑	↑	=	[48,52]

NI: not indicated
↑, ↓,= : increase, decrease or not changed, respectively, in comparison to a control batch.

oxidation, and deleterious effects on sensory features in the sausages added with fish oil emulsions. A partial substitution of pork back-fat by deodorized fish oil emulsions in fresh (15% replacement of back-fat) and dry-fermented sausages (25% replacement of back-fat) has been carried out by Valencia et al.[46,47] These authors also tested the inclusion of green tea catechin and green coffee antioxidants, and of butylhydroxytoluene (BHT) and BHA, respectively. The enriched products resulted in improved nutritional properties, with higher EPA and DHA contents and a lower ω-6/ω-3 ratio. In fresh sausages containing fish oil, higher levels of lipid oxidation and lower sensory scores for flavor and overall acceptability, compared to controls, were observed.[47] In these products, the addition of green tea catechin significantly reduced lipid oxidation and improved sensory quality. However, in dry-fermented sausages, sensory properties and oxidation status were unaffected by the addition of fish oil emulsions, which may be due to the inclusion of BHT and BHA in the experimental batch.

Lee et al.[44] incorporated an emulsion of algae oil into different meat products: ground turkey, pork sausages and restructured hams. These authors investigated the effect of cooking treatment and did not report enhanced levels of EPA and DHA in enriched products, but found higher lipid oxidation values and lower sensory quality as compared to the respective control batches. Valencia et al.[50] also enriched dry-cured fermented sausages by the addition of algae oil emulsion. These authors tested two levels of back-fat substitution (15% and 25%) and also included BHT and BHA as antioxidants. Increments

of EPA and DHA were detected in the enriched batches. The authors found that the higher the algae oil amount used in the formulation, the higher the ω-3 PUFA content in modified sausages was obtained. No signs of oxidation increment in algae oil added products with regard to control sausages were observed, which was related to the inclusion of antioxidants. Results on sensory analysis of this study determined that products with 25% algae oil substitution were not viable, due to a peculiar taste detected by panelists, which is not typical from this kind of meat products.

Some authors have tried to optimize lipid profiles by using oil combinations as fat replacers. An emulsion containing a mixture of linseed-algae oil has been included in Bologna sausages (100% replacement)[49] and dry fermented sausages formulation (25% replacement).[53] Besides, the addition of a *Melissa officinalis* extract as an antioxidant to these meat products enriched with the linseed-algae oil combination has also been analyzed. Other authors[48,52] used an emulsion of olive, linseed and fish oils as back fat replacers (100% replacement) in frankfurters without the addition of antioxidants. Products enriched with the linseed-algae oil emulsion experienced a concomitant increase in ALA and DHA, and frankfurters added with the emulsion of olive, linseed and fish oils contained more LA, ALA, EPA and DHA in comparison to the control batch. In addition, no oxidation problems were detected when adding linseed-algae oil emulsion plus the *Melissa officinalis* extract, whereas a slight increase in lipid oxidation was found when using the emulsion of olive, linseed and fish oils. However, any oil combination affected the sensory characteristics of products.

The use of emulsions of plant oil combinations stabilized in konjac gel has been tested in different meat products for fat reduction and improved lipid profile. For example, in pork liver pâté[54,55], dry-fermented sausages[56,57], frankfurters[58,59] and patties[60], pork back-fat has been partially replaced by an emulsion of olive, linseed and fish oil combination stabilized with konjac gel. In general, an increase of ALA, EPA, and DHA was reported in the re-formulated meat products, which also showed higher lipid oxidation values and poor sensory attributes in comparison to control batches. Thus, the authors of these investigations indicated the necessity of further modifications in product formulations to improve quality characteristics. Apart from the konjac, there are also some studies using carrageenan to stabilize the gels added to meat products. Poyato et al.[61] added sunflower oil emulsions stabilized in carrageenan gel to burger meat from pork, and Alejandre et al.[62,63] worked with linseed and algae oil emulsions in carrageenan gel that were incorporated in dry-fermented sausages and beef patties, respectively. These authors found that the content of ω-3 PUFA increased from control to enriched samples and did not find significantly influence lipid oxidation or acceptability.

In general, a healthier lipid profile can be achieved by adding ω-3 PUFA oil emulsions to a variety of meat products. However, lipid oxidation and sensory attributes are still serious challenges that should be faced before upscaling to industrial production, since even when antioxidants are included in the formula, meat products showed deleterious effects in most cases. Thus, it seems necessary to have an even stronger stabilization of oil emulsions for the enrichment of meat products in ω-3 PUFA. In addition, thinking ahead about a practical application of this strategy in meat industries, it is worth noting that, once stabilized, emulsions must be immediately added to the product. This represents an important logistical inconvenience, since most meat plants are not designed to have an

additional processing line aiming to produce emulsified oils, and thus, incorporating this enrichment procedure into the production lines might entail some difficulties.[63]

Meat enrichment by PUFA microencapsulation

Microencapsulation appears as an innovative and interesting strategy for protecting ω-3 PUFA against lipid oxidation. Microcapsules consist of dispersing oil droplets coated or embedded in a homogeneous or heterogeneous matrix. In this way, a physical barrier is put in between the active compounds (ω-3 PUFA in this case) and the environment, which minimizes their contact and reactivity with water, oxygen, iron and other oxidizing promoters.[64] However, the encapsulation of ω-3 PUFA rich oil requires a previous elaboration of oil-in-water emulsions, due to its lipophilic character, and a short exposition to high temperatures, due to their high susceptibility to oxidation.[65]

Encapsulation of ω-3 PUFA rich oil has been reported by using different techniques, such us complex coacervation, gelation, inclusion complexation or electrospinning, but no doubt, spray-drying is the most used one.[66] This fact can be ascribed to its short drying times (5–30 s), which makes it a useful technique for encapsulating heat-sensitive materials[67], and to the stability of feed emulsions in the spray-drying process, which plays an important role in the retention of the oil.[68] In addition, this method produces powder microparticles with low water activity, which, ensures a good microbiological quality, easing transport, handling, and storage of the products.[69]

Most studies have evaluated the use of fish oil microcapsules to enrich meat products in ω-3 PUFA (Table 4). There are important differences in the type of microcapsules used for addition as well as in the enriched products. Josquin et al.[71] and Pelser et al.[70] added commercial fish oil microcapsules to sausages, Jiménez-Martín et al.[72] and Aquilani et al.[73] elaborated fish oil microcapsules by spray-drying multilayered emulsions to enrich chicken nuggets and burgers, respectively, and Lorenzo et al.[74] prepared microcapsules from the monolayered emulsion in a konjac matrix that were added to Spanish salchichon, a dry-fermented sausage. In each study, different batches of meat products were compared by means of quality parameters, ω-3 PUFA content, lipid oxidation, and sensory traits.

Overall, EPA and DHA enrichment was successfully achieved in all products added with encapsulated fish oil. However, the consequence of such an enrichment on lipid oxidation is more irregular. Thus, the enrichment with commercial fish oil microcapsules[71] or microcapsules from multilayered emulsions of fish oil[72,73] led to meat products with lower lipid oxidation values in comparison to those enriched with bulk fish oil and/or pre-emulsified fish. On the contrary, Lorenzo et al.[74] found higher lipid oxidation values in products enriched with fish oil microcapsules in a konjac matrix than in control ones. Results on sensory analyses were also variable in the reported studies. The use of fish oil microcapsules in a konjac matrix negatively influences several sensory traits, increasing hardness, gumminess, and chewiness of meat products[74], whereas no negative effects were found when using microcapsules from multilayered emulsions and commercial fish oil microcapsules.[70–73]

Fish oil microcapsules in a konjac and carrageenan matrix were prepared as follows[74]: 1) a mixture of water, maltodextrin, gum arabic and caseinate was homogenized at 75°C to have a monolayered emulsion; 2) this solution was cooled to 40°C, and subsequently the

Table 4. Strategies for adding oil microcapsules to increase ω-3 content on meat products: effect on quality characteristics.

ω-3 SOURCE	EMULSIFIER/WALL MATERIAL	TYPE OF EMULSION	MICROENCAPSULATION TECHNIQUE	ENRICHED PRODUCT	MEAT PRODUCT QUALITY CHARACTERISTICS				REFERENCE
					C20:5 ω-3	C22:5 ω-3	Oxidation values	Sensory quality	
Fish oil	NI	NI	NI	Pork fermented sausage	↑	↑	↑	↑	[70]
	NI	NI	NI	Pork fermented sausage	↑	↑	=	↑	[71]
	Lecithin/Chitosan-Maltodextrine	Multilayered	Spray-drying	Chicken nuggets	**NI**	**NI**	=	=	[72]
	Lecithin/Chitosan-Maltodextrine	Multilayered	Spray-drying	Pork burgers	↑	↑	=	=	[73]
	Lecithin/Chitosan-Maltodextrine	Monolayer	Spray-drying	Pork Spanish salchichon	↑	↑	↑	↓	[74]

NI: not indicated

↑, ↓,= : increase, decrease or not changed, respectively, in comparison to a control batch.

fish oil was added, mixed and emulsified at 80°C for 2 h; 3) the homogenate was dried by spray drying to obtain the microencapsulated fish oil powder; 4) konjac flour was homogenized in water and microencapsulated fish oil powder; 5) the obtained mixture was again homogenized with i-carrageenan (10 g/kg), and with pre-gelled corn starch powder dispersed in water; 6) the mixture was cooled to 10°C; 7) a $Ca(OH)_2$ solution (1%) was added; 8) the final mixture was gently stirred at room temperature.

Fish oil microcapsules from multilayered emulsion have been selected after evaluation of different types of fish oil emulsions (double, monolayered and multilayered) and their corresponding microcapsules.[75–77] In this case, the authors prepared the monolayered emulsions with lecithin as emulsifier and maltodextrin as wall material. For that, a primary emulsion was prepared by homogenizing the water and oily (fish oil and lecithin solution) phases. This emulsion is first added with water and then with a water + maltodextrin solution to obtain the final emulsion. In this way, the oil is emulsified with lecithin and covered with a layer of maltodextrin. The multilayered emulsions were performed following the "layer-by-layer" technique.[78] It consists of the combination of an emulsifier and one or more polyelectrolyte of opposite charges. In this case, the primary emulsion was the same as in the monolayered emulsion, which has a negative charge. Then, two polyelectrolytes of positive charge were added, first a chitosan and acetic acid solution and then a water and maltodextrin solution, giving rise to the multilayered emulsion (Fig. 2). Thus, oil droplets are coated with a two-layer system based on electrostatic attraction that covers the drops of the previous emulsion. Regarding double emulsions, an oil-in-water-in-oil (O1/W/O2) emulsion was prepared by homogenizing the fish oil with a sodium caseinate solution, used as a hydrophilic emulsifier, and diluting with lactose monohydrate. Finally, this emulsion was dropped on to an oily solution made of olive oil and polyglycerol polyricinoleate, which act as a hydrophobic emulsifier.

Multilayered emulsions showed higher stability, measured by means of creaming index, than monolayered and double ones, which can be explained by the addition of chitosan in the multilayered emulsion.[75] The contact between lecithin layers of different droplets is avoided by chitosan and it also increases the electrostatic force and the viscosity. This leads to a flow resistance and makes droplet aggregation difficult.[79] In double emulsions, the droplet size may be slightly larger than in multiple emulsions, and the polyglycerol polyricinoleate would not allow stabilizing interfacial membranes as well as lecithin and chitosan. More differences among these emulsions were observed in the optical microscopic images, with double emulsions appearing to be more heterogeneous (droplet size

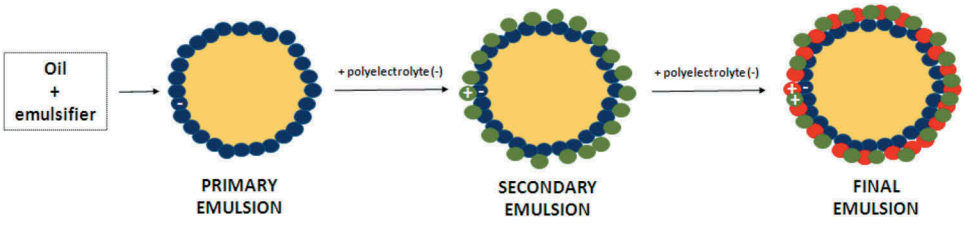

Figure 2. Example of the procedure for a multilayered emulsion with two layers: 1º, emulsifier (●) of negative charge; 2º, two polyelectrolytes (●, ●) of positive charge.

between 1 and 20 μm) than monolayered and multilayered emulsions (97% of the droplets being smaller than 2 μm).[77]

Microcapsules produced by spray-drying from the multilayered emulsions showed better quality characteristics (higher microencapsulation efficiency and EPA and DHA content) than those from monolayered or double ones. The structure formed in the multilayered microcapsules (lecithin-chitosan) could explain this result, since it increases the thickness of the coating that surrounds the oil droplets, preventing contact with pro-oxidants.[80]

More recently, a trial for improving the preparation of multilayered emulsions by applying high-pressure homogenization has been carried out[81], evaluating two homogenization pressures, 7.10^7 and 15.10^7 Pa. Improved quality characteristics in the obtained emulsion (the creaming index decreased) and microcapsules (higher encapsulated oil and lower lipid oxidation) were found when applying 1500 Ba. The scanning electronic microscopic (SEM) images of microcapsules from homogenized emulsions showed less pores and dimples, but also a different shape, with microcapsules from emulsions homogenized at 1500 Ba being less sphere-shaped (Fig. 3).

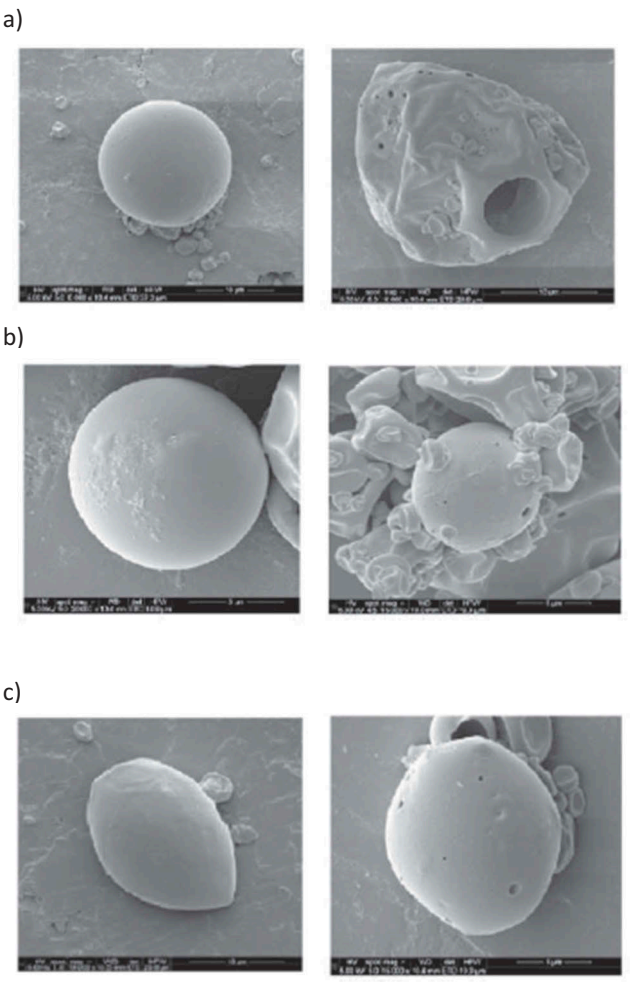

Figure 3. Scanning electron microscopic images of ω-3 microcapsules from different types of emulsions: (a) not homogenized, (b) homogenized at 700 Ba and (c) homogenized at 1500 Ba.

Conclusions

Four principal strategies have been carried out for increasing the content of ω-3 PUFA in meat products. These include adding ω-3 PUFA sources to the animal feeding (i), or to the meat products as bulk oil (ii), oil emulsions (iii), or oil microcapsules (iv).

Most reported strategies to enrich meat and meat products in ω-3 PUFA are those based on the animal feeding and addition of oil emulsions, followed by bulk oil addition, and with fewer researches about the use of oil microcapsules. Most of these studies used poultry and meat products from pigs, with sausages being the preferred in the case of oil emulsion addition. In general, linseed, fish, and algae oils are the most used ω-3 PUFA sources.

Overall, meat products with a healthier lipid profile (higher percentage of ω-3 PUFA) have been achieved with these strategies. However, no data about the content of these fatty acids expressed as mg per g sample has been reported, which does not allow labeling the enriched meat products as "source of ω-3" or "rich in ω-3" according to the European regulation (EU 2010).

The addition of ω-3 PUFA sources to the animal feeding or to the meat products as bulk oil or oil emulsion sometimes influenced negatively on lipid oxidation and sensory attributes, even when antioxidants are used in some cases. Nevertheless, these negative effects have not been found when meat products are enriched with oil microcapsules.

Considering these statements, future research should be focused on i) the microencapsulation techniques, developing and characterizing different fish oil microcapsules, with special attention on the quantification of ω-3 fatty acids as well as their behavior in a great variety of meat products; ii) the impact of the storage process and the different types of culinary process on the enriched meat products; and iii) studies on the digestion and absorption of the enriched meat products, performed in both *in vitro* and *in vivo* models. In addition, the long-term effects of regular consumption of ω-3 fatty acid fortified meat products should be evaluated.

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Capítulo 2

Improvement of encapsulation and stability of EPA and DHA from monolayered and multilayered emulsions by high-pressure homogenization

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Improvement of encapsulation and stability of EPA and DHA from monolayered and multilayered emulsions by high-pressure homogenization

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Abstract

This study aims to improve encapsulation and stability of eicosapentaenoic acid and docosahexaenoic acid by optimizing high-pressure homogenization conditions of monolayered (lecithin + maltodextrine) and multilayered (lecithin + chitosan-maltodextrine) fish oil emulsions. First, a positive influence of high-pressure homogenization on quality characteristics of emulsion and microcapsules was observed. Trials were conducted to get optimum combination conditions of pressure and number of passes of homogenization of both types of emulsions for improving microencapsulation yield and efficiency and lipid oxidation of the corresponded microcapsules. These studied variables showed a notable effect on monolayered microcapsules, being less influencing on multilayered ones. Finally, 1,200 Ba—3 passes and 1,100 Ba—2 passes were selected as optimum combination for monolayered and multilayered emulsions, respectively. This led to microcapsules with improved quality characteristics in terms of microencapsulation yield, microencapsulation efficiency, and oxidation stability, especially in the case of monolayered microcapsules.

Practical applications

The results of this study suppose an advance in the investigations about microencapsulation of bioactive compounds, being specifically useful to develop high stable vehicles of omega-3 fatty acids to enrich food products. To the best of our knowledge, this kind of homogenization procedure has been used to elaborate fish oil microcapsules from monolayered (lecithin + maltodextrine) and multilayered (lecithin + chitosan-maltodextrine) emulsions. The knowledge of the optimal homogenization conditions is important to achieve high quality microcapsules in terms of oxidative stability and efficiency of encapsulation.

1 | INTRODUCTION

Nowadays, high percentage of population is aware of the benefits of omega-3 polyunsaturated fatty acids (ω-3 PUFA), especially eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) (Patch, Tapsell, & Williams, 2017). Fish, seafood, and algae are main natural sources of EPA and DHA. However, these foods are not consumed in sufficient amount to reach the recommended intake of these bioactive compounds: 250 mg for EPA plus DHA (EFSA, 2010).

Concerning the strategies to increase the EPA and DHA content in food, the addition of fish oil, as a bulk or emulsified, has not been successful, mainly due to problems related to lipid oxidation and sensory quality. To solve this difficult, antioxidant addition and microencapsulation (Encina, Vergara, Giménez, Oyarzún-Ampuero, &

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Robert, 2016) have been mainly carried out. Antioxidants can have different behavior depending on the food matrix, being antioxidant in some products and prooxidant in others (Waraho, McClements, & Decker, 2011), and they do not remove unpleasant fish oil and rancid flavors (Shahidi & Zhong, 2010). Microencapsulation of fish oil protects ω-3 PUFA against oxidation, minimizes the contact with environmental conditions (light, temperature, oxygen, iron and other oxidizing promoters), increases the shelf life and masks of the displeasing fish flavor (Encina et al., 2016).

Several methods have been reported to microencapsulate fish oil, being spray drying the most used one (Encina et al., 2016), which requires the elaboration of oil-in-water emulsions. Monolayered emulsions with only a single layer of emulsifier around every single oil particle have been usually prepared for fish oil microencapsulation. However, some alternative modifications of the fish oil emulsion preparation have been developed to obtain multilayered emulsions (Jiménez-Martín, Antequera, Gharsallaoui, Ruiz, & Perez-Palacios, 2015; Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz, & Antequera, 2016). They are performed following the "layer-by-layer" technique (Grigoriev & Miller, 2009). It consists of the combination of an emulsifier (i.e., lecithin) and one or more polyelectrolyte of opposite charges (i.e., chitosan and maltodextrin). This technique starts with a primary emulsion using an ionic emulsifier to stabilize the oil particles in the aqueous phase. Then, a polyelectrolyte of opposite charge is added and surrounds the drops of the previous emulsion. In this way, a secondary emulsion with oil particles coated by a two-layered system is obtained. This process is simple (homogenization and mixing) and advantageous in terms of microcapsules stability and release of bioactive compounds (Guzey & McClements, 2006).

Fish oil emulsions are usually prepared by rotor–stator systems that favors the adsorption of the emulsifiers to oil particles and the decrease of the interfacial tension and prevents particle aggregation. This process is sometimes followed by a high-pressure homogenization step to diminish and standardize the size of oil particles, broke aggregates of particles and spread them evenly, and consequently, improve the emulsion stability, the efficiency of microencapsulation as well as the characteristics of the microcapsules (McClements, 2004). Nevertheless, homogenization of emulsions at high pressure could also have undesired side effects, such as viscosity, foam, changes in the surface area, and even increase lipid oxidation (Penbunditkul et al., 2012).

Overall, optimization of the different parameters of a given methodology is performed by univariate strategies, determining the conditions by means of assays and error studies. However, this kind of procedure does not consider interactions among parameters of the method nor with more than one quality measure. The response surface methodology can be applied to solve these limitations, since it explores the relationship between several explanatory variables and one or more response variables by means of a mathematical model able to properly predict the values of the response. This is a very useful tool for selecting the optimum conditions when there are interactions between variables (Ghafoor, Choi, Jean, & Jo, 2009; Leardi, 2009).

Thus, the main objective of the present study was to improve quality characteristics of ω-3 microcapsules from monolayered and multilayered fish oil emulsions by the optimization of high-pressure homogenization conditions.

2 | MATERIAL AND METHODS

2.1 | Experimental-statistical design

A preliminary assay was conducted to evaluate the influence of high-pressure homogenization on quality characteristics of monolayered and multilayered emulsions and their corresponding microcapsules, which were obtained by spray drying as below detailed. For that, emulsions were homogenized at 1,100 Ba with two passes, which can be considered as intermediate homogenization conditions according to results on a previous experiment (unpublished data). A control batch (without homogenization) of each type of emulsion was also prepared. Creaming index, pH, and particle size were analyzed in the emulsions. Microcapsules were evaluated by means of microencapsulation yield, moisture, microencapsulation efficiency, lipid oxidation, particle size, and scanning electron microscopy. Replicate experimental samples (n = 5) of each batch of emulsions and microcapsules were produced, and analysis were performed by duplicate. The effect of high-pressure homogenization was analyzed by one-way ANOVA using IBM SPSS Statistics v.19.

Following, a response surface methodology trial was carried out for each type of emulsion individually. They were conducted to optimize the high-pressure homogenization conditions for emulsions in order to achieve the best quality characteristics of their corresponding microcapsules. For that, it was applied a full factorial central composite design, consisting of a complete 2^3—factorial design with five center points and one axial point on the axis of each design variable at a distance of α = 1 from the design center. Hence the complete design had 13 combinations, including 5 replicates of the center point. Experimental parameters were pressure and passes of homogenization, varying from 700 to 1,500 Ba, and from 1 to 3 passes, respectively. Three responses were evaluated: microencapsulation yield, microencapsulation efficiency, and lipid oxidation of microcapsules. Table 1 shows the complete experimental design, which was performed by Design Expert trial-Version 7 (Stat-Ease Inc., Minneapolis, MN).

2.2 | Materials

Fish oil from cod liver was kindly provided by Biomega Nutrition (Galicia, Spain). Soybean lecithin (L-alpha-lecithin: 1-hexadecanoyl-2-(9E, (2E-octadececanoyl))-sn-glycero-3-phosphocholine) (Across Organics, Madrid, Spain), chitosan with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, Roquette, Lestrem, France), and food-grade glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions.

TABLE 1 Coded and uncoded values of the independent variables and obtained responses of the central composite design for optimisation the homogenization conditions of monolayered and multilayered fish oil emulsions

	Independent variables				Responses					
	Coded		Uncoded		Monolayered			Multilayered		
Run	*A* pressure (Ba)	*B* number of passes	*A* pressure (Ba)	*B* number of passes	MY (%)	MEE (%)	TBARs (mg MDA/kg sample)	MY (%)	MEE (%)	TBARs (mg MDA/kg sample)
1	−1	−1	700	1	64.93	81.34	230.80	33.95	44.495	24.81
2	−1	+1	700	3	54.21	86.49	184.71	37.50	58.18	27.27
3	0	−1	1,100	1	44.87	84.09	148.42	44.78	23.99	33.57
4	0	0	1,100	2	59.70	88.12	197.48	33.33	66.50	31.53
5	0	0	1,100	2	67.43	84.53	190.39	35.30	67.68	27.83
6	+1	0	1,500	2	45.48	81.49	188.99	43.00	35.252	32.94
7	0	+1	1,100	3	63.58	90.71	154.46	30.43	33.25	18.94
8	−1	0	700	2	57.58	90.37	202.15	47.68	26.70	31.45
9	0	0	1,100	2	36.26	92.52	199.15	38.81	67.80	23.12
10	0	0	1,100	2	64.50	88.82	190.98	38.89	64.18	19.54
11	+1	+1	1,500	3	47.97	93.41	90.35	48.68	48.51	32.38
12	0	0	1,100	2	58.71	82.22	198.42	44.06	62.91	33.03
13	+1	−1	1,500	1	40.83	81.33	151.69	44.29	34.94	24.58

Abbreviations: MEE, microencapsulation efficiency; MY, microencapsulation yield; TBARs, lipid oxidation.

Journal of Food Processing and Preservation

Hydrochloric acid and petroleum ether (Scharlau, Barcelona, Spain) were used for the oil extraction of the microcapsules. For the oxidative stability, 1-butanol and isopropanol (Scharlau, Barcelona, Spain) were used as solvents and 2-thiobarbituric acid (TBA, Serva, Heidelberg, Germany), trichloroacetic acid (Fisher, Barcelona, Spain), and 2,6-di-tert-butyl-4-methylphenol 99% (BHT, Across Organics, Madrid, Spain) as reagents.

2.3 | Preparation of emulsions and microcapsules

Two different types of fish oil emulsions (monolayered and multilayered) and their corresponded microcapsules were prepared according to Jiménez-Martin et al. (2015) with slight modifications.

Fish oil (20 g) and lecithin (6 g) were mixed with a magnetic stirrer overnight. Then water was added until a total weight of 200 g and homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion was obtained and then homogenized at high pressure (SPX, model APV-200a, Silkeborg, Denmark) under the conditions previously described.

The homogenized primary emulsion was blended with 200 g of water, in the case of the monolayered, and with 200 g of 1% of chitosan (wt/wt) in acetic acid 1%, in the case of the multilayered, by slowly agitating with a magnetic stirrer for 15 min. In both types of emulsions, the final step consists of adding 400 g of maltodextrin solution (120 g maltodextrin + 280 g water), to obtain the feed emulsion.

Feed emulsions (800 g) were dried in a laboratory-scale spray-dryer equipped with a 0.5-mm nozzle atomizer (Mini spraydryer B-290, Buchi, Switzerland). The emulsions, maintained at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray drying process. The aspirator rate was adjusted at 80%, feed rate was 1 L/h, inlet temperature was 180°C, and outlet temperature ranged 85–90°C. The collected dried powders were stored in containers at 4 ± 1°C until further analyses were performed.

2.4 | Analysis of emulsion characteristics

2.4.1 | pH

The pH of the feed emulsions was determined with a glass electrode pH meter model CyberScan pH 510 (Eutech Instruments, Illkirch, France), testing a 10 ml volume taken immediately after the production. The pH meter was calibrated with commercial buffer solutions (Crison, Barcelona, Spain) at pH 4.0 and 7.0 prior to use at the moment of the sampling.

2.4.2 | Creaming Index

Emulsions were transferred into a tube sealed with a plastic cap and stored for 1–7 days at room temperature. The cream layer (HC) and the total height (HE) of the emulsions were measured. Creaming index was calculated as creaming index = 100 (HE − HC/HE) (Surh, Decker, & McClements, 2006).

2.4.3 | Particle size of emulsions

Particle sizes of emulsions were measured by laser diffraction in a Mastersizer 3000 (Malvern Instruments, Ltd., Worcestershire, UK). Solutions were diluted in recirculating water (2,100 rpm), until it reached an obscuration of 7%. The refractive indices used for particle and dispersant were 1.520 and 1.330, respectively. Results were given in percentage volume, surface area mean diameter (D [3;2]) and percentile 10%, 50%, and 90% (Dv (10)), (Dv (50)), and (Dv (90), respectively).

2.5 | Analysis of microcapsule characteristics

2.5.1 | Microencapsulation yield

Microencapsulation yield provides the ratio between the quantity of microcapsules obtained and the solid content in the feed emulsions, and it was calculated according to Zhong, Jin, Davidson, and Zivanovic. (2009):

$$\text{Microencapsulation yield (\%)} = (\text{mass of collected product/non- solvent mass in the feed emulsions}) * 100 \quad (1)$$

2.5.2 | Moisture

Moisture was analyzed by following AOAC (2000) reference method 935.29.

2.5.3 | Microencapsulation efficiency

Microencapsulation efficiency was determined as a function of the encapsulated oil related to the total oil content of the microcapsules (Velasco, Marmesat, Dobarganes, & Marquez-Ruiz, 2006):

$$\text{Microencapsulation efficiency (\%)} = ((\text{total oil} - \text{external oil}) / \text{total fish oil}) * 100 \quad (2)$$

Total and external oil were calculated as described by Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz, and Antequera (2015).

Lipid oxidation

The lipid oxidation of the microcapsules was determined by the thiobarbituric acid reactive substances method, as described by Hu and Zhong (2010). It was expressed as mg malondialdehyde (MDA)/kg sample.

2.5.4 | Particle size

Mean diameter of microcapsules was measured using a laser light diffraction instrument, Mastersizer 3000 (Malvern Instruments, Malvern, UK) in dry-dispersion. Results were given in percentage volume, surface area mean diameter (D [3;2]), and percentile 10% (Dv (10)), 50% (Dv (50)) and 90% (Dv (90)).

2.5.5 | Scanning electron microscopy

The morphology of the microcapsules was examined with a scanning electron detector microscope FEI QUANTA 3D FEG (FEI Company, Hillsboro, EE.UU.) in high vacuum conditions mode using EDT (Everhart-Thornley Detector). Powder samples were mounted on stubs, fixed with a double adhesive-coated carbon conductive adhesive sheet, and then subjected to metallization (sputtering) with a thin layer of a conductive gold coating for 8 s in order to amplify the secondary electron signal. After metallization, the samples were imaged operating at 3 kV with focused electron beam of Ga^{+} (current of $<6^{e-4}$ Pa) and observed with magnifications comprised between 5,000 and 15,000.

3 | RESULTS AND DISCUSSION

3.1 | Influence of high-pressure homogenization

Table 2 shows results on monolayered and multilayered emulsion with and without homogenization and of the corresponding microcapsules. The main difference between them is the addition of chitosan in the monolayered emulsions. In both cases, oil particles are coated with a layer system based on electrostatic attraction. In monolayered emulsions, the oil is emulsified with lecithin, which has negative charge, and covered with a layer of maltodextrin, a polyelectrolyte with positive charge. In the case of multilayered emulsions, two polyelectrolytes of positive charge, chitosan, and maltodextrin surrounded the oil drop emulsified with lecithin (Jiménez-Martín et al., 2015).

In the case of pH of emulsions, it was higher in monolayered emulsion without homogenization (6.53) than in that homogenized (6.17), while homogenized multilayered emulsions showed higher pH values than multilayered emulsions without homogenization (4.78 and 4.26, respectively). The lower pH of multilayered emulsions can be ascribed to the addition of acetic acid 1%, which is not used for monolayered emulsion preparation. These results are quite in concordance with those found by Jiménez-Martin et al. (2015). The different effects of high-pressure homogenization on pH between monolayered and multilayered emulsions could be ascribed to the influence of high-pressure homogenization on particle size. As detailed below, homogenized monolayered emulsion showed smaller and higher homogeneity oil particles than monolayered emulsion without homogenization and homogenized multilayered emulsion had lower high size oil particles than not homogenized ones. Thus, the lower pH of homogenized monolayered emulsion and multilayered emulsion without homogenization could be explained by the higher surface contact area of their oil particles, which are surrounded by maltodextrine (with positive charge).

As for the creaming index of emulsions, monolayered emulsion without homogenization showed lower values (4.81) than homogenized ones (0.63), with no significant influence on multilayered emulsions. Creaming index and emulsion stability are inversely related, thus, the homogenization of emulsions at high pressure has a positive effect on monolayered ones, which may be more stable. This different effect may be ascribed to changes on the lecithin layer of particles due to high-pressure homogenization, modifying electrostatic attraction with chitosan and favoring contact between layers of different particles. In fact, high-pressure homogenization promotes loss of emulsifying capacity (Penbunditkul et al., 2012) and could lead to collisions of particles and make them sensitive to coalesce (Jafari, He, & Bhandari, 2007).

Particle size curves of homogenized monolayered emulsion and multilayered emulsion without homogenization are monomodal, with one peak representing a predominant size, while monolayered emulsion without homogenization and homogenized multilayered emulsion showed a bimodal distribution (Figure 1). In addition, smaller and higher homogeneity particle size is observed in homogenized monolayered emulsion (D [3;2] = 0.71 μm, Dv (10) = 0.34 μm, and Dv (90) = 9.99 μm) in comparison to not homogenized ones (D [3;2] = 1.52 μm, Dv (10) = 0.57 μm, and Dv (90) = 35.61 μm). However, multilayered emulsion with and without homogenization showed similar values for D [3;2] (9.53 and 12.80 μm, respectively) and Dv (10) (4.15

TABLE 2 Quality characteristics of monolayered (MO) and multi-layered (MU) emulsions and their corresponding microcapsules as affected by high pressure homogenization[a]

	MO			MU		
	NH	H	*p*	NH	H	*p*
Emulsions						
pH	6.53 ± 0.05	6.17 ± 0.04	.001	4.26 ± 0.00	4.78 ± 0.01	***
Creaming index	4.81 ± 0.78	0.63 ± 0.18	<.001	12.61 ± 2.07	11.71 ± 0.58	ns
Microcapsules						
Microencapsulation yield (%)	46.64 ± 0.96	50.71 ± 1.39	.014	41.62 ± 1.13	42.20 ± 1.30	ns
Moisture content (%)	1.71 ± 0.16	1.65 ± 0.03	.535	3.79 ± 0.21	2.88 ± 0.14	**
Microencapsulation efficiency (%)	46.72 ± 0.82	88.88 ± 0.46	<.001	32.97 ± 2.98	55.43 ± 4.60	**
Lipid oxidation (mg MDA/kg sample)	376.77 ± 58.9	231.87 ± 22.0	.016	34.19 ± 1.03	28.90 ± 0.21	***

[a]NH: no homogenized; H: homogenized.
**p < .01; ***p < .001; ns: non-significant.

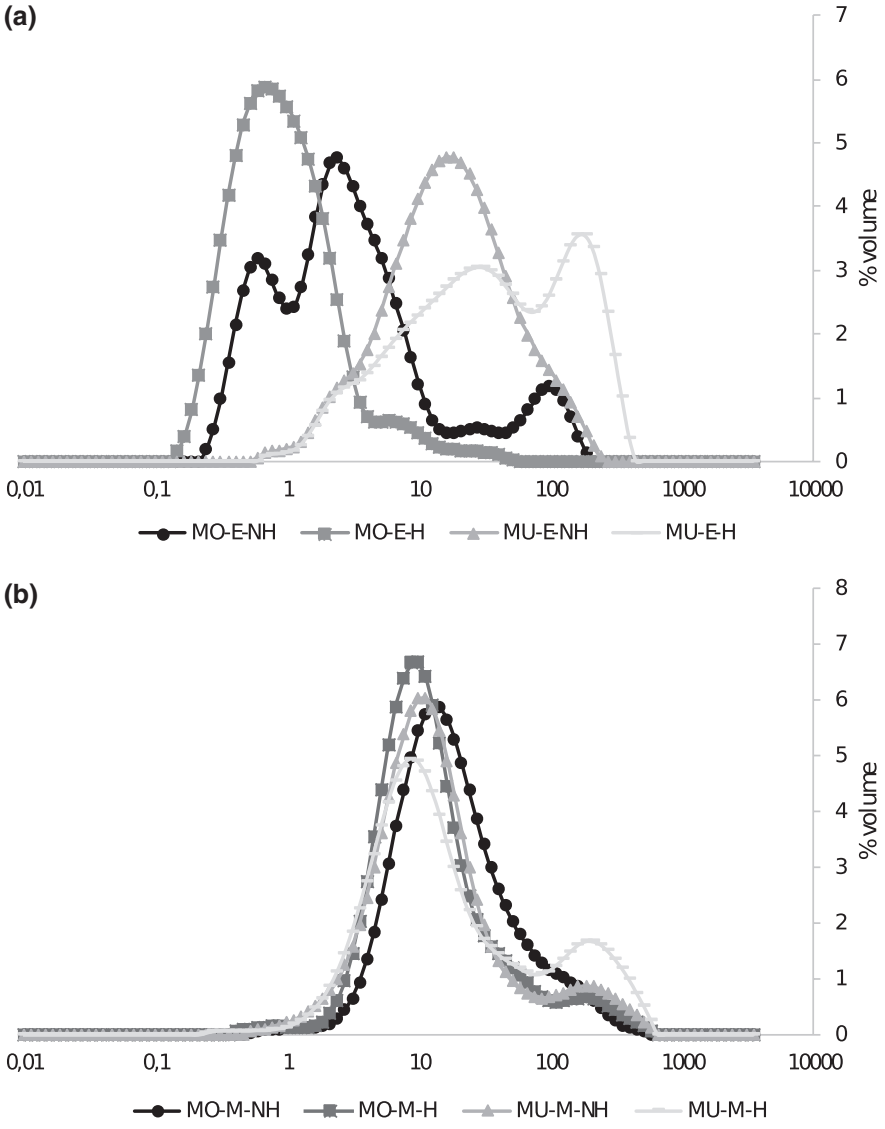

FIGURE 1 Particle size distribution of monolayered and multilayered emulsions (a) and their corresponding microcapsules (b) as affected by homogenization at middle conditions (1,100 Ba, 2 cycle)*. *E, emulsions; H, homogenized; M, microcapsules; MO, monolayered; MU, multilayered; NH, no homogenized

and 4.69 μm, respectively), and finding differences in Dv (90) (74.75 and 225 μm, respectively). As explained above, these results may be influenced on the pH values and again point out the major positive influence of high-pressure homogenization in monolayered than in multilayered fish oil emulsions, as previously discussed for creaming index. Size particles values found in the present study are within ranges previously described (Carneiro, Tonon, Grosso, & Hubinger, 2013; Hogan, McNameeb, O'Riordana, & O'Sullivan, 2001).

In the case of microcapsules, microencapsulation yield significantly differed between monolayered microcapsules without homogenization (46.64%) and homogenized ones (50.71%), while no statistical difference was found between multilayered microcapsules with and without homogenization (Table 2). Other authors have also observed that variations in the preparation procedure can vary the yield of the powders (Gallardo et al., 2013). Thus, in the monolayered emulsions of this study, the homogenization at high pressure may reduce and homogenize the particles of oil that favor the feed atomization and, consequently, the microencapsulation yield. This has a great importance from an economical point of view.

Moisture content of monolayered microcapsules was not significantly influenced by homogenization. However, multilayered microcapsules without homogenization showed higher moisture (3.79%) than homogenized ones (2.88%), which can be ascribed to the reduction of the size of oil particles caused by high-pressure homogenization, increasing the total surface area. Consequently, the water evaporation during spray drying should be improved. This is quite remarkable since lower moisture content is related to stability against microbiological spoilage and preservation of physico-chemical properties, which guarantees long shelf life during storage and final use (Drusch & Mannino, 2009).

Both types of microcapsules were significantly influenced by homogenization in terms of microencapsulation efficiency and lipid oxidation. Homogenized microcapsules showed higher microencapsulation efficiency and lower lipid oxidation than not homogenized ones. It is reported that microencapsulation efficiency is a high variable parameter (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006), which is also observed in the present study. According to Liu, Low, and Nickerson (2010), microencapsulation

efficiency is favored by materials that allow a rapid crust formation in oil particles, avoiding the diffusion of the encapsulated oil to the surface of the particle. If the time for the formation of this membrane increases during spray drying, the migration of some oil to the surface of the formed microcapsules would take place. This could be related to the increase of microencapsulation efficiency in homogenized emulsions of the present study, since the reduction of the size of oil particles positively influences on water evaporation (as corroborated by moisture results, lower in microcapsules from high-pressure homogenized emulsions [Table 2]) and, consequently, favors a rapid formation of the crust. It is also observed a higher microencapsulation efficiency in monolayered than in multilayered microcapsules, which may be related to the confluence of different factors, such as pH, droplet size, stability, which should influence the formation of the multilayer structure, leading to the loss of part of the oil during the spray drying process (Jiménez-Martín et al., 2015). So the optimization of the procedure and formulation to achieve the highest microencapsulation efficiency seems to be a challenge for each type of microcapsule.

As for oxidation stability, it is observed that homogenization of emulsion at high pressure reduces the values of lipid oxidation (Table 2). This quality parameter has been highly related to microencapsulation efficiency since it is inversely related to the external oil, more susceptible for lipid oxidation (Jiménez-Martin et al., 2015). This fact has been corroborated in the present study since microcapsules from high-pressure homogenized emulsions showed high microencapsulation efficiency and low lipid oxidation. Thus, Jafari, Assadpoor, He, and Bhandari (2008) highlighted the importance of controlling these factors to provide good protection and shelf life to the microencapsulated oil.

Homogenization of emulsion at high pressure did not exhibit a marked effect on particle mean diameters of microcapsules (D [3;2] = 8.44, 8.63, 11.82, and 8.20 μm for monolayered microcapsules without and with homogenization and multilayered microcapsules without and with homogenization, respectively), but it influenced on size homogeneity. Monolayered microcapsules with homogenization presented a monomodal distribution, and those not homogenized showed a bimodal distribution, with a second small peak (Figure 1). Besides, monolayered microcapsules without homogenization showed a wider distribution than homogenized ones, with Dv (10) and Dv (90) being 4.12 and 88.81 μm, respectively for monolayered microcapsules without homogenization, and 4.59 and 63.32 μm, respectively for monolayered microcapsules with homogenization. In the case of multilayered microcapsules, both had bimodal distribution, but homogenized multilayered microcapsules showed a second peak being more intense and a wider distribution than not homogenized ones (Dv (10) and Dv (90) = 6.09 and 81.12 μm, respectively for multilayered microcapsules without homogenization, and 3.93 and 209.11 μm, respectively for multilayered microcapsules with homogenization). Thus, homogenization of emulsions at high pressure led to more homogeneous particles in the case of monolayered microcapsules, but not favor particle size or distribution of multilayered microcapsules. Moreover, monolayered microcapsules particles were more homogeneous than multilayered microcapsules. Homogeneity of particle size has been previously related to stability of feed emulsions, resulting low homogeneity from low stable emulsions (Carneiro et al., 2013). This is quite in agreement with findings of the present study.

The scanning electron microscopy images of the microcapsules of this study are shown in Figure 2. As observed previously in most determinations, the influence of high-pressure homogenization is more notable in microstructure of monolayered microcapsules. Overall observations of this type of microcapsules showed microcapsule-bounds in the case of monolayered microcapsules without homogenization but not in those homogenized. However, both types of multilayered microcapsules have shown agglomeration of microcapsules. Regarding individual images, homogenized monolayered microcapsules have an egg-shape morphology of smooth surface, with shrivelling but not pores, wrinkles or dimples. However, monolayered microcapsules without homogenization are spherical and present pores and dimples and even some broken microcapsules. These findings could indicate that the high-pressure homogenization of the monolayered emulsions led to more flexible and softer microcapsules, avoiding the damage of the surface and favoring the protection of the core material. Multilayered microcapsules with and without homogenization showed spherical morphology with similar microstructural features: pores, wrinkles, and dimples. However, the different influence of the emulsion homogenization on the microcapsule morphology could be related to the inclusion of acetic acid and chitosan in multilayered emulsions, which diminish the pH values and strengthen the lecithin layer of the particles, and, consequently this may moderate the influence of the homogenization procedure.

To sum up, homogenization of monolayered and multilayered emulsions at high pressure favors most quality characteristics of both emulsions and microcapsules, specially microencapsulation efficiency and oxidation stability. This result makes it necessary to carry out the high-pressure homogenization process under the conditions that maximize microencapsulation efficiency, oxidation stability, and microencapsulation yield, given the economic importance of this last parameter.

3.2 | Prediction adequacy of models

Two variables of emulsion homogenization, pressure (Ba) and number of passes, were optimized in monolayered and multilayered emulsions with the objective of maximizing microencapsulation yield, microencapsulation efficiency, and lipid oxidation stability of ω-3 microcapsules. The two full-factorial central composite design involved 13 experiments, including 5 replicates of center point for verifying any change in the estimation procedure and measuring the precision property. Table 3 shows results on analysis of the variance by means of Fisher's *F* test for microencapsulation yield, microencapsulation efficiency, and lipid oxidation of monolayered and multilayered microcapsules. In the case of monolayered emulsions, the model *F* values were 0.91, 4.39, and 5.16 for microencapsulation yield, microencapsulation efficiency, and lipid oxidation, respectively. This indicates the

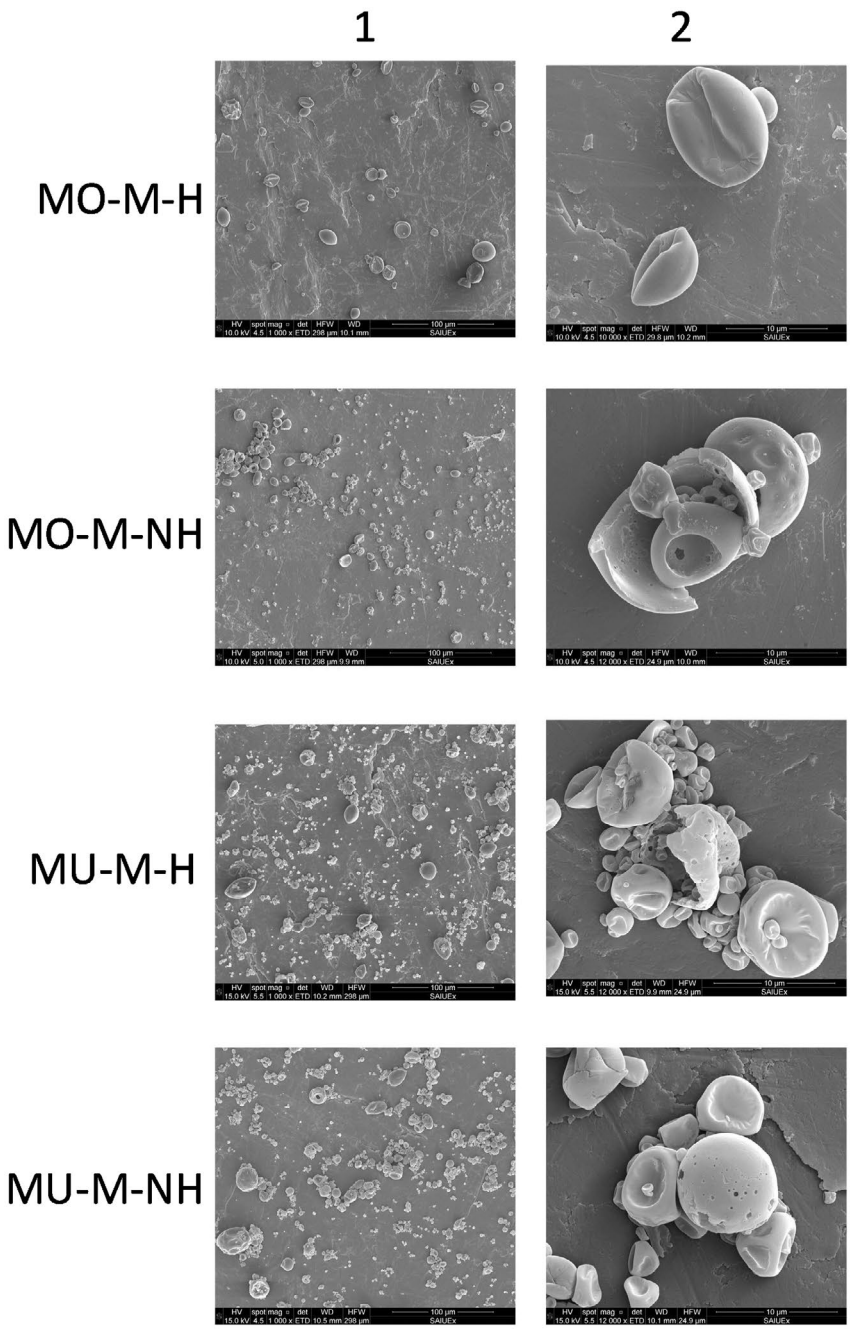

FIGURE 2 Microscopic scanning electron microscopy images of spray-dried microcapsules from monolayered and multilayered emulsions with and without high pressure homogenization (MO-M-H, MO-M-NH, MU-M-H and MU-M-NH, respectively), observed overall (1) and individually (2) with 1,000×–12,000× magnifications

significance of the model for microencapsulation efficiency and lipid oxidation, with a 3.33% and 2.66% chance, respectively, that a so large model *F* value could occur due to noise. Lower values than 0.05 were found for *B* (number of passes) and B^2 in microencapsulation efficiency, and *A* (pressure) and B^2 in lipid oxidation, which indicates that they are significant terms. The lack of fit *F* values of 1.09 and 1.44, respectively for microencapsulation efficiency and lipid oxidation, are good and show that the model fits. There is a 44.85% and 31.43% chance, respectively, for microencapsulation efficiency and lipid oxidation, that a so large lack of fit *F* value could take place due to noise. Moreover, the obtained R^2_{pred} and R^2_{adj} (0.6152 and 0.6647, and 0.7126 and 0.69982, for microencapsulation efficiency and lipid oxidation respectively) were in concordance, and the ratios of 5.176 and 8.335, respectively for microencapsulation efficiency and lipid oxidation, were attained, which indicates an adequate signal. Thus, the response surface quadratic models for microencapsulation efficiency and lipid oxidation of monolayered microcapsules are adequate and significant.

Regarding multilayered emulsions, the model *F*-value of 0.71, 0.85 and 0.27, for microencapsulation yield, microencapsulation efficiency, and lipid oxidation, respectively, implied the insignificance of models for these parameters. This lack of fit indicates the no existence of functional relationship between pressure and number passes of emulsion homogenization and the response variables. The differences in the composition and structure between monolayered (a layer of lecithin) and multilayered emulsions (a multilayer of

TABLE 3 Analysis of variance for response surface model for the microencapsulation yield (MY), microencapsulation efficiency (MEE) and lipid oxidation (TBARs) of fish oil microcapsules from monolayered and multilayered homogenized emulsions

		Monolayered		Multilayered	
Dependent variable	Source	*F* value	Prob. > *F*	*F* value	Prob. > *F*
MY	Model	0.91	.5248	0.71	.6341
	A	2.78	.1396	1.23	.3034
	B	0.35	.5706	0.18	.6854
	AB	0.74	.4189	4.659E-003	.9475
	A^2	0.42	.5355	2.14	.1872
	B^2	0.049	.8315	0.38	.5582
	Residual				
	Lack of fit	0.34	.8020	3.99	.1075
MEE	Model	4.39	.0332	0.85	.5584
	A	0.039	.8494	0.065	.8065
	B	7.73	.0179	0.76	.4132
	AB	0.73	.0523	1.127E-003	.9974
	A^2	0.39	.5523	0.83	.3934
	B^2	4.81	.0384	1.29	.2926
	Residual				
	Lack of fit	1.09	.4485	142.45	.0002
TBARs	Model	5.16	.0266	0.27	.9144
	A	12.79	.0090	0.17	.6887
	B	3.77	.0932	0.083	.7821
	AB	0.13	.7308	0.18	.6805
	A^2	0.25	.6330	0.71	.4258
	B^2	8.66	.0216	0.55	.4829
	Residual				
	Lack of fit	1.44	.3143	1.48	.3472

Note: *A* = pressure; *B* = number of passes.

lecithin-chitosan) could explain this different behavior. The presence of only one layer of lecithin makes it quite susceptible to moderate changes in the procedure, while the multilayer of lecithin–chitosan seems to be less vulnerable.

3.3 | Selection of the optimum conditions

The surface and contour plots on each response function (microencapsulation yield, microencapsulation efficiency, and lipid oxidation) as affected by two studied variables (pressure and number of passes of high-pressure homogenization) for monolayered and multilayered microcapsules are shown in Figures 3 and 4, respectively. In the case monolayered microcapsules, microencapsulation yield is not significantly affected by the studied variables but Figure 3a exposes that very high pressure decreases microencapsulation yield notably, microencapsulation efficiency increases with both the pressure and the number of passes (Figure 3b), and the lowest values of lipid oxidation (Figure 3c) are found with high pressure and 1 or 3 passes of homogenization. Thus, the final quality of monolayered microcapsules is affected by pressure and number of passes of homogenization. Next, optimization of these variables was developed to achieve the following goals: maximum microencapsulation yield and microencapsulation efficiency and minimum lipid oxidation, resulting on 1,200 Ba and 3 passes of emulsion homogenization.

Regarding multilayered microcapsules, although pressure and number of passes of homogenization are not significant models for microencapsulation yield, microencapsulation efficiency and lipid oxidation, surface and contour plots showed that microencapsulation yield increased with the pressure of homogenization (Figure 4a), while the highest percentage of microencapsulation efficiency (Figure 4b) and the lowest values of lipid oxidation (Figure 4c) are found at middle conditions of pressure and number of passes. Thus, optimum conditions to achieve maximum microencapsulation yield and microencapsulation efficiency and minimum lipid oxidation in multilayered microcapsules consists of homogenizing multilayered emulsions at 1,100 Ba during 2 passes.

3.4 | Validation of model prediction

To confirm the validity of the experimental models, the microencapsulation yield, microencapsulation efficiency, and lipid oxidation were analyzed in microcapsules from monolayered and multilayered

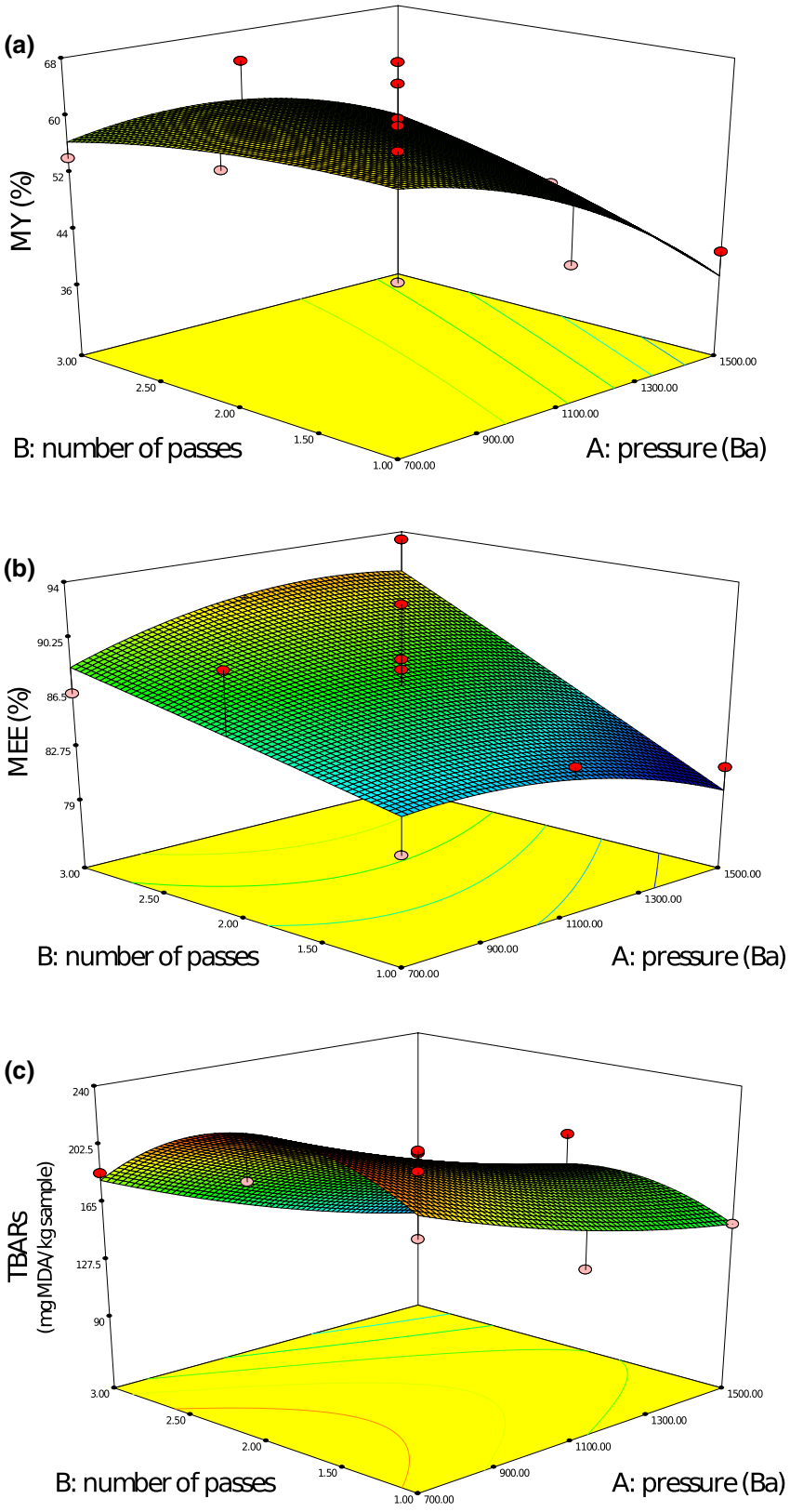

FIGURE 3 Response surface plots on the microencapsulation yield (a), microencapsulation efficiency (b) and lipid oxidation (c) of omega-3 microcapsules as affected by homogenization conditions (pressure and number of passes) of monolayered fish oil emulsions

emulsions homogenized at the selected optimum conditions, with a triplicate set. Experimental and predicted values of microencapsulation yield, microencapsulation efficiency, and lipid oxidation of monolayered and multilayered microcapsules from emulsions homogenized under the optimum combination of pressure and number passes showed a high consistency (Table 4).

It is also worth noting that homogenized monolayered microcapsules obtained higher values ($p < .001$) than multilayered ones

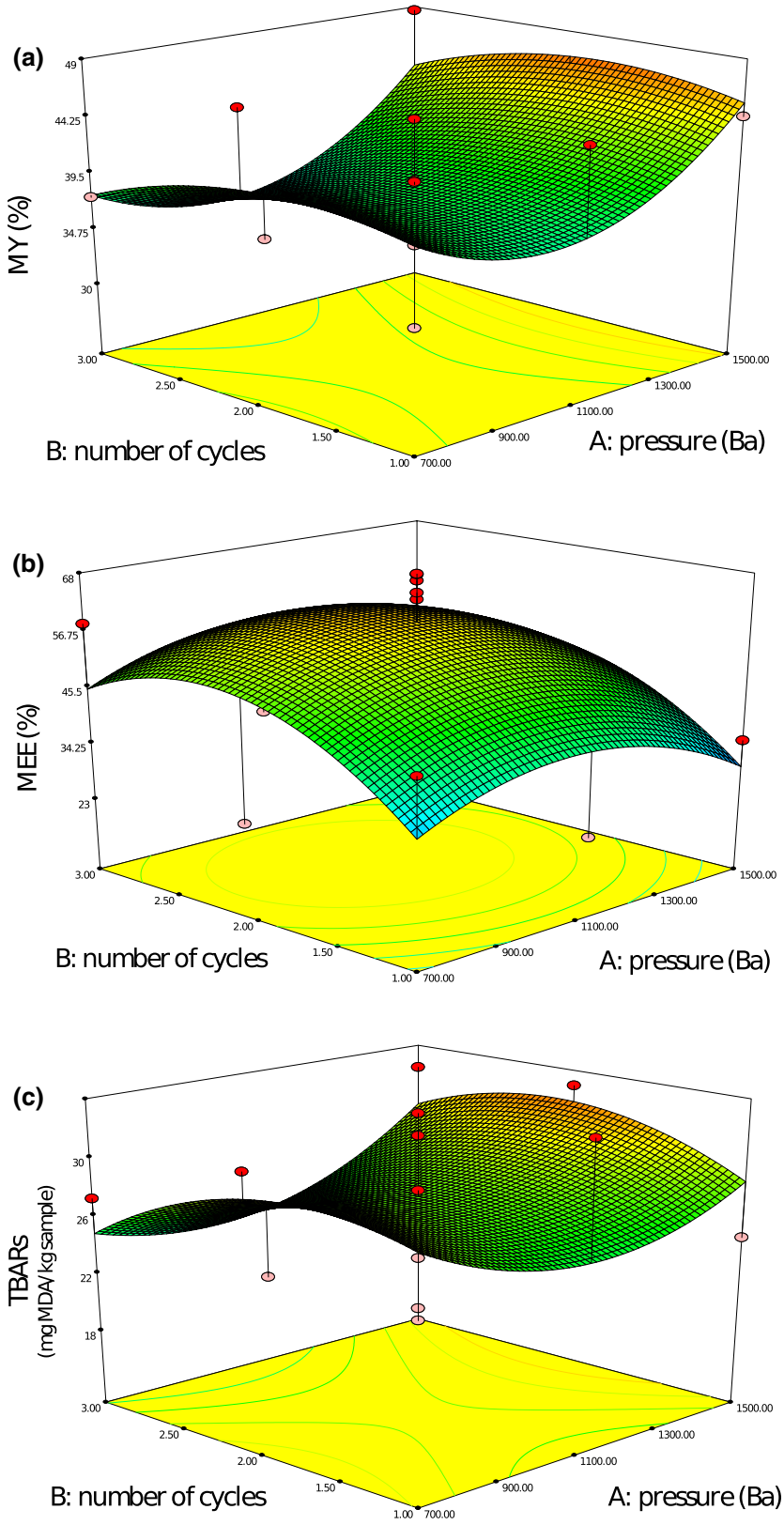

FIGURE 4 Response surface plots on the microencapsulation yield (a), microencapsulation efficiency (b) and lipid oxidation (c) of omega-3 microcapsules as affected by homogenization conditions (pressure and number of passes) of multilayered fish oil emulsions

for microencapsulation yield (56.93% and 40.79%, respectively), microencapsulation efficiency (87.39% and 56.45%, respectively) and lipid oxidation (131.47 and 29.01 mg MDA/kg sample, respectively) (Table 4). These results may indicate the great quality of homogenized monolayered microcapsules, since (a) high microencapsulation yield and microencapsulation efficiency are always desired and (b) the maximum concentration of MDA in fish oil for keeping its quality and acceptability for human consumption is 7–8 mg MDA/kg oil (Huss, 2011), which is far away from values of homogenized monolayered microcapsules (1.19 mg MDA/kg oil, as their total oil is 11%).

TABLE 4 Optimum homogenization conditions of monolayered and multilayered emulsions and their experimental and predicted response in the corresponding microcapsules

	Monolayered	Multilayered
Optimum homogenization conditions		
Pressure (Ba)	1,200	1,100
Number of cycles	3	2
Response		
Microencapsulation yield (%)		
Experimental	56.93 ± 4.41	40.78 ± 0.95
Predicted	56.23	38.67
Microencapsulation efficiency (%)		
Experimental	87.39 ± 1.39	56.45 ± 3.89
Predicted	91.54	58.48
Lipid oxidation (mg MDA/kg sample)		
Experimental	131.47 ± 18.91	29.01 ± 0.33
Predicted	125.71	27.57

Thus, homogenized monolayered microcapsules have, in general, more desired quality characteristics. However, the higher oxidation stability of homogenized multilayered microcapsules could be explained by the strong influence of the wall materials on the oxidative stability of powders, as reported by Carneiro et al. (2013), since the structured formed is different between microcapsules (chitosan-maltodextrine in multilayered microcapsules and maltodextrine in monolayered ones). In addition, Friedman and Juneja (2010) have suggested that chitosan (added in multilayered emulsions) could act as a free radical scavenger.

In addition, quality parameters of homogenized monolayered microcapsules from emulsion homogenized at optimized conditions (Table 4) are better than those from emulsion homogenized at middle conditions (Table 2). In the case of homogenized multilayered microcapsules, since optimum and middle conditions are the same (1,100 Ba, 2 passes), no differences were found between values for microencapsulation yield, microencapsulation efficiency, and lipid oxidation shown in Tables 2 and 4. This seems to indicate the appropriateness of using 1,100 Ba and 2 passes for high-pressure homogenization of multilayered fish oil emulsions.

4 | CONCLUSIONS

This study demonstrates the importance of optimizing conditions for high-pressure homogenization of fish oil emulsions to improve properties of omega-3 microcapsules, specially microencapsulation efficiency and oxidation stability.

Pressure and number of passes of homogenization have shown a notable effect on percentage of microencapsulation efficiency and lipid oxidation values of microcapsules from two types of emulsions (monolayered with maltodextrine and multilayer with chitosan-maltodextrine).

Optimum combination of pressure and number of passes of homogenization has been reached: 1,200 Ba-3 passes and 1,100 Ba-2 passes for monolayered and multilayered emulsions of fish oil, respectively, which led to microcapsules with great quality characteristics in terms of microencapsulation yield, microencapsulation efficiency and oxidation stability.

The positive effect of the high-pressure homogenization is less notable in microcapsules from multilayered than from monolayered emulsions, which showed, in general, better quality characteristics.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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Capítulo 3

Capítulo 3.1

Study on fish oil microcapsules as neat and added to meat model systems: Enrichment and Bioaccesibility of EPA and DHA

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Study on fish oil microcapsules as neat and added to meat model systems: Enrichment and bioaccesibility of EPA and DHA

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ABSTRACT

This study is mainly focused on evaluating the bioaccesibility of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) in different types of fish oil microcapsules, as a neat and delivered in different meat model systems. For that, lecithin-maltodextrine (MO) and lecithin + chitosan-maltodextrine (MU) microcapsules of fish oil were produced and added to two meat model systems: cooked (CK) and dry-cured (DC). The content of EPA and DHA was similar in MO and MU, but in meat model systems the highest quantity of these fatty acids was found in DC-MU, followed by DC-MO, CK-MU and CK-MO. The highest percentage of EPA and DHA released during the *in vitro* digestion was found in CK-MO, followed by CK-MU, MU and MO, and DC-MU and DC-MO. Thus, the characterisctics of the microcapsules and the meat model systems influence on both the enrichment and the bioaccesibility of EPA and DHA, but not in the same trend. This could point out the importance of analysing not only the quantity of EPA and DHA in the enriched food but also the bioaccesibility of these bioactive compounds in most products as possible.

1. Introduction

Beneficial effects of consuming omega-3 polyunsaturated fatty acids (ω-3 PUFA), mainly eicosapentaenoic acid (EPA, C20:5 ω-3) and docosahexaenoic acid (DHA, C22:6 ω-3), are well known, such as the decrease in the risk of cardiovascular diseases (Garcia-Almeida et al., 2010), inflammatory disorders (Yates, Calder, & Ed Rainger, 2014) and tumors (Kim, Park, Park, Chon, & Park, 2009), and the contribution to the development of infant brain and liver (Agostoni, 2008). Thus, dietary recommendations for the daily intakes of these fatty acids have been established by different health organizations, being around 0.25 g of EPA plus DHA per person per day (EFSA, 2010). However, the consumption of fish and seafood, which are the main sources of EPA and DHA, is not enough to reach their recommended intake.

The main challenge of enrichment with ω-3 PUFA is their high susceptibility to oxidation (Jacobsen, 2010), releasing unhealthy oxidized products. This involves nutritional loss and unacceptable rancidity and fishy off-flavor (Taneja & Singh, 2012). Among the evaluated strategies to enrich meat and meat products in ω-3 PUFA, the use of microcapsules seems to be the most appropriate one (Pérez-Palacios, Ruiz-Carrascal, Solomando, & Antequera, 2019). Different types of ω-3 microcapsules have been used to enrich meat and meat products. These studies have focused on evaluating the effect of adding the microcapsules on the quality characteristics of the meat products, mainly lipid oxidation and sensory attributes. However, the quantity of ω-3 PUFA (expressed as mg per g sample) has not been calculated, not being able to label those analysed meat products as “source of omega-3“ or “rich in omega-3” according to the European regulation (EU 2010).

The microencapsulation of fish oil has also been proved as an accurate strategy to release the encapsulated material at targeted sites (Chatterjee & Judeh, 2016). In this context, the selection of the coating wall materials and emulsifiers is decisive. The combination of lecithin, as emulsifier, and chitosan, as coating material, has been proven to secure the stability to oxidation (Hwa Shin, Kyun Chung, Tae Kim, Joung Joung, & Jin Park, 2013).

Besides, it is being claimed to study the stability of the bioactive compounds in the stomach and bioaccesibility in the intestine (Chew, Tan, & Nyam, 2018). The bioaccessibility of oils and food bioactive components delivered has been evaluated by using *in vitro* models in many studies (Leal-Calderon & Cansell, 2012). However, the studies on ω-3 PUFA enriched meat products have neither carried out digestibility analysis, there not being data about the releasing of EPA and DHA from microcapsules in the gastrointestinal tract (Pérez-Palacios et al., 2019).

This study is mainly focused on analyzing the use of different fish oil

Abbreviations: ω-3 PUFA, omega-3 polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MO, microcapsules from monolayered emulsions; MU, microcapsules from multilayered; CK, cooked meat model systems; DC, dry-cured meat model system; CON, control batch

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microcapsules as ω-3 PUFA vehicles by evaluating their enrichment capability and bioaccesibility of EPA and DHA during *in vitro* digestion, as a neat or delivered in different types of meat model systems.

2. Material and methods

2.1. Experimental design

Monolayered and multilayered emulsions of fish oil were spray-dried to obtain their corresponding microcapsules (MO and MU, respectively), following the methodology of Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz-Carrascal, and Antequera (2014) with slight modifications. Lecithin was used as emulsifier in both emulsions, which differed in the wall material composition, maltodextrine and chitosan-maltodextrine, respectively. The microcapsules were characterized by means of lipid composition and digestibility.

Two different meat model system were elaborated, cooked (CK) and dry-cured (DC), which were added with MO (MO-CK, MO-DC) and MU (MU-CK, MU-DC). A control batch (without enriching) of each model system was also prepared (CON-CK, CON-DC). The six meat model systems were elaborated in triplicate and analysed by lipid content, quantity of EPA and DHA and digestibility. The analyses were carried out in triplicate.

2.2. Elaboration of meat model systems

CK were made with minced pork (65%), minced belly fat (15%), water (20%), salt (20 g/kg), and the corresponding microcapsules in the case of the enriched batches. The mixture was kneaded by using a Thermomix, stuffed into 50 mL Falcon tubes, cooked in a water bath at 80 °C during 15 min and finally cooled.

DC were elaborated with minced pork (87%), minced belly fat (13%), salt (20 g/kg), and the corresponding microcapsules in the case of the enriched batches. The mixture was also blended in a Thermomix, stuffed into 50 mL Falcon tubes, and submitted to a dry-cured processing at 15 °C and 80% relative humidity during 12 days.

In both model systems, the quantity of MO and MU added was 3 and 5 g, respectively, per 100 g of dough. These figures were estimated to get the required quantity of EPA + DHA to label food as "source of omega-3" (40 mg per 100 g of sample), considering the EPA and DHA content, which was similar in both types of microcapsules, and the microencapsulation efficiency, which was higher in MO (Solomando, Antequera, Ruiz-Carrascal, & Pérez-Palacios, 2019).

2.3. Lipid analysis

The percentage of total oil of the microcapsules was calculated as described (Jiménez-Martín et al., 2014).

Fat content of meat model systems was determined gravimetrically with chloroform:methanol (2:1, vol/vol) (Pérez-Palacios, Ruiz, Martín, Muriel, & Antequera, 2008). Sample (5 g) were homogenized with chloroform:methanol (2:1, vol/vol) (100 mL), centrifuged (10 min, 1008 g) and filtered. Then, distilled water (5 mL) was added to the filtrate, shaken and centrifuged (10 min, 1008 g). The upper aqueous phase was eliminated, and the lower chloroformic phase was collected after filtering through anhydrous sodium sulphate. Chloroform was finally evaporated and the lipid content was gravimetrically determined.

Fatty acid methyl esters (FAME) of microcapsules and meat model systems were obtained by applying acidic trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992). FAME were analysed by gas chromatography, using an Agilent 6890N gas chromatograph, equipped with a flame ionisation detector (FID). Separation was carried out on a polyethylene glycol capillary column (60 m long, 0.32 mm wide and 0.25 mm film thickness) (DB-WAX; Agilent, Santa Clara, USA). Oven temperature programming started at 120 °C. Immediately, it was raised 3 °C min^{-1} to 250 °C, and held for the last 12 min at 250 °C. Injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 1.5 mL min^{-1}. EPA and DHA FAME peaks were identified, by comparing their retention times with those of standards (Sigma, St Louis, MO, USA), quantified, by using tridecanoic acid (C13:0) as internal standard and calibration curves for EPA and DHA, and expressed as mg FAME/g sample.

2.4. Simulated digestion

The release of EPA and DHA from the microcapsules and meat model systems was evaluated in simulated digestion conditions following the methodology of Wang, Gong, Huang, Yu, and Xue (2009) and Werner and Böhm, (2011) with slight modifications. Firstly, the digestion solutions were prepared as follows: 20 mg amylase (α-amylase from *Aspergillus oryzae*, 30U/mg, Sigma, St Louis, MO, USA) in 1 mL water at pH 6.5 for the oral solution, 3.2 g/L pepsin (from porcine gastric mucose, 2500U/mg protein, Sigma, St Louis, MO, USA) in 2 g/L NaCl at pH 1.5 for the gastric solucion, and 10 g/L pancreatin (from porcine pancreas, 4 × USP specifications, Sigma, St Louis, MO, USA) in 0.05 mol/L KH_2PO_4 at pH 7.4 for the intestinal solution. Then, the sample (5 g) was weighed, mixed with the oral fluid (1.25 mL) by vortex during 1 min, and stirred at 300 rpm during 5 min at 37 °C. The supernatant was separated from the residue by extraction with hexane (5 mL) and centrifugation (2285 g, 20 min). The residue was added with the gastric fluid (20 mL) and mixed by vortex. The mixture was incubated at 37 °C with shaking at 300 rpm during 2 h. Again, the supernatant was extracted with hexane, and the residue was incubated with the intestinal fluid (25 mL) at 37 °C with stirring at 300 rpm during 3 h. At each time point of 1, 2 and 3 h, three tubes were taken out to extract the supernatant. All supernatants were extracted in weighted glass tubes. After evaporating the solvent, the lipid content was calculated gravimetrically, analysing their fatty acid composition by GC-FID as previously described. Results were expressed as percentage of released fat, EPA and DHA in relation the initial content (before digestion) at the end of each digestion stage.

Two g of sample and 0.25, 8 and 10 mL of oral, gastric and intestine solutions, respectively, were used in the digestion analysis of the microcapsules.

2.5. Statistical analysis

Data of microcapsules and meat model systems were analysed by one-way analysis of variance (ANOVA). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. The statistics were run using the program IBM SPSS Statistics v.22.

3. Results and discussion

3.1. Fish oil microcapsules as neat

Both types of microcapsules (MO and MU) showed similar lipid content (7–9%) and EPA (4–4.5 mg/g samples) and DHA (8–9 mg/g samples) quantities. Fig. 1 shows the percentage of released fat throughout the *in vitro* digestion of MO and MU. Significant differences were found at the five digestion phases. In the case of MO, the highest percentage of released fat were found at the gastric phase, followed by the oral, and 1, 2 and 3 h intestine phases in decreasing order, while MU presented the highest percentage of released fat at the oral phase, followed by the 1 and 2 h intestine, the gastric and the 3 h intestine phases in decreasing order. Thus, in MO, a higher percentage of fat is released at the first phases of *in vitro* digestion, having a lower percentage in the intestine phase. In MU, the percentage of released fat at the first phases (oral and gastric) and at the intensitine phases are similar. Part of the fat released at the oral phase could derive from the

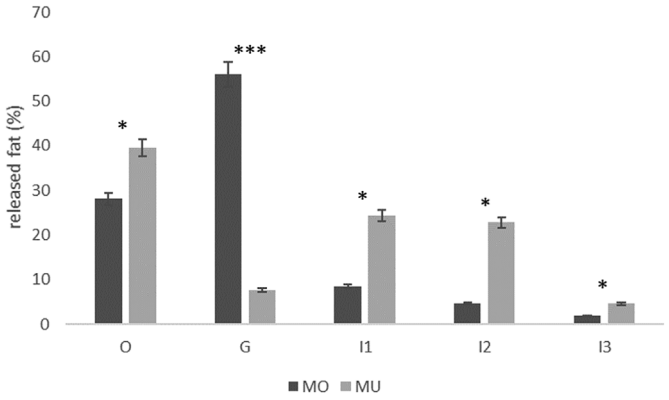

Fig. 1. Percentage of released fat throughout the *in vitro* digestion[1] of monolayered (MO) and multilayered (MU) microcapsules of fish oil.

external fat of the microcapsules, which was higher in MU than in MO (Solomando et al., 2019), since this fat should be firstly and easily liberated from the microcapsules during digestion. In fact, Calvo, Lozano, Espinosa-Mansilla, and González-Gómez (2012) have related the different amount of released oil to the microencapsulation efficiency, and hence to the content of external and internal oil. Differences between MO and MU at the gastric and intestine phases can be ascribed to the wall material of the microcapsules, being the multilayer structure of chitosan-maltodextrine of MU more resistant to the gastric conditions than maltodextrine layer of MO. This is in concordance with the results of the study of in-vitro evaluation of chitosan-coated microcapsules of kenaf seed oil that exposed the resistence of chitosan coating to the gastric environment (Chew et al., 2018). The protection of chitosan against the acidity of gastric observed have been also demonstrated by (Ma et al., 2008). This fact may be related to the great stability of chitosan-coated microencapsulated at pH 3 (Chew et al., 2018).

As for the releasing of EPA and DHA in MO and MU (Fig. 2), both types of microcapsules showed a similar behaviour, there being scarce significant differences. The highest percentage of EPA and DHA released were found at the 1 h intestine phase for MO and MU, followed in decreasing order by the 2 h intestine, oral, gastric and 3 h intestine phases. However, the behaviour of releasing of EPA and DHA from the microcapsules during digestion, which principaly took place at the intestine phase, is different from that previously decribed for the fat, which mainly ocured at oral and gastric phases. These findings are not easy to explain and could be related to different encapsulation efficiency between total oil and fatty acids. In fact, in the study of (Jiménez-Martín, Pérez-Palacios, Ruiz-Carrascal, and Antequera (2016)) with fish oil micrcocapsules, EPA and DHA showed higher encapsulation efficiency than total oil. These results are related to those found by (Calvo et al., 2012), who have also shown differences in the release of total oil and fatty acids during the *in vitro* digestion of different types of walnut oil microcapsules. Moreover, it is described a different rate of fatty acid hydrolysis depending on the length of the chain, with the short chain fatty acid having the fastest rates of hydrolysis (Mu & Høy, 2004), which could also explain the result of the present study.

The released of EPA and DHA was also significantly influenced by the type of microcapsules, with MO showing higher released of EPA at oral and gastric phases and of DHA at oral phase than MU, while higher percentage of DHA was found in MU at 1 and 2 h intestine phases in comparison to MO. Thus, the pecentage of release of EPA and DHA during the whole *in vitro* digestion analysis was higher in MO than in MU, while their bioaccesibility (at the intestine phase) was a bit higher in MU in comparison to MO. This finding again indicates the major resistence of the multilayer structure of chitosan-maltodextrine to the gastric conditions than of the maltodextrine layer of MO. In fact, it has been reported that chitosan provides protection against the acidity of gastric environment (Ma et al., 2008). However, in the study of Zhang, Alsarra, and Neau (2002) carried out with pancreatine and porcine pancreas, the chitosan was hydrolytically digested.

3.2. Meat model systems added with fish oil microcapsules

Results on lipid analysis of the different batches of meat model systems are shown in Table 1. In both CK and DC, the lipid content was similar in the control and in the enriched batches. This result is expected because of the small amount of microcapsule added to the enriched model systems (3 and 5 g of MO and MU per 100 g of dough, respectively), and it is in agreement with findings on chicken nuggets, pork Spanish salchichon and pork burgers enriched with fish oil microcapsules (Aquilani et al., 2018; Jiménez-Martín, Pérez-Palacios, Carrascal, & Rojas, 2016; Lorenzo, Munekata, Pateiro, Campagnol, & Domínguez, 2016).

Regards EPA and DHA, they were only found in the enriched batches, with significant higher content in CK-MU than in CK-MO, and in DC-MU in comparison to DC-MO. It is also worth noting the sum of EPA and DHA in the four batches of model systems, 0.31, 0.38, 0.62 and 0.79 mg/g sample in CK-MO, CK-MU, DC-MO and DC.MU, respectively. In this way, only the DC batches could be labelled as "source of omega-3 fatty acids", while CK-MU is near to get it, but not possible for CK-MO. Since the same quantity of EPA and DHA has been added to the four batches, the difference observed in the content of EPA + DHA may be ascribed to the combined influence of the type of microcapsule and model system. In MO, the coat surrounding the fish oil droplets of lecithin is a maltodextrine layer, while MU have a multilayer structure of chitosan-maltodextrine. As for the type of model system, water is included as ingredient in CK but not in DC. The existence of water in the CK dough may facilitate the solubilisation of the maltrodextrine that forms the wall material of the microcapsules, since maltodextrine had high soluble properties (Stephen, Phillips, & Williams, 2006). This could lead to the release of a proportion fish oil, which may increase the subceptibility to oxidation of its fatty acids, with their probably loss. However, this effect may be minimized in MU, where chitosan is included, since it can increase the electrostatic force and viscosity of the layer (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005). Thus, the chitosan could maintain the structure of the layer and/or reduce the maltodextrine solubilisation, and, consequently avoid the release of EPA and DHA. Moreover, it is noted that the EPA + DHA quatity in DC samples is higher than the estimated figures, which can be explained by de desicattion process (percentage of moisture of 72 and 65% in fresh and dry-cured, respectively) of this model system that concetrates the fatty acid levels.

Regarding to the simulated digestion assay on MO and MU added to meat model system, Fig. 3 exposes the percentage of released fat in CK (Fig. 3.1) and DC (Fig. 3.2). The release of fat in the meat model systems added with microcapsules is lower in comparison to the control one at the oral, gastric and 1 h intestine phases of CK and at the oral and gastric phases of DC. This is difficult to explain and could be related to influence of the physicochemical characteristics of the fat droplet of the emulsions, such as their size, the molecular structure of the triacylglicerols or the surface organization and composition on the gastrointestinal digestion (Berton et al., 2012). However, the mechanisms involved in lipid digestion of complex systems are not fully understood after three decades of studies on artificial emulsions. Instead of these differences, in general, in both model systems, the highest percentage of released fat was found at the 1 and 2 h intestine phases, while the oral, gastric and 3 h intestine phases showed lower percentage. It is also noted a higher percentage of total fat release in CK (around 100%) than in DC (around 50%). It is described that low surface areas available for lipase and the existence of solid fat decrease the rate of lipolysis (Golding & Wooster, 2010; McClements, Decker, & Park, 2008). In comparison to CK, which is a fine paste with fat particles distributed in an acuose phase with proteins and salt, DC presents small and solid cuts

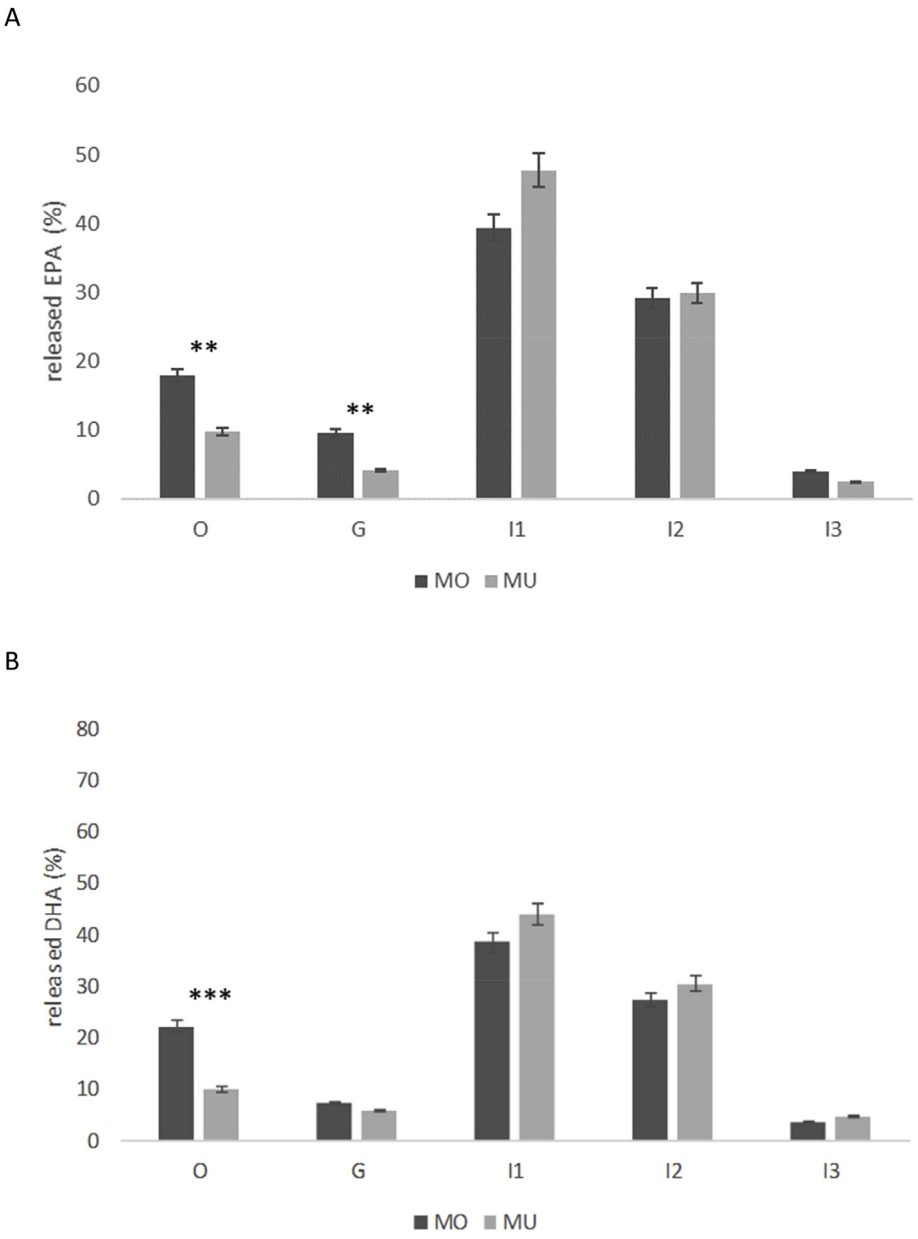

Fig. 2. Percentage of EPA (A) and DHA (B) released throughout the *in vitro* digestion[1] of monolayered (MO) and multilayered (MU) microcapsules of fish oil.

Table 1
Lipid content and quantity of EPA and DHA in cooked (CK) and dry-cured (DC) model systems as affected by the addition of different types of fish oil microcapsules (monolayered (MO) and multilayered (MU)).

		CON	MO	MU	*p*
CK	Lipid (%)	8.29 ± 0.79	8.70 ± 0.41	8.80 ± 0.81	NS
	EPA (mg/g sample)	< LD	0.11 ± 0.01	0.14 ± 0.02	***
	DHA (mg/g sample)	< LD	0.20 ± 0.01	0.24 ± 0.02	***
DC	Lipid (%)	12.60 ± 0.63	12.34 ± 0.83	12.57 ± 0.44	NS
	EPA (mg/g sample)	< LD	0.23 ± 0.01	0.30 ± 0.02	*
	DHA (mg/g sample)	< LD	0.39 ± 0.02	0.49 ± 0.03	***

LD: limit of detection.
NS: not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

of fat. These facts may explain differences in the percentage of fat released between CK and DC.

The release of EPA and DHA during the *in vitro* digestion of CK and DC is shown in Fig. 4. In CK, the major release of EPA took place at both 2 and 3 h intestine phases, whereas the highest percentage releae of DHA were found at 2 h intestine phase followed by the 3 h intestine phase. In the case of DC, the percentage release of EPA was also higher at 2 h intestine phase than at 3 h ones. Regarding the release of DHA in DC, it was quite different in comparison to previous ones, finding the highest percentage release at 1 and 2 h intestine phases. These differences between fatty acids could be related to the percentage of EPA and DHA sterified at the sn-1, sn-2 and sn-3 of triacylglicerols of the fish oil used in this study (unknown), since there is a high specificity of pancreatic lipase for fatty acids esterified at the sn-1 and sn-3 positions of triacylglycerols (Shen & Wijesundera, 2006). For both fatty acids, oral, gastric and 1 h intestine phases showed minor percentage of release in CK and DC. Thus, the released of EPA and DHA ocured principally at the intestine phases, finding a higher percentage in CK (around 90%) than in DC (around 50%), in concordance with results on lipid relasing

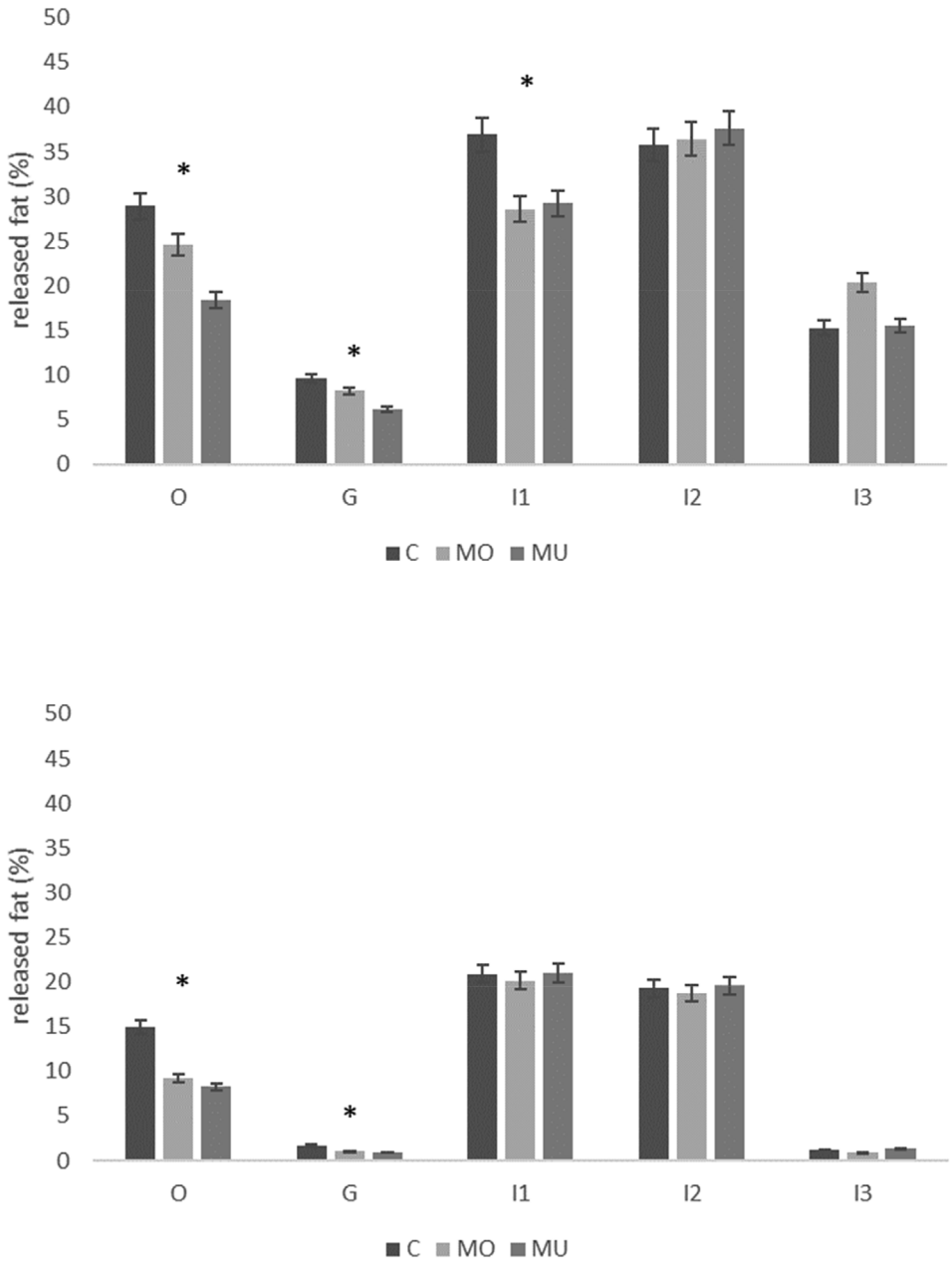

Fig. 3. Percentage of released fat throughout the *in vitro* digestion[1] of cooked (1) and dry-cured (2) model systems: not enriched (C) and enriched with monolayered (MO) and multilayered (MU) microcapsules of fish oil.

and also with previous studies (Shen, Apriani, Weerakkody, Sanguansri, & Augustin, 2011). These authors fortified orange juice, yogurt and cereal bar with microencapsulated tuna oil powder, and found that the type of food matrix affected the extent of lipolysis of omega-3 PUFA, being higher in orange juice and yogurt samples than in cereal bar ones. This result was ascribed to the larger lipid droplets in the digest of the cereal bar and, hence, to the low total surface area available for lipase attack, and could also explain the findings of this study.

The type of microcapsule added to the meat model system influence significantly the percentage of EPA and DHA released at most stages of the *in vitro* digestion process. A higher release of these fatty acids were found at the oral phase when enriching with MU, while at gastric and intestine phases, MO enriched models showed higher percentage of EPA and DHA released than those added with MU. Consequently, in both model systems, the bioaccesibility of EPA and DHA was higher when enriching with MO than with MU. As previously indicated, the differences in the characteristics between MO and MU would cause these results. The higher content of external fat of MU and its multilayer structure of chitosan-maltodextrine, more resistant to the gastric conditions, would explain the major fatty acids released at the oral phase and minor at intestine phases, respectively, in CK-MU and DC-MU.

Moreover, it is worth noting the higher bioaccesibility of EPA and DHA when microcapsules are delivered in CK than in DC or as a neat. However (Shen et al., 2011), did not found significant differences in lipolysis between tunal oil microencapsulates and the enriched orange juice and yogurt. In this study, the lower bioaccesibility of EPA and DHA from the microcapsules is due to the higher released of these fatty acids at the oral phase in these samples (Fig. 2) than in CK (Fig. 4). This could be related to a lower accesibitility of amilase when microcapsules are included in a food matrix. Nevertheless, the influence of oral enzimes on EPA and DHA releasing from microcapsules has not been considered in previous studies found among the scientific literature.

To sum up, among the samples of the present study the highest biodisponiblity for EPA + DHA was found in CK-MO, closely followed

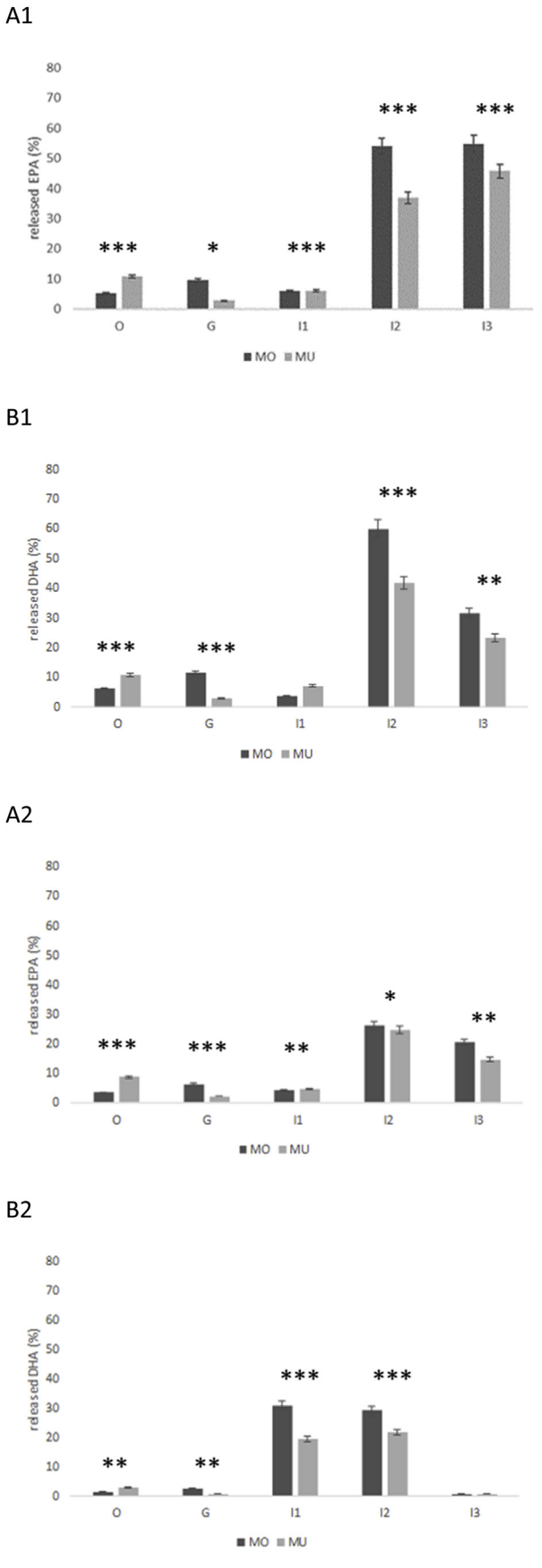

Fig. 4. Percentage of released of EPA (A) and DHA (B) throughout the *in vitro* digestion[1] of cooked (1) and dry-cured (2) model systems enriched with monolayered (MO) and multilayered (MU) microcapsules of fish oil.

by CK-MU, MU and MO and at some distance by DC-MO and DC-MU. As discussed with detail above, these results are due to characteristics of both the type of microcapsules and the characteristics of meat model system.

4. Conclusions

The releasing of EPA and DHA during the *in vitro* digestion of microcapsules as a neat followed the same trend in both types of microcapsules, but lecithin + chitosan-maltodextrine ones showed a higher bioaccesibility for these fatty acids.

The enrichment in EPA and DHA depends on the type of microcapsules and also on the type of meat model system, with fish oil microcapsules of lecithin + chitosan-maltodextrine added to dry-cured meat model system having the best results.

In the enriched meat model systems, the releasing of fat and EPA + DHA was highly affected by the meat model system, whereas the influence of the type microcapsule was less notable. The distribution of the fat particles in fine paste of the meat cooked model systems seems to favour the releasing of fat and the bioaccesibility of EPA and DHA.

Thus, the characterisctics of the microcapsules and of the meat model systems influence on both the enrichment and the bioaccesibility of EPA and DHA. Consequently, it could be pointed out the importance of analysing not only the quantity of EPA and DHA in the enriched food but also the bioaccesibility of these bioactive compounds in most products as possible.

Author contributions

Juan Carlos Solomando: formal analysis, investigation, writting.

Teresa Antequera: conceptualization, supervising, project administration.

Trinidad Perez-Palacios: conceptualization, methodology, resources, supervising, project administration, funding acquisition.

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Declaration of competing interest

None.

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Capítulo 3.2

Lipid digestion and oxidative stability in ω-3 enriched meat model systems: Effect of fish oil microcapsules and processing or culinary cooking

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Lipid digestion and oxidative stability in ω-3-enriched meat model systems: Effect of fish oil microcapsules and processing or culinary cooking

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ABSTRACT

This study evaluates the addition of monolayered (MO) and multilayered (MU) fish oil microcapsules to meat model systems and determines the effects of processing and culinary cooking. Adding MO and MU increased the content of EPA and DHA and the level of secondary oxidation products but being far away from the oxidation values that generate anomalous flavours. However, it did not lead to oxidative damage of the enriched batches or affect the moisture and lipid content during processing and/or culinary cooking. The type of fish oil microcapsules, the processing and/or culinary cooking and the type of meat matrix influenced the fatty acid digestion. The highest bioaccessibility of EPA and DHA occurred in cooked samples enriched with MO after processing and before culinary cooking. To optimize EPA and DHA enrichment and bioaccessibility, the type of fish oil microcapsules may be selected as a function of the type of added meat products.

1. Introduction

Meat and meat products are relevant food due to their nutritional impact because they are important sources of high-quality proteins and certain vitamins (especially vitamin B6 and B12) and minerals (iron, selenium and zinc) (Astiasarán & Alfredo Martínez, 2000, chap. 1). In addition, these products are highly valuable for consumers mainly due to their sensory properties. Nevertheless, meat and meat products are sometimes questioned in terms of their nutrition due to their high to moderate amount of saturated fatty acid (SFA) and low polyunsaturated fatty acid (PUFA) contents and high ω-6/ω-3 ratio (Jiménez-Colmenero, Carballo, & Cofrades, 2001). Therefore, different approaches have been developed to balance the lipid profile of meat and meat products, such as increasing the ω-3 PUFA content.

The importance of enriching meat and meat products with ω-3 PUFA is ascribed to the verified beneficial effects of consuming these fatty acids, mainly eicosapentaenoic acid (EPA, C20:5 ω-3) and docosahexaenoic acid (DHA, C22:6 ω-3); such effects include promoting the development of infant brain and liver and decreasing the risk of cardiovascular diseases, inflammatory disorders and tumours (Zhang, Xu, Wang, & Xue, 2019). Thus, dietary recommendations for the daily intake of EPA plus DHA have been established and are approximately 0.25–0.5 g per person per day (ISSFAL, 2004; EFSA, 2010; FAO, 2010).

A high percentage of the population is aware of these positive effects and understands the importance of incorporating EPA and DHA in their diet; however, the consumption of the main sources of EPA and DHA, i.e., fish and seafood, is not sufficient to reach the recommended intake for these fatty acids, for which the median daily intake in Europe is approximately 14 and 42 mg, respectively (EFSA, 2012). Therefore, increasing the content of EPA and DHA in different foodstuffs, including meat and meat products, has been suggested. The European Union has established legislation on the minimum levels of the sum of EPA + DHA required to label a food as a "source of ω-3 fatty acids" and "high in ω-3 fatty acids": 40 and 80 mg per 100 g and per 100 kcal, respectively (EU, 2010).

One of the main concerns of enrichment with ω-3 PUFA is their high susceptibility to oxidation (Jacobsen, 2010), which involves nutritional loss, unacceptable rancidity and off-flavour and the release of unhealthy oxidized products (Ye, Cui, Taneja, Zhu, & Singh, 2009). It can also lead to the perception of fish odour and flavour in the enriched products (Lopez-Ferrer, Baucells, Barroeta, & Grashorn, 2001). Another major challenge of this approach is the availability of ω-3 PUFA in the intestines; thus, their stability against gastric conditions is required (Chatterjee & Judeh, 2016).

Most reported strategies to enrich meat and meat products with ω-3 PUFA are based on adding linseed, fish and/or algae oils to the animal feed (directly) or to the products (directly, emulsified and microencapsulated). Microencapsulation of fish oil emulsions by spray-drying seems to be the most accurate strategy for increasing ω-3 PUFA in meat products (Perez-Palcios, Ruiz-Carrascal, Solomando, & Antequera,

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2019). In addition, this technique has been described as an appropriate technique to release encapsulated material at targeted sites (Chatterjee & Judeh, 2016).

Different authors have added ω-3 microcapsules to enrich meat products. Josquin, Linssen, and Houben (2012) and Pelser, Linssen, Legger, and Houben (2007) included commercial fish oil microcapsules in sausages; Jiménez-Martín, Pérez-Palacios, Ruiz-Carrascal, and Antequera (2016) and Aquilani et al. (2018) incorporated fish oil microcapsules by spray-drying multilayered emulsions of fish oil to enrich chicken nuggets and burgers, respectively; and Lorenzo, Munekata, Pateiro, Campagnol, and Domínguez (2016) prepared microcapsules from a monolayered emulsion of fish oil in a konjac matrix that were added to Spanish salchichon, a dry-fermented sausage. More recently, Vasile, Romero, Judis, and Mazzobre (2019) evaluated a patty beef product enriched with fish oil encapsulated in polyelectrolyte beads. In each study, different batches of meat products were compared based on quality parameters, ω-3 PUFA content, lipid oxidation and sensory traits. However, except for the work of Vasile et al. (2019), the quantity of EPA and DHA (expressed as mg per g sample) has not been calculated, and the analysed meat products cannot be labelled as a "source of ω-3" or "rich in ω-3" according to the European regulation (EU 2010). These studies have either evaluated the influence of the processing and/or cooking on the quality characteristics of the enriched meat products.

In addition, limited information is available on the release of ω-3 PUFA in the gastrointestinal tract during the digestion of meat products treated with microcapsules. The bioaccessibility of the delivered oils and food bioactive components has been evaluated using in vitro models in many studies (Leal-Calderon & Cansell, 2012). Augustin et al. (2014) reported that in vitro studies provide useful insights into the structure of the interface around the oil droplets and how it affects the accessibility of the oil within a droplet to digestive enzymes.

This study is mainly focused on evaluating the bioaccessibility and oxidative stability of EPA and DHA in meat model systems enriched with monolayered and multilayered fish oil microcapsules. The effect of processing and culinary cooking on these parameters was also analysed.

This study hypothesized that i) the use of different types of fish oil microcapsules and meat model systems might result in differences in the enrichment of EPA and DHA as well as the bioaccessibility of these fatty acids, ii) the effect of adding fish oil microcapsules, processing and culinary cooking may not affect the oxidation stability of the ω-3-enriched meat model systems.

2. Material and methods

2.1. Biological material

Fish oil from cod liver was kindly provided by Biomega Nutrition (Galicia, Spain). Soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95% deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, Roquette, Lestrem, France), and food-grade glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions. Minced pork, minced fat, salt and water used for developing the meat model systems were purchased from a local market.

2.2. Reagents

Chloroform and methanol (Scharlau) were used for the lipid extraction. To determine the oxidative stability, hexane, isopropanol, perchloric acid (Scharlau) and butylated hydroxytoluene (Sigma, St Louis, MO, USA) were used as solvents and 2-thiobartituric acid (TBA) (Serva, Heidelberg, Germany), 1,1,3,3-tetraethoxypropane (TEP) (Acros Organics, Madrid, Spain) were used as reagents. Sulphuric acid and methanol (Scharlau) were used for the transesterification of fatty acids. For the simulated digestion, α-amylase from *Aspergillus oryzae* 30 U/mg, pepsin from porcine gastric and pancreatin porcine pancreas (Sigma), and sodium chloride, potassium phosphate and hexane (Scharlau) were used.

2.3. Experimental design

Monolayered and multilayered microcapsules (MO and MU, respectively) were first elaborated from fish oil emulsions by spray-drying according to Jiménez-Martín et al. (2015) with some modifications: high pressure was used to homogenize the emulsions (1200 Ba-3 passes and 1100 Ba-2 passes of the MO and MU emulsions, respectively) (Solomando, Antequera, Ruiz-Carrascal, & Perez-Palacios, 2019).

Two different meat model systems were developed, namely, cooked (CK) and dry-cured (DC) systems, and they were treated with MO (MO-CK, MO-DC) and MU (MO-CK, MU-DC). A control batch (without enriching) of each model system was also prepared (CON-CK, CON-DC).

The CK system was made with minced pork (65%), minced pork belly (15%), water (20%), salt (20 g/kg), and the corresponding microcapsules for the enriched batches. The mixture was kneaded by using a Thermomix (Wuppertal, Germany), stuffed into 50 mL Falcon tubes, heated in a water bath at 80 °C for 15 min and finally cooled.

The DC system was made with minced pork (87%), minced pork belly (13%), salt (20 g/kg), and the corresponding microcapsules for the enriched batches. The mixture was also blended in a Thermomix, stuffed into 50 mL Falcon tubes, and processed at 15 °C and 80% relative humidity over 12 days. In this case, holes were made in the Falcon tubes to allow the release of water and promote the drying process.

In both cases, the quantity of added MO and MU was 3 and 5 g per 100 g of model system, respectively. These amounts were calculated to exceed 40 mg of EPA + DHA per 100 g of sample, which is the required quantity to label food as a "source of ω-3".

The processing and culinary cooking effects were evaluated by analysing the cooked model systems before (CON-CK-t0, MO-CK-t0 and MU-CK-t0) and after processing (CON-CK-t1, MO-CK-t1 and MU-CK-t1), and after being culinary cooked at 90 °C during 3 min (CON-CK-t2, MO-CK-t2 and MU-CK-t2). The dry-cured model systems were analysed before (CON-DC-t0, MO-DC-t0 and MU-DC-t0) and after processing (CON-DC-t1, MO-DC-t1 and MU-DC-t1). The meat model systems were developed in triplicate and analysed to determine the moisture and lipid contents, EPA and DHA contents, lipid oxidation and digestibility.

The analyses were carried out in triplicate.

2.4. Analysis of meat model systems

2.4.1. Moisture and lipid contents

The moisture and fat contents were analysed following the official methods of the Association of Official Analytical Chemist (AOAC, 2000), reference 950.46, and using chloroform:methanol (2:1, vol/vol) as described by Pérez-Palacios, Ruiz, Martín, Muriel, and Antequera (2008), respectively. Both parameters were determined gravimetrically and are expressed as a percentage.

2.4.2. Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared by acidic *trans*-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992) and analysed by gas chromatography using an Agilent 6890N gas chromatograph equipped with a flame ionisation detector (FID). Separation was carried out on a polyethylene glycol capillary column (60 m long, 0.32 mm wide and 0.25 mm film thickness) (DB-WAX; Agilent, Santa Clara, USA). The oven temperature programming started at 120 °C, was immediately raised 3 °C min^{-1} to 250 °C, and then was held for the last 12 min at 250 °C. The injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 1.5 mL min^{-1}. Individual FAME peaks were identified by comparing their retention times with those of standards (Sigma, St Louis, MO, USA). EPA and DHA FAME peaks were identified by

Table 1
Percentage of moisture and total fat (mean values ± standard deviation) in cooked (CK) and dry-cured (DC) meat model systems as affected by fish oil microcapsules addition (*p* (a)) and processing and/or culinary cooking (*p*)*.

		Moisture (%)				Total fat (%)			
		CON	MO	MU	*p (a)*	CON	MO	MU	*p (a)*
CK	t0	72.91 ± 0.70 z	72.63 ± 0.20 z	72.51 ± 0.58 z	0.660	8.22 ± 0.23	8.70 ± 0.28	8.60 ± 0.12 y	0.092
	t1	65.18 ± 0.17 y	65.88 ± 0.98 y	65.01 ± 0.93 y	0.929	8.81 ± 0.79	9.46 ± 0.41	10.19 ± 0.81 z	0.139
	t2	62.55 ± 0.48 x	62.56 ± 0.10 y	61.99 ± 0.29 x	0.135	9.09 ± 0.066	9.51 ± 0.56	10.30 ± 0.45 z	0.084
	p	< 0.001	0.014	< 0.001		0.314	0.109	0.015	
DC	t0	72.11 ± 0.36	72.45 ± 0.51	72.68 ± 0.41	0.335	12.94 ± 0.06	12.01 ± 0.44	12.74 ± 0.43	0.940
	t1	65.70 ± 1.01	65.48 ± 0.31	65.60 ± 0.80	0.144	13.27 ± 1.31	14.69 ± 0.20	14.53 ± 0.38	0.138
	p	< 0.001	< 0.001	< 0.001		0.009	0.001	0.006	

*CON, MO, MU: samples not enriched, and enriched with monolayered and multi-layered fish oil microcapsules, respectively; to, t1: samples before and after processing, respectively; t2, samples after culinary cooking; Different letters in the same column (x, y, z) indicated significant differences among batches due to processing and/or culinary cooking effects.

comparing their retention times with those of standards (Sigma, St Louis, MO, USA), and they were quantified using tridecanoic acid (C13:0) as an internal standard and the calibration curves for EPA and DHA. They are expressed as mg FAME/g sample.

2.4.3. Lipid oxidation

The measurement of conjugated dienes (CDs) was determined according to the procedure described by Juntachote, Berghofer, Siebenhandl, and Bauer (2006). The sample (0.5 g) was suspended in 5 mL of distilled water and homogenized to form a smooth slurry. A 0.5 mL aliquot of this suspension was mixed with 5 mL of extracting solution (3:1 (v/v) hexane/isopropanol) for 1 min. After centrifugation at 3500 rpm for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of CD was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1}, and the results are expressed as micromoles per kilogram of sample.

The thiobarbituric acid reactive substance (TBARs) content was measured by following the extraction method described by Salih, Smith, Price, and Dawson (1987). Each sample was minced in a kitchen blender, and 2.5 g was homogenized for 2 min with 7.5 mL of 3.86% perchloric acid and 0.5 mL of butylated hydroxytoluene. Tubes were kept on ice to avoid heat degradation. The homogenate was filtered and centrifuged (3 min, 3500 rpm), and the supernatant (2 mL) was mixed with 2 mL of 97% 1,1,3,3-tetraethoxypropane (TEP). Immediately, the mixture was heated to 90 °C for 30 min, cooled, and centrifuged again (2 min, 3500 rpm). Absorbance was measured at 532 and 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The measurement at 600 nm is considered contamination, and it was subtracted from the other measurement to obtain the final absorbance. The concentration of TBARs was calculated as malondialdehyde (MDA) from a standard curve, which was developed simultaneously with the samples using solutions of TEP (Merck, Schchardt, Germany). The TBARs content is expressed as mg MDA kg^{-1} sample.

2.4.4. Simulated digestion

The release of EPA and DHA in the meat model systems was evaluated by following the methodology of Wang, Gong, Huang, Yu, and Xue (2009) and Werner and Böhm (2011) with slight modifications. Firstly, oral, gastric and intestinal solutions were prepared with 20 mg amylase in 1 mL water at pH 6.5, 3.2 g/L pepsin in 2 g/L NaCl at pH 1.5, and 10 g/L pancreatin in 0.05 mol/L KH_2PO_4 at pH 7.4. Then, the sample (5 g) was weighed, mixed with the oral fluid (1.25 mL) by vortexing for 1 min, and stirred at 300 rpm for 5 min at 37 °C. The supernatant was separated from the residue by extraction with hexane (5 mL) and centrifugation (4000 rpm, 20 min). The residue was added to the gastric fluid (20 mL) and mixed by vortexing. The mixture was incubated at 37 °C with shaking at 300 rpm for 2 h. Again, the supernatant was extracted with hexane, and the residue was incubated with the intestinal fluid (25 mL) at 37 °C with stirring at 300 rpm for 3 h. At each time point of 1, 2 and 3 h, three tubes were taken out to extract the supernatant. All supernatants were extracted in weighted glass tubes. After evaporating the solvent, the lipid content was calculated gravimetrically, and their fatty acid compositions were analysed by GC-FID as previously described. The results are expressed as the percentage of fat or fatty acid released in relation to their content in the undigested model system.

2.5. Statistical analysis

The effects of adding fish oil microcapsules, processing and culinary cooking on the moisture and lipid content, EPA and DHA quantity, lipid oxidation and digestibility of the CK and DC meat model systems were analysed individually by a one-way analysis of variance (ANOVA). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using Tukey's test. The statistical analyses were run using the program IBM SPSS Statistics v.22.

3. Results and discussion

3.1. Lipid composition in ω-3 enriched meat model systems

Table 1 shows the percentage of moisture and total fat in the CK and DC meat model system based on the type of fish oil microcapsules added and the processing and/or culinary cooking. The inclusion of MO and MU did not significantly influence the percentage of moisture or fat in any batch, which was likely because a low quantity of microcapsules was included in the meat model systems (3 and 5 g of MO and MU per 100 g of model system). This result is consistent with previous findings in ω-3-enriched chicken nuggets (Jiménez-Martín et al., 2016). Nevertheless, other authors have published lower values of moisture in salchichon and fresh and cooked pork burgers enriched with fish oil microcapsules (Lorenzo et al., 2016; Aquilani et al., 2018) than in the corresponding control batches, which was explained by the addition of extra dry matter with the microcapsules. In the CK, the effect of processing and/or culinary cooking led to significant differences in the percentage of moisture of CK-CON, CK-MO and CK-MU, with higher values found in the samples before processing (t0) than after the processing (t1) and after the culinary cooking (t3). The decrease in moisture after the processing can be related to lack of phosphates, which are normally used to increase the water holding capacity of emulsion-type meat products (Wang, Xu, & Zhou, 2009). Consequently, a lower percentage of total fat was observed at t0 compared with the samples at t1 and at t2, although this effect was only significant for CK-MU. In the DC system, the effect of processing significantly decreased the percentage of moisture from t0 to t1, and, consequently, it significantly increased the total fat percentage. This result was expected because of the loss of water that takes place during the dry-cured processing. Thus, the addition of MO and MU did not influence the

Table 2
Quantity of EPA and DHA (mg/g sample (mean values ± standard deviation)) in cooked (CK) and dry-cured (DC) meat model systems as affected by fish oil microcapsules addition (*p* (a)) and processing *and/or* culinary cooking (*p*)*.

		EPA				DHA			
		CON	MO	MU	*p (a)*	CON	MO	MU	*p (a)*
CK	t0	nd b	0.11 ± 0.02 a x	0.15 ± 0.02 a x	< 0.001	nd b	0.23 ± 0.04 a x	0.30 ± 0.03 a x	< 0.001
	t1	nd b	0.16 ± 0.01b y	0.28 ± 0.04 a y	< 0.001	nd c	0.31 ± 0.02b y	0.57 ± 0.08 a y	< 0.001
	t2	nd b	0.25 ± 0.03b z	0.31 ± 0.02 a z	< 0.001	nd c	0.50 ± 0.05b z	0.61 ± 0.03 a z	< 0.001
	p	–	0.001	0.002		–	0.001	0.001	
DC	t0	nd b	0.20 ± 0.00 a	0.18 ± 0.04 a	< 0.001	nd b	0.40 ± 0.01 a	0.56 ± 0.03 a	0.007
	t1	nd b	0.27 ± 0.08 a	0.32 ± 0.07 a	0.007	nd b	0.47 ± 0.04 a	0.61 ± 0.04 a	0.001
	p	–	0.012	0.043		–	0.051	0.065	

*CON, MO, MU: samples not enriched, and enriched with monolayered and multi-layered fish oil microcapsules, respectively; to, t1: samples before and after processing, respectively; t2, samples after culinary cooking; nd, not detected.
Different letters in the same line (a, b, c) or column (x, y, z) indicated significant differences among batches due to fish oil microcapsules addition, and processing *and/or* culinary cooking, respectively.

percentage of moisture and total fat of the CK and DC or their changes during the processing and/or culinary cooking procedures.

The quantity of EPA and DHA in the CK and DC is presented in Table 2. Both types of microcapsules used in the present study increased the content of EPA and DHA in both model systems, and these fatty acids were only observed in the enriched batches but not in the control ones. Previous research on meat products supplemented with fish oil microcapsules have also reported an increase in the percentage of EPA and DHA (Pelser et al., 2007; Josquin et al., 2012; Aquilani et al., 2018; Lorenzo et al., 2016) in enriched batches of products; however, these authors did not quantify the EPA and DHA content. In addition, the present study showed that the quantity of EPA and DHA was higher with the addition of MU relative to MO in most cases. This result was not expected because the same quantity of EPA and DHA was added to the MO and MU microcapsules, although it could be ascribed to the different types of walls between the two types of microcapsules. In MO, the coating surrounding the fish oil droplets of lecithin is a maltodextrin layer, while in MU, the coating is a multilayer structure of chitosan-maltodextrine, which should more efficiently protect EPA and DHA. In fact, chitosan has been found to increase the electrostatic force and viscosity of the layers (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005), which contribute to the emulsion stability and could impact on oxidative damage. Additionally, the antioxidant properties of chitosan (Ngo & Kim, 2014) could explain the higher EPA and DHA quantity in the MU-enriched batches.

The effect of processing and/or culinary cooking significantly influenced the quantity of EPA and DHA (Table 2) in the batches enriched with both MO and MU. Thus, in the CK, the lowest quantities of EPA and DHA were found at t0, and they increased in order in the samples at t1 and at t2. In the DC, the samples at t0 showed significantly lower quantities of EPA than at t1, while a similar tendency was found for DHA but the differences were not significant. This increase in the quantities of EPA and DHA after the processing and culinary cooking of the meat model systems can be proportionally ascribed to the decrease in moisture as previously discussed, which leads to a concentration of the components. This result may also indicate that EPA and DHA are either not lost or only slightly reduced in the meat model systems. In fact, Jiménez-Martín et al. (2016) and Aquilani et al. (2018) stated that these fatty acids are protected within fish oil microcapsules. This is an interesting issue when the objective is to achieve a specific quantity of fatty acids, which is important when labelling a product as enriched or for the consumption of adequate amounts. Thus, in the present study, after processing (t1), the CK and DC systems contain 0.47, 0.85, 0.84 and 0.93 mg EPA + DHA/g sample for CK-MO, CK-MU, DC-MO and DC-MU, respectively. According to the European regulations, CK-MO could be labelled as a "source of ω-3" and CK-MU, DC-MO and DC-MU would be labelled as a "high content in ω-3" (EU, 2010). However, it is worth noting that the quantity of EPA + DHA intake when consuming the CK after culinary cooking is similar to that offered by the DC. These findings should be considered when labelling food with certain functional properties and to reach the adequate intake of EPA and DHA.

3.2. Oxidative stability of ω-3 enriched meat model systems

Fig. 1 shows the oxidation values of the CK (Fig. 1A) and DC (Fig. 1B) systems based on the type of fish oil microcapsules and the processing and/or culinary cooking procedures. Primary oxidation products, evaluated as CD, were not significantly modified by any of the studied microcapsules added to the CK and DC meat model systems, with values between 0.22 and 0.25 μmol CD/mg sample in the CON, MU and MO batches at t0, t1 and t2. Previous studies using chicken nuggets have also detected similar CD values between the control samples and those enriched with fish oil microcapsules (Jiménez-Martín et al., 2016; Pérez-Palacios, Ruiz-Carrascal, Jiménez-Martín, Solomando, & Antequera, 2018).

As for the secondary oxidation products, the addition of fish oil microcapsules significantly affected the TBARs values in the CK at t0 and t2 and in the DC at t0. In CK, MO batches showed higher values than the MU batches, while the lowest TBARs levels were found in the CON samples. In DC, lower TBARs levels were found in CON in comparison to MO and MU batches. Nevertheless, the TBARs values were within a narrow range (0.15–0.58 mg MDA/kg sample) and much lower than the values that lead to anomalous flavours (1–2 mg MDA/kg sample) that can be sensorially detected (Barbut, Kakuda, & Chan, 1990). In comparison to these values, the oxidation levels in the batches with fish oil microcapsules were low, which may be ascribed to the layers of MO and MU, minimizing the contact between the encapsulated fish oil and external factors that are oxidant promoters. The protective effect seemed to be maintained during the processing and cooking. In fact, the oxidative stability of powders is highly related to the wall materials (Costa de Conto, Fernandes, Grosso, Eberlin, & Gonçalves, 2013). In this study, samples enriched with MO showed higher TBARs values than samples enriched with MU, which can be explained by the influence of chitosan, which formed a multilayer structure (lecithin-chitosan-maltodextrin) in MU and could act as a free radical scavenger (Friedman & Juneja, 2010). In addition, microcapsules with chitosan have been reported to show higher oxidative stability (Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz-Carrascal, & Antequera, 2014). The processing and/or culinary cooking procedures also statistically influenced the TBARs values in the CK and DC. In CK, the oxidation values increased in the CON and MO batches from t0 to t1, keeping constant from t1 to t2, while in MU samples there was a progressive increase in the TBARs levels from t0 to t1 to t2. In DC, oxidation values increased from t0 to t1. Accordingly, Aquilani et al. (2018) also found an increase in the oxidation levels from fresh to cooked pork burger treated or not with fish oil microcapsules. In the study of Vasile et al. (2019), the

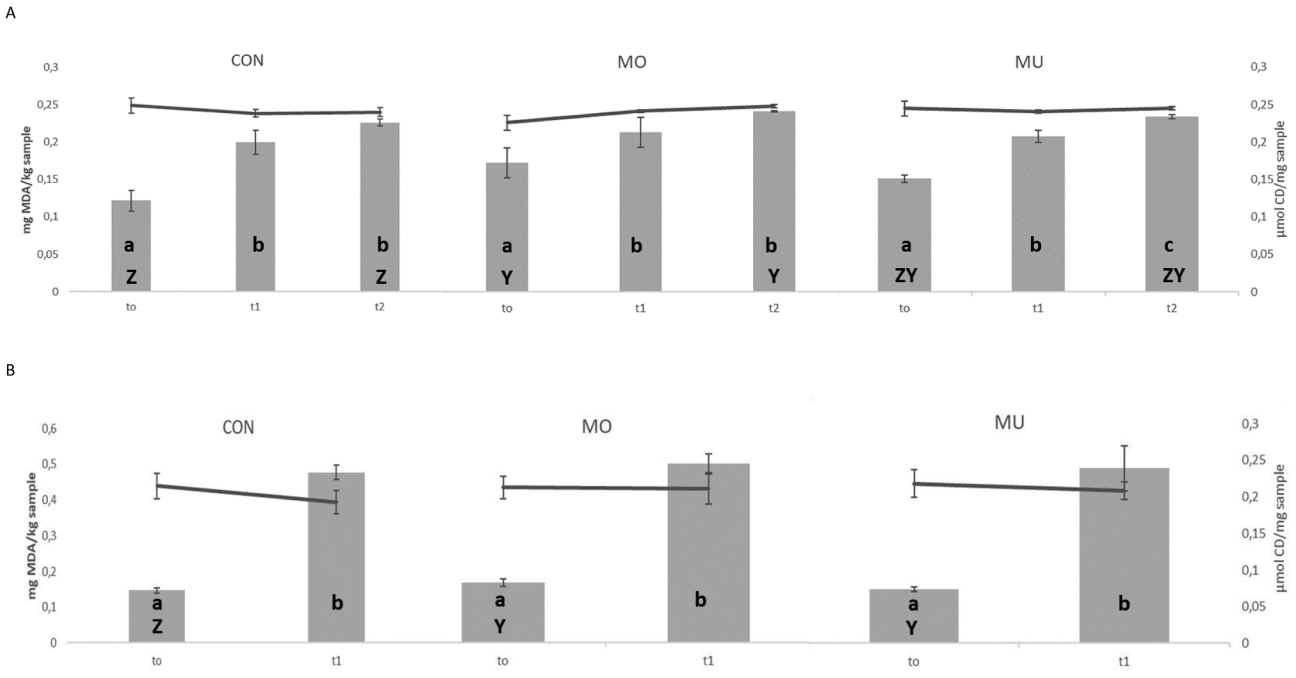

Fig. 1. Oxidation values (measured as TBARs (columns) and CD (lines)) in cooked (A) and dry-cured (B) meat model systems as affected by adding fish oil microcapsule and processing and/or culinary cooking*.

cooking process led to an increase in oxidation values in beef patties treated with alginate-chitosan and alginate-gum-chitosan encapsulates containing fish oil. In addition, a significant influence of both time and temperature on the TBARs values of fish oil microcapsules has also been found (Jiménez-Martín et al., 2014), which supports the results of this study of the meat model systems.

3.3. In vitro digestion of total fat, EPA and DHA in the enriched meat model systems

Fig. 2 shows the percentage of total fat (TF) released after in vitro digestion and the percentage of fat released from the intestinal phases (IF) in the CK (Fig. 2A) and DC (Fig. 2B) meat model systems. The results show that the addition of fish oil microcapsules significantly

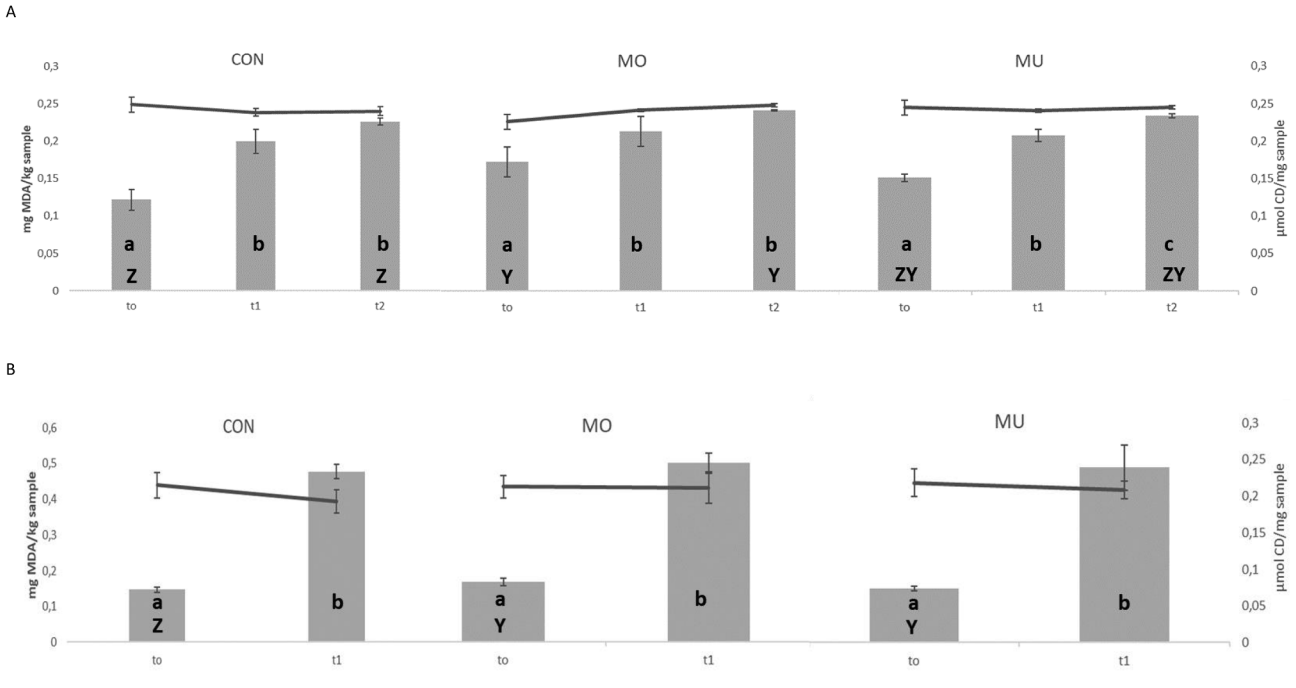

Fig. 2. Percentage of fat released throughout the in vitro digestion of cooked (A) and dry-cured (B) meat model systems as affected by adding fish oil microcapsules and processing and/or culinary cooking*.

influenced the TF and IF, with higher percentages in the CON than the MO and MU treatments. The processing and/or culinary cooking procedures significantly decreased the percentage of TF and IF from t0 to t1 and t2 in all batches of the CK and from t0 to t1 in the MO and MU batches of the DC. Thus, the lowest percentages of TF and IF were found in the enriched meat model systems at t2 for the CK and at t1 for the DC. These results may indicate a major difficulty in digesting samples treated with fish oil microcapsules and subjected to emulsification, dry-curing and cooking. A previous study in which pork fat was encapsulated with pectin and added to meat emulsions, also found a reduction in the digestibility of fat (Santiaguín-Padilla et al., 2019), which could be attributed to the alteration of the breakdown and the coalescence of lipid particles decreasing the exposed area of fat globules to the enzymatic activity of lipases (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014). The influence of the processing and/or culinary cooking could be related to the modifications in the structure of the meat model systems during these procedures from a mix of cuts of fat and meat to complex assemblages of protein gels containing lipids, where fat molecules are trapped in a solid food matrix (Dickinson, 2012), which should hinder the release of lipids. According to Dickinson (2012), lipolysis is conditioned by the rate and extent of proteolysis in this type of system: as the protein structure is broken down, the lipids are released from the matrix and exposed to lipases.

Fig. 2 also shows that the percentage of TF and IF is higher in the CK than the DC, which may be related the characteristics of the components and their conformation within the meat model systems. The DC has small and solid cuts of fat, while the CK is a fine paste with fat particles distributed in an aqueous phase with proteins and salt. This finding is consistent with the results found by Asensio-Grau, Calvo-Lerma, Heredia, and Andrés (2018), who related the rate of lipolysis with the structure of the meat products and showed that a higher release of free fatty acids and matrix degradation occurred during in vitro digestion of unstructured matrices. In fact, a low rate of lipolysis has been associated with low surface areas and the presence of solid fat (Golding & Wooster, 2010; McClements, Decker, & Park, 2008).

The percentage of fat released throughout the in vitro digestion of the CK and DC systems was evaluated to determine the total released and the amount released from the intestinal phase based on the type of fish oil microcapsule added and processing-cooking procedures, and then the bioaccessibility of EPA and DHA was evaluated by quantifying these fatty acids at the intestinal phases (Table 3). Regarding the addition of fish oil microcapsules, the release of EPA and DHA from the intestinal phase was significant in the model systems enriched with MO compared with those enriched in MU in most of the analysed batches. Consequently, the percentage of bioaccesible EPA and DHA in relation to their content in the undigested samples was higher for the MO type, which may be explained by the differences in the structure of the wall of the fish oil microcapsules. The multilayer structure of chitosan-maltodextrin of the MU microcapsule should be more resistant to digestion than the maltodextrin layer of the MO microcapsule. This hypothesis is supported by Ma et al. (2008), who reported that chitosan provides protection against the acidity of the gastric environment. In addition, chitosan has fat-binding properties (Muzzarelli, Frega, Miliani, Muzzarelli, & Cartolari, 2000)

The effect of the cooking procedures led to significant differences in the quantity of EPA and DHA released from the intestinal phase during the in vitro digestion of all batches of the CK, although the results were affected by the type of microcapsule. In the CK-MO, the highest quantities of bioaccessible EPA and DHA were found in samples at t1 and then in samples at t2 and t0. In the case of CK-MU, the samples at t0 showed higher quantities of EPA and DHA than those at t1 and t2. Again, the structure of the microcapsules could be responsible for this finding. The multilayer of chitosan-maltodextrin in the MU is firmly embedded to the structure of the fine paste of the CK, whereas the binding of the layer of maltodextrin in the MO may be weaker. In fact, chitosan is normally chosen as a coating material due to its high viscosity and adhesive properties that prevent contact between the encapsulated oil and the pro-oxidant agents (Shin, Chung, Kim, Joung, & Park, 2013). However, these properties do not seem to promote the release of EPA and DHA from the microcapsules used in this study during the in vitro digestion of meat model systems. In contrast, the influence of processing on the DC system was not significant for most batches, with a statistical decrease in the quantity of DHA only found from MO-t0 to MO-t1. This finding could indicate a similar embeddedness of MO and MU in this model system, which is a matrix of small and solid cuts of fat and meat.

To further evaluate the bioaccessibility of EPA and DHA in the MO and MU enriched meat models of the present work, the digestion results for EPA and DHA in the CK-t2 and DC-t1 samples should be assessed because these samples replicated the usual form of consumption of cooked and dry-cured sausages, respectively. In both cases, the percentage of EPA and DHA released at the intestine phase was higher relative to that of the undigested samples when MO was added compared with MU (52.94 and 40.86 vs 27.68 and 7.98% of EPA and DHA in CK-MO-t2 and CK-MU-t2, respectively; and 50.99 and 39.35 vs 45.15 and 23.38% of EPA and DHA in CD-MO-t1 and DC-MU-t1, respectively). Moreover, no marked differences were found in these percentages between the meat model systems.

Thus, all of these results confirm the initially posed hypothesis.

4. Conclusions

The addition of monolayered and multilayered microcapsules of fish oil to cooked and dry-cured meat model systems enriches the amount of EPA and DHA and does not lead to oxidative changes during cooking and processing or change the moisture and lipid contents of these samples.

Table 3
Quantity of EPA and DHA (mean values ± standard deviation) released from the intestinal phase of the in vitro digestion of cooked (CK) and dry-cured (DC) meat model systems as affected by fish oil microcapsules addition (p (a)) and processing and/or culinary cooking (p)*.

		EPA			DHA		
		MO	MU	p (a)	MO	MU	p (a)
CK	t0	0.12 ± 0.00 y	0.16 ± 0.00 z	< 0.001	0.17 ± 0.00 y	0.24 ± 0.00 z	< 0.001
	t1	0.13 ± 0.00 y	0.11 ± 0.00 y	0.108	0.20 ± 0.00 z	0.18 ± 0.00 y	0.017
	t2	0.05 ± 0.001 z	0.08 ± 0.00 x	0.017	0.09 ± 0.01 x	0.05 ± 0.01 x	0.018
	p	< 0.001	< 0.001		< 0.001	< 0.001	
DC	t0	0.14 ± 0.00	0.12 ± 0.00	0.006	0.23 ± 0.01	0.14 ± 0.01	< 0.001
	t1	0.13 ± 0.00	0.13 ± 0.00	0.081	0.18 ± 0.00	0.14 ± 0.01	0.014
	p	0.141	0.116		0.003	0.914	

*CON, MO, MU: samples not enriched, and enriched with monolayered and multi-layered fish oil microcapsules, respectively; to, t1: samples before and after processing, respectively; t2, samples after culinary cooking; Different letters in the same column (x, y, z) indicated significant differences among batches due to processing and/or culinary cooking effects.

The release of fat during in vitro digestion is affected by the type of fish oil microcapsules, especially by the wall material, and the processing and/or culinary cooking procedures, and it is also influenced by the characteristics of the meat model system.

The EPA and DHA quantities are influenced by the type of microcapsule, with multilayered microcapsules being more efficient, and by the processing-culinary cooking procedure, which lead to an increase in EPA and DHA. Nevertheless, the bioaccessibility of EPA and DHA is higher when using monolayered fish oil microcapsules as the ω-3 vehicle, and it is also affected by the processing and culinary cooking and the characteristics of the meat model system.

Thus, the type of fish oil microcapsule may be selected as a function of the type of meat product to optimize the enrichment and bioaccessibility of EPA and DHA.

CRediT authorship contribution statement

Juan Carlos Solomando: Formal analysis, Investigation. **Teresa Antequera:** Conceptualization, Project administration. **Trinidad Perez-Palacios:** Conceptualization, Methodology, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Capítulo 3.3

Evaluating the use of fish oil microcapsules as omega-3 vehicle in cooked and dry-cured sausages as affected by their processing, storage, and cooking

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Evaluating the use of fish oil microcapsules as omega-3 vehicle in cooked and dry-cured sausages as affected by their processing, storage and cooking

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ABSTRACT

This work evaluated the use of monolayered (Mo) and multilayered (Mu) fish oil microcapsules as vehicles of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in cooked and dry-cured meat products as affected by the storage and culinary heating or dry-cured processing. Proximate composition, oxidation, EPA and DHA quantity, acceptability and morphology were evaluated. Mo and Mu microcapsules increased the quantity of EPA and DHA, leading to cooked and dry-cured products susceptible to be labeled as "source of omega-3 fatty acids", without influencing physico-chemical characteristics, oxidative stability, acceptability or usual changes that take place during the culinary heating or dry-cured processing. The storage at refrigeration influenced on usual modifications on lipid oxidation and hedonic scores, but overall quality of the meat products enriched seems not to be impaired after storing. Thus, it could be indicated the viability of Mo and Mu as vehicles to enrich meat products subjected to low and high temperature for manufacturing, storage at refrigeration and culinary heating.

1. Introduction

A diet rich in omega-3 polyunsaturated fatty acids (ω-3 PUFA), principally eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), has been shown to reduce the risk of cardiovascular disease (Pourashouri et al., 2014), some types of cancer (Sioen et al., 2006) and to prevent of neurodegenerative and inflammatory diseases (Bahadori et al., 2010; Bazan, 2009; Caughey, James, Proudman, & Cleland, 2010).

Major sources EPA and DHA are fish, seafood and algae. However, the consumption of these products and their derivatives is low (Rubio-Rodríguez et al., 2010). Thus, the recommended minimum of two servings of fish per person per week are not accomplished, neither the adequate intake of 1.5 g of EPA plus DHA per week, which supposes the approximate consumption of 0.2–0.25 g per day (Kolanowski, Jaworska, Laufenberg, & Weißbrodt, 2007; Trautwein, 2001).

As response to this dilemma, the increasing of EPA and DHA in well-accepted food have been tried (Pérez-Palacios, Ruiz-Carrascal, Jiménez-Martín, Solomando, & Antequera, 2018). The inclusion of fish and algae oils, as bulk or emulsified, has been principally reported (Cáceres, García, & Selgas, 2008; Delgado-Pando et al., 2011; Josquin, Linssen, & Houben, 2012). However, these strategies have a negative impact on some sensory attributes and lipid oxidation (Pérez-Palacios, Ruiz-Carrascal, Solomando, & Antequera, 2019). This is due to the undesirable off-flavor and off-odor, specially fishy, and the ocurrence of lipid oxidation reactions (Jacobsen, 1999). To solve this problem, the addition of low amounts of fish oil and extra artificial flavorings or seasonings to foods has been tried to mask the unpleasant off-flavor of gradually oxidized fish oil products. However, this oxidation did not allow to maintain the sensory quality of the enriched products nor improved their nutritional properties (Gökmen et al., 2011; Hernandez, 2013) and decrease their shelf life (Hernandez, 2014; Kolanowski et al., 2007; Tamjidi, Nasirpour, & Shahedi, 2014). In this context, the microencapsulation of ω-3 PUFA rich oils has been used as a possible strategies to avoid ω-3 PUFA oxidation (Akanbi & Barrow, 2018; Carneiro, Tonon, Grosso, & Hubinger, 2013; Przybysz, Szterk, Zawiślak, & Dłużewska, 2014), based on creating a physical barrier between the active compounds and the environment, minimizing their contact and reactivity with water, oxygen, iron and other oxidizing promoters (Miyashita, Uemura, & Hosokawa, 2018; Onwulata, 2013), as well as the perception of off-flavors (Serfert, Drusch, & Schwarz, 2010).

Fish oil has been microencapsulated by different methods, being spray-drying the most used technique for the wide variety of materials that can be used, the simplicity of the procedure and its low cost (Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016), but involves the previous elaboration of an oil-in-water emulsion. Different

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types of fish oil emulsions have been used to make ω-3 PUFA microcapsules, such as monolayer, where the oil drops are covered by a single wall material, or multilayer (Jiménez-Martín, Antequera Rojas, Gharsallaoui, Ruiz Carrascal, & Pérez-Palacios, 2016; Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Rojas, 2014; Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz Carrascal, & Antequera Rojas, 2015; Jiménez-Martín, Pérez-Palacios, Carrascal, & Rojas, 2016; Pérez-Palacios et al., 2018). In this case the oil drops are coated by successive wall materials deposited by electrostatic charges. Its application offers several advantageous features such as the improvement in the physical and chemical stability of the microencapsulated components, the use of various food-grade materials and it allows a better control in the release of the bioactive compounds (Gharsallaoui et al., 2012). Emulsion stability is essential to control the encapsulation procedure. For that, emulsions are generally prepared using rotor-stator systems followed by a high pressure homogenization step.

Currently, there is a high consumption of meat products, 3–4 times per week (OMS, 2015). This is in part due to the growing intake of "ready-to-eat" products, mainly related to the current lifestyle focuses on saving time. Meat and meat products have high quality proteins and some vitamins (especially vitamin B6 and B12) and minerals (iron, selenium and zinc) (Santos, Hoz, Cambero, Cabeza, & Ordóñez, 2008), but their lipid profile is sometimes questioned, because of their high to moderate amount of saturated FA (SFA) and low PUFA contents and to the higher content in ω-6 PUFA than in ω-3 (Nuernberg et al., 2005). Thus, meat industries are interested in producing healthier meat products (Jiménez-Colmenero, 2007). In regards, the European Union legislation have established the minimum level required of the sum of EPA and DHA to label a food as "source of ω-3 fatty acids" and "high in ω-3 fatty acids": 40 and 80 mg of the sum of EPA and DHA per 100 g and per 100 kcal, respectively (EU, 2010).

Thus, the EPA and DHA enrichment in "ready-to-eat" meat products could be an interesting and promising strategy for increasing the intake of these fatty acids. Dry-cured and cooked sausages are a good example of these foods, being highly purchased in many European countries (Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2008; Polizer Rocha, de Noronha, & Trindade, 2019).

The enrichment in EPA and DHA by using fish oil microcapsules have been evaluated in some meat products: sausages (Josquin et al., 2012; Pelser, Linssen, Legger, & Houben, 2007), chicken nuggets (Jiménez-Martín, Pérez-Palacios, et al., 2016; Pérez-Palacios et al., 2018), burgers (Aquilani, Pérez-Palacios, et al., 2018; Aquilani, Pérez-Palacios, Sirtori, et al., 2018) and Spanish salchichon (Lorenzo, Munekata, Pateiro, Campagnol, & Domínguez, 2016). All these studies have been focused on analyzing proximate composition, percentage of EPA and DHA, lipid oxidation and sensory attributes. Nevertheless, the content of these fatty acid expressed as mg per g sample has not been determined, not being possible to know if the quantity of microcapsules added is enough to label the enriched meat products as "source of omega-3 "or "rich in omega-3". Besides, the effect of the industrial processes, storage and the culinary preparations have never been considered, which has been recently claimed (Pérez-Palacios et al., 2019).

Considering all these aspects, the objective of this work was to evaluate the viability of different types of fish oil microcapsules in meat products, to achieve a source of ω-3 food with appropriate quality characteristics. In addition, the impact of the processing, storing and culinary heating was also analyzed.

2. Material and methods

2.1. Biological material

Fish oil from cod liver was kindly provided by Biomega Nutrition (Galicia, Spain). Soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, kindly provided Roquette, Lestrem, France), and food-grade glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions.

2.2. Reagents

Commercial buffer solutions (Crison, Barcelona, Spain) at pH 4.0 and 7.0 were used to calibrate the pH meter. For the proximate composition, methanol, chloroform, sulfuric acid 95–98% and kjeldahl catalyst (Scharlau) were used as regents. Sulfuric acid and methanol (Scharlau) were used for the transesterification of fatty acids. For the oxidative stability, thiobarbituric acid (TBA), 2-propanol, perchloric acid 70% (Scharlau), malonaldehyde bis (diethyl acetal) 97% (Acros Organics, Madrid, Spain) and 2,6 di-tert-butyl-4-methyl-phenol (Sigma-Aldrich, Heidelberg, Germany) were used. And, di-potassium hydrogen phosphate trihydrate, potassium dihydrogen phosphate, acetone (Scharlau) and glutaraldehyde (Sigma-Aldrich, Heidelberg, Germany) were needed for preparing the sample to be analyzed by scan electron microscopy (SEM).

2.3. Experimental design

A scheme of experimental design is showed in Fig. 1. Monolayered and multilayered emulsions of fish oil were spray-dried to obtain their corresponding microcapsules (Mo and Mu, respectively). Two different meat products were elaborated, cooked (C-SAU) and dry-cured sausages (D-SAU), which were added with Mo (C-SAU-Mo, D-SAU-Mo) and Mu microcapsules (C-SAU-Mu, D-SAU-Mu), modifying the formulation of the batter by the addition of 2.75% (*w*/w) of Mo and 5.26% (w/w) of Mu, according to the microencapsulation efficiency for each type of microcapsule, optimized in a previous study (Solomando, Antequera, Ruiz-Carrascal, & Pérez-Palacios, 2019), and the minimum amount required to label a food as a "source of ω-3 fatty acids" (EU, 2010). A control batch (without enriching) of each meat product was also prepared (C-SAU-Co, D-SAU-Co). All batches of C-SAU were analyzed at the beginning (T0) and after 4 months (T4) of storage at refrigeration (0–5 °C), and in both cases before and after being heating (90 °C during 3 min) (BH and AH, respectively). D-SAU were analyzed before and after the dry-cured processing (BP and AP, respectively), and all dry-cured batches were analyzed at time 0 (T0) and after 4 months (T4) of storage at refrigeration temperature (0–5 °C). Thus, twelve batches were obtained for C-SAU (BH-Co-T0, BH-Co-T4, BH-Mo-T0, BH-Mo-T4, BH-Mu-T0, BH-Mu-T4, AH-Co-T0, AH-Co-T4, AH-Mo-T0, AH-Mo-T4, AH-Mu-T0 and AH-Mu-T4) and nine for D-SAU: BP-Co-T0, BP-Mo-T0, BP-Mu-T0, AP-Co-T0, AP-Co-T4, AP-Mo-T0, AP-Mo-T4, AP-Mu-T0 and AP-Mu-T4.

Samples were analyzed by means of proximate composition, lipid oxidation, EPA and DHA quantities, sensory analysis and scanning electron microscopy. All batches were done in triplicate and analyses were carried out in duplicate.

2.4. Preparation of emulsions and microcapsules

Emulsion and microcapsules of this study were prepared following the methodology of Jiménez-Martín et al. (Jiménez-Martín et al., 2014) with slight modifications.

Fish oil (20 g) and lecithin (6 g) were mixed with a magnetic stirrer overnight. Then, water was added until a total weight of 200 g and homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion was obtained and then homogenized at high-pressure (SPX, model APV-200a, Silkeborg, Denmark) under the conditions previously optimized, 1200 Ba-3 passes for Mo and 1100-2 passes for Mu.

The homogenized primary emulsion was blended with 200 g of water in Mo, and 200 g of 1% of chitosan (*w*/w) in acetic acid 1%, in Mu, by slowly agitation with a magnetic stirrer for 15 min. In both

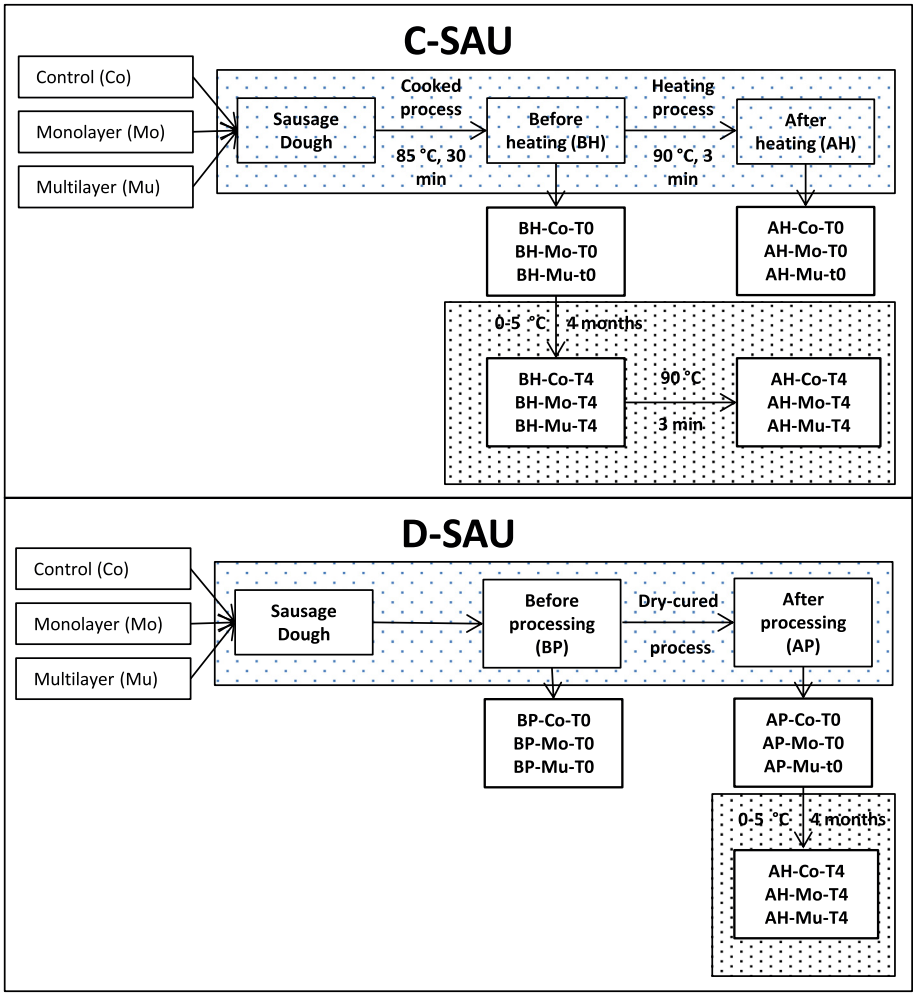

Fig. 1. Experimental design scheme of cooked (C-SAU) and dry-cured sausages (D-SAU).

types of emulsions, the final step consists on adding 400 g of maltodextrin solution (120 g maltodextrin + 280 g water).

Feed emulsions (800 g) were dried in a laboratory-scale spray drier equipped with a 0.5-mm nozzle atomizer (Mini spray-dryer B-290, Buchi, Switzerland). The emulsions, maintained at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray drying process. The aspirator rate was adjusted at 80%, feed rate was 1 L/h, inlet temperature was 180 °C, and outlet temperature ranged 85–90 °C. The collected dried powders were stored in containers at 4 °C until being added to the meat products.

2.5. Elaboration of meat products

C-SAU were elaborated with meat mechanically separated from chicken, water, pork fat, salt, pork plasma, stabilizer (E-450), aromas, vegetable fiber, spices, spice extracts, smoke flavor, antioxidant (E-316) and preservative (E-250) and the corresponding microcapsules in the case of the enriched batches, added in the knead phase. All C-SAU batches were pasteurized in a water bath at 85 °C during 30 min, vacuum packed and stored at refrigeration temperature (0–5 °C). Formulation and manufacture of these products were made in a meat industry (remain anonymous).

D-SAU were elaborated with Iberian pork meat and fat, which were ground through a 6 mm diameter mincing plate. The rest of ingredients: salt, dextrose, soy protein, spices, stabilizers (E-451 and E-450), antioxidant (E-301), preservatives (E-252 and E-250), enhancer flavor (E-621), coloring (E-120) and the corresponding microcapsules were added and mixed for 3 min and kept at 4 °C until stuffed. No starter culture was added. The obtained doughs were stuffed into collagen casings with a length of 40 cm and a diameter of 60 mm. The sausages followed a dry-cured process under controlled conditions of 4 °C and 82% of relative humidity for 3 days, then, 21 days in a drying-curing chamber at 8 °C and 80% of relative humidity and finally at 5 °C and 85% humidity, until reaching a percentage of loss of 38–40% (approximately 14 days).

In both products, the quantity of Mo and Mu added was 3 and 5 g per 100 g of dough, respectively. These figures were calculated to excess the required quantity of EPA + DHA to label a food as “source of ω-3 fatty acids”: at least 40 mg of the sum of EPA and DHA per 100 g and per 100 Kcal (EU, 2010).

2.6. Physico-chemical analysis

2.6.1. Water activity and pH

Water activity (a_w) was analyzed using a Novasina aw-Center meter (Novasina AG, Lanchen, Switzerland), and pH was measured directly in the samples with a pH-meter (micropH 2000) using a needle electrode (Crison Instruments SA, Barcelona, Spain) after calibration.

2.6.2. Protein, moisture, and fat content

Protein and moisture were analyzed following the official methods of the Association of Official Analytical Chemist (AOAC, 2000a,b), references 992.15 and 935.29, respectively. Moisture was determined gravimetrically. Protein was carried out following the Kjeldahl

methodology. Fat content was determined gravimetrically, carrying out the method of Gossert et al. (2011) and modified by Pérez-Palacios, Ruiz, Martín, Muriel, & Antequera (2008).

2.6.3. Instrumental color

Instrumental color was measured in all batches at T0 and T4 placing a transparent film on a cross section of the sample, determining luminosity (L*), redness (a^*) and yellowness (b^*). It was used a Minolta CR-300 colorimeter (Minolta Camera Corp., Meter Division, Ramsey, NJ) with illuminant D65, a 0° standard observer and one port / display area of 2.5 cm. The colorimeter was calibrated before use with a white tile having the following values: L* = 93.5, a^* = 1.0 and b^* = 0.8.

2.7. Oxidation assay

2.7.1. Conjugated dienes (CDs)

The measurement of CDs was determined according to the procedure described by (Juntachote, Berghofer, Siebenhandl, & Bauer, 2006). The CD concentration was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1}, and the results were expressed as μmol per mg of sample.

2.7.2. Thiobarbituric acid reactive substances (TBARs)

The measurement of TBARs was determined according to the procedure described by (Salih, Smith, Price, & Dawson, 1987). The absorbance at 532 nm was measured in a spectrophotometer Jenway 7305 (Roissy, France) using a mixture of perchloric acid and thiobarbituric acid as blank and glass cuvettes with a light path length of 1 cm. Results on TBARs were expressed as mg MDA/kg sample.

2.8. Analysis of EPA and DHA fatty acids

Firstly, the Fatty Acid Methyl Esthers (FAMEs) were obtained by acidic transesterification following the method described by (Sandler, Karo, Sandler, & Karo, 1992), using 10 mg of the total lipids extracted from samples. FAMEs were analyzed by gas chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethylene glycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA, USA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). The GC oven program temperature was as follows: initial temperature of 180 °C that increased at 5 °C/min to 200 °C, being maintained 40 min at this temperature; thereafter, it increased at 5 °C/min to 250 °C, and then kept for an additional 21 min. The injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO, USA). Peak areas were measured and FAMEs were expressed as mg FAMEs (EPA and DHA) per g of sample, by using internal standard (tridecanoic acid, C13) and calibration curves of FAMEs (Supelco 37 component FAME mix, PA, USA).

2.9. Scan electron microscopy

Firstly, C-SAU and D-SAU samples were cut into spheres of 1 × 0.5 cm and cleaned with buffer phosphate 0.1 M, pH = 7–7.4, and then fixed during 12 h in glutaraldehyde 2.5%. After this time, successive passes in buffer phosphate were carried out until obtain a crystalline solution. Finally, the samples were dehydrated through successive passes with increasing concentrations of acetone (25-50-80-95%) up to 100% acetone and subsequent desiccation by critical point.

A scan electron detector microscope FEI QUANTA 3D FEG (FEI Company, Hillsboro, EE.UU.) in high vacuum conditions mode using EDT (Everhart Thornley Detector) was used to explore the morphology of the microcapsules in the products. The desiccated samples were added on stubs, fixed with a double adhesive coated carbon conductive adhesive sheet, and then subjected to metallization (sputtering) with a thin layer of a conductive gold coating for 15 s in order to amplify the secondary electron signal. After metallization, the samples were imaged operating at 5 kV with focused ion beam of Ga+ (current of < 6e-4 Pa) and observed with magnifications comprised between 5000× and 15,000×.

2.10. Sensory analysis

A hedonic sensory analysis was carried out in this study with untrained volunteers (129), includes children, students, professors and staff recruited at the School of Veterinary Sciences (Extremadura University, Caceres, Spain), who rated the samples following an unstructured five-point line scale with the hedonic inscriptions “extremely unpleasant” and “extremely liked” on the left and right extremes of the scale, respectively. Each volunteer was presented three samples, marked with random three digit codes and served at room temperature on white plastic plates. Cookies (with no added salt) and about 200 mL of water were also provided to the panellists to rinse between samples. The panel sessions were held around 2 h before lunch in the hall of the Veterinary School of the University of Extremadura in Caceres (Spain). Data about the sex and age was also asked.

2.11. Sampling replication and statistical analysis

The effects of i) addition different types of fish oil microcapsules, ii) storage and iii) heating or dry-cured processing on C-SAU and D-SAU were analyzed by one-way analysis of variance (ANOVA). When a significant effect ($p < .05$) was detected, paired comparisons between means were conducted using the Tukey's test. For the hedonic test, the nonparametric analysis Friedman's test was applied. The statistics were run using the program IBM SPSS Statistics v.22.

3. Results and discussion

3.1. Proximate composition, a_W, pH and instrumental color coordinates of cooked and dry-cured sausages

Before starting the exposition and discussion of the results, it is precise to note that C-SAU-BH and D-SAU-AP at TO have been considered the starting batches to analyze the different effects, since they have the usual purchased format. Table 1 shows results on proximate composition (moisture, fat and protein), a_W, pH and instrumental color coordinates (L, a * and b *) of C-SAU and D-SAU as affected by type of fish oil microcapsule addition and culinary heating (in C-SAU) or dry-cured processing (in D-SAU). The percentage of moisture, fat, protein and a_w were not influenced by the enrichment effect in any meat product, being around 61.42–62.93%, 17.50–17.76%, 14.35–14.50% and 0.97 for C-SAU-BH, respectively, and 26.36–26.57%, 26.23–26.72%, 31.01% and 0.81–0.82, for D-SAU-AP, respectively. These are expected result since the amount of microcapsules added in the enriched batches is quite small (3 g of Mo and 5 g of Mu per 100 g of meat product) to significantly influence on the proximate composition and a_w content. These results are quite in concordance with previous study carried out in chicken nuggets (Jiménez-Martín, Pérez-Palacios, et al., 2016). Other studies in pork salchichon and fresh and cooked pork burgers enriched with fish oil microcapsules (Aquilani, Pérez-Palacios, Jiménez Martín, et al., 2018; Lorenzo et al., 2016) showed lower values of moisture than the control batch, which the authors have explained by the addition of extra dry matter. Besides, these values of moisture, fat, protein and a_w are those normally found in similar products (Bañón, Bedia, Almela, & Martínez, 2010; Pereira, Tarley, Matsushita, & de Souza, 2000). Regarding the culinary heating effect, no significant differences were found in these parameters between C-SAU-BH and C-SAU-AH in Co, Mo and Mu batches. These indicates that the behavior of C-SAU during the culinary heating is not modified by the fish oil microcapsules addition

Table 1
Proximate composition, a_W, pH and instrumental color coordinates of cooked and dry-cured sausages (C-SAU and D-SAU, respectively) as affected by fish oil microcapsules addition (*p*E) and culinary heating (*p*C) or dry-cured processing (*p*P)[a].

			Moisture (%)	Fat (%)	Protein (%)	aW	pH	L	a[a]	b[a]
C-SAU	BH	Co	62.93 ± 0.25	17.67 ± 0.20	14.35 ± 0.72	0.97 ± 0.00	6.54[a] ± 0.02	63.81 ± 0.34	14.66 ± 0.10	14.35 ± 0.33
		Mo	61.42 ± 0.31	17.50 ± 0.30	14.49 ± 0.20	0.97 ± 0.00	6.46[b] ± 0.01	63.51 ± 0.75	16.51 ± 0.62	14.86 ± 1.09
		Mu	61.53 ± 0.24	17.76 ± 0.26	14.50 ± 0.65	0.97 ± 0.00	6.38[c] ± 0.01	62.82 ± 0.37	16.66 ± 0.39	14.89 ± 0.77
		*p*E	0.282	0.221	0.576	0.299	< 0.001	0.198	0.457	0.646
	AH	Co	62.74 ± 0.86	17.60 ± 0.31	14.43 ± 0.28	0.97 ± 0.00	6.51[a] ± 0.02	61.44 ± 0.25	15.81[a] ± 0.14	13.28 ± 0.18
		Mo	61.57 ± 0.34	17.73 ± 0.08	14.17 ± 0.42	0.97 ± 0.00	6.45[b] ± 0.01	61.89 ± 0.83	14.96[b] ± 0.58	13.33 ± 0.69
		Mu	61.29 ± 0.57	17.85 ± 0.16	14.39 ± 0.28	0.97 ± 0.00	6.35[c] ± 0.03	61.07 ± 0.55	14.14[c] ± 0.40	13.54 ± 0.46
		*p*E	0.079	0.209	0.515	0.242	< 0.001	0.314	< 0.001	0.414
	*p*C	Co	0.224	0.731	0.328	0.087	0.325	< 0.001	< 0.001	< 0.001
		Mo	0.486	0.106	0.246	0.744	0.192	< 0.001	0.001	0.016
		Mu	0.193	0.629	0.206	0.303	0.108	< 0.001	< 0.001	< 0.001
D-SAU	BP	Co	59.76 ± 0.43	15.62 ± 0.28	17.58 ± 0.42	0.97 ± 0.01	5.98[a] ± 0.03	63.01 ± 0.51	17.13 ± 0.42	10.95 ± 0.31
		Mo	59.69 ± 0.81	15.78 ± 0.19	17.32 ± 0.75	0.97 ± 0.01	5.96[a] ± 0.01	62.98 ± 0.50	16.99 ± 0.41	11.44 ± 0.30
		Mu	60.07 ± 0.95	15.82 ± 0.17	16.66 ± 0.64	0.97 ± 0.01	5.90[b] ± 0.02	62.83 ± 0.78	17.04 ± 0.61	11.60 ± 0.29
		*p*E	0.206	0.142	0.154	0.971	< 0.001	0.364	0.252	0.135
	AP	Co	26.87 ± 0.33	26.23 ± 0.18	32.00 ± 0.82	0.81 ± 0.00	5.69[a] ± 0.02	46.54[a] ± 0.87	13.32 ± 0.69	5.34[b] ± 0.29
		Mo	26.53 ± 0.22	26.57 ± 0.25	31.01 ± 0.59	0.82 ± 0.00	5.66[a] ± 0.01	45.89[b] ± 1.19	10.61 ± 3.50	5.41[a] ± 0.56
		Mu	26.36 ± 0.15	26.72 ± 0.49	31.66 ± 0.42	0.81 ± 0.00	5.48[b] ± 0.03	46.63[b] ± 2.34	12.79 ± 1.13	5.81[a] ± 0.58
		*p*E	0.207	0.096	0.130	0.259	< 0.001	0.357	0.104	0.068
	*p*P	Co	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		Mo	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001
		Mu	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Bars with different letters (a, b, c) within the same formulations show significant differences ($p < 0.05$) due to enrichment effect.

[a] Cooked and dry-cured sausages not enriched (Co) and enriched with monolayer (Mo) or multilayered microcapsules (Mu); cooked sausages before and after heating (C-SAU-BH and C-SAU-AH, respectively); dry-cured sausage before and after dry-cured processing (D-SAU-AP and D-SAU-BF, respectively).

in regards to moisture, lipid, protein and a_w. Although there are no previous studies evaluating the effect of heating on sausages added with fish oil microcapsules, in the study carried out by (Aquilani, Pérez-Palacios, Jiménez Martín, et al., 2018) in pork burgers the cooking effect was not significant in aw, fat or protein but it significantly decreased the percentage of moisture.

As for the dry-cured processing effect on D-SAU, there were found significant differences on moisture, fat, protein and aw values of Co, Mo and Mu batches, which showed the same behavior: a decrease in the percentage of moisture with the consequent increase of fat and protein percentages, and a decrease of the aw. In this product, again, the addition of fish oil microcapsules does not significantly influence on the usual changes that take place during the dry-cured processing.

The pH values were significantly influenced by the addition of fish oil microcapsules in both studied products, showing Mu added batches the lowest pH levels (Table 1). This is observed in C-SAU-BH (6.54, 6.46 and 6.38 in Co, Mo and Mu, respectively), C-SAU-AH (6.51, 6.45 and 6.35 in Co, Mo and Mu, respectively), D-SAU-BP (5.98, 5.96 and 5.90 in Co, Mo and Mu, respectively), and in D-SAU-AP (5.69, 5.66 and 5.48 in Co, Mo and Mu, respectively). These results could be related to the composition of the Mu emulsions where the chitosan is dispersed in a solution of acetic acid at 1% that lead to a pH of 3.85 in the final emulsion (Jiménez-Martín et al., 2014). The influence of culinary cooking does not influence the pH values in any batch. These pH values have also found in similar sausages (Jiménez-Colmenero et al., 2010; Matulis, McKeith, Sutherland, & Brewer, 1995), however, there are no previous studies at evaluating pH changes as affected by the culinary heating.

In D-SAU, the dry-cured processing diminished pH values of Co, Mo and Mu batches (5.98, 5.96 and 5.90 vs. 5.69, 5.66 and 5.48 in D-SAU-BP and D-SAU-AP, respectively). This is an expected change due to the effect of exponential growth of lactic acid bacteria during the dry-cured processing, (Toldrá, 2008). As in C-SAU, the batches enriched with Mu showed the lowest pH values in both D-SAU-BP and D-SAU-AP which reaffirms that these differences could be caused by acetic acid content. These pH values are comparable to those found in similar products (Lorenzo, Gómez, & Fonseca, 2014).

As for the results on the instrumental color coordinates (Table 1), the addition of Mo and Mu microcapsules did not influence on values of L, a* and b* in C-SAU and D-SAU, being in concordance with findings in previous works (Bañón et al., 2010; Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, & Perez-Alvarez, 2003). However, the effect of culinary heating in C-SAU and dry-cured processing in D-SAU influenced on L, a* and b* values. In general, the values for these color coordinates decreased after the culinary heating and the dry-cured processing in all batches. In C-SAU, these results may be expected and ascribed to the thermal treatment of cooking at 90 °C for 3 min, but this effect has not been previously analyzed among the scientific literature. In the case of D-SAU, the color changes can be explained by the loss of water during the dry-cured process (Pérez-Alvarez, Sayas-Barberá, Fernández-López, & Aranda-Catalá, 1999), the addition of curing salts and the formation of nitric oxide and nitrosomyoglobin (Chasco, Lizaso, & Beriain, 1996). However, the addition of fish oil microcapsules led to a different effect after the culinary cooking on a*, which increase in Co and decrease in Mo and Mu. Thus, in C-SAU-AH, lower a* values were found in enriched samples (14.96 and 14.14 in Mo and Mu, respectively) than in control ones (15.81). This effect could be related to the white color of the powder that could slightly diminish the red color in the added samples. These findings are in agreement with the results reported by (Lorenzo et al., 2016; Ruiz-Capillas, Triki, Herrero, Rodriguez-Salas, & Jiménez-Colmenero, 2012).

3.2. Lipid oxidation on cooked and dry-cured sausages enriched with fish oil microcapsules

Fig. 2 exposes the values of CDs (Fig. 2a) and TBARs (Fig. 2b) of C-SAU and D-SAU as affected by the type of fish oil microcapsule added, storage and culinary heating or dry-cured processing. The storage effect was not analyzed in the case of D-SAU before dry-cured processing, since this sample corresponds to the dough. CDs values were not influenced by the enrichment effect in any experimental batches at T0 in C-SAU (0.13, 0.12 and 0.13 μmol/mg sample in Co, Mo and Mu, respectively) and D-SAU (0.15, 0.14 and 0.14 μmol/mg sample in Co, Mo and Mu, respectively). However, at T4, there were significant differences among Co, Mo and Mu batches in both products. In C-SAU, the lowest values of CDs were found in Co (0.22 μmol/mg sample),

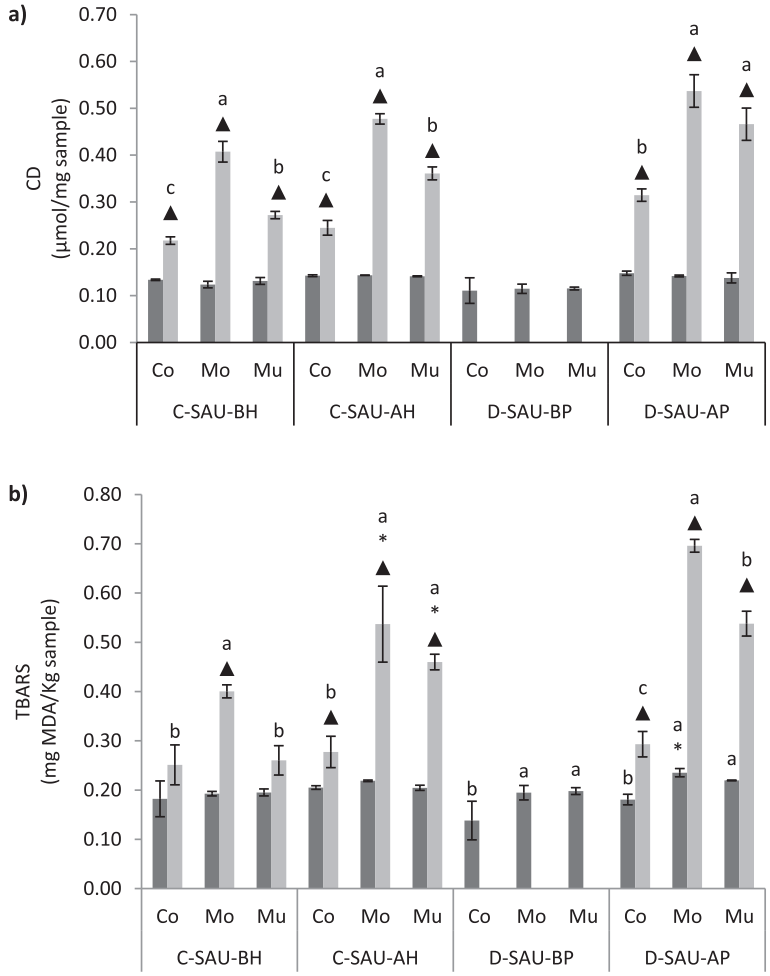

Fig. 2. Results on CDs (a) and TBARs (b) in cooked and dry-cured sausages (C-SAU and D-SAU), as affected by the type of microcapsule added, culinary heating or dry-cured processing and storage at refrigeration*.

* Samples not enriched (Co) and added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules; samples analyzed before and after the culinary cooking (BH and AH, respectively) or dry-cured processing (BP and AP, respectively); samples analyzed before (T0) and after 4 (T4) months of storage at refrigeration (dark and medium gray, respectively). Bars with different letters (a,b,c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect (Co vs Mo vs MU). *: significant differences ($p < 0.05$) due to culinary heating (BH vs AH) or dry-curing effects (BP vs AP). ▲: significant differences ($p < 0.05$) due to the storage effect. Data without symbols means no significant differences.

followed in increasing order by Mu (0.27 µmol/mg sample) and Mo (0.41 µmol/mg sample). In the case of D-SAU, CDs values were lower in Co batch (0.31 µmol/mg sample) than in Mu and Mo ones, which showed similar values (0.47 and 0.54 µmol/mg sample). These results are related to the influence of the storage effect on CDs, with higher values at T4 than at T0 in all batches of C-SAU and D-SAU samples. Besides, it is noted a higher increase of the CDs levels in the enriched products than in control ones. Nevertheless, the culinary heating, in C-SAU, and the dry-cured processing, in D-SAU, did not significantly influence on CDs values of the analyzed batches. Among the scientific literature, there are few studies analyzing CDs in meat products enriched with ω-3 PUFA or evaluating the effect of the processing, the culinary heating and the storage on this parameter. Most related works could be those investigating the rate of primary oxidation in frozen chicken nuggets enriched with fish oil microcapsules (Jiménez-Martín, Pérez-Palacios, et al., 2016; Pérez-Palacios et al., 2018). These authors also detected similar values of CDs between control and enriched samples with microcapsules as well as an increase after the freezing storage.

As for the values of TBARs in C-SAU and D-SAU (Fig. 2b), at T0, the addition of fish oil microcapsules was not significant in C-SAU (0.18, 0.19 and 0.20 mg MDA/kg sample in Co, Mo and Mu, respectively), however in D-SAU, the lowest values of TBARs were found in Co (0.18 mg MDA/kg sample), followed in increasing order by Mu (0.22 mg MDA/kg sample) and Mo (0.24 mg MDA/kg sample) batches. This is in concordance with previous findings in chicken nuggets, pork burgers and Spanish salchichon enriched with fish oil microcapsules (Aquilani, Pérez-Palacios, Sirtori, et al., 2018; Jiménez-Martín, Pérez-Palacios, et al., 2016; Lorenzo et al., 2016; Pérez-Palacios et al., 2018). As occurred with CDs, the storage led to a significant increase of the TBARs values in most batches, enriched and not enriched. In fact, the increase of TBARs in sausages stored at different temperatures has been described as a usual effect (Wenjiao, Yongkui, Yunchuan, Junxiu, & Yuwen, 2014). It is also noted a higher increase of TBARs in the enriched samples than in the control ones after the storage. Thus, at T4, in both C-SAU and D-SAU products, the lowest TBARs values were found in the Co batches (0.25 and 0.29 mg MDA/kg sample, respectively), followed in increasing order by Mu (0.26 and 0.54 mg MDA/kg sample, respectively) and Mo (0.40 and 0.70 mg MDA/kg sample, respectively) batches. In the study of (Aquilani, Pérez-Palacios, Jiménez Martín, et al., 2018) with burger enriched with fish oil microcapsules, the storage at refrigeration during five days did not significantly influence the TBARs values. Nevertheless, the different composition of the meat products and their processing and storage conditions makes the comparison difficult. In relation to the culinary heating and dry-cured processing effects, significant differences were found in C-SAU at T4 enriched with Mo (0.40 vs 0.54 mg MDA/kg sample in C-SAU-BH and C-SAU-AH, respectively) and Mu (0.26 vs 0.47 mg MDA/kg sample in C-SAU-BH and C-SAU-AH, respectively) and in D-SAU at T0 enriched with Mo (0.19 vs 0.24 mg MDA/kg sample in C-SAU-BH and C-SAU-AH, respectively).

So, in both products, the storage at refrigeration seems to be the most influencing effect on oxidation levels in the enriched samples, while the addition of fish oil microcapsules, the culinary cooking or the dry-cured processing are less influencing. However, values of both CDs and TBARs of the all studied samples showed a narrow range (0.11–0.54 µmol/mg sample and 0.18–0.70 mg MDA/kg sample, respectively). In addition, TBARs values of the enriched samples of this

Table 2
EPA and DHA quantities (mg FAMEs/g sample) on cooked sausages before (C-SAU-BH) and after (C-SAU-AH) culinary heating and dry-cured sausages before (DC-SAU-BP) and after (DC-SAU-AP) dry-cured processing as affected by enrichment with different fish oil microcapsules (*p*E), culinary cooking (*p*C) or dry-cured processing (*p*P) and storage at refrigeration (*p*S)[a].

			EPA			DHA		
			T0	T4	*p*S	T0	T4	*p*S
C-SAU	BH	Co	Nd[b]	Nd[c]	–	Nd[b]	Nd[b]	–
		Mo	0.16[a] ± 0.05	0.12[a] ± 0.01	0.364	0.31[a] ± 0.04	0.28[a] ± 0.03	0.241
		Mu	0.15[a] ± 0.01	0.14[a] ± 0.02	0.215	0.32[a] ± 0.05	0.29[a] ± 0.02	< 0.001
		pE	< 0.001	< 0.001		< 0.001	< 0.001	
	AH	Co	Nd[b]	Nd[b]		Nd[b]	Nd[b]	Nd
		Mo	0.14[a] ± 0.03	0.10[a] ± 0.01	0.173	0.29[a] ± 0.02	0.27[a] ± 0.01	0.098
		Mu	0.14[a] ± 0.03	0.12[a] ± 0.02	0.030	0.29[a] ± 0.02	0.27[a] ± 0.01	0.153
		pE	< 0.001	< 0.001		< 0.001	< 0.001	
	*p*C	Co	–	–		–	–	
		Mo	0.639	0.024		0.661	0.617	
		Mu	0.541	0.333		0.506	0.317	
D-SAU	BP	Co	Nd[b]	Na		Nd[b]	Na	
		Mo	0.14[a] ± 0.04	Na		0.32[a] ± 0.02	Na	
		Mu	0.15[a] ± 0.02	Na		0.34[a] ± 0.04	Na	
		pE	< 0.001			< 0.001		
	AP	Co	Nd[b]	Nd[b]	–	Nd[b]	Nd[b]	
		Mo	0.17[a] ± 0.01	0.13[a] ± 0.02	< 0.001	0.39[a] ± 0.02	0.37[a] ± 0.06	0.162
		Mu	0.19[a] ± 0.07	0.16[a] ± 0.03	0.129	0.42[a] ± 0.02	0.42[a] ± 0.07	0.314
		pE	< 0.001	< 0.001		< 0.001	< 0.001	
	*p*P	Co	–			–		
		Mo	< 0.001	–		< 0.001	–	
		Mu	< 0.001	–		< 0.001	–	

Bars with different letters (a,b,c) within the same formulations show significant differences ($p < 0.05$) due to enrichment effect.
Nd: not detected; Na: not analyzed.
[a] Sausages not enriched (Co) and enriched with monolayer (Mo) and multilayered (Mu) microcapsules: samples analyzed before and after the refrigeration storage for 4 months (T0 and T4, respectively).

study are quite lower than those related to the detection of anomalous flavors by a sensory panel as consequence of the accumulation of secondary products of lipid oxidation (1–2 mg MDA/kg sample) (Barbut, Kakuda and Chan, 1990). This is mainly due to the microcapsule layers that act as a barrier, minimizing the contact and reactivity of the fish oil with the oxidant promoters in the meat products, such as iron, oxygen. Moreover, in the case of being some ω-3 PUFA oxidation reaction, this microcapsule layers may avoid the propagation of the oxidation radicals through the meat product.

3.3. EPA and DHA quantity of cooked and dry-cured sausages enriched with fish oil microcapsules

Quantities of EPA and DHA in C-SAU and D-SAU were analyzed at the beginning and after 4 months of storage and are exposed in Table 2. As can be observed, in both evaluated meat products, the quantity of EPA and DHA increased from control (not detected) to the enriched batches, with no significant differences between Mo and Mu enriched products in C-SAU (0.16 and 0.15 mg EPA/g sample and 0.31 and 0.32 mg DHA/g sample, respectively) and D-SAU (0.17 and 0.19 mg EPA/g sample and 0.39 and 0.42 mg DHA/g sample, respectively). Previous studies in pork fermented sausages (Josquin et al., 2012; Pelser et al., 2007) pork burgers (Aquilani, Pérez-Palacios, Sirtori, et al., 2018) and pork Spanish salchichon (Lorenzo et al., 2016) have reported the increase in the percentage of EPA and DHA due to the addition of ω-3 microcapsules, but no quantification results were shown. The culinary heating of C-SAU only influences significantly in the EPA content in samples enriched with Mo at T4, with a loss of 0.02 mg EPA/g sample, whereas no significant differences were found in the rest of the enriched batches. These results are in concordance with a previous study in pork burgers enriched ω-3 microcapsules, where no significant differences were observed in the percentages of EPA and DHA between fresh and cooked burgers (Aquilani, Pérez-Palacios, Sirtori, et al., 2018). Regarding D-SAU, the dry-cured processing lead to a significant increase of EPA and DHA quantities in all batches, enriched with Mo and Mu microcapsules. This result may be related to the decreasing in the percentage of moisture in this product, with the consequent loss of weight (around 38–40%) and concentration of the components. Thus, it may indicate that both types of wall materials tested in the present study, maltodextrin and chitosan-maltodextrin effectively protects EPA and DHA during the culinary treatment and dry-cured processing.

The storage decreases significantly the quantity of EPA, in C-SAU-AH enriched with Mo (0.02 mg EPA/g sample) and D-SAU-AP enriched with Mo (0.04 mg EPA/g sample) and of DHA, in C-SAU-BH enriched with Mu (0.03 mg DHA/g sample). Nevertheless, most of the evaluated batches showed similar quantities of EPA and DHA before and after the storage for four months, which may indicate the accuracy of the microcapsules used in this study to avoid the degradation of EPA and DHA during the storage.

The fatty acids quantification make possible know if the products would be label as “as source of ω-3 fatty acids”, which requires a minimum content of 40 mg of the sum of EPA and DHA per 100 g of product (EU, 2010). Thus, all batches enriched with Mo and Mu subjected to be labeled (C-SAU-BH and D-SAU-AP) reached these quantities at T0 (0.47 and 0.47 mg EPA + DHA/g sample in C-SAU-BH with Mo and Mu, respectively; 0.56 and 0.61 mg EPA + DHA/g sample in D-SAU-AP with Mo and Mu, respectively) and at T4 (0.40 and 0.43 mg EPA + DHA/g sample in C-SAU-BH with Mo and Mu, respectively; 0.50 and 0.58 mg EPA + DHA/g sample in in D-SAU-AP with Mo and Mu, respectively).

3.4. Microstructure of cooked and dry-cured sausages enriched with fish oil microcapsules

SEM images of C-SAU and D-SAU are exposed in Fig. 3. In the case of C-SAU-Co, the SEM images showed a spongy structure characterized by the presence of numerous holes of different sizes and evenly distributed, which is in part due to the denaturation and aggregation of myofibrillar proteins during the heating processing (Carballo et al., 1996). A similar appearance have been described in previous studies

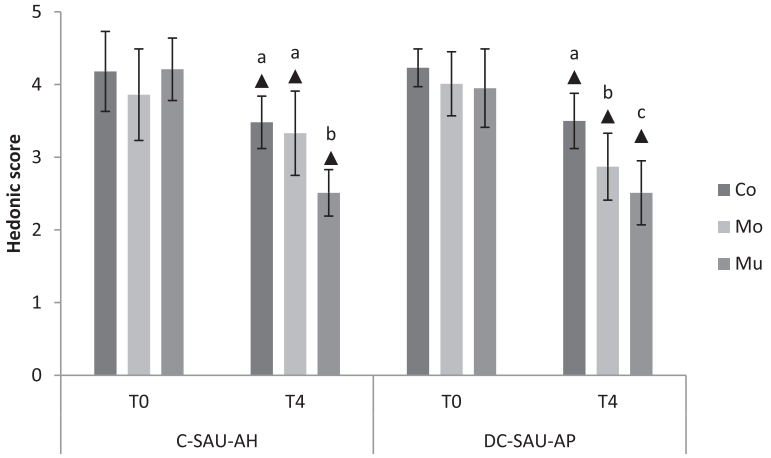

Fig. 3. Results on acceptability of cooked sausages after the culinary heating (C-SAU-AH) and dry-cured sausages after the dry-cured processing (D-SAU-AP) as affected by enrichment with different fish oil microcapsules and storage at refrigeration*.
* Batches not enriched (Co) and enriched with monolayer (Mo) and multilayered fish oil microcapsules (Mu). Bars with different letters (a,b,c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect. ▲: significant differences ($p < 0.05$) due to the storage effect.

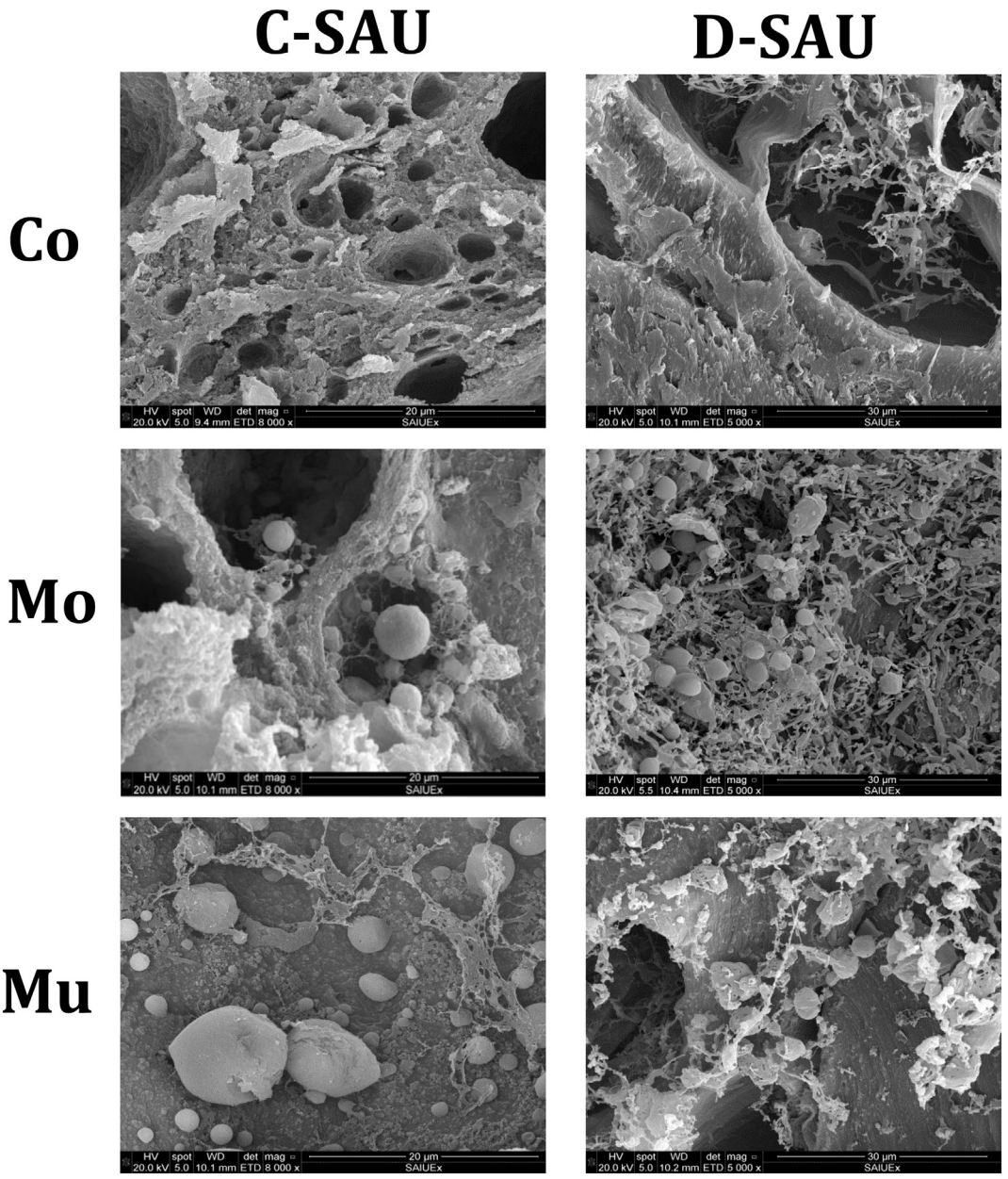

Fig. 4. Scaning electron microscopic images of cooked and dry-cured sausages (C-SAU and D-SAU, respectively), added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules and not enriched (Co), observed with ×8000 and ×5000 magnifications.

(Barretto, Pacheco, & Pollonio, 2015; Cáceres et al., 2008; Delgado-Pando et al., 2011; Yang et al., 2015). In the batches enriched with Mo and Mu microcapsules, the existence of microcapsules is evidenced. In these samples, partial loss of the spongy structure can be noted, which may be ascribed to the presence of the microcapsules that could impair this structure. Mo and Mu microcapsules appeared as oval particles of different sizes, with some wrinkles and holes and no apparent pores. The microcapsules produced by the spray drying method must have a spherical particle size and different particle size distribution types (Caparino et al., 2012). The microcapsules with oval morphology observed in this study could be attributed to the combination of two factors, the applied high pressure homogenization process and the viscoelastic properties of lecithin (Martiel, Sagalowicz, & Mezzenga, 2014). The absence of pores in the skin of the microcapsules is advantageous and supposed a high protection to the exposure to reactive oxygen species, avoiding the loss of ω-3 PUFA.

Regarding the SEM images of D-SAU, it is observed the sarcolemma breakage, as a consequence of the mincing procedure, within a dehydrated network structure and some fibers of connective tissue, which is quite in agreement with the description of (Dertli et al., 2016; Katsaras & Budras, 1992) for fermented sausages. As occurred in C-SAU, the SEM images of the enriched batches of D-SAU evidenced the existence of microcapsules, which showed similar characteristics to those previously described for C-SAU.

3.5. Results on sensory analysis of cooked and dry-cured sausages enriched with fish oil microcapsules

Sensory analyses were carried out in C-SAU after the culinary heating and in D-SAU after the dry-cured processing, since these products are usually consumed in these formats. Results on the hedonic test are exposed in Fig. 4. The enrichment effect did not show significant differences in C-SAU-AH with similar acceptability scores in Co batches than in Mo and Mu (4.18, 3.86 and 4.21 hedonic scores, respectively) and DC-SAU-AP at T0 (4.23, 4.01 and 3.95 hedonic scores, respectively), However, at T4, there were found significant differences due to the enrichment in both products. In, C-SAU-AH, the samples enriched with Mu showed lower acceptability scores (2.51 hedonic score) that Co (3.48 hedonic score) and Mo (3.33 hedonic score) ones, and in D-SAU-AP, the Co batch obtained the highest scores (3.5 hedonic score), followed in decreasing order by enriched samples with Mo (2.87 hedonic score) and Mu (2.51 hedonic score). This finding could be related to the observed changes in the oxidation values, but no studies to compare have been found. It is also noted that the storage effect is specially marked in Mu batches, which could be ascribed to the lower pH values observed, as consequence of the addition of acetic acid in Mu, and also to the flavor that can impart the chitosan, which has been described as bitter (Devlieghere, Vermeulen, & Debevere, 2004). Previous studies in enriched burgers (Aquilani, Pérez-Palacios, Sirtori, et al., 2018; Keenan et al., 2015) and Spanish salchichon (Lorenzo et al., 2016) have also reported the impact of adding fish oil microcapsules in the sensory quality of the enriched products. However, despite these differences, it may point out a good acceptability for all the batches of this study. Considering these results, a deeper sensory study should be indicated to clarify the effect of fish oil microcapsules addition on the sensory quality of these products.

4. Conclusions

The addition of monolayered and multilayered fish oil microcapsules achieved the enrichment in EPA and DHA in meat products, without affecting main quality characteristics.

Usual changes that take place during the culinary heating, dry-cured processing and refrigeration of meat products are not affected by the enrichment with fish oil microcapsules notably.

Therefore, the present study demonstrates the viability of the monolayered and multilayered microcapsules of fish oil, as EPA and DHA vehicles, to enrich meat products subjected to low and high temperature for manufacturing, storage at refrigeration and culinary heating.

Declaration of Competing Interest

None.

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Capítulo 3.4

Effect of omega-3 microcapsules addition on the profile of volatile compounds in enriched dry-cured and cooked sausages

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Effect of Omega-3 Microcapsules Addition on the Profile of Volatile Compounds in Enriched Dry-Cured and Cooked Sausages

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Abstract: The main goal of the present study was evaluating the effect of enriching meat products (cooked (C-SAU) and dry-cured sausages (D-SAU)) with monolayered (Mo) and multilayered (Mu) fish oil microcapsules on the profile of volatile compounds, with special interest in lipid oxidation markers. For that, Solid-Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) were used. Significant differences were found in the volatile compound profile between Mo and Mu, which was been reflected in the meat samples. Thus, in general, volatile compounds from lipid oxidation have shown higher abundance in Mo and C-SAU and D-SAU enriched with this type of microcapsule, indicating that the wall of Mu (chitosan-maltodextrine) might protect the encapsulated bioactive compounds more effectively than that of Mo (maltodextrine). However, this finding is not reflected in the results of previous studies evaluating the sensory perception and oxidation stability of C-SAU and D-SAU, but it should be considered since unhealthy oxidation products can be formed in the enriched meat products with Mo. Thus, the addition of Mu as an omega-3 vehicle for enriching meat products may be indicated.

Keywords: volatile compounds; fish oil microcapsule; dry-cured sausage; cooked sausage

1. Introduction

Numerous epidemiological studies suggest that a diet rich in omega-3 polyunsaturated fatty acids (ω-3 PUFAs), mainly eicosapentaenoic acid (EPA; C20:5 ω-3) and docosahexaenoic acid (DHA; C22:6 ω-3), has a marked influence in the avoidance and therapy of a series of chronic disorders [1], particularly coronary heart disease [2–5]. However, Western diets are poor in long chain ω-3 PUFAs and the liking of fish and seafood products (main sources of EPA and DHA) is currently static or declining [6]. Therefore, to improve the welfare state of the population, different professional organizations and health agencies have recommended the consumption of around 0.25 g EPA + DHA per person and day [7–10]. There also is a European Union law that sets up the minimum quantities of EPA + DHA (40 and 80 mg per 100 g and per 100 kcal) to label a food as a "source of ω-3 fatty acids" and "high in ω-3 fatty acids", respectively [11].

Nowadays, meat products are highly appreciated, with a habitual consumption of around 3–4 times per week [12], which is principally ascribed to the modern standards of life and the demand of "ready-to-eat" products. The content of high biological value proteins in meat products is valued; however, the percentage saturated fatty acids (SFA) and the ratio of ω-6/ω-3 PUFA is

not so desirable in some of these foods [13]. Hence, industries are focused on the developing of meat products with a healthier lipid profile [14], there being some investigations on the possibility of incorporating fish oils, as bulk or emulsified, in different foods [15–17]. However, the presence of numerous double bonds in ω-3 PUFAs causes a rapid oxidation in contact with oxidant promoters, like iron, light, oxygen and high temperatures [18,19] that accelerate the formation of primary oxidation products, such as hydroperoxides, which easily isomerize and degrade to volatile compounds. Some of them impart undesirable off-fishy and rancid odors and flavors such as, 4-heptenal, 3,5-octadiene or 2-ethylfuran [20–22].

In this context, several studies have investigated the possibility of producing stable foods enriched with ω-3 PUFA microcapsules [15,23–25]. The microencapsulation technique aims to form a wall around the active compounds, reducing the perception of off-flavors [26] and the contact with oxidant promoters [18,19]. Moreover, this technique is of easy application in the food industry, economic and scalable [27]. Recent studies have pointed out the possibility of adding fish oil microcapsules to enrich different meat products, which have been recently reviewed [28,29].

Most studies on enrichment of meat products with ω-3 PUFAs microcapsules have focused on the evaluation of the proximal composition, oxidative stability, fatty acid profile and sensory attributes of the enriched foods [24,25,30,31], without taking into account the influence of the different processing conditions and microcapsules addition on the volatile compounds profile of these meat derivatives, except for a previous study in chicken nuggets [32].

Authors of the present study have developed two types of fish oil microcapsules (from homogenized monolayered (Mo) and multilayered (Mu) fish oil emulsions) with high quality characteristics in terms of yield, microencapsulation efficiency and oxidative stability [29]. Results on meat products have shown similar quality characteristics in control and enriched batches with Mo and Mu fish oil microcapsules [28].

The main objective of the present study was to evaluate the profile of volatile compounds of cooked and dry cured meat products enriched with monolayered and multilayered fish oil microcapsules, paying special attention to those from oxidation processes. The profile of volatile compounds of the fish oil microcapsules was also investigated.

2. Materials and Methods

2.1. Experimental Design

This study was carried out with cooked (C-SAU) and dry-cured sausages (D-SAU). For each meat product, three batches were prepared: added with monolayered (Mo) (C-SAU-Mo and D-SAU-Mo) and multilayered (Mu) microcapsules (C-SAU-Mu and D-SAU-Mu), and a control batch (without enriching) (C-SAU-Co, D-SAU-Co). In the added bathes, the batter was modified by the addition of 2.75% (*w/w*) of Mo and 5.26% (*w/w*) of Mu. These are 3 and 5 g of Mo and Mu, respectively, per 100 g of dough. The different quantities of Mo and Mu added are due to the differences between Mo and Mu in the efficiency of fish oil encapsulation (87.39 and 56.43%, respectively) and consequently, in the quantity of EPA and DHA (2.75 and 5.26 g EPA + DHA per 100 g of microcapsule, respectively). The quantities of Mo and Mu were deliberately added in order to give 40 mg of EPA + DHA per 100 g of meat products and labelling with "source of ω-3 fatty acids".

The profile of volatile compounds was analyzed in the three batches of C-SAU and D-SAU, and also in Mo and Mu.

2.2. Preparation of Omega-3 Sources

The source of EPA and DHA was fish oil from cod liver, supplied by Biomega Nutrition (Silkeborg, Spain) and containing 5.96% EPA and 25.83% DHA. Mo and Mu microcapsules were prepared following the procedure described by [33], with slight modifications. Firstly, Mo and Mu emulsions were prepared, by mixing fish oil (20 g) and soybean lecithin (6 g), provided by Across Organics

(Madrid, Spain), with a magnetic stirring overnight. Water was added until the mixture reached a total weight of 200 g. The mixture was homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Königswinter, Germany), with a primary emulsion that was homogenized at high-pressure (SPX, model APV-200a, Silkeborg, Denmark). The high-pressure homogenization conditions have been previously optimized: 1200 Ba and 3 passes for Mo and 1100 and 2 passes for Mu [29]. The next step was different for each type of emulsion: water (200 g) was blended with Mo, while 200 g of 1% of chitosan (*w/w*) with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain) in acetic acid 1% were added to Mu. The mixtures were slowly agitated with a magnetic stirrer for 15 min. Finally, in Mo and Mu, 400 g of maltodextrin solution (120 g maltodextrin + 280 g water) with a dextrose equivalent of 12% (Glucidex 12, kindly provided Roquette, Lestrem, France) were added. Thus, feed emulsions (800 g) of Mo and Mu were obtained.

A laboratory-scale spray drier equipped with a 0.5-mm nozzle atomizer (Mini spray-dryer B-290, Buchi, Switzerland) was used to dry the feed emulsions, applying the following parameters (80% aspirator rate = 80%, feed rate = 1 L/h, inlet temperature = 180 °C, outlet temperature = 85–90 °C). During the spray-drying process, the emulsions were agitated in a magnetic stirrer at room temperature. The dried powders collected were refrigerated (4 °C) until they were added to the meat products. Quality characteristics of Mo and Mu emulsions and microcapsules have been previously analyzed [29].

2.3. Elaboration of Meat Products

The ingredients for the elaboration of C-SAU were meat mechanically separated from chicken (60%), water (20%), pork fat (12%), salt (14g/kg), pork plasma (8%), stabilizer (E-450), aromas, vegetable fiber, spices, spice extracts, smoke flavor, antioxidant (E-316) and preservative (E-250). Moreover, the enriched batches were added with the corresponding microcapsules in the knead phase. The mincer Asgo MVZ 600 (Oporto, Portugal) was used for mixing. All C-SAU batches followed the same processing: stuffing in 18 mm diameter cellulose casings, using a Stuffer Vernag HPE (Barcelona, Spain), heating at 85 °C for 30 min in a cooking pot Gaser MCA 200 (Girona, Spain), cooling at 7 °C for 1 h, and finally being vacuum packed. Samples were maintained in refrigeration (0–5 °C for 7–9 days), heated (90 °C, 3 min) and analyzed.

In the case of D-SAU, the ingredients were Iberian pork meat (80%) and fat (15%), salt (20 g/kg), dextrose, soy protein, spices, aromas, stabilizers (E-451 and E-450), antioxidant (E-301), preservatives (E-252 and E-250), flavor enhancer (E-621), coloring (E-120) and the corresponding microcapsules in the case of the enriched batches. The meat and the fat were minced (Sheydelman AU 200, Aalen, Germany), through a 6 mm diameter mincing plate, mixed with the rest of ingredients (Asgo MVZ 600, Porto, Portugal). No starter culture was added. Collagen casings (40 cm length, 60 mm diameter) and a Stuffer Vernag HPE (Barcelona, Spain) were used to stuff the dough. Once stuffing, the products were dry-cured processed in three consecutive stages: (i) 4 °C, 82% of relative humidity, 3 days; (ii) 8 °C, 80% of relative humidity, 21 days; (iii) 5 °C, 85% relative humidity, 14 days. At the end of this process, the percentage of loss was 38–40%. The dry-cured sausages were analyzed after storing at ambient temperature (18–20 °C for 7–9 days).

C-SAU and D-SAU batches were formulated and manufactured in a meat industry (remain anonymous).

2.4. Analysis of Volatile Compounds

Microcapsules and minced meat products were sampled (1 g) into a 10 mL glass flask (Hewlett–Packard, Palo Alto, CA, USA) sealed with an aluminum cap and polytetrafluoroethylene (PTFE) butyl septum (Perkin-Elmer, Foster City, CA, USA). A solid-phase microextraction (SPME) method [34] was first applied for the volatile compound extraction. A cross-linked carboxen/polydimethylsiloxane fiber (10 mm long, 100 µm thick; Supelco, Bellefonte, PA, USA) was used. It was conditioned (220 °C, 50 min) in the gas chromatograph (GC) injection port before use. For the absorption of the volatile compounds of samples, the fiber was introduced in the sealed vial,

being placed in a water bath a 40 °C, for 30 min. After that, SPME fiber was moved to the injection port and maintained for 30 min for desorption. Analyses were performed using a Hewlett–Packard 6890 series II GC coupled to a mass selective detector (HP 5973) (Hewlett–Packard, Wilmington, DE, USA). A 5% phenyl–95% polydimethylsiloxane column (30 m × 0.32 mm ID, 1.05 µm film thickness, Hewlett–Packard) was used, operating at 40 °C, with a column head pressure of 6 psi of and a flow of 1.3 mL min^{-1}. The mode of the injection port was splitless. The following temperature program was applied: isothermal for 15 min at 35 °C, increased to 150 °C at 4 °C min^{-1}, and then to 250 °C at 20 °C min^{-1}. Temperature of the transfer line to the mass spectrometer was 280 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of one scan over the m/z range of 30–550 u.m.a. The linear retention indexes (LRIs) of the volatile compounds were calculated by means of N-alkanes (Sigma R-8769), which were analyzed under the same conditions. Identification of volatile compounds of samples was done by comparison of mass spectra with databases (National Institute of Standards and Technology (NIST) and Wiley libraries) and by comparison of their LRI with those available in the literature [20,21,35–58]. Results from volatile analyses are provided in area units (AU).

2.5. Sampling Replication and Statistical Analysis

Replicate experimental samples (n = 3) of Mo and Mu microcapsules and of the three batches (Co, Mo and Mu) of meat products (C-SAU and D-SAU) were analyzed in triplicate. One-way analysis of variance (ANOVA) was applied to evaluate the addition of different types of fish oil microcapsules and the differences between microcapsules. In the case of being significant effects ($p < 0.05$), paired comparisons between means were conducted using Tukey's test. Furthermore, all volatile compounds that showed significant differences in the ANOVA analysis were included into a principal component analysis (PCA). The statistics were run using the program IBM SPSS Statistics v.22 (IBM, Armonk, NY, USA).

3. Results and Discussion

3.1. Volatile Compounds in Fish Oil Microcapsules

A total of 40 volatile compounds were identified in the Mo and Mu fish oil microcapsules, which were grouped in the following chemical families: aliphatic hydrocarbons, alcohols, aldehydes, ketones, furans and acids. Figure 1 shows the area percentage of each chemical family in Mo and Mu. In both types of fish oil microcapsules, aliphatic hydrocarbon was the major chemical family, followed in decreasing order by aldehydes, ketones, alcohols, furans and acids. This profile of volatile compounds is quite according to a previous study with double and multilayered fish oil microcapsules [35]. The percentage of aliphatic hydrocarbons has been used as an indicator of quality and stability of different commercial fish oils, from salmon, tuna, sardines and shrimp. Considering the relationship between the decrease in the percentage of this family of volatile compounds with an increase in lipid oxidation [51], the high percentage of aliphatic hydrocarbons in Mo and Mu may support the protective effect of the wall materials of these types of microcapsules, minimizing the contact and reactivity of fish oil with oxidizing promoters. Significant differences were detected in the percentage of the most chemical families of volatile compounds between the Mo and Mu of this study, showing Mo higher percentages of aldehydes (8.43% vs. 4.78%), ketones (3.82% vs. 2.65%), alcohols (1.97% vs. 1.10%) and lower of aliphatic hydrocarbon (87.89 vs. 90.43%) and acids (n.d. vs. 0.32%) than Mu. Accordingly, [59] also found significant differences in the percentage of chemical families of volatile compounds between different types of fish oil microcapsules.

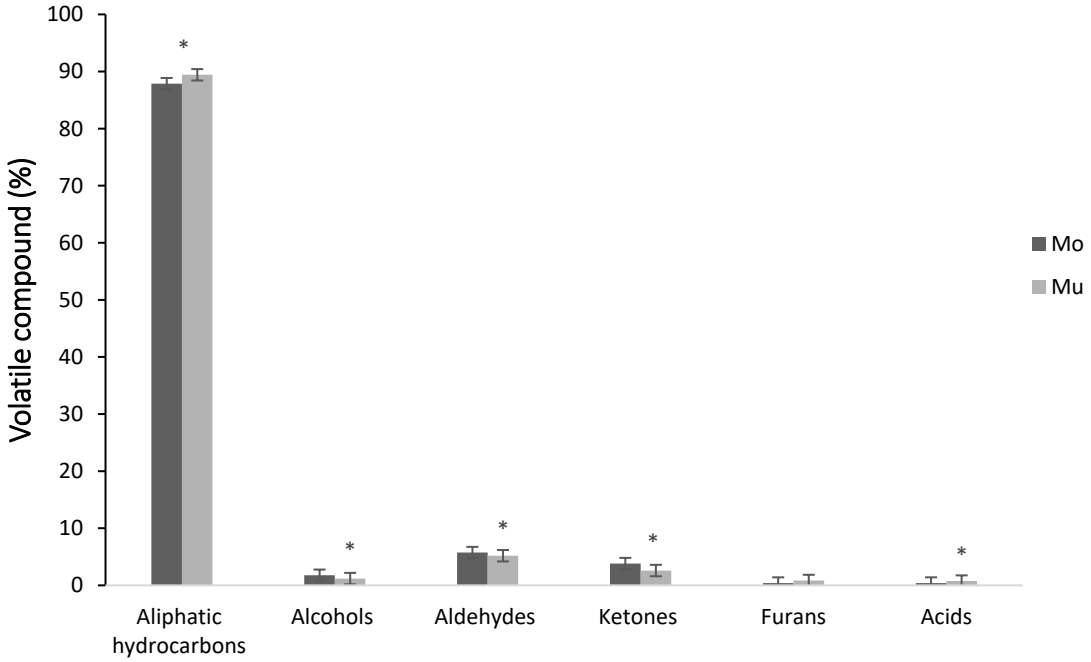

Figure 1. Volatile compounds of monolayered (Mo) and multilayered (Mu) fish oil microcapsules classified according to chemical families. Values are expressed as the average percentage of each family. *: significant differences ($p < 0.05$).

Table 1 lists the individual volatile compounds of Mo and Mu, being expressed as AU × 10^6. Hexane was the major volatile compound in Mo and Mu, followed by pentane, hexanal and 3-hydroxy-2-butanone, and the rest of individual volatile compounds showed less than 1 AU × 10^6. This agrees with results described for double and multilayered fish oil microcapsules [35]. From the 40 individual volatile compounds identified in the fish oil microcapsules of the present study, 9 of them were found in Mu but not in Mo (tridecane, 1,2,4-butanetriol, phenylethyl alcohol, 2-heptenal, 2-octenal, 2-nonanone, 2-butyltetrahydrofuran, heptanoic and sorbic acid) and 7 were only detected in Mo (1-heptene, heptane, decane, 2-propanol, 4-hexen-1-ol, 1-heptanol and 2-hexenal).

Significant differences were found in most individual volatile compounds between Mo and Mu (Table 1). Regarding the aldehydes, which have been described as the most important indicators of fish oil oxidation [51], Mo showed significant higher levels of propanal, pentanal, hexanal, 2-hexenal, heptanal, octanal and nonanal compared to Mu. It has been described that propanal comes from the lipid hydroperoxides derived from ω-3 PUFA while hydroperoxides derived from ω-6 PUFA mainly generate hexanal, as consequence of the breakdown of the first double bond of the n position of the ω-3 and ω-6 fatty acids, respectively [60]. In addition, hexanal has been used in previous studies as a marker to measure the quality and oxidative stability of fish oil microcapsules [20]. On the other hand, Mu had a significant higher AU of 2-pentenal and 2-octenal than Mo, but these two volatile compounds have not been related to the oxidation of ω-3 PUFAs. In addition, other relevant indicators of fish oil oxidation, such as 2,4-heptadienal and 2,4-decadienal, which have been associated to the perception of rancid flavor [20,48,60], or other aldehyde volatile compounds related to rancid odors, such as decanal or 2-nonenal [20,48,61], have not been identified in Mo or Mu.

Most individual alcohols and ketones have shown significant differences between Mo and Mu, with higher AU in Mo in comparison to Mu in most cases. However, as is our knowledge, either of the ketones detected in Mo and Mu are associated with the lipid oxidation process. The most important ketones from lipid autoxidation reactions are 3,5-octadien-2-one and 1-octen-3-one [49]. In fact, they have been detected in mayonnaise and milk enriched with fish oil, being strongly correlated with the strength of the oxidation process [48,50]. However, these ketones were not detected in Mo or Mu. Regarding the alcohols, 1-penten-3-ol and 2-penten-1-ol have been described as one of the

most characteristics oxidation markers for PUFA [48,49]. 1-penten-3-ol was detected in both types of microcapsules, with higher AU in Mo than in Mu, while 2-penten-1-ol was not found in these fish oil microcapsules. Another common oxidation product of ω-3 PUFA is 2-ethylfuran, which can be generated from the 12-hydroperoxide of EPA and 16-hydroperoxide of DHA [35,62]. This volatile compound has been identified in both types of microcapsules, Mo having significantly higher AU than Mu. Two volatiles compounds, heptanoic and sorbic acid, were found in Mu, but not in Mo. Heptanoic acid at high concentrations imparts unpleasant rancid odor, but the AU of this compound in Mu are very low.

Table 1. Volatile compounds from monolayered (Mo) and multilayered (Mu) fish oil microcapsules. Values are expressed as peak area × 10^6.

LRI	ID	Compound	Mo	Mu	SEM	*p*
Aliphatic hydrocarbons						
504	A	Pentane	16.15	23.17	2.11	0.090
602	A	Hexane	91.47	83.26	2.47	0.091
692	A	1-heptene	0.03	n.d.	0.00	<0.001
703	A	Heptane	0.02	n.d.	0.00	0.004
799	A	Octane	0.05	0.07	0.01	0.002
901	A	Nonane	0.08	0.17	0.02	0.002
1003	A	Decane	0.02	n.d.	0.00	0.001
1099	A	Undecane	0.14	0.17	0.01	0.494
1200	A	Dodecane	0.02	0.08	0.01	0.013
1305	A	Tridecane	n.d.	0.04	0.01	0.001
Alcohols						
614	A	1-propanol	0.06	0.03	0.01	0.153
621	A	2-propanol	0.02	n.d.	0.00	0.007
687	A	1-penten-3-ol	0.29	0.08	0.05	0.026
784	A	2,3-butanediol	0.42	0.34	0.04	0.362
921	A	1,2,4-butanetriol	n.d.	0.55	0.12	<0.001
927	A	1-hexanol	0.08	0.01	0.02	<0.001
1015	A	4-hexen-1-ol	0.21	n.d.	0.05	0.008
1032	A	1-heptanol	0.03	n.d.	0.01	<0.001
1092	A	2-ethyl-1-hexanol	1.07	0.33	0.17	<0.001
1210	A	Phenylethyl alcohol	n.d.	0.07	0.02	<0.001
Aldehydes						
522	A	Propanal	0.27	0.15	0.03	0.048
587	A	2-methylpropanal	0.41	0.40	0.04	0.909
628	A	Butanal	0.45	0.29	0.04	0.055
730	A	Pentanal	1.49	0.26	0.29	0.003
777	A	2-pentenal	0.02	0.16	0.03	<0.001
854	A	Hexanal	2.83	1.94	0.20	<0.001
914	A	2-hexenal	0.07	n.d.	0.01	<0.001
936	A	Heptanal	0.66	0.24	0.10	0.014
1022	A	2-heptenal	n.d.	0.07	0.01	<0.001
1034	A	Octanal	1.42	0.49	0.23	0.016
1127	A	2-octenal	n.d.	0.48	0.12	0.009
1147	A	Nonanal	0.81	0.38	0.10	0.008
Ketones						
820	A	3-hydroxy-2-butanone	2.68	1.59	0.25	0.001
1070	B	1,4-cyclohexanedione	0.15	0.24	0.03	0.133
1140	A	2-nonanone	n.d.	1.10	0.25	<0.001
Furans						
722	A	2-ethylfuran	0.55	0.24	0.07	0.001
954	A	2-butyltetrahydrofuran	n.d.	0.02	n.d.	0.001
Terpenes						
1040	A	Limonene	0.02	0.01	n.d.	0.327
Acids						
1165	A	Heptanoic acid	n.d.	0.17	0.04	0.003
1168	A	Sorbic acid	n.d.	0.17	0.04	0.008

n.d. not detected; SEM. standard error; LRI. Linear retention index of the compounds eluted from the GC-MS; ID. Method of identification: A. mass spectrum and retention time similar to previous publication data; B. tentative identification by mass spectrum. $p < 0.05$ indicates significant differences between microcapsules.

The higher AU in some individual volatile compounds related to lipid oxidation found in Mo in comparison to Mu could be explained by the different wall material of these fish oil microcapsules, being of maltodextrine and of chitosan plus maltodextrine, respectively. In fact, it has been described that chitosan increases the electrostatic force and viscosity of the layers [63], avoids the oxidative damage and could act as a free scavenger [64]. Thus, the Mu coating may be more effective than Mo to protect fish oil from oxidative damage. This aspect can be marked in the case of volatile compounds such as hexanal, 1-penten-3-ol and 2-ethylfuran, with low odor thresholds (4.5, 1 and 2.2 μg kg^{-1} oil, respectively) and associated with sensory defects [65,66].

Nevertheless, in comparison to the profile of volatile compounds in bulk fish oil, Mo and Mu have not shown to be polyunsaturated lipid oxidation products with rancid taste perceptions, such as 2,4-heptadienal, 2,4-decadienal, 2-nonenal, 3,5-octadien-2-one and 1-octen-3-one [51,67], which points out the effectiveness of the Mo and Mu microcapsules of the present study to minimize the contact and reactivity of fish oil encapsulated with oxidizing promoters.

3.2. Volatile Compounds in Dry-Cured and Cooked Sausages Enriched with Fish Oil Microcapsules

A total of 53 and 60 volatile compounds were identified in D-SAU and C-SAU of the present study, respectively, which were grouped in the following chemical families: aliphatic hydrocarbons, alcohols, aldehydes, furans, ketones, terpenes, acids, esters, aromatics, cyclic hydrocarbons and pyrazines. Figure 2A,B show the percentages of these chemical families of volatile compounds in D-SAU and C-SAU as affected by type of fish oil microcapsule addition, respectively. The most abundant families in all batches of D-SAU were acids and aldehydes, followed far behind by terpenes and esters. Minor percentages were found for aliphatic hydrocarbons, aromatics, ketones, cyclic hydrocarbons, alcohols and furans. This profile is quite in concordance with previous studies in fermented sausages [53,58]. Moreover, significant differences were detected in the chemical families of volatile compounds between the D-SAU batches of this study, with Mo showing higher percentages of aldehydes and terpenes (16.03 and 8.2) than Co and Mu batches, and Mu having a higher percentage of acids (69.63) and lower percentages of esters (5.58) than Co and Mo.

In C-SAU, the major family of volatile compounds was aldehydes, followed by aliphatic hydrocarbons, cyclic hydrocarbons and alcohols, while minor abundance was detected for terpenes, acids, esters, aromatics, ketones, furans and pyrazines. This agrees with the profile of volatile compounds previously reported in other studies in cooked sausages [42,46]. Moreover, significant differences were found in the chemical families of volatile compounds between the C-SAU batches of this study, showing in C-SAU-Mo higher percentages of aldehydes and alcohols than in C-SAU-Co and C-SAU-Mu; C-SAU-Mu obtained a higher percentage of acids and esters and a lower percentage of aliphatic hydrocarbons than C-SAU-Co and C-SAU-Mo, and the percentage of cyclic hydrocarbons was higher in C-SAU-Co than in the enriched batches. Thus, at first, considering the results on the percentage of volatile compounds, the differences between microcapsules are reflected in the bathes of meat products. Taking a step forward, the individual volatile compounds of the control and enriched batches of the meat products of the present study are analyzed in the following sections.

Table 2 lists the individual volatile compounds of D-SAU as affected by the type of fish oil microcapsule added. Acetic acid was the major volatile compound in all batches, followed by hexanal, methyl hexanoate, β-myrcene, pentanoic acid, butanoic acid and heptanal, the rest of the individual volatile compounds showing less than 10 AU × 10^6. This profile is quite in concordance with previous studies in dry fermented sausages [53,55]. The high content of acetic acid would be related to microbial fermentation of carbohydrates [55,68]. Others compounds also typical of carbohydrate fermentation, such as 3-hydroxy-2-butanone, were also detected in D-SAU batches [57]. The high AU of β-myrcene in these samples is also noted, which may be ascribed to the addition of species [69].

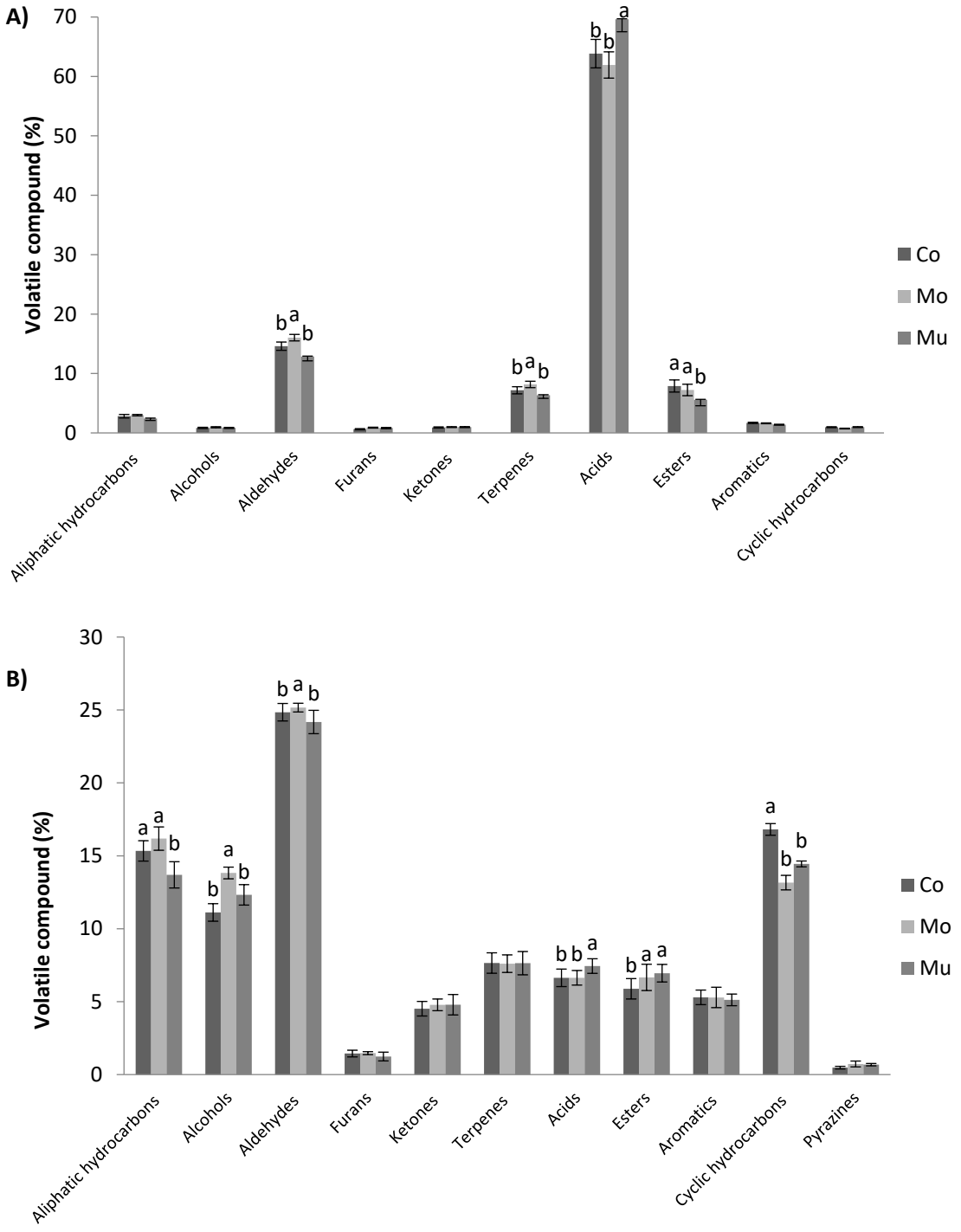

Figure 2. Volatile compounds of dry-cured (**A**) and cooked (**B**) sausages as affected by enrichment with omega-3 polyunsaturated fatty acids (ω-3 PUFA) (control: dark gray; enriched with multilayered fish oil microcapsules: medium gray; enriched monolayered fish oil microcapsules: light gray) classified according to chemical families. Values are expressed as the average percentage of each family. Bars with different letters (a, b) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect (Co vs. Mo vs. Mu).

Table 2. Volatile compounds (arbitrary area units × 10^6) on dry-cured sausages (D-SAU) as affected by enrichment with ω-3 PUFA (*p*) *.

LRI	ID	Compound	Co	Mo	Mu	*p*	SEM
Aliphatic hydrocarbons							
497	A	Pentane	0.49	0.44	0.65	0.409	0.15
599	A	Hexane	0.48	0.24	0.33	0.724	0.07
704	A	Heptane	0.41 ^{b}	0.46 ^{b}	0.92 ^{a}	<0.001	0.08
761	A	1-propene	n.d. ^{b}	0.43 ^{a}	n.d. ^{b}	<0.001	0.05
800	A	Octane	7.84	7.21	7.02	0.533	0.34
901	A	Nonane	0.52	0.39	0.63	0.057	0.04
997	A	Decane	0.89	1.01	0.92	0.319	0.07
1097	A	Undecane	1.95	1.81	1.89	0.756	0.11
Alcohols							
614	A	1-propanol	0.03 ^{b}	0.12 ^{a}	0.09 ^{a}	0.043	0.02
687	A	1-penten-3-ol	n.d. ^{b}	0.11 ^{a}	n.d. ^{b}	<0.001	0.26
820	A	1-pentanol	1.42	1.47	1.48	0.936	0.06
1024	A	1-heptanol	1.18	1.10	1.18	0.851	0.17
1031	A	1-octen-3-ol	2.58 ^{c}	3.53 ^{a}	3.03 ^{b}	0.028	0.19
1195	A	4-terpineol	1.82	1.84	1.75	0.318	0.03
Aldehydes							
591	A	2-methylpropanal	0.31	0.42	0.28	0.172	0.03
618	A	Butanal	0.29 ^{a}	0.10 ^{b}	0.32 ^{a}	0.002	0.05
667	A	2-methyl butanal	0.15	0.28	0.07	0.104	0.03
738	A	Pentanal	7.89 ^{c}	9.54 ^{a}	8.69 ^{b}	0.027	0.33
862	A	Hexanal	92.26	93.64	101.51	0.176	6.08
942	A	Heptanal	8.12	7.27	7.35	0.433	0.62
1050	A	Octanal	0.49	0.55	0.53	0.525	0.02
1147	A	Nonanal	5.59	5.11	4.94	0.295	0.13
1322	A	2-decenal	0.87	0.84	0.88	0.594	0.01
1395	A	2,4-decadienal	0.39	0.42	0.37	0.577	0.04
Ketones							
749	A	2,3-pentanedione	0.47	0.45	0.46	0.456	0.03
778	A	3-hydroxy-2-butanone	0.49 ^{a}	0.35 ^{a}	0.15 ^{b}	<0.001	0.04
933	A	2-heptanone	7.00	6.99	6.08	0.129	0.24
981	A	3-heptanone	3.34	3.76	4.95	0.330	0.40
1039	A	2-octanone	0.37	0.33	0.27	0.204	0.05
1063	A	3,5-octadien-2-one	n.d. ^{b}	0.09 ^{a}	n.d. ^{b}	0.001	0.31
Furans							
722	A	2-ethyl-furan	n.d. ^{b}	0.13 ^{a}	0.07 ^{a}	0.012	0.41
837	A	3-furaldehyde	0.39	0.26	0.22	0.053	0.02
1012	A	2-pentyl-furan	1.53 ^{a}	1.09 ^{b}	1.25 ^{b}	0.004	0.06
Terpenes							
982	A	Sabinene	8.19	7.79	7.78	0.498	0.10
1003	B	β-myrcene	36.06 ^{b}	36.84 ^{b}	40.64 ^{a}	0.023	0.70
1021	A	α-phellandrene	5.93	6.20	6.78	0.416	0.14
1037	A	ɒ-limonene	3.55	3.23	3.82	0.168	0.26
1066	A	gamma-terpinene	2.63	2.27	2.41	0.122	0.10
1105	A	Terpene	1.87	1.87	1.60	0.280	0.11
1404	A	α-cubebene	1.23	1.26	1.68	0.282	0.09
Acids							
717	A	Acetic acid	458.69 ^{b}	582.75 ^{a}	422.62 ^{b}	<0.001	43.49
895	A	Butanoic acid	30.15	26.94	27.08	0.094	1.26
986	A	Pentanoic acid	34.56	36.84	36.14	0.510	0.40
1362	A	Nonanoic acid	2.60	2.43	2.61	0.894	0.08
1472	A	Decanoid acid	3.05	2.58	2.61	0.409	0.17
Esters							

Table 2. *Cont.*

LRI	ID	Compound	Co	Mo	Mu	*p*	SEM
786	A	Methylpropyl acetate	0.27	0.28	0.26	0.992	0.02
853	A	Methyl hexanoate	65.15	51.94	57.08	0.345	2.55
Aromatics							
1018	A	Benzaldehyde	9.30	8.29	7.64	0.109	0.18
1190	A	4-methyl-phenol	0.53	0.59	0.60	0.517	0.02
1375	A	Eugenol	4.18	4.16	4.57	0.198	0.09
Cyclic hydrocarbons							
992	B	α-thujene	6.30	5.86	6.06	0.419	0.10
1495	A	Humulene	0.61	0.66	0.78	0.469	0.04
Other							
899	B	Allyl sulphide	2.41 [a]	2.68 [a]	1.90 [b]	0.003	0.15

* Not enriched (Co) and enriched with monolayer (Mo) and multilayered fish oil microcapsules (Mu). Bars with different letters (a, b, c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect. n.d., not detected; SEM, standard error; LRI, linear retention index of the compounds eluted from the GC-MS; ID, method of identification: A, mass spectrum and retention time identical with an authentic standard; B, tentative identification by mass spectrum.

The enrichment effect with Mo and Mu fish oil microcapsules significantly influence the volatile compounds of most chemical groups (Table 2), excluding esters, aromatics and cyclic hydrocarbons. Only 13 in 53 volatile compounds identified in D-SAU showed significant differences among batches: C-SAU-Mo showed higher AU in six volatile compounds (1-propanone, 1-penten-3-ol, 1-octen-3-ol, pentanal, 3,5-octadien-2-one and acetic acid) and lower in one (butanal) in comparison to C-SAU-Co and C-SAU-Mu; C-SAU-Mu obtained higher abundance in two volatile compounds (heptane and β-mycene) and lower in one (3-hydroxy-2-butanone) than in C-SAU-Co and C-SAU-Mo, and C-SAU-Co showed higher AU in one volatile compound (2-penthyl-furan) and lower in two (1-propanol and 2-ethylfuran) in comparison to the enriched batches. Figure 3a represents the score plots of the PCA of volatile compounds data from the D-SAU samples. The first principal component (PC1) comprised 55.48% of the total variance, and the second principal component (PC2) accounted for 29.47%. The score plot indicates a clear differentiation of samples as affected by the addition of fish oil microcapsules: those with high positive PC1 scores (D-SAU-Mo), those with high positive PC2 scores (D-SAU-Mu) and those with high negative PC2 scores (D-SAU-Co). Several volatile compounds (3,5-octadien-2-one, 1-propanol, pentanal, 1-octen-3-ol, 1-penten-3-ol, acetic acid, 2-ethylfuran, 1-propene, allyl sulphide) are located in the right quadrants (upper and lower), which correspond to high positive charges in PC1, associated with D-SAU-Mo batch. On the other hand, there were a few volatile compounds allocated in the PC2: heptane, β-myrcene, and butanal in the left upper quadrant, and 2-pentyl-furan and 3-hydroxy-2-butanone in the left lower quadrant, related to D-SAU-Mu and D-SAU-Co, respectively. Thus, in comparison to C-SAU-Co and C-SAU-Mu, C-SAU-Mo are more related to typical volatiles compounds of fatty acid oxidation, such as 1-propanol, 1-octen-3-ol and pentanal [45], and to characteristic oxidation markers for PUFA oxidation, such as 1-penten-3-ol, 2-ethyl-furan and 3,5-octadien-2-one, which have been previously observed in mayonnaise and chicken nuggets enriched with fish oil [23,48], and are correlated with the strength of the oxidation process [48,50]. The low odor thresholds some of these volatile compounds, such as 1-octen-3-ol and 3,5-octadien-2-one (1 and 0.45 μg kg^{-1} of oil, respectively) [70,71], would lead to the perception of anomalous odor and/or flavor, which may have a negative impact in the products enriched with Mo. Nevertheless, in a previous study carried out with the D-SAU samples of the present work [28], no significant differences in acceptability were found among Co, Mo and Mu samples. So, the influence of the fish oil microcapsules addition on the profile of volatile compounds does not seem to be reflected in the consumer's perception of D-SAU.

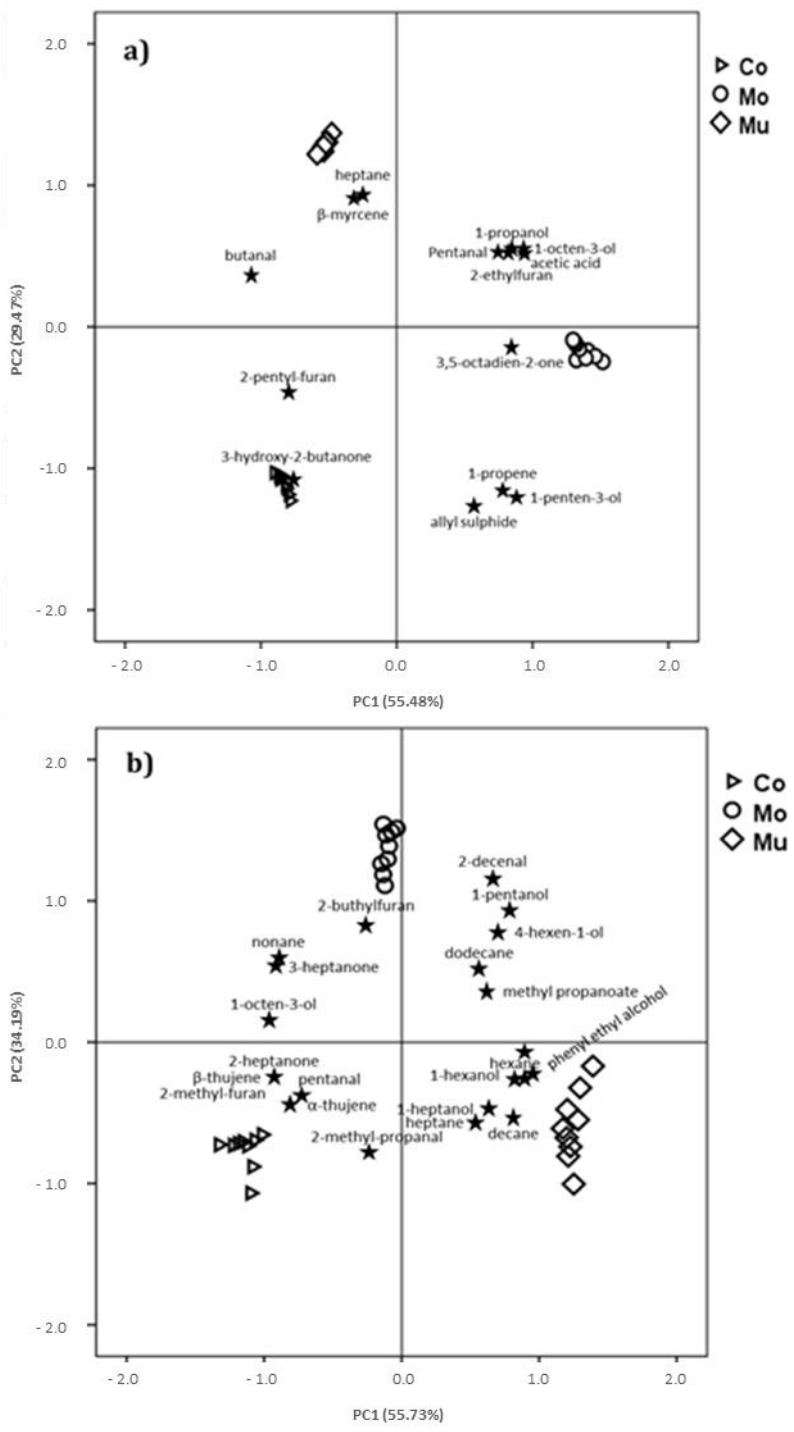

Figure 3. Principal component analysis (PCA) of the significant volatile compounds in dry-cured (**a**) and cooked (**b**) sausages. The plots represent, for the two first principal components, the loading of each volatile compound and the average scores of each one of batches. Control (▷); enriched with monolayered fish oil microcapsules (○); enriched with multilayered fish oil microcapsules (◊).

Table 3 showed the individual volatile compounds of C-SAU as affected by the type of fish oil microcapsule added. α-thujene was the major volatile compound in all batches (around 6.07 AU × 10^6), followed by pentanal (around 4.84 AU × 10^6), β-thujene (around 3.52 AU × 10^6), hexanal (around 3.5 AU × 10^6), 1-octen-3-ol (around 3.40 AU × 10^6), gamma-terpinene (around 3.20 AU × 10^6) and heptanal (around 3.15 AU × 10^6), and the rest individual volatile compounds showed less

than 3 AU × 10^6. In previous studies in cooked sausages, hexanal has been identified as the most abundant volatile compound, followed by heptanal, pentanal and volatiles compounds from the chemical families of alcohols (1-pentanol and 1-octen-3-ol) and terpenes (limonene, β-myrcene, and gamma-terpinene) [39,40,42], which is quite in agreement with the findings of the present study. However, in these previous works α-thujene and β-thujene were identified but with lower AU than in the present work. These compounds are associated with spicy flavor and have been found in a wide variety of medicinal herbs, essential oils, flavorings and spices such as nutmeg [72]; therefore, its abundance in the present study could be related to the addition of spices in the meat product formulation.

Table 3. Volatile compounds (arbitrary area units × 10^6) on cooked sausages (C-SAU) as affected by enrichment with fish oil microcapsules (*p*) *.

LRI	ID	Compound	Co	Mo	Mu	*p*	SEM
Aliphatic hydrocarbons							
499	A	Pentane	0.49	0.47	0.45	0.598	0.04
601	A	Hexane	0.44 ^{b}	0.45 ^{b}	0.78 ^{a}	0.021	0.09
703	A	Heptane	1.49 ^{b}	1.10 ^{b}	2.03 ^{a}	0.046	0.17
799	A	Octane	2.67	2.58	2.51	0.612	0.11
812	A	2-octene	2.05 ^{a}	2.06 ^{a}	1.67 ^{b}	0.032	0.16
901	A	Nonane	1.89 ^{a}	2.07 ^{a}	1.03 ^{b}	<0.001	0.13
1000	A	Decane	n.d. ^{b}	n.d. ^{b}	0.56 ^{a}	<0.001	0.17
1101	A	Undecane	0.60	0.60	0.74	0.165	0.03
1200	A	Dodecane	0.40 ^{b}	0.48 ^{a}	0.54 ^{a}	0.046	0.03
1296	A	Tridecane	n.d. ^{c}	0.24 ^{b}	0.46 ^{a}	0.021	0.03
1402	A	Tetradecane	0.51	0.54	0.68	0.465	0.15
Alcohols							
615	A	1-propanol	0.13	0.11	0.14	0.561	0.10
825	A	1-pentanol	1.39 ^{b}	1.73 ^{a}	1.41 ^{b}	0.028	0.04
923	A	1-hexanol	0.69 ^{b}	0.80 ^{b}	1.08 ^{a}	0.039	0.06
927	A	4-hexen-1-ol	0.96 ^{c}	1.90 ^{b}	1.54 ^{a}	0.026	0.12
1024	A	1-heptanol	0.66 ^{b}	0.86^{a}	0.91 ^{a}	0.041	0.06
1031	A	1-octen-3-ol	3.84 ^{a}	3.50 ^{a}	2.84 ^{b}	0.036	0.31
1088	A	2-ethyl-1-hexanol	0.44	0.44	0.57	0.134	0.14
1092	A	Phenyl ethyl alcohol	n.d. ^{c}	0.37 ^{b}	0.91 ^{a}	0.011	0.06
Aldehydes							
593	A	2-methyl propanal	1.47 ^{a}	1.21 ^{b}	1.66 ^{a}	0.048	0.11
667	A	2-methyl butanal	0.70	0.65	0.76	0.432	0.07
687	A	3-methyl butanal	2.04	2.19	2.25	0.686	0.53
738	A	Pentanal	6.06 ^{a}	4.58 ^{b}	3.89 ^{b}	0.017	0.61
862	A	Hexanal	3.41	3.44	3.65	0.490	0.16
939	A	Heptanal	3.31	2.82	3.11	0.082	0.22
1047	A	Octanal	0.38	0.35	0.43	0.372	0.04
1114	A	2-octenal	0.08	0.13	0.18	0.142	0.05
1147	A	Nonanal	0.51	0.54	0.75	0.260	0.25
1328	A	2-decenal	0.77 ^{b}	1.69 ^{a}	0.89 ^{b}	0.002	0.09
1390	A	2,4-decadienal	0.36	0.44	0.38	0.298	0.04
Ketones							
744	A	2,3-pentanedione	0.33	0.47	0.40	0.312	0.04
933	A	2-heptanone	0.84 ^{a}	0.55 ^{b}	0.38 ^{b}	0.041	0.10
979	A	3-heptanone	2.27 ^{a}	2.37 ^{a}	1.05 ^{b}	0.003	0.23
1039	A	2-octanone	1.11	1.35	1.21	0.087	0.09
Furans							
720	A	2-ethylfuran	0.38	0.26	0.21	0.446	0.08
908	A	2-butylfuran	0.37 ^{a}	0.42 ^{a}	0.19 ^{b}	0.033	0.03
1008	A	2-pentyl-furan	0.79 ^{a}	0.60 ^{b}	0.45 ^{c}	0.004	0.03
Terpenes							

Table 3. *Cont.*

LRI	ID	Compound	Co	Mo	Mu	*p*	SEM
1026	A	3-carene	1.56	1.38	1.42	0.311	0.12
1037	A	ɒ-limonene	2.60	2.41	2.36	0.298	0.13
1066	A	gamma-terpinene	3.16	3.13	3.29	0.589	0.16
1097	A	Terpene	0.20	0.16	0.27	0.178	0.08
1491	A	Isocayophillene	0.26	0.24	0.26	0.589	0.03
Acids							
716	A	Acetic acid	n.d. ^{b}	0.04 ^{b}	0.22 ^{a}	0.005	0.04
895	A	Butanoic acid	2.36	2.27	2.13	0.798	0.15
898	A	2-butenoic acid	0.25	0.29	0.26	0.636	0.03
986	A	Pentanoic acid	1.97 ^{a}	1.06 ^{b}	0.69 ^{c}	<0.001	0.08
1273	A	Octanoic acid	0.42 ^{a}	0.33 ^{a}	n.d. ^{b}	0.005	0.13
1366	A	Nonanoic acid	0.45 ^{b}	0.43 ^{b}	0.63 ^{a}	0.032	0.04
1461	A	Decanoic acid	0.41	0.36	0.18	0.168	0.18
Esters							
656	A	Methyl propanoate	0.04 ^{c}	0.22 ^{b}	0.33 ^{a}	<0.001	0.02
836	A	Ethyl butanoate	2.31	2.45	2.50	0.647	0.11
Aromatics							
1018	A	Benzaldehyde	1.59	1.94	1.67	0.109	0.19
1190	A	4-methyl-phenol	1.08	0.90	0.72	0.068	0.18
1305	B	Safrole	0.36	0.31	0.37	0.298	0.03
Cyclic hydrocarbons							
980	B	β-thujene	4.25 ^{a}	2.76 ^{c}	3.54 ^{b}	0.029	0.41
991	B	α-thujene	7.95 ^{a}	5.13 ^{b}	5.13 ^{b}	0.007	0.22
1422	B	cis-muurola-4(14),5-diene	0.20	0.19	0.19	0.469	0.02
1524	A	δ-cadinene	0.39	0.37	0.45	0.298	0.04
Pyrazines							
863	A	2-methylpyrazine	0.27	0.35	0.25	0.369	0.11
947	A	2,6-dimethylpyrazine	0.20	0.17	0.24	0.428	0.05

* Not enriched (Co) and enriched with monolayer (Mo) and multilayered fish oil microcapsules (Mu). Bars with different letters (a, b, c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect. n.d., not detected; SEM, standard error; LRI, linear retention index of the compounds eluted from the GC-MS; ID, method of identification: A, mass spectrum and retention time identical with an authentic standard; B, tentative identification by mass spectrum.

In C-SAU, the addition of Mo and Mu fish oil microcapsules significantly influenced the volatile compounds of most chemical families (Table 3), excluding terpenes and pyrazines, finding significant differences in 27 volatile compounds. Higher abundance was found in two volatile compounds in C-SAU-Mo (1-pentanol and 2-decenal) than in C-SAU-Co and C-SAU-Mu. A total of nine volatile compounds (hexane, heptane, decane, tridecane, 1-hexanol, phenyl ethyl alcohol, acetic acid, nonanoic acid, methyl propanoate and methyl propanoate) obtained higher AU in C-SAU-Mu than in C-SAU-Co and C-SAU-Mo. On the contrary, C-SAU-Mu showed lower abundance for 2-octene, nonane, 1-octen-3-ol, 2-methyl-propanal, 3-heptanone, 2-buthyl-furan and octanoic acid than C-SAU-Co and C-SAU-Mo. In comparison to the enriched batches, C-SAU-Co showed higher AU for six volatile compounds (pentanal, 2-heptanone, 2-methyl-furan, pentanoic acid, β-thujene and α-thujene) and lower for three (dodecane, 4-hexen-1-ol and 1-heptanol). A score plot of PCA of volatile compounds data from the C-SAU samples is shown in Figure 3b. The PC1 accounted for 54.78% of the total variance, and the PC2 comprised 34.19%. The score plot allowed a clear separation of the samples: those with high positive PC1 scores (C-SAU-Mu), those with high positive PC2 scores (C-SAU-Mo) and those with negative PC2 scores (C-SAU-Co). 2-decenal, 2-buthylfuran, 3-heptanone, 1-pentanol, 4-hexen-1-ol, 1-octen-3-ol, dodecane, nonane and methyl-propanoate were grouped and allocated in the upper quadrants (left and right), which correspond to the C-SAU-Mo batch, while 2-heptanone, pentanal, 2-methylpropanal, β-thujene, α-thujene, 2-methylfuran and hexane, heptane, decane, 1-hexanol, 1-heptanol, phenyl-ethyl-alcohol were in the lower left and right quadrants, related to C-SAU-Co

and C-SAU-Mu, respectively. Thus, in C-SAU, Mo enriched batches also showed a close relation with volatile compounds from lipid oxidation, such us 2-decenal, a characteristic volatile compound of ω-3 PUFA oxidation, and in 1-pentanol, 4-hexen-1-ol and 1-octen-3-ol, described as typical lipid oxidation products [42,46]. 2-decenal has been related in previous studies to rancid odors in fish oil enriched mayonnaise [48] and fish oil microcapsules [20]. These volatile compounds have a fatty and fishy aroma [71] with a low odor threshold, around 10 μg kg^{-1} oil [73], which may be detrimental the acceptability of the meat products added with Mo. However, as occurred in D-SAU, similar scores in the acceptability analysis have been found by [28] in the three batches of C-SAU, the differences in the profile volatile compounds not being reflected by the sensory results.

It is worth noting that the differences found in the present study in the profile volatile compounds depend on the type of fish oil microcapsules added. Anyway, a major protection of the microencapsulated fish oil against lipid oxidation when Mu are added could be indicated. This can be ascribed to the different wall material in Mo (maltodextrine) and Mu (chitosan plus maltodextrine). Thus, the multilayer structured of chitosan-maltodextrine may protect the encapsulated material more effectively than the simple coating of maltodextrine. Indeed, chitosan improves the emulsion stability, increasing the electrostatic force and viscosity of the layers, and can also act as an antioxidant [63,64]. Moreover, a high oxidative stability has been found in microcapsules with chitosan [33]. So, although no marked effects on sensory or oxidation stability have been previously found [28], differences in the volatile compounds should be considered since they could release to unhealthy oxidized products [74]. This can be the case of furans, such us 2-ethylfuran, 2-butylfuran, 2-acetylfuran, 2-pentylfuran, 2-furfural and furfural alcohol, which have been found in different fish products [75,76], and have revealed toxicity in animals and humans [77,78]. In fact, 2-ethylfuran and 2-buthylfuran were closely related to the D-SAU-Mo and C-SAU-Mo samples in this study. Considering this aspect, more studies should be conducted in this sense, for evaluating the formation of contaminants in different omega-3 enriched meat products.

4. Conclusions

The type of fish oil microcapsules influences its profile of volatile compounds and that of the enriched meat products. The use of multilayered microcapsules with chitosan-maltodextrine walls may be more protective to the formation of lipid oxidation products, especially from omega-3 fatty acids, than microcapsules with a maltodextrine layer. Thus, the use of multilayered fish oil emulsions to elaborate omega-3 microcapsules for enriching meat products may be indicated.

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Capítulo 3.5

Fish oil microcapsules as omega-3 enrichment strategy: changes in volatile compounds of meat products during storage and cooking

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Fish Oil Microcapsules as Omega-3 Enrichment Strategy: Changes in Volatile Compounds of Meat Products during Storage and Cooking

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Abstract: This work aims to analyze the effects of processing and storage on the volatile compound profile of different meat products enriched in ω-3 polyunsaturated fatty acids (PUFA). Monolayered (Mo) and multilayered (Mu) microcapsules of fish oil were tested. The profiles of volatile compounds were analyzed by solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). The enrichment with Mo significantly increases the abundance of volatile compounds from lipid oxidation and markers of ω-3 PUFA oxidation, which may be related to the multilayer structure of chitosan–maltodextrin in Mu that achieves greater fish oil protection than the simple coating of maltodextrin in Mo. Besides, the changes in volatile compounds during storage depends on the type of fish oil microcapsules and the meat products, having an increased abundance of ω-3 PUFA oxidation markers in dry-cured sausages added with Mo. However, the enrichment of these meat products with Mo and Mu does not modify the usual variations in the volatile compound profile during culinary cooking. Thus, the addition of multilayer fish oil microcapsules may be a suitable option for enrichment of meat products in ω-3 PUFA without modifying the abundance of volatile compounds, including oxidation markers.

Keywords: volatile compound; ω-3 PUFA microcapsule; cooked sausage; dry-cured sausage; storage; cooking

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1. Introduction

Meat is recognized by the content of nutrients of high biological value, mainly proteins [1]; however, the lipid profile of meat and meat products is often not well-regarded because of the content of saturated fatty acids (SFA), which may be high in some products, and the ratio of ω-6/ω-3 polyunsaturated fatty acids (PUFA) [2]. These components are related with an increase in the risk of suffering certain metabolic and cardiovascular diseases and different types of cancer [3–6]. Besides, a diet rich in ω-3 PUFA, mainly eicosapentaenoic acid (EPA; C20: 5 ω-3) and docosahexaenoic acid (DHA; C22:6 ω-3), is associated with the prevention and therapy of various chronic disorders, particularly those related to cardiovascular diseases [7]. In this sense, one of the current challenges for the meat industry is the improvement of the lipid profile of meat products [8], adapting to current health recommendations (250 mg EPA+DHA/person/day) established by different professional organizations and health agencies [9–12]. Moreover, there is a labelling regulation regarding nutritional claims on ω-3 PUFA: "source of ω-3 fatty acids" (minimum 48 mg EPA+DHA/100 g and 100 kcal) and "high in ω-3 fatty acids" (minimum 80 mg EPA+DHA/100 g and 100 kcal) [13].

Consequently, some previous works evaluated the possibility of incorporating oils rich in ω-3 PUFA, as an emulsion or directly, in some foods [14–16]. Nevertheless, due to

the existence of double bonds, ω-3 PUFA are highly susceptible to oxidation under pro-oxidant conditions, i.e., at high temperatures or in contact with oxygen, light or iron [17,18]. Lipid oxidation leads to primary oxidation products, mainly hydroperoxides, which are isomerized and degraded to volatile compounds. Oxidation of ω-3 PUFA may lead to detrimental sensory attributes [19–21]. Besides, the development of volatile compounds providing pleasant characteristics but being potentially harmful, such as furan and its derivatives, may also take part in this enriching of food with ω-3 PUFA.

Consequently, the microencapsulation of oils rich in ω-3 PUFA has been used as a possible strategy to avoid the oxidation of these beneficial compounds [22–24]. The basis of this technique is to build an impediment [17,18] to avoid contact and reactivity between the active compounds and oxidative promoters of the environment, and to minimize the perception of undesirable odors and flavors [25]. In addition, microencapsulation is easy to apply and economical [26].

In meat products, the generation of volatile compounds takes place through a complex process that involves the interactions of numerous factors, proteins, lipids and additives (salt, curing agents, aromas and spices) being the main precursors and influenced by the manufacturing process [27]. Storage time and conditions also influence the development of volatile compounds related to unpleasant flavors and loss of food quality, with rancidity being identified as the main cause of flavor deterioration and low acceptability by consumers [28]. In fact, the loss of sensory quality in meat products can be monitored through the profile of volatile compounds [29].

The influence of the addition of ω-3 PUFA microcapsules to meat products has been preferably evaluated by means of the percentage of fat and fatty acids, lipid oxidation and acceptability analyses [30–33], paying less attention to the effect on the profile of volatile compounds, which may be related to the sensory attributes and/or have harmful effects, or its changes during the processing or storing.

Therefore, identifying the effect of processing and storage condition on the generation of volatile compounds in different meat derivatives enriched in omega-3 with fish oil microcapsules was the aim in the present work, evaluating two types of fish oil microcapsules.

2. Material and Methods

2.1. Experimental Design

Figure 1 outlines the experimental design of the present study. Three batches of cooked (C-SAU) and dry-cured sausages (D-SAU) were elaborated: a control (C-SAU-Co and D-SAU-Co), and with the additions of Mo (C-SAU-Mo and D-SAU-Mo) and Mu microcapsules (C-SAU-Mu and D-SAU-Mu). Mo and Mu were added in excess to label C-SAU and D-SAU as "source of ω-3 fatty acids" [13]: 3 (2.75%, *w*/*w*) and 5 (5.26%, *w*/*w*) g microcapsules/100 g product, respectively. This was because the content of EPA+DHA was different in Mo (2.75 mg/100 g) and Mu (5.26 mg/100 g) [34].

The effects of storage and culinary heating were evaluated. Thus, C-SAU batches were sampled immediately after elaboration (T0), after being refrigerated at 2–4 °C for four months (T4) and before (Be) and after (Af) being heated (90 °C, 3 min). Therefore, there were 12 C-SAU batches (Co-T0-Be; Co-T0-Af; Co-T4-Be; Co-T4-Af; Mo-T0-Be; Mo-T0-Af; Mo-T4-Be; Mo-T4-Af; Mu-T0-Be; Mu-T0-Af; Mu-T4-Be; and Mu-T4-Af). D-SAU batches were sampled immediately after elaboration (T0) and after being stored at ambient temperatures (18–20 °C), resulting in six D-SAU batches (Co-T0; Co-T4; Mo-T0; Mo-T4; Mu-T0; and Mu-T4). All batches were evaluated by means of volatile compounds. Analyses were carried out in triplicate.

2.2. Fish Oil Microcapsules

Elaboration of Mo and Mu microcapsules was carried out according to [34]. Emulsions (with fish oil, lecithin, maltodextrin and water (for Mo) or 1% of chitosan in acetic acid 1% (for Mu)) were prepared, high-pressure homogenized and dried by spray-drying.

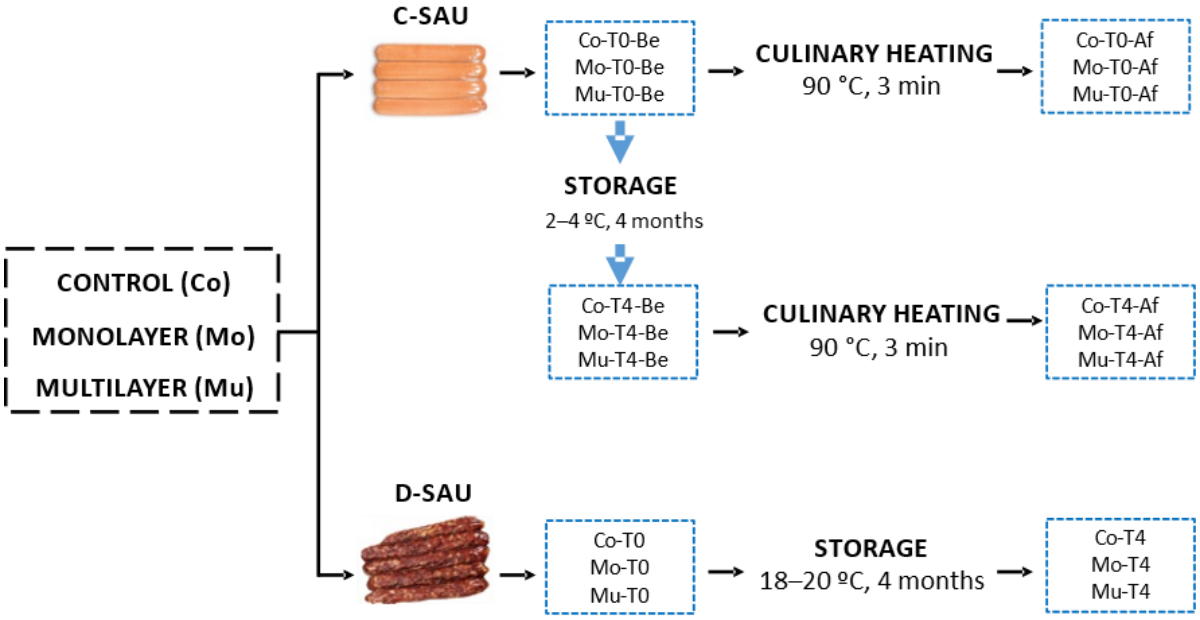

Figure 1. Scheme of the experimental design. C-SAU: cooked sausages; D-SAU: dry-cured sausages; Co: control batch; Mo: batch with added monolayered fish oil microcapsules; Mu: batch with added multilayered fish oil microcapsules; T0: batch sampled before storing; T4: batch sampled after storing for four months; Be: batch sampled before culinary heating; and Af: batch sampled after culinary heating.

2.3. Meat Products

Elaboration of C-SAU and D-SAU batches was carried out in a meat industry (remaining anonymous), as previously described in [35]. Therefore, the exact amounts of ingredients were not reported.

2.4. Analysis of Volatile Compounds

Solid-phase microextraction (SPME) and gas chromatography (GC) with a mass selective detector were applied to carry out the analysis of volatile compounds, according to [36], by using a cross-linked carboxen/polydimethylsiloxane fiber (10 mm long, 100 µm thick, Supelco, Bellefonte, PA, USA) and a Hewlett-Packard 6890 series II GC coupled to a mass selective detector (HP 5973) (Hewlett-Packard, Wilmington, DE, USA) with a 5% phenyl-95% polydimethylsiloxane column (30 m × 0.32 mm ID, 1.05 µm film thickness, Hewlett-Packard). Operating conditions were: 6 psi of column head pressure, 1.3 mL min^{-1} of flow at 40 °C, and a splitless mode. The analyses were carried out at random for two weeks. Compounds were identified by comparison of the mass spectrum with a database (National Institute of Standards and Technology (NIST) and Wiley libraries) and with linear retention (LRI) indexes available in the literature [19,21,37–43]. For calculating the LRI of the samples of the present work, n-alkanes (Sigma R-8769, Barcelona, Spain) were run under the same conditions. Results are expressed in area units (AU).

2.5. Statistical Design

Replicate experimental samples (n = 3) of three batches of each meat product (C-SAU and D-SAU) were analyzed in triplicate. One-way analyses of variance (ANOVA) and the Tukey's test (if $p < 0.05$) were applied to evaluate the effects of (i) enrichment, (ii) storage and iii) culinary heating. Data on volatile compounds showing significant differences were analyzed by principal component analysis (PCA). For that, the original data were normalized and the variables were classified in the first two components. The IBM SPSS Statistics v.22 program was used.

3. Results and Discussion

3.1. Profile of Volatile Compounds in D-SAU and C-SAU Batches

The results of D-SAU and C-SAU samples show a total of 53 and 76 volatile compounds, respectively, which have been classified into different chemical families (Figures 2 and 3, respectively), and Tables 1 and 2 show the abundance of individual volatile compounds, respectively.

In D-SAU batches, acids was the most abundant chemical family of volatile compounds (Figure 2), with aldehydes, esters and terpenes being in second place, and aliphatic hydrocarbons, aromatics, alcohols, ketones, furans and cyclic hydrocarbons showed percentages lower than 5%. As for individual volatile compounds (Table 1), acetic acid had the highest abundance, followed in decreasing order by hexanal, methyl hexanoate, β-myrcene, pentanoic acid and butanoic acid, with the rest of the volatile compounds having an abundance lower than 10 AU $\times$ 10^6. This finding agrees with results found by other authors in similar products [29,43–46]. The microbial fermentation of carbohydrates may explain the high content of acids (acetic, pentanoic and butanoic acids) [45,47]. Another characteristic compound of carbohydrate fermentation, 3-hydroxy-2-butanone, was also detected in D-SAU batches [48]. Moreover, the existence of β-myrcene, α-phellandrene and D-limonene was also noticeable and could be ascribed to the addition of spices [49].

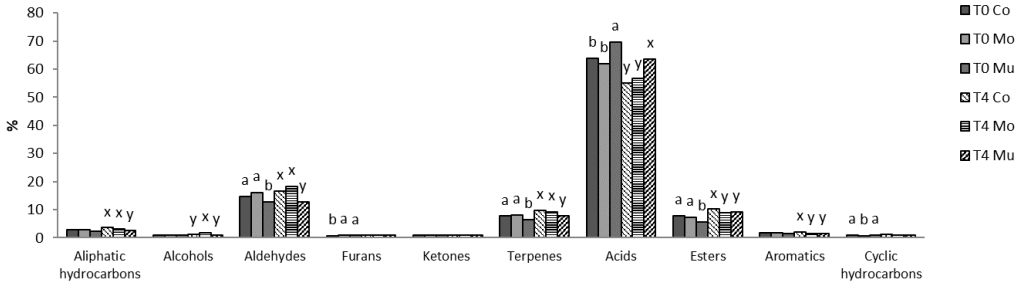

Figure 2. Percentage of families of volatile compounds in dry-cured sausages. Different letters indicate a significant enrichment effect among batches at T0 (a,b) and T4 (x,y). Co: control batch; Mo: batch with added monolayered fish oil microcapsules; Mu: batch with added multilayered fish oil microcapsules; T0: batch sampled before storing; T4: batch sampled after storing for four months.

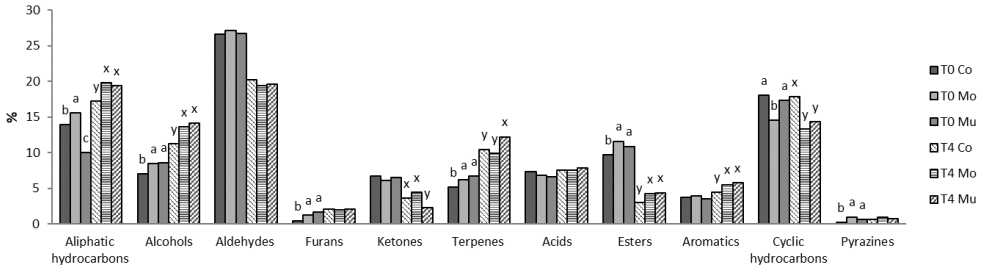

Figure 3. Percentage of families of volatile compounds in cooked sausages. Different letters indicate a significant enrichment effect among batches at T0 (a,b) and T4 (x,y). Co: control batch; Mo: batch with added monolayered fish oil microcapsules; Mu: batch with added multilayered fish oil microcapsules; T0: batch sampled before storing; T4: batch sampled after storing for four months.

Table 1. Storage effect (*p*S) on the abundance of volatile compounds (AU × 10^6) in omega-3-enriched dry-cured sausages (*p*E).

LRI	ID	Chemical Group/Compound	T0			*p*E	T4			*p*E	*pS*	SEM
			Co	Mo	Mu		Co	Mo	Mu			
						Aliphatic hydrocarbons						
497	A	Pentane	0.51	0.42	0.59	NS	1.51	1.47	1.31	NS	***	0.27
599	A	Hexane	0.41	0.36	0.31	NS	0.13	0.55	0.17	NS	NS	0.07
704	A	Heptane	0.42 ^{b}	0.48 ^{b}	0.97 ^{a}	***	0.49	0.45	0.32	NS	NS	0.08
761	A	1-propene	n.d.^{b}	0.54 ^{a}	n.d.^{b}	***	0.29 ^{a}	n.d.^{c}	0.13 ^{b}	*	NS	0.05
800	A	Octane	6.65	6.41	6.12	NS	8.23	6.95	7.91	NS	NS	0.32
901	A	Nonane	0.54	0.42	0.61	NS	0.37	0.58	0.31	NS	NS	0.04
997	A	Decane	1.06	0.96	0.92	NS	1.29	1.39	1.34	NS	***	0.07
1097	A	Undecane	1.92	1.78	1.86	NS	1.68	1.65	1.48	NS	NS	0.10
						Alcohols						
614	A	1-propanol	0.03 ^{b}	0.11 ^{a}	0.10 ^{a}	*	0.12	0.15	0.18	NS	*	0.02
687	A	1-penten-3-ol	n.d.^{b}	0.23 ^{a}	n.d.^{b}	***	0.31 ^{c}	8.51 ^{a}	6.47 ^{b}	***	***	0.25
820	A	1-pentanol	1.32	1.61	1.42	NS	3.24 ^{b}	4.53 ^{a}	3.33 ^{b}	***	***	0.06
1024	A	1-heptanol	1.08	1.13	1.10	NS	1.44	1.40	1.22	NS	NS	0.18
1031	A	1-octen-3-ol	2.78 ^{c}	3.73 ^{a}	3.21 ^{b}	NS	2.58	3.97	3.68	NS	NS	0.20
1195	A	4-terpineol	1.85	1.88	1.79	NS	1.68	1.64	1.58	NS	**	0.03
						Aldehydes						
591	A	2-methyl propanal	0.45	0.43	0.38	NS	0.30	0.37	0.29	NS	NS	0.03
618	A	Butanal	0.30 ^{a}	0.09 ^{b}	0.31 ^{a}	**	0.36	0.52	0.20	NS	NS	0.05
667	A	2-methyl butanal	0.15	0.17	0.11	NS	0.25	0.20	0.09	NS	NS	0.03
738	A	Pentanal	7.91 ^{c}	9.66 ^{a}	8.73 ^{b}	*	6.29 ^{c}	15.40 ^{a}	10.36 ^{b}	***	**	0.33
862	A	Hexanal	93.84	96.14	105.06	NS	181.49 ^{c}	265.60 ^{a}	242.11 ^{b}	***	***	6.23
942	A	Heptanal	8.01	7.19	7.25	NS	13.31	12.88	14.29	NS	***	0.62
1050	A	Octanal	0.45	0.57	0.54	NS	0.48	0.44	0.54	NS	NS	0.02
1147	A	Nonanal	5.63	5.24	5.09	NS	12.94 ^{c}	14.93 ^{a}	13.82 ^{b}	*	***	0.13
1322	A	2-decenal	0.96	0.89	1.05	NS	0.89 ^{b}	0.90 ^{b}	1.34 ^{a}	**	*	0.01
1395	A	2,4-decadienal	0.41	0.46	0.44	NS	0.38 ^{b}	0.66 ^{a}	0.42 ^{b}	**	NS	0.04

Table 1. *Cont.*

LRI	ID	Chemical Group/Compound	T0			*p*E	T4			*p*E	*pS*	SEM
			Co	Mo	Mu		Co	Mo	Mu			
						Ketones						
749	A	2,3-pentanedione	0.49	0.44	0.40	NS	0.54	0.32	0.41	NS	NS	0.03
778	A	3-hydroxy-2-butanone	0.56 ^{a}	0.39 ^{a}	0.11 ^{b}	***	0.34	0.29	0.30	NS	NS	0.04
933	A	2-Heptanone	7.00	6.99	6.58	NS	6.06	5.54	5.06	NS	**	0.27
981	A	3-Heptanone	3.51	3.88	4.69	NS	2.50	3.62	3.67	NS	NS	0.46
1039	A	2-Octanone	0.42	0.35	0.29	NS	n.d.	n.d.	n.d.	-	***	0.05
1063	A	3,5-octadien-2-one	n.d.^{b}	0.11 ^{a}	n.d.^{b}	***	n.d.^{b}	3.87 ^{a}	4.21 ^{a}	***	***	0.35
						Furans						
722	A	2-ethyl-furan	n.d.^{b}	0.15 ^{a}	0.09 ^{a}	*	n.d.^{c}	2.84 ^{a}	1.14 ^{b}	***	***	0.44
837	A	3-furaldehyde	0.41	0.29	0.26	NS	0.27	0.29	0.24	NS	NS	0.02
1012	A	2-pentyl-furan	1.93 ^{a}	1.19 ^{b}	1.37 ^{b}	**	1.60 ^{a}	1.31 ^{b}	1.26 ^{b}	*	NS	0.06
						Terpenes						
982	A	Sabinene	8.17	7.89	7.98	NS	7.71	7.49	7.56	NS	NS	0.11
1003	B	β-myrcene	36.01 ^{b}	36.62 ^{b}	46.78 ^{a}	*	38.84	40.05	41.90	NS	NS	0.75
1021	A	α-phellandrene	5.93	6.20	6.78	NS	8.72	8.83	8.17	NS	**	0.12
1037	A	D-Limonene	3.65	3.31	3.72	NS	1.67	1.76	1.22	NS	***	0.26
1066	A	γ-terpinene	2.41	2.06	2.21	NS	2.85	2.86	2.01	NS	NS	0.10
1105	A	Terpene	1.87	1.87	1.70	NS	1.65	1.51	1.41	NS	NS	0.11
1404	A	α-cubebene	1.43	1.36	1.78	NS	1.29	1.25	1.23	NS	NS	0.09
						Acids						
717	A	Acetic acid	478.69 ^{b}	602.75 ^{a}	452.62 ^{b}	***	374.50 ^{b}	439.17 ^{b}	543.82 ^{a}	***	*	43.89
895	A	Butanoic acid	29.05	27.74	27.48	NS	28.64 ^{b}	23.33 ^{a}	23.61 ^{a}	**	*	1.56
986	A	Pentanoic acid	35.66	36.94	36.54	NS	36.34	36.05	35.90	NS	NS	0.40
1362	A	Nonanoic acid	2.60	2.43	2.61	NS	2.42	2.42	2.63	NS	NS	0.08
1472	A	Decanoid acid	2.87	2.78	2.61	NS	2.91	3.20	3.79	NS	NS	0.19
						Esters						
786	A	Methylpropyl acetate	0.32	0.29	0.25	NS	0.19	0.19	0.13	NS	*	0.02
853	A	Methyl hexanoate	63.15	56.04	57.08	NS	63.64	63.33	73.61	NS	NS	2.76
						Aromatics						
1018	A	Benzaldehyde	9.30	8.29	7.64	NS	6.05	5.81	6.14	NS	***	0.19
1190	A	4-methyl-phenol	0.53	0.59	0.61	NS	0.88 ^{a}	0.57 ^{b}	0.52 ^{b}	**	NS	0.02
1375	A	Eugenol	4.18	4.16	4.34	NS	4.40	4.42	3.97	NS	NS	0.09

Table 1. *Cont.*

LRI	ID	Chemical Group/Compound	T0			*p*E	T4			*p*E	*pS*	SEM
			Co	Mo	Mu		Co	Mo	Mu			
						Cyclic hydrocarbons						
992	B	α-thujene	6.22	5.87	6.01	NS	6.34	6.59	6.37	NS	*	0.10
1495	A	Humulene	0.72	0.75	0.84	NS	0.35	0.26	0.27	NS	*	0.04
						Other						
899	B	Allyl sulphide	2.51 [a]	2.89 [a]	1.94 [b]	**	1.59	1.43	1.49	NS		0.14

[a–c] Different letters indicate a significant enrichment effect among batches. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS: no significant; n.d., not detected; SEM, standard error; LRI, linear retention index of the compounds eluted from the GC-MS; ID, method of identification; A, mass spectrum and retention time identical with an authentic standard; and B, tentative identification by mass spectrum. Co: control batch; Mo: batch with added monolayered fish oil microcapsules; Mu: batch with added multilayered fish oil microcapsules; T0: batch sampled before storing; T4: batch sampled after storing for four months.

Table 2. Storage (pS) and culinary heating (pC) effects on the abundance of volatile compounds (AU × 10^6) in omega-3-enriched cooked sausages (pE).

LRI	ID	Compound	T0						*p*E	*p*C	T4						*p*E	*p*C	*p*S	SEM
			Co		Mo		Mu				Co		Mo		Mu					
			Be	Af	Be	Af	Be	Af			Be	Af	Be	Af	Be	Af				
										Aliphatic hydrocarbons										
499	A	Pentane	0.53	0.47	0.44	0.45	0.39	0.43	NS	NS	0.88	0.39	0.86	0.56	0.63	0.61	NS	*	***	0.04
601	A	Hexane	0.23	0.43	2.12	0.46	0.84	0.79	*	*	0.12	0.74	0.25	0.85	1.25	0.58	*	***	NS	0.09
695	A	1-Heptene	0.80	n.d.	1.05	n.d.	0.74	n.d.	*	***	0.85	n.d.	1.01	n.d.	1.10	n.d.	NS	***	NS	0.08
703	A	Heptane	3.35	1.55	1.54	1.13	0.75	2.08	*	NS	3.67	1.35	0.76	2.37	0.82	2.24	*	NS	NS	0.17
799	A	Octane	1.27	2.75	2.47	2.68	1.51	2.50	NS	*	1.92	2.41	2.57	2.15	2.36	1.65	NS	NS	NS	0.11
812	A	2-octene	n.d.	2.08	n.d.	2.13	n.d.	1.59	NS	***	n.d.	1.76	n.d.	1.80	n.d.	1.62	NS	***	NS	0.16
901	A	Nonane	0.23	1.91	0.46	2.15	0.32	1.23	***	***	n.d.	2.36	0.38	1.27	0.43	0.72	NS	***	NS	0.13
1000	A	Decane	n.d.	n.d.	0.19	n.d.	n.d.	0.55	*	-	2.39	0.26	2.53	0.26	2.56	0.30	NS	***	*	0.17
1101	A	Undecane	0.52	0.71	0.43	0.63	0.57	0.77	NS	NS	0.85	0.68	0.76	0.68	0.81	0.67	NS	*	NS	0.03
1200	A	Dodecane	0.42	0.43	0.27	0.49	n.d.	0.58	**	*	n.d.	0.49	0.35	0.25	0.51	0.32	**	NS	NS	0.03
1296	A	Tridecane	n.d.	n.d.	n.d.	0.31	n.d.	0.54	NS	*	n.d.	n.d.	n.d.	0.24	n.d.	0.30	-	*	NS	0.03
1402	A	Tetradecane	0.58	0.52	0.16	0.53	n.d.	0.68	NS	NS	0.85	0.50	0.19	0.68	n.d.	0.45	NS	*	NS	0.15

Table 2. *Cont.*

LRI	ID	Compound	T0						*p*E	*p*C	T4						*p*E	*p*C	*p*S	SEM
			Co		Mo		Mu				Co		Mo		Mu					
			Be	Af	Be	Af	Be	Af			Be	Af	Be	Af	Be	Af				
Alcohols																				
615	A	1-propanol	0.11	0.14	0.17	0.13	0.21	0.16	**	NS	0.18	0.28	0.69	0.40	2.09	0.42	*	NS	*	0.10
681	A	2-methyl-1-propanol	0.98	n.d.	1.24	n.d.	1.48	n.d.	NS	**	2.92	n.d.	2.68	n.d.	3.25	n.d.	NS	*	NS	0.26
825	A	1- pentanol	1.33	1.65	1.23	1.92	1.22	1.64	**	*	1.45	1.56	1.34	1.69	1.38	1.57	NS	NS	NS	0.04
923	A	1-hexanol	0.22	0.78	0.26	0.88	0.22	1.11	*	***	0.88	0.97	1.37	0.74	1.23	0.88	NS	*	*	0.06
927	A	4-hexen-1-ol	n.d.	0.98	n.d.	1.96	n.d.	1.92	*	***	n.d.	1.42	n.d.	0.98	n.d.	1.49	NS	***	NS	0.12
1024	A	1-heptanol	1.10	0.74	0.78	0.89	0.82	0.89	NS	NS	1.49	1.36	1.04	0.99	1.28	1.50	*	NS	NS	0.06
1031	A	1-octen-3-ol	0.77	3.34	0.81	3.42	0.63	2.76	*	***	2.27	5.60	3.16	5.00	2.93	6.02	*	***	***	0.31
1088	A	2-ethyl-1-hexanol	0.71	0.40	0.74	0.46	0.77	0.89	NS	*	0.60	0.56	0.82	0.89	1.06	1.30	*	NS	NS	0.14
1092	A	Phenyl ethyl alcohol	n.d.	n.d.	0.25	0.41	0.24	0.96	**	**	n.d.	n.d.	0.24	0.36	0.32	0.94	**	*	NS	0.06
Aldehydes																				
521	A	Propanal	0.16	n.d.	0.17	n.d.	0.14	n.d.	***	**	0.52	0.78	0.64	1.26	0.49	0.94	-	*	***	0.09
593	A	2-methylpropanal	1.30	1.35	1.14	1.12	1.16	1.54	NS	*	2.16	1.75	2.37	1.36	1.93	1.09	*	*	***	0.08
621	A	Butanal	0.47	n.d.	0.75	n.d.	0.38	n.d.	NS	*	1.05	n.d.	1.76	n.d.	1.59	n.d.	NS	***	*	0.11
667	A	2-methyl butanal	n.d.	0.62	n.d.	0.43	n.d.	0.54	NS	***	n.d.	0.82	n.d.	0.81	n.d.	0.76	NS	***	NS	0.07
687	A	3-methyl butanal	1.98	2.01	2.25	2.10	2.69	2.21	NS	NS	7.49	7.59	7.72	8.53	8.50	7.61	NS	NS	**	0.53
738	A	Pentanal	5.86	6.00	5.89	4.66	4.44	3.34	*	NS	5.69	6.50	6.64	6.95	6.65	7.48	*	*	*	0.61
862	A	Hexanal	4.14	4.33	4.60	3.40	4.54	3.54	NS	NS	3.29	5.57	5.12	7.56	3.86	4.92	*	*	NS	0.16
904	A	2-hexenal	0.31	n.d.	0.44	n.d.	0.26	n.d.	-	***	0.20	n.d.	0.22	n.d.	0.54	0.56	*	*	NS	0.03
939	A	Heptanal	3.18	3.50	2.26	2.77	2.69	3.13	***	*	3.98	4.33	5.49	6.26	5.24	6.84	***	***	***	0.22
1047	A	Octanal	n.d.	0.38	n.d.	0.33	n.d.	0.43	NS	***	n.d.	0.66	n.d.	0.31	n.d.	0.40	*	**	NS	0.04
1011	A	2-heptenal	0.10	n.d.	0.41	n.d.	0.11	n.d.	-	***	0.23	n.d.	0.22	n.d.	0.26	n.d.	NS	***	-	0.02
1114	A	2-octenal	n.d.	0.09	n.d.	0.12	n.d.	0.18	-	***	0.57	0.44	0.43	0.76	0.61	0.85	*	NS	***	0.05
1147	A	Nonanal	0.35	0.50	0.67	0.55	0.35	0.87	NS	NS	0.73	0.50	1.04	0.68	0.99	0.45	NS	*	NS	0.25
1223	A	2-nonenal	n.d.	n.d.	0.08	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	0.09	n.d.	-	-	-	0.01
1286	A	2,4-nonadienal	n.d.	n.d.	0.04	n.d.	0.04	n.d.	-	-	n.d.	n.d.	0.04	n.d.	0.05	n.d.	-	-	-	n.d.
1328	A	2-decenal	0.43	0.79	0.40	1.79	0.40	1.09	*	*	0.66	1.07	1.92	3.51	0.75	1.60	*	***	*	0.09
1390	A	2,4-decadienal	n.d.	0.29	0.12	0.47	n.d.	0.37	NS	*	0.42	0.79	2.14	2.87	1.21	1.37	NS	NS	***	0.04

Table 2. *Cont.*

LRI	ID	Compound	T0						*p*E	*p*C	T4						*p*E	*p*C	*p*S	SEM
			Co		Mo		Mu				Co		Mo		Mu					
			Be	Af	Be	Af	Be	Af			Be	Af	Be	Af	Be	Af				
Ketones																				
735	A	2-pentanone	0.88	n.d.	0.78	n.d.	0.72	n.d.	-	***	0.89	n.d.	0.58	n.d.	0.64	n.d.	*	***	NS	0.07
744	A	2,3-pentanedione	n.d.	0.34	n.d.	0.47	n.d.	0.40	***	***	n.d.	0.36	n.d.	0.54	n.d.	0.43	-	***	NS	0.04
933	A	2-heptanone	1.86	0.84	1.62	0.55	1.22	0.38	*	**	1.03	1.18	0.40	0.19	0.33	n.d.	*	NS	*	0.10
979	A	3-heptanone	3.91	2.27	3.42	2.39	3.36	1.09	*	***	4.24	1.32	4.44	1.39	4.20	3.25	NS	*	*	0.23
1039	A	2-octanone	0.19	0.89	n.d.	1.35	n.d.	0.20	NS	***	0.27	0.29	0.31	0.30	0.63	0.33	***	NS	NS	0.09
1342	A	2-undecanone	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	0.05	n.d.	n.d.	n.d.	0.07	n.d.	-	-	-	n.d.
Furans																				
720	A	2-ethylfuran	n.d.	0.38	n.d.	0.26	n.d.	0.21	NS	***	0.82	0.60	1.29	1.65	0.92	1.16	*	NS	***	0.08
841	A	3-furaldehyde	0.49	n.d.	0.53	n.d.	0.45	n.d.	NS	*	0.41	n.d.	0.43	n.d.	0.35	n.d.	NS	*	NS	0.04
908	A	2-butylfuran	n.d.	0.35	0.39	0.41	0.32	0.13	*	NS	0.22	0.12	0.27	n.d.	0.21	n.d.	NS	-	NS	0.03
1008	A	2-pentyl-furan	0.29	0.70	0.49	0.50	0.71	0.40	NS	NS	0.52	0.64	0.54	0.28	0.27	0.30	NS	NS	NS	0.03
Terpenes																				
1026	A	3-carene	n.d.	1.50	n.d.	1.35	n.d.	1.47	NS	***	n.d.	1.47	n.d.	1.23	n.d.	1.31	NS	***	NS	0.12
1037	A	D-limonene	0.25	2.89	0.43	2.67	0.65	2.59	NS	***	1.51	1.86	1.55	1.38	1.70	1.25	NS	NS	NS	0.13
1066	A	γ-terpinene	1.97	3.33	2.30	3.53	2.04	3.44	NS	*	2.23	3.80	2.48	2.24	1.21	2.32	*	*	NS	0.16
1097	A	Terpene	0.26	0.28	0.19	0.17	0.17	0.37	NS	NS	0.28	0.66	0.16	0.45	0.15	0.43	NS	**	*	0.08
1136	A	Terpinolene	0.70	n.d.	0.67	n.d.	0.62	n.d.	NS	**	0.39	n.d.	0.58	n.d.	0.28	n.d.	*	*	*	0.05
1116	A	β-terpinene	n.d.	n.d.	0.31	n.d.	0.40	n.d.	NS	***	n.d.	n.d.	n.d.	n.d.	0.61	n.d.	-	-	NS	0.09
1195	A	4-terpineol	n.d.	n.d.	0.32	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	0.11	n.d.	n.d.	n.d.	-	-	-	0.02
1491	A	Isocayophillene	0.34	n.d.	0.26	n.d.	0.29	0.46	NS	*	n.d.	n.d.	n.d.	0.50	n.d.	n.d.	-	-	-	0.03
Acids																				
716	A	Acetic acid	n.d.	n.d.	n.d.	0.04	0.44	0.14	NS	-	n.d.	0.34	0.15	0.37	0.46	0.64	***	NS	NS	0.04
895	A	Butanoic acid	3.36	2.50	2.44	2.41	2.01	2.10	**	NS	3.43	2.14	3.47	2.90	5.19	2.98	*	**	*	0.15
898	A	2-butenoic acid	n.d.	0.27	n.d.	0.33	n.d.	0.51	-	***	n.d.	0.26	n.d.	0.17	n.d.	0.40	NS	*	NS	0.03
986	A	Pentanoic acid	1.14	1.99	0.73	1.09	0.63	0.72	***	**	0.45	1.27	0.65	1.05	0.57	0.69	NS	NS	NS	0.08
1273	A	Octanoic acid	n.d.	0.45	n.d.	0.35	n.d.	n.d.	NS	NS	n.d.	0.49	n.d.	0.42	n.d.	0.14	NS	*	NS	0.13
1366	A	Nonanoic acid	0.43	0.49	0.54	0.46	0.39	0.67	NS	NS	0.61	0.74	0.32	0.41	0.68	0.39	**	NS	NS	0.04
1461	A	Decanoid acid	0.33	0.44	0.31	0.37	0.66	0.23	*	NS	0.62	0.83	0.85	1.07	0.74	2.72	NS	*	*	0.18

Table 2. *Cont.*

LRI	ID	Compound	T0						*p*E	*p*C	T4						*p*E	*p*C	*p*S	SEM
			Co		Mo		Mu				Co		Mo		Mu					
			Be	Af	Be	Af	Be	Af			Be	Af	Be	Af	Be	Af				
Esters																				
656	A	Methyl propanoate	n.d.	0.07	n.d.	0.26	n.d.	0.37	***	***	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	0.02
750	A	Methyl butanoate	1.27	n.d.	1.04	n.d.	1.02	n.d.	NS	***	0.99	n.d.	1.08	n.d.	1.06	n.d.	NS	***	NS	0.20
836	A	Ethyl butanoate	2.31	2.34	2.56	2.49	2.81	2.62	**	NS	3.18	1.96	3.44	1.49	3.50	1.43	NS	***	NS	0.11
952	A	Methyl hexanoate	3.39	n.d.	3.95	n.d.	3.37	n.d.	NS	***	3.44	n.d.	3.76	n.d.	4.61	n.d.	*	***	NS	0.33
Aromatics																				
1018	A	Benzaldehyde	2.98	1.76	2.01	2.21	1.50	1.85	*	*	4.36	4.47	4.18	3.07	3.06	3.43	*	NS	***	0.19
1190	A	4-methyl-phenol	0.40	1.33	0.41	0.93	0.46	0.81	NS	*	0.24	0.23	0.38	0.36	0.36	0.50	NS	NS	*	0.18
1305	B	Safrole	0.05	0.45	0.04	0.51	0.03	0.40	-	***	0.05	0.55	0.03	0.31	0.04	0.21	*	*	NS	0.03
Cyclic hydrocarbons																				
980	B	β-thujene	5.20	4.56	3.90	2.99	4.90	3.67	*	**	4.90	4.79	4.79	2.20	5.30	2.75	*	***	NS	0.41
991	B	α-thujene	7.28	8.05	5.51	5.23	5.51	5.33	**	NS	8.26	7.18	6.13	5.17	6.45	5.91	**	*	NS	0.22
1422	B	cis-muurola-4(14),5-diene	n.d.	0.20	n.d.	0.19	n.d.	n.d.	-	-	n.d.	0.26	n.d.	n.d.	n.d.	0.24	-	.	-	0.02
1524	A	δ-cadinene	0.47	0.43	0.63	0.41	0.36	0.65	NS	NS	0.38	0.57	0.83	0.26	0.58	0.34	NS	NS	NS	0.04
Pyrazines																				
863	A	2-methylpyrazine	0.45	0.26	0.30	0.38	0.37	0.28	NS	NS	0.26	0.34	0.20	0.14	0.29	0.20	NS	NS	NS	0.11
947	A	2,6-dimethylpyrazine	n.d.	0.22	0.38	0.19	n.d.	0.24	NS	NS	n.d.	0.44	n.d.	0.41	n.d.	0.55	NS	*	NS	0.05

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS: no significant; n.d., not detected; SEM, standard error; LRI, linear retention index of the compounds eluted from the GC-MS; ID, method of identification; A, mass spectrum and retention time identical with an authentic standard; and B, tentative identification by mass spectrum. Co: control batch; Mo: batch with added monolayered fish oil microcapsules; Mu: batch with added multilayered fish oil microcapsules; T0: batch sampled before storing; T4: batch sampled after storing for four months; Be: batch sampled before culinary heating; and Af: batch sampled after culinary heating.

In C-SAU (Figure 3), the most abundant family of volatile compounds was aldehydes, followed by cyclic and aliphatic hydrocarbons. Lower percentages were found for alcohols, esters, acids, ketones, terpenes, aromatics, furans and pyrazines, in decreasing order. This is similar to the profile previously found in other studies of cooked sausages [40,50]. α-thujene was the most abundant volatile compound in C-SAU, followed by pentanal, β-thujene and hexanal (Table 2). Other authors have found the highest abundance in hexanal, followed by heptanal, pentanal, benzaldehyde, nonanal alcohols (1-octen-3-ol, 1-pentanol, 2-ethyl-1-hexanol and 1-heptanol) and terpenes (limonene and β-myrcene and γ-terpinene) [50–52], as observed in the present work. Nevertheless, the abundance of α and β-thujene in this study was higher in comparison to previous studies. Since α and β-thujene are characteristic of medicinal herbs, essential oils, flavorings and spices, such as nutmeg [53,54], their high abundance in the samples of the present study may be ascribed to the addition of spices to C-SAU batches.

The profile of volatile compounds in D-SAU and C-SAU remained largely the same in control and enriched batches. Nevertheless, some statistical differences were detected in the percentages of chemical families (Figures 2 and 3, respectively) and in the abundance of individual volatile compounds (Tables 1 and 2, respectively). The most marked effects are described as follows. Overall, in D-SAU, lower percentages of aldehydes, terpens, esters and aliphatic hydrocarbons, and higher percentages of acids were found in D-SAU-Mu than in the other two batches; and both enriched batches showed higher percentages of furans and lower percentages of esters and aromatics than D-SAU-Co. In C-SAU, most chemical families of volatile compounds showed higher percentages in the enriched batches than in C-SAU-Co. Despite these differences, the addition of Mo and Mu fish oil microcapsules only influenced significantly the abundance of 13 and 14 of the 53 volatile compounds identified in D-SAU at T0 and T4, respectively, and of 27 and 30 of the 76 volatile compounds identified in C-SAU at T0 and T4, respectively. In general, Mo-added meat products had the highest abundance of volatile compounds from fatty acid oxidation, such as pentanal, hexanal and 1-pentanol [39], and of characteristic markers of ω-3 PUFA oxidation, such as 2-ethylfuran, 2,4-decadienal and 2-decenal [54]. Other foods (mayonnaise and nuggets) added fish oil have also shown these volatile compounds [54,55], which are related to rancid flavor and ω-3 PUFA oxidation. This effect can be ascribed to the type of fish oil microcapsule with different wall materials, maltodextrin in Mo and chitosan–maltodextrin in Mu, with the multilayer structure of chitosan–maltodextrin giving a higher protection than the simple coating of maltodextrin of Mo.

In the study of Yang et al. [20], who carried out a chemometric analysis to characterize volatile compounds from oxidized ω-3 PUFA rich oils, three groups of volatile compounds were differentiated depending on their relationship with the degree of oxidation of these oils: not related (3-hexenal, hexanal, 1,4-octadien-1-ol and 2,6-nonadienal), an intermediate relationship (ethylbenzene, 1,2-dimethylbenzene, cyclohexanone, 1,3-dimethylbenzene, 1-ethyl-4-methyl benzene, 2-penylfuran, decane and undecane) and high-quality markers of the oxidation of ω-3 PUFA rich oils (2,4-heptadienal and 2-propenal). In fact, these authors made a point to identify 2,4-heptadienal as the most sensitive marker volatile compound of ω-3 PUFA rich oils oxidation. In the samples of this study, 2,4-heptadienal was not found, which may disprove a powerful ω-3 PUFA oxidation, even when Mo was added to the meat products. However, other specific oxidation markers associated with rancid flavors in fish oil such as 2,4-decadienal [19] were found in the present study in both the C-SAU and D-SAU enriched batches

In the work of Resconi et al. [56], with raw meat stored under high oxygen conditions, eight volatile compounds were proposed as shelf-life markers (pentanoic, hexanoic and heptanoic acids, 1-hexanol, 2-undecenal, ethyl octanoate, 2-heptanone and 2-pentyl furan). In the samples of the present study, some of these markers (decane, undecane, pentanoic acid, 1-hexanol, 2-heptanone, 2-penylfuran) were found, with enriched samples showing a significantly lower abundance of three of them (pentanoic acid and 2-heptanone in C-SAU and 2-pentylfuran in D-SAU) in comparison to the control batches. The reason behind this

finding may be the use of maltodextrin as a wall material in the fish oil microcapsules, since a high antioxidant capacity of this carrier agent was proved previously in gelatin powders of golden goatfish [57]. This fact is worth noting in the case of the 2-pentylfuran, because furan and its derivatives, apart from providing pleasant characteristics, are associated with potential harmful effects [58,59]

3.2. Storage Effect on the Profile of Volatile Compounds of D-SAU and C-SAU Enriched with Fish Oil Microcapsules

In D-SAU, the storage for 4 months at ambient temperatures did result in significant differences in any family of volatile compounds (Figure 2). However, the analysis of individual volatile compounds showed significant differences in 23 compounds (Table 1), finding a similar trend in Co, Mo and Mu batches. The abundance of pentane, decane, 1-propanol, 1-penten-3-ol, 1-pentanol, 4-terpineol, pentanal, hexanal, heptanal, nonanal, 2-decenal and 3,5-octadien-2-one increased from T0 to T4, and 4-terpineol, 2-heptanone, 2-octanone, phellandrene, D-limonene, acetic acid, butanoic acid, methylpropil acetate, benzaldehyde, humulene and ally sulphide decreased from T0 to T4. A similar pattern of changes was previously observed during the refrigeration storage of ripened sausages [29]. Most of the volatile compounds with increased abundance from T0 to T4 were lipid oxidation products, which agrees with the increases in the lipid oxidation values from T0 to T4 previously observed in the same samples [60]. The decrease in the abundance of acetic and butanoic acids, which are generated from the microbial fermentation of carbohydrates, could be explained by the gradual reduction in the homofermentative activity of staphylococci and lactic acid bacteria [61]. In fact, this finding has been previously observed in similar studies of dry-cured sausages [29,46]. Moreover, the decreases in volatile compounds generated from spices, belonging to the terpene (α-phellandrene and d-limonene) and cyclic hydrocarbons families (humulene) agree with previous studies on ripened sausages stored at refrigeration temperatures [29,62].

Figure 4a represents a bi-plot of the PCA of the volatile compounds data from D-SAU samples as affected by enrichment and storage. The first principal component (PC1) comprised 42.11% of the total variance, and the second principal component (PC2) accounted for 20.73%. The score plot indicates a clear classification of samples as a function of the enrichment and the storage: those with high negative PC1 (D-SAU-Co-T0, in the axis; D-SAU-Mo-T0, far from the axis and D-SAU-Mu-T0, very far from the axis), those with high positive PC2 (D-SAU-Co-T4), those with high positive PC1 (D-SAU-Mu-T4) and those with low positive PC2 (D-SAU-Mo-T4). As for the loading plot, 2,4-decadienal, hexanal, 1-penten-3-ol and 2-ethylfuran are located in the in the upper right quadrant, which corresponds to low positive charges in PC2, and near to D-SAU-Mo-T4. These volatile compounds come from fish oil oxidation and have, in general, low threshold odors [63,64], which may negatively influence the odor and flavor of the samples. On the other hand, pentanal and allyl-sulphyde are located in the low left quadrant, which corresponds to high negative charges in PC1, associated with D-SAU-Co-T0, D-SAU-Mo-T0 and D-SAU-Mu-T0. This is quite in agreement with differences found in individual volatile compounds (Table 2) and is consistent with the changes during storage. The association of PUFA oxidation markers with D-SAU-Mo-T4 reinforces our previous hypothesis, pointing out the more protective effect against the reactivity of fish oil of the wall of Mu (chitosan and maltodextrin) than of Mo (maltodextrin). Despite these results on the profile of volatile compounds, no marked differences were found in the sensory quality among D-SAU batches as affected by refrigeration storage [65].

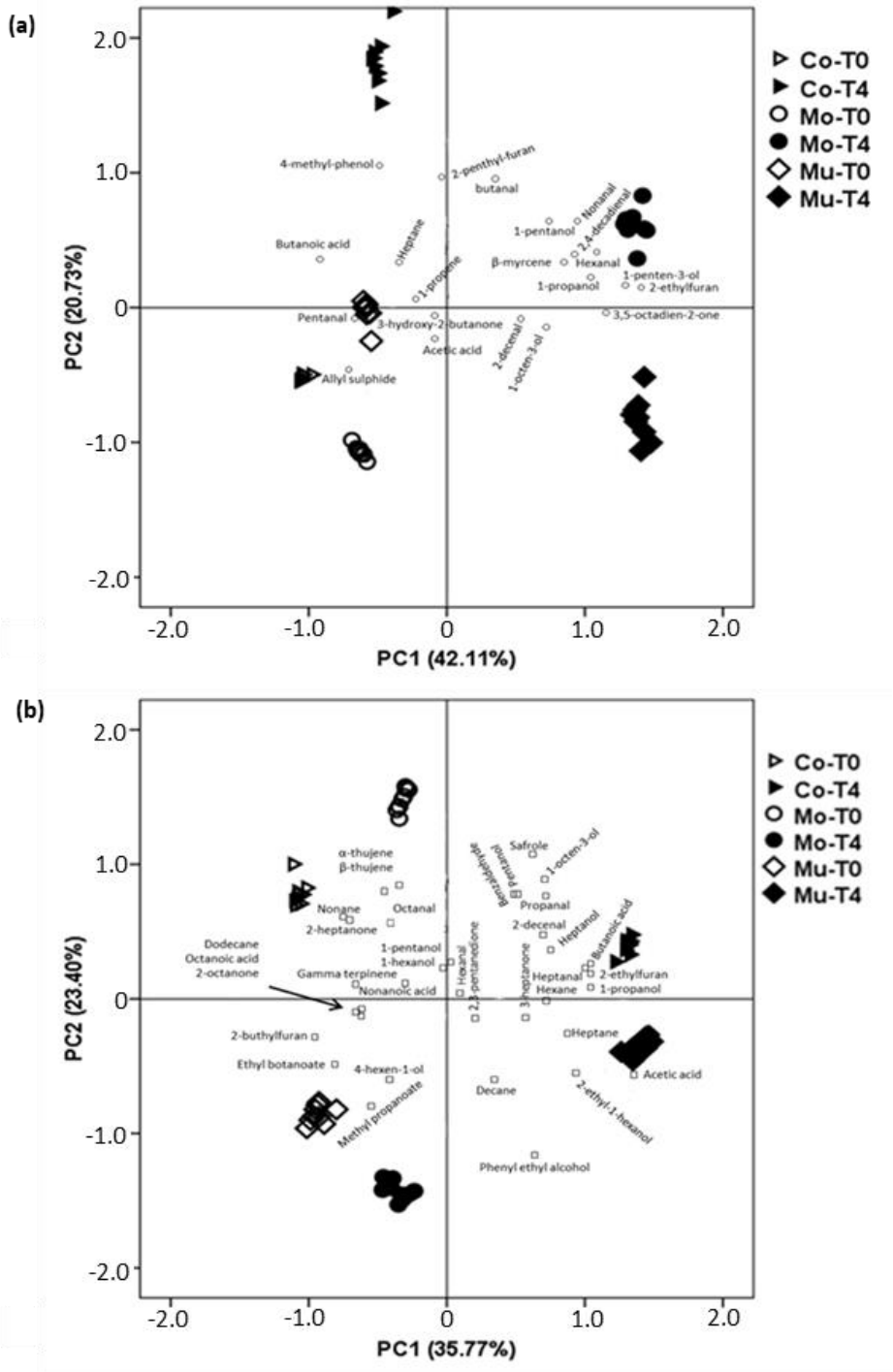

Figure 4. Bi-plot of principal component analysis (PCA) of volatile compounds in dry-cured (**a**) and cooked (**b**) sausages. Co: control batch; Mo: batch with added monolayered fish oil microcapsules; Mu: batch with added multilayered fish oil microcapsules; T0: batch sampled before storing; T4: batch sampled after storing for four months.

In C-SAU, the storage at refrigeration showed significant differences in six families of volatile compounds (Figure 3), showing higher percentages at T4 of aliphatic hydrocarbons, alcohols and terpenes, and lower percentages of aldehydes, ketones and esters than at T0. The analysis of individual volatile compounds showed significant differences in 23 compounds (Table 2). In Co, Mo and Mu batches, the abundance of most of them increased from T0 to T4: pentane, decane, 1-propanol, 1-hexanol, 1-octen-3-ol, propanal, 2-methylpropanal, butanal, 3-methylbutanal, pentanal, heptanal, 2-octenal, 2-decenal,

2,4-decadienal, 3-heptanone, 2-ethylfuran, terpene, butanoic acid, decanoic acid and benzaldehyde, while 2-heptanone, terpinolene and 4-methyl-phenol decreased from T0 to T4. A similar pattern of changes was previously observed in irradiated cooked pork sausages, vacuum packed and stored for 4 and 8 days at refrigeration temperature [66]; however, the comparison is complicated due to the existence of great differences between meat products, i.e., composition, processing and storage conditions. As in D-SAU, most of the volatile compounds with increased abundance from T0 to T4 were lipid oxidation products [67], which agrees with the increases in the lipid oxidation values from T0 to T4 previously observed in the same samples [60]. However, those volatile compounds previously identified in bulk fish oil [41,68] and related to PUFA oxidation and rancid flavor perceptions, such as 2,4-heptadienal, 3,5-octadien-2-one and 1-octen-3-one, have not been detected in any of the batches analyzed at T0 or T4. This is in concordance with the volatile profiles of Mo and Mu fish oil microcapsules [54]. The storage also significantly increased the abundance of acetic, butanoic and nonanoic acids, which could be explained by an increase in the growth rate of lactic acid bacteria (LAB). These bacteria are psychotrophic, microarophilic and able to resist high concentrations of salt and smoke [69]. In fact, LAB have been identified in previous studies as the main population of microorganisms in cooked vacuum-packed meat emulsions stored at refrigerated temperatures [69,70].

Figure 4b represents a bi-plot of the PCA of the volatile compounds data from the C-SAU samples as affected by enrichment and refrigeration storage. The first principal component (PC1) comprised 35.77% of the total variance, and the second principal component (PC2) accounted for 23.40%. The score plot indicates a clear classification of samples as a function of the enrichment and the storage: those with high negative PC2 (C-SAU-Mu-T0 and C-SAU-Mo-T4), those with high positive PC2 (C-SAU-Co-T0 and C-SAU-Mo-T0), those with high positive PC1 (C-SAU-Mu-T4) and those with low positive PC2 (C-SAU-Co-T4). In this meat product, according to the loading plot, volatile compounds markers of fish oil oxidation, such as propanal, pentanal, hexanal, 2-decenal and 2-ethylfuran, were not specifically associated with any batch. Thus, in contrast to D-SAU, in C-SAU the different wall material in Mo and Mu fish oil microcapsules seems not to have an effect during the refrigeration storage. In fact, a previous study found different effects on meat products of the addition of these types of microcapsules depending on the meat matrix [71].

3.3. Culinary Heating Effect on the Profile of Volatile Compounds of Cooked Sausages Enriched with Fish Oil Microcapsules

In C-SAU, the culinary heating significantly influenced 45 and 47 volatile compounds at T0 and T4, respectively (Table 2), with the Co, Mo and Mu batches experiencing similar changes. In general, culinary heating increased aliphatic hydrocarbons, alcohols, aldehydes, acids and furans, while esters and ketones decreased. These changes are quite in agreement with previous studies [59,72,73], and are associated with the thermal degradation of lipids, the Maillard reactions and the interaction between the products of the Maillard reaction with the lipids' oxidized products [74].

Previous results [60] showed an increase in lipid oxidation values after the culinary cooking, with low values that varied in a narrow range (0.18–0.54 mg MDA/Kg sample). In this respect, it is worth mentioning that the numerical increase in the abundance of the most volatile compounds during culinary heating was quite scarce, even in those markers of PUFA oxidation. This may be ascribed to the short period of time required for the culinary heating of C-SAU samples, 3 min at 90 °C, since time was described as a greater influence than temperature in the development of volatile compounds during cooking [72].

4. Conclusions

The general profile of volatile compounds in cooked and dry-cured sausages remained largely the same in control and ω-3 PUFA enriched batches, but the type of fish oil microcapsule influenced the generation of individual volatile compounds, especially those considered as markers of ω-3 PUFA oxidation. Thus, multilayer microcapsules (Mu) with chitosan–maltodextrin as wall materials reduced the formation of volatile compounds

characteristic of ω-3 fatty acid oxidation to a greater extent than microcapsules with a simple coating of maltodextrin (Mo). This suggests a better oxidative stability of the meat product enriched with multilayer microcapsules of fish oil.

The enrichment of cooked and dry-cured sausages with different fish oil microcapsules does not modify the usual changes in the profile of volatile compounds during culinary cooking in cooked sausages. However, its impact during the refrigeration storage depends on the type of fish microcapsule and on the meat product, with a significant increase in the volatile compound indicators of ω-3 PUFA oxidation in dry-cured sausages with added monolayered fish oil microcapsules.

Therefore, the addition of multilayer fish oil microcapsules may be an applicable option to increase the content of ω-3 PUFA in dry-cured and cooked meat products without modifying the abundance of volatile compounds, including oxidation markers.

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Capítulo 3.6

Lipid digestion products in meat derivatives enriched with fish oil microcapsules

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Lipid digestion products in meat derivatives enriched with fish oil microcapsules

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ABSTRACT

This study analyzes the potential application of monolayered (Mo) and multilayered (Mu) fish oil microcapsules as EPA and DHA vehicles in cooked (C-SAU) and dry-cured (D-SAU) meat products and the bioaccesibility of their fatty acids. The quantities of EPA and DHA in all batches (44–64 mg EPA + DHA/100 g sample) exceeded the level established by the European Union to label a food as "source of ω-3 fatty acids" (40 mg EPA + DHA/100 g product). The highest percentages of released fat were observed in the intestinal phase. The amount of EPA and DHA bioaccesible was higher in C-SAU-Mu and D-SAU-Mu batches (0.35 and 0.33 mg EPA + DHA per gram of sample digested), in contrast to C-SAU-Mo and D-SAU-Mo batches (0.25 and 0.24 mg EPA + DHA per gram of sample digested). Therefore, the types of microcapsule of fish oil do not influence the EPA + DHA enrichment, but it did in their bioaccessibility, being better when using Mu.

1. Introduction

The addition of bioactive compounds, such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), to food has become increasing important in the last years (Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2016; Tocher, Betancor, Sprague, Olsen, & Napier, 2019). This is mainly due to two reasons, the well-known beneficial effects of these fatty acids (Kris-Etherton, Harris, & Appel, 2002; Swanson, Block, & Mousa, 2012; Zhang, Xu, Wang, & Xue, 20019) and the insufficient consumption of fish, seafood or algae to reach the recommended intake EPA plus DHA, which is around 0.25 g per person and day (EFSA, 2016). Besides, the European Union legislation have established the minimum level required of the sum of EPA and DHA to label a food as "source of ω-3 fatty acids" and "high in ω-3 fatty acids": 40 and 80 mg per 100 g and per 100 kcal, respectively (EU, 2010).

Meat and meat products are valuable foods with high quality proteins, vitamins (especially vitamin B6 and B12) and minerals (iron, selenium and zinc) (Santos, Hoz, Cambero, Cabeza, & Ordóñez, 2008). However, these products are sometimes questioned because of their lipid profile, especially in relation to their high to moderate percentage of saturated fatty acids (SFA) and low polyunsaturated FA (PUFA) contents (Nuernberg et al., 2005). On the other hand, the consumption of meat products is around 3–4 times per week (OMS, 2015), which is associated to the growing intake of "ready-to-eat" products.

Consequently, different strategies have been tested in well-accepted meat products to increase the content of EPA and DHA. The inclusion of fish and algae oils, as bulk or emulsified, has been principally reported. However, these approaches have a detrimental influence on some sensory attributes and lipid oxidation stability, even when antioxidants are used in some cases (Bolger, Brunton, & Monahan, 2017; Lee et al., 2006; Valencia, Ansorena, & Astiasarán, 2007). This is due to the undesirable off-flavor and off-odor of fish and algae oils and to the high suceptibiliy of EPA and DHA to oxidation, leading to unhealthy secondary oxidation products (Fetterman & Zdanowicz, 2009; Shahidi & Zhong, 2010).

The addition of fish oil microcapsules to meat products has also been tested, not finding marked negative effects (Trinidad Pérez-Palacios, Ruiz-Carrascal, Solomando, & Antequera, 2019). In fact, the microencapsulation is based on creating a physical barrier between the encapsulated compounds and the environment, reducing the contact and reactivity with water, oxygen, iron and other oxidizing promoters (Miyashita, Uemura, & Hosokawa, 2018; Onwulata, 2013). Different types of fish oil microcapsules have been used to enrich meat products: commercials (Josquin, Linssen, & Houben, 2012; Pelser, Linssen, Legger, & Houben, 2007) and from spray-dried monolayered (Lorenzo,

Abbreviations: Co, control; C-SAU, cooked sausages; DHA, docosahexaenoic acid; D-SAU, dry-cured sausages; EPA, eicosapentaenoic acid; FAMEs, fatty acid methyl esthers; GC, gas chromatography; Mo, monolayered fish oil microcapsules; Mu, multilayered fish oil microcapsules; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids

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Munekata, Pateiro, Campagnol, & Domínguez, 2016) and multilayered emulsions (Aquilani et al., 2018; Jiménez-Martín, Pérez-Palacios, Carrascal, & Rojas, 2016) . All these studies have evaluated the influence of adding fish oil microcapsules on the proximal composition of the enriched products, the lipid oxidation stability, sensory attributes and on the percentage of EPA and DHA. However, the content of these fatty acids expressed in mg per g of sample has not been determined, not being possible to know if the amount of microcapsules added to the food products is enough to label the enriched meat products as "source of omega-3 fatty acids" or "rich in omega-3" according to European regulations (EU, 2010).

The gastrointestinal stability and bioavailability of the bioactive compounds added to the products should be one of the major concerns in the omega-3 enrichment studies. When new vehicles of bioactive lipid compounds are developed, it is necessary to know their behavior during the digestion process (Dimagno, Go, & Summerskill, 1973) as well as to their bioaccessibility. This information will allow to evaluate the fraction of the ingested compound available to be absorbed (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). A bioaccessibility study requires the use of an adequate in vitro digestion model that reliably simulates human digestion. The usual models described in the literature for lipids are generally hydrolytic (Dahan & Hoffman, 2006; Kaukonen, Boyd, Porter, & Charman, 2004), static (Porter et al., 2004; Sek, Porter, Kaukonen, & Charman, 2002) and with distinction of the digestive compartment and enzymes at all stages (Guerra et al., 2012). (Chatterjee & Judeh, 2016) have evaluated microencapsulated and un-encapsulated fish oil under simulated gastrointestinal condition, determining the percentage of released oil and finding a major stability and delivery with the microcapsules. However, there are no published data on the gastrointestinal release of EPA and DHA from microcapsules, neither meat products enriched with fish oil microcapsules

Considering all these aspects, the objective of this work was evaluating the bioaccesibility of the fatty acids, especially EPA and DHA of the different meat products enriched with fish oil microcapsules.

2. Material and methods

2.1. Biological material

Fish oil from cod liver was kindly provided by Biomega Nutrition (Galicia, Spain). Soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, kindly provided Roquette, Lestrem, France), and food-grade glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions. Iberian pork and chicken meat, water, pork fat, salt, dextrose, soy protein, pork plasma, stabilizer (E-450 and E-451), flavors, vegetable fiber, spices, spice extracts, smoke flavor, antioxidant (E-316), preservative (E-250 and E-252) enhancer flavor (E-621) and colorant (E-120) used for the elaboration of cooked and dry-cured products were kindly provided by a factory (remain anonymous).

2.2. Reagents

Sulphuric acid, methanol, sodium metal and hexane 96% (Scharlau) were used for the transesterification of fatty acids. For the simulated digestion, α-amylase from *Aspergillus oryzae* (30U/mg), pepsin from porcine gastric mucose (2500U/mg protein) and pancreatin from porcine pancreas (4 × USP specifications) (Sigma, St Louis, MO, USA), sodium chloride, potassium phosphate and hexane 96% (Scharlau) were used.

2.3. Experimental design

Monolayered and multilayered emulsions of fish oil were spray-dried to obtain their corresponding microcapsules (Mo and Mu, respectively). Two different meat products were elaborated, cooked (C-SAU) and dry-cured sausages (D-SAU), which were added with Mo (C-SAU-Mo and D-SAU-Mo) and Mu microcapsules (C-SAU-Mu and D-SAU-Mu), modifying the formulation of the batter by the addition of 2.75% (w/w) of Mo and 5.26% (w/w) of Mu. A control batch (without enriching) of each meat product was also prepared (C-SAU-Co, D-SAU-Co). Moreover, all batches were analyzed at time 0 (T0) and after 4 months (T4) of storage at refrigeration (0–5 °C). The six meat products were elaborated in triplicate and analyzed by fatty acid composition and digestibility. The analyses were carried out in triplicate.

2.4. Preparation of emulsions and microcapsules

Emulsion and microcapsules of this study were prepared following the methodology of (Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Rojas, 2014) with slight modifications.

Fish oil (20 g) and lecithin (6 g) were mixed with a magnetic stirrer overnight. Then, water was added until a total weight of 200 g and homogenized (20000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion was obtained and then homogenized at high-pressure (SPX, model APV-200a, Silkeborg, Denmark) under the conditions previously optimized, 1200 Ba-3 passes for Mo and 1100–2 passes for Mu (J. C. Solomando, Antequera, Ruiz-Carrascal, & Pérez-Palacios, 2019).

The homogenized primary emulsion was blended with 200 g of water, in the case of Mo, and with 200 g of 1% of chitosan (w/w) in acetic acid 1%, in the case of Mu, by slowly agitation with a magnetic stirrer for 15 min. In both types of emulsions, the final step consists on adding 400 g of maltodextrin solution (120 g maltodextrin + 280 g water) to obtain the feed emulsion.

Feed emulsions (800 g) were dried in a laboratory-scale spray drier equipped with a 0.5-mm nozzle atomizer (Mini spray-dryer B-290, Buchi, Switzerland). The emulsions, maintained at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray drying process. The aspirator rate was adjusted at 80%, feed rate was 1 L/h, inlet temperature was 180 °C, and outlet temperature ranged 85–90 °C. The collected dried powders were stored in containers at 4 °C until being added to the meat products (Jiménez-Martín et al., 2014).

2.5. Elaboration of meat products.

Formulation and manufacture of meat products were made in a meat industry (remain anonymous), following their procedures.

C-SAU were elaborated with meat mechanically separated from chicken, water, pork fat, salt, pork plasma, stabilizer (E-450), aromas, vegetable fiber, spices, spice extracts, smoke flavor, antioxidant (E-316) and preservative (E-250) and, in the case of the enriched batches, the corresponding microcapsules, which were added in the knead phase. All C-SAU batches were pasteurized in a water bath at 85 °C during 30 min, vacuum packed and stored at refrigeration temperature (0–5 °C). All batches were analyzed at time 0 (T0) and after 4 months (T4) of storage at refrigeration (0–5 °C).

D-SAU was elaborated with Iberian pork meat and fat, which were ground through a 6 mm diameter mincing plate. The rest of ingredients: salt, dextrose, soy protein, spices, aromas, stabilizers (E-451 and E-450), antioxidant (E-301), preservatives (E-252 and E-250), enhancer flavor (E-621), coloring (E-120) and the corresponding microcapsules in the case of the enriched batches were added, mixed for 3 min and kept at 4 °C until stuffed. No starter culture was added. The obtained dough was stuffed into collagen casings (40 cm length × 60 mm diameter). The sausages followed a dry-cured process under controlled conditions of 4 °C and 82% of relative humidity for 3 days. Then, the

Table 1
Fatty acid composition (mg FAMEs/g sample) on cooked sausages (C-SAU) as affected by enrichment with fish oil microcapsules (*p*E) and storage (*p*S)*.

Fatty acids		Co	Mo	Mu	*p*E	*p*S		
						Co	Mo	Mu
C14:0	T0	0.72 ± 0.01	0.67 ± 0.09	0.52 ± 0.19	0.081	0.569	0.201	0.056
	T4	0.70 ± 0.07	0.71 ± 0.08	0.70 ± 0.06	0.204			
C16:0	T0	2.49 ± 0.23	2.51 ± 0.17	2.65 ± 0.11	0.065	0.057	0.041	0.035
	T4	2.83 ± 0.18	2.87 ± 0.09	3.21 ± 0.18	0.044			
C16:1n-7	T0	0.67 ± 0.09	0.79 ± 0.03	0.72 ± 0.10	0.081	0.486	0.136	0.569
	T4	0.62 ± 0.12	0.87 ± 0.09	0.79 ± 0.09	0.256			
C17:0	T0	0.39 ± 0.03	0.37 ± 0.05	0.25 ± 0.06	0.209	0.105	0.236	0.436
	T4	0.27 ± 0.8	0.33 ± 0.10	0.21 ± 0.03	0.296			
C17:1n-7	T0	0.20 ± 0.01	0.20 ± 0.02	0.20 ± 0.03	0.130	0.786	0.852	0.866
	T4	0.21 ± 0.02	0.20 ± 0.01	0.20 ± 0.02	0.169			
C18:0	T0	3.94 ± 0.22	3.86 ± 0.19	3.97 ± 0.17	0.471	0.411	0.285	0.058
	T4	4.06 ± 0.26	3.99 ± 0.27	4.21 ± 0.19	0.302			
C18:1n-9	T0	5.87 ± 0.16	6.09 ± 0.17	6.14 ± 0.14	0.089	0.044	0.156	0.039
	T4	5.62 ± 0.10^{b}	5.87 ± 0.23^{a}	5.93 ± 0.13^{a}	0.024			
C18:2n-6	T0	4.92 ± 0.09	5.02 ± 0.16	5.06 ± 0.13	0.103	0.368	0.589	0.758
	T4	4.96 ± 0.05	5.09 ± 0.07	5.03 ± 0.09	0.052			
C18:3n-6	T0	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.682	0.032	0.029	0.044
	T4	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.166			
C18:3n-3	T0	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.147	0.041	0.625	0.758
	T4	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.222			
C20:0	T0	0.16 ± 0.01	0.14 ± 0.04	0.18 ± 0.01	0.179	0.215	0.113	0.352
	T4	0.14 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.331			
C20:1n-9	T0	0.92 ± 0.04	0.88 ± 0.24	0.85 ± 0.06	0.382	0.425	0.154	0.102
	T4	0.90 ± 0.12	0.86 ± 0.28	0.91 ± 0.09	0.101			
C20:2n-6	T0	0.39 ± 0.02	0.33 ± 0.09	0.31 ± 0.02	0.294	0.536	0.125	0.253
	T4	0.40 ± 0.11	0.36 ± 0.04	0.36 ± 0.03	0.421			
C20:3n-6	T0	0.08 ± 0.00	0.08 ± 0.02	0.06 ± 0.00	0.208	< 0.001	0.246	< 0.001
	T4	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.165			
C20:3n-3	T0	0.21 ± 0.00	0.27 ± 0.07	0.21 ± 0.00	0.789	0.136	0.045	0.255
	T4	0.16 ± 0.06	0.17 ± 0.06	0.19 ± 0.03	0.352			
C20:4n-6	T0	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.183	0.456	0.256	0.225
	T4	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.0	0.096			
C20:5n-3	T0	Nd^{b}	0.18 ± 0.01^{a}	0.19 ± 0.01^{a}	< 0.001	–	0.022	0.042
	T4	Nd^{b}	0.14 ± 0.02^{a}	0.15 ± 0.03^{a}	< 0.001			
C24:0	T0	0.06 ± 0.02	0.09 ± 0.04	0.06 ± 0.00	0.244	0.365	0.106	0.126
	T4	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.120			
C22:6n-3	T0	Nd^{c}	0.41 ± 0.02^{b}	0.44 ± 0.02^{a}	< 0.001	–	0.289	0.469
	T4	Nd^{c}	0.37 ± 0.06^{b}	0.43 ± 0.07^{a}	< 0.001			
Σ SFA	T0	7.90 ± 0.42	8.00 ± 0.33	7.89 ± 0.24	0.275	0.098	0.063	0.356
	T4	7.56 ± 0.23	7.55 ± 0.29	7.76 ± 0.31	0.296			
Σ MUFA	T0	7.66 ± 0.29	7.96 ± 0.44	7.91 ± 0.52	0.078	0.059	0.328	0.279
	T4	7.35 ± 0.48^{b}	7.80 ± 0.29^{a}	7.83 ± 0.22^{a}	0.023			
Σ PUFA	T0	5.70 ± 0.51^{b}	6.37 ± 0.94^{a}	6.36 ± 0.78^{a}	0.019	0.371	0.289	0.211
	T4	5.63 ± 0.44^{b}	6.25 ± 0.56^{a}	6.27 ± 0.20^{a}	< 0.001			
n3	T0	0.23 ± 0.09^{b}	0.88 ± 0.21^{a}	0.86 ± 0.14^{a}	< 0.001	0.106	0.067	0.088
	T4	0.17 ± 0.14^{b}	0.70 ± 0.11^{a}	0.79 ± 0.17^{a}	< 0.001			
n6	T0	5.47 ± 0.54	5.52 ± 0.96	5.51 ± 0.66	0.410	0.597	0.492	0.438
	T4	5.46 ± 0.71	5.56 ± 0.49	5.49 ± 0.84	0.346			
n6/n3	T0	23.78 ± 0.33^{a}	6.49 ± 0.27^{b}	6.48 ± 0.09^{b}	< 0.001	< 0.001	< 0.001	0.003
	T4	32.12 ± 0.36^{a}	8.06 ± 0.29^{b}	7.04 ± 0.28^{c}	< 0.001			
SFA/UFA	T0	0.59 ± 0.11	0.56 ± 0.13	0.55 ± 0.06	0.976	0.542	0.393	0.601
	T4	0.58 ± 0.08	0.54 ± 0.08	0.55 ± 0.11	0.685			
MUFA/PUFA	T0	1.34 ± 0.25	1.25 ± 0.62	1.24 ± 0.37	0.749	0.375	0.788	0.690
	T4	1.31 ± 0.45	1.25 ± 0.44	1.25 ± 0.41	0.428			
Σ EPA + DHA	T0	Nd^{c}	0.59 ± 0.06^{b}	0.64 ± 0.09^{a}	< 0.001	–	0.067	0.079
	T4	Nd^{c}	0.51 ± 0.07^{b}	0.58 ± 0.05^{a}	< 0.001			

* Not enriched (Co) and enriched with monolayer (Mo) and multilayered microcapsules (Mu): samples analyzed before and after the refrigeration storage for 4 months (T0 and T4, respectively). Bars with different letters (a, b, c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect. Nd: not detected. Myristic acid (C14:0); palmitic acid (C16:0); palmitoleic acid (C16:1n-7); margaric acid (C17:0); margaroleic acid (C17:1n-7); stearic acid (C18:0); elaidic acid (C18:1n-9); linoleic acid (C18:2n-6); ɣ-Linolenic acid (C18:3n-6); α-Linolenic acid (C18:3n-3); arachidic acid (C20:0); eicosenoic acid (C20:1n-9); eicosadenoic acid (C20:2n-6); dihomo-γ-linolenic acid (C20:3n-6); 5,8,11-eicosatrienoic acid (C20:3n-3); arachidonic acid (C20:4n-6); eicosapentaenoic acid (C20:5n-3); lignoceric acid (C24:0); docosahexaenoic acid (C22:6n-3).

product was 21 days in the drying-curing chamber at 8 °C and 80% of relative humidity, and finally they rested in a cellar at 5 °C and 85% humidity until reaching a percentage of weight loss around 38–40%. D-SAU were analyzed at time 0 (T0) and after 4 months (T4) of storage at room temperature (0–5 °C)

In both products, the quantity of Mo and Mu added was 3 and 5 g per 100 g of dough, respectively. These amounts were calculated to excess the required quantity of EPA + DHA to label a food as "source of ω-3 fatty acids": at least 40 mg of the sum of EPA and DHA per 100 g and per 100 Kcal (EU, 2010).

2.6. Measure of lipid content.

Fat content was determined gravimetrically, following the method

Table 2
Fatty acid composition (mg FAMEs/g sample) on dry-cured sausages (D-SAU) as affected by enrichment with ω-3 PUFA (*p*E) and storage (*p*S).*

Fatty acids		Co	Mo	Mu	*p*E	*p*S		
						Co	Mo	Mu
C14:0	T0	0.40 ± 0.08	0.37 ± 0.9	0.40 ± 0.08	0.816	0.041	0.137	0.169
	T4	0.26 ± 0.06	0.26 ± 0.04	0.31 ± 0.02	0.360			
C16:0	T0	8.33 ± 0.14	8.46 ± 0.17	8.38 ± 0.06	0.371	0.269	0.331	0.369
	T4	8.11 ± 0.17	8.37 ± 0.14	8.29 ± 0.33	0.480			
C16:1n-7	T0	1.12 ± 0.05	1.20 ± 0.16	1.07 ± 0.03	0.220	0.086	0.346	0.038
	T4	1.28 ± 0.10	1.23 ± 0.06	1.24 ± 0.10	0.466			
C17:0	T0	0.26 ± 0.05	0.28 ± 0.04	0.29 ± 0.04	0.563	0.038	0.021	0.030
	T4	0.17 ± 0.02	0.17 ± 0.03	0.16 ± 0.06	0.774			
C17:1n-7	T0	0.13 ± 0.03	0.12 ± 0.05	0.13 ± 0.03	0.152	0.097	0.249	0.101
	T4	0.09 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.883			
C18:0	T0	3.50 ± 0.29	3.71 ± 0.41	3.42 ± 0.19	0.167	0.498	0.204	0.274
	T4	3.49 ± 0.33	3.65 ± 0.26	3.58 ± 0.24	0.276			
C18:1n-9	T0	10.05 ± 0.32^{b}	11.22 ± 0.56^{a}	10.21 ± 0.68^{b}	< 0.001	0.069	0.034	0.019
	T4	9.41 ± 0.45^{c}	10.78 ± 0.22^{b}	11.65 ± 0.28^{a}	< 0.001			
C18:2n-6	T0	8.11 ± 0.75	7.94 ± 1.02	8.22 ± 0.69	0.067	< 0.001	0.031	0.019
	T4	6.55 ± 0.38	6.24 ± 0.29	6.50 ± 0.53	0.081			
C18:3n-6	T0	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.926	0.075	0.136	0.247
	T4	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.104			
C18:3n-3	T0	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.432	0.671	0.111	0.257
	T4	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.090			
C20:0	T0	0.10 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	0.653	0.002	< 0.001	0.051
	T4	0.06 ± 0.01	0.06 ± 0.03	0.11 ± 0.04	0.075			
C20:1n-9	T0	0.66 ± 0.04	0.71 ± 0.03	0.87 ± 0.15	0.236	< 0.001	< 0.001	< 0.001
	T4	0.39 ± 0.06	0.42 ± 0.15	0.40 ± 0.05	0.092			
C20:2n-6	T0	0.25 ± 0.03	0.25 ± 0.06	0.28 ± 0.05	0.874	< 0.001	0.090	0.064
	T4	0.14 ± 0.02	0.23 ± 0.05	0.14 ± 0.08	0.141			
C20:3n-6	T0	0.12 ± 0.01	0.11 ± 0.03	0.11 ± 0.04	0.907	< 0.001	0.233	0.307
	T4	0.06 ± 0.01	0.08 ± 0.02	0.10 ± 0.03	0.186			
C20:3n-3	T0	0.35 ± 0.09	0.32 ± 0.08	0.34 ± 0.14	0.788	< 0.001	< 0.001	0.186
	T4	0.16 ± 0.04	0.17 ± 0.03	0.24 ± 0.09	0.225			
C20:4n-6	T0	0.04 ± 0.02	0.06 ± 0.02	0.08 ± 0.01	0.108	0.136	0.003	0.001
	T4	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.054			
C20:5n-3	T0	Nd^{b}	0.15 ± 0.03^{a}	0.14 ± 0.03^{a}	< 0.001	–	0.057	0.121
	T4	Nd^{b}	0.11 ± 0.01^{a}	0.11 ± 0.02^{a}	< 0.001			
C24:0	T0	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.549	0.169	0.834	0.256
	T4	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.081			
C22:6n-3	T0	Nd^{b}	0.31 ± 0.02^{a}	0.30 ± 0.02^{a}	< 0.001	–	0.213	0.351
	T4	Nd^{b}	0.27 ± 0.01^{a}	0.29 ± 0.01^{a}	< 0.001			
Σ SFA	T0	12.59 ± 0.81	12.81 ± 1.23	12.69 ± 0.45	0.304	0.099	0.132	0.375
	T4	12.15 ± 0.58	12.58 ± 0.35	12.52 ± 1.09	0.080			
Σ MUFA	T0	11.96 ± 0.39	13.25 ± 0.74	12.28 ± 0.21	0.286	0.095	0.048	0.024
	T4	11.37 ± 0.17^{b}	12.71 ± 0.21^{a}	13.58 ± 1.08^{a}	0.031			
Σ PUFA	T0	8.96 ± 0.25	9.01 ± 0.27	9.57 ± 0.24	0.105	0.057	< 0.001	0.033
	T4	7.00 ± 0.33^{c}	7.17 ± 0.09^{b}	7.50 ± 0.18^{a}	0.039			
n3	T0	0.37 ± 0.02^{b}	0.81 ± 0.02^{a}	0.81 ± 0.05^{a}	< 0.001	0.020	0.041	0.029
	T4	0.18 ± 0.01^{b}	0.56 ± 0.10^{a}	0.66 ± 0.03^{a}	< 0.001			
n6	T0	8.59 ± 0.63	8.23 ± 0.42	8.77 ± 0.49	0.087	< 0.001	< 0.001	< 0.001
	T4	6.82 ± 0.17	6.62 ± 0.15	6.84 ± 0.16	0.361			
n6/n3	T0	23.22 ± 1.35^{a}	10.55 ± 1.04^{b}	10.96 ± 0.95^{b}	< 0.001	< 0.001	0.088	0.164
	T4	37.89 ± 2.07^{a}	12.04 ± 0.66^{b}	10.36 ± 1.33^{b}	< 0.001			
SFA/UFA	T0	0.60 ± 0.03	0.58 ± 0.26	0.58 ± 0.12	0.595	0.107	0.253	0.365
	T4	0.66 ± 0.27	0.63 ± 0.14	0.59 ± 0.09	0.468			
MUFA/PUFA	T0	1.33 ± 0.28	1.47 ± 0.08	1.28 ± 0.45	0.180	0.070	0.088	< 0.001
	T4	1.62 ± 0.17	1.77 ± 0.24	1.81 ± 0.13	0.422			
Σ EPA + DHA	T0	Nd^{b}	0.46 ± 0.02^{a}	0.44 ± 0.02^{a}	< 0.001	–	0.044	0.127
	T4	Nd^{b}	0.38 ± 0.08^{a}	0.40 ± 0.04	< 0.001			

* Not enriched (Co) and enriched with monolayer (Mo) and multilayered microcapsules (Mu): samples analyzed before and after the refrigeration storage for 4 months (T0 and T4, respectively). Bars with different letters (a, b, c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect. Nd: not detected. See the caption of the table 1 for the names of the fatty acids.

of (Pérez-Palacios, Ruiz, Martín, Muriel, & Antequera, 2008).

2.7. Analysis of fatty acids

Firstly, the Fatty Acid Methyl Esthers (FAMEs) of fat (10 mg) were prepared by acidic transesterification, as described by (Sandler, Karo, Sandler, & Karo, 1992). In the case the digestion phases, the total amount of extracted fat was esterified, adjusting the volume of the methylation reagents. FAMEs were analyzed by gas chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethylene glycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA, USA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). The GC oven program temperature was as follows: initial temperature of 180 °C that increased at 5 °C/min to 200 °C, being maintained 40 min at this temperature; thereafter, it increased at 5 °C/min to 250 °C, and then kept for an additional 21 min. The injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of

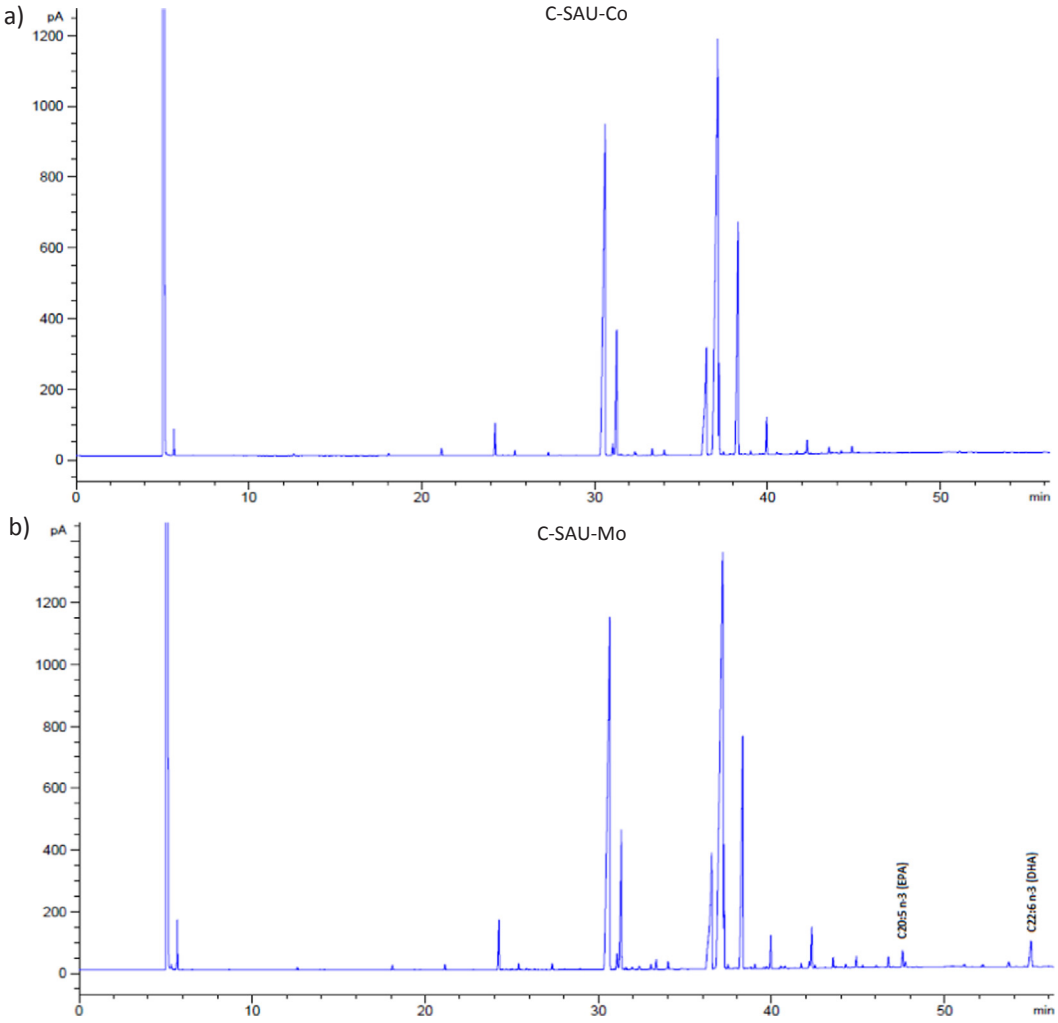

Fig. 1. Chromatograms of fatty acid methyl esters peaks from control (a) and enriched (b) cooked sausages.

0.8 mL/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO, USA). Peak areas were measured and FAMEs were expressed as mg FAMEs per g of sample, by using internal standard (tridecanoic acid, C13) and calibration curves of FAMEs (Supelco 37 component FAME mix, PA, USA).

2.8. Simulated digestion

The release of fatty acids was evaluated under simulated digestion conditions following the methodology of (Wang, Gong, Huang, Yu, & Xue, 2009) and (Werner & Böhm, 2011) with slight modifications. Firstly, the oral, gastric and intestinal solutions were prepared with 20 mg amylase in 1 mL water at pH 6.5, 3.2 g/L pepsin in 2 g/L NaCl at pH 1.5, and 10 g/L pancreatin in 0.05 mol/L KH_2PO_4 at pH 7.4. Then, the sample (2 g) was weighed, mixed with the oral fluid (0.5 mL) by vortex during 1 min, and stirred at 300 rpm during 5 min at 37 °C. The supernatant was separated from the residue by extraction with hexane (5 mL) and centrifugation (4000 rpm, 20 min). The residue was added with the gastric fluid (8 mL) and mixed by vortex. The mixture was incubated at 37 °C with shaking at 300 rpm during 2 h. Again, the supernatant was extracted with hexane, and the residue was incubated with the intestinal fluid (10 mL) at 37 °C with stirring at 300 rpm during 3 h. At each time point of 1, 2 and 3 h, three tubes were taken out to extract the supernatant. All supernatants were extracted in weighted glass tubes. After evaporating the solvent of each digestion stage, the lipid content was calculated gravimetrically and their fatty acid composition was analyzed by GC-FID, as previously described. Results were expressed as mg of FAMEs per gram of sample released at each digestion stage. Besides, the percentage of released fat in relation the initial fat content (before digestion) was also calculated.

2.9. Sampling replication and statistical analysis

The effects of fish oil microcapsules addition and storage on composition and bioaccessibility of fatty acids were analyzed by one-way analysis of variance (ANOVA). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. The statistics were run using the program IBM SPSS Statistics v.22 (IBM Co., New York, USA).

3. Results and discussion

3.1. Lipid composition of dry-cured and cooked sausages as affected by enrichment with fish oil microcapsules and storage

The addition of ω-3 fish oil microcapsules did not affect the percentages of fat in C-SAU and D-SAU, being around 17.59–17.84% and 26.31–26.87%, respectively. These results are expected result since the amount of microcapsules added in the enriched batches is quite small (3 and 5 g per 100 g of meat product in Mo and Mu respectively).

Tables 1 and 2 show the fatty acid composition of C-SAU and D-SAU

batches, respectively, as affected by the addition with fish oil microcapsules and refrigeration storage for four months. In the case of C-SAU, the sum of saturated (SFA) and monounsaturated fatty acids (MUFA) showed similar quantities, which were higher than the sum polyunsaturated fatty acids (PUFA). In these batches, oleic acid (C18:1n-9) showed the highest quantity, followed in decreasing order by, linoleic (C18:2n-6), stearic (C18:0) and palmitic (C16:0) acids, the rest of fatty acids showed concentrations lower than 1 mg FAMEs/g sample. This fatty acid profile reflected the fatty acid composition of the ingredients used to manufacture this product, mainly made with chicken meat and pork fat. Moreover, the fatty acid composition of C-SAU is quite in agreement with that found in other previous studies on similar meat products (Pereira, Tarley, Matsushita, & de Souza, 2000; Yilmaz, Şimşek, & Işikli, 2002).

In relation to the effect of enrichment with fish oil microcapsules, as example, Fig. 1 shows the FAME peak chromatograms from C-SAU-C and C-SAU-Mo bathes, evidencing the existence of EPA and DHA FAME peaks in the enriched batches but not in the control ones. In C-SAU, the addition of Mo and Mu lead to significant differences in some fatty acids (Table 1). As expected, EPA and DHA quantities significantly increased from Co (not detected) to the enriched batches ($p < 0.001$), with no significant differences between Mo and Mu in the case of EPA at T0 (0.18 and 0.19 mg EPA/g sample, respectively) and at T4 (0.14 and 0.15 mg EPA/g sample, respectively). However, the batches enriched with Mu showed higher quantities of DHA at T0 and at T4 (0.44 and 0.43 mg DHA/g sample, respectively) than Mo (0.41 and 0.37 mg DHA/g sample, respectively). This could be explained by the additional layer of chitosan on wall of Mu, which avoids contacts and reactivity with water, oxygen, iron and other oxidant promoters during cooking process and storage, minimizing the oxidation of ω-3 PUFA encapsulated (Jiménez-Martín et al., 2014; Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005). Thus, C-SAU enriched with Mo and Mu exceeded the minimum level established by the European Union legislation to label a food as "source of ω-3 fatty acids" (EU, 2010), with values of 0.59 and 0.64 mg EPA + DHA/g sample, respectively, at T0 and of 0.51 and 0.58 mg EPA + DHA/g sample, respectively, at T4. The addition of Mo and Mu microcapsules also significantly increased the quantities of oleic acid (C18:1n-9) (5.87 and 5.93 mg C18:1n-9/g sample, respectively) in comparison with Co (5.62 mg C18:1n-9/g sample) at T4 ($p = 0.024$), and this tendency was also observed at T0. These differences could be ascribed to the moderate percentage of oleic acid in fish oil (15.81 g/100 g FAMEs) (Jiménez-Martín, Antequera Rojas, Gharsallaoui, Ruiz Carrascal, & Pérez-Palacios, 2016). As consequence of these effects, the n-6/n-3 ratio significantly diminished from Co to Mo and Mu at T0 ($p < 0.001$) of (23.78, 6.49 and 6.48 in Co, Mo and Mu, respectively) and at T4 ($p < 0.001$) (32.12, 8.06 and 7.04 in Co, Mo and Mu, respectively). This effect has also been previously reported by (Aquilani, Pérez-Palacios, Jiménez Martín, et al., 2018) in pork burgers enriched with fish oil microcapsules.

The effect of storage at refrigeration of C-SAU for four months significantly influence on some fatty acids, leading to a slight decrease ($p < 0.05$) from T0 to T4 of oleic acid (C18:1n-9), γ-linolenic acid (C18:3n-6), α-linolenic acid (C18:3n-3), eicosatrienoic acid (C20:3n-3) and EPA with independence of the experimental batches (Co, Mo or Mu). Nevertheless, the sum of SFA, MUFA, PUFA and EPA + DHA was similar at T0 and T4. This may indicate the appropriateness of both of wall materials of both types of microcapsules tested in the present study (maltodextrin in the case of Mo and chitosan-maltodextrin in the case of Mu) to protect the fatty acids of the encapsulated fish oil to oxidation during the refrigeration storage for four months. Besides, this finding is quite in concordance with previous studies in similar dry-cured sausages (Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008; Summo, Caponio, & Pasqualone, 2006), which showed scarce differences in the profile of fatty acid after storage.

Table 2 exposes the fatty acid composition of D-SAU batches. As occurred in C-SAU, SFA and MUFA showed similar quantities and higher than the sum PUFA. Again, oleic acid (C18:1n-9) was the major fatty acid, followed in decreasing order by palmitic (C16:0), linoleic (C18:2n-6), stearic (C18:0) and palmitoleic (C16:1n-7) acids, and the rest of fatty acids showed concentrations lower than 1 mg FAMEs/g sample. This fatty acid profile is in concordance with the fatty acid composition of the ingredients used to manufacture this product, mainly made with Iberian pork meat and fat. Besides, it agrees with other previous studies on similar dry-cured sausages (Bañón, Bedia, Almela, & Martínez, 2010; Navarro, Nadal, Izquierdo, & Flores, 1997). The enrichment effect by the addition of Mo and Mu fish oil microcapsules in D-SAU significantly increased the quantities of EPA and DHA ($p < 0.001$) from Co (not detected) to Mo (0.15 and 0.11 mg EPA/g sample and 0.31 and 0.27 mg DHA/g sample at T0 and T4, respectively) and Mu batches (0.14 and 0.11 mg EPA/g sample and 0.30 and 0.29 mg DHA/g sample at T0 and T4, respectively). No significant differences were found between the enriched batches. Previous studies in pork fermented sausages and pork Spanish salchichon (Lorenzo et al., 2016; Muguerza, Ansorena, & Astiasarán, 2004) have reported the increase in the percentage of EPA and DHA in the batches enriched with bulk and microencapsulated fish oil extract respectively. Nevertheless, these works did not show quantification results, not being possible to compare their results with that obtained in present study. The addition of Mo and Mu microcapsules also significantly increased the quantities of oleic acid (C18:1n-9) ($p = 0.034$ and $p = 0.019$, respectively), being also higher at T0 and at T4 in the enriched batches (11.22 and 10.78 mg/g sample for Mo, respectively and 10.21 and 11.65 mg/g sample for Mu, respectively) in comparison to the control one (10.05 and 9.41 mg/g sample, respectively). As previously explained, these results may be ascribed to the high content of oleic acid in the fish oil. A significant decrease in the n6/n3 ratio has also been observed at T0 ($p < 0.001$) and at T4 ($p < 0.001$) from Co batches (23.22 and 37.89, respectively) to Mo (10.55 and 12.04, respectively) and Mu ones (10.96 and 10.36, respectively). This means a decrease factor around 13 in the n6/n3 ratio, higher than that found in previous studies in Spanish salchichon (Lorenzo et al., 2016), dry-fermented sausages (Muguerza et al., 2004) and Dutch-style fermented sausages (Josquin et al., 2012; Pelser et al., 2007), which showed decreased factors around of 2–6, 8–11, 5–12 and 7–9, respectively. The n6/n3 ratio is considered an important health parameter associated with cardiovascular illness, cancer and inflammatory and autoimmune diseases (Simopoulos, 2002; Wood et al., 2004), and their decreased by the addition of fish oil microencapsulates in D-SAU may be a notable aspect from a healthy point of view.

The effect of the storage at refrigeration for four months on D-SAU significantly decreased the quantities of most fatty acids and of the sum MUFA, PUFA and n-3 in most samples. However, the quantity of EPA and DHA were not influenced by storing, in concordance with previous studies in similar dry-cured sausages (Rubio et al., 2008; Summo et al., 2006). The general maintenance of EPA and DHA quantities in C-SAU and D-SAU batches may point out the protection effect of Mo and Mu during processing, storing and cooking. This supports the findings of a previous study showing low levels of primary and secondary oxidation products in control and enriched C-SAU and D-SAU with Mo and Mu. Besides, similar oxidation values were observed among these batches (Solomando, Antequera, & Perez-Palacios, 2020).

3.2. Fat and fatty acids released through in vitro digestion of cooked and dry-cured sausages as affected by enrichment with fish oil microcapsules

Regarding to the in vitro digestion assay on meat products, Fig. 2.a exposes the percentages of released fat in C-SAU and D-SAU. In general, in both meat products, the highest percentages of released fat were found in the intestinal phase (around 34 and 71%, respectively) followed in decreasing order by oral (around 8 and 19%, respectively) and gastric phases (around 3 and 7%, respectively). These results are in concordance with previous fat digestion studies, which have shown

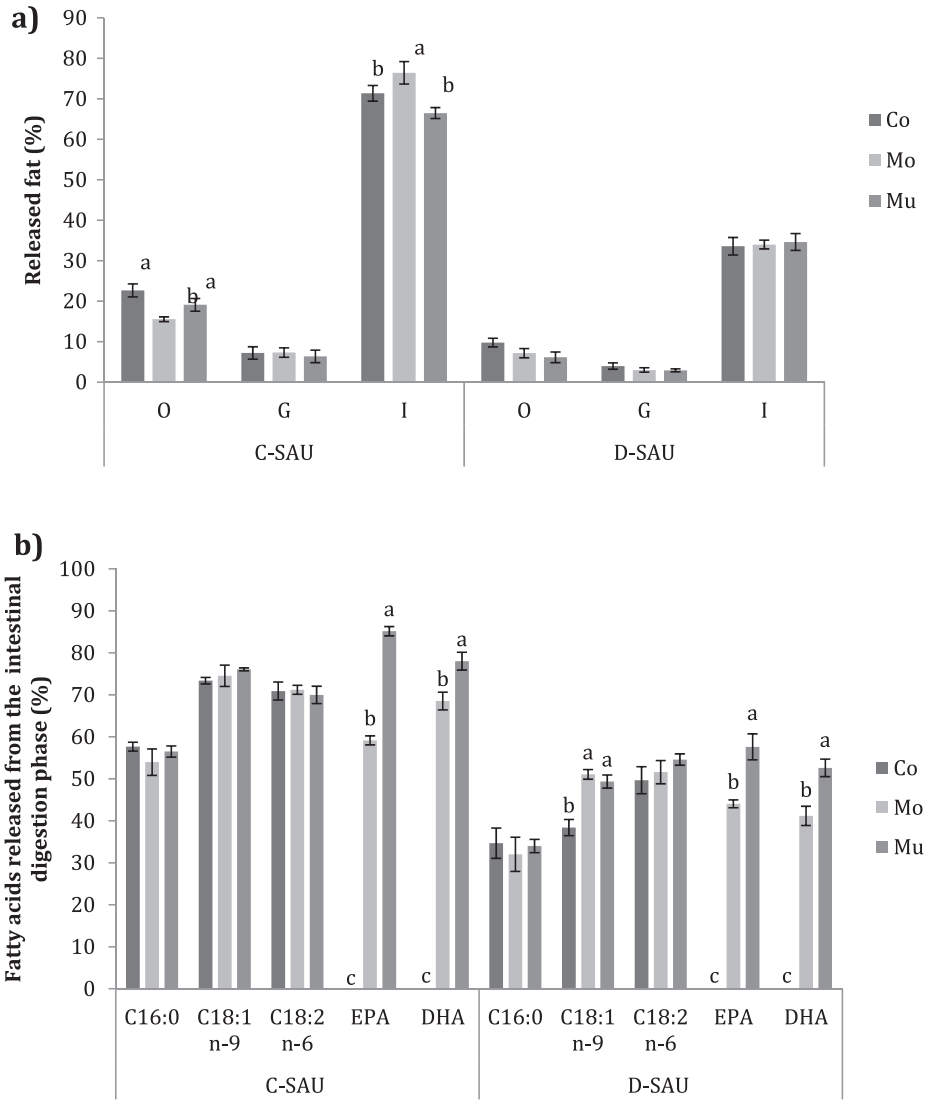

Fig. 2. Results on released of fat (%) through the in vitro digestion phases (O: oral, G: gastric and I: intestinal) (a) and of individual fatty acids (%) in the intestinal digestion phase (b) of cooked (C-SAU) and dry-cured sausages (D-SAU) as affected by enrichment with fish oil microcapsules *.Sausages not enriched (Co, dark gray), enriched with multilayered (Mu, medium gray) and monolayer fish oil microcapsules (Mo, light gray). Bars with different letters (a,b,c) show significant differences ($p < 0.05$) due to enrichment effect (Co vs Mo vs Mu).

percentages of hydrolysis around 10–30% in the stomach phase and 50–90% in the intestine phase, depending on the nature of the lipid compound (Friedman & Nylund, 1980; Gunstone, 2001; Lairon, 2009). This is normal since all digestive enzymes, including lipolytic enzymes (pancreatic lipase, phospholipase and sterol esterase), are secreted at the intestinal level, producing most of the hydrolyzed lipid compounds.

The microcapsules addition led to significant differences in C-SAU, with samples added with Mo showing lower percentage of released fat in the oral phase (15.54%) and higher in intestinal phase (76.42%) in comparison to Co (22.66 and 71.36%, respectively) and Mu (19.11 and 66.47%). However, no significant differences were found between D-SAU batches. It is also noted that the percentage of released fat from C-SAU is higher than from D-SAU at each phase of the digestion, being the total released fat around 100 and 45% for C-SAU and D-SAU, respectively. It is described that the presence of solid fat and low areas of exposure to lipolytic enzymes leads to a lower lipolysis rate (Golding & Wooster, 2010; McClements, Decker, & Park, 2009). This may explain the results of this study since the chopping process of the ingredients until obtain a fine paste in C-SAU decrease the size of the fat globules (Barretto, Pacheco, & Pollonio, 2015), facilitating the action of lipolytic enzymes.

Table 3 shows the quantity of fatty acids released in the different phases of the in vitro digestion of C-SAU. In Co batches, the highest quantities of fatty acids were found in the intestinal phase, followed in decreasing order by oral and gastric phases. This is in concordance with the results on the percentage of fat released, previously explained. In general, the major fatty acid released was oleic acid (C18: 1n-9), following in decreasing order by palmitic (C16: 0) and linoleic acids (C18: 2n-6), and stearic acid (C18: 0), with the rest of fatty acids having less than 0.5 mg/g sample digested. This behavior agrees with the profile of fatty acids described in Table 1 for C-SAU. These results are hardly comparable since there are no previous studies of in vitro digestibility of enriched meat products. Nevertheless, the effects of digestibility on the amount and type of fatty acids have been studied in millet (Annor, Marcone, Corredig, Bertoft, & Seetharaman, 2015), prawns fed with diets enriched with different oils (cod liver, olive, coconut, sesame, canola, flaxseed and fish) (Glencross, Smith, Thomas, & Williams, 2002), red hybrid tilapia fed with palm oil (Bahurmiz & Ng, 2007) and broiler chickens fed with a commercial diet (Tancharoenrat, Ravindran, Zaefarian, & Ravindran, 2014). All these investigations showed that the digestibility of total lipids was not affected by the composition of fatty acids, except when the levels of PUFA in the diet exceeded 17 g kg^{-1}.

Table 3
Fatty acids released through in vitro digestion of cooked sausages as affected by the addition of different types of fish oil microcapsules: monolayered (Mo) and multilayered (Mu)*.

Fatty acids	Co			Mo			Mu			*p*E		
	O	G	I	O	G	I	O	G	I	O	G	I
C14:0	0,09 ± 0.01	0,03 ± 0.00	0,29 ± 0.05	0,08 ± 0.00	0,03 ± 0.00	0,28 ± 0.03	0,08 ± 0.01	0,02 ± 0.00	0,31 ± 0.07	0.321	0.562	0.196
C16:0	2,66 ± 0.24	0,58 ± 0.05	5,24 ± 0.16	2,54 ± 0.12	0,58 ± 0.02	5,58 ± 0.31	2,39 ± 0.09	0,51 ± 0.03	5,74 ± 0.40	0.207	0.126	0.097
C16:1n-7	0,25 ± 0.07	0,08 ± 0.01	0,82 ± 0.10	0,25 ± 0.04	0,08 ± 0.02	0,91 ± 0.22	0,20 ± 0.04	0,07 ± 0.01	0,83 ± 0.13	0.321	0.436	0.224
C17:0	0,06 ± 0.01	0,02 ± 0.00	0,19 ± 0.06	0,06 ± 0.00	0,02 ± 0.00	0,21 ± 0.04	0,06 ± 0.00	0,02 ± 0.00	0,23 ± 0.03	0.789	0.825	0.510
C17:1n-7	0,03 ± 0.00	0,01 ± 0.00	0,10 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.00	0,02 ± 0.00	0,01 ± 0.00	0,10 ± 0.00	0.621	0.987	0.778
C18:0	0,89 ± 0.13	0,33 ± 0.03	2,07 ± 0.26	0,86 ± 0.09	0,35 ± 0.02	2,20 ± 0.37	0,75 ± 0.05	0,31 ± 0.06	1,97 ± 0.29	0.109	0.204	0.326
C18:1n-9	2,27 ± 0.41	0,71 ± 0.11	7,37 ± 0.56^{b}	2,30 ± 0.34	0,76 ± 0.09	8,46 ± 0.76^{a}	1,94 ± 0.08	0,62 ± 0.02	7,96 ± 0.58^{b}	0.153	0.065	0.041
C18:2n-6	1,83 ± 0.22	0,58 ± 0.10	5,95 ± 0.84^{b}	1,59 ± 0.13	0,53 ± 0.09	5,84 ± 0.56^{b}	1,56 ± 0.08	0,50 ± 0.06	6,41 ± 0.43^{a}	0.231	0.069	0.037
C18:3n-6	0,02 ± 0.00	Nd	0,05 ± 0.00	0,01 ± 0.00	Nd	0,05 ± 0.00	0,02 ± 0.00	Nd	0,06 ± 0.01	0.458	–	0.495
C18:3n-3	Nd	Nd	0,01 ± 0.00	0,01 ± 0.00	Nd	0,02 ± 0.00	0,01 ± 0.00	Nd	0,02 ± 0.00	0.105	–	0.079
C20:0	0,02 ± 0.00	0,01 ± 0.00	0,07 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.02	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.01	0.462	0.945	0.101
C20:1n-9	0,15 ± 0.03	0,05 ± 0.00	0,48 ± 0.09	0,15 ± 0.02	0,05 ± 0.01	0,54 ± 0.08	0,17 ± 0.04	0,05 ± 0.00	0,68 ± 0.11	0.174	0.906	0.206
C20:2n-6	0,06 ± 0.01	0,02 ± 0.00	0,18 ± 0.03	0,05 ± 0.00	0,02 ± 0.00	0,19 ± 0.03	0,05 ± 0.01	0,02 ± 0.00	0,22 ± 0.04	0.398	0.864	0.422
C20:3n-6	0,03 ± 0.00	0,01 ± 0.00	0,09 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,08 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.02	0.378	0.789	0.736
C20:3n-3	0,08 ± 0.03	0,02 ± 0.00	0,26 ± 0.06	0,07 ± 0.01	0,02 ± 0.00	0,24 ± 0.07	0,06 ± 0.00	0,02 ± 0.00	0,27 ± 0.04	0.208	0.836	0.501
C20:4n-6	0,01 ± 0.00	Nd	0,03 ± 0.00^{b}	0,01 ± 0.00	Nd	0,05 ± 0.00^{a}	0,02 ± 0.01	Nd	0,06 ± 0.00^{a}	0.471	–	< 0.001
C20:5n-3	Nd^{b}	Nd^{b}	Nd^{c}	0,04 ± 0.00^{a}	0.02 ± 0.00^{a}	0,08 ± 0.04^{b}	0,03 ± 0.00^{a}	0,02 ± 0.00^{a}	0,12 ± 0.04^{a}	< 0.001	< 0.001	< 0.001
C24:0	0,02 ± 0.00	Nd	0,05 ± 0.02	0,01 ± 0.00	Nd	0,05 ± 0.00	0,02 ± 0.01	Nd	0,06 ± 0.02	0.169	–	0.071
C22:6n-3	Nd^{b}	Nd^{b}	Nd^{c}	0,06 ± 0.00^{a}	0,05 ± 0.00^{a}	0,17 ± 0.04^{b}	0,06 ± 0.00^{a}	0,02 ± 0.00^{a}	0,23 ± 0.07^{a}	< 0.001	< 0.001	< 0.001
Σ SFA	2.84 ± 0.21	0.89 ± 0.17	9.21 ± 0.55	2.67 ± 0.16	0.68 ± 0.10	9.81 ± 0.62	2.61 ± 0.20	0.77 ± 0.08	10.38 ± 0.49	0.324	0.269	0.276
Σ MUFA	2.70 ± 0.18	0.80 ± 0.15	8.57 ± 0.71^{c}	2.62 ± 0.21	0.85 ± 0.11	9.29 ± 1.06^{b}	2.24 ± 0.63	0.75 ± 0.07	9.98 ± 0.84^{a}	0.096	0.061	< 0.001
Σ PUFA	2.02 ± 0.13	0.64 ± 0.07	6.57 ± 0.46	1.85 ± 0.27	0.61 ± 0.11	6.80 ± 0.33	1.82 ± 0.21	0.58 ± 0.08	7.47 ± 1.13	0.065	0.116	0.084

Data are expressed as mg FAMEs / g sample digested.
Bars with different letters (a,b,c) within the same digestion phase show significant differences ($p < 0.05$) due to enrichment effect.
See the caption of the Table 1 for the names of the fatty acids.
* O, G, I: oral, gastric and intestine digestion phases, respectively. Nd: not detected.

The enrichment with Mo and Mu did not lead to marked differences in the quantities of fatty acid released throughout in vitro digestion. As for the quantities of EPA and DHA released, they followed the same trend as the rest of fatty acids, with higher values in the intestinal than in the oral and gastric phases. This may indicate than the encapsulated fatty acids within the meat matrix are digested as the rest of fatty acids of the products, and point out the appropriateness of the wall materials of both types microcapsules (maltodextrin in combination or not with chitosan) to protect against the acidity of the gastric environment, which is supported by results on previous studies on different wall material (Aranaz et al., 2012; Klinkesorn, Sophanodora, Chinachoti, & McClements, 2004; Yongsheng et al., 2008). Moreover, the type of fish oil microcapsules significantly influence on the quantities of these fatty acids that are released in the intestinal phase, being higher when Mu are added to C-SAU in comparison to Mo, for both EPA (0.08 and 0.12 mg EPA/g sample digested, respectively) and DHA (0.17 and 0.23 mg DHA/g sample, respectively). This result can be attributed to the combination of the protective effect of the maltodextrin wall together with the chitosan in Mu, since their electrostatic characteristic depends on the relative pH. At pH between 1 and 3 the chitosan tends to charge positively, having a high electrostatic attraction with the anionic molecules, however the chitosan losses its positive charge at above 6.5, not having electrostatic interaction (Hur, Lim, Decker, & McClements, 2011; McClements & Li, 2010). Thus, during the acid digestion in the stomach, it may take place a high electrostatic interaction between the chitosan and the lipid drops, decreasing the amount of exposed lipid surface, but in the intestinal digestion phase (pH between 6 and 7.5) that electrostatic interaction diminishes, which may favor the release of the lipid molecules to be digested by the pancreatic lipase enzyme.

In the case of the batches enriched with Mo the protection of the ω-3 PUFA is lower than Mu because the fish oil is only covered by a single wall of maltodextrin and, although the polysaccharides disperse easily and quickly in water, the compact molecular structure of maltodextrin prevents rapid absorption of water from the food, providing a partial protection against oxidizing agents in the early stages of the gastrointestinal tract. At the gastric level, the decrease in pH catalyzes the hydrolysis of the glycosidic bonds that bind the monosaccharide molecules, which induce the beginning of the microcapsule wall degradation. However, the microcapsule may not be completely digested until the intestine by the action of pancreatin (Damodaran, Parkin, & Fennema, 2008).

According to (Calvo, Lozano, Espinosa-Mansilla, & González-Gómez, 2012), the amount of oil released is related to the efficiency of the microencapsulation and therefore with the external and internal oil content of the microcapsules. This statement could also be related to the higher quantities of EPA and DHA released when Mu are added, since the external fat of Mu is higher than of Mo (Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Antequera, 2014).

Results on the quantities of fatty acids released throughout the in vitro digestion of D-SAU batches are shown in Table 4. As occurred in C-SAU, the major release of fatty acids took place in the intestinal phase and the major fatty acids are the most released ones. However, no significant differences were found between D-SAU samples enriched with Mo and Mu in the quantity of EPA and DHA, whereas this effect was observed in C-SAU. Moreover, comparing results from Table 3 and 4, it is observed a higher quantity of fatty acid released from C-SAU than from D-SAU. This is in concordance with results on the percentage of fat released (Fig. 2a), which were previously discussed.

Once quantified the fatty acids of C-SAU and D-SAU batches as well as their released throughout the different phases of the in vitro digestion, the bioaccessibility of major fatty acids (palmitic acid (C16:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6)) and of added fatty acids (EPA and DHA) has been calculated (Fig. 2.b), in order to know about the percentage of the ingested fatty acids available for absorption. As can be seen, the type of fish oil microcapsules significantly influenced on the bioaccessibility of EPA and DHA, being lower when added Mo than Mu in C-SAU (59 and 85% EPA, 68 and 78% DHA, respectively) and in D-SAU (44 and 57% EPA, 41 and 52% DHA, respectively). Moreover, the addition of fish oil microcapsules significantly increased the bioaccessibility of oleic acid (C19:1n-9) (38, 51 and 49% in Co, Mo and Mu, respectively), which is an extra but positive effect from a nutritional point of view. As occurred with the quantities of fatty acid released, their bioaccesibility was also higher in C-SAU than in D-SAU, which can be ascribed to the differences in the meat matrix, as previously explained. Moreover, these results are in agreement with those found by Shen, Apriani, Weerakkody, Sanguansri, and Augustin (2011) who added microencapsulated tuna oil powder to orange juice, yogurt and cereal bar with. These authors found a higher lipolysis extent of omega-3 PUFA in orange juice and yogurt samples than in cereal bar ones, which was explained by the larger lipid droplets in the digest of the cereal bar and, hence, to the low total surface area available for lipase attack. This statement could be the reason behind the findings of this study. It is also noted differences in the bioaccesibility between fatty acids in all analyzed batches, being lower for palmitic acid (C16:0) (around 60 and 40% in C-SAU and D-SAU, respectively) in comparison to oleic acid (C18:1n-9), linoleic acid (C18:2n-6), EPA and DHA (around 70 and 50% in C-SAU and D-SAU, respectively). These differences could be related to the specific position (sn-1, sn-2 or sn-3) at which the different fatty acids are sterified, since the pancreatic lipase has a high specificity of for fatty acids esterified at the sn-1 and sn-3 positions of triacylglycerols (Shen & Wijesundera, 2006)

4. Conclusions

The addition of fish oil microcapsules made of lecithin + maltodextrin and lecithin + chitosan-maltodextrin to cooked and dry-cured meat products achieves the enrichment in EPA and DHA of these products, not being influenced by the refrigeration storage but susceptible to be labelled as “source of ω-3 fatty acids” according to European Union legislation. Besides, the enrichment with these types of vehicles of omega-3 fatty acids did not influence on the lipid composition of the analyzed meat products.

The quantity of fatty acid released at the different phases of the in vitro digestion of meat products added with microcapsules is firstly described in the present study. The release of fat and fatty acids of the meat products is not affected by the addition of fish oil microcapsules, but it influenced on the bioaccesibility of EPA and DHA. Moreover, the type meat matrix seems to be a significant effect on the released of fat and fatty acids. In this way, the addition of fish oil microcapsules of lecithin + chitosan-maltodextrin as wall material to cooked sausages should be more appropriate than fish oil microcapsules of lecithin + maltodextrin and dry-cured sausages to maximize the percentage of EPA and DHA available for absorption. Therefore, it could be pointed out the importance of analyzing not only the quantity of EPA and DHA in the enriched foods, but also the bioavailability of these bioactive compounds in most of the possible products. These could be used to develop functional foods that provide healthier lipid profiles and promote health and welfare.

CRediT authorship contribution statement

Juan Carlos Solomando: Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Teresa Antequera:** Methodology, Resources, Writing - review & editing. **Trinidad Perez-Palacios:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Table 4
Fatty acids released through in vitro digestion of dry-cured sausages as affected by the addition of different types of fish oil microcapsules: monolayered (Mo) and multilayered (Mu)*.

Fatty acids	Co			Mo			Mu			pE		
	O	G	I	O	G	I	O	G	I	O	G	I
C14:0	0.07 ± 0.01	0.03 ± 0.00	0.28 ± 0.07	0.06 ± 0.01	0.04 ± 0.00	0.33 ± 0.09	0.04 ± 0.00	0.03 ± 0.00	0.27 ± 0.06	0.352	0.263	0.195
C16:0	0.55 ± 0.06	0.10 ± 0.03	0.71 ± 0.31^{b}	0.57 ± 0.06	0.16 ± 0.02	1.12 ± 0.19^{a}	0.54 ± 0.05	0.15 ± 0.02	1.18 ± 0.14^{a}	0.236	0.046	< 0.001
C16:1n-7	0.06 ± 0.00	0.03 ± 0.00	0.26 ± 0.11	0.07 ± 0.00	0.04 ± 0.00	0.39 ± 0.08	0.06 ± 0.01	0.04 ± 0.00	0.38 ± 0.04	0.411	0.569	0.102
C17:0	0.04 ± 0.00	0.02 ± 0.00	0.15 ± 0.03	0.03 ± 0.00	0.02 ± 0.00	0.18 ± 0.06	0.02 ± 0.00	0.01 ± 0.00	0.13 ± 0.03	0.201	0.236	0.302
C17:1n-7	0.02 ± 0.00	0.01 ± 0.00	0.08 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.10 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.11 ± 0.03	0.732	0.653	0.721
C18:0	0.38 ± 0.08	0.17 ± 0.04	1.06 ± 0.29	0.36 ± 0.07	0.22 ± 0.04	1.16 ± 0.41	0.34 ± 0.07	0.20 ± 0.05	1.09 ± 0.57	0.521	0.098	0.085
C18:1n-9	0.56 ± 0.11	0.25 ± 0.06	2.25 ± 0.43^{b}	0.56 ± 0.13	0.34 ± 0.07	3.11 ± 0.67^{a}	0.53 ± 0.18	0.31 ± 0.14	3.03 ± 0.54^{a}	0.186	0.092	0.039
C18:2n-6	0.47 ± 0.09	0.21 ± 0.01	2.69 ± 0.47	0.46 ± 0.11	0.28 ± 0.06	2.49 ± 0.21	0.43 ± 0.10	0.26 ± 0.04	2.76 ± 0.39	0.236	0.312	0.142
C20:0	0.02 ± 0.00	0.01 ± 0.00	0.06 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.07 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.09 ± 0.02	0.421	0.721	0.206
C20:1n-9	0.09 ± 0.01	0.04 ± 0.00	0.35 ± 0.07	0.08 ± 0.00	0.05 ± 0.00	0.44 ± 0.11	0.07 ± 0.01	0.04 ± 0.00	0.45 ± 0.13	0.645	0.695	0.158
C20:2n-6	0.04 ± 0.00	0.02 ± 0.00	0.15 ± 0.02	0.03 ± 0.00	0.02 ± 0.00	0.16 ± 0.03	0.03 ± 0.00	0.02 ± 0.01	0.16 ± 0.04	0.462	0.430	0.671
C20:3n-6 + C21	0.01 ± 0.00	Nd	0.03 ± 0.00	0.01 ± 0.00	Nd	0.04 ± 0.00	0.01 ± 0.00	Nd	0.03 ± 0.00	0.853	–	0.409
C20:3n-3	0.02 ± 0.00	0.01 ± 0.00	0.08 ± 0.02	0.03 ± 0.00	0.02 ± 0.01	0.12 ± 0.03	0.02 ± 0.00	0.01 ± 0.00	0.11 ± 0.04	0.512	0.301	0.096
C20:4n-6	0.01 ± 0.00	Nd	0.02 ± 0.00	0.01 ± 0.00	Nd	0.03 ± 0.00	0.01 ± 0.00	Nd	0.03 ± 0.01	0.802	–	0.723
C20:5n-3	Nd^{b}	Nd^{b}	Nd^{b}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.08 ± 0.03^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.11 ± 0.05^{a}	< 0.001	< 0.001	< 0.001
C24:0	0.01 ± 0.00	Nd^{b}	0.02 ± 0.00^{c}	0.01 ± 0.00	0.01 ± 0.00^{a}	0.04 ± 0.01^{a}	0.01 ± 0.00	Nd^{b}	0.03 ± 0.00^{b}	0.326	< 0.001	< 0.001
C22:6n-3	Nd^{b}	Nd^{b}	Nd^{b}	0.05 ± 0.01^{a}	0.03 ± 0.00^{a}	0.16 ± 0.06^{a}	0.04 ± 0.01^{a}	0.02 ± 0.00^{a}	0.23 ± 0.07^{a}	< 0.001	< 0.001	< 0.001
Σ SFA	0.76 ± 0.13	0.33 ± 0.08	3.03 ± 0.59	0.74 ± 0.15	0.45 ± 0.06	3.97 ± 0.71	0.67 ± 0.23	0.40 ± 0.08	4.10 ± 0.39	0.098	0.075	0.106
Σ MUFA	0.74 ± 0.08	0.32 ± 0.08	2.94 ± 0.09^{b}	0.74 ± 0.09	0.45 ± 0.05	3.95 ± 0.82^{a}	0.68 ± 0.11	0.41 ± 0.06	4.16 ± 0.74^{a}	0.136	0.083	< 0.001
Σ PUFA	0.55 ± 0.06	0.27 ± 0.04	2.19 ± 0.33^{b}	0.59 ± 0.21	0.36 ± 0.07	3.16 ± 0.51^{a}	0.55 ± 0.11	0.33 ± 0.07	3.35 ± 0.27^{a}	0.263	0.102	< 0.001

Data are exposed as mg FAMEs / g sample digested.
Bars with different letters (a,b,c) within the same digestion phase show significant differences ($p < 0.05$) due to enrichment effect. See the caption of the Table 1 for the names of the fatty acids.
* O, G, I: oral, gastric and intestine digestion phases, respectively. Nd: not detected.

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Capítulo 3.7

Sensory profile and consumer perception of meat products enriched with EPA and DHA using fish oil microcapsules

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Sensory profile and consumer perception of meat products enriched with EPA and DHA using fish oil microcapsules

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Summary The sensory properties of cooked and dry-cured sausages enriched with monolayered and multilayered fish oil microcapsules, as EPA and DHA vehicles, were investigated, by using quantitative descriptive analysis (QDA), temporal dominance of sensations (TDS) and hedonic and purchase intent tests. The enrichment effect was slight, mainly influencing flavour attributes, which led to lower scores in the QDA and lower dominance rate in TDS. The effect on acceptability and purchase intent depends on the meat product, being more noticeable in cooked sausages. It is worth noting the impact of the label information that increased the scores of hedonic and purchase intent tests. All these results may point out the use of fish oil microcapsules, the adjustment of the content of salt and flavour additives and the inclusion of accurate label information to enrich meat products. The importance of combining different sensory techniques to achieve a complete sensory evaluation of meat products has also been highlighted in the present study.

Keywords Fish oil microcapsules, meat product, omega-3 enrichment, purchase intent, quantitative descriptive analysis, temporal dominance of sensations.

Introduction

Globally, consumers are very aware of the beneficial health effects of eating foods rich in omega-3 polyunsaturated fatty acids (ω-3 PUFA), which reduce the risk of cardiovascular diseases and prevent certain types of neurodegenerative and inflammatory diseases (Pourashouri *et al*., 2014; Roke *et al*., 2018). Besides, different health organizations have established dietary recommendations for the daily intake of these beneficial fatty acids (International Society for the Study of Fatty Acids and Lipids, 2004; EFSA, 2010; European Food Safety Authority, 2010), which is around 0.25–0.5 g of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) per person per day. However, the consumption of fish and seafood, which are the main sources of EPA and DHA, is not enough to reach their recommended intake, with a median daily intake in Europe of around 0.056 g (EFSA, 2012). In addition, the European Union legislation has established the minimum level required for the sum of EPA and DHA to label a food as 'source of ω-3 fatty acids' and 'high in ω-3 fatty acids': 40 and 80 mg per 100 g and per 100 kcal, respectively (EU, 2010). All these aspects have led to an increase in the interest of the food industry in the development of omega-3-enriched products.

The use of fish oil microcapsules seems to be an appropriate strategy to enrich meat products in ω-3 PUFA (Pérez-Palacios *et al*., 2019), limiting the high susceptibility to oxidation of these fatty acids and their consequences. In fact, several publications are evaluating the effect of adding fish oil microcapsules on some quality parameters of different meat products (Pelser *et al*., 2007; Josquin *et al*., 2012; Jiménez-Martín *et al*., 2016; Lorenzo *et al*., 2016; Aquilani *et al*., 2018; Pérez-Palacios *et al*., 2018). These studies have principally applied quantitative descriptive analysis (QDA) and hedonic techniques to evaluate the effect of fish oil microcapsules addition on the sensory quality of meat products.

Quantitative descriptive analysis is the sensory technique routinely used to profile a product in all its sensory characteristics of appearance, odour, texture, mouthfeel, flavour and after flavour (Stone *et al*., 2008; Sidel *et al*., 2017). It is a highly detailed and valid method commonly used to establish the sensory profile of the food in the industry (Murray *et al*., 2001). However, QDA is static, because the food evaluation is only performed at a single point of time, and

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it is also a fairly expensive technique, since a trained panel is required. Therefore, the application of traditional QDA would be unable to evaluate subtle changes in flavour and texture in the sensory perception of the foods during consumption, since it only provides a global impression of the maximum intensity of the attribute (Pineau *et al*., 2009).

Sensory perception is a complex and dynamic process with changes in the oral cavity during the mastication and salivation that cause sensory differences in the flavour and texture perception (Piggott, 2000; Ng *et al*., 2012); however, these changes cannot be detected by static techniques such as QDA. Thus, it is not surprising that, in recent years, techniques such as temporal dominance of sensations (TDS) have been developed in order to measure the dynamic process involved in the flavour and taste perception throughout the tasting of the food.

Temporal dominance of sensations has been developed (Pineau *et al*., 2003, 2009; Le Révérend *et al*., 2008) based on a simultaneous evaluation of several sensory attributes and provides a temporary sequence of dominant attributes. This methodology has been successfully implemented in cooked and dry-cured sausages (Paulsen *et al*., 2014; Braghieri *et al*., 2016) and dry-cured loins and hams (Lorido, Hort, Estévez, & Ventanas, 2016; Lorido, Estévez, & Ventanas, 2018), but there are no data about the application of this technique to meat products enriched with omega-3 sources.

On the other hand, although it has been shown that the choice of a food is determined by intrinsic and extrinsic sensory stimuli related to the brand, label or health claim with a multilevel approach (Enneking *et al*., 2007; Langan *et al*., 2017), the traditional sensory analysis only focuses on intrinsic product attributes, not being sufficient to meet current market requirements (Sun *et al*., 2009). In this respect, the evaluation of the purchase intention may make sense.

Therefore, the objective of this work was focused on evaluating the sensory characteristics, acceptability and purchase intention of cooked and dry-cured meat products as affected by the enrichment in EPA and DHA with fish oil microcapsules, by static (QDA), dynamic (TDS) and hedonic sensory techniques.

Material and methods

Preparation of omega-3 microcapsules

Fish oil from cod liver (with initial peroxide value < 10 meq kg^{-1} and a percentage of 5.96% EPA and 25.83% DHA) kindly provided by Biomega Nutrition (Galicia, Spain) was used as a source of ω-3 PUFA to prepare two types of microcapsules (monolayered (Mo) and multilayered (Mu)) by the spray-drying technique, according to the methodology of Jiménez-Martín *et al*. (2014) with slight modifications (Solomando, Antequera, Ruiz-Carrascal, & Pérez-Palacios, 2019). The process started with the production of the Mo and Mu fish oil emulsions. Accordingly, fish oil (20 g) and soybean lecithin (6 g), provided by Across Organics, Madrid, Spain, were mixed with a magnetic stirrer overnight. Then, water was added to a total weight of 200 g and homogenised (8960 ***g***, 10 min) using an Ultra Turrax T 18 basic (IKA, Germany). In this way, the primary emulsion was obtained and then homogenised at high pressure (SPX, model APV-200a, Silkeborg, Denmark) under the conditions previously optimised (pressure and number of cycles), 120 MPa-3 passes for Mo and 110 MPa-2 passes for Mu (Solomando *et al*., 2019). The primary emulsion was blended with 200 g of water, in the case of Mo, and with 200 g of 1 % of 95 % deacetylated chitosan (w/w) (ChitoClear FG 95, kindly provided by Trades, Murcia, Spain) in acetic acid 1 %, in the case of Mu, by slow agitation with a magnetic stirrer for 15 min. In this way, the lipid droplets are surrounded by lecithin and lecithin-chitosan in Mo and Mu, respectively. In both types of emulsions, the final step consists of adding 400 g of maltodextrin solution (120 g maltodextrin + 280 g water) with a dextrose equivalent of 12 % (Glucidex 12, kindly provided by Roquette, Lestrem, France), to obtain the feed emulsion. The Mo and Mu emulsions obtained (800 g) were driedin a laboratory-scale spray-dryer equipped with a 0.5-mm nozzle atomiser (Mini spray-dryer B-290, Buchi, Switzerland). The emulsions, maintained at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray-drying process. The aspirator rate was adjusted at 80 %, feed rate was 1 L h^{-1}, inlet temperature was 180 °C, and outlet temperature ranged over 85–90 °C. In this way, the Mo and Mu microcapsules were obtained and then stored in containers at 4 °C until being added to the meat products.

Experimental design

Two different meat products were elaborated, cooked (C-SAU) and dry-cured sausages (D-SAU), to which were added Mo (C-SAU-Mo and D-SAU-Mo) and Mu microcapsules (C-SAU-Mu and D-SAU-Mu). A control batch (without enrichment) of each meat product was also prepared (C-SAU-Co, D-SAU-Co).

C-SAU were elaborated with meat mechanically separated from chicken, water, pork fat, salt, pork plasma, stabiliser (E-450), aromas, vegetable fibre, spices, spice extracts, smoke flavour, antioxidant (E-316) and preservative (E-250) and the corresponding microcapsules in the case of the enriched batches, which were added during the kneading phase. The

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obtained dough was stuffed into edible collagen casings with a length of 15 cm and a diameter of 13 mm. All C-SAU batches were pasteurised in a water bath at 85 °C for 30 min, vacuum-packed and stored at refrigeration temperature (0–5 °C). Formulation and manufacture of these products were made by triplicate in a meat industry (remain anonymous). All batches were sensory analysed after heating at 90 °C for 3 min.

D-SAU were elaborated with Iberian pork meat and fat, which were ground through a 6-mm-diameter mincing plate. The rest of ingredients: salt, dextrose, soy protein, spices, aromas, stabilisers (E-451 and E-450), antioxidant (E-301), preservatives (E-252 and E-250), enhancer flavour (E-621), colouring (E-120) and the corresponding microcapsules, in the case of the enriched batches, were added, mixed for 3 minutes and kept at 4 °C until being stuffed. No starter culture was added. The obtained dough was stuffed into collagen casings with a length of 40 cm and a diameter of 60 mm. The sausages followed a dry-cured process under controlled conditions of 4 °C and 82% relative humidity for 3 days; after this period, they were maintained for 21 days in a drying-curing chamber at 8 °C and 80% relative humidity; finally, they were in a cellar at 5 °C and 85% humidity for around 14 days, until reaching a percentage weight loss of 38–40%. The replicate for each one of the D-SAU batches ($n = 3$) was stored at ambient temperature (18–20 °C).

In both products, the quantity of Mo and Mu added was 2.75 and 5.26 g per 100 g of dough, respectively. These figures were calculated to exceed the required quantity of EPA + DHA to label a food as 'source of ω-3 fatty acids': at least 40 mg of the sum of EPA and DHA per 100 g and per 100 Kcal (EU, 2010) and considering the quantity of EPA and DHA in Mo and Mu (Solomando, Antequera, & Perez-Palacios, 2020).

Sensory evaluation

All sessions were held in sensory panel rooms with the conditions specified in the UNE-EN-ISO 8589, 2010 regulation (UNE-EN-ISO 8589, 2010). The software used to carry out the sensory sessions and record scores was FIZZ Network (version 1.01: Biosystems, France). The panel rooms are equipped with white fluorescent lighting and conditioned to a temperature of 22 ± 2 °C. The evaluation sessions were performed and held at mid-morning, around 11–13 h. In each session, three portions of approximately 5 grams of samples were evaluated. Three samples, marked with random three-digit codes, were served, hot (45–50 °C) in the case of C-SAU and at room temperature for the D-SAU samples, on white plastic plates to the panellists in each session. Crackers or toast (with no added salt) and about 200 mL of mineral water at room temperature were provided to the panellists to rinse between samples.

Quantitative descriptive analysis

C-SAU and D-SAU were assessed by a trained panel of 14 staff members at the University of Extremadura with ages between 22–41 years old, selected previously using individual taste, flavour and aroma recognition thresholds (Pérez-Palacios *et al.*, 2018). The sensory attributes to be evaluated in C-SAU and D-SAU batches were selected after a total of four training sessions. Firstly, a list of descriptors for both products was provided to each panellist, and then, the redundant descriptive terms were removed using dichotomous responses; the selected attributes were evaluated using an unstructured scale of 0–10 with verbal anchors 'little' and 'very much'; the obtained results were analysed by an analysis of variance (ANOVA) to evaluate the panellist effect; an additional training session was finally performed on the most variable attributes. In this way, the quantitative descriptive sensory analysis was finally conducted with 17 sensory traits for C-SAU (homogeneity, brightness, colour intensity, colour homogeneity, spicy odour, smoky odour, rancid odour, saltiness, smoky flavour, rancid flavour, flavour intensity, hardness, chewiness, juiciness, elasticity, granularity and oiliness) and 13 for D-SAU (homogeneity of surface, brightness, red lean intensity, spicy odour, saltiness, sourness, spicy flavour, rancid flavour, flavour intensity, hardness, chewiness, juiciness and oiliness), which were evaluated using an unstructured scale of 0–10 anchored with verbal extremes 'little' to 'very much'. Each panellist evaluated three samples in each session, and the replicates were served in three different sessions, recording in each session the average of the panel for each attribute per sample evaluated.

Hedonic and purchase intent test

The hedonic sensory analysis and the purchase intent test were carried out with untrained volunteers (154) that are regular consumers of cooked and dry-cured sausages, including students, professors and staff recruited at the Faculty of Veterinary and at the Research Institute of Meat and Meat Products of the University of Extremadura. Besides, the hedonic test was also carried out with children at a school. Most panellists (64%) were delimited in an age range between 19 and 24 years, followed in decreasing order by the range of 25–32 years (11%) and ≥ 33 years (8%), the three remaining categories (<6, 7–12 and 13–18 years) showed a similar percentage, around 5–6%. In relation to the sex, more than half of the population surveyed was women (64%) and the rest men. Moreover, a high percentage of them (around 55%)

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consider themselves as habitual consumers of sausages and dry-cured meat products.

The samples were rated in a structured five-point scale for the hedonic test, with the inscriptions 'extremely unpleasant' and 'extremely liked' on the left and right extremes, respectively. A structured five-point scale was used to evaluate the purchase intention, with the inscriptions 'I definitely would not buy' and 'I would definitely buy' on the left and right extremes, respectively. Data about consumption habits, sex and age were also asked.

To evaluate the effect of nutritional information on hedonic sensory analysis and purchase intent responses, a nutritional label was designed and presented to participants including the content of energy, fat, sugar, protein and salt per 100 g of product was shown. This information was total energy 923.63 kJ/220.7 kcal, fats 17.7 g, of which saturated 7.9 g, carbohydrates 1.0 g, of which sugars 0.5 g, proteins 14.35 g, salt 2.0 g, for C-SAU, and energy 1561 kJ/373 kcal, fat 26.6 g, of which saturated 12.6 g, carbohydrates 1.4 g, of which sugars 0.6 g, proteins 32 g, salt 4.0 g, for D-SAU. The list of ingredients, described in the experimental design section, also accompanied the nutritional information. Besides, in the case of the enriched batches, the term 'fish oil' was written with a different typography from the rest of ingredients, according to regulation 1169/2011 of labelling of substances that cause allergies and intolerances (EU, 2011), and the health claim 'source of omega-3 fatty acids' were also included in the label.

Temporal dominance of sensations analysis (TDS)

The 17 panellists selected for this analysis had previous experience in TDS procedure (Lorido *et al.*, 2016). Even then, they attended two training sessions of 1 h. In these sessions, the concept of 'dominant sensation' was firstly explained and the use of the computerised data capture system TDS (FIZZ v 2.40A) was experienced by evaluating different meat products with similar characteristics to the samples of the present study (turkey, pork and chicken cooked sausages and cured loin, salami and turkey dry-cured), following the protocol described in previous studies (Pineau *et al.*, 2009; Lorido, Ventanas, & Ventanas, 2014).

Thus, TDS analysis was finally conducted as follows: panellists put the sample in their mouths and press the start button to begin the evaluation. After 15 s (25 % of standardised time), they were requested to swallow the sample by a text message displayed on the screen and continue their evaluation until a final period of 60 s (100% of standardised time) or press the stop button of data acquisition when they do not perceive any attribute as dominant. The evaluation consists of selecting the dominant attribute from a list of 8 attributes for C-SAU (saltiness, smoked flavour, rancid flavour, meat flavour, tenderness, juiciness, oiliness and fish flavour) and 9 for D-SAU (saltiness, acidness, fish flavour, spicy flavour, meat flavour, juiciness, oiliness, rancid flavour and hardness) throughout the evaluation period. Panellists did not have to select all the sensory attributes that appear in the list, and they could choose the same attribute as dominant several times during the course of food tasting. The order of appearance of the different attributes on the screen differed between the panellists to counteract the possible effects of the order of presentation. However, each panellist had the same sequence of attributes in all sessions to facilitate the location of the descriptor. Three different batches were evaluated per session, which were presented monadically, with 1 min between batches to ensure no drag effects. Six sessions were carried out, being analysed each batch in triplicate.

The TDS analysis was carried out using the FIZZ software, which gives the graphical results as curves that represent the dominance rates of each attribute. The time data of each panellist was standardised to a score between 0 and 100, 0 representing the time they clicked start and 100 the stop click. Besides, to facilitate the interpretation of TDS curves, the chance level and the level of significance were also included on each curve. The chance level represents the dominance rate that an attribute can obtain by chance (P0 = 1/ number of attributes) (Pineau *et al.*, 2009), and the level of significance represents the smallest value of the proportion, being significantly ($P = 0.05$) higher than the chance level ($Ps = P0+1.645[P0\ (1 - P0)/n]1/2$, n is the number of runs: judges × replicates). Moreover, two TDS parameters were calculated from TDS curves for each attribute: Std time (time of first citation as dominant) and Std duration (total duration of dominance over citations).

Statistical analysis

For both products, there were three batches of samples, two with added fish oil microcapsules (Mo and Mu) and one control batch without enrichment (Co), which were analysed after production (T0) and after four months of storage at refrigerated conditions (T4). The effects of enrichment (pE) and storage (pS) on results from QDA, hedonic, purchase intent and TDS analyses were evaluated by one-way analysis of variance (ANOVA). When a significant effect ($P < 0.05$) was detected, paired comparisons between means were conducted using the post hoc Tukey's test. The statistics were run using the program IBM SPSS Statistics v.22.

Results and discussion

Quantitative descriptive analysis

Figure 1 shows results on QDA carried out in C-SAU (Figs 1a and 2a) and D-SAU (Figs 1b and 2b) as

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affected by the type of fish oil microcapsule addition and storage. Some differences were found between control and enriched batches. In C-SAU at T0, Co batches showed significantly higher scores for spicy odour (4.09 ± 1.15) compared with Mo and Mu batches (3.72 ± 1.29 and 3.46 ± 1.32, respectively) and flavour intensity (6.70 ± 0.67) compared with Mo and Mu batches (5.84 ± 0.95 and 6.10 ± 0.84, respectively). At T4, Co batches showed significant lower scores for smoky odour (5.36 ± 0.93) compared with Mo and Mu batches (4.73 ± 1.12 and 4.71 ± 0.98, respectively) and higher scores for flavour intensity (6.78 ± 0.79) compared with Mo and Mu batches (5.81 ± 0.74 and 6.06 ± 0.72, respectively). No significant differences were found between both enriched batches. In D-SAU, at T0, Co batches showed significantly lower scores for saltiness (4.59 ± 0.87) compared with Mo and Mu batches (5.33 ± 0.90 and 5.62 ± 1.12, respectively) and spicy flavour (5.24 ± 0.97) compared with Mo and Mu batches (6.86 ± 0.85 and 6.93 ± 1.12, respectively). At T4, Co batches showed significant lower scores for homogeneity of surface (2.76 ± 0.95) compared with Mo and Mu batches (3.54 ± 1.07 and 3.89 ± 1.02, respectively) and significantly higher scores for flavour intensity (7.29 ± 0.63) compared with Mo and Mu batches (6.98 ± 0.61 and 6.49 ± 0.77, respectively), hardness (7.17 ± 0.81) compared with Mo and Mu batches (5.62 ± 0.90 and 5.06 ± 1.02, respectively) and chewiness (7.14 ± 0.76) compared with Mo and Mu batches (6.08 ± 0.93 and 5.73 ± 0.97, respectively). These results were not expected since the same formulation was used in the three batches.

Nevertheless, the findings in C-SAU are quite in accordance with previous studies. In the research of (Aquilani *et al*., 2018) with burgers enriched with microencapsulated fish oil, a significant decrease in odour (odour intensity and cooked meat odour) and flavour attributes (cooked meat flavour) were observed in the enriched batches in comparison to the control one, without enrichment. Similarly, in the study carried out in Dutch-style fermented sausages by Josquin *et al*. (2012), a decrease in spicy flavour was observed in the batch enriched with encapsulated fish oil compared with the control batch. These results could be associated with a possible masking effect of the original tastes and flavours in the batches enriched with omega-3 fish oil microcapsules that would have led to a decrease in their perception (Salminen *et al*., 2013).

The increase in the scores of saltiness in enriched D-SAU batches is also in concordance with previous results from a study carried out on chicken nuggets enriched with fish oil microcapsules (Jiménez-Martín *et al*., 2016), which can be related to the hypothesis of (Lad *et al*., 2012). These authors established that significant variations in saltiness among batches with a constant amount of salt could be related to an increase in the oil phase, which would improve the mixing with the saliva, accelerating the salt transport to the taste buds. This may take place in the D-SAU-Mo and D-SAU-Mu batches of the present study, because of the added fish oil, specially the one with microcapsules. This finding could be interesting from a nutritional point of view since it may allow reducing the salt content in meat products with added fish oil microcapsules.

Differences in the surface homogeneity and texture attributes in D-SAU between control and enriched batches are difficult to explain. The extra mixing process after adding the microcapsules to the enriched batches could have affected these attributes because the added kneaded phase could have caused a decrease in the size of solid fat and muscle bundles. In fact, it has been reported that the degree of chopping could affect on texture attributes (Honikel & Hamm, 1994).

Effect of storage for four months, at 0–5 °C and at 18–20 °C in C-SAU and D-SAU, respectively, significantly decreased the oiliness and rancid flavour scores in the three batches studied of both, C-SAU and D-SAU. Nevertheless, these statistical differences were not significant from a practical point of view, since the scores for these attributes in the three batches were very close. These findings highlight the high stability of the fish oil microcapsules used in this study under storage for four months at 0–5 °C and 18–20 °C. Similarly, previous studies on burgers (Pelser *et al*., 2007; Jiménez-Martín *et al*., 2016; Aquilani *et al*., 2018) have shown that the addition of fish oil microcapsules does not cause an increase in sensory rancidity scores after storage.

Temporal dominance of sensations analysis

Figure 2 shows average TDS curves of selected texture, taste and flavour attributes during the consumption of C-SAU and D-SAU control batches, without enrichment (Fig. 2a) and enriched with Mo and Mu microcapsules (Fig. 2b,c respectively). Besides, TDS parameters calculated from these curves (Std time and Std duration) are shown in Table 1.

In general, texture attributes dominated during the chewing period, while the period from swallowing to the end of the analysis was characterised by dominant flavour attributes. These results are quite in concordance with the consumption process. It starts with the chewing, which makes it possible to perceive the textural properties of the food. The chewing process also leads to components of lower size, allowing the swallowing and the stimulation of the taste and odour receptors (Tunick, 2011). In C-SAU, the TDS curves revealed some differences between Co and enriched batches throughout the chewing-food period.

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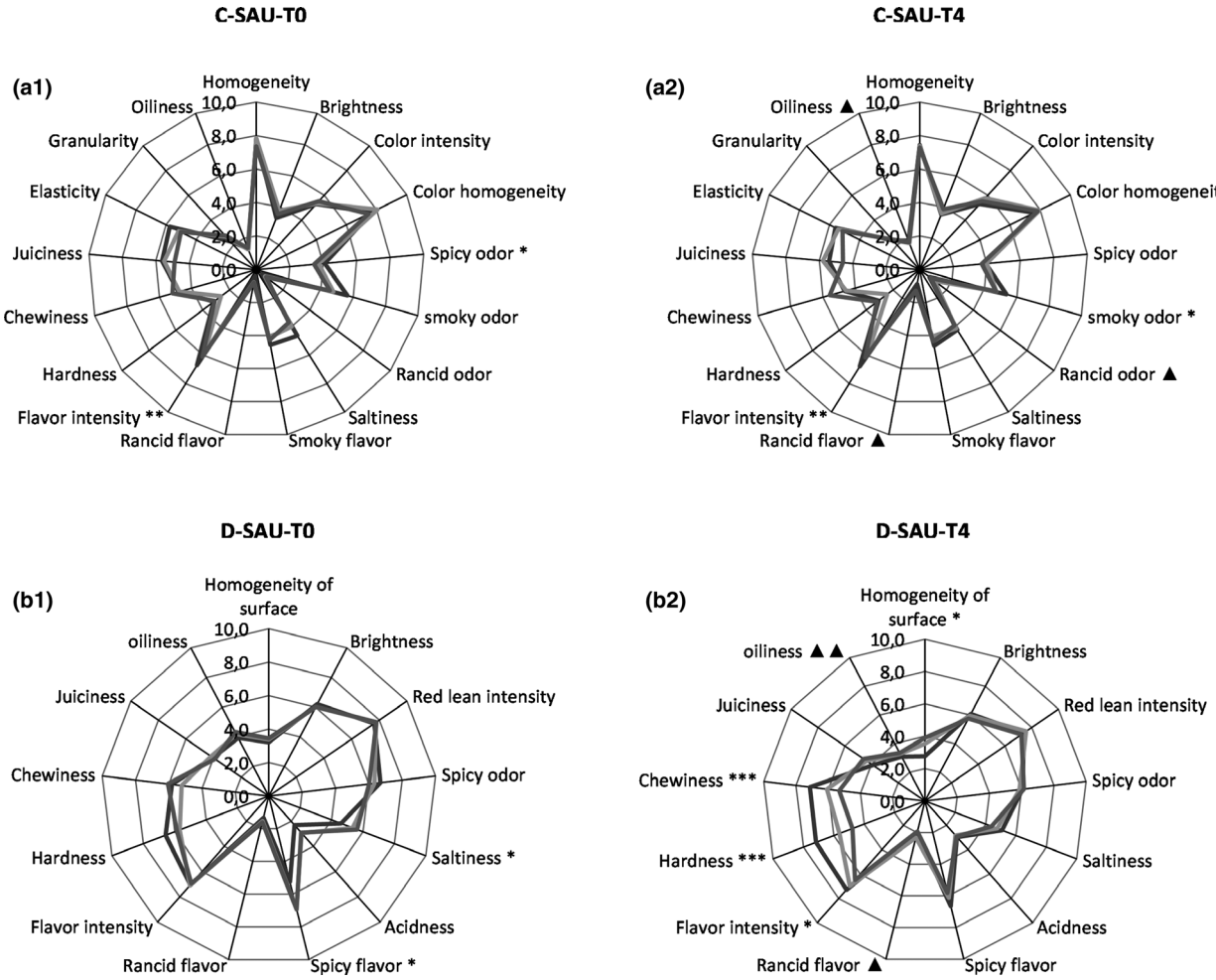

Figure 1 Results on the Quantitative descriptive sensory analysis of cooked (C-SAU) and dry-cured (D-SAU) sausages (a and b, respectively) as affected by enrichment with ω-3 PUFA (control: dark grey; enriched with multilayered fish oil microcapsules: medium grey; enriched monolayered fish oil microcapsules: light grey) and analysed after production (T0) and after four months of storage (T4) (1 and 2, respectively). Significant enrichment effect is indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$); significant storage effect is indicated by ▲ ($P < 0.05$) and ▲▲ ($P < 0.01$).

From the beginning of the evaluation to the time of swallowing (from 0 to 25–30% of standardised time, approximately), in Co batches, the attributes of tenderness and meat flavour showed significant dominant perceptions, while C-SAU-Mo batch did not show significant dominant attributes during this period, and C-SAU-Mu presented meat flavour as the only dominant attribute. As for the time from swallowing to the end of the test (from 25–30 to 100% of standardised time), in C-SAU-Co batches, meat and smoked flavours were perceived as significantly dominant sensations during this whole period (25–100% and 45–100% of standardised time, respectively) with a maximum dominance rate of approximately 45% for both significant attributes. C-SAU-Mo batches showed the same trend but with lower dominance rate (approximately 35% at maximum) for meat and smoked flavours. In C-SAU-Mu, meat and smoked flavours were also the dominant attributes but exhibited a different curve during this phase. Meat flavour was only found at the beginning of this period (from 25 to 40% of standardised time, approximately) and smoke flavour at the end (from 75 to 100% of standardised time approximately), with dominance rates around 38%, similar to those obtained for C-SAU-Mo. In relation to the fish flavour attribute, it appeared as dominant in C-SAU-Mu, with two peaks, after swallowing (from 40 to 65% of standardised time, approximately) and during the aftertaste period (from 80 to 100% of standardised time,

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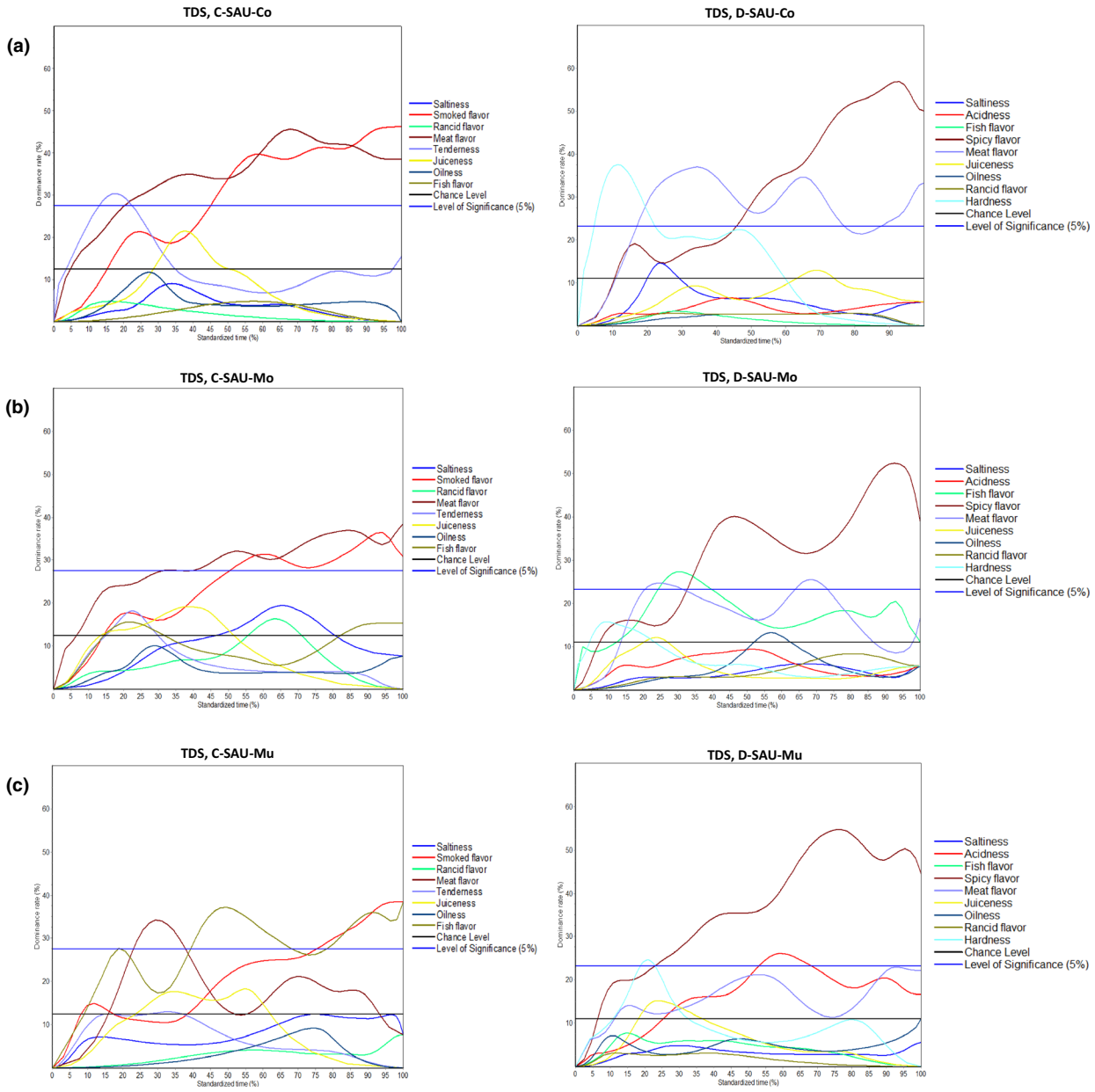

Figure 2 TDS curves of cooked (C-SAU) and dry-cured (D-SAU) sausages as affected by enrichment with fish oil microcapsules (control (a); enriched with monolayered (b) and multilayered (c) fish oil microcapsules). [Colour figure can be viewed at wileyonlinelibrary.com]

approximately), showing a dominance rate of around 35%.

The dominance of the meat flavour in C-SAU batches is expected, since this attribute has been evaluated with high scores in previous studies with similar meat products (Valencia *et al.*, 2008; Aquilani *et al.*, 2018). In the same trend, the dominance of smoked flavour is intended due to the use of smoked flavour additives in the formulation of C-SAU. In concordance, (Paulsen *et al.*, 2014) have also found this attribute as dominant in sausages. The higher dominance rate for these dominance attributes in C-SAU-Co than in the enriched batches are in concordance with QDA results and may be due to a possible masking effect of the original tastes and flavours caused by the addition of fish oil microcapsules, as previously explained. Besides, in the case of the C-SAU-Mu samples, the dominance of the fish flavour

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Table 1 Parameters of temporal dominance sensations analysis of taste (a), flavour (b) and texture (c) attributes of cooked (C-SAU) and dry-cured sausages (D-SAU) (means ± SD) as affected by enrichment with ω-3 PUFA (*p*E).

	C-SAU				D-SAU			
	Co	Mo	Mu	*p*E	Co	Mo	Mu	*p*E
(a) Taste								
Saltiness								
StdTime%	25.67 ± 0.57^{b}	37.67 ± 4.93^{a}	46.33 ± 5.50^{a}	0.003	38.33 ± 3.78	35.33 ± 10.40	33.00 ± 2.00	0.625
StdDuration%	10.00 ± 7.24	7.33 ± 2.08	7.03 ± 1.23	0.659	5.00 ± 1.73	6.00 ± 1.00	4.00 ± 0.89	0.18
Acidness								
StdTime%	–	–	–		25.00 ± 8.71	47.00 ± 5.56	37.33 ± 14.05	0.101
StdDuration%	–	–	–		4.00 ± 2.36	2.33 ± 1.52	4.67 ± 2.96	0.766
(b) Flavour								
Rancid								
StdTime%	5.33 ± 4.16^{b}	42.67 ± 19.50^{a}	56.67 ± 12.42^{a}	0.009	22.67 ± 16.77	34.00 ± 20.42	24.00 ± 10.53	0.671
StdDuration%	0.33 ± 0.29	5.00 ± 1.23	4.00 ± 2.28	0.058	12.33 ± 8.32	3.00 ± 1.56	6.33 ± 1.15	0.160
Spicy								
StdTime%	–	–	–		33.67 ± 8.50	38.00 ± 7.00	34.00 ± 5.58	0.722
StdDuration%	–	–	–		16.00 ± 8.12	11.67 ± 7.02	11.67 ± 8.97	0.915
Meat								
StdTime%	21.67 ± 8.96	34.33 ± 13.05	30.00 ± 7.02	0.353	26.67 ± 15.17	25.00 ± 4.35	30.33 ± 8.93	0.821
StdDuration%	33.00 ± 5.58	28.00 ± 10.00	18.67 ± 2.88	0.102	9.00 ± 6.24	8.67 ± 4.04	12.00 ± 3.46	0.657
Fish								
StdTime%	31.67 ± 9.22	26.33 ± +9.02	21.33 ± 3.51	0.361	21.67 ± 1.15^{b}	28.30 ± 5.71^{b}	44.14 ± 7.27^{a}	0.038
StdDuration%	3.67 ± 2.08^{b}	14.67 ± 9.86^{b}	26.00 ± 6.00^{a}	0.019	2.00 ± 0.23	10.67 ± 9.29	8.00 ± 6.21	0.536
Smokedness								
StdTime%	33.33 ± 2.08	28.33 ± 4.04	42.33 ± 10.91	0.113	–	–	–	
StdDuration%	25.33 ± 6.24	19.00 ± 3.60	20.67 ± 1.15	0.253	–	–	–	
(c) Texture								
Hardness								
StdTime%	–	–	–		21.33 ± 8.93	26.00 ± 1.73	25.00 ± 4.00	0.605
StdDuration%	–	–	–		26.00 ± 6.08	18.33 ± 12.01	26.33 ± 9.01	0.532
Tenderness								
StdTime%	20.67 ± 3.51	14.33 ± 8.08	17.33 ± 7.63	0.549	–	–	–	
StdDuration%	7.67 ± 3.78	5.33 ± 0.57	6.00 ± 2.64	0.579	–	–	–	
Juiciness								
StdTime%	32.00 ± 7.81	26.33 ± 5.85	25.67 ± 6.50	0.495	25.33 ± 13.01	26.00 ± 9.16	18.00 ± 6.08	0.574
StdDuration%	8.00 ± 3.41	6.67 ± 3.05	7.33 ± 1.52	0.848	0.67 ± 0.02^{c}	16.00 ± 5.52^{a}	4.00 ± 2.35^{b}	0.012
Oiliness								
StdTime%	42.00 ± 13.52	76.33 ± 17.42	43.67 ± 24.54	0.193	29.33 ± 12.70	30.00 ± 3.606	22.67 ± 6.80	0.546
StdDuration%	2.33 ± 2.08	1.67 ± 2.08	1.33 ± 0.57	0.780	16.67 ± 12.09	11.67 ± 4.51	11.33 ± 9.07	0.736

StdTime% (average time of the first citation of an attribute) and StdDuration% (average of the total duration of a attribute over citations).
Not enriched (Co) and enriched with monolayer (Mo) and multilayered microcapsules (Mu). Bars with different letters (a, b, c) within the same formulation show significant differences ($P < 0.05$) due to enrichment effect.

could also influence the lower dominance rate of meat and smoked flavours. In fact, when the fish flavour attribute becomes dominant, the dominance of the meat flavour disappears (Fig. 2c). Also, the dominance of fish flavour in C-SAU-Mu but not in C-SAU-Mo could be associated with the higher content of external fat (fish oil not encapsulated) in Mu than in Mo (Solomando *et al.*, 2019) since it can be more easily detected than the internal fat (fish oil encapsulated). It is also worth noting that panellists did not consider including the fish flavour attribute in the QDA; however, it was selected as dominant in the C-SAU-Mu samples. This reinforces the importance of the TDS to detect differences in the sensory perception during all the stages of consumption. As for texture, as previously indicated, differences in these attributes between control and enriched batches are difficult to be justified. Thus, the existence of tenderness as the dominant attribute in C-SAU-Co but not in C-SAU-Mo or C-SAU-Mu during the chewing period may be related to the influence of the degree of chopping on texture attributes reported by Honikel & Hamm (1994), since the additional mixing process was carried out on the enriched batches.

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Regarding the Std time and Std duration in C-SAU (Table 1), significant differences were found in Std time for saltiness and rancid flavour; with higher values in enriched samples than in control ones. Nevertheless, these attributes are not dominant (they did not exceed the level of significance of dominance rate (5%) in the TDS graphs (Fig. 2)). As for Std duration, only fish flavour showed significant differences with higher values in C-SAU-Mu than in C-SAU-Mo and C-SAU-Co batches. This is in concordance with TDS curves, which have shown this attribute as dominant in C-SAU-Mu but not in C-SAU-Mo and C-SAU-Co.

In D-SAU, TDS curves also showed some differences between Co and enriched batches with fish oil microcapsules (Fig. 2). In D-SAU-Co batches, hardness and meat flavour were showed as dominant attributes from the beginning of the evaluation to the time of swallowing the samples (0–25% of standardised time approximately). However, the batches enriched with Mo and Mu fish oil microcapsules did not show significant dominant attributes in this period. As for the time from swallowing to the end of the test, in D-SAU-Co batches, meat flavour was perceived as a dominant attribute with three peaks, during the swallowing time (25–40% of standardised time, approximately), after swallowing (55–70% of standardised time, approximately) and at the end of the analysis (85–100% of standardised time, approximately), showing a maximum dominance rate around 35%. Besides, the spicy flavour was perceived as a dominant attribute from 50 to 100% of the standardised time, approximately, with a maximum dominance rate around 55%. In the D-SAU batches enriched with Mo and Mu fish oil microcapsules, the spicy flavour was the only attribute perceived as dominant, having a longer standardised time than Co samples (35–100% and 25–100% of standardised time, respectively) and with a maximum dominance rate approximately of 55% for both enriched batches.

The fact that the spicy flavour was the temporal dominant sensation of all D-SAU batches may be due to the addition of spices in the formulation of this meat product, as detailed in the material and methods section. Besides, this result agrees with a previous study in sausages (Paulsen *et al.*, 2014). The sourness attribute would expect to be dominant, due to the exponential growth of lactic acid bacteria during the dry-cured processing of this type of meat products (Toldrá, 2008). However, this result has not been observed, which could be ascribed to the masking effect of the spicy flavour.

Regarding the Std time and Std duration in D-SAU (Table 1), significant differences were found in Std time for fish flavour, with significantly higher values in D-SAU-Mu compared to D-SAU-Mo and D-SAU-Co. As previously explained, it could be ascribed to the higher external fat content in Mu than in Mo (Solomando *et al.*, 2019). Nevertheless, although the fish flavour was cited as dominant first in D-SAU-Mu, this attribute was not dominant (it did not exceed the level of significance (5%) in the TDS graphs (Fig. 2)). As for Std duration, only juiciness showed significant differences, reaching the highest values in D-SAU-Mo, followed by D-SAU-Mu and D-SAU-Co batches, but this attribute does not seem to influence on the global perception of this product because it is well below the level of significance in the TDS graphs.

Finally, it also worth noting that higher dominance rates were reached in D-SAU in comparison to C-SAU (approximately 45 vs. 55%, 38 vs. 50% and 38 vs. 55% in Co, Mo and Mu batches, respectively) (Fig. 2). This fact may be explained by the different food matrix in these meat products. In fact, according to some authors, the structure of the food is an essential factor that affects the release of flavouring compounds during chewing (Foster *et al.*, 2011), and therefore, it could also influence on the dominance ratio.

Hedonic and purchase intent

Fig. 3 shows results on hedonic analysis (Fig. 3a) and purchase intent (Fig. 3b) of C-SAU and D-SAU, as affected by the fish oil microcapsules addition and the label information (nutritional composition, ingredients and health claim). In products without previous nutritional information, the enrichment with fish oil microcapsules led to significant differences in the hedonic and purchase intent scores of C-SAU, whereas this effect was not detected in D-SAU. C-SAU-Co samples showed higher hedonic scores (4.40) than Mo and Mu enriched batches (3.22 and 2.69, respectively). Regarding the purchase intent, C-SAU-Co and C-SAU-Mo (3.47 and 3.05, respectively) showed significantly higher scores than C-SAU-Mu (1.34). These results can be related to the characteristics of the wall material of the microcapsules and to the ingredients of this meat product. The existence of water in the C-SAU dough may facilitate the solubilisation of the maltodextrin that forms the wall material of the microcapsules, since maltodextrin exhibits properties of high solubility (Stephen, Phillips, & Williams, 2016; Solomando *et al.*, 2020). This could lead to the release of part of the fish oil, with the consequent lower acceptability. Besides, in the case of C-SAU-Mu, the existence of chitosan in the wall of Mu could also explain the lowest scores of this batch of samples, since chitosan can impart a bitter taste (Devlieghere *et al.*, 2004). In this sense, the nonexistence of significant differences between D-SAU-Co (4.30) and Mo and Mu enriched batches (3.99 and 4.05, respectively) can be ascribed to not adding of water in the elaboration of

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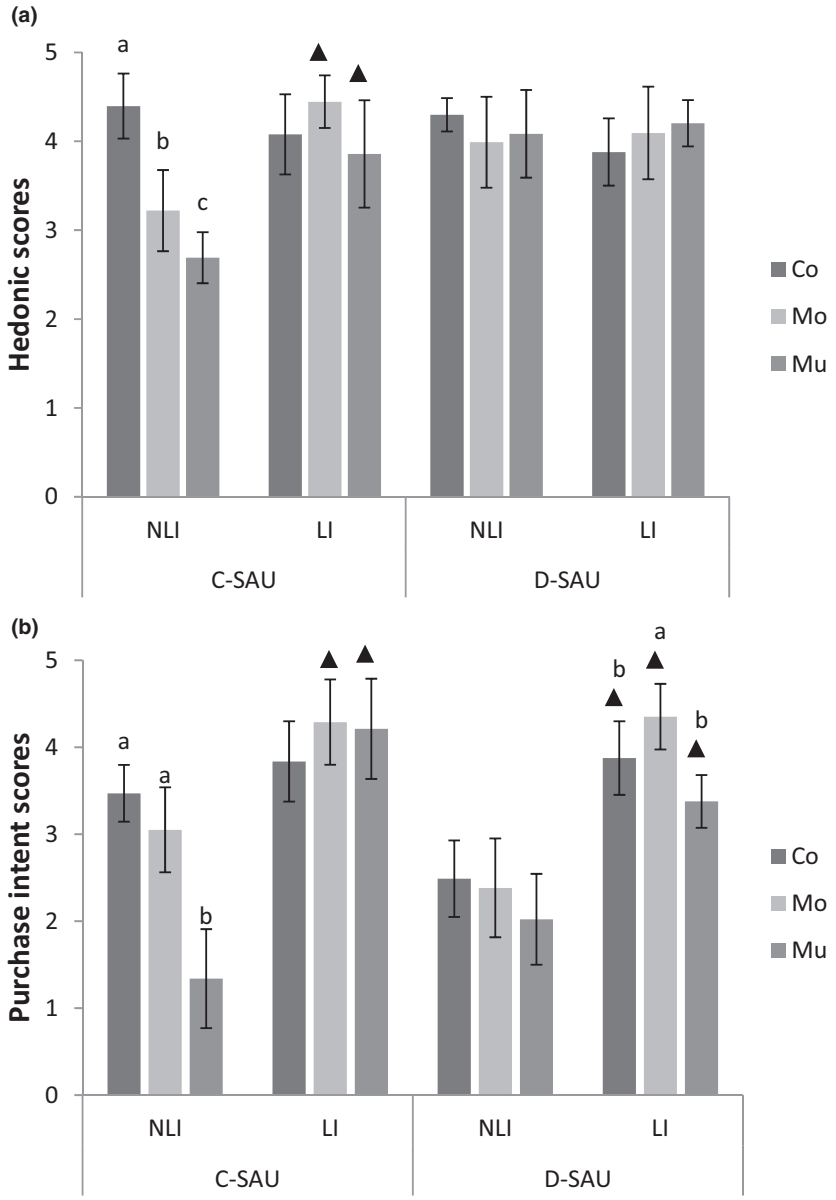

Figure 3 Results on hedonic (a) and purchase intent (b) sensory analyses of cooked (C-SAU) and dry-cured (D-SAU) sausages as affected by enrichment with fish oil microcapsules (mean values ± standard deviation) (control: dark grey; enriched with multilayered fish oil microcapsules: medium grey; enriched monolayered fish oil microcapsules: light grey) and data on nutritional information and claims (nonlabel information (NLI); label information (LI)); significant storage effect is indicated by ▲ ($P < 0.05$).

this type of dry-cured meat product and the addition of spices, which could mask and/or diminish the perception of the rest of ingredients (Salminen *et al.*, 2013). A previous study on Spanish salchichon enriched with ω-3 fish oil microcapsules (Lorenzo *et al.*, 2016) detected a loss of the sensory quality in the enriched batches.

The nutritional information and the nutritional claim 'source of ω-3 fatty acids' led to a significant increase in the hedonic scores of C-SAU-Mo (from 3.22 to 4.45) and C-SAU-Mu (from 2.69 to 3.85) and in purchase intent of all batches of C-SAU (from 3.47, 3.05 and 1.34 to 3.84, 4.29 and 4.21 in Co, Mo and Mu batches, respectively) and D-SAU (from 2.49, 2.38

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and 2.02 to 3.88, 4.35 and 3.38 in Co, Mo and Mu batches, respectively). These results could be explained by the sum of several factors. For example, the increase of consumer awareness in recent years about the beneficial health effects of consuming EPA and DHA; education campaigns to disseminate information, that is cardiovascular diseases, are the leading cause of death in the European continent (European Heart Network, 2019), responsible for 3.9 million deaths every year and almost 49 million people live with these diseases; the policies of the World Health Organization to promote the development of foods with a better nutritional value and evidence based on health claims (Waxman, 2004); the use of precise nutritional labelling accompanied by nutritional declarations and health properties is easily understood by the consumer, without requiring precise nutritional knowledge (Feunekes *et al*., 2008; Van Kleef *et al*., 2008; Draper *et al*., 2013). These facts could influence the increase in purchase intention scores in the omega-3 enriched batches of both meat products after the presentation of nutritional information and the declaration of healthy properties, reaching values very close to control batches.

Conclusions

The enrichment of cooked and dry-cured sausages with monolayered and multilayered fish oil microcapsules is firstly revealed by results from static, dynamic and hedonic sensory analyses and purchase intent evaluation.

In general, fish oil microcapsules addition has a slight effect on sensory traits, principally influencing flavour attributes and saltiness, and on acceptability and purchase intent results, which depend on the meat product and are favoured by the label information.

In this sense, the enrichment in EPA and DHA of cooked and dry-cured meat products may be carried out by using of fish oil microcapsules, adjusting the content of salt and flavour additives and showing accurate label information.

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Conflict of interest

Authors (juancarlosg@unex.es, tantero@unex.es, sanvenca@unex.es, triny@unex.es) state that there are no known conflicts of interest associated with this publication.

Author contribution

Juan Carlos Solomando: Data curation (equal); Formal analysis (equal); Methodology (equal); Writing-original draft (equal). **Sonia Ventanas:** Methodology (equal); Writing-review & editing (equal). **Teresa Antequera:** Methodology (equal); Supervision (equal); Writing-review & editing (equal). **Trinidad Pérez-Palacios:** Funding acquisition (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal).

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.14932.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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5. Discusión

El punto de partida de la presente tesis doctoral consistió en una revisión bibliográfica sobre las estrategias más relevantes para enriquecer derivados cárnicos con AGPI ω-3 (capítulo 1). Al tratarse de un estudio de revisión, parte de lo que aquí se debería de mostrar ya ha quedado reflejado en gran medida en la introducción, en concreto, en el subapartado 1.1.3, y se muestra por completo en el capítulo 1. A rasgos generales se han desarrollado principalmente cuatro estrategias para aumentar el contenido de AGPI ω-3 en derivados cárnicos, que varían desde la suplementación a través de la alimentación animal hasta la adición directa de vegetales y aceites líquidos a distintos alimentos o la incorporación de estos últimos en forma de pre-emulsión o microcápsulas. Las estrategias más estudiadas han sido las que se basan en el enriquecimiento a través de la alimentación animal y la adición de emulsiones oleosas en derivados cárnicos, seguido por la adición directa de aceites y encontrando un reducido número de publicaciones donde se emplea la microencapsulación como estrategia de enriquecimiento. Por otro lado, las aves de corral y derivados cárnicos de cerdo aparecen como los productos más utilizados en este tipo de estudios y los aceites de linaza, pescado y algas como las fuentes más comúnmente utilizadas de AGPI ω-3. Con estas estrategias, en la mayor parte de los casos, se han obtenido derivados cárnicos con un perfil lipídico más saludable, pero sin mostrar ninguno de ellos datos sobre el contenido de dichos ácidos grasos expresado como mg por gramo de muestra, lo cual no permite el etiquetado de ninguno de ellos como “fuente de ácidos grasos omega-3” o “ricos en ácidos grasos omega-3” según normativa Europea [14]. En otro orden de cosas, la adición de AGPI ω-3 a través de la alimentación animal o a derivados cárnicos mediante adición directa o emulsionada de aceites en ocasiones influye negativamente en los niveles de oxidación lipídica y calidad sensorial de los derivados resultantes, incluso cuando se emplean distintos antioxidantes. Sin embargo, dichos efectos negativos no se han encontrado cuando los derivados cárnicos son enriquecidos con microencapsulados de aceite de pescado. Por todo ello, las investigaciones futuras deberían centrarse en el empleo de la microencapsulación como técnica para el desarrollo y caracterización de distintos tipos de microcápsulas de AGPI ω-3, prestando especial atención a la cuantificación de los AGPI ω-3 y a su comportamiento en distintos derivados cárnicos, evaluando en la medida de lo posible el impacto de las condiciones de procesado y almacenamiento, y su comportamiento durante el proceso de digestión gastrointestinal, con el fin de obtener derivados cárnicos enriquecidos estables, con características sensoriales similares a la de sus análogos no fortificados y alta bioaccesibilidad de los AGPI ω-3. Estas conclusiones obtenidas en el capítulo 1 sentaron las bases para el diseño y desarrollo de la presente tesis doctoral.

En el segundo capítulo se han desarrollado microcápsulas mono y multicapa mediante el empleo de altas presiones de homogeneización. En primer lugar, se evalúo el efecto de la homogeneización a alta presión de los dos tipos de emulsiones de aceite de pescado: monocapa (lecitina + maltodextrina) y multicapa (lecitina + quitosano-maltodextrina) sobre las características de calidad de las emulsiones y las microcápsulas obtenidas a partir de las mismas (MO and MU, respectivamente). Los resultados obtenidos mostraron una mejora en la mayoría de las características de calidad de las emulsiones (mayor

estabilidad y homogeneidad y menor tamaño de partícula) y de las microcápsulas (mayor rendimiento, eficiencia de la microencapsulación, estabilidad oxidativa y homogeneidad de tamaño, y menor agregación de partículas y presencia de poros) en relación con las microcápsulas obtenidas en trabajos previos, donde no se aplicó homogeneización a alta presión en las emulsiones [86]. Esta mayor estabilidad en las emulsiones como consecuencia del efecto de la homogeneización podría aumentar la superficie de contacto de las partículas de aceite durante el proceso de secado por atomización, facilitando la evaporación del agua y, por lo tanto, la formación de la pared que recubre al material bioactivo, obteniendo un polvo más estable al reducir la difusión pasiva del aceite encapsulado hacia la superficie, preservando sus características físico-químicas al adicionarlas a matrices alimentarias y someterlas a las condiciones habituales de procesado y almacenamiento del derivado. En el presente estudio, este efecto positivo de la homogeneización a alta presión fue más notable en el caso de las emulsiones-microcápsulas monocapa que en las multicapa, lo que podría explicarse por la modificación de la atracción electrostática tras la adición de quitosano, favoreciendo el contacto entre las capas de las diferentes partículas de aceite, ocasionando un aumento de coalescencia [128]. Esto dificultaría la evaporación del agua durante el proceso de secado por atomización, aumentando el tiempo requerido para la formación de la pared y permitiendo la difusión pasiva de parte del aceite encapsulado hacia la superficie. Este hecho se ha observado en este trabajo, donde las MU mostraron mayor porcentaje de aceite externo (no encapsulado) en comparación con las MO. Una vez contrastado el efecto positivo de la homogenización a alta presión de las emulsiones y microcápsulas monocapa y multicapa de nuestro estudio, el siguiente paso fue la optimización de las condiciones. Para ello se realizó un ensayo de metodología de superficie de respuesta, donde los parámetros experimentales fueron la presión (Ba) y número de ciclos de homogeneización. Los resultados obtenidos indicaron como combinaciones optimas, 1200 Ba-3 ciclos en el caso de MO y 1100 Ba-2 ciclos en el caso de MU.

Las microcápsulas MO y MU elaboradas bajo estas condiciones optimizadas, mostraron características similares: mayor rendimiento y eficiencia de microencapsulación y menor oxidación lipídica, al compararlas con las no homogeneizadas. Una vez optimizadas las condiciones de elaboración de emulsiones y microcápsulas se establecieron las cantidades requeridas para su posterior adición a derivados cárnicos, teniendo en cuenta la cantidad mínima requerida de la suma de EPA y DHA para etiquetar un alimento como "fuente de ácidos grasos omega-3" (40 mg por 100 g de muestra), y considerando el contenido de EPA y DHA (similar en ambos tipos de microcápsulas) y la eficiencia de la microencapsulación, mayor en MO en comparación con MU, estableciendo finalmente una cantidad de microcápsulas MO y MU de 3 y 5 gramos, respectivamente, por cada 100 gramos de derivado.

Las microcápsulas de este estudio también fueron sometidas a un análisis de digestión *in vitro* (Capítulo 3.1) y de perfil de compuestos volátiles (capítulo 3.5). Con relación a la digestión, pudo observarse como en MO se liberó un mayor porcentaje de grasa en la fase en las primeras fases de la digestión (oral y

gástrica) que, en la fase intestinal, en comparación con MU, donde el porcentaje de grasa liberada en la fase intestinal fue superior al obtenido en las primeras fases de la digestión (oral y gástrica). En cuanto a EPA y DHA, la mayor liberación de estos ácidos grasos se produjo en la fase intestinal en ambos tipos de microcápsulas, encontrando una mayor bioaccesibilidad intestinal de dichos ácidos grasos en MU en comparación con MO. En ambos tipos de microcápsulas parte de la grasa liberada en la fase oral probablemente provendría del aceite externo de las microcápsulas (no encapsulado) y, por tanto, fácilmente extraída. El diferente comportamiento de MO y MU durante las fases gástrica e intestinal se relaciona con los materiales de pared empleados en la microencapsulación, siendo la estructura multicapa de quitosano-maltodextrina más resistente a las condiciones gástricas que la envoltura monocapa de maltodextrina [128].

Al analizar el perfil de compuestos volátiles, se observaron claras diferencias entre microcápsulas, al mostrar MO niveles más altos de pentanal, hexanal, 2-hexenal, heptanal, octanal, nonanal, 1-penten-3-ol y 2-etilfurano en comparación con MU, siendo estos compuestos importantes indicadores de oxidación del aceite de pescado [130] al estar relacionados con la ruptura del primer doble enlace de la posición n de los ácidos grasos ω-3 y ω-6, lo cual indica que el recubrimiento de MU fue más eficaz a la hora de proteger al aceite de pescado del daño oxidativo. Sin embargo, otros indicadores relevantes de oxidación en el aceite de pescado como 2,4-heptadienal, 2,4-decadienal y 2-penten-1-ol, asociados con la percepción del sabor a rancio, aldehídos relacionados con olores rancios como decanal o 2-nonenal y cetonas como 3,5-octadien-2-ona y 1-octen-3-ona no fueron identificados en ninguno de los dos tipos de microcápsulas estudiadas [131] pero si en otros estudios donde se analizó el perfil de compuestos volátiles del aceite de pescado no encapsulado [132], pudiendo afirmar la idoneidad del proceso de microencapsulación a la hora de minimizar el contacto y reactividad de los AGPI ω-3 con promotores oxidantes.

En el tercer capítulo de la presente tesis doctoral, las microcápsulas MO y MU previamente optimizadas se emplearon para enriquecer diferentes matrices cárnicas y poder evaluar el efecto de su adición sobre las mismas. En primer lugar, se evalúo la influencia del enriquecimiento sobre dos sistemas modelos cárnicos, uno cocido y otro curado, y se determinó el efecto de diferentes tratamientos (procesado, cocinado) en estos sistemas enriquecidos con microcápsulas (Capítulos 3.1 y 3.2). La adición de microcápsulas (capítulo 3.1) no influyó de forma significativa en el porcentaje de humedad y grasa de los sistemas modelo estudiados, lo que podría ser debido a la pequeña cantidad de microcápsulas adicionada (3 y 5 g de MO y MU por 100 g de sistema modelo), no siendo lo suficientemente elevada como para causar variaciones en estas características físico-químicas. Las cantidades de EPA y DHA, como era esperable, aumentaron en todos los sistemas modelo enriquecidos, siendo más acusada en los lotes enriquecidos con MU en comparación con MO, y la oxidación lipídica se mantuvo dentro de un rango estrecho y con valores muy inferiores a los asociados a sabores y flavores anómalos detectados a nivel sensorial [133].

Por otro lado, el efecto del secado-maduración y/o cocinado no influyó sobre la estabilidad oxidativa de ninguno de los sistemas modelos estudiados y como consecuencia de la disminución progresiva del porcentaje de humedad durante estos tratamientos, el porcentaje de grasa y las cantidades de EPA y DHA aumentaron, lo que indicaría la eficacia de los materiales de pared a la hora de limitar la susceptibilidad a la oxidación de los AGPI ω-3 durante el procesado, un aspecto extremadamente interesante cuando se busca llegar a una cantidad determinada de EPA y DHA para poder etiquetar un derivado como "fuente de ácidos grasos omega-3".

Tras el ensayo de digestión *in vitro* de los sistemas modelo enriquecidos (capítulo 3.2), a nivel general se observó una mayor liberación de EPA y DHA en la fase intestinal en comparación con la oral y gástrica, viéndose favorecida la bioaccesibilidad de EPA y DHA cuando los derivados eran enriquecidos con MO en comparación con MU, lo cual podría estar relacionado con el mayor contenido de grasa externa en MU y su estructura multicapa de quitosano-maltodextrina, más resistente a las condiciones gástricas, que explicaría la mayor cantidad de EPA y DHA en la fase oral y su descenso en las fases intestinales. Entre ambos sistemas modelo también se observaron diferencias en relación a la liberación del aceite encapsulado, en el caso del sistema modelo cocido enriquecido con MO se encontró una mayor liberación intestinal de EPA y DHA en comparación con MU lo cual podría ser debido a que la estructura multicapa de quitosano-maltodextrina quedaría firmemente incrustada en la disposición de la pasta fina, mientras que en MO existiría una unión más débil que favorecería la liberación del aceite encapsulado. Por otro lado, en el sistema modelo curado, al no emplearse agua en su elaboración, la maltodextrina no se solubilizaría, obteniendo una integración similar de MO y MU en la matriz cárnica y liberación de EPA y DHA en la fase intestinal. Por otro lado, el efecto del secado-maduración y/o cocinado influyó en la liberación de EPA y DHA al encontrarse una reducción en la bioaccesibilidad de ambos ácidos grasos asociado a la modificación en la estructura de los sistemas modelos cárnicos durante estos procedimientos, donde las moléculas de grasa quedarían firmemente atrapadas en una matriz cárnica sólida debido a fenómenos de coalescencia entre partículas, disminuyendo el área expuesta de los glóbulos grasos a la actividad enzimática de las lipasas ya que la lipólisis está condicionada por la velocidad de la proteólisis: a medida que se descompone la estructura proteica, los lípidos se liberan de la matriz cárnica y quedan expuestos a las lipasas [134].

En segundo lugar, las MO y MU previamente optimizadas se emplearon para evaluar la influencia del enriquecimiento sobre dos derivados cárnicos: salchicha cocida tipo Viena y fuet elaborados a nivel industrial y se determinó el efecto de los diferentes tratamientos (secado-maduración, cocción, cocinado y almacenamiento) en estos derivados enriquecidos con microcápsulas (capítulos 3.3, 3.4, 3.5, 3.6 y 3.7).

La adición de microcápsulas no influyó de forma significativa sobre la composición nutricional, actividad de agua y coordenadas de color instrumental en los derivados cárnicos estudiados, ni tampoco afectó a las modificaciones que suelen sufrir estos parámetros durante el procesado, cocinado o almacenamiento

(capítulo 3.3). En cuanto a la estabilidad oxidativa (Capítulo 3.3), en ambos derivados cárnicos pudo observarse como el efecto del almacenamiento es el que más influye en los niveles de oxidación lipídica de las muestras enriquecidas, mientras que la adición de microcápsulas, calentamiento culinario y procesado no ejercieron un efecto relevante. Al igual que ocurría en los sistemas modelo, los valores de productos primarios (dienos conjugados) y secundarios (TBARs) de oxidación lipídica de todas las muestras analizadas se posicionaron en un rango estrecho y más bajos que los relacionados con la detección de sabores anómalos, de la misma forma que ocurrió en los sistemas modelo [135]. Mediante el enriquecimiento con los dos tipos de microcápsulas (MO y MU) se obtuvieron derivados cárnicos con cantidades apreciables de EPA y DHA, de forma que las salchichas tipo Viena superaron el nivel mínimo establecido para etiquetar un alimento como "fuente de ácidos grasos omega-3" con valores de 0,47 mg EPA + DHA /100 g y en fuet se obtuvieron valores entre 0,56-0,61 mg EPA + DHA / 100 g de muestra, hecho que hizo disminuir la relación n-6/n-3 en ambos derivados cárnicos. Por otro lado, ni las condiciones de procesado y cocinado ni el almacenamiento modificaron significativamente las cantidades de EPA y DHA. Estos resultados concuerdan con las imágenes de microestructura obtenidas a través de SEM, evidenciándose la presencia de microcápsulas como partículas ovaladas de diferentes tamaños, con algunas arrugas, pero sin poros aparentes ni roturas en la pared, lo cual indicaría la eficacia de los materiales de pared empleados en la microencapsulación a la hora de proteger al aceite de pescado frente a la exposición de especies reactivas de oxígeno, evitando la pérdida de AGPI ω-3, en concreto, de EPA y DHA.

El análisis del perfil de compuestos volátiles de ambos derivados cárnicos (capítulo 3.4 y 3.5) reveló una mayor abundancia en algunos compuestos asociados a oxidación lipídica cuando los derivados cárnicos eran enriquecidos con MO, como pentanal, 1-penten-3-ol, 1-octen-3-ol, 2-etil-furano y 2-decenal en comparación con MU, mientras que otros relacionados con la oxidación de AGPI ω-3 y previamente identificados en el aceite de pescado, como 2,4-heptadienal, 3,5-octadien-2-ona y 1-octen-3-ona no han sido detectados en ninguno de los lotes estudiados. El almacenamiento y calentamiento culinario dan lugar a cambios similares en todos los lotes, no teniendo marcadas consecuencias en el perfil volátil de los derivados cárnicos fortificados, lo cual es indicativo del importante efecto protector de los materiales de pared empleados en la protección del aceite de pescado.

Tras el ensayo de digestión *in vitro* (capítulo 3.6), en ambos derivados cárnicos se observó una mayor liberación de EPA y DHA en la fase intestinal, en comparación con la oral y gástrica, lo cual indica una alta bioaccesibilidad de estos AG a la hora de ser absorbidos por la mucosa intestinal, logrando proteger los materiales de pared empleados en la microencapsulación al aceite de pescado y por consiguiente al EPA y DHA de la acidez gástrica, siendo dicha protección más marcada en MU en comparación con MO. Esto puede ser debido a la carga electrostática del quitosano, que depende del pH relativo, y por lo tanto, durante la digestión ácida del estómago (pH 1-3) el quitosano tiende a cargarse positivamente, lo que da

lugar a una mayor interacción entre este y las gotas lipídicas, disminuyendo la cantidad de lípidos expuestos, sin embargo, a pH mayores de 6.5 el quitosano pierde su carga positiva y por lo tanto, durante la fase de digestión intestinal se favorecería la liberación de las moléculas lipídicas y su posterior digestión por la enzima lipasa pancreática [136]. Por otro lado, el tipo de microcápsula empleada influye en la bioaccesibilidad de EPA y DHA en función del tipo de derivado cárnico enriquecido, siendo más adecuado el empleo de MU en salchichas cocidas tipo Viena. Esto puede estar relacionado con la adición de agua en derivados cárnicos cocidos, que puede ocasionar una menor protección del aceite de pescado encapsulado en MO al estar recubierto por una única pared de maltodextrina; aunque la estructura molecular compacta de la maltodextrina evita la rápida absorción de agua en el alimento, puede inducir el inicio temprano de la hidrólisis de los enlaces glucosídicos que facilitará la degradación de la pared de la microcápsula en las etapas iniciales de la digestión [137].

Los resultados de las distintas pruebas sensoriales realizadas en ambos tipos de derivados cárnicos (capítulo 3.7) revelaron menores puntuaciones en los derivados enriquecidos en algunos atributos como intensidad de olor y flavor y una mayor percepción del sabor salado. Este resultado fue inesperado ya que se empleó la misma formulación en todos los lotes y podría estar asociado con un posible efecto de enmascaramiento de estos atributos como consecuencia del enriquecimiento, dando lugar a una disminución en la percepción de estos. Por otro lado, el aumento en la percepción del sabor salado podría asociarse con la hipótesis de Lad y cols., [138] quienes postularon que un aumento de la fase oleosa en los alimentos mejoraría la mezcla del cloruro sódico con la saliva y aceleraría el transporte de la sal a las papilas gustativas. Este hallazgo resulta muy interesante desde el punto de vista nutricional, ya que podría permitir reducir el contenido de sal en derivados cárnicos enriquecidos con microcápsulas de aceite de pescado. Por otro lado, el análisis de las sensaciones temporales dominantes mostró en ambos derivados una dominancia de los atributos de textura durante el periodo de masticación, mientras que el periodo comprendido entre la deglución hasta la finalización del análisis se caracterizó por el dominio de atributos asociados al flavor, lo cual está bastante relacionado con el proceso de consumo habitual de un alimento. En salchichas cocidas tipo Viena, desde el inicio de la evaluación hasta la deglución, los atributos de terneza y flavor cárnico fueron dominantes en los lotes no enriquecidos, mientras que en los enriquecidos con MO no se encontró dominancia en ninguno de los atributos estudiados y en MU solo se identificó el flavor cárnico como dominante. Desde la deglución hasta el final del análisis, el flavor cárnico y ahumado fueron dominantes en los lotes no enriquecidos, al igual que en los enriquecidos, pero con menores tasas de dominancia, lo cual concuerda con el posible efecto de enmascaramiento por la adición de microcápsulas anteriormente explicado. Además, en los lotes enriquecidos con MU, el atributo flavor a pescado fue identificado como dominante en dos periodos concretos: tras la deglución y durante el regusto, lo cual podría estar asociado con el mayor contenido de grasa externa en este tipo concreto de microcápsula, como ya que explicó previamente en el capítulo 2, pudiendo llegar a ser más fácilmente detectado por los consumidores que la grasa interna (aceite de pescado encapsulado). En el caso del fuet,

al igual que para la salchicha tipo Viena, el enriquecimiento llevó a algunas diferencias. Desde el inicio de la evaluación hasta la deglución, los atributos de dureza y flavor cárnico se mostraron como dominantes en los lotes no enriquecidos, mientras que los enriquecidos no mostraron dominancia de ninguno de los atributos estudiados en este periodo y, desde la deglución hasta el final del análisis, los flavores cárnico y especiado fueron los dominantes en el grupo control, mientras que en los lotes enriquecidos solo dominó el flavor especiado, siendo importante recalcar que ninguno de los dos tipos de microcápsulas adicionadas en el fuet dieron lugar a dominancia para el atributo flavor a pescado. En último lugar, se realizó un análisis de aceptabilidad e intención de compra con consumidores habituales de ambos derivados cárnicos, observando variaciones de ambos parámetros en salchichas tipo Viena, pero no en fuet. En las salchichas tipo Viena el test hedónico reveló menores puntuaciones en ambos lotes enriquecidos, con similar intención de compra entre el lote control y el enriquecido con MO, pero inferior en MU, lo cual podría asociarse con la mayor cantidad de aceite externo en este tipo concreto de microcápsulas y al empleo de quitosano, ya que puede impartir ciertas connotaciones de sabor amargo [139]. En fuet, la inexistencia de diferencias podría estar relacionada con la no adición de agua en la elaboración de este tipo de embutido y con el uso de especias, que podrían enmascarar y/o disminuir la percepción del resto de ingredientes. Además, ambos test se repitieron pero acompañando cada una de las muestras con la información nutricional y declaración nutricional “fuente de ácidos grasos omega-3”, lo cual llevó a un marcado aumento en las puntuaciones hedónicas e intención de compra de todos los lotes en ambos derivados cárnicos y que podría explicarse por la suma de varios factores como la toma de conciencia en los últimos años sobre los efectos beneficiosos para la salud del consumo de EPA y DHA, las campañas de educación para difundir dicha información, las políticas de la Organización Mundial de la Salud para promover el desarrollo de alimentos con mejor valor nutricional y evidencias basada en declaraciones de propiedades saludables, el uso de un etiquetado nutricional preciso acompañado de declaraciones nutricionales y propiedades saludables fácilmente comprensible para el consumidor.

La **figura 6** muestra un resumen de los resultados obtenidos tras la adición de microcápsulas MO y MU tanto en sistemas modelo como en derivados cárnicos.

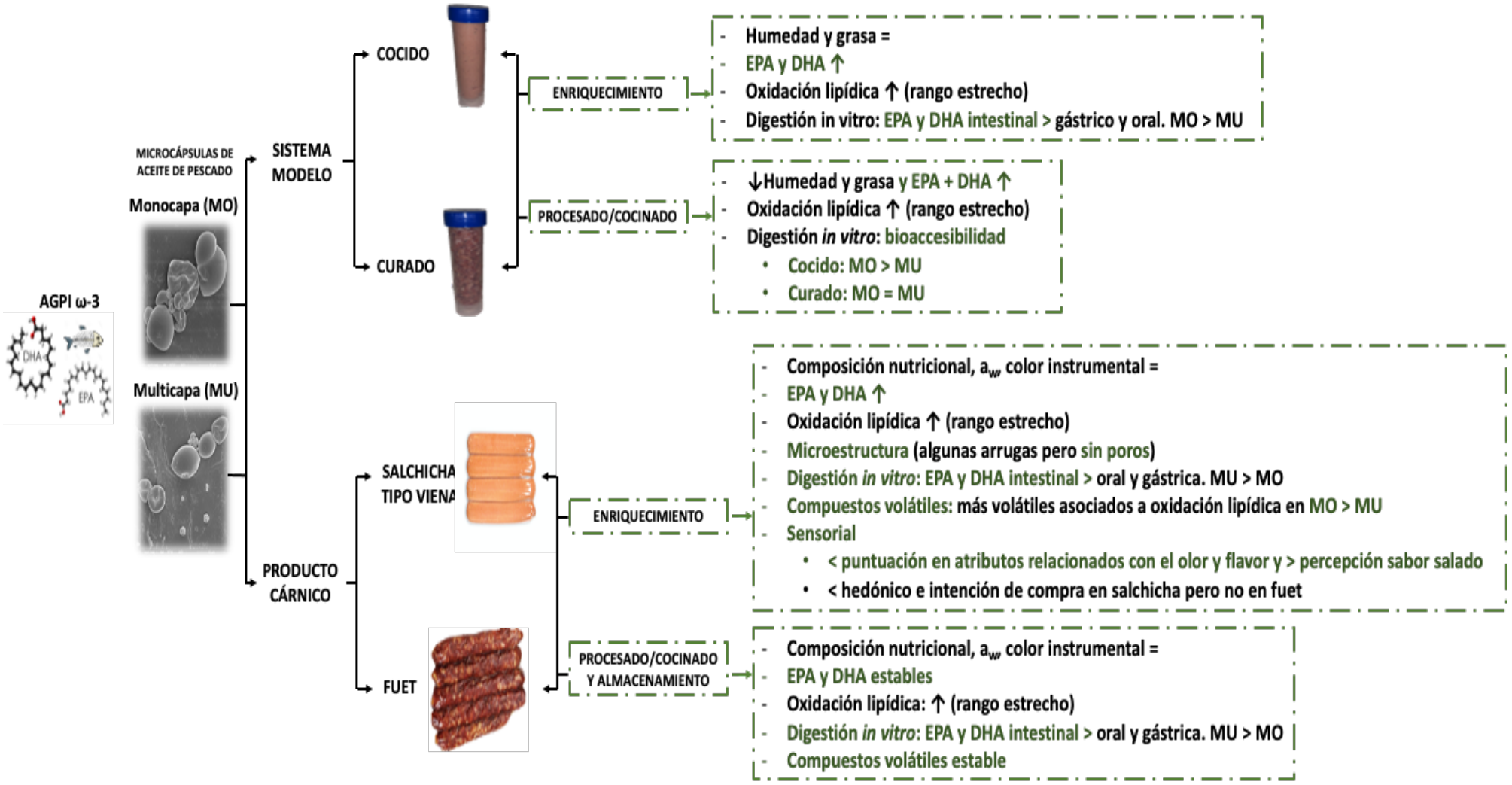

Figura 6. Resumen de los principales resultados obtenidos en sistemas modelo y derivados cárnicos enriquecidos con microcápsulas monocapa (MO) y multicapa (MU).

6. Conclusiones

1. Es recomendable implementar la homogeneización por altas presiones en el proceso de elaboración tanto de emulsiones monocapa y multicapa como sus correspondientes microcápsulas mediante spray-drying, ya que este proceso influye positivamente en la mayoría de las características de calidad tanto de las emulsiones como de las microcápsulas,
2. El tipo de microcápsula influye de forma significativa en la bioaccesibilidad de EPA y DHA cuando las microcápsulas se someten a digestión *in vitro*, obteniéndose una mayor cantidad de estos ácidos grasos en el caso de las multicapa, probablemente debido a que la estructura multicapa de quitosano-maltodextrina es más resistente a las condiciones gástricas que la envoltura monocapa de maltodextrina.
3. La adición de microcápsulas monocapa y multicapa de aceite de pescado a sistemas modelos cocidos y sometidos a un proceso de secado-maduración enriquece en EPA y DHA, sin producir cambios oxidativos importantes durante los procesados. No obstante, el tipo de microcápsulas influye sobre las cantidades de dichos ácidos grasos y su bioaccesibilidad. Además, el tipo de sistema modelo influye en el enriquecimiento y liberación de EPA y DHA. Así, resulta importante seleccionar el tipo de microcápsula más adecuada para cada matriz alimentaria con el fin de optimizar el enriquecimiento y la bioaccesibilidad de EPA y DHA.
4. El empleo de microcápsulas monocapa y multicapa de aceite de pescado en salchichas cocidas tipo Viena y fuet permite enriquecer estos productos en EPA y DHA y etiquetarlos como fuente de ácidos grasos omega-3 sin afectar a sus principales características de calidad, incluso después de su almacenamiento.
5. El tipo de microcápsula adicionada influye sobre la concentración de compuestos volátiles en los derivados cárnicos enriquecidos, siendo mayor al añadir microcápsulas monocapa. La liberación de EPA y DHA a nivel gastrointestinal se ve afectada tanto por el tipo de microcápsula como por el derivado cárnico, encontrándose la mayor bioaccesibilidad cuando se añaden microcápsulas multicapa a salchichas cocidas tipo Viena.
6. El enriquecimiento con microcápsulas monocapa y multicapa de aceite de pescado en los derivados cárnicos de este estudio aumenta el sabor salado y disminuye la intensidad de algunos atributos de flavor. La aceptabilidad e intención de compra se ven ligeramente disminuidos en algunos casos por este enriquecimiento, sin embargo, aumentan cuando el consumidor dispone de la información sobre la composición y declaración nutricional “fuente de ácidos grasos omega-3”.
7. El uso de microcápsulas de ácidos grasos omega-3 elaboradas mediante spray-drying a partir de emulsiones monocapa y multicapa de aceite de pescado es recomendable para enriquecer derivados cárnicos cocidos y curados previo ajuste del contenido de sal.

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8. Anexos

Anexo I

Otras publicaciones

1. Journal of the Science of Food and Agriculture (2018), 98 (14), 5302-5312
2. Journal of the Science of Food and Agriculture (2020), 100, 1875-1886
3. Journal of Food Science and Technology (2020), 57 (1), 60-69
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Improving the lipid profile of ready-to-cook meat products by addition of omega-3 microcapsules: effect on oxidation and sensory analysis

Trinidad Pérez-Palacios,* Jorge Ruiz-Carrascal, Estefanía Jiménez-Martín, Juan Carlos Solomando and Teresa Antequera

Abstract

BACKGROUND: The omega-3 enrichment of ready-to-cook meat products by microencapsulated fish oil (MFO) addition was analyzed. Accordingly, three batches of chicken nuggets were prepared: (i) control (C); (ii) enriched in bulk fish oil (BFO); and (iii) with added MFO. Sensory features, acceptability, oxidative stability and volatile compounds were analyzed.

RESULTS: MFO nuggets did not differ from C ones with respect to any sensory trait. BFO showed increased juiciness and saltiness but decreased meat flavor. Acceptability was not affected by enrichment. Consumers were not able to differentiate between C and MFO in a triangle test, although they could clearly identify BFO nuggets. Higher levels of lipid and protein oxidation indicators and of volatile compounds from fatty acid oxidation were found in BFO nuggets compared to C and MFO nuggets.

CONCLUSION: Enrichment of ready-to-cook meat products in omega-3 fatty acids with MFO provides both lipid and protein oxidative protection without changes in sensory quality.
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Keywords: microencapsulation; ω-3 fatty acids; chicken nuggets; sensory analysis; oxidation; volatile compounds

INTRODUCTION

Omega-3 (ω-3) are polyunsaturated fatty acids (PUFA) of great interest as a result of their bioactivity. They are related to the promotion of human health in terms of a reduction of the risk of cardiovascular disease, chronic diseases and tumors.[1] Consequently, there is growing interest among consumers, food authorities and food industry with respect to increasing ω-3 in food products and the diet.

Fish and seafood are food sources with a high content of ω-3 PUFA, such as eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3).[1] However, *per capita* fish consumption is quite low compared to that of meat and meat products.[2] Meat industries have a growing interest in producing meat products with a healthier profile.[3] Thus, the development of meat products enriched in ω-3 fatty acids appears to provide a good opportunity for increasing the intake of this type of fatty acids. Enrichment of meat products in ω-3 fatty acids[4,5] and the effect of their intake on health of consumers has been the focus of recent studies.[6]

The increased content of ω-3 PUFA in meat products could be considered advantageous from a nutritional point of view. However, it can lead to several drawbacks resulting from the susceptibility of PUFA to oxidation processes. The breakdown of the ω-3 PUFA chain during oxidation involves nutritional loss and a detrimental sensory, with an unacceptable rancidity and fishy off-flavor.[7] In the particular case of meat products enriched in ω-3 PUFA, which are indeed highly susceptible to oxidative reactions,[8] the method used to add a source of these bioactive compounds was found to influence the development of these reactions and their adverse consequences.[9]

A possible strategy for protecting ω-3 PUFA from oxidation is microencapsulation, which limits the adverse food processing environment, such as contact with water and oxygen, by packaging bioactive functional food components within protective matrix structures.[10] Fish oil is currently microencapsulated using different methods, with the most popular one being spray-drying.[11] Microencapsulation of fish oil by spray-drying using multilayered emulsions of lecithin–chitosan combined with carbohydrates as a coating material was reported to demonstrate successful effects with respect to protection from oxidation.[12–14] However, although enrichment with bulk fish oil (BFO) has been assayed, the literature concerning food enriched with microencapsulated ω-3 PUFA is scarce, and only some bakery products,[15,16] beverages,[17–19] lactic products[20–22] and meat products[23–26] have been produced.

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Fish oil/lycopene microcapsules as a source of eicosapentaenoic and docosahexaenoic acids: a case study on spreads

Juan Carlos Solomando,* Teresa Antequera, Alberto González-Mohíno and Trinidad Perez-Palacios

Abstract

BACKGROUND: The consumption of omega-3 fatty acids has many beneficial effects for human health, but the intake of foods rich in these fatty acids is not enough to achieve the recommended quantity per person and per day, and their direct addition in foods cause oxidation and unacceptable rancidity and off-flavor. Taking account of all these aspects, this study was aimed to develop stable microcapsules of fish oil (omega-3 polyunsaturated fatty acids) and lycopene (antioxidant) and to investigate their effect on different spreads.

RESULTS: The inclusion of different proportions of lycopene in fish oil did not show great benefits in the quality characteristics of emulsions and microcapsules. After the addition of fish oil and fish oil + lycopene microcapsules to dry-cured ham and cheese spreads, no significant differences were found in the proximal composition and oxidative stability, whereas fatty acids composition and sensory analysis were influenced. The eicosapentaenoic and docosahexaenoic acids content increased with the fish oil content in both products, but it decreased significantly after storage in the cheese spreads. Addition of microcapsules did not significantly influence on quantitative–descriptive and acceptability sensory analyses in dry-cured spreads, but it negatively affected the flavor of cheese spreading creams.

CONCLUSION: There is no need to add antioxidants to improve the stability of the fish oil microcapsules in the present study, which are appropriate as eicosapentaenoic acid and docosahexaenoic acid vehicles to enrich meat-derived spreading creams.
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Keywords: omega-3 enrichment; fish oil microcapsules; lycopene; spreads; storage

INTRODUCTION

The consumption of omega-3 polyunsaturated fatty acids (ω−3 PUFAs), especially eicosapentaenoic acid (EPA; C20:5(n−3)) and docosahexaenoic acid (DHA; C22:6(n−3)), has many health benefits for human health,[1] such as a decrease in the risk of cardiovascular disease[2] and the prevention of neurodegenerative and inflammatory diseases (Alzheimer's, allergies, and rheumatoid arthritis).[3–6]

Major sources of EPA and DHA are fish, seafood, and algae. However, the consumption of these foods is insufficient to achieve the recommended quantity of EPA + DHA per person and per day (0.2–0.5 g).[7] Thus, in recent years, dietary supplements and foods fortified with these fatty acids are in high demand. However, the main challenge of enrichment with ω−3 PUFAs is their high susceptibility to oxidation,[8] with the consequent nutritional loss and unacceptable rancidity and off-flavor.[9] In this context, microencapsulation and the addition of antioxidant have been used as possible strategies to avoid ω−3 PUFA oxidation.[10–13]

Microencapsulation is based on creating a physical barrier between the active compounds and the environment, minimizing their contact and reactivity with water, oxygen, iron, and other oxidizing promoters.[14] Spray-drying has been the technique used most to encapsulate ω−3 PUFA-rich oil.[15] The encapsulation of ω−3 PUFAs requires a previous elaboration of oil-in-water emulsions, and their stability is essential to guarantee the encapsulation procedure. Different types of fish oil emulsions have been used to elaborate ω−3 PUFA microcapsules, such as monolayered, multilayered, and double.[16–20] The use of high pressure to homogenize the emulsions has recently been indicated to improve the quality characteristics of emulsions and microcapsules.[21]

Different antioxidants have also been used for counteracting the oxidative susceptibility of ω−3 PUFA sources to enrich food; for example, butylhydroxyanisole, tocopherols, and rosemary extract were evaluated in ground-beef patties fortified with fish oil.[22] In other studies, frankfurters, cooked ham, and cooked turkey breast were enriched with deodorized salmon oil with rosemary extract and vitamin E,[23] and the addition of different antioxidants to fish oil emulsions has been tasted in fresh and dry-cured sausages.[24]

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ORIGINAL ARTICLE

Microencapsulation of oil and protein hydrolysate from fish within a high-pressure homogenized double emulsion

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Abstract In this study, the effect of high-pressure homogenization on the water-in-oil-in-water ($W_1/O/W_2$) double emulsions containing fish protein hydrolysate and fish oil encapsulated within a complex of whey protein concentrate and inulin were investigated in order to produce stable double emulsion. After adequacy of the positive influence of high-pressure homogenization at W_1/O (one pass) and $W_1/O/W_2$ (three passes), the double emulsions were produced with (H) and without (HS) high-pressure homogenization. H samples were demonstrated lower CI of double emulsion and higher amounts of yield, total oil, encapsulated oil, EPA and DHA of microcapsules in comparison with HS samples. At subsequent step, response surface methodology were applied to optimize the high-pressure homogenization conditions (700–1500 Ba) of double emulsions in terms of minimum CI of emulsions and maximum microencapsulation efficiency and oxidation stability. Optimal conditions were obtained by using high-pressure homogenization at 1000 and 1100 Ba on W_1/O and $W_1/O/W_2$, respectively.

Keywords Double emulsion · Fish protein hydrolysate · Fish oil · High-pressure homogenization · Response surface methodology

Introduction

The development of functional foods by using marine sources is a concerned issue. This is mainly due to the high content of omega-3 polyunsaturated fatty acids (ω-3 PUFA), especially eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively), whose consumption has been related to some beneficial effects on human health, such as decreasing the risk of cardiovascular diseases or improving nervous system functions (Jiménez-Martín et al. 2016). Moreover, the use of fish protein hydrolysates (FPH) is an alternative method for using fish-by-products and some under-utilized fish species, i.e. pacific whiting (*Merluccius productus*), anchovy sprat (*Clupeonella engrauliformis*), and bigeye ilisha (*Ilisha megaloptera*) (Pacheco-Aguilar et al. 2008; Ovissipour et al. 2012). In addition, FPH is credited with antioxidant and anti-hypertension properties, and has high digestibility proteins with a balanced amino acid composition (Pacheco-Aguilar et al. 2008). Nevertheless, the addition of marine sources to food is challenging because of the high susceptibility to oxidation of ω-3 PUFA as well as the undesirable bitter taste, rancid and/or fishy flavours that they can impart.

The microencapsulation technique is a highly reported strategy to avoid the inconveniences of adding fish oil to food (Jiménez-Martín et al. 2016). It consists in retaining the oil droplets in an inner matrix by surrounding them with a protective coating. However, the encapsulation of ω-3 PUFA rich oil requires a previous treatment of oil-in-

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Improvements in the methodology for fatty acids analysis in meat products: One-stage transmethylation and fast-GC method

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Meat products
Performance

Chemical compounds:
Chloroform (PubChem CID 6212)
Methanol (PubChem CID 887)
Sodium chloride (PubChem CID 5234)
Anhydrous sodium sulphate (PubChem CID 24436)
Sulphuric acid (PubChem CID 1118)
Sodium metal (PubChem CID 5360545)
Chlorotrimethylsilane (PubChem CID 6397)
Hydrochloric acid (PubChem CID 313)

ABSTRACT

The quantification of fatty acids (FA) in meat products is frequently carried out by two-stage methylation procedures followed by long gas chromatography (GC) runs. This work aimed to simplify this methodology by means of a one-stage transmethylation method and a fast GC run, evaluating the influence of sample preparation, reagents and type of heating on the amount of FA in different meat products and optimizing a fast GC-FID (flame ionization detector) run. This allowed to establish the optimum combination of parameters (methanol + chlorotrimethylsilane, lyophilized samples and oven heating) to achieve the quantification of the highest possible amount of FA and to reduce the time of GC run from 60 to 10 min. The quality evaluation of this method obtained satisfactory results. Thus, the quantification of FA in meat products was achieved in a straightforwardly and quickly way by using a one-stage transmethylation procedure followed by a fast GC-FID run.

1. Introduction

Fatty acid (FA) composition in meat products strongly influences their quality characteristics, such as firmness, oxidative stability, shelf life or flavour. In fact, in dry-cured hams, the FA profile has been used to predict some sensory traits (Pérez-Palacios, Ruiz, Ferreira, Petisca, & Antequera, 2012). The nutritional implications of dietary FA acid composition are also worth noting, since its relationship with promotion and prevention of different diseases, i.e. the consumption of low contents of saturated FA (SFA) and high of monounsaturated and polyunsaturated FA (MUFA and PUFA, respectively) seems to significantly reduce cardiovascular risk factors (Tindall et al., 2019).

Besides, in packaged products, the analysis of FA is required in the case of labelling with nutritional claims about the lipid profile, i.e. "LOW SATURATED FAT: *a claim that a food is low in saturated fat, and any claim likely to have the same meaning for the consumer, may only be made if the sum of saturated fatty acids and trans-fatty acids in the product does not exceed 1.5 g per100 g for solids or 0.75 g/100 ml for liquids and in either case the sum of saturated fatty acids and trans-fatty acids must not provide more than 10 % of energy*." or "HIGH MONOUNSATURATED FAT: *a claim that a food is high in monounsaturated fat, and any claim likely to have the same meaning for the consumer, may only be made where at least 45 % of the fatty acids present in the product derive from monounsaturated fat under the condition that monounsaturated fat provides more than 20 % of energy of the product*" (EU, 2010).

The methodology for the analysis of FA in meat products is not simple, requiring an exhaustive extraction of lipids from the sample, followed by the derivatization of the FA to obtain their volatile FA methyl esters (FAMEs) derivatives, and the analysis of such FAMEs, which is normally carried out by gas chromatography (GC). The usual process initiates with a solvent extraction of lipids followed by their transmethylation. Nowadays, this kind of two-stage procedures are

Abbreviations: FA, fatty acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FAME, fatty acid methyl ester; GC, gas chromatography; FID, flame ionization detector; CTMS, chlorotrimethylsilane; IS, internal standard; LOD, limit of detection; LOQ, limit of quantification; RDS, relative standard deviation; R^2, coefficient of determination; ANOVA, one way analysis of variance.

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5. Foods (2022), 11 (2), 205-211

foods

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Editorial

Improvements in the Procedures to Encapsulate Diverse Bioactive Compounds

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Published articles within the "Microencapsulation of Bioactive Compounds: Techniques and Applications" special issue have been mainly focused on the evaluation of variables affecting the encapsulation of healthy compounds, aiming to achieve accurate quality properties in the encapsulates. Most publications have analyzed variables related to the encapsulation process, applying different techniques and carrying out several determinations to evaluate the quality of the encapsulates themselves. There are few articles at evaluating the influence of the addition of the encapsulates to food.

Five main groups of publications can be differentiated depending on the bioactive compounds they have focused on: with recognized antioxidant properties (tomato oil as a source of lycopene, thyme essential oil as a source of phenolic compounds, oleoresin of Haematococcus pluvialis as a source of astaxanthin, Cornelian cherry fruits as a source of anthocyanins and resveratrol solutions), with high content in polyunsaturated fatty acids (hempseed oil and fish oil as source of omega-3 fatty acids), flavones (tangerine oil as a source of tangeretin and a commercial genistein solution), flavorings (ethyl acetate and vanilla) and probiotics (*Lactobacillus rhamnosus* and *L. casei*).

Table 1 summarizes the encapsulation variables evaluated for the different bioactive compounds that have been studied in the publication of this issue. Starting with those compounds with recognized antioxidant properties, the ability of α-, β- and γ-cyclodextrins to stabilize emulsions with tomato oil and to form powders has been evaluated by Durante et al. [1]. α-cyclodextrins achieved the highest encapsulation efficiency, while β-cyclodextrins showed the best oil dispersion. α- and β-cyclodextrins demonstrated better performance than γ-cyclodextrins in relation to the antioxidant activity of emulsions and powders, although their stability was not affected by the cyclodextrin type, with a rapid decline in the carotenoid content. On the other hand, the capability of γ-cyclodextrins to form a complex with resveratrol has been confirmed by Silva et al. [2], who showed a true inclusion complex with the resveratrol inside the cavity of the γ-cyclodextrin channels. In the study of González-Reza et al. [3], the influence of different encapsulating (poly-ε-caprolactone and ethylcellulose) and stabilizing (polyvinyl alcohol and Pluronic® F-127) polymers on quality parameters of thyme essential oil nanocapsules has been evaluated. The use of poly-ε-caprolactone and polyvinyl alcohol had a positive effect on physical, antioxidant and stability properties of the nanocapsules of thyme essential oil, being inferred its potential application in food processing and preservation. The effect of including dodecanol as a membrane stabilized in resveratrol niosomes formulated with mixtures of Tween 80 and Span 80 has been evaluated by Machado et al. [4]. The presence of dodecanol preserved the antioxidant capacity and release of resveratrol without affecting the general properties of niosomes, such as size or shape. The use of lupin protein isolate, carrageenan and chitosan as ionic interfacial layers to stabilize multilayer emulsions of astaxanthin oleoresin has been evaluated by Morales et al. [5]. In this study, lupin protein isolate has been used to obtain the first layer, which covers the oily phase. Then, carrageenan and chitosan were added over, obtaining second and third layers, respectively. Although all emulsions

check for updates

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6. Eurocarne (2019), 276, 39-46

Nuggets de pollo enriquecidos con microcápsulas de ácidos grasos omega-3

En este artículo se presentan los resultados de un estudio centrado en evaluar el efecto sobre las características físico-químicas, estabilidad oxidativa y calidad sensorial del enriquecimiento en omega-3 mediante la adición de microcápsulas de aceite de pescado a nuggets *de pollo.*

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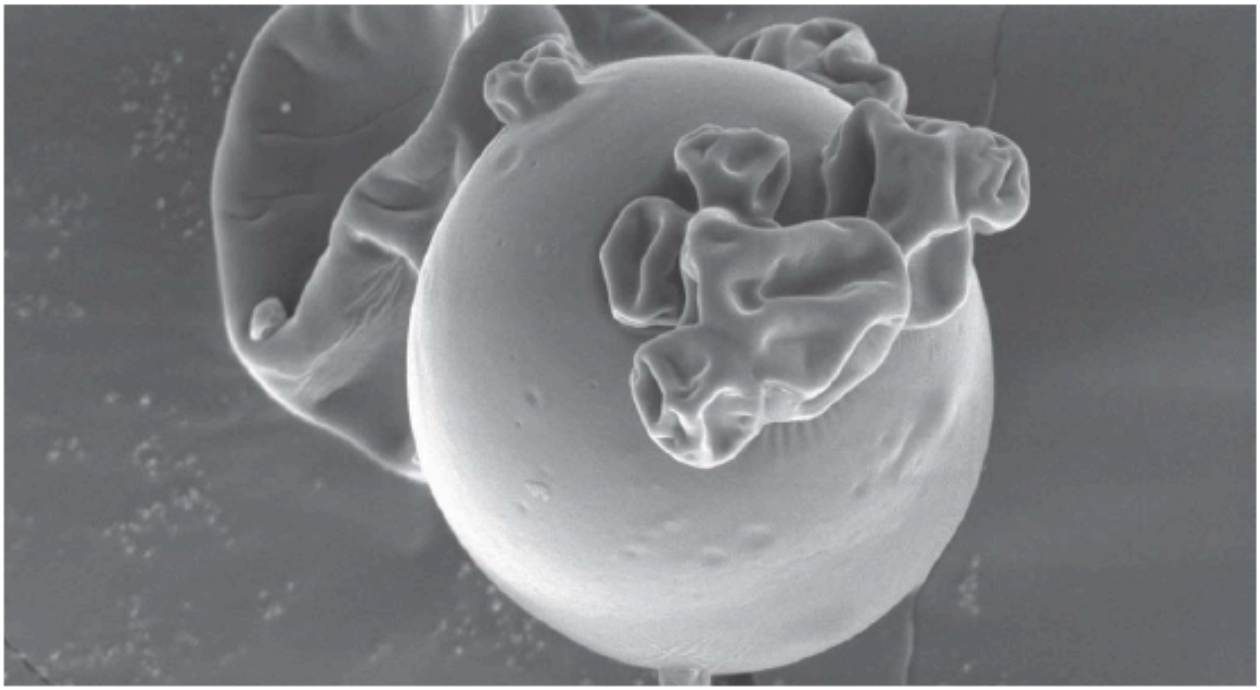
Resumen

En el presente trabajo se ha evaluado el efecto del enriquecimiento en omega-3 mediante la adición de microcápsulas de aceite de pescado sobre las características físico-químicas, estabilidad oxidativa y calidad sensorial de *nuggets* de pollo. Los resultados encontrados han permitido probar que la adición de microcápsulas de aceite de pescado a la masa de *nuggets* es una estrategia adecuada para enriquecer este producto en ácidos grasos omega-3. Las prácticas industriales (prefritura y congelación durante 3

Palabras clave: enriquecimiento en omega-3, microcápsulas, nuggets de pollo, calidad tecnológica, calidad sensorial.

7. Eurocarne (2021), 294, 49-56



Derivados cárnicos cocidos y curados enriquecidos con microcápsulas de ácidos grasos omega-3

En el presente artículo se muestran los resultados obtenidos al evaluar el efecto del enriquecimiento en ácidos grasos omega-3 mediante la adición de diferentes tipos de microcápsulas (monocapa y multicapa) de aceite de pescado a dos tipos de derivados cárnicos listos para su consumo, uno cocido y otro curado.

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Resumen

En el presente estudio se ha evaluado en diferentes derivados cárnicos, el efecto del enriquecimiento en omega-3 mediante la adición de distintos tipos de microcápsulas (monocapa y multicapa) de aceite de pescado sobre las características físico-químicas, estabilidad oxidativa, perfil de ácidos grasos y bioaccesibilidad. Los resultados obtenidos han permitido probar que la adición de los dos tipos de microcápsulas desarrolladas a derivados cárnicos cocidos (ti-

Palabras clave:
Microcápsulas, enriquecimiento en omega-3, derivados cárnicos, calidad tecnológica, estabilidad oxidativa, bioaccesibilidad.

Marzo 2021

8. Eurocarne (2021), 295, 48-56

Derivados cárnicos cocidos y curados enriquecidos con microcápsulas de ácidos grasos omega-3: aspectos sensoriales

En este artículo se ha evaluado el efecto del enriquecimiento en ácidos grasos omega-3 sobre la calidad sensorial de diferentes derivados cárnicos mediante la adición de diferentes tipos de microcápsulas (monocapa y multicapa) de aceite de pescado, empleando para ello técnicas estáticas y dinámicas de evaluación sensorial y considerando el efecto de estímulos extrínsecos.

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Resumen

En el presente estudio se han evaluado las propiedades sensoriales de salchichas tipo Viena y fuet enriquecidos con microcápsulas monocapa y multicapa de aceite de pescado como vehículos de ácidos grasos omega-3 mediante la combinación de diferentes técnicas sensoriales: análisis cuantitativo descriptivo, dominio temporal de sensaciones, estudio hedónico e intención de compra, para lograr una evaluación

Palabras clave:
Microcápsula, enriquecimiento en omega-3, salchicha tipo Viena, fuet, análisis sensorial, intención de compra, efecto información nutricional.

Abril 2021

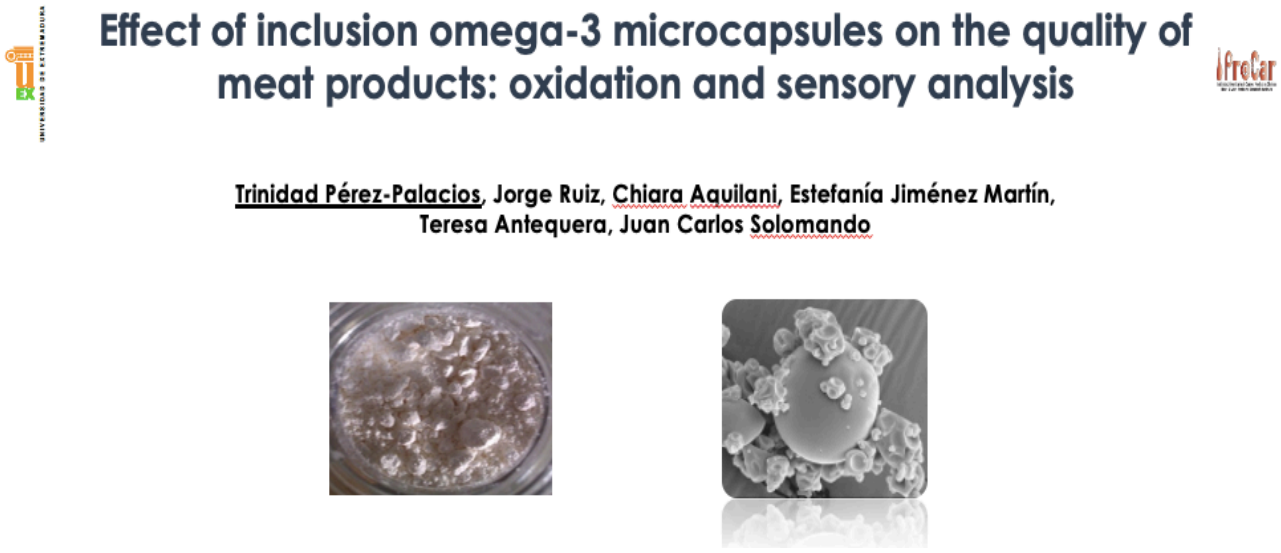
Anexo II

Comunicaciones orales y posters en congresos nacionales e internacionales

1. 31st EFFoST International Conference (2017). Sitges, España
2. 32nd EFFoST International Conference (2018). Nantes, Francia
3. 32nd EFFoST International Conference (2018). Nantes, Francia
4. I Congreso Nacional de Investigadores en Formación (2018). Granada, España
5. X Congreso Nacional CyTA-CESIA (2019). León, España
6. X Congreso Nacional CyTA-CESIA (2019). León, España
7. X Congreso Nacional CyTA-CESIA (2019). León, España
8. V Jornadas de Ciencias de la Salud (2019). Cáceres, España.
9. 17th Euro Fed Lipid Congress (2019). Sevilla, España
10. 17th Euro Fed Lipid Congress (2019). Sevilla, España
11. 17th Euro Fed Lipid Congress (2019). Seville, España
12. 33rd EFFoST International Conference (2019). Rotterdam, Holanda.

1. 31st EFFoST International Conference (2017). Sitges, España

Effect of inclusion omega-3 microcapsules on the quality of meat products: oxidation and sensory analysis



2. 32nd EFFoST International Conference (2018). Nantes, Francia

Microencapsulation of lycopene and omega-3 fatty acids by spray-drying

32nd EFFoST International Conference

Microencapsulation of lycopene and omega-3 fatty acids by spray-drying

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INTRODUCTION

The health effects of consumption of omega-3 polyunsaturated fatty acids (ω-3 PUFA) are scientifically proven, but the consumption of foods rich in these fatty acids is not high enough. This is leading to food added with ω-3 PUFA. Main concern for enriching food ω-3 PUFA is their high susceptibility to oxidation. Microencapsulation of fish oil or antioxidant addition to sources of ω-3 PUFA have been used to solve this problem. This study tries to combine these two strategies.

OBJECTIVE

The objective of this study was to develop microcapsules of fish oil and lycopene.

METHODOLOGY

PRIMARY EMULSION (E) 200 g ← Fish oil (F) + Olive oil with lycopene (L) (20 g) + Lecithin (6 g) + Distilled water (174 g)

↓ High pressure homogenization

↓ + Distilled water (200 g)

↓ + Maltodextrin solution (400 g)

FEED EMULSION (E) 800 g → Creaming index (CI)

↓ Spray-drying

MICROCAPSULES (M) MFL20; MFL15; MFL10; MFL5; MFL0 → Efficiency [2]; Oxidation (TBARs) [3]; Fatty acid composition (GC-FID) [4]; Scanning electron microscopy (SEM)

EXPERIMENTAL BATCHES:
1. EFL20 (100% F)
2. EFL15 (75% F-25% L)
3. EFL10 (50% F-50% L)
4. EFL5 (25% F-75% L)
5. EFL0 (100% L)

ACHIEVEMENTS

The inclusion of L does not influence the CI of the emulsions, efficiency (Figure 1) nor morphology of the microcapsules (Figure 2). As expected, the content of eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) in microcapsules increased with the content of F. Values of TBARs (Figure 3) significantly decreased as the percentage of L increased. Storage during 1 month at 25 ºC led to higher TBARs level in all analysed batches of microcapsules.

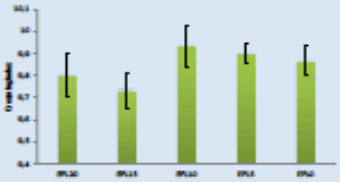


Figure 1. Efficiency of microcapsules with different percentage of F and L.

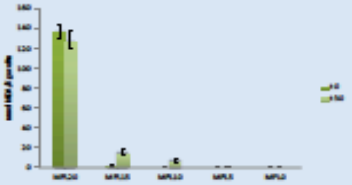
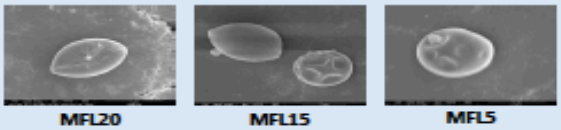


Figure 3. Values of TBARs of microcapsules with different percentage of F and L at day 0 (t0) and after 30 days storing (t30).



MFL20 MFL15 MFL5

Figure 2. SEM of microcapsules with different percentage of F and L

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Acknoledgements

CONCLUSIONS

The addition of L does not influence stability of emulsions nor efficiency and morphology of microcapsules and allow increasing their oxidation stability. Thus, the addition of microcapsules of FL could be a strategy to enrich food in ω-3 PUFA avoiding oxidation problems.

3. 32nd EFFoST International Conference (2018). Nantes, Francia

Effect of inclusion microcapsules of lycopene and omega-3 fatty acids on pork pâté

32nd EFFoST International Conference

Effect of inclusion microcapsules of lycopene and omega-3 fatty acids on pork pâté

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INTRODUCTION

The beneficial effects of omega-3 polyunsaturated fatty acids (ω-3 PUFA) on human health have been demonstrated for years. Main sources of these fatty acids are fish and seafood, which have a low consumption. Main concern to add ω-3 PUFA to food is their high susceptibility to oxidation. The use of antioxidant and microencapsulation are some strategies to solve this problem.

OBJECTIVE

The aim of this study was to analyse meat products (pork pâté) added with lycopene and omega-3 microcapsules in terms of oxidative stability and sensory properties.

METHODOLOGY

Effect of omega-3 and lycopene enrichment

Pork pâté

Effect of storage time: 0 days (t0) and 4 months at 20-22 ºC (t4)

Microcapsules were prepared according to [1].
Samples were analysed by means of:

- Fatty acid composition (GC-FID) [2]
- Lipid oxidation (TBARs) [3]
- Sensory analysis (acceptability)

ACHIEVEMENTS

The highest content of ω-3 PUFA (eicosapentaenoic and docosahexaenoic acids) were found in F samples, followed by FL ones, whereas C pâtés did not show these fatty acids. Oxidation values were higher in C than in F and FL samples at t4 (Figure 1), with no differences found at t0. FL samples obtained lower scores for acceptability than C and F ones at t0 and t4 (Figure 2).

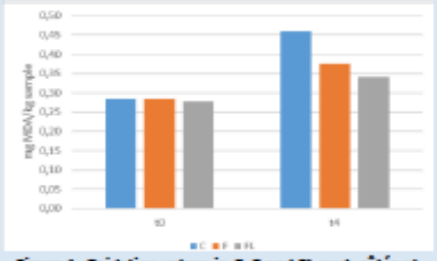

Figure 1. Oxidation values in C, F and FL pork pâtés at time 0 (t0) and after four months of storage (t4).

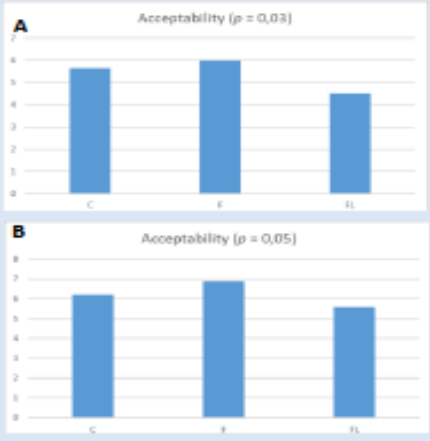

Figure 2. Acceptability scores for C, F and FL pork pâtés at time 0 (A) and after four months of storage (B).

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Acknoledgements

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CONCLUSIONS

Addition of microcapsules of F and FL is appropriate to enrich pork pâtés in ω-3 PUFA and avoids lipid oxidation during storage at ambient temperature. However, the enrichment with FL microcapsules seems to decrease the acceptability of samples. More investigations on FL microencapsulation should be carried out.

4. I Congreso Nacional de Investigadores en Formación (2018). Granada, España

Avances en el estudio de la microencapsulación de ácidos grasos Omega-3 para su incorporación a derivados cárnicos

5. X Congreso Nacional CyTA-CESIA (2019). León, España.

Mejora del perfil lipídico de derivados cárnicos mediante la adición de microcápsulas de aceite de pescado

MEJORA DEL PERFIL LIPÍDICO DE PRODUCTOS CÁRNICOS MEDIANTE LA ADICIÓN DE MICROCÁPSULAS DE ACEITE DE PESCADO

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El enriquecimiento de productos cárnicos mediante la adición de microcápsulas de aceite de pescado ha sido probado en diferentes productos cárnicos, mostrando resultados satisfactorios en cuanto a su estabilidad oxidativa y atributos sensoriales. No obstante, es necesario la evaluación de otros parámetros y efectos.

INTRODUCCIÓN

Existen evidencias científicas sobre los efectos beneficiosos que los ácidos grasos EPA y DHA tienen sobre la salud humana [1]. Sin embargo, el consumo de la principal fuente de estos compuestos bioactivos (pescado) no es suficiente para alcanzar la dosis recomendada [1]. Consecuentemente, se vienen llevando a cabo numerosos trabajos con el objetivo de enriquecer diferentes alimentos en omega-3.

La carne y los productos cárnicos son alimentos muy valorados por los consumidores, debido a sus características sensoriales. Además, aportan proteínas de alto valor biológico, vitaminas (B6 and B12) y minerales (hierro, selenio y cinc). Sin embargo, el perfil lipídico de la carne y los productos cárnicos no es nutricionalmente deseable en algunos casos, debido a que contiene un alto porcentaje de ácidos grasos saturados y bajo de poliinsaturados. Por ello, la modificación de dicho perfil en este tipo de alimentos mediante el enriquecimiento en omega-3 resulta de especial interés. Las principales estrategias empleadas para este fin han sido el enriquecimiento a través de la alimentación animal, la adición de aceites ricos en EPA y DHA a los productos cárnicos de forma directa, como emulsión o en forma de microcápsulas.

El objetivo del presente trabajo fue realizar una revisión sobre estudios centrados en enriquecer productos cárnicos en omega-3 mediante la incorporación de microcápsulas.

MÉTODOS

Se realizó una búsqueda en Web of Science, Scopus y Science Direct, empleando como palabras clave: omega-3, encapsulation, meat. Se seleccionaron los artículos que cumplieron el criterio de búsqueda de acuerdo con el objetivo indicado, todos ellos publicado en el Journal Citation Report

RESULTADOS Y DISCUSIÓN

Tras la búsqueda bibliográfica realizada se han encontrado seis artículos científicos sobre enriquecimiento de productos cárnicos en omega-3 mediante la adición de microcápsulas (Tabla 1). En todos los estudios la fuente de ácidos grasos omega-3 fue aceite de pescado, aunque se emplearon diferentes tipos de microcápsulas. En general, los resultados encontrados fueron aceptables. No obstante, en ninguno de los artículos se ha cuantificado la cantidad de EPA+DHA ni se ha evaluado su digestibilidad, y solo en dos de ellos se ha evaluado el efecto del procesado y/o cocinado [3,4].

Tabla 1. Principales resultados sobre el enriquecimiento en omega-3 de productos cárnicos mediante la adición de microcápsulas.

PRODUCTO	TIPO DE MICROCÁPSULA	CARACTERÍSTICAS DE LOS PRODUCTOS				REFERENCIA
		EPA	DHA	NIVEL DE OXIDACIÓN	CALIDAD SENSORIAL	
Embutido fermentado	Comercial	↑	↑	↑	↑	6
Embutido fermentado	Comercial	↑	↑	=	↑	7
Nugget de pollo	Elaboradas mediante spray-drying a partir de emulsiones	NI	NI	=	=	2,3
Burguer meat de cerdo	Elaboradas mediante spray-drying a partir de emulsiones	↑	↑	=	=	4
Embutido curado	Elaboradas mediante spray-drying a partir de emulsiones y dentro de una matriz de konjac	↑	↑	↑	↓	5

NI: no se indica; ↑, ↓, = : aumento, disminución o sin cambios, respectivamente, frente a un lote control.

CONCLUSIONES

Para conseguir un enriquecimiento en omega-3 en productos cárnicos, la adición de microcápsulas de aceite de pescado parece ser una estrategia adecuada. Sin embargo, son necesarios futuros estudios que i) cuantifiquen la cantidad exacta de EPA+DHA en la matriz cárnica, ii) que analicen la digestibilidad y iii) evalúen el efecto del procesado y/o cocinado

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AGRADECIMIENTOS: Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER) por la financiación del proyecto AGL2016-313 73260-JIN (AEI/FEDER/UE). Junta de Extremadura, Consejería de Economía e Infraestructuras- Ayuda GR18104.

6. X Congreso Nacional CyTA-CESIA (2019). León, España

Caracterización de microcápsulas procedentes de diferentes tipos de emulsiones de aceite de pescado homogeneizadas a alta presión

X Congreso Nacional CyTA · CESIA
León, 15-17 mayo 2019
Hotel Real Colegiata San Isidoro
CIENCIA Y TECNOLOGÍA DE LOS ALIMENTOS • INGENIERÍA DE LOS ALIMENTOS

CARACTERIZACIÓN DE MICROCÁPSULAS PROCEDENTES DE DIFERENTES TIPOS DE EMULSIONES DE ACEITE DE PESCADO HOMOGENEIZADAS A ALTA PRESIÓN

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Se han evaluado diferentes tipos de emulsiones de aceite de pescado (simple vs multicapa), homogeneizadas a alta presión, y sus microcápsulas, obtenidas mediante spray-drying. Se han encontrado mejores características de calidad en las simples, lo que podría deberse a un mayor efecto de la homogeneización a alta presión en estas emulsiones.

INTRODUCCIÓN

La obtención de microcápsulas de aceite de pescado mediante spray-drying requiere la elaboración de una emulsión previa [1], que puede ser simple o modificada, como es el caso de las emulsiones multicapa [2]. La homogeneización a presión de estas emulsiones puede mejorar su estabilidad y las características de las microcápsulas [3].

El objetivo de este trabajo es comparar las características de calidad emulsiones simples y multicapa, homogeneizadas a alta presión, y de sus correspondientes microcápsulas.

MATERIALES Y MÉTODOS

Se prepararon emulsiones simples (ES) y multicapa (EM) siguiendo la metodología descrita por Jiménez-Martín et al. [2]. En primer lugar, se elaboró una emulsión primaria con aceite de pescado y lecitina, que fue homogeneizada a alta presión bajo condiciones previamente optimizadas (datos no publicados). Seguidamente, se le añadió a la emulsión primaria homogeneizada agua, en el caso de la ES, y chitosan en ácido acético, en el caso de la EM. Finalmente, en ambos casos, como material de pared se utilizó maltodextrina para obtener la emulsión final. Esta emulsión se sometió a un proceso de spray-drying, obteniéndose así las correspondientes microcápsulas, MS y MM.

En las emulsiones se determinó el Creaming Index (CI), y en las microcápsulas el rendimiento (R), la eficiencia de la microencapsulación (EM), la humedad (H), la estabilidad oxidativa (TBARs), y también se observaron mediante microscopía electrónica de barrido (SEM), tal y como se describe en [2].

Tabla 1. Características de calidad de emulsiones (E) simples y multicapa y sus correspondientes microcápsulas (M)

		Simple	p	Multicapa	p
E	CI	0.63 ± 0.18	<0.001	11.71 ± 0.58	0.187
M	R	50.71 ± 1.39	0.014	42.20 ± 1.30	0.591
	EM	1.65 ± 0.03	0.535	2.88 ± 0.14	0.003
	H	66.88 ± 0.46	<0.001	55.43 ± 4.60	0.002
	TBARs	231.87 ± 22.0	0.016	28.90 ± 0.21	<0.001

RESULTADOS Y DISCUSIÓN

En las ES de este estudio las gotas de grasa se encuentran emulsificadas con lecitina y recubiertas con una pared de maltodextrina, mientras que en las EM la lecitina, con carga negativa al pH de la emulsión, se une electrostáticamente al chitosan y a la maltodextrina, (carga +). Se encontraron diferencias estadísticamente significativas en todos los parámetros analizados debido al efecto del tipo de emulsión. Las ES presentaron un menor CI que las EM, y las MS obtuvieron mayores valores de R, EM, TBARs y menor porcentaje de H que las MM. Además, en las imágenes de SEM se observa que las MS no presentan muchas aglomeraciones de partículas, tienen una forma ahuevada, con algunas arrugas, pero sin poros ni hoyuelos. Sin embargo, en las MM se observan aglomeraciones de partícula, y tienen poros y hoyuelos. Estos resultados indican, en general, mejores características de calidad en las ES y MS homogeneizadas. Sin embargo, los resultados obtenidos previamente en estudios sin homogeneización a alta presión no han mostrado este efecto [2]. Esto podría estar relacionado con la homogeneización a alta presión, que parece influir de forma positiva en las ES pero no a las EM, probablemente debido a la adición de chitosan en estas emulsiones, que disminuye el pH e intensifica la unión de los componentes de la pared.

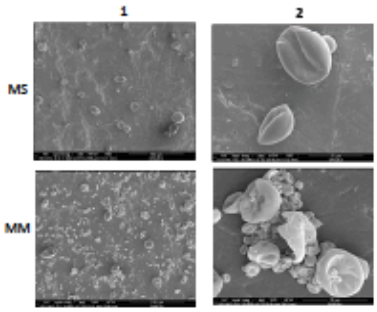

Figura 1. Microscopía electrónica de barrido de microcápsulas simples (MS) y multicapa (MM) a nivel general (1) e individual (2) a x1000 y x12.000 aumentos, respectivamente.

CONCLUSIONES

El tipo de emulsión homogeneizada a alta presión (S vs M) influye sobre las características de calidad de las emulsiones y sus correspondientes microcápsulas. Las ES y MS presentan mejores características de calidad que las EM y MM, respectivamente y el efecto de la homogeneización a alta presión parece ser más efectivo en las ES que en las EM.

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AGRADECIMIENTOS: Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER) por la financiación del proyecto AGL2016-313 73260-JIN (AEI/FEDER/UE). Junta de Extremadura, Consejería de Economía e Infraestructuras-Ayuda GR18104.

7. X Congreso Nacional CyTA-CESIA (2019). León, España

Cuantificación de ácidos grasos Omega-3 microencapsulados en diferentes sistemas modelo: estudio de la digestibilidad

CUANTIFICACIÓN DE ÁCIDOS GRASOS OMEGA-3 MICROENCAPSULADOS EN DIFERENTES SISTEMAS MODELO: ESTUDIO DE LA DIGESTIBILIDAD

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El enriquecimiento en ácidos grasos omega-3 mediante la adición de microcápsulas de aceite de pescado es más eficaz en un sistema modelo cárnico que en uno lácteo. Sin embargo, la digestibilidad de estos compuestos bioactivos es similar en ambos sistemas, obteniéndose importantes cantidades de omega-3 biodisponible.

INTRODUCCIÓN

La ingesta EPA y DHA tiene efectos positivos sobre la salud, estando establecidas dosis adecuadas de ingesta (250 mg por persona y día) [1]. Sin embargo, el consumo actual de la principal fuente de estos compuestos bioactivos (pescado) es insuficiente para alcanzar estas cantidades. Existen dos declaraciones nutricionales que podrían indicarse en el etiquetado de los alimentos "Fuente de ácidos grasos omega-3" y "Rico en ácidos grasos omega-3" (mínimo 40 y 80 mg EPA+DHA/100 g producto, respectivamente). Para conseguir estos niveles, puede incorporarse aceite de pescado microencapsulado [2]. Los trabajos científicos dedicados a este fin han evaluado principalmente el efecto de la incorporación de las microcápsulas sobre el porcentaje de EPA y DHA, la estabilidad oxidativa y calidad sensorial en los productos enriquecidos. Sin embargo, es necesaria una correcta cuantificación de estos ácidos grasos para poder llegar a etiquetar los alimentos con alguna de las declaraciones nutricionales.

El objetivo del presente trabajo fue cuantificar la cantidad de EPA y DHA y además evaluar su digestibilidad en dos sistemas modelos (cárnico y lácteo) enriquecidos con microcápsulas de aceite de pescado.

MATERIALES Y MÉTODOS

Se elaboraron microcápsulas de omega-3 (Figura 1) a partir de emulsiones simples de aceite de pescado [2]. Se elaboraron dos sistemas modelos, uno cárnico (salchicha cocida) y otro lácteo (yogur), a los que se adicionaron las cantidades correspondientes de microcápsulas para poder etiquetarlos como "Fuente de ácidos grasos omega-3". En los sistemas modelos se cuantificó la cantidad de EPA y DHA mediante GC-FID, y un estudio de digestibilidad [3].

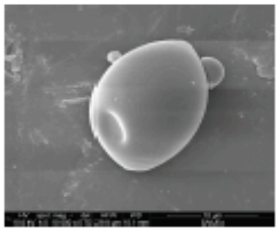

Figura 1. Microcápsula de aceite de pescado vista mediante microcopía electrónica de barrido.

RESULTADOS Y DISCUSIÓN

La cantidad de EPA+DHA en los sistemas modelos cárnico y lácteo fue de 47.80 y 13.99 mg/100 g producto, respectivamente. Esto indicaría que el sistema modelo cárnico cocido podría etiquetarse como "Fuente de ácidos grasos omega-3", pero no el sistema modelo lácteo, en el que se podría haber producido una pérdida de EPA y DHA durante el procesado. El consumo de una ración de estos dos sistemas alimentarios (3 salchichas cocidas, 102 g; 1 yogur, 125 g) supondría un 20 y 7 % de la dosis adecuada de ingesta de EPA+DHA establecida, respectivamente. Esto supone un importante aporte de estos compuestos bioactivos, sobre todo en el caso del sistema modelo cárnico. En los ensayos de digestibilidad se ha encontrado que las cantidades de EPA y DHA son inferiores en el fluido gástrico que en el intestinal, tanto en el sistema modelo cárnico (0.05 y 0.32 mg EPA+DHA/g muestra, respectivamente) como en el lácteo (0.09 y 0.15 mg EPA+DHA/g muestra, respectivamente) (Figura 2). El porcentaje de digestibilidad de EPA+DHA en intestino fue del 66.78 y 62.81 %, en los sistemas modelo cárnico y lácteo, respectivamente, y por lo tanto el porcentaje de EPA y DHA que se encuentra biodisponible para ser absorbido, en relación a la cantidad ingerida, es alto e en los dos sistemas modelo ensayados.

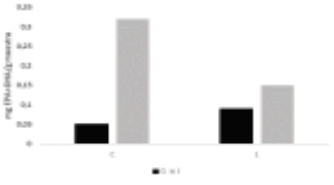

Figura 1. Cantidad de EPA+DHA en los fluidos gástrico (G) e intestinal (I) de la digestión in vitro de los sistemas modelo cárnico (C) y lácteo (L) enriquecidos con microcápsulas de aceite de pescado.

CONCLUSIONES

La adición de microcápsulas procedentes de emulsiones simples de aceite de pescado consigue enriquecer en omega-3 un sistema modelo cárnico cocido de forma eficiente, mientras que su incorporación a un sistema lácteo de yogur no resulta totalmente eficaz. No obstante, en ambos sistemas modelo, la digestibilidad de EPA y DHA es notable.

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AGRADECIMIENTOS: Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER) por la financiación del proyecto AGL2016-313 73260-JIN (AEI/FEDER/UE). Junta de Extremadura, Consejería de Economía e Infraestructuras- Ayuda GR18104.

8. V Jornadas de Ciencias de la Salud (2019). Cáceres, España.

Enriquecimiento en Omega-3 mediante microcápsulas de aceite de pescado: estudio en sistemas modelos cárnicos

9. 17th Euro Fed Lipid Congress (2019). Sevilla, España

Fish oil microcapsules as a vehicle to enrich cooked sausages

17th Euro Fed Lipid Congress and Expo
20 – 23 October 2019
Seville · Spain

FISH OIL MICROCAPSULES AS A VEHICLE TO ENRICH COOKED SAUSAGES

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INTRODUCTION

A diet rich in omega-3 polyunsaturated fatty acids (ω-3 PUFA) has been demostrated to reduce the risk of cardiovascular disease, some types of cancer and to prevent of neurodegenerative and inflammatory diseases. Fish, seafood and algae are the main sources of eicosapentaenoic (EPA; C20:5 n-3) and docosahexaenoic (DHA; C22:6 n-3) acids, but their consumption is quite low, not being enough to reach the adequate intake of 1.5 g of EPA + DHA per week [1]. Currently, the meat industry is interested in producing healthier meat products with ω-3 PUFA, but these fatty acids are highly susceptible to oxidation. The microencapsulation seems to be a good strategy to avoid it [2].

This study aims to evaluate the viability of different types of fish oil microcapsules as vehicle of EPA and DHA in cooked sausages.

MATERIAL AND METHODS

Three batches of sausages (C-SAU) were prepared: control (Co), enriched with monolayered (Mo) and multilayered microcapsules (Mu). All batches were analyzed before and after heating (BH and AH, respectively) at 90 °C during 3 min. Samples were analyzed by TBARs lipid oxidation, EPA and DHA quantities and scan electron microscopy.

RESULTS AND DISCUSSION

The addition of Mo and Mu increased the quantity of EPA and DHA, which were not affected by the heating of the sausages. The lipid oxidation stability were not significantly influenced by the type of microcapsule or the heating procedure. The scan electron microscopy images showed the integrity of the microcapsules wall in sausages enriched with Mo and Mu.

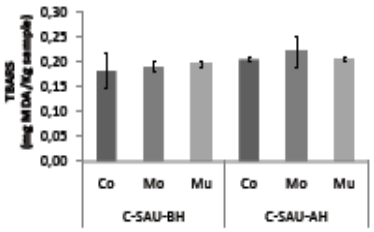

Figure 1. Lipid oxidation of cooked sausages as affected by the type of fish oil microcapsules added and heating process*
* not enriched (Co) and added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules; before and after heating (BH and AH)

Table 1. Fatty acid composition (mg FAMEs/g sample) in sausages (C-SAU) as affected by enrichment with fish oil microcapsules (pE) and heating process (pH)*
* not enriched (Co) and added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules; before and after heating (BH and AH)

mg FAMEs/g sample	EPA				DHA			
	Co	Mo	Mu	pE	Co	Mo	Mu	pE
C-SAU-BH	Nd[b]	0.16±0.05[a]	0.15±0.01[a]	<0.001	Nd[b]	0.30±0.04[a]	0.32±0.05[a]	<0.001
C-SAU-AH	Nd[b]	0.14±0.03[a]	0.14±0.03[a]	<0.001	Nd[b]	0.28±0.02[a]	0.29±0.02[a]	<0.001
pH	-	0.639	0.541		-	0.661	0.506	

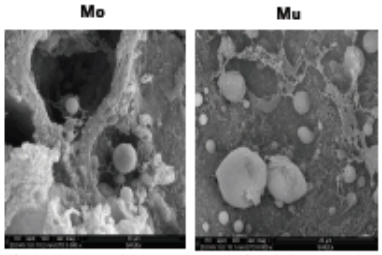

Figure 1. Scaning electron microscopy of cooked sausages (C-SAU) added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules.

CONCLUSIONS

The effectiveness of Mo and Mu microcapsules to enrich cooked meat products undergoing thermal cooking treatments, which allows them to be labeled as "source of omega-3 fatty acids" since all the batches analyzed exceeded the minimum amounts of 40 mg. of the sum of EPA and DHA per 100 grams and per 100 Kcal of product [3].

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ACKNOWLEDGEMENTS

Agencia Estatal de Investigación (AEI) and the Fondo Europeo de Desarrollo Regional (FEDER) the funding for this study, which was supported by the project AGL2016-73260-JIN (AEI/FEDER/UE).

European Federation for the Science and Technology of Lipids

10. 17th Euro Fed Lipid Congress (2019). Sevilla, España

Fish oil microencapsulated as a source of EPA and DHA in Yogurt model system

17th Euro Fed Lipid Congress and Expo
20 – 23 October 2019
Seville · Spain

FISH OIL MICROENCAPSULATED AS A SOURCE OF EPA AND DHA IN YOGURT MODEL SYSTEM

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INTRODUCTION

Consumers in developed countries are increasingly interested in the consumption of healthy foods of animal origin, particulairly those enriched with omega-3 polyunsaturated fatty acids (ω-3 PUFA). The main sources of these fatty acids are fish, and seafood, however, the consumption of these products is not sufficient to achieve a recomended intake of 0.2-0.25 g of EPA + DHA per person and day mainly due to their low acceptance among the population [1]. Dairy should be adequate foods to be enriched in ω-3 PUFA, because of their high frecuency of consumption (2-4 portions per day) [2]. However, direct addition of EPA and DHA sources should not be adequate, due to their negative impact on sensory attributes and their high susceptibility to oxidation [3]. The microencapsulation seems to be a good strategy to avoid these drawbacks [4].

The objective of this study was to evaluate the viability of different types of microcapsules to enrich in EPA and DHA a yogurt model system.

MATERIAL AND METHODS

Three batches of yogurt model systems (YMS) were prepared: control (Co), enriched with monolayered (Mo) and multilayered microcapsules (Mu). All batches were analyzed BF and AF fermentation procedure at 40 °C during 12 hours. Primary and secondary lipid oxidation products and EPA and DHA quantities were analyzed in the samples.

RESULTS AND DISCUSSION

The quantity of EPA and DHA increased with the addition of Mo and Mu, which did not influenced on the lipid oxidation values. The fermentation did not affect lipid oxidation values, but it significantly decreased EPA and DHA quatities. This could be releated to high solubilization properties of the the wall material of the microcapsules (maltodextrine).

Table 1. Fatty acid composition (mg FAMEs/g sample) in yogurt model system (YMS) as affected by enrichment with ω-3 PUFA (pE) and fermentation process (pF)*
* not enriched (Co) and added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules; before and after fermentation procedure (BF and AF)

mg FAMEs/g	EPA				DHA			
sample	Co	Mo	Mu	pE	Co	Mo	Mu	pE
YMS-BF	Nd[b]	0.13±0.03[a]	0.14±0.04[a]	<0.001	Nd[b]	0.28±0.04[a]	0.30±0.09[a]	<0.001
YMS-AF	Nd[b]	0.05±0.03[a]	0.07±0.02[a]	<0.001	Nd[b]	0.16±0.06[a]	0.14±0.04[a]	<0.001
pF	-	<0.001	<0.001		-	<0.001	<0.001	

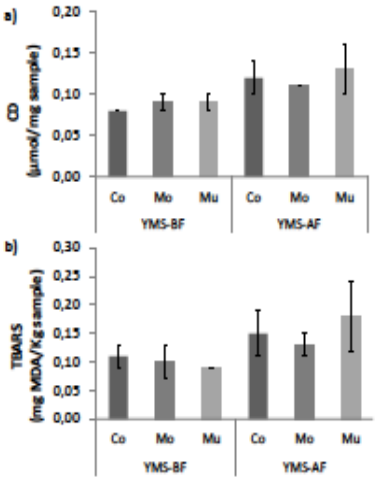

Figure 1. Lipid oxidation, determined by CDs (a) and TBARs (b), in yogurt model system, as affected by the type of fish oil microcapsules added and fermentation procedure*
* not enriched (Co) and added with monolayered (Mo) and multilayered (Mu) fish oil microcapsules; before and after fermentation procedure (BF and AF)

CONCLUSIONS

The addition of the Mo and Mu would not be appropriate to enrich yogurt type products in EPA and DHA.

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European Federation for the Science and Technology of Lipids

11. 17th Euro Fed Lipid Congress (2019). Seville, España

Effect of the meat matrix on the Omega-3 enrichment with fish oil microcapsules

Effect of the meat matrix on the omega-3 enrichment with fish oil microcapsules

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12. 33rd EFFoST International Conference (2019). Rotterdam, Holanda

EPA and DHA in fish oil microcapsules and enriched meat model systems: quantification and release during *in vitro* digestion

EPA and DHA in fish oil microcapsules and enriched meat model systems: quantification and release during in vitro digestion

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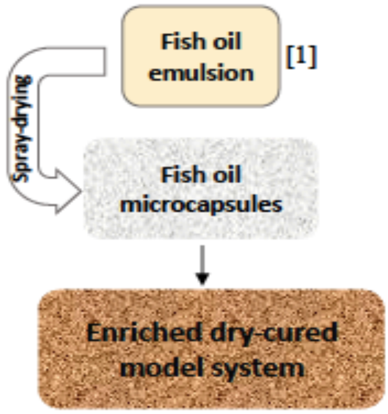
INTRODUCTION

Different types of omega-3 microcapsules have been used to enrich meat and meat products, but no data about the quantity of EPA and DHA (expressed as mg per g sample) and the releasing of these fatty acids in the gastrointestinal tract has been published.

OBJECTIVE

This study aimes to quantify the content of EPA and DHA in fish oil microcapsules (M) and in an enriched dry-cured meat model system (DC) and to evaluate their release during in vitro digestion.

METHODOLOGY



- ✓ Quantification of EPA and DHA (GC-FID)
- ✓ In vitro digestion [2,3]

ACHIEVEMENTS

The content of EPA and DHA was 4.01 ± 0.51 and 8.32 ± 0.22 mg/100 g in M, and 19.84 ± 0.45 and 49.86 ± 0.74 mg/100 g in DC, respectively. Figures 1 and 2 show the release of EPA and DHA in M and DC, respectively, during the in vitro digestion in the mouth (M), stomach (S) and intestine at 1 (I1), 2 (I2) and 3 hours (I3). Major quantity of EPA and DHA were found in the intestine phase, at 1 and 2 hours in the case of M and at 2 and 3 hours in DC.

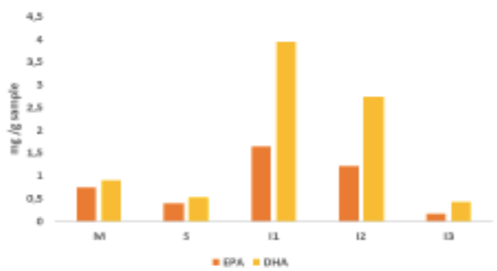


Figure 1. Releasing of EPA and DHA during the in vitro digestion of fish oil microcapsules.

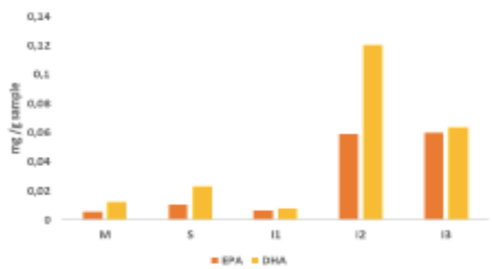


Figure 2. Releasing of EPA and DHA during the in vitro digestion of enriched dry-cured model system.

References

[1] Food Bioprocess Tech. 8, 100-111; [2] J. Appl. Microbiol. 107, 1781–1788; [3] J. Agric. Food Chem. 59, 1163–1170

Acknoledgements

CONCLUSIONS

The quantity of M added (3 g per 100 g of dough) is quite enough to label DC as "source of omega-3" (40 mg EPA+DHA per 100 g of sample). The enrichment of DC with M allows a high bioavailability of EPA and DHA.