UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE VETERINARIA

DEPARTAMENTO DE NUTRICIÓN, BROMATOLOGÍA Y TECNOLOGÍA DE LOS ALIMENTOS

TESIS DOCTORAL

Agente de carga a base de *konjac* y partículas de hidrogel como nuevos sistemas de incorporación de aceites en productos cárnicos

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Jenny Lorena Salcedo Sandoval

Directores

Susana Cofrades Barbero
Claudia Ruiz-Capillas Pérez
Francisco Jiménez Colmenero

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Madrid, junio de 2015

Universidad Complutense de Madrid
Facultad de Veterinaria
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Tecnología de los Alimentos

Consejo Superior de Investigaciones Científicas
Instituto de Ciencia y Tecnología
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Departamento de Productos

Agente de carga a base de konjac y partículas de hidrogel como nuevos sistemas de incorporación de aceites en productos cárnicos

Memoria que presenta **J. Lorena Salcedo Sandoval** para optar al grado de Doctor por la Universidad Complutense de Madrid

Bajo la dirección de la Dra. Susana Cofrades Barbero, la Dra. Claudia Ruiz-Capillas Pérez y el Dr. Francisco Jiménez Colmenero
Instituto de ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC)

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Que la presente memoria titulada "**Agente de carga a base de konjac y partículas de hidrogel como nuevos sistemas de incorporación de aceites en productos cárnicos**", cuya autora es *Jenny Lorena Salcedo Sandoval* para optar al grado de Doctor ha sido realizada en el Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC) bajo su dirección, y que hallándose concluida, autorizan su presentación para que pueda ser juzgada por el tribunal correspondiente.

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RESUMEN

Los derivados cárnicos tienen una enorme relevancia en la industria alimentaria en España, entre ellos las salchichas tipo frankfurt y las hamburguesas ocupan un papel destacado, por cuanto se trata de alimentos muy populares que gozan de amplia aceptación. Sin embargo, su consumo presenta algunas connotaciones negativas sobre la salud, asociadas a su contenido en grasa y perfil lipídico, pudiendo llegar a ser percibidos por los consumidores como alimentos menos atractivos. Es por esto que el desarrollo de productos cárnicos con una composición lipídica más saludable constituye una excelente opción de mejorar su imagen y de satisfacer las necesidades del consumidor actual. En este sentido, las estrategias de reformulación son los procedimientos más utilizados a la hora de diseñar nuevos productos cárnicos saludables, ya que son más rápidas al incidir directamente en la composición del producto final. Tales estrategias se han llevado a cabo fundamentalmente a dos niveles: reducción del contenido en grasa y/o mejora del perfil de ácidos grasos, limitando la presencia de ácidos grasos saturados (AGS) y favoreciendo la de ácidos grasos monoinsaturados (AGM) y poliinsaturados n-3 (AGP n-3), en especial los de cadena larga. En relación a la mejora del perfil lipídico, varias de las estrategias de reformulación se basan en la incorporación de aceites vegetales y/o marinos cuya composición es más saludable en comparación con la grasa animal habitualmente presente en estos productos. Tal incorporación se ha llevado a cabo mediante diferentes opciones tecnológicas, entre las más comunes se encuentran la adición directa de aceites, la interesterificación y la pre-emulsificación. Sin embargo, recientemente han surgido nuevas alternativas tecnológicas, entre ellas las basadas en el empleo de lípidos estructurados. Se trata de implementar procesos en los que aceites con una composición lipídica más saludable, son dotados de propiedades sólidas o viscosas, muy similares a los de la grasa animal a la que se va a reemplazar. Pese a que estas estrategias ofrecen interesantes posibilidades, su aplicación en derivados cárnicos es muy limitada.

En este contexto, el objetivo general trazado en la presente memoria, consistió en el **diseño y desarrollo de derivados cárnicos con mejor composición lipídica mediante procesos de reformulación encaminados a la obtención de productos más saludables.** Para ello**,** se eligieron dos sistemas distintos de lípidos estructurados escasamente empleados: **agente de carga de aceite a base de konjac y partículas de hidrogel**.

La utilización de un agente de carga de aceite a base de konjac estabilizando una mezcla de aceites de oliva, lino y pescado, se ensayó como estrategia en el desarrollo de salchichas tipo frankfurt y de hamburguesas, aplicando procesos de reformulación encaminados a la reducción de grasa y a la mejora del perfil de ácidos grasos. Para ello, dicho agente de carga se empleó en la sustitución de grasa animal de los productos: a) 19,1% en salchichas tipo frankfurt (sustitución parcial) y b) 6,8% y 13,4% en hamburguesas (sustitución parcial y total, respectivamente). Además en el caso de las hamburguesas, se evaluó el efecto de los métodos de cocción (plancha y fritura) sobre los componentes mayoritarios y el perfil lipídico.

Por otro lado, el empleo de partículas de hidrogel encapsulando aceite de pescado, se estudió como estrategia de desarrollo dirigida a la mejora del perfil lipídico en productos tipo gel/emulsión (sistema modelo y salchichas tipo frankfurt), enriquecidos en AGP n-3 de cadena larga (EPA y DHA). En primer lugar se evaluó su estabilidad oxidativa como ingredientes, sometidos a condiciones de procesado propias de los productos tipo gel/emulsión, posteriormente se determinó la estabilidad oxidativa en una matriz cárnica (sistema modelo) formulada con tales partículas, y finalmente se estudió su incorporación (23,5%) en salchichas tipo frankfurt.

En ambos planteamientos, se analizaron los efectos de la reformulación y la conservación en refrigeración sobre las propiedades nutricionales, tecnológicas, sensoriales y vida útil de los productos.

Los resultados obtenidos señalan que el uso de agente de carga como estrategia de reformulación en salchichas tipo frankfurt y hamburguesas dio lugar a la obtención de productos con contenido reducido en grasa (50% en salchichas, 38% y 72% en hamburguesas), y con perfil lipídico más en línea con los objetivos nutricionales. Esto es, disminuyendo el contenido en AGS, incrementando los AGP n-3 (1,12 g y 0,36/100 g producto, en salchichas y hamburguesas, respectivamente), ajustándose a las recomendaciones de diversas organizaciones internacionales. En relación a los métodos de cocción en hamburguesas (plancha y fritura), si bien estos afectaron la composición, no implicaron una alteración sobre las proporciones relativas de ácidos grasos de los productos reformulados, por lo que la mejora nutricional conseguida mediante esta estrategia siguió presente. Los procesos de reformulación con agente de carga tuvieron diferentes efectos sobre parámetros de composición, propiedades tecnológicas y sensoriales en salchichas y hamburguesas. Tales variaciones, en algunos casos estuvieron relacionadas con la proporción de agente de carga incorporado (composición, propiedades ligantes, color,

textura), mientras que en otros, estuvieron condicionadas por la naturaleza de la matriz cárnica (oxidación lipídica y parámetros sensoriales). En general, la aplicación de esta estrategia permitió la obtención de derivados cárnicos con adecuada viabilidad tecnológica y sensorial, sin más limitaciones en términos de vida útil que las propias de un derivado cárnico de naturaleza análoga.

Por otra parte, el empleo de las partículas de hidrogel como sistema de estabilización de aceite de pescado presentó una notable mejora sobre la estabilidad oxidativa de este material lipídico, tanto como ingrediente, como formando parte de una matriz cárnica. El enriquecimiento en EPA y DHA de salchichas tipo frankfurt a través del empleo de partículas de hidrogel llevó a la obtención de productos con perfil lipídico mejorado y con un aporte significativamente alto de AGP n-3 (0,99 g/100 g), conformado principalmente por n-3 de cadena larga (0,87 g/100 g EPA y DHA). Esto supone que, aunque las recomendaciones dietéticas de consumo de ácidos grasos n-3 de cadena larga varían en función de diversos factores, los productos enriquecidos harían una contribución muy significativa a dicha ingesta en comparación con los productos no enriquecidos. La aplicación de esta estrategia de reformulación dio lugar a la formación de una matriz proteica estable, con capacidad adecuada para inmovilizar agua y grasa, sin condicionar las propiedades tecnológicas y sensoriales ni la vida útil.

Cabe destacar que los cambios de composición ocasionados permitieron dotar a los productos de distintas declaraciones nutricionales y de propiedades saludable, en el marco establecido por el Reglamento (CE) 1924/2006 del Parlamento Europeo.

Como conclusión general se puede señalar que, en consonancia con los objetivos planteados, la aplicación de lípidos estructurados (agente de carga y partículas de hidrogel) como nuevos sistemas de estabilización de aceites abren nuevas posibilidades dentro de las estrategias de reformulación dirigidas a la producción de derivados cárnicos saludables. Se trata de propuestas novedosas que permiten la obtención de productos, con un contenido lipídico mejorado a nivel cuantitativo y/o cualitativo, con un perfil de ácidos grasos más acorde con los objetivos nutricionales recomendados, además de dotarlos de propiedades tecnológicas y sensoriales apropiadas, así como de una estabilidad comparable a productos de naturaleza análoga.

ABSTRACT

Meat products have a prominent importance in the food industry in Spain, among them frankfurters and patties occupy an outstanding role, since they are very popular and widely accepted products. However their consumption has some negative connotations on health, associated with their fat content and lipid profile and may be perceived by consumers as less attractive foods. That is why the development of meat products with a healthier lipid composition is an option to improve their image and meet the needs of consumers. In this regard, reformulation strategies are the most commonly used procedures to design new healthy meat products, because they are faster to influence the composition of final product. Such strategies have been carried out primarily at two levels: reducing fat content and/or improving fatty acid profile, limiting the concentration of saturated fatty acids (SFA) and favoring the presence monounsaturated fatty acids (MUFA) and polyunsaturated (PUFA), especially n-3 long chain PUFA. In relation to the lipid profile improvement, several of the reformulation strategies are based on the incorporation of vegetable and/or marine oils whose composition is healthier compared to animal fat normally present in these products. Such incorporation has been carried out through different technological options, e.g. direct addition of oils, interesterification and pre-emulsification. However, recently have emerged novel alternative technologies, including those based on the use of structured lipid systems. These are processes that provide to oils with healthier composition solid or viscous functionalities, very similar to those of animal fat that is to replace. Although these strategies offer interesting possibilities, its application in meat products is very limited.

In this context, the general goal set out in the present dissertation consisted on the **design and development of meat products with better lipid composition through reformulation strategies aimed to obtain healthier products**. For this, two different structured lipid systems barely used were chosen: **Konjac-based oil bulking agent** and **hydrogel particles**.

The use of konjac-based bulking agent stabilizing a mixture of olive oil, flax and fish, was evaluated as a strategy in the development of frankfurters and patties, applying reformulation processes leading to reduce their fat content and improve their fatty acid profile. For this, such bulking agent was employed in the substitution of animal fat normally present in these products as follows: a) 19.1% frankfurter type sausages (partial replacement) b) 6.8% and 13.4%

(partial substitution and total, respectively) on patties. Additionally in the case of patties, the effect of cooking methods commonly used for their consumption (grilling and pan-frying) on proximate composition and lipid profile was evaluated in a second study.

On the other hand, the use of hydrogel particles encapsulating fish oil was studied as a development strategy aimed at improvement of the lipid profile of gel/emulsion meat matrices (meat systems and frankfurters), rich in long chain n-3 PUFA (EPA and DHA). Firstly, oxidative stability was evaluated in hydrogel particles under typical processing conditions of gel/emulsion products, then lipid oxidation was determined in a basic meat matrix (model system) containing such particles, and finally its incorporation was studied (23.5 %) in frankfurters.

In both approaches, the effects of reformulation and chilling storage on nutritional, technological and sensory properties, as well as shelf life of products were analyzed.

Results indicate that the use of a konjac-based oil bulking agent as reformulation strategy in frankfurters and patties resulted in the development of products with reduced fat content (reductions of 50% frankfurters, 38% and 72% in patties), improved lipid profile, associated to SFA decreased values, increment of n-3 PUFA levels (1.12 g and 0.36/100 g product in frankfurters and patties, respectively), in line with the recommendations from several international organizations, and therefore with better relationships with fatty acids, which were within the suggested ranges. In relation to cooking methods of patties (grilling and pan-frying), though these affect the composition, they did not alter fatty acid ratios of the products containing konjac-based oil bulking agent, thus the nutritional improvement achieved by this strategy remained stable. Reformulating processes with the bulking agent had different effects on parameters of composition, technological and sensory properties in frankfurters and patties. Such variations in some cases (composition, binding properties, color, texture) were related to the proportion of agent incorporated, while in other cases (lipid oxidation and sensory parameters), they were conditioned by the nature of the meat matrix. In any case, the implementation of this strategy allowed obtaining meat products with appropriate technological and sensory viability, without limitations in terms of shelf life than those characteristics of products with similar nature.

Moreover, the employment of hydrogel particles as stabilization system of fish oil provided a significant improvement on the oxidative stability of the lipid material, as an ingredient as well as part of a meat matrix. The enrichment of frankfurters with EPA and DHA through the use of hydrogel particles led to obtaining products with enhanced lipid profile, whose contribution of n-3 PUFA was significantly high (0.99 g/100 g product), comprising mainly by n-3 long chain PUFA (0.87 g EPA and DHA/100 g product). This entails that although the dietary recommendations of consumption of n-3 long chain fatty acids vary depending on various factors, enriched products would make a very significant contribution to the intake, compared to non-enriched products. The implementation of this reformulation strategy led to the formation of a stable protein matrix, with adequate capacity to immobilize water and fat, without conditioning technological and sensory properties neither product shelf life.

It is worth to highlight that composition changes achieved in the present dissertation would allow labeling of the reformulated products with several nutrition and health claims, according to the established by Regulation (EC) 1924/2006 of the European Parliament.

As a general conclusion it can be noted that in line with the goals, incorporation of structured lipids (konjac-based oil bulking agent and hydrogel particles) as new stabilization oil systems opens up new possibilities within the reformulation strategies leading to obtain healthy meat products. These innovative proposals enable the development of products with improved lipid content in quantitative and/or qualitative terms, presenting a fatty acid profile more in line with the recommended nutritional goals, besides of providing them with adequate technological and sensory properties, as well as shelf life stability, comparable to those of products of similar nature.

1. Introducción

1. INTRODUCCIÓN

1.1 CARNE Y SALUD

1.1.1 Producción y consumo de carne y derivados a nivel mundial y en España

Globalmente el consumo de carne y sus derivados ha ido aumentando de forma progresiva en las últimas décadas, asociado generalmente a cambios relacionados con el progreso económico y social. En tal sentido, la diferencia en el consumo per cápita entre los países industrializados (78,3 kg/año) y en vías desarrollo (32,2 kg/año) se ha reducido. En los últimos 20 años el consumo de carne per cápita en los países en vías de desarrollo ha crecido más de un 70%, mientras que en los países industrializados apenas ha aumentado un 2%, según datos de la Organización de las Naciones Unidas para la Agricultura y la Alimentación (*Food and Agriculture Organization of the United Nations*, FAO, 2014). En términos de tasas de crecimiento de los principales productos agrícolas, se prevé que para el año 2021 a nivel mundial, el crecimiento del consumo de carne sólo será superado por el de aceite vegetal (OECD/FAO, 2012).

El sector de alimentos y de bebidas en la Unión Europea es la principal actividad de la industria manufacturera tanto en términos de volumen de negocios como en puestos de trabajo (FoodDrink-Europe, 2014). Concretamente en España, de acuerdo a los datos proporcionados por el Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA, 2015), la industria de alimentación y bebidas es la primera rama industrial que además ocupa el quinto puesto en valor de ventas tras Alemania, Francia, Italia y Reino Unido. Dentro de la industria alimentaria española, el sector

cárnico ocupa el primer lugar en total de ventas netas y en volumen de exportaciones, y el segundo puesto en número de empresas.

La carne y sus derivados en España suponen alrededor del 22% del gasto total destinado a alimentación, siendo el porcentaje más alto de entre todos los productos alimentarios. En 2014 el gasto total en España en carne y productos cárnicos ascendió a 14.573,0 millones de euros. En este mismo año los hogares españoles consumieron 2.287,2 millones de kg de carne y productos cárnicos, lo que supuso 51 kg/año per cápita (MAGRAMA, 2014). Dentro de los distintos tipos de carne, cabe destacar que el consumo de derivados cárnicos anual asciende hasta 11,9 kg/persona. En la **Figura 1.1** se puede observar la distribución de consumo por tipo de carne, ocupando la de pollo el primer lugar (27%) seguido de los transformados cárnicos (24%) y la carne de cerdo (21%). Así mismo, dentro de los transformados se destacan la categoría de productos curados, los fiambres y las salchichas.

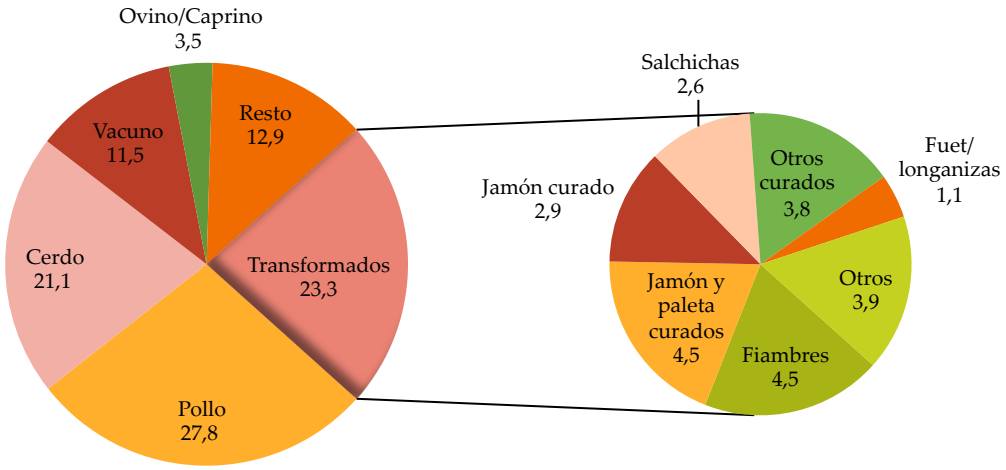


Figura 1.1. Distribución (%) de consumo en los hogares españoles de los diferentes tipos de carne.
Fuente: MAGRAMA (2014)

Lo anterior pone de manifiesto que el sector cárnico tiene un enorme peso en la economía, donde las carnes transformadas presentan un papel muy relevante. Es por ello que son necesarios continuos esfuerzos de innovación y desarrollo de nuevos productos capaces de cubrir las demandas de los consumidores y crear nuevas oportunidades de mercado.

1.1.2 La salud como factor determinante en el consumo

La manera en que los consumidores valoran diversos aspectos de los alimentos es determinante en los patrones de consumo. Los principales factores que influyen en la preferencia de ciertos productos, y por ende en la demanda de alimentos, incluyen aspectos relacionados, entre otros, con la salud, la edad, el género y la cultura. Los tres últimos son generalmente estables, modificándose lentamente con el tiempo, mientras que la salud puede actuar como un desencadenante que cambia rápidamente la demanda ya que los consumidores reaccionan positiva y negativamente frente a las noticias relacionadas con la salud (Catlett, 2011). En este sentido, durante las últimas décadas especialmente en los países desarrollados, se ha apreciado un cambio en los hábitos de consumo cuando estudios científicos han señalado la existencia de potenciales problemas de salud asociados a determinados alimentos (Siro et al., 2008). En este contexto, los continuos avances en el conocimiento de las relaciones entre alimentación/salud y alimentación/enfermedad, y su traslación (no siempre adecuada), a los medios de comunicación ha puesto a la carne en una situación no muy ventajosa. Por un lado, la carne es fuente importante de una amplia gama de nutrientes tales como proteínas, grasas, vitaminas y minerales (**Tabla 1.1**). En el caso de los derivados cárnicos, las condiciones de procesado a las que son sometidos otorgan ventajas tales como la diversificación, extender su vida útil y en algunos casos mejorar sus cualidades sensoriales a un coste más asequible (de

Barcellos et al., 2011). Por otro lado, muchos de estos productos tienen niveles elevados de grasa, sal y en algunos otros casos, compuestos, bien, adicionados intencionadamente como por ejemplo los nitritos, o bien, formados durante el procesado como es el caso de las aminas biógenas y los compuestos de oxidación, entre otros. Estos componentes a menudo se ha descrito que presentan implicaciones negativas para la salud. Por tanto el consumo de carne y sus derivados se ha considerado un factor de riesgo en relación con el desarrollo de enfermedades cardiovasculares (ECV) (Micha et al., 2010) y algunos tipos de cáncer (Linseisen et al., 2006; Cross et al., 2010).

De todos los componentes "polémicos" de la carne podría decirse que la grasa es posiblemente el que mayor atención ha recibido, por su elevada presencia, especialmente en algunos productos cárnicos, como es el caso por ejemplo, de salchichas tipo frankfurt o chorizo con niveles de entre 20-40% (Jiménez-Colmenero, 2000). Todo ello contribuye a dotar a los productos cárnicos de una "imagen" de alimentos poco saludables. No obstante, a pesar de estas implicaciones negativas, puestos en evidencia a distintos niveles (consumidores, medios de comunicación y organizaciones de salud), la grasa es un ingrediente clave en la carne y sus derivados. Además de su contribución nutricional, su presencia condiciona características como el sabor, la jugosidad, la textura, el aroma, etc. Poner a disposición de los consumidores productos cárnicos más saludables sin que esto suponga una alteración de sus características, supone uno de los desafíos más grandes de la industria alimentaria en la actualidad.

1.1.3 La grasa en los productos cárnicos: composición, valor nutricional e implicaciones en la salud.

El contenido de grasa de la carne puede variar ampliamente dependiendo de factores como la especie, raza, edad, sexo, estado sexual, alimentación,

estación del año, ejercicio y parte anatómica del animal, así como de la formulación en el caso de los productos cárnicos (**Tabla 1.1**). Cambios en materia de producción animal así como en la preparación y el corte de la carne, han generado una importante reducción en su contenido, llegando incluso a presentar niveles inferiores al 5% (Webb y O'Neill, 2008). En contraste, algunos productos cárnicos contienen elevadas proporciones de grasa, muy superiores a las de la carne de la cual proceden (**Tabla 1.1**).

Tabla 1.1. Principales componentes de la carne y derivados.

Nutriente	Vacuno[1]	Porcino[1]	Salchichas tipo frankfurt	Hamburguesas
Proteína (g/100 g)	17,0 - 20,7	15,4 - 21,5	10,7 - 12,8	14,2 - 15,4
Lípidos (g/100 g)	5,4 - 21,0	2,6 - 29,5	14,9 - 30,1	14,1 - 24,8
AGS	2,2 - 8,5	0,9 - 9,4	6,3 - 12,5	3,7 - 7,6
AGM	2,4 - 9,3	1,1 - 12,3	7,5 - 14,6	5,0 - 8,4
AGP	0,3 - 3,3	0,6 - 4,5	1,0 - 2,7	0,8 - 2,6
Colesterol (mg/100g)	59 - 65	58 - 72	49 - 66	57 - 73
Minerales (mg/100 g)				
Na	61	70 - 76	778 - 1079	494 - 740
Fe	1,6 - 2,1	0,8 - 1,8	1,1 - 3,7	0,9 - 1,2
Zn	3,3 - 3,8	1,6 - 2,5	1,8 - 2,2	2,0 - 2,4
P	170 - 200	16 - 170	86 - 171	130 - 162
K	350	270 - 370	158 - 401	231 - 307
Ca	7 - 8	8 - 13	11 - 21	8 - 13
Vitaminas (mg /100g)				
Tiamina	0,05 - 0,06	0,6 - 0,8	0,2 - 0,6	0,2 - 0,7
Riboflavina	0,1 - 0,2	0,1 - 0,2	0,1 - 0,2	0,1 - 0,2
Niacina	7,2 - 8,1	4,1 - 8,8	2,6 - 2,8	5,3 - 2,6
Vit. B_6	0,2 - 0,3	0,3 - 0,5	0,1 - 0,3	0,2
Ácido fólico (µg/100g)	8 - 10	3 - 5	3 - 4	2 - 3
Vit. B_{12} (µg/100g)	1 - 2	2 - 3	0,5 - 1,3	0,8 - 0,9

[1]. Correspondientes a distintos cortes comerciales.
AGS: Ácidos grasos saturados; AGM: Ácidos grasos monoinsaturados; AGP: Ácidos grasos poliinsaturados. Fuente: Moreiras et al. (2008); USDA (2014)

Los lípidos en la carne están constituidos mayoritariamente por triglicéridos (ácidos grasos unidos mediante enlaces éster a una molécula de glicerol) con un bajo contenido en fosfolípidos, esteroles (como el colesterol y los fitoesteroles) e isoprenoides.

El **colesterol** presente en la carne (generalmente entre 58 y 75 mg/100 g, **tabla 1.1**) varía en función de distintos factores, principalmente de la procedencia cárnica. En España la contribución de la carne al colesterol ingerido en la dieta alcanza el 28% (Jiménez-Colmenero et al., 2012b). Este componente se encuentra presente en el organismo en dos formas: libre y esterificado. El colesterol libre es un componente fundamental de las membranas celulares, mientras que los ésteres de colesterol circulan por el plasma. Debido a que los esteres de colesterol no son solubles en agua, para que puedan ser transportados por el torrente sanguíneo tienen que combinarse con sustancias que sean solubles en agua como algunos fosfolípidos y proteínas. Esta combinación da lugar a varios tipos de lipoproteínas que varían en composición, peso y función. Las lipoproteínas que mayor importancia tienen para la salud son las de alta densidad (*high density lipoproteins*, HDL) y las de baja densidad (*low density lipoproteins*, LDL) (Gotto Jr et al., 1986). Las LDL son las encargadas de transportar la mayoría del colesterol esterificado (aproximadamente dos tercios del colesterol circulante). Cuando los niveles de colesterol llegan a ser elevados, se depositan en las paredes de las arterias, lo cual puede dar origen a la ateroesclerosis, lo que puede dar lugar a infartos y accidentes cerebrovasculares. Por su parte, las HDL intervienen en la movilización del colesterol desde las arterias hacia el hígado, para que este sea transportado al intestino a través de la bilis, presentando un efecto antiaterogénico (Tall, 1998). Es por ello que las concentraciones elevadas de colesterol total y colesterol LDL, así como niveles bajos de colesterol HDL, son factores de riesgo de ECV. En general, según algunos autores, el colesterol de la dieta aumenta el colesterol LDL y el total, aunque este aumento está más condicionado por la ingesta de grasa saturada y grasas *trans* que por el colesterol dietético (Beynen y Katan, 1985; Howell et al., 1997).

En la actualidad es ampliamente reconocido el papel que juegan los lípidos como factor de riesgo en el desarrollo de algunas enfermedades, donde ya no se trata tanto de la cantidad de grasa ingerida, sino del tipo de ácido graso que es consumido (European Food Safety Authority, EFSA, 2010). Los ácidos grasos se pueden clasificar dependiendo del número de dobles enlaces que presenten en: ácidos grasos saturados (AGS) cuando no contienen ningún doble enlace, ácidos grasos monoinsaturados (AGM) que contienen un doble enlace y ácidos grasos poliinsaturados (AGP) que contienen dos o más dobles enlaces. Dentro de los AGP conviene señalar la importancia de los AGP n-3 y n-6 (también llamados omega 3 y omega 6, respectivamente), donde su primer doble enlace se encuentra en la posición 3 para los AGP n-3 y en la 6 y 7 para los AGP n-6, respecto al metilo terminal (O'Keefe, 2008).

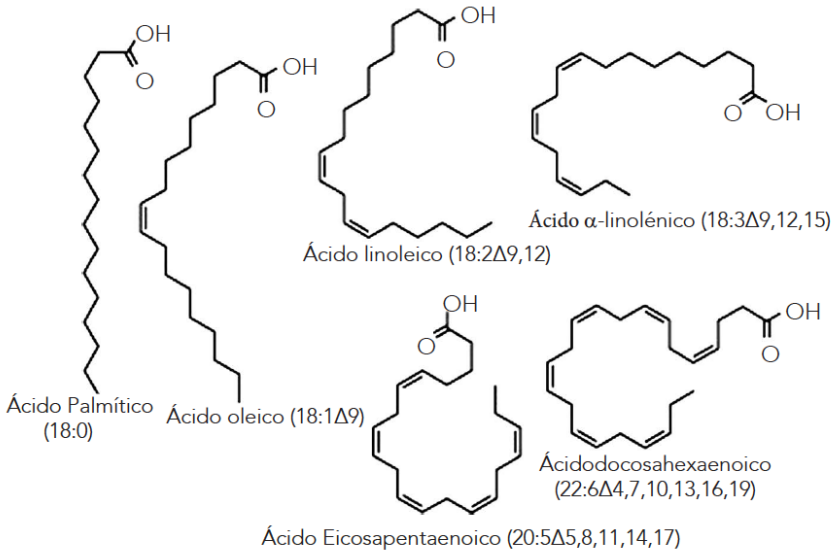


Figura 1.2. Estructuras de algunos ácidos grasos saturados e insaturados.
Adaptado de Hernandez y Kamal-Eldin (2013)

Como se ha comentado anteriormente, la composición de ácidos grasos en la carne depende fundamentalmente de la especie animal y de su alimentación. Las especies rumiantes (vacuno, ovino, etc.) durante la digestión en el rumen, convierten a la mayoría de los ácidos grasos insaturados

consumidos en AGS (Lunn y Theobald, 2006). Los animales no rumiantes, como el cerdo, incorporan más fácilmente a sus tejidos los ácidos grasos de la dieta, por lo que el perfil lipídico puede modificarse fácilmente mediante la alimentación (Wood et al., 2004). La cantidad de AGS y AGM presentes en la carne es muy similar, varía según la especie y el corte. Es en la fracción de los AGP donde existe una mayor diferencia entre especies.

Los **AGS** constituyen alrededor del 30-40% de los ácidos grasos presentes en la carne (**Tabla 1.1**), la cual aporta aproximadamente el 29% de estos ácidos a la dieta en España (**Tabla 1.2**). En la carne se encuentran predominantemente el ácido palmítico (C16:0), el esteárico (C18:0) y en una menor cantidad el ácido mirístico (C14:0). Aunque se ha sugerido que la mezcla de AGS incrementa la concentración total de colesterol, a nivel tanto de la fracción LDL, como de la HDL en algunos casos (EFSA Panel on Dietetic Products, 2010), diversos estudios han demostrado que cada ácido graso tiene efectos marcadamente distintos. Así, los ácidos mirístico y palmítico se han asociado con un aumento de valores de colesterol total y LDL-colesterol (Temme et al., 1996; Kris-Etherton y Yu, 1997). El ácido mirístico es el más aterogénico, con un potencial cuatro veces mayor para elevar el colesterol sérico que el ácido palmítico (Ulbricht y Southgate, 1991). Sin embargo, dado que el ácido palmítico se encuentra en mayor cantidad en la carne y en la dieta en general, es el que más efecto ejerce sobre los niveles de colesterol en la dieta occidental (Keys et al., 1965). El ácido esteárico se considera un ácido graso neutro con respecto a las ECV debido a que no aumenta los niveles de colesterol sanguíneos (Bonanome y Grundy, 1988), ni contribuye a otros factores de riesgo trombogénico (Kelly et al., 2001).

Recientemente, el efecto de los ácidos grasos saturados sobre la salud ha sido objeto de debate (Siri-Tarino et al., 2010). En tal sentido, y a pesar de lo expresado por las organizaciones de salud, estos autores no encontraron

ninguna relación entre el consumo de grasas saturadas y un mayor riesgo de ECV. En cualquier caso, cabe señalar que los AGS, individualmente o en su conjunto, no tienen los mismos beneficios positivos que los AGM y especialmente los AGP n-3.

Dentro de los ácidos grasos insaturados de la carne y sus derivados, la proporción de **AGM** representa en torno al 44-50 % (**Tabla 1.1**). La contribución de la carne y los productos cárnicos a la ingesta de AGM en algunos países llega a ser hasta del 40% (**Tabla 1.2**). El ácido oleico (C18:1 n-9) es el ácido graso más abundante de la carne, constituyendo entre el 20 y 47% del total de ácidos grasos, y junto con el ácido palmitoleico (C16:1) y cis-vaccénico (C18:1 n-7) conforman los principales AGM (Keeton et al., 2014). A raíz de los efectos beneficiosos observados de los AGM en la denominada *dieta mediterránea*, en la que el aceite de oliva (rico en ácido oleico) es uno de sus principales componentes, se ha enfatizado mucho interés el papel de estos ácidos grasos. Recientemente se ha descrito que la incidencia de los principales problemas cardiovasculares, se redujeron en individuos cuya dieta era rica en ácido oleico (Estruch et al., 2013). Así mismo, dietas ricas en este tipo de ácidos grasos originan descenso de los niveles de colesterol LDL y de colesterol total a la vez que se mantienen los niveles de colesterol HDL (Kris-Etherton y Nutrition, 1999). En relación con los AGP, los AGM presentan una menor susceptibilidad oxidativa, ya que sólo poseen un doble enlace. Este hecho podría trasladarse a la oxidación de las LDL, donde existen importantes evidencias de que los AGP aumentan la posibilidad de oxidación de las LDL comparados con los AGM. La presencia de LDL oxidadas puede inducir una respuesta inflamatoria y estimular la producción de otras especies reactivas de oxígeno, que contribuyen al desarrollo de aterosclerosis (Bhupathiraju y Tucker, 2011).

Tabla 1.2. Contribuciones (%) de la carne y derivados a la ingesta de grasa total y ácidos grasos en la dieta de varios países europeos.

País	Grasa total	AGS	AGM	AGP
Bélgica	26,7	25,1	32,2	19,4
Finlandia	23	19,5	28,5	23
Francia	23,4	20,2	30	19,1
Alemania	28,9	18,2	43	21,9
Italia	13,3	15,3	12,6	6,9
Países Bajos	21,2	20,1	29,5	12,9
España	23,7	29	24,2	15,8
Reino Unido	18,5	17,1	19,2	12,2

AGS: Ácidos grasos saturados; AGM: Ácidos grasos monoinsaturados; AGP: Ácidos grasos poliinsaturados. Fuente: Hulshof et al. (1999).

Los **AGP** representan del 6 al 40 % del total de ácidos grasos en la carne y derivados (**Tabla 1**), los cuales proporcionan en España casi el 16 % de los AGP ingeridos en la dieta (**Tabla 1.2**). Entre estos destacan el ácido linoleico (C18:2 n-6), que es el AGP mayoritario en la carne, y el ácido α-linolénico (ALA, C18:3 n-3), ambos ácidos grasos esenciales (Simopoulos, 1999). En el organismo estos dos ácidos se elongan y desaturan para obtener derivados de cadena larga como son los ácidos grasos araquidónico (C20:4 n-6) y docosapentaenoico (C22:5 n-6) a partir del ácido linoleico, y los ácidos eicosapentaenoico (EPA, C20:5 n-3) y docosahexaenoico (DHA, C22:6 n-3) a partir del ALA. Sin embargo, la conversión de ALA en EPA y DHA compite con la conversión del ácido linoleico en ácido araquidónico, ya que utilizan las mismas enzimas y puesto que el ácido linoleico es mucho más prevalente que el ALA en la mayoría de dietas, el metabolismo AGP n-6 es cuantitativamente más importante. Adicionalmente, la tasa de conversión de ALA en EPA y DHA es muy limitada (Simopoulos, 2002a). Se ha puesto en evidencia que los AGP n-6 ejercen efectos reductores directos sobre el colesterol LDL (Howard et al., 1995; Hu et al., 2001). No obstante, otros estudios señalan una reducción de los niveles de colesterol HDL al reemplazar AGS por AGP n-6, de ahí que a veces se haya recomendado preferencialmente a los AGM sobre los AGP n-6 (Gardner y Kraemer, 1995; Lunn y Theobald, 2006). Un AGP n-6 que también

está presente en la carne en cantidades relevantes es el ácido araquidónico, el cual incrementa el riesgo de trombosis (Budowski y Crawford, 1985).

Especial atención merecen los AGP n-3, dada la evidencia de su impacto beneficioso sobre diferentes ámbitos de la salud: desarrollo infantil, ECV, agregación plaquetaria, hipertensión, cáncer, depresión, inflamación, demencia y la enfermedad de Alzheimer (Riediger et al., 2009; Ruxton y Derbyshire, 2009; McManus et al., 2011). El aumento de los niveles de estos ácidos grasos en la población puede reducir la incidencia de esas enfermedades crónicas responsables de la mayor parte de la tasa mundial de morbilidad (Hibbeln et al., 2006). Principalmente, tres son los AGP n-3 que se encuentran comúnmente en los alimentos: por un lado el ALA, presente en semillas como la canola y el lino y por otro lado el EPA y el DHA (AGP n-3 de cadena larga), muy abundantes en el pescado y otras fuentes marinas. La evidencia de los beneficios sobre la salud se asocia principalmente a los n-3 de cadena larga. Por ejemplo, una revisión reciente sobre la eficacia de los AGP n-3 en la prevención de ECV, reveló que la disminución de las tasa de mortalidad por muerte cardiaca estaba asociada al consumo ácidos grasos provenientes del pescado y no por el consumo de ALA (Wang et al., 2006a). En la mayoría de los casos se recomienda que para contribuir a una salud optima y reducir el riesgo de enfermedades crónicas como las ECV, es necesaria la ingesta de 500 mg de EPA y DHA (Meyer, 2011) o entre 3 a 5,5 g para el total de AGP n-3 por día (Kolanowski et al., 1999).

Según las recomendaciones sobre dieta total, los lípidos deberían proporcionar entre el 20% y el 35% de la energía total, de ellos los AGS no deberían suponer más del 10 , los AGP entre el 6 % y el 11 % y los AGM entre el 15 % y el 20 % dependiendo del aporte calórico total de la grasa (FAO/WHO, 2010). Atendiendo a los datos de la Encuesta Nacional de Ingesta Dietética acerca de la energía total ingerida por los españoles, los AGS

suponen el 12 %, los AGM el 18 % y los AGP el 4,7 % (MSSSI/AESAN, 2012), por lo que sólo se ajustó a los objetivos nutricionales la ingesta de AGM. Así pues, a pesar de que con frecuencia se afirma que menos cantidad de grasa en la dieta, es más beneficioso para la salud, aspectos cualitativos deben tenerse en cuenta, incluyendo el hecho de que algunos ácidos grasos son esenciales en nuestra dieta.

Puesto que algunas de las consecuencias del consumo de grasas sobre la salud vienen determinadas por las proporciones entre los diferentes ácidos grasos, varias de las recomendaciones en este sentido se han realizado sobre la base de relaciones de ácidos grasos específicos (Jiménez-Colmenero, 2007). En consecuencia, la relación recomendada de AGP/AGS puede oscilar entre 0,4 y 1,0, mientras que la relación recomendada de AGP n-6/n-3 no debe exceder de 4 (Enser, 2000; Wood et al., 2004). Las cantidades excesivas de AGP n-6 y valores altos de la proporción AGP n-6/n-3 promueven el desarrollo de ECV, cáncer, enfermedades inflamatorias y autoinmunes, mientras que mayores niveles de AGP n-3 (y por ende relaciones disminuidas de n-6/n-3) ejercen efectos supresores (Simopoulos, 2002b). Sin embargo la realidad es que la dieta occidental generalmente es deficiente en AGP n-3 (especialmente en los de cadena larga) y contiene cantidades excesivas de AGP n-6, con ratios n-6/n-3 entre 15-20, en oposición al intervalo recomendado de 1-4 (Simopoulos, 2002b). Adicionalmente, la tendencia de consumo de alimentos que contienen AGP n-3 se encuentra estancada o en retroceso (Lee et al., 2006). Por consiguiente, con el fin de mejorar el estado de salud de la población, instituciones implicadas en este sentido y organizaciones profesionales han emitido recomendaciones para aumentar el consumo de alimentos ricos en AGP n-3, como un medio de promover una reducción en la relación de AGP n-6/n-3 (Kolanowski et al., 1999; Simopoulos, 2002a; Garg et al., 2006; Taneja y Singh, 2012). Del mismo modo, las dietas ricas en grasas monoinsaturadas se

han asociado con beneficios para la salud (Mattson y Grundy, 1985), recomendándose en consecuencia su incorporación en la dieta. Aunque estas recomendaciones se refieren a la dieta total, dado que la carne y los productos cárnicos constituyen una de las fuentes más importantes de grasa en la dieta (**Tabla 1.2**) y que tanto el contenido como las proporciones AGP/AGS y AGP n-6/n-3 de algunas carnes están naturalmente algo alejados de los valores recomendados (Wood et al., 2004), promover cambios en la cantidad y el perfil lipídico de estos productos podría ayudar a mejorar la calidad nutricional de la dieta occidental (Jiménez-Colmenero et al., 2001; Fernandez-Gines et al., 2005; Arihara, 2006; Jiménez-Colmenero, 2007). Por su elevada frecuencia y alto consumo, notable contribución a los niveles de ingesta de distintos nutrientes, gran versatilidad de presentación, amplio grado de aceptación y aptitud para experimentar procesos de reformulación (cambios de composición) usando ingredientes de diversas procedencias, etc., los elaborados cárnicos son alimentos excepcionalmente convenientes para actuar como vehículo y condicionar la presencia de compuestos bioactivos sin modificar los hábitos de consumo (Jiménez-Colmenero et al., 2012b)

1.2 OPTIMIZACIÓN DE LA COMPOSICIÓN LIPÍDICA EN PRODUCTOS CÁRNICOS MEDIANTE ESTRATEGIAS DE REFORMULACIÓN

Como se ha señalado anteriormente, la carne y sus derivados pueden ser percibidos por los consumidores como alimentos poco atractivos, debido en gran parte a la grasa que contiene. De ahí la necesidad de disponer de diferentes estrategias capaces de ofertar productos más saludables, contrarrestando dichas asociaciones negativas. Entre tales estrategias se encuentran, por un lado las asociadas a las **prácticas de producción animal** (genéticas y nutricionales), que ofrecen oportunidades interesantes para

modificar los niveles de distintos compuestos bioactivos en carnes. En relación a la alimentación animal, los esfuerzos se han centrado principalmente en la reducción del contenido de grasa y en el aumento de los AGM y AGP mediante el uso de fuentes de alimentación enriquecidos con estos compuestos. Estrategias de selección y cruzamiento favorecidas recientemente por el empleo de marcadores genéticos, también se han empleado para reducir el contenido de grasa de la canal. En este sentido se están produciendo nuevos avances asociados a procesos de transgénesis y clonación (Olmedilla-Alonso et al., 2013).

Sin embargo, **las estrategias de reformulación de derivados cárnicos** son las más utilizadas a la hora de diseñar nuevos productos cárnicos saludables, ya que son más rápidas al incidir directamente en el desarrollo del producto final (Jiménez-Colmenero, 2007; Grasso et al., 2014). Dependiendo del tipo de derivado cárnico, durante la preparación del mismo, su composición puede ser fácilmente alterada optimizando la presencia de componentes con implicaciones en la salud del consumidor. La posibilidad de desarrollar productos cárnicos con un contenido lipídico óptimo va a depender de varios factores como son el nivel de grasa deseado, la naturaleza del producto a reformular (sistemas gel/emulsión, características estructurales como grado de desintegración, coexistencia de estructuras con diferente granulometría, untuosidad, etc.) y el tipo de procesado requerido por el mismo (formación de la emulsión, tratamientos térmicos, entre otros) (Jiménez-Colmenero et al., 2001).

La optimización del contenido lipídico de los derivados cárnicos empleando estrategias de reformulación se ha llevado a cabo fundamentalmente a dos niveles: reducción del contenido en grasa y/o modificación del perfil de ácidos grasos, reduciendo la presencia de AGS y favoreciendo la de AGM y AGP, en especial los de cadena larga. A

continuación se analizan ambos planteamientos, así como sus múltiples posibilidades de aplicación.

1.2.1 Reducción del contenido en grasa animal

La grasa juega un papel importante en las características tecnológicas, sensoriales y nutricionales de los alimentos. La grasa interactúa con otros ingredientes contribuyendo a la generación de textura, jugosidad, etc., proporcionando un perfil de sabor único (Giese, 1992). La grasa presente y añadida en los productos cárnicos también tiene una función destacada en las propiedades reológicas y estructurales, (Rakosky, 1970; Hughes et al., 1998; Jiménez-Colmenero, 2007; Caceres et al., 2008). Por todo lo anterior, formular un producto bajo en grasa, con características similares a las de su homólogo con alto contenido en grasa no es una tarea fácil. La grasa habitualmente presente en estos productos ha de ser reemplazada por ingredientes capaces de contribuir a impartir los atributos de calidad aportados por la misma.

La elaboración de productos cárnicos con menor nivel de grasa generalmente responde a dos criterios básicos, la utilización de materias primas cárnicas más magras (lo que encarecería el costo de la formulación) y la disminución de la densidad de grasa y calorías mediante la adición de agua y otros ingredientes con escaso o nulo poder calórico. Su potencial aplicación puede ser llevada a cabo especialmente en productos en donde existe cierta desintegración estructural de las materias primas y por lo tanto es posible el íntimo contacto entre los diversos constituyentes (Youssef y Barbut, 2011). Las sustancias empleadas como sustitutos de grasa han de suponer por una parte, una aportación escasa de calorías, y por otra, como se ha mencionado anteriormente, contribuir a impartir al producto las características deseadas. Aunque los sustitutos de grasa son conocidos por diversos sinónimos en función de distintos factores asociados a su papel en el alimento (**Tabla 1.3**). A

lo largo de esta memoria se hará uso indistinto de algunas de estas denominaciones.

Tabla 1.3. Diferentes términos usados como "Sustituto de la grasa" y sus significados.

Reemplazantes de grasa
- Pueden ejercer algunas o todas las funciones de la grasa
- Generalmente, contienen menos calorías que la grasa
- Pueden o no proveer los mismos nutrientes que la grasa que sustituyen

Sustitutos de la grasa
- Se asemejan a las grasas y ofrecen todas las funciones de estas en el alimento
- Reemplazan grasa en proporción 1:1
- Por lo general son estables a temperaturas de cocción/fritura
- Valor calórico < 9 kcal/g
- En algunos casos, no se absorben totalmente, aportando menos calorías

Análogos de grasa
- Proporcionan alimentos con muchas de las características de la grasa
- Posen menor digestibilidad que la grasa de la dieta
- Alteran el valor nutricional

Extensores de grasa
- Optimizan la funcionalidad de la grasa
- Permiten una disminución de la cantidad habitual de grasa en el producto

Miméticos de grasa
- Imitan una o más de las funciones físicas y sensoriales de la grasa en el alimento
- Compuestos de carbohidratos, proteínas, grasas, solos o en combinación
- Proporcionan de 0 a 9 kcal/g
- Proporcionan lubricidad y otras características de la grasa mediante el atrapamiento de agua
- Inadecuado para funciones de grasa, como la fritura, debido a la presencia de agua
- Pueden presentar oscurecimiento por someterse a altas temperaturas

Adaptado de Jones y Jonnalagadda (2006)

Además del agua, la mayoría de los ingredientes y/o aditivos empleados para disminuir el nivel de grasa se pueden clasificar como: 1) proteínas de origen no cárnico (soja, surimi, proteínas de origen lácteo, harina de trigo, albúminas, etc.), y 2) carbohidratos (gomas o hidrocoloides, almidones y maltodextrinas y derivados de la celulosa). En general, estos ingredientes aportan ventajas adicionales ya que incrementan el rendimiento debido a sus propiedades ligantes de agua y grasa, lo que en consecuencia permite rebajar los costos de formulación (Pietrasik y Duda, 2000).

Sustitutos de origen proteico

La disminución de grasa en productos cárnicos mediante el empleo de derivados proteicos tanto de origen animal como vegetal ha sido ampliamente discutido. Entre las proteínas vegetales más utilizadas se destaca la soja y entre las de origen animal, las proteínas del suero lácteo (Dexter et al., 1993; Casas et al., 1998b; Pietrasik y Duda, 2000; Brewer, 2012). En la **Tabla 1.4** se presentan algunos ejemplos de sustitutos proteicos de grasa animal utilizados en productos cárnicos. Además de sus contribuciones tecnológicas, algunos sustitutos pueden presentar efectos saludables, proporcionando un valor añadido a su empleo. La soja, por ejemplo, presenta efectos beneficiosos en relación con la prevención y el tratamiento de ECV, cáncer y osteoporosis, así como en el alivio de los síntomas menopáusicos (Lichtenstein, 1998).

Tabla 1.4. Ejemplos de sustitutos proteicos empleados para la reducción de grasa animal en productos cárnicos.

Sustitutos a base de proteínas	**Producto en el que se ha sustituido**	**Referencia**
Soja	Carne picada (vacuno)	Brewer et al. (1992) Dignam et al. (1979); Ziprin et al. (1981); Ali et al. (1982); Deliza et al. (2002); Kilic et al. (2010)
	Hamburguesa	Angor y Al-Abdullah (2010); Kassem y Emara (2010)
	Salchichas cocidas	Pietrasik y Duda (2000)
Suero	Albóndigas	Serdaroğlu (2006)
	Hamburguesa de cerdo	Peña-Ramos y Xiong (2003); Cheng et al. (2009).
	Hamburguesa de vacuno	El-Magoli et al. (1996); Hale et al. (2002) Desmond et al. (1998); Andïç et al. (2010)
	Salchichas	Yetim et al. (2001); Wang et al. (2006b); Marchetti et al. (2014)
Colágeno	Carne picada (vacuno)	Chavez et al. (1986); Graves et al. (1993); Campbell et al. (1996)
	Reestructurado de vacuno	Kenney et al. (1992)
	Salchichas frankfurt	Arganosa et al. (1988); Eilert et al. (1996)

Sustitutos basados en carbohidratos

Los carbohidratos que se han empleado en la reformulación de productos cárnicos para reducir el contenido en grasa son básicamente fibras, gomas o hidrocoloides de distintas procedencias (Casas et al., 1998a; Brewer, 2012), entre los que se encuentran fibra de trigo, derivados de maíz, avena, diversos tipos de almidón, pectinas, maltodextrinas, carragenatos, etc. (**Tabla 1.5**).

Tabla 1.5. Ejemplos de sustitutos basados en carbohidratos para la reducción de grasa animal en productos cárnicos.

Sustituto a base de carbohidrato	**Producto en el que se ha sustituido**	**Referencia**
Almidón patatas	Salchichas de vacuno cocidas	Liu et al. (2008)
Almidón de trigo	Hamburguesas de vacuno	Rocha-Garza y Zayas (1996)
Almidón de maíz	Carne de vacuno picada	Khalil (2000)
Harina de garbanzos	Albóndigas	Serdaroğlu et al. (2005)
	Carne de vacuno picada	Shaner y Baldwin (1979)
Harina de frijol	Albóndigas	Serdaroğlu et al. (2005)
Remolacha	Salchichas frankfurt	Vural et al. (2004)
Fibra de avena	Hamburguesa de vacuno	Desmond et al. (1998) Troy et al. (1999); Chevance et al. (2000); Pinero et al. (2008)
	Salchichas frankfurt	Hughes et al. (1997)
Fibra de avellana	Hamburguesa de vacuno	Turhan et al. (2005)
Fibra de cítrico	Salchichas frankfurt	Cengiz y Gokoglu (2007)
Fibra de soja	Salchichas tipo bologna	Cofrades et al. (2000)
Fibra de arroz	Emulsión cárnica	Choi et al. (2010)
Celulosa	Hamburguesa de vacuno	Hill y Prusa (1988)
Tapioca	Hamburguesa de vacuno	Berry (1997); Desmond et al. (1998); Troy et al. (1999)
	Salchichas frankfurt	Chevance et al. (2000)
Carragenato	Hamburguesa de vacuno	Troy et al. (1999)
	Salchichas frankfurt	Hughes et al. (1997) Candogan y Kolsarici (2003a); Cierach et al. (2009)
	Salchichas cocidas	Pietrasik y Duda (2000)
Beta-glucano	Hamburguesa de vacuno	Pinero et al. (2008)
Maltodextrina	Carne de vacuno picada	Garzon et al. (2003)
	Salchichas frankfurt	Crehan et al. (2000)
Polidextrosa	Carne de vacuno picada	Troutt et al. (1992)
Inulina	Salchichas	Archer et al. (2004)
	Chorizo	(Mendoza et al., 2001)
Konjac glucomanano	Salchichas frankfurt	Lin y Huang (2003); Kao y Lin (2006); Jiménez-Colmenero et al. (2010a)
	Mortadela	Chia-Cherng et al. (1998); Chin et al. (1998)
	Salchichas frescas	Osburn y Keeton (1994); Triki et al. (2013a)
	Chorizo	Ruiz-Capillas et al. (2012)

1.2.2 Mejora del perfil lipídico: incorporación de aceites con composición más saludable

Los procesos de reformulación dirigidos a la mejora en el perfil lipídico se basan, generalmente, en la sustitución en mayor o menor medida de la grasa animal normalmente adicionada durante la elaboración del producto, por otra cuyas características estén más acordes con las recomendaciones nutricionales, es decir, menor proporción de AGS y mayor de AGM y AGP (especialmente AGP n-3 de cadena larga), mejores relaciones AGP/AGS y AGP n-6/n-3. Con tal propósito se han empleado tanto aceites vegetales (ricos en AGM y AGP) como aceites de pescado (ricos en AGP n-3 de cadena larga) en muchos tipos de productos cárnicos (**Figura 1.3**). Entre estos tipos de aceites, cabe destacar el aceite de pescado por su elevado valor biológico debido a su alto contenido en EPA y DHA (Kolanowski et al., 1999). Interesantes posibilidades ofrece la combinación de aceites (vegetales y de pescado) con el objetivo de reducir las relaciones AGS/AGM y AGP n-6/n-3 en los productos cárnicos reformulados (Delgado-Pando et al., 2010b).

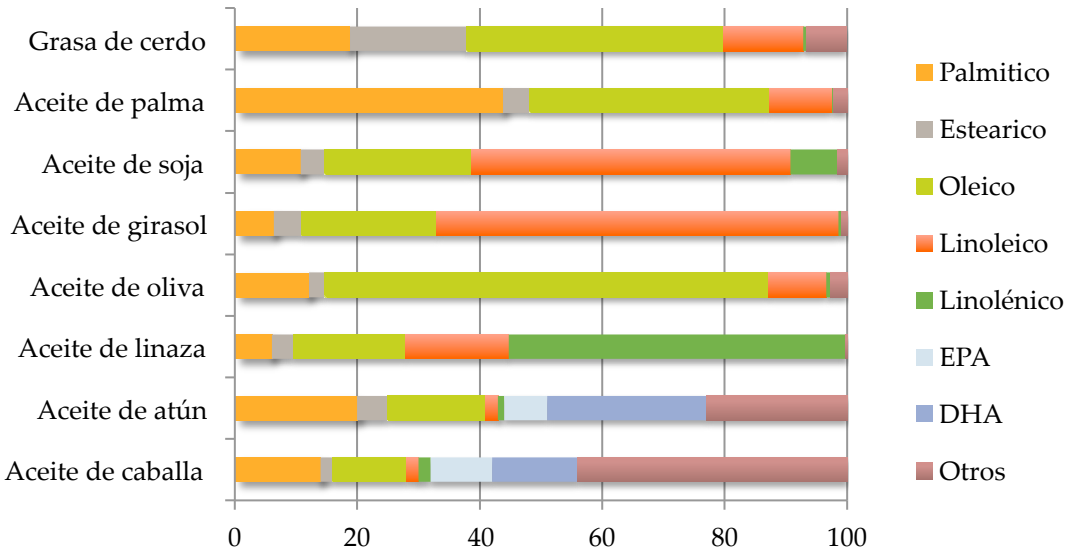


Figura 1.3. Principales ácidos grasos (%) que componen la grasa de cerdo (tocino) y algunos aceites vegetales y marinos.
Fuente: Frankel et al. (2002); Dubois et al. (2007); Gong et al. (2007)

Aceite de pescado

El aceite de pescado es la principal fuente dietética de AGP n-3 de cadena larga (EPA y DHA). A menudo, este aceite se obtiene como subproducto de la industria pesquera, y a través de técnicas de purificación se ha podido adaptar a las demandas sensoriales del consumidor. Aunque la cantidad y el tipo de ácidos grasos varía por el tipo de procesado y la especie procedencia, la mayoría de los aceites de pescado tras su procesado tienen alrededor de 30 g EPA y DHA/100 g (Taneja y Singh, 2012). La asociación entre el consumo de pescado y riesgo de padecer ECV se ha estudiado extensamente, y aunque con resultados no siempre concluyentes, en la mayoría de los casos se ha constatado el efecto cardioprotector del consumo pescado. Pese a que parece fuera de toda duda que son los AGP n-3 de cadena larga presentes en el aceite de pescado los responsables de ejercer este efecto beneficioso, también parece probable que otros compuestos actúen sinérgicamente con los ácidos grasos, produzciendo el efecto protector (Meyer, 2011). La suplementación con aceite de pescado está asociada con una reducción de muertes por causas cardiacas (Kris-Etherton et al., 2003; Kolanowski, 2010). El empleo del aceite de pescado para mejorar el perfil lipídico se ha llevado a cabo en diferentes productos cárnicos, como se verá más adelante. No obstante, la alta susceptibilidad a la oxidación de lípidos del aceite de pescado y de otros alimentos ricos en AGP n-3 de cadena larga puede ocasionar tres problemas principales: 1) da lugar a la formación de sabores extraños, 2) los radicales libres formados durante la oxidación puede contribuir en el desarrollo de la aterosclerosis, y 3) reduce el valor nutricional de los productos (Jacobsen, 2010). Todo lo anterior constituye un reto a la hora de incorporar este tipo de ácidos grasos en una matriz cárnica por lo que se deben buscar opciones tecnológicas que minimicen los procesos de oxidación y sus efectos secundarios.

Combinación de aceites de origen vegetal y marino

Si bien, debido a su composición de ácidos grasos, la adición individual de aceites de origen vegetal y marino (**Figura 1.3**), puede mejorar el perfil lipídico de los productos cárnicos, una aproximación más acertada para lograr un perfil lipídico óptimo (desde un punto de vista saludable), podría alcanzarse usando una combinación de los mismos como sustituto de grasa animal. Es por ello que numerosos autores han empleado este tipo de estrategia para mejorar el perfil de ácidos grasos en los productos cárnicos (Paneras et al., 1998; Valencia et al., 2008; García-Íñiguez de Ciriano et al., 2009; Delgado-Pando et al., 2010a; Martínez et al., 2011; Triki et al., 2013c). Entre estos estudios, cabe destacar los realizados por Delgado-Pando et al. (2010b), empleando una combinación conformada por aceites vegetales (de oliva y de lino) y de pescado en proporciones y niveles adecuados para proporcionar un perfil de ácidos grasos ajustado a los objetivos de ingesta saludable. Se trata de un material lipídico con proporciones reducidas de AGS y elevadas cantidades de AGM y AGP (incluyendo los n-3 de cadena larga) y una relación equilibrada de n-6/n-3 AGP y AGP/AGS. La sustitución de grasa habitualmente presente en los productos cárnicos utilizando una combinación de aceites permitiría obtener un perfil lipídico optimizado, más en la línea con las recomendaciones nutricionales.

1.2.2.1 Opciones tecnológicas convencionales

A causa de las diferentes características físico-químicas que presentan los aceites de origen vegetal y marino en comparación con la de las grasas de origen animal, para su sustitución en productos cárnicos, resulta necesario ajustar las condiciones de procesado para poder obtener un producto con similares características (Grasso et al., 2014). En tal sentido, en la reformulación de productos cárnicos con perfil lipídico mejorado se han

utilizado tanto grasas (materia sólida) como aceites (materia líquida) (Jiménez-Colmenero, 2007). La incorporación de estos lípidos se ha realizado de diferentes maneras dependiendo por lo general del producto al que van a ser incorporados. En tal sentido, se han ensayado fundamentalmente tres distintos procedimientos para incorporar aceites vegetales y marinos en la reformulación de productos cárnicos: la adición de manera directa ya sea líquida o sólida (incluyendo la interesterificación), la adición como aceites encapsulados y la adición como aceites pre-emulsionados.

Adición directa de aceites líquidos

Habitualmente los aceites de origen vegetal y marino se encuentran en estado líquido a temperatura ambiente e incluso por debajo de temperaturas de refrigeración. Esto es debido a la presencia de cantidades elevadas de ácidos grasos insaturados en su composición. Niveles elevados de insaturación no solo conllevan una disminución del punto de fusión sino que aumentan su susceptibilidad a la oxidación. Varios autores han llevado a cabo la incorporación directa de aceites en diferentes tipos de productos (**Tabla 1.6**). Las condiciones de adición varían en función de factores relacionados con el tipo de producto (músculo entero, productos frescos, productos tipo gel/emulsión, etc.) y con las características y cantidad del aceite incorporado (Jiménez-Colmenero, 2007). En productos cárnicos constituidos por músculos enteros, el aceite líquido se ha añadido por micro-inyección, no obstante, este procedimiento por lo general puede requerir la presencia de otros ingredientes y la aplicación de procesos mecánicos con el fin de favorecer a las características del producto (Domazakies, 2005). Bloukas et al. (1997) reportaron que la incorporación directa de 3,3 y 6,6% aceite de oliva en embutidos fermentados produjo un aspecto inaceptable y una textura muy suave. La sustitución del 50% de tocino de cerdo por adición directa de aceites de oliva, girasol y aguacate en hamburguesas produjo diferentes efectos sobre

la textura, mientras que los aceites de aguacate y girasol dieron lugar a productos más blandos. Por el contrario, el aceite de oliva no provocó ningún cambio en textura (Rodríguez-Carpena et al., 2012).

Solidificación de aceites

Algunas de las grasas vegetales como el aceite de palma, tienen consistencia sólida a temperatura ambiente, sin embargo debido a su alto contenido en AGS (especialmente palmítico), esta opción no resulta apropiada para procesos de reformulación de productos cárnicos con mejor perfil lipídico.

Otra tecnología que se ha empleado para solidificar aceites es la hidrogenación parcial, que consiste en aumentar el punto de fusión de las grasas mediante la adición directa de hidrógeno a los ácidos grasos insaturados eliminando los dobles enlaces. Sin embargo, y pese a que se han utilizado aceites vegetales parcialmente hidrogenados como sustitutos de grasa animal para el desarrollo de productos cárnicos (**Tabla 1.6**), esta tecnología presenta importantes limitaciones derivadas de la presencia de ácidos grasos *trans*, los cuales se ha demostrado que presentan implicaciones negativas en la salud (Hu et al., 2001).

Un procedimiento alternativo para aumentar el punto de fusión de aceites es la interesterificación que puede realizarse por métodos químicos o enzimáticos, con el fin de reorganizar los ácidos grasos dentro de un triglicérido o entre moléculas de triglicéridos (**Figura 1.4**).

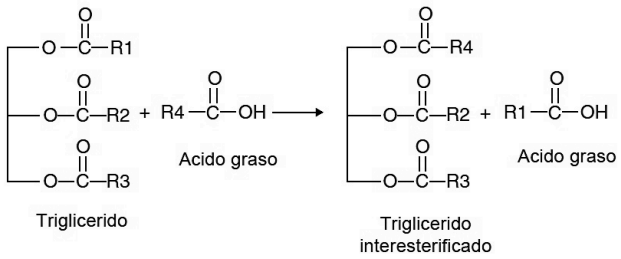


Figura 1.4. Reacción general de interesterificación.
Adaptado de Hernandez y Kamal-Eldin (2013)

La ventaja de esta tecnología es que permite el aumento del punto de fusión sin la generación de AGS y ácidos grasos *trans*, por lo que carece de los efectos adversos de la hidrogenación. Diversos aceites vegetales interesterificados se han utilizado como sustitutos de grasa para modificar la composición lipídica de distintos tipos de productos cárnicos (**Tabla 1.6**).

Aceites encapsulados

La encapsulación de aceites permite su adición en pequeñas cantidades, si bien les dota de una gran protección frente a la oxidación lipídica (Kolanowski et al., 1999). El sistema de encapsulación más básico se genera a partir de una emulsión O/W, en donde la fase acuosa se evapora por atomización en una corriente de aire seco a alta temperatura, convirtiendo a la fase dispersa en polvo seco, este procedimiento se denomina *spray drying*. Fundamentalmente se ha aplicado esta tecnología para fortificar alimentos de amplio consumo (pan, sopa, zumos, etc.) con AGP n-3 de cadena larga (Garg et al., 2006). Su uso en productos cárnicos, aunque limitado, ha sido también llevado a cabo (**Tabla 1.6**).

Tabla 1.6. Ejemplos de mejora del perfil lipídico en productos cárnicos mediante la adición de aceites líquidos, solidificados y encapsulados.

Material lipídico	Producto	Contenido de grasa (g/100 g)	Contenido de aceite (g/100 g)	AGM/ AGS	AGP n-6/n-3	AGP/AGS	Referencia
Liquido							
Girasol alto oleico	Salchicha frankfurt	11-28	6-24	2,6-3,8	-	3.1-4.5	Park et al. (1990)
Soja	Salchicha frankfurt	22	19	-	-	-	Ambrosiadis et al. (1996)
Oliva	Salchicha frankfurt	12	5	-	-	-	Lurueña-Martinez et al. (2004)
Oliva + Alga	Salchicha frankfurt	10	5	2,1	1,8	0,5	López-López et al. (2009)
Pescado (Extracto)	Chorizo	25	5,3-10,7	-	0,5-0,6	5,3-7,8	Muguerza et al. (2004a)
Soja	Hamburguesa de vacuno	14-45	5-40	-	-	-	Shiota et al. (1995)
Maní	Hamburguesa de vacuno	17	14	-	-	-	Dzudie et al. (2004)
Aguacate	Hamburguesa de vacuno	10	5	2,2	1,9	0,74	Rodríguez-Carpena et al. (2012)
Parcialmente hidrogenado							
Soja	Hamburguesa de vacuno	9,6	5	-	-	-	Liu et al. (1991)
Semilla de algodón	Hamburguesa de vacuno	9,9	5	-	-	-	Liu et al. (1991)
Cacahuete	Hamburguesa de vacuno	9,6	5	-	-	-	Liu et al. (1991)
Interesterificado							
Oliva	Salchicha frankfurt	18-21	6-10	1,2-1,3	9,3-10,7	0,08	Vural et al. (2004)
Avellana	Salchicha frankfurt	10	10	2,1	-	0,20	Özvural y Vural (2008)
Semilla de algodón	Salami	10	6-10	-	-	0,4-0,7	Javidipour et al. (2005)
Encapsulado							
Pescado	Chorizo	39	4,5		9,59	0,31	Pelser et al. (2007)
Lino	Chorizo	39	4,5		1,06	0,59	Pelser et al. (2007)

Aceites emulsificados

Es una emulsión O/W estabilizada generalmente con proteína no cárnica, que se emplea como ingrediente en la emulsión cárnica, de ahí que con frecuencia se denomine pre-emulsión. Mediante el uso de esta tecnología, es posible incrementar la capacidad ligante de grasa en el producto reformulado, ya que el aceite se estabiliza en la matriz proteica. Esto deja más proteína cárnica disponible para actuar en el sistema, reduciéndose así las posibilidades de que el aceite se separe de la estructura del producto cárnico durante las etapas de procesado, conservación y consumo (Djordjevic et al., 2004). Las emulsiones O/W constituyen un excelente medio para aumentar la estabilidad oxidativa de aceites insaturados, aunque también, como medida de protección complementaria pueden adicionarse antioxidantes (Djordjevic et al., 2004).

Distintos procedimientos se han descrito para producir emulsiones O/W destinadas a la incorporación en derivados cárnicos (Jiménez-Colmenero, 2007). El más comúnmente aplicado es el propuesto por Hoogenkamp (1989) que ha sido utilizado en numerosas aplicaciones (Bloukas y Paneras, 1993; Bloukas et al., 1997; Ansorena y Astiasaran, 2004; Pelser et al., 2007). Brevemente, el procedimiento consiste en mezclar ocho partes de agua caliente (50-60 °C) con una parte de caseinato de sodio (SC) o aislado de proteína de soja (SPI) durante dos minutos. La mezcla se emulsiona con diez partes de aceite durante otros tres minutos. Generalmente, el SC se ha utilizado como emulsificante en productos tratados por el calor, mientras que el SPI se ha utilizado en productos fermentados. Se han empleado distintos aceites de origen vegetal (como oliva, maíz, linaza, algodón, etc.) o marino (algas o pescado) para la mejora del perfil lipídico del producto final (**Tabla 1.7**).

Tabla 1.7. Ejemplos de mejora del perfil lipídico en productos cárnicos mediante la incorporación de aceites emulsificados.

Material lipídico/ emulsificante	Contenido de grasa (g/100 g)	Contenido de aceite (g/100 g)	AGM/AGS	AGP n-6/n-3	AGP/AGS	Referencia
Productos frescos						
Alga/WPI	3	1.1	-	-	-	Lee et al. (2005)
Oliva/SPI	10	2,6-5,3	1,2-1,6	14,1-16,4	0,18-0,20	López-López et al. (2011)
Oliva + Maiz + Pescado/SPI	8	7,3	1,7	2,1	0,7	Martínez et al. (2012)
Lino + Pescado/SPI	25	3,1	1,1	1,6	0,7	Valencia et al. (2008)
Canola/SPI	10,0-17,5	5,5-11,8	-	-	-	Youssef y Barbut (2011)
Productos cocidos						
Oliva/SC	10	6.7	2,9	14.8	0.33	Paneras y Bloukas (1994)
Oliva/SC	16,8	13	-	-	-	Jiménez-Colmenero et al. (2010b)
Oliva/SPI	20	13	-	-	-	Herrero et al. (2012)
Girasol	10	6,7	1,4	21,5	1,9	Paneras y Bloukas (1994)
Oliva + Soja + Algodón/SC	10	4	1,1	24	0,6	Paneras et al. (1998)
Pescado/SC	14-20	1-6	1,2-1,4	1,8-4,9	0,5-0,6	Caceres et al. (2008)
Oliva + Lino + Pescado/SC	10	9,5	1,4	0,47	1,74	Delgado-Pando et al. (2010a)
Pescado/WPI	20	1,5	-	-	-	Salminen et al. (2013)
Productos fermentados						
Pescado/SPI	29-33	0,5-1,1	1,2-1,3	5,3-7,7	0,5-0,6	Muguerza et al. (2004b)
Lino/SPI	30-32	3,3	1,1	1,7-2,1	0,6-0,7	Ansorena y Astiasaran (2004)
Lino/SC	35	6	-	0,87	0,7	Pelser et al. (2007)
Lino/SC	25	3,3	-	1,8	-	García-Íñiguez de Ciriano et al. (2009)
Lino + alga/SC	25	3,3	1,2	2,0	0,6	García-Íñiguez de Ciriano et al. (2010a)

WPI, aislado de proteína de suero; SPI, aislado de proteína de soja; SC, caseinato de sodio.

1.2.2.2 Nuevas alternativas tecnológicas: lípidos estructurados

Recientemente han surgido nuevas propuestas en relación con el desarrollo de sistemas de estabilización de aceites líquidos a fin de mejorar las características de los sistemas reformulados. La modificación o estructuración de aceites para producir grasas plásticas que exhiban propiedades sólidas a la vez que posean un perfil de ácidos grasos más saludable, actualmente representa un área muy importante de investigación tanto en el campo científico como en el industrial (Dickinson, 2012; Co y Marangoni, 2012; Zetzl et al., 2012; Patel et al., 2014).

El concepto de lípidos estructurados se ha asociado a triglicéridos cuta composición de ácidos grasosa sido establecida por un proceso de laboratorio, o industrial y cuyo objetivo es modificar la biodisponibilidad del producto con fines nutricionales y/o tecnológicos especificos (Hernandez y Kamal-Eldin, 2013). Típicamente esto ha sido llevado a cabo mediante procesos de hidrogenación e interesterificación (Sección 1.2.4.1). Este concepto de lípidos estructurados limita las modificaciones estructurales en aceites a aquellas que ocurren a nivel del esqueleto del triglicérido. No obstante, recientemente Jiménez-Colmenero et al. (2015) sugirieron los procesos de gelificación de fases orgánicas o inorgánicas para estabilizar material lipídico en redes tridimensionales como una nueva forma de estructurar aceites para su empleo en matrices cárnicas. En tal sentido, los ***lípidos estructurados*** son materiales constituidos por aceites pero con propiedades similares a los de una grasa sólida (o con mayor viscosidad). Esto puede ir asociado a una composición en lípidos que puede ser rica en AGM y AGP, con niveles reducidos de AGS y libre de ácidos grasos *trans*. Las nuevas estrategias utilizadas para estabilizar y estructurar aceites líquidos comestibles incluyen los oleogeles, los agentes de carga, y las emulsiones estructuradas, todos ellos implicando procesos de gelificación.

Se denomina gel a una estructura de red tridimensional con capacidad de inmovilizar un líquido. Está formado por dos componentes, una fase liquida solvente (que puede ser polar o apolar) y un agente gelificante, responsable de la estructura del sistema. Sin embargo, dependiendo de la polaridad del líquido inmovilizado en la red, los geles se clasifican en hidrogeles si el solvente es polar (generalmente agua), o en organogeles si el solvente es orgánico (Sagiri et al., 2014).

Un **oleogel** es un organogel cuya fase orgánica es aceite comestible, que se encuentra atrapado dentro de una red tridimensional inducida por un agente gelificante (organogelificante) por lo que su estructura se define como O + Org (*Oil + organogelator*) (**Figura 1.5)**. Esto simplemente significa la transformación de un aceite líquido en una estructura similar a la de un gel con propiedades viscoelásticas (Rogers et al., 2009; Stortz et al., 2012). Estos sistemas se pueden producir por dos mecanismos de estructuración: por auto-ensamblaje (formado por la auto-organización a nivel molecular en la fase oleosa) y por cristalización (partículas de cristal que se producen a través de nucleación y posterior crecimiento de los cristales en la fase oleosa). Es importante señalar que muchos geles requieren cantidades relativamente pequeñas de agentes gelificantes (organogelificantes) y por lo tanto, pueden ser considerados como materiales grasos con proporciones de aceite líquido incluso por encima del 97% en peso (Patel et al., 2014). Los organogelificantes se clasifican en dos tipos: poliméricos y de bajo peso molecular. Los poliméricos (entre los que se destaca la etilcelulosa), presentan mayor potencial para aplicaciones alimentarias ya que muchos son de grado alimentario y de bajo costo en comparación con los organogeles de bajo peso molecular (Co y Marangoni, 2012; Stortz et al., 2012; Zetzl et al., 2012).

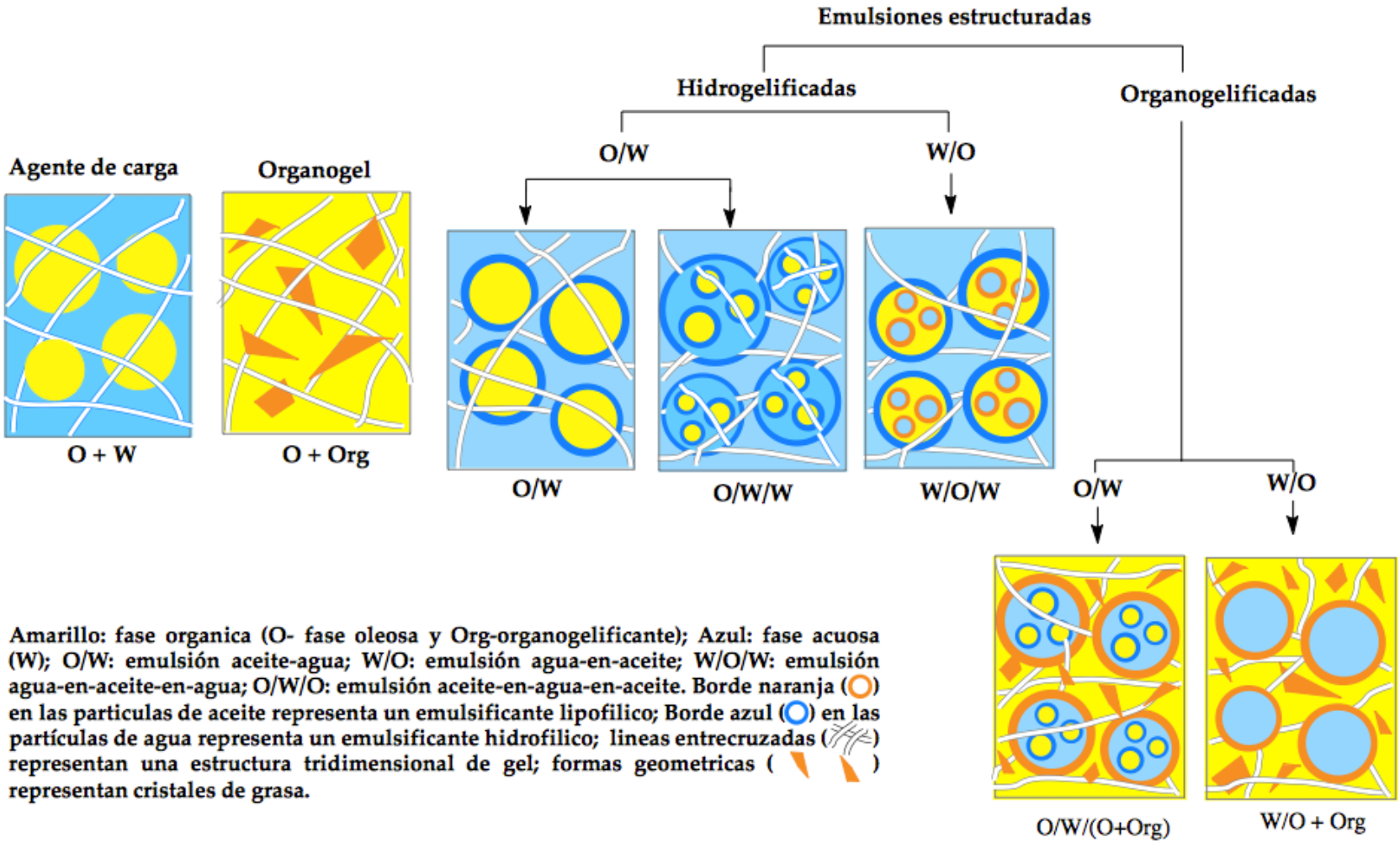


Figura 1.5. Esquema general de los diferentes sistemas de estabilización y estructuración de lípidos.
Adaptado de Jiménez-Colmenero et al. (2015)

En general, los oleogeles se forman en un sólo paso, mediante la combinación de organogelificantes y aceites comestibles en condiciones térmicas (alta temperatura) y de cizallamiento específicas, que varían dependiendo del tipo de oleogel.

Otro sistema empleado para estructurar y estabilizar aceite son los denominados **agentes de carga,** los cuales están constituidos por partículas de aceite, inmovilizadas en una red de hidrogelificada, por lo que su estructura es de tipo O + W (*Oil + Water*) (**Figura 1.5**). Esta estrategia será descrita en detalle más adelante.

Las emulsiones convencionales se componen de dos fases inmiscibles (generalmente aceite y agua), con una fase dispersa en la otra en forma de pequeñas partículas y se clasifican generalmente de acuerdo con la disposición de las dos fases inmiscibles, ya sea como sistemas de aceite-en-agua O/W o de agua-en-aceite W/O. Sin embargo, tales sistemas son generalmente propensos a fenómenos de inestabilidad física como coalescencia, separación de fases, etc., no siendo capaces de proporcionar una textura sólida, a menos que la concentración de gotas en la emulsión resulte en una emulsión estrechamente empaquetada (McClements et al., 2007; McClements, 2010). Esta limitación ha conducido al diseño de **emulsiones estructuradas** más complejas, con propiedades funcionales novedosas y diversas aplicaciones industriales (Dickinson, 2012; McClements, 2012). El proceso de estructuración se realiza esencialmente mediante tres mecanismos: capas, agrupación e incrustación, mejor conocidas por sus términos en ingles *layering*, *clustering* y e*mbedding*, respectivamente (McClements, 2012) (**Figura 1.6**). Sin embargo, el enfoque que parece garantizar una modificación fácil y exitosa del aceite para proveer una emulsión con una textura más sólida es la incrustación.

Figura 1.6. Mecanismos generales para la creación de emulsiones estructuradas
Adaptado de McClements, 2012

Basados en sistemas de estructuración por incrustación, resulta posible condicionar características de emulsiones (O/W o W/O), mediante su incorporación como partículas embebidas en una fase continua hidrogelificada u organogelificada, dando lugar a materiales complejos en los que coexisten estructuras de emulsión y de gel (**Figura 1.5**). Estas emulsiones estructuradas se producen a través de un procedimiento que consta de dos pasos. En general, la primera etapa implica la producción de una emulsión líquida estabilizada mediante proteínas. La segunda fase supone inducir la gelificación de la fase continua (de forma térmica, enzimática o química) (Dickinson, 2012). Las propiedades reológicas de este material (sólido o viscoso) están determinadas principalmente por las propiedades de la fase hidrogelificada u organogelificada.

En base a lo anteriormente descrito, la elección de uno u otro sistema dependerá del tipo de mejora lipídica a llevar a cabo en el producto. Si el objetivo es sustituir totalmente o parcialmente la grasa animal y por lo tanto en proporción considerable, es necesario recurrir a estructuras que permitan contener un gran volumen de material lipídico. Por otra parte, si de lo que se trata es de enriquecer un producto en lípidos bioactivos muy lábiles, como por ejemplo los AGP n-3 de cadena larga, los principales criterios para seleccionar el sistema serían la alta protección que pueda ofrecer frente al deterioro, especialmente el oxidativo y/o el favorecimiento de su biodisponibilidad (Chen et al., 2013).

En general, las diferentes estrategias de estructuración de lípidos han sido estudiadas fundamentalmente a nivel de diseño, formación, estructura y propiedades finales. Sin embargo, a pesar del elevado número de potenciales aplicaciones (varias de ellas prometedoras), actualmente existen pocos ejemplos de incorporación de lípidos estructurados en alimentos, incluidos los productos cárnicos. Así, el conocimiento del comportamiento de estos sistemas en matrices alimentarias es todavía limitado (**Tabla 1.8**).

Tabla 1.8. Ejemplos de aplicaciones de aceites estructurados en la mejora del contenido de grasa en productos cárnicos.

Tipo de aceite estructurado	**Aceite**	**Producto**	**Referencia**
Oleogel: etilcelulosa (10%)	Soja, lino y canola	Salchicha frankfurt	Zetzl et al. (2012)
Oleogel: monoacilgliceroles (0.5-2.5%) o lecitina de soja (2.5%)	Mezcla de oliva virgen y girasol	Suspensión cárnica	Lupi et al. (2012); Lupi et al. (2014)
Oleogel: etilcelulosa (15%) o etilcelulosa (11%) and monoestearato de sorbitán (3.67%)	Canola	Salchicha frankfurt de vacuno y salchicha frescas de cerdo	Wood (2013)
Agente de carga a base de konjac	Combinación de oliva, lino y aceite	Embutido fermentado	Triki et al. (2013c); Salcedo-Sandoval et al. (2013a)
Agente de carga a base de alginato	Oliva	Emulsión cárnica	Ruiz-Capillas et al. (2013)
Agente de carga a base de alginato	Oliva	Salchicha frankfurt	Herrero et al. (2014b)
Emulsión gel: transglutaminasa microbiana (MTG)	Combinación de oliva, lino y aceite	Salchicha frankfurt	Delgado-Pando et al. (2010a)
Emulsión gel: k-carragenato	Lino	Salchicha bologna	Poyato et al. (2014)
Emulsión gel: MTG, alginato o gelatina	Oliva	Salchicha frankfurt	Pintado et al. (2015)

De entre las distintas opciones disponibles para utilizar en procesos de reformulación encaminados a mejorar la composición lipídica de productos cárnicos se encuentran los agentes de carga y las partículas de hidrogel (una emulsión estructurada tipo $O/W_1/W_2$). Estos pueden ser empleados para sustituir grasa animal y enriquecer con AGP n-3 de cadena larga, respectivamente, ofreciendo interesantes oportunidades de desarrollo escasamente exploradas, por lo que a continuación serán revisados en detalle.

1.2.2.2.1 Agentes de carga de aceite (Sistema O+W)

Como se ha señalado anteriormente, una forma de estabilizar y estructurar el material lipídico es la dispersión de un gran número de gotas de aceite en una fase acuosa continua gelificada, originando los denominados agentes de carga (*oil bulking agent*). En este caso, el aceite líquido está atrapado físicamente en una red de gel, que proporciona una estructura sólida al sistema, lo que lo hace apto para su uso como análogo de grasa (Herrero et al., 2014a).

La tecnología requerida para elaborar estos sistemas es relativamente simple y económica. En primer lugar el aceite se dispersa y homogeniza en la fase acuosa y a continuación se induce la gelificación de la fase acuosa usando un agente gelificante. Existe un gran número de hidrocoloides que se pueden utilizar como gelificantes, ya sea individualmente o en combinación, para crear una variedad de estructuras de gel. Tales estructuras ayudan a inmovilizar las partículas de aceite y por lo tanto, a actuar como agentes de carga. Los agentes de carga a base de konjac glucomanano (**Figura 1.7**) son un ejemplo representativo del empleo de esta tecnología, dirigida al desarrollo de productos cárnicos con mejor contenido lipídico **(Tabla 1.8)**.

Konjac glucomanano

El konjac glucomanano (en adelante konjac), es el nombre genérico de la harina formada a partir de moler la raíz seca de la planta *Amorphophallus konjac*, la cual

contiene un polisacárido neutro de alto peso molecular (200.000 y 2.000.000 daltons) (Tye, 1991). En Estados Unidos, la Administración de Alimentos y Medicamentos (*Food and Drug Administration,* FDA) considera el konjac un ingrediente generalmente reconocido como seguro (*generally recognized as safe*, GRAS). Está permitido en productos cárnicos y avícolas en que se autorizan las harinas vegetales, que no exceda el 3,5% de la fórmula del producto de forma individual o colectiva con otros ligantes. La Comisión Europea incluye al konjac dentro de los ingredientes emulsionantes, espesantes, estabilizantes y gelificantes aprobados, con el número de referencia E-425. Este ingrediente natural se ha utilizado en Asia durante siglos en alimentos tradicionales como fideos y otros productos que requieren estabilidad a temperaturas de ebullición. Su estructura molecular se compone de cadenas de manosa y glucosa en una relación molar del 1,6 a 1, respectivamente, unidas mediante enlaces glucosídicos β (1→4). La molécula de glucomanano, componente funcional de la harina de konjac, tiene ramas laterales cortas y grupos acetilo presentes de forma aleatoria en la posición C-6 de una unidad de azúcar, considerándose que es la presencia de este grupo la que le confiere su solubilidad en el agua (Huang et al., 2002; Ratcliffe et al., 2005).

El konjac es una fibra muy soluble que posee una excepcional capacidad de retención de agua, hasta más de 100 veces su peso, proporcionando una elevada viscosidad a las soluciones que forma (Tye, 1991; González Canga et al., 2004). Esta fibra es capaz de formar películas muy estables en agua fría y caliente, en medios ácido o básico (Tye, 1991; Zhang et al., 2001), así mismo presenta interesantes propiedades gelificantes condicionadas principalmente por su peso molecular y su contenido en grupos acetilo. Al disolverse en medio alcalino (por ejemplo, por adición de hidróxido cálcico o carbonato potásico o sódico) se forman geles térmicamente estables (incluso a 200° C) por liberación de los grupos acetilo, permitiendo a la molécula interaccionar, dando lugar a las

estructuras poliméricas que constituyen los geles (Tye, 1991). Existen efectos sinérgicos entre el konjac y los almidones e hidrocoloides (carragenatos, goma xantan, goma gellan, etc.), que hace posible la formación de geles con diferentes propiedades físico-químicas y por tanto con distintas oportunidades de aplicación en tecnología de alimentos (Tye, 1991; Miyoshi et al., 1996; Huang y Lin, 2004).

La harina de konjac se considera un ingrediente escasamente calórico que dado su contenido en fibra no digestible, presenta numerosos efectos fisiológicos y aplicaciones terapéuticas (Tye, 1991; Zhang et al., 2001; Al-Ghazzewi et al., 2007). Según Pittler y Ernst (2004), la ingestión del konjac contribuye a reducir el apetito debido a su elevada capacidad de absorción de agua que proporciona sensación de saciedad. Este hecho aumenta la viscosidad del contenido gastrointestinal retrasando el vaciado gástrico y prolongando así la sensación de plenitud. Estos fenómenos conllevan una reducción del peso corporal. El konjac se ha mostrado también eficaz para el tratamiento de estreñimiento crónico por el incremento en el volumen de heces (Marsicano et al., 1994). Ha sido además, empleado para el control dietético de diabetes dada su capacidad de reducir los niveles sanguíneos de glucosa e insulina, probablemente debido al hecho de retrasar el vaciado gástrico se dificulta el acceso de la glucosa a la mucosa intestinal (McCarty, 2002). Varios autores también han señalado el efecto hipocolesteromiante del konjac, ya que provoca un descenso del colesterol y de la fracción LDL (Arvill y Bodin, 1995; Martino et al., 2005), así como de triaciglicerol (Takigami et al., 2009). Este hecho le ha atribuido la propiedad de prevenir ECV.

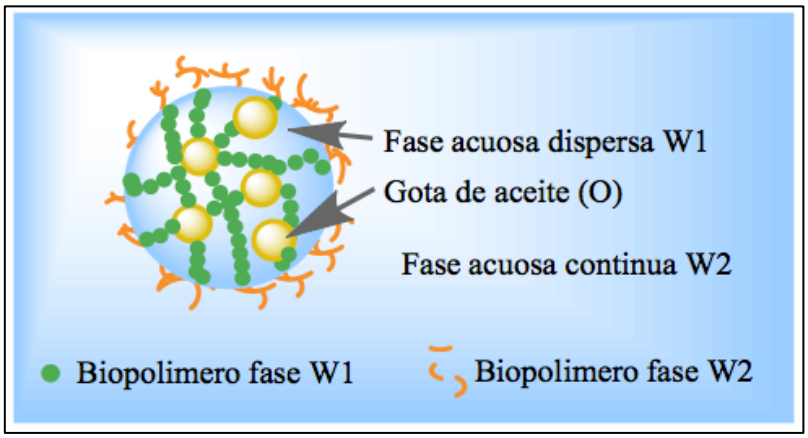
Figura 1.7. Agente de carga a base de konjac conteniendo 20% de una mezcla de aceites de oliva, lino y pescado
Fuente: Jiménez-Colmenero et al., 2015

Los geles de konjac son capaces de simular las propiedades organolépticas de la grasa (sensación en la boca) y tejido conectivo en sistemas cárnicos (Tye, 1991; Triki et al., 2013a), por lo que su empleo para la sustitución de grasa animal se ha llevado a cabo en derivados cárnicos de distinta naturaleza como fue descrito en la sección 1.2.1. y tabla 1.4. Sin embargo, su uso como agente de carga ha sido más bien limitado. Con tal propósito, aceite de oliva y una combinación de los aceites de oliva, semilla de lino y de pescado (20% p/p) fueron estabilizados en agentes de carga a base de konjac glucomanano (**Figura 1.7**). Estas matrices han sido empleadas como sustitutos de grasa de cerdo para reducir contenido y mejorar el perfil de ácidos grasos en diferentes productos cárnicos como salchichas frescas (Triki et al., 2013a), productos fermentados (Triki et al., 2013c) y paté (Delgado-Pando et al., 2012a), sin afectar significativamente las propiedades tecnológicas y sensoriales de los productos.

1.2.2.2.2 Partículas de hidrogel (Sistemas O/W_1/W_2)

Las partículas de hidrogel cargadas (*filled hydrogel particles*), que para efectos de esta memoria se denominaran partículas de hidrogel son esferas, donde el

aceite emulsionado se ha incorporado dentro de una fase acuosa dispersa gelificada (W_1) que a su vez está contenida en una fase acuosa continua (W_2). En tales condiciones, dichas gotas quedan encapsuladas dentro de la matriz de hidrogel formando una estructura tipo $O/W_1/W_2$ (McClements, 2010) (**Figura 1.8)**. Aunque en general se utilizan polímeros sintéticos para el desarrollo de este tipo sistemas en aplicaciones farmacéuticas y biomédicas, la mayoría de ellos no están permitidos en alimentos y bebidas (Chen et al., 2006). Por tanto, en su lugar se han venido utilizando biopolímeros como polisacáridos y



algunas proteínas para obtener hidrogeles de calidad alimentaria.

Figura 1.8. Estructura de una partícula de hidrogel.
Adaptado de Matalanis (2012)

Existen distintos métodos para la formación de partículas de hidrogel. Como primer paso, la mayoría de estos métodos requiere la preparación de una emulsión O/W. El tamaño, la concentración, y la carga de las gotas de estas emulsiones pueden ser controlados mediante la selección de un emulsificante apropiado (tipo y concentración) y de un procedimiento de homogeneización adecuado (tipo homogeneizador y condiciones de funcionamiento). Una partícula de hidrogel puede ser creada por la combinación de una emulsión O/W con una solución de biopolímeros, ajustando posteriormente las condiciones que promuevan su formación. De acuerdo con McClements (2010),

son varios los métodos que se pueden utilizar para formar partículas de hidrogel, como se resume a continuación:

Métodos de inyección: Las partículas de aceite de una emulsión O/W se combinan con una solución biopolimérica (capaz de formar gel) antes de la gelificación. La mezcla resultante se inyecta en otro líquido que promueve la rápida gelificación del biopolímero (**Figura 1.9a**).

Método disruptivo: Consiste en la ruptura macroscópica de la red de hidrogel por aplicación de una fuerza. La manera más común de realizar este método es llevar una solución hidrocoloide (que contenga las partículas O/W) cerca del punto gelificación, y en ese momento ejercer una fuerza para formar las partículas (**Figura 1.9b**).

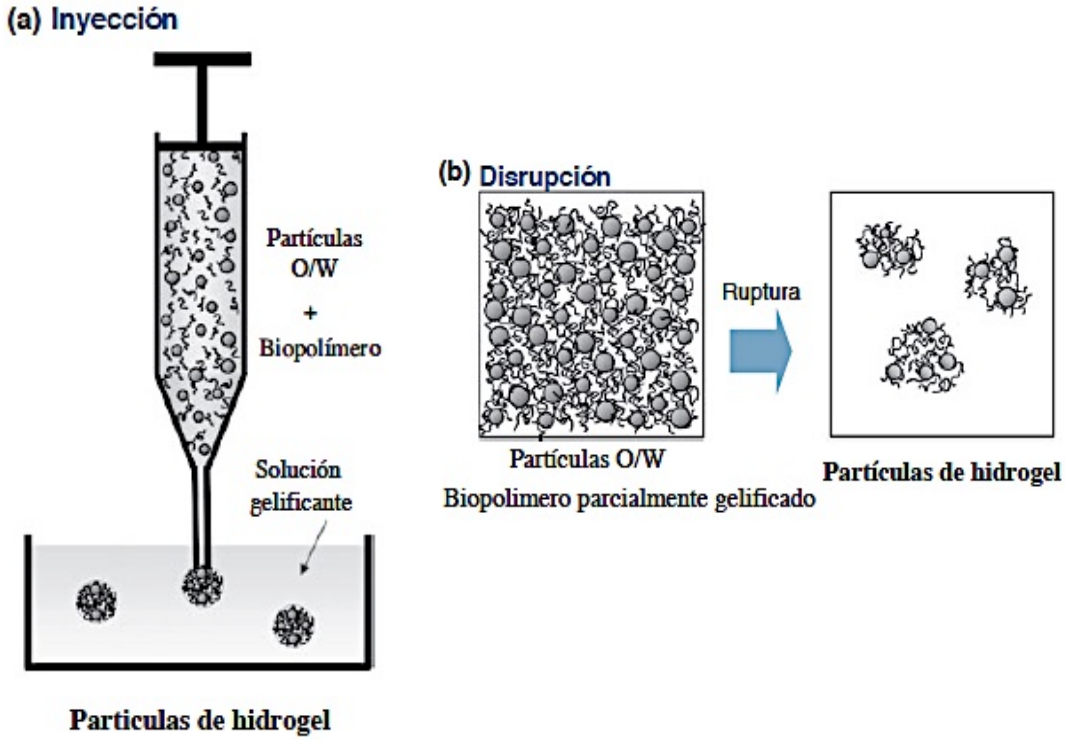


Figura 1.9. Formación de partículas de hidrogel a través de métodos de inyección y disruptivo
Adaptado de McClements (2010)

Método de separación de fases biopoliméricas: Se pueden formar partículas de hidrogel a partir de combinaciones biopoliméricas en las que puede ocurrir separación de fases. Cuando un sistema conformado por dos biopolímeros y un disolvente como el agua, es mezclado hay tres resultados posibles: miscibilidad, asociación y segregación (**Figura 1.10**).

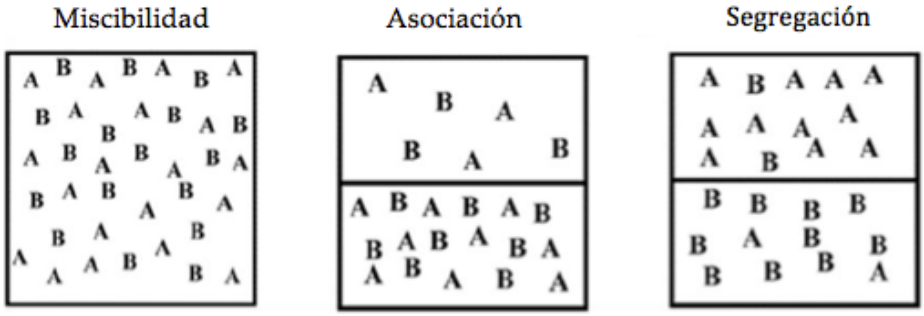


Figura 1.10. Posibles resultados de la mezcla de un sistema que consiste de un biopolímero A, un biopolímeros B y un disolvente.
Adaptado de Matalanis (2012)

La miscibilidad se refiere a la propiedad de algunos líquidos para mezclarse en cualquier proporción, formando una fase homogénea. La asociación tiende a ocurrir cuando hay fuerte atracción entre las moléculas de biopolímeros. Por ejemplo, atracción electrostática entre biopolímeros con carga opuesta que conduce a la formación de un sistema de dos fases que consiste en una fase acuosa rica en ambos biopolímeros, y otra fase acuosa pobre en ambos biopolímeros (**Figura 1.10**). La segregación tiende a ocurrir cuando existe repulsión neta entre las moléculas de biopolímeros, lo cual conduce a la formación de un sistema de dos fases con una fase acuosa rica en uno de los biopolímeros, y otra fase acuosa rica en el otro biopolímero. El tipo de comportamiento exhibido por una combinación particular de biopolímeros dependerá de la composición del sistema (por ejemplo, concentraciones de los biopolímeros), las características de biopolímero (por ejemplo, peso molecular, carga eléctrica, la conformación) y condiciones de la solución (por ejemplo, pH,

fuerza iónica, temperatura, fuerzas mecánicas) (McClements, 2010; Matalanis et al., 2010).

Cuando un sistema de fases separadas se mezcla, tiende a formar una especie de "emulsión" W_1/W_2, en donde típicamente, la fase que ocupa el mayor volumen se convertirá en la fase continua mientras que la fase que ocupa menos volumen se convertirá en la fase dispersa (Norton y Frith, 2001). En estos sistemas, las partículas O/W se mezclan con la emulsión W_1/W_2 bajo condiciones donde las partículas de aceite se ubican preferentemente en la fase biopolimérica dispersa. El sistema resultante es una emulsión $O/W_1/W_2$ que consiste en partículas de aceite dispersas dentro de una fase acuosa, que a su vez está dispersa dentro de otra fase acuosa (Matalanis et al., 2010). Sin embargo, estos sistemas son inestables y con el paso del tiempo tienden a separarse (Norton y Frith, 2001). Para detener este proceso de separación y preservar la estructura de la emulsión $O/W_1/W_2$, las condiciones de la solución se pueden ajustar de tal manera que una de las dos fases acuosas del sistema W_1/W_2 forme un gel (**Figura 1.11**) (McClements et al., 2007; McClements, 2010). Por ejemplo, si la emulsión contiene un biopolímero capaz de gelificar en frío, entonces la fase constituida por ese biopolímero podría ser gelificada mediante la disminución de la temperatura de la mezcla. La gelificación en frio puede ser llevada a cabo mediante la adición de iones o por métodos enzimáticos. La gelificación enzimática de proteína implica la formación de enlaces químicos entre las cadenas de proteínas para formar un gel. La enzima más conocida y ampliamente disponible para la gelificación de proteínas es la transglutaminasa de origen microbiano (MTG). Esta enzima es capaz de formar enlaces cruzados inter e intramoleculares entre el grupo γ-carboxy amida de glutamina y el grupo ε-amino de lisina de las proteínas (DeJong y Koppelman, 2002). La capacidad de la MTG para gelificar fuertemente una proteína depende de la accesibilidad de los residuos de glutamina y lisina. Por esta razón, las proteínas

con estructuras flexibles, tales como la caseína y la gelatina son buenos sustratos para la gelificación con MTG mientras que las proteínas con estructuras más rígidas, tales como α-lactoalbúmina nativa son sustratos pobres para la transglutaminasa (DeJong y Koppelman, 2002). Las condiciones de reacción también son importantes para lograr suficiente gelificación proteica mediante la MTG. En el caso de transglutaminasa procedente de *Streptomyces mobaraensis*, las condiciones óptimas se encuentra a pH entre 6 y 7 a 50 °C, aunque 90-100% de la actividad enzimática se mantiene en un rango de pH entre 5 y 9 (Dube et al., 2007).

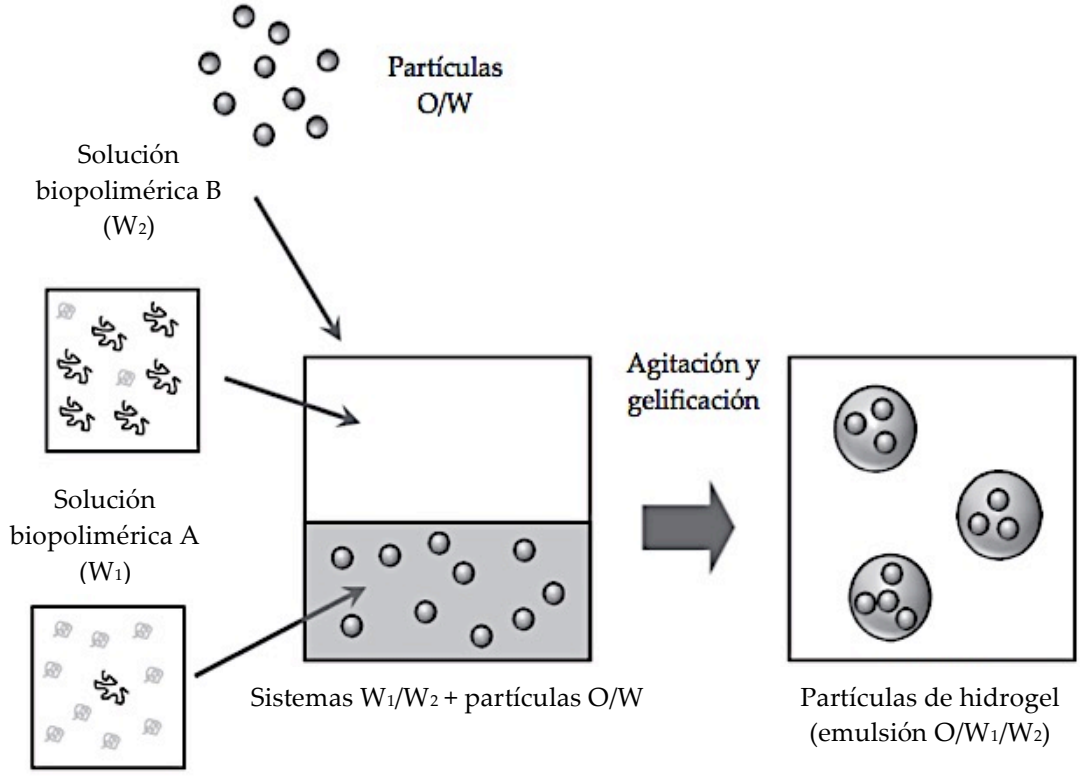


Figura 1.11. Representación esquemática de la producción de una emulsión $O/W_1/W_2$ a partir de un sistema de dos fases biopoliméricas
Adaptado de McClements (2014)

Las partículas de hidrogel han encontrado muchas aplicaciones en la industria farmacéutica, para proteger y administrar fármacos a lugares específicos en el cuerpo. Teniendo en cuenta su éxito en esta área, existe un

notable interés en el desarrollo de partículas de hidrogel con atributos deseables para su aplicación en alimentos y bebidas (McClements et al., 2007).

1.3 DISEÑO DE PRODUCTOS CÁRNICOS MÁS SALUDABLES

De entre los distintos productos cárnicos susceptibles de ser mejorados en su composición, algunos resultan especialmente interesantes en relación con el desarrollo de alimentos más saludables. Entre ellos se encuentran productos tratados por calor como las salchichas tipo frankfurt y productos frescos como las hamburguesas. Se trata de alimentos de gran aceptación por determinados sectores de la población, atribuible, entre otros, a factores de conveniencia, precio o marketing. Sin embargo, estos alimentos suelen presentar limitaciones en su composición, asociado a aspectos cualitativos y cuantitativos de su material lipídico (**Tabla 1.1**). Por sus características (elevado grado de desintegración estructural y homogenización) estos productos son más susceptibles de ser sometidos a procesos de reformulación, lo que ayudaría a dotarlos de propiedades más convenientes en relación con la salud. A fin de entender mejor la aplicación de estrategias de reformulación sobre tales alimentos, a continuación se detallan algunos aspectos específicos de los productos seleccionados.

1.3.1 Productos tipo gel/emulsión: salchichas tipo frankfurt

Un producto cárnico tipo gel/emulsión como consiste en una emulsión cárnica (pasta fina donde el picado de la carne ha sido muy intenso) gelificada por calor. Los glóbulos de grasa constituyen la fase discontinua, mientras que la fase continua está formada por una disolución acuosa de sales y proteínas solubles e insolubles en suspensión, así como porciones de fibra musculares y restos de tejido conectivo (**Figura 1.12**).

Los principales agentes emulsificantes son las proteínas cárnicas (miofibrilares) solubles en soluciones salinas, principalmente la miosina, la cual debido a su carácter polar permite establecer uniones hidrófilas con la matriz acuosa e hidrófobas con las grasas, formando una matriz proteica o película alrededor del glóbulo de grasa. Al tratarse de proteínas solubles en soluciones salinas, la cantidad de sal presente a la hora de formarse la emulsión es un factor determinante. La estabilidad de dicha emulsión dependerá del grado de extracción y solubilización de estas proteínas, lo cual además de la concentración de sal depende de los siguientes factores: el pH de la carne, la temperatura, el grado de picado (tamaño de las glóbulos de grasa) y la viscosidad de la emulsión.

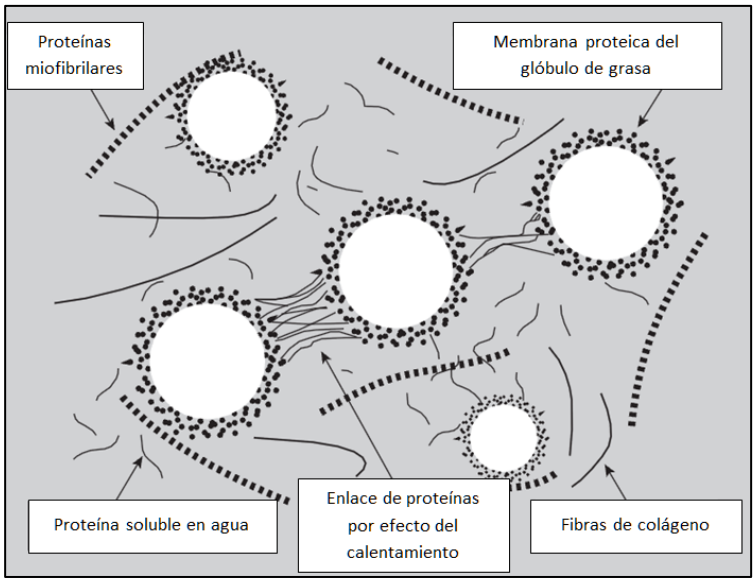


Figura 1.12. Representación esquemática de una emulsión cárnica.
Fuente: Xiong (2004)

Para la elaboración de salchichas, tras la realización de la emulsión cárnica tiene lugar su embutido en tripas de pequeño diámetro, que son sometidas a tratamiento térmico, que conduce a la formación de estructura de gel. Cuando la emulsión cárnica alcanza unos 45 °C, las proteínas miofibrilares inician el proceso de desnaturalización, produciéndose un despliegue de las cadenas polipeptídicas que posteriormente se asocian mediante distintos tipos

de enlace, dando lugar a redes tridimensionales. En el interior de estas redes se encuentran inmovilizadas agua y partículas de grasa, además de otros componentes del sistema. La estabilidad del gel formado dependerá fundamentalmente de factores como temperatura, pH, concentraciones de sal y proteína. El tratamiento térmico generalmente finaliza al alcanzar los 70 °C en el centro térmico, para asegurar la destrucción de la carga microbiana patógena. Estos productos generalmente se comercializan bajo refrigeración.

1.3.2 Productos frescos: hamburguesas

Estos productos son carnes picadas a las que se pueden incorporar otros ingredientes, principalmente tejido adiposo, y sal por razones tecnológicas y organolépticas. Con frecuencia a estos productos se les adicionan otro tipo de ingredientes como pan, vegetales, fibras, etc., y es por lo que, en estos casos es más acertado denominarlos "tipo hamburguesa". Sin embargo a lo largo de esta memoria se denominarán "hamburguesas". Suelen presentar un grado de picado grueso (5-8 mm) para dotarlos de una textura fibrosa y desmenuzable. Pertenecen a los productos cárnicos frescos y se pueden comercializar refrigerados, congelados o cocinados. El hecho de que los productos picados lleven asociada una elevada carga microbiana capaz de crecer con rapidez, hace que su estabilidad (vida útil), sea limitada.

1.3.3 Posibles declaraciones nutricionales y de propiedades saludables de acuerdo con la legislación europea

Tras la elaboración de un producto diseñado para conferir una serie de beneficios en la salud, resulta esencial comunicar tales beneficios al consumidor. Es a través del etiquetado la manera en que éste puede informarse sobre los beneficios que la ingestión del alimento aporta. El Reglamento (CE) nº 1924/2006 del Parlamento Europeo y del Consejo, de 20 de diciembre de 2006, relativo a las declaraciones nutricionales y de propiedades saludables en los

alimentos, está encaminado específicamente a establecer las reglas básicas para la creación y utilización de las declaraciones nutricionales y de propiedades saludables. Está dirigido a servir de referencia legal, en la publicidad y el etiquetado de aquellos alimentos, que además de nutrir, tienen un beneficio específico para la salud, científicamente demostrado. En tal sentido dicho reglamento, por un lado define los principios generales que deben cumplir todas las declaraciones, así como las condiciones específicas para su uso, y por otro, establece una lista positiva de declaraciones nutricionales. De igual modo, plantea las bases para proceder a la petición de autorización de declaraciones de propiedades saludables.

Según se establece en el Reglamento (CE) 1924/2006 (Art. 2.), se entiende por declaración nutricional "*cualquier declaración que afirme, sugiera o dé a entender que un alimento posee propiedades nutricionales benéficas específicas con motivo de su aporte energético y los nutrientes u otras sustancias que: contiene, no contiene, o contiene en proporciones reducidas o incrementadas*". Como se ha señalado anteriormente, este Reglamento estableció una lista positiva (y por lo tanto limitada) de declaraciones nutricionales, la cual ha sufrido posteriormente varias ampliaciones, caso del Reglamento (UE) nº 116/2010 relativo a los ácidos grasos omega-3, las grasas monoinsaturadas, las grasas poliinsaturadas y las grasas insaturadas, así como el Reglamento (UE) nº 1047/2012 en relación con las declaraciones "sin sodio o sin sal añadidos" y "contenido reducido de grasas saturadas" (**Tabla 1.9**). Las posibilidades de utilización de tales declaraciones por el sector cárnico son amplias y directas ya que no requieren autorización específica, y su verificación se produce meramente por métodos analíticos. En la **Tabla 1.10** se recogen ejemplos de productos cárnicos en los que se ha modificado la composición en grasa mediante el uso de estrategias de reformulación con la asignación de declaraciones nutricionales de acuerdo a la normativa.

Tabla 1.9. Declaraciones nutricionales autorizadas de acuerdo al Reglamento (CE) nº 1924/2006 y sus modificaciones posteriores (Reglamentos nº 116/2010 y 1047/2012).

Bajo valor energético (1924/2006)
Valor energético reducido (1924/2006)
Sin aporte energético (1924/2006)
Bajo contenido en grasa (1924/2006)
Sin grasa (1924/2006)
Bajo contenido en grasas saturadas (1924/2006)
Sin grasas saturadas (1924/2006)
Fuente de ácidos grasos omega-3 (116/2010)
Alto contenido de ácidos grasos omega-3 (116/2010)
Alto contenido de grasas monoinsaturadas (116/2010)
Alto contenido de grasas poliinsaturadas (116/2010)
Alto contenido de grasas insaturadas (116/2010)
Fuente de fibra (1924/2006)
Alto contenido en fibra (1924/2006)
Fuente de proteínas (1924/2006)
Alto contenido en proteínas (1924/2006)
Contiene (nutriente u otra sustancia) (1924/2006)
Mayor contenido de (nutriente) (1924/2006)
Contenido reducido de (nutriente) (1924/2006) (1047/2012)
Light/lite (ligero) (1924/2006)

*Nutrientes: Proteínas, grasas, hidratos de carbono, vitaminas, minerales y fibra

En relación a las declaraciones de propiedades saludables el Reglamento (CE) 1924/2006 (Art. 2.) las define como "*cualquier declaración que afirme, sugiera o dé a entender que existe una relación entre una categoría de alimentos, un alimento o uno de sus constituyentes, y la salud"*. Las declaraciones de propiedades saludables establecidas son de tres tipos y se detallan a continuación.

a) *Declaraciones distintas a las relativas a la reducción de riesgo de enfermedad y al desarrollo y salud de los niños (Art. 13)*

Para ser establecidas este tipo de declaraciones se plantean dos posibilidades: 1) Basadas en datos científicos generalmente aceptados, y que sean bien comprendidas por el consumidor medio (Art. 13.1), y 2) Basadas en datos científicos recientemente obtenidos y/o que incluyan una solicitud de protección de los datos sujetos a derechos de propiedad industrial (Art 13.5). En relación a las solicitudes de propiedades saludables de los alimentos presentadas en el marco del artículo 13.1, la Autoridad Europea de Seguridad Alimentaria (EFSA) estableció que, en el caso de algunas declaraciones de propiedades saludables, podía determinarse una relación causa-efecto entre una categoría de alimentos, un alimento o uno de sus constituyentes, y el efecto declarado. Esto dio lugar a que en mayo de 2012, la Comisión Europea, a través del Reglamento (UE) nº 432/2012 publicara una lista de declaraciones autorizadas de propiedades saludables de los alimentos distintas de las relativas a la reducción del riesgo de enfermedad y al desarrollo y la salud de los niños. Esta lista inicial de más de 200 declaraciones fue posteriormente ampliada en tres ocasiones (Reglamentos (UE) nº 536/2013, nº 851/2013 y nº 1018/2013) . En dicha lista existen muchas posibilidades para dotar con este tipo de declaraciones a los productos cárnicos reformulados en su composición lipídica, ya que sus condiciones de uso hacen referencia básicamente a la presencia/ausencia de nutrientes y sustancias contenidas en los productos, de acuerdo a lo establecido en las declaraciones nutricionales. Todo ello abre una amplia gama de posibilidades de establecer diseños específicos sujetos a declaraciones saludables deseadas. En la **Tabla 1.11** se recogen ejemplos de declaraciones de propiedades saludables distintas a las relativas a la reducción de riesgo de enfermedad y al desarrollo y salud de los niños, que de acuerdo con la reglamentación pueden asignarse a distintos productos cárnicos obtenidos mediante estrategias de reformulación (Jiménez-Colmenero, 2014).

Tabla 1.10. Ejemplos de productos cárnicos con composición en grasa modificada mediante estrategias de reformulación y las declaraciones nutricionales reportadas, correspondientes de acuerdo con el Reglamento (CE) 1924/2006

Derivados cárnicos enriquecidos con:	**Declaraciones**	**Referencia**
Salchichas: aceite de lino o pescado	Alto contenido omega-3	Valencia et al. (2008)
Mortadela: aceite de lino	Alto contenido omega-3 Alto contenido de proteínas	(Berasategi et al., 2011)
Paté: konjac y mezcla de aceites (oliva+lino+pescado)	Valor energético reducido Alto contenido de proteínas Alto contenido omega-3, AGM y AGI	Delgado-Pando et al. (2011)
Chorizo: aceite de lino	Alto contenido proteínas Alto contenido omega-3	García-Íñiguez de Ciriano et al. (2009)
Chorizo: mezcla de aceites (lino+alga)	Alto contenido proteínas Alto contenido omega-3	García-Íñiguez de Ciriano et al. (2010b)
Chorizo: aceite de lino, sal yodada y ascorbato cálcico	Alto contenido proteínas Fuente de omega-3	García-Íñiguez de Ciriano et al. (2013)
Chorizo: konjac y mezcla de aceites (oliva+lino+pescado)	Valor energético reducido Alto contenido proteínas Alto contenido omega-3, AGM y AGI	Salcedo-Sandoval et al. (2013a)
Salchichón: calcio	Fuente de calcio Contenido reducido de grasa	Selgas et al. (2009)
Salchichón: fructooligosacáridos	Fuente de fibra Contenido reducido de grasa	Selgas et al. (2009)
Merguez (salchicha fresca tunecida)	Fuente de proteínas Alto contenido de ácidos grasos monoinsaturados. Contenido reducido en sodio	Triki et al. (2013b)

AGI: ácidos grasos insaturados. Adaptado de Jiménez-Colmenero (2014)

El artículo 13.5 ofrece otra vía para establecer este tipo de declaraciones, aunque en este caso, las oportunidades para el sector cárnico son muy limitadas. De las cinco declaraciones que figuran en el registro de la UE, cuatro de ellas tienen derecho de uso restringido (sujeta a protección de datos). Sólo el

empleo de fibra de remolacha queda abierto y es aplicable a la fibra naturalmente presente y a la adicionada a los alimentos (EFSA, 2011). Las condiciones de uso indican que la declaración aplicable en este caso, "*la fibra de remolacha azucarera aumenta el volumen fecal*", y debe ser utilizada sólo para los alimentos que puedan ser etiquetados como alto contenido en fibra (> 6 g de fibra/100 g de producto) de acuerdo al anexo del Reglamento (CE) n 1924/2006. Concretamente fibra de remolacha azucarera ha sido empleada en varios productos cárnicos como por ejemplo, salchichas tipo frankfurt o productos similares a hamburguesas, tanto como fuente de fibra como por su aptitud tecnológica para reducir grasa (Troutt et al., 1992; Vural et al., 2004).

Tabla 1.11. Declaraciones de propiedades saludables para algunos de los elaborados cárnicos incluidos en la Tabla 1.10, según el artículo 13.1 del Reglamento (CE) 1924/2006 y de acuerdo a lo establecido en el Reglamento (UE) 432/2012

Declaración nutricional	**Declaración saludable**
Fuente de ácidos grasos omega-3 (Mín 0,3 g de ácido alfa-linolénico por 100 g)	El ácido linolénico contribuye a mantener niveles normales de colesterol sanguíneo.
Fuente de ácidos grasos omega-3 (Mín 80 mg EPA+DHA por 100 g)	Los ácidos EPA y DHA contribuyen al funcionamiento normal del corazón.
Fuente de ácidos grasos omega-3 (Mín 40mg DHA/100g y 100kcal)	El ácido DHA contribuye a mantener el funcionamiento normal del cerebro y al mantenimiento de la visión en condiciones normales.
Alto contenido en grasas insaturadas	La sustitución de grasas saturadas por grasas insaturadas contribuye a mantener niveles normales de colesterol sanguíneo. El ácido oleico es una grasa insaturada.
Alto contenido en grasas insaturadas	La sustitución de grasas saturadas por grasas insaturadas contribuye a mantener niveles normales de colesterol sanguíneo (los AGM y AGP son grasas insaturadas).

Adaptado de Jiménez-Colmenero (2014)

b) Declaraciones de reducción del riesgo de enfermedad (Art. 14.1.a)

Estas declaraciones se centran en la actividad de un grupo muy reducido de sustancias. Excluyendo las declaraciones relativas a la goma de mascar con xilitol y a los chicles sin azúcar, las demás son autorizadas teniendo en cuenta la bioactividad y presencia en el alimento de compuestos tales como fitoesteroles y ésteres de fitoestanol, así como de betaglucanos de cebada y avena. En el caso de los fitoesteroles y los ésteres de fitoestanol sólo podrá hacerse referencia del efecto a la magnitud para los alimentos incluidos en las siguientes categorías: grasas amarillas para untar, productos lácteos, mayonesa y aliños para ensaladas, así en lo que respecta a los productos cárnicos reformulados, sólo podrían ser etiquetados con este tipo de declaración si llevaran en su composición betaglucanos de cebada (1 g por porción), los cuales podrían llevar las siguientes declaraciones:

- *Se ha demostrado que el betaglucano disminuye/reduce el colesterol sanguíneo. Una tasa elevada de colesterol constituye un factor de riesgo en el desarrollo de cardiopatías coronarias.*
- *Se ha demostrado que el betaglucano de cebada disminuye/reduce el colesterol sanguíneo. Una tasa elevada de colesterol constituye un factor de riesgo en el desarrollo de cardiopatías coronarias.*

Distintos derivados de la avena (salvado, harina, fibra de avena, etc.), han sido empleados para reducir grasa (como sustituto de grasa) o como ingrediente funcional en la elaboración de productos cárnicos tipo hamburguesa, en albóndigas, etc., (Jiménez-Colmenero y Delgado-Pando, 2013). Más concretamente, fibra soluble de avena (betaglucano) ha sido incorporada en un producto cárnico tipo hamburguesa bajo en grasa (Pinero et al., 2008). Con propósitos similares, varios derivados de la cebada (salvado, harina, etc.) han sido empleados en procesos de reformulación de productos tanto frescos como tratados por el calor (Jiménez-Colmenero y Delgado-Pando, 2013), utilizándose

específicamente betaglucanos de cebada en salchichas frescas con reducido contenido en grasa (Morin et al., 2004). De todo lo expuesto se desprende que no existe razón alguna para que el sector cárnico no pueda desarrollar derivados con niveles de betaglucano de avena o cebada apropiados y poder etiquetarlos con las correspondientes declaraciones saludables autorizadas relativas a la reducción del riesgo de enfermedad.

c) Declaraciones relativas al desarrollo y salud de los niños (Art. 14.1.b).

Estas declaraciones están basadas en la bioactividad y presencia de determinados compuestos en los alimentos, particularmente en relación a compuestos lipídicos, varios de estos podrían estar contenidos en diversos productos cárnicos reformulados en los niveles exigidos en las condiciones de uso (**Tabla 1.12**).

Tabla 1.12. Declaraciones saludables autorizadas relativas al desarrollo y salud de los niños (art. 14.1.b del Reglamento (CE) 1924/2006) que podrían ser utilizadas en productos cárnicos con composición lipídica mejorada

Nutriente	**Declaración**	**Condiciones de uso**
Ácido α-linolénico y ácido linoleico, ácidos grasos esenciales	Los ácidos grasos esenciales son necesarios para el crecimiento y el desarrollo normales de los niños.	El efecto beneficioso se obtiene con una ingesta diaria de 2 g de ácido α-linolénico (ALA) y una ingesta diaria de 10 g de ácido linoleico (LA).
Ácido docosahexaenoico (DHA)	La ingesta de ácido docosahexaenoico (DHA) contribuye al desarrollo visual normal de los niños hasta los 12 meses de edad.	Puede emplearse en alimentos que contienen al menos un 0,3 % del total de ácidos grasos como DHA.
Ácido docosahexaenoico (DHA)	La ingesta materna de ácido docosahexaenoico (DHA) contribuye al desarrollo normal de los ojos del feto y del lactante alimentado con leche materna.	La declaración puede ser utilizada solamente para aquellos alimentos que aporten una ingesta diaria de al menos 200 mg de DHA.

En base a las declaraciones nutricionales recogidas en la **Tabla 1.9**, distintos productos cárnicos podrían ser sujetos de varios tipos de declaraciones de propiedades saludables relativas al desarrollo y la salud de los niños (**Tabla 1.12**).

Por todo lo expuesto anteriormente, la industria cárnica dispone de amplias posibilidades de utilizar en su etiquetado tanto declaraciones nutricionales como de propiedades saludables. Partiendo de productos concretos (ya disponibles o diseñados específicamente y obtenidos mediante distintos tipos de estrategias), resulta fundamental conocer su composición, y en base a ella identificar las declaraciones (nutricionales o de propiedades saludables), que podrían aplicarse de acuerdo al registro comunitario, asegurándose de que se cumplen las condiciones de uso requeridas. Para decidir entre las distintas opciones posibles, resulta necesario valorar el interés y grado de atracción que podría suscitar en el consumidor una determinada declaración. Generalmente la valoración de los consumidores sobre alegaciones nutricionales y de salud varía según los países. Por ejemplo, en Bélgica, Países Bajos y Francia, y en el caso de la carne de vacuno, las declaraciones nutricionales y de propiedades saludables relativas a grasas saturadas despertaron un mayor interés que aquellas otras relacionadas con proteína y/o hierro, mientras que lo contrario se encontró entre los consumidores del Reino Unido (Van Wezemael et al., 2014). Así pues parece evidente la necesidad de tener en cuenta el tipo de sociedad a la que van destinadas, a la hora de plantear diferentes propuestas a nivel de mercado.

2. Objetivos

2. OBJETIVOS

El **objetivo general** de la presente memoria consiste en el **diseño y desarrollo de derivados cárnicos con mejor composición lipídica llevados a cabo mediante procesos de reformulación encaminados a la obtención de productos más saludables**. Para ello se han planteado estrategias de optimización de la composición de productos cárnicos basadas en la utilización de aceites de origen vegetal y/o marino estructurados como un agente de carga a base de konjac y en forma de partículas de hidrogel. Estas estrategias están dirigidas tanto a reducir el contenido de grasa como a mejorar el perfil de ácidos grasos disminuyendo la proporción de AGS y favoreciendo la de AGP, especialmente los de AGP n-3 de cadena larga, además de mejorar la relación AGP n-6/n-3 y AGP/AGS. En este contexto, y de acuerdo con la legislación de la Unión Europea, los productos reformulados podrían estar sujetos a alegaciones nutricionales y de propiedades saludables.

La realización de esta propuesta supone un notable reto tecnológico, ya que los productos reformulados han de poseer características similares a las que habitualmente muestran los productos cárnicos convencionales.

Para abordar este objetivo general, se plantearon los siguientes objetivos específicos:

1. Evaluar la utilización de un agente de carga de aceite a base de konjac como estrategia en el desarrollo de salchichas tipo frankfurt y de hamburguesas. En el marco de este objetivo se han aplicado procesos de reformulación encaminados a la reducción de grasa y a la mejora del perfil de ácidos grasos de estos productos. Para tal fin, se plantea la sustitución de la grasa animal, habitualmente empleada, por un agente de carga a base de konjac glucomanano conteniendo una combinación de aceites de oliva, lino y

pescado específicamente establecida para dotar al producto con altas cantidades AGM y AGP, por tanto de un perfil lipídico más ajustado en relación con las recomendaciones nutricionales. En este sentido, se han estudiado los efectos de la reformulación y la conservación en refrigeración sobre las propiedades tecnológicas, nutricionales, microbiológicas y sensoriales de los productos. Adicionalmente se aborda como los distintos métodos de cocción habitualmente empleados, afectan la composición de las hamburguesas haciendo particular referencia al perfil de ácidos grasos.

2. Analizar el empleo las partículas de hidrogel encapsulando aceite de pescado como estrategia en el desarrollo de productos cárnicos más saludables. Este objetivo se ha centrado en la aplicación de procesos de reformulación dirigidos a la mejora del perfil lipídico, basados en la presencia de partículas de hidrogel conteniendo aceite de pescado en productos tipo gel/emulsión (sistema modelo y salchichas tipo frankfurt). Estas partículas se han diseñado especialmente para vehiculizar altas concentraciones de EPA y DHA, permitiendo el desarrollo de productos enriquecidos en ácidos grasos n-3 de cadena larga. En tal sentido, los efectos sobre las propiedades tecnológicas, nutricionales y sensoriales en los productos debidos a la reformulación y la conservación en refrigeración han sido evaluados.

3. Materiales y métodos

3. MATERIALES Y MÉTODOS

Para una mejor comprensión del diseño experimental, y a fin de evitar la repetición de aspectos descritos en las publicaciones, en esta sección se expondrán de manera esquematizada y resumida los materiales y métodos empleados en el desarrollo experimental (**Figura 3.1**)

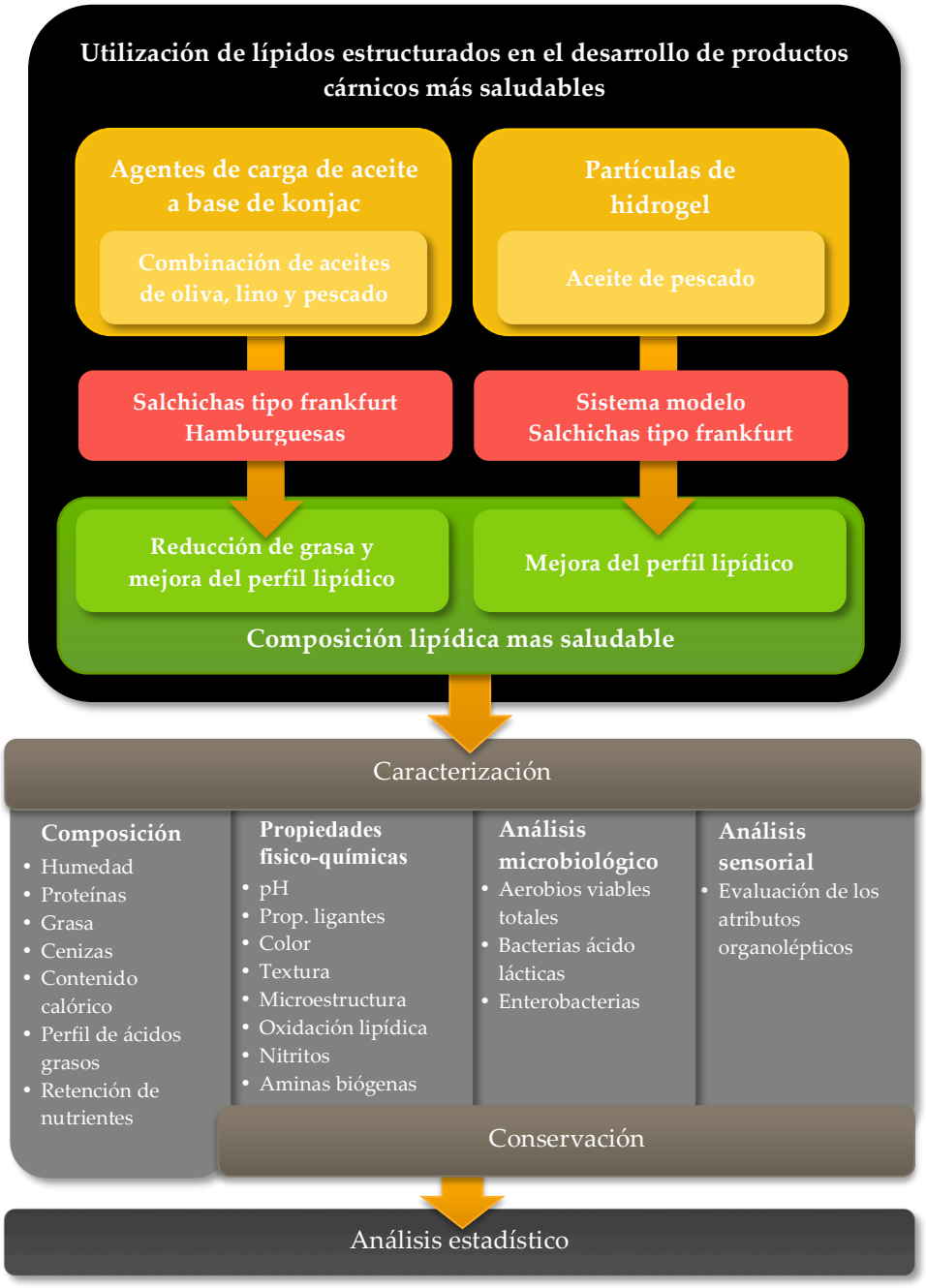


Figura 3.1. Esquema general del desarrollo experimental y de la metodología empleada en esta memoria.

3.1 MATERIAS PRIMAS

Para la preparación del **agente de carga a base de konjac**, se utilizó harina de konjac (83% de glucomanano, 120 mesh, Trades SA, Barcelona, España), i-carragenato (Secolata IP Hispanagar; Burgos, España), almidón de maíz pre-gelificado (Amigel, Julio Criado Gómez SA; Alcorcón, España) y dihidróxido de calcio (Panreac Química SA; Barcelona, España). La combinación de aceites estabilizada en este sistema estuvo constituida por: 44,39% de aceite de oliva virgen extra (Carbonell, SOS Cuétara SA; Madrid, España), 37,87% de aceite de lino (Natursoy SL; Casterçol, España) y 17,74% de aceite de pescado (Omevital 18/12 TG Gold, Cognis GmbH; Illertissen, Alemania). También se usó gel de konjac en el desarrollo de algunas salchichas tipo frankfurt (**capítulos 4.1.1** y **4.1.2**) y en los estudios con hamburguesas (**capítulos 4.1.3** y **4.1.4**), y sus ingredientes (exceptuando la mezcla de aceites) son los mismos que los requeridos para elaborar el agente de carga.

En la fabricación de **partículas de hidrogel**, se usó caseinato de sodio con 90,5% de proteína (Excellion EM 7, DMV Campina BV; Veghel, Holanda), pectina de alto metoxilo con 85.3% de ácido galacturonico y un contenido mínimo en metoxilo del 6.7% (GRINDSTED Pectin USP, Danisco; Grindsted, Dinamarca), transglutaminasa microbiana (Activa WM, 99% maltodextrina y 1% transglutaminasa; Ajinomoto Co; Tokio, Japón). El aceite de pescado (Omevital™ 1812 TG Gold, BASF SE, Ludwigshafen, Alemania) fue usado como fuente de ácidos grasos n-3 de cadena larga y de acuerdo con el proveedor contenía 169 mg de EPA/g and 110 mg de DHA/g más una mezcla de tocoferoles. Soluciones de búfer fosfato, hidróxido de sodio y ácido cítrico fueron preparadas usando reactivos suministrados por Panreac Química, SA (Barcelona, España).

Para la elaboración de los diferentes **productos cárnicos** (sistema modelo, salchichas tipo frankfurt y hamburguesas) se empleó carne magra de cerdo y tocino de cerdo que se adquirieron durante todo el estudio en un mercado local de Madrid. Manualmente se eliminó la grasa superficial de la carne magra y junto con el tocino se sometieron a un picado a 6 mm en una picadora (Mainca; Granollers, España) y se dividieron en lotes de aproximadamente 500 g que se envasaron al vacío en bolsas de plástico (Cryovac BB3050), para ser almacenados en cámara de congelación (-20 ± 2 °C) hasta su utilización.

Otros ingredientes empleados en la elaboración de salchichas tipo frankfurt fueron: tripolifosfato de sodio (Manuel Riesgo, SA; Madrid, España), nitrito de sodio (Sigma-Aldrich Company Ltd, GmbH; Steinheim, Alemania), y saborizantes (Gewürzmüller, GmbH; Münichingen, Alemania). También se empleó en el desarrollo de algunas salchichas tipo frankfurt (**capítulos 4.1.1** y **4.1.2**) una emulsión O/W con la mezcla de aceites de oliva, lino y pescado descrita anteriormente y estabilizada mediante caseinato de sodio con 86,4% de proteína (Julio Criado Gómez SA; Alcorcón, España). En la elaboración de todos los productos se uso cloruro de sodio (Panreac Química, SA; Barcelona, España).

3.2 ELABORACIÓN DE LOS LÍPIDOS ESTRUCTURADOS

3.2.1 Agente de carga de aceite a base de konjac

Como se muestra en la **Figura 3.1**, el agente de carga fue empleado en la reformulación de salchichas tipo frankfurt (**capítulos 4.1.1** y **4.1.2**) y hamburguesas (**capítulo 4.1.3** y **4.1.4**), por lo que una descripción más detallada de su fabricación se encuentra en los capítulos mencionados. De manera esquematizada, la obtención del agente de carga se realizó según se muestra a continuación (Figura 3.2).

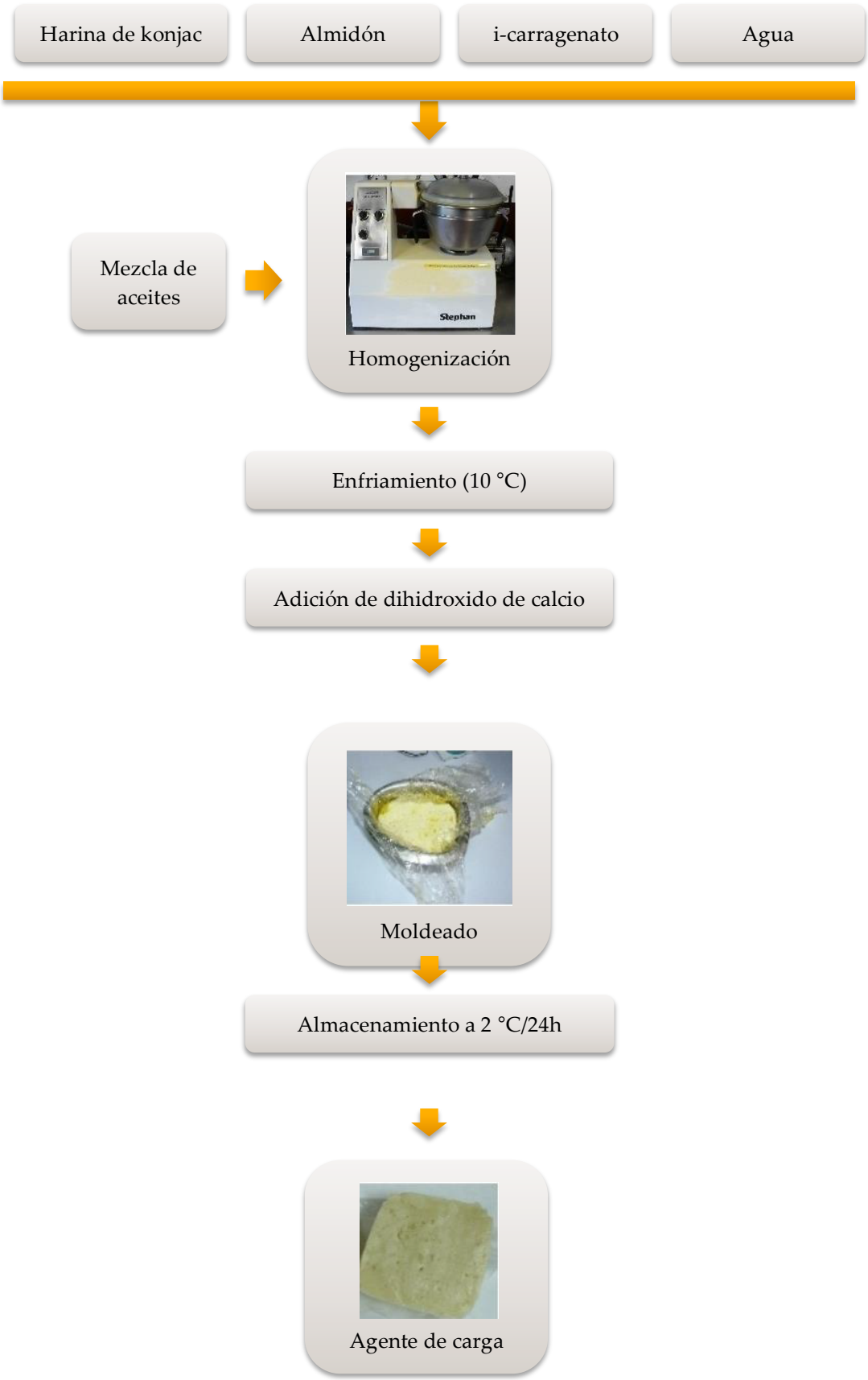


Figura 3.2. Proceso de obtención del agente de carga a base de konjac estabilizando una mezcla de aceites de oliva, lino y pescado.

La harina de konjac fue homogeneizada (Stephan Universal Machine UM5, Stephan U. Söhne; Hameln, Alemania) con agua durante 5 minutos; a continuación se añadió después el i-carragenato para ser todo homogeneizado durante otros 3 minutos. Seguidamente se incorporó la mezcla de aceites y todo el conjunto fue homogeneizado durante 3 minutos. A la mezcla anterior se adicionó una disolución de almidón de maíz pre-gelificado en agua, procediendo a su homogenización nuevamente durante 3 minutos. La mezcla se enfrió hasta los 10 °C. A continuación, con el fin de inducir la gelificación del sistema, se añadió una solución de hidróxido cálcico (1%), agitando vigorosamente durante 3 minutos. La mezcla fue colocada en un contenedor adecuado y dejada en reposo durante 24 horas a 2 ± 2 °C para su uso posterior.

3.2.2 Partículas de hidrogel

Las partículas de hidrogel se utilizaron en la reformulación de sistemas modelo (**capítulo 4.2.1**) y salchichas tipo frankfurt (**capítulo 4.2.2**), por lo que información detallada acerca del proceso de obtención de estos sistemas puede ser encontrada en tales capítulos. Sin embargo, a continuación se ofrece una breve descripción del proceso de fabricación de partículas de hidrogel (**Figura 3.3**). En primer lugar, se prepararon, una emulsión O/W con aceite de pescado (50%) estabilizada con caseinato de sodio (SC), y dos soluciones de pectina y SC, una de ellas rica en pectina (W_1) y la otra rica en SC (W_2); tanto la emulsión como las soluciones biopoliméricas fueron llevadas a pH 7. Las soluciones se mezclaron con un agitador (RW 20; Janke & Kunkel IKA Laboratortechnick; Staufen, Alemania) para formar un sistema W_1/W_2. Después la emulsión O/W fue adicionada al sistema W_1/W_2 y el conjunto fue mezclado durante 30 minutos. Dado que las partículas de aceite migran hacia la fase dispersa, a partir de esta mezcla se produjo una emulsión $O/W_1/W_2$. Debido a que la carga electrostática del SC cambia a medida que se aproxima a su punto isoeléctrico

(pH 4,6), (lo que atraería a las moléculas de pectina de carga negativa alrededor de las moléculas de SC), el pH del sistema fue reducido hasta 5 usando ácido cítrico 1 M. Posteriormente se añadió transglutaminasa microbiana para promover la gelificación de la fase W_1, y de esta manera estabilizar a las partículas de aceite en su interior. El sistema fue incubado en un baño de agua a 50 °C durante 15 minutos con agitación constante. Después, el pH fue aumentado con hidróxido de sodio 1 M hasta 7 y el sistema fue almacenado en refrigeración (2 ± 2 °C) hasta el día siguiente. Las partículas de hidrogel se lavaron para remover los restos de la fase rica en pectina (W_2), mezclando una parte del sistema de partículas de hidrogel con 4 partes de búfer fosfato pH 7 y centrifugando a 10000 g durante 1 hora (Sorvall Evolution RC Centrifuge, Kendro Laboratory Products; Asheville, NC, USA). La solución de lavado junto con los restos de la fase W_2 se decantaron y las partículas de hidrogel fueron conservadas en refrigeración (2 ± 2 °C) hasta su utilización (**Figura 3.3**).

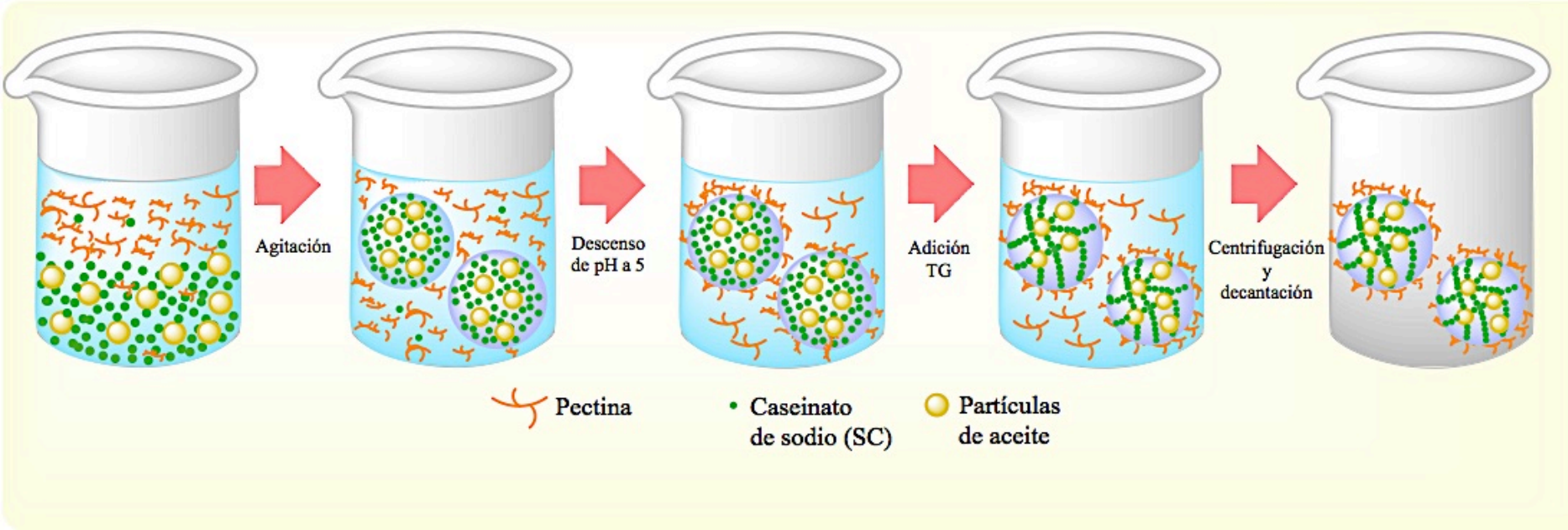


Figura 3.3. Proceso de fabricación de partículas de hidrogel estabilizando aceite de pescado.

3.3 ELABORACIÓN DE LOS PRODUCTOS CÁRNICOS

3.3.1 Sistemas modelo y salchichas tipo frankfurt

El proceso de elaboración de los productos tipo gel/emulsión (sistemas modelo y salchichas tipo frankfurt) presenta las mismas etapas hasta el proceso de embutido y cocción (**Figura 3.4**). Inicialmente, tanto la carne de cerdo como el tocino ibérico (previamente picados como fue señalado en la sección 3.1) fueron descongelados durante 18 h a 2 ± 2 °C hasta alcanzar una temperatura entre -1 y -2 °C. Posteriormente fueron sometidos a picado y homogeneización en una picadora-homogeneizadora refrigerada a 2 °C (Stephan Universal Machine UM5, Stephan U. Söhne; Hameln, Alemania). La incorporación de los diferentes ingredientes fue realizada en el siguiente orden: en primer lugar la carne fue homogeneizada durante 1 minuto, seguidamente se adicionaron la mitad de los ingredientes (incluidos el tocino y/o los sistemas de lípidos estructurados u otra materia grasa, agua y aditivos) y mezclaron durante otro minuto, se terminaron de añadir el resto de ingredientes y mezclaron durante otro minuto, para finalizar con 2 minutos más de mezcla en condiciones de vacío. Por lo tanto, el tiempo total de homogeneización fue de 5 minutos, asegurándose de que la temperatura final de la masa siempre estuviera por debajo de los 14 °C. La mezcla cárnica obtenida fue trasvasada a una embutidora manual (Mainca; Barcelona, España). A partir de esta etapa ambos productos siguieron un proceso distinto (**Figura 3.4**).

- Los sistemas modelo fueron envasados en tubos de plástico (diámetro 3,5 cm, altura 7 cm), cerrados herméticamente y sometidos a tratamiento térmico en baño de agua (80 °C), hasta alcanzar una temperatura interna de 70 °C. Posteriormente se almacenaron en refrigeración hasta los análisis. Dicho proceso fue el empleado en el trabajo experimental del **capítulo 4.2.1** de la presente memoria.

- Las salchichas tipo frankfurt fueron embutidas en tripas de celulosa de 20 mm de diámetro (Viscase SA; Bagnold Cedex, Francia) y sometidas a tratamiento térmico en un horno ahumador a 80 °C (Unimatic 1000, Micro 40, Eller; Merano, Italia) hasta alcanzar 70 °C en el centro térmico. Una vez terminado el proceso térmico las salchichas fueron sacadas del horno ahumador y se dejadas a temperatura ambiente durante aproximadamente 15 minutos, después fueron almacenadas en refrigeración (2 °C ± 2) durante 14 h. Pasado este tiempo se eliminaron las tripas y las salchichas fueron envasadas en bolsas (Cryovac BB3050) a vacío y conservadas en refrigeración (2 °C ± 2) hasta sus posteriores análisis. Este proceso fue el empleado en los trabajos experimentales de los **capítulos 4.1.1, 4.1.2 y 4.2.2** de la presente memoria.

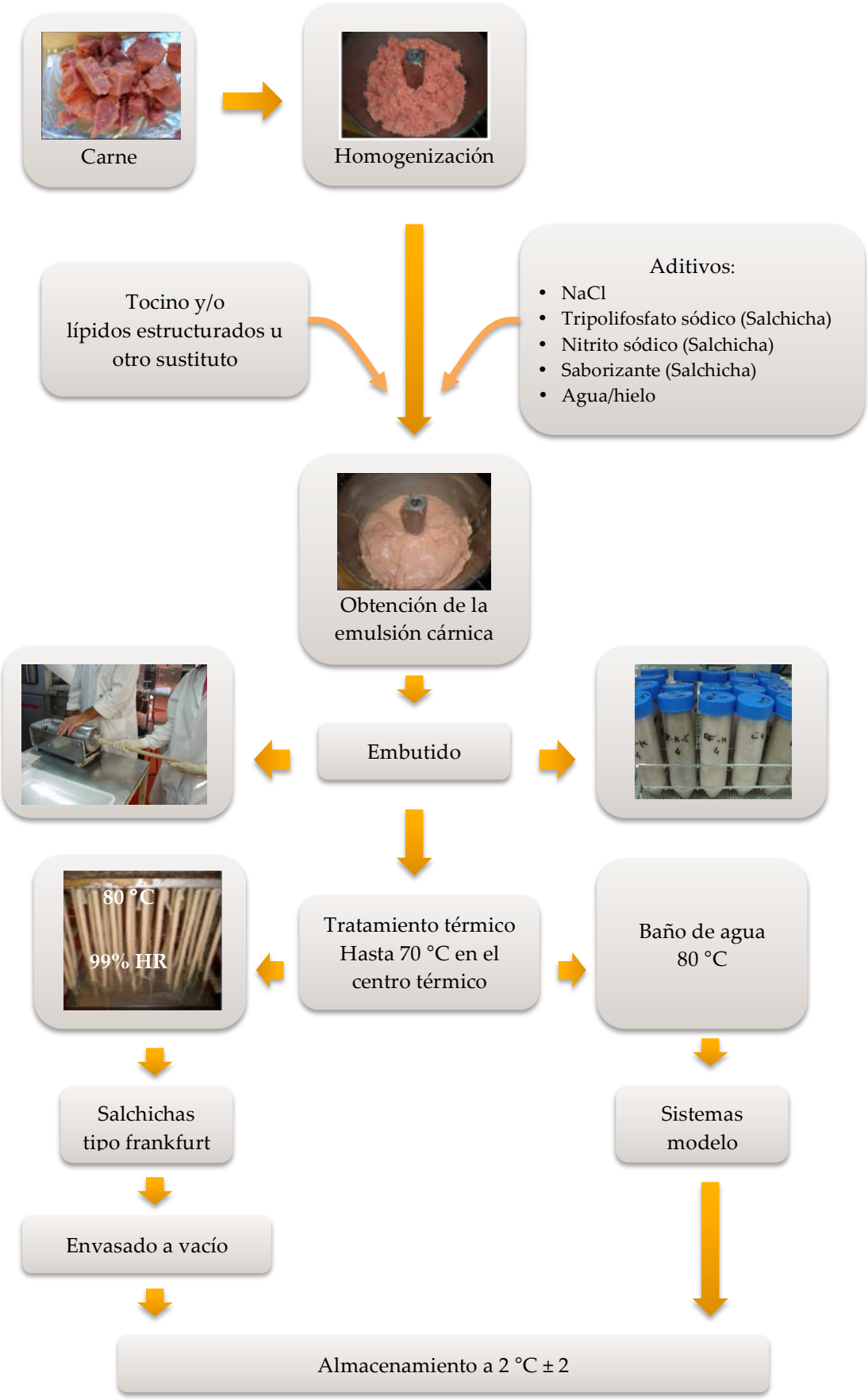


Figura 3.4. Proceso de elaboración de productos tipo gel/emulsión (sistemas modelo y salchichas tipo frankfurt).

3.3.2 Hamburguesas

La carne de cerdo y el tocino ibérico fueron descongelados (18 h a 2 ± 2 °C) antes de su uso. Seguidamente, la carne junto con el tocino y/o el agente de carga a base konjac fueron picados a (4,5 mm, Vam. Dall. Srl. Modelo FTSIII; Treviglio, Italia). La carne y la mitad de los ingredientes (incluidos el tocino y según fuera el caso, el gel de konjac o el agente de carga a base de konjac, cloruro de sodio y agua) fueron mezclados (mezcladora Hobart N-506, Hobart MFG. Co., Troy, USA) por 1 minuto, se terminaron de añadir el resto de ingredientes y mezclaron durante otro minuto, estandarizándose el tiempo de mezclado a 2 minutos. En el trabajo experimental del **capítulo 4.1.3**, las hamburguesas se elaboraron usando una formadora automática (Formatic standardmodel, Deighton Engineering, Bradford, UK), y después fueron colocadas en bolsas plásticas (Cryovac® BB3050) en condiciones aeróbicas, debido a que en este estudio, uno de los parámetros a evaluar fue la textura y el envasado a vacío hubiera podido deformar las muestras, alterando los resultados de esta medición. Posteriormente fueron almacenadas a 2 ± 2 °C hasta análisis. En el trabajo experimental del **capítulo 4.1.4**, las hamburguesas se moldearon en una formadora de hamburguesas manual y se introdujeron en bolsas de plástico envasadas al vacío y conservadas a -20 ± 2 °C hasta su análisis.

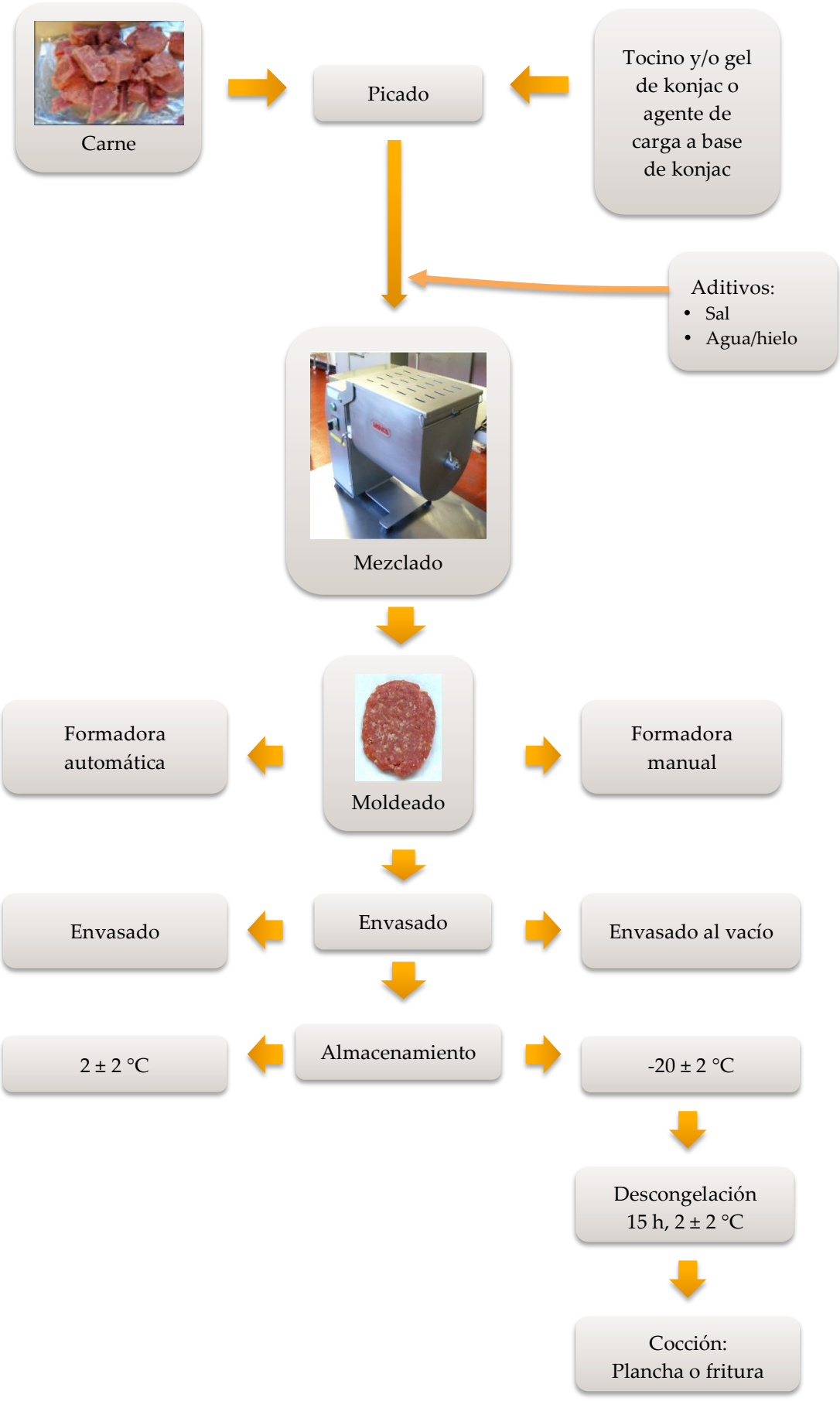


Figura 3.5. Proceso de elaboración de hamburguesas.

3.4 CARACTERIZACIÓN DE LOS PRODUCTOS

Los análisis de distintas propiedades de los productos fueron llevados a cabo con el fin de determinar su calidad nutricional, viabilidad tecnológica, microbiológica y sensorial de los mismos. En tal sentido, se realizó su caracterización así como también se evaluó su estabilidad durante la conservación en refrigeración. Igualmente, se llevaron a cabo análisis sensoriales para valorar diversos atributos organolépticos.

3.4.1 Composición

3.4.1.1 Componentes mayoritarios

La determinación de humedad y cenizas (%) se realizó según la metodología descrita por la AOAC (2005). La cantidad de proteína (%) se midió en un analizador automático de nitrógeno (LECO FP-2000, Leco Corporation; St Joseph, MI, EEUU). El contenido en grasa (%) se determinó siguiendo el método descrito por Bligh y Dyer (1959). Estas determinaciones fueron realizadas en todos los trabajos experimentales (**Capítulo 4**). El contenido en hidratos de carbono se estimó teóricamente en los productos formulados con el agente de carga, teniendo en cuenta la composición y la formulación (**Capítulos 4.1.1 y 4.1.3**).

3.4.1.2 Contenido calórico

El contenido calórico fue estimado en los productos utilizando los factores de conversión de 9,1 kcal/g para la grasa y 4,1 kcal/g para la proteína y los hidratos de carbono (**Capítulos 4.1.1, 4.1.3 y 4.1.4**).

3.4.1.3 Perfil de ácidos grasos

La determinación del perfil de ácidos grasos se realizó por cromatografía de gases siguiendo el método descrito por López-López et al. (2009). La

preparación de los ésteres metílicos con trifluoruro de boro/metanol se realizó siguiendo el método descrito por Sánchez-Muniz et al. (1998). Se utilizó un cromatografo Shimadzu (Modelo GC-2014; Kyoto, Japón) equipado con una columna capilar SP-2330 (60 m x 0,25 mm x 0,2 μm) (Supelco, Inc; Bellefonte, EEUU) y un detector de ionización de llama. Los ácidos grasos fueron identificados por comparación con una mezcla estándar de ésteres metílicos (Supelco, Alltech Associated, Inc; Deerfield, IL, EEUU). Los resultados fueron expresados en mg/100 g de muestra utilizando el factor de conversión de lípidos (MAFF, 1998) (**Capítulos 4.1.1, 4.1.4** y **4.1.2**).

3.4.1.4 Retención de nutrientes

Los factores de retención de humedad, proteína, grasa, cenizas y ácidos grasos en hamburguesas, en función del tipo de tratamiento térmico (**Capítulo 4.1.4**) fueron calculados de acuerdo con Murphy et al. (1975).

3.4.2 Propiedades físico-químicas

3.4.2.1 pH

Se determinó el pH por triplicado homogeneizando 10 g de muestra con 100 mL de agua destilada, utilizando un pH-metro 827 Metrohm (Metrohm AG, Suiza) a temperatura ambiente (**Capítulos 4.1.1, 4.1.3 y 4.2.2**).

3.4.2.2 Propiedades ligantes de agua y grasa

La metodología utilizada para evaluar las propiedades ligantes de agua y grasa varió en función del tipo de producto, aunque el fundamento es el mismo en todos ellos. Se basa en analizar cambios de peso asociados al tratamiento térmico y/o la conservación, expresado como porcentaje respecto al peso inicial del producto.

Pérdidas durante el procesado

Las pérdidas durante el procesado se calcularon para las salchichas tipo frankfurt como la suma de las pérdidas de peso durante el tratamiento térmico y el almacenamiento en refrigeración durante la primera noche (**Capítulos 4.1.1 y 4.2.2**).

Pérdidas de peso por cocción

Las pérdidas por cocción se midieron en los hamburguesas como cambios de peso por efecto del tratamiento térmico (**Capítulos 4.1.3 y 4.1.4**). La diferencia entre el peso antes y después de la cocción, fue expresada como porcentaje.

Pérdidas de peso por descongelación

En hamburguesas se evaluaron las pérdidas de peso por descongelación. Para ello se descongelaron los productos (15 h, 2 °C ± 2), eliminando el exudado superficial manualmente con papel absorbente (**Capítulo 4.1.4**). La diferencia entre el peso inicial y el peso final fue expresada como porcentaje.

Pérdidas de peso durante la conservación

Se determinaron las pérdidas de peso durante la conservación en refrigeración para salchichas y hamburguesas. Cada día de análisis se secó el producto superficialmente, se pesó una vez atemperado y las pérdidas por conservación fueron calculadas por diferencia de peso y expresadas como porcentaje del peso inicial (**Capítulos 4.1.1, 4.1.3 y 4.2.2**).

Pérdidas de peso totales

Las pérdidas de peso totales se midieron en hamburguesas como la suma de las pérdidas por descongelación y las pérdidas por cocción (**Capítulo 4.1.4**).

3.4.2.3 Medida objetiva del color

Esta determinación se realizó por el método de reflectancia utilizando el sistema de coordenadas CIELab (CIE, 1978; Young y Whittle, 1985; CIE, 1995) mediante

un Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Se determinaron los parámetros L*, a* y b*, donde L* representa la luminosidad (0 es negro y 100 es blanco), a* la tendencia al rojo (-60 es verde y +60 rojo) y b* la tendencia al amarillo (-60 es azul y +60 amarillo). En salchichas tipo frankfurt se midió el color sobre la superficie del corte transversal (**Capítulos 4.1.1 y 4.2.2**) y en hamburguesas sobre la superficie externa (**Capítulo 4.1.3**).

3.4.2.4 Determinación instrumental de la textura

Para la evaluación de la textura se empleó un texturómetro TA.XT2i (Stable Microsystems Ltd.; Surrey, Inglaterra). En el caso de las salchichas tipo frankfurt, se llevó a cabo el análisis del perfil de textura (TPA). Mientras que en las hamburguesas se utilizó el test de Kramer. El motivo para la elección de uno u otro radicó en la propia naturaleza de los productos. Pese a que el ensayo del perfil de textura proporciona datos más completos, el test de Kramer se consideró el más adecuado para las hamburguesas debido a su estructura menos sólida.

Análisis del Perfil de Textura (Texture Profile Analysis, TPA)

La evaluación de las características texturales salchichas tipo frankfurt (**Capítulos 4.1.1** y **4.2.2**) se realizó mediante TPA de acuerdo al procedimiento descrito por Bourne (1978). Las medidas se realizaron a temperatura ambiente (22 °C). Se prepararon porciones de 20 mm de diámetro y 20 mm de altura, que posteriormente se sometieron a doble compresión hasta un 40% de su altura original. Se utilizó una célula de carga de 5 kg a una velocidad del cabezal de 1 mm/s para obtener las curvas de fuerza-tiempo de deformación, a partir de estas, se calcularon los siguientes parámetros:

- Dureza (N): definida como la altura máxima obtenida en el primer ciclo de compresión. Este parámetro sirve para evaluar la fuerza máxima necesaria para producir una cierta deformación.
- Cohesividad (adimensional): calculada como la relación entre el área positiva de la primera y segunda compresión.
- Elasticidad (mm): corresponde a la altura recuperada por la muestra tras la primera compresión.
- Masticabilidad (N x mm): calculada como el producto de dureza, elasticidad y cohesividad.

Test de Kramer

El test de Kramer se realizó en hamburguesas (**Capítulo 4.1.3**). La fuerza de corte Kramer (*Kramer shear force*, KSF) fue determinada en porciones (5 x 5 cm), utilizando una célula de corte de Kramer con una velocidad de cabezal de 120 mm/min. Los resultados se expresaron como la fuerza máxima por unidad de peso de muestra (N/g).

3.4.2.5 Microestructura

Se determinó la microestructura de las salchichas tipo frankfurt (**Capítulo 4.1.1**) mediante microscopía electrónica de barrido (Scanning Electron Microscopy, SEM, Jeol, JSC 6400; Tokio, Japón) siguiendo la metodología propuesta por Jiménez-Colmenero et al. (1995).

3.4.2.6 Determinación del tamaño de partícula

El efecto del tratamiento térmico sobre la distribución del tamaño de partícula (*particle size distribution*, PSD) de hidrogeles (**Capítulo 4.2.1**) fue determinado de acuerdo con la metodología descrita por Cofrades et al. (2013).

3.4.2.7 Oxidación lipídica

Al ser la primera vez que las partículas de hidrogel se evaluaban en productos cárnicos y que una de sus mayores funcionalidades era la protección que podría ofrecer a un aceite altamente insaturado (como el aceite de pescado), frente a la oxidación lipídica, se decidió evaluar los compuestos de oxidación primaria en los productos enriquecidos con EPA y DHA mediante el índice de peróxidos. En todos los productos, los compuestos de oxidación secundaria, fueron evaluaron a través de la determinación de las sustancias reactivas con el ácido 2-tiobarbitúrico (Thiobarbituric reactive substances, TBARS).

Índice de hidroperóxidos

El índice de hidroperóxidos como medida de determinación de productos de oxidación primaria, se llevó a cabo siguiendo la metodología de Matalanis et al. (2012) con algunas modificaciones (**Capítulos 4.2.1** y **4.2.2**). A partir de la muestra se preparó un extracto en cloroformo/metanol que a su vez fue mezclado con metanol/1-butanol. Luego esta mezcla se hizo reaccionar con soluciones de tiocianato de amonio, sulfato ferroso y cloruro de bario (Panreac Química, SA; Barcelona, España). La reacción se llevó a cabo en completa oscuridad durante 20 minutos a temperatura ambiente, produciendo un compuesto coloreado que fue medido espectrofotométricamente a 510 nm (espectrofotómetro Shimadzu UV-1800, Shimadzu Inc.; Kioto, Japón). La concentración de hidroperóxidos fue calculada empleando una curva de calibración hecha con hidroperóxido de cumeno (Sigma Chemical Co.; St. Louis, MO, USA) y los resultados fueron expresados en mmoles de hidroperóxido por kg de muestra.

Determinación de las sustancias reactivas con el ácido 2-tiobarbitúrico (TBARS)

La formación de productos de oxidación secundaria fue medida mediante la determinación de TBARS, de acuerdo a la metodología descrita por Serrano et al. (2006), y se analizó tanto en las partículas de hidrogel (**Capítulo 4.2.1**), como en todos los productos cárnicos evaluados en esta memoria **(Capítulos 4.1.2, 4.1.3, 4.2.1** y **4.2.2**). Se realizó una extracción ácida con una solución de ácido tricloroacético (Panreac Química S.A., Barcelona, España) y posteriormente se añadió ácido 2-tiobarbitúrico 20 mM (Merck KGaA, Dannstadt, Alemania). La reacción se llevó a cabo en completa oscuridad durante 20 h a 20 ± 2 °C. La coloración característica fue medida a 532 nm en un espectrofotómetro (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, USA) para el estudio de las salchichas tipo frankfurt con agente de carga y en los demás estudios con el espectrofotómetro Shimadzu UV-1800. Los resultados se calcularon en una curva de calibración realizada con 1,1,3,3-tetraetoxipropano (Sigma Chemical Co.; St. Louis, MO, USA), expresando los resultados en mg de malonaldehído por kg de muestra.

3.4.2.8 Determinación de nitritos

La determinación del contenido de nitrito residual durante la conservación de salchichas tipo frankfurt (**Capítulo 4.1.2**) se determinó mediante análisis de inyección de flujo (FIA), de acuerdo con el método descrito por Ruiz-Capillas et al. (2007).

3.4.2.9 Determinación de aminas biógenas

La determinación de tiramina, feniletilamina, histamina, putrescina, cadaverina, agmatina, espermidina y espermina fue realizada en salchichas (**Capítulo 4.1.2**), mediante cromatografía de alta resolución (HPLC) siguiendo la metodología de Triki et al. (2012).

3.4.3 Análisis microbiológico

Se hicieron recuentos de aerobios viables totales, bacterias ácido lácticas y enterobacterias, por duplicado tanto en salchichas (**Capítulo 4.1.2**) como en hamburguesas (**Capítulo 4.1.3**). Se utilizó el medio de cultivo Plate Count Agar (PCA) para el recuento de microorganismos aerobios totales y la incubación se realizó a 30 °C durante 72 h. Para la determinación de bacterias acido lácticas se utilizó el medio De Mann Rogosa Sharp Agar (MRS) e incubación a 30 °C entre 3 y 5 días. Para el recuento de enterobacterias se utilizó el medio de cultivo Violet Red Bile Glucose Agar (VRBG) incubando a 37 °C durante 24 h. Los resultados fueron expresados por logaritmo de unidades de

3.4.4 Análisis sensorial

El análisis sensorial, específico para cada producto cárnico, se llevó a cabo para evaluar sus propiedades organolépticas y comparar su grado de aceptación frente a los respectivos controles. La selección de los catadores, se realizó con diversas sesiones de entrenamiento en las que los panelistas se familiarizaron con los productos y los atributos a medir. Finalmente se eligieron 15 panelistas para el análisis sensorial. Para cada formulación se evaluaron diferentes parámetros en una escala no estructurada de 0 a 10 sin extremos fijos. Los parámetros evaluados en salchichas (**Capítulos 4.1.1 y 4.2.2**) fueron: jugosidad, dureza, textura, sabor y aceptabilidad general. Mientras que en el caso de las hamburguesas (**Capítulo 4.1.3**) se evaluaron sabor, textura y aceptabilidad general.

3.4.5 Análisis estadístico

El análisis estadístico de los resultados se realizó empleando el SPSS Statistics 19, 20, 21 y 22 (SPSS Inc; Chicago, IL, USA). Para determinar el efecto de la formulación sobre los diferentes parámetros estudiados, se realizó un análisis

de la varianza (*Analysis of variance*, ANOVA) de una vía. Se utilizó el test "post hoc" de la diferencia significativa de Tukey (*Honest Significant Difference*, HSD) para determinar las diferencias entre grupos. Para establecer el efecto de las diferentes formulaciones y el tiempo de conservación en los parámetros medidos, se utilizó un ANOVA de dos vías (formulación y tiempo) donde se empleó el test HSD de Tukey para determinar las diferencias. También se utilizó el cálculo de la correlación de Pearson para establecer la posible relación entre dos variables. El nivel de significación quedó establecido en 95% para todos los análisis.

4. Publicaciones

4.1 UTILIZACIÓN DE UN AGENTE DE CARGA DE ACEITE A BASE DE KONJAC COMO ESTRATEGIA EN EL DESARROLLO DE PRODUCTOS CÁRNICOS MÁS SALUDABLES

4.1.1 Healthier oils stabilized in konjac matrix as fat replacers in n – 3 PUFA enriched frankfurters

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Healthier oils stabilized in konjac matrix as fat replacers in n − 3 PUFA enriched frankfurters

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ABSTRACT

Nutritional, sensory and technological properties of frankfurters as affected by reformulation processes designed to reduce fat content and improve fatty acid profile were investigated. Healthier oils stabilized in oil in water emulsion or in konjac matrix gel were used as fat replacers. Results showed that improved fat content by the replacement of pork backfat with konjac gel and by the addition of healthier oils stabilized by various different systems, both resulted in products with very similar characteristics. From a nutritional standpoint, reformulated frankfurters with konjac gel and/or added a healthier oil combination may claim "*reduced fat content*" and/or "*high omega 3 fatty acid content*" according to European Regulation, since they could contain less than 30% of the fat in the reference product and more than 0.6 g of ALA/100 g and more than 80 mg of the sum of EPA plus DHA per 100 g, respectively. Chill storage over 40 days generally had little effect on the technological characteristics of frankfurters.

1. Introduction

Because of their importance, lipids are among the bioactive components that have received most attention (in quantitative and qualitative terms), particularly with respect to the development of healthier meat products (Jimenez-Colmenero, 2007; Jimenez-Colmenero, Carballo, & Cofrades, 2001; Muguerza, Gimeno, Ansorena, & Astiasaran, 2004). Healthier lipid formulation based on processing strategies is one of the most important current approaches to the development of potential meat based functional foods, including frankfurter-type products. Frankfurters have a large market, especially among a particular sector of the population, and their fat contents normally diverge (quantitatively and qualitatively) from dietary goals. To overcome this limitation, reformulation has been used to achieve better lipid compositions of frankfurters by reducing fat content and/or replacing (to a greater or lesser extent) the animal fat normally present in the product with another fat (of plant and/or marine origin) whose characteristics (fatty acid profile) are more in line with health recommendations (Jimenez-Colmenero, 2007).

Various different approaches have been used to minimize problems associated with fat reduction in meat products, including the use of ingredients such as proteins, carbohydrates or lipids (Keeton, 1994). One such ingredient is konjac (glucomannan)-based fat analogue. Konjac gels have been used to simulate fat characteristics and reduce the fat content in different meat products, including frankfurters (Jiménez-Colmenero et al., 2012; Kao & Lin, 2006; Lin & Huang, 2003; Osburn & Keeton, 2004). As a strategy to improve fatty acid profiles (decrease SFA, and increase MUFA and PUFA levels) of frankfurters, various vegetable oils (olive, cottonseed, corn, soya bean, peanut, etc.) have been used as partial substitutes for meat fats (Bloukas & Paneras, 1993; Luruena-Martinez, Vivar-Quintana, & Revilla, 2004) (among others). Although individual addition of any of these oils improves the fatty acid profile of meat products, a better approximation to optimal lipid profiles (that is, more in line with health recommendations) can be achieved by using combinations of them to replace animal fats. Combinations of vegetable oils (olive, cottonseed and soya bean) have been used in frankfurter formulation following dietary guidelines for fatty acids (Paneras, Bloukas, & Filis, 1998). A healthier lipid formulation (algal and olive oils) produced a low-fat frankfurter enriched with high levels of long n − 3 PUFA and MUFA and a good balance of MUFA/SFA, PUFA/SFA and n − 6/n − 3 ratios (Lopez-Lopez, Cofrades, & Jimenez-Colmenero, 2009; Lopez-Lopez, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2009). Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, and Jimenez-Colmenero (2010a) designed a healthier lipid combination formed by vegetable oils (olive and linseed) and fish oils in suitable amounts and proportions to provide a fatty acid profile adjusted to healthier intake goals, and this combination was used to produce healthier-lipid low-fat frankfurters (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a).

One essential aspect of a strategy for achieving healthier lipid composition is the procedure used to incorporate the chosen oil combination in meat products, ranging from direct addition as liquid oils or as solids (including interesterified oils), to incorporation in encapsulated or

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pre-emulsified form or as part of plant ingredients (Jimenez-Colmenero, 2007). Because of their physicochemical characteristics, pre-emulsions are the most suitable technological option to stabilize the oils used as animal fat replacers in meat derivates. A pre-emulsion is an oil-in-water emulsion containing an emulsifier, typically a non-meat protein. Oil-in-water emulsion technology with a non-meat protein improves the system's fat binding ability, since the oils can be stabilized or immobilized in a protein matrix. This leaves more meat protein available to act in the system and reduces the chances of bulk oil physically separating from the structure of the meat product, so that the product remains stable throughout the range of environmental conditions that it is likely to encounter during processing, storage and consumption (Djordjevic, McClements, & Decker, 2004). A number of procedures have been reported for producing an oil-in water emulsion for incorporation in meat derivatives, most of them using sodium caseinate, soy protein isolate or whey proteins as emulsifiers. Of these, sodium caseinate has generally been used to produce oil-in-water emulsions as animal fat replacers in cooked sausage-type products (Jimenez-Colmenero, 2007). Oil-in-water emulsions of this type are added to meat products as fat ingredients, and hence their composition and physicochemical characteristics affect different quality properties of the reformulated product. Therefore, various studies have been carried out to evaluate the physicochemical characteristics of pre-emulsion systems used as animal fat replacers and their effects on products characteristics (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010b; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a; Youssef & Barbut, 2011). In this context, one unexplored technological option for animal fat replacement in meat production as a means of stabilizing the oil is inclusion in a konjac matrix. These ingredients would help to reduce fat content and improve the fatty acid profile (associated with the oil present) conferring additional health benefits associated with the presence of konjac. As far as the authors are aware no studies have been reported on the use of konjac as a lipid stabilization system in a healthier-lipid frankfurter formulation.

The objective of this research was thus to evaluate the nutritional, sensory and technological properties of frankfurters as affected by reformulation processes designed to improve fat content by: a) reducing fat content (replacing pork back fat with konjac gel), b) reducing fat/improving fatty acid through partial substitution of pork backfat by a healthier oil combination stabilized as an oil-in-konjac matrix or the healthier oil combination stabilized as oil-in-water emulsion or a combination of KG and oil-in-water emulsion. The oil combination used was designed with suitable amounts and proportions of fatty acids in order to achieve healthier intake goals (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010b). The influence of chill storage (40 days at 2 °C) on frankfurter characteristics as affected by oil stabilization system was also studied.

2. Materials and methods

2.1. Meat raw materials, non-meat ingredients and additives

Fresh post-rigor pork from different animals (21.2% ± 0.5 protein, 4.77% ± 0.7 fat) (mixture of muscles *biceps femoris*, *semimembranosus*, *semitendinosus*, *gracilis* and *adductor*) and pork backfat (4.80% ± 0.9 protein, 90.64% ± 2.5 fat) were obtained from a local meat market. The meat was trimmed of visible fat and connective tissue. Meat and backfat were passed through a grinder with a 0.6 cm plate (Mainca, Granollers, Spain). Lots of approximately 500 g were vacuum packed, frozen and stored at −20 °C until used.

Ingredients used for the preparation of lipid combinations included olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), linseed oil (Natursoy S.L., Alimentos Ecológicos, Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold from Cognis GmbH, Illertissen, Germany). According to supplier information, the latter contained 160 mg of eicosapentenoic acid (EPA)/g and 115 mg of docosahexaenoic acid (DHA)/g plus a combination of tocopherols as antioxidants.

Konjac materials were made with konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain), pre-gelled corn starch (Amigel, Julio Criado, S.A., Madrid, Spain), i-carrageenan (Hispanagar S.A., Burgos, Spain) and $Ca(OH)_2$ (Panreac Química S.A., Barcelona, Spain). Sodium caseinate (SC) containing 86.4% protein (Julio Criado Gómez SA, Alcorcón, Spain) was used for the preparation of oil-in-water emulsions.

Other additives used included sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie GmbH, Buchs, Germany) and flavoring (Gewürzmüller, GmbH, Münichingen, Germany).

2.2. Preparation of the healthier oil combination, konjac materials and oil-in-water emulsion

The oil combination, which was the same in all reformulated frankfurters, consisted of olive (44.39%), linseed (37.87%) and fish (17.74%) oils. It was designed to produce a healthier lipid formulation with a small proportion of SFAs, large proportions of MUFAs and PUFAs (including LC n − 3 PUFAs) and balanced n − 6/n − 3 PUFA and PUFA/SFA ratios (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010b).

Two types of konjac materials were prepared: one a konjac gel (KG) and another with 20% of a healthier oil mixture added to the konjac matrix (oil-in-konjac matrix/OKM). KG was prepared as described by Osburn and Keeton (2004) with modifications (Jiménez-Colmenero et al., 2010a). Briefly, (for each 1000 g of KG preparation) 50 g of konjac flour was homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) at 2850 rpm with water (648 mL) for 3 min, left to rest for 5 min then homogenized at 1400 rpm for a further 3 min. The i-carrageenan (10 g) was then added and the mixture homogenized again at 2850 rpm for 3 min. The pre-gelled corn starch powder (30 g) was dispersed in 162 mL of water, homogenized at 2850 rpm with the mixture of konjac flour and i-carrageenan, left to rest for 5 min and then homogenized again at 1400 rpm for a further 3 min. The mixture was cooled to 10 °C, then 100 mL of $Ca(OH)_2$ solution (1%) was added with a gentle stirring at room temperature. Oil-in-konjac matrix (OKM) was prepared in the same way as KG, except that 20% w/w of the healthier oil combination was added just after the addition of i-carrageenan and the mixture was homogenized for 3 min. Then the total konjac matrix contains 20% of the oil combination. In both types of the konjac materials (KG, OKM) the same proportions of the components used to prepare them were maintained with respect to the water base (hence not including the added oil). The preparation conditions and the technological viability of adding these proportions of oils to konjac materials (OKM) were established earlier.

Oil-in-water emulsion (OWE) was prepared as described by Delgado-Pando, Cofrades, Ruiz-Capillas and Jimenez-Colmenero (2010b), mixing eight parts of water (at room temperature) with one part of SC for 2 min in the Stephan Universal Machine. The mixture was emulsified with 10 parts of the oil combination for another 3 min. The oil-in-water emulsion contained 52.63% of the oil combination.

Both konjac materials and oil-in-water emulsion were placed in suitable containers (like metal ham molds), covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until used (within 24 h of preparation). These ingredients were prepared in duplicate.

2.3. Design and preparation of frankfurters

Five different frankfurters were formulated (Table 1): a control sample (C) prepared with a normal fat content (20%); a reduced-fat content (10%) frankfurter (F/KG) in which pork backfat was partially replaced by KG. Three samples were formulated with a reduced fat

Table 1
Formulation (g) of frankfurters.

Sample	Meat	Backfat	Substituents			Water
			KG	OKM	OWE	
C	2281.25	762.56	–	–	–	843.71
F/KG	2381.74	315.97	762.56	–	–	427.26
F/OKM	2421.93	137.33	–	762.56	–	565.70
F/OWE	2421.93	137.33	–	–	304.00	1024.26
F/OWE + KG	2421.93	137.33	220.40	–	304.00	803.86

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. The following were also added to all samples: 2.0 g/100 g sodium chloride; 0.30 g/100 g sodium tripolyphosphate; 0.012 g/100 g sodium nitrite; and 0.50 g/100 g flavoring.

content (10%) and improved fatty acid profile in which pork backfat was partially replaced by a) the healthier oil combination stabilized in oil-in-konjac matrix (F/OKM), b) the healthier oil combination stabilized in oil-in-water emulsion (F/OWE), and c) a combination of KG and oil-in-water emulsion (F/KG + OWE). The reformulated products were designed to have similar fat levels, irrespective of the type of lipid material and/or the mode of incorporation. Whereas in C and F/KG samples all the fat was pork fat, part of the fat content in F/OKM, F/OWE and F/KG + OWE came from added olive, linseed and fish oils. These three samples were designed to contain the same proportion and type of fat (both pork fat and added oils), so that they only differed in the mode of oil incorporation (oil stabilization system). One fundamental requirement of design and reformulation of these products as regards potential health benefits is that the lipid content and profile be such as to make a serious contribution to the recommended intake levels when consumed in normal quantities. For this reason, reducing the fat content more in frankfurters was not considered.

Meat and fat packages were thawed (~18 h at 2 ± 2 °C) prior to use. Preparation of the frankfurters was as described by Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al. (2010a). Briefly, raw meat material was homogenized and ground for 1 min in a chilled cutter (Stephan Universal Machine UM5, Stephan U. Söhne, Hameln, Germany). Half of the pork backfat or konjac gel or oil-in-water emulsion or oil-in-konjac matrix (depending on the formulation), NaCl, sodium tripolyphosphate and sodium nitrite (the last two previously dissolved in the added water) were added to the ground meat and mixed again for 1 min. The rest of the additives, the pork backfat and konjac gel or oil-in-water emulsion or oil-in-konjac matrix (depending on the formulation) were added and the whole homogenized for 1 min. Finally the whole meat batter was homogenized under a vacuum for 2 min. Mixing time was standardized at 5 min. The final batter temperature was below 14 °C in all cases.

The meat batter was stuffed into 20 mm diameter Nojax cellulose casings (Viscase S.A., Bagnold Cedex, France) and hand-linked. Frankfurters were heat processed in an Eller smokehouse (model Unimatic 1000, Micro 40, Eller, Merano, Italy) until the core of the product reached 70 °C. Heat processing conditions were established beforehand, and the internal temperature was monitored throughout heating by means of thermocouples inserted in each frankfurter (thermal center) and connected to a temperature recorder (Yokogawa Hokuskin Electric YEM, Mod. 3087, Tokyo, Japan). Once heating was complete, the frankfurters were cooled (at room temperature), kept in a cold room (2 °C for 14 h), vacuum packed (Cryovac® BB3050) and stored at 2 °C (±1 °C). Analyses were carried out at 1, 12, 16 and 40 days.

2.4. Proximate analysis and fatty acid composition

Moisture (no. 950.46) and ash (no. 923.03) contents of the frankfurters were determined in triplicate according to the AOAC methods (2002). Fat content was evaluated (in triplicate) according to Bligh and Dyer (1959). The protein content was measured in quadruplicate by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Carbohydrate was estimated from the konjac material carbohydrate content used.

Fatty acid composition of the frankfurters was determined (in quintuplicate) by GC as reported by Lopez-Lopez, Cofrades, Ruiz-Capillas, et al. (2009). Briefly, boron trifluoride/methanol was used for fatty acid methyl ester (FAME) preparation. A Shimadzu gas chromatograph (Model GC-2014, Kyoto, Japan) fitted with a capillary column SP™-2330 (60 m × 0.25 mm × 0.2 μm id) (Supelco, Bellefonte, USA) was used with a flame ionization detector. Injector and detector temperatures were 250 and 260 °C respectively. The oven temperature was 140 °C for 5 min, followed by an increase to 240 °C at 4 °C/min, which was held for 20 min. The carrier gas was helium, and nitrogen was used as the make-up gas. Fatty acids were identified by comparison of the retention times with a standard of 37 fatty acids (Supelco™ 37 FAME Mix 47885-U, USA). Quantification was done by normalization and transformation of the area percentage into mg/100 g of the edible portion, by using the lipid conversion factor for pork fat and oils (MAFF, 1998).

Based on the FAME results, the atherogenic index (AI) and thrombogenic index (TI) were computed according to Ulbricht and Southgate (1991) as a ratio between some saturated and unsaturated fatty acids:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / (MUFA + n{-}3\ PUFA + n{-}6\ PUFA);$$

$$TI = (C14:0 + C16:0 + C18:0) / (0.5 \times MUFA + .0.5 \times n{-}6\ PUFA + 3 \times n{-}3\ PUFA + n{-}3\ PUFA / n{-}6\ PUFA)$$

2.5. Processing loss, purge loss and pH determination

Processing loss of frankfurters was calculated in sextuplicate, as the weight loss (expressed as % of initial sample weight) occurring after heat processing and chilling overnight at 2 °C.

Three vacuum packs per formulation were used to determine purge loss during chill storage. After the frankfurters were removed from the package, the surface exudate (tiny drops) was wiped off with paper towels and the frankfurters were weighed again. The purge loss was calculated by weight difference and expressed as a percentage of the initial weight.

The pH was determined on a Radiometer model PHM 93 pH-meter (Orion 3 Star, Thermo Fisher Scientific, Waltham MA, USA) at room temperature on homogenates of frankfurters in water in a ratio of 1:10 w/v. Four determinations were performed per sample.

2.6. Color measurement

Color, CIE-LAB tristimulus values, lightness, L*; redness, a* and yellowness, b* of the frankfurter cross-sections were immediately evaluated on a CR-400 Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan). Ten determinations were performed from each formulation.

2.7. Texture profile analysis (TPA)

TPA was performed in a TA-XT.plus Texture Analyzer (Texture Technologies Corp, Scarsdale, NY, USA) as described by Bourne (1978). Ten frankfurter cores (diam = 20 mm, height = 20 mm) were axially compressed to 40% of their original height. Force-time deformation curves

were obtained with a 5 kg load cell, applied at a crosshead speed of 1 mm/s. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) in that the sample recovers after the first compression; and chewiness (Cw) = Hd × Ch × Sp (N × mm). Measurements were carried out at room temperature.

2.8. Microstructure

Microstructure was analyzed by scanning electron microscopy (SEM). The frankfurters were fixed with a mixture (1:1, v/v) of paraformaldehyde (4 g/100 g) and glutaraldehyde (0.2 g/100 g) in 0.1 M phosphate buffer, pH 7.2, post-fixed with OsO_4, washed, dehydrated in increasing concentrations of acetone, critical-point-dried, sputter-coated with gold/palladium in a metallizer (Blazer, SCD004) and scanned by SEM (Jeol, JSC 6400, Akishima, Tokyo, Japan) at 20 kV. A large number of micrographs were taken in order to select the most representative ones.

2.9. Sensory evaluation

Frankfurters were assessed by a 10-member panel. The panel was selected in preliminary sessions from the staff who had received training (two sessions) with the products and terminology. After heating the frankfurters in a microwave for 15 s, samples (2.5 cm long) from each formulation were immediately presented to panelists in a random order. An intensity scale test of 9 points was used to evaluate the following parameters for each sample: juiciness (0 = very dry, 9 = very juicy), hardness (0 = soft, 9 = firm), off-flavor (0 = none, 9 = very intense). In addition, a hedonic scale rating test was carried out where the panelist evaluated the texture and flavor (1 = dislike extremely, 9 = like extremely). The evaluation was made on a non-structured scale with fixed extremes. Each point was later converted to a numerical scale. Sensory analysis was performed 5 days after the preparation of the frankfurters.

2.10. Statistical analysis

Each product was prepared in duplicate. The repeated measures test was used for statistical comparisons between samples. Data were analyzed by using SPSS Statistics (v. 19, IBM SPSS Inc., Chicago, IL) for one-way and two-way ANOVA. Least squares differences were used for comparison of mean values between treatments and Tukey's HSD test to identify significant differences ($P<0.05$) between formulations and storage time.

3. Results and discussion

3.1. Proximate analysis

Formulation affected ($P<0.05$) the proximate composition of frankfurters (Table 2). Protein content of frankfurters ranged between 15.34 and 17.04%. These relatively small differences in protein content were due mainly to formulation (Table 1) and were associated mainly with the meat protein and the SC present in the oil-in-water emulsion; hence, the protein level was lowest ($P<0.05$) in C, medium in the F/KG and F/OKM samples, and highest ($P<0.05$) in the F/OWE and F/OWE + KG samples (Table 2). Fat reduction was accompanied by an increase ($P<0.05$) in moisture, which ranged between 67.7 and 70.06% in reformulated sausages. These variations may be attributed to the fact that fat was basically reduced at the expense of water (protein variation was very limited) either added or forming part of the konjac materials used in as pork fat replacers (Table 1). Ash contents differed significantly ($P<0.05$) but only slightly.

Table 2
Proximate analysis (%) of different frankfurters.

Sample	Moisture	Protein	Fat	Ash
C	59.54 ± 0.25^{a}	15.34 ± 0.10^{a}	18.88 ± 0.08^{b}	3.17 ± 0.02^{a}
F/KG	67.70 ± 0.25^{b}	16.20 ± 0.10^{b}	10.01 ± 0.34^{a}	3.33 ± 0.04^{c}
F/OKM	70.06 ± 0.17^{d}	16.27 ± 0.11^{b}	10.30 ± 0.18^{a}	3.29 ± 0.01^{c}
F/OWE	68.37 ± 0.35^{c}	17.04 ± 0.17^{c}	10.27 ± 0.51^{a}	3.23 ± 0.02^{b}
F/OWE + KG	68.26 ± 0.08^{bc}	17.01 ± 0.18^{c}	10.26 ± 0.77^{a}	3.34 ± 0.07^{c}

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. Means ± SD. Different letters in the same column indicate significant differences ($P<0.05$).

In accordance with the target composition, controls had higher ($P<0.05$) fat levels (near 19%) than the formulated products, in all of which the fat content was similar ($P>0.05$) (between 10.01 and 10.30%). This translates as a fat reduction of more than 45%, but there is an additional aspect that needs to be considered regarding the type of fat present in the sausages, which varied with the formulation (Table 1). In C and F/KG samples, all the fat was pork fat, whereas in terms of ingredient composition and formulation in the reduced fat frankfurters F/OKM, F/OWE, F/OWE + KG, around 40% of the total fat was supplied by the added oils, with each 100 g of product containing 1.7 g of olive, 1.5 g of linseed and 0.7 g of fish oils. A number of studies have been conducted to reduce fat and improve the lipid profile of frankfurters by using a large variety of plant and marine oils (olive, cottonseed, sunflower, soyseed, high-oleic-acid sunflower, palm, fish, etc.). These oils have been added in variable proportions (2–20 g oil/100 g of product) and in different ways, including directly during product manufacture (in liquid or solid form at the end of the process), oil-in-water emulsified or interesterified (Alvarez et al., 2011; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a; Jimenez-Colmenero, 2007). When oil combinations (as oil-in-water emulsion with SC) have been incorporated to improve fat content in frankfurters, the oil contents have varied between 4 (Paneras et al., 1998) and 9.5 g (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a) per 100 g of product, in the latter case by using the same oil combination as in this experiment. Higher concentrations of oil combination (15 g canola and 5 g olive oils/100 g) incorporated in liquid form have been reported by Alvarez et al. (2011), but in the formulation of the normal fat content (26%) frankfurter-type sausages.

The energy content (based on 9.1 kcal/g for fat and 4.1 kcal/g for protein and carbohydrates) of the normal fat control (C) was 235 kcal/100 g, of which 73% was supplied by pork fat, while in the reduced fat frankfurters the energy content ranged between 163 and 167 kcal/100 g (around 30% lower than the C sample), more than 50% of which was supplied by the fat. In the case of samples containing non-meat fat, the added oils supplied around 22% of the total energy content.

3.2. Fatty acid content

Table 3 reports the result of fatty acid content (mg/100 g product). The frankfurters formulated with added oils (F/OKM, F/OWE and F/KG + OWE) contained similar proportions and types of fat (both pork fat and added oils), and differed only in the way the oil was incorporated, and therefore the data reported in Table 3 are the mean values of these frankfurters. Fatty acid content varied with the formulation, as affected by fat reduction and substitution of pork backfat by

Table 3
Fatty acid profile (g/100 g product) and nutritional significant ratios of different frankfurters.

Fatty acid	Frankfurters		
	With normal fat content	With reduced fat content	With reduced and improved fat content
	C	F/KG	F/OKM, F/OWE, F/OWE + KG
Myristic C 14:0	0.22 ± 0.00^{b}	0.12 ± 0.00^{a}	1.11 ± 0.00^{a}
Palmitic C16:0	4.09 ± 0.03^{c}	2.15 ± 0.01^{b}	1.77 ± 0.02^{a}
Stearic C18:0	2.09 ± 0.08^{c}	1.05 ± 0.00^{b}	0.85 ± 0.02^{a}
Arachidic C20:0	–	–	0.03 ± 0.01
Other SFAs	0.05 ± 0.00^{c}	0.03 ± 0.00^{b}	0.02 ± 0.00^{a}
Σ SFA	6.45 ± 0.10^{c}	3.34 ± 0.00^{b}	2.79 ± 0.04^{a}
Palmitoleic C16:1	0.34 ± 0.00^{b}	0.21 ± 0.00^{a}	0.22 ± 0.00^{a}
Oleic C18:1n9	8.78 ± 0.09^{c}	4.49 ± 0.02^{b}	4.43 ± 0.01^{a}
Vaccenic C18:1n7c	0.64 ± 0.01^{c}	0.36 ± 0.01^{b}	0.34 ± 0.00^{a}
Eicosenoic C20:1n9c	0.25 ± 0.00^{c}	0.12 ± 0.00^{b}	0.08 ± 0.00^{a}
Σ MUFA	10.01 ± 0.10^{c}	5.18 ± 0.02^{b}	5.07 ± 0.01^{a}
Linoleic C18:2n6	1.41 ± 0.02^{c}	$0.76 \pm 0.00a$	0.83 ± 0.00^{b}
Linolenic C18:3n3	0.08 ± 0.00^{b}	0.04 ± 0.00^{a}	0.88 ± 0.00^{c}
Eicosadienoic C20:2n6	0.08 ± 0.00^{c}	0.04 ± 0.00^{b}	0.03 ± 0.00^{a}
Eicosapentaenoic C20:5n3	–	–	0.12 ± 0.00
Docosahexaenoic C22:6n3	–	–	0.07 ± 0.00
Other PUFAs	0.07 ± 0.00^{c}	0.04 ± 0.00^{a}	0.05 ± 0.00^{b}
Σ PUFA	1.64 ± 0.02^{b}	0.88 ± 0.00^{a}	1.98 ± 0.00^{c}
PUFA/SFA	0.26 ± 0.01^{a}	0.26 ± 0.00^{a}	0.71 ± 0.01^{b}
Σ n−3	0.16 ± 0.00^{a}	0.08 ± 0.00^{a}	1.12 ± 0.00^{b}
Σ n−6	1.49 ± 0.02^{c}	0.80 ± 0.00^{a}	0.85 ± 0.00^{b}
n−6/n−3	9.61 ± 0.18^{b}	9.58 ± 0.07^{b}	0.76 ± 0.00^{a}
Atherogenic index	0.42 ± 0.01^{b}	0.43 ± 0.00^{b}	0.31 ± 0.00^{a}
Thrombogenic index	1.01 ± 0.03^{b}	0.99 ± 0.00^{b}	0.36 ± 0.01^{a}

Sample denomination: C, control sample prepared with normal fat content; F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG. Since the three frankfurters (F/OKM, F/OWE and F/OWE + KG) were formulated with the same lipid material, data reported are the mean values of these frankfurters. Means ± SD. Different letters in the same row (a, b, c) indicate significant differences ($P<0.05$).

the healthier oil combination. In all samples the most abundant fatty acids were MUFAs, followed by SFAs and PUFAs (Table 3). SFAs and MUFAs accounted for around 90% of the total fatty acid content of C and F/KG (all pork fat), while pork backfat replacement by the healthier oils reduced this proportion to 80%, meaning a 10% increase of PUFAs in these modified samples.

SFA contents were affected ($P<0.05$) by formulation, in which the most abundant were palmitic and stearic acids. As compared with control samples, fat reduction (F/KG) reduced SFA contents by 47%; when the pork backfat was replaced by the oil combination the reduction was even greater (56%). These results are consistent with those reported by Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a, who found that concentrations of these fatty acids (palmitic and myristic acids) in frankfurters decreased from 24.5 to 12.5% when pork backfat was replaced by a vegetable and marine oil combination. Although SFAs are considered to be the chief risk factor because of their hypercholesterolemic effect, not all of them act in the same way. While stearic acid is neutral, palmitic and myristic acids produce the greatest atherogenic effect (Hu, Manson, & Willett, 2001).

Control samples had the highest ($P<0.05$) MUFA and oleic contents, with concentrations around 5.2 and 4.5 g/100 g respectively in all reduced fat sausages (F/KG). Since both samples, C and F/KG, had the same type of fat the differences found can only be related to the fat content. Modified frankfurters containing a combination of healthier oils had lower ($P<0.05$) MUFA contents than the F/KG samples, although the differences were not large (Table 3). This is consistent with the high MUFA content (oleic acid) in the oil combination (due to olive oil) used to compensate for the reduction of pork backfat. Use of the selected oil combination as an ingredient ensures that a gradual reduction of pork fat in low fat products does not reduce the MUFA (oleic acid) content.

F/KG samples contained less ($P<0.05$) PUFAs than controls (Table 3). This decrease, of about 50%, was mainly due to the fat reduction process, since PUFA levels were related to the fat content of each sample. However, when the reformulation process to improve the fat content included a partial replacement of the pork backfat by an oil combination (irrespective of the stabilization system), the PUFA content increased ($P<0.05$). As a result of the composition of the oils making up the oil combination and the amount used in the formulation (Table 1), these modified samples (F/OKM, F/OWE and F/KG + OWE) contained more ($P<0.05$) PUFAs than the normal fat content sample (C) and twice the amount measured in the frankfurter with a similar fat content (F/KG). This is due mainly to the high concentration of α-linolenic acid (ALA) (from linseed oil), and to a lesser extent to long chain n−3 PUFAs (EPA and DHA from fish oil). These results show a total of 1.12 g n−3 PUFA/100 g of product, of which around 0.9 g/100 g was supplied by ALA and 190 mg/g by the sum of EPA and DHA (Table 3). Considering that the dietary recommendation (variable depending on different factors) for total n−3 PUFAs is estimated to be between 1.4 and 3 g/day or even higher, the estimated daily range for the long chain n−3 PUFAs is between 180 and 1000 mg (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a). When consumed on a regular basis the oil-enriched frankfurters can supply a significant proportion of the recommended n−3 PUFA (including long chain n−3 PUFA) intakes, higher than most of the reformulated cooked meat products reported in the literature, which generally contain less than 150 mg/100 g of n−3 PUFAs (Jimenez-Colmenero, 2007). However, Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a reported the formulation of frankfurters (around 11.5% fat content) in which total n−3 PUFAs were around 2.5 g/100 g (of which approximately 2 g/100 g was ALA and 500 mg/100 g were long chain n−3 PUFAs, EPA, docosapentaenoic acid and DHA).

The PUFA/SFA ratio is one of the main parameters used to evaluate the nutritional quality of the lipid fraction in foods. It is recommended that the index should be greater than 0.4 (Wood et al., 2004). The ratio in all the pork fat samples was 0.26 (Table 3), which is consistent with the results reported in other conventional meat products

(Ayo et al., 2007; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a), whereas partial replacement of the pork fat by the combination of vegetable and fish oils raised that ratio to 0.74 due to the reduced proportion of SFAs and the increased proportion of PUFAs (Table 3). Similar ratios have been reported in meat products incorporating different vegetable and marine oils (Lopez-Lopez, Cofrades, Ruiz-Capillas, et al., 2009; Paneras et al., 1998).

There is evidence suggesting that a high n−6/n−3 PUFA ratio is associated with the pathogenesis of numerous disorders, among them cardiovascular diseases (CVD) and cancer, while increased n−3 PUFA concentrations to reduce the n−6/n−3 PUFA ratio has a suppressive effect (Simopoulos, 2002). The nutritional recommendation for the prevention of CVD is to reduce this ratio to less than 4. Since not all n−3 and n−6 PUFAs have the same effect on health, the value of this ratio has been questioned, suggesting that more importance be placed on the linolenic acid and long-chain n−3 (McAfee et al., 2010). Control and reduced samples had n−6/n−3 PUFA ratios around 10, which are comparable to the ratios reported by other authors (Caceres, Garcia, & Selgas, 2008). The addition of the oil combination considerably reduced this ratio, bringing it down to 0.76 (Table 3). This is because the n−3 PUFA content increased around 14-fold in the reduced and improved frankfurters, while the % of the n−6 PUFAs remained similar in all samples. These results are consistent with the findings of other authors (Caceres et al., 2008).

The atherogenic index (AI) and the thrombogenic index (TI) were greater ($P<0.05$) in the samples containing only added animal fat (C, F/KG). Similar AI and TI index values have been reported in all-pork-fat (normal and low-fat) frankfurters (Ayo et al., 2007) and low-fat frankfurters with a healthier oil combination (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a).

3.3. Processing loss, purge loss and pH determination

Processing loss of frankfurters, which ranged from 13.88 to 16.75% (Table 4), was not affected ($P>0.05$) by the fat content, the type of fat or the technological strategy used to incorporate the oil combination as a pork backfat replacer. Ranges of processing loss (including samples made with vegetable and marine oils) between 10 and 20% have been reported in the formulation of frankfurters with improved fat content (Lopez-Lopez, Cofrades, & Jimenez-Colmenero, 2009; Paneras & Bloukas, 1994). It has generally been reported that frankfurters prepared with vegetable oils have higher cooking losses than those prepared with animal fat (Ambrosiadis, Vareltzis, & Georgakis, 1996; Lopez-Lopez, Cofrades, & Jimenez-Colmenero, 2009; Paneras & Bloukas, 1994; Park, Rhee, Keeton, & Rhee, 1989; Townsend, Ackerman, Witnauer, Palm, & Swift, 1971), but it has also been reported that cooking loss in meat batters decreased when animal fat was replaced by vegetable oils, and this effect was affected by the type of oil (Youssef & Barbut, 2011). It has also been reported that the cooking loss of frankfurters was not affected by the type of fat (pork or beef fat or vegetable oils) used in the formulation (Alvarez et al., 2011; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a; Marquez, Ahmed, West, & Johnson, 1989). Purge accumulation in the packaged product during retail refrigerated storage is undesirable for aesthetic and microbiological reasons. Purge loss levels were relatively low (Table 4) and similar to those reported in low-fat frankfurters by other authors (Candogan & Kolsarici, 2003; Paneras & Bloukas, 1994), indicating good storage stability in terms of fat and water binding of the meat matrix. Variations in purge loss were not dependent ($P>0.05$) on the type of lipid used (pork backfat versus plant and fish oils), or on the system (oil-in-water emulsion or oil-in-konjac matrix) used to stabilize the oils. In support of these results there have been various studies (Pappa, Bloukas, & Arvanitoyannis, 2000) showing that replacing pork backfat with vegetable and marine oils in low-fat frankfurters has no effect on purge loss. In contrast, Bishop, Olson, and Knipe (1993) reported that purge loss was higher in bologna sausage containing emulsified oil than in bologna sausage containing pork backfat. Generally, chill storage affected purge loss very little; even when significant, and were unlikely to be of practical importance. Similar behavior has been found by Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a, but increasing purge loss with storage time has been reported in low-fat frankfurters (Bloukas & Paneras, 1993; Paneras & Bloukas, 1994). The apparent discrepancies in the water and fat binding properties in frankfurters reported in the literature may be due to a variety of factors such as differences in the product composition (e.g., fat level, protein/moisture ratio and/or type of proteins present in the meat matrix, parameters which affect the characteristics of the matrix formed according to the fat reduction strategy), in the form in which the oil is added (as a liquid or stabilized in an oil-in-water emulsion by using different non-meat proteins), the simultaneous action of other non-meat ingredients, or even the processing conditions. In the present study, animal fat replacement (simultaneous fat reduction and improvement of fatty acid profiles) by oils stabilized with SC and konjac matrix had a similar effect on water and fat binding properties (evaluated through processing yield or purge loss). This behavior may be favored by the relatively high protein content in frankfurters and the fact that part of the added water was bound in the konjac gel.

The pH values of the frankfurters were not influenced ($P>0.05$) by formulation or chill storage. Mean values ranged between 6.25 and 6.36, which may be considered normal in products of this kind. As in the present study, Ambrosiadis et al. (1996) reported no changes in the pH of the frankfurters as affected by the pork backfat replacement with vegetable oils. However, while Lopez-Lopez, Cofrades and Jimenez-Colmenero (2009) found that pork fat replacement by olive oil reduced the pH value of low-fat frankfurters, Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a reported the opposite effect using a combination of olive, linseed and fish oils, but that the pH was not affected by the particular emulsified oil stabilizing system used in the frankfurter reformulation.

Table 4
Processing loss (%), purge loss (%) and pH during chilling storage of different frankfurters.

Sample	Processing loss	Purge loss (storage days)				pH
		1	12	26	40	
C	13.88 ± 1.20^{a}	1.11 ± 0.08^{a1}	2.35 ± 0.59^{a1}	1.41 ± 0.21^{a1}	1.33 ± 0.24^{a1}	6.36 ± 0.11^{a}
F/KG	14.54 ± 0.57^{a}	0.96 ± 0.08^{a1}	1.37 ± 0.24^{a12}	1.82 ± 0.20^{a2}	1.80 ± 0.21^{a2}	6.36 ± 0.10^{a}
F/OKM	15.11 ± 0.49^{a}	1.16 ± 0.06^{a1}	2.40 ± 0.13^{a2}	1.80 ± 0.49^{a12}	1.83 ± 0.30^{a12}	6.35 ± 0.11^{a}
F/OWE	16.75 ± 1.12^{a}	1.17 ± 0.06^{a1}	1.98 ± 0.19^{a2}	2.00 ± 0.09^{a2}	1.83 ± 0.17^{a2}	6.27 ± 0.15^{a}
F/OWE + KG	16.12 ± 1.05^{a}	0.99 ± 0.09^{a1}	1.62 ± 0.62^{a1}	1.53 ± 0.02^{a1}	1.86 ± 0.08^{a1}	6.25 ± 0.16^{a}

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. Since pH was not influenced ($P>0.05$) by chilling storage, data reported are the mean values of pH over the storage period. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P<0.05$).

3.4. Color

Color parameters of the frankfurters were affected ($P<0.05$) by the type of formulation and by chill storage, with an interaction ($P<0.05$) between both factors (Table 5). Generally fat reduction had little effect on the color parameters. As compared with the normal-fat control (C), partial replacement of the pork backfat by konjac gel (F/KG) did not affect ($P>0.05$) lightness (L*) and produced a slight but significant decrease of a* and b*. Jiménez-Colmenero et al. (2010a), Kao and Lin (2006) and Lin and Huang (2003) found that the effect of the addition of konjac gel on the color of the frankfurters was affected by the proportion of this fat replacer, so that in formulations containing 19% konjac gel to reduce fat, L* and b* values decreased and a* values increased compared with full-fat frankfurters. No differences in the color parameters were observed between full-fat products and reduced-fat products containing konjac (2%)–potato starch (4%) mixed gels (Kao & Lin, 2006). Compared to the reduced-fat sample (KG), the addition of oils (irrespective of the procedures used) had little effect on the color perception of the frankfurters (F/OWE, F/OKM and F/ OWE + KG). It has been reported that the replacement of the pork backfat by olive oil reduced the redness of the frankfurters (Lopez-Lopez, Cofrades & Jimenez-Colmenero, 2009), although Paneras and Bloukas (1994) found that oil substitution had no effect on color. Differences have been reported in the color parameters of the frankfurters as affected by the protein system (SC versus soy protein isolate) used for oil-in-water emulsion stabilization (Delgado-Pando, Cofrades, Ruiz-Capillas, et al., 2010a,b; Jiménez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010b). Although with some significant variations, chill storage (over 40 days) generally had little effect on color (Table 5). Similar results were reported by Bloukas and Paneras (1993), who found that storage time (5 weeks) had no effect on the color of low-fat frankfurters with olive oil.

Table 5
Color parameters during chilling storage of the different frankfurters.

Sample	Storage (days)	Color parameter		
		Lightness (L*)	Redness (a*)	Yellowness (b*)
C	1	72.30 ± 0.66^{b3}	9.87 ± 0.20^{b12}	8.55 ± 0.24^{b1}
	12	71.43 ± 0.74^{b12}	9.89 ± 0.30^{bc2}	8.56 ± 0.28^{b1}
	26	71.07 ± 0.53^{a1}	9.83 ± 0.27^{c12}	8.62 ± 0.19^{bc1}
	40	71.68 ± 0.37^{a2}	9.66 ± 0.21^{b1}	8.51 ± 0.21^{b1}
F/KG	1	71.79 ± 0.84^{ab12}	9.22 ± 0.29^{a1}	8.04 ± 0.26^{a1}
	12	71.94 ± 0.63^{bc2}	9.59 ± 0.27^{a2}	8.09 ± 0.22^{a1}
	26	71.19 ± 0.87^{a1}	9.56 ± 0.26^{b2}	7.92 ± 0.13^{a1}
	40	71.44 ± 0.53^{a12}	9.26 ± 0.13^{a1}	8.09 ± 0.19^{a1}
F/OKM	1	71.15 ± 1.16^{a1}	9.23 ± 0.43^{a1}	8.70 ± 0.47^{bc2}
	12	70.57 ± 0.67^{a1}	9.99 ± 0.33^{c2}	8.50 ± 0.22^{b12}
	26	70.72 ± 0.73^{a1}	9.98 ± 0.18^{c2}	8.39 ± 0.15^{b1}
	40	71.31 ± 0.88^{a1}	9.97 ± 0.47^{c2}	8.49 ± 0.18^{b12}
F/OWE	1	72.35 ± 0.76^{b3}	8.94 ± 0.52^{a1}	8.91 ± 0.40^{c2}
	12	71.91 ± 0.85^{bc23}	9.63 ± 0.30^{ab2}	8.48 ± 0.19^{b1}
	26	71.17 ± 0.62^{a1}	9.53 ± 0.21^{b2}	8.46 ± 0.25^{bc1}
	40	71.57 ± 0.60^{a12}	9.39 ± 0.32^{ab2}	8.59 ± 0.21^{b1}
F/OWE + KG	1	72.17 ± 0.76^{b2}	9.01 ± 0.39^{a1}	8.94 ± 0.12^{c1}
	12	72.22 ± 0.79^{c2}	9.38 ± 0.21^{a2}	8.96 ± 0.26^{c1}
	26	71.35 ± 0.43^{a1}	9.21 ± 0.18^{a12}	8.78 ± 0.27^{c1}
	40	71.85 ± 0.39^{a12}	9.18 ± 0.14^{a12}	8.78 ± 0.13^{c1}

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. Means ± SD. Different letters for samples at the same storage (a, b, c) time and different numbers for each sample during the storage time (1, 2, 3) indicate significant differences ($P<0.05$).

3.5. Texture

Texture parameters of the frankfurters were affected ($P<0.05$) by the type of formulation and by chill storage, with an interaction ($P<0.05$) between both factors (Table 6). Reducing the fat content by replacing the pork backfat with konjac gel (C as compared with F/KG) increased ($P<0.05$) all TPA parameters. This may be due to the effect of two factors: fat reduction strategy and the presence of konjac gel as a fat analogue. The effect produced by the differing fat content has been attributed to the characteristics of the matrix formed in each case. Generally speaking, where low fat is compensated for by increased protein, textures tend to be harder. However as fat content is reduced by increasing the proportion of water while keeping the amount of protein essentially constant (as in this case), the structure of low-fat systems becomes softer (Claus, Hunt, Kastner, & Kropf, 1990; Jimenez-Colmenero, Barreto, Fernandez, & Carballo, 1996). Such behavior was not observed in this experiment (Table 6), a fact that has been attributed to the water retention and gelling properties of the konjac gel (Jiménez-Colmenero et al., 2010a). According to the present results, the replacement of the pork backfat by an equal amount of a mixed konjac/gellan gum gel in a reduced fat frankfurter generally produces a product that is harder and chewier than a regular high-fat frankfurter (Lin & Huang, 2003). However, the effect of replacing the pork backfat with konjac gels can vary according to the nature of the gel and the proportion of the fat replaced (Kao & Lin, 2006; Lin & Huang, 2003; Osburn & Keeton, 2004).

Fat reduction associated with the incorporation of oils affected ($P<0.05$) the TPA parameters, although these effects were conditioned by the oil stabilization system used (Table 6). The samples formulated with oil-in-water emulsion as an animal fat replacer (F/OWE) had similar hardness values ($P>0.05$) than the control. However, when the konjac materials were included (F/OKM and F/OWE + KG samples), Hd and Cw increased ($P<0.05$) as compared to the control (C), but these values were lower ($P<0.05$) than in the F/KG samples. There are several factors that can contribute to such behavior. One is the oil content in the fat replacer (20% in OKM versus 53% in OWE), even though the final content in the product is the same (Table 1). Another are the characteristics of the different oil stabilization systems: while OKM presented a stronger structure typical of a gel, the oil-in-water emulsion stabilized with SC (OWE) behaved like a viscous material but lacked gel-like behavior (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al., 2010a). In any case these results suggest that the presence of oils in the frankfurters limits the effect induced by the presence of konjac on the hardness of the products. Youssef and Barbut (2011) reported that fat reduction reduced the hardness of comminuted meat products; however the effect of the reformulation process on the TPA parameters was affected by the form in which the canola oil was incorporated: in a liquid form or in an oil-in-water emulsion stabilized with sodium caseinate, soy protein isolate or whey protein isolate. Substitution of pork fat by olive oil (oil-in-water emulsion with SC) in reduced-fat frankfurter (as compared to a normal-fat product) has been reported to produce harder/firmer products (Paneras & Bloukas, 1994; Paneras et al., 1998) or to have no influence (Bloukas & Paneras, 1993). However, other authors have observed that olive oil addition combined with fat reduction (with similar protein content) caused a decrease in the hardness and chewiness of the frankfurters (Luruena-Martinez et al., 2004). The apparent discrepancies regarding the effect of substituting olive oil for animal fat may be related to the composition factors (moisture and protein content).

In general, the TPA parameters were affected ($P<0.05$) by chill storage. For instance, with the passage of time Hd, cohesiveness and Cw increased ($P<0.05$), with less significant changes in Sp (Table 6). The influence of chill storage on the TPA parameters was affected by formulation (interactive effect), although variations were of minor relevance. Kao and Lin (2006) found that shear force increased gradually in reduced-fat frankfurters with an increase in storage time. And similarly,

Table 6
TPA parameters during chilling storage of the different frankfurters.

Sample	Storage (days)	Hardness (N)	Springiness (mm)	Cohesiveness (dimensionless)	Chewiness (N x mm)
C	1	14.95 ± 0.48^{a1}	6.38 ± 0.20^{a1}	0.652 ± 0.005^{a1}	62.41 ± 0.85^{a1}
	12	15.72 ± 0.48^{a12}	6.41 ± 0.19^{a1}	0.662 ± 0.007^{a12}	66.31 ± 1.89^{a2}
	26	17.45 ± 1.12^{a3}	6.57 ± 0.14^{a12}	0.665 ± 0.012^{a2}	77.04 ± 0.59^{a4}
	40	16.36 ± 1.31^{a23}	6.65 ± 0.26^{a2}	0.684 ± 0.008^{a3}	72.77 ± 1.19^{a3}
F/KG	1	23.15 ± 1.22^{d2}	6.69 ± 0.34^{b1}	0.693 ± 0.002^{bc1}	101.19 ± 1.68^{e1}
	12	21.65 ± 0.68^{d1}	6.79 ± 0.28^{b1}	0.699 ± 0.008^{bc2}	104.42 ± 0.71^{e2}
	26	24.81 ± 0.73^{c3}	6.73 ± 0.10^{ab1}	0.693 ± 0.003^{b1}	116.49 ± 0.71^{d3}
	40	24.87 ± 1.41^{d3}	6.99 ± 0.30^{ab1}	0.711 ± 0.004^{b3}	123.77 ± 1.93^{d4}
F/OKM	1	20.62 ± 0.68^{c1}	6.84 ± 0.17^{b1}	0.697 ± 0.008^{c1}	96.45 ± 1.95^{d1}
	12	20.55 ± 0.50^{c1}	6.83 ± 0.16^{b1}	0.707 ± 0.005^{c2}	98.97 ± 1.35^{d2}
	26	24.34 ± 1.01^{c2}	6.93 ± 0.09^{b1}	0.701 ± 0.003^{b12}	117.91 ± 1.30^{d2}
	40	24.46 ± 0.74^{d2}	7.15 ± 0.22^{ab2}	0.723 ± 0.004^{c3}	129.09 ± 0.72^{e3}
F/OWE	1	14.90 ± 0.78^{a1}	6.79 ± 0.19^{b1}	0.687 ± 0.007^{b1}	72.05 ± 1.57^{b1}
	12	16.26 ± 0.46^{a2}	6.88 ± 0.20^{b1}	0.692 ± 0.004^{b1}	75.55 ± 0.43^{b2}
	26	18.16 ± 0.49^{a3}	6.88 ± 0.14^{b1}	0.693 ± 0.006^{b1}	89.32 ± 1.24^{b3}
	40	17.73 ± 0.67^{b3}	6.81 ± 0.13^{ab1}	0.708 ± 0.006^{b2}	85.49 ± 1.65^{b4}
F/OWE + KG	1	17.03 ± 0.75^{b1}	6.56 ± 0.19^{ab1}	0.693 ± 0.006^{bc1}	76.62 ± 1.82^{c1}
	12	19.36 ± 0.67^{b2}	6.77 ± 0.17^{b12}	0.697 ± 0.008^{b1}	91.10 ± 1.71^{c2}
	26	21.04 ± 1.86^{b3}	6.85 ± 0.26^{b12}	0.700 ± 0.007^{b1}	101.48 ± 1.74^{c3}
	40	21.22 ± 0.88^{c3}	7.33 ± 0.92^{b2}	0.713 ± 0.003^{b2}	109.67 ± 1.71^{c4}

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. Means ± SD. Different letters for samples at the same storage time and different numbers for each sample during the storage time indicate significant differences ($P<0.05$).

hardness has been reported to increase during chill storage, a development attributed to changes in purge loss (Andres, Garcia, Zaritzky, & Califano, 2006; Candogan & Kolsarici, 2003). Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, et al. (2010a) reported that there was some loss of hardness in the low-fat frankfurters with several oil-in-water emulsions.

3.6. Microstructure

Fig. 1 shows micrographs of the meat products. The morphology of the control frankfurter (Fig. 1a) was characteristic of cooked gel/emulsion systems (Katsaras & Peetz, 1989), showing the formation of numerous cavities, producing structures with a spongy (honeycomb-like) appearance. The formation of these cavities may have been due to the expansion of a number of constituents, mainly fat, water or air (Katsaras & Peetz, 1989).

The morphology of the frankfurters was affected by reformulation. F/KG presented a compact structure without a spongy appearance (Fig. 1b). Samples prepared with the oil combination lost the spongy honeycomb-like structures (Fig. 1c, d and e), whereas F/OWE had a more continuous and compact appearance and F/OKM and F/OWE + KG exhibited increased disorganization caused by the presence of the konjac gel. The morphological characteristics observed in the frankfurters containing the konjac gel conferred greater consistency on the product and promoted textural changes, so that these were harder and chewier than the control sample and F/OWE (Table 6). Similar findings on the microstructure have been reported. Jiménez-Colmenero et al. (2010a) found changes in the structure of reduced-fat frankfurter formulations with added konjac gel. Delgado-Pando et al. (2011)

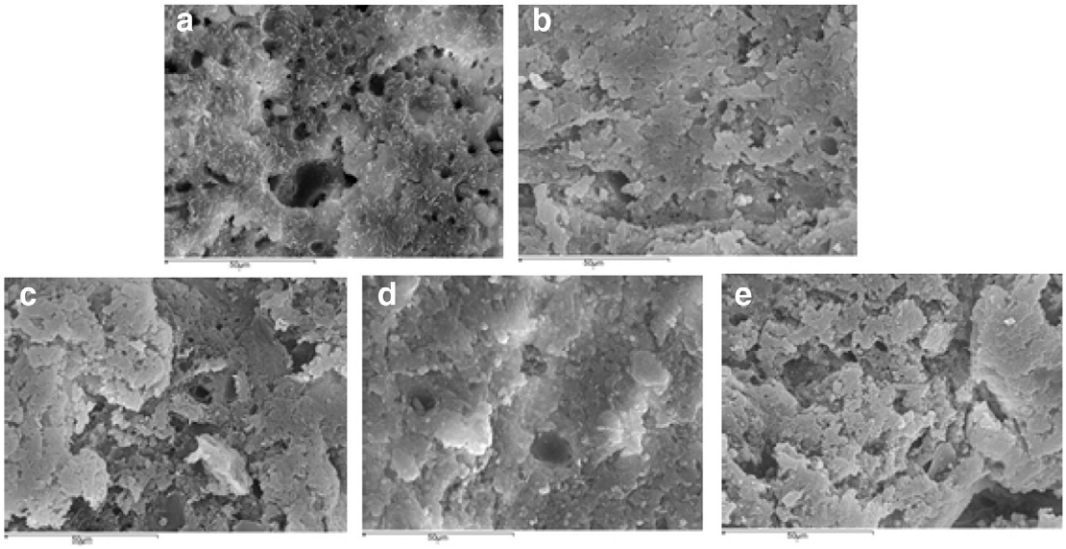

Fig. 1. Scanning electron micrographs of the different frankfurters: a) C, frankfurter prepared with normal fat content (all pork fat); b) F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); c) F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); d) F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); e) F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. Bar represents 50 μm.

Table 7
Sensory evaluation of frankfurters.

Sample	Juiciness	Hardness	Texture	Off flavor	Flavor
C	6.5 ± 2.2^{a}	3.1 ± 1.5^{a}	5.7 ± 2.1^{a}	2.7 ± 2.6^{a}	7.0 ± 1.5^{a}
F/KG	4.8 ± 2.5^{a}	5.6 ± 1.8^{b}	6.2 ± 2.1^{a}	2.3 ± 2.2^{a}	6.4 ± 2.1^{a}
F/OKM	4.7 ± 1.8^{a}	5.0 ± 1.7^{ab}	5.8 ± 1.8^{a}	4.0 ± 2.5^{a}	5.4 ± 2.0^{a}
F/OWE	5.0 ± 2.1^{a}	4.8 ± 2.0^{ab}	6.1 ± 2.0^{a}	2.9 ± 2.3^{a}	6.1 ± 2.1^{a}
F/OWE + KG	6.1 ± 2.3^{a}	5.0 ± 1.8^{ab}	6.9 ± 1.4^{a}	2.1 ± 2.2^{a}	7.1 ± 1.3^{a}

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat by KG (all pork fat); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. Means ± SD. Different letters in the same column (a, b, c) indicate significant differences ($P<0.05$).

reported that the morphology of frankfurters containing oil-in-water emulsions as pork backfat replacers indicated that the characteristics of the continuous protein matrix and the fat globules were affected by the type of oil-in-water emulsions used in the product formulation.

3.7. Sensory evaluation

Fat reduction (partial replacement of the pork backfat by konjac gel, C versus F/KG) did not affect ($P>0.05$) the sensory parameters except for the hardness (Table 7). F/KG was the sample that was scored the highest by the panelists ($P<0.05$). These results are consistent with the results for instrumental hardness (Table 6). Otherwise sensory quality was not affected ($P>0.05$) by the presence of oils in the frankfurter formulation or the way in which they were incorporated (Table 6). In general, the panelists considered that all the products were satisfactory. Osburn and Keeton (2004) reported that low-fat sausages with konjac flour (10 and 20 g/100 g) slightly reduced the sensory panel values. Bloukas and Paneras (1993) reported that the low-fat frankfurters produced by replacing the pork backfat with olive oil had lower overall palatability or acceptability than the high-fat frankfurters produced with the pork backfat. In contrast, Jiménez-Colmenero, Herrero, Pintado, Solas (2010b) found no significant differences in the overall acceptability between the pork backfat replacement by olive-oil-in water in frankfurters. These findings are consistent with the results of this study.

4. Conclusions

The results show the technological viability of the procedure used to improve the fat content (reducing fat and improving the fatty acid profile) of frankfurters. Fat reduction by replacement of the pork backfat with konjac gel and by the addition of a healthier oil combination stabilized by different systems both resulted in products with similar water and fat binding properties, color and sensory characteristics. From a nutritional standpoint the changes in composition are such as to allow products reformulated in this way (with less than 30% of the fat in the reference product) to carry a "reduced fat content" claim pursuant to Regulation (EC) No 1924/2006 of the European Parliament and of the Council (2006). Similarly (pursuant to Commission Regulation No 116/2010), frankfurters with added oil may claim "high omega 3 fatty acid content" since they contain more than 0.6 g of ALA/100 g and more than 80 mg of the sum of EPA plus DHA per 100 g. Chill storage had little effect on the technological characteristics of the frankfurters.

Additional studies on microbiology, biogenic amine formation, residual nitrite, volatile compounds and lipid oxidation will be reported later.

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4.1.2 Shelf-life of n-3 PUFA enriched frankfurters formulated with a konjac-based oil bulking agent

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Shelf-life of n-3 PUFA enriched frankfurters formulated with a konjac-based oil bulking agent

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ABSTRACT

Frankfurters with improved-fat content were manufactured following two strategies: a) reduction of fat content by replacing pork backfat with konjac gel (KG); and b) reduction of fat/improvement of fatty acid profiles through partial substitution of pork backfat by an oil combination (olive, linseed and fish oils) stabilized in a -konjac matrix (oil bulking agent) (KG), in an oil-in-water emulsion (OWE), or a combination of both of these (KG and OWE).

Their shelf-life characteristics were studied on the basis of nitrite, microbiological, biogenic amine profile and lipid oxidation analyses during chilling storage. Nitrite levels decreased over storage in all samples and were highest ($P < 0.05$) in the sample with OWE. No clear relationship was observed between microbiota and reformulation. High initial histamine levels were observed in frankfurters containing OWE and/or KG. Over chilling storage, histamine and tyramine increased the most, with the highest levels occurring in the samples containing the oil combination. Initial levels of lipid oxidation were lower in the control sample. The samples containing the oil combination presented similar lipid oxidation patterns over storage, with higher levels in F/OWE. No reformulation-dependent factor that might limit shelf-life was detected during storage.

1. Introduction

Healthier lipid formulation based on processing strategies is one of the most important current approaches to the development of potential meat-based functional foods, including frankfurter-type products. One of the strategies that has received most attention in this connection entails reformulation processes designed to replace animal fat with other oils of plant and/or marine origin which comply better with health recommendations. These oils have been incorporated in cooked meat products like frankfurters in liquid or solid forms or encapsulated, but the most widely-used form has been as oil-in-water emulsions. Oil-in-water emulsion technology using a non-meat protein improves the system's fat binding ability, since the oils can be stabilized or immobilized in a protein matrix. Oil-in-water emulsions prepared with different proteins (mostly with sodium caseinate) as emulsifiers and various types of oils, have been used as fat ingredients in frankfurter formulation (Bloukas & Paneras, 1993; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jimenez-Colmenero, 2010). Strategies for incorporation of oils in a gel-like matrix to form an oil bulking agent (in which this new ingredient acts as an animal fat replacer) could offer new possibilities for improving the fat content of meat products (Ruiz-Capillas, Carmona, Jimenez-Colmenero, & Herrero, 2013).

In a previous paper (Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas, & Jiménez-Colmenero, 2013) our group assessed the suitability of an oil combination stabilized in an oil-in-water emulsion and in an oil-in-konjac matrix as pork backfat replacers in n-3 polyunsaturated fatty acids (PUFA)-enriched frankfurters. The authors reported that the procedure used to improve fat content was viable in technological and sensory terms: it both reduced fat and improved the fatty acid profile of frankfurters by replacing pork backfat with konjac gel and by an oil combination stabilized by a konjac matrix (oil bulking agent) and an oil-in-water emulsion. From a nutritional standpoint the changes in composition are such as to allow products reformulated in this way to carry a “reduced fat content” claim pursuant to Regulation (EC) No 1924/2006 of the European Parliament and of the Council (2006). Similarly (pursuant

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to Commission Regulation No 116/2010), frankfurters with added oil may claim "high omega 3 fatty acid content".

However, other aspects such as safety and shelf-life characteristics need to be considered in order to gain a clearer understanding of these products and a more accurate assessment of the suitability of this strategy for a healthier reformulation of frankfurters (Delgado-Pando et al., 2011). Lipid oxidation is one of the processes that most limit the shelf life of foods, affecting the use of nutritionally beneficial lipids in functional foods. Susceptibility to lipid oxidation can be augmented by increasing concentrations of unsaturated fatty acids and processing conditions. Several factors affect the rate and extent of lipid oxidation (Decker & Xu, 1998). Some of these factors, such as the reactivity of antioxidants and prooxidants, interaction with other food components, molecular environmental conditions, physical location, etc., are dependent on the strategy used to incorporate healthier lipids in a product. No studies have been reported about the effect that a stabilized oil combination (e.g. oil-in-water emulsion or bulk oil in a konjac matrix) used as a pork backfat replacer has on cooked meat products such as frankfurters. And again, the microbiota is known to affect the safety and shelf life of meat products during chilling storage. This factor is important because of the microbial population and any compounds that they may generate, such as biogenic amines (BA), which are toxic compounds with negative health implications (headaches, urticaria, migraine, nausea, etc.). Their formation (by decarboxylation of free amino acids through the action of amino acid decarboxylase enzymes, mainly of microbiological origin) is conditioned by different factors implicit in the process of reformulating these meat products (ingredient modifications, handling and processing conditions, etc.) (Ruiz-Capillas & Jimenez-Colmenero, 2004). Also, it has been reported that some biogenic amines, mainly physiological amines like spermidine and spermine, may act as antioxidants, reducing lipid oxidation (Løvaas, 1991). Nitrite contributes to microbiological safety and also to the flavour, colour and antioxidative stability of cured meat products. Nitrite may react with amines (mainly biogenic amines) to produce nitrosamines, which are carcinogens (De Mey et al., 2014).

Therefore, additional studies were carried out in parallel to Salcedo-Sandoval et al. (2013) to assess the effect of reformulation processes and chilling storage (40 days at 2 °C) on lipid oxidation, nitrite content, microbiological changes and biogenic amine formation in frankfurters. The reformulation processes designed to improve fat content were: a) reduction of fat content (replacing pork backfat with konjac gel), b) reduction of fat/improvement of fatty acid profiles through partial substitution of pork backfat by an oil combination stabilized in an oil-in-konjac matrix (oil bulking agent) or the same oil combination stabilized in an oil-in-water emulsion, or a combination of konjac gel and oil-in-water emulsion. The oil combination used was designed with suitable amounts and proportions of fatty acids to achieve healthier intake goals (Delgado-Pando et al., 2010).

2. Materials and methods

2.1. Materials, frankfurters preparation and chilled storage

The meat products (and consequently material and procedures) used to make the oil combination, konjac materials and oil-in-water emulsion were as reported by Salcedo-Sandoval et al. (2013). Briefly, sufficient fresh post-rigor pork meat from different animals (mixture of M. biceps femoris, M. semimembranosus, M. semitendinosus, M. gracilis and M. adductor) and pork backfat were obtained from a local meat market. The oil combination consisted of olive (44.39%), linseed (37.87%) and fish (17.74%) oils. It was designed to produce a healthier lipid formulation with a small proportion of saturated fatty acids (SFA), large proportions of monounsaturated fatty acids (MUFA) and PUFA (including long chain n-3 PUFA) and balanced n-6/n-3 PUFA and PUFA/SFA ratios (Delgado-Pando et al., 2010). Two types of konjac materials were prepared: one a konjac gel (KG) and another with 20% of an oil mixture added to the konjac matrix (oil-in-konjac matrix/OKM). The preparation conditions and the technological viability of adding these proportions of oils to konjac matrix (OKM) were established earlier at our laboratory (Salcedo-Sandoval et al., 2013). Oil-in-water emulsion (OWE) was prepared according to the procedure described by Delgado-Pando et al. (2010), mixing eight parts of water (at room temperature) with one part of sodium caseinate for 2 min in a Stephan Universal Machine (Model UM5, Stephan Machinery GmbH and Co., Hameln, Germany). The mixture was emulsified with 10 parts of the oil combination for another 3 min. The oil-in-water emulsion contained 52.63% of the oil combination. Both konjac materials and oil-in-water emulsion were placed in suitable containers (like metal ham moulds), covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until used (within 24 h of preparation). These ingredients were prepared in duplicate.

Five different frankfurters were formulated (Table 1): a control sample (C) prepared with normal fat content (20%), and a reduced-fat (10%) frankfurter (F/KG) in which pork backfat was partially replaced by KG. In addition, three samples were formulated with reduced fat content (10%) and improved fatty acid profile in which pork backfat was partially replaced by a) the oil combination stabilized in an oil-in-konjac matrix (F/OKM), b) the oil combination stabilized in an oil-in-water emulsion (F/OWE), and c) a combination of KG and oil-in-water emulsion (F/OWE + KG). The reformulated products were designed to have similar fat levels irrespective of the type of lipid material and/or the mode of incorporation. Whereas in C and F/KG samples all the fat was pork fat, part of the fat in F/OKM, F/OWE and F/OWE + KG came from added olive, linseed and fish oils. These three samples were designed to contain the same proportion and type of fat (both pork fat and added oils), so that they only differed in the mode of oil incorporation (oil stabilization system). The specific conditions for frankfurters preparation are detailed in Salcedo-Sandoval et al. (2013). Vacuum-packed (Cryovac® BB3050) frankfurters were stored (in dark) at 2 °C (±1 °C) and analysed periodically (1, 12, 26 and 40 days). The entire frankfurters processing procedure was replicated twice at two different days.

2.2. Determination of residual nitrite

Residual nitrite contents were determined using the flow injection analysis according to Ruiz-Capillas, Aller-Guiote, and

Table 1
Formulation (g) of frankfurters.

Sample	Meat	Backfat	Substituents			Water
			KG	OKM	OWE	
C	2281	762	–	–	–	843
F/KG	2381	315	762	–	–	427
F/OKM	2421	137	–	762	–	565
F/OWE	2421	137	–	–	304	1024
F/OWE + KG	2421	137	220	–	304	803

Sample denomination: C, control sample prepared with normal fat content (all pork fat); F/KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with konjac gel (KG); F/OKM, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil in konjac matrix (OKM); F/OWE, reduced fat content frankfurter prepared by partial replacement of pork backfat with oil-in-water emulsion (OWE); F/OWE + KG, reduced fat content frankfurter prepared by partial replacement of pork backfat with OWE and KG. The following were also added to all samples: 2.0 g/100 g sodium chloride; 0.30 g/100 g sodium tripolyphosphate; 0.012 g/100 g sodium nitrite; 0.50 g/100 g flavouring.

Jimenez-Colmenero (2007). Three determinations were performed per sample.

2.3. Microbiological analysis

Samples were prepared in a vertical laminar-flowcabinet (model AV 30/70, Telstar, Madrid, Spain). For each sample, 10 g (in replicate) was taken and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of peptone water (0.1%) and 0.85% NaCl (Panreac Química, S.A. Barcelona, Spain). After 1 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on the following media: Plate Count Agar (PCA) (Merck, Germany) for total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharpe Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). The results were expressed as logarithms of colony forming units per gram (Log cfu/g).

2.4. Analysis of biogenic amines (BA) by ion-exchange chromatography

Tyramine, phenylethylamine, histamine, putrescine, cadaverine, agmatine, spermidine and spermine were determined following the methodology of Triki, Jiménez-Colmenero, Herrero, and Ruiz-Capillas (2012). The results are averages of at least 2 determinations from two extractions per sample.

2.5. Lipid oxidation

Oxidative stability was evaluated from changes in thiobarbituric acid reactive substances (TBARs). The procedure for measurement of TBARs was based on methods used by Serrano, Cofrades, and Jimenez-Colmenero (2006). Three determinations were performed per sample.

2.6. Statistical analysis

The entire trial was replicated. Two-way analyses of variance (ANOVA) to evaluate the statistical significance ($P < 0.05$) of the formulation and storage time were carried out using general linear model (GLM) procedure of SPSS Statistics (v.19, IBM SPSS Inc., Chicago, IL). Formulation and storage time were assigned as fixed effects and replicate as random effect. Least squares differences were used for comparison of mean values between treatments and Tukey's HSD test to identify significant differences ($P < 0.05$) between formulations and storage time. To examine relationships between parameters Pearson product moment correlation (r) was performed.

3. Results and discussion

Lipid modification of meat products such as frankfurters by fat reduction and/or substitution of animal fat with other lipid sources has been shown to be a good strategy to improve nutritional quality (Delgado-Pando et al., 2010). However, the effect of predetermined composition changes on processing and product characteristics can be affected by the reformulation strategy used. In a previous paper Salcedo-Sandoval et al. (2013) reported the proximate composition, nutritional benefits and technological and sensory properties of improved lipid frankfurters reformulated by reducing fat/improving fatty acid content through partial substitution of pork backfat by an oil combination stabilized in an oil-in-konjac matrix or in an oil-in-water emulsion. The following sections deal with different aspects relating to shelf-life characteristics and safety which are essential to gain a clearer understanding of these products.

3.1. Residual nitrite

Residual nitrite was affected ($P < 0.05$) by the formulation and storage (Table 2). The initial levels of residual nitrite in samples varied within a narrow range (78–81 mg/kg), with the highest ($P < 0.05$) concentration in F/OWE + KG sample. Depending on the frankfurter formulation, between 65 and 67% of the added nitrite was detectable initially in the final product after processing and 23–45% after storage for 40 days at 2 °C (Table 2). Residual nitrite levels decline more rapidly during heating than during storage (Honikel, 2008). The levels of nitrite processing loss (33–35%), mainly associated with the manufacturing and heating processes, are within the range generally reported for these products (20–80%), which varies in response to factors related to product characteristics and heating schedule (Cassens, Greaser, Ito, & Lee, 1979). Residual nitrite processing loss in reformulated cooked sausages has been found to vary over a wide range, for example 20–39% (Jiménez-Colmenero et al., 2010), 39–49% (Delgado-Pando et al., 2011) in frankfurters, and by as much as 94% in bolognas formulated with citrus fibres (Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, & Perez-Alvarez, 2003). As storage progressed, residual nitrite decreased ($P < 0.05$) depending on the formulation. The pattern of the residual nitrite decrease was quite similar in all samples, except for F/OWE, which showed the greatest reduction after 12 days of storage (Table 2). At the end of the storage period, control samples had the highest and F/OWE the lowest residual nitrite values, coinciding with the highest TBARs value (Table 5). The level of storage loss ranged between 19 and 43%.

The reason for the disappearance of added nitrite during processing and storage is that nitrite is a known reactive chemical which participates in numerous reactions or binds to constituents (lipids, proteins, etc.) of the meat. The rate of depletion is dependent upon various factors, such as pH, initial nitrite concentration, product composition (meat-to-water ratio, fat level, presence of reductants, etc.), processing (mainly thermal treatment) and storage conditions (time and temperature, light exposure, etc.) (Cassens et al., 1979). Because of its technological importance and potential human health implications, considerable effort has been devoted to monitoring residual levels of nitrite in a range of meat products. This is particularly relevant in reformulation processes like the one assayed in this experiment, where some of those factors are modified, in ways that depend on the strategy used in healthier meat product formulation, and this may affect the nitrite depletion pattern. In this experiment, the main differences among the samples lay in the quantitative and qualitative aspects of fat content (Fig. 1), and the stabilization system used to incorporate the oil combinations in the meat matrix. While the control sample

Table 2
Concentration of residual nitrite (mg/kg) of frankfurters during chilling storage.

Sample	Storage (days) at 2 °C			
	1	12	26	40
C	78.0 ± 1.0^{ab4}	64.3 ± 0.7^{a3}	58.2 ± 0.5^{b2}	54.7 ± 1.1^{e1}
F/KG	78.9 ± 0.7^{b3}	62.4 ± 6.5^{a2}	60.4 ± 0.5^{d2}	41.1 ± 0.5^{b1}
F/OKM	77.9 ± 1.0^{a4}	65.8 ± 1.2^{a3}	60.0 ± 0.7^{d2}	47.2 ± 0.5^{c1}
F/OWE	78.5 ± 0.9^{ab4}	71.1 ± 2.5^{b3}	41.3 ± 0.5^{a2}	27.3 ± 0.7^{a1}
F/OWE + KG	80.9 ± 0.7^{c4}	72.3 ± 1.4^{b3}	59.0 ± 0.5^{c2}	51.8 ± 1.0^{d1}

For sample denomination see Table 1. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$).

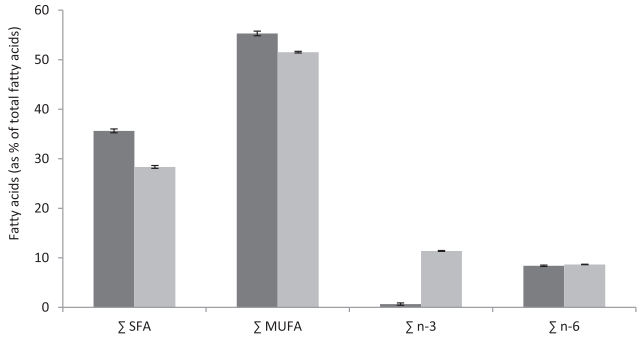

Fig. 1. Fatty acid profile (as % of total fatty acids) of frankfurters. ■ C, F/KG F/OKM ■ F/OKM, F/OWE, F/OWE + KG. Data extracted from Salcedo-Sandoval et al. (2013).

contained 20% (all pork) fat, the other frankfurters contained around 10%. Of these low-fat products, F/KG contained all pork fat, while in F/OKM, F/OWE and F/OWE + KG animal fat was partially replaced by the same combination of olive, linseed and fish oils added in an oil-in-water emulsion or in a konjac-based bulking agent. Since the nitrite reacts with lipid components of meat (Cassens et al., 1979), fat replacer characteristics (associated with both the lipid source and the presence of some compounds associated with the stabilization system used) may affect the residual nitrite content in reformulated frankfurters. Higher residual nitrite levels have been reported in low-fat sausages as compared with high-fat products (Jiménez-Colmenero et al., 2010). However, no such behaviour was observed in this experiment; high-fat sample (C) also had the maximum residual nitrite concentration at the end of storage (Table 2). It has been reported that the replacement of pork fat by olive oil reduced the residual nitrite as compared with all pork fat sample (López-López, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2009), but other authors (Delgado-Pando et al., 2011; Paneras & Bloukas, 1994) found no differences in the residual nitrite content of frankfurters with added oils. In this experiment, nitrite depletion was similar in samples containing konjac gel (F/KG, F/OKG and F/OWE + KG) and in the F/OWE sample (Table 2). Therefore, the presence of konjac gel seems to influence nitrite depletion in frankfurters more than other factors such as the type of fat. Various authors have found that addition of konjac to cooked sausage (wieners and frankfurters) formulations did not influence the residual nitrite in the finished product (Jiménez-Colmenero et al., 2010; Kilic, Cassens, & Borchert, 2002). However, it is also the case that the rate of nitrite depletion has been related to the pH of the meat system (Honikel, 2008). In this work no effect can be attributed to this factor since the pH level was similar (6.3) in all products irrespective of formulation and storage (Salcedo-Sandoval et al., 2013).

3.2. Microbiological analysis

Microbial levels of the samples are shown in Table 3. As the table shows, the microbiota was affected ($P < 0.05$) by storage and formulation. In general, total viable counts (TVC) and lactic acid bacteria (LAB) levels showed no significant differences in the initial analyses (less than 3 and 2 Log cfu/g respectively) (Table 3). These results are consistent with reports by other authors in similar products (Delgado-Pando, Cofrades, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2012; Ruiz-Capillas, Carballo, & Jimenez-Colmenero, 2007).

Microbial growth increased ($P < 0.05$) in all samples during storage, especially by day 26 (Table 3). At that time the samples with the largest microbial populations (TVC and LAB) were F/OWE and F/OWE + KG (Table 3). However, the same behaviour was not observed at the end of storage, when the control sample and F/OWE presented the highest levels of TVC. In the case of LAB, the main microbiota in products of this kind kept in vacuum (Korkeala & Björkroth, 1997), the highest levels were detected in samples F/OKM and F/OWE + KG, possibly because KG stimulates the growth of these microorganisms (LAB) in the presence of high moisture levels and carbohydrates. This has also been reported by other authors (Triki, Herrero, Rodríguez-Salas, Jiménez-Colmenero, & Ruiz-Capillas, 2013). Initial *Enterobacteriaceae* levels were <1 Log cfu/g and remained below 3 Log cfu/g over storage. In general in general over storage there was no clear correlation between microbial population and product formulation (Table 3). Similar levels of microorganisms and behaviour during storage were observed in low-fat frankfurter and pate reformulated with a healthier lipid combination in an oil-in-water emulsion (Delgado-Pando et al., 2011; Delgado-Pando et al., 2012).

Table 3
Total viable count (TVC) and Lactic acid bacteria (LAB) counts (Log cfu/g) of frankfurters during chilling storage.

Microorganism	Sample	Storage (days) at 2 °C			
		1	12	26	40
TVC	C	2.8 ± 0.3^{1}	3.1 ± 0.0^{a1}	5.7 ± 0.1^{b2}	6.8 ± 0.0^{d3}
	F/KG	2.7 ± 0.4^{1}	2.8 ± 0.2^{a1}	4.8 ± 0.0^{a2}	6.5 ± 0.1^{c3}
	F/OKM	3.1 ± 0.7^{1}	4.7 ± 0.1^{b12}	6.1 ± 0.0^{c2}	6.1 ± 0.2^{b2}
	F/OWE	2.7 ± 0.5^{1}	4.7 ± 0.3^{b2}	6.7 ± 0.0^{e3}	6.6 ± 0.0^{cd3}
	F/OWE + KG	3.0 ± 0.8^{1}	3.2 ± 0.1^{a1}	6.4 ± 0.1^{d2}	5.5 ± 0.0^{a2}
LAB	C	1.7 ± 0.4^{a1}	2.2 ± 0.2^{1}	5.2 ± 0.0^{ab2}	6.4 ± 0.0^{a3}
	F/KG	1.7 ± 0.4^{a1}	2.4 ± 0.6^{1}	4.3 ± 0.0^{a2}	6.4 ± 0.0^{a3}
	F/OKM	1.0 ± 0.0^{b1}	2.9 ± 0.2^{2}	5.8 ± 0.0^{b3}	7.6 ± 0.2^{b4}
	F/OWE	1.0 ± 0.0^{b1}	2.8 ± 0.1^{2}	6.1 ± 0.1^{b3}	6.1 ± 0.1^{a3}
	F/OWE + KG	1.7 ± 0.5^{a1}	2.1 ± 0.1^{1}	5.8 ± 0.5^{b2}	7.4 ± 0.0^{b3}

For sample denomination see Table 1. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$).

3.3. Biogenic amines

Biogenic amines were affected ($P < 0.05$) by formulation and storage (Table 4), with interaction ($P < 0.05$) between the two factors. Physiological amines, chiefly spermine and spermidine, were the most abundant initially, presenting levels of around 19 mg/kg and 1 mg/kg respectively, with no significant differences between the various reformulated samples. These values were comparable to values reported by other authors in similar pork products (Delgado-Pando et al., 2011; Ruiz-Capillas, Carballo, et al., 2007). Other biogenic amines were present at levels below 1 mg/kg. Some amines, such as phenylethylamine, cadaverine and tryptamine, were not detected at all initially. On the other hand, high initial histamine levels were recorded in the sample containing the non-animal fat emulsion (F/OWE and F/OWE + KG) (Table 4). This could have been due to the fact that the oil combination contained fish oil, in which histamine is the principal biogenic amine. Other authors (Delgado-Pando et al., 2011) have also reported high histamine levels in samples of sausages made with an emulsion and a similar oil mix.

Spermidine and spermine levels changed slightly but significantly in the course of storage, never exceeding 3 and 23 mg/kg respectively. Putrescine and agmatine levels were also very low (<2 mg/kg) throughout storage. Histamine and tyramine concentrations increased ($P < 0.05$) over storage (Table 4). The increase of biogenic amines during storage was mainly a consequence of microbial activity (Ruiz-Capillas & Jimenez-Colmenero, 2004). Histamine levels were generally higher in the lots containing the oil

Table 4
Biogenic amines content (mg/kg) in frankfurters sausages during chilling storage.

Biogenic amines	Samples	Storage (days) at 2 °C			
		1	12	26	40
Tyramine	C	0.7 ± 0.1^{ab1}	1.6 ± 0.0^{a2}	4.9 ± 0.3^{a3}	6.4 ± 0.0^{a4}
	F/KG	1.1 ± 0.1^{b1}	2.1 ± 0.1^{b2}	5.1 ± 0.0^{a3}	7.3 ± 0.4^{b4}
	F/OKM	0.9 ± 0.2^{ab1}	3.4 ± 0.0^{c2}	6.4 ± 0.0^{b3}	11.0 ± 0.4^{d4}
	F/OWE	0.5 ± 0.0^{a1}	3.3 ± 0.0^{c2}	5.2 ± 0.2^{ab3}	8.4 ± 0.5^{c4}
	F/OWE + KG	0.6 ± 0.0^{a1}	3.2 ± 0.0^{c2}	5.1 ± 0.1^{a3}	8.6 ± 0.2^{c4}
Histamine	C	0.4 ± 0.0^{a1}	1.7 ± 0.0^{a2}	3.4 ± 0.2^{a3}	6.5 ± 0.1^{b4}
	F/KG	0.2 ± 0.0^{a1}	1.8 ± 0.0^{a2}	3.6 ± 0.3^{a3}	4.7 ± 0.7^{a4}
	F/OKM	0.5 ± 0.0^{a1}	2.6 ± 0.0^{b2}	4.0 ± 0.3^{a3}	11.5 ± 0.1^{e4}
	F/OWE	4.5 ± 0.2^{c1}	6.2 ± 0.8^{d2}	8.9 ± 0.6^{c3}	10.3 ± 0.0^{d4}
	F/OWE + KG	3.0 ± 0.1^{b1}	4.2 ± 0.7^{c2}	6.6 ± 0.9^{b3}	8.6 ± 0.9^{c4}
Putrescine	C	0.2 ± 0.0^{b1}	0.4 ± 0.0^{b2}	0.5 ± 0.0^{c3}	0.6 ± 0.0^{c4}
	F/KG	0.3 ± 0.1^{c1}	0.5 ± 0.0^{c2}	0.7 ± 0.0^{d3}	0.8 ± 0.0^{d4}
	F/OKM	0.2 ± 0.0^{b1}	0.4 ± 0.0^{b2}	0.7 ± 0.0^{d3}	0.8 ± 0.0^{d4}
	F/OWE	0.1 ± 0.0^{a1}	0.1 ± 0.0^{a2}	0.3 ± 0.0^{a3}	0.3 ± 0.0^{a4}
	F/OWE + KG	0.1 ± 0.0^{a1}	0.1 ± 0.0^{a2}	0.3 ± 0.0^{b3}	0.5 ± 0.0^{b4}
Agmatine	C	0.5 ± 0.0^{a1}	0.6 ± 0.0^{b2}	0.6 ± 0.0^{a3}	0.7 ± 0.0^{a4}
	F/KG	0.4 ± 0.1^{a1}	0.5 ± 0.1^{ab12}	0.6 ± 0.0^{a2}	0.6 ± 0.1^{a2}
	F/OKM	0.4 ± 0.0^{a1}	0.5 ± 0.0^{a2}	1.0 ± 0.0^{b3}	1.0 ± 0.0^{b3}
	F/OWE	1.0 ± 0.0^{c1}	1.2 ± 0.0^{c2}	1.4 ± 0.0^{c3}	1.5 ± 0.0^{c4}
	F/OWE + KG	0.9 ± 0.0^{b1}	1.1 ± 0.1^{c2}	1.6 ± 0.1^{d3}	2.1 ± 0.0^{d4}
Spermidine	C	0.9 ± 0.1^{12}	0.9 ± 0.0^{a1}	0.9 ± 0.0^{b12}	1.0 ± 0.0^{a2}
	F/KG	1.1 ± 0.1^{1}	1.2 ± 0.0^{c2}	1.2 ± 0.0^{c12}	1.3 ± 0.1^{b2}
	F/OKM	1.2 ± 0.1^{2}	1.0 ± 0.0^{b1}	1.4 ± 0.1^{d2}	2.2 ± 0.0^{d3}
	F/OWE	1.2 ± 0.1^{2}	1.2 ± 0.0^{c2}	0.6 ± 0.0^{a1}	1.7 ± 0.1^{c3}
	F/OWE + KG	1.0 ± 0.1^{2}	1.1 ± 0.0^{b2}	0.6 ± 0.0^{a1}	1.3 ± 0.1^{b3}
Spermine	C	18.9 ± 0.2^{1}	18.3 ± 0.4^{a1}	19.7 ± 1.2^{ab1}	22.1 ± 0.8^{c2}
	F/KG	19.0 ± 0.1^{1}	21.3 ± 0.1^{b2}	20.2 ± 0.0^{b1}	19.5 ± 0.5^{a1}
	F/OKM	19.0 ± 1.1^{12}	18.1 ± 0.1^{a1}	20.4 ± 1.4^{ab23}	21.4 ± 0.2^{bc3}
	F/OWE	19.6 ± 0.5^{12}	20.7 ± 1.0^{b2}	18.5 ± 0.7^{a1}	19.6 ± 0.1^{a12}
	F/OWE + KG	18.5 ± 0.2^{2}	23.8 ± 0.9^{c3}	18.7 ± 1.0^{a1}	20.8 ± 0.3^{b2}

Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$). β-phenylethylamine, cadaverine and tryptamine were not detected during the study.

combination, possibly due to the fish oil in the mix. This could also explain the fact that histamine levels were higher at the end of storage and exceeded the levels of tyramine, which is generally more characteristic of meat products (Ruiz-Capillas & Jimenez-Colmenero, 2004). Other authors (Delgado-Pando et al., 2011, 2012) have also reported that reformulated low-fat cooked products containing an oil-in-water emulsion and an oil combination similar to the one described here contained higher levels of tyramine and histamine than the control. However, in the case of dry fermented products reformulated with an oil combination (containing fish oil) in a konjac matrix, there was no clearly observable relationship between the biogenic amines (mainly histamine) and the reformulation strategy (Triki, Herrero, Rodríguez-Salas, et al., 2013). Histamine levels were lowest in all-pork fat products (C and F/KG), and especially in low fat sample formulated with konjac gel (F/KG) (Table 4). Then again, other authors (Triki, Herrero, Jimenez-Colmenero, & Ruiz-Capillas, 2013) have found higher histamine levels in products where fat was replaced by konjac gel; however, these were fresh merguez sausages, which are very different from the cooked product in terms of microbial growth and selection.

The fact that reformulation of meat products affects this flora and causes augmented production of these amines has been established in the case of cooked (Delgado-Pando et al., 2012; Ruiz-Capillas et al., 2007b), fresh (Triki, Herrero, Jimenez-Colmenero et al., 2013) and fermented products (Triki, Herrero, Rodríguez-Salas, et al., 2013).

In no case did the levels of these biogenic amines exceed 12 mg/kg at day 40 of storage (Table 4). That is well below the legal limits for these amines, which in the case of histamine is set at 50 mg/kg in the USA (FDA, 2011) and at 100 mg/kg in the EU (EFSA, 2011). Such products therefore pose no risk for consumers.

3.4. Lipid oxidation

The TBARs values in the healthier reformulated frankfurter were affected ($P < 0.05$) by formulation and chilling storage (Table 5). The lowest initial levels of TBARs were observed in the all-pork fat products (C and F/KG) irrespective of fat content (18.9 and 10.0% respectively) (Salcedo-Sandoval et al., 2013), but the highest levels were observed in the samples with oils (F/OKM, F/OWE and F/OWE + KG) irrespective of the system used to stabilize the oil in the fat replacer incorporated in the reformulated frankfurter (all containing 10.3% fat).

TBARs values increased with chilling storage in all samples (Table 5); however, these changes were dependent on the reformulation. All-pork fat samples (C and F/KG) presented the lowest ($P < 0.05$) lipid oxidation. Since F/KG sample contained less fat than the control sample, in this experiment fat reduction could not have affected the rate and extent of lipid oxidation. The samples containing the oil combination (F/OKM, F/OWE and F/OWE + KG)

Table 5
Lipid oxidation (TBARs values, expressed as mg MDA/kg sample) of frankfurters during chilling storage.

Sample	Storage (days) at 2 °C			
	1	12	26	40
C	0.014 ± 0.003^{a1}	0.040 ± 0.000^{a2}	0.042 ± 0.003^{a2}	0.055 ± 0.003^{a3}
F/KG	0.024 ± 0.005^{a1}	0.044 ± 0.004^{a2}	0.048 ± 0.003^{a23}	0.057 ± 0.004^{a3}
F/OKM	0.038 ± 0.003^{b1}	0.072 ± 0.007^{b2}	0.103 ± 0.003^{c3}	0.128 ± 0.002^{b4}
F/OWE	0.042 ± 0.003^{b1}	0.074 ± 0.003^{b2}	0.088 ± 0.006^{b3}	0.138 ± 0.003^{c4}
F/OWE + KG	0.036 ± 0.006^{b1}	0.077 ± 0.004^{b2}	0.082 ± 0.006^{b2}	0.127 ± 0.004^{b3}

For sample denomination see Table 1. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$).

presented similar lipid oxidation patterns over storage, with higher ($P < 0.05$) TBARs values than in all-pork fat products (Table 5). The TBARs values of F/OKM, F/OWE and F/OWE + KG frankfurters remained below 0.14 mg MDA/kg, with the highest ($P < 0.05$) level occurring in sample F/OWE (0.138 mg MDA/kg) at 40 days of storage (Table 5). Variations in the initial oxidation levels (an effect of processing, mainly heating) and in the course of storage could be due to differences in polyunsaturated fatty acid (PUFA) content induced by reformulation, and also some processing conditions (e.g. grinding, cooking) which facilitate interaction between fatty acids and oxygen and reaction rates, result in increased susceptibility to lipid oxidation.

As reported previously (Salcedo-Sandoval et al., 2013), in these experimental conditions saturated (SFA) and monounsaturated (MUFA) fatty acids accounted for around 90% of total fatty acid content of C and F/KG (all pork fat), while replacement of pork backfat by the oil combination reduced this proportion to 80%, meaning a 10% increase of PUFA in these modified samples (see Fig. 1).

Although the use of an oil combination to replace animal fat in frankfurter formulation promoted slightly more lipid oxidation than in the control sample (Table 5), the TBARS values of these frankfurters were lower than have been found in other frankfurters formulated with vegetable and/or fish oils to improve the lipid fraction, which ranged generally between 0.1 and 0.9 mg MDA/kg (Bloukas & Paneras, 1993). The similarity of the lipid oxidation patterns of F/OWE and F/OKM indicates that, in the given conditions, the nature of the fat replacer had no effect. Sodium caseinate is reported to protect against lipid oxidation in oil-in-water emulsions through a combination of free radical scavenging and/or metal chelation (Faraji, McClements, & Decker, 2004). However, in the present experiment the TBARs values showed no sign of such activity (Table 5); indeed, sample F/OWE registered the highest ($P < 0.05$) TBARs value (0.138 mg MDA/kg sample) at the end of storage. This particular finding may additionally be associated with the relatively low residual levels of nitrite (a compound with antioxidant activity) detected in the latter stages of storage (as noted earlier).

The antioxidant activity of biogenic amines is already known (Løvaas, 1991). The polyamines putrescine, spermidine and spermine all inhibit lipid oxidation by free radical inactivation (they can act as free radical scavengers) and inhibition of iron catalysed reactions (Decker & Xu, 1998). These substances may inhibit chain reactions during lipid oxidation. In this case, however, no clear relationship was detected between the levels of these amines in the different samples and the observed TBARs data ($r = 0.31$, 0.59 and 0.29 for putrescine, spermidine and spermine respectively, $P < 0.01$). This is possibly because the putrescine levels in these lots were very low (<1 mg/kg) (Table 5) and the levels of spermidine and spermine, although within the expected range for these samples, differed only slightly, if significantly.

4. Conclusions

This experiment examined the shelf-life of n-3 PUFA-enriched frankfurters formulated with a konjac-based oil bulking agent and oil-in-water emulsion (as pork backfat replacers) in order to establish the real viability of such products during prolonged storage. The reformulation strategies studied do not impose any kind of limitations on the shelf life of n-3 PUFA-enriched frankfurters during chilled storage including the microbial point of view. Although the use of an oil combination to replace animal fat in frankfurter formulation promoted slightly more lipid oxidation than in the control sample, the rate and extent of the oxidation were small. In no case did the levels of biogenic amines exceed 12 mg/kg, which is well within the legal limits for these amines. Therefore, these products should not pose any risk to consumers.

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4.1.3 Konjac-based oil bulking system for development of improved-lipid pork patties: Technological, microbiological and sensory assessment.

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Konjac-based oil bulking system for development of improved-lipid pork patties: Technological, microbiological and sensory assessment

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ABSTRACT

Improved-lipid pork patties were manufactured following two different reformulation strategies: fat reduction by replacement of pork backfat with konjac gel (KG), and fat reduction/lipid improvement by replacement of pork backfat with an improved oil combination (olive, linseed and fish oils) bulking system based on konjac gel (O-KG). Technological, microbiological and sensory properties were analyzed as affected by the type of formulation and by chilled storage (9 days, 2 °C). Fat was reduced by between 30 and 86%. In the cases where O-KG was incorporated, 12 and 41% of total fat in patties came from the oil combination. There was no observable effect on color parameters in samples with O-K. Higher KG levels produced harder cooked patties. Animal fat replacement in patties promoted an increase in lipid oxidation, which was more pronounced in samples with an oil combination. In general, during chilled storage no major changes were observed in the studied properties as a result of the different treatments.

1. Introduction

Meat and meat products are some of the most important sources of dietary fat; however, their lipid composition diverges (quantitatively and qualitatively) from nutritional goals. This has prompted an interest in manipulating their composition by modifying the fat content and/or fatty acid profile to make healthier products. This approach is of particular interest in the case of products like burgers or patties, since they are widely accepted in certain population groups, and changes of composition can readily be induced to improve their nutritional value and their health-beneficial properties (Rodríguez-Carpena, Morcuende & Estévez, 2012).

As a strategy to achieve a healthier lipid composition in meat products, reformulation offers a possible means to improve the nutritional value of the fat by altering its composition in the product. There are two aspects to be considered in this connection: reduction of fat content and improvement of the fatty acid profile. Fat reduction in meat products is usually achieved by adding water and other ingredients to lower the fat density (dilution). These ingredients are selected for their low calorie content and their ability to impart desired characteristics to the product (Keeton, 1994). One such ingredient is konjac (glucomannan)-based fat analogs. These form gels which, when combined with other ingredients (starch, carrageenates, gellan gum), can be used as "fat analogs" in the formulation of reduced/low-fat meat products (Osburn & Keeton, 2004). As regards the improvement of fatty acid profiles, addition of individual lipids (of plant or marine origin) improves the fatty acid profile of meat products; however, a better result from a health standpoint can be achieved using healthier oil combinations as animal fat replacers.

In previous papers, our research group assessed the suitability of a healthier oil combination formed by olive, linseed and fish oils in suitable proportions to provide a fatty acid profile better adjusted to healthier intake goals. This combination was designed to produce a lipid material with a small proportion of saturated fatty acids (SFAs), large proportions of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) and balanced n–6/n–3 PUFA and PUFA/SFA ratios (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas & Jimenez-Colmenero, 2010a). The improvement of several CVD risk markers like total and LDL cholesterol has been reported in volunteers at risk of CVD through the consumption of meat products formulated with this oil combination (Delgado-Pando, Celada, Sánchez-Muniz, Jiménez-Colmenero & Olmedilla-Alonso, 2014). However, some recent studies conclude that there is no evidence that high levels of saturated fat in the diet are epidemiologically associated with either heart disease or cardiovascular problems (Malhotra, 2013; Siri-Tarino, Sun, Hu & Krauss, 2010). In contrast, according to the European Food Safety Authority (EFSA), the evidence provided by consensus opinions/reports from authoritative bodies and reviews shows that there is good consensus that a mixture of SFAs increases blood total and LDL-cholesterol concentrations (EFSA Panel on Dietetic Products, 2010).

Incorporation of oils in a gel-like matrix to form an oil bulking system (in which this new ingredient acts as an animal fat replacer) could offer new possibilities for improving the fat content of meat products. In this regard, oils in a konjac matrix have been used to improve fat content in dry fermented sausage (Jiménez-Colmenero, Triki, Herrero, Rodríguez-Salas & Ruiz-Capillas, 2013), fresh merguez sausages (Triki,

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Herrero, Jimenez-Colmenero & Ruiz-Capillas, 2013a) and frankfurters (Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas & Jiménez-Colmenero, 2013). As far as the authors are aware, the use of an oil combination stabilized in a konjac matrix as a functional ingredient and animal fat replacer in the development of low-fat and PUFA enriched patties has not been explored.

Therefore, the purpose of this study was to evaluate technological, microbiological and sensory characteristics of improved-lipid pork patties, which were reformulated following two different approaches: 1) reduction of fat content by replacement of animal fat with konjac gel; and 2) reduction of fat/improvement of fatty acid profile through the replacement of animal fat by a healthier lipid combination made with olive, linseed and fish oils stabilized in a konjac gel matrix (oil bulking system). The influence of chilled storage (9 days at 2 ± 2 °C) on pork patty characteristics as affected by formulation was also considered.

2. Materials and methods

2.1. Meat raw material, ingredients and additives

Sufficient fresh post-rigor pork meat from different animals (mixture of *bíceps femoris*, *semimembranosus*, *semitendinosus*, *gracilis* and *adductor* muscles) and pork backfat were obtained from a local supermarket. Pork meat was trimmed of visible fat and connective tissue and cut in squares of approximately 5 × 5 cm. Backfat was passed through a grinder with a 6 mm plate (Mainca, Granollers, Spain). Both were vacuum packed in lots of approximately 500 g. All the materials were frozen and stored at −20 °C until use (less than 1 week).

Konjac materials used to replace pork backfat were made with konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain), pre-gelled cornstarch (Amigel, Julio Criado, S.L. Madrid. Spain), i-carrageenan (Hispanagar S.A, Burgos, Spain) and $Ca(OH)_2$ (Panreac Química S.A., Barcelona, Spain). Olive oil (Carbonell Virgen Extra, Cuétara SA, Madrid, Spain), linseed oil (Natursoy S.L., Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold, Cognis GmbH, Illertissen, Germany) containing 160 mg eicosapentaenoic acid (EPA)/g and 115 mg docosahexaenoic acid (DHA)/g according to the producer, were used to prepare the healthier oil combination for incorporation in the konjac matrix. This oil combination was prepared with 44.39% olive oil, 37.87% linseed oil and 17.74% fish oil (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas & Jimenez-Colmenero, 2010a).

2.2. Preparation of konjac materials

Two types of konjac materials were manufactured: a konjac gel (KG) and another material with 20% of a healthier oil mixture stabilized in a konjac gel matrix (oil bulking system based on konjac gel, O-KG), both prepared as described by Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas and Jiménez-Colmenero (2013). Briefly, KG was prepared with konjac flour (5.0%) homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with 64.8% of the water and i-carrageenan (1.0%). The mixtures were then homogenized with pre-gelled corn starch powder (3.0%) previously dispersed in 16.2% of water. The mixture was cooled to 10 °C and 10% of a $Ca(OH)_2$ solution (1%) was added with gentle stirring. Samples were then placed in suitable containers, covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until use (within 24 h of preparation). O-KG was prepared in the same way as KG, except that 20% of water was replaced by the same proportion of the healthier oil combination, which was added just after the i-carrageenan.

2.3. Design and production of pork patties

Modified pork patties were formulated in such a way that pork backfat was replaced with KG (to reduce fat content) or O-KG (to reduce fat content and improve the lipid profile). Six different formulations were prepared (Table 1). A pork patty made with normal fat content (100% pork backfat) as control (CFP); three reduced-fat samples: medium, low and very low fat pork patties (MFP, LFP and VLFP) in which pork backfat was replaced with the same proportion of KG (38%, 78% and 100% respectively); and two reduced-fat/improved lipid profile samples: improved medium and improved low fat pork patties (IMFP and ILFP) in which pork backfat was replaced with O-KG (49% and 100% respectively). Formulations with the same fat levels (medium and low fat samples) were designed to possess similar fat content but different lipid material. Thus, in MFP and LFP samples all the fat came from animal sources, whereas in IMFP and ILFP samples, the fat source was also provided in different proportions by the healthier oil combination. All samples contained a similar amount of lean meat (and therefore basically of muscle protein).

Before preparing formulations (7 kg each one), pork meat and pork backfat packages were thawed for 18 h at 2 ± 2 °C. Pork meat, backfat and KG and O-KG were passed through a grinder with a 4.5 mm plate (Vam. Dall. Srl. Modelo FTSIII, Treviglio, Italy). Meat and half of the ingredients (pork backfat, KG or O-KG, water and NaCl) were placed in a mixer (Hobart mixer N-506, Hobart MFG. Co., Troy, USA) for 1 min. After this, the other half of the ingredients was added and the whole mixed again for 1 min. Then, round patties (103 g, 12 mm thick, 115 mm diameter) were prepared using an automatic burger former (Formatic standard model, Deighton Engineering, Bradford, UK). Patties were weighed (initial weight) and placed in aerobic conditions in plastic bags (Cryovac® BB3050) and stored at 2 ± 2 °C for 9 days. The various analyses were performed at days 1, 3, 6 and 9 of chilled storage. Eight patties were randomly taken for the analyses on the abovementioned days: four for raw sample evaluation and four for cooked sample evaluation. The entire patty processing procedure was replicated twice on two different days.

2.4. Proximate analysis

Moisture and ash contents were determined on raw patties following the AOAC (2005). Protein content was measured with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St. Joseph, MI, USA) and fat content was evaluated according to Bligh and Dyer (1959). All analyses were done in triplicate. The energy content was calculated based on 9.1 kcal/g for fat and 4.1 kcal/g for protein and carbohydrates.

2.5. Purge loss, cooking loss and pH determination

The purge loss (PL) of pork patties was evaluated during chilled storage as follows: eight patties from each formulation were tempered for 10 min (at room temperature) and then removed from the bag, and their surfaces were wiped with a paper towel to eliminate surface

Table 1
Formulation (%) of pork patties.

Samples	Meat	Backfat	KG	O-KG
CFP	80.06	13.44	0.00	0.00
MFP	80.06	8.26	5.18	0.00
LFP	80.06	2.97	10.47	0.00
VLFP	80.06	0.00	13.44	0.00
IMFP	80.06	6.82	0.00	6.62
ILFP	80.06	0.00	0.00	13.44

Sample denomination: CFP, control pork patty prepared with normal fat content (all pork fat); MFP and LFP, medium and low fat pork patties prepared by partial replacement of pork backfat (38% and 78% respectively) with KG; VLFP, very low fat pork patty prepared by total replacement of pork backfat (100%) with KG; IMFP and ILFP, improved medium and improved low fat pork patties prepared by partial or total replacement of pork backfat (49% and 100% respectively) with O-KG. All samples also contained 5% added water and 1.5% NaCl.
KG: Konjac gel; O-KG: oil bulking agent based on konjac gel, as konjac material containing 20% of healthier oil combination (olive, linseed and fish oils).

exudate before weighing. The purge loss was calculated by the weight difference and expressed as a percentage of the initial weight.

Cooking loss was measured in quadruplicate. Patties were wrapped in aluminum foil and cooked for 7.5 min in a forced air oven (Rational CM6, Groβküchentechnik GmbH, Landsberg a. Lech) at 170 °C to bring the temperature at the center of the product up to 70 °C (López-López, Cofrades, Yakan, Solas & Jiménez-Colmenero, 2010). These cooking conditions were monitored by inserting thermocouples connected to a temperature recorder (DaqPRO 5300 Data recorder; OMEGA Engineering, Inc., Stamford CT, USA). The cooked patties were weighed again to measure the cooking loss by difference.

The pH was determined on raw patties using a pH meter (827 pH Lab Methrom, Herisau, Switzerland) at room temperature on 10 g homogenate samples in 100 ml of distilled water. Six determinations were performed per sample.

2.6. Texture

Kramer shear force (KSF) was determined in quadruplicate on raw and cooked samples. Measurements were made in eight portions (5 × 5 cm) per formulation at room temperature using a TA-XT.plus Texture Analyzer (Texture Technologies Corp. Scarsdale, NY) with a Kramer shear cell attached to a 2.5 kN load cell (crosshead speed 120 mm/min). KSF was expressed as maximum load per gram of sample (N/g).

2.7. Color measurement

Color, CIE-LAB tristimulus values, lightness (L^*), redness (a^*) and yellowness (b^*) were measured on the surface of raw and cooked patties with a CR-400 Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan). Before use, the colorimeter was standardized using the white calibration plate (C: $Y = 93.6$, $x = 0.3130$, $y = 0.3193$). Eight readings were taken per sample.

2.8. Lipid oxidation

Oxidative stability, evaluated from changes in thiobarbituric acid-reactive substances (TBARs) as described by Serrano, Cofrades and Jimenez-Colmenero (2006), was determined in raw pork patties. Briefly, 5 g of each sample was homogenized in 35 ml of 7.5% trichloroacetic acid (Panreac Química S.A., Barcelona, Spain) for 1 min at high speed in an Omnimixer blender (ES Homogenizer, OMNI International Inc., Gainsville, VA, USA). After centrifugation (3000 *g*, 2 min) (Multifuge 3L-R, Kendro Laboratory Products GmbH, Hanau, Germany) and filtration (Whatman No. 1), 5 ml of the supernatant was mixed with 5 ml of 20 mM thiobarbituric acid (Merck KGaA, Darmstadt, Germany), and finally the solution was mixed and kept in the dark for 20 h at 20 ± 1.5 °C. The pink color that formed was measured sing a UV–vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 532 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to obtain the malonaldehyde (MDA) concentration and results were expressed as mg MDA/kg of sample. TBARs determinations for each sample were performed in triplicate.

2.9. Microbiological analyses

Analyses were carried out on raw patties. Samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain). For each sample, 10 g (per replicate) was taken and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of peptone water (0.1%) and 0.85% NaCl (Panreac Química, S.A. Barcelona, Spain). After 1 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on the following media: Plate Count Agar (PCA) (Merck, Germany) for total viable count (TVC) (30 °C for 72 h); De Man–Rogosa–Sharpe Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). The results were expressed as logarithms of colony forming units per gram (log cfu/g).

2.10. Sensory evaluation

Patties were assessed by a 10-member panel. The panel was selected in preliminary sessions from staff who had received training (two sessions) with the products and terminology. Patties were cooked for 3 min on a grill until the center of the product reached 70 °C. Temperature monitoring was done by inserting thermocouples as described previously in cooking loss. A quarter portion of each patty was given to the panelists with a three-digit randomized code to identify the samples. The sensory attributes were measured on unstructured scales (10 cm) with descriptors at either end. These were: flavor, texture, and overall acceptability (from extremely unacceptable to extremely acceptable). Each point marked was converted to a numerical value from 0 to 10 according to location. The sensory analyses were performed at day 2 of the storage.

2.11. Statistical analysis

The entire trial was replicated. One-way analysis of variance (ANOVA) to evaluate the statistical significance ($P < 0.05$) of the formulation and two-way ANOVA as a function of formulation and storage time were carried out using the SPSS Statistics general linear model (GLM) procedure (v.20, IBM SPSS Inc., Chicago, IL). Formulation and storage time were assigned as fixed effects and replication as a random effect. Least squares differences were used for comparison of mean values between treatments, and Tukey's HSD test to identify significant differences ($P < 0.05$) between formulations and storage time.

3. Results and discussion

3.1. Proximate analysis

Reformulation significantly ($P < 0.05$) affected the proximate composition of the different raw pork patties (Table 2). The moisture content of modified patties increased significantly with decreasing fat levels since pork backfat was replaced by konjac material which held entrapped water (Table 1). In general, reformulation did not affect protein content (values were around 17%) since all formulations were prepared with the same meat content (Table 1). Even when significant, the differences observed in ash content were not important ($P < 0.05$). The highest value was recorded in VLFP sample. By target composition, the highest ($P < 0.05$) fat content was detected in control sample (14.07%). Patties with medium (MFP and IMFP; ≈10%) and low (LFP and ILFP; ≈4%) fat levels were statistically similar ($P > 0.05$), while VLFP presented the lowest ($P < 0.05$) value (1.98%). A considerable level of fat reduction was achieved in reformulated samples with respect to the control, approximately 30% in MFP and IMFP samples, 70% in LFP and ILFP samples, and more than 86% in VLFP. Consequently, under the current Regulation (EC) No. 1924/2006 of the European Parliament and of the Council on nutrition claims, a “reduced fat content” claim could be made for MFP, IMFP, LFP and ILFP patties because the fat content in these samples was reduced by at least 30 % compared to a similar product. Similarly, under the same regulation, VLFP sample could qualify for a “low-fat” claim since it contained no more than 3 g of fat per 100 g of solids (Table 2).

A number of studies on fat reduction in ground meat products have shown similar trends when various non-meat ingredients were used as fat substitutes, such as soy protein isolate and mixed konjac/gellan gum (Akesowan, 2010) or a sodium alginate–tapioca starch combination (Berry, 1997), among others. However the authors have found no report about the use of a bulking system based on konjac gel to replace pork backfat in patties.

Table 2
Proximate analysis (%) and energy values (kcal/100 g) of raw pork patties.

	CFP	MFP	LFP	VLFP	IMFP	ILFP
Moisture	66.23 ± 0.17^{a}	70.28 ± 0.34^{b}	75.83 ± 0.23^{c}	77.61 ± 0.16^{d}	70.67 ± 0.25^{b}	75.54 ± 0.18^{c}
Protein	17.31 ± 0.20^{ab}	17.20 ± 0.24^{ab}	17.68 ± 0.49^{ab}	17.76 ± 0.14^{b}	16.97 ± 0.25^{a}	17.19 ± 0.06^{ab}
Fat	14.07 ± 1.61^{d}	9.98 ± 1.42^{c}	3.99 ± 0.48^{b}	1.98 ± 0.88^{a}	8.77 ± 2.03^{c}	3.91 ± 1.51^{b}
Ash	2.34 ± 0.01^{b}	2.33 ± 0.01^{b}	2.18 ± 0.02^{a}	2.39 ± 0.02^{c}	2.37 ± 0.01^{bc}	2.30 ± 0.00^{b}
Fat reduction (%)	–	29.1	71.6	85.9	37.7	72.2
Energy value	199.0	161.3	108.8	90.8	149.4	106.1
From total fat	128.0 (64.3)	90.8 (56.3)	36.3 (33.4)	18.0 (19.8)	79.8	35.6
From pork fat	128.0 (64.3)	90.8 (56.3)	36.3 (33.4)	18.0 (19.8)	67.8 (85.0)	11.2 (31.5)
From oils	–	–	–	–	12.0 (15.0)	24.4 (68.5)
Energy value reduction (%)	–	18.9	45.3	54.4	24.9	46.7

For sample denomination see Table 1. In brackets, percentage of energy content of the fat source with respect to the total fat. Means ± SD. Different letters in the same row indicate significant differences ($P < 0.05$).

As regards the type of lipid present in the pork patties, while in CFP, MFP, LFP and VLFP patties the lipid fraction was made up entirely of animal fat, in IMFP and ILFP samples around 12% and 40% respectively of total fat was supplied by the healthier oil combination. A more in-depth discussion of the nutritional aspects of patties manufactured with the present healthier oil combination will be reported in another paper. However, improvement of the lipid profile using this healthier lipid combination has been achieved in other meat products (Delgado-Pando, Cofrades, Ruiz-Capillas & Jimenez-Colmenero, 2010b; Jiménez-Colmenero, Triki, Herrero, Rodríguez-Salas & Ruiz-Capillas, 2013).

Several authors have reported the use of different oils with different modes of incorporation to improve the lipid profile of patties. Replacement of 50% of pork backfat has been achieved by direct incorporation of olive oil (Hur, Jin & Kim, 2008) and olive, avocado and sunflower oils (Rodríguez-Carpena, Morcuende & Estévez, 2011) in pork patties. Lowder and Osburn (2010) formulated healthier beef patties with 10% or 20% fat content by addition of blended lipid sources (beef tallow combined with 43% safflower, olive or corn oil). López-López, Cofrades, Yakan, Solas and Jiménez-Colmenero (2010) replaced approximately 25% and 60% of pork backfat by incorporation of 5 and 10% of an olive oil-in-water emulsion (52.6% oil content) in beef patties (10% fat content). Patties have been made (9% fat content) with a pre-emulsified mix of olive, corn and fish oils (Martínez et al., 2012).

In the present study, an additional effort was made to optimize fat content of patties in terms of amount and type. Thus, by replacing backfat by a healthier lipid combination stabilized in a bulking system (O-KG), it was possible to reduce fat content by more than 70% and simultaneously improve the lipid profile with respect to control samples.

As regards energy content (Table 2), irrespective of the konjac material used (KG or O-KG), fat substitution produced a reduction of energy values compared to control patty, ranging from 19% in MFP to 55% in VLFP samples. In the case of patties with O-KG in particular, besides the calorie content being reduced, the healthier oil combination accounted for 15% in IMFP and 69% in ILFP of the energy content from total fat.

3.2. Purge loss (PL), cooking loss (CL) and pH determination

Purge accumulation during chilled storage is one of the main problems with fresh marketed products as it not only gives the product an unpleasant appearance but can also limit its shelf life. PL of patties was affected ($P < 0.05$) by formulation and storage time (Table 3), with significant interaction between them ($P < 0.05$). PL during storage ranged between 0.85 and 1.83% and was generally higher in reformulated than in control samples. However, there were no variations in PL clearly attributable to the different reformulation strategies pursued in this study (fat reduction with KG or fat reduction and lipid improvement with O-KG). There was no clear trend in the behavior of PL levels over storage; in some cases the differences were significant ($P > 0.05$), but in none were these important. Lopez-Caballero, Carballo and Jiménez-Colmenero (1999) indicated that the appearance of commercially available meat products with PL levels around 3.5–4.5% tended to deteriorate; however, the levels found in this study (0.85–1.83%) seem to be acceptable.

The loss of specific components during cooking of the products could limit their potential health benefits (Jiménez-Colmenero, 2007). CL (Table 3) was influenced by formulation ($P < 0.05$) but not by chilled storage ($P > 0.05$), and therefore the data for each formulation over the storage period were pooled. Cooking losses varied between 12.59 (ILFP) and 18.89% (LFP). They were higher ($P < 0.05$) in reduced-fat samples in which pork backfat was partially replaced by KG (MFP and LFP), whereas replacement of the pork backfat with O-KG produced the lowest CL values (12–14%). Values ranging from 27 to 28% have been reported in reformulated pork patties (5% pork backfat and 5% olive oil) using different mixtures of additives (isolated soy protein, carrageenan and maltodextrin) (Hur, Jin & Kim, 2008). In general, the CL values in the present experiment are lower than reported by other authors in similar meat products. Given that all the patties contained similar amounts of protein, cooking losses might be expected to depend on the levels of water and fat in the product and on how effectively the fat replacer used in the reformulated patties retained fluids. The results here suggest that cooking losses were affected by the type of fat and

Table 3
Purge loss (%), cooking loss (%) and pH during chilled storage of pork patties.

Sample	Purge loss Storage (days at 2 °C)				Cooking loss	pH
	1	3	6	9		
CFP	0.88 ± 0.05^{a1}	1.08 ± 0.15^{a12}	1.20 ± 0.08^{a2}	0.85 ± 0.24^{a1}	14.42 ± 1.20^{b}	5.69 ± 0.00^{b}
MFP	1.03 ± 0.05^{ab12}	1.00 ± 0.12^{a1}	1.23 ± 0.13^{a12}	1.28 ± 0.17^{ab2}	18.13 ± 1.61^{c}	5.64 ± 0.01^{a}
LFP	1.25 ± 0.24^{b1}	1.30 ± 0.00^{a1}	1.63 ± 0.26^{bc1}	1.45 ± 0.13^{b1}	18.89 ± 1.20^{c}	5.63 ± 0.03^{a}
VLFP	1.08 ± 0.05^{ab1}	1.33 ± 0.19^{a12}	1.40 ± 0.12^{ab2}	1.25 ± 0.21^{ab12}	14.42 ± 1.90^{b}	5.64 ± 0.01^{a}
IMFP	1.23 ± 0.10^{b1}	1.28 ± 0.05^{a1}	1.83 ± 0.15^{c2}	1.65 ± 0.25^{b2}	14.04 ± 1.28^{ab}	5.79 ± 0.01^{c}
ILFP	1.30 ± 0.16^{b12}	1.15 ± 0.24^{a1}	1.63 ± 0.21^{bc2}	1.00 ± 0.22^{a1}	12.59 ± 1.24^{a}	5.81 ± 0.01^{d}

For sample denomination see Table 1. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$). Since cooking loss and pH were not influenced ($P > 0.05$) by chilled storage, data reported are the mean values over the storage period.

the mode of stabilizing it rather than by fat levels. Thus, although similar in composition (Table 2), the medium-fat (MFP, IMFP) and the low-fat (LFP, ILFP) samples did not present similar loss values. This is possibly because stabilization of the lipid material in the bulking system improved water- and fat-binding properties. Water and fat binding properties of different oil bulking agents made with alginate/inulin or dextrin were optimal, with no noticeable release of exudates after heating (Herrero, Carmona, Jiménez-Colmenero & Ruiz-Capillas, 2014). The fact that CL was lower in VLFP sample (highest content of KG) than in the other patties reformulated with KG could be explained on the basis of findings reported by Jiménez-Colmenero et al. (2012). These authors indicated that heating losses in ground pork backfat were much greater (77%) than in ground konjac gel (1%), confirming that the latter had excellent thermal water binding properties despite containing very high proportions of water (around 90%).

Since pH values in raw patties were not influenced ($P > 0.05$) by chilled storage, Table 3 shows only the mean values over the storage period. These ranged between 5.63 and 5.81, which is comparable to other reports for this type of products (Choi et al., 2012). The highest pH was observed in samples with the added oil mixture bulking system (O-KG), possibly through the effect of the lipid material. These results agree with Delgado-Pando, Cofrades, Ruiz-Capillas and Jimenez-Colmenero (2010b), who found that replacement of pork backfat by an oil in water emulsion made with a vegetable and marine lipid combination increased the pH of low-fat frankfurters.

3.3. Texture

Texture of raw and cooked pork patties measured as Kramer shear force (KSF) is shown in Table 4. In raw patties, differences in formulation and chilled storage time produced significant changes ($P < 0.05$) in texture values. At the beginning of storage (day 1) of raw patties, the reformulation process barely affected the texture, and slightly higher values of KSF ($P < 0.05$) were only observed when 38% of pork backfat was replaced with KG (MFP). KSF values decreased as KG increased in the reformulated patties; this is in line with the results reported by Osburn and Keeton (1994). Compared to control patties (CFP), KSF values were not affected ($P > 0.05$) by partial or total replacement of pork backfat with the oil bulking system based on konjac gel (IMFP and ILFP). During chilled storage KSF values increased ($P < 0.05$) only in CFP and VLFP, and then only slightly.

In cooked samples, no differences ($P > 0.05$) were detected in texture as a function of storage time, and therefore the values presented are the means of each formulation over the storage period (Table 4). Addition of KG as pork fat replacer produced harder cooked patties the higher the percentage of fat replaced (78% in LFP and 100% in VLFP). This is consistent with reports on cooked meat products such as frankfurters, where reduction of the fat content by replacement of pork backfat with konjac gel increased texture parameters (Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas & Jiménez-Colmenero, 2013). Product texture was unaffected by the level of KG in MPF (lowest of all), or by the incorporation of O-KG irrespective of the level (IMFP and ILFP). The reason why sample ILFP, which contained even more konjac material than LFP, was softer (Tables 1 and 4) could be that the above-mentioned hardening effect of konjac gel is overcome when the healthier oil combination is incorporated in a konjac matrix as reported by Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas and Jiménez-Colmenero (2013).

3.4. Color

Color parameters in raw and cooked patties are shown in Table 5. Formulation and chilled storage time significantly affected color parameters in raw patties ($P < 0.05$). In general, fat reduction with KG affected lightness (L^*), which was significantly lower in patties with higher levels of pork backfat replacement (LFP and VLFP) than in CFP. Samples incorporating healthier lipid material (IMFP and ILFP) had similar L^* values ($P > 0.05$) to control patties. Redness (a^*) values ($P < 0.05$) were highest in the control sample, and generally speaking reformulation reduced a^* levels, particularly in those products where pork backfat was totally replaced with either KG or O-KG (VLFP and ILFP). Yellowness (b^*) decreased significantly ($P < 0.05$) when pork backfat was replaced with KG irrespective of fat level reduction. As in the case of L^*, b^* values did not vary ($P > 0.05$) in response to fat reduction and lipid improvement by addition of O-KG as compared with the control formulation (CFP = IMFP = ILFP). This behavior could be related to the fact, reported by Jiménez-Colmenero et al. (2012), that konjac gels (intact or ground) are darker and less red than pork fats. Regarding the use of vegetable oils, the incorporation of olive, corn and deodorized fish oil in a beef hamburger patty contributed to a significantly higher L^* value and lower a^* and b^* values compared with a conventional product (Martínez et al., 2012). Discrepancies between studies may be due, among other things, to differences in the mode of stabilizing the lipids.

Chilled storage (over 9 days) of raw patties generally had little effect on L^*, although some significant variations were detected (Table 5). However, the reformulation-dependent differences noted at the outset had disappeared by the end of storage ($P > 0.05$). Redness decreased as storage time progressed in raw patties where pork backfat was replaced with KG (MFP, LFP and VLFP), but this effect was not observed in samples where fat was replaced with O-KG. These results suggest that KG reduced redness during storage of raw patties but the effect was offset by addition of the oil to the konjac matrix. No variations in b^* values ($P > 0.05$) were observed during chilled storage of raw patties.

Color in cooked patties was affected ($P < 0.05$) only by formulation, and therefore the reported data are averages for each formulation over the storage time (Table 5). When pork backfat was replaced by high levels of KG or O-KG (LFP, VLFP and ILFP), lightness values were higher ($P < 0.05$) than in the control patty. Replacement of pork backfat by KG reduced a^* values. The decrease was greater the higher the level of substitution, while a^* values in IMFP and ILFP were statistically similar ($P > 0.05$) to control sample. No changes were observed in yellowness when pork backfat was replaced by KG ($P > 0.05$), but b^* values increased in samples containing O-KG. Several studies support these results. Choi, Choe, Cho, and Kim (2012) reported that total replacement of pork backfat by surimi-like material in cooked pork patties

Table 4
Kramer shear force (KSF) of raw and cooked pork patties.

Sample	Raw pork patties Storage (days at 2 °C)				Cooked pork patties
	1	3	6	9	
CFP	0.73 ± 0.06^{ab1}	0.72 ± 0.07^{ab1}	0.71 ± 0.06^{bc1}	0.86 ± 0.02^{b2}	5.84 ± 0.61^{a}
MFP	0.88 ± 0.03^{c2}	0.78 ± 0.04^{b1}	0.77 ± 0.06^{c1}	0.87 ± 0.07^{b12}	5.56 ± 0.44^{a}
LFP	0.80 ± 0.07^{bc12}	0.83 ± 0.07^{b12}	0.74 ± 0.07^{c1}	0.90 ± 0.05^{b2}	6.56 ± 0.65^{b}
VLFP	0.64 ± 0.04^{a1}	0.63 ± 0.05^{a1}	0.61 ± 0.03^{ab}	0.83 ± 0.09^{ab2}	6.48 ± 0.75^{b}
IMFP	0.67 ± 0.04^{a1}	0.72 ± 0.05^{ab1}	0.72 ± 0.03^{c1}	0.76 ± 0.05^{a1}	5.51 ± 0.47^{a}
ILFP	0.73 ± 0.06^{ab2}	0.61 ± 0.05^{a12}	0.55 ± 0.04^{a1}	0.72 ± 0.08^{a2}	5.34 ± 0.43^{a}

For sample denomination see Table 1. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$). Since KSF in cooked patties was not influenced ($P > 0.05$) by chilled storage, data reported are the mean values over the storage period.

Table 5
Color parameters during chilled storage of raw and cooked pork patties.

Color parameter	Sample	Raw pork patties Storage (days at 2 °C)				Cooked pork patties
		1	3	6	9	
Lightness (L^*)	CFP	51.90 ± 1.59^{c23}	50.48 ± 1.28^{b12}	49.31 ± 0.73^{a1}	52.97 ± 1.34^{c3}	60.44 ± 2.10^{a}
	MFP	50.26 ± 1.54^{bc1}	49.62 ± 0.57^{ab1}	50.24 ± 0.83^{ab1}	48.40 ± 1.40^{b1}	61.25 ± 2.08^{a}
	LFP	48.11 ± 0.92^{ab12}	49.65 ± 1.27^{ab2}	48.85 ± 1.32^{a12}	47.42 ± 0.42^{ab1}	63.77 ± 1.11^{b}
	VLFP	46.39 ± 1.51^{a12}	47.48 ± 1.01^{a2}	48.08 ± 0.98^{a2}	45.50 ± 0.48^{a1}	64.79 ± 1.86^{b}
	IMFP	53.48 ± 0.63^{c2}	53.44 ± 2.09^{c2}	51.72 ± 1.20^{bc2}	48.96 ± 1.78^{b1}	61.39 ± 1.15^{a}
	ILFP	53.97 ± 1.29^{c2}	52.80 ± 1.07^{c2}	52.57 ± 1.49^{c12}	50.83 ± 0.91^{b1}	63.33 ± 1.66^{b}
Redness (a^*)	CFP	13.00 ± 0.50^{c1}	12.92 ± 0.38^{c1}	12.30 ± 0.71^{d1}	12.37 ± 0.71^{c1}	6.11 ± 0.77^{c}
	MFP	11.25 ± 0.85^{b3}	11.19 ± 0.50^{b23}	6.14 ± 0.56^{ab1}	10.04 ± 0.95^{b2}	5.55 ± 0.44^{b}
	LFP	11.39 ± 0.65^{b3}	8.60 ± 1.05^{a2}	6.15 ± 0.26^{ab1}	7.03 ± 0.56^{a1}	5.30 ± 0.52^{ab}
	VLFP	9.98 ± 0.59^{a3}	8.77 ± 1.02^{a3}	5.19 ± 0.90^{a1}	7.20 ± 0.98^{a2}	5.01 ± 0.65^{a}
	IMFP	12.64 ± 0.83^{c3}	8.94 ± 0.82^{a2}	7.15 ± 0.93^{b1}	11.73 ± 0.52^{c3}	6.12 ± 0.50^{c}
	ILFP	11.18 ± 0.45^{ab23}	8.92 ± 0.35^{a1}	10.23 ± 1.02^{c2}	12.01 ± 0.42^{c3}	6.19 ± 0.73^{c}
Yellowness (b^*)	CFP	6.76 ± 0.69^{b2}	4.50 ± 0.60^{c1}	5.08 ± 0.57^{cd1}	6.80 ± 0.92^{d2}	7.43 ± 0.66^{ab}
	MFP	3.70 ± 0.82^{a2}	2.04 ± 0.40^{ab1}	3.80 ± 0.52^{b2}	4.63 ± 0.57^{bc2}	7.09 ± 0.68^{a}
	LFP	3.97 ± 0.79^{a2}	2.89 ± 0.66^{b1}	3.46 ± 0.58^{b12}	3.79 ± 0.39^{b12}	7.72 ± 0.55^{b}
	VLFP	2.70 ± 0.87^{a2}	1.60 ± 0.38^{a1}	2.24 ± 0.56^{a12}	2.46 ± 0.37^{a12}	7.48 ± 0.81^{ab}
	IMFP	6.28 ± 0.79^{b3}	4.76 ± 0.50^{c12}	4.22 ± 0.75^{c1}	5.41 ± 0.80^{c23}	8.35 ± 0.89^{c}
	ILFP	6.88 ± 0.70^{b2}	6.50 ± 0.59^{d12}	5.87 ± 0.41^{d1}	6.94 ± 0.74^{d2}	8.89 ± 0.55^{c}

For sample denomination see Table 1. Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$). Since color parameters in cooked patties were not influenced ($P > 0.05$) by chilled storage, data reported are the mean values over the storage period.

produced higher L^* values than in full fat control, whereas the measured redness and yellowness were similar. Jung and Joo (2013) reported that the replacement of pork back fat with vegetable oils in cooked pork patties led to significantly higher L^* and b^* values.

In general, the results suggest that the use of an oil bulking system based on konjac in improved-lipid patties caused no major variations in color as a function of reformulation and chilling storage.

3.5. Lipid oxidation (TBARs)

TBARs values of the different raw patties were affected ($P < 0.05$) by formulation and storage time, with significant interaction ($P < 0.05$) between the two factors (Table 6). Fat reduction by replacement of pork backfat with different levels of KG produced a significant decrease in lipid oxidation, which seems to be influenced by the amount of KG in the formulations; thus, TBARs values were lower in samples with more KG (VLFP < LFP < MFP). These differences could be due to the different fat content in the patties, considering that higher fat levels speed up lipid oxidation. Although there are no reports on patties, the effect of fat replacement by konjac gel on lipid oxidation has been reported in other meat products. Triki, Herrero, Jimenez-Colmenero and Ruiz-Capillas (2013a) found a decrease in TBARs values when pork backfat was replaced by different amounts of KG. The partial or total replacement of pork backfat with the oil bulking system produced the highest lipid oxidation values ($P < 0.05$), with levels rising as the proportion of oil in the formulation increased (ILFP > IMFP). Since the oil combination used in this experiment was designed to provide a considerable amount of PUFAs (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas & Jimenez-Colmenero, 2010a), these results are consistent with the different levels of unsaturation in meat products. The increasing of unsaturated fatty acid levels (especially PUFAs), and also some processing conditions used in this study (grinding, aerobic storage packing, no additives) would facilitate interaction between fatty acids and oxygen, resulting in increased susceptibility to lipid oxidation (Lee, Faustman, Djordjevic, Faraji & Decker, 2006).

Table 6
Lipid oxidation (TBARS values expressed as mg MDA/kg sample) of raw pork patties during chilled storage.

Sample	Storage (days)			
	1	3	6	9
CFP	0.55 ± 0.01^{c1}	0.81 ± 0.00^{b2}	0.96 ± 0.01^{c3}	1.23 ± 0.01^{c4}
MFP	0.53 ± 0.01^{c1}	0.80 ± 0.02^{b2}	1.07 ± 0.01^{d3}	1.19 ± 0.02^{c4}
LFP	0.42 ± 0.01^{b1}	0.72 ± 0.01^{b2}	0.84 ± 0.03^{b3}	0.87 ± 0.02^{b4}
VLFP	0.17 ± 0.01^{a1}	0.21 ± 0.01^{a1}	0.28 ± 0.02^{a2}	0.35 ± 0.03^{a3}
IMFP	0.80 ± 0.03^{d1}	1.20 ± 0.03^{c2}	1.62 ± 0.01^{e3}	1.88 ± 0.01^{d4}
ILFP	1.41 ± 0.03^{e1}	3.31 ± 0.10^{d4}	2.82 ± 0.07^{f3}	2.09 ± 0.04^{e2}

Means ± SD. Different letters in the same column (a, b, c) and different numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$).

TBARs values increased significantly ($P > 0.05$) during storage in all samples (Table 6) and generally presented the same trend, except in ILFP. There, lipid oxidation increased until day 3 of storage and values decreased significantly ($P < 0.05$) thereafter until the end of chilled storage (9 days). This pattern, a peak followed by a decline of the TBARs value, has been reported during frozen storage of ground beef by Bhattacharya, Hanna and Mandigo, 1988, who suggested that the decline in TBARs values may have been due to the formation of malonaldehyde as an intermediate product; up to a certain time, the rate of malonaldehyde formation was greater than the rate of disappearance of the product. In the present case TBARs values of CFP and ILFP at the end of storage ranged between 0.35 and 2.09 mg MDA/kg sample respectively. Similar results were reported by Martínez et al. (2012) in beef hamburger and by Stelzleni, Ponrajan and Harrison (2013) in ground beef patties.

3.6. Microbiological analysis

Changes in total viable count (TVC), lactic acid bacteria (LAB) and enterobacteria are shown in Table 7. The microbial counts were affected by differences in tested formulations and by storage time ($P < 0.05$).

At the beginning of storage all the microbial counts were below 6 log cfu/g in all samples, which is the acceptable total microbial quality standard for this kind of product (Ruiz-Capillas, Cofrades, Serrano & Jiménez-Colmenero, 2004). LAB ranged between 3.38 and 4.25 log cfu/g and enterobacteria did not exceed 3.24 log cfu/g in the samples studied. These results agree with microbial levels detected in pork patties in other studies (Bradford, Huffman, Egbert & Jones, 1993; Lorenzo, Sineiro, Amado & Franco, 2014) and are even slightly lower than in restructured beef steak (Ruiz-Capillas, Cofrades, Serrano & Jiménez-Colmenero, 2004). There was a considerable increase in all studied microorganisms during storage. At day 3 of chilled storage, TVC levels in particular had increased to above 6 log cfu/g, except in ILFP, and Enterobacteria increased significantly ($P > 0.05$) up to 4 log cfu/g. At the end of storage

Table 7
Microbiological counts (log CFU/g) of raw pork patties during chilled storage.

Microorganisms	Sample	Storage (days at 2 °C)			
		1	3	6	9
Total viable counts	CFP	5.89 ± 0.74^{a1}	6.22 ± 0.15^{ab1}	7.78 ± 0.05^{a2}	8.15 ± 0.04^{ab2}
	MFP	5.68 ± 0.85^{a1}	7.68 ± 0.31^{c2}	7.80 ± 0.02^{a2}	8.64 ± 0.13^{b2}
	LFP	5.41 ± 0.00^{a1}	7.16 ± 0.49^{bc2}	7.84 ± 0.04^{a2}	7.84 ± 0.04^{a2}
	VLFP	5.38 ± 0.00^{a1}	6.09 ± 0.12^{a2}	8.08 ± 0.00^{a3}	8.15 ± 0.22^{ab3}
	IMFP	5.38 ± 0.08^{a1}	6.27 ± 0.10^{ab2}	7.42 ± 0.14^{a3}	8.36 ± 0.08^{ab4}
	ILFP	4.95 ± 0.74^{a1}	5.85 ± 0.00^{a12}	7.37 ± 0.67^{a23}	8.22 ± 0.32^{ab3}
Lactic acid bacteria	CFP	4.25 ± 0.92^{a1}	4.78 ± 0.05^{d12}	7.06 ± 0.31^{c3}	6.45 ± 0.21^{a23}
	MFP	3.70 ± 0.01^{a1}	3.77 ± 0.10^{a1}	5.78 ± 0.04^{a2}	6.33 ± 0.04^{a3}
	LFP	3.49 ± 0.02^{a1}	3.65 ± 0.07^{a1}	6.58 ± 0.11^{bc2}	7.02 ± 0.64^{a2}
	VLFP	3.38 ± 0.14^{a1}	3.80 ± 0.14^{ab1}	6.54 ± 0.10^{bc3}	6.04 ± 0.06^{a2}
	IMFP	3.49 ± 0.16^{a1}	4.22 ± 0.02^{c1}	6.74 ± 0.04^{bc2}	7.05 ± 0.43^{a2}
	ILFP	3.52 ± 0.06^{a1}	4.13 ± 0.07^{bc1}	6.21 ± 0.19^{ab2}	7.45 ± 0.52^{a3}
Enterobacteriaceae	CFP	2.42 ± 0.14^{a1}	4.55 ± 0.45^{a2}	5.18 ± 0.00^{b23}	5.92 ± 0.11^{a3}
	MFP	2.51 ± 0.27^{a1}	4.28 ± 0.24^{a2}	4.16 ± 0.68^{ab12}	5.87 ± 0.38^{a2}
	LFP	2.44 ± 0.23^{a1}	4.54 ± 0.23^{a3}	3.76 ± 0.03^{ab2}	6.18 ± 0.04^{ab4}
	VLFP	3.05 ± 0.39^{a1}	4.73 ± 0.02^{a2}	3.65 ± 0.01^{a1}	6.78 ± 0.02^{bc3}
	IMFP	3.24 ± 0.09^{a1}	4.68 ± 0.31^{a2}	4.27 ± 0.02^{ab1}	7.22 ± 0.06^{c3}
	ILFP	2.88 ± 0.10^{a1}	4.24 ± 0.05^{a2}	3.22 ± 0.56^{a1}	5.51 ± 0.26^{a2}

Means ± SD. Different letters for samples at the same storage (a, b, c) time and different numbers for each sample during the storage time (1, 2, 3) indicate significant differences ($P < 0.05$).

the microorganism levels were very high — above 8 log cfu/g (except in LFP) and 6 log cfu/g in TVC and LAB respectively. Generally speaking, the results do not show a clear connection between microbial growth and the fat improving strategy, either in formulations where pork fat was replaced by KG (reduction of fat content) or where a healthier oil combination stabilized in a konjac matrix was added (fat reduction plus addition of healthier lipids). These results are similar to the report by Moroney, O'Grady, O'Doherty and Kerry, 2013 in pork patties stored at 4 °C. The reason why microorganism levels were highest in this sample could be that products of this kind undergo considerable manipulation and have a large exposed surface area facilitating spoilage, and also there are no antimicrobial additives or compounds. The addition of compounds with antimicrobial activity is one of the most common practices to ensure the microbiological safety and prolong the shelf life of products. Since the goal of this study was to evaluate the influence of fat improvement on some characteristics, the substances with antimicrobial activity (e.g. spices and additives) incorporated in the formulations were not considered so as to minimize factors that could interfere with the effect attributable exclusively to the lipid reformulation. However, the use of some additives, for instance sulphites in this case, could help to prolong storage life in products of this kind, as reported in other experiments with similar kinds of products (Triki, Herrero, Jimenez-Colmenero & Ruiz-Capillas, 2013b).

3.7. Sensory evaluation

Table 8 shows the sensory evaluation of the different pork patties. Different levels of fat reduction by replacement of pork backfat with konjac gel (CFP versus MFP, LFP and VLFP) did not affect ($P > 0.05$) any of the sensory attributes evaluated. VLFP was the best rated of the reformulated patties (Table 8). These results agree with a report by Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas and Jiménez-Colmenero (2013) on reduced-fat frankfurters, where 50% of pork fat was replaced by konjac gel. All products achieved similar ($P > 0.05$) texture acceptability scores irrespective of the formulation. Compared to control samples, the addition of the oil bulking system to the patties (IMFP and ILFP) produced lower scores ($P < 0.05$) for flavor and overall acceptability (Table 8). The patty in which pork backfat was entirely replaced with O-KG scored the lowest for overall acceptability. As reported in this study, Valencia, O'Grady, Ansorena, Astiasaran and Kerry (2008) found that addition of fish oil resulted in lower sensory scores for flavor and overall acceptability in fresh sausages. This is probably related to the presence of fish oil in the lipid material, lending a detectable "fishy" flavor. Briefly then, fat reduction in pork patties using KG (regardless of the amount of pork backfat replaced) was successfully achieved without significant alteration of sensory attributes as compared with a control normal-fat patty. Fat reduction/improvement of patties did not affect texture, but flavor and overall acceptability scores were affected when the proportion of the oil bulking system in the formula increased. However, this can be mitigated by reformulating the seasoning.

4. Conclusions

The reformulation strategies described, based on replacement of pork backfat by konjac gel or a healthier oil combination (olive, linseed and fish oils) stabilized in a konjac matrix (oil bulking system) as fat replacers in pork patties, made it possible to develop healthier products with suitable technological characteristics. Fat and energy values were reduced considerably (up to 86% and 55% respectively) as compared with normal fat product. This means that reformulated pork patties could carry a "reduced fat content" claim under Regulation (EC) No. 1924/2006 of the European Parliament and of the Council (2006). Additionally, under the same regulation, VLFP sample could qualify for a "low fat" claim. The reformulated products also had satisfactory technological characteristics (binding properties, texture and color), with no appreciable change in storage stability. Sensory quality of patties was acceptable except in product where the animal fat was entirely replaced with an oil bulking system based on konjac gel. Nonetheless, sensory attributes could be further improved by slight modifications to the product formulation (addition of spices).

Table 8
Sensory evaluation of pork patties.

Sample	Flavor acceptability	Texture acceptability	Overall acceptability
CFP	7.6 ± 1.4^{b}	7.5 ± 1.2^{a}	7.7 ± 1.2^{c}
MFP	6.3 ± 1.5^{ab}	5.6 ± 2.3^{a}	6.0 ± 2.0^{bc}
LFP	6.7 ± 1.9^{ab}	5.9 ± 2.0^{a}	6.6 ± 1.4^{bc}
VLFP	7.0 ± 2.0^{ab}	7.3 ± 1.7^{a}	7.5 ± 0.9^{bc}
IMFP	5.3 ± 2.5^{a}	6.4 ± 2.0^{a}	5.4 ± 2.2^{ab}
ILFP	4.8 ± 1.8^{a}	5.6 ± 1.9^{a}	3.5 ± 2.4^{a}

For sample denomination see Table 1. Means ± SD. Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$).

Conflict of Interest

The authors have no conflict of interest to declare.

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4.1.4 Effect of cooking method on the fatty acid content of reduced-fat and PUFA-enriched pork patties formulated with a konjac-based oil bulking system.

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Effect of cooking method on the fatty acid content of reduced-fat and PUFA-enriched pork patties formulated with a konjac-based oil bulking system

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ABSTRACT

The effect of cooking methods (electric grilling and pan-frying in olive oil) on the composition of reduced-fat and reduced-fat/PUFA enriched pork patties was studied. Fat reduction was performed by replacing pork backfat (38% and 100%) with konjac gel and PUFA-enrichment by replacing pork backfat (49%) with a konjac-based oil bulking system stabilizing a healthier oil combination (olive, linseed and fish oils). Cooking losses (13%–27%) were affected ($p < 0.05$) by formulation and cooking procedure. Compared with raw products, cooked samples had higher ($p < 0.05$) concentrations of MUFAs and PUFAs (both n-3 and n-6); the difference was greater ($p < 0.05$) in the pan-fried patties. Fatty acid retention was generally better in pan-fried than in grilled samples. When cooked, the PUFA levels in the medium-fat/improved sample containing the oil bulking system ranged between 1.4 and 1.6 g/100 g (0.47–0.51 from n-3 PUFAs), with EPA + DHA concentrations of around 75 mg/100 g. Konjac materials were successfully used to produce pork patties with a better lipid composition.

1. Introduction

In recent years there has been a considerable accumulation of studies concerning strategies to optimize the presence of specific components with health implications for designing of potential meat-based functional foods. In this regard, fat is one of the components of meat and meat products that have received special attention owing to the fact that their amount and fatty acid composition exceed the limits set in nutritional recommendations and therefore need to be modulated. Different strategies have been considered for achieving healthier lipid meat products. Most of them entail reducing fat and/or replacing animal fat with vegetable and/or marine oils to produce a food more in line with health recommendations: reducing saturated fatty acids (SFAs) and cholesterol and increasing monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs, specially n-3). Work on strategies to improve fat content is of particular interest in the case of products like burgers or patties, since they are one of the world's most popular processed meat products. These products are widely accepted in certain population groups, but they are also the subject of some negative perceptions associated with factors such as fat content and fatty acid profiles. These products further offer numerous possibilities for the use of technological strategies to modify their composition and improve their nutritional value and potential health-beneficial properties (López-López, Cofrades, Yakan, Solas, & Jiménez-Colmenero, 2010).

A wide variety of ingredients have been proposed for the purpose of fat reduction, based on the use of proteins, carbohydrates or lipids (Jiménez-Colmenero, 1996). These include konjac (glucomannan)-based fat analogues, which open up interesting possibilities. The interest of this ingredient lies in its valuable technological properties (water retention capacity, gelling and thickening activity) and potential health implications (e.g. reduction of cholesterol, insulin and glucose levels or its satiating and laxative effect). Its use as a food additive is authorized in Europe (E-425) and it is classified as GRAS by the FDA. Konjac has been used to reduce fat in products including pork patties (Akesowan, 2010).

To modify lipid profiles, vegetables oils have been added to products like pork or beef patties in liquid form (Dzudie, Kouebou, Essia-Ngang, & Mbofung, 2004; Hur, Jin, & Kim, 2008; Jung & Joo, 2013; Rodríguez-Carpena, Morcuende, & Estévez, 2012; Shiota et al., 1995), as solid fat (Shiota et al., 1995), in frozen state (Lowder & Osburn, 2010) or as part of an oil-in-water emulsion (Lee, Faustman, Djordjevic, Faraji, & Decker, 2006; López-López et al., 2011; Martínez et al., 2012).

Recently there have been novel suggestions regarding technological strategies to improve fat content in meat products. One new way to enhance the nutritional value of meat products is to use a fat replacer like konjac gel to immobilize lipid material (in what is called a konjac-based oil bulking system) and so improve the fat content of meat

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products. This would make it possible, on the one hand to reduce fat content and on the other hand to alter the lipid profile by adding healthier fats. Several authors have reported the use of healthy oils in a konjac matrix to improve fat content in products like dry fermented sausage (Jiménez-Colmenero, Triki, Herrero, Rodríguez-Salas, & Ruiz-Capillas, 2013), merguez fresh sausages (Triki, Herrero, Jimenez-Colmenero, & Ruiz-Capillas, 2013) and frankfurters (Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Solas, & Jiménez-Colmenero, 2013).

Pork patties, like other foods, will normally be cooked prior to consumption. The process of cooking in itself may affect composition. Depending on cooking method employed, varying amounts of different meat components (water, fat, minerals) are lost during cooking and this affects nutrient consumption and energy intakes (Badiani et al., 2002; Librelotto et al., 2008; Serrano, Librelotto, Cofrades, Sánchez-Muniz, & Jiménez-Colmenero, 2007; Sheard, Nute, & Chappell, 1998). There have been some studies on the effect of heat treatment on the composition and fatty acid profiles of healthier-lipid formulations for beef and pork patties with vegetables lipid sources (López-López et al., 2011; Martínez et al., 2012); however, the authors have found no reports dealing with the effect of cooking on the chemical composition of pork patties reformulated to improve fat content using a healthier oil combination stabilized in a konjac-based oil bulking system. Cooking-induced modifications need to be considered in order to achieve a more realistic assessment of the potential nutritional/functional benefits of foods.

Therefore, the objective of this study was to determine how different cooking methods affect the chemical composition, with particular reference to the fatty acid profile, of pork patties reformulated to improve their lipid composition. The reformulation processes studied were i) reduction of fat content by replacing animal fat (38% and 100% of pork backfat) with the same proportion of konjac gel; and ii) improvement of the fatty acid profile by replacing pork backfat (49%) with a healthier oil combination stabilized in a konjac-based oil bulking system. Reformulated samples were compared with a control patty in which the added fat was all pork backfat. The cooking processes assayed were electric grilling and pan-frying, selected as the most commonly employed procedures for this kind of product.

2. Materials and methods

2.1. Materials

Sufficient fresh post-rigor pork meat (16 kg per trial), from different animals (mixture of muscles bíceps femoris, semimembranosus, semitendinosus, gracilis and adductor) and pork backfat (1.5 kg per trial) were purchased from a local market. The meat was trimmed of visible fat and connective tissue. Pork meat was cut in squares of 5 × 5 cm approximately and backfat was passed through a grinder with a 6 mm plate (Mainca, Granollers, Spain). Lots of ~500 g were vacuum packed, frozen and stored at −20 °C until used (less than 2 weeks).

Konjac materials used as animal fat replacers were made with konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain), pre-gelled cornstarch (Amigel, Julio Criado, S.L. Madrid. Spain), i-carrageenan (Hispanagar S.A, Burgos, Spain) and $Ca(OH)_2$ (Panreac Química S.A., Barcelona, Spain). A healthier oil combination was made according to Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, and Jimenez-Colmenero (2010), mixing 44.39% olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), 37.87% linseed oil (Natursoy S.L., Alimentos Ecológicos, Castellterçol, Spain) and 17.74% fish oil (Omevital 18/12 TG Gold, Cognis GMBH, Illertissen, Germany), the latter containing 160 mg eicosapentaenoic acid (EPA)/g and 115 mg docosahexaenoic acid (DHA)/g according to the manufacturer's specifications. This oil combination was prepared for incorporation in the konjac matrix. Sodium chloride was supplied by Panreac Química, S.A. (Barcelona, Spain).

2.2. Konjac materials preparation

Two types of konjac materials were prepared for use in patty formulation: one without added oil (KG) and another one with 20% of a healthier oil mixture stabilized in a konjac gel matrix (konjac-based oil bulking system, O-KG) as described by Salcedo-Sandoval et al. (2013). The oil combination used in the present study was designed to produce a lipid material with a small proportion of SFAs, large proportions of MUFAs and PUFAs (including long chain n-3 PUFAs) and balanced n-6/n-3 PUFAs and PUFA/SFA ratios (Delgado-Pando et al., 2010).

Both KG and O-KG were prepared in duplicate and placed in suitable containers, covered, manually overpressured to eliminate air and stored at 2 ± 2 °C until used (within 24 h of preparation).

2.3. Preparation of pork patties

Four formulations were selected based on their technological and sensory characteristics from a previous study (Salcedo-Sandoval, Cofrades, Ruiz-Capillas Pérez, Carballo, & Jiménez-Colmenero, submitted for publication) to assess the effect of cooking treatments on their composition with special reference to fatty acid content. These pork patties were formulated in such a way that pork backfat was replaced with KG (to reduce fat content) or O-KG (to reduce fat content and improve the lipid profile). A pork patty was prepared with normal fat content (15%) with all pork backfat as control (CFP). A medium fat content patty (MFP; 10%) was prepared by replacing 38% of pork backfat with the same proportion of KG; a low fat content patty (LFP; 3%) was formulated by replacing all the pork backfat with KG and a medium fat content (IMFP; 10%) was prepared by replacing 49% of pork backfat with the same proportion of O-KG. The detailed formulation of pork patties is presented in Table 1.

Before preparing formulations (2 kg each one), pork meat and pork backfat packages were thawed for approximately 18 h at 2 ± 2 °C. Pork meat, backfat and KG or O-KG (depending on the formulation) were passed through a grinder with a 4.5 mm plate (Vam.Dall. Srl. Model FTSIII, Treviglio, Italy). Meat and half of the ingredients (pork backfat, KG or O-KG, water and NaCl) were placed in a mixer (Hobart mixer N-506, Hobart MFG. Co., Troy, USA) for 1 min. The rest of these ingredients were added and the sample mixed for 1 min again. Oval-shaped patties (85 g, 12 mm thick, 8 cm wide and 11 cm long) were prepared using a manual burger former, then weighed, placed in plastic bags (Cryovac® BB3050), vacuum packed and stored at −20 ± 2 °C until analysis (within 2 weeks of preparation). The entire patties processing procedure was replicated twice at two different days.

2.4. Thawing loss

Twelve patties from each formulation were randomly taken, thawed (15 h, 2 °C ± 2) and manually wiped with a paper towel to remove visible exudates. The thawing loss was calculated as the weight difference

Table 1
Formulation (%) of pork patties.

Samples	Meat	Backfat	KG	O-KG
CFP	80.06	13.44	0.00	0.00
MFP	80.06	8.26	5.18	0.00
LFP	80.06	0.00	13.44	0.00
IMFP	80.06	6.82	0.00	6.62

Sample denomination: CFP, control pork patty prepared with normal fat content (all pork fat); MFP, medium fat pork patty prepared by partial replacement of pork backfat (38%) with KG; LFP, low fat pork patty prepared by total replacement of pork backfat (100%) with KG; IMFP, improved medium fat pork patty prepared by partial of pork backfat (49%) with O-KG. All samples also contained 5% of added water and 1.5% of NaCl.
KG: Konjac gel. O-KG: oil bulking agent based on konjac gel, as konjac material containing 20% of healthier oil combination (olive, linseed and fish oils).

between initial and thawed samples, expressed as a percentage of the initial weight.

2.5. Cooking methods and cooking loss

Two cooking methods – electric grilling and pan-frying – were chosen, as the ones most commonly used for this kind of products. Preliminary cooking trials were performed to establish the cooking conditions required to achieve a meat core temperature of 70 °C. The final cooking temperatures in grilling and pan-frying were determined beforehand by inserting thermocouples connected to a temperature recorder (DaqPRO 5300 Data recorder; OMEGA Engineering, Inc., Stamford CT, USA).

2.5.1. Electric grilling

Samples were cooked 3 min per side at 210 ± 4 °C in a contact grill (Princess classic multigrill type 2321, The Netherlands).

2.5.2. Pan-frying

Samples were cooked at 170 °C for a total of 5 min (2 min 30 s each side) in a preheated 24 cm diameter pan containing 25 ml olive oil (extra virgin olive oil, Carbonell, Sos, Cuetara S.A., Madrid, Spain).

Four thawed pork patties were used for each formulation and cooking method. Following cooking, all patties were cooled at room temperature (20–22 °C) for 30 min, wiped off with a paper towel to remove visible exudate, and then weighed. Cooking loss was calculated by weight difference and expressed as a percentage of the initial weight. Total losses were calculated as the sum of thawing and cooking losses.

2.6. Proximate composition and energy content

Moisture and ash contents in the raw and cooked samples were determined in quadruplicate according to AOAC (2005). Fat content was evaluated in triplicate following the method of Bligh and Dyer (1959). Protein was measured in quadruplicate with a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St Joseph, MI. USA).

Energy content (kcal) was calculated based on 9.1 kcal/g for fat and 4.1 kcal/g for protein.

2.7. Fatty acid profile

The fatty acid contents of raw and cooked formulated patties were determined from their lipid extracts (Bligh & Dyer, 1959) by gas chromatography. Boron trifluoride/methanol was used to prepare fatty acid methyl esters (FAME), according to Sánchez-Muniz, Oubiña, Benedí, Ródenas, and Cuesta (1998). A Shimadzu gas chromatograph (Model GC-2014, Kyoto, Japan) fitted with a capillary column SP™-2330 (60 m × 0.25 mm × 0.2 μm id) (Supelco, Bellefonte, USA) was used with a flame ionization detector. Injector and detector temperatures were 250 and 260 °C respectively. The oven temperature was set at 140 °C for 5 min then raised to 240 °C at 4 °C/min and held there for 20 min. The carrier gas was helium and nitrogen was used as the make-up gas. Fatty acids were identified by comparison of the retention times with a standard of 37 fatty acids (Supelco™ 37 FAME Mix 47885-U, USA). Quantification was done by normalization and transformation of the area percentage into mg/100 g of the edible portion, using the lipid conversion factor for pork fat and oils (MAFF, 1998).

Based on the FAME results, the atherogenic (AI) and thrombogenic indexes (TI) were calculated from the fatty acid results according to Ulbricht and Southgate (1991):

$$AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0)/(MUFA + n\text{-}3\ PUFA + n\text{-}6\ PUFA);$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/(0.5 \times MUFA + 0.5\ x\ n\text{-}6\ PUFA + 3 \times n\text{-}3\ PUFA + n\text{-}3\ PUFA/n\text{-}6\ PUFA).$$

2.8. Nutrient retention

Retention factors for moisture, protein, fat, ashes and fatty acids were calculated according to Murphy, Criner, and Gray (1975) as follows:

$$\text{Retention}\ (\%) = \frac{(\text{g of cooked product} \times \text{g of component in cooked product})}{(\text{g of raw product} \times \text{g component in raw product})} \times 100$$

2.9. Statistical analysis

The entire trial was replicated twice. Data were analyzed using general linear model (GLM) procedure of SPSS Statistics (v. 21, IBM SPSS Inc., Chicago, IL) for one-way analyses of variance (ANOVA) to evaluate the statistical significance ($P < 0.05$) of the formulation and two-way ANOVA as a function of formulation and cooking method. Formulation and cooking method were assigned as fixed effects and replicate as random effect. Least squares differences were used for comparison of mean values between treatments and Tukey's HSD test to identify significant differences ($p < 0.05$) between formulations and cooking method.

3. Results and discussion

For a better exposition and understanding, the discussion of the results was divided in two main sections: one related to the effect of the formulation (raw patties) and another regarding to the effect of the cooking methods.

3.1. Composition and energy content of raw pork patties as affected by patties formulation

3.1.1. Proximate composition of raw pork patties

Proximate analysis and energy content of raw patties showed some significant differences between formulations (Table 2). All modified pork patties had similar protein content (close to the target level), ranging between 17.35% and 17.61%, roughly 1% lower than the control sample, irrespective of the reformulation process. With no change in the proportion of lean meat used (Table 1), this difference must be due essentially to the replacement of pork backfat (containing almost 5% of meat protein) with konjac material. The ash content of the samples ranged between 2.17% and 2.39%. Moisture and fat contents were generally close to the target levels. Moisture content increased and fat level decreased when pork backfat was replaced by konjac gel (Table 2), a fat replacer containing a very high proportion of water (over 95%). Thus, moisture content was lowest in the control sample (CFP) and highest in LFP; the opposite was true in the case of the fat level. These results are consistent with the experimental design, which defined three fat levels – normal fat (15%), medium fat (10%) and low fat (3%) – producing fat reductions of up to 80%. The lipid content in control sample and in the patties in which pork backfat was partially (MFP) and totally (LFP) replaced by konjac gel came from the lean pork meat and pork backfat. IMFP also had medium fat content (10%), similar to MFP, but its origin was different and so the product was formulated by partially replacing pork backfat with a konjac material (as oil bulking system) containing a combination of olive, linseed and fish oils. According to the formulation conditions, the IMFP sample

Table 2
Proximate analysis (%) and energy content (kcal/100 g) of raw and cooked formulated pork patties.

	CFP	MFP	LFP	IMFP
Moisture				
Raw	64.05 ± 0.14 ^{c1}	70.60 ± 0.32^{b2}	77.54 ± 0.30^{b3}	70.54 ± 0.17^{b2}
Electric grill	58.79 ± 0.66 ^{b1}	65.00 ± 0.45^{a2}	73.57 ± 0.21^{a3}	64.49 ± 0.34^{a2}
Pan-frying	57.27 ± 0.23 ^{a1}	65.24 ± 0.11^{a3}	72.86 ± 0.33^{a4}	63.84 ± 0.49^{a2}
Protein				
Raw	18.40 ± 0.20 ^{a2}	17.35 ± 0.21^{a1}	17.61 ± 0.22^{a1}	17.45 ± 0.14^{a1}
Electric grill	24.62 ± 0.33 ^{c3}	22.26 ± 0.08^{c2}	20.45 ± 0.11^{c1}	21.62 ± 0.47^{c2}
Pan-frying	23.05 ± 0.21 ^{b3}	20.90 ± 0.13^{b2}	19.92 ± 0.10^{b1}	20.13 ± 0.12^{b1}
Fat				
Raw	15.13 ± 0.55^{a3}	10.03 ± 0.80^{a2}	2.93 ± 0.20^{a1}	9.94 ± 0.67^{a2}
Electric grill	13.79 ± 0.90^{a4}	9.80 ± 0.41^{a2}	3.84 ± 0.23^{ab1}	11.34 ± 0.16^{b3}
Pan-frying	17.95 ± 0.64^{b4}	11.14 ± 0.95^{a2}	4.47 ± 0.49^{b1}	13.79 ± 0.21^{c3}
Ash				
Raw	2.36 ± 0.02^{a2}	2.39 ± 0.01^{a2}	2.17 ± 0.01^{a1}	2.35 ± 0.02^{a2}
Electric grill	2.89 ± 0.01^{c3}	2.91 ± 0.01^{c3}	2.65 ± 0.01^{b1}	2.79 ± 0.03^{c2}
Pan-frying	2.63 ± 0.03^{b1}	2.76 ± 0.09^{b1}	2.68 ± 0.03^{b1}	2.66 ± 0.04^{b1}
Energy content				
Raw	213.20 ± 5.58^{a3}	162.42 ± 6.52^{a2}	98.88 ± 2.56^{a1}	162.06 ± 5.94^{a2}
Electric grill	226.49 ± 6.80^{a4}	180.43 ± 3.40^{b2}	118.83 ± 2.28^{b1}	191.82 ± 1.25^{b3}
Pan-frying	257.92 ± 5.76^{b4}	187.02 ± 8.21^{b2}	122.38 ± 4.89^{b1}	208.05 ± 2.46^{c3}
% ***calories from fat***				
Raw	64.60 ± 0.68^{b3}	56.14 ± 2.23^{b2}	26.95 ± 1.17^{a1}	55.81 ± 1.71^{a2}
Electric grill	55.39 ± 1.96^{a3}	49.40 ± 1.12^{a2}	29.42 ± 1.19^{ab1}	53.78 ± 0.84^{a3}
Pan-frying	63.34 ± 0.89^{b3}	54.12 ± 2.24^{ab2}	33.18 ± 2.38^{b1}	60.33 ± 0.23^{b3}
% ***calories from SFAs***				
Raw	61.58 ± 0.10^{b4}	36.43 ± 0.12^{c3}	9.07 ± 0.03^{a1}	33.33 ± 0.03^{a2}
Electric grill	49.20 ± 0.11^{a4}	32.98 ± 0.09^{a2}	12.90 ± 0.04^{c1}	36.73 ± 0.01^{b3}
Pan-frying	67.09 ± 0.12^{c4}	36.18 ± 0.09^{b2}	12.45 ± 0.07^{b1}	43.98 ± 0.24^{c3}
% ***calories from MUFAs***				
Raw	57.98 ± 0.08^{a4}	39.14 ± 0.06^{a2}	10.91 ± 0.08^{a1}	41.82 ± 0.08^{a3}
Electric grill	58.39 ± 0.11^{b4}	42.29 ± 0.12^{b2}	14.81 ± 0.05^{b1}	47.24 ± 0.02^{b3}
Pan-frying	79.53 ± 0.12^{c4}	49.69 ± 0.12^{c2}	20.31 ± 0.12^{c1}	60.88 ± 0.06^{c3}
% ***calories from PUFAs***				
Raw	10.17 ± 0.04^{a4}	7.42 ± 0.04^{a2}	3.38 ± 0.02^{a1}	9.81 ± 0.12^{a3}
Electric grill	10.43 ± 0.03^{b3}	8.37 ± 0.08^{b2}	3.73 ± 0.03^{b1}	12.56 ± 0.08^{b4}
Pan-frying	12.34 ± 0.10^{c3}	9.36 ± 0.07^{c2}	4.32 ± 0.01^{c1}	14.58 ± 0.12^{c4}

For sample description see Table 1. Different letters in the same column and different numbers in the same row indicate significant differences ($p < 0.05$).

contained almost 1.3% of blended oils, which is around 13% of product fat content.

There are reports of the use of different oils to improve the lipid content in patties. Hur et al. (2008) incorporated olive oil directly in pork patties (15%–17% fat) to replace 50% of backfat. Lowder and Osburn (2010) formulated healthier beef patties with fat contents of 10% or 20% by addition of blended lipid solutions (57% beef tallow combined with 43% of safflower, olive or corn oil). The above-cited studies reported enhancement of the quality of lipid content in patties through the incorporation of better fat sources. In this experiment an additional effort was made to optimize fat content of patties in terms of type and amount. Thus, in IMFP sample the use of oil stabilized in a bulking system based on konjac gel made it possible to partially replace backfat with a healthier lipid source and simultaneously lower the fat content to 67% of the control.

3.1.2. Energy content of raw pork patties

As expected from the experimental design, the main factor influencing the energy content of the formulated patties was the fat content in the product (Table 2). The energy contents of the raw pork patties ranged from 213 kcal/100 g for the control sample (CFP) to 162 kcal/100 g for both reduced fat samples (MFP and IMFP) and 99 kcal/100 g for the low fat product (LFP). In these samples the fat accounted for between 64% (CFP) and 27% (LFP) of the total calorie content. The reduction in calorie content of medium fat samples was higher than reported for beef hamburger (9% fat) made with pre-emulsified olive, corn and fish oils as lipid material (Martínez et al., 2012). In the case of LFP, the energy value was reduced by more than 30% with respect to the control and therefore that product could legitimately carry an "energy-reduced" claim under Regulation (EC) No 1924/2006 of the European Parliament & of the Council (2007). The contribution of specific fatty acids to total product energy was again related basically to the product fat content and to a lesser extent to the replacement of pork fat with a konjac-based oil bulking system (Table 2). As fat content was reduced, there was a proportional decrease ($p < 0.05$) in the energy contribution of all fatty acids. Where fat levels were similar (MFP versus IMFP), the presence of non-pork fat caused a reduction in the proportion of energy supplied by SFAs and an increase ($p < 0.05$) in the proportion supplied by MUFAs and PUFAs (Table 2).

3.1.3. Fatty acid content and nutritional ratios of raw pork patties

The fatty acid content of raw patties, shown in Tables 3 and 4, was affected by formulation. As was to be expected, the main differences in fatty acid concentrations in the reformulated raw patties were due to the fat content in the product; there was generally a direct relationship between the fat level in the product and the concentration of each fatty acid. Therefore, following the enunciated fat reduction strategy, the SFA concentration was reduced from 6.7 g/100 g (CFP, normal fat patty) to 0.99 g/100 g (LFP, low-fat patty) and MUFA and PUFA contents were respectively reduced from 6.3 and 1.1 g/100 g (CFP) to 1.2 and 0.37 g/100 g (LFP) (Table 4).

Table 3
Main fatty acid contents (mg/100 g product) of raw and cooked pork patties.

Fatty acid		CFP	MFP	LFP	IMFP
Myristic	Raw	191.85 ± 1.43^{b3}	131.31 ± 1.63^{a2}	38.19 ± 0.88^{a1}	133.70 ± 1.59^{a2}
C 14:0	Electric grill	175.50 ± 2.81^{a4}	133.36 ± 0.75^{a2}	43.07 ± 2.06^{b1}	145.61 ± 0.53^{b3}
	Pan-frying	217.45 ± 1.69^{c4}	138.00 ± 0.85^{b2}	43.83 ± 0.39^{b1}	162.18 ± 1.63^{c3}
Palmitic	Raw	3929.63 ± 10.09^{b3}	2269.45 ± 7.57^{a2}	584.79 ± 1.89^{a1}	2258.75 ± 4.51^{a2}
C16:0	Electric grill	3331.34 ± 14.50^{a4}	2352.84 ± 6.25^{b2}	870.19 ± 4.92^{c1}	2495.69 ± 3.18^{b3}
	Pan-frying	4224.19 ± 8.22^{c4}	2510.41 ± 16.87^{c2}	858.08 ± 2.92^{b1}	2915.10 ± 17.19^{c3}
Stearic	Raw	2513.61 ± 3.93^{b4}	1514.54 ± 7.61^{c3}	361.08 ± 0.63^{a2}	1189.11 ± 3.23^{a1}
C18:0	Electric grill	1819.07 ± 8.71^{a4}	1081.11 ± 4.28^{a2}	470.61 ± 2.94^{c1}	1305.21 ± 2.36^{b3}
	Pan-frying	2769.31 ± 8.12^{c4}	1223.71 ± 8.08^{b2}	414.91 ± 4.74^{b1}	1622.81 ± 3.57^{c3}
Arachidic	Raw	41.72 ± 1.37^{a2}	25.41 ± 0.01^{b1}	–	24.03 ± 0.47^{a1}
C20:0	Electric grill	–	–	–	26.89 ± 0.53^{b}
	Pan-frying	54.15 ± 2.76^{b4}	20.65 ± 0.52^{a2}	8.75 ± 0.78^{1}	36.26 ± 1.06^{c3}
Other SFAs	Raw	89.86 ± 1.65^{b4}	62.60 ± 1.22^{b3}	12.73 ± 1.35^{a1}	56.94 ± 2.29^{a2}
	Electric grill	80.60 ± 2.12^{a4}	57.02 ± 1.30^{a2}	33.20 ± 2.28^{b1}	62.82 ± 0.87^{b3}
	Pan-frying	107.03 ± 2.54^{c4}	82.85 ± 1.79^{c2}	42.91 ± 0.23^{c1}	96.14 ± 6.38^{c3}
Palmitoleic	Raw	276.36 ± 1.37^{a4}	199.55 ± 2.17^{a2}	73.19 ± 0.46^{a1}	208.84 ± 1.40^{a3}
C16:1	Electric grill	294.46 ± 4.57^{b4}	226.02 ± 0.88^{b2}	97.67 ± 0.45^{b1}	242.51 ± 2.93^{b3}
	Pan-frying	364.67 ± 3.24^{c4}	242.55 ± 4.27^{c2}	103.12 ± 0.53^{c1}	268.69 ± 0.75^{c3}
Oleic	Raw	5424.10 ± 7.67^{a4}	3629.15 ± 2.82^{a2}	960.00 ± 3.23^{a1}	3918.74 ± 1.94^{a2}
C18:1n9	Electric grill	5429.92 ± 15.83^{a4}	3900.24 ± 12.93^{b2}	1335.91 ± 0.57^{b1}	4436.86 ± 4.87^{b3}
	Pan-frying	7507.08 ± 11.34^{b4}	4644.99 ± 14.21^{c2}	1892.60 ± 11.18^{c1}	5809.48 ± 4.90^{c3}
Vaccenic	Raw	449.66 ± 3.93^{a4}	304.04 ± 1.09^{a2}	114.32 ± 6.15^{a1}	320.14 ± 2.29^{a3}
C18:1n7*c*	Electric grill	485.24 ± 5.45^{b4}	356.85 ± 3.97^{b2}	145.80 ± 3.71^{b1}	372.42 ± 1.82^{b3}
	Pan-frying	610.47 ± 3.49^{c4}	404.07 ± 4.56^{c2}	165.45 ± 0.69^{c1}	437.02 ± 7.81^{c3}
Eicosenoic	Raw	156.54 ± 2.14^{a3}	104.01 ± 0.54^{a2}	20.66 ± 1.85^{a1}	102.44 ± 2.99^{a2}
C20:1n9c	Electric grill	163.48 ± 0.65^{b3}	111.05 ± 0.88^{b2}	26.47 ± 1.41^{b1}	110.74 ± 0.87^{b2}
	Pan-frying	200.10 ± 2.89^{c4}	114.48 ± 0.60^{c2}	26.34 ± 0.90^{b1}	120.42 ± 1.50^{c3}
Other MUFAs	Raw	64.90 ± 3.59^{b3}	64.71 ± 6.31^{b3}	30.93 ± 1.02^{b1}	45.73 ± 2.53^{b2}
	Electric grill	42.90 ± 0.01^{a3}	52.65 ± 0.46^{a4}	21.84 ± 2.59^{a1}	29.02 ± 0.53^{a2}
	Pan-frying	57.11 ± 7.10^{b2}	53.84 ± 1.05^{a2}	44.74 ± 4.42^{c1}	54.71 ± 0.65^{c2}
Linoleic	Raw	951.74 ± 5.38^{a4}	704.79 ± 3.87^{a3}	297.83 ± 0.90^{a1}	688.36 ± 3.28^{a2}
C18:2n6	Electric grill	970.15 ± 3.25^{b4}	774.16 ± 6.39^{b2}	342.10 ± 2.44^{b1}	868.63 ± 3.41^{b3}
	Pan-frying	1143.94 ± 7.18^{c4}	869.82 ± 5.60^{c2}	392.74 ± 0.53^{c1}	1049.50 ± 7.66^{c3}
Linolenic	Raw	50.99 ± 1.79^{a3}	40.00 ± 0.54^{a2}	13.93 ± 1.14^{a1}	272.31 ± 9.44^{a4}
C18:3n3	Electric grill	51.03 ± 1.95^{a3}	41.16 ± 1.16^{a2}	12.67 ± 1.35^{a1}	336.49 ± 4.99^{b4}
	Pan-frying	68.53 ± 3.52^{b3}	48.88 ± 0.52^{b2}	19.63 ± 1.54^{b1}	363.21 ± 2.24^{c4}
Eicosapentaenoic	Raw	–	–	–	32.90 ± 1.40^{a}
C20:5n3	Electric grill	–	–	–	40.73 ± 1.02^{b}
	Pan-frying	–	–	–	44.67 ± 0.75^{c}
Docosahexaenoic	Raw	–	–	–	21.70 ± 1.40^{a}
C22:6n3	Electric grill	–	–	–	33.54 ± 0.61^{c}
	Pan-frying	–	–	–	30.75 ± 0.65^{b}
Other PUFAs	Raw	114.82 ± 2.47^{a3}	70.83 ± 1.41^{a2}	60.13 ± 0.34^{b1}	63.24 ± 2.45^{a1}
	Electric grill	125.45 ± 2.25^{b3}	104.16 ± 3.39^{b2}	55.47 ± 2.18^{a1}	101.16 ± 1.74^{b2}
	Pan-frying	143.42 ± 4.45^{c3}	109.77 ± 2.56^{b2}	62.44 ± 0.53^{b1}	113.95 ± 2.36^{c2}

For sample description see Table 1. For each fatty acid different letters in the same column and different numbers in the same row indicate significant differences ($p < 0.05$).

In the reformulated samples SFA levels dropped and MUFA and PUFA levels increased. The most abundant fatty acids were MUFAs (46.7%–49.2%), contrasting with the control, where the most abundant were SFAs (47.5%). Oleic acid (C18:1) was the most abundant fatty acid in all patties, followed by palmitic (C16:0) and then stearic (C18:0) and linoleic (C18:2) acids. The most abundant PUFA was linoleic acid (Table 3). In the case of the patties with all pork fat (CFP, MFP and LFP), differences between fatty acid profiles were possibly due to the different proportions of fat from meat and pork backfat used in their formulation (Ayo et al., 2007). For instance, the proportion of intramuscular fat was higher in LFP samples and that of depot fat (backfat) higher in CFP (Table 1). Intramuscular fat contains smaller proportions of MUFAs and higher proportions of PUFAs than removable depot fat (Raes, De Smet, & Demeyer, 2004) and leaner cuts have higher percentages of PUFAs (Badiani et al., 2002).

Fatty acid concentrations in the IMFP sample differed from those in all-pork fat patties due to the partial replacement of pork backfat by a konjac-based bulking system containing the healthier oil combination (Tables 3 and 4). With similar fat levels (MFP versus IMFP), the patty containing vegetable and marine oil (IMFP) contained a lower ($p < 0.05$) concentration of SFAs and higher ($p < 0.05$) concentrations of MUFAs and n-3 PUFAs, for example linolenic (C18:3), eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids, than MFP, where the values were 272.3, 32.9 and 21.7 mg/100 g, respectively (Tables 3 and 4).

The PUFA/SFA ratio is one of the main parameters used to assess the nutritional quality of the lipid fraction in foods (Table 4). In the case of raw patties this ratio was similar to those reported elsewhere for pork products (López-López, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2009; López-López et al., 2011), ranging between 0.17 and 0.37 (Table 4). The PUFA/SFA ratio has been reported to increase in pork patties made with olive oil replacing backfat (Hur et al., 2008; Rodríguez-Carpena et al., 2012). There is abundant evidence associating a higher n-6/n-3 ratio with the promotion of pathogenesis of many diseases, including cardiovascular diseases (CVD), cancer, etc. and lower ratios with a suppressive effect (Simopoulos, 2002). The dietary recommendation is therefore to reduce this value to less than 4 for prevention of CVD. The ratios found in the present experiment for all-pork fat patties diverged to some extent from the recommended values, with the highest ratio in the case of MFP (Table 4). However, the n-6/n-3 ratio was much lower ($p < 0.05$) in patties where backfat was partially replaced by an oil. In products with similar fat levels (MFP versus IMFP), this effect would appear to be due mainly to an increase of n-3 PUFAs, with levels up to five times greater in IMFP than in MFP (Table 4). The atherogenicity and thrombogenicity indexes (AI and TI) are shown in Table 5. Both indexes decreased ($p < 0.05$) in all reformulated samples.

Table 4
Fatty acid group (SFAs, MUFAs and PUFAs) contents (mg/100 g of product) and nutritional ratios of raw and cooked pork patties.

Fatty acid		CFP	MFP	LFP	IMFP
∑ SFAs	Raw	6766.66 ± 10.61^{b4}	4003.31 ± 12.65^{b3}	996.80 ± 3.33^{a1}	3662.53 ± 3.73^{a2}
	Electric grill	5406.52 ± 11.79^{a4}	3624.33 ± 10.08^{a3}	1417.07 ± 4.07^{c1}	4036.22 ± 1.37^{b2}
	Pan-frying	7372.13 ± 12.85^{c4}	3975.63 ± 10.36^{b2}	1368.48 ± 7.37^{b1}	4832.50 ± 26.64^{c3}
∑ MUFAs	Raw	6371.56 ± 9.10^{a4}	4301.46 ± 6.80^{a2}	1199.10 ± 9.03^{a1}	4595.89 ± 9.18^{a3}
	Electric grill	6416.00 ± 12.21^{b4}	4646.81 ± 13.06^{b2}	1627.69 ± 5.31^{b1}	5191.56 ± 1.84^{b3}
	Pan-frying	8739.44 ± 13.29^{c4}	5459.92 ± 12.96^{c2}	2232.25 ± 12.84^{c1}	6690.33 ± 6.72^{c3}
∑ PUFAs	Raw	1117.55 ± 4.28^{a4}	815.63 ± 4.64^{a2}	371.88 ± 2.22^{a1}	1078.50 ± 12.94^{a3}
	Electric grill	1146.63 ± 3.18^{b3}	919.48 ± 8.86^{b2}	410.24 ± 2.87^{b1}	1380.55 ± 8.72^{b4}
	Pan-frying	1355.89 ± 11.26^{c3}	1028.47 ± 7.75^{c2}	474.80 ± 0.96^{c1}	1602.09 ± 13.14^{c4}
∑ n-6	Raw	1006.30 ± 5.01^{a4}	742.21 ± 3.48^{a3}	311.69 ± 0.90^{a1}	726.86 ± 2.07^{a2}
	Electric grill	1025.40 ± 3.25^{b4}	797.39 ± 7.20^{b2}	351.19 ± 1.64^{b1}	910.69 ± 2.36^{b3}
	Pan-frying	1211.20 ± 11.26^{c4}	913.21 ± 4.64^{c2}	406.57 ± 0.53^{c1}	1093.85 ± 8.68^{c3}
∑ n-3	Raw	111.26 ± 1.16^{a3}	73.42 ± 1.72^{a2}	60.19 ± 1.38^{a1}	351.65 ± 11.08^{a4}
	Electric grill	121.23 ± 2.68^{b2}	122.09 ± 1.74^{c2}	59.05 ± 1.48^{a1}	469.85 ± 6.53^{b3}
	Pan-frying	144.68 ± 4.48^{c3}	115.26 ± 3.35^{b2}	68.24 ± 0.84^{b1}	508.24 ± 4.55^{c4}
PUFAs/SFAs	Raw	0.17 ± 0.00^{a1}	0.20 ± 0.00^{a2}	0.37 ± 0.00^{c4}	0.29 ± 0.00^{a3}
	Electric grill	0.21 ± 0.00^{c1}	0.25 ± 0.00^{b2}	0.29 ± 0.00^{b3}	0.34 ± 0.00^{c4}
	Pan-frying	0.18 ± 0.00^{b1}	0.26 ± 0.00^{b2}	0.35 ± 0.00^{a4}	0.33 ± 0.00^{b3}
n-6/n-3	Raw	9.05 ± 0.13^{b3}	10.11 ± 0.21^{c4}	5.18 ± 0.11^{a2}	2.07 ± 0.06^{b1}
	Electric grill	8.46 ± 0.20^{a3}	6.53 ± 0.04^{a2}	5.95 ± 0.13^{b2}	1.94 ± 0.02^{a1}
	Pan-frying	8.38 ± 0.29^{a4}	7.93 ± 0.20^{b3}	5.96 ± 0.07^{b2}	2.15 ± 0.00^{c1}
Atherogenic index	Raw	0.63 ± 0.00^{c4}	0.55 ± 0.00^{c3}	0.47 ± 0.00^{b1}	0.49 ± 0.00^{c2}
	Electric grill	0.53 ± 0.00^{b3}	0.52 ± 0.01^{b23}	0.51 ± 0.00^{c2}	0.47 ± 0.00^{b1}
	Pan-frying	0.50 ± 0.00^{a4}	0.47 ± 0.00^{a3}	0.38 ± 0.00^{a1}	0.43 ± 0.00^{a2}
Thrombogenic index	Raw	1.65 ± 0.00^{c4}	1.43 ± 0.01^{c3}	1.05 ± 0.01^{b2}	0.96 ± 0.01^{b1}
	Electric grill	1.30 ± 0.00^{a4}	1.16 ± 0.01^{b2}	1.19 ± 0.01^{c3}	0.88 ± 0.00^{a1}
	Pan-frying	1.33 ± 0.01^{b3}	1.10 ± 0.01^{a2}	0.86 ± 0.00^{a1}	0.87 ± 0.01^{a1}

For sample description see Table 1. Different letters in the same column and different numbers in the same row indicate significant differences ($p < 0.05$).

Similar results have been reported in low-fat (10%) beef patties where pork backfat was replaced with an olive oil-in-water emulsion (López-López et al., 2011).

3.2. Effect of cooking methods on weight losses and composition of patties

3.2.1. Weight losses

Since pork patties must be cooked before they are consumed, it is essential to take into account losses of matter (mainly water and fat) that occur during preparatory procedures like thawing and cooking, which will alter the composition. Thawing losses (TLs) of pork patties were affected ($p < 0.05$) by formulation (Table 5). TLs in all-pork fat samples ranged between 3.39% and 8.11%. The TLs increased as the fat level decreased and the proportion of konjac gel (Table 1) and the moisture content (Table 2) increased as per the design. In this respect a decreased water-holding capacity has been reported, caused by freezing/thawing of konjac gels. This is because the formation of ice crystals reduces the amount of water entrapped in the network, causing some dislocation which is not wholly reversed after thawing (Jiménez-Colmenero, Cofrades, Herrero, Solas, & Ruiz-Capillas, 2013). In samples with the same fat level (MFP and IMFP) TLs were not affected by the type of fat ($p > 0.05$) (Table 5). TLs ranging between 2% and 3% have been reported in low-fat beef patties formulated with partial or total replacement of pork backfat by olive oil-in-water emulsion (López-López et al., 2010). Miller, Andersen, Ramsey, and Reagan (1993) reported TLs around 5% in low-fat ground beef patties (10% fat content).

Cooking losses (CLs) of meat products were mainly influenced by the ability to retain water and fat during thermal treatment. This ability depends on the mass transfer process during thermal treatment (Vittadini, Rinaldi, Chiavaro, Barbanti, & Massini, 2005), which in turn is influenced by the characteristics of the cooking procedure (i.e. heating rate, final cooking temperature, etc.) and of the meat systems (i.e. moisture, fat and protein composition and size, shape, pH, degree of structural disintegration, etc.). Cooking led to significant loss of matter, a process affected ($p < 0.05$) by formulation and cooking procedure (Table 5). Weight losses during cooking of patties reported in the literature vary widely, but the values recorded in this experiment (between 13% and 27%), are within the normal range (15% and 40%) given for comparable ground meat products (Boles & Shand, 1999). Hur et al. (2008) reported cooking loss values around 27% in pork patties formulated with 5% olive oil (added in liquid form). López-López et al. (2011) found CLs ranging between 28% and 34% in low-fat beef patties in which pork backfat was partially or totally replaced by an olive oil-in-water emulsion. Significant interaction between cooking methods and type of pork patties was observed in the case of CLs. These losses tended to be higher ($p < 0.05$) after grilling than after pan-frying (Table 5). Unlike the case of weight losses caused by freezing/thawing, in all-pork fat samples fat reduction caused CLs to decrease ($p < 0.05$). With the same fat level, replacement of pork fat by a konjac-based oil bulking system caused a decrease of CLs in the products using either cooking method (Table 5); this suggests that a relatively high level of the fat and water present in the konjac-based oil bulking system was retained after

Table 5
Weight losses (%) during freezing/thawing and cooking of pork patties.

Sample	Thawing losses	Cooking losses		Total losses	
		Electric grill	Pan-frying	Electric grill	Pan-frying
CFP	3.39 ± 0.35^{a}	26.99 ± 1.46^{c2}	20.89 ± 1.45^{c1}	30.59 ± 1.60^{b2}	24.06 ± 1.24^{b1}
MFP	6.10 ± 0.55^{b}	23.13 ± 1.09^{b1}	21.28 ± 1.38^{c1}	29.51 ± 1.43^{b2}	27.10 ± 1.40^{c1}
LFP	8.11 ± 0.89^{c}	17.80 ± 0.75^{a2}	13.55 ± 1.57^{a1}	26.67 ± 0.63^{a2}	20.90 ± 1.32^{a1}
IMFP	7.03 ± 1.26^{bc}	20.27 ± 1.81^{a2}	17.27 ± 0.71^{b1}	28.01 ± 0.74^{ab2}	23.59 ± 0.32^{b1}

For sample description see Table 1. Means ± SD. Different letters in the same column and different numbers in the same row indicate significant differences ($p < 0.05$).

thermal treatment. These results are consistent with the fact that some effects of freezing/thawing on fat analogues seem to be overcome by heating (Jiménez-Colmenero, Confrades, et al., 2013). The fact that the patty absorbed some fat during frying could account for lower CLs measured with this method as compared with electric grilling (Librelotto et al., 2008). Increased cooking losses have been reported in patties with increasing fat content (Berry, 1997, 1998; Choi et al., 2012; Hoelscher, Savell, Harris, Cross, & Rhee, 1987). Differences in cooking losses between fat levels were highly dependent on the formulation and the cooking method employed.

Total weight losses (TLs + CLs) were affected ($p < 0.05$) by formulation and cooking method and ranged between 21% and 31% (Table 5). As in the case of CLs, total losses were higher ($p < 0.05$) after grilling than after pan-frying. In general, total losses tended to decrease with the reduction of fat, although this was less apparent than in the case of CLs owing to the contribution of TLs, which has the opposite effect. Miller et al. (1993) reported total losses ranging between 31% and 38% in ground beef patties with 10% and 22% fat content, and López-López et al. (2011) recorded 29%–37% TLs in low-fat beef patties in which pork backfat was partially or totally replaced by an olive oil-in-water emulsion.

3.2.2. Proximate composition of cooked pork patties

Patty composition was affected by the formulation and by the cooking method (Table 2), mainly as a consequence of the weight losses occurring in each case (Table 5). Moisture content decreased ($p < 0.05$) after cooking regardless of the formulation or the cooking method. Moisture retention values (Table 6) were higher ($p < 0.05$) in pan-fried (67%–74%) than in grilled (63%–69%) patties. Fat reduction increased moisture retention (Table 6) regardless of cooking technique. Similar results have been reported in beef patties cooked on an electric grill (Tekin, Saricoban, & Yilmaz, 2010). Cooking increased ($p < 0.05$) the protein percentage in all patties, but the increase was greater in grilled products regardless of formulation (Table 2). This protein increase was consistent with the cooking losses (Table 6), which were possibly the result of moisture loss (drip and evaporation) and fat melting during cooking; in the case of pan-frying, there is in addition some exchange of fat with the frying oil. Although some water soluble proteins could be lost in the thawing process, protein is not susceptible to migration, so the combined effects of patty treatment (freezing/thawing and cooking) had little impact on protein content, as reflected by retention levels of around 90% (Table 6). Protein retention levels of around 100% have been reported in different meat products subjected to various cooking techniques (López-López et al., 2011; Maranesi et al., 2005; Serrano et al., 2007).

The fat content in cooked products was affected by the formulation and the thawing/cooking method (Table 2). Pan frying increased the fat content ($p < 0.05$) except for MFP patties, in which fat retention ranged between 81% and 120% (Table 6). Similar results have been reported by Garcia-Arias, García-Linares, Capita, García-Fernández, and Sanchez-Muniz (2003), who found that the fat content increased in pan-fried chicken-burgers cooked with olive or sunflower oils. Clausen and Ovesen (2005) reported that pork patties with 18% fat initially lost 2.7% when pan-fried with margarine, while pork patties (11% fat initially) gained 0.4%. However Danowska-Oziewicz (2009) reported a loss of fat in pan-fried pork patties irrespective of the fat level. Also, Hoelscher et al. (1987) reported higher fat losses during pan-frying of beef patties as the fat level increased. Grilling only produced a significant increase of fat content in IMFP (Table 2). These results agree with the report by Serrano et al. (2007) in grilled restructured beef steaks with added walnut as fat source. Retention values ranged between 63% and 96% (Table 6), which are similar to the values found by Hur et al. (2008) in patties formulated with 5% pork backfat and 5% olive oil (in liquid form). Fat retention was higher ($p < 0.05$) in low-fat samples (LFP) with both cooking procedures (Table 6). It has been reported (Serrano et al., 2007; Sheard et al., 1998) that fat retention depends on different factors, among them the fat level: high-fat products tend to lose large amounts of fat during cooking while low-fat products lose relatively little fat. With similar fat levels (MFP versus IMFP), fat retention was greater ($p < 0.05$) (106%) in the sample with the oil bulking system (IMFP) (Table 6), indicating that there was no noticeable fat loss during cooking in those samples. These results confirm the good oil stabilization provided by the konjac-based oil bulking system, which helps to retain the fat and reduce fat loss during cooking.

The ash percentage increased ($p < 0.05$) in all patties with thawing/cooking, although the increase was generally greater with grilling (Table 2). Ash retention (ranging between 84% and 98%) was dependent on the fat level, with the highest values recorded in low-fat patties (LFP). Our results agree with reports by (Serrano et al., 2007) and Sheard et al. (1998) on grilled restructured steaks.

3.2.3. Energy content of cooked pork patties

Cooking produced an increase of energy values in all patties (Table 2). The contribution of fat to these energy values decreased with decreasing fat content and was generally smaller in grilled patties (Table 2). The contribution of specific fatty acids to total product energy was again related basically to the fat content in the cooked product, and to a lesser extent to the replacement of pork fat by the oil bulking system (Table 4). Generally speaking, the energy contribution of all groups of fatty acids declined as fat content decreased, irrespective of the cooking method ($p < 0.05$). In products with similar fat levels (MFP versus IMFP), the two cooking methods produced an increase in the contribution of MUFAs and PUFAs to the energy value, particularly in pan-frying and in IMFP sample (Table 4).

Table 6
Component retentions (%) of pork patties.

	CFP	MFP	LFP	IMFP
Moisture				
Electric grill	62.70 ± 0.93^{a1}	64.90 ± 0.47^{a2}	69.58 ± 0.40^{a3}	64.66 ± 0.28^{a2}
Pan-frying	66.83 ± 0.21^{b1}	68.12 ± 0.42^{b12}	74.33 ± 0.47^{b3}	69.16 ± 0.44^{b2}
Protein				
Electric grill	92.85 ± 0.51^{a3}	90.43 ± 0.79^{a2}	85.15 ± 0.58^{a1}	87.62 ± 1.41^{a12}
Pan-frying	95.12 ± 0.22^{b2}	88.78 ± 0.61^{a1}	89.49 ± 0.86^{b1}	88.15 ± 0.19^{a1}
Fat				
Electric grill	63.24 ± 2.81^{a1}	69.01 ± 2.90^{a1}	96.24 ± 1.90^{a3}	80.79 ± 4.21^{a2}
Pan-frying	90.23 ± 6.18^{b1}	81.87 ± 2.35^{b1}	120.43 ± 5.68^{b3}	106.19 ± 5.34^{b2}
Ashes				
Electric grill	85.04 ± 0.49^{a1}	85.90 ± 0.14^{a1}	89.74 ± 0.14^{a2}	83.99 ± 0.14^{a1}
Pan-frying	84.67 ± 0.81^{a1}	85.24 ± 2.57^{a1}	97.87 ± 0.60^{b2}	86.61 ± 1.17^{a1}

For sample description see Table 1. Different letters in the same column and different numbers in the same row indicate significant differences ($p < 0.05$).

Table 7
Fatty acid retention (%) of pork patties.

	CFP	MFP	LFP	IMFP
SFAs				
Electric grill	55.46 ± 0.17^{a1}	63.54 ± 0.60^{a2}	104.25 ± 0.32^{a4}	77.94 ± 0.10^{a3}
Pan-frying	82.73 ± 0.09^{b2}	73.21 ± 0.37^{b1}	108.59 ± 0.34^{b4}	100.82 ± 0.49^{b3}
MUFAs				
Electric grill	69.89 ± 0.17^{a1}	76.15 ± 0.19^{a2}	99.54 ± 0.53^{a4}	79.89 ± 0.16^{a3}
Pan-frying	104.16 ± 0.26^{b2}	93.57 ± 0.12^{b1}	147.26 ± 1.83^{b4}	111.23 ± 0.33^{b3}
PUFAs				
Electric grill	71.22 ± 0.12^{a1}	79.52 ± 0.71^{a2}	80.89 ± 0.36^{a2}	90.53 ± 0.52^{a3}
Pan-frying	92.14 ± 1.06^{b1}	92.96 ± 1.18^{b1}	100.99 ± 0.75^{b2}	113.51 ± 0.44^{b3}
n-6				
Electric grill	70,73 ± 0,55^{a1}	75,73 ± 0,69^{a2}	82,62 ± 0,25^{a2}	88,61 ± 0,14^{a3}
Pan-frying	91,40 ± 0,55^{b1}	90,71 ± 0,76^{b2}	103,18 ± 0,26^{b3}	114,99 ± 0,66^{b4}
n-3				
Electric grill	75,64 ± 2,24^{a1}	117,24 ± 1,52^{a3}	71.95 ± 1.08^{a1}	94,53 ± 1,72^{a2}
Pan-frying	98,77 ± 3,40^{b2}	115,72 ± 0.99^{a4}	89.72 ± 3.09^{b1}	110,50 ± 2,51^{b3}

For sample description see Table 1. Different letters in the same column and different numbers in the same row indicate significant differences ($p < 0.05$).

3.2.4. Fatty acid content and nutritional ratios of cooked pork patties

Fatty acid concentrations in cooked patties were affected by the formulation and the cooking method (Tables 3 and 4). Compared with raw products, cooked samples generally had higher ($p < 0.05$) concentrations of MUFAs and PUFAs (both n-3 and n-6); this difference was larger ($p < 0.05$) in pan-fried patties. Cooking affected SFA content differently; concentrations increased in LFP and IMFP samples but decreased in samples in which pork backfat was the only lipid source, as indicated by SFA retentions (Tables 3, 4 and 7). Increases in fatty acid concentrations as a result of cooking have been reported in trimmed beef muscles (Badiani et al., 2002) and in restructured beef steaks (Librelotto et al., 2008), although this effect has been reported to vary (with fatty acid content remaining stable or decreasing) depending on the composition of the burgers (López-López et al., 2011; Rodríguez-Carpena, Morcuende, & Estévez, 2011). In general, the differences in behavior of fatty acid content were due mainly to weight losses during thawing/cooking (Table 5), and hence to changes in fat and fatty acid retentions (Tables 6 and 7). Fatty acid retention (Table 7) was affected by the formulation and the cooking method ($p < 0.05$). In all the reformulated patties, fatty acid retention (SFAs, MUFAs and PUFAs) was generally higher than in normal fat patties (CFP) (Table 7). As a function of lipid material, the samples with the konjac-based oil bulking system showed higher fatty acid retention than samples in which the proportion of lipids was the same but the fat source was animal. These results confirm the good retention properties of the bulking system. As a function of cooking method, fatty acid retention was higher in pan-fried than in grilled samples. Additionally, fatty acid retention was higher in grilled reformulated patties ($p < 0.05$) than in control sample (CFP). It has been suggested that during frying changes in fatty acids occur due to the existence of fatty acid gradients from the frying medium (olive oil) to the food and vice versa. However, the proportion of fatty acid exchanged is not all the same (Librelotto et al., 2008). Of the major fatty acids and fatty acid groups, MUFAs (particularly oleic acid) were the ones whose concentrations increased most in all patties (Tables 3 and 4). SFA concentrations varied in line with sample fat contents; between 5406 and 7372 mg/100 g in CFP and between 1417 and 1368 mg/100 g in LFP (grilled and pan-fried respectively), and a similar effect was observed in the case of MUFAs and PUFAs. Pan fried control sample contained the most ($p < 0.05$) oleic acid (7507 mg/100 g) (Table 3). In cooked IMFP, PUFA contents ranged from 1380 to 1602 (469–508 n-3 PUFAs) mg/100 g for grilled and pan-fried products respectively (Table 4), with EPA + DHA concentrations of around 75 mg/100 g (Table 3).

PUFA/SFA ratios were generally higher in cooked than in raw samples (Table 5). Other authors reporting similar behavior in grilled beef patties (Gerber, Scheeder, & Wenk, 2009; Scheeder et al., 2001) suggested that unsaturated fatty acids, especially PUFAs, are less affected by cooking since they are more fully a part of the membrane structure than SFAs. Therefore, the proportional change in fatty acid composition may thus be explained by loss of lipids containing mainly triacylglycerols of adipose tissues with relatively more saturated than unsaturated fatty acids. However, in the case of beef patties, the PUFA/SFA ratio has been found to be higher in raw than in grilled samples (Bilek & Turhan, 2009). On the other hand, no changes were observed in PUFA/SFA ratios in beef muscle as affected by various cooking methods (boiling, broiling, oven roasting and microwaving) (Badiani et al., 2002). Of all the cooked patties, only the ones containing the konjac-based oil bulking system (IMFP) had n-6/n-3 ratios within the recommended range (<4) (Table 4). As in the present study, Librelotto et al. (2008) found that the n-6/n-3 ratio increased in restructured beef steak after pan-frying. Cooking reduced ($p < 0.05$) the atherogenicity and thrombogenicity indexes in all samples except for grilled LFP (Table 4). Similar results have been reported in pan-fried restructured beef steak (Librelotto et al., 2008) and oven-cooked beef patty (López-López et al., 2011).

4. Conclusion

Strategies based on the use of fat replacers such as konjac gel to reduce fat and a konjac-based oil bulking system to improve fatty acid profiles were successfully used to produce pork patties with healthier lipid formulations. The composition of these products was conditioned by the formulation and the cooking method (electric grilling or pan-frying in olive oil). Cooking caused loss of matter (mainly fat), more so in the case of grilling. Fat loss significantly affected the fat and fatty acid contents and the energy values of products. These results confirm that the konjac-based oil bulking system may be considered a good oil stabilization procedure since it helps to retain the fat and reduce fat loss during cooking. By using fat replacers such as konjac gel and konjac-based oil bulking system to improve fatty acid profiles, it was possible to produce pork patties with healthier lipid composition.

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4.2 EMPLEO DE PARTÍCULAS DE HIDROGEL ENCAPSULANDO ACEITE DE PESCADO COMO ESTRATEGIA EN EL DESARROLLO DE PRODUCTOS CÁRNICOS MÁS SALUDABLES

4.2.1 Oxidative stability of n-3 fatty acids encapsulated in filled hydrogel particles and of pork meat systems containing them.

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Oxidative stability of n-3 fatty acids encapsulated in filled hydrogel particles and of pork meat systems containing them

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ABSTRACT

The effect of storage time (2 °C, 19 days) and heating (70 °C, 30 min) on physical characteristics and oxidative stability of fish oil encapsulated in filled hydrogel particles was determined and compared with a conventional oil-in-water (O/W) emulsion with the same oil content (8.5%). Subsequently they were used to enrich meat systems with n-3 LCPUFAs, and their lipid oxidation was evaluated and compared with two other meat systems: one containing all animal fat and another with fish oil added directly. Filled hydrogel particles were more effective in lowering the oxidation rate than O/W emulsion, even when thermal treatment was applied. Oxidative stability over the storage time was best in the n-3 LCPUFA-enriched meat system containing filled hydrogel particles, in which TBARS levels were up to 62% lower than other systems containing fish oil. Hydrogel particles offer a promising means of controlling lipid oxidation in n-3 LCPUFA-enriched meat products.

1. Introduction

Health benefits provided by consumption of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, have been widely documented. Indeed, most nutritional guidelines now include recommendations to increase the intake of n-3 LCPUFA (Aranceta & Pérez-Rodrigo, 2012). However, dietary intake of these fatty acids is generally low in western societies due to low consumption of the richest sources (fish oil and seafood) (Meyer, 2011). One possible strategy for enhancing the intake of these fatty acids is to develop food products fortified with fish or algae oil, the most abundant and cheapest sources of EPA and DHA. Nevertheless, although attractive, this alternative has a drawback in the high oxidative instability of n-3 LCPUFA. Lipid oxidation of LCPUFA-rich foods is a serious problem that often negatively affects shelf-life, consumer acceptability, functionality, nutritional value and safety (Arab-Tehrany et al., 2012). Providing the means to deliver n-3 LCPUFA effectively to the body through food therefore requires novel approaches.

Emulsion technology is particularly suited to the design and fabrication of delivery systems for encapsulating bioactive lipids such as n-3 LCPUFA. Oil-in-water (O/W) emulsions are the most common type of emulsion-based systems for delivery of bioactive lipids because of their relative ease of preparation and low cost. However, O/W emulsions have limited potential in terms of their ability to afford bioactive protection. This has led to the development of more complexly-structured emulsions such as filled hydrogel particles. These systems consist of oil droplets (O) trapped inside a hydrogel matrix (W_1), which is dispersed in an aqueous medium (W_2) that may be described as an $O/W_1/W_2$ type of structured emulsion (Matalanis & McClements, 2013). Filled hydrogel particles have the potential to provide certain benefits over conventional O/W emulsions, such as enhanced stability, or targeted delivery in the body (McClements, Decker, & Weiss, 2007). In this connection it has been reported that the lipid material present in filled hydrogel particles is highly accessible for digestion and absorption in the body and therefore this delivery system would be appropriate for incorporating lipid-based bioactives in foods (Matalanis & McClements, 2012b). Nevertheless, there is still significant room for improvement, particularly in the area of increasing lipid loading capacity and particle yields (Matalanis & McClements, 2013). Thus, such delivery systems, designed to make an important contribution to dietary n-3 LCPUFA recommended intakes, may be incorporated in real foods.

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LCPUFA enrichment is especially challenging in meat products. High concentrations of prooxidants (e.g. transition metal and haeme-containing proteins), meat processing operations like grinding and thermal treatment, and addition of potential prooxidant ingredients (e.g. sodium chloride) are conditions that reduce oxidative stability (Lee, Decker, Faustman, & Mancini, 2005).

Other omega-3 delivery systems have been tested in meat systems. However, to our knowledge, the ability of filled hydrogels to protect omega-3 fatty acids in meats has not been tested. The filled hydrogels used in this study could have several advantages for meat systems because they are structurally more robust than conventional emulsions (Matalanis & McClements, 2012b) and thus the integrity of the particle is more likely to survive the thermal processing of meats. In addition, the filled hydrogels used in this study are stabilized with casein and pectin. These biopolymers could potentially play a due role in both physically and chemically stabilizing the omega 3 fatty acids since both proteins and anionic polysaccharides have been reported to have antioxidant activity (Chen, McClements, & Decker, 2010; Elias, Kellerby, & Decker, 2008). Therefore the goals of this study were (1) to assess physical characteristics and oxidative stability of filled hydrogel particles (fresh and heated 70 °C/30 min), that contain nutritionally significant amounts of n-3 LCPUFA when used as a food ingredient. This system was compared with an O/W emulsion containing the same type and amount of lipid material. (2) To evaluate the lipid oxidation of n-3 LCPUFA-enriched pork meat systems by incorporation of fish oil added in three different ways: (a) directly; (b) stabilized in a O/W emulsion; and (c) stabilized in filled hydrogel particles. These were compared with a pork meat system containing all animal fat. The influence of chilled storage (19 days at 2 ± 2 °C) was also considered.

2. Materials and methods

2.1. Materials

Fish oil (Omevital™ 1812 TG Gold, BASF SE, Ludwigshafen, Germany) was used as a n-3 LCPUFA source and contained 169 mg of EPA/g and 110 mg of DHA/g plus mixed tocopherols, as specified by the supplier. Sodium caseinate (90.5% protein and 5.5% moisture, information provided by the manufacturer) was from DMV (Excellion EM 7, DMV Campina B.V.; Veghel, The Netherlands). Highly methyl-esterified pectin (GRINDSTED® Pectin USP, Danisco; Grindsted, Denmark) was used. Composition according to the supplier: 9% moisture, 85.3% galacturonic acid and minimum 6.7% methoxy content. The enzyme used was transglutaminase (TG) (Activa® WM – 99% maltodextrine and 1% TG – Ajinomoto Co.; Tokyo, Japan) with an activity of approx. 100 U/g of powder. Cumene hydroperoxide and 1,1,3,3-tetraethoxypropane were obtained from Sigma–Aldrich (Steinheim, Germany). 2-Thiobarbituric acid was purchased from Merck (Darmstadt, Germany). All other chemicals used in this study were purchased from Panreac Química, S.A (Barcelona, Spain). Distilled water was used throughout the study. Sufficient fresh post-rigor pork meat (mixture of M. biceps femoris, M. semimembranosus, M. semitendinosus, M. gracilis and M. adductor) and pork backfat, both from different animals were obtained from a local market.

2.2. Preparation and characterization of delivery systems

2.2.1. Preparation of conventional O/W emulsion

To prepare the O/W emulsion an emulsifier solution (2%, w/w) was first prepared by dispersing and stirring sodium caseinate into a buffer solution (0.04% sodium azide, 10 mM phosphate buffer, pH 7) at room temperature until fully dissolved and then storing the solution overnight at 4 °C. A coarse pre-emulsion was formed by homogenizing 20% (w/w) of fish oil with 80% (w/w) of emulsifier solution using a high-speed hand blender (Taurus, Barcelona, Spain) at medium speed for 2 min. Thereafter, the coarse emulsion was passed twice through a two-stage high pressure homogenizer at 750/50 bars (first-stage pressure/s-stage pressure; Panda Plus 1000, GEA NiroSoavi, Parma, Italy). The O/W emulsion was kept chilled (2 °C) until use.

2.2.2. Preparation of filled hydrogel particles

2.2.2.1. Preparation of solutions and O/W emulsion. Solutions were prepared according to Matalanis and McClements (2013) using pectin and sodium caseinate. Briefly, a pectin-rich solution containing 4.03% pectin and 0.22% sodium caseinate and a sodium caseinate-rich solution with 0.52% pectin and 14.2% sodium caseinate were prepared in 10 mM phosphate buffer pH 7 with 0.04% sodium azide using a mechanical stirrer (RW 20; Janke & Kunkel IKA Laboratortechnick, Staufen, Germany). Following dispersion, the pH of the solutions was adjusted to pH 7 with 4 M sodium hydroxide. An oil-in-water emulsion with 50% fish oil was prepared as previously described for conventional O/W emulsion.

2.2.2.2. Formation of filled hydrogel particles. Filled hydrogel particles formation was a multistep process carried out according to Matalanis and McClements (2013) with modifications to increase the lipid loading capacity of the delivery system. The general scheme for forming these particles involved creating an O/W emulsion of fish oil using sodium caseinate as a surfactant. This emulsion was then mixed with a water-in-water (W_1/W_2) emulsion made up of two biopolymer phases where the continuous phase was rich in polysaccharides (pectin-rich) while the dispersed phase was rich in protein (caseinate-rich). It was found that emulsified oil droplets added to this system preferentially partition into the dispersed (casein-rich) phase, forming an $O/W_1/W_2$ emulsion (Matalanis, Lesmes, Decker, & McClements, 2010).

The procedure was as follows: 5% (v/v) of casein-rich phase, 90% (v/v) of pectin-rich phase, and 5% (v/v) of a 50% (v/v) fish O/W emulsion were mixed with a stirrer (RW 20; Janke & Kunkel IKA Laboratortechnick, Staufen, Germany) at 300 rpm for 30 min to ensure homogeneity. During mixing, the pH of the mixture was measured and adjusted to 7. Environmental conditions were then altered by lowering the pH to 5 by adding one drop of 1 M citric acid every 10 s as the mixture was stirred at 300 rpm, allowing adsorption of the pectin-rich phase (W_2) around the casein-rich W_1 droplets, thus forming the $O/W_1/W_2$ system. After that, transglutaminase was used to cross-link the casein present in the W_1 phase so as to further stabilize these particles. To aid in the dispersion of transglutaminase, 10 g of powdered Activa® WM were homogenized for 30 s in 20 ml of 10 mM phosphate buffer adjusted to pH 5, at high speed in an Omnimixer blender (ES Homogenizer, OMNI International Inc., Gainsville, VA, USA). This solution was then added to the mixture of filled hydrogel particles in a proportion of 50 Units of enzyme activity per gram of protein using an overhead stirrer at a speed of 300 rpm for 10 min. This mixture was then transferred to a 50 °C water bath and incubated for 15 min with constant stirring at 300 rpm. The mixture was then removed from the water bath and cooled at room temperature for 20 min, still under constant stirring. After cooling, the pH of the sample was adjusted from 5 to 7 using sodium hydroxide (1 M), then the mixture was stirred for a further 20 min. Sample was stored overnight at 2 °C. At the end of the overnight storage, hydrogel particles were washed to remove the pectin-rich phase and thus concentrate the fat in the delivery system. One part hydrogel particles to four parts 10 mM phosphate buffer (pH 7) was mixed for 1 h and then centrifuged using a Sorvall Evolution RC Centrifuge (Kendro Laboratory Products; Asheville, NC, USA)

at 10,000*g*. The washing solution (along with most of the continuous phase) was decanted and the washed particles were collected in a glass beaker at 2 °C until use.

2.2.3. Determination of delivery system oil content

The oil content in the O/W emulsion and filled hydrogel particles was determined to ensure that both had the same oil concentration. Lipid quantification was performed in triplicate according to the method of Iverson, Lang, and Cooper (2001). Briefly, 1 g of sample was vigorously vortexed three times with 7.5 ml of chloroform–methanol (2:1 v/v) followed by centrifugation for 30 min at 2400*g*. Following centrifugation, 2 ml from the lower solvent phase was placed in pre-weighed test tubes and caps, and the solvent was evaporated under a stream of nitrogen. The amount of extracted oil was then determined gravimetrically. As the O/W emulsion was more concentrated, it was diluted with phosphate buffer (10 mM, pH 7) to achieve the same oil concentration as the filled hydrogel particles (8.5%, w/w).

2.2.4. Sample treatments and storage conditions

Since heating is used in most meat processing operations, it was decided to observe the effect of thermal treatment on the physical and oxidative stability of the delivery systems in time and temperature conditions similar to those used in meat processing.

Seven grams of each delivery system was weighed into 15 ml test tubes and then half of them were heated in a water bath (70 °C for 30 min). The other half were unheated. The test tubes were then stored in the dark at 2 °C for 19 days. Test tubes were randomly taken for different analyses, which were performed at days 1, 6, 12 and 19 of storage.

2.2.5. O/W emulsion and filled hydrogel analyses

2.2.5.1. Particle size measurement. Particle size distribution (PSD) of oil droplets in both delivery systems was measured by static light scattering using a Malvern Mastersizer S laser diffraction particle size analyzer (Malvern Instrument Ltd., Worcestershire, UK). The measurement range was 0.05–900 μm. Obscuration was in the range of 8–15%. Particle size calculations were based on the Mie Scattering theory. Average diameter ($d_{3,2}$ and $d_{4,3}$) was measured immediately after addition to the dispersion unit. Results as reported were averages of at least three measurements.

2.2.5.2. Determination of lipid oxidation in delivery systems. In this study, the lipid hydroperoxide and thiobarbituric acid-reactive substance (TBARS) concentrations were used as measures of the primary and secondary oxidation products respectively.

Lipid hydroperoxides were measured in triplicate according to Matalanis, Decker, and McClements (2012) with some minor modifications. Briefly, 1 g of sample was vigorously vortexed three times with 7.5 ml of chloroform–methanol (2:1, v/v) followed by centrifugation for 30 min at 3000*g*. Following centrifugation, 0.2 ml of the chloroform/methanol extract (lower layer) was carefully removed and mixed with 2.8 ml of methanol/1-butanol (2:1, v/v). Depending on the concentration of hydroperoxides, the chloroform/methanol extract was diluted with solvent so that the final absorbance of the reacted sample remained below 1.0. This mixture (3 ml) was then reacted with 15 μl of 3.94 M ammonium thiocyanate and 15 μl of ferrous iron solution (prepared by reacting 0.132 M barium chloride and 0.144 M ferrous sulfate). The mixture was vortexed and allowed to react for 20 min at room temperature in the dark, then the absorbance of the sample was measured at 510 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan). The concentration of hydroperoxides was determined based on a standard curve of cumene hydroperoxide (0–20 μM). Results were expressed as mmol hydroperoxides/kg sample.

TBARS were measured in triplicate by a method adapted from Serrano, Cofrades, and Jimenez-Colmenero (2006). Briefly, 3 g of each sample were homogenized for 1 min in 35 ml of 7.5% trichloroacetic acid at high speed in an Omnimixer blender. The blender sample was centrifuged at 3000*g* for 30 min (Heraeus multifuge 3L-R DJB Labcare Ltd.; Buckinghamshire, UK) and 5 ml of the supernatant were mixed with 5 ml of 20 mM thiobarbituric acid. Finally the solution was mixed and kept in the dark at 20 °C for 20 h. The pink color that formed was measured spectrophotometrically at 532 nm. A calibration curve was plotted using 1,1,3,3-tetraethoxypropane as the malondialdehyde (MDA) source, and results were expressed as mg MDA eq/kg of sample.

2.3. Pork meat systems

2.3.1. Design and preparation

Once the oxidative stability of the O/W emulsion and filled hydrogel particles was assessed, the next step was to establish how lipid oxidation of a meat system was affected by the incorporation of these n-3 LCPUFA delivery systems. To that end the impact of processing and storage on oxidative stability of encapsulated n-3 LCPUFA in the meat systems was examined. The meat systems were formulated so that the final products would contain adequate nutritional intakes of EPA and DHA. Recommended intakes of EPA and DHA range between 0.3 and 1 g/day for healthy people (Meyer, 2011; WHO, 2003). Therefore, in order to provide nutritionally significant amounts of n-3 LCPUFA, the meat systems were designed to contain 0.6 g of EPA and DHA/100 g of product (Table 1). This formulation was achieved by replacing the fat content from pork backfat with the same proportion of fat from the delivery systems. Thus, four formulations were prepared with the same protein and fat contents: a control meat system (MS/C) with all pork fat; a meat system with fish oil added directly (MS/O); a meat system with fish oil stabilized in the conventional O/W emulsion (MS/E); and a meat system with fish oil incorporated in filled hydrogel particles (MS/H) (Table 1).

Pork meat and pork backfat were passed through a grinder with a 0.6 cm plate (Mainca, Granollers, Spain). Lots of approximately 500 g were vacuum packed and frozen (at −18 °C) until use. For meat system preparation, the meat and fat were thawed for about 18 h at 2 ± 2 °C up to a temperature between −1 and −2 °C. The meat was homogenized for 1 min in a grinder/homogenizer connected to a cooling bath (2 °C) (Stephan Universal Machine UM5, Stephan u. Söhne GmbH and Co., Hameln, Germany). Half of the fat or delivery system (depending on the formulation) was mixed for 1 min with sodium chloride and water. Then the remaining half of the ingredients was added and the whole mixture was homogenized for 1 min. The final mass was homogenized under vacuum for 2 min. The final temperature was below 12 °C in all cases. Portions of each meat system (approximately 70 g) were placed in plastic containers (diameter 3.5 cm, height 7 cm), which were hermetically sealed. They were then heated for 30 min in a water bath at 70 °C. Thermocouples connected to a temperature recorder were used to establish the thermal conditions required to reach an internal temperature of 70 °C (DaqPRO 5300 Data recorder; OMEGA Engineering, Inc.; Stamford, CT, USA). Samples were stored at 2 °C and analyzed on days 1, 6, 12 and 19. The entire meat system processing procedure was replicated twice on two different days.

2.3.2. Proximate analysis

Moisture and ash contents of the cooked meat systems were determined according to AOAC (2005). Protein content was measured with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Fat content was evaluated

Table 1
Formulation (%) of meat systems.

Sample	Meat	Backfat	Fish oil	n-3 LCPUFAs delivery systems	Added water
MS/C	70.0	2.3	–	–	25.7
MS/O	70.0	–	2	–	4.5
MS/E	70.0	–	–	23.5	4.5
MS/H	70.0	–	–	23.5	26.0

Sample denomination: MS/C, control meat system (MS/C) with all pork fat; MS/O, meat system with fish oil added directly; MS/E, meat system with fish oil stabilized in a O/W emulsion; MS/H, meat system with fish oil incorporated in filled hydrogel particles. All samples also contained 2% NaCl.

following the method reported by Bligh and Dyer (1959). All analyses were done in triplicate.

2.3.3. Determination of lipid oxidation in the meat systems

For meat samples, lipid hydroperoxides were measured in triplicate as described in Section 2.2.5.2, but with some modifications. Two grams of sample were homogenized for 30 s with 25 ml of chloroform–methanol (1:1, v/v) using a high speed Omnimixer blender. The mixture was then transferred to falcon tubes and 6 ml of a 0.5% NaCl solution was added and the whole centrifuged for 30 min at 3000*g*. The rest of the method was as described above (Section 2.2.6). Results were expressed as mmol hydroperoxides/kg sample.

TBARS were determined in triplicate as reported by Serrano et al. (2006) and results were expressed as mg MDA eq/kg sample.

2.4. Statistical analysis

The entire trial was replicated. One-way analysis of variance (ANOVA) to evaluate the statistical significance ($P < 0.05$) of the formulation, and two-way ANOVA as a function of formulation and storage time were carried out using general linear model (GLM) procedure of SPSS Statistics (v.20, IBM SPSS Inc.; Chicago, IL, USA). Formulation and storage time were assigned as fixed effects and replicate was assigned as random effect. Least squares differences were used for comparison of mean values between treatments and Tukey's HSD test to identify significant differences ($P < 0.05$) between formulations and storage time.

3. Results

3.1. Filled hydrogel particles

3.1.1. Physical characteristics of filled hydrogel particles

The PSD and mean particle diameters ($d_{3,2}$ and $d_{4,3}$) of the samples are shown in Fig. 1 and Table 2 respectively. Since PSD and mean particle diameter values were not influenced ($P > 0.05$) by low temperature storage, Fig. 1 shows the PSD of the samples at day 1 and Table 2 shows the mean values over the entire storage period. As was to be expected, O/W emulsions contained smaller ($P < 0.05$) particles than the filled hydrogel particles. This could be due to the fact that O/W emulsions were formed by intense homogenization conditions (750 bars), whereas hydrogel particles were produced under relatively mild shearing conditions (300 rpm). Emulsion particle sizes displayed a unimodal distribution with sizes between 0.1 and 10 μm, while hydrogel particles presented a trimodal distribution (Fig. 1), with particles distributed in three clearly defined areas: a small peak (~16%, v/v) composed of small particles ranging between 0.4 and 2.6 μm; a predominant peak (~72%, v/v) with particle sizes ranging from 3.0 to 56.2 μm; and finally a small peak (~12%, v/v) with large particles, sized between 65.5 and 301.7 μm.

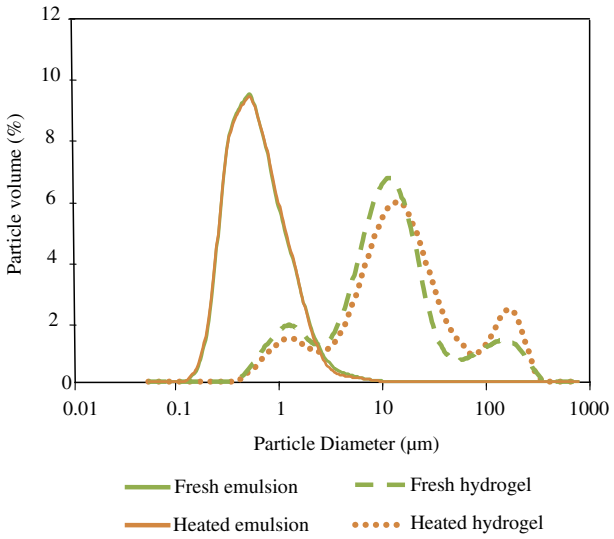

Fig. 1. Particle size distribution at day 1 of fresh and heated O/W emulsions and filled hydrogel particles.

Table 2
Particle size diameter (μm) in fresh and heated O/W emulsions and filled hydrogel particles.

Sample	$d_{3,2}$	$d_{4,3}$
Fresh emulsion	0.49 ± 0.01[a]	0.79 ± 0.07[a]
Fresh hydrogel	4.89 ± 0.48[b]	31.19 ± 7.63[b]
Heated emulsion	0.48 ± 0.02[a]	0.72 ± 0.08[a]
Heated hydrogel	5.14 ± 0.21[b]	33.12 ± 3.16[b]

Means ± SD. Different letters in the same column (a, b) indicate significant differences ($P < 0.05$). Since D[3,2] and D[4,3] were not influenced ($P > 0.05$) by chilled storage, data reported are the mean values over the storage period.

The region of small particles could reflect emulsion droplets that were not embedded in the hydrogel system while the large particles could be the result of aggregation of particles produced by the action of the transglutaminase used in the fabrication of the system as reported by Matalanis and McClements (2013). The distributions found in this study are in line with findings reported in sodium caseinate-stabilized oil-in-water emulsion and filled hydrogel particles, both containing 1% of fish oil (Matalanis et al., 2012).

In the case of emulsions, no differences between samples were observed in PSD, $d_{3,2}$ or $d_{4,3}$ diameters after heating (Fig. 1 and Table 2), indicating that thermal treatment did not affect particle size in this system. In the case of the hydrogel particles, heated sample registered a slight increase of volume of the largest particles (~3% more than the fresh hydrogel), coinciding with a decrease in the population of smaller particles. The volume of heated particles in the main peak (72.9%, v/v) was practically the same as in the fresh sample; the particle size range incremented respect to the fresh sample, recording values up to 76.0 μm (Fig. 1). However, no such differences were observed between mean particle size diameters ($P > 0.05$) (Table 2) in fresh and heated hydrogels, which would suggest that the changes produced by heating were not important. Matalanis and McClements (2012a) reported that thermal treatments (40–90 °C, 20 min) had no impact on the particle size of filled hydrogel particles containing 1% corn oil. Therefore, the hydrogel studied should be able to withstand the conventional thermal stresses occurring in meat processing.

3.1.2. Oxidative stability of filled hydrogel particles

The concentrations of primary oxidation products (lipid hydroperoxides) in fresh and heated O/W emulsions and filled hydrogel particles are presented in Fig. 2a. Initial hydroperoxide values (day 1) were similar ($P > 0.05$) in both fresh emulsion and fresh hydrogel systems.

Differences in hydroperoxide formation during storage seemed to be associated particularly with the kind of delivery system, and to a lesser extent with the thermal treatment (Fig. 2a). In general, during storage more hydroperoxides ($P > 0.05$) were formed in the O/W emulsions than in the hydrogel particles. Concentrations of hydroperoxides were slightly higher in O/W emulsions that were heated. At the end of storage, hydroperoxide levels in O/W emulsions decreased significantly ($P < 0.05$), possibly due to conversion from primary to secondary oxidation products. In the case of hydrogel particles, on the other hand, oxidation rates over the storage time were lower. Heating of hydrogel particles produced only minor increases of hydroperoxide during storage.

TBARS were chosen firstly because to assess lipid oxidation because they are effective at determining secondary lipid oxidation aldehydes, especially those produced from the oxidation of omega-3 fatty acids (Guillén-Sans & Guzmán-Chozas, 1998). Second, TBARS has been found to correlate strongly with sensory analysis for the determination of oxidative rancidity as well as hexanal content of cooked ground pork (Shahidi & Hong, 1991; Shahidi, Yun, Rubin, & Wood, 1987). Finally, headspace analysis of aldehydes in meats is limited by the interactions of the meat proteins with compounds such as hexanal resulting in decreased headspace concentrations meaning that these techniques can underestimate oxidation (Pignoli, Bou, Rodriguez-Estrada, & Decker, 2009). The acid environment under which TBARS analysis is performed helps to release aldehydes from proteins making of this an excellent methods to measure lipid oxidation in meats. However, one can criticize TBARS for its lack of specificity, thus lipid hydroperoxides were also determined in all samples.

As in the case of primary oxidation compounds, no major differences were detected in TBARS between fresh O/W emulsion and hydrogel particles at the outset of storage (Fig. 2).

As with hydroperoxides, TBARS values were higher ($P < 0.05$) in O/W emulsions than in hydrogels. Fresh O/W emulsion and hydrogel particles both increased sharply until day 6 and thereafter increased moderately up to day 12.

Thermal treatment produced an increase of TBARS concentrations in both systems (Fig. 2b). However, the increase in both the velocity and the amount of TBARS was more pronounced in heated emulsions than in heated hydrogel particles. TBARS concentrations in the emulsion samples declined during the later stages of storage, presumably due to secondary products reacting with other components such as proteins.

3.2. Pork meat systems: oxidative stability

Since hydrogel particles are more oxidatively stable than traditional emulsions in an aqueous system, the next step was to assess the lipid oxidation in meat systems with a hydrogel containing n3-LCPUFA (MS/H) and compare it with meat systems containing the same amount of fish oil but incorporated either by direct addition (MS/O) or as an O/W emulsion (MS/E). In addition, lipid oxidation was monitored in the meat system where all the lipid came from the pork (MS/C). All the meat systems showed similar proximate composition, with moisture values between 77.0% and 78.2%, protein ranging from 16.5% to 17.6%, fat levels between 2.4% and 2.7% and ash values around 2.8%.

Hydroperoxide results in meat systems are shown in Fig. 3a. At the outset of the experiment, all samples with fish oil registered high hydroperoxide levels ($P < 0.05$) indicating that the omega-3

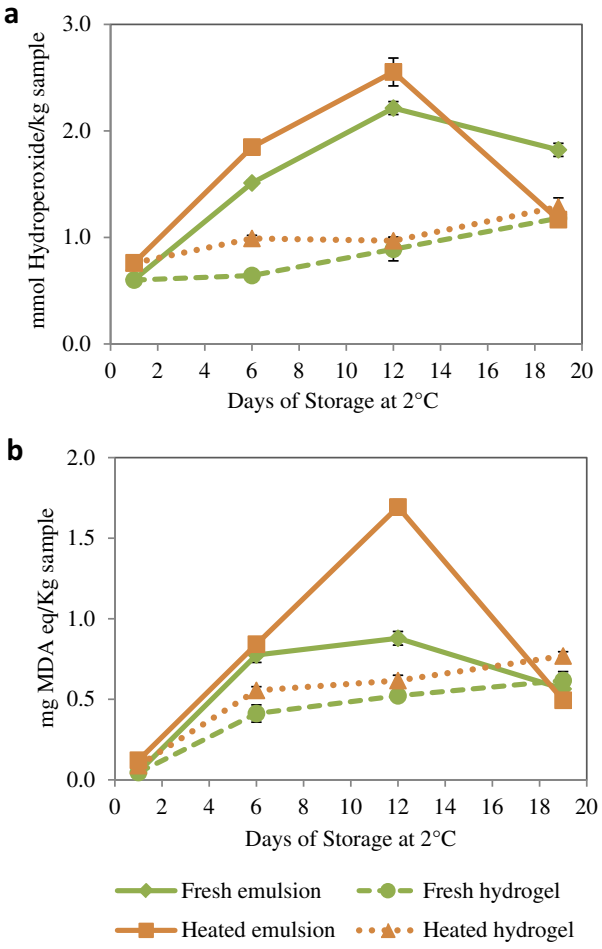

Fig. 2. Concentration of (a) primary reaction products (hydroperoxides) and (b) secondary reaction products (TBARS expressed as malondialdehyde equivalents) in fresh and heated O/W emulsions and filled hydrogel particles during chilled storage (2 °C).

fatty acids could quickly oxidize. At day 6, the recorded hydroperoxide concentrations in emulsions were, in descending order: emulsions > direct oil addition > hydrogels > pork fat. The hydroperoxides in the samples with direct oil or emulsion addition declined from this point until the end of storage, indicating that these oils were oxidizing faster than the others.

TBARS levels are presented in Fig. 3b. TBARS in the all-pork-fat control system cannot be compared directly with samples containing fish oil given that oxidation of fish oil will produce a much higher yield of TBARS due to their higher degree of unsaturation. However, the low TBARS values ($P < 0.05$) in the all-pork-fat control is consistent with the hydroperoxide data, indicating that oxidation proceeded more slowly in these samples than in the samples with fish oil. At days 1 and 6, TBARS levels were highest in the samples with oil added directly or in an emulsion. This is again consistent with the hydroperoxide results, indicating that these samples were less oxidatively stable. TBARS levels were lower in the samples with hydrogels, but oxidation increased during storage.

4. Discussion

Regarding the oxidative stability of the delivery systems, both primary and secondary oxidation products indicated that the filled hydrogel particles were more effective in lowering the rate than O/

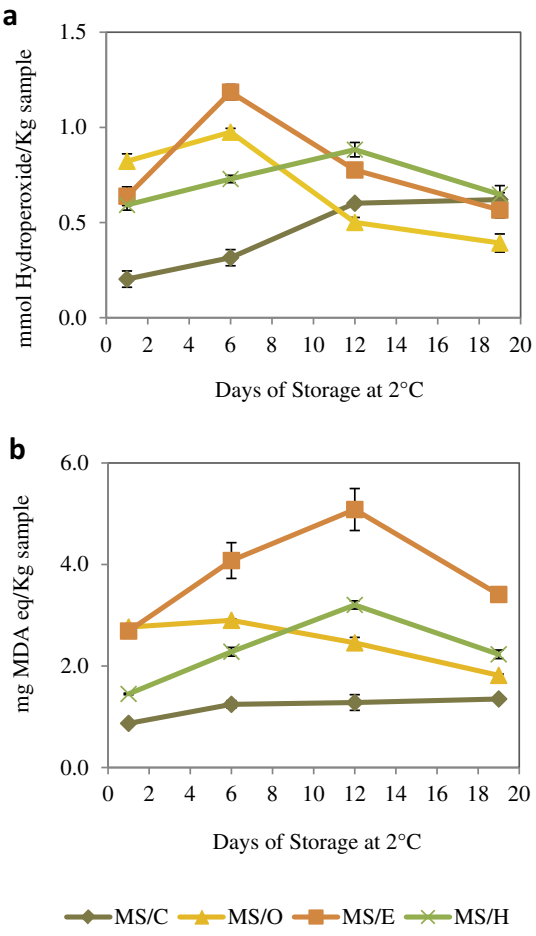

Fig. 3. Concentration of (a) primary reaction products (hydroperoxides) and (b) secondary reaction products (TBARS expressed as malondialdehyde equivalents) in pork meat systems during chilled storage (2 °C).

W emulsion, even when thermal treatment was applied (Fig. 2a and b).

Studies on oxidation in food emulsions have shown that both interfacial proteins and proteins in the continuous phase of an emulsion can enhance oxidative stability (Faraji, McClements, & Decker, 2004; Waraho, McClements, & Decker, 2011). Casein has antioxidant properties due to its ability to scavenge free radicals (Díaz & Decker, 2004) and to chelate transition metals through its phosphorylated serine residues (Díaz, Dunn, McClements & Decker, 2003). At pH 7.0, the casein would produce a negative charge at the emulsion droplet interface that could attract prooxidative metals and accelerate lipid oxidation (Waraho et al., 2011). However, any protein not adsorbed to the emulsion droplet surface or entrapped in the hydrogel particles would partition into the continuous phase. According to Faraji et al. (2004), this aqueous phase protein could bind transition metals and inhibit them from interacting with the lipids in the emulsion droplets. However, in the case of the hydrogel particles, since the emulsified lipid droplets were embedded in a casein-rich hydrogel particle as depicted in Fig. 4, there would be more protein near the emulsion droplet and this higher casein concentration could potentially be more effective at scavenging free radicals and chelating metals. In addition, the hydrogels contain pectin, which could also chelate metals and protect the lipid (Chen et al., 2010). Thus, the higher oxidative stability of the hydrogel particles could be due to differences in biopolymer concentrations as well as close proximity of the biopolymers to the lipid droplets.

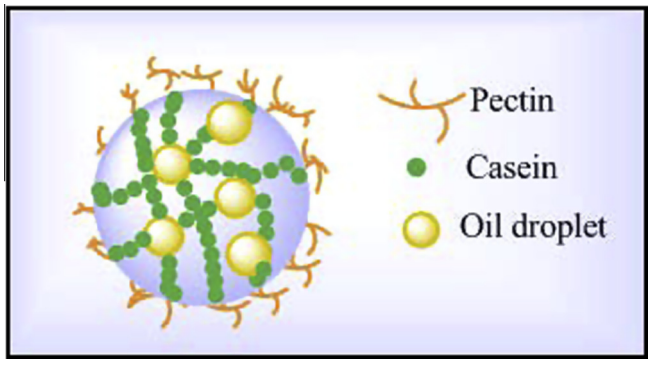

Fig. 4. Structure of a filled hydrogel particle.

Matalanis et al. (2012) concluded that there was no significant benefit to using filled hydrogel particles over a conventional casein-stabilized O/W emulsion (both systems containing 1% fish oil), as both systems had similar oxidative stability. The authors attributed this to the possibility that the difference in protein concentration between the filled hydrogel particles and the casein stabilized emulsion (~2.7% and 0.09%, respectively) was not large enough for the delivery systems to show differences in their ability to inhibit lipid oxidation. In the present study, the amount of casein was greater in both systems (O/W emulsion 0.8% and hydrogel particles 3.2%) due to the increased oil content, but the caseinate/oil ratios in our delivery systems were similar to those reported by Matalanis et al. (2012). Nevertheless, some modifications were made in order to enhance the loading capacity of the systems: the oil concentration was increased by incorporation of an O/W emulsion containing more fish oil (50% versus 20% reported by Matalanis et al. (2012), and in the present study the filled hydrogel particles were centrifuged to increase their concentrations. This washing step could have removed prooxidants, thus increasing oxidative stability.

Finally, one of the goals of this paper was to evaluate oxidative stability in meat systems formulated with filled hydrogel particles as food ingredients to enhance the stability of n-3 LCPUFAs. Oxidation is a particular problem in enriched n-3 LCPUFA meat products. Composition factors like the presence of salts, metal ions, varying amounts of proteins and polyunsaturated lipids, as well as processing conditions like grinding, heating, storage, etc., render these products more susceptible to lipid oxidation. The meat systems formulated in this experiment contained high levels of unsaturated fatty acids (over 600 mg of EPA and DHA/100 g of product); they also presented structural disintegration, promoting the exposure and accessibility of labile compounds to oxygen; and finally the specific heating conditions used in this experiment (70 °C, 30 min) would make them prone to lipid oxidation. Moreover, lipid oxidation in heterophasic systems such as emulsions is generally recognized as being very complex as it may include oxidation in all the different phases of the systems, so that the mechanisms of lipid oxidation in emulsions are very different and significantly more complex than lipid oxidation in bulk oil systems (Jacobsen, Let, Nielsen, & Meyer, 2008).

Two recent papers have reported the use of complex structured-emulsions like multiple emulsions ($W_1/O/W_2$) to improve meat product formulations as their lipid phases incorporate unsaturated fatty acid sources (Cofrades et al., 2014; Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasarán, 2014). However, in both cases the oxidative stability of the meat systems was enhanced by adding antioxidants (hydroxytyrosol and butylated hydroxyanisole) in their delivery systems. In the present case, the use of sodium caseinate and/or pectin provided a good antioxidative effect both in the delivery systems and in the meat system.

Oxidation results in n-3 LCPUFA-enriched meat systems are consistent with those from the delivery systems (Fig. 3a and b). The incorporation of bioactive compounds through a filled hydrogel in a meat system (MS/H) resulted in substantially less lipid oxidation than in the other samples containing fish oil, reducing the rate and extent of hydroperoxide values and reducing the TBARS concentration by an average of 59% over the storage time with respect to oxidation values in sample containing a conventional delivery system (O/W emulsion, MS/E).

5. Conclusions

Fish oil in filled hydrogel particles was more oxidatively stable than a conventional n-3 LCPUFA delivery system (O/W emulsion), even under heating conditions. The filled hydrogel particles were also more oxidatively stable than conventional emulsions and bulk oil when added to a meat system with levels of omega-3 fatty acids recommended by many health organizations. These results suggest that it is worth considering the use of filled hydrogel particles in the development of healthier meat products. Nevertheless, further studies are needed to determine their effect on physicochemical properties and sensory characteristics.

Acknowledgements

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4.2.2 Filled hydrogel particles as a delivery system for n-3 long chain PUFA in low-fat frankfurters: consequences for product characteristics with special reference to lipid oxidation

Enviado a Meat Science

Filled hydrogel particles as a delivery system for n-3 long chain PUFA in low-fat frankfurters: consequences for product characteristics with special reference to lipid oxidation

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12 **Abstract**

13 This article examines the suitability of filled hydrogel particles for use as a
14 delivery system for n-3 long chain PUFAs in low-fat frankfurters. Their effects on
15 product characteristics over chilled storage were compared with those of frankfurters
16 containing all-pork fat (control) or a comparable amount of fish oil (n-3 LCPUFA)
17 incorporated in liquid form or in an oil-in-water emulsion. In modified samples n-3 fatty
18 acids ranged between 801.34 to 996.37 mg/100 g as opposed to 66 mg/100 g in all-pork
19 fat product. As compared with the control, hardness and chewiness values were similar
20 ($P>0.05$) in filled hydrogel frankfurter. The presence of fish oil favoured lipid oxidation
21 to varying degrees depending on delivery system, in descending order: direct oil
22 addition>oil-in-water emulsion>hydrogels. Sensory evaluation demonstrated the
23 advantages, from a sensory point of view, of hydrogel filled particles as n-3 delivery
24 systems in frankfurters.

25

26 **Keywords**: Filled hydrogel particles; O/W emulsion; n-3 LCPUFA delivery systems;
27 frankfurters; lipid oxidation; chilled storage

28

1. Introduction

There is growing evidence that dietary fat may play a role in the prevention and therapy of several of the most important chronic diseases affecting our society, among them cardiovascular diseases. Recommendations for optimal intake of total and saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids have been proposed by a number of scientific authorities and nutritional organizations (McNeill, 2014; WHO, 2003). These recommendations include increasing the consumption of foods rich in n-3 PUFAs, especially long chain (n-3 LCPUFAs), such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, as a means of promoting a reduction in the n-6/n-3 PUFA ratio (Kolanowski, Swiderski, & Berger, 1999; Simopoulos, 2002; Garg, Wood, Singh, & Moughan, 2006).

Given that meat and meat products are some of the most important sources of dietary fat, changes in the amounts and the lipid profiles of such products could help to improve the nutritional quality of Western diets (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014; Jiménez-Colmenero, 2007; McNeill, 2014). In response to these considerations, numerous researchers are endeavouring to optimize the amounts of lipids and the fatty acid profiles of various meat products in order to achieve a composition more in line with nutrient intake goals. In this regard, various different technology options for meat fat replacement in meat products have been assayed, and of these oil-in-water (O/W) emulsions are the type of systems most commonly used to deliver bioactive lipids because of their capacity for oil stabilization, relative ease of preparation and low cost (McClements, Decker, & Weiss, 2007). In fact, O/W emulsions have been shown to be suitable for incorporating n-3 fatty acids of land and marine origin in fresh, cooked and fermented meat products (Jiménez-Colmenero, 2007). Nevertheless, although O/W emulsions constitute an excellent means of

54 enhancing the oxidative stability of lipids in bulk oils, lipid oxidation can occur
55 relatively rapidly in these systems due to their large surface area, which facilitates
56 interactions between lipids and water-soluble prooxidants (Waraho, McClements, &
57 Decker, 2011).

58 In order to improve the potential of O/W emulsions in terms of their ability to
59 afford bioactive protection, new and more complex-structured emulsions such as filled
60 hydrogel particles have been developed. These systems consist of oil droplets (O)
61 trapped inside a hydrogel matrix (W_1), which is dispersed in an aqueous medium (W_2)
62 that may be described as an $O/W_1/W_2$ type of structured emulsion (Matalanis &
63 McClements, 2013). These filled hydrogels could offer a number of advantages over
64 other n-3 fatty acid delivery systems tested in meat systems in that they are structurally
65 more robust than conventional emulsions (Matalanis, Decker, & McClements, 2012)
66 and the biopolymers used in their preparation (casein and pectin) could help to stabilize
67 n-3 fatty acids both physically and chemically (McClements et al., 2007; Salcedo-
68 Sandoval et al., 2013a). In this regard, it has been shown that fish oil in filled hydrogel
69 particles is more oxidatively stable than a conventional n-3 LCPUFA delivery system
70 (O/W emulsion), even when heated. Additionally, the filled hydrogel particles are more
71 oxidatively stable than conventional emulsions and bulk oil when added to a meat
72 model system with nutritionally significant amounts of n-3 LCPUFA in line with health
73 recommendations (Salcedo-Sandoval et al., 2015). These results suggest that it is worth
74 considering the use of filled hydrogel particles in the development of healthier meat
75 products. However, as far as the authors know, no studies have reported the use of filled
76 hydrogel particles as ingredients in a real meat product.

77 The goal of this study was to assess, for the first time, the suitability of filled
78 hydrogel particles as a delivery system for n-3 LCPUFAs in frankfurters. This aim was

addressed through evaluation of fatty acid profiles, technological properties (processing and purge losses, texture and colour), sensory characteristics and lipid oxidation of n-3 LCPUFA-enriched frankfurters as affected by the strategy used to incorporate the fish oil: a) directly; b) stabilized in an O/W emulsion; and c) stabilized in filled hydrogel particles. The effects of these n-3 fatty acid delivery systems were compared with frankfurters containing all pork fat and assessed during chilled storage (40 days at 2 ± 2°C). The frankfurters were designed and formulated so that the final products would provide nutritionally adequate intakes of EPA and DHA.

2. Materials and methods

2.1 Materials

Fish oil (Omevital^{TM} 1812 TG Gold, BASF SE, Ludwigshafen, Germany) was used as n-3 LCPUFA source and contained 169 mg of EPA/g and 110 mg of DHA/g plus mixed tocopherols, as specified by the supplier. Sodium caseinate (SC) (90.5% protein and 5.5% moisture, information provided by the manufacturer) was from DMV (Excellion EM 7, DMV Campina B.V.; Veghel, The Netherlands). Highly methyl-esterified pectin (GRINDSTED® Pectin USP, Danisco; Grindsted, Denmark) was used. Composition according to the supplier: 9% moisture, 85.3% galacturonic acid and minimum 6.7% methoxy content. The enzyme used was transglutaminase (TG) (Activa® WM - 99% maltodextrine and 1% TG - Ajinomoto Co.; Tokyo, Japan) with an activity of approx. 100 U/g of powder. Cumene hydroperoxide and 1,1,3,3-tetraethoxypropane were obtained from Sigma-Aldrich (Steinheim, Germany). 2-Thiobarbituric acid was purchased from Merck (Darmstadt, Germany). All other chemicals used in this study were purchased from Panreac Química, S.A (Barcelona, Spain). Distilled water was used throughout the study. Sufficient (35 Kg) fresh post-rigor pork meat (mixture of *biceps femoris*,

104 *semimembranosus*, *semitendinosus*, *gracilis* and *adductor* M) (21.1% ± 0.5 protein,
105 4.57% ± 0.7 fat) and pork backfat (4.58% ± 0.9 protein, 90.24% ± 2.5 fat), both from
106 different animals, were obtained from a local market. Other additives used in product
107 formulation included soy protein isolate (SPI) (92.1% protein content) (Trades SA,
108 Barcelona, Spain), sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium
109 tripolyphosphate (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie
110 GmbH, Buchs, Germany) and flavouring (Gewürzmüller, GmbH, Münichingen,
111 Germany).
112
113 *2.2 Preparation of delivery systems*
114 *2.2.1 Preparation of O/W emulsion*
115 To prepare the O/W emulsion a solution (2%, w/w) was first prepared by dispersing and
116 stirring SC into a buffer solution (10 mM phosphate buffer, pH 7) at room temperature
117 until fully dissolved and then storing the solution overnight at 4 °C. A coarse pre-
118 emulsion was formed by homogenizing 20% (w/w) of fish oil with 80% (w/w) of
119 emulsifier solution using a high-speed hand blender (Taurus, Barcelona, Spain) at
120 medium speed for 2 min. Thereafter, the coarse emulsion was passed twice through a two-
121 stage high pressure homogenizer at 750/50 bars (first-stage pressure/second-stage
122 pressure; Panda Plus 1000, GEA NiroSoavi, Parma, Italy). The O/W emulsion was kept
123 chilled (2 °C) until use.
124
125 *2.2.2. Preparation of filled hydrogel particles*
126 *2.2.2.1. Preparation of solutions and O/W emulsion*
127 Solutions were prepared according to Matalanis & McClements (2013) using pectin
128 and SC. Briefly, a pectin-rich solution containing 4.03% pectin and 0.22% SC and a SC-

129 rich solution with 0.52% pectin and 14.2% SC were prepared in 10 mM phosphate buffer
130 pH 7 using a mechanical stirrer (RW 20; Janke & Kunkel IKA Laboratortechnick,
131 Staufen, Germany). Following dispersion, the pH of the solutions was adjusted to pH 7
132 with 4 M sodium hydroxide. An oil-in-water emulsion with 50% fish oil was prepared as
133 previously described for conventional O/W emulsion.
134
135 *2.2.2.2. Formation of filled hydrogel particles*
136 Filled hydrogel particle formation was carried out in a multistep process according
137 to Salcedo-Sandoval et al. (2015). Briefly, 5% (v/v) of casein-rich phase, 90% (v/v) of
138 pectin-rich phase and 5% (v/v) of a 50% (v/v) fish O/W emulsion were mixed with a
139 stirrer (RW 20; Janke & Kunkel IKA Laboratortechnick, Staufen, Germany) at 300 rpm
140 for 30 min to ensure homogeneity. During mixing, the pH of the mixture was measured
141 and adjusted to 7. Then, pH was lowered to 5 by adding one drop of 1 M citric acid every
142 10 s as the mixture was stirred at 300 rpm, allowing adsorption of the pectin-rich phase
143 (W_2) around the casein-rich (W_1) droplets, thus forming the $O/W_1/W_2$ system. After that**,**
144 10 g of powdered transglutaminase, previously homogenized, was used to cross-link the
145 casein present in the W_1 phase so as to further stabilize these particles. This mixture was
146 then transferred to a 50 °C water bath and incubated for 15 min with constant stirring at
147 300 rpm. The mixture was then removed from the water bath and cooled at room
148 temperature for 20 min, still under constant stirring. After cooling, the pH of the sample
149 was adjusted from 5 to 7 using sodium hydroxide (1 M), and then the mixture was stirred
150 for a further 20 min. Sample was stored overnight at 2 °C. At the end of overnight
151 storage, hydrogel particles were washed to remove the pectin-rich phase and thus
152 concentrate the fat in the delivery system. One part hydrogel particles to four parts 10 mM
153 phosphate buffer (pH 7) was mixed for 1 h and then centrifuged using a Sorvall Evolution

154 RC Centrifuge (Kendro Laboratory Products; Asheville, NC, USA) at 10000 g. The
155 washing solution (along with most of the continuous phase) was decanted and the washed
156 particles were collected in a glass beaker at 2 °C until use.
157
158 *2.2.3. Determination of delivery system oil content*
159 The oil content in the O/W emulsion and filled hydrogel particles was determined
160 to ensure that both had the same oil concentration. Lipid quantification was performed
161 in triplicate according to the method of Iverson, Lang, & Cooper (2001). Briefly, 1 g of
162 sample was vigorously vortexed three times with 7.5 ml of chloroform-methanol (2:1
163 v/v) followed by centrifugation for 30 min at 2400 g (Sorvall RTB6000B, DuPont;
164 Wilmington, DE, USA). Following centrifugation, 2 ml from the lower solvent phase
165 was placed in pre-weighed test tubes and caps, and the solvent was evaporated under a
166 stream of nitrogen. The amount of extracted oil was then determined gravimetrically.
167 As the O/W emulsion was more concentrated, it was diluted with phosphate buffer (10
168 mM, pH 7) to achieve the same oil concentration as the filled hydrogel particles (8.5%,
169 w/w).
170
171 *2.3. Design and preparation of frankfurters*
172 Four different frankfurters (Table 1) were prepared with the same protein and fat
173 contents: a control frankfurter (F/C) prepared with all pork fat; and three frankfurters
174 reformulated to contain significant amounts of n-3 LCPUFAs (0.6 g of EPA and
175 DHA/100 g of product), by replacement of the fat content from pork backfat with the
176 same proportion of fat from fish oil added directly (F/O); stabilized in a conventional
177 O/W emulsion (F/E); or encapsulated in filled hydrogel particles (F/H).

178 Frankfurters were prepared according to (Delgado-Pando, Cofrades, Ruiz-
179 Capillas, & Jimenez-Colmenero, 2010). Briefly, pork meat and pork backfat were
180 passed through a grinder with a 0.6 cm plate (Mainca, Granollers, Spain) and lots of
181 approximately 500 g were vacuum packed and frozen (at −18 °C) until use (less than 2
182 weeks). For frankfurter preparation, the meat and fat were thawed for 18 h at 2 ± 2 °C.
183 Meat was homogenized for 1 min in a grinder/homogenizer connected to a cooling bath
184 (2 °C) (Stephan Universal Machine UM5, Stephan u. Söhne GmbH and Co., Hameln,
185 Germany). Half of the pork backfat or delivery system (depending on the formulation),
186 SPI, NaCl, sodium tripolyphosphate and sodium nitrite (the last two previously
187 dissolved in the added water) were added to the ground meat and mixed again for 1 min.
188 Then the remaining half of the ingredients was added and the whole mixture was
189 homogenized for 1 min. Finally the whole meat batter was homogenized under vacuum
190 for 2 min. The final batter temperature was below 14°C in all cases.
191 The meat batter was stuffed into 20 mm diameter Nojax cellulose casings (Viscase
192 S.A., Bagnold Cedex, France) and hand-linked. Frankfurters were heat processed in an
193 Eller smokehouse (model Unimatic 1000, Micro 40, Eller, Merano, Italy) until the core
194 of the product reached 70 °C, which was controlled by means of thermocouples
195 connected to a temperature recorder (DaqPRO 5300 Data recorder; OMEGA
196 Engineering, Inc., Stamford CT, USA). Once heating was complete, the frankfurters
197 were cooled (at room temperature), kept in a cold room (2 °C for 14 h), vacuum packed
198 (Cryovac® BB3050) and stored at 2 ± 2 °C. Analyses were carried out at the following
199 days: 1, 12, 26 and 40 days. The entire meat system processing procedure was
200 replicated twice on two different days.
201
202 *2.4. Proximate analysis and fatty acid composition*

Proximate composition was determined before (on meat batter) and after cooking (frankfurters). Moisture and ash contents were determined in quadruplicate according to AOAC (2005). Fat content was evaluated in triplicate following the method of Bligh & Dyer (1959). Protein was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI. USA).

Fatty acid composition of frankfurters was determined (in quintuplicate) from their lipid extracts by gas chromatography (GC) as reported by Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, (2013b). Briefly, boron trifluoride/methanol was used to prepare fatty acid methyl esters (FAME). A Shimadzu gas chromatograph (Model GC-2014, Kyoto, Japan) fitted with a capillary column SP™-2330 (60 m × 0.25 mm × 0.2 μm id) (Supelco; Bellefonte, PA, USA) was used with a flame ionization detector. Injector and detector temperatures were 250 and 260 °C respectively. The oven temperature was set at 140 °C for 5 min then raised to 240 °C at 4 °C/min and held there for 20 min. The carrier gas was helium and nitrogen was used as the make-up gas. Fatty acids were identified by comparison of the retention times with a standard of 37 fatty acids (Supelco™ 37 FAME Mix 47885-U, USA). The results were expressed as mg/100 g of edible portion.

Based on the FAME results, the atherogenic (AI) and thrombogenic indexes (TI) were calculated from the fatty acid results according to Ulbricht & Southgate (1991):

AI = (C12:0 + 4 x C14:0 + C16:0)/(MUFA + n-3 PUFA + n-6 PUFA);

TI = (C14:0 + C16:0 + C18:0) / (0.5 x MUFA + 0.5 x n-6 PUFA + 3 x n-3 PUFA + n-3 PUFA/n-6 PUFA).

2.5 Processing loss, purge loss and pH determination

Processing loss was calculated in sextuplicate, as the weight loss (expressed as % of initial sample weight) occurring after heat processing and chilled overnight at 2°C.

Purge loss during chilled storage was measured using three vacuum packs per formulation. After the frankfurters were removed from the package, the surface exudate (tiny drops) was wiped off with paper towels and the frankfurters weighed again. The purge loss was calculated by weight difference and expressed as a percentage of the initial weight.

The pH was determined on homogenates of frankfurters in water in a ratio of 1:10 w/v at room temperature using a pH meter (827 pH Lab Methrom, Herisau, Switzerland). Six determinations were performed per sample.

2.6. Colour measurement

Colour, CIE-LAB tristimulus values, lightness, L^*; redness, a^* and yellowness, b^* of frankfurter cross-sections were evaluated on a CR-400 Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan). Before use, the colorimeter was standardized using the white calibration plate (C: Y=93.6, x=0.3130, y=0.3193). Ten determinations were performed from each sample.

2.7. Texture profile analysis (TPA)

TPA was performed in a TA-XT.plus Texture Analyzer (Texture Technologies Corp, Scarsdale, NY, USA) as described by Bourne (1978). Ten frankfurter cores (diam = 20 mm, height = 20 mm) were axially compressed to 40% of their original height. Force-time deformation curves were obtained with a 5 Kg load cell, applied at a crosshead speed of 1 mm/s. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done

252 under the second compression curve to that done under the first compression curve
253 (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first
254 compression; chewiness (Cw) = Hd × Ch × Sp (N·mm). Measurement of samples was
255 carried out at room temperature.
256
257 *2.8. Lipid oxidation evaluation*
258 Oxidative stability in frankfurters was assessed by changes in lipid
259 hydroperoxides and thiobarbituric acid-reactive substance (TBARS) concentrations as
260 measures of primary and secondary oxidation products respectively.
261 Lipid hydroperoxides were measured in triplicate as described by Salcedo-
262 Sandoval et al. (2015). Briefly, 1 g of sample was vigorously vortexed three times with
263 7.5 ml of chloroform-methanol (2:1, v/v) followed by centrifugation for 30 min at
264 3000g. Following centrifugation, 0.2 ml of the chloroform/methanol extract (lower
265 layer) was carefully removed and mixed with 2.8 ml of methanol/1-butanol (2:1, v/v).
266 Depending on the concentration of hydroperoxides, the chloroform/methanol extract
267 was diluted with solvent so that the final absorbance of the reacted sample remained
268 below 1.0. This mixture (3 ml) was then reacted with 15 µl of 3.94 M ammonium
269 thiocyanate and 15 µl of ferrous iron solution (prepared by reacting 0.132 M barium
270 chloride and 0.144 M ferrous sulphate). The mixture was vortexed and allowed to react
271 for 20 min at room temperature in the dark, then the absorbance of the sample was
272 measured at 510 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc.,
273 Kyoto, Japan). The concentration of hydroperoxides was determined based on a
274 standard curve of cumene hydroperoxide (0–20 µM). Results were expressed as mmol
275 hydroperoxides/Kg sample.

TBARS were determined in triplicate as reported by Serrano, Cofrades, & Jimenez-Colmenero (2006). Briefly, 3 g of each sample was were homogenized for 1 min in 35 ml of 7.5% trichloroacetic acid at high speed in an Omnimixer blender (ES Homogenizer, OMNI International Inc., Gainsville, VA, USA). The blender sample was centrifuged at 3000 g for 30 min (Heraeus multifuge 3L-R DJB Labcare Ltd.; Buckinghamshire, UK) and 5 ml of the supernatant was were mixed with 5 ml of 20 mM thiobarbituric acid. Finally the solution was mixed and kept in the dark at 20 °C for 20 h. The pink colour that formed was measured spectrophotometrically at 532 nm. A calibration curve was plotted using 1,1,3,3-tetraethoxypropane as the malondialdehyde (MDA) source, and results were expressed as mg malondialdehyde eq/Kg sample.

2.9. Sensory evaluation

Frankfurters were assessed by a 10-member panel. The panel was selected in preliminary sessions from staff who had received training (two sessions) with the products and terminology. After heating in a microwave for 15 s, frankfurter samples (2.5 cm long) from each formulation were immediately presented to panellists with a three-digit randomized code to identify the samples. An intensity scale test of 9 points was used to evaluate the following parameters for each sample: juiciness (0 = very dry, 9 = very juicy), firmness (0 = very soft, 9 = very hard). In addition, a hedonic scale rating test was carried out where the panellist evaluated texture, flavour and overall acceptability (0 = dislike extremely, 9 = like extremely). The evaluation was made on a non-structured scale with fixed extremes. Each point was later converted to a numerical scale. Sensory analysis was performed 4 days after the preparation of the frankfurters.

2.10. Statistical analysis

301 The entire trial was replicated. One-way analysis of variance (ANOVA) was carried
302 out to evaluate the statistical significance ($P<0.05$) of the proximate composition, and
303 two-way ANOVA as a function of formulation and storage time using the SPSS
304 Statistics general linear model (GLM) procedure (v.22, IBM SPSS Inc.; Chicago, IL,
305 USA). Formulation and storage time were assigned as fixed effects and replicate was
306 assigned as random effect. Least squares differences were used for comparison of mean
307 values between treatments and Tukey's HSD test to identify significant differences
308 ($P<0.05$) between formulations and storage time.
309
310 **3. Results and discussion**
311 It has been suggested that foods enriched with healthier fatty acids can be used to
312 achieve desired biochemical effects without changes in dietary habit. Lipid modification
313 of meat products by substitution of animal fat with other lipid sources has been shown
314 to be a good strategy to improve their nutritional quality (Jiménez-Colmenero, 2007).
315 Different reformulation approaches of cooked products such as frankfurters or bologna
316 sausage have been used to improve lipid content (reduce fat level and/or fatty acid
317 composition) for better adaptation to nutrient intake goals, but only a few of them use
318 fish oils. In these cases fish oil (alone or combined with other oils) has been added in
319 liquid form (Park, Rhee, Keeton, & Rhee, 1989), stabilized in a konjac matrix (Salcedo-
320 Sandoval et al., 2013b), or pre-emulsified with caseinates (Caceres, Garcia, & Selgas,
321 2008; Delgado-Pando et al., 2011), soy protein (Delgado-Pando et al., 2011), or
322 carrageenans and milk proteins (Marchetti, Andrés, & Califano, 2014). However, recent
323 research has proposed a new and interesting possibility (Matalanis & McClements,
324 2013). In a previous paper Salcedo-Sandoval et al. (2015) reported the use of filled
325 hydrogel particles as a n-3 LCPUFA delivery system in a meat model system,

326 concluding that it is worth considering the use of filled hydrogel particles in the
327 development of healthier meat products. The following sections deal with a number of
328 aspects bearing on the effect of these particles on different properties and characteristics
329 of frankfurters, which are essential to a clearer understanding of the potential uses of
330 these hitherto unexplored reformulation strategies.
331
332 *3.1. Proximate analysis and fatty acid composition*
333 Control (F/C) and modified frankfurters (F/O, F/E and F/H) presented some
334 differences in proximate analysis (Table 2) and all were consistent with product
335 formulation (Table 1). The differences between samples in moisture, protein and ash
336 contents, which were relatively small in quantitative terms, even when significant, can
337 be attributed mainly to the changes induced by variations in processing loss (described
338 below). Fat content was generally close to the target level, ranging between 4.09 % and
339 5.34 % (Table 2), with the highest ($P<0.05$) value in the sample prepared with filled
340 hydrogel particles. Since all uncooked samples (meat batter) had similar ($P>0.05$) fat
341 contents (3.70% ± 0.4), the differences between final products may be associated with
342 variations of fat loss during cooking, which were affected by the strategy used to
343 incorporate fish oil. Depending on the formulation (Table 1), while in control sample all
344 the fat content was supplied by pork fat, in modified frankfurters about 2% of the fat
345 was supplied by the fish oil in association with the different n-3 delivery systems used.
346 On the whole, the results of fatty acid composition are consistent with the type
347 of ingredients used in the formulation, and so the fatty acid profile of frankfurters
348 differed in control and modified samples (Table 3). The most abundant fatty acids in the
349 control sample (all pork fat) were MUFAs (oleic acid), followed by SFAs and PUFAs;
350 MUFAs and PUFAs together accounted for 63% of total fatty acids. These results are

consistent with reports for fatty acid composition of frankfurters (Delgado-Pando et al., 2010) and for pork fat (Wood et al., 2004). As compared with control sample, the reformulated products contained less ($P<0.05$) SFAs (among them myristic and stearic acids) and MUFAs (mainly oleic acid), but more ($P<0.05$) total PUFAs (linolenic acid, EPA, and DHA), with n-3 PUFAs between 12-15 times higher (especially due to EPA and DHA input) and little observable difference in n-6 (Table 3). In modified samples n-3 PUFAs ranged between 801.34 and 996.37 mg/100 g (of which approximately 692 to 867 mg/100 g were n-3 LCPUFAs) as opposed to around 66 mg/100 g in all-pork fat product. This means that although dietary recommendations vary depending on different factors (population, desired disease prevention, etc.), these products make a nutritionally significant contribution to dietary intake as compared to non-fortified frankfurters. Recommended intakes of EPA and DHA vary between 180 and 1000 mg/day for healthy people (Garg et al., 2006; WHO, 2003). In addition to considerations of individual fatty acids, scientific evidence suggests that ratios such as PUFA/SFA (recommended > 0.4) and n-6/n-3 PUFAs (recommended < 4) are the main parameters currently used to assess the nutritional quality of the lipid fraction of foods. Whereas the PUFA/SFA ratio in control sample was 0.34, which is consistent with reports by other authors in conventional meat products (Delgado-Pando et al., 2010), replacement of pork fat by different n-3 LCPUFA delivery systems increased ($P<0.05$) this ratio to 1.0 (Table 3). Control samples presented a n-6/n-3 PUFA ratio of 6.7; the presence of fish oil considerably reduced this ratio, almost down to 0.5 (Table 3). Atherogenic index (AI) and thrombogenic index (TI) values were lower ($P<0.05$) in modified samples than in control product. Similarly, reductions of AI and TI index values have been reported when pork backfat was replaced by an oil (combination of

olive, linseed and fish oils)-in-water emulsion (Delgado-Pando et al., 2010) or walnut (Ayo et al., 2007) in frankfurters.

Some differences were observed in fatty acid content as affected by the n-3 LCPUFA delivery systems used (Table 3), due basically to differences in fat content (Table 2). Generally the SFAs (palmitic and stearic acid), MUFA (oleic and vaccenic acids) and PUFAs (linolenic acid, EPA and DHA) contents increase in the following order in frankfurters formulated using liquid < O/W < filled hydrogel particles. This means that compared to other tested n-3 fatty acid delivery systems, the filled hydrogels provide nutritional advantages deriving from the presence of these bioactive lipids.

3.2. Physicochemical properties

Processing loss (PL) of frankfurters ranged from 14.94 to 17.22 %, with PL values lowest ($P<0.05$) in the frankfurter formulated with filled hydrogel (F/H) and no significant variations among the other samples (Table 4). Ranges of processing loss for low-fat frankfurters (including those made with vegetable oils) between 10-20 % have been reported (Delgado-Pando et al., 2010; Lopez-Lopez, Cofrades, & Jimenez-Colmenero, 2009; Paneras & Bloukas, 1994). Purge accumulation in the packaged product during retail storage, undesirable for aesthetic and microbiological reasons, was relatively low, ranging from 0.13 to 0.58 % (Table 4), without any clear effect from product formulation. This means that all samples had good storage stability in terms of fat and water binding properties of the meat matrix. This stability was not dependent on the type of lipid used (pork backfat versus fish oil), nor did it vary with the n-3 LCPUFA delivery system used to stabilize the fish oil. The pH values of frankfurters were not influenced ($P>0.05$) by formulation or chilled storage. This parameter ranged between 6.37 and 6.45 and may be considered normal in products of this kind (Delgado-

400 Pando et al., 2010). The relatively small loss of fluid during processing and storage
401 indicated the formation of a stable protein matrix, irrespective of formulation, with
402 adequate ability to immobilize both fat and water.

403 Colour parameters were affected by formulation and storage time (Table 5).
404 Lightness values were lower ($P<0.05$) in the control than in the samples with fish oil
405 incorporated. There were also some differences in lightness depending on the delivery
406 system used and on chilled storage. The replacement of pork backfat by fish oil caused
407 changes in redness, which was affected ($P<0.05$) by the incorporation strategy; thus,
408 redness values were lowest in F/O sample and highest in frankfurter made with filled
409 hydrogel particles (Table 5). Generally the changes in colour parameters (lightness,
410 redness and yellowness) induced by formulation and storage, even when significant,
411 were quantitatively small and of very little practical relevance. Colour differences in
412 frankfurters can be attributed to variations in the colour of pork backfat and fish oil, but
413 more importantly to the system (presence of biopolymers such as caseinate) used to
414 stabilize the oil-in-water emulsions for oil delivery (Salcedo-Sandoval et al., 2013a). In
415 fact, the conflicting results concerning the effect of pork backfat replacement by
416 different lipid sources on product colour have been associated largely with the type of
417 sausage, the product formulation, the characteristics of the oils, and the delivery system
418 assayed (Delgado-Pando et al., 2010).

419 Texture profile analyses of frankfurters were affected ($P<0.05$) by the type of
420 formulation and by chilled storage (Table 6). As compared with the control, hardness
421 and chewiness values were similar ($P>0.05$) in filled hydrogel sample (F/H), while F/O
422 and F/E products exhibited higher ($P<0.05$) values. F/H sample had the lowest ($P<0.05$)
423 springiness, while F/E product had the highest cohesiveness. There are various factors
424 that may help explain this behaviour. For instance, the greater ($P<0.05$) hardness of F/E

425 samples (as compared with the control) was probably due to emulsification of the fish
426 oil with non-meat protein, so that more meat protein became available to contribute to
427 gel formation (Bishop, Olson, & Knipe, 1993); additionally, proteins such as caseinate
428 used as emulsifying ingredients produce a very strong link between the components of
429 the fat emulsion, increasing the consistency and hence the hardness of the product
430 (Caceres et al., 2008). Then again, it has been reported that liquid oils (e.g. F/O) achieve
431 a better distribution than animal fat in meat emulsion matrixes, thus producing firmer
432 sausages due to improved association with the protein (Hammer, 1992). However
433 despite this, conflicting results have been reported for the effect of vegetable oils on the
434 texture of frankfurters (Delgado-Pando et al., 2010). Chilled storage had some effects
435 on TPA parameters (Table 6). As storage progressed, hardness and chewiness increased,
436 in very similar patterns, in control and F/H samples, with F/O and F/E registering
437 greater increases. Chilled storage generally had only a minor effect on springiness,
438 while cohesiveness decreased only at the end of storage time. Similarly, hardness or
439 shear force has been reported to increase in frankfurters during chilled storage (Andrés,
440 García, Zaritzky, & Califano, 2006; Candogan & Kolsarici, 2003; Kao & Lin, 2006), an
441 effect that in this experiment cannot be attributed to changes in purge loss (Table 2).
442 The results therefore indicate that the use of a filled hydrogel (to replace pork backfat)
443 had only limited effects on product texture.
444
445 *3.3 Lipid oxidation*
446 Lipid oxidation is a major cause of deterioration in the quality of stored meat
447 products. One of the main potential problems associated with healthier lipid
448 formulations in meat products is how they may influence the rate and extent of lipid
449 oxidation, which in turn affects final quality characteristics and has health implications.

Since fish oil in filled hydrogel particles is more oxidatively stable than in a conventional n-3 LCPUFA delivery system (O/W emulsion), even under heating conditions, and the filled hydrogel particles are also more oxidatively stable than conventional emulsions and bulk oil when added to a meat system (Salcedo-Sandoval et al., 2015), the next step was to assess the lipid oxidation in real meat products such as low-fat, n-3 enriched frankfurters. Composition factors like the presence of salts, metal ions or polyunsaturated lipids, as well as processing conditions like grinding, heating, storage, etc., render these products more susceptible to lipid oxidation. However, the antioxidant activity of compounds such as nitrite limits the oxidative process. For that purpose, both primary and secondary oxidation products were evaluated in frankfurters formulated using different n-3 delivery systems. Hydroperoxide contents in frankfurters were affected ($P<0.05$) by formulation and storage (Fig. 1a). Compared with control sample, at the outset of the experiment all samples with fish oil registered high hydroperoxide levels ($P<0.05$) indicating that the n-3 fatty acids underwent oxidation during product processing. Differences in hydroperoxide formation during storage seemed to be associated with the kind of delivery system. After 12 days of storage, the recorded hydroperoxide concentrations in frankfurters were, in descending order: direct oil addition > oil-in-water emulsion> hydrogels > pork fat. The hydroperoxides in the samples with direct oil or emulsion addition declined from this point until the end of storage, indicating that these lipid materials were oxidizing faster than the others. Over storage the lowest hydroperoxide contents were observed in control sample.

TBARS values were affected ($P<0.05$) by formulation and storage (Fig. 1b). In line with the hydroperoxide results, the F/O sample (direct fish oil addition) initially registered the highest TBARS levels. Over storage the rate and extent of TBARS formation in frankfurters were, in descending order: direct oil addition > oil-in-water

475 emulsion> hydrogels > pork fat. The lower TBARS values in the control product
476 indicate that oxidation progressed more slowly in these samples than in the samples
477 with fish oil. In the present experiment, although the presence of fish oil promoted more
478 lipid oxidation than in the control sample (Fig. 1b), the observed TBARS values (< 0.2
479 mg/kg in the control and <0.8 in filled hydrogel sample) were lower than have been
480 reported as the minimum needed to detect objectionable flavours in processed meat
481 products (Caceres et al., 2008; Mercadante, Capitani, Decker, & Castro, 2010).
482 Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, & Perez-Alvarez (2003)
483 reported TBARS levels of 4-6 mg MDA/kg in vacuum-packed sliced bologna sausages
484 without any rancid taste being detected.

485 There are some interesting conclusions to be drawn from analysis of both the
486 primary and the secondary oxidation products: the presence of fish oil favours lipid
487 oxidation, and the relative susceptibility of n-3 enriched samples to lipid oxidation was,
488 in descending order, direct oil addition > oil-in-water emulsion> hydrogels. The first is
489 consistent with the well-known fact that the increment in unsaturated fatty acids,
490 particularly polyunsaturated, renders the meat matrix more prone to oxidation.
491 Reporting on the use of liquid fish oil in low-fat frankfurter formulation, Park et al.
492 (1989) found that the resulting product had undesirable sensory properties, and no
493 further studies was carried out. Regarding incorporation of a fish oil O/W emulsion, it
494 has been reported that the replacement of animal fat by healthier oil combinations
495 (including fish oil) in low-fat frankfurter formulation did promote a slight increase in
496 lipid oxidation as compared with similar all-pork fat products, which is consistent with
497 the results of this experiment (Delgado-Pando et al., 2011). However, Caceres et al.
498 (2008) reported low lipid oxidation in bologna sausage containing fish oil, mainly due
499 to the combined effect of the antioxidants (α-tocopherol) included in the fish oil to

500 stabilize the n-3 PUFAs and the ones in the mixture of spices (ascorbate, nitrite) used in
501 sausage manufacture.

502 The fact that of the n-3 LCPUFA delivery systems assayed in frankfurter
503 formulation, filled hydrogel particles were the most oxidatively stable is consistent with
504 the report by Salcedo-Sandoval et al. (2015) on meat model systems. Several factors
505 may be involved in this behaviour. The filled hydrogels used are structurally more
506 robust than conventional emulsions (Matalanis et al., 2012) and thus the particle is more
507 likely to survive thermal processing of the product intact. In addition, these filled
508 hydrogels are stabilized with casein and pectin. Since the emulsified lipid droplets were
509 embedded in a casein-rich hydrogel particle, there would be more protein near the
510 emulsion droplet and this higher casein concentration could potentially be more
511 effective at scavenging free radicals and chelating metals. In addition, the hydrogels
512 contain pectin, which could also chelate metals and protect the lipid (Chen,
513 McClements, & Decker, 2010). Thus, the greater oxidative stability of the hydrogel
514 particles could be due to differences in biopolymer concentrations as well as close
515 proximity of the biopolymers to the lipid droplets (Salcedo-Sandoval et al., 2015).

516

517 *3.4 Sensory analysis*

518 Sensory evaluation indicated that incorporation of fish oil through different
519 strategies affected ($P<0.05$) some sensory attributes of the frankfurters (Table 7). As
520 compared with the control (all pork fat), scores for juiciness, texture, flavour and overall
521 acceptability were generally lower in F/E sample, while firmness was greater ($P<0.05$)
522 in frankfurters formulated with liquid fish oil (F/O) and fish oil-in-water emulsion
523 (F/E). There were no significant differences in any sensory parameters between the
524 control and frankfurters made with a filled hydrogel. Sensory evaluations of firmness

525 and flavour acceptability are generally consistent with the TPA results (Table 6) and
526 lipid oxidation levels (Fig. 1) respectively. The control and filled hydrogel samples
527 scored highest, and so both products were equally acceptable. Interestingly, unlike
528 addition of fish oil in liquid form or as O/W, sensory evaluations revealed that the
529 enrichment of frankfurter with n-3 LCPUFA-filled hydrogel particles would not be a
530 problem for consumers. Undesirable sensory properties have been reported in low-fat
531 frankfurters made with liquid fish oil (Park et al., 1989). However, (Caceres et al.,
532 2008) reported that their panellists found all reduced-fat bologna sausages acceptable,
533 regardless of the amount of pre-emulsified fish oil added. The incorporation of a
534 healthier oil (including fish oil) combination stabilized with different non-meat protein
535 systems seems to be a good option to obtain sensorily acceptable and healthier
536 frankfurters (Delgado-Pando et al., 2010). The results of this study demonstrate the
537 advantages, from a sensory point of view, of hydrogel filled particles as n-3 fatty acid
538 delivery systems in frankfurters.
539
540 *3.5 Possible nutrition and health claims*
541 Nutrition and health claim are an added value that can position and differentiate a
542 product. The European Union introduced a regulation (Regulation EC 1924/2006 and
543 Commission Regulation EU 432/2012) to ensure the effective functioning of the market
544 whilst providing a high level of consumer protection. In light of the nutrition and health
545 claims authorized under those European Regulations (EU Register of nutrition and
546 health claims made on foods, 2015), the changes made to the modified frankfurters
547 would warrant a number of nutrition and health claims (Table 8). These include some
548 health claims other than ones referring to the reduction of disease risk and to children's

549 development and health (Art. 13.1) and claims relating to children's development and
550 health (Art.14(1)(b)).
551
552 **4. Conclusions**
553 The n-3 PUFA contents of low-fat (4-5%) frankfurters made with n-3 fatty acid
554 delivery systems as pork backfat replacers ranged from 801.34 to 996.37 mg/100 g, of
555 which approximately 692 to 867 mg/100 g were n-3 LCPUFAs. This means that,
556 although dietary recommendations vary depending on different factors, the products
557 provide a nutritionally significant contribution to dietary intake as compared to non-
558 fortified frankfurters. The incorporation of fish oil had some effect on the technological
559 and sensory characteristics and the stability of reformulated products. The results
560 showed the formation of a stable protein matrix, irrespective of formulation, with
561 adequate ability to immobilize both fat and water. The use of a filled hydrogel
562 (replacing pork backfat) had only limited effects on low-fat frankfurter texture.
563 Although the presence of fish oil favours lipid oxidation, of the n-3 LCPUFA delivery
564 systems assayed to achieve a low-fat frankfurter formulation, filled hydrogel particles
565 were the most oxidatively stable. Compared to the other n-3 delivery systems tested, the
566 filled hydrogels provide nutritional advantages deriving from the presence of these
567 bioactive lipids. These results suggest that the incorporation of fish oil-filled hydrogel
568 particles seems to be a good option for the addition of n-3 LCPUFAs to frankfurters to
569 achieve sensorily acceptable and healthier stable products.
570
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577

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691

692 **Figure caption**

693

694 **Fig. 1.** Concentration of a) hydroperoxides and b) thiobarbituric acid-reactive substance

695 (TBARS) over storage (2 °C) of frankfurters. Error bars represent standard deviations.

696 For sample denomination see Table 1.

Highlights

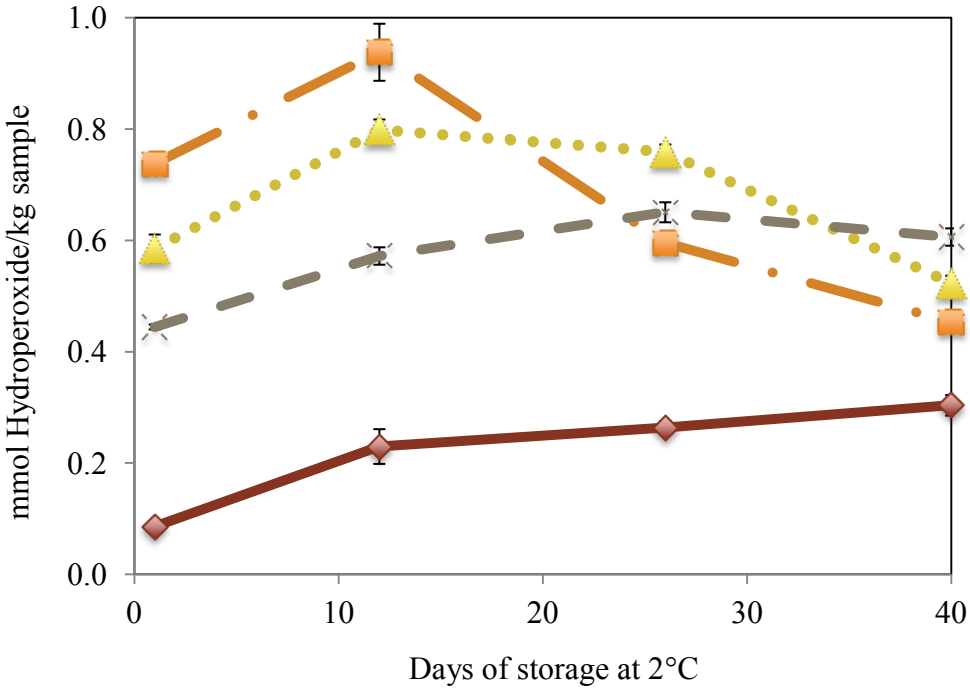
Filled hydrogel particles are used to develop n-3 PUFA in low-fat frankfurters

The strategy of incorporation of fish oil affects the lipid oxidation stability

Filled hydrogel particles frankfurters were the most oxidatively stable

Modified frankfurters would warrant a number of nutrition and health claims

Figure


1.0
0.8
0.6
0.4
0.2
0.0
mmol Hydroperoxide/kg sample
0
10
20
30
40
Days of storage at 2°C

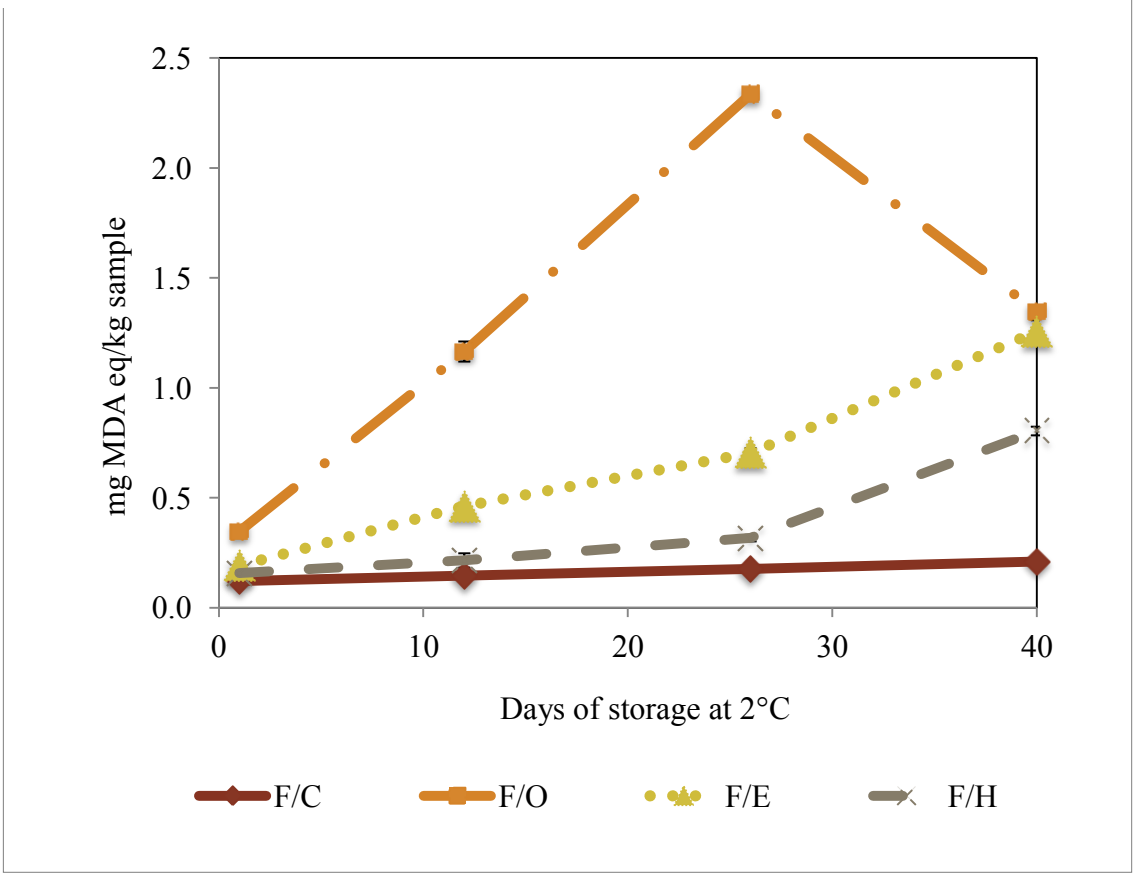

2.5
2.0
1.5
1.0
0.5
0.0
mg MDA eq/kg sample
0
10
20
30
40
Days of storage at 2°C
F/C
F/O
F/E
F/H

Table 1. Formulation (%) of frankfurters.

Sample	Meat	Backfat	Strategy used to incorporate the fish oil			Water
			Liquid oil	O/W emulsion	Filled hydrogel particles	
F/C	58.87	2.16				33.96
F/O	58.87	-	2.00			34.12
F/E	58.87	-		23.50		12.62
F/H	58.87	-			23.50	12.62

Sample denomination: F/C, control frankfurter (F/C) with all pork fat; F/O, frankfurter with fish oil added directly, as liquid form; F/E, frankfurter with fish oil added stabilized in a O/W emulsion; F/H, frankfurter with fish oil incorporated in filled hydrogel particles. O/W emulsion and filled hydrogel particles contained 8.50% fish oil. The following were also added to all samples: 2.50% soy protein isolate; 1.70% sodium chloride; 0.50% flavouring; 0.30% sodium tripolyphosphate and 0.012% sodium nitrite.

Table 2. Proximate analysis (%) of frankfurters.

	F/C	F/O	F/E	F/H
Moisture	72.80 ± 0.09^{1}	73.10 ± 0.03^{2}	73.30 ± 0.09^{3}	73.05 ± 0.07^{2}
Protein	19.37 ± 0.32^{3}	18.68 ± 0.07^{2}	18.71 ± 0.06^{2}	18.05 ± 0.03^{1}
Fat	4.13 ± 0.13^{1}	4.09 ± 0.13^{1}	4.81 ± 0.20^{2}	5.34 ± 0.24^{3}
Ash	3.44 ± 0.01^{2}	3.37 ± 0.04^{1}	3.32 ± 0.01^{1}	3.58 ± 0.02^{3}

For sample denomination see Table 1. Means ± SD. Different numbers in the same row indicate significant differences ($P<0.05$).

Table 3. Fatty acid profile (mg/100 g product) and nutritional significant ratios of frankfurters.

Fatty acid	F/C	F/O	F/E	F/H
Myristic C14:0	41.87 ± 0.90^{2}	19.97 ± 2.65^{1}	20.90 ± 1.06^{1}	21.19 ± 0.82^{1}
Palmitic C16:0	871.42 ± 12.19^{2}	766.10 ± 15.83^{1}	826.44 ± 15.68^{2}	902.14 ± 20.44^{3}
Stearic C18:0	538.02 ± 20.81^{3}	243.02 ± 6.58^{2}	282.33 ± 12.08^{1}	298.16 ± 6.24^{1}
Other SFAs	78.43 ± 17.27^{1}	105.90 ± 1.32^{2}	115.57 ± 7.60^{2}	138.62 ± 5.59^{3}
∑ SFA	1491.49 ± 6.75^{4}	1134.99 ± 23.79^{1}	1245.24 ± 18.84^{2}	1360.11 ± 31.47^{3}
Palmitoleic C16:1	72.26 ± 3.63^{1}	315.50 ± 3.42^{2}	474.63 ± 25.32^{3}	517.85 ± 29.78^{4}
Oleic C18:1n9	1774.16 ± 46.64^{3}	884.37 ± 18.98^{1}	1027.19 ± 42.92^{2}	1025.76 ± 43.07^{2}
Vaccenic C18:1n7*c*	122.00 ± 12.27^{1}	144.03 ± 1.63^{2}	159.48 ± 7.38^{2}	184.34 ± 4.99^{3}
Eicosenoic C20:1n9c	41.91 ± 2.60^{3}	33.04 ± 0.59^{1}	37.95 ± 0.60^{2}	43.76 ± 1.10^{3}
Other MUFA	38.92 ± 4.93^{1}	60.34 ± 0.18^{2}	71.60 ± 8.88^{3}	81.82 ± 3.20^{3}
∑ MUFA	2049.25 ± 66.65^{3}	1437.26 ± 24.23^{1}	1770.84 ± 34.50^{2}	1853.53 ± 71.42^{2}
Linoleic C18:2n6	400.84 ± 13.43^{3}	260.80 ± 2.73^{1}	311.42 ± 18.64^{2}	288.23 ± 6.42^{2}
Linolenic C18:3n3	20.46 ± 0.97^{1}	26.23 ± 0.30^{2}	29.67 ± 1.53^{3}	34.79 ± 0.42^{4}
Eicosapentaenoic C20:5n3	--	417.42 ± 8.23^{1}	466.67 ± 24.41^{2}	539.10 ± 22.15^{3}
Docosahexaenoic C22:6n3	--	275.17 ± 35.29^{1}	294.47 ± 11.91^{12}	328.52 ± 12.67^{2}
Other PUFA	85.57 ± 4.99^{1}	174.93 ± 1.83^{2}	222.62 ± 13.78^{3}	212.29 ± 8.21^{3}
∑ PUFA	506.87 ± 19.35^{1}	1154.55 ± 32.99^{2}	1324.85 ± 49.55^{3}	1402.93 ± 42.02^{3}
∑ n-6	440.87 ± 19.80^{3}	353.22 ± 3.22^{1}	443.43 ± 22.97^{3}	406.56 ± 6.69^{2}
∑ n-3	66.00 ± 1.78^{1}	801.34 ± 30.15^{2}	881.42 ± 40.05^{3}	996.37 ± 38.72^{4}
∑ n-3 LCPUFA	--	692.59 ± 29.14^{1}	761.14 ± 35.54^{2}	867.62 ± 34.80^{3}
n-6/n-3	6.69 ± 0.39^{2}	0.44 ± 0.01^{1}	0.50 ± 0.03^{1}	0.41 ± 0.01^{1}
PUFA/SFA	0.34 ± 0.01^{1}	1.02 ± 0.05^{2}	1.06 ± 0.05^{2}	1.03 ± 0.01^{2}
Atherogenic index	0.40 ± 0.02^{3}	0.33 ± 0.01^{2}	0.29 ± 0.01^{1}	0.30 ± 0.01^{12}
Thrombogenic index	0.98 ± 0.04^{2}	0.31 ± 0.01^{1}	0.30 ± 0.01^{1}	0.30 ± 0.00^{1}

For sample denomination see Table 1. Means ± SD. Different numbers in the same row indicate significant differences ($P<0.05$).

Table 4. Processing loss (%), purge loss (%) of frankfurters.

Sample	Processing loss	Purge loss			
		Storage (days at 2 °C)			
		1	12	26	40
F/C	17.22 ± 0.32^{b}	0.15 ± 0.05^{a1}	0.36 ± 0.08^{a23}	0.44 ± 0.02^{ab3}	0.23 ± 0.07^{ab12}
F/O	17.14 ± 1.31^{b}	0.16 ± 0.03^{a1}	0.54 ± 0.07^{b2}	0.49 ± 0.09^{b2}	0.26 ± 0.06^{b1}
F/E	16.61 ± 0.63^{b}	0.17 ± 0.06^{a1}	0.58 ± 0.06^{b2}	0.28 ± 0.08^{a1}	0.18 ± 0.02^{ab1}
F/H	14.94 ± 0.49^{a}	0.18 ± 0.05^{a1}	0.20 ± 0.03^{a1}	0.39 ± 0.04^{ab2}	0.13 ± 0.04^{a1}

For sample denomination see Table 1. Means ± SD. Different letters in the same column and different numbers in the same row indicate significant differences ($P<0.05$).

Table 5. Colour parameters of frankfurters.

Colour parameter	Sample	Storage (days at 2 °C)			
		1	12	26	40
Lightness (*L**)	F/C	72.97 ± 0.31^{a2}	72.00 ± 0.24^{a1}	71.93 ± 0.69^{a1}	71.74 ± 0.26^{a1}
	F/O	74.46 ± 0.31^{c2}	73.03 ± 0.30^{c1}	73.26 ± 0.28^{c1}	73.05 ± 0.24^{c1}
	F/E	75.38 ± 0.37^{d3}	74.13 ± 0.15^{d2}	73.67 ± 0.34^{c1}	73.74 ± 0.22^{d1}
	F/H	73.57 ± 0.28^{b2}	72.68 ± 0.19^{b1}	72.60 ± 0.18^{b1}	72.64 ± 0.17^{b1}
Redness (*a**)	F/C	5.21 ± 0.28^{c1}	5.77 ± 0.11^{c2}	5.93 ± 0.26^{b2}	6.20 ± 0.14^{c3}
	F/O	4.37 ± 0.15^{a2}	4.12 ± 0.14^{a12}	3.91 ± 0.06^{a1}	4.37 ± 0.44^{a2}
	F/E	4.96 ± 0.13^{b1}	5.57 ± 0.15^{b2}	5.87 ± 0.13^{b2}	5.75 ± 0.47^{b2}
	F/H	6.15 ± 0.14^{d1}	6.35 ± 0.10^{d2}	6.26 ± 0.12^{c12}	6.24 ± 0.23^{c12}
Yellowness (*b**)	F/C	10.20 ± 0.31^{a1}	10.48 ± 0.22^{b12}	10.54 ± 0.43^{a12}	10.59 ± 0.28^{a2}
	F/O	10.90 ± 0.15^{b1}	12.02 ± 0.21^{c2}	12.27 ± 0.32^{b2}	11.88 ± 0.59^{b2}
	F/E	9.68 ± 0.21^{a1}	10.04 ± 0.18^{a2}	10.19 ± 0.36^{a2}	10.38 ± 0.38^{a2}
	F/H	11.86 ± 0.24^{c1}	12.55 ± 0.18^{d2}	12.81 ± 0.22^{c23}	12.93 ± 0.30^{c3}

For sample denomination see Table 1. Means ± SD. Different letters in the same column and different numbers in the same row indicate significant differences ($P<0.05$).

Table 6. TPA parameters of the frankfurters.

Texture parameter	Sample	Storage (days at 2 °C)			
		1	12	26	40
Hardness (N)	F/C	11.54 ± 0.31^{a1}	12.07 ± 0.24^{a12}	12.39 ± 0.33^{a2}	14.39 ± 0.47^{a3}
	F/O	14.17 ± 0.15^{c1}	14.78 ± 0.27^{b12}	15.48 ± 0.20^{c2}	20.34 ± 0.86^{c3}
	F/E	13.17 ± 0.27^{b1}	14.27 ± 0.72^{b2}	14.84 ± 0.71^{c2}	18.29 ± 0.84^{b3}
	F/H	11.50 ± 0.30^{a1}	12.40 ± 0.30^{a2}	13.95 ± 0.27^{b3}	15.13 ± 0.66^{a4}
Springiness (mm)	F/C	7.07 ± 0.10^{bc1}	7.08 ± 0.04^{b1}	7.04 ± 0.08^{a1}	7.00 ± 0.07^{b1}
	F/O	6.97 ± 0.06^{b1}	7.13 ± 0.07^{b2}	7.01 ± 0.08^{a1}	6.95 ± 0.05^{b1}
	F/E	7.12 ± 0.05^{c2}	7.25 ± 0.02^{c3}	7.10 ± 0.10^{a2}	6.99 ± 0.03^{b1}
	F/H	6.83 ± 0.07^{a1}	6.95 ± 0.04^{a2}	7.06 ± 0.04^{a3}	6.75 ± 0.04^{a1}
Cohesiveness (dimensionless)	F/C	0.744 ± 0.004^{a2}	0.743 ± 0.004^{a2}	0.757 ± 0.008^{b3}	0.697 ± 0.009^{b1}
	F/O	0.744 ± 0.007^{a2}	0.742 ± 0.002^{a2}	0.744 ± 0.006^{a2}	0.679 ± 0.011^{a1}
	F/E	0.763 ± 0.005^{b2}	0.764 ± 0.004^{b2}	0.768 ± 0.003^{c2}	0.709 ± 0.002^{c1}
	F/H	0.741 ± 0.002^{a2}	0.741 ± 0.005^{a2}	0.743 ± 0.005^{a2}	0.684 ± 0.003^{a1}
Chewiness (N.mm)	F/C	60.64 ± 1.85^{a1}	63.55 ± 1.70^{a12}	66.03 ± 1.91^{a2}	70.16 ± 1.56^{a3}
	F/O	73.55 ± 1.90^{b1}	78.22 ± 1.77^{b2}	80.69 ± 1.55^{c2}	96.02 ± 2.79^{c3}
	F/E	71.55 ± 1.40^{b1}	79.03 ± 3.92^{b2}	80.89 ± 3.84^{c2}	90.65 ± 4.28^{b3}
	F/H	58.22 ± 1.97^{a1}	63.13 ± 1.97^{a2}	73.14 ± 1.83^{b3}	69.84 ± 2.78^{a3}

For sample denomination see Table 1. Means ± SD. Different letters in the same column and different numbers in the same row indicate significant differences ($P<0.05$).

Table 7. Sensory evaluation of frankfurters.

Sample	Juiciness	Firmness	Texture acceptability	Flavor acceptability	Overall acceptability
F/C	6.32 ± 1.60^{a}	5.17 ± 1.57^{a}	6.97 ± 1.59^{b}	7.15 ± 2.13^{b}	6.95 ± 1.83^{c}
F/O	5.21 ± 1.16^{a}	7.00 ± 1.07^{b}	4.48 ± 1.55^{a}	2.97 ± 1.23^{a}	2.45 ± 0.95^{a}
F/E	3.98 ± 1.60^{b}	6.78 ± 1.16^{b}	4.69 ± 0.81^{a}	4.75 ± 1.39^{a}	4.73 ± 1.57^{b}
F/H	6.08 ± 1.24^{a}	4.67 ± 1.55^{a}	6.65 ± 1.66^{b}	7.08 ± 1.33^{b}	6.83 ± 1.48^{c}

For sample denomination see Table 1. Means±SD. Different letters in the same column indicate significant differences ($P<0.05$).

Table 8. Examples of nutrition and health claims authorized in the reformulated frankfurters according to EU Register of nutrition and health claims made on foods (2015).

Claim type	Nutrient	Health claim	Conditions of use[1]
Art.13 (1)	Protein	Protein contributes to a growth in muscle mass.	Food with at least nutrition claim SOURCE OF PROTEIN (> 12 % of the energy value)
		Protein contributes to the maintenance of muscle mass.	Food with at least nutrition claim SOURCE OF PROTEIN (> 12 % of the energy value)
Art.14 (1)(b)		Protein contributes to the maintenance of normal bones.	Food with at least nutrition claim SOURCE OF PROTEIN (> 12 % of the energy value)
		Protein is needed for normal growth and development of bone in children	Food with at least nutrition claim SOURCE OF PROTEIN (> 12 % of the energy value)
Art.13 (1)	MUFA and/or PUFA	Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels	Food with nutrition claim HIGH UNSATURATED FAT (>70% of the fatty acids present in the product derive from unsaturated fat under the condition that unsaturated fat provides more than 20% of energy of the product)
Art.13 (1)	DHA	DHA contributes to maintenance of normal brain function	Food with at least 40 mg of DHA/100 g
		DHA contributes to the maintenance of normal vision	Food with at least 40 mg of DHA/100 g
Art.14 (1)(b)		DHA maternal intake contributes to the normal brain development of the foetus and breastfed infants	Food shall provide a daily intake of at least 200 mg DHA
		DHA maternal intake contributes to the normal development of the eye of the foetus and breastfed infants	Food shall provide a daily intake of at least 200 mg DHA
Art.13 (1)	EPA/ DHA	EPA and DHA contribute to the normal function of the heart	Food with at least nutrition claim SOURCE OF OMEGA 3 FATTY ACIDS (>40 mg of the sum of EPA and DHA/100 g)

[1] The information required to stablish these claims was estimated from data of Table 2 and 3.

5. Discusión integradora

5. DISCUSIÓN INTEGRADORA

Estrategias de reformulación se han aplicado como respuesta a la necesidad de modificar la composición lipídica de productos cárnicos cuyo contenido en grasa y perfil de ácidos grasos divergen de aquellos recomendados por diferentes organizaciones de salud. En este sentido, algunas de estas estrategias involucran la incorporación de aceites vegetales y marinos cuya composición es más saludable en comparación con la grasa animal habitualmente presente en estos productos. Tal incorporación se ha llevado a cabo mediante diferentes opciones tecnológicas, encontrándose entre las más comunes la adición directa de aceites, la interesterificación y la pre-emulsificación (Jiménez-Colmenero, 2007; Grasso et al., 2014). Recientemente han surgido nuevas alternativas tecnológicas, caso de los denominados lípidos estructurados, basados en procesos de estabilización de aceites con una composición lipídica más saludable, mediante la formación de sistemas con propiedades sólidas o viscosas. Pese a que por sus características, ofrecen interesantes posibilidades de aplicación en derivados cárnicos, su empleo aún es muy limitado. Una amplia información acerca de los diferentes sistemas de lípidos estructurados, sus propiedades, aplicaciones reales (y en algunos casos potenciales), se puede encontrar en un artículo de revisión elaborado por nuestro grupo de investigación y recientemente publicado (Jiménez-Colmenero et al., 2015), el cual se adjunta en el **Anexo**.

Con el fin de cumplir el objetivo general trazado en la presente memoria, que consiste en el **diseño y desarrollo de derivados cárnicos con mejor composición lipídica mediante procesos de reformulación encaminados a la obtención de productos más saludables,** se eligieron dos sistemas distintos de lípidos estructurados escasamente empleados: agente de carga de aceite a base de konjac y partículas de hidrogel. Tales sistemas presentan diferente

estructura, composición y funcionalidad, por lo que su utilización en la reformulación de productos cárnicos provoca cambios a nivel lipídico de distinta naturaleza.

El agente de carga de aceite a base de konjac fue desarrollado recientemente en nuestro grupo de investigación, presentando resultados interesantes en su aplicación en productos frescos (salchichas tipo merguez) en donde se estabilizó aceite de oliva (Triki et al., 2013a), y en productos fermentados (chorizo) (Salcedo-Sandoval et al., 2013a), donde fue empleado para contener una mezcla de aceites similar a la utilizada en esta memoria. Este sistema posee características sólidas que se asemejan bastante a aquellas aportadas por la grasa animal empleada en la elaboración de derivados cárnicos, lo que lo hace muy apropiado a la hora de elaborar productos con contenido reducido en grasa. Otro rasgo a destacar del agente de carga es que puede contener cantidades elevadas de aceite, que para el caso concreto de esta memoria fue del 20%. Esta capacidad de carga permite estabilizar material lipídico con una composición adecuada, como es el caso de la mezcla de aceites de oliva, lino y pescado desarrollada por Delgado-Pando et al. (2010a) y empleada en esta memoria. Esta mezcla contiene una importante proporción de AGM y AGP n-3, (que incluye un significativo aporte de AGP n-3 de cadena larga), logrando unas relaciones de AGP/AGS y de AGP n-6/n-3 ajustadas a las recomendaciones de ingesta óptima de ácidos grasos insaturados y de ácidos grasos totales (WHO, 2003). Así pues, de acuerdo con lo anteriormente mencionado, la sustitución de grasa animal típicamente presente en productos cárnicos por un agente de carga de aceite a base de konjac podría conducir a la reducción del contenido de grasa y simultáneamente, a la mejora del perfil lipídico de productos reformulados. Con el fin de observar el efecto de esta estrategia de reformulación sobre la composición, propiedades tecnológicas, microbiológicas, sensoriales y la conservación en refrigeración, se elaboraron

dos tipos de productos cárnicos distintos como salchichas tipo frankfurt (producto cocido) y hamburguesas (producto fresco). En ambos casos fueron sustituidos diferentes niveles de grasa animal por cantidades similares de agente de carga a base de konjac.

Las partículas de hidrogel se clasifican dentro de la categoría de "emulsiones estructuradas", tratándose de un sistema $O/W_1/W_2$ (McClements, 2012; Jiménez-Colmenero et al., 2015). La idea de aplicar este tipo de sistema en la optimización lipídica de productos cárnicos se apoyó en gran medida en las actividades llevadas a cabo durante una estancia predoctoral realizada en un laboratorio con amplia y reconocida trayectoria en el desarrollo de estos y otros tipos de emulsiones estructuradas[1]. Las partículas de hidrogel han sido ampliamente usadas en la industria farmacéutica, donde se han utilizado como sistemas de entrega (*delivery systems)* de principios activos en zonas específicas del cuerpo humano, perfilandose actualmente como ingredientes potenciales en el desarrollo de productos alimenticios (McClements, 2010). Estos sistemas pueden ser diseñados para proveer de protección física y química a compuestos bioactivos lipofílicos como los AGP n-3 de cadena larga, a la vez que pueden utilizarse en el desarrollo de productos bajos en grasa debido a su estructura viscosa (Chung et al., 2013). Las partículas de hidrogel utilizadas en el desarrollo de esta memoria fueron previamente estudiadas en relación a su formación, propiedades físicas, reológicas y digestibilidad in vitro (Matalanis et al., 2010; Matalanis y McClements, 2012a, b). En estos estudios, se confirmó que la fase lipídica presente en este sistema era altamente accesible para la digestión y absorción en el cuerpo, siendo las partículas de hidrogel, apropiadas para la entrega de compuestos activos lipídicos. No obstante, uno de los principales inconvenientes para la aplicación de las mismas en el desarrollo de productos

[1] Department of Food Science, University of Massachusetts. Amherst, MA, USA. Estancia realizada bajo la tutoría del Profesor Eric. A. Decker. Marzo-Agosto, 2012.

es su limitada capacidad de carga (Matalanis y McClements, 2013). En este sentido, para cumplir con el objetivo planteado en esta memoria, fue necesario replantear la formación de partículas de hidrogel estabilizando aceite de pescado, a fin de hacerlas apropiadas para su aplicación en el desarrollo de productos cárnicos enriquecidos con EPA y DHA. Teniendo en cuenta que las recomendaciones de ingesta diaria de EPA y DHA establecidas por diversos organismos varían en un rango de 190 a 1000 mg (Garg et al., 2006; Meyer, 2011; Taneja y Singh, 2012), la preparación de las partículas de hidrogel requirió la realización de varias modificaciones dirigidas a aumentar la capacidad de carga de estos sistemas, de forma tal que permitiera desarrollar productos con niveles superiores a 600 mg de EPA y DHA/100 g, y así poder realizar una mejora relevante en el perfil lipídico de productos cárnicos .

Ambos planteamientos descritos anteriormente suponen la mejora del perfil de ácidos grasos mediante sustitución de grasa animal. Ciertamente, el agente de carga pueden considerarse un sustituto de grasa animal, puesto que este tipo de sistema permite reemplazar grasa en un amplio rango de niveles. Sin embargo, las partículas de hidrogel no son estrictamente sistemas adecuados para sustituir grasa, puesto que es difícil reemplazar niveles superiores a los llevados a cabo en esta memoria, más bien deben ser considerados como ingredientes para el enriquecimiento de productos en EPA y DHA. Por lo tanto, a lo largo de esta discusión se hablará por un lado de la sustitución de grasa animal mediante el uso del agente de carga y por otro lado del enriquecimiento en EPA y DHA a través del empleo de partículas de hidrogel, las cuales se abordarán por separado. Ambas estrategias utilizadas tienen un común denominador: La obtención de productos con composición lipídica más saludable.

Por otra parte, este estudio se ha realizado con dos tipos de producto que difieren entre sí por sus características y condiciones de procesado: productos

tipo gel/emulsión (salchichas tipo frankfurt) y productos frescos (hamburguesas). Un sistema modelo fue empleado para evaluar la estabilidad oxidativa de las partículas de hidrogel en una matriz cárnica, puesto que tales partículas hasta entonces, no habían sido estudiadas en este tipo de matrices. La elección de las salchichas tipo frankfurt y las hamburguesas se hizo por dos razones. Por un lado, ambos son productos muy populares, frecuentemente consumidos, gozan de una amplia aceptación y tienen una considerable importancia económica. De otro lado, su contenido en grasa y perfil de ácidos grasos generalmente divergen de las recomendaciones nutricionales (Jiménez-Colmenero, 2007). Por consiguiente, llevar a cabo procesos de reformulación en productos con alto índice de consumo y susceptibles de ser optimizados en su composición lipídica, podría multiplicar el impacto positivo de los resultados recogidos en esta memoria.

Con el propósito de discutir los resultados desde un enfoque diferente al proporcionado por las publicaciones incluidas (**Capítulo 4**), en esta sección se propone un análisis integral de los principales resultados obtenidos a lo largo del estudio. Se trata de aportar una visión general de las consecuencias producidas por efecto de las estrategias de reformulación aplicadas sobre las distintas características evaluadas. Para facilitar la lectura y compresión de la discusión integradora, en la **Figura 5.1** se representa esquemáticamente la estructura con la que ha sido planteada.

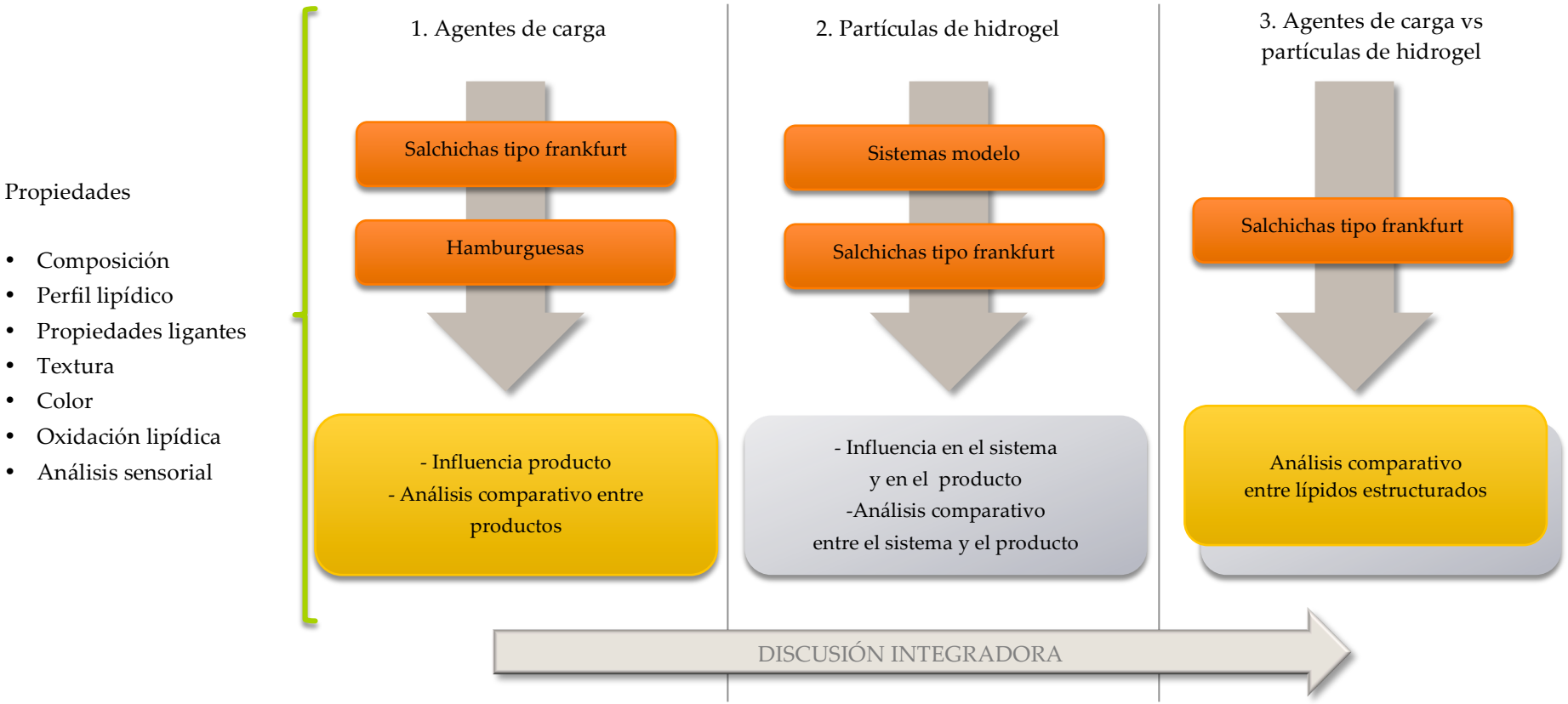


Figura 5.1. Esquema de la estructura con la que se aborda la discusión integradora

5.1 COMPOSICIÓN

5.1.1 Efecto de la reformulación asociado al empleo de agente de carga

La utilización de un agente de carga a base de konjac, estabilizando una mezcla de aceites de oliva, lino y pescado se empleó en primera instancia, en la sustitución parcial de tocino de cerdo en salchichas tipo frankfurt (F/OKM), diseñadas para contener un 10% de grasa y una mejor composición en ácidos grasos (**Capítulo 4.1.1**). Estas salchichas fueron comparadas con a) un producto control (F/C, 20% de grasa, toda de procedencia animal); b) una formulación con contenido reducido en grasa mediante sustitución parcial de tocino de cerdo por gel de konjac, (F/KG, 10% de grasa, toda de procedencia animal), y c) dos formulaciones en las que se redujo el contenido en grasa y se mejoró el perfil lipídico a través de la sustitución parcial de tocino de cerdo por la mezcla de aceites anteriormente mencionada, estabilizada en un caso con una emulsión O/W (F/OWE) y en otro caso con una emulsión O/W junto con gel de konjac (F/OWE+KG), ambas formulaciones diseñadas para contener 10% de grasa (**Tabla 1, capítulo 4.1.1**). El empleo de emulsiones O/W en las dos últimas formulaciones mencionadas se debe a que este sistema es la opción más utilizada a la hora de estabilizar aceites destinados a la sustitución de grasa animal en matrices cárnicas (Lee et al., 2006; Caceres et al., 2008; Delgado-Pando et al., 2010a; Martínez et al., 2012; Salminen et al., 2013; entre otros)

Los cambios en composición estuvieron afectados principalmente por la reducción de grasa, independientemente del sustituto empleado (**Tabla 2, capítulo 4.1.1**). En la **Figura 5.2** se presentan los resultados de composición tanto del producto control, como de la muestra reformulada con agente de carga. Los parámetros más afectados fueron la humedad y la grasa, mientras que la proteína y las cenizas no presentaron diferencias relevantes. Puesto que la reducción de grasa en los productos se llevó a cabo a expensas de un aumento en la cantidad de agua añadida (manteniendo básicamente constante

la proporción de proteína), las formulaciones con menores niveles de grasa tuvieron mayores niveles de humedad, siendo del 60% en la muestra control y de alrededor del 70% en las muestras reformuladas. En consonancia con la formulación propuesta, la muestra control presentó un contenido de grasa de aproximadamente 19%, mientras que todas las muestras reformuladas, incluida la elaborada con agente de carga a base de konjac presentaron niveles del 10,0-10,3%. En las muestras en las que fue sustituido tocino de cerdo por la mezcla de aceites saludable (F/OKM, F/OWE y F/OWE+KG), cerca de un 40% del contenido total de grasa fue aportado por dicha mezcla.

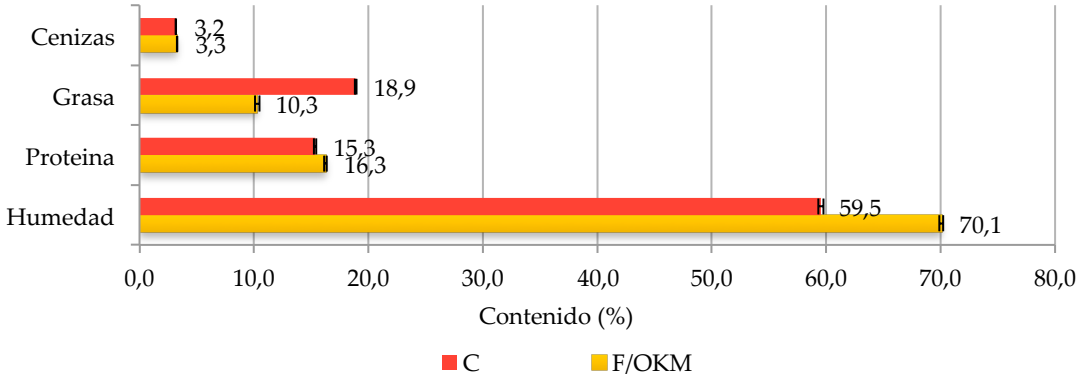


Figura 5.2. Composición (%) de salchichas tipo frankfurt convencionales y reformuladas con agente de carga.

Adaptado de la **tabla 2, capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b).

Denominación de las muestras: C, control; F/OKM, reducción de grasa y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga.

Una gran diversidad de aceites vegetales y marinos se ha utilizado en la reducción y mejora del contenido lipídico en salchichas tipo frankfurt. Estos han sido incorporados generalmente de forma individual, en proporción variable (1-20 g aceite/100 g producto) y mediante distintas estrategias (adición directa, interesterificación, emulsificación) (Paneras y Bloukas, 1994; Vural et al., 2004; Álvarez et al., 2011). En relación a la incorporación de mezcla de aceites en productos análogos, Paneras et al. (1998), describieron la adición de una combinación de aceites de oliva, maíz y soja (4,0 g/100 g), mientras que Delgado-Pando et al. (2010a) utilizó una combinación de aceites similar a la empleada en este estudio (9,5 g/100 g), para la sustitución total de grasa animal

añadida. En ambos casos, las mezclas de aceites fueron estabilizadas en emulsiones O/W y el contenido total de grasa en los productos fue de alrededor del 10%.

En el caso de las hamburguesas (**Capítulo 4.1.3)**, el agente de carga de aceites a base de konjac se empleó en la reducción de grasa y mejora del perfil lipídico mediante sustitución parcial (49%) y total (100%) de tocino de cerdo, obteniendose niveles medio y bajo en grasa, respectivamente (muestras IMFP e ILFP). Estas hamburguesas fueron comparadas con una muestra control (CFP), diseñada con contenido normal de grasa (toda de cerdo), así como con tres muestras en las que se redujo el contenido en grasa a niveles medio (MFP), bajo (LFP) y muy bajo (VLFP), a través de la sustitución de tocino de cerdo por cantidades proporcionales de gel de konjac (38%, 78%, 100% respectivamente) (**Tabla 1, capítulo 4.1.3)**. Todas las hamburguesas fueron formuladas con cantidad similar de carne magra.

Al igual que las salchichas tipo frankfurt, la reformulación influyó considerablemente sobre los contenidos de humedad y grasa de las diferentes hamburguesas. En tal sentido, la reducción de grasa estuvo acompañada de un incremento en los niveles de humedad, mientras que los parámetros de proteína y cenizas no mostraron diferencias importantes (**Tabla 2 del capítulo 4.1.3** y **Figura 5.3**). Las muestras con niveles medio y bajo en grasa elaboradas con gel de konjac o agente de carga, presentaron composiciones similares (MFP = IMFP y LFP = ILFP, $P>0,05$). Los porcentajes de grasa fueron de alrededor del 15% para la muestra control, 9% para las muestras con nivel medio en grasa, 4% en las muestras con nivel bajo en grasa y 2% en la muestra con nivel muy bajo en grasa. En el caso de las hamburguesas en las que se incorporó agente de carga (IMFP e ILFP), alrededor del 12% y 40% de la fracción lipídica fue aportada por la mezcla de aceites.

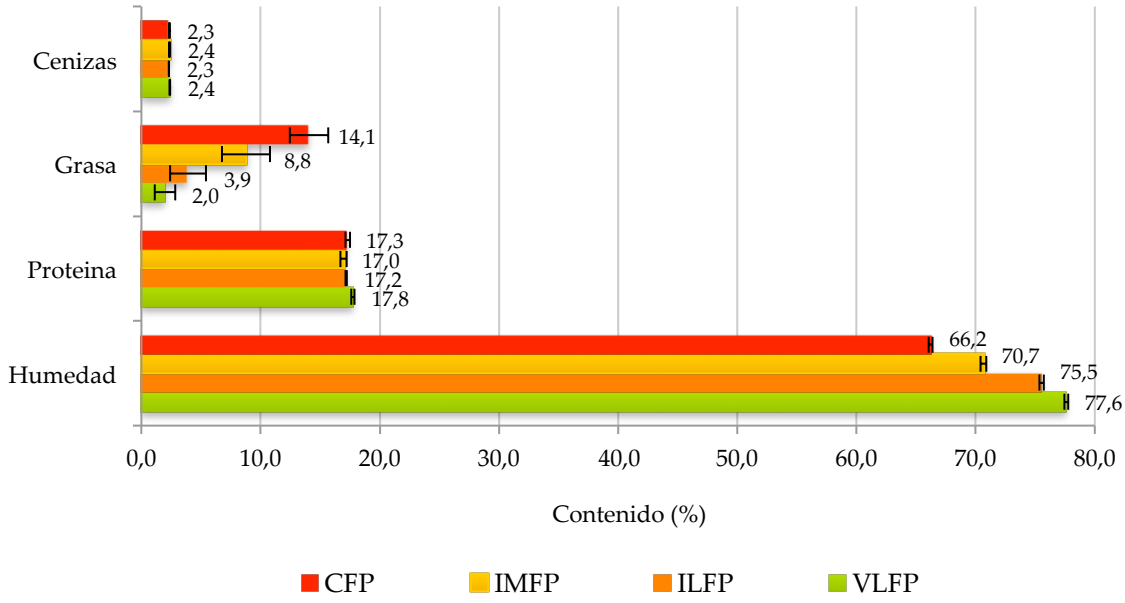


Figura 5.3. Composición (%) de hamburguesas convencionales y reformuladas con agente de carga y con gel de konjac.

Adaptado de la **tabla 2, capítulo 4.1.3** (Salcedo-Sandoval et al., 2015a).

Denominación de las muestras: CFP, control con contenido normal de grasa; IMFP, reducción de grasa (nivel medio) y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga; ILFP, reducción de grasa (nivel bajo) y mejora del perfil mediante sustitución total de tocino de cerdo con agente de carga; VLFP, reducción de grasa (nivel muy bajo) mediante sustitución total de tocino de cerdo con gel de konjac.

Con el objeto de mejorar el contenido lipídico en hamburguesas, varios autores han reportado el empleo de diferentes aceites incorporados de distintas formas. Hur et al. (2008) sustituyeron 50% de tocino de cerdo por aceite de oliva adicionado directamente en hamburguesas con aproximadamente 15% de grasa. López-López et al. (2010) reemplazaron en hamburguesas de vacuno aproximadamente 25% y 60% de tocino de cerdo a través de la adición de 5 y 10% de emulsión O/W de aceite de oliva (52,6% aceite). La utilización de una mezcla de aceites oliva, maíz y pescado en la elaboración de hamburguesa de vacuno con 9% de contenido en grasa, ha sido descrita por Martínez et al. (2012).

Por otro lado, en un segundo estudio de hamburguesas (**Capítulo 4.1.4**), se evaluó el efecto de la reformulación y los métodos de cocción (plancha y fritura) sobre los componentes mayoritarios y el perfil lipídico de los productos. Dicho estudio se llevó a cabo en cuatro muestras seleccionadas previamente

(**Capítulo 4.1.3**), en base a sus características tecnológicas y sensoriales. Tales muestras fueron CFP, MFP, IMFP y LFP (esta última en el capítulo 4.1.3 está denominada como VLFP).

La composición de las hamburguesas estuvo influenciada tanto por la reformulación como por los métodos de cocción (plancha y fritura) (**Tabla 5.1**). El contenido de humedad disminuyó en todas las muestras después de la cocción, aunque los valores de retención de agua aumentaron por efecto de la reducción de grasa y la cocción en fritura ($P<0,05$). Estos resultados son similares a los encontrados por otros autores, quienes observaron que el gel de konjac presenta excelentes propiedades de retención incluso a altas temperaturas, pese a que contiene grandes proporciones de agua (Jiménez-Colmenero et al., 2012a). El contenido en proteína se incrementó en todas las muestras como resultado de la cocción, aunque de manera más acusada cuando las hamburguesas fueron preparadas a la plancha, resultados que se corresponden con los datos de pérdidas por cocción. No obstante, la reformulación y la cocción tuvieron un impacto menor sobre la proteína de las hamburguesas, como se reflejan los valores de retención cercanos al 90% (**Tabla 5.1**). Niveles de retención de proteína cercanos al 100% han sido descritos en varios productos cárnicos sometidos a distintos métodos de cocción (Maranesi et al., 2005; López-López et al., 2011). El contenido en grasa se incrementó ($P<0,05$) por efecto de la fritura en todos los productos (excepto en MFP), mientras que la cocción a la plancha sólo produjo un incremento significativo en la muestra con agente de carga (IMFP). Con independencia del método de cocción, los valores de retención de grasa fueron superiores en la muestra con menor contenido en grasa (LFP). Se ha descrito que la retención de grasa en este tipo de productos depende de varios factores, entre ellos su porcentaje: productos con alto contenido en grasa tienden a perder mayor cantidad durante la cocción, en tanto que productos con bajo contenido en grasa pierden

relativamente poca cantidad (Sheard et al., 1998; Serrano et al., 2007). En hamburguesas formuladas con niveles similares de grasa (MFP e IMFP), la retención fue significativamente mayor en las muestras con agente de carga, independientemente del método de cocción (**Tabla 5.1**). Estos resultados confirman la buena estabilización que el agente de carga proporciona al aceite, contribuyendo a retener y a evitar las pérdidas de grasa durante la cocción.

Tabla 5.1. Composición y retención de nutrientes (%) en hamburguesas.

	CFP	MFP	LFP	IMFP
Humedad				
Crudo	64.05 ± 0.14 ^{c1}	70.60 ± 0.32^{b2}	77.54 ± 0.30^{b3}	70.54 ± 0.17^{b2}
Plancha	58.79 ± 0.66 ^{b1}	65.00 ± 0.45^{a2}	73.57 ± 0.21^{a3}	64.49 ± 0.34^{a2}
Fritura	57.27 ± 0.23 ^{a1}	65.24 ± 0.11^{a3}	72.86 ± 0.33^{a4}	63.84 ± 0.49^{a2}
Proteína				
Crudo	18.40 ± 0.20 ^{a2}	17.35 ± 0.21^{a1}	17.61 ± 0.22^{a1}	17.45 ± 0.14^{a1}
Plancha	24.62 ± 0.33 ^{c3}	22.26 ± 0.08^{c2}	20.45 ± 0.11^{c1}	21.62 ± 0.47^{c2}
Fritura	23.05 ± 0.21 ^{b3}	20.90 ± 0.13^{b2}	19.92 ± 0.10^{b1}	20.13 ± 0.12^{b1}
Grasa				
Crudo	15.13 ± 0.55^{a3}	10.03 ± 0.80^{a2}	2.93 ± 0.20^{a1}	9.94 ± 0.67^{a2}
Plancha	13.79 ± 0.90^{a4}	9.80 ± 0.41^{a2}	3.84 ± 0.23^{ab1}	11.34 ± 0.16^{b3}
Fritura	17.95 ± 0.64^{b4}	11.14 ± 0.95^{a2}	4.47 ± 0.49^{b1}	13.79 ± 0.21^{c3}
Cenizas				
Crudo	2.36 ± 0.02^{a2}	2.39 ± 0.01^{a2}	2.17 ± 0.01^{a1}	2.35 ± 0.02^{a2}
Plancha	2.89 ± 0.01^{c3}	2.91 ± 0.01^{c3}	2.65 ± 0.01^{b1}	2.79 ± 0.03^{c2}
Fritura	2.63 ± 0.03^{b1}	2.76 ± 0.09^{b1}	2.68 ± 0.03^{b1}	2.66 ± 0.04^{b1}
Retención humedad				
Plancha	62.70 ± 0.93^{a1}	64.90 ± 0.47^{a2}	69.58 ± 0.40^{a3}	64.66 ± 0.28^{a2}
Fritura	66.83 ± 0.21^{b1}	68.12 ± 0.42^{b12}	74.33 ± 0.47^{b3}	69.16 ± 0.44^{b2}
Retención proteína				
Plancha	92.85 ± 0.51^{a3}	90.43 ± 0.79^{a2}	85.15 ± 0.58^{a1}	87.62 ± 1.41^{a12}
Fritura	95.12 ± 0.22^{b2}	88.78 ± 0.61^{a1}	89.49 ± 0.86^{b1}	88.15 ± 0.19^{a1}
Retención grasa				
Plancha	63.24 ± 2.81^{a1}	69.01 ± 2.90^{a1}	96.24 ± 1.90^{a3}	80.79 ± 4.21^{a2}
Fritura	90.23 ± 6.18^{b1}	81.87 ± 2.35^{b1}	120.43 ± 5.68^{b3}	106.19 ± 5.34^{b2}
Retención cenizas				
Plancha	85.04 ± 0.49^{a1}	85.90 ± 0.14^{a1}	89.74 ± 0.14^{a2}	83.99 ± 0.14^{a1}
Fritura	84.67 ± 0.81^{a1}	85.24 ± 2.57^{a1}	97.87 ± 0.60^{b2}	86.61 ± 1.17^{a1}

Adaptado de las **tablas 2 y 6, capítulo 4.1.4** (Salcedo-Sandoval et al., 2014).

Medias ± desviación estándar. Diferentes letras en la misma columna y diferentes números en la misma fila indican diferencias significativas (P<0,05).

Denominación de las muestras: CFP, control con contenido normal de grasa; MFP, reducción de grasa (nivel medio) mediante sustitución parcial de tocino de cerdo con gel de konjac; IMFP, reducción de grasa (nivel medio) y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga; LFP, reducción de grasa (nivel muy bajo) mediante sustitución total de tocino de cerdo con gel de konjac.

A la luz de los resultados obtenidos, se puede señalar que las consecuencias que produce en la composición la presencia de agente de carga no están asociadas al tipo de producto, sino más bien a la estrategia de reformulación planteada en cada estudio. Las cantidades de agente de carga empleadas en los productos fueron 19,1% en salchichas tipo frankfurt, 6,8% en hamburguesas con nivel medio de grasa y sustitución parcial de tocino y 13,4% en hamburguesas con nivel bajo en grasa y sustitución total de tocino. Estos niveles de incorporación permitieron tanto reducir el contenido en grasa, como favorecer la presencia de un material lipídico más acorde con las recomendaciones nutricionales (**Figura 5.4**). De igual forma, la utilización del agente de carga de aceites a base de konjac permitió llevar a cabo reducciones de niveles de grasa superiores al 30% en los productos reformulados con respecto a las formulaciones control (diseñadas con contenido normal de grasa), por lo que estos podrían llevar en su etiquetado la alegación "Contenido reducido en grasa", de acuerdo con el Reglamento 1924/2006 de la Comisión Europea (**Figura 5.4**).

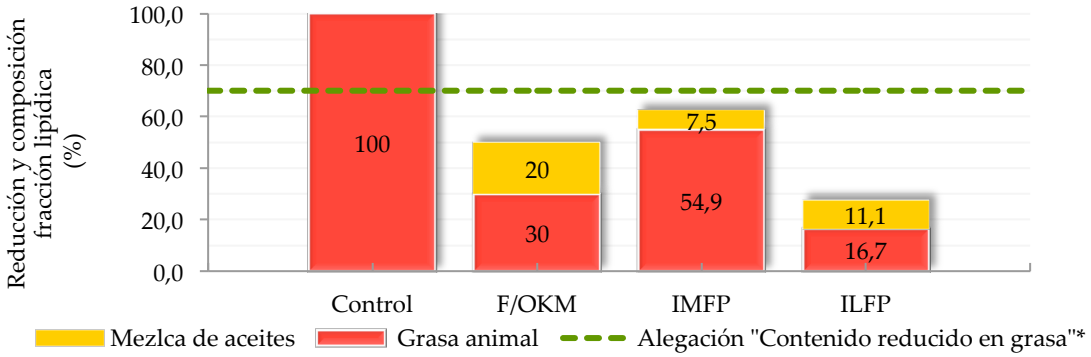


Figura 5.4. Porcentajes de reducción de grasa con respecto a la muestra control (fijada como 100%) en salchichas tipo frankfurt y hamburguesas reformuladas por incorporación del agente de carga de aceites a base de konjac y proporción de los tipos de grasa que componen los productos.

Adaptado del **capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b) y **capítulo 4.2.2** (Salcedo-Sandoval et al., 2015b)

Formulaciones con reducciones por debajo de la línea verde punteada (30%), pueden llevar la alegación "Contenido reducido en grasa" de acuerdo con el reglamento 1924/2006 de la Comisión Europea.

Denominación de las muestras reformuladas mediante sustitución de tocino de cerdo con agente de carga: F/OKM, salchicha con sustitución parcial; IMFP, hamburguesa con sustitución parcial; ILFP, hamburguesa con sustitución total.

5.1.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

Existen diversos estudios sobre partículas de hidrogel que describen su buen comportamiento estabilizando y protegiendo compuestos bioactivos lipofílicos (Matalanis et al., 2012; Matalanis y McClements, 2012b; Chung et al., 2013; Matalanis y McClements, 2013). En base a esta actuación, las partículas de hidrogel se perfilan como una buena opción para incorporar AGP n-3 de cadena larga en productos alimenticios, que pueden presentar limitaciones de estabilidad frente a procesos de oxidación (McClements, 2012). Sin embargo, su utilización como parte de una formulación cárnica no había sido anteriormente estudiada. Factores intrínsecos de los productos cárnicos (presencia de sal, iones metálicos de transición, etc.), junto con otros relacionados con sus condiciones de procesado (picado, mezclado, aumento de temperatura, entre otros), favorecen la oxidación lipídica en los derivados elaborados con AGP n-3 de cadena larga (Lee et al., 2006). Por tanto, la protección que las partículas de hidrogel podrían ofrecer a un producto cárnico conteniendo altas concentraciones de AGP n-3 de cadena larga frente a procesos de oxidación, aunque potencialmente relevante, no había sido explorada. En consecuencia, como primer paso al diseño y desarrollo de un producto cárnico enriquecido en EPA y DHA, se planteó la evaluación de estabilidad oxidativa en una matriz cárnica (sistema modelo), cuya composición sólo contuvo carne, material lipídico, sal y agua, con el fin de evitar interferencias que impidieran observar el efecto de las partículas de hidrogel en la oxidación lipídica.

La utilización de partículas de hidrogel estabilizando aceite de pescado en sistemas modelo (MS/H) no produjo cambios en la composición, con respecto a las otras muestras evaluadas en el estudio (**Tabla 1, capítulo 4.2.1**). Este es el caso del producto control, elaborado con grasa animal (MS/C), y otros dos preparados con aceite de pescado (en lugar de tocino de cerdo) adicionado: 1)

directamente (MS/O); y 2) estabilizado en una emulsión O/W (MS/E). Esto se debió a que todas las muestras fueron formuladas con igual contenido de grasa y proteína, de manera que la única diferencia entre las formulaciones fue el origen de su material lipídico. Por tanto, todos los sistemas presentaron similar composición, con valores de humedad entre 77-78%, proteína en un rango de 16,5-17,6%, niveles de grasa entre 2,4% y 2,7% y ceniza de alrededor de 2,8%.

A partir de los resultados obtenidos, el segundo paso fue la utilización de partículas de hidrogel en la reformulación de salchichas tipo frankfurt, analizando el efecto sobre su composición, sus propiedades tecnológicas y sensoriales. La composición de salchichas tipo frankfurt formuladas con partículas de hidrogel (F/H), mostraron ligeras diferencias cuantitativas respecto a las demás muestras (formuladas siguiendo el mismo planteamiento del estudio de los sistemas modelo descrito anteriormente, F/C, F/O y F/E) (**Tabla 2, capítulo 4.2.2**). Tales diferencias pueden ser principalmente atribuidas a las pérdidas de peso, como se discutirá más adelante (**apartado 5.2**, propiedades ligantes de agua y grasa). El nivel de humedad se situó entre 72,8 y 73,3%, mientras que la proteína alcanzó un rango entre 18,05-19,37%. El contenido en grasa fue generalmente muy cercano al establecido en el diseño del estudio, situándose entre 4,09 y 5,34%, siendo el valor más alto ($P<0,05$) el mostrado por las salchichas que contenían partículas de hidrogel. Mientras que en la formulación control la grasa de la muestra era procedente de cerdo, en las otras tres muestras, cerca del 40% fue proporcionada por el aceite de pescado, incorporado de distintas formas. Así pues, las diferencias en el contenido de grasa de los distintos lotes pueden estar asociadas con variaciones de pérdida de grasa producidas durante el proceso de cocción, las cuales pudieron ser afectadas tanto por el tipo de material lipídico, como por la estrategia utilizada para incorporar el aceite de pescado en las formulaciones.

Las diferencias de composición entre matrices (sistemas modelo y salchichas tipo frankfurt) en las que se emplearon partículas de hidrogel como estrategia para mejorar el perfil lipídico, parecen atribuibles básicamente a diferencias en la sistemática de evaluación. Mientras en el caso de los sistemas modelo, se cuantificó la composición de la masa total, en las salchichas tipo frankfurt, se determinó la composición del producto después de experimentar las correspondientes pérdidas durante el procesado.

5.1.3 Agente de carga vs partículas de hidrogel

Cuando se trata de comparar el mismo tipo de producto (salchichas tipo frankfurt), pero elaborado mediante las dos estrategias planteadas, se observan algunas diferencias. Como punto de partida, conviene señalar que los productos elaborados con agente de carga (F/OKM) y con partículas de hidrogel (F/H) muestran ligeras diferencias en las proporciones de carne magra y agua añadida (**Figura 5.5**). Por otro lado, las cantidades incorporadas de uno u otro sistema de lípidos estructurados (agente de carga o partículas de hidrogel), no estuvieron muy lejanas entre sí, aunque una de ellas (F/OKM) incluye grasa animal añadida.

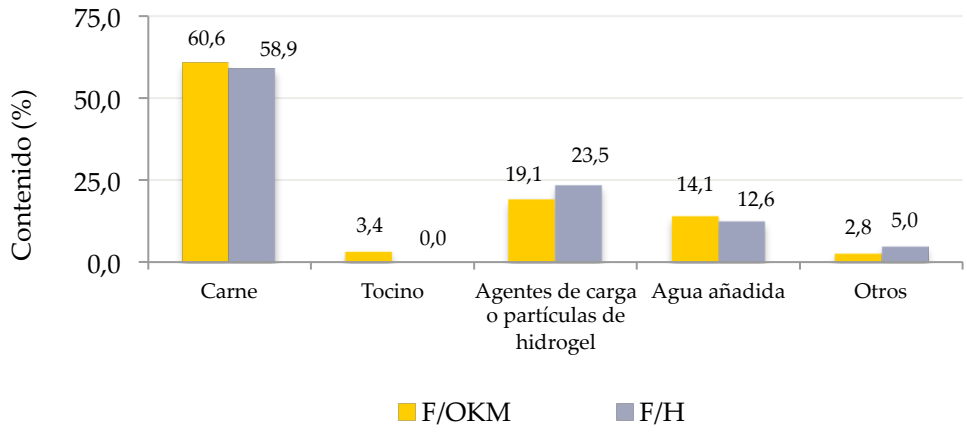


Figura 5.5. Formulaciones de salchichas tipo frankfurt en las que se utilizaron agente de carga (F/OKM) o partículas de hidrogel (F/H).
Adaptado de **tabla 1, capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b) y **tabla 1, capítulo 4.2.2** (Salcedo-Sandoval et al., 2015b).

De acuerdo a lo anterior, y teniendo en cuenta los resultados de composición de ambas formulaciones (**Figura 5.6**), se puede apreciar que, como no podría ser de otra manera, los cambios en la composición de salchichas están relacionados principalmente con el tipo de sistema de lípidos estructurados incorporado en la formulación. Por ejemplo, los porcentajes de humedad y proteína fueron mayores en el caso de las salchichas formuladas con partículas de hidrogel, lo cual se puede asociar a un mayor contenido de humedad de este (aproximadamente 91%) con respecto al agente de carga (alrededor de 79%) y a la presencia de proteína en este sistema (caseinato de sodio). No obstante, es en el contenido en grasa donde se observó la diferencia más notable. Así, las formuladas con agente de carga presentan mayor contenido de grasa frente a las preparadas con partículas de hidrogel. Sin embargo, en los dos casos, la proporción de material lipídico más saludable (mezcla de aceites o aceite de pescado), constituyó alrededor del 40% de la grasa del producto.

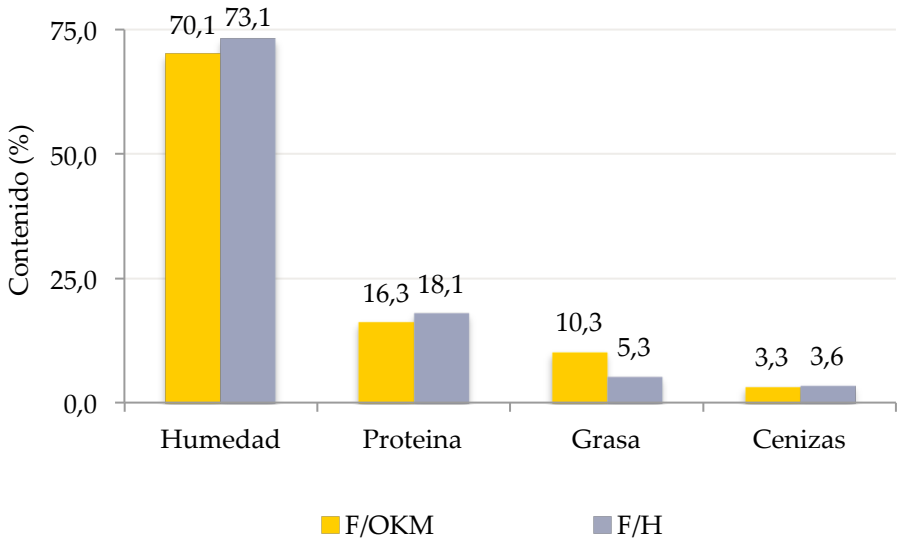


Figura 5.6. Composición (%) de salchichas tipo frankfurt formuladas con agente de carga (F/OKM) o partículas de hidrogel (F/H).
Adaptado de **tabla 2, capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b) y **tabla 2, capítulo 4.2.2** (Salcedo-Sandoval et al., 2015b).

5.2 PERFIL LIPÍDICO

El potencial efecto saludable que puede proporcionar la presencia de material lipídico en los derivados cárnicos estudiados en esta memoria, se fundamenta principalmente en los cambios originados sobre el perfil de ácidos grasos, lo que llevaría asociadas implicaciones en la mejora del estado de salud y bienestar y/o reducción del riesgo de enfermedad. En este sentido se ha analizado cómo la presencia de la mezcla de aceites estabilizada en un agente de carga o el aceite de pescado encapsulado en partículas de hidrogel condicionan el perfil lipídico de los productos reformulados.

5.2.1 Efecto de la reformulación asociado al empleo de agente de carga

La incorporación de material lipídico con composición más saludable mediante un agente de carga para sustituir parcialmente grasa animal, produjo una mejora importante del perfil de ácidos grasos de salchichas tipo frankfurt (**Tabla 3, capítulo 4.1.1**). Tanto en las salchichas control como en las reformuladas, los AGM fueron los ácidos más abundantes. La muestra control mostró el mayor contenido en AGS y AGM. La reducción de grasa con gel de konjac o con agente de carga produjo un descenso en la presencia de ácidos grasos, no obstante, la disminución de grasa con gel de konjac no alteró las proporciones relativas de ácidos grasos con respecto a la muestra control (**Figura 5.7**). En la formulación en la que se sustituyó parcialmente grasa animal por la combinación de aceites estabilizada en un agente de carga (F/OKM), tuvo lugar la mayor disminución de AGS (57 %) respecto a la muestra control, además de un notable incremento en los niveles de AGP (en especial los n-3), produciendo un aumento de casi 3 veces en la relación AGP/AGS y una disminución de aproximadamente 13 veces en la proporción AGP n-6/n-3 (**Figura 5.7**). Estas proporciones son similares a las descritas por otros autores en salchichas reformuladas con aceites vegetales y marinos (Paneras et al., 1998; López-López et al., 2009).

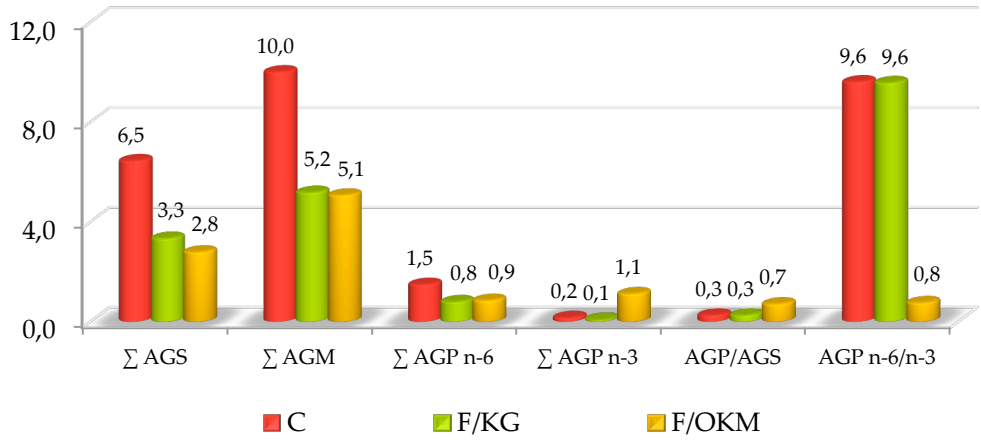


Figura 5.7. Composición en ácidos grasos (g/100 g de producto) y relaciones de ácidos grasos tipo frankfurt.

Adaptado de la **Tabla 3, capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b).

Denominación de las muestras: C, control; F/KG, reducción de grasa mediante sustitución parcial de tocino de cerdo con gel de konjac; F/OKM, reducción de grasa y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga.

En un primer estudio se evaluaron las propiedades tecnológicas y sensoriales de las hamburguesas (**Capítulo 4.1.3**). En base a los resultados obtenidos se seleccionaron aquellas que presentaron mejores características, evaluándose su composición nutricional con especial referencia al perfil de ácidos grasos (**Capítulo 4.1.4**). Tal evaluación se realizó considerando dos factores: la aplicación de la estrategia de reformulación (reducción de grasa empleando gel de konjac o reducción de grasa y mejora del perfil lipídico utilizando el agente de carga de aceite a base de konjac) y el impacto de los métodos de cocción comúnmente utilizados en la preparación de este tipo de productos (plancha y fritura). Es bien conocido que los tratamientos térmicos previos a su consumo modifican la composición de los productos, alterando entre otros, su contenido lipídico (Sheard et al., 1998; Badiani et al., 2002; Serrano et al., 2007).

Tanto los procesos de reformulación como los distintos métodos de cocción produjeron un notable efecto sobre el perfil lipídico de las hamburguesas **(Figura 5.8)**. La concentración de ácidos grasos en las

hamburguesas crudas estuvo directamente relacionada con los niveles de grasa (**Tabla 4, capítulo 4.1.4**). En las muestras reformuladas (MFP, LFP e IMFP), los niveles de AGS y AGM disminuyeron proporcionalmente a la reducción de grasa, siendo estos últimos los más abundantes en dichas muestras, en contraste con el control, donde los AGS fueron mayoritarios. Aunque formuladas con niveles de grasa similares, la composición de ácidos grasos en hamburguesas con gel de konjac o agente de carga difirieron, especialmente en el contenido de AGP. En este sentido, la presencia de la mezcla de aceites (oliva, lino y pescado) en las hamburguesas formuladas con agente de carga (IMFP) dio lugar a una mejora en el perfil lipídico, reflejada en una leve disminución de AGS, un ligero incremento en las concentraciones de AGM y niveles 5 veces más altos de AGP n-3. Esto condujo por un lado, a un aumento del 45% y 70% en la relación AGP/AGS con respecto a la muestra con similar contenido en grasa (formulada con gel de konjac, MFP) y al control, respectivamente. En la muestra donde el tocino fue totalmente sustituido por gel de konjac (LFP), la grasa fue básicamente intramuscular, caracterizada por presentar menores proporciones de AGM y mayores de AGP que la grasa de depósito (Raes et al., 2004), exhibiendo la mayor relación AGP/AGS **(Figura 5.8)**. Por otro lado, la reformulación con agente de carga produjo un descenso de entre un 60 y 79% de la relación AGP n-6/n-3.

En general, y en concordancia con lo señalado en otros productos cárnicos (Badiani et al., 2002; Librelotto et al., 2008), aunque con escasa relevancia cuantitativa, los niveles de ácidos grasos se incrementaron significativamente por efecto de la cocción (básicamente debido al aumento del contenido en grasa), sin variaciones importantes en sus proporciones relativas, por lo que las relaciones entre ellos apenas se vieron afectadas (**Figura 5.8**).

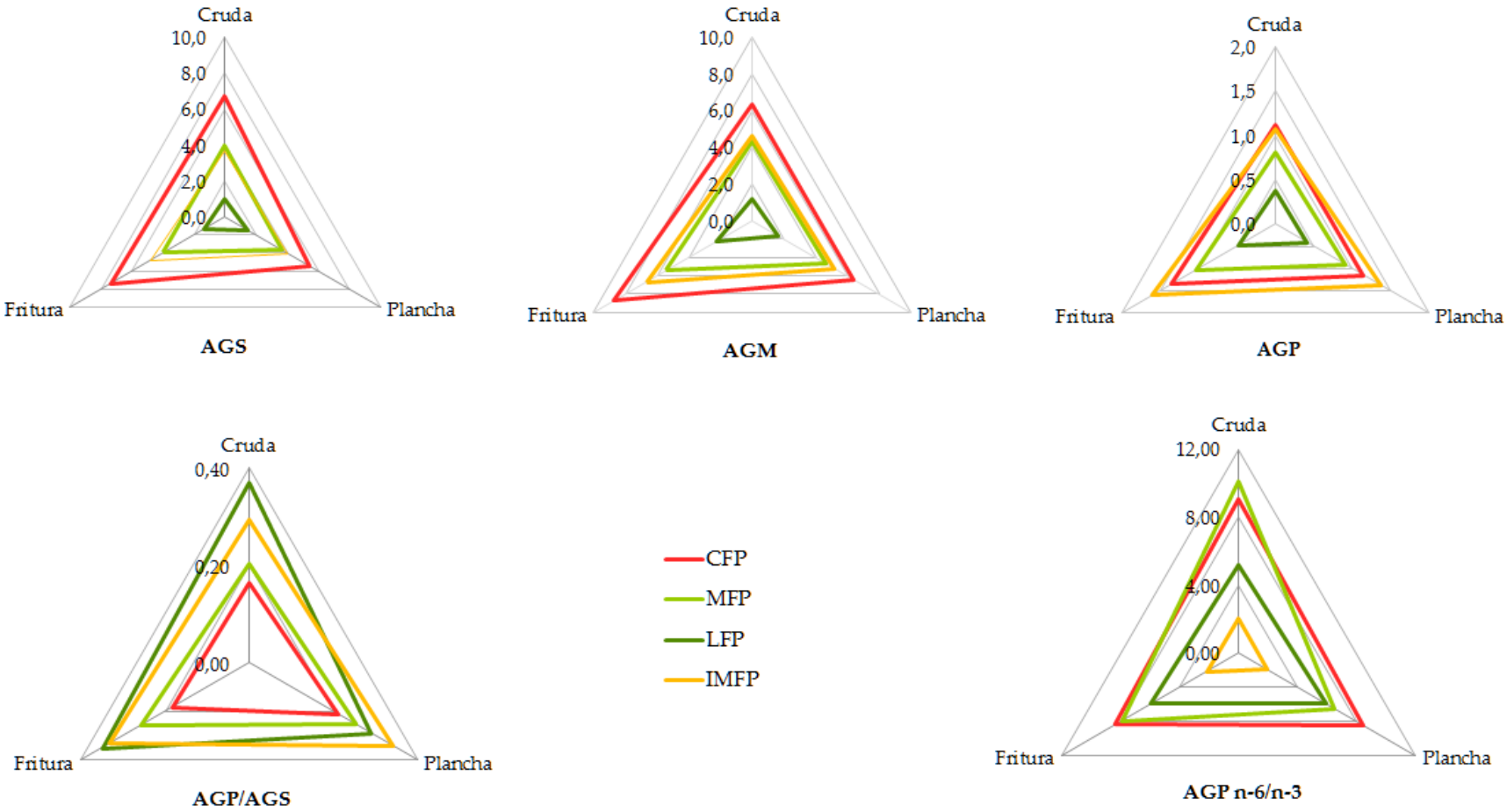


Figura 5.8. Efecto de los métodos de cocción (plancha y fritura) sobre la composición en ácidos grasos (g/100 g de producto) y relaciones de ácidos grasos de hamburguesas control y reformuladas con gel de konjac o agente de carga.

Adaptado de la **tabla 4, capítulo 4.1.4** (Salcedo-Sandoval et al., 2015a).

Denominación de las muestras: CFP, control con contenido normal de grasa; MFP, reducción de grasa mediante sustitución parcial de tocino de cerdo con gel de konjac; LFP, reducción de grasa mediante sustitución total de tocino de cerdo con gel de konjac; IMFP, reducción de grasa y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga.

Con independencia del tipo de producto, la incorporación de la mezcla de aceites estabilizada en un agente de carga a base de konjac, produjo disminución de los AGS y aumento de los AGP n-3, debido principalmente a la presencia de ALA y en menor medida a los EPA y DHA. Hu et al. (2001) afirmaron que la sustitución de AGS por AGP es más efectiva en la reducción del colesterol sérico y el riesgo de padecer ECV, que la simple reducción de la cantidad de grasa. Respecto a sus controles, los productos reformulados presentaron menores AGM, lo cual está relacionado con el contenido de tocino en las formulaciones, puesto que su principal ácido graso es el oleico (Wood et al., 2004). No obstante, al comparar las muestras formuladas con el mismo nivel de grasa con gel de konjac y agente de carga, se puede apreciar que la presencia de aceite de oliva en la mezcla de aceites ha limitado dicho descenso. Todo ello indica que la utilización de la mezcla de aceites como ingrediente en la formulación de productos con contenido reducido en grasa no afecta dramáticamente el contenido de AGM.

La cantidad total de AGP n-3 en los productos reformulados con agente de carga fue alrededor de 1,12 g/100 g (0,9 g ALA, 190 mg EPA y DHA) en salchichas y 0,35 g/100 g (0,3 g ALA, 66 mg EPA y DHA) en hamburguesas. Los niveles de AGP n-3 para los productos en los que toda su grasa era de origen animal fueron 0,16 y 0,11 en salchichas y hamburguesas control respectivamente, y 0,08 y 0,07 g/100 g en salchichas y hamburguesas respectivamente, conteniendo aproximadamente 10% de grasa y reformuladas con gel de konjac. Teniendo en cuenta que las recomendaciones sobre la ingesta de AGP n-3 se han situado entre 1,4 y 3 g/día o incluso superiores (Kolanowski et al., 1999; EFSA, 2005; Garg et al., 2006), el consumo de los productos elaborados en esta memoria con contenido normal y reducido en grasa (toda de cerdo) supone una contribución de 11,4% y 5,71% respectivamente, mientras que las salchichas y hamburguesas reformuladas con agente de carga

aportarían el 80% y el 25% respectivamente, del valor de ingesta mínimo recomendado de AGP n-3.

La calidad nutricional de la fracción lipídica de los alimentos se puede evaluar mediante la **relación AGP/AGS**. Se ha señalado que un aumento de esta ratio puede conducir a una reducción del colesterol total en plasma, siendo por ello aconsejable aumentarlo en la carne y productos cárnicos (McAfee et al., 2010). En este sentido, la relación recomendada de AGP/AGS se situa entre 0,4 y 1,0 (Wood et al., 2004). Tal ratio en salchichas tipo frankfurt sólo estuvo dentro del rango recomendado cuando se incorporó el agente de carga (0,7). En el caso de las hamburguesas, pese a que la presencia del agente de carga aumentó entre 31% y 41% esta relación, frente a las muestras conteniendo similar o superior niveles de grasa (toda de procedencia animal), dicha relación fue cercana a 0,30, aumentando hasta 0,34 por efecto de la cocción.

Otro de los índices que ayuda a evaluar la calidad lipídica de los alimentos es la **relación** entre los AGP **n-6 y n-3**. Se ha sugerido que valores muy altos de este índice inducen la patogénesis de muchas enfermedades (ECV, cáncer, etc.), mientras que un incremento de los niveles de AGP n-3 (y por ende un valor bajo de n-6/n-3) ejerce un efecto supresor (Simopoulos, 2002a). En este sentido, se recomienda que la ratio de AGP n-6/n-3 no exceda de 4 (Enser, 2000; Wood et al., 2004). En contraste con sus productos análogos, pero conteniendo únicamente grasa animal, tanto las salchichas como hamburguesas reformuladas con agente de carga, mostraron valores de esta relación que no excedieron el límite recomendado (0,8 y 2,1 respectivamente).

Por consiguiente, el uso de un agente de carga de aceite como estrategia de reformulación en salchichas tipo frankfurt y hamburguesas permite obtener productos con perfil lipídico más en línea con los objetivos nutricionales. Esto es, disminuyendo el contenido en AGS, incrementando el de AGP (incluyendo

AGP n-3), de acuerdo a las recomendaciones de diversas organizaciones internacionales (WHO, 2003; EFSA Panel on Dietetic Products, 2010). De igual modo, la utilización de este agente dió lugar a productos cárnicos con mejores relaciones de ácidos grasos, los cuales se situaron generalmente dentro de los rangos recomendados.

Por otra parte, como resultado de los distintos niveles de adición del agente de carga, el efecto de las modificaciones anteriormente descritas en el perfil de ácidos grasos fue más acusado en las salchichas tipo frankfurt, dado que se incorporó mayor cantidad de agente de carga y por tanto, mayor contenido de mezcla de aceites que en las hamburguesas. Por esta razón, los productos ensayados en esta memoria presentaron diferentes distribuciones en sus proporciones de ácidos grasos (**Figura 5.9**).

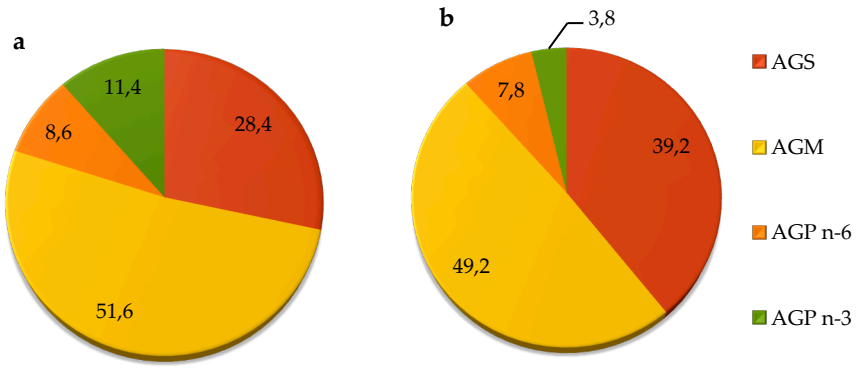


Figura 5.9. Distribución (%) de la composición de ácidos grasos en productos reformulados con agente de carga de aceite a) salchichas tipo frankfurt (F/OKM) y b) hamburguesas (IMFP).
Adaptada de la **tabla 3, capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b) y **tabla 4, capítulo 4.1.4** (Salcedo-Sandoval et al., 2014).

5.2.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

La incorporación de partículas de hidrogel en salchichas tipo frankfurt provocó modificaciones en el perfil lipídico. Si bien estas fueron notables con respecto a la muestra control, resultaron de escasa importancia en relación con las demás

formulaciones conteniendo aceite de pescado. En la **Figura 5.10** se representan los resultados de las salchichas control (F/C) y de aquellas conteniendo partículas de hidrogel (F/H).

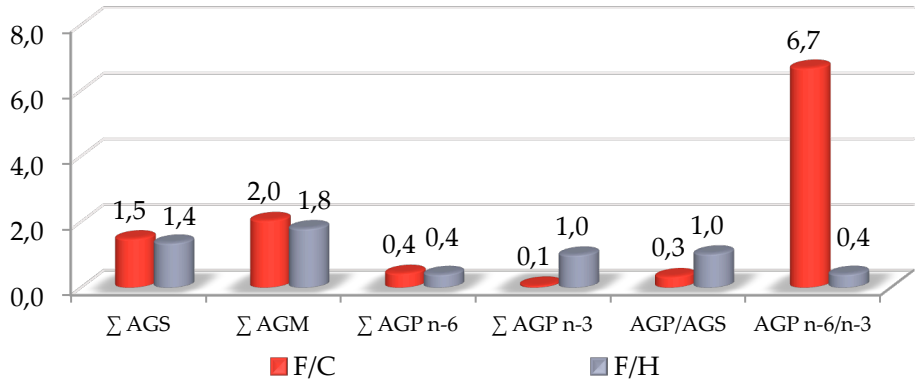


Figura 5.10. Composición en ácidos grasos (g/100 g de producto) y relaciones de ácidos grasos de salchichas tipo frankfurt control (F/C) y reformulada con partículas de hidrogel (F/H).
Adaptado de **Tabla 3, capítulo 4.2.2** (Salcedo-Sandoval et al., Enviado).

Los productos reformulados mostraron menor contenido de AGS y AGM y mayor de AGP. Este aumento, al no haber diferencias en el nivel de AGP n-6, es atribuible a la presencia de AGP n-3 (principalmente EPA y DHA), que resultó ser aproximadamente 10 veces más alta que la registrada en la muestra control (**Figura 5.10**). Es importante señalar que la concentración final de EPA y DHA superó los niveles establecidos en el diseño experimental (600 mg EPA+DHA/100 g de producto), por lo que la incorporación de partículas de hidrogel hizo posible la obtención de un producto rico en AGP n-3 de cadena larga. Dependiendo de factores tales como el tipo de población, la patología a prevenir, entre otros, la ingesta recomendada de EPA y DHA oscila entre 180-1000 mg/día (WHO, 2003; Garg et al., 2006; Taneja y Singh, 2012). Esto supone que los productos enriquecidos harían una contribución muy significativa a dicha ingesta, en comparación con los productos no fortificados. Así mismo, el notable incremento en los EPA y DHA produjo variaciones considerables en las relaciones de ácidos grasos, aumentando la de AGP/AGS desde 0,3 hasta 1,0 y

disminuyendo la de AGP n-6/n-3 desde 6,7 hasta 0,4 (**Figura 5.10**). Estos valores están en concordancia con los señalados en productos de naturaleza similar (Delgado-Pando et al., 2010a; Poyato et al., 2014).

5.2.3 Agente de carga vs partículas de hidrogel

La incorporación de ambos tipos de lípidos estructurados dio lugar a salchichas tipo frankfurt con mejores perfiles lipídicos, aunque estos tuvieron diferencias entre sí, como no podría ser de otra forma (**Figura 5.11**). La concentración de AGS y AGP n-6 presentó valores similares, mientras que la proporción de AGM fue mayor en las salchichas reformuladas con agente de carga, lo que estaría asociado al ácido oleico proporcionado de una parte por el aceite de oliva presente en la mezcla de aceites, y de otra parte por el tocino de cerdo adicionado. No obstante, las mayores diferencias entre los perfiles de salchichas se observaron en la proporción total y composición de los AGP n-3 (**Figura 5.11**).

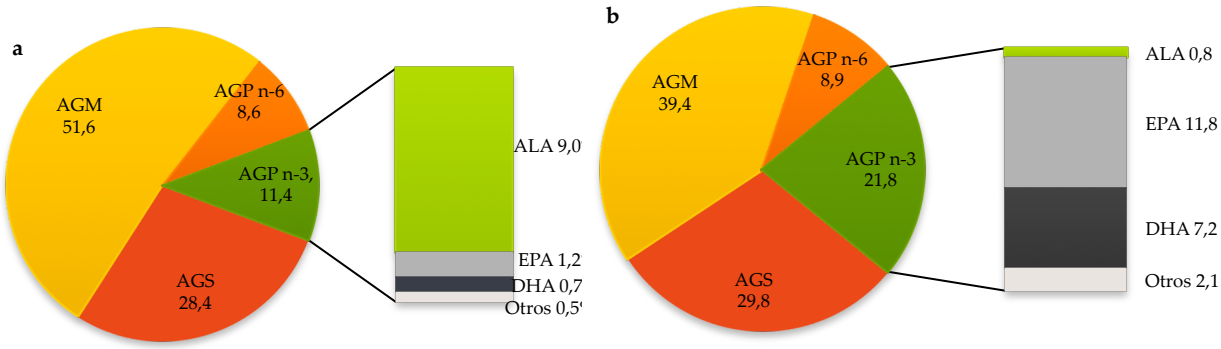


Figura 5.11. Distribución (%) de ácidos grasos en salchichas tipo frankfurt reformuladas con: a) agente de carga de aceite a base de konjac (F/OKM), y b) partículas de hidrogel (F/H).
Adaptado de **tabla 3, capítulo 4.1.1** (Salcedo-Sandoval et al., 2013b) y **tabla 3, capítulo 4.2.2** (Salcedo-Sandoval et al., 2015b).

En el caso de las muestras reformuladas con agente de carga, la proporción de AGP n-3 representa el 11,4% del total de ácidos (1,1 g/100 g producto) siendo el ALA, el ácido graso n-3 mayoritario. Paralelamente, en las

salchichas desarrolladas con partículas de hidrogel, la fracción de AGP n-3 representa un 21,8% (aproximadamente 1 g/100 g de producto), la cual está mayoritariamente formada por AGP n-3 de cadena larga (EPA y DHA) procedentes del aceite de pescado (**Figura 5.11**). Estos resultados están en consonancia con la composición del material lipídico empleado en cada caso. Por tanto las diferencias en los distintos perfiles son atribuibles principalmente al tipo de sistema de lípido estructurado utilizado (agente de carga o partículas de hidrogel).

Así pues, mientras las salchichas reformuladas con agente de carga aportarían a la dieta cantidades significativas de AGM y AGP n-3, las salchichas enriquecidas en AGP n-3 de cadena larga mediante el uso partículas de hidrogel proporcionarían una cantidad altamente significativa de este tipo de ácidos grasos, en relación con la ingesta recomendada por distintas organizaciones, que se encuentra en promedio, en un valor de 500 mg/día (Jiménez-Colmenero, 2007; Meyer, 2011).

Los cambios en la composición lipídica de los productos reformulados a través del empleo de sistemas de lípidos estructurados fueron tales que podrían ser etiquetados con varias declaraciones nutricionales y propiedades saludables, las cuales se recogen en las **Tabla 5.1** y **5.2**, respectivamente. Todo ello abre importantes expectativas para el consumidor con los consiguientes beneficios a nivel comercial.

Tabla 5.1. Posibles ejemplos de declaraciones nutricionales aplicables a salchichas tipo frankfurt y a hamburguesas reformuladas empleando lípidos estructurados, según el Reglamento (CE) 1924/2006.

Declaración	Condiciones de uso	Valor en el producto		
		Salchicha con agente de carga (F/OKM)	**Hamburguesa con agente de carga (IMFP)**	**Salchicha con partículas de hidrogel (F/H)**
Alto contenido de grasas monoinsaturadas	Sólo puede usarse si al menos 45 % de los ácidos grasos presentes en el producto proceden de grasas monoinsaturadas y las grasas monoinsaturadas aportan más del 20 % del valor energético del producto.	51,6% de los ácidos grasos y 40,0% del valor energético	49,2% de los ácidos grasos y 25,8% del valor energético	-
Alto contenido grasas insaturadas	Sólo podrá utilizarse si al menos un 70 % de los ácidos grasos presentes en el producto proceden de grasas insaturadas y las grasas insaturadas aportan más del 20 % del valor energético del producto.	71,6% de los ácidos grasos y 40,0% del valor energético	-	70,1% de los ácidos grasos y 27,7% del valor energético
Fuente de ácidos grasos omega 3	Sólo puede usarse con alimentos que contienen al menos 0,3 g de ALA por 100 g y por 100 kcal o al menos 40 mg de la suma de EPA y DHA por 100 g y por 100 kcal.	0,9 g de ALA/100 g y 190 mg de EPA + DHA / 100 g	66 mg de EPA + DHA / 100 g	868 mg de EPA + DHA / 100 g
Alto contenido de ácidos grasos omega 3	Sólo puede usarse con alimentos que contienen al menos 0,6 g de ALA por 100 g y por 100 kcal o al menos 80 mg de la suma de EPA y DHA por 100 g y por 100 kcal.	0,9 g de ALA/100 g y 190 mg de EPA + DHA / 100 g	-	868 mg de EPA + DHA / 100 g

Tabla 5.2. Posibles ejemplos de declaraciones de propiedades saludables aplicables a salchichas tipo frankfurt y a hamburguesas reformuladas empleando lípidos estructurados, según los artículos 13.1 y 14.1 del Reglamento (CE) 1924/2006.

Compuesto y declaración asociada	**Condiciones de uso**	**Valor en el producto**		
		Salchicha con agente de carga (F/OKM)	**Hamburguesa con agente de carga (IMFP)**	**Salchicha con partículas de hidrogel (F/H)**
AGM y AGP *La sustitución de grasas saturadas por grasas insaturadas contribuye a mantener niveles normales de colesterol sanguíneo (los ácidos grasos monoinsaturados o poliinsaturados son grasas insaturadas).* ***Art 13.1***	Solo puede utilizarse respecto a alimentos con alto contenido de ácidos grasos insaturados, de acuerdo con la declaración ALTO CONTENIDO DE GRASAS INSATURADAS* del Reglamento (CE) no 1924/2006.	71,6% de los ácidos grasos y 40,0% del valor energético	-	70,1% de los ácidos grasos y 27,7% del valor energético
ALA *El ácido alfa-linolénico contribuye a mantener niveles normales de colesterol sanguíneo.* ***Art 13.1***	Sólo puede usarse para los alimentos considerados como FUENTE DE ÁCIDOS GRASOS OMEGA-3* del Reglamento (CE) no 1924/2006. Se informará al consumidor de que el efecto beneficioso se obtiene con una ingesta diaria de 2 g de este ácido graso.	0,9 g de ALA/100 g y 190 mg de EPA + DHA /100 g	-	-
DHA *El ácido docosahexaenoico contribuye a mantener el funcionamiento normal del cerebro y al mantenimiento de la visión en condiciones normales.* ***Art 13.1***	El alimento debe contener al menos 40mg de DHA por 100 g y por 100 kcal. Debe informarse al consumidor que el beneficio se obtiene con una ingesta diaria de 250 mg de DHA	70 mg de DHA/100 g	-	328 mg de DHA/100 g
DHA *La ingesta materna de ácido docosahexaenoico (DHA) contribuye al desarrollo normal de los ojos del feto y del lactante alimentado con leche materna y al desarrollo normal del cerebro del feto y del lactante alimentado con leche materna.* ***Art 14.1***	La declaración puede ser utilizada solamente para aquellos alimentos que aporten una ingesta diaria de al menos 200 mg de DHA.	-	-	328 mg de DHA/100 g
EPA/DHA *Los ácidos eicosapentaenoico y docosahexaenoico contribuyen al funcionamiento normal del corazón.* ***Art 13.1***	Sólo puede usarse para los alimentos considerados como FUENTE DE ÁCIDOS GRASOS OMEGA-3* del Reglamento (CE) no 1924/2006. Se informará al consumidor de que el efecto beneficioso se obtiene con una ingesta diaria de 250 mg de EPA y DHA.	190 mg de EPA + DHA /100 g	66 mg de EPA + DHA /100 g	868 mg de EPA + DHA /100 g

*Las condiciones para obtener tal consideración se encuentran en la tabla 5.1.
Fuente: EU Register of nutrition and health claims made on foods: http://ec.europa.eu/nuhclaims/

5.3 PROPIEDADES LIGANTES DE AGUA Y GRASA

Las propiedades ligantes de agua y grasa se encuentran dentro de los parámetros más importantes a valorar en productos cárnicos, puesto que afectan a distintas características del producto, incluyendo la calidad final.

Tal y como se ha descrito en la sección de materiales y métodos (**Capítulo 3.4.2.2**), estas propiedades fueron determinadas de manera distinta en función del tipo de producto y de factores de procesado tales como: tratamiento térmico a altas temperaturas, descongelación y conservación. A continuación se discutirá el efecto de las estrategias de reformulación planteadas en esta memoria sobre las pérdidas de peso tanto como consecuencia del tratamiento térmico a altas temperaturas (a partir de ahora se mencionará simplemente como tratamiento térmico), como de la conservación, puesto que ambas fueron medidas en todos los productos.

5.3.1 Pérdidas de peso por efecto del tratamiento térmico

El comportamiento durante el tratamiento térmico es de gran relevancia, ya que lleva asociado mayores pérdidas de peso, y por tanto incide de manera directa sobre las características finales del producto (composición, textura, jugosidad, color, etc), condicionando la aceptabilidad y los costes de producción. En las salchichas tipo frankfurt, las pérdidas por efecto del tratamiento térmico están incluidas en las pérdidas por procesado (junto con las pérdidas por enfriamiento), mientras que en las hamburguesas, las pérdidas fueron medidas por efecto de distintos métodos de cocción (plancha y fritura).

5.3.1.1 Efecto de la reformulación asociado al empleo de agente de carga

En las salchichas tipo frankfurt, las pérdidas por procesado no estuvieron influenciadas ($P>0,05$) por la reducción de grasa y tampoco por la estrategia para incorporar la mezcla de aceites en los productos**,** presentando valores entre 13,88 a 16,75% (**Tabla 4, capítulo 4.1.1)**. Niveles de pérdidas por procesado

entre 10 y 20% se han encontrado en productos similares en los que se han incorporado aceites vegetales y marinos (Paneras et al., 1998; Lopez-Lopez et al., 2009).

Por otro lado, en el primer estudio de hamburguesas (**Capítulo 4.1.3**), se analizó la influencia de los procesos de reformulación sobre las pérdidas por cocción al horno. En tales condiciones, los valores variaron entre 12,59 y 18,89%, estando claramente afectados por la estrategia de reformulación. Mientras la reducción de grasa a niveles medio y bajo con gel de konjac (MFP y LFP) produjo los niveles más altos de pérdidas por cocción al horno ($P<0,05$), en muestras con similares niveles de grasa, pero reformuladas con agente de carga, tales pérdidas fueron más bajas ($P<0,05$) (**Tabla 3, capítulo 4.1.3**). Esto se debe posiblemente a que la estabilización del material lipídico en el agente de carga mejora las propiedades de retención de agua y grasa. En este sentido, Herrero et al. (2014a), encontraron que las propiedades de retención de agua y grasa en agentes de carga de aceite de oliva (55%) a base de alginato/inulina o dextrina fueron optimas, sin apenas liberación de exudado después de calentamiento a 70 °C.

En relación al segundo estudio de hamburguesas (**Capítulo 4.1.4**), se observó que tanto el efecto de la reformulación, como los distintos métodos de cocción (plancha y fritura) condicionaron este parámetro. La incorporación del agente de carga (muestra IMFP), mejoró las propiedades ligantes de agua y grasa durante ambos procesos de cocción, en comparación con muestras cuyos niveles de grasa fueron mayor y similar (CFP y MFP, respectivamente), si bien conteniendo únicamente grasa animal (**Tabla 5, capítulo 4.1.4 y Tabla 5.3**). Esto pone de manifiesto nuevamente que el empleo del agente de carga mejora la capacidad para retener agua y grasa en este tipo de matrices. En cuanto a los métodos de cocción, la preparación de las hamburguesas a la plancha ocasionó las mayores pérdidas (**Tabla 5.3**). El hecho de que durante la fritura los

productos absorben algo de grasa, puede ser la razón de los menores valores de pérdidas respecto a la cocción a la plancha (Librelotto et al., 2008). Los niveles de pérdidas de cocción registrados (13 y 27%) estuvieron dentro del rango habitualmente observado para este tipo de productos (15-40%) (Boles y Shand, 1999). Pérdidas por cocción cercanas al 27% fueron descritas en hamburguesas formuladas con 5% de aceite de oliva, adicionado directamente (Hur et al., 2008). López-López et al. (2011) determinaron valores de pérdidas por cocción entre 28 y 34% en hamburguesas de vacuno bajas en grasa, en las cuales el tocino de cerdo fue parcial y totalmente sustituido por una emulsión O/W de aceite de oliva. Varios autores han señalado que las pérdidas por cocción en hamburguesas están directamente relacionadas, entre otros factores, con el aumento en el nivel de grasa (Hoelscher et al., 1987; Berry, 1997, 1998; Choi et al., 2012). Las diferencias en las pérdidas por cocción determinadas en este estudio fueron altamente dependientes de la estrategia de reformulación empleada y del método de cocción.

Tabla 5.3. Pérdidas (%) por efecto del tratamiento térmico de los distintos productos cárnicos reformulados con sistemas de lípidos estructurados.

Tratamientos	Salchichas*	Hamburguesa a la plancha	Hamburguesa en fritura
Control	C 13,9 ± 1,2^{a}**	CFP 27,0 ± 1,5^{c1}	CFP 20,9 ± 1,4^{b2}
Reducción de grasa (Gel de konjac)	F/KG 14,5 ± 0,6^{a}	MFP 23,1± 1,1^{b2}	MFP 21,3 ± 1,4^{b1}
Reducción grasa y mejora del perfil (Agente de carga)	F/OKM 15,1 ± 0,5^{a}	IMFP 20,3 ± 1,8^{a2}	IMFP 17,3 ± 0,7^{a1}
Mejora del perfil (Partículas de hidrogel)**	F/H 14,9 ± 0,5^{a}	--	--

Datos tomados de tabla 4, capítulo 4.1.1 (Salcedo-Sandoval et al., 2013b); tabla 5, capítulo 4.1.4 (Salcedo-Sandoval et al., 2014) y tabla 4, capítulo 4.2.2 (Salcedo-Sandoval et al., Enviado)

*En salchichas tipo frankfurt los valores corresponden a las pérdidas por procesado.

**Valor del control correspondiente al estudio de salchichas formuladas con agente de carga.

Medias ± Desviación estándar. Diferentes letras en la misma columna y diferentes números en la misma fila indican diferencias significativas (P<0,05).

Denominación de las salchichas: C, control; F/KG, reducción de grasa mediante sustitución parcial de tocino de cerdo con gel de konjac; F/OKM, reducción de grasa y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga; F/H, mejora del perfil mediante incorporación de partículas de hidrogel.

Denominación de las hamburguesas: CFP, control con contenido normal de grasa; MFP, reducción de grasa mediante sustitución parcial de tocino de cerdo con gel de konjac; IMFP, reducción de grasa y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga.

Comparando los valores de pérdidas por efecto del tratamiento térmico entre los distintos productos formulados con agente de carga, se observa que estos son mayores en hamburguesas. Los productos elaborados mediante sustitución parcial de tocino por agente de carga y que presentan niveles de grasa alrededor del 10%, exhiben porcentajes de humedad y de proteína próximos (**Figura 5.2 y 5.3**). Sin embargo, las diferencias en los niveles de agua y material lipídico estabilizado en el agente de carga (19% en salchichas y 7% en hamburguesas), ayuda a explicar las mejores propiedades ligantes de agua y grasa en el sistema gel/emulsión. En todo caso, con independencia del tipo de producto los resultados señalan que el agente de carga es una buena opción tecnológica para mejorar las propiedades ligantes de agua y grasa en una matriz cárnica con composición lipídica mejorada (**Tabla 5.3**).

5.3.1.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

Las pérdidas por procesado en salchichas enriquecidas con AGP n-3 de cadena larga presentaron los valores más bajos ($P > 0,05$) en la muestra en la que fueron incorporadas las partículas de hidrogel, F/H, sin diferencia significativa entre las demás muestras (**Tabla 4, capítulo 4.2.2**). Los valores de pérdidas por procesado estuvieron entre 14,9 y 17,2%, siendo consistentes con los reportados en salchichas tipo frankfurt bajas en grasa, los cuales se situaron en un rango entre 10-20% (Paneras y Bloukas, 1994; Lopez-Lopez et al., 2009; Delgado-Pando et al., 2010a).

5.3.1.3 Agente de carga vs partículas de hidrogel

Los valores de pérdidas por procesado en salchichas tipo frankfurt conteniendo partículas de hidrogel fueron similares a aquellos obtenidos en el mismo producto elaborado con agente de carga (**Tabla 5.3**). Esto indica que el tipo de lípido estructurado no condicionó este parámetro. Resultados contradictorios han sido descritos en relación al efecto de la sustitución de grasa animal por

aceites vegetales, sobre las pérdidas de peso asociadas al tratamiento térmico. Se ha descrito que las salchichas preparadas con aceites vegetales tienen pérdidas por cocción más altas que las elaboradas con grasa animal (Park et al., 1989; Paneras y Bloukas, 1994; Ambrosiadis et al., 1996; Lopez-Lopez et al., 2009). No obstante, también se ha señalado que las pérdidas por cocción en sistemas cárnicos tipo gel/emulsión disminuyen cuando la grasa animal se sustituye por aceites vegetales, efecto influenciado por el tipo de aceite (Youssef y Barbut, 2011). En coherencia con los resultados obtenidos en este estudio, varios autores han señalado que las pérdida por cocción en salchichas tipo frankfurt no se afectan por el tipo de grasa utilizada en la formulación (grasa de vacuno, grasa de cerdo o aceites) (Marquez et al., 1989; Delgado-Pando et al., 2010a; Álvarez et al., 2011).

5.3.2 Pérdidas de peso durante la conservación

Los cambios en las propiedades ligantes de agua y grasa durante la conservación de los productos son un aspecto esencial. La liberación de exudado al envase durante el almacenamiento en refrigeración favorece el crecimiento microbiano y disminuye la aceptabilidad por parte del consumidor.

5.3.2.1 Efecto de la reformulación asociado al empleo de agente de carga

La presencia del agente de carga en salchichas tipo frankfurt (F/OKM) no influyó sobre las pérdidas durante la conservación (P>0,05), variando en un rango entre 1,16% y 2,40% (**Tabla 4, capítulo 4.1.1**). Tales niveles son similares a los reportados en salchichas con bajo contenido en grasa (Paneras y Bloukas, 1994; Candogan y Kolsarici, 2003b).

Las pérdidas durante la conservación en hamburguesas presentaron valores desde 0,85 hasta 1,83% y fueron generalmente más altas en las muestras reformuladas que en la muestra control (CFP) (**Tabla 3, capítulo 4.1.3)**. Sin embargo, no existieron variaciones claramente atribuibles a la estrategia de

reformulación empleada (reducción de grasa con gel de konjac o reducción de grasa y mejora del perfil con agente de carga). Tampoco fue apreciada una tendencia clara en el comportamiento de estas pérdidas durante el tiempo de almacenamiento. En cualquier caso se observaron algunas diferencias significativas, estas fueron de escasa relevancia cuantitativa. Así mismo, los distintos niveles de incorporación de agente de carga generalmente no afectaron los valores de pérdidas durante el almacenamiento (**Tabla 3, capítulo 4.1.3)**. Lopez-Caballero et al. (1999) indicaron que productos cárnicos comercialmente disponibles con pérdidas durante la conservación en un rango del 3,5-4,5%, tendían a presentar deterioro en su apariencia. Los niveles de pérdidas encontrados durante la conservación tanto en salchichas como hamburguesas, permiten señalar que tales productos presentan adecuadas propiedades ligantes de agua y grasa.

5.3.2.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

El empleo de partículas de hidrogel en salchichas dio lugar a valores muy bajos en las pérdidas durante la conservación (0,13-0,39%), que no representaron cambios respecto a la muestra control, ni tampoco variaciones significativas entre los días de medición.

5.3.2.3 Agente de carga vs partículas de hidrogel

Considerando los resultados obtenidos en relación a las pérdidas de peso durante la conservación, se puede afirmar que la utilización de agente de carga o partículas de hidrogel confieren adecuadas propiedades ligantes de agua y grasa en las salchichas tipo frankfurt, las cuales se mantienen durante la conservación. Estos resultados son consistentes con lo descrito por Pappa et al. (2000), quienes encontraron que al sustituir tocino de cerdo por aceites de origen vegetal y marino no se producían efectos sobre las pérdidas durante la conservación. En oposición a esto, Bishop et al. (1993) afirmaron que las

pérdidas durante la conservación fueron mayores en salchichas tipo bologna conteniendo aceite emulsificado que en aquellas formuladas con grasa animal.

5.4 TEXTURA

La textura es uno de los factores que más condiciona la aceptación del alimento por parte del consumidor. En salchichas tipo frankfurt, el efecto de la reformulación fue evaluado a través de distintos parámetros (**Tabla 6, capítulo 4.1.1**). Ya que no resulta posible analizar todos ellos, se ha seleccionado la dureza para visualizar el efecto de las estrategias aplicadas. Así mismo, la fuerza Kramer será empleada como medida de textura instrumental en hamburguesas crudas y cocidas (**Tabla 4, capítulo 4.1.3**).

5.4.1 Efecto de la reformulación asociado al empleo de agente de carga

La textura en productos reformulados con agente de carga varió en función del tipo de producto (**Figura 5.12**). Las salchichas tipo frankfurt registraron un incremento en la dureza como resultado de la reducción de grasa con matrices de konjac ($P<0,05$). Dicho aumento fue notable cuando se utilizó gel de konjac (F/KG), mientras que con la sustitución de grasa animal por agente de carga (F/OKM), el efecto fue menor (**Figura 5.12**). Sin embargo, la reformulación de salchichas mediante la incorporación de la mezcla de aceites, estabilizada en emulsiones O/W, no ocasionó diferencias ($P>0,05$) en la dureza respecto al control. Existen varios factores que pueden contribuir a este tipo de comportamiento. Uno de ellos es el contenido de aceite en el sustituto de grasa (20% en el agente de carga frente al 53% en la emulsión O/W), a pesar de que el contenido final de grasa en el producto es el mismo (**Tabla 2, capítulo 4.1.1**). Otro factor fue la diferencia en las características de los sistemas de estabilización de aceite empleados: mientras el agente de carga presenta una estructura más firme, típica de un gel, la emulsión O/W se comportó como un

material viscoso, careciendo de comportamiento similar a un gel. Youssef y Barbut (2011) señalaron que la sustitución de grasa animal con aceite de canola disminuyó la dureza en productos tipo gel/emulsión con contenido reducido en grasa; sin embargo, este efecto fue afectado por la forma en la que el aceite fue incorporado (adición directa o en emulsiones O/W estabilizadas con distintos emulsificantes). En comparación con un producto con contenido normal de grasa, la sustitución de la grasa de cerdo por aceite de oliva emulsificado en salchichas tipo frankfurt (con contenido reducido en grasa), dio lugar a productos más duros (Paneras y Bloukas, 1994; Paneras et al., 1998) o no tuvo influencia (Bloukas y Paneras, 1993). Sin embargo, otros autores (Lurueña-Martinez et al., 2004), han observado que la adición de aceite de oliva en salchichas tipo frankfurt con bajo contenido en grasa disminuyó la dureza. Estas discrepancias respecto al efecto de la sustitución de grasa animal por aceites pueden estar relacionadas con la composición de los distintos productos (contenido de humedad y proteína).

En hamburguesas crudas, la incorporación de gel de konjac (muestra MFP), también dio lugar a los valores más altos de textura ($P<0,05$), estando en línea con los resultados descritos por Osburn y Keeton (1994) y Triki et al. (2013b). Aunque la adición de distintos niveles de agente de carga (IMFP e ILFP) produjo un descenso de estos valores, estos fueron similares a los de la muestra control, con independencia del nivel agente de carga incorporado ($P>0,05$) (**Figura 5.12**).

Excepto en la hamburguesa con bajo contenido en grasa, formulada con gel de konjac (LFP), la cocción redujo las diferencias en los valores de textura de las hamburguesas reformuladas respecto al control (**Figura 5.12**).

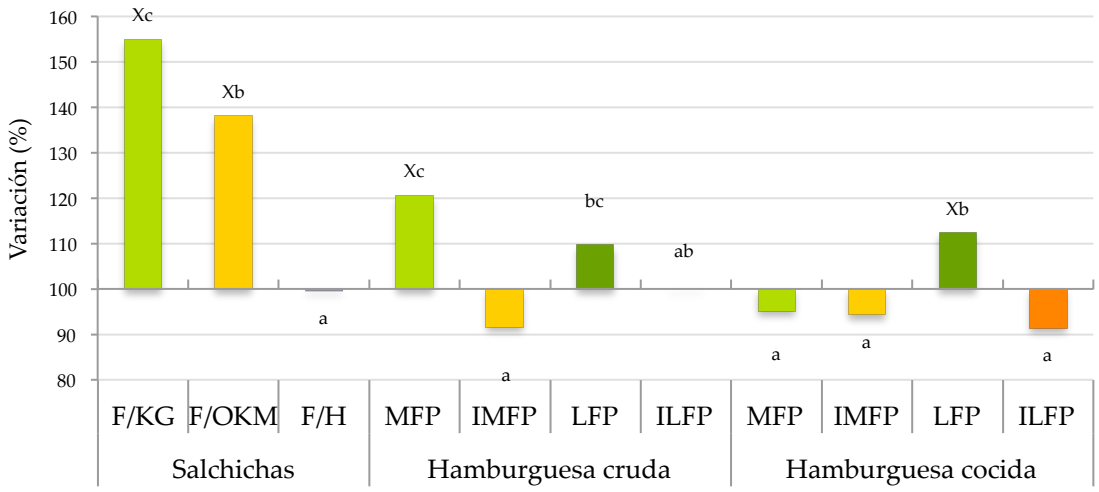


Figura 5.12. Variaciones relativas de textura (%) en salchichas y hamburguesas reformuladas con gel de konjac, agente de carga y partículas de hidrogel.

Las variaciones fueron calculadas respecto al control con un valor asignado del 100% en el eje Y. Datos calculados de la tabla 6, capítulo 4.1.1 (Salcedo-Sandoval et al., 2013b); tabla 4, capítulo 4.1.3 (Salcedo-Sandoval et al., 2015a) y tabla 6, capítulo 4.2.2 (Salcedo-Sandoval et al., Enviado). Diferentes letras (a,b) en un mismo producto indican diferencias significativas reformulaciones de un mismo producto. X indica diferencias significativas respecto al control.

Denominación de las salchichas: F/KG, reducción de grasa mediante sustitución parcial de tocino de cerdo con gel de konjac; F/OKM, reducción de grasa y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga; F/H, mejora del perfil mediante sustitución total de tocino de cerdo con partículas de hidrogel.

Denominación de las hamburguesas: MFP, reducción de grasa (nivel medio) mediante sustitución parcial de tocino de cerdo con gel de konjac; IMFP, reducción de grasa (nivel medio) y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga; LFP, reducción de grasa (nivel bajo) mediante sustitución parcial de tocino de cerdo con gel de konjac; ILFP, reducción de grasa (nivel bajo) y mejora del perfil mediante sustitución parcial de tocino de cerdo con agente de carga.

Puesto que en cada caso, los productos fueron formulados con cantidades similares de carne magra, la reducción de grasa se llevó a cabo aumentando la proporción de agua. Normalmente este tipo de estrategia trae consigo la obtención de productos más blandos (Claus et al., 1990). Tal comportamiento no se observó en las salchichas y hamburguesas con agente de carga, hecho atribuible a la estructura del gel de konjac, que además posee una alta capacidad de retención de agua (Jiménez-Colmenero et al., 2010a). Así pues, la presencia de gel de konjac produce estructuras más firmes que la grasa animal, lo cual está en consonancia con lo descrito por diversos autores acerca del empleo de fibras dietéticas en productos cárnicos produce incrementos en los parámetros de textura (Fernandez-Gines et al., 2005; Brewer, 2012). No obstante, la estabilización de la mezcla de aceites en la red tridimensional

formada por el gel de konjac suaviza este efecto endurecedor (**Figura 5.12**), lo cual representa una ventaja tecnológica en productos con contenido reducido en grasa. Las diferencias en el efecto del agente de carga sobre los parámetros de textura en los distintos productos estarían relacionadas con el nivel del mismo en los productos, que como se ha mencionado anteriormente, fue considerablemente mayor en las salchichas tipo frankfurt. A este respecto, varios autores han encontrado que el efecto producido por la sustitución de grasa animal con geles de konjac puede variar de acuerdo a la naturaleza del gel y a la proporción de grasa sustituida (Lin y Huang, 2003; Osburn y Keeton, 2004; Kao y Lin, 2006)

La conservación en refrigeración afecta de manera distinta los parámetros de textura de los productos elaborados con agente de carga. Con independencia del tipo de formulación al final del periodo de conservación, las salchichas presentaron mayor dureza que al inicio ($P<0,05$). Sin embargo, estos cambios no se pueden explicar en base a las pérdidas de peso durante la conservación. Distintos autores han descrito resultados similares en salchichas tipo frankfurt con reducido contenido graso (Kao y Lin, 2006; Lopez-Lopez et al., 2009). A diferencia de la muestra control, en general la textura de las hamburguesas con agente de carga se mantiene constante a lo largo del periodo de conservación, tanto en el producto crudo como tras el proceso de cocción ($P>0,05$).

5.4.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

La dureza en salchichas tipo frankfurt enriquecidas con EPA y DHA varió en función de la forma de adición del aceite de pescado en estos productos, siendo las muestras con partículas de hidrogel (F/H) las únicas que obtuvieron valores similares de dureza a los de la muestra control. En contraposición, las demás muestras reformuladas con aceite de pescado (F/E y F/O) presentaron valores

más altos (**Tabla 6, capítulo 4.2.2**). Varios factores pueden ayudar a explicar este comportamiento. Por ejemplo, los mayores valores de dureza (P <0,05) en la muestra con aceite estabilizado en una emulsión O/W (F/E), en comparación con el control, este hecho se debió probablemente a que la emulsificación del aceite de pescado con proteína no cárnica hizo que hubiera más proteína cárnica disponible para contribuir a la formación de la estructura de gel del producto (Bishop et al., 1993). En este sentido se ha descrito que, proteínas emulsificantes como el caseinato producen enlaces fuertes entre los componentes de la emulsión, aumentando la consistencia y por lo tanto la dureza del producto (Caceres et al., 2008). Por otra parte, en relación a la muestra F/O, se ha descrito que la incorporación de aceites líquidos de forma directa conlleva una mejor distribución en matrices cárnicas (comparada con la grasa animal), debido a que tales aceites presentan una mejor asociación con la proteína, dando lugar a salchichas más firmes (Hammer, 1992).

Adicionalmente, el almacenamiento en refrigeración provocó un aumento paulatino de los valores de dureza, independientemente del tipo de formulación, lo cual, en el caso de las salchichas con partículas de hidrogel, no puede ser asociado a las pérdidas durante la conservación. No obstante, los resultados de F/H presentan un comportamiento similar al exhibido por las salchichas con agente de carga (F/OKM) (**Tablas 6, capítulo 4.1.1 y capítulo 4.2.2**).

5.4.3 Agente de carga vs partículas de hidrogel

Los resultados obtenidos muestran que para un mismo producto, la estrategia de reformulación (empleo de agente de carga o partículas de hidrogel), condiciona la textura. El hecho de que las salchichas tipo frankfurt con agente de carga presenten mayores valores de dureza respecto a su control, y que las que contienen partículas de hidrogel no muestren variaciones (**Figura 5.12**),

puede ser atribuido tanto a la composición, como a la naturaleza de los lípidos estructurados empleados. Mientras el agente de carga presenta propiedades de un material sólido, las partículas de hidrogel son un carácter viscoso. Estas diferencias habrían influenciado de forma distinta la textura de salchichas tipo frankfurt. Por otro lado, ambas estrategias de reformulación provocaron un endurecimiento gradual ($P<0,05$) del producto durante el tiempo de almacenamiento en refrigeración. Cabe señalar que este comportamiento se observó en todas las muestras, incluyendo la control, por lo que tales cambios no pueden ser atribuidos al efecto de la reformulación. Estos resultados están en línea con los señalados por varios autores en salchichas con contenido reducido en grasa (Kao y Lin, 2006; Delgado-Pando et al., 2010a).

5.5 COLOR

La apariencia es quizás el primer factor que toma en consideración el consumidor a la hora de elegir un producto, siendo el **color** uno de sus elementos básicos.

5.5.1 Efecto de la reformulación asociado al empleo de agente de carga

Los atributos de color en salchichas tipo frankfurt se vieron influenciados por las estrategias de reformulación aplicadas (sustitución parcial o total de tocino de cerdo con gel de konjac o agente de carga) (**Tabla 5, capítulo 4.1.1**). En el caso de la luminosidad (L^*), sólo los productos con agente de carga (F/OKM) presentaron variaciones respecto a la muestra control, que aunque significativas fueron de escasa magnitud. La tendencia al rojo (a^*) disminuyó ($P<0,05$) en todos los productos reformulados, lo cual estaría relacionado en parte con la dilución de los pigmentos de la carne. Sin embargo, en las salchichas con agente de carga, tal diferencia no se apreció a lo largo del almacenamiento. La tendencia al amarillo (b^*), disminuyó ($P<0,05$) en las muestras reducidas en

grasa mediante la adición de gel de konjac (F/KG), aumentó significativamente en las muestras con presencia de emulsión O/W (F/OWE y F/OWE+KG), y no presentó diferencias (P>0,05) en las salchichas con agente de carga (F/OKM). Se ha descrito que en salchichas tipo frankfurt, la sustitución de tocino de cerdo por aceite de oliva adicionado directamente, redujo la tendencia al rojo (Lopez-Lopez et al., 2009), aunque en contraposición a esto, Paneras y Bloukas (1994) encontraron que la sustitución de grasa animal por aceite no tuvo ningún efecto en el color.

Los parámetros de color en hamburguesas crudas se vieron afectadas por el tipo de reformulación (**Tabla 5, capítulo 4.1.3**). La reducción de grasa con gel de konjac (MFP, LFP y VLFP) produjo una disminución de los parámetros de color. La estrategia de reducir y mejorar la composición lipídica mediante el empleo de agente de carga (muestra ILFP), no supuso cambios en la luminosidad ni en la tendencia al amarillo, en tanto que la tendencia al rojo, sólo disminuyó (P<0,05), aunque de forma leve, cuando dicho sistema se utilizó en la sustitución total de la grasa animal añadida. Estos resultados discrepan de los reportados por Martínez et al. (2012), quienes encontraron que la incorporación de una emulsión O/W conteniendo una combinación de aceites de oliva, maíz y pescado en hamburguesas de vacuno, contribuyó a incrementar significativamente los niveles de L^* y a^* y disminuir los valores de b^*, en comparación con un producto convencional. Las variaciones entre los resultados obtenidos en tales estudios pueden atribuirse, entre otras cosas, a diferencias en el modo de estabilización de los lípidos.

Por otro lado, al igual que en salchichas y hamburguesas en fresco, las variaciones en el color de hamburguesas cocidas estuvieron condicionadas por las estrategias de reformulación. La luminosidad registró un ligero aumento (P<0,05) en muestras con sustitución total de grasa animal por gel de konjac (VLFP) o agente de carga (ILFP), mientras la tendencia al rojo presentó los

valores más bajos en las muestras con gel de konjac, no observando diferencias (P>0,05) por efecto de la reformulación con agente de carga (IMFP e ILFP). La tendencia al amarillo sólo aumentó en las hamburguesas cocidas reformuladas con agente de carga. Dichos resultados están en línea con los reportados por Jung y Joo (2013), quienes encontraron que el hecho de reemplazar en hamburguesas cocidas, tocino de cerdo por aceites vegetales condujo a un significativo aumento de los valores de L^* y b^*.

Entre todos los parámetros de color medidos, el más relevante en productos cárnicos es la tendencia la rojo. Con independencia del tipo de producto, los resultados sugieren que la reducción de grasa con gel de konjac disminuye los valores de a^*. Este comportamiento podría está en consonancia con lo descrito por Jiménez-Colmenero et al. (2012a), quienes observaron que los geles de konjac resultaron más oscuros y menos rojos que la grasa de cerdo. Sin embargo, este efecto generalmente fue contrarrestado por la presencia de la mezcla de aceites en la matriz de konjac (agente de carga). En consecuencia, se puede señalar que las limitadas discrepancias entre los parámetros de color de productos reformulados con agente de carga podrían estar asociadas a la distinta composición de los productos.

Respecto al efecto de la conservación, los parámetros de color en salchichas con agente de carga no se vieron afectados, excepto en el caso del parámetro a^*. Por otra parte, en hamburguesas con agente de carga, se produjo un ligero descenso (P<0,05) en la luminosidad, mientras que la tendencia al rojo y al amarillo no presentaron cambios.

En general los resultados indican que el uso de agente de carga en los distintos productos no causó variaciones importantes en el color, el cual apenas fue afectado a lo largo del periodo de conservación.

5.5.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

El enriquecimiento en EPA y DHA de salchichas tipo frankfurt condicionó los parámetros de color, siendo tales variaciones influenciadas por el modo de incorporación del aceite de pescado (**Tabla 5, capítulo 4.2.2**). La luminosidad fue menor (P<0,05) en la muestras control (F/C) que en las muestras formuladas con aceite de pescado. De igual forma, la luminosidad varió en función de la estrategia utilizada para incorporar el aceite de pescado en las salchichas (F/H < F/O < F/E). La tendencia al rojo presentó la mayor disminución en las salchichas en las que el aceite de pescado se añadió directamente (F/O), mientras que tal parámetro registró los mayores valores en los productos reformulados con partículas de hidrogel (F/H).

Todas las salchichas presentaron un descenso de L^* en el día 12 de conservación, el cual se mantiene hasta el final del almacenamiento. Generalmente los valores de L^* y b^*, presentaron variaciones significativas inducidas por la formulación y la conservación en refrigeración, aunque estas fueron cuantitativamente pequeñas y de poca relevancia. Las diferencias en el color de las distintas salchichas pueden ser debidas en parte a las variaciones entre el color del tocino y el aceite de pescado, aunque la presencia de caseinato y/o pectina en los sistemas empleados para estabilizar los aceites puede tener un papel relevante.

5.5.3 Agente de carga vs partículas de hidrogel

Las diferencias entre el efecto del agente de carga y las partículas de hidrogel sobre los atributos de color de salchichas tipo frankfurt pueden relacionarse básicamente con variaciones en la composición, en la proporción de grasa animal sustituida, y en el sistema utilizado para estabilizar el material lipídico. De hecho, resultados conflictivos en los atributos de color de este tipo de

productos, por efecto de la sustitución de grasa animal con diferentes fuentes de lípidos, se han asociado en gran medida al tipo de producto, la formulación del mismo y a las características de los aceites y del sistema de estabilización empleado (Delgado-Pando et al., 2010a).

5.6 OXIDACIÓN LIPÍDICA

Uno de los principales problemas potenciales en relación a productos cárnicos formulados con lípidos más saludables, es como estas modificaciones en la composición de la materia grasa pueden incrementar la velocidad y extensión de la oxidación lipídica. Esto además de afectar las características de calidad, puede traer consigo implicaciones negativas en la salud. Factores de composición como son la presencia de sal, iones metálicos, y AGP, así como las condiciones de procesado (picado, tratamiento térmico, almacenamiento, etc.), contribuyen a que estos productos sean más susceptibles a los fenómenos de oxidación.

5.6.1 Efecto de la reformulación asociado al empleo de agente de carga

En salchichas tipo frankfurt, la reducción del 50% de grasa respecto a la muestra control a través de la incorporación de gel de konjac (F/KG), no produjo modificaciones en la velocidad y la extensión de la oxidación lipídica (**Tabla 5, capítulo 4.1.2** y **Figura 5.13a**). En contraste, esta misma reducción llevada a cabo mediante la sustitución parcial de grasa animal por la mezcla de aceites supuso un aumento en la formación de compuestos de oxidación tanto en las salchichas con agente de carga (F/OKM) como en las demás muestras en las que se incorporó la mezcla de aceites (F/OWE y F/OWE+KG), no habiendo diferencias ($P>0,05$) en sus niveles iniciales (**Figura 5.13a**). Tales variaciones caben atribuirlas al aumento en el contenido de AGP en las muestras reformuladas (**Figura 5.7**). Las salchichas reducidas en grasa y mejoradas en su perfil por la

presencia de la mezcla de aceites tuvieron patrones de oxidación similares mostrando durante el tiempo de conservación valores más altos (P<0,05) de TBARS que los de las salchichas con sólo grasa animal.

La similitud de los modelos y niveles de oxidación de las salchichas formuladas con emulsión (F/OWE) y las elaboradas con agente de carga (F/OKM), indican que la naturaleza del agente estabilizante de la mezcla de aceites no tuvo efecto sobre este parámetro (**Figura 5.13a**). Los valores de TBARS obtenidos en estos productos fueron inferiores a los mostrados en este tipo de productos, en los que se han incorporado aceites vegetales y/o de pescado para mejorar la fracción lipídica (Bloukas y Paneras, 1993; Caceres et al., 2008; Berasategi et al., 2011).

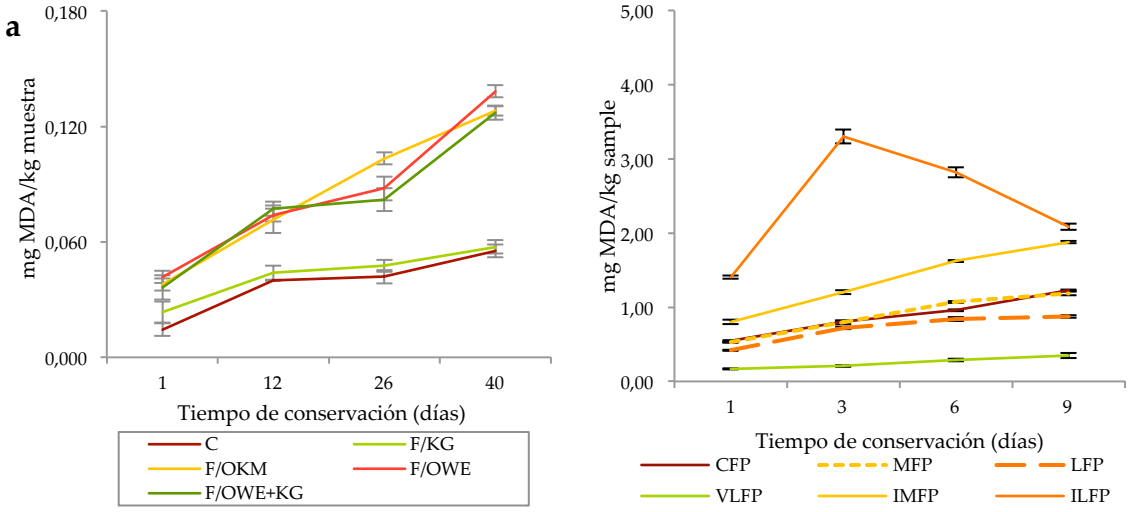


Figura 5.13. Oxidación lipídica (valores de TBARS expresados como mg de MDA/kg de muestra) en a) salchichas y b) hamburguesas frescas.

Adaptado de la tabla 5, capítulo 4.1.2 (Salcedo-Sandoval et al., 2015c) y tabla 6, capítulo 4.1.3 (Salcedo-Sandoval et al., 2015a).

Las barras representan la desviación estándar.

Denominación de las salchichas: C, control con contenido normal de grasa; F/KG, reducción de grasa con gel de konjac; F/OKM, F/OWE y F/OWE+KG, reducción de grasa y mejora del perfil con la mezcla de aceites estabilizada en agente de carga, emulsión O/W y emulsión O/W más gel de konjac, respectivamente.

Denominación de las hamburguesas: CFP, control con contenido normal de grasa; MFP, LFP y VLFP, reducción de grasa a niveles medio, bajo y muy bajo con gel de konjac; IMFP y ILFP, reducción de grasa y mejora del perfil con la mezcla de aceites estabilizada en agente de carga, niveles medio y bajo.

La reformulación de hamburguesas con varios niveles de sustitución de grasa animal por diferentes niveles de gel de konjac (reducción de grasa) y agente de carga (reducción de grasa y mejora del perfil), alteró la estabilidad oxidativa de los productos (**Tabla 6 del capítulo 4.1.3 y Figura 5.13b**). En general, los mayores niveles de reducción de grasa con gel de konjac (LFP y VLFP) presentaron los valores más bajos de oxidación ($P<0,05$). De forma inversa, la incorporación de la mezcla de aceites en el agente de carga para obtener productos con nivel medio y bajo de grasa mediante sustitución parcial y total de tocino (IMFP y ILFP, respectivamente), produjo los niveles de oxidación más altos ($P<0,05$), tal incremento estuvo directamente relacionado con el nivel de adición de agente de carga (IMFP<ILFP) (**Figura 5.13b**). Los patrones de oxidación fueron similares en todas las muestras, menos en ILFP, que registró un máximo el día 3, seguido por un descenso en los niveles de TBARS. Bhattacharya et al. (1988) sugirieron que esta tendencia podría deberse a que el MDA es un producto intermedio que interacciona con otros compuestos, por lo que una vez transcurrido cierto tiempo, disminuye su concentración en el medio.

Las variaciones en la oxidación lipídica por efecto de la reformulación de salchichas y hamburguesas con agente de carga con respecto a las exhibidas por la muestra control y las muestras reducidas al mismo contenido en grasa con gel de konjac se representan en la **Figura 5.14.** En todos los productos reformulados con gel de konjac, la oxidación lipídica fue menor que en aquellos elaborados con agente de carga, hecho que está en concordancia con el nivel de insaturación de los lípidos presentes en los productos. Las muestras elaboradas con agente de carga y con un contenido en grasa de alrededor del 10% (F/OKM y IMFP), presentaron variaciones distintas respecto a sus controles (**Figura 5.14**). El mayor nivel de agente de carga en salchichas, así como también el tipo de producto, pudieron determinar estas diferencias. Las salchichas tipo

frankfurt son productos con un grado de desintegración mayor que el de las hamburguesas, además de que su elaboración requiere de tratamiento térmico. En tal sentido, se ha descrito que las condiciones de procesado (picado, tratamiento térmico, tipo de envasado) facilitan la interacción entre los ácidos grasos y el oxígeno, lo que resulta en un incremento de la susceptibilidad a la oxidación lipídica (Lee et al., 2006). De otro lado, aspectos que pudieron potenciar el aumento de los valores de oxidación en hamburguesas pueden ser el envasado aeróbico de las mismas y la ausencia de cualquier aditivo con actividad antioxidante, en tanto que las salchichas contenían nitritos en su formulación. Cabe aclarar que la diferencia entre los valores de TBARS de salchichas y hamburguesas (**Figura 5.13**), se debió en cierta parte a las variaciones en los procedimientos de medición (**Capítulo 3.4.2.7**).

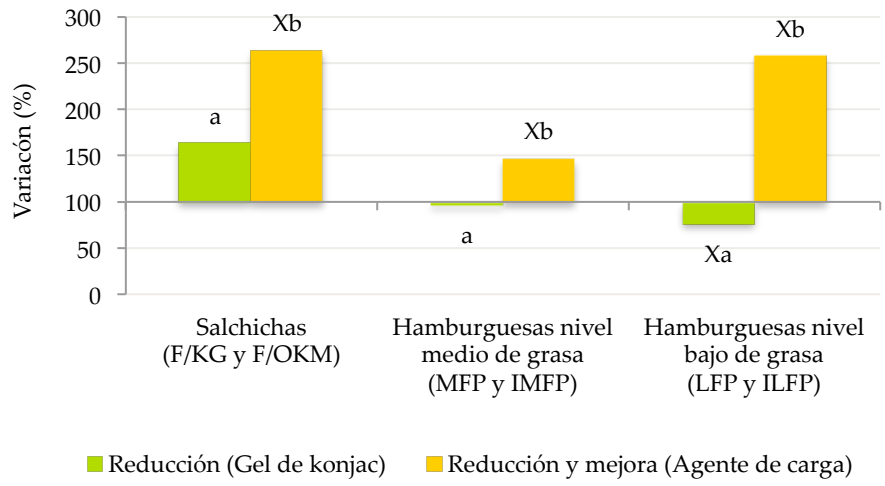


Figura 5.14. Variación en la oxidación lipídica (TBARS) al inicio de la conservación por efecto de a) reducción de grasa con gel de konjac y b) reducción y mejora del perfil con agente de carga en salchichas y hamburguesas.

Datos calculados de la tabla 5, capítulo 4.1.2 (Salcedo-Sandoval et al., 2015c) y tabla 6, capítulo 4.1.3 (Salcedo-Sandoval et al., 2015a)

Las variaciones fueron calculadas respecto al control con un valor asignado del 100% en el eje Y. Diferentes letras (a,b) en un mismo producto indican diferencias significativas reformulaciones de un mismo producto. X indica diferencias significativas respecto al control.

5.6.2 Efecto de la reformulación asociado al empleo de partículas de hidrogel

Como se ha señalado, la estabilidad oxidativa es un aspecto crítico en los productos enriquecidos con AGP n-3 de cadena larga. En este sentido, las partículas de hidrogel son componentes que pueden dotar de protección a lípidos susceptibles a la oxidación, puesto que estos se encuentran rodeados de una estructura biopolimérica concentrada con capacidad antioxidante (caseinato) que pueden limitar las interacciones entre prooxidantes de la fase acuosa y lípidos (Waraho et al., 2011).

Para valorar esa posibilidad, en los estudios relativos a la utilización de partículas de hidrogel, se analizaron la formación de compuestos primarios y secundarios de oxidación (índice de hidroperóxidos y TBARS, respectivamente). En primer lugar se evaluó la estabilidad oxidativa de las partículas de hidrogel, sometidos a condiciones de procesado propias de los productos tipo gel/emulsión (tratamiento térmico a 70 °C y conservación a 2 °C) (**Capítulo 4.2.1**). Estos sistemas fueron comparados con una emulsión O/W con similar contenido de aceite de pescado. Los resultados mostraron que las partículas de hidrogel fueron más efectivas al disminuir la formación de compuestos primarios y secundarios de oxidación que la emulsión O/W, y ello a lo largo del periodo de conservación, incluso cuando se aplicó el tratamiento térmico (**Figura 2, capítulo 4.2.1**). Estos resultados son consistentes con los obtenidos en sistemas modelo, en donde el enriquecimiento en AGP n-3 de cadena larga a través del empleo de partículas de hidrogel (F/H), dio lugar a niveles de oxidación lipídica sustancialmente menores que los mostrados por las otras muestras conteniendo aceite de pescado (F/O y F/E). Tal fenómeno se evidenció en una reducción de la concentración de hidroperóxidos en torno al 59% durante el tiempo de almacenamiento, respecto a la muestra conteniendo

un sistema convencional de estabilización de aceite (emulsión O/W, F/E) (**Figura 3, capítulo 4.2.1**).

Los resultados de oxidación de salchichas enriquecidas con AGP n-3 fueron consistentes con los obtenidos en sistemas modelo. Sin embargo, los niveles fueron inferiores en salchichas, lo que se atribuye a la acción de antioxidantes (nitritos) presentes en la formulación (**Figura 1, capítulo 4.2.2**). Varios factores pueden contribuir a explicar por qué las partículas de hidrogel reducen la formación de compuestos de oxidación. En primer lugar estos sistemas son más robustos que una emulsión convencional (Matalanis et al., 2012). Adicionalmente las partículas de hidrogel fueron estabilizadas con caseinato y pectina. Dado que las partículas de grasa fueron embebidas dentro de una estructura rica en caseinato, habría alta concentración de proteína muy próxima a la partícula de grasa emulsificada, en tales condiciones el caseinato podría actuar de manera efectiva en la eliminación de radicales libres, así como en la quelación de iones metálicos (Díaz y Decker, 2004). Además, la presencia de pectina en la estructura podría también ejercer actividad antioxidante protegiendo el material lipídico (Chen et al., 2010). Por consiguiente, la mayor estabilidad oxidativa proporcionada por las partículas de hidrogel podría atribuirse a la concentración de biopolímeros así como también a su proximidad estructural a la partícula de grasa. Los valores de TBARS obtenidos en las salchichas con partículas de hidrogel (<0,8 mg MDA/kg producto) fueron más bajos que los valores descritos en la literatura como capaces de producir sabor desagradable en productos cárnicos procesados (Caceres et al., 2008; Mercadante et al., 2010).

5.6.3 Agente de carga vs partículas de hidrogel

La optimización de la composición de salchichas tipo frankfurt mediante el empleo de agente de carga o partículas de hidrogel tuvo efectos marcadamente distintos sobre la oxidación (**Figura 5.15**). Los resultados señalan

que en las salchichas formuladas con partículas de hidrogel, la tasa de formación de compuestos de oxidación fue mucho menor que en el agente de carga. Esto posiblemente responde a dos factores. Por un lado, y teniendo en cuenta que la composición de AGP juega un papel determinante en la estabilidad oxidativa, al hecho de que el contenido en AGP fue mayor en salchichas con agente de carga (2,0 g/100 g, **Figura 5.7**) que en salchichas con partículas de hidrogel (1,4 g/100 g, **Figura 5.10**). De otro lado, como ha sido mencionado anteriormente, las partículas de hidrogel son estructuras diseñadas para proporcionar protección frente a la oxidación, mientras que el agente de carga no posee esta funcionalidad.

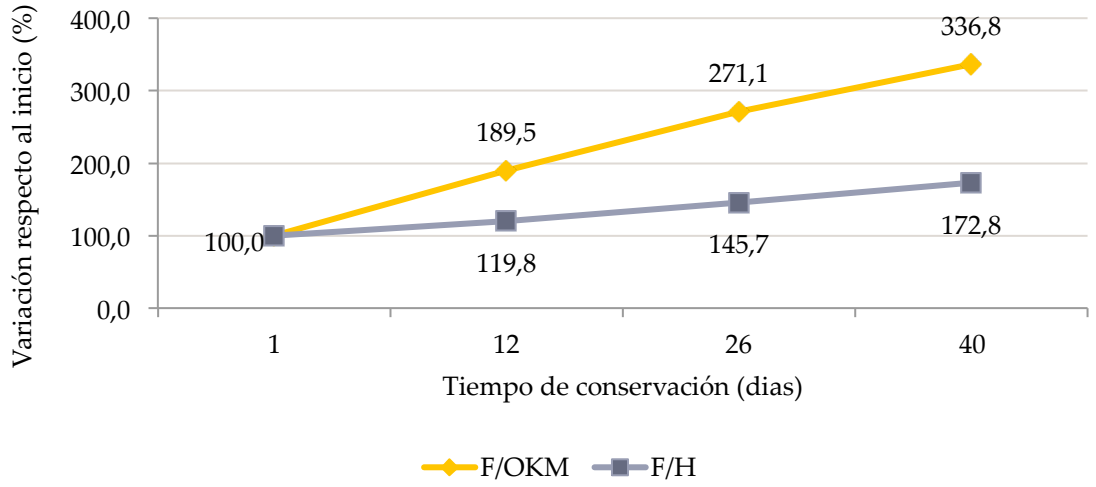


Figura 5.15. Variación en la oxidación lipídica (TBARS) durante el tiempo de conservación respecto a los valores iniciales de salchichas tipo frankfurt formuladas con agente de carga (F/OKM) o partículas de hidrogel (F/H).
Datos calculados de la tabla 5, capítulo 4.1.2 (Salcedo-Sandoval et al., 2015c) y figura 1 capítulo 4.2.2 (Salcedo-Sandoval et al., Enviado).
Las variaciones fueron calculadas fijando en cada caso los valores iniciales como 100%.

5.7 ANALISIS SENSORIAL

Uno de los aspectos primordiales a tener en cuenta a la hora de valorar un nuevo producto, es su evaluación sensorial. En coherencia con los resultados de

textura instrumental, la reducción de grasa con gel de konjac en salchichas tipo frankfurt produjo valores significativamente más altos de dureza que el control. Sin embargo esta concordancia no fue similar en las salchichas con agente de carga, en las que los catadores no detectaron diferencias en la dureza en comparación con la muestra control. La calidad sensorial de estos productos no fue afectada por la presencia de la mezcla de aceites o por la estrategia utilizada para la estabilización del mismo (P>0,05) (**Tabla 7, capítulo 4.1.1**).

Por otra parte, con independencia del nivel de grasa sustituido, los parámetros sensoriales de hamburguesas (aceptabilidad de textura, aceptabilidad de sabor y aceptabilidad general) no se vieron alterados (P>0,05) por la reducción de grasa con gel de konjac. La reformulación con agente de carga no afectó significativamente la aceptabilidad de textura, aunque si provocó una disminución en la aceptabilidad de sabor y la aceptabilidad general, las cuales decrecieron a medida que la proporción de agente de carga aumentó en el producto (**Tabla 8, capítulo 4.1.3**). Este comportamiento está en relación con aquellos obtenidos en la oxidación lipídica. Esto plantea la conveniencia de reformular el producto empleando agentes naturales, caso por ejemplo de algunos aceites esenciales, que de acuerdo con varios autores, han mostrado ser adecuados para aumentar la estabilidad de productos formulados con fracción lipídica más saludable (Dzudie et al., 2004; Lee et al., 2005; Lee et al., 2006).

En relación a los productos ricos en AGP n-3 de cadena larga, no se detectaron diferencias en ningún atributo sensorial (jugosidad, firmeza, aceptabilidad de textura, aceptabilidad de sabor y aceptabilidad general) entre las muestras control y la que fue reformulada empleando partículas de hidrogel, ambas presentaron los mayores niveles de aceptabilidad general. En contraste con las salchichas reformuladas con adición directa de aceite o con emulsión O/W, el enriquecimiento de salchichas en EPA y DHA mediante la

utilización de partículas de hidrogel no mostró efectos limitantes en relación a la aceptabilidad de los consumidores.

Según lo expuesto, en general, en el marco de los procesos de reformulación, la incorporación de agente de carga y partículas de hidrogel como estrategias para optimizar la fracción lipídica de salchichas tipo frankfurt no conlleva consecuencias negativas sobre los atributos sensoriales de los productos.

6. Conclusiones

6. CONCLUSIONES

De todo lo expuesto anteriormente se pueden extraer las siguientes conclusiones:

1. La aplicación de una estrategia de reformulación basada en la sustitución parcial de grasa animal por agente de carga de aceite (combinación de oliva, linaza y pescado) a base de konjac en salchichas tipo frankfurt puede considerarse adecuada para mejorar aspectos cuantitativos y cualitativos de su fracción lipídica. Dicho procedimiento, además de reducir el contenido en grasa y en ácidos grasos saturados, favorece la presencia de ácidos grasos poliinsaturados n-3. Adicionalmente, si bien la aplicación de tal estrategia originó modificaciones en la naturaleza de la matriz (sistema gel/emulsión) con consecuencias sobre alguna de sus características (textura y oxidación lipídica), permitió la obtención de productos con adecuada viabilidad tecnológica y sensorial, sin más limitaciones en términos de vida útil que las propias de un derivado cárnico de naturaleza análoga.

2. El proceso de reformulación encaminado al desarrollo de hamburguesas más saludables en base a un contenido lipídico mejorado (reducción de grasa y ácidos grasos saturados, y aumento de poliinsaturados n-3), resultó viable empleando agente de carga de aceites preparado con konjac como estrategia de sustitución de grasa animal. Tal proceso afectó a la naturaleza del sistema, que exhibió mejores propiedades ligantes de grasa y agua, aunque sin consecuencia sobre otras características tecnológicas (textura, color), y sobre la vida útil de los productos. Sin embargo, limitaciones asociadas a la oxidación lipídica y a algunos atributos sensoriales en función de los niveles de agente de carga empleados (básicamente asociados a la presencia de aceite de pescado) plantean la conveniencia de introducir ciertos cambios para paliar tales inconvenientes.

3. Los procedimientos de cocción (plancha y fritura) comúnmente empleados para el consumo, aplicados a hamburguesas formuladas con un contenido lipídico mejorado mediante la utilización de agente de carga de aceites, afectaron a su composición. Si bien tales cambios, condicionados por el método de cocción, influyeron en aspectos cuantitativos del material lipídico (presencia de grasa y de ácidos grasos), no produjeron modificaciones en las proporciones relativas de ácidos grasos. Tal comportamiento parece estrechamente relacionado con la naturaleza de la matriz, y en especial con la capacidad de estabilización de aceites del agente de carga, incluso cuando se aplican altas temperaturas. El hecho de que tales efectos se mantengan aún después del proceso térmico permite resaltar lo apropiado de la estrategia para elaborar este tipo de productos con implicaciones saludables.

4. Con independencia del tipo de derivado (salchicha o hamburguesa), el agente de carga puede considerarse como un excelente ingrediente (análogo de grasa) para mejorar su contenido y perfil lipídico. La disminución de la presencia de grasa y AGS junto con el incremento de AGP (en especial n-3), hace posible la obtención de productos más en línea con los objetivos nutricionales recomendados. Sin embargo, las consecuencias del proceso sobre propiedades tecnológicas y/o atributos sensoriales variaron con la proporción de agente de carga incorporado, así como con la naturaleza de la matriz cárnica (tipo de producto).

5. Las partículas de hidrogel constituyen un sistema de entrega adecuado para ser empleado como medio de estabilización de ácidos grasos poliinsaturados de cadena larga (EPA, DHA de pescado) ya que limitan los procesos de oxidación lipídica, proporcionando mayor estabilidad que los sistemas convencionales. Su mecanismo de actuación cabe atribuirlo tanto a aspectos estructurales como aquellos otros relacionados con la composición de las partículas de hidrogel. Por cuanto este comportamiento se observó también

cuando se incorporan en una matriz cárnica (sistema modelo), tales hidrogeles constituyen una opción adecuada y no explorada para llevar a cabo el desarrollo de derivados cárnicos enriquecidos con ácidos grasos n-3 de cadena larga, y ello con un menor riesgo de experimentar deterioro oxidativo.

6. El empleo de partículas de hidrogel en procesos de reformulación de salchichas tipo frankfurt, si bien no tuvo consecuencias sobre el contenido de grasa supuso un aporte muy notable de ácidos grasos poliinsaturados n-3 (0,99 g/100 g), principalmente n-3 de cadena larga (0,87 g/100 g EPA y DHA). Su presencia dio lugar a la formación de una matriz proteica estable, con capacidad adecuada para inmovilizar agua y grasa sin condicionar sus propiedades tecnológicas, atributos sensoriales, estabilidad oxidativa y vida útil. Todo ello permite establecer que el empleo de partículas de hidrogel constituye un proceso apropiado para el enriquecimiento en ácidos grasos de cadena larga en sistemas gel/emulsión (salchichas tipo frankfurt).

7. Los lípidos estructurados (agente de carga de aceites y partículas de hidrogel) pueden ser empleados, aunque con distintos propósitos, en el marco de las estrategias de reformulación en salchichas tipo frankfurt. Su aplicación, ligada al tipo de modificación lipídica propuesta, conduce a la obtención de productos con adecuada viabilidad tecnológica y sensorial, sin limitaciones adicionales de vida útil

CONCLUSIÓN GENERAL

Como conclusión general se puede señalar que, en consonancia con los objetivos planteados, la aplicación de lípidos estructurados (agente de carga y partículas de hidrogel) como nuevos sistemas de estabilización de aceites abren nuevas posibilidades dentro de las estrategias de reformulación de derivados cárnicos saludables. Se trata de propuestas novedosas que permiten la

obtención de productos con un contenido lipídico mejorado a nivel cuantitativo y/o cualitativo, con un perfil de ácidos grasos más acorde con los objetivos nutricionales recomendados, además de dotarlos de propiedades tecnológicas y sensoriales apropiados, así como de una estabilidad comparable con productos de naturaleza análoga.

Los cambios de composición originados permiten conferir a los productos distintas declaraciones nutricionales y de propiedades saludable, en el marco establecido por el Reglamento (CE) 1924/2006 del Parlamento Europeo y del Consejo relativo a las declaraciones nutricionales y de propiedades saludables en los alimentos.

7. Resumen ampliado[1]

[1] Este resumen ampliado se presenta en cumplimiento de las directrices de la normativa de desarrollo del Real Decreto 99/2011, de 28 de enero, que regula los estudios de doctorado en la Universidad Complutense de Madrid (UCM) (BOUC nº 14, de 21 de diciembre de 2012) y de acuerdo con las especificaciones establecidas por la Comisión de Doctorado de la UCM.

7. RESUMEN AMPLIADO

INTRODUCCIÓN

Los productos cárnicos son una de las fuentes más importantes de grasa en la dieta. Sin embargo, su composición se encuentra algo alejada (cuantitativa y cualitativamente) de las recomendadas por diversas organizaciones de salud. Esto ha planteado la conveniencia de poner en práctica diversas estrategias para modificar su contenido en grasa y/o perfil de ácidos grasos y obtener productos más saludables (Jiménez-Colmenero, 2007). Esto es de particular interés en el caso de productos como salchichas tipo frankfurt y hamburguesas, por ser muy populares, frecuentemente consumidos y gozar de una amplia aceptación en determinados sectores de la población. En este sentido, las estrategias de reformulación ofrecen interesantes posibilidades para favorecer la incorporación de aceites vegetales y marinos cuya composición es más saludable que la de la grasa animal habitualmente presente en estos productos. La sustitución, en mayor o menor medida, de la grasa animal se ha llevado a cabo mediante diferentes opciones tecnológicas, encontrándose entre las más comunes la adición directa de aceites, la aplicación de procesos de interesterificación y de pre-emulsificación (Jiménez-Colmenero, 2007; Grasso et al., 2014).

En este contexto, recientemente se han planteado nuevas alternativas tecnológicas, entre ellas las basadas en el empleo de lípidos estructurados. Se trata de la formación de sistemas capaces de estabilizar aceites, creando estructuras plásticas con propiedades similares (de sólido/viscoso) a las que exhibe la grasa animal a la que va a reemplazar, si bien con una composición lipídica más saludable. Pese a que por sus características, ofrecen interesantes posibilidades de aplicación en derivados cárnicos, su empleo es aún muy limitado (Jiménez-Colmenero et al., 2015). De entre las distintas opciones

disponibles los agentes de carga y las partículas de hidrogel (emulsión estructurada tipo $O/W_1/W_2$) ofrecen interesantes y no exploradas oportunidades para sustituir grasa animal y enriquecer con ácidos grasos poliinsaturados (AGP) n-3 de cadena larga, respectivamente.

Un agente de carga (en inglés *oil bulking agent*), se forma mediante dispersión de un gran número de gotas de aceite en una fase acuosa continua gelificada. En este caso, el aceite líquido está atrapado físicamente en una red de gel, que proporciona una estructura sólida al sistema, lo que lo hace apto para su uso como análogo de grasa (Herrero et al., 2014a). Estos sistemas poseen características sólidas que se asemejan bastante a aquellas aportadas por la grasa animal empleada en la elaboración de derivados cárnicos (generalmente tocino de cerdo), lo que los hace muy apropiados a la hora de elaborar productos con contenido reducido y mejorado en grasa. Un aspecto a destacar de los agentes de carga es la cantidad de aceite que es capaz de estabilizar. Delgado-Pando et al. (2012b), describe la formación de un agente de carga a base de konjac (preparado con una combinación de aceites de oliva, lino y pescado), específicamente seleccionada para contener proporciones de ácidos grasos monoinsaturados (AGM) y AGP n-3, ajustadas a las recomendaciones de ingesta óptima de ácidos grasos insaturados y de ácidos grasos totales (WHO, 2003). Así pues, de acuerdo con lo anteriormente mencionado, la sustitución de la grasa animal típicamente presente en productos cárnicos por agentes de carga de aceites a base konjac podría conducir a la reducción del contenido de grasa y simultáneamente, a la mejora del perfil lipídico de productos reformulados.

Las partículas de hidrogel son esferas donde el aceite emulsionado se ha incorporado dentro de una fase acuosa dispersa gelificada (W_1), que a su vez está contenida en una fase acuosa continua (W_2). En tales condiciones, dichas partículas quedan encapsuladas dentro de la matriz de hidrogel formando una

estructura tipo $O/W_1/W_2$ (McClements, 2010). Las partículas de hidrogel han sido ampliamente usadas en la industria farmacéutica como sistemas de entrega (*delivery systems)* de principios activos. Actualmente se perfilan como ingredientes potenciales en el desarrollo de productos alimenticios (McClements, 2010). Estos sistemas pueden ser diseñados para proveer de protección física y química a compuestos bioactivos lipofílicos como los AGP n-3 de cadena larga (EPA y DHA), a la vez que pueden utilizarse en el desarrollo de productos bajos en grasa debido a su estructura viscosa (Chung et al., 2013). Por tanto la utilización de este sistema para estabilizar aceite de pescado podría considerarse para el desarrollo de productos cárnicos enriquecidos en AGP n-3 de cadena larga

OBJETIVOS

El **objetivo general** de la presente memoria consiste en el **diseño y desarrollo de derivados cárnicos con mejor composición lipídica llevados a cabo mediante procesos de reformulación encaminados a la obtención de productos mas saludables**. Para ello se han planteado estrategias de optimización de la composición de productos cárnicos basadas en la utilización de aceites de origen vegetal y/o marino estructurados como un agente de carga a base de konjac y en forma de partículas de hidrogel. Estas estrategias están dirigidas tanto a reducir el contenido de grasa como a mejorar el perfil de ácidos grasos disminuyendo la proporción de AGS y favoreciendo la de AGP, especialmente los de AGP n-3 de cadena larga, además de mejorar la relación AGP n-6/n-3 y AGP/AGS. En este contexto, y de acuerdo con la legislación de la Unión Europea, los productos reformulados podrían estar sujetos a alegaciones nutricionales y propiedades de salud.

Para abordar este objetivo general, se plantearon los siguientes objetivos específicos:

1. **Evaluar la utilización de un agente de carga de aceite a base de konjac como estrategia en el desarrollo de salchichas tipo frankfurt y de hamburguesas.** En el marco de este objetivo se han aplicado procesos de reformulación encaminados a la reducción de grasa y a la mejora del perfil de ácidos grasos de estos productos. Para tal fin, se realizó la sustitución de la grasa animal, habitualmente empleada, por un agente de carga a base de konjac glucomanano conteniendo una combinación de aceites de oliva, lino y pescado específicamente establecida para dotar al producto con altas cantidades AGM y AGP, por tanto de un perfil lipídico más ajustado en relación con las recomendaciones nutricionales. En este sentido, se han estudiado los efectos de la reformulación y la conservación en refrigeración sobre las propiedades tecnológicas, nutricionales, microbiológicas y sensoriales de los productos. Adicionalmente se aborda como los distintos métodos de cocción habitualmente empleados afectan la composición de las hamburguesas haciendo particular referencia al perfil de ácidos grasos.

2. **Analizar el empleo las partículas de hidrogel encapsulando aceite de pescado como estrategia en el desarrollo de productos cárnicos más saludables.** Este objetivo se ha centrado en la aplicación de procesos de reformulación dirigidos a la mejora del perfil lipídico, basados en la presencia de partículas de hidrogel conteniendo aceite de pescado en productos tipo gel/emulsión (sistema modelo y salchichas tipo frankfurt). Estas partículas se han diseñado especialmente para vehiculizar altas concentraciones de EPA y DHA, permitiendo el desarrollo de productos enriquecidos en ácidos grasos n-3 de cadena larga. En tal sentido, los efectos sobre las propiedades tecnológicas, nutricionales y sensoriales en los productos debidos a la reformulación y la conservación en refrigeración han sido evaluados.

PRINCIPALES RESULTADOS Y DISCUSIÓN

El uso de agente de carga como estrategia de reformulación en salchichas tipo frankfurt y hamburguesas ha dado lugar a la obtención de productos con contenido reducido en grasa (50% en salchichas y 38% y 72% en hamburguesas), con perfil lipídico en línea con los objetivos nutricionales, disminuyendo el contenido en ácidos grasos saturados, incrementando los ácidos grasos poliinsaturados n-3 (1,12 g y 0,36/100 g producto, en salchichas y hamburguesas, respectivamente). Se ha estudiado el efecto que conlleva la aplicación de diversos tratamientos térmicos (fritura y plancha) sobre la composición de las hamburguesas. Se ha observado que si bien estos afectaron la composición, no implicaron una alteración sobre las proporciones relativas de ácidos grasos de los productos reformulados, por lo que la mejora nutricional conseguida mediante esta estrategia permaneció estable. Valorando la aplicación de los procesos de reformulación con agente de carga en función del tipo de producto, se puso de manifiesto la existencia de diferentes efectos sobre parámetros de composición, propiedades tecnológicas y sensoriales. Tales efectos, en algunos casos tuvieron relación con la proporción de agente de carga incorporado (composición, propiedades ligantes, color, textura), mientras que en otros estuvieron asociados con la naturaleza de la matriz cárnica (oxidación lipídica y parámetros sensoriales). Adicionalmente, aunque la aplicación de esta estrategia condicionó ciertas características, permitieron la obtención de derivados cárnicos con adecuada viabilidad tecnológica y sensorial, sin más limitaciones en términos de vida útil que las propias de un derivado cárnico de naturaleza análoga.

Por otro lado, el empleo de las partículas de hidrogel como sistema de estabilización de aceite de pescado presentó una notable mejora sobre la estabilidad oxidativa de este material lipídico, cuyo comportamiento se mantuvo formando parte de una formulación cárnica (sistemas modelo). El enriquecimiento en EPA y DHA de salchichas tipo frankfurt a través del empleo

de partículas de hidrogel conllevó a la obtención de productos con perfil lipídico mejorado, con un aporte significativamente alto de ácidos grasos poliinsaturados n-3 (0,99 g/100 g), conformado principalmente por n-3 de cadena larga (0,87 g/100 g EPA y DHA). Esto significa que aunque las recomendaciones dietarías de consumo de ácidos grasos n-3 de cadena larga varíen en función de diversos factores, los productos enriquecidos harían una contribución muy significativa a dicha ingesta, en comparación con los productos no fortificados. La aplicación de esta estrategia de reformulación dio lugar a la formación de una matriz proteica estable, con capacidad adecuada para inmovilizar agua y grasa, sin condicionar las propiedades tecnológicas, la vida útil y los atributos sensoriales.

Los cambios de composición originados permitieron dotar a los productos de distintas declaraciones nutricionales y de propiedades saludable, en consonancia con el marco establecido por el Reglamento (CE) 1924/2006 del Parlamento Europeo.

CONCLUSIONES

Como conclusión general se puede señalar que, en consonancia con los objetivos planteados, la aplicación de lípidos estructurados (agente de carga y partículas de hidrogel) como nuevos sistemas de estabilización de aceites abren nuevas posibilidades dentro de las estrategias de reformulación de derivados cárnicos saludables. Se trata de propuestas novedosas que permiten la obtención de productos con un contenido lipídico mejorado a nivel cuantitativo y/o cualitativo, con un perfil de ácidos grasos más acorde con los objetivos nutricionales recomendados, además de dotarlos de propiedades tecnológicas y sensoriales convenientes, así como de una estabilidad comparable con productos de naturaleza análoga.

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8. Extended abstract[1]

[1] This extended abstract is included in fulfillment of the directives of the regulation of development of the Real Decreto 99/2011, 28th of January, which regulates the studies of doctorate at the Universidad Complutense de Madrid (UCM) (BOUC no 14, 21st of December 2012) and in agreement with the specifications established by the Commission of Doctorate of the UCM.

8. EXTENDED ABSTRACT

INTRODUCTION

Meat products are one of the most important sources of dietary fat. However, its composition diverges (quantitatively and qualitatively) of those recommended by various health organizations. This has raised the convenience of implementing different strategies to change their fat and/or fatty acid profile and thus obtain healthier products (Jiménez-Colmenero, 2007). This is of particular interest in the case of products such as frankfurters and patties, since they are very popular, frequently consumed and enjoy wide acceptance in certain sectors of the population. In this regard, the reformulation strategies offer interesting possibilities for promoting the incorporation of vegetable and marine oils whose composition is healthier than animal fat usually present in these products. Substitution (in greater or lesser extent) of the animal fat has been carried out by various technology options, among the most common are direct addition of oils, interesterification and pre-emulsification processes (Jiménez-Colmenero, 2007; Grasso et al., 2014).

In this context, recently novel technological alternatives have emerged, including those based on the use of structured lipids. It is about the formation of systems capable of stabilizing oils, creating plastic with similar properties (solid / viscous) that exhibits the animal fat which will be replaced, but with a healthier lipid composition. Though for their characteristics these systems offer interesting possibilities for application in meat products, their use is still very limited (Jiménez-Colmenero et al., 2015). Among the available options, konjac-based oil bulking agents and hydrogel particles ($O/W_1/W_2$ emulsion) offer interesting and unexplored opportunities to replace animal fat and enriched with n-3 long chain polyunsaturated fatty acids (n-3 long chain PUFA) respectively.

An oil bulking agent is formed by dispersing a large number of oil droplets in a continuous gelled aqueous phase. Thus, the liquid oil is physically entrapped in a gel network, which provides a solid structure to the system, making it suitable for use as fat analogue (Herrero et al., 2014a). This system is a solid-like material whose characteristics strongly resemble to those showed by animal fat used in the preparation of meat products (usually pork backfat), what makes it very appropriate when it comes to developing products with reduced and improved fat content. A notable aspect of the bulking agent is the amount of oil that is capable of stabilizing. Delgado-Pando et al. (2012a), reported the formation of a konjac-based oil bulking agent (prepared with a combination of olive, flaxseed and fish oils), specifically selected to contain proportions of monounsaturated fatty acids (MUFA) and n-3 PUFA adjusted to the recommendations of optimal intake of unsaturated fatty acids and total fatty acids (WHO, 2003). Thus, according to the above, the substitution of animal fat typically present in meat products by a konjac-based oil bulking agent could lead to reduced fat content and simultaneously improve the lipid profile of reformulated products.

Hydrogel particles are spheres where emulsified oil is incorporated into a dispersed gelled aqueous phase (W_1), which in turn is contained in a continuous aqueous phase (W_2). Under such conditions, these oil particles are encapsulated within the hydrogel matrix forming a structure type $O/W_1/W_2$ (McClements, 2010). Hydrogel particles have been widely used in the pharmaceutical industry as delivery systems of active compounds. Currently they are emerging as potential ingredients in the development of food (McClements, 2010). These systems can be designed to provide physical and chemical protection to lipophilic bioactive compounds such as n-3 long chain PUFA (EPA and DHA), and simultaneously may be used in the development of low-fat products because of their viscous structure (Chung et al., 2013).

Therefore the use of such particles to stabilize fish oil may be considered for the development of n-3 long chain PUFA enriched-meat products.

OBJETIVES

The overall objective of this thesis dissertation is **to study of designing possibilities and the development of meat products with better lipid composition by means of reformulation processes aimed at obtaining healthier products**. To this aim, two different optimization strategies based on meat products composition have been proposed. These strategies involve the use of vegetable and/or marine oils that were structured in a konjac-based oil bulking agent and in hydrogel particles. These strategies are aimed both at reducing the fat content and to improve the fatty acid profile by decreasing the proportion of SFA and at the same time rising up the content in PUFA, especially very long-chain n-3 PUFA, and thus improving n-6 PUFA/n-3 PUFA and PUFA/SFA ratios. In this context, and according to the European Union regulations, the reformulated products may be subjected to nutrition and health claims associated with the change of their composition.

These modifications represent a significant technological challenge as they involve the application of novel reformulation strategies to obtain products with similar characteristics to those that normally show conventional meat products.

To address this overall objective, the following specific objectives were proposed:

1. **Evaluate the use of a konjac-based oil bulking agent as a strategy in the development of frankfurters and pork patties**. In this frame, the reformulation process aimed at both reducing the fat content and improving the fatty acid profile of these products have been assessed. For this, the substitution of animal fat was carried out by using a bulking agent based on konjac gel containing a combination of olive, flax and fish oils that were specifically established to

provide the product with high amounts MUFA and PUFA and, in consequence, resulting in a lipid profile that is more in line with health recommendations. In this regard, the effects of the reformulation and chilling storage on nutritional, technological and sensory properties, as well as shelf life of products have been studied.

2. **Analyze the use hydrogel particles encapsulating fish oil as a strategy for the development of healthier meat products**. This objective has focused on the implementation process of reformulation aimed at improving the lipid profile, based on the presence of hydrogel particles containing fish oil in gel/emulsion products (meat systems and frankfurters). These particles are designed especially for delivering high concentrations of EPA and DHA, enabling the development of products rich in n-3 long chain fatty acids. In this regard, the effects of reformulation and chilling storage on nutritional, technological and sensory properties, as well as shelf life of products were analyzed.

MAIN RESULTS AND DISCUSSION

The use of the konjac-based oil bulking agent as reformulation strategy has led to the development of products with reduced fat (50% in frankfurters and 38% and 72% in patties), with lipid profile in line with nutritional goals, limiting the content of saturated fatty acids, increasing n-3 polyunsaturated fatty acid (1.12 g and 0.36 g/ 100 g product, frankfurters and patties, respectively). Moreover, the effect that involves the application of different cooking methods (grilling and pan-frying) on the composition of patties was studied. It has been observed that although these affected the composition, did not alter fatty acids ratios of reformulated products, thus nutritional improvement achieved by this approach remained stable. The implementation of reformulating processes with bulking agents depending on the type of product revealed the existence of different effects on composition parameters, technological and sensory properties. Such effects, in some cases (composition, binding properties, color,

texture), were related to the proportion of oil bulking agent, while others (lipid oxidation and sensory parameters), were associated with the nature of the meat matrix. Additionally, although the implementation of this strategy conditioned certain characteristics, it allowed obtaining meat products with appropriate technological and sensory viability, without limitations in terms of shelf life than those exhibited by products with similar nature.

Furthermore, the use of hydrogel particles as fish oil stabilization system showed a remarkable improvement in the oxidative stability of the lipid material, whose behavior is maintained as part of a meat formulation (model systems). The EPA and DHA enrichment of frankfurters by means of the use of hydrogel particles led to the development of products with improved lipid profile, with a significantly higher intake of n-3 PUFA (0.99 g/100 g), consisting mainly of n-3 long chain PUFA (0.87 g/100 g EPA and DHA). This means that although the dietary recommendations of n-3 long chain PUFA vary depending on several factors, enriched products would make a significant contribution to this intake, compared to non-enriched products. The implementation of this reformulation strategy led to the formation of a stable protein matrix, with adequate capacity to immobilize water and fat, without conditioning technological and sensory properties, as well as shelf life of the products.

It is worth to highlight that composition changes achieved in the present dissertation would allow labeling of the reformulated products with several nutrition and health claims, according to the established by Regulation (EC) 1924/2006 of the European Parliament.

CONCLUSIONS

In line with the goals, incorporation of structured lipids (konjac-based oil bulking agent and hydrogel particles) as new stabilization oil systems opens up new possibilities within the reformulation strategies leading to obtain healthy

meat products. These innovative proposals enable the development of products (of different nature), with improved lipid content in quantitative and/or qualitative terms, presenting a fatty acid profile more in line with the recommended nutritional goals, besides of providing them with adequate technological and sensory properties, as well as self life stability, comparable to those of products of similar nature.

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Anexo

Novel applications of oil-structuring methods as a strategy to improve the fat content of meat products[1]

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[1] Esta publicación se incluye como anexo ya que ha sido inspirada parcialmente en el trabajo desarrollado en esta memoria, y elementos del mismo figuran en la introducción.

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TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Novel applications of oil-structuring methods as a strategy to improve the fat content of meat products

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This paper reviews the approaches taken to stabilize and structure edible oils in order to promote solid-lipid functionality for use as an alternative to animal fat for the development of healthy lipid meat products. Interesterification and organogelation processes, the formation of oil bulking agents and the creation of structured emulsions (hydrogelled emulsion and organogelled emulsion) are described as strategies for the stabilization and structuring of edible liquid oils. Different aspects related to their composition, preparation and structural organization are described as well as their utilization in meat product formulation.

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Introduction

There is growing evidence that dietary fat may play a role in the prevention and therapy for a number of chronic disorders, such as coronary heart disease or type 2 diabetes. Promoting health through nutrition is an increasingly important objective in public health programs in a number of countries. Recommendations for optimal intake of total and unsaturated fatty acids have been proposed by a number of scientific authorities and nutritional organizations (McNeill, 2014; WHO, 2003). These recommendations refer to the overall diet but, given that meat and meat products are some of the most important sources of dietary fat (Givens, Kliem, & Gibbs, 2006), changes in the amounts and the lipid profiles of such products could help to improve the nutritional quality of the Western diet (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014; Jimenez-Colmenero, 2007; Muguerza, Gimeno, Ansorena, & Astiasaran, 2004; Oostindjer *et al*., 2014). Consumer unwillingness to change their dietary habits suggests that there is a considerable potential market for frequently-consumed foods such as meats which have been reformulated to produce health benefits. Also, owing to their broad range of presentations, the possibility of modifying their composition using non-meat ingredients, high consumer acceptability, etc., meat products are an excellent vehicle to delivery bioactive compounds in the diet and come closer to meeting dietary recommendations.

Reformulation of meat products (with different level of structural disintegration: comminuted, ground, restructured), is one of the most important approaches to remove, reduce, increase, add and/or replace different components in order to develop healthy meat products. Three main goals related to the improvement of fat content using meat reformulation strategies have been identified: reduction of total fat (and energy content), reduction of cholesterol and modification of fatty acid profiles. Technological strategies to improve the fatty acid profile generally entail replacement of the animal fat normally present in the product with a different lipid more in line with health recommendations—*i.e.* with smaller proportions of saturated fatty acids (SFAs), *trans* fatty acids (TFAs) and larger proportions of monounsaturated (MUFAs), n-3 polyunsaturated (n-3 PUFAs, especially long-chain) fatty acids or conjugated linoleic acid (CLA), better n-6/n-3 PUFA and PUFA/SFA ratios and, if possible, reduced cholesterol (Jimenez-Colmenero, 2007). Notwithstanding the above,

the true effect of saturated fatty acids on human health has recently come under debate and they are not considered as harmful as once believed (Siri-Tarino, Sun, Hu, & Krauss, 2010). However, it can still be concluded that, individually or as a whole, they do not have the same positive benefits as mono and especially polyunsaturated fatty acids. Many attempts have been made and numerous studies conducted to replace animal fat and improve the fat content of meat products (Grasso *et al*., 2014; Jimenez-Colmenero, 2007; Muguerza *et al*., 2004). Specifically, the use of technological approaches to replace meat fat with non-meat fat has been widely reviewed by Jimenez-Colmenero (2007). The technology developed to incorporate natural or processed plant and marine lipids into meat products ranges from direct addition in the form of liquid oils or solids (high-saturated and interesterified oils) to incorporation in encapsulated or emulsified form or as part of plant ingredients. Under these conditions, different vegetable oils (olive, cottonseed, corn, soybean, peanut, etc.), marine oils (fish and algae), or combinations of these, have been used to partially replace animal fat in fresh, cooked and fermented meat products. It is well documented that compared to habitually used meat fats, lipid materials of plant or marine origin have different physicochemical characteristics which may have a negative effect on the desired quality attributes in the reformulated product (Grasso *et al*., 2014; Jimenez-Colmenero, 2007; Muguerza *et al*., 2004). The functionality and texture of lipid phase (solid animal fat) present in meat products has a major effect on several product characteristics (mouthfeel, juiciness, texture, bite, heat transfer, etc.). Therefore, reducing or replacing animal fat with liquid oils presents a considerable technical challenge.

In this context, novel proposals for liquid phase oil stabilization and structuring has recently been reported to develop fat alternatives which can be used to improve the quality of the reformulated systems. The modification or structuring of the oils to create a plastic fat which retains solid-like properties while possessing a healthier fatty acid profile is a very important area of research from both an academic and industrial point of view (Co & Marangoni, 2012; Dickinson, 2012; Patel, Cludts, Sintang, Lesaffer, & Dewettinck, 2014; Zetzl, Marangoni, & Barbut, 2012). This paper reviews the approaches taken to stabilize and structure edible oil to form soft matter structures with solid-lipid functionality and studies their application as fat alternatives in order to improve the fat content of meat products. Many reviews have dealt with the topic of healthy meat products, including the technological strategies used to design and develop them (Arihara, 2006; Grasso *et al*., 2014; Jimenez-Colmenero, 2007; Muguerza *et al*., 2004, and others). However, to the best of our knowledge, there is lack of studies focusing on the novel approaches of stabilizing and structuring liquid oils as a way to develop healthy lipid meat products. Through these approaches, it is possible to create a solid-like material with a healthier lipid composition which may be rich in MUFA and PUFA and with reduced SFA levels and zero *trans* fatty acids.

Stabilization and structuring of edible oils: approaches and application in meat product formulation

The structuring of organic phases has received considerable attention in different fields including food science. Structuring techniques for liquid oils can be subdivided into two broad categories, dispersion of a foreign phase (small inert particles, crystallized solids, separated droplets), and specific molecular mechanisms such as self-assembly (Pernetti, van Malssen, Flöter, & Bot, 2007). In both cases, different structuring elements which act as building blocks are present for the formation of three-dimensional networks required to structure oil. Oil structuration can be also reached by means of interestification and hydrogenation processes in which the nature of the native oil is changed. However, the aim of this review is to describe those approaches that confer solid-like properties and at the same time being in line with health recommendations. Therefore the strategies used for the stabilization and structuring of liquid oils include those based on the application of interesterification and organogelation processes, the creation of structured emulsions (Zetzl *et al*., 2012), and the formation of oil bulking agents.

Since the properties of the lipid material determine those of the product in which it can be used, they must be considered when animal fat alternatives are designed. Color, consistency, lipid oxidation stability, taste, etc., are among the main quality attributes associated with meat fat. In meat products, the use of alternative animal fat of lower technological quality could lead to insufficient/excessive drying of products, oily appearance, rancidity, etc. Therefore quality criteria, in terms of fatty acid composition and physical properties, must be considered for the new fat materials (Hugo & Roodt, 2007).

Interesterification

Interesterification is an acyl rearrangement reaction used to improve the functionality and physical properties of fats and oils. This acyl rearrangement of fatty acids occurs within, and between triacylglycerols (TAGs) on a glycerol backbone thus resulting in the formation of a new TAG that may not have existed in the original fat. This process, which can be achieved by either chemical or enzymatic means, does not have the adverse effects of hydrogenation related to the formation of SFAs and particularly TFAs (which have a number of unhealthy effects). The chemical process is carried out by a first hydrolysis of fatty acyl groups from the mixture of acylglycerols and a subsequent random reesterification onto the glycerol backbone, being this reaction catalyzed by alkali metals or alkali metal alkylates. Enzymatic technology (milder and less aggressive than chemical reactions) makes possible to incorporate a desired fatty acid onto a specific position of the glycerol

backbone, so it is possible to obtain specific lipids with a defined chemical composition and structure. On the contrary, chemical methods for lipid modifications do not allow those possibilities due to the random nature of the reaction. Interesterification process shows the drawback of deterioration of oxidative quality in most cases, especially when susceptible oil sources are used. This phenomenon seems to be independent of the performed technology (Martin, Reglero, & Señorán, 2010). This technology can be used to modify the melting point of triglycerides and their crystallization properties to create plastic fats while maintaining their nutritional characteristics. Hence, this is an alternative technique for the creation of plastic fats with a healthy fatty acid profile which can be used as attractive fat replacers in meat product formulations (Fig. 1).

Combinations of chemically interesterified vegetable oils (sunflower, soybean, palm, canola, cotton, safflower, olive and maize oils in different proportions) have been tailored to simulate the characteristics of pork backfat (hard fat) and meet the technological requirements of the meat processing industry. These materials have been used as an alternative to animal fat and have lowered SFA content resulting in safe, tasty and healthier meat products (Ospina-E, Cruz-S, Pérez-Álvarez, & Fernández-López, 2010). Moreover, interesterified vegetable oils (and oil blends) prepared from different plant sources (palm, cottonseed, olive, hazelnut) have been used as fat replacers to modify the fatty acid composition of frankfurters and semi-dry fermented sausages (Javidipour & Vural, 2002; Javidipour, Vural, Özbaş, & Tekin, 2005; Özvural & Vural, 2008; Vural, 2003; Vural, Javidipour, & Ozbas, 2004) (Table 1). Cheong *et al.* (2010) produced sausages from enzymatically interesterified blends of lard and rapeseed oil. None of the sausages produced from interesterified blends of lard and rapeseed oil had apparent fat excretion and they were rated as having acceptable sensory attributes as compared to reference sausage produced from pure lard. Moreover, chemical and enzymatic interesterification of fats such as mutton tallow and vegetable oils has recently been used to create new fats containing certain amounts of mono- and diacylglycerols (MAG and DAG, respectively) with potential applications in the food industry (Kowalska, Żbikowska, & Kowalski, 2014).

Partial hydrogenation was the most common method to structure vegetable oils in the past. To produce a solid form, vegetable oils are hydrogenated to eliminate double bonds by direct addition of hydrogen to unsaturated fatty acids. It is well known that the hydrogenation process, in which SFAs are formed, presents various adverse effects on health (formation of TFAs). Partially hydrogenated plant oils (corn, cottonseed palm, peanut and soybean) have been substituted for beef fat in lean (10% fat) ground beef patties (Liu, Huffman, & Egbert, 1991). Also, partially hydrogenated palm oil has been used in the formulation of beef burgers (Babji *et al.*, 1998).

Organogelation

Gels are three-dimensional networked structures with the ability to immobilize a liquid phase. These are composed of two components, namely a liquid solvent

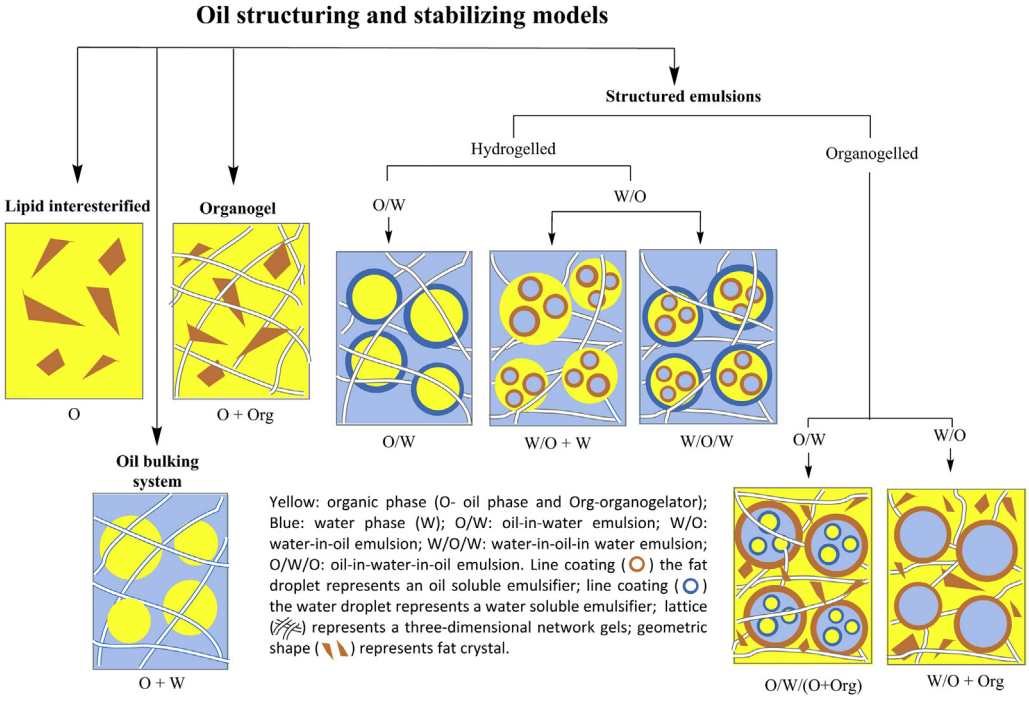

Fig. 1. Schematic presentation of structural arrangement of the structuring/stabilizing systems of edible oils in solid-like materials suitable as meat fat alternatives.

Table 1. Examples of application of oil-structuring strategies to improve the fat content of meat products.

Meat system	Oil	Oil-structuring strategy	Reference
Frankfurters	Palm, palm stearin, cottonseed and hazelnut oils, and their blended forms	Chemical interesterification	Özvural & Vural, 2008
Semy-dried fermented sausage	Palm and cottonseed oils	Chemical interesterification	Vural, 2003
Pork sausages	Blends of lard and rapeseed oil	Enzymatic interesterification	Cheong *et al.*, 2010
Frankfurters	Soy oil, flax oil and canola oil	Oleogel: ethylcellulose (10%)	Zetzl *et al.*, 2012
Meat suspensions	Mixture of virgin olive oil and sunflower oil	Oleogel: monoacylglycerols (0.5–2.5%), fatty alcohols (0.5–2.5%) or soy lecithin (2.5%)	Lupi *et al.*, 2012; Lupi, Gabriele, Seta, Baldino, & de Cindio, 2014
All-beef frankfurters and pork breakfast sausages	Canola oil	Oleogel: ethylcellulose (15%) or ethylcellulose (11%) and sorbitan monostearate (3.67%)	Wood, 2013
Frankfurters	Combination of olive, linseed and fish oils	Konjac-based oil bulking system	Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Solas *et al.*, 2013
Dry fermented sausages	Combination of olive, linseed and fish oils	Konjac-based oil bulking system	Jimenez-Colmenero *et al.*, 2013
Pork patties	Combination of olive, linseed and fish oils	Konjac-based oil bulking system	Salcedo-Sandoval *et al.*, 2014
Meat batters	Olive oil	Alginate-based oil bulking system	Ruiz-Capillas *et al.*, 2013
Frankfurter	Olive oil	Alginate-based oil bulking system	Herrero, Ruiz-Capillas, *et al.*, 2014
Frankfurter	Combination of olive, linseed and fish oils	Emulsion gel: microbial transglutaminase (MTG)	Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, *et al.* 2010
Bologna-type sausage	Linseed oil	Emulsion gel: k-carrageenan	Poyato *et al.*, 2014
Frankfurter	Olive oil	Emulsion gel: MTG, alginate or gelatin	Pintado, Herrero, *et al.*, in press; Pintado, Ruiz-Capillas, *et al.*, submitted for publication

phase (either polar or non-polar) and a gelling agent responsible for forming the three-dimensional networked structure. However, depending on the polarity of the liquid immobilized within the networked structure, gels may be regarded either as hydrogels (polar solvent-water) or organogels (organic solvent) (Sagiri *et al.*, 2014). Organogels (referred to as oleogels if the organic phase is an edible oil), can be defined as an organic liquid entrapped within a generally thermo-reversible, anhydrous and structured visco-elastic material by a three-dimensional gel network. This simply means transformation of a liquid oil into a 'gel-like' structure with visco-elastic properties (Rogers, Wright, & Marangoni, 2009; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012). It is worth noting that many gels require relatively small amounts of organogelators and thus can be considered as bulk-like fat materials as they contain a large amount of edible liquid oil (even $>$ 97 wt%) (Patel, Cludts, *et al.*, 2014).

Organogels may be categorized either as physical or chemical gels depending on the type of chemical interactions involved during the gelation process (Sagiri *et al.*, 2014). Oleogelators can be classified into two groups: self-assembly systems and crystal particles systems. In the former the oleogelator involves a molecular-level self-organization in the oil phases, whereas in the latter involves crystal particles occurring through nucleation and subsequent growth of crystals in the oil phase (Co & Marangoni, 2012; Dassanayake, Kodali, & Ueno, 2011). Another classification differentiates between polymeric and low-molecular weight organogelators. Among others, TAGs, DAGs, MAGs, fatty acids, fatty alcohols, waxes, wax esters and sorbitan monostearate, have been identified as low molecular weight organogelators. Phytosterol-based organogelators have interesting possibilities, since besides their good structuring properties, they can also help to lower blood cholesterol having been used in the formulation of margarines (Duffy *et al.*, 2009). Polymeric organogelators show the greatest potential for food applications as many are food grade and inexpensive compared with the former. Among them, ethylcellulose shows particularly interesting potential (Co & Marangoni, 2012; Stortz *et al.*, 2012; Zetzl *et al.*, 2012).

Oleogel preparation methods have a significant effect on final gel properties. In general, they are formed in one single step by combining organogelators and edible oils at specific thermal (high temperature) and shearing conditions, which vary depending on the type of organogelators. Alternatively, oleogels can be formed via drying of oil-in-water emulsions. Therefore, this indirect approach includes a two-step process that firstly, involves the fabrication of a concentrated oil-in-water emulsion stabilized by a combination of water soluble food polymers, followed by the complete removal of the water phase to drive the structure formation where oil droplets are tightly packed in the

formed network. Oleogel is obtained during emulsion drying and results in the formation of the oil-rich powder (Co & Marangoni, 2012; Patel, Cludts, *et al*., 2014). In general, to effectively induce gelation of the oil and the organogelators, the oil must be heated to melt the gelator (≈140 °C in the case of ethylcellulose). In this regard it is important to consider susceptibility to oxidation of the unsaturated lipid source to be structured.

Organogelation is one of the most promising techniques to give liquid oils solid-fat functionality and has been widely used in the pharmaceutical and cosmetics industries over the last two decades with notable developments as drug delivery matrices (Hughes, Marangoni, Wright, Rogers, & Rush, 2009; Sagiri *et al*., 2014; Stortz *et al*., 2012). Additionally, organogels have a great many different potential functionalities in food products including restriction of oil mobility and migration, replacement of saturated and *trans* fats, stabilization of emulsions (including entrapping water droplets inside the oleogel network). Moreover, the development of such structures can also improve the formation of micelles during digestion thus improving the bioavailability of lipid soluble bioactive compounds (Stortz *et al*., 2012), and additionally attenuate postprandial fat levels in the blood (Marangoni *et al*., 2007). The formation of organogels based on edible lipid materials has been thoroughly reviewed on the basis of their design, formation, structure and final properties (Co & Marangoni, 2012; Dassanayake *et al*., 2011; Hughes *et al*., 2009). However, despite numerous promising applications, including molecular gastronomy (Rogers *et al*., 2014), there are few real food applications and therefore there is only limited knowledge of the behavior of oleogels in the development of new food products such as cookies, creams, chocolate and meat-based foods (Hwang, Singh, Winkler-Moser, Bakota, & Liu, 2014; Stortz *et al*., 2012; Yılmaz & Öğütcü, 2014; Zulim Botega, Marangoni, Smith, & Goff, 2013).

As for meat products, the use of oleogels is novel, very limited and focused to comminuted/chopped meat products (Table 1). Organogelation has been proposed by Lupi *et al*. (2012) as a suitable technique for the stabilization and control of texture in food suspension. The purpose was to improve the stability of heterogeneous meat/fat sauces by structuring the oil phase (mixtures of virgin olive and sunflower oils) with glycerol monostearate and monopalmitate (at 25 °C) or by a liquid oil/lecithin system. The authors concluded that organogelators can successfully be used to stabilize edible oil-based suspensions. Zetzl *et al*. (2012) reported the formation and use of an oleogel (canola oil and ethycellulose as gelator) as an animal fat replacer (100%) in comminuted frankfurters (25% fat), in order to substantially reduce the amount of saturated fat. A comparison with frankfurters made with un-gelled canola oil showed that these were much harder and chewier than those made with beef fat. However, when the canola oil was added as an oleogel in the formulation of these frankfurters, neither hardness nor chewiness were significantly different from those containing beef fat. This textural improvement over products made with un-gelled oil appears to be caused by the increased size of the fat/oil globules in the cooked meat batter. Wood (2013) studied the effectiveness of saturated fat replacement in all-beef frankfurters (26% fat) and pork breakfast sausages (20.8% fat) by means of a canola oil oleogel formed by using ethylcellulose as the gelator and sorbitan monostearate as a plasticizer. This organogel technology shows promising results in overcoming the textural and sensory issues in these meat products associated with the simple replacement of saturated fat with vegetable oils. It was concluded that oleogels can produce a frankfurter with a healthier lipid profile, similar processing characteristics, economic feasibility and acceptable sensorial qualities. However, since the target temperature for gels was 140 °C, excessive oxidation of the oil was occurring and sensory scores showed that rancidity continued to be one of the major flavor detractors. The inclusion of antioxidants (butylated hydroxytoluene and rosemary oleoresin) to the oleogels was able to lower the rancid and chemical off flavors to an acceptable level.

To the best of our knowledge, there are no applications other than fresh breakfast sausages and cooked frankfurters as concerns the development of meat products. However, the specific design of oleogels opens up a promising area of research for the formulation and development of a wide range of meat products (e.g. dry fermented sausages, patties, etc.).

Oil bulking systems

An alternative way to stabilize and structure lipid material is the dispersion of a large number of oil droplet particles in a continuous aqueous gel matrix (oil bulking system). In this case, the liquid oil is physically trapped in a hydrogel network structure (O + W) (Fig. 1), which provides a solid structure to the system making it apt for use as a fat analog. The technology behind the formation of these fat analogs is relatively simple and inexpensive. The edible oil is first homogenized/dispersed in the aqueous phase and then the gelation of the aqueous phase is induced by the gelling agent. There are a great number of hydrocolloids that can be used as gelators, either individually or in combination, to create a variety of gel structures. These structures may be suitable for immobilizing oil droplets thus acting as oil bulking agents. Konjac glucomannan and alginate are representative examples of this approach in the development of healthy meat products (Table 1).

Konjac glucomannan (KGM) is a neutral polysaccharide produced from the tuber of *Amorphophallus konjac* C. Koch. It has been widely used in food industry because of its good swelling, gelling and other features. When konjac flour is dissolved in alkaline coagulant (such as calcium hydroxide, sodium or potassium carbonate), deacetylation occurs and a thermally stable gel is formed (Lin & Huang, 2003). Konjac gel properties (color, mechanical/rheological behavior and thermal properties) have been

compared to those of different animal fat types used in the production of meat derivatives (Jimenez-Colmenero *et al*., 2012), as well as their use as fat analogs (animal fat replacement) in the formulation of various types of reduced/low-fat meat products (Lin & Huang, 2003; Ruiz-Capillas, Triki, Herrero, Rodriguez-Salas, & Jimenez-Colmenero, 2012). In this regard, olive oil and a combination of olive, linseed and fish oils (20% w/w) were stabilized in a konjac matrix (konjac-based oil bulking system, Fig. 2) and were used as pork backfat replacers to reduce fat content and/or improve fatty acid profile in different meat products such as frankfurters (Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2013), fresh sausage (Triki, Herrero, Jimenez-Colmenero, & Ruiz-Capillas, 2013), dry sausage (Jimenez-Colmenero, Triki, Herrero, Rodríguez-Salas, & Ruiz-Capillas, 2013) and pork patties (Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Carballo, & Jimenez-Colmenero, 2015; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2014).

Alginate forms gels in the presence of calcium salts. This is consequence of the cross-linking of alginate with calcium to form a three-dimensional macromolecular network containing a large fraction of water within the structure while exhibiting mechanical rigidity. This particular network offers interesting possibilities as an oil bulking agent. Herrero, Carmona, Jiménez-Colmenero, and Ruiz-Capillas (2014) reported the development of two different olive oil bulking systems (Fig. 3) based on polysaccharide gels (alginate/inulin and alginate/dextrin), in order to obtain optimal characteristics for use as animal fat replacers in the development of healthier meat products. This was suggested as a feasible strategy to produce olive oil bulking agents based on polysaccharide gel matrices with desirable nutritional and technological properties that make them suitable for use as fat replacers. Studies have been conducted on the technological and structural effects of incorporating these alginate-based oil bulking systems (55% olive oil) as animal fat replacers in meat matrices. In

Fig. 2. Konjac-based oil bulking system containing 20% (w/w) of a combination of olive, linseed and fish oils.

Fig. 3. Alginate-based oil bulking system containing 55% (w/w) of olive oil and prepared with: a) alginate/inulin; b) alginate/dextrin.

meat batters, the replacement of pork backfat with oil bulking agents produces an increase in β-sheet structures accompanied by enhanced water and fat binding properties and stronger textural characteristics, specifically hardness and chewiness (Ruiz-Capillas, Carmona, Jimenez-Colmenero, & Herrero, 2013). The technological and structural characteristics of frankfurters containing olive oil bulking systems based on polysaccharide gels as fat replacers were also examined during chilled storage (Herrero, Ruiz-Capillas, Jimenez-Colmenero, & Carmona, 2014). These authors reported that the new products are appropriately stable in terms of fat and water binding properties during storage. These reformulation processes affect the textural properties of the final product (higher hardness and chewiness values) irrespective of fat content. In addition, in these meat products, reformulation influences the structures of proteins and lipids. The relationship between structural and technological properties of the reformulated meat product could be decisive to understand the implications of both protein and lipid interactions on specific technological properties such as texture, which are very important for the consumers acceptance.

Structured emulsions

Emulsions consist of at least two immiscible phases (usually oil and water), with one phase being dispersed in the other as tiny droplets. Conventional emulsions are usually classified according to the arrangement of the two immiscible liquids as either oil-in-water (O/W) or water-in-oil (W/O) systems. Emulsions exhibit a wide variety of different rheological behaviors depending on their composition, structure, and droplet interactions (McClements, 1999). However, emulsions are generally prone to physical instability (coalescence, phase separation, etc.) and are not able to provide a solid-like texture unless droplet concentration results in a closely packed emulsion (McClements, 2010; McClements, Decker, & Weiss, 2007). This limitation in food applications has led to the development of more complex structured emulsions with novel functional properties and many industrial applications (Dickinson,

2012; McClements, 2012). These structural design approaches have been grouped into three main categories: *layering*, *embedding* and *clustering* (McClements, 2012), whose characteristics are mainly determined by composition, preparation conditions and especially by their structural organization. The structural design that seems to guarantee an easy and successful modification of the oil to provide a fat analog is *embedding*. However, different structural designs can be conveniently combined to structure liquid oils into solid-like plastic materials which can be used as food ingredients, particularly useful for the development of healthier meat products. Some of the more interesting possibilities for this type of application, structured by hydrogel or oleogel approaches, are considered below.

Hydrogelled emulsions (emulsion gels)

The functional performance of emulsions can also be controlled by embedding the emulsified (W/O or O/W) droplets within a continuous hydrogel matrix resulting in the formation of hydrogelled emulsions. It is therefore a complex colloidal material where emulsion and gel structures co-exist. These structured emulsions are produced using a two-step procedure. Typically, the initial stage in emulsion gel formulation involves producing a protein-stabilized liquid emulsion. A solid-like emulsion gel may be generated from a stable liquid-like emulsion by gelling (using thermal, enzymatic or chemical means) the continuous phase and/or aggregating the emulsion droplets. The solid-like rheological properties of emulsion gel are mostly determined by the network properties of the hydrogel matrix (Dickinson, 2012). Different polymers (protein, polysaccharides and their combination with other ingredients) can be used to promote hydrogel formation. The gelation process depends, to a great extent, on the nature of the system. For instance, numerous proteins (from milk, soy, egg) have been used in protein-stabilized emulsion gels, being heat treatment, acidification, and enzyme treatment (transglutaminase) the main protein gelation methods (Dickinson, 2012). Salts (calcium) addition is also a common way to promote protein gelation in cold-set processes. Examples of polysaccharide-stabilized emulsion gels are those prepared with alginate (gelation produced by the addition of calcium ions), carrageenan and inulin (Paradiso *et al*., 2015). Depending on the emulsion type (W/O or O/W), two different possible stabilization and structuring techniques must be considered (Fig. 1).

O/W emulsion hydrogels. Although O/W emulsions have been widely used as non-meat ingredient to provide healthy lipids in the development of healthier meat products, most of these applications fail to mimic the rheological and textural characteristics of the replaced animal fats (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jimenez-Colmenero, 2010; Jimenez-Colmenero, 2007). Their stabilization and structuring in hydrogel, however, offer new opportunities. While there are many studies dealing with the formation and characterization of these new systems, their application in foods is quite limited despite their proven utility in a wide range of food products (yoghurt, cheese, sauces), including meat products (sausages, patés, etc.) (Dickinson, 2012). Some preliminary considerations need to borne in mind when describing this technology in meat-based foods. It is well known that meat emulsions (batters) are heterogeneous composite materials composed of fat droplets dispersed in an aqueous phase of aggregated biopolymer molecules, where the salt-soluble meat protein acts as a hydrophilic emulsifier (coating the fat globules). Structural reinforcement is produced by heat-induced formation and physical entrapment within a continuous meat protein gel matrix, forming the final emulsion gel network. Incentive to develop novel meat emulsion gels based on emulsified oils (prepared in advance) containing a healthy combination of fatty acids is an important consumer-driven trend encouraging further research in this area.

In this regard, most meat product reformulation studies employ O/W emulsions prepared with a non-meat origin emulsifier (mainly sodium caseinate and soy protein, hereafter referred as SC and SP, respectively) whereas a wide variety of oils (from plant and marine origin) have been used as partial substitutes for fats of animal origin (Jimenez-Colmenero, 2007). In general, these O/W emulsions, made prior to meat product manufacture, are not hydrogelled emulsions since the necessary step to form solid-like emulsion gel is only produced during heating (thermal gelling) of the meat matrix where it has been added. There are reports of examples including the formation of emulsion gels prepared with alginate and/or gelatin aimed at formulating emulsion hydrogels (Dickinson, 2012; Sato, Moraes, & Cunha, 2014). In real food applications, different emulsion gels were prepared with linseed oil (polysorbate 80 as the hydrophobic emulsifier) and k-carrageenan (both phases at 70 °C), which polymerized at room temperature. The optimum emulsion gel formulation (maximizing the hardness and minimizing the syneresis) was applied as a partial fat replacer in a Bologna-type sausage, proving to be a suitable lipophilic delivery system for n-3 PUFA compounds and applicable in food formulations as a fat replacer (Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasarán, 2014). In addition, various O/W emulsions containing a mixture of vegetable (olive and linseed) and fish oils and stabilized by protein systems containing SC, SP, microbial transglutaminase (MTG) and meat protein were studied (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010). The authors reported that MTG plays an important role in the protein matrix by inducing stiff, elastic structures through a cold-set gelling process. In fact, MTG is known to covalently bind different protein residues thus enabling the formation of relatively thermostable gels. A complex particle-gel network is formed under those emulsion conditions

(Fig. 1) which favors a number of characteristics that resemble those of solid fat. Lipid and protein structural characteristics of olive oil-in-water emulsion gel formulated with MTG, SC and SP (Herrero, Carmona, Pintado, Jimenez-Colmenero, & Ruíz-Capillas, 2011a) or MTG and SC (Herrero, Carmona, Pintado, Jimenez-Colmenero, & Ruíz-Capillas, 2011b) have been reported, revealing that the enzymatic action of MTG induced the formation of lipid protein interactions and the formation of a gel structure in the emulsion. These types of materials have been used as animal fat replacers in frankfurters with improved fat content (Carmona, Ruiz-Capillas, Jiménez-Colmenero, Pintado, & Herrero, 2011; Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010; Herrero, Carmona, Pintado, Jimenez-Colmenero, & Ruiz-Capillas, 2012; Jimenez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010). However, further work is needed to improve the solid structure for use in fresh and dry meat products.

Recently, emulsion gels formulated with olive oil and chia products as hydrophilic emulsifiers and MTG, alginate or gelatin as cold gelling agents have been developed and characterized at structural and physico-chemical level (Pintado, Ruiz-Capillas, Jimenez-Colmenero, Carmona, & Herrero, 2015). Due to their convenient technological characteristics and their nutritional properties (thanks to the olive oil and chia), these emulsion gels have been used as animal fat replacers in the formulation of healthier frankfurters (Pintado *et al.*, in press; Pintado *et al.*, 2015). The incorporation of these emulsion gels improved the fat content of frankfurters (low level of SFAs and high levels of MUFAs and n-3 PUFAs), exhibiting acceptable technological properties and sensory attributes. Another potential alternative approach is the development oil-filled hydrogel particles (Chung, Degner, Decker, & McClements, 2013). Fish oil encapsulated in filled hydrogel particles (O/W/W) emulsion has been prepared with an O/W emulsion (fish oil/SC) mixed with two biopolymer phases, and used to enrich meat systems with n-3 LCPUFAs (Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Carballo, 2015; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Matalanis, *et al.*, 2013; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Matalanis, *et al.*, 2015). The filled hydrogel particles were oxidatively more stable than conventional emulsions and bulk oil when added to meat systems. New developments are needed to give these systems the composition and solid-like properties to make them more apt for use in meat products as animal fat replacers.

W/O emulsion hydrogels. The functional performance of W/O emulsions can be controlled by embedding the droplets in larger particles comprised of a different material. The properties of the new material depend on preparation conditions and the ingredients used. Double emulsions (DEs) offer interesting possibilities for structuring emulsions which can be used to create novel functional attributes (McClements, 2012). DEs are multi-compartmentalized systems in which oil-in-water (O/W) and water-in-oil (W/O) co-exist and where the globules of the dispersed phase themselves contain even smaller dispersed droplets (Garti, 1997). The most common forms are water-in-oil-in-water (W/O/W), but oil-in-water-in-oil (O/W/O) emulsions can also be used in specific applications. Multiple emulsions offer numerous food applications, including their use as food ingredients to modify qualitative and quantitative aspects of the lipid material in foods (including meat-based products). Because of their properties, multiple emulsions can be used to improve food lipid content through two main approaches: by reducing the fat content and by providing healthy fatty acid profiles. Multiple emulsions are normally produced using a two-step procedure. Typically, a water-in-oil emulsion (W/O) is formed by homogenizing an aqueous phase (W) and a lipid phase (O) in the presence of a lipophilic emulsifying agent. In the second stage, the W/O emulsion is homogenized with a new aqueous phase (W) with the help of a hydrophilic emulsifying agent thus producing a double emulsion (W/O/W).

When certain structural properties are needed, multiple emulsions can be designed for the intended food. In this regard, aspects relating to the characteristics of the different emulsifiers as well as the oil and water phases of W/O/W emulsions are the main composition factors for the purpose of stabilizing and structuring. To increase the viscosity of these structures, macromolecular substances such as sugars, protein and polysaccharides are incorporated into the inner aqueous phase (Dickinson, 2011; Su, Flanagan, Hemar, & Singh, 2006). When biopolymers that are readily converted to the gel state (e.g. by thermal processing) are present, the inner emulsion droplets are converted into soft solid-like particles (Dickinson, 2011). Furthermore, several approaches have been proposed to modify lipid phase characteristics for structuring purposes (Garti, 1997; Kukizaki & Goto, 2007; Weiss, Scherze, & Muschiolik, 2005). However, the most promising procedure to obtain a solid material is intervention on the outer aqueous phase. The strategies reported in the formation of hydrogels are also valid in this case. Various soluble polysaccharides (pectin, alginate, gellan gum, etc.) were added in the outer aqueous phase of the DEs to act as thickening and gelling agents (Dickinson, 2011; Hattrem, Dille, Seternes, & Draget, 2014).

To date, there has been very little use of DEs in food applications including as fat replacers (food ingredients) in meat reformulation processes (Jimenez-Colmenero, 2013). Cofrades, Antoniou, Solas, Herrero, and Jiménez-Colmenero (2013) were the first to improve fat content (in quantitative and qualitative terms) by replacing pork backfat in a meat system with W/O/W prepared with olive oil. Consequently, these authors confirmed the feasibility of using double emulsion (although in liquid form) as a technological strategy for the development of healthier meat

products. Later on, the effect on frankfurter properties of replacing pork backfat with two different W/O/W emulsions prepared using perilla oil and pork backfat as lipid phases was studied by Freire, Bou, Cofrades, Solas, and Jimenez-Colmenero (2015). This study confirms the technological viability of these approaches, showing that major fat reduction (over 60%) can be achieved, and that products with perilla oil may qualify for labeling with specific nutritional and health claims. In any case, new preparation of DEs is required with rheological characteristics similar to those of the animal fat being replaced. When DEs are not formed due to the homogenization conditions and/or the absence of a hydrophilic emulsifier, alternatively the W/O emulsion can be embedded in the hydrogel matrix (W/O + W) (Fig. 1). Structurally it would resemble that of an oil-bulking system but using W/O emulsion instead of oil.

Organogelled emulsions

The functional performance of emulsions can also be controlled by embedding the emulsified (W/O or O/W) droplets in a continuous organogel matrix. Hence, organogelled W/O and O/W emulsions can be produced by means of this approach (Fig. 1).

Organogelation of W/O emulsions. The previously reported organogelation process may also occur at the lipid (outer) phase of W/O emulsion. Due to the amphiphilic nature of some low molecular weight organogelator molecules, it has been proposed that they may be able to simultaneously stabilize and provide structure for W/O emulsions. Therefore, it is possible to immobilize water droplets within a continuous gelled oil phase (Hughes *et al.*, 2009). Two main steps may be required to produce this structured lipid (W/O + Org, Fig. 1); one related to the combination of edible oil and the organogelator and the other related to their homogenization (as melted oleogels) with an aqueous phase (Patel, Cludts, *et al.*, 2014). In these systems featuring different phases, different stabilization and structuring mechanisms such as lipid crystallization (fat) of the continuous lipid phase and stabilization from the Pickering effect at the interfacial level are very likely to occur at the same time. In this regard, a highly structured W/O emulsion network was obtained by Lupi, Gabriele, de Cindio, Sánchez, and Gallegos (2011) through oil phase crystallization by organogelator agents (MAG and DAG). These emulsions exhibited suitable rheological properties for their potential use as solid fats. An organogelled emulsion was obtained by homogenizing melted organogel (β-sitosterol:γ-oryzanol in olive, corn and sunflower oils at 90 °C) with the aqueous phase (Duffy *et al.*, 2009). Patel, Rajarethinem, *et al.* (2014) identified shellac (a food-grade resin) as a new structuring agent capable of gelling edible oil. This organogelator can be used to prepare structured W/O emulsions (spreads) which then can be used as a stabilizer in emulsifier-free spread formulation, a replacer for oil binders in chocolate pastes and a structurant for shortening alternatives in cake preparation. Organogelled emulsions have been developed with candelilla wax, safflower oil, and MAG (Toro-Vazquez *et al.*, 2013). The emulsions were prepared by solubilizing candelilla wax and MAG in the oil (at 90 °C) and this was combined with water at 70 °C which, thereafter was emulsified with a high pressure homogenizer. Although no applications have been described in meat products, the design of specific organogelled emulsions could be used in the development of healthier meat products.

Organogelation of O/W emulsions. Organogelation processes can be also used to prepare healthier structured DEs (O/W/O) using convenient organic phases and emulsifiers. Solid O/W/O emulsions can be obtained (using similar methodology as previously reported for DE preparation) using an outer organic phase (oil, and if appropriate an organogelator) to meet the technological and sensory requirements to simulate a meat fat replacer, whereas the inner lipid phase can be used to deliver fatty acids that are more in line with health recommendations (Fig. 1). Jahaniaval, Kakuda, and Abraham (2003) reported a method to disperse an O/W emulsion in plastic fat and stabilize the resulting O/W/O emulsion. When DEs are not formed due to the homogenization conditions and/or the absence of a lipophilic emulsifier, alternatively the W/O emulsion can be embedded in the oleogel matrix (W/O + Org) (Fig. 1).

Interesting applications, including the use of O/W/O emulsions and the formation of solid lipid nanoparticles consisting of a solid shell and liquid oil core in O/W emulsions to microencapsulate and prevent omega-3 fatty acid oxidation, were recently reported (Liao, Luo, Zhao, & Wang, 2012; Salminen, Aulbach, Leuenberger, Tedeschi, & Weiss, 2014; Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013). However, to the best of our knowledge, there are no studies using this technology to create a solid-fat for use in the food industry. However, this type of emulsion may be further transformed into a more solid system by other means (e.g. gelation) that have been reviewed here and which are in line with those described in certain pharmaceutical applications (Souto, Wissing, Barbosa, & Muller, 2004).

Further considerations and conclusions

There is growing interest in healthier foods which depend on technology for their production. The idea of improving the lipid component (reduce fat content and provide better fatty acid profile) by reformulating traditional foods is challenging not only technologically but also from a consumer acceptance point of view. Given that meat and meat products are some of the most important sources of dietary fat, new approaches for the stabilization

and structuring of edible oil to use as animal fat alternatives for the development of healthier lipid meat products is especially interesting. Novel structuring materials can be developed to wholly or partially replace traditional structurants. These strategies include interesterification and organogelation, the creation of structured emulsions (hydrogelled and organogelled emulsions) and the formation of oil bulking systems. There are numerous studies, mostly focusing on their characterization. However, a multi-disciplinary approach is required to consider the different essential aspects necessary for their real use as ingredients in food applications, as in the case of meat products. The first step is to determine the characteristics of the novel material in order to achieve the appearance and the technological, rheological and sensorial properties required for use as raw materials (ingredients) to replace animal fats. Structured lipids are added to meat products as alternatives to animal fat, and therefore their composition and physicochemical characteristics need to be considered since they affect the quality of the reformulated products. A deeper understanding of structured lipid characteristics will therefore facilitate their use, help to elucidate their role in the protein matrix structure and help to improve the quality of the healthy meat-based food systems to which they are added (as fat analogs). It is also important to gain a thorough understanding of the behavior of structured lipids during processing, aspects which must be considered when designing a delivery system for a particular food application. In addition to these important aspects, it is essential to understand their role as part of the food product. As structural and functional components, lipids are involved in most food properties and have a decisive effect on meat product quality even at low concentrations. It is therefore essential to assess the consequences of adding these elements to new foods, bearing in mind that the latter differ in terms of their composition, structural properties (degree of disintegration, emulsion, gels, etc.), processing conditions (heating, drying, smoking, etc.) and storage conditions (chilling, freezing, etc.). Their suitability depends on how well they are able to mimic the characteristic requisites of the fat type (ground/comminuted to a desired particle size depending on the type of meat product) in the meat matrix, including color, texture and rheological behavior. Therefore, in order to broaden application possibilities, different aspects related to their composition, preparation, structural organization, properties of lipid structure designed for specific food uses and their application in real food need to be studied.

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