TESIS DOCTORAL

Desarrollo y optimización de microcápsulas de ácidos grasos poliinsaturados mediante spray-drying. Efecto de su incorporación sobre las características de calidad de precocinados cárnicos

Development and optimization of polyunsaturated fatty acid microcapsules by spray-drying. Effect of their incorporation on the quality characteristics of precooked meat products

Estefanía Jiménez Martín

Departamento de Producción Animal y Ciencia de los Alimentos

2016

UNIVERSIDAD DE EXTREMADURA

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Los Dres. Mª Teresa Antequera Rojas, Jorge Ruíz Carrascal y Mª Trinidad Pérez Palacios expresan su conformidad para tramitación de la presente Tesis Doctoral.

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2016

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JUNTA DE EXTREMADURA
Consejería de Economía e Infraestructuras

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El trabajo expuesto en esta Tesis Doctoral ha sido subvencionado por la Junta de Extremadura-Consejería de Economía e Infraestructuras y el Fondo Europeo de Desarrollo Regional (FEDER) mediante la concesión de ayudas económicas a grupos de investigación. Referencia: GR15113.

Estefanía Jiménez Martín ha disfrutado de una beca de Formación de Personal Investigador (FPI) concedida por el Gobierno de Extremadura para la realización de la tesis doctoral en el departamento de Producción Animal y Ciencia de los Alimentos, Área de Tecnología de los Alimentos de la Facultad de Veterinaria de Cáceres, de la Universidad de Extremadura. Referencia: PD10026.

Resumen/Summary

RESUMEN

El objetivo de esta tesis doctoral fue i) optimizar la microencapsulación de aceite de pescado mediante spray-drying a partir de emulsiones monocapa, multicapa y múltiples como una estrategia ventajosa para obtener una fuente rica en AGPI ω-3 y estable desde el punto de vista oxidativo, ii) desarrollar nuggets de pollo enriquecidos con las microcápsulas de aceite de pescado optimizadas y iii) comprender el efecto del enriquecimiento sobre las características de calidad de los nuggets.

Aunque todos los tipos de microcápsulas preparadas son alternativas posibles a la adición directa de aceite de pescado como método de enriquecimiento de ácidos grasos ω-3, las microcápsulas preparadas con emulsiones multicapa de lecitina-quitosano (1% w/w quitosano) y maltodextrina son más adecuadas en cuanto a cantidad de aceite encapsulado, microestructura y protección de ácidos grasos frente a la oxidación, especialmente ácido eicosapentaenoico (C20:5 ω-3, EPA) y ácido docosahexaenoico (C22:6 ω-3, DHA), en las condiciones ensayadas (almacenamiento a temperatura ambiente, 30 ºC y 60 ºC). Por ello, estas microcápsulas fueron seleccionadas para ser usadas como fuente de AGPI ω-3 para el enriquecimiento de nuggets de pollo.

Se prepararon 3 lotes de nuggets: control (C), enriquecidos mediante adición directa de aceite de pescado (BFO) y enriquecidos mediante adición de microcápsulas de aceite de pescado (MFO), observándose la influencia del tipo de enriquecimiento en los parámetros físico-químicos y sensoriales de los nuggets, así como en los cambios que ocurren durante la fritura y el almacenamiento a congelación durante 1 y 3 meses, especialmente la oxidación lipídica y la composición en ácidos grasos.

Los nuggets de pollo enriquecidos con microcápsulas presentaron un mayor contenido de EPA y DHA y menores niveles de oxidación lipídica en comparación con los nuggets enriquecidos mediante adición directa de aceite de pescado. Sin embargo, el tipo de enriquecimiento no influyó de forma notable sobre las características sensoriales ni la aceptabilidad general.

Las pérdidas de EPA y DHA y las reacciones de oxidación que tienen lugar durante la fritura de los nuggets fueron inferiores en los nuggets enriquecidos con

microcápsulas. También se observó que el almacenamiento a congelación favorece la oxidación lipídica, y que ésta aumenta cuando se adiciona aceite de pescado a los nuggets.

La microencapsulación es una técnica efectiva de protección de AGPI ω-3 frente a la oxidación, pudiendo ser utilizadas las microcápsulas de aceite de pescado como una forma estable de almacenamiento de estos AGPI ω-3 previamente al enriquecimiento de alimentos. Así mismo, la adición de microcápsulas de aceite de pescado, obtenidas a partir de emulsiones multicapa, es una estrategia adecuada para el enriquecimiento de productos cárnicos pre-fritos congelados, permitiendo que estos productos conserven sus características de calidad y protegiendo a los AGPI ω-3 durante los procesos de pre-fritura, almacenamiento a congelación durante 3 meses y fritura final previa al consumo.

SUMMARY

The aim of this PhD Thesis was i) to optimize the microencapsulation of fish oil by spray-drying of monolayer, multilayer and multiple emulsions, as an advantageous strategy to obtain a rich source of oil high ω-3 PUFA which is stable from the oxidative point of view, ii) to develop chicken nuggets enriched with the optimized fish oil microcapsules and iii) to understand the consequences of such enrichment on the quality characteristics of nuggets.

While all types of developed microcapsules within this work appeared as feasible alternatives to the direct addition of fish oil as a method of enriching in ω-3 fatty acids. However, the microcapsules produced from multi-layered emulsions of lecithin-chitosan (1% w/w chitosan) and maltodextrin were more appropriate in terms of quantity of encapsulated oil, microstructure and protection of fatty acids against oxidation, especially eicosapentaenoic acid (C20: 5 ω-3, EPA) and docosahexaenoic acid (C22: 6 ω-3, DHA), under the conditions tested (storage at ambient temperatures, 30 ° C and 60 ° C). Therefore, these microcapsules were selected as a source of ω-3 PUFA enrichment for chicken nuggets.

In order to address the effect of adding ω-3 rich microcapsules, 3 batches of nuggets were prepared: control (C), enriched by direct addition of fish oil (BFO) and enriched with addition of microcapsules of fish oil (MFO). This allowed to discern the potential influence of the type of enrichment on physicochemical and sensory parameters of the nuggets, as well as on the changes that take place during frying and frozen storage for 1 and 3 months, especially those related to lipid oxidation and fatty acid composition.

Chicken nuggets enriched with microcapsules had higher content of EPA and DHA and lower levels of lipid oxidation compared to nuggets enriched by direct addition of fish oil and their sensory characteristics were not affected. On the other hand, direct addition of fish oil affected sensory attributes of the chicken nuggets. However, type of enrichment did not influenced overall acceptability. Losses of EPA and DHA and oxidative reactions that occur during frying of the nuggets were lower on nuggets enriched with microcapsules as compared to those enriched in pure oil. A promoting effect of frozen storage on lipid oxidation was detected, which was more intense in nuggets containing fish oil.

Microencapsulation appears as an effective technique to protect ω-3 PUFA against oxidation, so that microcapsules of fish oil can be used as a method for store of such PUFAs ω-3 prior to food enrichment. In addition to that, the addition of fish oil microcapsules is an appropriate strategy for the enrichment of pre-fried frozen meat products, allowing these products to preserve their quality characteristics and protecting ω-3 PUFAs during the processes of pre-frying, frozen storage for 3 months and final frying previous to consumption.

Agradecimientos

Quisiera agradecer en unas líneas a todas aquellas personas que han tomado parte de forma directa o indirecta en que esta Tesis Doctoral se lleve a cabo.

Al Gobierno de Extremadura y Fondos FEDER por la beca pre-doctoral de personal investigador FPI que disfruté durante el desarrollo de la presente tesis y por las ayudas a grupos con la que se financiaron parte de los aparatos y material utilizados en este trabajo y que así mismo me permitió la participación en congresos nacionales e internacionales y la realización de una estancia de investigación.

A Biomega Natural Nutrients, y en especial a Virginia Villaverde, por proporcionarnos desinteresadamente el aceite de pescado utilizado como fuente de omega-3. A Trades S.A. por proporcionarnos el quitosano y a Roquette-Laisa por proporcionarnos el Glucidex 12, ambos utilizados durante los experimentos. A Ana Mayoral por su valiosa ayuda con la microscopía de las emulsiones.

A los directores y a todos los profesores y compañeros del Área de Tecnología de los Alimentos y de Higiene y Bromatología de los Alimentos con los que he coincidido durante la realización de mi tesis, a los que estuvieron y los que están, por los momentos de trabajo y las experiencias compartidas.

En especial a mis directores, Teresa Antequera, Jorge Ruiz y Trinidad Pérez, por el asesoramiento, paciencia y ánimos infundidos a lo largo de estos años.

A los Prof. Adem Gharsallaoui y Pascal Degraeve, gracias por permitirme realizar una estancia de investigación predoctoral en el laboratorio Biodymia (Bioingénierie et Dynamique Microbienne Aux Interfaces Alimentaires) de la Universidad de Lyon 1, y a todo el personal de dicho departamento y compañeros durante mi estancia por la acogida y la ayuda.

(À Prof. Adem Gharsallaoui et à Prof. Pascal Degraeve, je vous remercie pour permettre un séjour de recherche pré-doctorale dans le laboratoire Biodymia (Bioingénierie et Dynamique Microbienne Aux Interfaces Alimentaires) de l'Université de Lyon 1, et à tout le personnel du laboratoire et des partenaires pendant mon séjour pour leur accueil et leur aide).

A mis padres y hermanos, porque sin ellos no estaría donde estoy, por su cariño incondicional, por compartir mis alegrías y soportar mis agobios e inquietudes, por ayudarme con sus consejos aunque sepan que al final no siempre acabo

siguiéndolos, por entenderme y quererme como soy. A la familia de Álvaro, por su apoyo y por sus palabras de ánimo. A mis amigos, Lidia y Sergio, por los momentos que hemos vivido y porque sean muchos más.

A Álvaro, por lo que eres para mí, mi todo, mi carga positiva, mi contrapeso para alcanzar el equilibrio, mi dosis de cordura y serenidad, porque esta Tesis sin tu ayuda no sólo no habría llegado a materializarse, si no que no tendría sentido. Porque mi esfuerzo y trabajo tienen como único incentivo y recompensa una vida compartida contigo.

1. Introducción

1. ÁCIDOS GRASOS OMEGA-3 (ω-3)

1.1. Efectos beneficiosos y enriquecimiento

Los ácidos grasos omega-3 (ω-3) son ácidos grasos poliinsaturados (AGPI) con dos o más dobles enlaces en su cadena, teniendo en común que presentan un doble enlace en el tercer carbono contando desde el grupo metilo terminal. Los ácidos grasos ω-3 más importantes son el ácido α-linolénico (ALA, C18:3 ω-3), ácido eicosapentaenoico (EPA, 20:5 ω-3) y ácido docosahexaenoico (DHA, 22:6 ω-3) (Figura 1).

Figura 1. Estructura de los principales ácidos grasos ω-3.*

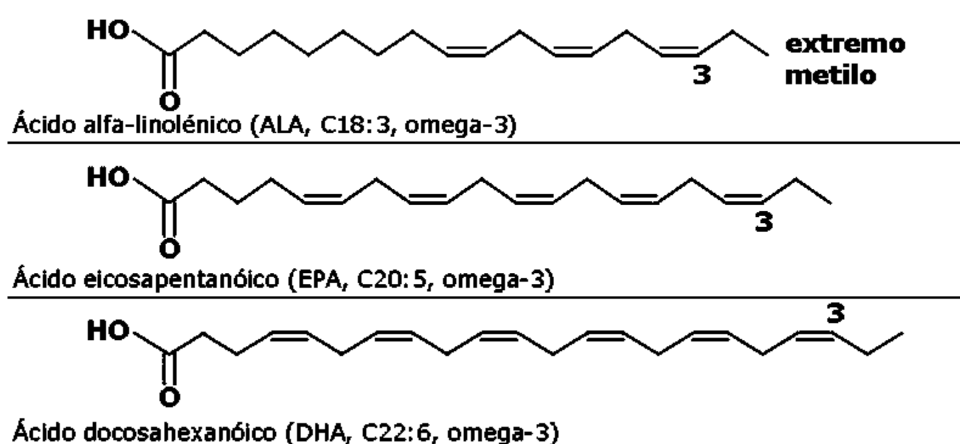

*Imagen extraída de la página web del Consejo Europeo de Información sobre la Alimentación (EUFIC) (http://www.eufic.org/).

Los AGPI ω-3 de cadena larga ácido eicosapentaenoico (EPA, 20:5 ω-3) y ácido docosahexaenoico (DHA, 22:6 ω-3) tienen propiedades bioactivas y efectos beneficiosos para la salud humana [1]. Algunas de las ventajas que se han relacionado con su consumo incluyen la mejora del desarrollo neurológico en edades tempranas, la disminución del riesgo de enfermedades cardiovasculares (mediante la reducción de la agregación plaquetaria, hipertensión e hiperlipidemia), o efectos positivos sobre enfermedades del sistema inmune, enfermedades neurodegenerativas (como el Alzheimer) y varios tipos de tumores [2].

El EPA y DHA pueden sintetizarse en el organismo a partir de otro ácido graso ω-3, el ácido alfa-linolénico (ALA, 18:3 ω-3). Sin embargo, ALA es un ácido graso esencial, y como tal no puede ser sintetizado por el organismo humano, siendo sólo posible su obtención a partir de la dieta. Esto se debe a que el organismo humano carece de las enzimas 12-desaturasa y 15-desaturasa, encargadas de la síntesis de ALA a partir del ácido esteárico. La conversión de ALA en EPA y DHA es limitada, por lo que se recomienda incluir en la dieta alimentos que contengan directamente estos PUFA. La importancia del aumento de la ingesta de EPA y DHA ha sido reconocida por varios organismos de salud en todo el mundo [3]. Como consecuencia, se ha producido un mayor interés de los consumidores por aumentar la ingesta de estos ácidos grasos ω-3 [4]. La Comisión Europea considera a partir del Reglamento 432/2012 de 16 de mayo de 2012 que para obtener un beneficio de la ingesta de EPA y DHA habría que hacer un consumo diario como mínimo de 250 miligramos de estos AGPI ω-3.

Las principales fuentes de ALA son aceites de semillas, principalmente de chía, perilla, semilla de lino (linaza) y canola (aceite de colza). No obstante, en la mayoría de estas semillas (excepto en el caso de las de lino y chía) y sus aceites, la proporción de ácido linoleico (C18:2 ω-6) es elevada, y por lo tanto el ratio ω-6/ω-3 no es el más adecuado. Por ello, la ingestión de fuentes directas de EPA y DHA supone una ventaja frente a la ingestión de fuentes de ALA.

La principal fuente de EPA y DHA en la dieta es el aceite de origen animal de ambiente marino. Sin embargo, como consecuencia del estilo de vida actual y las tendencias en los hábitos de consumo, la ingesta de alimentos con alto contenido de EPA y DHA, como el salmón, la sardina, el atún o la caballa no es suficiente para alcanzar la dosis diaria de EPA y DHA recomendada [5]. Por esta razón, hay un interés creciente en el desarrollo de alimentos funcionales y suplementos como fuente de EPA y DHA, siendo Australia y España los países más involucrados [6].

Se han realizado algunos estudios aplicando distintas fuentes de ácidos grasos ω-3 para mejorar el perfil lipídico de varios alimentos con el objetivo de obtener productos más saludables [7]. En este aspecto, existen numerosos estudios en la bibliografía científica tratando la fortificación de alimentos con AGPI ω-3 y los efectos beneficiosos de su consumo a largo plazo [8].

1.2. Métodos de enriquecimiento de alimentos en ácidos grasos ω-3

El Reglamento (UE) Nº 116/2010 DE LA COMISIÓN de 9 de febrero de 2010 por el que se modifica el Reglamento (CE) N 1924/2006 del Parlamento Europeo y del Consejo en lo relativo a la lista de declaraciones nutricionales, indica que:

- "Solamente podrá declararse que un alimento es fuente de ácidos grasos omega-3 o efectuarse cualquier otra declaración que pueda tener el mismo significado para el consumidor, si el producto contiene al menos 0,3 g de ácido alfa-linolénico por 100 g y por 100 kcal, o al menos 40 mg de la suma de ácido eicosapentaenoico y ácido docosahexaenoico por 100 g y por 100 kcal."

- "Solamente podrá declararse que un alimento tiene un alto contenido de ácidos grasos omega-3 o efectuarse cualquier otra declaración que pueda tener el mismo significado para el consumidor, si el producto contiene al menos 0,6 g de ácido alfa-linolénico por 100 g y por 100 kcal, o al menos 80 mg de la suma de ácido eicosapentaenoico y ácido docosahexaenoico por 100 g y por 100 kcal."

Los procedimientos utilizados para incorporar AGPI ω-3 a los alimentos varían desde la suplementación a través de la alimentación animal en el caso de alimentos como carne o productos cárnicos y huevos, hasta la adición directa de vegetales y aceites líquidos a distintos alimentos o la incorporación de estos últimos en forma de pre-emulsión o microencapsulados.

En cuanto a la suplementación a través de la alimentación animal, se han utilizado forrajes, semillas y aceites tanto ricos en ALA como en EPA y DHA [9]. En cuanto a la suplementación de los piensos con ALA, se han utilizado como fuentes de este ácido graso aceites de linaza o de colza, para aumentar la biosíntesis de EPA y DHA en el animal y de esta forma obtener carnes enriquecidas. Sin embargo, la biosíntesis de estos ácidos grasos a partir del ALA es limitada y poco efectiva no sólo en el organismo humano, como ya se ha mencionado previamente, si no también en la mayoría de especies animales utilizadas en la industria cárnica [10]. Debido a esto, en otros estudios se han añadido a los piensos aceites ricos en EPA

y DHA como aceite de pescado y extractos de algas [11]. No obstante, la cantidad de estos aceites que se puede añadir a la dieta animal es muy limitada, ya que un incremento de estos AGPI en las canales da lugar a modificaciones negativas, dando lugar al desarrollo de flavores y aromas “a rancio” y “a pescado”, así como efectos negativos en la textura, produciendo un incremento de la oleosidad (“carnes aceitosas”). Por lo tanto, estas carnes serían inadecuadas para ser utilizadas como materias primas de productos procesados.

En cuanto a la adición directa para enriquecer alimentos con ácidos grasos ω-3, se han utilizado principalmente fuentes como nueces, aceites vegetales ricos en ALA y aceites de pescado o algas ricos en EPA y DHA [12]. Igualmente, los alimentos obtenidos son más susceptibles a la oxidación, pudiendo desarrollarse aromas desagradables que producirían un rechazo por parte de los consumidores [13], incluso con cantidades moderadas de aceite. Park et al. (1989) observaron que el reemplazo de grasa por una cantidad de un 5 % de aceite de pescado en salchichas tipo Frankfurt producía puntuaciones muy bajas en los análisis sensoriales, debido a la aparición de un aroma indeseable a pescado [14].

Más recientemente, se han utilizado aceites pre-emulsionados. La pre-emulsificación es una posible opción tecnológica para estabilizar los aceites con el objetivo de ser utilizados para reemplazar parte de la grasa original de los alimentos procesados, en lugar de añadir el aceite como una cantidad de grasa adicional. Poyato et al. [15] han optimizado una emulsión gelificada de aceite de lino en agua para ser usada como sustituta de grasa en la formulación de salchichas enriquecidas con AGPI ω-3. Salcedo-Sandoval et al. [16] utilizaron una mezcla de aceites de oliva, de lino y de pescado estabilizados en una emulsión de aceite en agua como sustituta de grasa en salchichas enriquecidas en ω-3. No obstante, a pesar de la emulsificación, en la mayoría de los casos se detectó la necesidad de estabilización adicional del producto frente a procesos oxidativos [17].

De hecho, un prerrequisito para el desarrollo exitoso de alimentos con alto contenido en AGPI ω-3 es la prevención de la oxidación lipídica o al menos retrasarla lo máximo posible. En el caso particular de alimentos enriquecidos en AGPI ω-3, es necesario tener en cuenta no sólo los procesos de oxidación que se producen en la propia fuente de estos ácidos grasos (principalmente aceite de

pescado) durante el almacenamiento previo al enriquecimiento, sino también los procesos de oxidación de estos ácidos grasos una vez añadidos al alimento enriquecido, que pueden inducir la oxidación de los lípidos del propio alimento enriquecido.

Como consecuencia de los procesos de oxidación en el producto enriquecido, puede producirse una disminución de los AGPI ω-3 y su capacidad funcional debido a su degradación, siendo además algunos de los compuestos de degradación nocivos para la salud [18, 19]. La ruptura de la cadena de los ácidos grasos ω-3 durante la oxidación implica la pérdida de valor nutricional y un deterioro de la calidad, dando lugar a compuestos de oxidación con aromas desagradables y otras alteraciones sensoriales, entre las que se encuentran olores “a rancio” y “a pescado” [4, 20], poniendo en riesgo la aceptabilidad por parte de los consumidores de estos productos.

Este hecho determina que el principal reto en cuanto a la producción de alimentos enriquecidos con AGPI ω-3 sea la prevención de la oxidación lipídica [21]. La forma en la que se añade la fuente de estos compuestos bioactivos es un factor clave, ya que puede influir en el desarrollo de las reacciones de oxidación y sus consecuencias adversas [22].

Existen varias estrategias para prevenir las reacciones de oxidación lipídica de los AGPI ω-3. Entre ellas, el método más común es el uso de antioxidantes naturales o sintéticos. Sin embargo, su principal inconveniente es el diferente comportamiento que pueden mostrar cuando interactúan con los distintos componentes de la matriz alimentaria [23]. De hecho, el mismo antioxidante puede mostrar distinta eficacia dependiendo de la matriz alimentaria a la que es añadido, o tener efecto protector frente a la oxidación en algunos alimentos y efecto prooxidante en otros, haciendo necesario la verificación de la eficacia de un antioxidante concreto para la prevención de la oxidación en cada matriz alimentaria [24]. En el caso de alimentos enriquecidos en ω-3, los mecanismos de actuación de los antioxidantes son complejos, por tratarse de sistemas alimentarios multifase.

Otra estrategia posible para proteger los ácidos grasos ω-3 frente a la oxidación es la microencapsulación de éstos, limitando así el contacto del sustrato fácilmente

oxidable con el agua, O_2, Fe^{3+} y otros catalizadores de la oxidación, envolviendo los ingredientes funcionales en una matriz que actúa como estructura protectora [25].

2. MICROENCAPSULACIÓN

2.1. Generalidades

La microencapsulación puede definirse como un proceso para recubrir una sustancia (sustancia activa o núcleo) con , cubierta, membrana, pared o matriz (generalmente un material polimérico), produciendo partículas que en función de su morfología y estructura interna se denominan "microcápsulas", "microesferas" o "micropartículas" cuando su tamaño es micrométrico y "nanocápsulas", "nanoesferas" o "nanopartículas" cuando su tamaño es nanométrico. De esta forma, una microcápsula consiste en una partícula de tamaño micrométrico con una estructura de membrana o pared semipermeable, esférica y sólida alrededor de un núcleo o sustancia encapsulada que puede ser sólido o líquido. [26, 27]. Esta tecnología se utiliza ampliamente en la industria alimentaria [28].

La protección de los AGPI ω-3 frente a la oxidación mediante la técnica de microencapsulación se basa en obtener un producto (microcápsulas) con un aspecto macroscópico en forma de polvo. Esto se consigue mediante el recubrimiento de pequeñas partículas de aceite rico en AGPI ω-3 con una matriz que tiene función de pared y que puede variar desde hidratos de carbono a fosfolípidos y proteínas o ser una combinación de estos componentes.

La aplicación del método de microencapsulación permite la producción de un producto en polvo, más fácil de manejar y almacenar que el producto sin encapsular. Además, la microencapsulación proporciona una barrera física entre el compuesto microencapsulado y el ambiente, evitando o reduciendo el contacto y la reactividad con factores ambientales promotores de la oxidación, tales como la luz, la temperatura y los iones metálicos [29]. La microencapsulación de ácidos grasos ω-3 con una matriz protectora produce una barrera que retrasa la autooxidación, ya que la movilidad de los radicales libres en las microcápsulas está limitada por el material de pared. Esto podría disminuir la propagación de las reacciones de oxidación en los productos enriquecidos. Además, la difusión del oxígeno es más

lenta en la matriz deshidratada del material que actúa como matriz de las microcápsulas que en el propio aceite si se utiliza como fuente de enriquecimiento [30]. En consecuencia, la microencapsulación ha demostrado ser un buen método para la estabilización y conservación de compuestos bioactivos.

Al mismo tiempo, la microencapsulación puede utilizarse para conseguir objetivos que no pueden alcanzarse con el simple uso de antioxidantes, tales como como facilitar la inclusión de los compuestos en la matriz del alimento, evitando efectos negativos sobre el perfil sensorial del producto, la liberación controlada de compuestos bioactivos, y la mejora de la absorción de los compuestos microencapsulados, debido a la liberación específica y controlada en el tracto gastrointestinal de los consumidores [1, 31].

En el caso de los productos enriquecidos con AGPI ω-3, el incremento en la retención de compuestos volátiles de aceite de pescado en las microcápsulas podría prevenir el efecto negativo en el perfil sensorial que de otro modo podría producirse si el producto se enriquece mediante la adición directa de aceite de pescado.

Varios investigadores han estudiado la producción de partículas sólidas mediante la tecnología de microencapsulación como estrategia potencial para evitar la degradación de aceite de pescado como fuente de AGPI ω-3 [4, 32]. La degradación de los ácidos grasos puede reducirse mediante la encapsulación de emulsiones de aceites ricos en ω-3 (aceite en agua), que dan lugar a un producto en forma de polvo en el que los AGPI ω-3 se encuentran protegidos por el material de pared [33].

La viabilidad del proceso de microencapsulación está determinada por la eficiencia de la microencapsulación y la estabilidad de los ácidos grasos ω-3 en las microcápsulas durante el proceso y el almacenamiento, y éstas dependen del tipo y composición de las emulsiones utilizadas previamente a la encapsulación [34]. Por lo tanto, además del método de microencapsulación y las condiciones de almacenamiento, las características del aceite rico en AGPI ω-3 y el material de la matriz de las microcápsulas, así como su ratio y el tipo de emulsión son factores

importantes que influyen en las características y la estabilidad de las microcápsulas de AGPI ω-3 [30].

2.2. Microencapsulación mediante spray drying

La microencapsulación de ácidos grasos ω-3 u otros compuestos bioactivos puede realizarse utilizando varios métodos. Existen numerosas técnicas para la producción de microcápsulas, siendo las más utilizadas “spray-cooling”, “spray-chilling”, extrusión, y freeze-drying entre otras [26].

Una de las técnicas de microencapsulación de ingredientes alimentarios más popular en cuanto a su uso industrial es la atomización mediante secado por aspersión o “spray-drying”. Esto se debe, en parte, a las ventajas de este método, que incluyen entre otras la gran variedad de materiales que pueden ser utilizados como pared, la simplicidad de la técnica y el coste relativamente bajo del equipo, que hace que sea la opción más sencilla y económica de producir microcápsulas con fines alimentarios [26].

El proceso general de spray-drying se encuentra esquematizado en la Figura 2. Implica en primer lugar la preparación de una emulsión o dispersión donde se encuentra el material a encapsular y el material de pared. Esta mezcla es aspirada hasta llegar al atomizador donde se produce la atomización/pulverización de la mezcla y la deshidratación de las gotas atomizadas, produciendo las microcápsulas, que son separadas mediante un ciclón y recogidas en un recipiente o vaso colector.

La rápida evaporación de la fase acuosa de la emulsión se produce mediante el aporte de aire a temperaturas elevadas (160-210ºC). No obstante, el impacto en la estabilidad de los ácidos grasos parece ser bajo, ya que las gotas se exponen apenas unos segundos a estas temperaturas y, además, mientras el agua de la emulsión se evapora, las partículas se enfrían, encontrándose su temperatura superficial en el rango de los 50-60ºC [35].

Figura 2. Principio de funcionamiento del método de spray-drying (Spray Dryer Buchi B-290).

El tamaño de las microcápsulas producidas mediante spray drying es muy variable y depende de la concentración de sólidos en la emulsión, del tamaño de las gotas de la fase dispersa en la emulsión y de las condiciones de atomización seleccionadas, pudiendo encontrarse microcápsulas en el rango de 10-50 µm las más pequeñas y 2-3 mm las de mayor tamaño [26].

2.3. Materiales para microencapsulación

El proceso para la producción de emulsiones es simple (homogeneización y mezclado). No obstante, la preparación de las emulsiones y su formulación debe optimizarse para evitar la desestabilización mediante sedimentación, coalescencia o floculación. De esta forma, la cantidad de aceite en la emulsión está limitada por estos procesos de desestabilización.

La microencapsulación puede considerarse realizada con éxito cuando se obtiene una elevada retención del material a encapsular. Es decir, en el caso de aceite rico en AGPI ω-3, se puede considerar que la microencapsulación se ha realizado con éxito cuando la mayoría del aceite se encuentra encapsulado, con una baja cantidad de aceite en la superficie del material en formato de polvo que constituyen las microcápsulas [36]. Este objetivo puede ser alcanzado mediante el uso de materiales con propiedades adecuadas, optimizando la formulación y la preparación de las emulsiones y ajustando las condiciones del proceso de spray-drying [26].

La selección de los emulsificantes y el material de pared de las emulsiones a partir de las cuales se producirán las microcápsulas representa un paso muy importante durante la optimización del procedimiento de microencapsulación mediante spray-drying, ya que es el factor principal que influye en la eficiencia del proceso y la estabilidad del producto obtenido [37]. Los emulsificantes más comunes utilizados en la industria alimentaria son proteínas, polisacáridos, fosfolípidos y pequeñas moléculas que actúan como surfactantes, variando las propiedades emulsificantes de los mismos, así como la estabilidad de las emulsiones producidas.

En cuanto a materiales de pared para la microencapsulación, los polisacáridos y las proteínas son los más utilizados, seguidos por lípidos y ceras, así como combinaciones de todos ellos. Es importante obtener un balance entre el aspecto económico, facilidad de uso y propiedades de encapsulación. Los materiales elegidos como encapsulantes deben reunir una serie de requisitos, como tener la capacidad para formar films, ser flexibles, no ser higroscópicos y no tener olor o aroma que puedan modificar las características del producto al que se añadan las microcápsulas. La solubilidad en medio acuoso o en solventes, así como la

capacidad para tener un cambio de fase, como fusión o gelificación, son a veces deseables, dependiendo del objetivo que se pretenda conseguir con la microencapsulación del ingrediente cuando se incorpora al alimento.

El material de encapsulación también puede modificarse mediante procesos físicos o químicos para conseguir la funcionalidad necesaria de matriz de microencapsulación. La elección del material encapsulante depende por tanto de numerosos factores, incluyendo sus propiedades físicas y químicas, su compatibilidad con el alimento que se va a enriquecer y la influencia en las propiedades sensoriales del producto final [27].

Los carbohidratos han sido ampliamente utilizados en la microencapsulación de compuestos bioactivos, en especial el almidón y sus derivados [38]. Las ciclodextrinas y sus complejos se han utilizado también para la encapsulación molecular de compuestos bioactivos de naturaleza lipofílica, pese a que su coste es elevado [39]. La maltodextrina es un polisacárido que actúa como matriz de relleno, relativamente económica, con elevada solubilidad en agua y capacidad para formar emulsiones estables [40]. Se ha comprobado que proporciona buena estabilidad a los aceites encapsulados, así como protección frente a la oxidación [41]. Otro polisacárido muy utilizado por su excelente capacidad para formar films es el quitosano [42]. Se trata de un polisacárido lineal, que se clasifica como un polielectrolito de carga catiónica, que no se encuentra cargado permanentemente, ya que su carga depende del pH del medio. Debido a que su valor pKa es aproximadamente 6.5, el quitosano se encuentra cargado positivamente y es soluble sólo en medios ácidos-neutros.

Las proteínas se utilizan como encapsulantes, sobre todo aquellas que presentan excelente solubilidad en agua, capacidad de formar geles y films y propiedades emulsificantes [43]. La microcápsulas realizadas con proteínas pueden ser fácilmente rehidratadas o solubilizadas en agua, lo que a menudo da lugar a la liberación inmediata del material encapsulado. Por ello, normalmente se combinan las proteínas con carbohidratos para la microencapsulación de aceites y compuestos bioactivos solubles en aceite. Se ha observado que en la elaboración de aceites microencapsulados, la eficiencia de la microencapsulación era mayor cuando la matriz de la encapsulación era una mezcla de proteínas lácteas y

carbohidratos, en comparación con el uso solamente de proteínas [44]. Entre las proteínas comúnmente utilizadas para la microencapsulación se encuentran las proteínas lácteas, como las caseínas, que son extremadamente estables frente al calor, no produciéndose su coagulación. De los productos obtenidos, el caseinato sódico es la caseína soluble en agua más utilizada [42].

Los lípidos se utilizan generalmente como una cubierta secundaria aplicada a las microcápsulas o a materiales bioactivos en forma de polvo para mejorar sus propiedades de barrera frente a la humedad o son incorporados a la formulación de la emulsión para formar una matriz o capa alrededor del compuesto bioactivo a microencapsular [45]. Normalmente se adicionan lípidos polares a las emulsiones que darán lugar a las microcápsulas, tales como la lecitina o los ésteres de ácidos grasos, que actúan como emulsificantes. Estos emulsificantes pueden ser usados solos o en combinación e integrarse a la formulación como agentes utilizados para la preparación de las microcápsulas o como estabilizantes de la dispersión durante su almacenamiento o aplicación.

2.4. Emulsiones para microencapsulación

La microencapsulación de AGPI ω-3 mediante spray-drying comienza con la producción de una emulsión de aceite en agua ("feed emulsion"). La fase de aceite consiste en un aceite rico en AGPI ω-3, como el aceite de pescado, y el material de pared se disuelve en la fase acuosa. La producción de la emulsión implica un proceso de homogeneización, que permite a los emulsificantes adsorber las gotas de aceite, reduciendo la tensión interfacial y produciendo una capa protectora que evita la agregación de las gotas. Este método simple da lugar a emulsiones tradicionales, emulsiones simples o "monocapa" de aceite-en-agua (O/W) o agua-en-aceite (W/O) (Figura 2). Sin embargo, como alternativa a estas emulsiones, se han desarrollado algunos procedimientos especiales de preparación de emulsiones previamente a la microencapsulación para incrementar la protección de los ácidos grasos ω-3 en las microcápsulas. Ejemplos de este tipo especial de emulsiones son las emulsiones múltiples (como las emulsiones dobles) y las emulsiones multicapa [46, 47].

2.4.1. Emulsiones dobles

Las emulsiones dobles se producen cuando una emulsión simple se homogeneiza para formar gotas dentro de una fase de hidrofobicidad/hidrofilicidad opuesta que se añade para formar la fase continua de la emulsión simple original (y de forma similar a la fase continua de la emulsión simple original). De esta forma, se producen “gotas dentro de gotas”. Por ejemplo, en una emulsión doble de aceite-en agua-en aceite (“oil-in-water-in-oil” o “O-W-O”) hay gotas muy pequeñas de aceite dentro de gotas más grandes de agua que se encuentran rodeadas por una fase continua de aceite (Figura 3).

Figura 3. Emulsiones simples y dobles.*

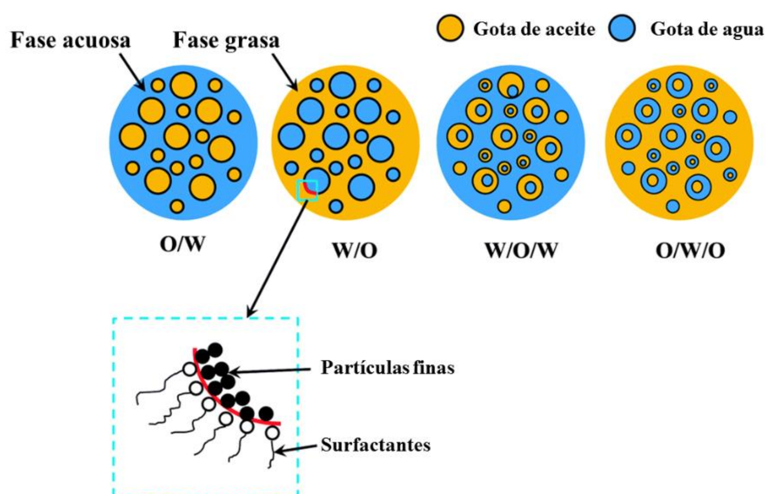

*Figura adaptada de He et al. [48].

La encapsulación de aceite mediante esta técnica se produce a partir de una emulsión de aceite-en agua-en aceite (O1/W/O2), en la que el aceite encapsulado constituye la fase de aceite interna (O1) y se añade otra fase de aceite de la misma o distinta naturaleza (O2) a la emulsión como fase externa protectora [49-51]. Finalmente se genera una emulsión O1/W/O2/W a partir de la emulsión que se somete al proceso de spray-drying. El proceso de fabricación de este tipo de emulsión requiere la combinación de dos tipos de emulsificantes: emulsificantes de naturaleza hidrofílica para la estabilización de la interfaz O1/W y O2/W y un emulsificante de naturaleza hidrofóbica para la estabilización de la interfaz W/O2 en la emulsión final.

2.4.2. Emulsiones multicapa

Las emulsiones multicapa pueden definirse como emulsiones en las que las gotas lipídicas están rodeadas por múltiples capas de material de cobertura, estando formadas estas capas por una combinación de emulsificante y uno o más biopolímeros o polielectrolitos de cargas opuestas. Esta técnica se denomina como capa a capa (LbL, del inglés “layer-by-layer”) y ha sido desarrollada recientemente, siendo aplicada con éxito a la microencapsulación de aceites ricos en AGPI ω-3 [32, 46, 52-54]. Las capas de emulsificantes y/o biopolímeros o polielectrolitos son depositadas y estabilizadas mediante cargas eléctricas, compensando cada capa la carga de la capa anterior y permitiendo la deposición de una capa adicional debido a un efecto de sobrecarga [47].

Figura 4. Producción de emulsiones multicapa.*

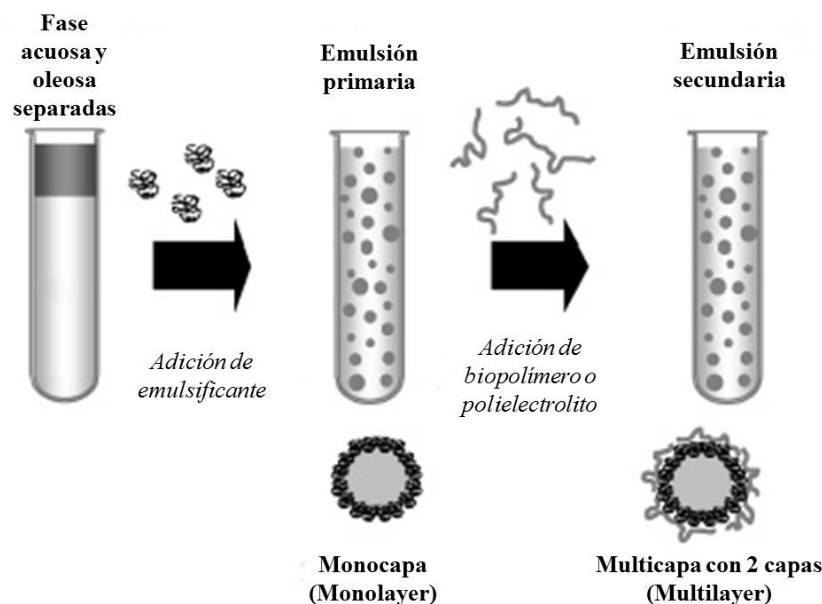

*Figura adaptada de Weiss et al. [55].

El método de producción de emulsiones multicapa (Figura 4) comienza produciendo una emulsión primaria, en la que las gotas de aceite se encuentran estabilizadas en la fase acuosa mediante un emulsificante iónico. Después, se produce una emulsión secundaria mediante la adición de un biopolímero o polielectrolito de carga opuesta. Debido a la atracción electrostática que rodea a las gotas de la emulsión previa, se obtiene un sistema en el que las gotas de aceite se encuentran rodeadas por dos capas. Mediante la repetición de este procedimiento pueden

obtenerse gotas rodeadas con más de dos capas. El proceso para la producción de la técnica LbL ofrece varias características ventajosas: mejora la estabilidad física y química de los componentes microencapsulados y permite la liberación específica y controlada de los compuestos bioactivos.

3. MICROENCAPSULACIÓN DE AGPI ω-3 MEDIANTE SPRAY-DRYING

Varios investigadores han utilizado la microencapsulación mediante spray-drying para producir microcápsulas de aceites ricos en AGPI ω-3 a partir de distintos tipos de emulsiones compuestas por materiales como proteínas, carbohidratos y lípidos, con formulaciones específicas para obtener características óptimas [34], con el objetivo de prevenir los problemas derivados de la oxidación de los AGPI ω-3 [26, 56]. Algunos autores han indicado que la combinación de distintos materiales, que actúan como emulsificantes y como matriz, es el procedimiento óptimo para emulsificar aceite de pescado como paso previo a la microencapsulación [57].

Diversos estudios han investigado la microencapsulación aceite de pescado utilizando emulsiones multicapa mediante la técnica LbL, y la combinación lecitina-quitosano con distintos carbohidratos como material de recubrimiento [32, 47, 52-54]. En estos estudios, se observó que las emulsiones multicapa de aceite de pescado preparadas utilizando lecitina-quitosano tenían mayor estabilidad que el aceite de pescado frente a factores de estrés ambiental, tales como tratamiento térmico (30-90 ºC durante 30 min), ciclo de congelación, descongelación (−18 °C for 22 h/30 °C durante 2 h), altos contenidos de sodio (200 mM NaCl) y liofilización, todos ellos factores que pueden darse durante el procesado de alimentos. Se observó que las microcápsulas obtenidas a partir de estas emulsiones multicapa de aceite de pescado eran sistemas estables y con buenas propiedades físico-químicas y de estabilidad oxidativa en comparación con el aceite de pescado, por lo que se sugirió que podían ser un buen sistema de enriquecimiento en ω-3. Esto da lugar a expectativas para probar diferentes tipos de materiales de encapsulación y distintas formulaciones de emulsiones multicapa para producir las correspondientes microcápsulas.

Sin embargo los datos sobre microencapsulación a partir de emulsiones múltiples son escasos en la bibliografía científica. Hasta la fecha, sólo el trabajo de Liao et

al. (2012) [58] ha abordado la microencapsulación de aceite de pescado a partir de emulsiones múltiples (O/W/O). Estos autores estudiaron la microencapsulación de aceite de pescado a partir de una emulsión doble producida con gluten de trigo deaminado con ácido succínico y consiguiente polimerización. En este estudio se observó que en la evaluación de la digestión in vitro con simulación de fluidos gástricos como en la estabilidad durante el almacenamiento, la matriz protegía al aceite tanto del oxígeno como de los fluidos gástricos, con una mejora en la estabilidad y las propiedades de liberación del mismo. Por lo tanto, este sistema de emulsión puede ser utilizado para encapsular aceite de pescado con fines de enriquecimiento y obtención de alimentos funcionales.

4. ALIMENTOS ENRIQUECIDOS CON MICROCÁPSULAS DE AGPI ω-3

El enriquecimiento de alimentos mediante la adición directa de aceite de pescado ha sido ampliamente estudiado [17]. Sin embargo, en cuanto a la microencapsulación de AGPI ω-3, la mayoría de los estudios se centran en el estudio de las características de las microcápsulas, mientras que el uso de estas microcápsulas para el enriquecimiento de alimentos con AGPI ω-3 ha sido poco estudiada. En la Tabla 2 se recogen las referencias de los artículos científicos que han realizado estudios de alimentos enriquecidos con microcápsulas ω-3. Si bien algunos autores han realizado microcápsulas utilizando ALA como fuente de ácidos grasos ω-3, la mayoría de los trabajos han realizado el enriquecimiento de los alimentos mediante aceite de pescado microencapsulado rico en EPA y DHA. En estos estudios, varían tanto la concentración del aceite en las microcápsulas, como los materiales utilizado para la microencapsulación y la cantidad de microcápsulas añadida a los alimentos. En cuanto a los alimentos enriquecidos, se encuentran tan sólo algunos ejemplos de productos de panadería [59-61], bebidas [41, 62, 63] y productos lácteos [64-66].

En lo que se refiere a productos cárnicos sólo se han encontrado en la bibliografía dos referencias de alimentos enriquecidos con microcápsulas de AGPI ω-3, en ambos casos salchichas de cerdo. En el estudio de Josquin et al. (2012) se realizó un reemplazo de un 15 % y un 30 % de la grasa de las salchichas por aceite de pescado en forma de microcápsulas (12.80 % y 23.50 % de microcápsulas respectivamente) [67]. Por otro lado, en el estudio de Pelser et al. (2007) [68] se

estudiaron dos formulaciones con microcápsulas: una formulación con un reemplazo del 15 % de grasa de las salchichas por aceite pescado microencapsulado y una formulación con un reemplazo del 15 % de grasa de las salchichas de aceite de lino microencapsulado, suponiendo esto un 9 % de microcápsulas en las salchichas en ambos casos. En estos dos estudios se obtuvo una mejora en la composición de ácidos grasos de los productos cárnicos, con una disminución del índice ω-6/ω-3, y menores valores de oxidación que en los productos enriquecidos con aceite sin microencapsular. Estos resultados son prometedores, ya que indican la posibilidad de enriquecer un tipo de producto muy consumido, como los productos cárnicos, utilizando la microencapsulación para proteger los ácidos grasos frente a la oxidación. De hecho, en el momento de realizar la elección del tipo de alimento para ser enriquecido con ingredientes funcionales es necesario considerar las demandas y requerimientos del mercado. El estilo de vida actual, con una creciente falta de tiempo para el cocinado tradicional, está dando lugar al consumo generalizado de alimentos listos para el consumo o listos para calentar ("ready-to-eat"/"ready to heat"), tales como los productos empanados pre-fritos [69-71]. Los productos pre-fritos son alimentos de conveniencia sujetos a un proceso de cocinado incompleto (precocinado o pre-fritura) que a continuación se almacenan a temperaturas de congelación (alrededor de los -18 ºC), conservando propiedades similares a los productos frescos, con la mejora de la vida útil, que puede verse prolongada varios meses dependiendo del producto. Las ventajas de estos productos incluyen, entre otras, la posibilidad de revalorizar materias primas, como sub-productos o cortes cárnicos de bajo valor comercial [72], aumentando la variedad de productos ofrecidos y obteniendo alimentos con atributos sensoriales y propiedades tecnológicas ajustadas a las demandas de los consumidores [73]. Los productos empanados pre-fritos son muy aceptados, especialmente entre los jóvenes, debido a sus atributos sensoriales, y la rapidez y facilidad de preparación, por lo que su consumo ha aumentado en los últimos años [70]. Esta demanda creciente en los alimentos pre-fritos empanados hace que estos alimentos de conveniencia sean una elección interesante para el enriquecimiento en ω-3.

Tabla 2. Bibliografía de alimentos enriquecidos con microcápsulas de ácidos grasos omega-3 (ω-3).

Alimento enriquecido	Fuente de ω-3	Material de microencapsulación	ω-3 en las microcápsulas (%)	Microcápsulas en el alimento (%)	ω-3 en el alimento (mg/100 g)	Ref
Sopa en polvo	ALA	Goma arábiga y maltodextrina	35 % ALA	2.25 %	790**	[41]
Galletas	ALA	Concentrado de proteína de suero	8.12 % ALA	20.00 %	1620**	[61]
Pan	ALA	Almidón de maíz con alto contenido de amilosa	6.5 % ALA	1.00 % 2.50 % 5.00 % 10.00 %	70 160 330* 650**	[60]
Pan	ALA	Goma arábiga y lecitina de soja	12.5 % ALA	3.80 %	480*	[74]
Pan	EPA y DHA	Fórmula comercial (No especificado)	3.27% EPA 4.68% DHA	1.00 % 1.75 % 2.50 %	80** 140** 200**	[75]
Pan	EPA y DHA	Fórmula commercial (No especificado)	12.9 % EPA + DHA	5.00 %	650**	[76]
Alimento para bebés	EPA y DHA	Caseinato de sodio y lecitina	6.75 % EPA 2.15 % DHA	2.60 %	230**	[66]
Zumo de fruta	EPA y DHA	Caseinato de sodio y goma arábiga	2.05 % EPA 2.61 % DHA	0.92 % 1.15 % 1.38 %	40* 50* 60*	[63]
Zumo de naranja Yogur Barra de cereales	EPA y DHA	Caseinato de sodio,glucosa monohidrato y almidón de maíz con alto contenido de amilosa	8.75 % EPA 6.75 % DHA	2.00 % zumo de naranja 2.00 % yogur 6.70 % barra de cereales	Zumo de naranja: 310** Yogur: 310** Barra de cereales: 1040**	[77]
Yogur	EPA y DHA	Gelatina y goma de acacia	9 % EPA 6 % DHA	0.53 %	80**	[65]
Queso fresco Queso cheddar Mozzarella	EPA y DHA	Fórmula comercial (No especificado)	Fórmula comercial (No especificado)	No especificado	Queso fresco: 850** Cheddar: 870** Mozarella: 270**	[64]
Salchichas	EPA y DHA	Fórmula comercial (No especificado)	3.2 % EPA 2.5 % DHA	-12.80 % -23.50 %	-730** -1340**	[67]
Salchichas	-EPA y DHA -ALA	Fórmula comercial (Glucosa, caseinato, proteína de soja y lecitina)	-11.30 % EPA 11.30 % DHA -28.8 % ALA	9 %	-EPA: 743** DHA: no detectado -ALA: 4022**	[68]

ALA = Ácido alfa-linolénico; EPA = Ácido eicosapentaenoico; DHA = Ácido docosahexaenoico; * = Fuente de ácidos grasos omega-3 (al menos 300 mg 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, ** = Alto contenido de ácidos grasos omega-3 (al menos 600 mg 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 (REGLAMENTO (UE) Nº 116/2010 DE LA COMISIÓN EUROPEA de 9 de febrero de 2010 por el que se modifica el Reglamento (CE) no 1924/2006 del Parlamento Europeo y del Consejo en lo relativo a la lista de declaraciones nutricionales); Ref = Referencia

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2. Planteamiento y objetivos

PLANTEAMIENTO Y OBJETIVOS

El consumo de los AGPI ω-3 de cadena larga EPA y DHA se relaciona con efectos beneficiosos para la salud humana. Debido a esto, existe un interés creciente entre los consumidores en aumentar la ingesta de estos AGPI. No obstante, en las dietas actuales los alimentos ricos en EPA y DHA no se consumen en cantidad suficiente como para alcanzar la ingesta recomendada de estos compuestos bioactivos.

Por ello, se han desarrollado diversas estrategias de enriquecimiento de alimentos en EPA y DHA. Sin embargo, uno de los principales inconvenientes que tienen los AGPI es son especialmente susceptibles a la oxidación lipídica, por lo que es necesario protegerlos para evitar que se desarrollen estos procesos oxidativos, o al menos retrasar su aparición lo máximo posible para obtener alimentos enriquecidos con una vida útil adecuada. Así mismo, la principal fuente de EPA y DHA es el aceite de pescado, cuya cantidad a añadir se ve limitada por el rechazo que causa en los consumidores la detección de aromas a pescado o a rancio en los alimentos enriquecidos.

Una estrategia que se puede utilizar para resolver estos inconvenientes es la microencapsulación mediante spray-drying. Esta técnica ha demostrado su efectividad para la protección de AGPI ω-3 y se caracteriza por ser un procedimiento sencillo, versátil y económico. Es nuestra hipótesis que la microencapsulación es una estrategia adecuada para proteger a los AGPI w-3 de los procesos oxidativos durante el almacenamiento de los alimentos.

En relación con la microencapsulación de AGPI ω -3, la mayoría de los estudios se han centrado en el análisis de las características de las microcápsulas. Por el contrario, la información científica disponible sobre la aplicación de estas microcápsulas con fines de enriquecimiento es escasa, sin referentes en la literatura científica de productos cárnicos de empanados pre-fritos enriquecidos con microcápsulas de AGPI ω-3.

El enriquecimiento en AGPI ω-3 microencapsulados de los productos empanados pre-fritos afectará al valor nutricional y saludable. Sin embargo, también podría limitar el tiempo de almacenamiento y la vida útil de estos productos, debido a la oxidación que los AGPI ω-3 pueden sufrir durante el

proceso de elaboración y almacenamiento. Es importante tener en cuenta este aspecto en el caso de enriquecer en ácidos grasos ω-3 este tipo de productos.

Teniendo en cuenta los planteamientos mencionados anteriormente, los objetivos de esta tesis doctoral fueron los siguientes:

1) Optimizar la microencapsulación de aceite de pescado mediante spray-drying a partir de emulsiones monocapa, multicapa y múltiples como una estrategia ventajosa para obtener una fuente rica en AGPI ω-3 y estable desde el punto de vista oxidativo.

2) Conocer la influencia de la composición y estructura de las emulsiones monocapa, multicapa y múltiple, sobre las características fisicoquímicas y la estabilidad a la oxidación de dichas emulsiones y sus microcápsulas correspondientes, así como el efecto del almacenamiento a diferentes temperaturas.

3) Desarrollar un producto cárnico empanado pre-frito (nuggets de pollo) enriquecido con microcápsulas de aceite de pescado como fuente de ácidos grasos ω-3, y comprender el efecto del enriquecimiento sobre las características de calidad (parámetros físico-químicos, estabilidad oxidativa y propiedades sensoriales) en comparación con el enriquecimiento mediante la adición directa de aceite de pescado.

4) Establecer los cambios durante el proceso tecnológico (elaboración, pre-fritura, almacenamiento a congelación y fritura) de los nuggets de pollo enriquecidos con aceite de pescado microencapsulado en comparación con el enriquecimiento mediante la adición directa de aceite de pescado.

APPROACH AND OBJECTIVES

The intake of the long chain ω-3 PUFA, EPA and DHA, is related to beneficial effects for human health. Because of that, consumers have shown an growing interest in enhancing the intake of these PUFA. However, with the current dietary habits, the consumption of food rich in EPA and DHA is not enough to allow reaching the intake of these bioactive compounds required to obtain the potential beneficial effect.

Thus, different food enrichment strategies have been developed aiming to increase the content of EPA and DHA in diverse food. Nevertheless, one of the main drawbacks of PUFA is that they are highly susceptible to lipid oxidation. Thus, it is necessary to prevent these oxidation processes, or at least delay its onset as long as possible, to obtain enriched food with with proper shelf life. On top of that, the main source of EPA and DHA is fish oil, whose amount to be added is limited by the rejection that the detection of fishy or rancid odors in fortified foods causes on consumers.

A strategy that has been used to attempt solving these problems is microencapsulation of oil by spray-drying. This strategy methodology has proven its effectiveness in the protection of ω-3 PUFA and being a simple, versatile and relatively low cost process. It is our hypothesis that microencapsulation is an appropriate strategy to protect ω-3 PUFA oxidative processes during storage.

Regarding microencapsulation of ω-3 PUFA, most studies have focused on the analysis of the characteristics of the microcapsules. On the contrary, the available scientific information on the application of these microcapsules for enrichment purposes is scarce, with no references in the literature of pre-fried breaded meat products enriched with ω-3 PUFA microcapsules.

Enrichment of pre-fried breaded meat products with microencapsulated ω-3 PUFA will positively affect their nutritional and health value. However, it could also limit the storage time and shelf-life of these products due to the oxidation that ω-3 PUFAs might suffer during production and storage. It is important to consider this aspect in the case of the enrichment of such products with ω-3 fatty acids.

Considering the approaches mentioned above, the objectives of this thesis were:

1) Optimize the microencapsulation of fish oil by spray-drying of monolayer, multilayer and multiple emulsions as an advantageous strategy to obtain a rich source of ω-3 PUFAs that are stable in terms of oxidation.

2) To determine the influence of the composition and structure of the emulsions (either monolayer, multilayer or multiple) on the physicochemical characteristics and oxidative stability of these emulsions and their corresponding microcapsules, as well as the effect of storage at different temperatures.

3) Developing a pre-fried breaded meat product (chicken nuggets) enriched with microcapsules of fish oil as a source of ω-3 fatty acids, and evaluating the effect of enrichment on their quality characteristics (physico-chemical parameters, oxidative stability and sensory properties) compared to enrichment by direct addition of fish oil.

4) Establishing the changes during the technological process (manufacture, pre-frying, frying and frozen storage) of chicken nuggets enriched with microencapsulated fish oil compared to enrichment by direct addition of fish oil.

3. Diseño experimental

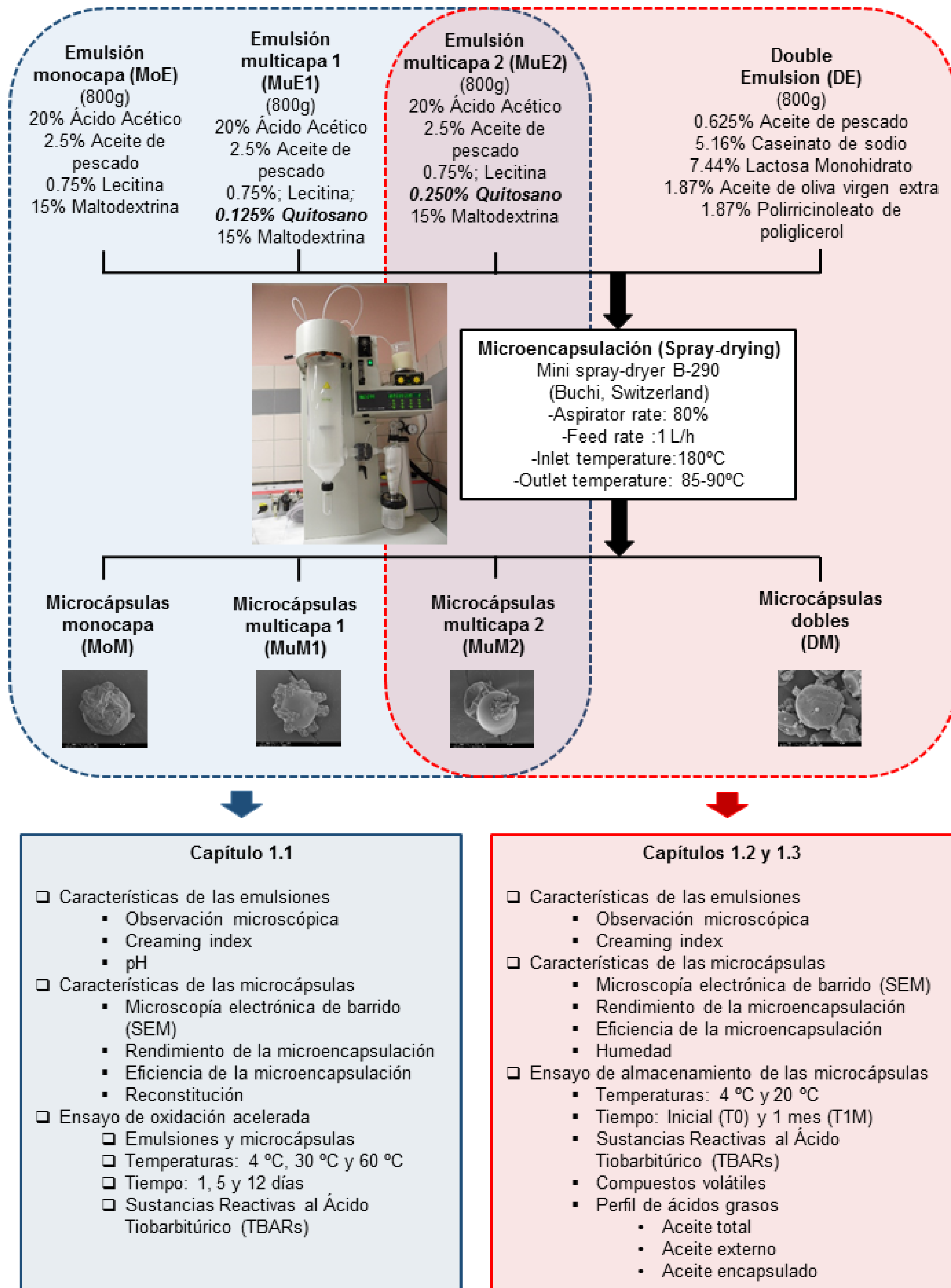
La presente Tesis Doctoral está dividida en dos partes bien diferenciadas, una primera en la que estudia la viabilidad de elaborar microcápsulas de aceite de pescado rico en ácidos grasos ω-3 mediante spray-drying a partir de diferentes tipos de emulsiones, y una segunda en la se evalúa la adición de dichas microcápsulas a un producto cárnico (nuggets de pollo).

SECCIÓN 1

En esta sección se incluyen los capítulos 1.1, 1.2 y 1.3 (Figura 1). En ellos se estudió la microencapsulación de aceite de pescado rico en ácidos grasos ω-3 mediante spray-drying a partir de emulsiones monocapa (MoE), multicapa (MuE1, y MuE2) y múltiples (dobles) (DE) de dicho aceite. El aceite de pescado que se utilizó para los experimentos fue proporcionado por Biomega Natural Nutrients S.L. (Galicia, España) y su formulación en ácidos grasos ω-3 fue aproximadamente de un 31.5 % (6.9 % EPA, 24.6 % DHA).

En el capítulo 1.1 se optimizó la formulación de emulsiones monocapa y multicapa de lecitina-quitosano y maltodextrina con diferente concentración de quitosano (0.125 % w/w vs. 0.250 % w/w en las emulsiones), y se elaboraron sus correspondientes microcápsulas (MoM, MuM1 y MuM2) mediante spray-drying. Se compararon la estabilidad de las emulsiones y las características microscópicas y físico-químicas de las microcápsulas. Así mismo, se evaluó la estabilidad oxidativa de las emulsiones y microcápsulas mediante un ensayo de oxidación acelerada a diferentes combinaciones de temperaturas (4, 30 y 60 ºC) y tiempos (1, 5 y 12 días). En los capítulos 1.2 y 1.3 se compararon emulsiones multicapa (lecitina-quitosano y maltodextrina con un 0.250 % w/w de quitosano) y emulsiones dobles (caseinato, lactosa, polirricinoleato de poliglicerol y aceite de oliva virgen extra) y sus correspondientes microcápsulas elaboradas mediante spray-drying (MuM2 y DM), evaluando la estabilidad de las emulsiones y sus características microscópicas, físico-químicas y la estabilidad oxidativa de las microcápsulas. En estos capítulos (1.2 y 1.3) las microcápsulas se sometieron a un ensayo de almacenamiento a temperatura ambiente (20ºC) y a temperatura de refrigeración (4ºC) durante 1 mes, analizando su estabilidad frente a la oxidación, el perfil de ácidos grasos y de compuestos volátiles de las microcápsulas.

Figura 1. Diseño experimental de la Sección 1.

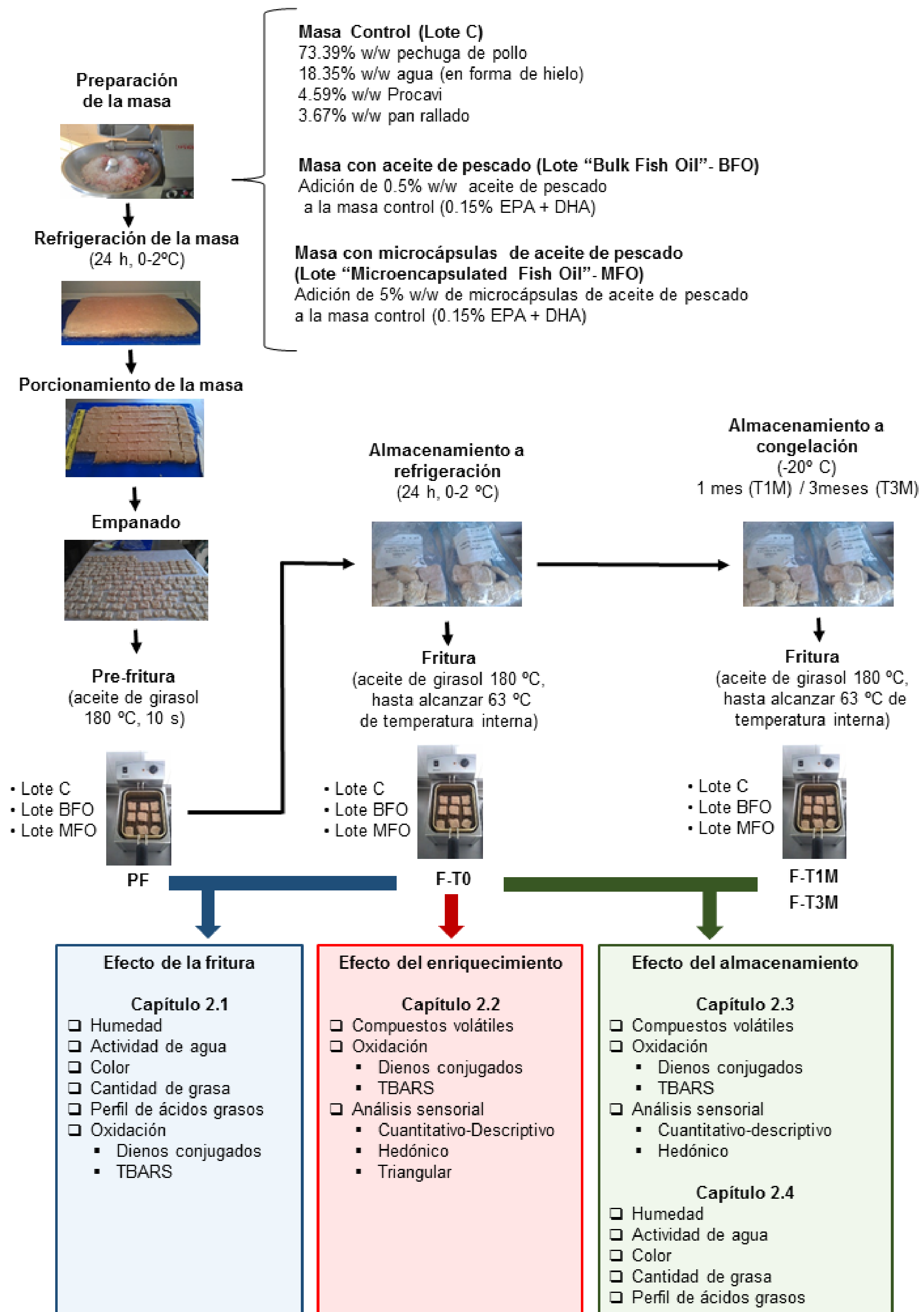


SECCIÓN 2

En esta sección se incluyen los trabajos relacionados con la adición del aceite de pescado rico en ácidos grasos ω-3 a nuggets de pollo, que se corresponden con los capítulos 2.1, 2.2, 2.3 y 2.4 (Figura 2) de esta tesis doctoral. Se elaboraron tres tipos de nuggets de pollo: un lote control (Lote C), un lote enriquecido con aceite de pescado sin encapsular (Lote "Bulk Fish Oil" o BFO) y un lote enriquecido con microcápsulas multicapa de aceite de pescado (Lote "Microencapsulated Fish Oil" o MFO). Para cada formulación se prepararon 3 masas.

La formulación y elaboración de los nuggets de pollo control se realizó siguiendo la metodología optimizada por Medina et al. (2014), utilizando pechuga de pollo como materia prima. Una vez elaborados, los nuggets se sometieron a un proceso de pre-fritura en aceite de girasol a 180 ºC durante 10 segundos, y se dejaron 10 minutos sobre papel absorbente para eliminar el exceso de aceite externo. Los nuggets pre-fritos se almacenaron en bolsas de plástico durante 24 horas a temperatura de refrigeración (0-2 ºC). Y tras el periodo de refrigeración se sometieron al proceso de fritura, que se realizó a 180 ºC en aceite de girasol hasta obtener en el interior del producto una temperatura de 63 ºC. La temperatura interna de los nuggets se registró utilizando un termómetro de sonda (Testo 735-2, Lenzkirch, Alemania). Además del efecto del tipo de enriquecimiento, que se evalúa en el Capítulo 2.2, se estudió i) el efecto de la fritura (nuggets pre-fritos vs. nuggets fritos) (Capítulo 2.1) y ii) el efecto del almacenamiento a congelación durante 1 y 3 meses sobre las características físico-químicas, composición y estabilidad frente a la oxidación de los nuggets (Capítulos 2.3 y 2.4). La figura 2 muestra el diseño experimental de la sección 2, especificándose las determinaciones analíticas realizadas en cada capítulo. El material y métodos utilizados para el desarrollo de esta sección están descritos en cada uno de los trabajos.

Figura 2. Diseño experimental de la Sección 2.



4. Capítulos

Capítulo 1.1

Suitability of using monolayered and multilayered emulsions for microencapsulation of ω-3 fatty acids by spray drying: effect of storage at different temperatures

Food Bioprocess Technol 8, 100-111 (2015)

Food Bioprocess Technol (2015) 8:100–111
DOI 10.1007/s11947-014-1382-y

ORIGINAL PAPER

Suitability of Using Monolayered and Multilayered Emulsions for Microencapsulation of ω-3 Fatty Acids by Spray Drying: Effect of Storage at Different Temperatures

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Received: 20 January 2014 / Accepted: 27 July 2014 / Published online: 10 August 2014
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Abstract Microencapsulation of ω-3 fatty acids by spray drying was studied using both monolayered (lecithin) and multilayered (lecithin-chitosan) fish oil emulsions with maltodextrin as wall material. Stability of the multilayered emulsions was higher than the monolayered ones, and increased with the increase of the concentration of chitosan. No differences were detected in the moisture or MY of the microcapsules related to the different composition of the corresponding emulsions. On the contrary, MEE was significant higher in the case of the microcapsules produced with the multilayered emulsions with the highest concentration of chitosan (1 % w/w), being related with lower detection of TBARS at high storage temperatures. Overall, this study shows the suitability of microencapsulating ω-3 fatty acids by spray drying using both monolayered and multilayered fish oil emulsions with maltodextrin as wall material. Multilayered microcapsules prepared with lecithin-chitosan emulsions provide a great protective effect against lipid oxidation of fish oil during storage at moderate to high temperatures (30 °C and 60 °C). These multilayered microcapsules could be therefore successfully used as a fish oil protection approach for storage before its use as an ingredient in food products.

Keywords Omega-3 · Multilayered · Monolayered · Emulsions · Microcapsules · Spray-drying · Storage · Temperature

Electronic supplementary material The online version of this article (doi:10.1007/s11947-014-1382-y) contains supplementary material, which is available to authorized users.

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Introduction

Microencapsulation is defined as a process in which particles or droplets of a compound are surrounded by a coating or embedded in a homogeneous or heterogeneous matrix (Gharsallaoui et al. 2007). This technology is widely used in food industry (Garg et al. 2006). The application of the microencapsulation methodology allows the production of a powder easier to handle and store than the non-encapsulated product. It provides a physical barrier between the microencapsulated compounds and the environment, reducing the contact and reactivity with environmental oxidizing promoters such as light, heat, and metal ions (Ye et al. 2009). Consequently, microencapsulation has been proved to be an excellent way for stabilization of bioactive compounds. It is also suitable for reaching other objectives, as the feasibility of inclusion of the compounds in the food matrix, preventing negative effect on the sensory profile of the product, the controlled release of bioactive compounds, and the enhancement of the absorption of the microencapsulated compounds, due to targeted and triggered release in the gastrointestinal tract of consumers (Drusch and Mannino 2009; McClements et al. 2007).

Since few years ago, the most used microencapsulation technique in food industry is spray drying (Gharsallaoui

et al. 2007). It consists on the rapid evaporation of the water phase of an emulsion by atomization with a pressure nozzle or a centrifugal wheel. The wide variety of materials that can be used, the simplicity of the procedure, its low cost, and accessible equipment makes spray drying the easiest and cheapest option to produce microencapsulated materials for food (Gharsallaoui et al. 2007).

The production of the emulsion involves a homogenization process, which allows the emulsifiers to adsorb the oil droplets, reducing the interfacial tension and producing a protective layer that avoids droplet aggregation. This simple method leads to traditional emulsions with only a single layer of emulsifier around every single oil droplet, namely, monolayered emulsions. As an alternative strategy, a new microencapsulation technique known as "layer-by-layer" (LBL) has been recently developed (Grigoriev and Miller 2009). It consists on the successive deposition of layers around oil droplets, allowing the formation of a multilayered stabilized system, namely, multilayered emulsions. Layers of emulsifiers and/or polyelectrolytes are deposited and stabilized by electrostatic charges, compensating the charge of the previous layer and allowing the deposition of an additional layer due to an overcharging effect (Shaw et al. 2007). The method starts with the production of a primary emulsion, in which oil droplets are stabilized in the aqueous phase by an ionic emulsifier. After that, a secondary emulsion is produced by the addition of a polyelectrolyte of opposite charge. Due to electrostatic attraction surrounding the drops of the previous emulsion, oil droplets coated by a two-layered system are obtained. Droplets coated with more than two layers can be achieved by the repetition of the process. The process for the performance of the LBL technique is simple (homogenization and mixing) and offer several advantageous characteristics: improves physical and chemical stability of the microencapsulated components and allows the control and trigger of the bioactive compound release and the use of food-grade materials. Special care is needed for the composition and preparation of the emulsions to avoid destabilization by bridging, depletion, flocculation, or coalescence (Gharsallaoui et al. 2012), the quantity of added oil to the primary emulsion being limited before droplet aggregation occurs.

Selection of the emulsifier and coating wall materials represents a very important step during the optimization of the microencapsulation by spray drying and has a major influence on the final efficiency and stability of the global process (Anwar and Kunz 2011). Emulsifiers commonly used in the food industry are proteins, polysaccharides, phospholipids, and small-molecule surfactants that differ in their emulsifying properties and the stability of the produced emulsions. The combination of lecithin-chitosan has proven to increase the thickness of the coating that surrounds the oil droplets, preventing contact with pro-oxidant agents and maintaining the distance between lecithin layers of different droplets, stabilizing the structure of the emulsion, and has also been reported to be an effective delivery system, with better stability to oxidation and environmental stress as well as a better controlled release (Shaw et al. 2007; Klinkesorn et al. 2005a, 2005b, 2006). As for the coating materials, polysaccharides and proteins are the most used materials. It is important to obtain a balance between economical aspect, ease of use, and encapsulating properties. In that sense, maltodextrin is a polysaccharide that acts as a filler matrix, with is relatively not expensive, highly soluble in water, and has the capacity to form stable emulsions (Rosenberg et al. 1993). Moreover, it has been reported to provide good stability to encapsulated oil and good protection from oxidation (Rubilar et al. 2012).

Among the wide range of different types of food-related compounds that can be advantageously microencapsulated, the case of the omega-3 (ω-3) fatty acids is noteworthy. These polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3), are compounds with bioactive characteristics and several beneficial effects for human health (McClements et al. 2007). Nevertheless, ω-3 PUFA are exceptionally susceptible to oxidation processes. This fact leads to the main challenge for the production of ω-3 PUFA enriched products: the prevention of lipid oxidation (Jacobsen 2008). The breakdown of the ω-3 chain during oxidation involves nutritional loss and a detrimental sensory, with an unacceptable rancidity and fishy off-flavor (Taneja and Zhu 2006). Microencapsulation of ω-3 has been widely used as a strategy to solve these drawbacks. Fish oil has been microencapsulated by different methods as spray granulation, freeze drying, and spray drying (Anwar and Kunz 2011). It has been established that combining different coating materials, which act as emulsifier and as carrier matrix, is the optimal way of emulsifying fish oil as a previous step to microencapsulation (Sheu and Rosenberg 1998). Different fish oils (menhaden oil, tuna oil) have been successfully spray-dried by several authors using as emulsifier the mentioned lecithin-chitosan combination with corn syrup solids as coating material (Shaw et al. 2007; Klinkesorn et al. 2005a, 2005b, 2006). However, as our knowledge, there are no studies using the combination of lecithin-chitosan as emulsifier with maltodextrin as coating material for the microencapsulation of fish oil by spray drying and considering the effect of the subsequent storage of microcapsules at different temperatures.

This work was aimed to optimize the microencapsulation of ω-3 PUFA by spray drying from monolayered lecithin and multilayered lecithin-chitosan emulsions both with maltodextrin as coating material, and to study for the first time the effect of the composition on the characteristics and oxidative stability of emulsions and microcapsules under storage at different temperatures.

Material and Methods

Material

Fish oil (kindly provided by Biomega Nutrition, Galicia, Spain) was used as source of ω-3 PUFA (5.96 % EPA, 25.83 % DHA, 0.02 % BHT). Soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95 % of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12 % (Glucidex 12, Roquette, Lestrem, France), and food-grade glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions. Hydrochloric acid and petroleum ether (Scharlau, Barcelona, Spain) were used for the oil extraction of the microcapsules. For the oxidative stability, 1-butanol and isopropanol (Scharlau, Barcelona, Spain) were used as solvents and 2-thiobartituric acid (TBA, Serva, Heidelberg, Germany), trichloroacetic acid (Fisher, Barcelona, Spain), and 2,6-di-tert-butyl-4-methylphenol 99 % (BHT, Across Organics, Madrid, Spain) as reagents.

Methods

Preparation of Monolayered and Multilayered Fish Oil Emulsions

A scheme of the production of the emulsions is showed in Fig. 1. Two different types of fish oil emulsions were prepared: monolayered emulsions (with lecithin as emulsifier to coat the oil droplets and maltodextrin as wall material) and multilayered emulsions (with lecithin-chitosan as emulsifier and maltodextrin as wall material).

Primary Emulsions First, fish oil (20 g) and lecithin (6 g) were mixed with a magnetic stirrer overnight. Then, acetic acid 1 % (*w/w*) was added until a total weight of 200 g and homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion (10 % fish oil and 3 % lecithin) was obtained.

Secondary Emulsions From primary emulsions, three types of secondary emulsions were prepared: one monolayered emulsion (Mo) and two multilayered ones (MuE1 and MuE2). For

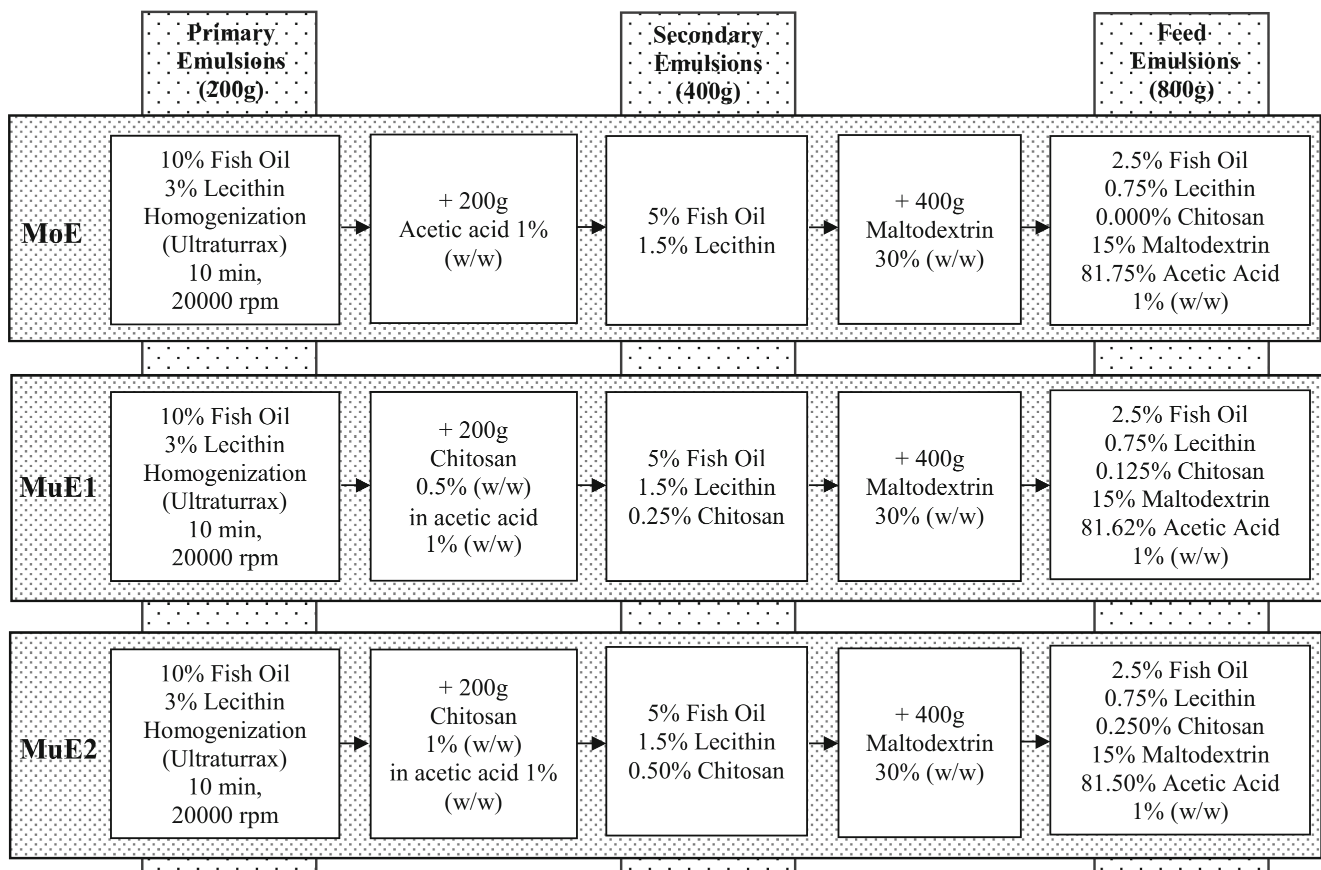

Fig. 1 Scheme of the production of each type of primary, secondary, and feed emulsions. *MoE* monolayered emulsion, *MuE1* multilayered emulsion with 0.5 % of chitosan, *MuE2* multilayered emulsion with 1 % of chitosan

the secondary Mo emulsion, primary emulsion was blended with 200 g of acetic acid (1 % *w/w*) by slowly agitation with a magnetic stirrer for 15 min. For the two secondary Mu emulsions, primary emulsion was blended with 200 g of 0.5 and 1 % of chitosan (*w/w*) in acetic acid 0.5 % *w/w* (for MuE1 and MuE2, respectively) and 200 g of chitosan in acetic acid 1 %.

Feed Emulsions Mo feed emulsions (MoE) and Mu feed emulsions with the two different concentrations of chitosan solution, 0.5 % *w/w* (MuE1) and 1 % *w/w* (MuE2), were produced by mixing secondary emulsions with 400 g of maltodextrin solution (30 % *w/w* in acetic acid 1 % *w/w*).

Preparation of Microencapsulated Fish Oil Powders

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

Reconstitution

Reconstitution of the spray-dried powders was used to characterize the properties of the reconstituted emulsions. Briefly, acetic acid (1 % *w/w*) was added to each batch of powders until reaching the moisture of the original feed emulsion.

Emulsion Characteristics

Microscopic Observations Samples of the feed and reconstituted emulsions were observed at the microscope to provide assessment of their respective microstructures. Immediately after the production and before spray drying process, emulsions were kept under gently agitation on a magnetic stirrer to maintain homogeneity, and a drop of sample was then taken with a Pasteur pipette, placed on a microscope slide, and covered by a cover slip. An optical microscope Axiovert 25 CFL (Prolabo, France) was used to examine the microstructure of the feed and reconstituted emulsions through a×100 oil immersion objective. Digital image files were acquired with a Nikon F90X camera connected to a digital image processing system (AxioVision Release 4.8, Zeiss, Germany).

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

Creaming Index For the measurement of the stability of the feed emulsions, 20 g of the emulsions was transferred into a tube sealed with a plastic cap and stored for 1 and 7 days at room temperature. Following the creaming index method (Surh et al. 2006), the cream layer (H_C) that corresponds to the top opaque layer, with respect to the total height of the emulsions in the tubes (H_E), was measured. Creaming index (CI) was calculated as $CI=100\ (H_E-H_C/H_E)$. As the CI increases, the emulsion stability decreases.

Microcapsules Characteristics

Microencapsulation Yield Microencapsulation yield (MY) provides the ratio between the quantity of microcapsules obtained and the solid content in the feed emulsions, and it was calculated using the following equation reported by Zhong et al. (2009):

$$MY(\%) = (\text{mass of collected product}/\text{non-solvent mass in the feed emulsions}) \times 100$$

Microencapsulation Efficiency Microencapsulation efficiency (MEE) was determined as a function of the encapsulated oil related to the total oil content of the microcapsules. For that, the external and total oil of the microcapsules were quantified, and the MEE was calculated with the equation provided by Velasco et al. (2006):

$$MEE(\%) = ((\text{total oil}-\text{external oil})/\text{total fish oil}) \times 100$$

For the extraction of total fish oil in the microcapsules, 5 g of the powders was placed into a sealed flask with 50 g of hydrochloric acid 1 N and, after digestion during 20 min at 96 °C, filtration was done with a Whatman no. 1 filter. Then, solvent was evaporated with a rotary evaporator Buchi R-210 coupled to a vacuum pump V-700 (Buchi, Switzerland) and oil content was determined gravimetrically.

For the quantification of the external fish oil in the microcapsules, 2 g of the powders was placed into an Erlenmeyer flask with 25 mL of petroleum ether and softly stirred. The flask was sealed using a cap with air cooler tube and introduced in a bath at 65 °C during 20 min. After that, filtration was done with a Whatman no. 3 filter. Finally, solvent was evaporated as described before for the total oil.

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

Scanning Electron Microcopy The morphology of the microcapsules was examined with a scanning electron detector microscope FEI QUANTA 3D FEG (FEI Company, Hillsboro, EE.UU.) in high vacuum conditions mode using EDT (Everhart Thornley Detector). Powder samples were mounted on stubs, fixed with a double adhesive coated carbon conductive adhesive sheet, and then subjected to metallization (sputtering) with a thin layer of a conductive gold coating for 8 s in order to amplify the secondary electron signal. After metallization, the samples were imaged operating at 3 kV with focused electron beam of Ga + (current of <6e-4 Pa) and observed with magnifications comprised between 5000 and 15,000.

Assay of Accelerated Oxidation

The susceptibility to oxidation of the fish oil, feed emulsions, and microcapsules was evaluated by an assay of accelerated oxidation at nine combinations of temperature (4, 30, and 60 °C) and time (1, 5, and 12 days). The oxidative level was determined by the thiobarbituric acid reactive substance (TBAR) method, as described by Hu and Zhong (2010). To completely dissolve the microcapsule matrix and fish oil, a ternary solvent mixture composed of 1-butanol/isopropanol/HCl 0.5 M (2:2:1, *v*/*v*/*v*) was used. TBA stock solution was obtained by mixing 15 g of trichloroacetic acid, 0.75 g of TBA, and a solution of 0.8 g of BHT dissolved in 100 mL of the ternary solvent mixture. Forty milligrams of samples was placed into a centrifuge tube and, subsequently, 10 mL of TBA stock solution was added and mixed using a vortex. Then, tubes were introduced in a water bath at 95 °C for 2 h and, after that, immediately cooled at room temperature. Concurrently, a calibration curve with solutions of 1,1,3,3-tetramethoxypropane (TMP) was prepared ranging from 0.2 to 20.0 μM, which were processed as the samples. Finally, absorbance at 532 nm was measured in an spectrophotometer Jenway 7305 (Roissy, France) using ternary solvent mixture as a blank. Malondialdehyde (MDA) equivalence was calculated from the calibration curve and TBARs level was expressed as nmol MDA/kg oil.

Sampling Replication and Statistical Analysis

Replicate experimental samples ($n=5$) of each batch of emulsions and microcapsules were produced, and analysis were performed by duplicate. Data were analyzed by one-way ANOVA using IBM SPSS Statistics v.19 and, when significant differences were observed ($p<0.05$), they were evaluated by a Tukey's test.

Results and Discussion

Preparation of Monolayered and Multilayered Fish Oil Emulsions

In this study, Mo and Mu emulsions of fish oil were produced and dried by spray drying to obtain Mo and Mu microcapsules. The main difference between Mo and Mu emulsions was the emulsifier used: lecithin in the case of MoE and lecithin-chitosan for MuE. In addition, and as explained in the "Material and Methods" section, two concentrations of the chitosan solution were added, 0.5 and 1.0 % *w*/*w* (MuE1 and MuE2, respectively). Consequently, the composition of MoE, MuE1, and MuE2 was slightly different, as can be observed in Table 1. From these emulsions, the corresponding microcapsules were produced, the main differences being the type of coat surrounding the fish oil droplets and the electronic charge (conferred by the coating materials themselves). It was a lecithin layer, of anionic nature, in the MoM and a lecithin-chitosan multilayer structure, with positive charge, in the case of MuM.

Emulsion Characteristics

The pH measurement results of primary, secondary, feed, and reconstituted emulsions are shown in Table 2. As expected, the pH of all primary emulsions used to produce the corresponding secondary emulsion was similar (6.54). In the secondary ones, feed and reconstituted emulsions, significant differences were found in the pH values. In general, MuE2 presented higher values of pH (4.25, 4.33, and 4.64 for secondary, feed, and reconstituted emulsions, respectively) than MuE1 (3.80, 3.85, and 4.01 for secondary, feed, and reconstituted emulsions, respectively) and MoE (3.38, 3.61, and 4.08 for secondary, feed, and reconstituted emulsions, respectively). These differences could be related to the composition of the emulsion, specifically with the chitosan concentration, as the highest pH values were observed in the

Table 1 Composition of the different feed emulsions

	MoM	MuM1	MuM2
Fish oil (%)	2.5	2.5	2.5
Soybean Lecithin (%)	0.75	0.75	0.75
Chitosan (%)	–	0.125	0.250
Maltodextrin (%)	15	15	15
Acetic acid 1 % (*w*/*w*) (%)	81.75	81.62	81.50
Total weight of feed emulsion	800	800	800
Solid (%) (*w*/*v*)	18.25	18.37	18.50
Wall material/oil ratio (*w*/*w*)	6.30	6.35	6.40

MoE monolayered emulsion, *MuE1* multilayered emulsion with 0.5 % of chitosan, *MuE2* multilayered emulsion with 1 % of chitosan

Table 2 pH and creaming index (CI) of the emulsions (values are expressed as mean±standard deviation)

		MoE	MuE1	MuE2	p
pH	Primary emulsion	6.54±0.11	6.54±0.11	6.54±0.11	1.000
	Secondary emulsion	3.38±0.08[c]	3.80±0.25[b]	4.25±0.04[a]	<0.001
	Feed emulsion	3.61±0.13[b]	3.85±0.12[b]	4.33±0.03[a]	<0.001
	Reconstituted emulsion	4.08±0.1[b]	4.01±0.12[b]	4.64±0.07[a]	<0.001
CI (%)	Feed emulsion	2.16±0.00[a]	0.80±0.00[b]	0.10±0.00[c]	0.005

Different letters in the same row indicate values significantly different ($p<0.05$)

MoE monolayered emulsion, *MuE1* multilayered emulsion with 0.5 % of chitosan, *MuE2* multilayered emulsion with 1 % of chitosan

emulsion with the highest chitosan concentration. The addition of chitosan may have shown a deacidification effect in the emulsions by means of an increase in the amount of amino groups. Chitosan has been previously reported to show a deacidification effect for low pH solutions, increasing the pH level and decreasing the acidity in coffee oils (Scheruhn et al. 1999). Chitosan has also been used for deacidification of fruit juices (Rwan and Wu 1996). In these studies, the referred effect on pH properties of chitosan depended on its concentration, which is in agreement with our results.

The creaming index (CI) was determined in the feed emulsions (Table 2). Given that the same values for CI were obtained after 1 and 7 days, only those for day 1 are shown in Table 2. There was a significant effect of the type of emulsion on the CI ($p<0.005$), the lowest CI values were found in the MuE2 (0.10 %), followed by MuE1 (0.80 %), and the highest levels were those of the MoE (2.16 %). Previous studies have obtained similar stability for multilayered emulsions produced with the combination lecithin-chitosan (Klinkesorn and McClements 2009). The CI is inversely related to the stability of the emulsions; thus, the addition of chitosan seems to influence positively the stability of emulsions. The higher emulsion stability produced by chitosan is related to the prevention of the contact between the lecithin layers of different droplets, and also to the increase of the electrostatic force and the viscosity, which leads to a flow resistance and a decrease in the mobility of the emulsions droplets, avoiding their aggregation (Klinkesorn et al. 2005a, b; Ogawa et al. 2003).

With the increase of the chitosan concentration and its consequent adsorption to the droplet surfaces, the electrical charge of the droplets of the lecithin-chitosan secondary emulsions is supposed to increase, ensuring a high positive charge and generating strong electrostatic repulsive forces, which helps maintaining the stability of the layers (Ogawa et al. 2003). However, some authors postulated that also lecithin/chitosan ratio needs to be taken into account to obtain an optimal stability, as precipitation and formation of insoluble particles were detected in lecithin/chitosan emulsions when the ratio of lecithin/chitosan above 4 was reached (Magdassi et al. 1997). In our multilayered emulsions, this ratio was 6 in the MuM1 and 3 in the MuM2, which could be related with the better stability of the MuM2 emulsions. Also, the mentioned differences of the pH of the emulsions could have also influenced in the stability differences indicated by the CI assay. Lecithin-chitosan emulsions at values of pH lower than 3.5 were observed to be simple emulsions, while increasing the pH to 3.5 produced the appearance of a rigid coating ordered structure typical of multilayer emulsions (Magdassi et al. 1997). The values of pH of the secondary and the feed MuM1 emulsions (3.80 and 3.85, respectively) were closer to this critical value than those of the secondary and feed MuM2 emulsions (4.25 and 4.33, respectively). However, Fig. 2 shows the microscopic images of the feed emulsions after 24 h of their production, and apparently, no signs of destabilization were observed.

Microcapsules Characteristics

Results on the characterization of the microcapsules (moisture, microencapsulation yield, microencapsulation efficiency) are presented in Table 3. Percentage of moisture in the MoM, MuM1, and MuM2 were 4.74, 1.89, and 4.16 %, respectively. These values are quite in agreement with results obtained in most dried powders used for food purposes (Gallardo et al. 2013). There were no significant differences in the moisture content between microcapsules. Besides spray drying itself, the final moisture in the microcapsules has been also related to the composition of the feed emulsions that subsequently constitutes the matrix material of the microcapsules (Gharsallaoui et al. 2012). Since the spray drying process conditions were identical for the three types of microcapsules produced in this work, it seems that the addition of chitosan to the emulsion as well as its concentration had no influence on moisture retention during the spray drying process.

There was no statistical difference for MY (46.84 % for MoM, 44.25 % for MuM1, and 39.76 % for MuM2). So, it seems that the different composition of the microcapsules (the inclusion of chitosan and its concentration) does not influence this characteristic. This contrasts with the findings reported by other authors, which have stated a dependence of the

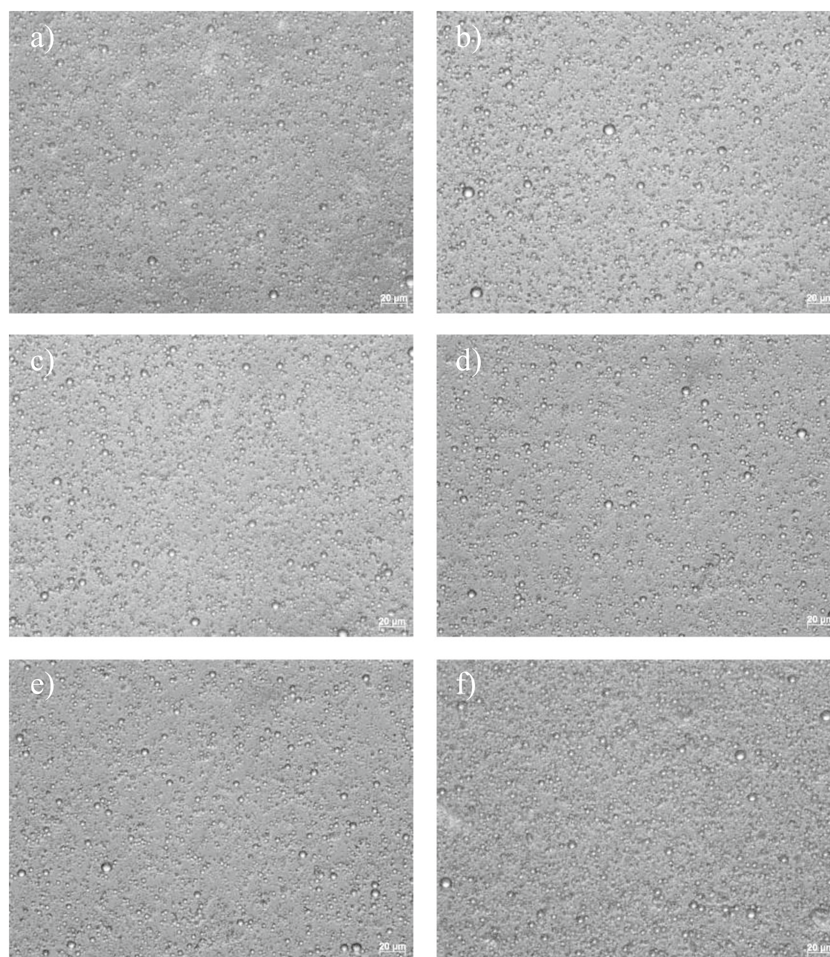

Fig. 2 Optical microscope images of feed and reconstituted emulsions observed at × 1,000 magnifications. **a** Feed monolayered emulsion (MoE); **b** reconstituted monolayered emulsion; **c** feed multilayered emulsion with 0.5 of chitosan (MuE1); **d** reconstituted multilayered emulsion with 0.5 % of chitosan; **e** feed multilayered emulsion with 1 % of chitosan (MuE2); and **f** reconstituted multilayered emulsion with 1 % of chitosan

microencapsulation yield on the composition (Botrel et al. 2012). However, it has also been discussed that the differences of the nature of the oil and variations in the preparation procedure can vary the yield of the powders (Gallardo et al. 2013). MY is an index of the efficacy of the spray drying processing, and so, being of great importance from an economical point of view.

Significant differences ($p<0.001$) were found in MEE between the different groups of microcapsules, with the highest values found in MuM2 (61.90 %), followed by MoM (52.03 %) and MuM1 microcapsules showing the lowest level (45.75 %). Then, both the addition of chitosan to the emulsions and its concentration seem to be important parameters affecting the MEE. In fact, the value of MEE is highly variable and depends, among other factors, on the ratio of core material to wall material (Klinkesorn et al. 2006). This can be in agreement with our results, as the wall material/oil was slightly different between emulsions (Table 1). Lower oil contents in the feed emulsion are related to a faster formation of the semipermeable surface membrane around the droplet surface

Table 3 Characteristics of the monolayered and multilayered microcapsules (values are expressed as mean±standard deviation)

	MoM	MuM1	MuM2	p
Moisture (%)	4.75±0.88	1.89±0.22	4.16±1.92	0.070
External oil (%)	3.66±1.65	2.03±0.62	3.60±0.55	0.060
Total oil (%)	7.64±1.18^{b}	3.74±0.65^{c}	9.45±1.03^{a}	<0.001
Microencapsulation yield (%)	46.84±7.85	44.25±1.38	39.76±3.64	0.246
Microencapsulation efficiency (%)	52.03±6.51^{b}	45.72±6.68^{c}	61.90±7.85^{a}	<0.001

Different letters in the same row indicate values significantly different ($p<0.05$)

MoM monolayered microcapsules, *MuM1* multilayered microcapsules prepared with MuE1 emulsions (0.5 % *w/w* chitosan), *MuM2* multilayered microcapsules prepared with MuE2 emulsions (1 % *w/w* chitosan)

that will eventually constitute the microcapsule wall, during the spray drying process. The early formation of this membrane avoids oil leaking from the microcapsules, which in turn increases efficiency (Huynh et al. 2008). A lengthening in the time needed for the formation of this membrane during spray drying would allow the migration of some oil to the surface of the formed microcapsules. Accordingly, in our results, those emulsions with the lower oil/solids ratio (MuE2) led to the highest MEE values in the obtained microcapsules (MuM2). However, such a behavior was not followed by the MuE1/MuM1, since they showed the lowest MEE, while having intermediate oil/solids ratios. The behavior of MuE1 during the spray drying process may have been also influenced by the confluence of other factors (as pH, droplet size, stability, or wall material/oil ratio) that could have affected the multilayer structure during the formation of the semipermeable membrane, decreasing the retention of part of the oil that seems to have been partially lost in the drying chamber. It is challenging to optimize the formulation of the emulsions to obtain the highest possible solid concentration and oil load that leads to the best oil retention and MEE. Maybe, the values of MEE could be improved by adjusting the oil load or varying the wall material/oil ratio and the spray drying conditions (as, for example, using lower inlet air temperature) (Jafari et al. 2008).

Figure 3 shows the SEM images of the microcapsules. In all cases (MoM, MuM1, and MuM2), microcapsules appeared as spherical particles of different sizes, which is expected in the powders produced by the spray drying method (Carneiro et al. 2013), and they were constituted by skin-forming particles with no apparent pores. Skin-forming particles are defined as particles composed of a polymeric or sub-

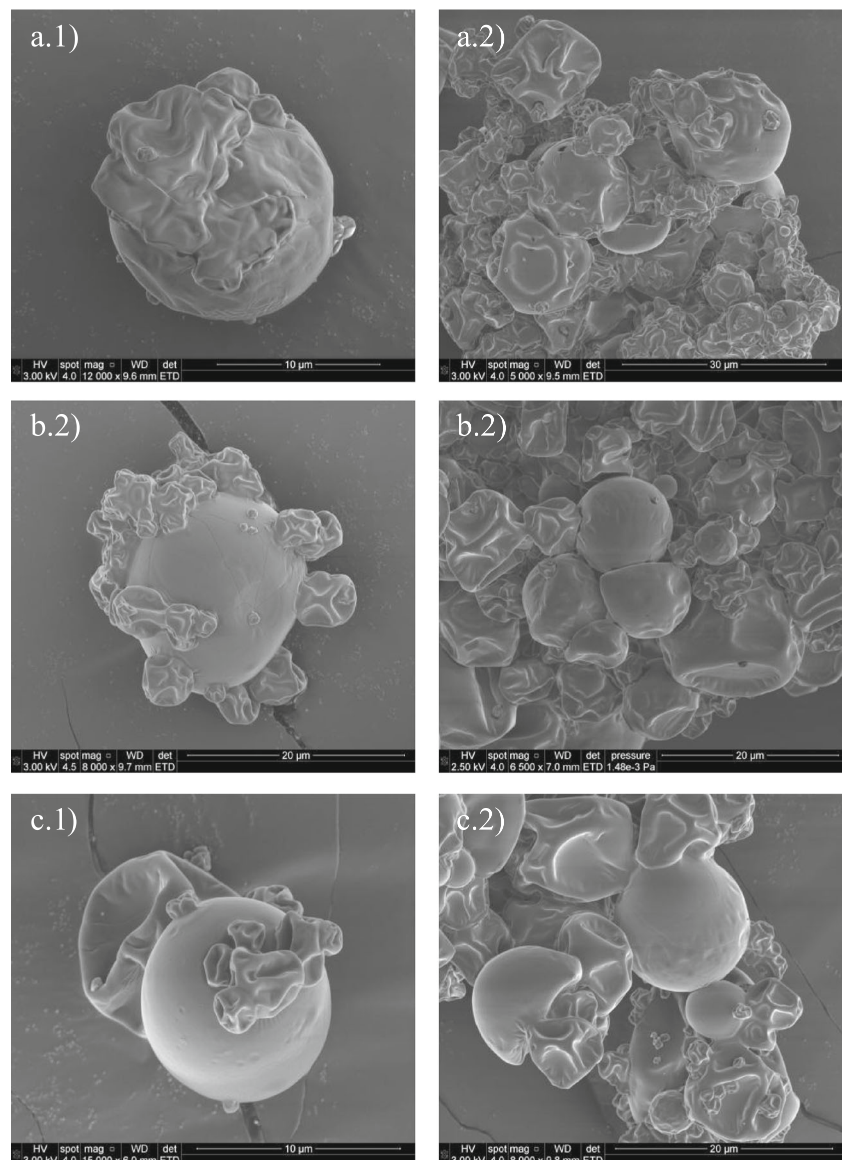

Fig. 3 Microscopic SEM images of spray-dried microcapsules observed with × 5,000–× 15,000 magnifications. **a1** Individual image of a monolayered microcapsule (MoM); **b1** individual image of a multilayered microcapsule with 0.5 % of chitosan (MuM1); **c1** individual image of a multilayered microcapsule with 1 % of chitosan (MuM2); **a2** overall image of monolayered microcapsules (MoM); **b2** overall image of multilayered microcapsules with 0.5 % of chitosan (MuM1); **c2** overall image of multilayered microcapsules with 1 % of chitosan (MuM2)

microcrystalline continuous non-liquid phase; this is the most common of the three distinct morphologies in which spray-dried food can be found, the other two being crystalline and agglomerate particles (Walton and Mumford 1999). The absence of pores in the skin of the microcapsules has been highlighted as an advantage, since it ensures better protection and retention of the encapsulated fish oil, as the presence of pores can lead to an increase in the permeability of the wall material, decreasing the protective effect of the core (Carneiro et al. 2013).

Some visual differences between microcapsules can be observed. Thus, MuM2 were formed, in general, by spherical defined particles of smoother surface, which is a sign of a better stability of the microcapsules. MoM and MuM1 showed some common microstructural features, such as particle collapse or shriveling, which have been evidenced to vary with the composition of encapsulating material and drying parameters (Rubilar et al. 2012). Other common morphological features for these two types of capsules were visible wrinkles or dimples on the surface that have been attributed to rapid evaporation of the drops during atomization in the drying process (Rosenberg et al. 1985). These characteristics could be caused by the different composition of the feed emulsions, as drying parameters were controlled to be the same during the performance of the atomization to produce the microcapsules. However, the difficulty of understanding the effect of each specific variable of the nature and composition of the materials of the emulsions and of the spray drying process (feed, drying, atomization conditions) on the morphology of the microcapsules has been previously highlighted (Chen and Mujumdar 2009).

Reconstituted Emulsions

The obtained powders were reconstituted into emulsions to compare their stability characteristics with those of the original feed emulsions. This is important since the microcapsules should be a stable system able to resist the stresses of spray drying process and environmental factors without affecting the characteristics of the original emulsions. Microscopic observation ""(Fig. 2) showed that, after the reconstitution of MoM, MuM1, and MuM2, the general aspect of the three corresponded reconstituted emulsions did not suffer noticeable changes in comparison to the feed emulsions, as reported by other researchers (Klinkesorn et al. 2005a). Some signs of depletion flocculation were only visualized in those from MuM2. However, this flocculation can be reverted by stirring or mild sonication (Rubilar et al. 2012).

Oxidative Stability

Results for ""TBAR values of fish oil, feed emulsions (MoE, MuE1, and MuE2), and microcapsules (MoM, MuM1, and MuM2) after storage at nine combinations of temperature (4, 30, and 60 °C) and time (1, 5, and 12 days) are shown in Table 4.

The effect of the time of storage on the oxidative stability was not so remarkable, and only significant differences for TBAR values in MoM and MuM1 samples were found, but not showing the same trend for all samples (i.e., at 4 °C, the highest TBAR values were found in MoM at 12 days and in MuM1 at 5 days). On the other hand, the storage temperature significantly influenced TBARs in all types of samples, with higher TBAR values as the temperature increase. In fact, temperature has been established as one of the most important factors determining the stability of dried powders (Klinkesorn et al. 2005b). The time × temperature effect was also significant for all samples, so that the oxidation levels increased with the time in those samples storage at higher temperatures, while at 4 °C, the behavior was not that clear for all the types of samples.

Significant differences in the oxidation levels when comparing TBAR values between all samples (fish oil vs. MoE vs. MuE1 vs. MuE2 vs. MoM vs. MuM1 vs. MuM2) at each combination of time and temperature of storage were found. In general, most of the remarkable differences in lipid oxidation between fish oil, emulsions, and microcapsules were found at 60 °C of storage temperature whereas the differences at 4 and 30 °C were less clear. Nevertheless, MuM2 capsules showed a much better stability at both 30 and 60 °C, which would be more realistic temperatures of storage for this kind of products during transportation and storage. In this sense, some companies have measured temperatures close to 60 °C in shipping containers (Weiskircher 2008).

When storing at 30 °C for 1 day, a clear increase in the TBAR values in the emulsions could be observed. This can be related to the stress suffered by the emulsions during their production (mechanical stress and exposition to air during homogenization) (Klinkesorn et al. 2006). However, at 60 °C, lower TBAR values were found in the microcapsules in comparison to the fish oil and the emulsion. This result indicates the protective effect of the microcapsules against oxidation at high temperatures and, consequently, the effectiveness of microencapsulation against ω-3 fatty acid oxidation. This suggests the possibility of using microencapsulation to enable the transportation and direct addition of ω-3 fatty acids to enriched food products.

Oxidative stability of powders is strongly influenced by the wall materials (Carneiro et al. 2013). Comparing the three types of emulsions, in general, the oxidation levels were similar in MoE, MuE1, and MuE2. With respect to the microcapsules, oxidation levels showed significant differences, the highest TBAR values being found in most cases in MuM1, followed by MoM, and with MuM2 having the lowest values. It could indicate the influence of the chitosan concentration added to the emulsion as well as the structure formed in the

Table 4 Oxidative level (expressed in nmol MDA/kg oil) in the fish oil, the feed emulsions, and their corresponded microcapsules after storage at different combinations of time (t) and temperature (T°) (values are expressed as mean±standard deviation)

	Day 1			Day 5			Day 12					
	4 °C	30 °C	60 °C	4 °C	30 °C	60 °C	4 °C	30 °C	60 °C	*p* (t)	*p* (T^{a})	*p* (txT^{a})
FO	$10.9\pm2.2^{a}_{\alpha}$	$29.1\pm2.9^{a}_{\alpha}$	$61.7\pm1.2^{c}_{\alpha}$	$22.0\pm1.1^{a}_{\alpha}$	$57.5\pm3.3^{a}_{\alpha}$	$245.7\pm14.4^{b}_{\beta}$	$34.4\pm4.2^{ab}_{\alpha}$	$99.6\pm2.6^{c}_{\alpha}$	$957.5\pm78.0^{e}_{\gamma}$	0.127	0.018	<0.001
MoE	$24.6\pm1.5^{ab}_{\alpha}$	$166.3\pm9.5^{cd}_{\alpha}$	$321.7\pm9.1^{c}_{\gamma}$	$29.6\pm8.6^{a}_{\alpha}$	$47.3\pm1.6^{a}_{\alpha\beta}$	$112.9\pm6.0^{a}_{\alpha\beta}$	$26.7\pm5.1^{a}_{\alpha}$	$69.8\pm4.1^{b}_{\alpha\beta}$	$818.3\pm17.9^{d}_{\delta}$	0.259	0.001	<0.001
MuE1	$35.3\pm2.4^{b}_{\alpha}$	$173.4\pm3.9^{d}_{\gamma}$	$415.5\pm8.9^{d}_{\delta}$	$33.5\pm7.4^{a}_{\alpha}$	$64.4\pm0.8^{a}_{\alpha\beta}$	$108.5\pm19.1^{a}_{\beta\gamma}$	$49.9\pm5.3^{bcd}_{\alpha\beta}$	$97.1\pm1.5^{c}_{\alpha\beta}$	$587.4\pm44.5^{c}_{\varepsilon}$	0.246	<0.001	<0.001
MuE2	$30.6\pm0.1^{b}_{\alpha}$	$129.7\pm5.8^{bc}_{\alpha}$	$171.5\pm12.0^{b}_{\alpha}$	$32.5\pm1.8^{a}_{\alpha}$	$64.2\pm1.8^{a}_{\alpha}$	$138.9\pm48.4^{a}_{\alpha}$	$70.3\pm9.8^{d}_{\alpha}$	$144.7\pm26.7^{d}_{\alpha}$	$997.2\pm96.7^{e}_{\beta}$	0.109	0.038	<0.001
MoM	$24.0\pm5.5^{ab}_{\alpha}$	$43.5\pm5.6^{a}_{\alpha\beta\gamma}$	$60.1\pm1.6^{b}_{\beta\gamma}$	$37.2\pm6.2^{a}_{\alpha\beta}$	$48.0\pm5.0^{a}_{\alpha\beta\gamma}$	$94.0\pm37.5^{a}_{\delta}$	$40.1\pm9.0^{abc}_{\alpha\beta}$	$68.9\pm7.2^{b}_{\gamma\delta}$	$71.2\pm15.7^{a}_{\gamma\delta}$	0.047	<0.001	<0.001
MuM1	$55.0\pm4.6^{c}_{\alpha}$	$92.1\pm11.0^{b}_{\varepsilon}$	$144.6\pm13.6^{b}_{\zeta}$	$70.5\pm6.7^{b}_{\beta\gamma\delta}$	$59.4\pm1.9^{a}_{\alpha\beta}$	$74.4\pm2.9^{a}_{\gamma\delta}$	$59.6\pm5.9^{cd}_{\alpha\beta\gamma}$	$81.3\pm5.2^{bc}_{\delta\varepsilon}$	$170.6\pm9.7^{b}_{\eta}$	<0.001	<0.001	<0.001
MuM2	$30.4\pm6.4^{b}_{\alpha\beta}$	$29.6\pm7.1^{a}_{\alpha\beta}$	$44.2\pm7.4^{a}_{\alpha\beta}$	$32.3\pm8.4^{a}_{\alpha\beta}$	$52.6\pm1.5^{a}_{\beta\gamma}$	$87.2\pm4.7^{a}_{\gamma}$	$24.7\pm5.8^{a}_{\alpha}$	$40.9\pm3.7^{a}_{\alpha\beta}$	$68.6\pm3.0^{a}_{\gamma}$	0.058	<0.001	<0.001
p (S)	0.000	<0.001	<0.001	<0.001	0.027	0.001	<0.001	<0.001	<0.001			
p (E)	0.017	0.046	0.036	0.838	0.339	0.772	0.063	0.051	0.016			
p (M)	<0.001	<0.001	<0.001	<0.001	0.014	0.602	<0.001	<0.001	<0.001			

Different letters (a, b, c, d, e, f, g) in the same column indicate significant differences ($p<0.05$) between samples, *p*(S). Different letters (α, β, γ, δ, ε, ζ, η) in the same row indicates significant differences due to the combined effect of time and temperature during storage, *p* (txT^{a}). Differences between the different types of emulsions and microcapsules are indicated by *p* (E) and *p* (M), respectively

FO fish oil, *MoE* monolayered emulsion, *MuE1* multilayered emulsion with 0.5 % of chitosan, *MuE2* multilayered emulsion with 1 % of chitosan. *MoM* monolayered microcapsules, *MuM1* multilayered microcapsules prepared with MuE1 emulsions (0.5 % *w/w* chitosan), *MuM2* multilayered microcapsules prepared with MuE2 emulsions (1 % *w/w* chitosan)

microcapsules (the lecithin-chitosan multilayer in MuM and the lecithin layer in MoM) on the oxidative stability of the microcapsules, which increased with the chitosan concentration and with the number of layers. In fact, chitosan, besides its well-known properties as improver of emulsification capacity and fatty acid absorbing functionality, has also shown antioxidant properties, which could be an additional benefit for its use as wall material for microencapsulation. Antioxidant activity of chitosan has been reviewed (Friedman and Juneja 2010), concluding that low-molecular-weight chitosan at a pH below 6.0 presents optimal conditions to act as antioxidant in both liquid and solid food. These authors suggested that chitosan could act as a free radical scavenger.

Moreover, it seems that, in agreement with other authors, lipid oxidation in spray-dried powders was not influenced by the amount of free fat (Klinkesorn et al. 2005b), since the content of external fat was similar in MoM, MuM1, and MuM2 (Table 3). However, as discussed before, total extracted oil was different for the three microcapsules. Because of that, although quantity of external fat was the same for the microcapsules, the different quantities of encapsulated oil caused difference in MEE. Interestingly, there was a close relationship between MEE and lipid oxidation. This has been previously detected by other studies (Jafari et al. 2008) and highlights the importance of controlling the factors that influence MEE to provide good protection and shelf life to the microencapsulated oil.

Conclusions

Multilayered emulsions produced with chitosan and maltodextrin show higher stability than monolayered ones without chitosan. Moreover, higher concentrations of chitosan lead to increased emulsion stability. In the subsequent microcapsules obtained by spray drying, MEE can be highly affected by composition of the original emulsions, multilayered emulsions with increased concentrations of chitosan leading to better MEE. Moreover, such higher MEE may lead to lower levels of lipid oxidation during the storage at high temperatures. This evidences the importance of adjusting the composition of the emulsions to obtain optimal values of MEE in microencapsulated fish oil produced by spray drying.

Overall, this study shows the suitability of microencapsulating ω-3 fatty acids by spray drying using both monolayered and multilayered fish oil emulsions with maltodextrin as wall material. Multilayered microcapsules prepared with lecithin-chitosan emulsions provide a great protective effect against lipid oxidation of fish oil during storage at moderate to high temperatures (30 and 60 °C). These multilayered microcapsules could be therefore successfully used as a fish oil

protection approach for storage before its use as an ingredient in food products.

Acknowledgments Estefanía Jiménez-Martín thanks the Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo for supporting the pre-doctoral grant (PD10026), equipment funding, and support to research groups and Biodymia Laboratory (University of Lyon) for making possible the development of the work during a research stay. Authors acknowledge "Biomega Nutricion" for kindly providing the fish oil and "Trades S.A." for kindly providing the chitosan, as well as technical and human support provided by Ana-Isabel Mayoral (Anatomy and Embriology Department, Faculty of Veterinary Sciences, University of Extremadura) and Facility of Analysis and Characterization of Solids and Surfaces of SAIUEx (funded by UEX, Junta de Extremadura, MICINN, FEDER, and FSE).

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Capítulo 1.2

Volatile compounds and physicochemical characteristics during storage of microcapsules from different fish oil emulsions

Food Bioprod Process 96, 52-64 (2015)

Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Volatile compounds and physicochemical characteristics during storage of microcapsules from different fish oil emulsions

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ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 28 June 2015
Accepted 6 July 2015
Available online 14 July 2015

Keywords:
Fish oil
Multilayered emulsions
Multiple emulsions
Microcapsules
Spray-drying
Oxidation volatile compounds

ABSTRACT

Spray-drying of double (DE) and multilayered (ME) fish oil emulsions, was used to produce two different types of microcapsules (DM and MM, respectively). Stability of emulsions and physicochemical characteristics, oxidative stability and volatile profile of the microcapsules at initial time and after 1 month of storage at 20 and 4 °C were analyzed. ME showed better stability and held higher amount of fish oil than DE (2.5 vs 0.625% w/w). Microencapsulation yield was similar for both types, whereas moisture, microencapsulation efficiency and oxidative stability were higher for MM. The type of microcapsule influenced the volatile profile and specifically the 28 selected volatiles related to oxidation. Both types of microcapsules appear as feasible alternatives to the bulk fish oil as a way to provide ω-3 storage and for enrichment purposes, but MM seems to be more appropriate in terms of oxidation during storage.

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1. Introduction

Polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3), are compounds with bioactive properties and several beneficial effects for human health (McClements et al., 2007). The importance of increasing EPA and DHA intake has been recognized by several health agencies worldwide (Barrow et al., 2007). As a result, consumer's interest in boosting the intake of ω-3 fatty acids (FA) has increased. The main dietary source of ω-3 FA is fish oil. However, as a consequence of current trends in eating habits, this source is not enough to reach the recommended daily dose of EPA and DHA (Taneja and Singh, 2012). For this reason, there is a growing interest in the development of functional foods and supplements as a source of EPA and DHA (McManus et al., 2011).

Several researchers have examined the possibility of incorporating fish oil (and thereby the beneficial ω-3 FA) into food products (Carneiro et al., 2013). Nevertheless, ω-3 FA are exceptionally susceptible to oxidation processes. The breakdown of the ω-3 chain during oxidation involves nutritional loss and a detrimental sensory, with an unacceptable rancidity and fishy off-flavour (Taneja and Zhu, 2006). This fact leads to the main challenge for the production of ω-3 FA

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http://dx.doi.org/10.1016/j.fbp.2015.07.005

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enriched products: the prevention of lipid oxidation (Jacobsen, 2008). Microencapsulation of ω-3 by spray-drying has been suggested as a strategy to solve these drawbacks. This technique involves the production of an emulsion by means of a homogenization process, which allows the emulsifiers adsorb the oil droplets, reducing the interfacial tension and producing a protective layer that avoids droplet aggregation. This simple method leads to traditional emulsions with only a single layer of emulsifier around every single oil droplet, namely monolayered emulsions. Alternative strategies, such as the modification of the emulsion preparation to obtain multiple or multilayer emulsions have also been developed (Grigoriev and Miller, 2009; Shaw et al., 2007).

Double emulsions occur when a single emulsion is itself homogenized to form droplets within another (added) continuous phase of the opposite hydrophobicity/hydrophilicity to that making up the continuous phase of the original single emulsion (and similar to the discrete phase of the original single emulsion). One thus produces "droplets within droplets". For example, in an "oil-in-water-in-oil" double emulsion (O-W-O), there are very small droplets of oil inside larger droplets of water which are themselves surrounded by a continuous oil phase. The encapsulation of oil by this technique is prepared as an oil-in-water-in-oil (O1/W/O2) emulsion, in which the encapsulated oil consists on the inner oil phase (O1) (Edris and Bergnstahl, 2001; Dwyer et al., 2013; O'Dwyer et al., 2013). It requires the combination of two emulsifiers: an emulsifier of hydrophilic nature for the stabilization of the O1/W interface and an emulsifier of hydrophobic nature for the stabilization of the O2/W interface. Multilayered emulsions can be defined as emulsions in which lipid droplets are surrounded by multiple layers instead of a single layer of coating material, these layers being formed by a combination of an emulsifier and one or more polyelectrolyte of opposite charges. This technique is known as "layer-by-layer" (LbL) and has been recently developed (Grigoriev and Miller, 2009; Jiménez-Martín et al., 2015; Klinkesorn et al., 2005a, b, c, 2006; Shaw et al., 2007).

Microencapsulation of fish oil as a source of ω-3 by spray-drying can be considered successfully performed when high retention of the core material is achieved and the majority of the oil is encapsulated, with low amounts of surface oil on the microcapsule powders (Jafari et al., 2008). This objective can be reached by the use of wall and core materials with adequate properties, optimizing formulation and preparation of emulsions and adjusting the drying process conditions (Gharsallaoui et al., 2007). Selection of the emulsifier and coating wall materials represents a very important step during the optimization of the microencapsulation by spray-drying and has a main influence on the final efficiency and stability of the global process (Anwar and Kunz, 2011). Emulsifiers commonly used in the food industry are proteins, polysaccharides, phospholipids and small molecule surfactants that differ in their emulsifying properties and the stability of the produced emulsions. As for the coating materials, polysaccharides and proteins are the most used materials. It is important to obtain a balance between economical aspect, ease of use and encapsulating properties.

Most studies on fish oil microcapsules by spray-drying are focused on their physical stability rather than on their volatile profile. Volatile compound profile or specific volatiles extracted by solid-phase microextraction (SPME) and analyzed with gas chromatography-mass spectrometry (GC/MS) appears as a useful tool for addressing lipid oxidation (Jacobsen, 1999). The complete volatile profile of the microcapsules, besides providing information of the oxidation level of the powders, can be also very useful as an approach to predict their flavor. Indeed, a recent study showed that sensory properties of fish oils were predicted using key oxidative volatiles, the results being comparable to the evaluation with a sensory panel (Ritter and Budge, 2012).

The stabilization of ω-3 FA by spray-drying microencapsulation has been assessed by complementary methods, such as peroxide value (PV), TBARs (Thiobarbituric Acid Reactive substances) index (Jiménez-Martín et al., 2015) and p-anisidine value determination (Omar et al., 2009), headspace propanal determination (Augustin et al., 2006) and non-isothermal differential scanning calorimetry (Pedroza-Islas et al., 2002). Dobarganes et al. (Dobarganes et al., 2009) also applied and validated the accelerated test Rancimat to evaluate the oxidative stability of dried microencapsulated oils.

Several authors have studied different microencapsulated fish oils (menhaden oil, tuna oil) using multilayered emulsions (LbL technique) and lecithin-chitosan emulsifier combination with different carbohydrates as coating material (Jiménez-Martín et al., 2015; Klinkesorn et al., 2005a, b, c, 2006; Shaw et al., 2007). On the other hand, to date, researches on microencapsulation of O/W/O emulsions that can be found in the literature are scarce, and, to the best of our knowledge, only two works have tackled this issue. In the study of Edris and Bergnstahl (Edris and Bergnstahl, 2001), orange oil was encapsulated in the inner compartment of a O1-W-O2 double emulsion, where O1 was orange oil, W was water and O2 was vegetable oil and was secondarily coated with lactose and caseinate as wall materials using spray-drying. Liao et al. (Liao et al., 2012) encapsulated fish oil in a O/W/O double-emulsion produced with succinic acid deamidated wheat gluten (SDWG) followed by heat-polymerization of emulsified SDWG.

This work was aimed to study the effect of microencapsulating fish oil by spray-drying from multiple and multilayered emulsions and to provide for the first time a comparison of both types of emulsions and their resulting microcapsules in terms of physicochemical characteristics and oxidative stability, including the analysis of the volatile profile by SPME-GC/MS during storage at ambient and refrigeration temperatures.

2. Material and methods

2.1. *Material*

Fish oil extracted by cold pressing from cod liver (kindly provided by Biomega Natural Nutrientes S.L., Galicia, Spain) was used as source of ω-3 FA (5.96% EPA, 25.83% DHA, and 0.02% BHT). Sodium caseinate (kindly provided by Anvisa S.A., Madrid, Spain), lactose monohydrate (Scharlau, Sentmenat, Spain), extra virgin olive oil (Hacendado, Madrid, Spain), polyglycerolpolyricinoleate (kindly provided by Cargill, Barcelona, Spain), soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, Roquette, Lestrem, France) and glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions. Hydrochloric acid and petroleum ether (Scharlau, Barcelona, Spain) were used for the oil extraction of the microcapsules. For the oxidative stability, 1-butanol and isopropanol (Scharlau, Barcelona, Spain) were purchased as solvents and 2-thiobartituric acid (TBA, Serva, Heidelberg, Germany), trichloroacetic acid (Fisher,

Barcelona, Spain) and 2, 6-di-tert-butyl-4-methylphenol 99% (BHT, Across Organics, Madrid, Spain) as reagents.

2.2. Methods

2.2.1. Experimental design

A scheme of the production of microcapsules and experimental design of the study is provided as supplementary material (Supplementary Fig. 1). Two different types of fish emulsions were prepared: double and multilayered emulsions (DE and ME, respectively). DE were prepared following the method of Edris and Bergnstahl (Edris and Bergnstahl, 2001) with some modifications, whereas ME formulation was optimized in our previous work (Jiménez-Martín et al., 2015). Two types of microcapsules were obtained: double (DM) and multilayered (MM). The collected dried powders were analyzed at initial time (t_0). The batches used for the storage assay were stored at 4 and 20 °C immediately after their production and kept stored for a month, in order to simulate refrigeration (t_1 m 4 °C) and room (t_1 m 20 °C) conditions, respectively.

2.2.2. Preparation of fish oil double emulsions (DE)

2.2.2.1. Primary O-W emulsions. To start with primary emulsion, sodium caseinate (6 g), used as hydrophilic emulsifier, and distilled water (74 g) were mixed in a magnetic stirrer overnight. Then, fish oil (20 g) was added and homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion (20% fish oil and 6% sodium caseinate) was obtained.

2.2.2.2. Secondary emulsions. To obtain the secondary emulsions, two different solutions were previously prepared. In the first place, a diluted primary emulsion was prepared adding 25 g of the primary emulsion to 25 g of lactose monohydrate (14% w/w) mixing with a magnetic stirrer. Then, an oily solution was prepared with 15 g of extra virgin olive oil and 15 g of polyglycerol polyricinoleate, a hydrophobic emulsifier, and subsequently mixed with a magnetic stirrer. Finally, the secondary emulsion was produced by dropping the diluted primary emulsion on to the oily solution, which was continuously agitated by magnetic stirrer.

2.2.2.3. Feed emulsions. The feed emulsion (800 g) was obtained by mixing the 80 g of the secondary emulsion with 624 g of distilled water containing 56 g of lactose monohydrate and 40 g of sodium caseinate (previously mixed in a magnetic stirrer overnight).

2.2.3. Preparation of fish oil multilayered emulsions (ME)

2.2.3.1. Primary O-W emulsions. First, fish oil (20 g) and lecithin (6 g) were mixed in a magnetic stirrer overnight. Then, acetic acid solution (1% w/w) was added until a total weight of 200 g and homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion (10% fish oil and 3% lecithin) was obtained.

2.2.3.2. Secondary emulsions. The secondary emulsion was obtained by blending primary emulsion with 200 g chitosan solution (1% w/w in acetic acid solution (1% w/w)). Briefly, primary emulsion was magnetically stirred and chitosan solution was slowly added on the inner face of the beaker (15 min). Then, the obtained secondary emulsion was maintained under agitation overnight.

2.2.3.3. Feed emulsions. Multilayered feed emulsions were produced by mixing secondary emulsions with 400 g of maltodextrin (30% w/w in acetic acid 1% w/w).

2.2.4. Preparation of microencapsulated fish oil powders

Both types of feed emulsions (800 g) were dried in a laboratory scale spray-drier equipped with a 0.5 mm nozzle atomizer (Mini spray-dryer B-290, Buchi, Switzerland). The emulsions, kept at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray-drying process. The aspirator rate was adjusted at 80%, feed rate was 1 L/h, inlet temperature was 180 °C and outlet temperature ranged 85–90 °C. Half of the collected dried powders of each batch were stored in plastic containers at −80 °C until analysis; the other half was subjected to the specific conditions of the oxidative stability storage assay.

2.2.5. Emulsion characteristics

2.2.5.1. Microscopic observations. Samples of the feed and reconstituted emulsions were observed with a microscope to provide assessment of their respective microstructures. Immediately after the production and before spray-drying process, emulsions were kept under gently agitation with a magnetic stirrer to maintain homogeneity, and a drop was then taken with a Pasteur pipette, placed on a microscope slide and covered by a cover slip. An optical microscope Axiovert 25 CFL (Prolabo, France) was used to examine the microstructure of the feed and reconstituted emulsions through a ×100 oil immersion objective. Digital image files were acquired with a Nikon F90X camera connected to a digital image processing system (AxioVision Release 4.8, Zeiss, Germany). Size distribution was obtained measuring the particle diameter of a total of 500 droplets using the image processing system.

2.2.5.2. Creaming index. For the measurement of the stability of the feed emulsions, 20 g of the emulsions were transferred into a tube sealed with a plastic cap and stored for 1 day and 7 days at room temperature. Following the creaming index method (Surh et al., 2006), the cream layer (HC), which corresponded to the superior opaque layer, with respect to the total height of the emulsions in the tubes (HE) were measured. Creaming index (CI) was calculated as: $CI = 100(HE - HC/HE)$. As the CI increases the emulsion stability decreases.

2.2.6. Microcapsules characteristics

2.2.6.1. Microencapsulation yield. Microencapsulation yield (MY) provides the ratio between the quantity of microcapsules obtained and the solid content in the feed emulsions and it was calculated using the following equation reported by Zhong et al. (Zhong et al., 2009):

$$MY(\%) = (\text{mass of collected product}/\text{non-solvent mass in the feed emulsions}) \times 100$$

2.2.6.2. Encapsulation efficiency. Microencapsulation efficiency (MEE%) was determined as a function of the encapsulated oil related to the total oil content of the microcapsules. For that, the external and total oil of the microcapsules were quantified, and the MEE% was calculated

with the equation provided by Velasco et al. (Velasco et al., 2006):

$$MEE(\%) = ((\text{total oil} - \text{external oil})/\text{total fish oil}) \times 100$$

2.2.6.3. *Extraction of total oil*.

For the extraction of total fish oil in the microcapsules, 5 g of the powders were placed into a sealed flask with 50 g of hydrochloric acid 1 mol/L and, after digestion during 20 min at 96 °C, filtration was done with a Whatman no. 1 filter. Then, solvent was evaporated with a rotary evaporator Buchi R-210 coupled to a vacuum pump V-700 (Buchi, Switzerland) and oil content was determined gravimetrically and expressed as g of total oil/100 g of microcapsules.

2.2.6.4. *Extraction of external oil*.

For the quantification of the external fish oil in the microcapsules, 2 g of the powders were placed into an Erlenmeyer flask with 25 mL of petroleum ether, and softly stirred. The flask was sealed using a cap with air cooler tube, and introduced in a bath at 65 °C during 20 min. After that, filtration was done with a Whatman no. 3 filter. Finally, solvent was evaporated as described before for the total oil, and external oil content was determined gravimetrically and expressed as g of external oil/100 g of microcapsules.

2.2.6.5. *Moisture*.

Moisture was analyzed following AOAC official reference 935.29 method (AOAC, 1999).

2.2.7. *Oxidative stability of fish oil microcapsules: storage assay*

The stability of DE and ME microcapsules was evaluated by an assay of storage during 1 month at different temperatures (4 and 20 °C) in order to simulate refrigeration and room temperature conditions, respectively. Powders were protected from light and moisture by storing them in sealed plastic tubes. One batch of each type of microcapsules was also analyzed before starting the assay of temperature storage (t_0), and they were considered the control batches. The thiobarbituric acid reactive substances (TBARs) method and the volatile compound analysis were carried out for determining the oxidative stability of the microcapsules.

2.2.7.1. *Thiobarbituric acid reactive substances (TBARs)*.

The TBARs method was carried out as described by Hu and Zhong (2010). To completely dissolve the microcapsules matrix, a ternary solvent mixture composed of 1-butanol:isopropanol:HCl 0.5 M (2:2:1, v/v/v) was used. TBA stock solution was obtained by mixing 15 g of trichloroacetic acid, 0.75 g of TBA and a solution of 0.8 g of BHT dissolved in 100 mL of the ternary solvent mixture. 40 mg of samples were placed into a centrifuge tube, and, subsequently, 10 mL of TBA stock solution were added and mixed using a vortex. Then, tubes were introduced in a water bath at 95 °C for 2 h and, after that, immediately cooled at room temperature. Concurrently, a calibration curve with solutions of 1,1,3,3-tetramethoxypropane (TMP) was prepared ranging from 0.2 to 20.0 μM, which were processed as the samples. Finally, absorbance at 532 nm was measured in a spectrophotometer Hitachi U-2000 (Giralt, Madrid, Spain) using ternary solvent mixture as a blank. Malondialdehyde (MDA) equivalence was calculated from the calibration curve and TBARs level was expressed as mg MDA/kg oil, taking into account the total oil content of each type of microcapsule.

2.2.7.2. *Analysis of volatile compounds*.

Headspace-solid phase microextraction coupled with gas chromatography/mass spectrometry (HS-SPME-GC/MS) was used to analyze the volatile compounds in the fish oil microcapsules during the storage assay. A method based on that described by Garcia-Esteban et al. (Garcia-Esteban et al., 2004) was carried out. Briefly, 1 g of each powder sample were placed in a 20 mL glass vial, screw-capped with a laminated Teflon-rubber disk and kept in a water bath at 60 °C for 25 min to reach the equilibrium of the volatile compounds in the headspace. Consecutively, after perforation of the vial septum with a SPME needle, a 2 cm Carboxen/PDMS/DVB fiber (75 μm thickness, Supelco, Bellefonte, PA, USA) was introduced into the headspace and exposed for 30 min while keeping the vial in the 60 °C water bath. Finally, the GC analysis was performed inserting the fiber into an Agilent 6890 series gas chromatograph (Agilent, Avondale, PA USA) coupled to a mass selective detector (Agilent 5973). The fiber was desorbed for 15 min at 250 °C in the injection port (splitless time: 3 min). The SPME fiber was conditioned prior to use by heating in the injection port of a gas chromatograph (GC) system under the conditions recommended by the manufacturer (at 270 °C for 1 h).

Analytes were separated using a 5% phenyl-methyl silicone (HP-5) bonded phase fused silica capillary column (Hewlett-Packard, 50 m × 0.32 mm i.d., film thickness 1.05 μm), operating at 45 kPa of column head pressure, resulting in a flow of 1.3 mL/min at 40 °C. The temperature program was 40 °C during 10 min, raised to 200 °C at a rate of 5 °C/min and then raised to 250 °C at a rate of 20 °C/min, maintained at this temperature for 5 min. The transfer line to the mass spectrometer was hold at 280 °C. The mass spectra were obtained by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan/s over the m/z range 30–300. n-Alkanes (Sigma R-8769, Sigma-Aldrich, Stainhein, Germany) were analyzed under the same conditions to calculate the retention indices (RI) for the volatiles. The compounds were identified by comparison with the mass spectrum and RI of commercial reference compounds (Sigma-Aldrich), by comparison of mass spectrum and RI with those described on the web NIST Chemistry and WebBook (2014) and by comparison of their mass spectra with those contained in the Wiley library. Results from the volatile analysis are given in area units (AU).

2.2.8. *Sampling replication and statistical analysis*

Replicate experimental samples ($n = 5$) of each batch of emulsions and microcapsules were produced, and analyses were performed in duplicate. The statistical study was done with the program IBM SPSS Statistics v.19. Data of emulsion and microcapsules characteristics at day 0 were analyzed by one-way ANOVA to detect significant differences ($p < 0.05$) related to the type of emulsion (DE vs ME) or microcapsule (DM vs MM). Data of oxidation (TBARs) and volatile compounds were analyzed by one-way ANOVA to detect significant differences ($p < 0.05$) between microcapsules at day 0 and after a month of storage at refrigeration (t_0 vs 4 °C) or room (t_0 vs 20 °C) temperature and between microcapsules after a month of storage at different temperature (4 °C vs 20 °C). Finally, in the same way, it were also analyzed global effects of temperature and type of microcapsule among all the batches. Pearson bivariate correlation was used to compare the relationship between TBARs and each group of volatile compounds and detect significant ($p < 0.05$) correlations among them. A Data Reduction

procedure (Factor analysis) was used to create two-dimensional principal component analysis score plots (PCA) on the selected volatile compounds related to oxidation. The original data were normalized and orthogonal and linear combinations of the original variables were classified depending on the level of information they produced in the first two components: PC1 (the axis, containing the largest possible amount of information of the variance of the data) and PC2 (perpendicular to PC1). The loading of each selected volatile compounds on the two first principal components were plotted and the average scores of the six batches of microcapsules (both types of microcapsules at initial time and after a month of storage at 4 °C and 20 °C) on the two first principal components were plotted. The similar spot locations in both scatter plots were taken into account to analyze the potential relationships between the volatile compounds and the different group of microcapsules.

3. Results and discussion

3.1. *Double and multilayer fish oil emulsion characteristics*

In this work we made DE and ME emulsions that were spray-dried to produce the corresponding DM and MM microcapsules. In the case of the DE, the fish oil is encapsulated in the inner phase of an oil-in-water-in-oil (O/W/O) emulsion. Two emulsifiers were used: sodium caseinate (hydrophilic emulsifier) to stabilize the O/W emulsion and poliglycerol polyricinoleate (hydrophobic emulsifier) to stabilize the W/O emulsion. In the case of the ME, the fish oil is surrounded by multiple layers of an emulsifier and a polyelectrolyte with opposite charges. The multilayer structure was formed by the combination of a coat of lecithin (an anionic emulsifier) and chitosan (a cationic polyelectrolyte) to surround this coat, using maltodextrin as carrier matrix. The use of chitosan in this emulsion required the use of acetic acid as solvent for the emulsion materials, as chitosan is not soluble in neutral or alkali conditions but it is soluble in acidic solutions.

Composition of DE and ME is shown in Table 1. Remarkable differences can be observed, mainly in the percentage of fish oil emulsified, which was lower in DE than in ME (0.625 and 2.50% w/w, respectively) and in the solid content (17.00 and 18.50% w/w, respectively), which also varied the wall material/total oil ratio and the wall material/fish oil ratio. So, in ME all the total oil was fish oil, so that the wall material/total oil ratio was the same as the wall material/fish oil ratio (6.40% w/w), while in DE, with the same total oil but less solid content, the wall material/total oil ratio was lower (5.80% w/w) and the wall material/fish oil ratio was higher (23.10% w/w), due to the fact that only part of the total oil was fish oil, and the rest was extra virgin olive oil from the secondary emulsion. The selection of extra olive virgin oil as the oily phase of the secondary emulsion in DE was made taking into account the low susceptibility to oxidation of this oil, as a consequence of the presence of natural antioxidants (tocopherols, carotenoids and polar phenolic compounds) but also, the healthy FA composition due to the high content of oleic acid (C18:9 n-9) (Velasco and Dobarganes, 2002). Special care is needed with composition, formulation and preparation of the DE to avoid destabilization by bridging, depletion, flocculation or coalescence (Gharsallaoui et al., 2012). Consequently, although the ratio of added fish oil to the primary emulsion in DE (20% w/w) was higher than in ME (10% w/w). In the latter all the primary emulsion was used for the preparation of the secondary and feed emulsions, while the preparation of the secondary emulsion in DE was limited before droplet aggregation occurred, and it was only possible to use 1/4 of the total amount of the primary emulsion prepared. The same total weight of feed emulsion contains higher weight of fish oil in the case of ME compared to DE, as this emulsion has significant quantity of extra olive virgin oil.

The visual separation of an emulsion can be considered a sign of physical instability (Chung and McClements, 2014). The tendency of emulsions to separate into two phases, as a measure of the stability of the feed emulsions, was determined by the creaming index (CI) assay (Table 2) in the feed emulsions of DE and ME, both of them showing no variations after 7 days. There were significant differences ($p = 0.002$) between DE and ME (3.10 vs 0.10%, respectively). Similar results for emulsion stability have been reported previously in multilayered emulsions prepared with lecithin-chitosan (Klinkesorn et al., 2005a). Given that the CI is inversely related to the stability of the emulsions, it seems that ME are more stable than DE. The droplet size in the DE may be slightly higher than that of ME because of the complex composition of DE.

Table 1 – Composition of the double (DE) and multilayered (ME) feed emulsions produced.

	DE	ME
Fish oil (%w/w)	0.625	2.500
Sodium caseinate (%w/w)	5.16	–
Lactose monohydrate (%w/w)	7.44	–
Extra virgin olive oil (%w/w)	1.87	–
Polyglycerol polyricinoleate (%w/w)	1.87	–
Water (%w/w)	83	–
Soybean lecithin (%w/w)	–	0.75
Chitosan (%w/w)	–	0.25
Maltodextrin (%w/w)	–	15.00
Acetic acid 1% (%w/w)	–	81.50
Total weight of feed emulsion (g)	800	800
Solid (%w/w)	17.00	18.50
Wall material/total oil ratio (w/w)	5.80	6.40
Wall material/fish oil ratio (w/w)	23.10	6.40

Table 2 – Characteristics of the double (DE) and multilayered (ME) emulsions and the double (DM) and multilayered (MM) microcapsules at initial time (t_0). Values are expressed as mean ± standard deviation. Statistical significance (*p*-value) for the effect of the type of emulsion (*p*E) and microcapsule (*p*M) are included.

	DE	ME	*p*E
Creaming index (%)	3.10	0.10	<0.001
	DM	**MM**	***p*M**
Moisture (%)	1.89 ± 0.22	4.16 ± 1.92	<0.001
Microencapsulation yield (%)	42.57 ± 5.60	39.76 ± 3.64	0.785
External oil (g/100 g microcapsules)	2.02 ± 0.43	3.60 ± 0.55	0.004
Total oil (g/100 g microcapsules)	4.91 ± 1.82	9.45 ± 1.03	<0.001
Encapsulation efficiency (%)	58.86 ± 5.59	61.90 ± 7.85	<0.001

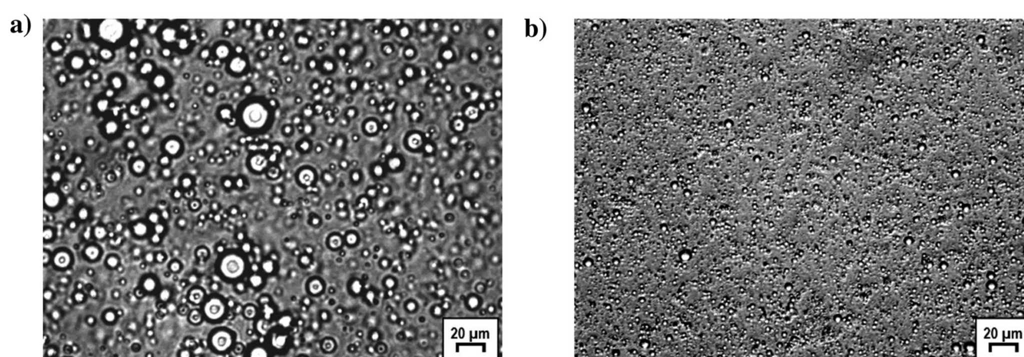

Fig. 1 – Optical microscope images of double (DE) (a) and multilayered (ME) (b) feed emulsions observed at 1000 magnifications.

Moreover, the interfacial membranes stabilized by polyglycerol polyricinoleate (DE) could be less rigid than that stabilized by lecithin and chitosan (ME). Also, the higher physical stability of ME could be ascribed to the chitosan content. At optimal concentration, chitosan prevents the contact between the lecithin layers of different droplets, and also increases the electrostatic forces and the viscosity of the continuous phase of the emulsions, which leads to higher flow resistance and decreases the mobility of the emulsions droplets, avoiding their aggregation and reducing the gravitational separation, leading to lower CI rate (Klinkesorn et al., 2005a). This information is important since the stability of emulsions is related to the subsequent encapsulation efficiency of oils and flavors, the efficiency being higher with better emulsion stability (Jafari et al., 2008).

Fig. 1 shows the microscopic images of the feed emulsions after 24 h of their production. Apparently, no signs of destabilization were observed, indicating the accuracy of the processing of the emulsions. However, visual differences can be noticed between the two types of feed emulsions. ME was apparently formed by a homogeneous scattering of little droplets, while DE appeared to be more heterogeneous, with some droplets of higher size than in the case of ME. Size distribution varied between the two types of emulsions. ME resulted in a monodisperse size distribution with 97% of the droplets being smaller than 2 μm, and only 3% of the droplets between 2 and 7 μm. Similar low particle diameter results were obtained by other authors with the preparation of lecithin-chitosan multilayered emulsions at pH < 5.0, suggesting that these emulsions were stable to droplet aggregation (Klinkesorn et al., 2005a). This is in concordance with the obtained results, as the pH of the multilayered emulsion studied in the present work was determined to be lower than 5.0 in a previous work (Jiménez-Martín et al., 2015). On the other hand, DE was highly heterogeneous, with an interval of droplet size between 1 and 20 μm (27% of the droplets smaller than 2 μm, 41% ranging 2–5 μm, 19% ranging 5–10 μm and 13% ranging 10–20 μm). In a study dealing with orange oil double emulsion prepared using similar procedure to that of the present work (Edris and Bergnstahl, 2001), it was reported 6.4 μm as mean diameter of the droplets, which mainly agrees with the values of DE. It has been proved that the size of the droplets plays an important role into gravitational separation of emulsions. The rate of creaming increases with the square of the particle radius (Chung and McClements, 2014) and thus, stability of the emulsions can be increased by reducing the size of the droplets (McClements et al., 2009). This makes sense with the results obtained, since ME, with smaller droplets was proved to be more physically stable by means of a better CI.

3.2. *Microcapsules characteristics*

3.2.1. *Physicochemical characteristics*

Table 2 presents the characteristics of the microcapsules (moisture, MY, MEE). No significant differences were observed in MY considering the two types of microcapsules (42.57% and 39.76% for DM and MM, respectively). So, it seems that there is no influence of the type of microcapsules or its composition on this characteristic. Quite recently we have found no effect of the microcapsules composition (chitosan concentration) on MY of the microcapsules from multilayer emulsions (Jiménez-Martín et al., 2015). However, other authors have stated differences in the yield of powders due to variations in the preparation procedure and composition (Botrel et al., 2012).

Percentage of moisture was significantly higher in MM (4.16%) in comparison to DM (1.89%). Moisture values were similar to those reported for most dried food powders (Gallardo et al., 2013) and those of the moisture of fish oil microencapsulated by spray-drying found by other authors (2.0–3.7%) (Drusch et al., 2007). It seems that type of emulsion influenced the moisture content of the microcapsules, which in fact, has been mainly related to the composition of the feed emulsions that thereafter constitutes the matrix material in the microcapsules (Gharsallaoui et al., 2012). In the case of DE, the interfacial membranes that stabilize the inner emulsion are not accessible to water. Lower moisture contents of microcapsules are usually related with lower water activity (Klaypradit and Huang, 2008) and associated to a longer shelf life during storage due to the preservation of the physical and chemical characteristics and the stability against microbiological spoilage (Drusch et al., 2007) during and after their final use.

As for the oil content, total oil and also external oil were higher ($p < 0.001$) for the MM (9.45 g/100 g microcapsules and 3.60 g/100 g microcapsules, respectively) than for the DM (4.91 g/100 g microcapsules and 2.02 g/100 g microcapsules, respectively). A significant difference ($p < 0.001$) was found for microencapsulation efficiency (MEE%) between MM and DM (61.90 vs 58.86%, respectively). MEE% is highly variable, with values in the range of 0–95% due to variations on the inner properties of the microcapsules (Pourashouri et al., 2014), such as wall material composition (total solids, viscosity, droplets size of the emulsion) and microencapsulation conditions during spray-drying (Jafari et al., 2008). The reported MEE% values for microcapsules produced from multilayer emulsions varied among different studies. In a study by Pourashouri et al. (2014), the MEE% ranged from a minimum value of 67.35% to a maximum value of 88%. The lower values corresponded to

microcapsules produced from cod liver oil emulsions prepared with chitosan-maltodextrin in similar amounts to those in our study. Thus, the MEE% value for the MM in our study was very similar to those reported values. Higher MEE% can be obtained using materials that can act as emulsifier and film-forming materials, allowing a rapid crust formation in droplets and reducing the diffusion of the entrapped oil to the surface of the particles (Liu et al., 2010).

Differences on MEE% between MM and DM are most likely related to the type emulsions, and specifically to the wall material/oil ratio, which was higher in ME feed emulsions than in DE ones (Table 1). This is in accordance with other studies that have obtained better MEE% with increasing amounts of wall material in the spray-drying process (Ramakrishnan et al., 2013). A higher amount of encapsulated oil in the obtained microcapsules is considered a positive feature (Jafari et al., 2008). In this sense, in MM the encapsulation seems to be better than in DM, since the difference between total oil and external oil, which is the encapsulated oil, is higher in MM, more amount of the oil being therefore protected against the environmental conditions, and so, less exposed to oxidation.

3.2.2. *Oxidative stability during storage*

Results for TBARs values of the microcapsules after storage during 1 month at 4 and 20 °C are shown in Fig. 2. Significant differences ($p < 0.001$) were found between the two types of microcapsules (Supplementary Table 1), with higher TBARs values for DM than for MM in all cases: in the day 0 (4.53 vs 1.64 mg MDA/kg oil, respectively), and after 1 month of storage at 4 °C (5.24 vs 1.50 mg MDA/kg oil, respectively) and 20 °C (4.38 vs 2.64 mg MDA/kg oil, respectively). The significant effect of the type of microcapsule (DM and MM) on TBARs could be related to the previously discussed differences in MEE%. Some authors have reported better oxidation stability in microcapsules with higher MEE% and have related these fact to the increase of the amount of wall material (Ramakrishnan et al., 2013). These results are in accordance with the results obtained in this study, as higher MEE% and also lower TBARs values were found in MM than in DM. As observed in Table 1, also the wall material/total oil ratio was higher in MM than in DM. The higher initial TBARs value for DM compared to that of MM could be explained by the differences in the formulation of the emulsions. The combination of lecithin-chitosan used in ME has previously proved to increase the thickness of the coating that surrounds the oil droplets. This in turn prevents contact with pro-oxidants and keeps the lecithin layers of different droplets separated, making it possible a quite stable structure of the emulsion, with good stability to oxidation and environmental stress (Shaw et al., 2007). Also, maltodextrin, used as wall material in MM, is a polysaccharide that acts as a filler matrix and has the capacity to help forming stable emulsions (Rosenberg et al., 1993), and improves the stability of encapsulated oil providing protection to lipid oxidation (Rubilar et al., 2012).

This could be explained by the further interaction of some of the secondary oxidation products quantified by TBARs with other compounds, like amino compounds, leading to the development of non-enzymatic browning reactions.

In DM and MM, there were found differences between the initial time group (t_0) and the storage microcapsules. DM after a month of storage at 4 °C and MM after a month of storage at 20 °C showed higher TBARs values than their respective microcapsules at initial time. The effect of the temperature of storage was significant, but it did not follow the same trend in DM and MM. DM microcapsules had higher TBARs value at 4 °C than at 20 °C, while MM microcapsules showed the opposite behavior, with the highest TBARs value after the storage at room temperature. Thus, there is a different behavior in the development of oxidation reactions between microcapsules storage at different temperatures. This could be explained by the further interaction of secondary oxidation products quantified by the TBAR method with other compounds, especially amino compounds, leading to the development of non-enzymatic browning reactions (Thomsen et al., 2013). Because of that, it is appropriate to complete the TBARs determination with other oxidation measurements. Thus, in the present work, the volatile oxidation products have been analyzed to have more information about the oxidative stability.

Huss (2011) established a limit of 7–8 mg MDA/kg oil as the maximum concentration of MDA in fish oil for keeping its quality and acceptability for human consumption. In our study, the highest value (5.24 mg MDA/kg oil), measured in the DM stored at refrigeration temperature for 1 month, did not exceed that limit. This result indicates the stability of both types of microcapsules produced, that can be stored at room temperature for a month without having negative effects due to lipid oxidation, not being necessary refrigeration for this length of storage. Boran et al. (2006) analyzed changes in the quality of oils from various species of fishes as influenced by the storage temperature and time, most of the oils being more oxidized than the MM microcapsules of our study after the same time of storage at room temperature. In previous studies, we have analyzed the same fish oil after storage for 12 days at different temperatures (Jiménez-Martín et al., 2015) obtaining values of 2.48 mg MDA/kg oil at 4 °C and 7.17 mg MDA/kg oil at 30 °C. These results indicate that MM are less susceptible to oxidation than DM. So, MM produced by spray-drying of ME provides good protection against oxidation, being an alternative to the storage of bulk fish oil.

3.2.3. *Volatile compounds profile*

A high number of volatile compounds (73) were detected in DM and MM. They were expressed in area units $\times 10^6$ and grouped in the following chemical families: acids, alcohols, aldehydes, aromatic and aliphatic hydrocarbons, ketones and furans (Supplementary Fig. 2). Aliphatic hydrocarbons were the major chemical family in the overall profile of volatile compounds of the DM and MM. In MM, they were followed by acids, alcohols and aldehydes, whereas, in DM, they were followed by alcohols, aldehydes and acids. Lower amounts of other compounds, as aromatic hydrocarbons, ketones and furans were detected in both types of microcapsules. So, there were some statistical differences due to the type of microcapsules and to the storage temperature (Supplementary Table 2). Differences between both types of microcapsules were statistically significant for alcohols (which highly increased in DM at both temperatures, while in MM only slightly increased at 4 °C), ketones (which increased in DM with increasing storage temperature, showing opposite behavior in MM), and furans (which did not suffer any change in DM, whereas in MM increased at 20 °C).

As previously mentioned, at initial time there was detected a high amount of aliphatic hydrocarbons in both types microcapsules, the amounts of the other families of volatile compounds being much lower. The area units of aromatic hydrocarbons, on the contrary, were low. The low amount of this group of volatile compounds can be related to the oxidative behavior of the microcapsules. In fact, aliphatic

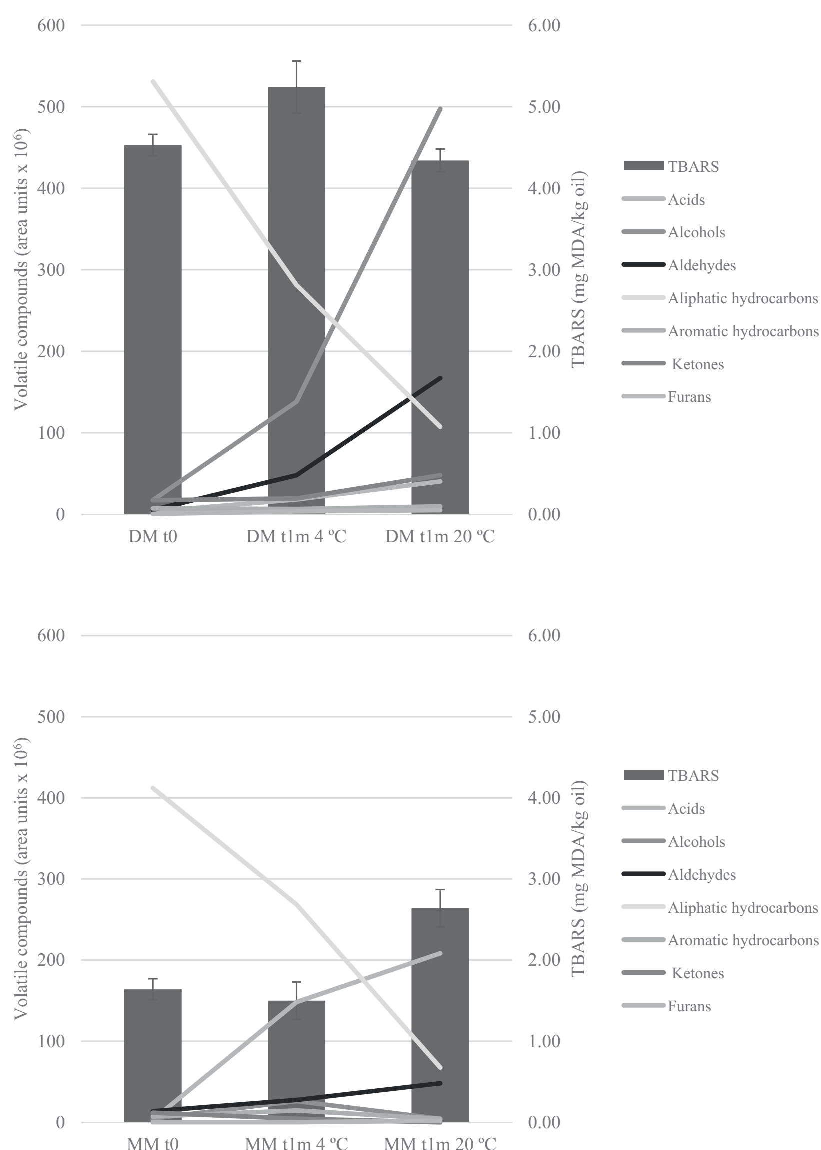

Fig. 2 – Oxidative level expressed as TBARs (mg MDA/kg oil) and profile of volatile compounds (area units × 10^6) in the double (DM) and multiple (MM) at initial time (t_0) and after storage during 1 month at 4 and 20 °C (t_1 m 4 °C and t_1 m 20 °C, respectively).

hydrocarbons can be used as a negative index for measuring the quality of fish products (Giogios et al., 2009). After storage for a month at 4 and 20 °C, aliphatic hydrocarbons decreased in both DM and MM. However, different behavior was found for the total amounts of the other various groups of volatile compounds detected in the two types of microcapsules after storage. In DM, there was a high increase of alcohols at both storage temperatures. At 20 °C, aldehydes were also increased. In MM, there was a high increase of acids at both temperatures. Alcohols increased at 4 °C, but no changes in this group of volatile compounds were detected at 20 °C. At 20 °C, aldehydes and furans were also increased in MM. Also, in MM ketones decreased at both storage temperatures. These results cannot be compared with other studies, as, to the best of our knowledge; there are no reports of the volatile profile of microcapsules from double neither multilayered fish oil emulsions.

Fig. 2 shows the tendency followed by each group of volatile compound in DM and MM, expressed in area units × 10^6, and its correspondence to the results of the secondary oxidation products (TBARs) previously discussed. Pearson bivariate correlation was used to compare the relationship between TBARs and each group of volatile compounds. There was no correlation between TBARs and any of the groups of volatile compounds in DM. On the contrary, in MM, TBARs were strong positively correlated with the amount of aldehydes ($r = 0.781$, $p = 0.001$) and furans ($r = 0.843$, $p < 0.001$) and negatively correlated with aliphatic hydrocarbons ($r = -0.553$, $p = 0.033$).

Opposite behavior showed by aliphatic hydrocarbons and aldehydes in both types of microcapsules can be explained by the oxidation reactions: cleavage of hydroperoxides of FA give place to the formation of carbonyls and, when the oxidation reactions continue during storage, these carbonyls are oxidized into saturated aldehydes (Frankel, 1998). Because of that, the increase of saturated aldehydes (that comprise the majority of the aldehydes of our samples) could be related to a decrease of the aliphatic hydrocarbons. The increase of alcohols in DM can be also associated with lipid oxidation (Jónsdóttir et al., 2005).

3.2.4. *Volatile compounds related to oxidation*

Typical GC–MS chromatograms portraying the characterization of volatile profile of DM and MM can be seen in Supplementary Figs. 3 and 4 respectively. These figures are a comparison of the volatile components and responses found

in both types of microcapsules at initial time and after a month of storage at refrigeration and room temperature. The portion of interest of the total ion chromatograms (TIC) has been enlarged in all the chromatograms in order to show the peaks with lowest area response. Visual differences can be noticed between chromatograms due to the type of microcapsules and storage. Thus, in general, more number of peaks and also higher peaks can be seen in DM (Supplementary Fig. 3) in comparison with MM (Supplementary Fig. 4), both at initial time and after storage at both temperatures. Moreover, for each type of microcapsule, larger number of peaks and peaks with higher areas can be found in the chromatograms after one month of storage at 20 °C (Supplementary Figs. 3c and 4c) compared to the chromatograms of the respective microcapsule after a month of storage at 4 °C (Supplementary Figs. 3b and 4b). Chromatograms with fewest peaks and peaks with the lowest areas are those of the microcapsules at initial time (Supplementary Figs. 3a and 4a).

A total of 28 volatile compounds of interest were chosen as specific indicators of oxidation, labeled in the chromatograms of Supplementary Figs. 3 and 4, and listed in Table 3. The choice was made based on the literature (Giogios et al., 2009; Iglesias et al., 2007; Jacobsen et al., 2000; Jónsdóttir et al., 2005; Medina et al., 1999; Pop, 2011; Roberts et al., 2000; Venkateshwarlu et al., 2004), the height of the peak areas of the eluted compounds and the occurrence in the samples. Most of them have been detected by other authors in fish oils and fish oil related and enriched products (Giogios et al., 2009; Iglesias et al., 2007; Jacobsen et al., 2000; Jónsdóttir et al., 2005; Medina et al., 1999; Venkateshwarlu et al., 2004). The selected oxidation volatile compounds of this study were also grouped in chemical families: alcohols (1-propanol, 1-penten-3-ol, 2,3-butanediol, 2-heptanol, 1,2,4-butanetriol), aldehydes (propanal, pentanal, 2-pentenal, hexanal, 2-hexenal, heptanal, octanal, 2-octenal, nonanal), aliphatic hydrocarbons (ethane, pentane, hexane, heptane, octadecane, docosane), ketones (2-propanone, 3-hydroxy-2-propanone, 1,4-cyclohexadione, 3,5-octadien-2-one, 2-nonanone) and furans (2-ethylfuran, 2-butyltetrahydrofuran, 2-pentylfuran).

Aldehydes are well known to be important indicators of the oxidation of fish oil. Most of the selected aldehydes were only detected in DM and not in any of the MM microcapsules (neither in the initial time ones nor after the storage period). This is the case of heptanal, octenal, nonanal, 2-pentenal and 2-octenal. Also, 2,4-heptadienal, which is a very typical compound derived of fish oil oxidation (Giogios et al., 2009), has not been detected in the samples of this study. Some authors have established that the major volatile aldehyde resulting from the degradation of ω-3 FA is propanal (Serfert et al., 2009), but in this study the major aldehyde detected is hexanal, which decrease has been correlated with the oxidation of linoleic acid (18:2 ω6) (Iglesias et al., 2007). Also, 2-hexenal presented low amount in the analyzed microcapsules, and it has not even been detected in some of them, and only was detected in small amounts in DM at 4 °C and in MM at 20 °C. Among ketones, 3,5-octadien-2-one has been widely detected in fish oil-enriched mayonnaise and milk, and it is strongly correlated with the strength of the oxidation process (Jacobsen et al., 2000; Venkateshwarlu et al., 2004), and indeed, it has been reported to be derived from lipid autoxidation reactions (Roberts et al., 2000). This compound was detected in some microcapsules of this study but it did not show high area units. In relation to alcohols, the most representative alcohol among those detected in our samples was 1-penten-3-ol, which has been described as one of the most characteristic oxidation marker for PUFA (Roberts et al., 2000). Other common oxidation product of ω-3 FA is 2-ethylfuran, which can be formed from the 12-hydroperoxide of EPA and the 16-hydroperoxide of DHA (Medina et al., 1999). As propanal and 3,5-octadien,2-one, 1-penten-3-ol and 2-ethylfuran did not show high levels in the microcapsules of this study. Considering these observations, it seems that the oxidation process taken place during the storage of the microcapsules in this study is not so remarkable.

The effect of type of microcapsule was clearly observed: 27 of the 28 selected oxidation volatile compounds in DM were detected, while MM showed only 11 of these compounds, most of them with lower area units than those in DM. This is in concordance with results on TBARs, and again points out to an improved feasibility of MM for encapsulation of fish oil. In relation to the influence of the temperature of storage in DM, most of the selected volatile oxidation compounds showed lower amount in microcapsules at initial time than in microcapsules stored at 4 and 20 °C. It was also observed higher area units in samples stored at 20 °C than in those stored at 4 °C. Pentane, hexane and 2-propanone showed the opposite behavior, with the lowest amount in microcapsules stored at 20 °C and the highest in the ones at the initial time. MM showed a similar effect of the temperature storage, but it was less remarkable.

PCA was used to identify groups among samples based on peak areas of the volatile compounds related to oxidation (Fig. 3). All 28 chosen volatile compounds were used in this PCA as variables. The loading of each selected volatile compound are shown in Fig. 3a, and the score plots of both types of microcapsules at initial time and after a moth of storage at 4 °C and 20 °C are shown in Fig. 3b. The first principal component (PC1) comprised 61.24% of the total variance, and the second principal component (PC2) comprised 12.40%. The loading plot and the scores plot of the PCA analysis indicate that both types of microcapsules were clearly different in their content of volatile compounds from oxidation. According to the loading plot (Fig. 3a), three main groups of volatile compounds can be distinguished: those with high positive PC1 scores, those with high positive PC2 scores and those with high negative PC2 scores. Many of the selected volatile compounds were grouped and represented in the direction of the right quadrants (both upper and lower), which corresponds to high positive loadings on the PC1. On the other hand, there were only a few of the compounds with high loadings on the PC2: pentane, hexane, heptane and 2-propanone, with high positive loadings (left upper quadrant), and 2-butyltetrahydrofuran and 2-hexenal with high negative loadings (left lower quadrant). The score plot (Fig. 3b) shows that DM at initial time and both types of microcapsules stored at 20 °C were separated from the other groups of samples, which remained all together. This seems to indicate that the samples of DM at initial time, with high positive scores in the PC2, may be distinguished by its content of pentane, hexane, heptane and 2-propanone. In the case of the microcapsules stored at 20 °C, there is a clear different behavior caused by the type of microcapsule. So, DM stored at 20 °C showed high positive scores in the PC1, while MM stored at 20 °C showed high negative scores in the PC2. So, DM stored at room temperature may be associated to the main group of volatile compounds related to oxidation, whereas the key role of the separation of MM stored at room temperature seemed to be the compounds 2-butyltetrahydrofuran and 2-hexenal. As MM had higher content of fish oil, the compounds associated to MM after storage at room temperature are also expected to be compounds more related to the specific

Table 3 – Volatile compounds related to oxidation of the double (DM) and multilayered (MM) microcapsules at initial time (t_0) and after 1 month of storage at 4 °C (t_1 m 4 °C) and 20 °C (t_1 m 20 °C). Values are expressed as peak area × 10^6. Global effect of the type of microcapsule (*p*M) among all the values and storage temperature (*p*T[a]) for both double and multilayer microcapsules were analyzed. Differences between microcapsules at initial time and microcapsules stored at 4 °C (*p*4 °C) and 20 °C (*p*20 °C) were also detected for each type of microcapsule.

IK	ID	Compound	Double microcapsules (DM)						Multilayered microcapsules (MM)						
			t_0	t_1 m 4 °C	t_1 m 20 °C	*p*4 °C	*p*20 °C	*p*T[a]	t_0	t_1 m 4 °C	t_1 m 20 °C	*p*4 °C	*p*20 °C	*p*T[a]	*p*M
Alcohols															
555	B	1-Propanol	n.d.	0.23	0.45	0.074	0.061	0.196	n.d.	n.d.	n.d.	–	–	–	0.007
681	B	1-Penten-3-ol	n.d.	1.69	35.12	0.149	0.197	0.121	n.d.	0.16	0.70	0.347	0.209	0.340	0.137
782	B	2,3-Butanediol	0.40	17.10	146.70	0.357	0.086	0.058	n.d.	n.d.	n.d.	–	–	–	0.046
856	B	2-Heptanol	n.d.	n.d.	1.29	–	0.081	0.031	n.d.	n.d.	1.28	–	0.347	0.347	0.946
933	C	1,2,4-Butanetriol	n.d.	n.d.	0.07	–	0.407	0.297	0.20	n.d.	n.d.	0.065	0.065	–	0.380
Aldehydes															
502	B	Propanal	n.d.	2.97	9.57	0.080	0.137	0.167	n.d.	n.d.	0.38	–	0.090	0.090	0.041
699	B	Pentanal	0.19	2.73	15.14	0.015	0.054	0.038	2.15	2.33	10.43	0.736	0.001	0.001	0.615
750	B	2-Pentenal	n.d.	0.33	9.51	0.407	0.121	0.060	n.d.	n.d.	n.d.	–	–	–	0.086
800	A	Hexanal	2.32	21.08	76.63	0.048	0.074	0.073	2.64	5.07	19.89	0.074	<0.001	0.001	0.064
862	B	2-Hexenal	n.d.	0.12	n.d.	0.407	–	0.389	n.d.	n.d.	4.22	–	0.010	0.010	0.061
901	A	Heptanal	0.72	3.50	7.73	0.235	0.150	0.231	n.d.	n.d.	n.d.	–	–	–	0.010
1002	A	Octanal	0.14	2.81	17.97	0.087	0.019	0.010	n.d.	n.d.	1.41	–	0.007	0.007	0.018
1061	B	2-Octenal	n.d.	n.d.	3.29	–	0.073	0.027	n.d.	n.d.	n.d.	–	–	–	0.069
1108	B	Nonanal	0.52	5.80	20.45	0.036	0.006	0.006	n.d.	n.d.	n.d.	–	–	–	0.002
Aliphatic hydrocarbons															
617	B	Ethane	30.13	34.84	39.66	0.937	0.736	0.997	35.14	12.68	26.00	0.275	0.631	0.457	0.270
500	A	Pentane	16.35	7.75	2.05	0.152	0.030	0.058	24.35	13.36	1.57	0.005	<0.001	<0.001	0.298
600	A	Hexane	201.65	134.73	8.13	0.217	0.015	<0.001	111.39	134.39	n.d.	0.560	0.006	<0.001	0.340
700	A	Heptane	0.18	n.d.	2.23	0.030	0.349	0.194	n.d.	n.d.	n.d.	–	–	–	0.186
1052	B	Octadecane	n.d.	n.d.	0.64	–	0.107	0.046	n.d.	n.d.	n.d.	–	–	–	0.090
1103	B	Docosane	n.d.	n.d.	2.36	–	0.079	0.030	n.d.	n.d.	n.d.	–	–	–	0.073
Ketones															
<500	C	2-propanone	7.50	n.d.	0.46	0.013	0.019	0.297	4.74	1.16	0.01	0.072	0.006	0.351	0.682
711	B	3-hydroxy-2-butanone	5.51	15.58	13.61	0.316	0.437	0.954	n.d.	n.d.	n.d.	–	–	–	0.006
1034	B	1,4Cyclohexanedione	n.d.	n.d.	12.25	–	0.033	0.009	n.d.	n.d.	n.d.	–	–	–	0.045
1073	A	3,5-Octadien, 2-one	n.d.	0.34	6.37	0.407	0.015	0.004	n.d.	n.d.	n.d.	–	–	–	0.023
1093	A	2-Nonanone	n.d.	0.42	10.09	0.407	0.005	0.001	n.d.	n.d.	n.d.	–	–	–	0.017
Furans															
705	A	2-ethylfuran	0.47	2.72	3.90	0.305	0.123	0.488	0.04	0.03	0.77	0.927	0.012	0.011	0.017
893	B	2-butyltetrahydrofuran	n.d.	n.d.	n.d.	–	–	–	n.d.	n.d.	0.91	–	0.007	0.007	0.048
992	B	2-pentylfuran	n.d.	0.73	1.08	<0.001	0.011	0.146	n.d.	n.d.	0.22	–	0.007	0.007	0.001

n.d., not detected; IK, linear retention index; ID, method of identification: A, mass spectrum and IK identical to a reference compound; B, mass spectrum and IK in accordance withliterature; C, tentative identification by mass spectrum.

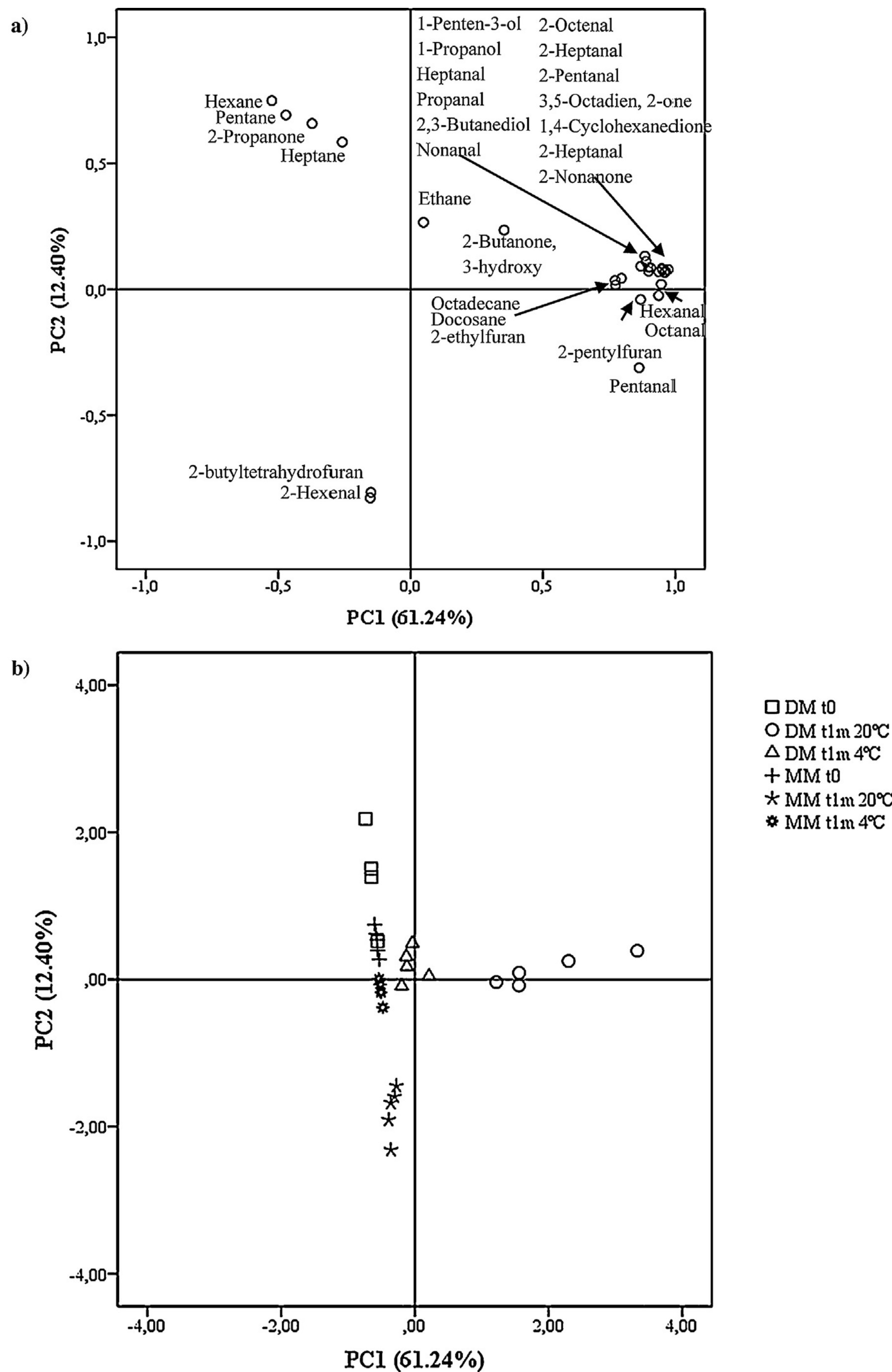

Fig. 3 – Principal component analysis (PCA) of the volatile compounds related to oxidation. The plots represent, for the two first principal components, the loading of each volatile compound (a) and the average scores (b) of double microcapsules (DM) and multilayered microcapsules (MM) at initial time (t_0) and after a month of storage at 4 °C (t_1 m 4 °C) and 20 °C (t_1 m 20 °C) on the two first principal components.

oxidation of fish oil. In fact, 2-hexenal has been reported as a possible marker of oxidation in fish-oil enriched milk emulsions (Venkateshwarlu et al., 2004). On the other hand, DM seem to be related to unspecific compounds from lipid oxidation, or compounds arising from the oxidation of oleic acid (the major one in olive oil) such as octanal o nonanal.

4. Conclusions

Microencapsulation of fish oil by spray-drying using double and multilayered emulsions successfully controls its susceptibility to oxidation. Multilayered emulsions appear as more suitable, since they can hold higher amounts of fish oil than double emulsions, and show better physical stability. The production of double emulsions and multilayered emulsions is simple, as it implies only homogenization and mixing processes and it offers several improved characteristics on physical and chemical stability of the microencapsulated components with the use of food-grade materials. Microcapsules produced by spray-drying from multilayered emulsions seem to be more appropriate in terms of oxidation during storage than those produced from double emulsions. These advantages make microcapsules produced from multilayered emulsions a promising approach to provide protection against oxidation of fish oil during storage and further technological use as a source of ω-3 for enrichment purposes.

Acknowledgment

Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo for supporting the pre-doctoral grant (PD10026), equipment funding and support to research groups (GR10180). Biodymia Laboratory (University of Lyon) for making possible the development of the work during a research stay. "Biomega Natural Nutrients S.L." for kindly providing the fish oil and "Trades S.A." for kindly providing the chitosan, both used during the experiments.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fbp.2015.07.005.

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Capítulo 1.3

Fatty acid composition in double and multilayered microcapsules of ω-3 as affected by storage conditions and type of emulsions

Food Chem 194, 476-486 (2016)

Food Chemistry 194 (2016) 476–486

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fatty acid composition in double and multilayered microcapsules of ω-3 as affected by storage conditions and type of emulsions

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ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 21 July 2015
Accepted 13 August 2015
Available online 14 August 2015

Keywords:
Multilayer emulsions
Double emulsions
Spray-dried microcapsules
ω-3 fatty acids
Storage

ABSTRACT

Spray-dried microcapsules from double (DM) and multilayered (MM) fish oil emulsions were produced to evaluate the effect of type of emulsion on the fatty acid composition during the microencapsulation process and after one month of storage at refrigeration (4 °C) and room (20 °C) temperature. Encapsulation efficiency, loading and loading efficiency were significantly higher in MM than in DM. C20:5 n-3 (EPA) and C22:6 n-3 (DHA) showed higher proportions in MM than in DM. Some differences in microstructural features were detected, with DM showing cracks and pores. The influence of the storage was significant, decreasing the content of polyunsaturated fatty acids in both MM and DM, above all at 20 °C. This decrease was more notable in DM. Multilayered emulsions are more suitable to encapsulate fish oil in terms of quantity of encapsulated oil, microstructure of the microcapsules and protection of fatty acids, especially EPA and DHA, during storage.

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1. Introduction

The interest in the intake of ω-3 long-chain polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), has increased in the last decades, due to its bioactive beneficial effects for human health (Pourashouri et al., 2014). The main sources of EPA and DHA in diet are marine lipids. However, due to the current life style, the consumption of food with high EPA and DHA content, such as salmon or sardine, is not so high to get levels of these ω-3 fatty acids that produce positive healthy effects. Consequently, the enrichment in DHA and EPA of commonly consumed food, mainly ready-to-eat and ready-to-cook products, is a hot topic nowadays in food technology (Kaushik, Dowling, Barrow, & Adhikari, 2014). The limiting factor for the enrichment in ω-3 fatty acids is their high susceptibility to oxidation. As a consequence of such oxidative phenomena, there is a decrease in the ω-3 fatty acids due to their degradation. Moreover, this can result in the formation of harmful compounds (Zunin, Boggia, Turrini, & Leardi, 2015) and degradation products leading to off-flavours and other sensory alterations (Pourashouri et al., 2014), risking the acceptance of the product by consumers. Thus, susceptibility to oxidation of ω-3 fatty acids make their storage an important challenge (Augustin et al., 2014).

Various researchers have studied the production of solid particles by microencapsulation technology as a potential solution to avoid the degradation of fish oil as source of ω-3 fatty acids (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005; Pourashouri et al., 2014). Fatty acid degradation can be minimized with the fast encapsulation of the ω-3 oil-in-water emulsions, that would lead to powders in which ω-3 fatty acids can be effectively protected by the microcapsule wall (Calvo, Castaño, Lozano, & González-Gómez, 2012).

Microencapsulation of ω-3 fatty acids into a wall material that constitutes a protective barrier delay lipid autoxidation, as mobility of free radicals in microcapsules is limited by the wall. This decreases the propagation of the oxidation reactions, and oxygen diffusion is slower in the dehydrated matrix of the wall material than in bulk oil (Ishido, Minemoto, Adachi, & Matsuno, 2003). The feasibility of the microencapsulation process is determined by the encapsulation efficiency and the stability of the ω-3 oil microcapsules upon storage, which in turn depends on the type and composition of feed emulsions to be spray-dried (Kaushik et al., 2014). Thus, besides the drying and storage conditions, the

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http://dx.doi.org/10.1016/j.foodchem.2015.08.046
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characteristics of the ω-3 oil and the wall material, as well as their ratio and the type of emulsions are important factors that influence the stability of ω-3 microcapsules (Ishido et al., 2003).

Microencapsulation by spray-drying is currently the most used microencapsulation technology due to its relatively low cost, also being not much time consuming and easier to use than other techniques, such as spray-cooling, spray-chilling, air suspension coating, extrusion, centrifugal extrusion, freeze-drying, and coacervation, among others (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Researchers have tested spray drying to produce ω-3 oil microcapsules from a number of emulsions composed by wall materials such as proteins, carbohydrates, lipids and gums, with specific formulations to obtain the optimal characteristics (Kaushik et al., 2014), with the objective of protecting the ω-3 fatty acids from degradation during storage and the further enrichment of foods (Augustin et al., 2014; Gharsallaoui et al., 2007).

Moreover, as a substitute to traditional feeding emulsions, some alternatives, such as multiple or multilayered emulsions have also been developed (Grigoriev & Miller, 2009; Shaw, McClements, & Decker, 2007). Multiple oil-in-water-in-oil (O1/W/O2) emulsions are prepared with two emulsifiers: a hydrophilic emulsifier that stabilizes the O1/W interface and a hydrophobic emulsifier that stabilizes the O2/W interface. In these emulsions, the encapsulated oil is in the inner oil phase (O1), and another oil of the same or different nature (O2) is added to the emulsion as an external protective phase (Edris & Bergnstahl, 2001; O'Dwyer, O'Beirne, Ni Eidhin, Hennessy, & O'Kennedy, 2013; O'Dwyer, O'Beirne, Eidhin, Hannon, & O'Kennedy, 2013). Multilayered emulsions have recently been successfully used for encapsulation of ω-3 oils (Grigoriev & Miller, 2009; Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz Carrascal, & Antequera Rojas, 2015; Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006; Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005a, 2005b; Klinkesorn et al., 2005; Shaw et al., 2007). As its name indicates, these are emulsions in which the oil is surrounded by multiple layers of coating material stabilized by electrical charges.

After spray-drying of the emulsions, the total oil in the microcapsules can be classified in two lipid fractions based on their location: a fraction of external, free or non-encapsulated oil (a minor fraction that is located on the surface, in pores and in oil droplets close to the surface) and a fraction of encapsulated oil (the major fraction), that is embedded in the inner matrix of the microcapsules (Drusch & Berg, 2008). Usually, total, external and/or encapsulated oil are separated from the microcapsules by various extraction methods and weighted in order to calculate encapsulation efficiency or analyzed to provide fatty acid composition of the microcapsules. However, there are no studies addressing the potential effect of storage on the changes in the fatty acid profile of the ω-3 oil microcapsules.

The main objective of this study was to analyze for first time the suitability of double and multilayered emulsion to produce spray-dried microcapsules of fish oil, determining the effect of both, type of emulsion (double vs. multilayered) and the storage at refrigeration and room conditions, on the fatty acid composition of spray-dried microcapsules. The influence of the type of emulsion on encapsulation efficiency, loading efficiency and microstructure of the microcapsules was also analyzed.

2. Material and methods

2.1. Material

Cod liver oil, stabilized with tocopherols (E-306) (0.40 g/100 g oil) and with an initial peroxide value <10 meq/kg (kindly provided by Biomega Natural Nutrients S.L., Galicia, Spain), was used as source of ω-3 FA. Sodium caseinate (kindly provided by Anvisa S.A., Madrid, Spain), lactose monohydrate (Scharlau, Sentmenat, Spain), extra virgin olive oil (Hacendado, Madrid, Spain), polyglycerolpolyricinoleate (kindly provided by Cargill, Barcelona, Spain), soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, kindly provided by Roquette-Laisa, Spain) and glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions. Hydrochloric acid and petroleum ether (Scharlau, Barcelona, Spain) were used for the oil extraction of the microcapsules. For the oxidative stability, 1-butanol and isopropanol (Scharlau, Barcelona, Spain) were purchased as solvents and 2-thiobartituric acid (TBA, Serva, Heidelberg, Germany), trichloroacetic acid (Fisher, Barcelona, Spain) and 2,6-di-tert-butyl-4-methylphenol 99% (BHT, Across Organics, Madrid, Spain) as reagents.

2.2. Experimental design

Two different types of fish oil emulsions were prepared: double and multilayered. From these emulsions the corresponding double and multilayered microcapsules (DM and MM, respectively) were produced as described below. The microcapsules were subjected to a storage trial during 1 month at different temperatures (4 and 20 °C) in order to simulate refrigeration and room temperature conditions, respectively. One batch of each type of microcapsules was also analyzed before starting the storage (time = 0) and they were considered the control batches.

2.3. Methods

DE were prepared following the method of Edris and Bergnstahl (2001) with some modifications, whereas ME formulation was optimized in a previous work (Jiménez-Martín et al., 2015).

2.3.1. Preparation of fish oil double emulsions (DE)

2.3.1.1. Primary emulsions. To start with primary emulsion, sodium caseinate (6 g), used as hydrophilic emulsifier, and distilled water (74 g) were mixed in a magnetic stirrer overnight. Then, fish oil (20 g) was added and homogenized (20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Staufen, Germany). In this way, the primary emulsion (20% fish oil and 6% sodium caseinate) was obtained.

2.3.1.2. Secondary emulsions. To obtain the secondary emulsions, two different solutions were previously prepared. In the first place, a diluted primary emulsion was prepared adding 25 g of the primary emulsion to 25 g of lactose monohydrate (14%, w/w) mixing with a magnetic stirrer. Then, an oily solution was prepared with 15 g of extra virgin olive oil and 15 g of polyglycerol polyricinoleate, a hydrophobic emulsifier, and subsequently mixed with a magnetic stirrer. Finally, the secondary emulsion was produced by dropping the diluted primary emulsion on to the oily solution, which was continuously agitated by magnetic stirrer.

2.3.1.3. Feed emulsions. The feed emulsion (800 g) was obtained by mixing the 80 g of the secondary emulsion with 624 g of distilled water containing 56 g of lactose monohydrate and 40 g of sodium caseinate (previously mixed in a magnetic stirrer overnight).

2.3.2. Preparation of fish oil multilayered emulsions (ME)

2.3.2.1. Primary emulsions. First, fish oil (20 g) and lecithin (6 g) were mixed in a magnetic stirrer overnight. Then, acetic acid 1% (w/w) was added until a total weight of 200 g and homogenized

(20,000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA). In this way, the primary emulsion (10% fish oil and 3% lecithin) was obtained.

2.3.2.2. Secondary emulsions. The secondary emulsion was obtained by blending primary emulsion with 200 g chitosan 1% (w/w) dissolved in acetic acid 1%.

2.3.2.3. Feed emulsions. Multilayered feed emulsions were produced by mixing secondary emulsions with 400 g of maltodextrin (30% w/w in acetic acid 1% w/w).

2.3.3. Preparation of microencapsulated fish oil powders

Both types of feed emulsions (800 g) were dried in a laboratory scale spray-drier equipped with a 0.5 mm nozzle atomizer (Mini spray-dryer B-290, Buchi, Flawil, Switzerland). The emulsions, kept at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray-drying process. Spray drying conditions were selected based on a previous work (Jiménez-Martín et al., 2015). The aspirator rate was adjusted at 80%, feed rate was 1 L/h, inlet temperature was 180 °C and outlet temperature of 85–90 °C. Two types of microcapsules were obtained: double (DM) and multilayered (MM). The collected dried powders used as control batch were analyzed immediately after their production. The batches used for the storage assay were stored at 4 and 20 °C immediately after their production, in order to simulate refrigeration and room conditions, respectively. Powders were protected from light and moisture by storing them in sealed plastic tubes.

2.3.4. Analysis of the microcapsules

2.3.4.1. Scanning electron microscopy. The morphology of the microcapsules was examined with a scanning electron detector microscope FEI QUANTA 3D FEG (FEI Company, Hillsboro, USA) in high vacuum conditions mode using EDT (Everhart Thornley Detector). Powder samples were mounted on stubs, fixed with a double adhesive coated carbon conductive adhesive sheet, and then subjected to metallization (sputtering) with a thin layer of a conductive gold coating for 8 s in order to amplify the secondary electron signal. After metallization, the samples were imaged operating at 3 kV with focused electron beam of Ga + (current of <6e^{-4} Pa) and observed with magnifications comprised between 5000 and 15,000.

Table 1
Fatty acid profile (g/100 g FAME) of the two types of oil used for the production of the microcapsules.

Fatty acid (g/100 g FAME)	Fish oil	Extra virgin olive oil
C14	8.74 ± 0.95	0.04 ± 0.00
C14:1	0.20 ± 0.00	0.00 ± 0.00
C16	20.16 ± 1.74	8.29 ± 0.87
C16:1	1.82 ± 0.06	0.34 ± 0.00
C17	3.54 ± 0.09	0.10 ± 0.00
C17:1	0.12 ± 0.00	0.11 ± 0.00
C18	3.68 ± 0.18	2.35 ± 0.10
C18:1	15.81 ± 0.95	81.41 ± 1.94
C18:2 n-6	2.42 ± 0.10	6.16 ± 1.05
C18:3 n-6	2.65 ± 0.09	0.00 ± 0.00
C18:3 n-3	1.93 ± 0.04	0.37 ± 0.00
C20	0.36 ± 0.00	0.35 ± 0.00
C20:1	2.77 ± 0.06	0.26 ± 0.00
C20:3 n-3	1.89 ± 0.01	0.00 ± 0.00
C20:4 n-6	1.65 ± 0.01	0.00 ± 0.00
C20:5 n-3	6.90 ± 0.62	0.00 ± 0.00
C22:0	0.09 ± 0.00	0.16 ± 0.00
C22:1	0.28 ± 0.00	0.00 ± 0.00
C24	0.40 ± 0.01	0.06 ± 0.00
C22:6 n-3	24.59 ± 1.42	0.00 ± 0.00
∑SFA	36.97 ± 1.90	11.35 ± 0.83
∑MUFA	21.00 ± 1.56	82.12 ± 1.90
∑PUFA	42.03 ± 1.87	6.53 ± 0.85
n-6/n-3	0.26 ± 0.00	16.65 ± 0.97
SFA/UFA	0.59 ± 0.01	0.13 ± 0.00
MUFA/PUFA	0.50 ± 0.02	12.58 ± 0.72

2.3.4.2. Extraction of total oil. For the extraction of total fish oil in the microcapsules, 5 g of the powders were placed into a sealed flask with 50 g of hydrochloric acid 1 M and, after digestion during 20 min at 96 °C, filtration was carried out using Whatman no 1 filter. Then, oil was extracted from the filter and quantified as described for external oil.

2.3.4.3. Extraction of external oil. For the quantification of the external fish oil in the microcapsules, 2 g of the powders were placed into an Erlenmeyer flask with 25 mL of petroleum ether, and softly stirred. The flask was sealed using a cap with an air condenser and introduced in a bath at 65 °C during 20 min. After that, filtration was carried out using Whatman no 3 filter. Finally, solvent was evaporated as described before for the total oil, and external oil content was determined gravimetrically and expressed as g of external oil/100 g of microcapsules.

2.3.4.4. Extraction of encapsulated oil. Encapsulated oil was extracted from the residue remaining in the Whatman filter after removal of external oil, following the method described for extraction of total oil.

2.3.4.5. Fatty acid analysis. *2.3.4.5.1. Fish oil and extra virgin olive oil.* The fatty acid methyl esters (FAMEs) of samples of bulk fish and extra virgin olive oils (10 mg) were obtained by acidic transesterification following the method described by Sandler and Karo (1992). FAMEs were analyzed by gas chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethylene glycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA, USA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). The GC oven program temperature was as follows: initial temperature of 180 °C, 5 °C/min to 200 °C; 40 min at this temperature and thereafter 5 °C/min to 250 °C, and then kept for an additional 21 min. The injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO, USA). Peak areas were measured and FAMEs were expressed as percentage of total FAMEs (%). Table 1 shows the fatty acid composition of the two oils used in the experiments for the preparation of the emulsions before spray drying

2.3.4.5.2. Total oils from the microcapsules. The FAMEs of the total oil from DM and MM were obtained applying transesterification method directly to the microencapsulate powders (instead of to extracted oil) for the determination of the fatty acid composition, as suggested by Costa de Conto, Fernandes, Grosso, Eberlin, and Gonçalves (2013), who recommended avoiding a previous fat extraction. Briefly, 100 mg of microcapsules were weighed and treated following the acidic transesterification method of Sandler and Karo (1992) and analyzed as described for bulk oils.

2.3.4.5.3. External and encapsulated oils. External oil (10 mg) extracted with petroleum ether as described in 2.3.4.2 was transesterified and analyzed following the method described for bulk oils in 2.3.4.5.1. For the analysis of encapsulated oil, 100 mg of samples remaining in the Whatman filters after removal of external oil were weighted, treated and analyzed following the method described for fatty acid analysis of total oils in 2.3.4.5.2.

2.3.4.6. Encapsulation efficiency of total oil, EPA and DHA. The amount of EPA and DHA in encapsulated and in total oil was

obtained from FAMEs analysis. The amount of EPA and DHA in MM was calculated taking into account proportion of EPA and DHA in the total oil extracted.

$$\text{Encapsulation efficiency (\%) (EE)} = (\text{weight of encapsulated oil/weight of total oil}) \times 100$$

where weight of encapsulated oil is the amount of encapsulated oil (for total oil EE) or encapsulated EPA or DHA (for EE of EPA and DHA, respectively) extracted from the microcapsules; and weight of total oil is the amount of total oil extracted from the microcapsules.

Encapsulation efficiency of EPA and DHA was calculated differently for DM and MM. Thus, in DM it was obtained by the data from the fatty acid analysis of encapsulated oil and total oil, while in MM, it was calculated the proportion of encapsulated EPA and DHA in encapsulated oil with data of fatty acid analysis of EPA and DHA in total oil and encapsulation efficiency value of total oil.

2.3.4.7. Loading and loading efficiency of total oil, EPA and DHA. Loading and loading efficiency of total oil, EPA and DHA was calculated in the DM and MM microcapsules obtained at initial time.

$$\text{Loading (\%)} = (\text{weight of oil/ weight of microcapsules}) \times 100$$

where weight of oil is the amount of total oil extracted (for Total Oil Loading) or the amount of EPA or DHA on the total oil extracted (for EPA and DHA Loading, respectively); and weight of the microcapsules is the amount of microcapsules used for extraction of the total oil.

$$\text{Loading efficiency (\%) (LE)} = (\text{weight of encapsulated oil/weight of microcapsules}) \times 100$$

where weight of encapsulated oil is the amount of encapsulated oil extracted (for Total Oil LE) or the amount or EPA or DHA on the encapsulated oil (for EPA and DHA LE, respectively); and weight of the microcapsules is the amount of microcapsules used for extraction of the encapsulated oil.

2.3.4.8. Remaining of EPA and DHA during storage. The remaining of EPA and DHA in DM and MM after storage at 4 and 20 °C was calculated from analysis of FAMEs in total oil with the following equations:

$$\text{Remaining at } T\,^{\circ}\text{C} = (\text{weight of } \omega\text{-3 FA at } T\,^{\circ}\text{C/weight of } \omega\text{-3 FA at initial time}) * 100$$

where T °C corresponds to each of the temperatures of the storage assay (4 and 20 °C) and FA corresponds to each of the fatty acids of interest (EPA and DHA).

2.3.5. Sampling replication and statistical analysis

Replicate experimental samples ($n = 5$) of each batch of emulsions and microcapsules were produced, and analyses were performed in duplicate. The statistical study was done with the program IBM SPSS Statistics v.19. One-way ANOVA was conducted to analyze the effect of the type of microcapsules (DM vs MM) on efficiency, loading and fatty acid profile, and also the influence of (i) storage at room or refrigeration during a month, (ii) the temperature of storage (4 vs 20 °C), and (iii) oil location (external vs encapsulated) on the fatty acid composition of microcapsules.

A Data Reduction procedure (Factor analysis) was used to create two-dimensional principal component analysis score plots (PCA) on the fatty acids from the extracted oils. The original data were normalized and orthogonal and linear combinations of the original variables were classified depending on the level of information they produced in the first two components: PC1 (the axis, containing the largest possible amount of information of the variance of the data) and PC2 (perpendicular to PC1). The loadings of each fatty acid on the two first principal components were plotted and the average scores of the six batches of microcapsules (both types of microcapsules at initial time and after a month of storage at 4 and 20 °C) on the two first principal components were plotted. The similar spot locations in both scatter plots were taken into account to analyze the potential relationships between the different fatty acids and the different types of microcapsules.

3. Results and discussion

3.1. Scanning electron microscopy

Analysis of the SEM images of the microcapsules (Fig. 1) revealed that DM and MM powders were characterized by their polydisperse particle size, as it is usual in the microcapsules produced by spray-drying (Carneiro, Tonon, Grosso, & Hubinger, 2013). As for the surface and morphology, they could be defined as skin forming particles (the most common of spray-dried food morphologies) (Walton & Mumford, 1999), with similar spherical morphology regardless of the differences in composition. Polavarapu, Oliver, Ajlouni, and Augustin (2012) also obtained particles with similar morphology when testing different compositions of feed emulsions. Also, in both DM and MM, there were visible wrinkles (dents) or dimples on the surface, that have been attributed to rapid shrinkage of the emulsion drops during atomization in the drying process (Jimenez, García, & Beristain, 2006). Although this wrinkles and dimples can have an adverse effect on the flowing ability of the powder, no effect on the stability is expected (Rosenberg, Kopelman, & Talmon, 1985).

Despite the similarities in the general morphology of the powders, also some differences in appearance and micro-structural features were detected between DM and MM. Thus, in the overall images, it could be observed that DM were more agglomerated than MM, and that MM included a higher number of particles with smooth spherical surface (Fig. 1). On the contrary, more signs of particle collapse or shriveling could be detected in DM ones. These features have been related to the composition of the encapsulating material and to the drying parameters (Rubilar et al., 2012). As drying conditions were the same during formation of the microcapsules, such differences are expected to be caused by the composition and structure (multiple or multilayered) of the emulsions. However, it is difficult to establish a clear link between the morphology of the microcapsules and specific variables of their production, such as the nature and composition of the materials of the emulsions or the spray-drying process parameters (Bhandari, Patel, & Chen, 2009).

The micrographs also showed that there are neither cracks nor pores present on the surface of the MM microcapsules, which is expected to contribute to the protection of the core material by decreasing oxygen diffusion into the microcapsules (Edris & Bergnstahl, 2001). On the other hand, some DM capsules were broken or damaged (Fig. 1); the presence of visible cracks and pores or small holes, might be a consequence of the aeration of the emulsion previous to the spray drying process, poor film-forming and/or uneven shrinkage of the material during the final drying period (Polavarapu et al., 2012).

Shaw et al. obtained microcapsules with similar good visual appearance using multilayered ω-3 emulsions produced with the combination of lecithin–chitosan layers resembling those of the MM in the present study, but adding corn syrup instead of maltodextrin (Shaw et al., 2007). However, Sheu and Rosenberg

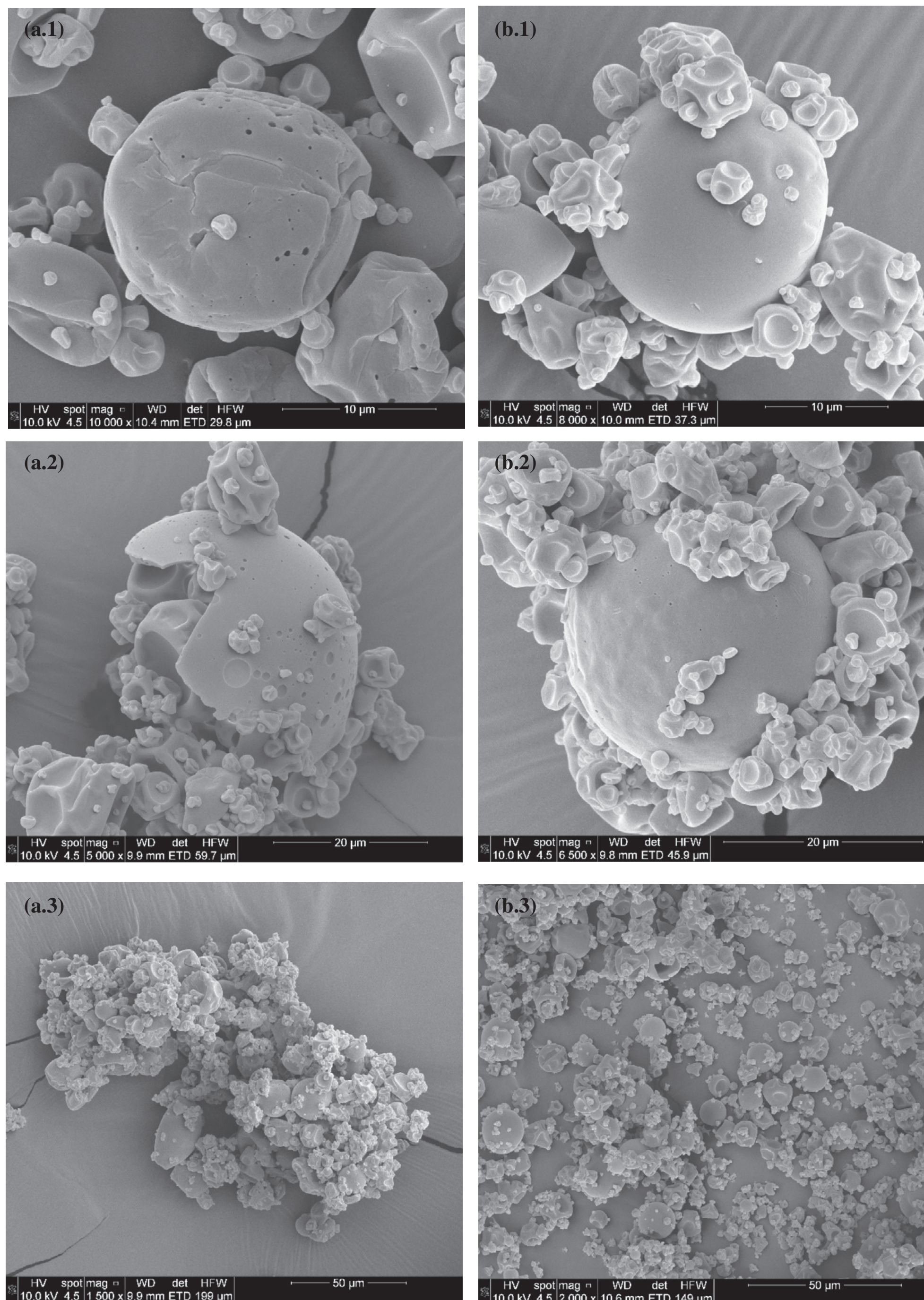

Fig. 1. Microscopic SEM images of spray-dried microcapsules observed with 1500–10,000 magnifications a.1, a.2 = individual image of a double microcapsule (DM); a.3 = overall image of double microcapsules (DM); b.1, b.2 = individual image of a multilayered microcapsule (MM); b3.1 = overall image of multilayered microcapsules (MM).

(1998) reported that maltodextrin improves the drying properties of the wall matrix, probably by enhancing the formation of a dried crust around the drying droplets. So, MM capsules show features pointing out to a good stability and protection to the ω-3 oil core, while in DM ones the presence of pores could decrease the protective effect of the core (Carneiro et al., 2013).

3.2. Encapsulation efficiency, loading and loading efficiency

Fig. 2 shows encapsulation efficiency (EE), loading and loading efficiency (LE) of the total oil, of EPA and of DHA of the microcapsules produced.

EE is a value that indicates the amount of the total oil that is actually efficiently encapsulated. That is, the encapsulated oil embedded in the matrix of the powder. Thus, EE calculation implies the quantification of the different oil fractions of the microcapsules: external, encapsulated and total. EE of the total oil was significantly higher ($p < 0.001$) in MM (61.90%) than in DM (58.86%). Velasco, Holgado, Dobarganes, and Márquez-Ruiz (2009) obtained similar results (around 70% of EE) in the microencapsulation of fish oil with milk proteins by freeze drying. The value for EE is important, as the superficial lipids might be more prone to oxidation due to their location in the microcapsule, which makes them more exposed to air. However, EE can only explain

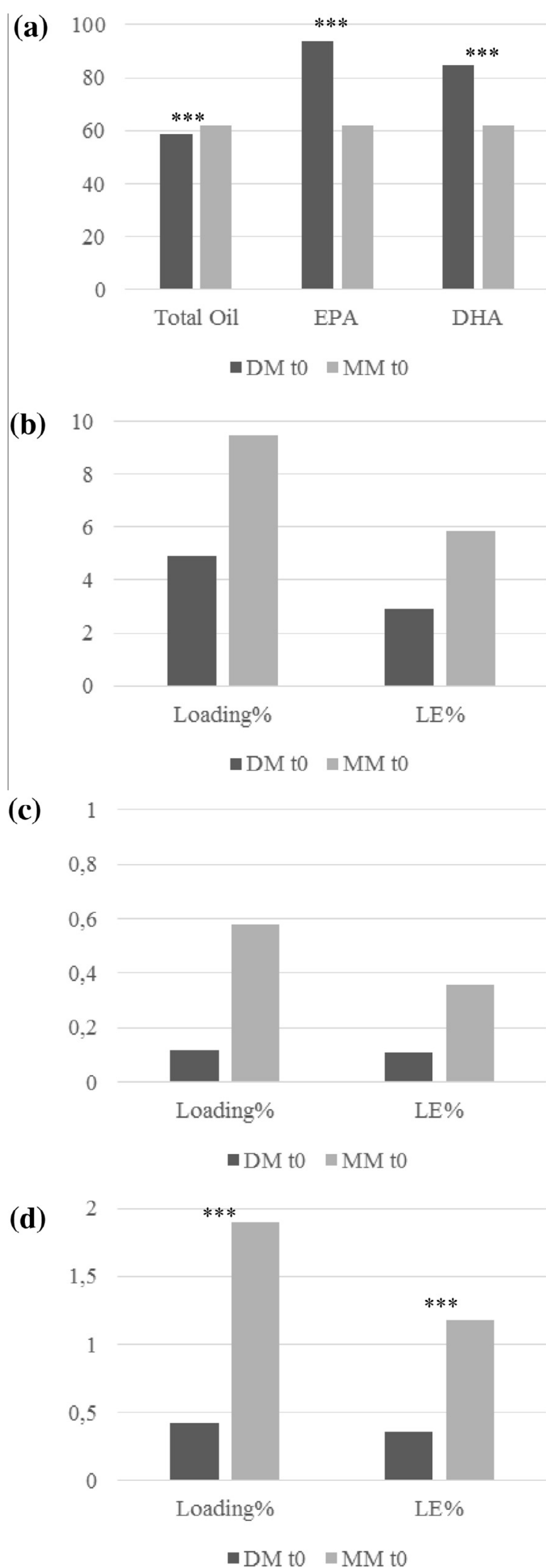

Fig. 2. Encapsulation efficiency (EE%) (a) of the total oil, EPA and DHA and Loading% and loading efficiency (LE%) of the total oil (b), EPA (c) and DHA (d) on the microcapsules from double (DM, dark grey) and multilayered (MM, light grey) emulsions at initial time (T0). *** = $p < 0.001$.

part of the differences in stability and shelf life of microencapsulated oils, as some authors have suggested that the amount of extractable oil could also protect other fractions of the oil of the microcapsules (Drusch & Berg, 2008). Therefore, higher EE is not necessarily always related to higher stability.

Loading and LE of the total oil were higher ($p < 0.001$) in MM (9.45% and 5.85%, respectively) than in DM (4.91% and 2.89%, respectively). This means that, although total oil proportion in the feed emulsions was the same in DM and in MM (2.50%), quantity of total oil extracted in MM was higher than in DM. Moreover, remarkable difference was detected in the quantity of oil that was effectively encapsulated in DM with respect to MM. Also, in DM total oil was a mix of fish oil and extra virgin olive oil, so that differences in the quantity of fish oil between both microcapsules were expected to be high.

EE of EPA and DHA was higher ($p < 0.001$) in DM (93.93% and 84.62%, respectively) than in MM (61.90% both). However, the loading proportion of EPA and DHA was higher ($p < 0.001$) in MM (0.58% and 1.90%, respectively) than in DM (0.12% and 0.42%, respectively). The weight of an active ingredient per 100 g of the powder is referred to as loading. When an encapsulation process achieves a higher load, the industrial use of the microcapsules becomes feasible in practice, and the process becomes more economically viable (Kaushik et al., 2014). High loading proportion means that a lower amount of powder is required for enrichment purposes when added to other foodstuff. Loading efficiency of EPA and DHA was also higher ($p < 0.001$) in MM (0.36% and 1.18%, respectively) than in DM (0.11% and 0.36%, respectively). Moreover, as it can be seen from the data, loading and LE of DHA were much higher than those of EPA in both types of microcapsules. Producing microcapsules by double emulsification and enzymatic gelation, Costa de Conto et al. (2013) obtained lower values for loading of EPA and DHA than those shown for MM capsules in this study, but higher values than that those for DM ones. However, they used as core for microencapsulation a fish oil ethyl ester with 62% of EPA + DHA, while the fish oil in the present study had 31.49% of EPA + DHA.

3.3. Fatty acid composition of the microcapsules

3.3.1. Fatty acid composition of the total oil of DM and MM

The composition of the total oil extracted from the two types of microcapsules is detailed in Table 2. In the microcapsules analyzed right after their production (initial time, or T0), the main fatty acids in DM were C18:1 n-9 (64.73%), followed by C16:0 (9.62%), DHA (8.58%), C18:2 n-6 (4.90%) and EPA (2.47%). At the same initial time, in MM, the main fatty acids were C16:0 (23.79%), DHA (20.19%), C18:1 n-9 (12.57%), C14:0 (9.43%) and EPA (6.13%).

The differences between fatty acid composition of the samples due to the type of microcapsule and inner composition of DM and MM and due to the storage for a month at simulated refrigeration (4 °C) and room (20 °C) temperatures were analyzed. Differences in the fatty acid composition between DM and MM were maintained along storage when we compare the two types of microcapsules within each batch of temperatures (the batch stored at refrigeration temperature and the batch stored at room temperature).

In the initial time, the differences between DM and MM were determined by the high amount of MUFA (66.89%) in DM, while in MM the higher values were for SFA (41.74%) and PUFA (41.30%). Thus, at initial time SFA/UFA was higher in MM (0.72) than in DM (0.17), whereas MUFA/PUFA was higher in DM (3.58)

Table 2
Fatty acid profile (g/100 g FAME) of the total oil of the two types of microcapsules at initial time (T0) and after a month of storage at refrigeration (T1m 4 °C) and room temperature (T1m 20 °C).

Fatty acid (g/100 g FAME)	Double microcapsules			pT			Multilayer microcapsules			pT			
	T0	T1m 4 °C	T1m 20 °C	p4 °C	p20 °C	p4–20 °C	T0	T1m 4 °C	T1m 20 °C	p4 °C	p20 °C	p4–20 °C	pMicrocapsule
C14	1.76 ± 0.04	2.30 ± 0.03	2.75 ± 0.04	<0.001	<0.001	<0.001	9.43 ± 0.05	10.23 ± 0.04	11.40 ± 0.05	<0.001	<0.001	<0.001	<0.001
C14:1	–	–	–	–	–	–	0.32 ± 0.00	0.20 ± 0.00	0.16 ± 0.00	<0.001	<0.001	0.002	<0.001
C16	9.62 ± 0.07	10.23 ± 0.10	14.32 ± 0.11	<0.001	<0.001	<0.001	23.79 ± 0.13	24.18 ± 0.15	26.42 ± 0.19	<0.001	<0.001	<0.001	<0.001
C16:1	0.91 ± 0.02	0.87 ± 0.03	0.71 ± 0.05	<0.001	<0.001	<0.001	1.12 ± 0.03	1.03 ± 0.05	1.08 ± 0.05	<0.001	<0.001	<0.001	<0.001
C17	1.04 ± 0.02	3.62 ± 0.04	4.16 ± 0.08	<0.001	<0.001	<0.001	3.30 ± 0.14	4.51 ± 0.13	5.31 ± 0.09	<0.001	<0.001	<0.001	<0.001
C17:1	0.09 ± 0.00	–	–	<0.001	<0.001	–	0.15 ± 0.00	0.14 ± 0.00	0.24 ± 0.00	<0.001	<0.001	<0.001	<0.001
C18	1.87 ± 0.04	4.91 ± 0.06	5.48 ± 0.13	<0.001	<0.001	<0.001	3.43 ± 0.09	4.76 ± 0.04	5.23 ± 0.08	<0.001	<0.001	<0.001	<0.001
C18:1	64.73 ± 1.89	62.88 ± 1.94	60.61 ± 1.39	<0.001	<0.001	<0.001	12.57 ± 0.79	14.33 ± 0.24	15.08 ± 0.35	0.081	0.058	0.096	<0.001
C18:2 n-6	4.90 ± 0.27	5.16 ± 0.45	4.68 ± 0.68	0.315	0.467	0.210	2.32 ± 0.08	1.89 ± 0.07	1.56 ± 0.08	<0.001	<0.001	<0.001	<0.001
C18:3 n-6	1.03 ± 0.09	0.70 ± 0.02	0.30 ± 0.02	<0.001	<0.001	<0.001	2.21 ± 0.12	1.32 ± 0.09	0.94 ± 0.08	<0.001	<0.001	<0.001	<0.001
C18:3 n-3	0.64 ± 0.04	0.73 ± 0.07	0.72 ± 0.04	<0.001	<0.001	<0.001	2.66 ± 0.14	2.34 ± 0.07	1.61 ± 0.04	<0.001	<0.001	<0.001	<0.001
C20	0.12 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	<0.001	<0.001	<0.001	0.10 ± 0.00	1.49 ± 0.05	1.15 ± 0.03	<0.001	<0.001	<0.001	<0.001
C20:1	1.16 ± 0.04	0.95 ± 0.01	0.55 ± 0.01	<0.001	<0.001	<0.001	2.07 ± 0.08	1.78 ± 0.02	1.26 ± 0.01	<0.001	<0.001	<0.001	<0.001
C20:3 n-6	0.67 ± 0.02	0.23 ± 0.00	0.26 ± 0.00	<0.001	<0.001	<0.001	1.63 ± 0.05	0.90 ± 0.02	0.93 ± 0.02	<0.001	<0.001	0.001	<0.001
C20:4 n-6	0.81 ± 0.02	0.64 ± 0.02	0.32 ± 0.00	<0.001	<0.001	<0.001	1.16 ± 0.06	0.83 ± 0.05	0.58 ± 0.03	<0.001	<0.001	<0.001	<0.001
C20:5 n-3	2.47 ± 0.06	1.55 ± 0.04	1.17 ± 0.05	<0.001	<0.001	<0.001	6.13 ± 0.16	5.81 ± 0.07	4.19 ± 0.11	<0.001	<0.001	<0.001	<0.001
C22:0	–	–	0.09 ± 0.00	–	<0.001	<0.001	0.98 ± 0.01	0.55 ± 0.02	0.68 ± 0.01	<0.001	<0.001	<0.001	<0.001
C22:1	–	–	–	–	–	–	0.73 ± 0.01	0.60 ± 0.01	0.57 ± 0.01	<0.001	<0.001	<0.001	<0.001
C24	–	–	0.13 ± 0.00	–	<0.001	<0.001	0.71 ± 0.02	0.54 ± 0.02	0.40 ± 0.01	<0.001	<0.001	<0.001	<0.001
C22:6 n-3	8.58 ± 1.03	4.62 ± 0.57	3.44 ± 0.08	<0.001	<0.001	<0.001	20.19 ± 1.74	18.57 ± 1.90	17.21 ± 1.65	0.463	0.442	0.635	<0.001
∑SFA	14.41 ± 0.08	21.37 ± 0.09	27.24 ± 0.07	<0.001	<0.001	<0.001	41.74 ± 0.16	46.26 ± 0.13	50.59 ± 0.18	<0.001	<0.001	<0.001	<0.001
∑MUFA	66.89 ± 1.83	64.7 ± 1.85	61.87 ± 1.64	0.324	0.042	0.106	16.96 ± 0.89	18.08 ± 0.93	18.39 ± 0.98	0.532	0.481	0.574	<0.001
∑PUFA	18.70 ± 0.15	13.93 ± 0.13	10.89 ± 0.14	<0.001	<0.001	<0.001	41.30 ± 0.19	35.66 ± 0.21	31.02 ± 0.18	<0.001	<0.001	<0.001	<0.001
n-6/n-3	0.66 ± 0.02	0.93 ± 0.04	1.04 ± 0.02	<0.001	<0.001	<0.001	0.22 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	<0.001	<0.001	0.086	<0.001
SFA/UFA	0.17 ± 0.00	0.27 ± 0.00	0.37 ± 0.00	<0.001	<0.001	<0.001	0.72 ± 0.01	0.86 ± 0.04	1.02 ± 0.06	<0.001	<0.001	<0.001	<0.001
MUFA/PUFA	3.58 ± 0.09	4.64 ± 0.12	5.68 ± 0.12	<0.001	<0.001	<0.001	0.41 ± 0.01	0.51 ± 0.01	0.59 ± 0.01	<0.001	<0.001	<0.001	<0.001

than in MM (0.41). This is a result of the incorporation of extra virgin olive oil in the case of DM, which led to a subsequent decrease in the concentration of EPA and DHA and an increase in the values for the fatty acids of olive oil, similar to what has been previously reported with the incorporation of corn oil to fish oil microcapsules (Costa de Conto et al., 2013). Comparison of the composition of the total oil extracted from DM and MM with the composition of the bulk oils points out to the fact that in DM the composition of the total oil was the result of a mixture of fish oil and extra virgin olive oil, and in MM the composition of the total oil was more related to that of the fish oil. However, in the total extracted oil from MM, higher SFA and lower MUFA than in the fish oil were detected, while PUFA remained similar to that of the fish oil. This contrasts with the results obtained by other authors that reported a slightly lower proportion of SFA and PUFA but higher MUFA after encapsulation of tuna oil (Klaypradit & Huang, 2008).

The composition in EPA and DHA in DM was 2.47% and 8.58%, respectively; and in MM it was 6.13% and 20.19%, respectively. Thus the concentration of EPA and DHA in DM was remarkably lower than the correspondent of the fish oil (6.90% and 24.59%, respectively). In MM, the difference was not so high, with a loss of only 0.77% of EPA and 4.4% of DHA from the bulk fish oil. Thus, total oil of MM had 88.84% of the EPA and 82.11% of the DHA from the bulk fish oil used in the production of the emulsion. In other study dealing with multilayered tuna oil microcapsules, EPA and DHA compositions decreased from 28.3% in the bulk fish oil to moreover 25% in the total oil extracted (Klinkesorn et al., 2005), which constitutes approximately the same loss shown in MM in this study. A decrease in the proportion of EPA and DHA from that in bulk oil has been also detected after spray-drying of linseed oil, and it was ascribed to the degradation of unsaturated fatty acids during microcapsule preparation (Gallardo et al., 2013; Rubilar et al., 2012). As for the n-6/n-3 ratio in microcapsules, it was higher in DM (0.66) than in MM (0.22), due to the higher concentration of EPA and DHA in MM.

Within each type of microcapsule, temperature also had a role on the changes in the fatty acid composition. In DM, there were differences caused by the storage at 4°, at 20 °C and also between both storage temperatures in all fatty acids except for C18:2 n-6, which was not affected by storage. In MM, the only fatty acids that were not affected by storage were C18:1 n-9 and DHA, the rest of them being strongly influenced by storage temperature. The differences observed due to the temperature effect were caused by the general tendency towards an increase in SFA as a result of the decrease in PUFA, this effect being similar in the two types of microcapsules after storage. Differences were also found between the two temperatures (4 and 20 °C), as the mentioned increase in SFA and decrease in PUFA were significant more evident at 20 °C than at 4 °C. As a consequence, SFA/UFA and MUFA/PUFA ratios increased with the storage temperature. This decrease of PUFA could be a consequence of the ease breakage of double bond of these fatty acids, due to the high susceptibility of these fatty acids to oxidation (Ng, Jessie, Tan, Long, & Nyam, 2013). On the contrary, the level of MUFA remained unchanged in the case of MM and DM after a month of refrigeration storage with respect to initial time, and in the case of MM after a month of room storage with respect to initial time. Moreover, the different temperature (refrigeration *vs* room) had no significant effect on MUFA in DM, neither in MM. It is remarkable that in the case of the MUFA/PUFA ratio, this increase was more pronounced in DM than in MM, this being attributable to the higher MUFA content of DM. The n-6/n-3 ratio had an opposite behavior in DM (where it increased with the storage temperature) and in MM (where it decreased after storage, and this decrease was the same at 4 and 20 °C). This contrast with other studies that have found no modification of the fatty acid composition of formulas with high content of PUFAs after 30 days of storage at 22 °C (Zunin et al., 2015) or 24 days of storage at 65 °C (Ng et al., 2013). However, differences in the composition of the oil and the capsules should be taken into account. Moreover, despite the changes in the microcapsules due to the storage effect, the protective effect against oxidation is noticeable, as bulk oils rich in PUFAs have shown much higher susceptibility to oxidation under accelerated storage (Jiménez-Martín et al., 2015).

3.3.2. External and encapsulated oil of DM

Multiple emulsions of this work had a particular composition, where the total oil includes the mixture of two different oils (fish oil and extra virgin olive oil). This makes necessary to analyze separately the composition of external and encapsulated oils of the correspondent DM (Table 3), in order to obtain information about the distribution of each oil in the structure of these microcapsules. Thus, the differences in fatty acid composition between external and encapsulated oils at the initial time and at each storage time were assessed. In addition to that, the changes in the fatty acid composition due to storage temperature were also studied individually for external oil and encapsulated oil.

Significant differences between the composition of external oil and encapsulated oil was evidenced by the value of pO in the Table 3, which pointed to significant differences for most of the fatty acids detected. However, some fatty acids, such as C18:2 n-6, C18:1 n-9, C18:3 n-3 and C20:0 were present in the same concentration in both extracted oils. There were higher SFA and PUFA in encapsulated oil than in external oil, while there were no differences in MUFA levels. In a study of microencapsulated fish oil, similar to results were obtained for MUFA and SFA, while for PUFA the authors found higher proportion in the external oil than in the encapsulated oil (Pourashouri et al., 2014). In our study, results point out to the fact that the encapsulated oil of DM is more related to the composition of fish oil. Nevertheless, a great proportion of the main fatty acids coming from extra virgin olive oil were found in DM encapsulated oil. Calvo et al. (2012) analyzed different wall compositions for microencapsulation of extra virgin olive oil, and observed that protein-based models helped to keep the relative levels of monounsaturated fatty acids particularly, while carbohydrate-based models were more appropriate to maintain high concentrations of linoleic fatty acids and PUFAs.

Moreover, in the encapsulated oil lower n-6/n-3 and MUFA/PUFA ratios were detected for all batches. Also, SFA/UFA was higher in encapsulated oil than in external oil at initial time and in DM stored at 20 °C, while this index was lower in encapsulated oil than in external oil in DM stored at 4 °C. This could be related to the fast oxidation rate of this type of oils, making oxygen diffusion through the solid matrix a key factor in the oxidation rate in the encapsulated fraction and, therefore, the external oil getting oxidize more rapidly than the encapsulated oil (Velasco et al., 2009).

At the initial time, more EPA was detected in the external oil than in encapsulated oil. Thus, it might be possible that DHA, a fatty acid with longer chain than EPA, was less prone to attain the surface of the microcapsules at the assayed spray-drying temperatures. However, after the storage at any of the two tested temperatures, a higher level of EPA was detected in the encapsulated than in the external oil, which could points out to the oxidative degradation of this PUFA in the outer location of the capsules. The amount of DHA was higher in the encapsulated oil for all the batches. This behavior of DHA has been also detected in a study of Pourashouri et al. (2014) dealing with microencapsulation of fish oil with different coating materials. Murrieta-Pazos, Gaiani, Galet, and Scher (2012) determined the fatty acid composition for whole milk powder, showing that fatty acids with long chains were more present in the encapsulated fat fractions, concurrently being fatty acids with short and medium chains more abundant in the free fat fractions.

Table 3
Fatty acid profile (g/100 g FAME) of the external (Ext) and encapsulated (Enc) oil of the microcapsules from double emulsions (DM) at initial time (T0) and after a month of storage at refrigeration (T1m 4 °C) and room temperature (T1m 20 °C).

Fatty acid (g/100 g FAME)	T0		T1m 4 °C		T1m 20 °C			p4 °C		p20 °C		p4–20 °C	
	Ext	Enc	Ext	Enc	Ext	Enc	pO	Ext	Enc	Ext	Enc	Ext	Enc
C14	1.39 ± 0.04	1.82 ± 0.02	2.91 ± 0.05	2.44 ± 0.02	2.37 ± 0.03	2.43 ± 0.04	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.150
C14:1	–	–	–	–	–	–	–	–	–	–	–	–	–
C16	9.14 ± 0.09	9.44 ± 0.12	9.76 ± 0.15	10.08 ± 0.06	13.40 ± 0.07	13.79 ± 0.09	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C16:1	1.17 ± 0.02	0.85 ± 0.03	0.80 ± 0.04	0.73 ± 0.05	0.79 ± 0.06	0.72 ± 0.04	<0.001	<0.001	<0.001	<0.001	<0.001	0.059	0.095
C17	1.38 ± 0.07	0.71 ± 0.05	3.94 ± 0.09	3.21 ± 0.08	4.23 ± 0.05	4.13 ± 0.12	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C17:1	0.16 ± 0.00	0.07 ± 0.00	–	–	–	–	<0.001	–	–	–	–	–	–
C18	1.19 ± 0.03	1.76 ± 0.04	5.47 ± 0.07	4.52 ± 0.12	5.21 ± 0.03	5.17 ± 0.06	<0.001	<0.001	<0.001	0.436	<0.001	0.046	<0.001
C18:1	67.15 ± 1.67	65.32 ± 1.20	61.63 ± 1.52	62.60 ± 1.25	64.69 ± 1.92	60.92 ± 1.28	0.139	0.191	0.094	<0.001	<0.001	0.163	0.061
C18:2 n-6	5.76 ± 0.48	6.26 ± 0.98	5.60 ± 0.40	5.28 ± 0.81	4.23 ± 0.49	4.77 ± 0.64	0.078	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C18:3 n-6	0.85 ± 0.04	1.22 ± 0.09	0.48 ± 0.06	1.10 ± 0.06	0.61 ± 0.04	0.36 ± 0.02	<0.001	<0.001	0.438	<0.001	<0.001	<0.001	<0.001
C18:3 n-3	1.09 ± 0.07	1.08 ± 0.06	0.92 ± 0.06	0.73 ± 0.05	0.76 ± 0.04	0.79 ± 0.05	0.290	0.015	<0.001	<0.001	<0.001	<0.001	0.031
C20	0.14 ± 0.00	0.14 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.13 ± 0.00	0.16 ± 0.00	0.471	<0.001	<0.001	0.091	0.016	<0.001	<0.001
C20:1	1.25 ± 0.02	1.12 ± 0.02	0.54 ± 0.01	0.46 ± 0.00	0.38 ± 0.00	0.58 ± 0.01	0.039	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C20:3 n-6	0.73 ± 0.02	0.46 ± 0.01	0.59 ± 0.04	0.31 ± 0.01	0.10 ± 0.00	0.23 ± 0.00	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C20:4 n-6	1.14 ± 0.04	0.93 ± 0.04	0.93 ± 0.04	0.76 ± 0.02	0.16 ± 0.00	0.35 ± 0.00	0.027	0.023	<0.001	<0.001	<0.001	<0.001	<0.001
C20:5 n-3	2.50 ± 0.05	2.32 ± 0.05	1.30 ± 0.05	1.56 ± 0.09	0.79 ± 0.03	1.50 ± 0.08	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.251
C22:0	–	–	–	–	–	–	–	–	–	–	–	–	–
C22:1	–	–	–	–	–	–	–	–	–	–	–	–	–
C24:0	–	–	–	–	–	–	–	–	–	–	–	–	–
C22:6 n-6	5.14 ± 0.83	7.26 ± 0.62	3.85 ± 0.55	5.39 ± 0.71	2.15 ± 0.06	3.89 ± 0.09	0.022	0.036	<0.001	<0.001	<0.001	<0.001	<0.001
∑SFA	13.24 ± 0.15	13.87 ± 0.13	22.15 ± 0.14	20.34 ± 0.10	25.34 ± 0.08	25.89 ± 0.11	0.047	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
∑MUFA	69.73 ± 1.29	67.36 ± 1.42	62.97 ± 1.39	63.79 ± 1.53	65.86 ± 1.87	62.22 ± 1.53	0.135	<0.001	0.046	<0.001	0.036	<0.001	0.418
∑PUFA	17.03 ± 0.12	18.77 ± 0.10	14.88 ± 0.12	15.87 ± 0.18	8.8 ± 0.09	11.89 ± 0.15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
n-6/n-3	0.99 ± 0.05	0.90 ± 0.04	1.04 ± 0.05	0.88 ± 0.03	1.38 ± 0.06	0.92 ± 0.06	<0.001	0.246	0.028	<0.001	0.195	<0.001	0.157
SFA/UFA	0.15 ± 0.00	0.16 ± 0.00	0.28 ± 0.00	0.26 ± 0.00	0.34 ± 0.00	0.35 ± 0.00	0.028	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MUFA/PUFA	4.09 ± 0.18	3.59 ± 0.12	4.23 ± 0.18	4.02 ± 0.10	7.48 ± 0.18	5.23 ± 0.14	<0.001	0.134	<0.001	<0.001	<0.001	<0.001	<0.001

The storage had also an overall significant effect in DM nearly for all initial time and temperature groups in external and encapsulated oils. It is particularly noteworthy that no differences were detected for C18:1 n-9 between DM at the initial time and after storage at 4° or between DM stored at the two temperatures (4 vs 20 °C). However, significant differences were detected for the level of C18:1 n-9 between DM at 20 °C when compared to that of the initial time.

With higher storage temperature, higher SFA, SFA/UFA and MUFA/PUFA and lower MUFA and PUFA were observed in all DM. Surprisingly, n-6/n-3 ratio did not follow a consistent behavior. Therefore, in the external oil of DM stored at 4 and 20 °C n-6/n-3 increased. However, for encapsulated oil, n-6/n-3 in DM stored at 4 °C decreased, while in that stored at 20 °C suffered no changes.

The content of the omega-3 fatty acids, EPA and DHA, in external and encapsulated oils underwent different changes due to the storage of DM. Velasco et al. (2009) stated that the free and encapsulated oil fractions displayed different oxidative behavior, the free oil being oxidized as lipids in continuous phase while oxidation in the encapsulated oil is the result of individual oil droplets oxidizing at different reaction rates. For DHA in the external and encapsulated oil, and for EPA in the external oil, a decrease was observed with the storage, and this decrease was higher at 20 °C than at 4 °C. However, in the encapsulated oil, EPA decreased after storage at 4 °C in the same way than after storage at 20 °C, the concentration between DM stored at the two temperatures assayed being non-significant. This points out to a lower oxidative degradation of EPA than of DHA, which is not surprising due to its lower unsaturation level. It is difficult to compare these results with data reported in other microencapsulation studies, due to differences in the source of ω-3 and the wall material used, as well as the encapsulation technology and the storage conditions (Drusch & Berg, 2008).

3.4. PCA of fatty acids

PCA was used to identify groups among samples based on the composition of fatty acids (Fig. 3). All analyzed fatty acids were used in this PCA as variables. The loading of each fatty acid in the two main PC are shown in Fig. 3a, and the score plots of the extracted and analyzed oils of both types of microcapsules at initial time and after a moth of storage at 4 and 20 °C are shown in Fig. 3b. The first principal component (PC1) comprised 68.74% of the total variance, and the second principal component (PC2) comprised 20.02%. According to the loading plot (Fig. 3a), four main groups of fatty acids can be distinguished: those with high positive PC1 scores, those with high negative PC1 scores, those with high negative PC2 scores, and those with intermediate values of PC1 and PC2.

Many of the fatty acids, including EPA and DHA, were grouped and represented in the direction of the right quadrants (both upper and lower), which corresponds to high positive loadings on the PC1. On the other hand, there were only two compounds with high negative loadings on the PC1, C18:1 n-9 and C18:2 n-6 (left upper quadrant), and two compounds with high negative loadings on the PC2, C17:0 and C18:0 (left and right lower quadrants). Finally, C20:0 and C16:0, in the right lower quadrant, had intermediate values of PC1 and PC2. The score plot of the PCA analysis (Fig. 3b) indicates that both types of microcapsules were clearly different in their composition of fatty acids. In that sense, all MM oils had high scores in the PC1, and all DM oils had high scores in the PC2.

MM capsules at the initial time had positive scores in PC2, and after storage at both 4 and 20 °C had negative scores in PC2. This seems to indicate that MM with high positive scores in the PC1 both at initial time and after storage, may be in general more related to the main group of fatty acids, among which there are included EPA and DHA. However, in the specific case of MM after storage at both temperatures, the negative scores in the PC2 may show that they can be more associated to contents of C16:0, C17:0, C18:0 and C20:0.

While MM samples were separated depending on the different storage (initial time, storage at 4 °C and storage at 20 °C), DM samples were separated by the location of the different extracted oils (encapsulated, external, and total). So, for each type of extracted oil of DM, the samples analyzed at initial time and stored at both

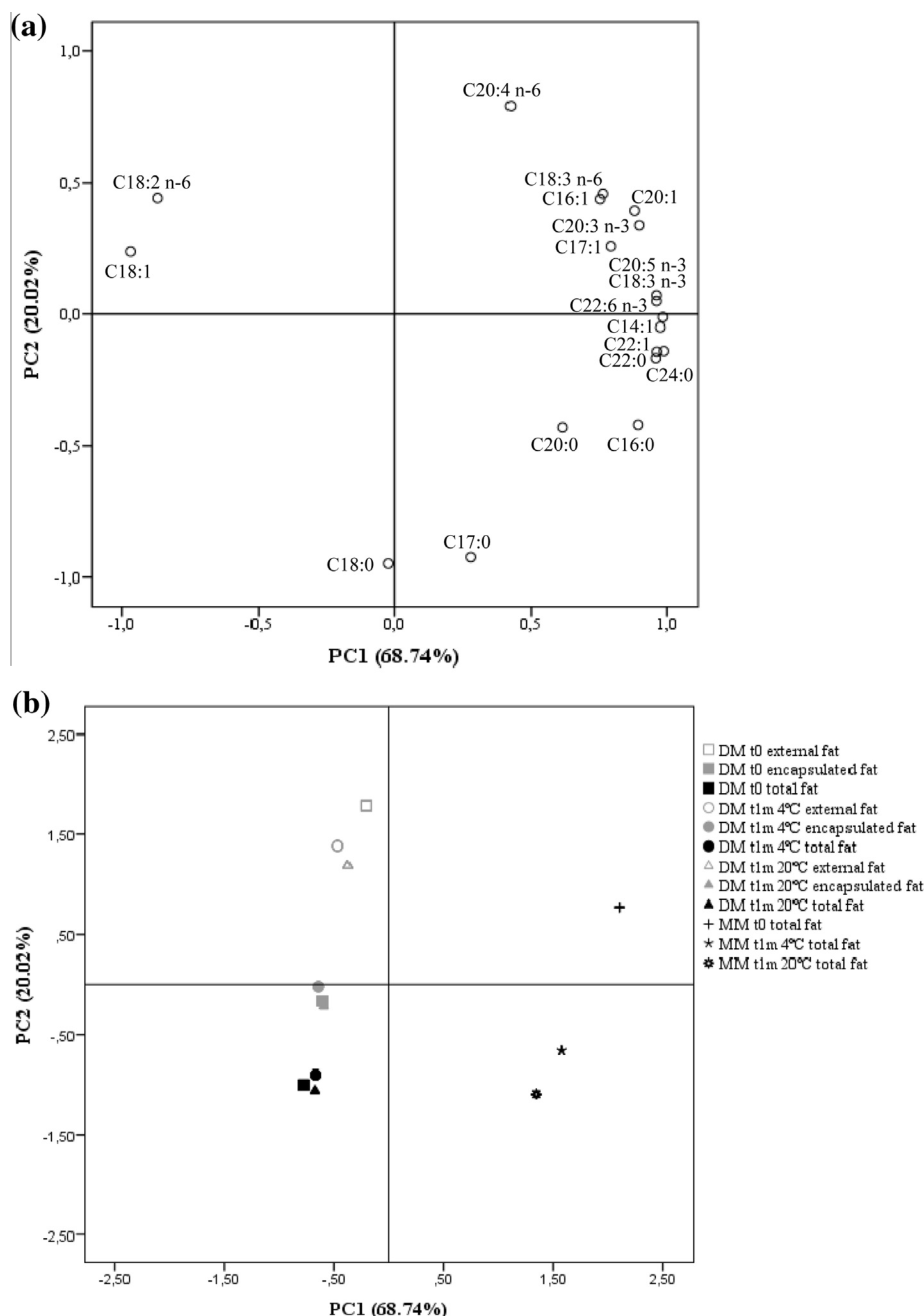

Fig. 3. Principal component analysis (PCA) of the fatty acids from the microcapsules. The plots represent, for the two first principal components, the loading of each fatty acid (a) and the average scores (b) of both types of microcapsules (double microcapsules (DM) and multilayered microcapsules (MM) at initial time (T0) and after a month of storage at 4 °C (T1m 4 °C) and 20 °C (T1m 20 °C) on the two first principal components.

temperatures were grouped. This indicates that in DM the variability caused by storage was smaller than the variability caused by location of the oil in the microcapsule. External oil (left upper quadrant) was more separated from the other two oils (right upper quadrant). It is noteworthy, as it suggests that external oil of DM may be distinguished by its content of C18:2 n-6 and C18:1 n-9. This, again, seems to be connected to the structure of DM, indicating that the location of the extra virgin olive oil in these microcapsules was mainly the external oil.

3.5. Remaining of EPA and DHA

There was analyzed the proportion of remaining EPA and DHA in total extracted oil of the microcapsules after storage at 4 and 20 °C with respect to those at the initial time (Supplementary material). Overall, there was a higher remaining proportion of EPA and of DHA in MM than in DM. Remaining proportion of DHA was higher than for EPA both in MM and DM microcapsules kept at both temperatures, this difference being more remarkable in MM. This could be due to the higher LE of DHA compared to that of EPA in MM, while differences in the values of LE for EPA and DHA in DM were lower. So, in MM, a higher proportion of DHA was encapsulated, which could have been the cause of the higher protection of DHA, with more remaining of this PUFA. In a study of 50% fish oil powders produced with different formulations of skim milk and buttermilk, a higher remaining DHA (80–90%) was detected in all the formulations tested with respect to remaining EPA (75–85%) after storage at 40 °C for 26 days (Augustin et al.,

2014). Chen, Zhong, Wen, McGillivray, and Quek (2013) obtained high remaining EPA and DHA with co-encapsulation of fish oil by milk proteins during storage at 45 °C and 30% relative humidity under saturated oxygen for 7 days, while the retention of EPA and DHA on the bulk fish oil under the same storage conditions was only about 11–14%. For each type of microcapsule, there was more remaining EPA and DHA, and, as a consequence, of ω-3, in the microcapsules stored at 4 °C than in those stored at 20 °C, being a clear effect of the storage temperature, which agrees with the results of the fatty acid profile of the microcapsules.

4. Conclusions

This study showed the suitability of using both double and multilayered emulsion to produce spray-dried microcapsules of fish oil. The type of emulsion (double or multilayered) influences significantly encapsulation efficiency, loading, loading efficiency, fatty acid composition and microstructure of the microcapsules, with multilayered emulsions giving better results. The storage at refrigeration and room conditions affects significantly the fatty acid composition, being this effect not so notable in microcapsules from multilayered emulsions, which seem to protect fatty acids, especially ω-3 fatty acids, more efficiently.

Acknowledgments

Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo for supporting the predoctoral Grant (PD10026), equipment funding and support to research groups. Biodymia Laboratory (University of Lyon) for making possible the development of the work during a research stay. "Biomega Natural Nutrients S.L." for kindly providing the fish oil and "Trades S.A." for kindly providing the chitosan and "Roquette-Laisa" for kindly providing the maltodextrin, all used during the experiments.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.08.046.

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Capítulo 2.1

Effect of type of omega-3 enrichment from fish oil on physico-chemical parameters of chicken nuggets

Enviado a: International Journal of Food Science and Technology

Noviembre 2015 (IJFST-2015-19174)

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Journal:	*International Journal of Food Science and Technology*
Manuscript ID	IJFST-2015-19174
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	24-Nov-2015
Complete List of Authors:	Jiménez-Martín, Estefanía; Research Institute of Meat and Meat Products (IproCar), University of Extremadura, Antequera, Teresa; Research Institute of Meat and Meat Products (IproCar), University of Extremadura Cáceres, Cáceres, ES 10003 Ruiz Carrascal, Jorge; University of Copenhagen, Department of Food Science Perez Palacios, Mª Trinidad; Research Institute of Meat and Meat Products (IproCar), University of Extremadura Cáceres, Cáceres, ES 10003
Keywords:	Omega 3, Fish, Microencapsulation, Poultry, Frying, Lipid Oxidation

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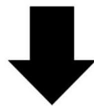
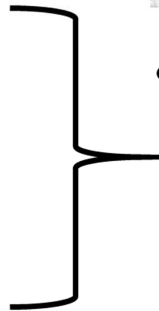


PRE-FRYING

FRYING



- **Physico-chemical parameters**
- **Compositional changes**

1 TITLE: Effect of type of omega-3 enrichment from fish oil on physico-chemical parameters of chicken nuggets.

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For Peer Review

ABSTRACT

This work evaluates the influence of type of ω-3 enrichment (bulk fish oil vs microencapsulated fish oil) on physico-chemical parameters and composition during deep-frying, analyzing pre-fried (PF) and fried (F) chicken nuggets. Addition of fish oil microcapsules led to lower moisture and water activity and higher instrumental redness in PF and F chicken nuggets, and lower fat uptake during deep-frying. In PF samples, higher eicosapentaenoic (EPA, C20:5 n-3) and docosahexaenoic (DHA, C22:6 n-3) acid and lower lipid oxidation were observed when enriching with fish oil microcapsules. During deep-frying, EPA and DHA losses and lipid oxidation rate was more marked in chicken nuggets enriched with bulk fish oil. F chicken nuggets enriched with fish oil microcapsules contained higher EPA and DHA and were less prone to lipid oxidation. Addition of fish oil microcapsules to chicken nuggets protects EPA and DHA from lipid oxidation and avoids losses during the manufacturing and deep-frying.

KEYWORDS: omega 3; fish oil; microencapsulation; poultry; frying; lipid oxidation.

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INTRODUCTION

There is growing interest in increasing the intake of foods rich in polyunsaturated fatty acids (PUFA), mainly long chain ω-3 fatty acids, specially eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acids (DHA, C22:6 n-3), due to their positive effects on human health (Sioen *et al,*. 2006).

Fish and seafood are food sources with high content of long chain ω-3 fatty acids, however, per capita fish consumption worldwide is quite low. The ω-3 fatty acids enrichment of well accepted and highly consumed products constitutes an interesting chance for increasing the intake of this PUFA. The main drawback for ω-3 fatty acids addition is their high susceptibility to oxidation, (Jacobsen 1999).

Microencapsulation has emerged as an effective strategy for protection of ω-3 PUFA from oxidation, spray-drying being the most popular method to achieve it (Gharsallaoui *et al,*. 2007). The use of multilayered emulsions of lecithin-chitosan combined with carbohydrates as coating material for fish oil microencapsulation by spray-drying has demonstrated to be effective in terms of protection from oxidation (Jiménez-Martín *et al,*. 2015).

There is not many scientific literature about food enriched with microencapsulated ω-3 fatty acids, and it is mainly focused on beverage (Ilyasoglu and El 2014, Rubilar *et al,*. 2012), dairy (Bermúdez-Aguirre and Barbosa-Cánovas 2012, Tamjidi *et al,*. 2012) and bakery products (Gökmen *et al,*. 2011, Umesha *et al,*. 2015). Only two works have studied the enrichment of meat products with microencapsulated ω-3 PUFA (Josquin *et al,*. 2012, Pelser *et al,*. 2007), and none of those deal with deep-fried coated products.

This work aimed to determine in which extent deep-fried coated chicken products are affected by enriching them with microencapsulated fish oil, bearing in mind the physico-chemical changes during deep-frying, specially lipid oxidation and fatty acid composition, in comparison to enrichment with bulk fish oil.

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MATERIAL AND METHODS

EXPERIMENTAL DESIGN

Three different types of nuggets were prepared: control with no fish oil addition (C), enriched with bulk fish oil (BFO) and enriched with microencapsulated fish oil (MFO). Fish oil (kindly provided by Biomega Natural Nutrients S.L., Galicia, Spain) was used as source of ω-3 FA (6.9 % EPA, 24.6 % DHA).

Formulation and manufacture of the chicken nuggets of the control batch was made following a methodology optimised previously (Medina *et al,*. 2014), using chicken breast as the meat raw material. As for the enriched batches, the methodology used was the same, but formulation of the batter was modified by the addition of 0.5% (w/w) of fish oil in the BFO batch and by the addition of 5 % (w/w) of multilayered microcapsules of fish oil optimised in a previous work (multilayered microcapsules prepared with lecithin-chitosan and maltodextrin containing 10% of fish oil) (Jiménez-Martín *et al,*. 2015). The enrichment level in EPA+DHA in BFO and MFO nuggets was 150 mg per 100 g (enough to exceed the minimum level required by the European Union regulation (EU 2010) to label a food as "high in ω-3 fatty acids": at least 80 mg of the sum of EPA and DHA per 100 g and per 100 kcal).

Chicken breast was purchased in a local market (Mercadona, Cáceres, Spain) and minced using a meat mincer (SEB IBERICA, Barcelona, Spain). It was mixed and further minced with the ingredients in a domestic kitchen processor Thermomix (Vorwerk Wuppertal, Germany) according to each formulation. A commercial preparation (Procavi; ANVISA, Madrid, Spain) (60 g/kg meat) was added. The obtained butter was stored at 0-2 ºC during 24h and it was portioned (4 x 4 x 1 cm, 25 g) and coated, first with a commercial stick solution (Avigum, ANVISA, Madrid, Spain) dissolved in cold water (25 g/L), and then coated with breadcrumbs (35 g/kg nugget). All nuggets were subjected to a pre-fried process in sunflower oil during 10 sec. Thereafter, the nuggets were immediately removed from the fryer and placed on paper towel to remove excess of external oil.

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3 79 Half of the pre-fried nuggets (PF) were stored at -80ºC in order to be further analysed. The other half of
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5 80 pre-fried nuggets followed a conventional storage and further processing. They were stored in a tray
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7 81 covered with a plastic wrap during 24h at refrigeration temperature (0-2 ºC). After that storage, nuggets
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9 82 were fried at 180 ºC in sunflower oil until the product reached 63 ºC of internal temperature. Temperature
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11 83 of nuggets during frying was recorded using a thermometer probe (Testo 735-2, Lenzkirch, Germany).
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13 84 Nine nuggets were fried at the same time in the fryer. The oil was replaced every four frying sessions.
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15 85 After each deep-frying, nuggets were drained, and placed on paper towel for removing external oil. Fried
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17 86 nuggets (F) were immediately stored at -80ºC until further analysis. For each one of the three
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19 87 formulations (C, BFO and MFO) two groups of chicken nuggets were obtained: PF and F.
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23 89 *METHODS*
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25 90 *Colour*
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27 91 Colour was measured across the surface of the chicken nuggets. L* value (lightness), a* value (redness)
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29 92 and b* value (yellowness) were obtained using a Minolta Colorimeter CR-300 (Minolta Camera Co.,
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31 93 Osaka, Japan) programmed to use the built-in internal illuminant D65. Means of readings on three
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33 94 locations on each sample were determined. Before each series of measurements, the instrument was
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35 95 calibrated using a white ceramic tile.
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37 96 *Moisture*
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39 97 Moisture content was determined by drying minced samples (5 g) at 102 °C according to the Association
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41 98 of Official Analytical Chemists (reference 935.29) (AOAC 1999).
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43 99 *Water activity.*
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45 100 For the water activity, the system LabMaster-aw (NOVASINA AG, Switzerland) was used after
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47 101 calibration.
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49 102 *Fat content*
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3 103 Fat content was analyzed gravimetrically with chloroform/methanol (2:1, vol/vol) (Pérez-Palacios *et al,.*
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5 104 2008).
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7 105 *Fatty acid profile*
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10 106 Fatty acid methyl esters (FAME) from extracted fat were prepared by trans-esterification in presence of
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12 107 sodium metal (0.1 N) and sulphuric acid in methanol (Sandler and Karo 1992). Fatty acid methyl esters
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14 108 were analyzed by gas chromatography, using a Hewlett-Packard HP-5890-II gas chromatograph,
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16 109 equipped with an on-column injector and a flame ionization detector (FID). Separation was carried out on
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19 110 a polyethylenglycol capillary column (60 m long, 0.32 mm id, 0.25 mm film thickness) (Supelcowax-10;
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21 111 Supelco, Bellafonte, PA, USA) maintained at 230 ºC for 60 min. Injector and detector temperatures were
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23 112 kept at 230 ºC. The carrier gas was nitrogen at a flow-rate of 0.8 ml/min. Individual compounds were
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25 113 identified by comparing their retention times with those of standards (Sigma, St. Louis, MO, USA).
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28 114 Quantification was based on the relative area percentages of the FAME analysed.
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30 115 *Oxidation*
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32 116 *Conjugated Dienes*
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34 117 The measurement of conjugated dienes (CD) was determined (Juntachote *et al,.* 2006). Absorbance was
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37 118 read at 233 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The concentration of CD were
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39 119 expressed as µmol per kg of sample.
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42 120 *Thiobarbituric Acid Reactive Substances*
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44 121 Thiobarbituric acid-reactive substances (TBARS) were measured (Salih *et al,.* 1987). The concentration
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46 122 of malonaldehyde (MDA) was calculated from a standard curve of TEP (Merck, Schchardt, Germany).
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48 123 TBARs were expressed as mg MDA kg^{-1} sample.
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51 124 *Protein Carbonyls*
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54 125 Protein oxidation in chicken nuggets was followed by measuring the formation of protein carbonyls
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56 126 according to method outlined (Oliver *et al,.* 1987). The amount of carbonyls was expressed as nmol of
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58 127 carbonyl per milligram of protein.
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3 128 *Sampling replication and Statistical analysis*
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6 129 Three batches were prepared (n=3) for each chicken nugget formulation (C, BFO and MFO). For each
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8 130 analysis, five experimental samples of each of pre-fried and fried nugget groups (C-PF, C-F, BFO-PF,
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10 131 BFO-F, MFO-PF, MFO-F) were analyzed in duplicate. The effect type of fish oil addition and deep-
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12 132 frying were analysed using a one-way analysis of variance (ANOVA) according to the General Linear
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14 133 Model procedure. When detecting significance of the effect ($p < 0.05$), the Tukey`s test was
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16 134 performed.The statistical study was done with the program IBM SPSS Statistics v.19.
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22 136 RESULTS
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25 137 *Moisture, lipid and colour measurements in fish oil enriched nuggets*
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27
28 138 Table 1 shows moisture, water activity, lipid content, and instrumental colour determination in PF and F
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30 139 chicken nuggets from C, BFO and MFO batches. Type of fish oil addition led to some significant
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32 140 differences in PF samples, with PF-MFO nuggets having lower water activity in comparison to PF-C and
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34 141 PF-BFO ones, and PF-BFO showing lower values for a* colour coordinate. In F samples, it was observed
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36 142 a significant lower water activity and moisture content and higher values for a* coordinates in F-MFO
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38 143 nuggets than in F-C and F-BFO ones. Nevertheless, the type of fish oil added did not significantly
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40 144 influence fat content of nuggets, finding similar lipid content in F-C, F-BFO and F-MFO nuggets.
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44 145 In relation to the deep-frying effect, as expected, F coated products had significant lower moisture and
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46 146 water activity levels and higher lipid content than PF ones in C, BFO and MFO (Table 1). The differences
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48 147 of moisture and fat content between PF and F suppose around 6.44 %, 7.19 % and 9.72 % (for C, BFO
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50 148 and MFO, respectively) of water loss and 3.26 %, 3.59 % and 2.34 (for C, BFO and MFO, respectively)
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52 149 of oil uptake during deep-frying.
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150 Colour changes during deep-frying followed a similar pattern in all studied batches. Thus, a* and b*
151 significantly increased, and L* decreased, being only significant this decrease in MFO nuggets.

152 *Fatty acid profile in fish oil enriched nuggets*

153 Table 2 shows the fatty acid profile of PF and F nuggets of C, BFO and MFO batches. In all groups of
154 nuggets, linoleic acid (C18:2 n-6) was the major FA (39.99-50.4 6g/100g FAME), followed in decreasing
155 order by oleic acid (C18:1 n-9) (29.54-33.67 g/100g FAME), palmitic acid (C16:0) (10.31-15.11 g/100g
156 FAME) and stearic acid (C18:0) (4.23-6.13 g/100g FAME), with the rest of FA being less than 1 g/100g
157 FAME.

158 As for the influence of type of fish oil enrichment in PF samples, significant differences were found in the
159 proportion of most fatty acids between batches. The percentage of SFA was higher in PF-BFO than in PF-
160 C and PF-MBO samples, while PF-MFO showed a lower percentage of MUFA and a higher of PUFA in
161 comparison to PF-C and PF-BFO chicken nuggets. This is mainly due to the higher percentage of
162 palmitic (C16:0) and stearic (C18:0) acids in PF-BFO, and to the lower percentage of oleic acid (C18:1 n-
163 9) and higher percentage of linoleic acid (C18:2 n-6) in PF-MFO samples. It is worth noting differences
164 in EPA and DHA, with PF-C showing lower percentages of these fatty acids than PF-BFO and PF-MFO
165 samples, as a direct consequence of the fish oil enrichment in BFO and MFO. In addition, the percentage
166 of DHA was higher in PF-MFO than in PF-BFO samples.

167 The effect of type of fish oil addition in the fatty acid profile of F chicken nuggets was similar as that
168 observed for PF: F-BFO showed a higher proportion of SFA than C and MFO batches, which is mainly
169 due to the percentage of palmitic (C16:0). Nevertheless, MFO had lower percentage of oleic acid (C18:1
170 n-9) and MUFA and higher of linoleic acid (C18:2 n-6) and PUFA than C and BFO. It is also remarkable
171 the significant differences in long chain ω-3 PUFA, with EPA and DHA being higher in MFO than in C
172 and BFO.

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During deep-frying only some minor significant changes were found. In C, the percentage of linoleic acid (C18:2 n-6) significantly increased from PF to F, as direct result of deep-frying in sunflower oil. Consequently, the percentage of palmitic (C16:0) and stearic (C18:0) acids decreased and the percentage of some minor fatty acids (C21:0, C22:0, C20:1 (n-9), C18:3 (n-3) and C20:2) also significantly changed by deep-frying, but not EPA nor DHA. Similar changes were observed in BFO, with the additional significant decrease of EPA and DHA. However, in MFO, the only significant changes were the decrease of eicosatrienoic acid (C20:3 n-3) and DHA. There were EPA and DHA losses during the deep-frying of chicken nuggets enriched with bulk fish oil, whereas in the case of microcapsules addition such losses only affected DHA.

Lipid and protein oxidation in fish oil enriched nuggets

Oxidative stability in PF and F samples of C, BFO and MFO was measured by CD (Figure 1.1) and TBARs (Figure 1.2) as indicators of primary and secondary oxidation products from lipids, respectively, and DNPH analysis for protein oxidation (Figure 1.3).

The type of fish oil enrichment significantly influenced lipid oxidation in PF but not protein oxidation. Higher CD and TBARs values were found in PF-BFO in comparison to PF-C and PF-MFO, while similar values for protein oxidation were found in all PF samples.

As expected, deep-frying led to a significant increase in all oxidation markers in C, BFO and MFO. The extent of primary lipid oxidation was similar in C, BFO and MFO, with CD values increasing around 0.20-0.25 µmol/mg for all samples. However, in the case of TBARs, the increase from PF to F samples was more notable in BFO (1.2 mg MDA/kg sample) than in C and MFO (0.3 mg MDA/kg sample). As for protein oxidation, it showed the same trend than CD, with a similar increase from PF to F in C, BFO and MFO (2.0-2.2 nmol/mg protein).

The influence of type of fish oil enrichment on lipid and protein oxidation in F nuggets was also analyzed. It could be observed higher values for CD and TBARs in F-BFO than in F-C and F-MFO,

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3 197 which showed close values. As for protein oxidation, there were not found differences in the carbonyl
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5 198 values between F-C, F-BFO and F-MFO.
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8 199 DISCUSSION
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11 200 *Moisture, lipid and colour measurements in fish oil enriched nuggets*
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14 201 The absence of differences on fat content between the chicken nuggets enriched using bulk fish oil or
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16 202 microencapsulated fish oil could be explained by the low quantity of fish oil added. The results of
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18 203 moisture content are in agreement with those found by some authors (Josquin *et al,*. 2012), who found
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20 204 that moisture contents of sausages with pure fish oil was significantly higher compared to sausages with
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22 205 encapsulated oil, which they ascribed to the limited amount of water loss during drying due to a smearing
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24 206 effect of the oil. It has been reported that the ingredients of the batter and even the constituents in the
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26 207 flour affect the characteristics of the batter (Firdevs Dogan *et al,*. 2005). In our study, the composition of
27
28 208 the microcapsules used to enrich MFO batch, with sodium caseinate, lactose monohydrate and
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30 209 polyglycerolpolyricinoleate (as hydrophobic emulsifier), could also be related to the lower moisture and
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32 210 water activity and higher a* values in this group of samples. The values of oil uptake during deep-frying
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34 211 are quite in agreement with previous studies (Perez-Palacios *et al,*. 2013). It seems that MFO suffered
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36 212 higher water loss and lower fat uptake during deep-frying than C and BFO. Some authors argue that fat
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38 213 uptake is inversely related to the total water volume (Southern *et al,*. 2000, Perez-Palacios *et al,*. 2013).
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40 214 This fact could explain the obtained results, since lower moisture content is observed in F-MFO than in F-
41
42 215 C and F-BFO.
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45 216 As for the colour changes after deep-frying, they are the common modifications in deep-fried coated
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47 217 products, which became progressively more golden brown by increasing redness and yellowness while
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49 218 decreasing lightness values (Medina *et al,*. 2014).
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52 219 *Fatty acid profile in fish oil enriched nuggets*
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The high content of linoleic acid (C18:2 n-6) is a clear consequence of the FA profile of the vegetable oils (sunflower oil) used for pre-frying and frying process. Similar results have been found in other deep-fried coated products (Perez-Palacios *et al,.* 2013).

The lower percentage of DHA in PF-BFO compared to PF-MFO points out to a loss of this fatty acid during the manufacture and pre-frying process when using bulk fish oil addition. Accordingly, some authors observed higher EPA and DHA percentages in baby food containing microencapsulated menhaden oil in comparison to baby food containing bulk menhaden oil or not enriched samples (Wang *et al,.* 2011). However, other authors did not found differences in PUFA content of sausages enriched with microencapsulated or bulk oil (Josquin *et al,.* 2012).

As for the losses of EPA and DHA during the deep-frying, this effect of releasing long chain ω-3 PUFA due to high temperature reached during the processing of enriched products was also observed by other authors (Henna Lu and Norziah 2011). These authors observed losses for both EPA and DHA during baking of whole wheat breads enriched with microencapsulated ω-3 PUFA. However, in our study, despite the losses, microencapsulation allowed significant higher percentage of EPA and DHA after frying than bulk fish oil addition.

Lipid and protein oxidation in fish oil enriched nuggets

The increase in all oxidation markers in F compared to PF nuggets is the consequence of the boosting effect of high temperatures on the oxidation processes that take place during deep-frying.

The higher TBARs values found in PF-BFO compared to PF-C and PF-MFO highlights the potential protective effect of the microcapsules against lipid oxidation during the manufacture and pre-frying process of the nuggets. Accordingly, some authors found much higher values of lipid oxidation parameters in sausages with pure fish oil than in products with encapsulated oil, which the authors related to the effect of the encapsulated layer of proteins that could have protected the fish oil from lipid oxidation (Josquin *et al,.* 2012). Similarly, in biscuits enriched with garden cress seed oil (rich in linolenic

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acid (C18:2 n-3)), oxidation rate was high in bulk fish oil supplemented biscuits compared to microencapsulated oil ones (Umesha *et al,.* 2015).

From the results of CD and carbonyls, it seems that primary lipid oxidation and protein oxidation stability is not influenced by the type of fish oil addition in the chicken nuggets Lipid oxidation products promote protein oxidation and consequently protein carbonylation (Estévez 2011). Results on this study show the concordance in the increase in carbonyl content and CD values during deep-frying, with independence of the type of fish oil addition. However, it seems that this is an influencing factor on the rate of formation of secondary lipid oxidation products, with TBARs being enhanced by enrichment with bulk fish oil, whereas the addition of ω-3 microcapsules does not increase secondary lipid oxidation rate in deep-fried chicken nuggets.

The results related to the influence of type of fish oil enrichment on lipid and protein oxidation on F samples are the consequence of the influence of type of fish oil addition on PF samples and oxidation rate during deep-frying. In fact, in an assay of accelerated oxidation, the susceptibility to oxidation of the fish oil was higher in comparison to microcapsules of fish oil (Jiménez-Martín *et al,.* 2015). It has been stated that the limit of TBARs value that could be considered as the threshold for detection of oxidation flavors by general population is around 2.0 mg/kg sample (Greene and Cumuze 1982). At this respect, TBARS values of the F-C and F-MFO deep-fried chicken nuggets of this study were lower than the established threshold value, whereas deep-fried F-BFO samples had TBARs values near to this limit. So, the type of fish oil enrichment influences lipid oxidation, with the bulk fish oil addition promoting it in PF and F chicken nuggets. However, the addition of microcapsules of fish oil almost did not modify CD and TBARs values in comparison to not enriched nuggets.

CONCLUSIONS

The way in which fish oil is added to chicken nuggets influences the physico-chemical parameters of pre-fried and fried products and also the changes taking place during deep-frying, especially lipid-related

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268 ones. During the manufacturing and deep-frying processes of chicken nuggets, bulk fish oil addition 269 promotes lipid oxidation while enrichment with fish oil microcapsules protects from lipid oxidation. The 270 addition of fish oil microcapsules allows the production of chicken nuggets with higher content of EPA 271 and DHA in comparison to bulk fish oil addition. Thus, spray-dried multilayered microcapsules of fish oil 272 seems to be an appropriate vehicle for enrichment of chicken nuggets with ω-3 fatty acids.

273 ACKNOWLEDGMENT

274 Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo for 275 supporting the pre-doctoral grant (PD10026), equipment funding and support to research groups. 276 Biodymia Laboratory (University of Lyon) for making possible the development of the work during a 277 research stay. "Biomega Natural Nutrients S.L." for kindly providing the fish oil.

278 CONFLICT OF INTEREST

279 The authors declare no conflict of interest

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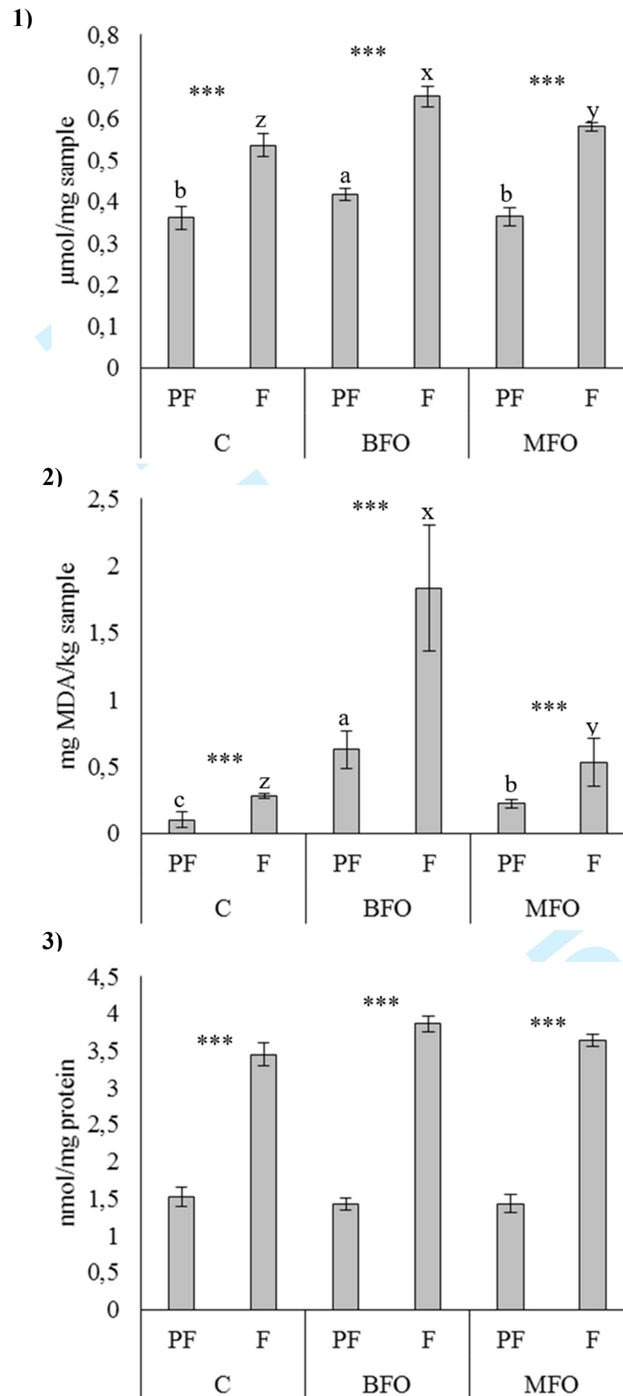
Figure 1. Results on conjugated dienes (1), TBARs (2) and carbonyls (3) in pre-fried (PF) and fried (F) chicken nuggets not enriched (C) and enriched with bulk fish oil (BFO) or with microcapsules of fish oil (MFO). The effects of frying (PF vs. F) within each batch, and of enrichment (C vs. BFO vs. MFO) in PF and F samples were analyzed.



Asterisks indicate significant differences (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) between PF and F within each batch. Different letters (a, b, c) among PF samples indicates significant differences. Different letters (x, y, z) among F samples indicates significant differences.

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6 Table 1. Moisture, water activity, fat content and instrumental colorin pre-fried (PF) and fried (F) chicken nuggets not enriched (C) and enriched with bulk fish oil (BFO) or with
7 microcapsules of fish oil (MFO). The effects of frying (p) within each batch, and of enrichment in PF (p (PF)) and F (p(F)) were analyzed.
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		C		*p*	BFO		*p*	MFO		*p*	*p*(PF)	*p*(F)
		PF	F		PF	F		PF	F			
Moisture (g/100 g)		64.35 ± 1.98	57.91 ± 2.53[x]	*	65.12 ± 0.91	57.93 ± 0.86[y]	***	63.80 ± 1.89	54.08 ± 1.05[y]	***	ns	***
Water activity		0.938±0.004[b]	0.925±0.004[x]	***	0.949±0.004[a]	0.926±0.004[x]	***	0.903±0.004[c]	0.864±0.004[y]	***	***	***
Fat content (g/100 g dry matter)		9.01 ± 0.75	12.27 ± 1.94	*	7.74 ± 1.26	11.33 ± 1.03	*	9.65 ± 0.90	11.99 ± 1.64	ns	ns	ns
Color	L*	55.22 ± 3.12	56.35 ± 3.47	ns	57.61 ± 1.00	54.73 ± 1.78	ns	57.82 ± 1.46	51.41 ± 3.45	*	ns	ns
	a*	3.45 ± 0.41[a]	6.68 ± 1.29[y]	**	2.44 ± 0.12[b]	5.87 ± 0.51[y]	***	3.11 ± 0.30[a]	9.21 ± 1.11[x]	***	***	***
	b*	20.64 ± 1.35	30.07 ± 2.77	***	19.92 ± 1.28	28.75 ± 1.61	***	22.08 ± 1.07	31.89 ± 1.54	***	ns	ns

17 Asterisks indicate significant differences (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). ns: not significant effect.
18 Means with different letters (a, b, c) among PF samples differ significantly.
19 Means with different letters (x, y, z) among F samples differ significantly.
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Table 2. Fatty acid profile (g FAME/100 g FAME) in pre-fried (PF) and fried (F) chicken nuggets not enriched (C) and enriched with bulk fish oil (BFO) or with microcapsules of fish oil (MFO). The effects of frying (p) within each batch, and of enrichment in PF (p(PF)) and F (p(F)) were analyzed.

	C			BFO			MFO				
	PF	F	*p*	PF	F	*p*	PF	F	*p*	*p*(PF)	*p*(F)
C14:0	0.38 ± 0.08^{b}	0.33 ± 0.07	ns	0.52 ± 0.48^{a}	0.43 ± 0.08	ns	0.42 ± 0.02^{b}	0.34 ± 0.09	ns	**	ns
C16:0	12.56 ± 1.35^{b}	10.61 ± 0.74^{xy}	*	15.11 ± 1.04^{a}	12.03 ± 0.48^{x}	***	11.39 ± 0.80^{b}	10.31 ± 1.51^{y}	ns	***	*
C18:0	4.93 ± 0.43^{b}	4.23 ± 0.18^{y}	**	6.13 ± 0.81^{a}	4.68 ± 0.47^{xy}	**	4.68 ± 0.26^{b}	4.80 ± 0.17^{x}	ns	**	*
C20:0	0.21 ± 0.04	0.20 ± 0.01	ns	0.23 ± 0.05	0.20 ± 0.02	ns	0.20 ± 0.01	0.20 ± 0.02	ns	ns	ns
C21:0	0.08 ± 0.01^{b}	0.05 ± 0.02	*	0.11 ± 0.02^{a}	0.08 ± 0.01	**	0.06 ± 0.02^{b}	0.06 ± 0.02	ns	**	ns
C22:0	0.36 ± 0.05	0.45 ± 0.05^{x}	*	0.43 ± 0.05	0.36 ± 0.02^{y}	*	0.36 ± 0.06	0.35 ± 0.04^{y}	ns	ns	**
C24:0	0.46 ± 0.24	0.56 ± 0.27^{x}	ns	0.31 ± 0.02	0.48 ± 0.09^{xy}	**	0.20 ± 0.08	0.25 ± 0.08^{y}	ns	ns	*
SFA	18.98 ± 1.42^{b}	16.43 ± 0.79^{xy}	**	22.86 ± 1.51^{a}	18.27 ± 0.74^{x}	***	17.40 ± 1.04^{b}	16.32 ± 1.59^{y}	ns	***	*
C14:1	0.02 ± 0.01	0.01 ± 0.00	ns	0.03 ± 0.02	0.01 ± 0.01	ns	0.01 ± 0.00	0.01 ± 0.00	ns	ns	ns
C16:1 (n-7)	0.85 ± 0.14^{b}	0.69 ± 0.19^{y}	ns	1.34 ± 0.14^{a}	0.98 ± 0.09^{x}	**	0.77 ± 0.05^{b}	0.66 ± 0.15^{y}	ns	***	**
C18:1 (n-9)	33.56 ± 0.79^{a}	33.67 ± 0.52^{x}	ns	33.40 ± 0.69^{a}	33.01 ± 0.92^{x}	ns	29.53 ± 0.45^{b}	30.95 ± 1.47^{y}	ns	***	**
C20:1 (n-9)	0.21 ± 0.03^{b}	0.17 ± 0.01^{z}	*	0.46 ± 0.08^{a}	0.31 ± 0.04^{x}	**	0.26 ± 0.03^{b}	0.23 ± 0.04^{y}	ns	***	***
C22:1	0.04 ± 0.02	0.05 ± 0.02	ns	0.05 ± 0.02	0.03 ± 0.01	ns	0.04 ± 0.01	0.07 ± 0.03	ns	ns	ns
MUFA	34.68 ± 0.71^{a}	34.60 ± 0.46^{x}	ns	35.29 ± 0.61^{a}	34.35 ± 0.91^{x}	ns	30.61 ± 0.42^{b}	31.93 ± 1.29^{y}	ns	***	***
C18:2 (n-6)	45.35 ± 1.18^{b}	48.26 ± 0.68^{y}	***	39.99 ± 1.60^{c}	46.11 ± 1.45^{z}	***	50.12 ± 1.28^{a}	50.46 ± 0.99^{x}	ns	***	***
C18:3 (n-3)	0.46 ± 0.05^{a}	0.35 ± 0.08	*	0.49 ± 0.04^{a}	0.37 ± 0.04^{yz}	**	0.35 ± 0.05^{bc}	0.28 ± 0.05^{z}	ns	**	ns
C20:2	0.09 ± 0.01^{b}	0.06 ± 0.02^{y}	*	0.13 ± 0.02^{a}	0.09 ± 0.01^{x}	*	0.08 ± 0.02^{b}	0.08 ± 0.02^{xy}	ns	**	*
C20:4 (n-6)	0.35 ± 0.05^{ab}	0.22 ± 0.13	ns	0.42 ± 0.06^{a}	0.32 ± 0.04	*	0.28 ± 0.06^{b}	0.27 ± 0.08	ns	**	ns
C20:3 (n-3)	0.01 ± 0.00^{b}	0.01 ± 0.00	ns	0.03 ± 0.00^{a}	0.01 ± 0.00	***	0.02 ± 0.00^{a}	0.01 ± 0.00	*	**	ns
C20:5 (n-3) EPA	0.02 ± 0.00^{b}	0.02 ± 0.22^{y}	ns	0.09 ± 0.03^{a}	0.02 ± 0.01^{y}	**	0.08 ± 0.03^{a}	0.08 ± 0.02^{x}	ns	***	***
C22:6 (n-3) DHA	0.05 ± 0.01^{c}	0.04 ± 0.02^{z}	ns	0.69 ± 0.15^{b}	0.44 ± 0.09^{y}	*	1.04 ± 0.27^{a}	0.56 ± 0.08^{x}	**	***	***
PUFA	46.34 ± 1.25^{b}	48.96 ± 0.60^{y}	**	41.85 ± 1.55^{c}	47.38 ± 1.49^{y}	***	51.98 ± 1.45^{a}	51.75 ± 0.86^{x}	ns	***	***

Asterisks indicate significant differences (* = p < 0.05; ** = p < 0.01; *** = p < 0.001). ns: not significant effect.
Means with different letters (a, b, c) among PF samples differ significantly.
Means with different letters (x, y, z) among F samples differ significantly.

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Capítulo 2.2

Enrichment of fried chicken nuggets with microencapsulated fish oil: effect on sensory properties, volatile compounds and oxidative stability

Enviado a: Journal of the Science of the Food and Agriculture

Octubre 2015 (JSFA-15-2727)

Journal of the
Science of
Food and Agriculture
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Enrichment of fried chicken nuggets with microencapsulated fish oil: effect on sensory properties, volatile compounds and oxidative stability

Journal:	*Journal of the Science of Food and Agriculture*
Manuscript ID	JSFA-15-2727
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	15-Oct-2015

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Key Words:	microencapsulation, ω-3 fatty acids, chicken nuggets, sensory analysis, oxidation, volatile compounds

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1 TITLE

2 Enrichment of fried chicken nuggets with microencapsulated fish oil: effect on sensory
3 properties, volatile compounds and oxidative stability

4 RUNNING TITLE

5 Enrichment of fried chicken nuggets with microencapsulated fish oil

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ABSTRACT

Background

Enrichment of chicken nuggets with microencapsulated ω-3 PUFA is a potential approach for obtaining a functional meat product that could maintain these fatty acids with protection from oxidation. This work studies the addition of microencapsulated fish oil rich in ω-3 fatty acids to chicken nuggets, and evaluates their oxidative stability and sensory properties in comparison to bulk fish oil addition.

Results

Three batches of chicken nuggets were prepared: control (C), enriched in bulk fish oil (BFO) and with added microencapsulated fish oil (MFO). Sensory features, acceptability, oxidative stability and volatile compounds were analysed. MFO nuggets did not differ in any sensory trait with C ones. BFO showed increased juiciness and saltiness but decreased meat flavour. Acceptability was not affected by enrichment. Consumers were not able to differentiate between C and MFO in a triangle test, but they could clearly identify BFO nuggets. No volatile compound related to ω-3 fatty acids oxidation was detected. Higher levels of lipid and protein oxidation indicators were found in BFO nuggets compared to C and MFO ones.

Conclusion

Enrichment of chicken nuggets with microencapsulated fish oil gives as a result a ω-3 PUFA enriched product that could be commercially well accepted with protein and lipid oxidative protection.

KEYWORDS: microencapsulation; ω-3 fatty acids; chicken nuggets; sensory analysis; oxidation; volatile compounds

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45 INTRODUCTION

46 Omega-3 (ω-3) are essential polyunsaturated fatty acids (PUFA) that have become of 47 great interest because of their bioactivity. They have been related to promotion of 48 human health in terms of reduction of the risk of cardiovascular disease, chronic 49 diseases and tumours. Due to that, there is a growing interest among consumers, food 50 authorities and food industry in increasing ω-3 in food products and diet.

51 Fish and seafood are food sources with high content of long chain ω-3 fatty acids, such 52 as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n- 53 3). However, per capita fish consumption is quite low compare with meat and meat 54 products [1]. Meat industries have a growing interest at producing meat products with a 55 healthier profile. Thus, the development of meat products enriched in ω-3 fatty acids 56 appears as a good opportunity for increasing the intake of this type of fatty acids. 57 Enrichment of meat products in ω-3 fatty acids [2] and the effect of their intake on health 58 of consumers has been the focus of some recent studies [3].

59 The increased content of ω-3 PUFA in meat products could be considered advantageous 60 from a nutritional point of view. However it can lead to several drawbacks caused by 61 the susceptibility of PUFA to oxidation processes. The breakdown of the ω-3 PUFA 62 chain during oxidation involves nutritional loss and a detrimental sensory, with an 63 unacceptable rancidity and fishy off-flavour. In the particular case of meat products 64 enriched in ω-3 PUFA, which are indeed highly susceptible to oxidative reactions, the 65 way to add the source of these bioactive compounds could influence the development of 66 these reactions and its adverse consequences.

67 A possible strategy to protect ω-3 PUFA from oxidation is microencapsulation, which 68 limits the adverse food processing environment such as the contact with water and

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oxygen by packaging bioactive functional food components within protective matrix structures. Fish oil is currently microencapsulated by different methods, the most popular method being spray-drying [4]. Microencapsulation of fish oil by spray-drying using multilayered emulsions of lecithin-chitosan combined with carbohydrates as coating material has been reported to have successful effects on protection from oxidation [5]. However, whereas enrichment with bulk fish oil has been assayed by some authors, the literature of food enriched with microencapsulated ω-3 PUFA is scarce, especially for meat products [6], with no examples in the literature of pan-fried convenience meat products enriched with ω-3 microcapsules.

The growing demand for "ready-to-heat" breaded pre-fried frozen products makes these convenience products an interesting and promising target for enrichment in ω-3. Among these type of products, chicken nuggets are perhaps the most popular ones, widely purchased in stores and consumed in fast food restaurants [7]. Enrichment of pan-fried frozen products, such as chicken nuggets, with microencapsulated ω-3 PUFA is a potential approach for obtaining functional food that could maintain these PUFA in the product with protection of these fatty acids from lipid oxidation during all the stages of the production (from manufacture to the final domestic cooking) avoiding the undesired fishy flavour.

Thus, the aim of the present work was to enrich chicken nuggets with fish oil omega-3 microencapsulates, and evaluate their oxidative stability and sensory properties in comparison to bulk fish oil addition.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

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3 92 Three different types of nuggets were prepared: a control batch (C), a batch enriched
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5 93 with bulk fish oil (BFO) and a batch enriched with microencapsulated fish oil (MFO).
6
7 94 Fish oil (kindly provided by Biomega Natural Nutrients S.L., Galicia, Spain) was used
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9 95 as source of ω-3 FA (6.9 % EPA, 24.6 % DHA).
10
11 96 Formulation and manufacture of the chicken nuggets of the control batch was made
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13 97 following the methodology optimised by Medina et al. [8] using chicken breast as the
14
15 98 meat raw material. As for the enriched batches, formulation of the batter was modified
16
17 99 by the addition of 0.5% (w/w) of fish oil in the batch BFO and by the addition of 5 %
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19 100 (w/w) of multilayered microcapsules of fish oil optimised in a previous work [5]. Thus,
20
21 101 the enrichment level in EPA+DHA in BFO and MFO nuggets was 150 mg per 100 g,
22
23 102 enough to exceed the minimum level required by the European Union law (Commision
24
25 103 Regulation (EU) Nº 116/2010 of 9 February 2010 amending Regulation (EC) Nº
26
27 104 1924/2006 of the European Parliament and of the Council with regard to the list of
28
29 105 nutrition claims to label a food as "high in ω-3 fatty acids"): at least 80 mg of the sum
30
31 106 of EPA and DHA per 100g and per 100 kcal [9].
32
33 107 Chicken breast was purchased in a local market (Mercadona, Cáceres, Spain) and
34
35 108 minced using a meat mincer (SEB IBERICA, Barcelona, Spain). Then, it was mixed
36
37 109 and further minced with the ingredients in a domestic kitchen processor Thermomix
38
39 110 (Vorwerk Wuppertal, Germany) according to each formulation, also a commercial
40
41 111 preparation (Procavi; ANVISA, Madrid, Spain) containing soya flour, salt, flavourings,
42
43 112 soya protein, milk protein, dextrose, spices, pentasodium triphosphate,
44
45 113 tetrasodiumdiphosphate, locust bean gum, guar gum and sodium ascorbate (60 g/kg
46
47 114 meat) was added.
48
49 115 The obtained dough was stored at 0-2 ºC during 24h and it was subsequently portioned
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51 116 to obtain each chicken nugget (4 x 4 x 1 cm, 25 g) and coated, first with a commercial
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117 stick solution containing wheat starch, wheat corn, guar gum, wheat starch and
118 powdered turmeric (Avigum, ANVISA, Madrid, Spain) dissolved in cold water (25
119 g/L), and subsequently with breadcrumbs (35 g/kg nugget). Subsequently, all nuggets
120 were subjected to a pre-fried process in sunflower oil during 10 sec. Thereafter, the
121 nuggets were immediately removed from the fryer and placed on paper towel to remove
122 excess of external oil. Then, pre-fried nuggets were stored at refrigeration temperature
123 (0-2 ºC) in a tray covered with a plastic wrap during 24h and subsequently fried at 180
124 ºC in sunflower oil until the product reached 63 ºC of internal temperature. Some of
125 these fried nuggets from the three batches (C, BFO, MFO) were immediately used for
126 the sensory test and the rest were stored at -80ºC until analysis.

127 Temperature of nuggets during frying was recorded using a thermometer probe (Testo
128 735-2, Lenzkirch, Germany). Nine nuggets were fried at the same time. The oil was
129 replaced every four frying sessions. After each deep-frying, nuggets were drained, and
130 placed on paper towel for removing external oil. Oxidation assays, volatile compounds
131 and sensory test were analysed on the fried nuggets. For volatile compounds and
132 oxidation assays, five replicates (whole nugget, including meat and crust) from each
133 batch were minced and analysed by duplicate.

134

135 *METHODS*

136 *Sensory analysis*

137 All sessions were done in sensory panel rooms with the conditions specified in UNE-
138 EN-ISO 8589:2010 Regulation [10]. Panel rooms were equipped with white fluorescent
139 lighting (220-230 V, 35 W). Nuggets were served hot on white plastic plates to
140 panelists, marked with random three-digit codes. The panel sessions were held around

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1-2 h before lunchtime. Salt free crackers and a glass of water at room temperature were provided to each panelist to rinse between samples.

Quantitative-Descriptive

Chicken nuggets were assessed by a trained panel of 15 members using a descriptive analysis method. The panelists were trained in four sessions for 1 h. In the first session, panelists tested chicken nuggets and were asked to express terms that describe their personal observations. In the second session, the redundant descriptive terms were eliminated and the samples were tested to include new attributes. In the third session, all the attributes selected were used in an unstructured scale of 0–10. At the fourth session, initially the different attributes were evaluated individually by panelists in unknown representative samples and then panelists responses were analyzed collectively. Data were gathered and analyzed by analysis of variance (ANOVA), and panelist deviations were assessed to determine where additional training was needed. Nineteen sensory traits of nuggets grouped under appearance, texture, odor and flavor were assessed. Their definitions and extremes are explained in Table 1. Questions were presented to assessors in the normal perception order, as follows: visual analysis, texture, taste and flavor. Sensory traits of the samples of chicken nuggets were assessed by each panelist in a 10 cm unstructured line.

Hedonic test

A hedonic test was carried out using an unstructured 9-point line scale with hedonic inscriptions at the lower extreme "disliked extremely" and upper extreme "liked extremely" of the scale. 30 untrained consumers (students and staff of the University) were randomly selected as volunteers to evaluate hedonic score of the chicken nuggets. The test took place in the tasting rooms, under the same conditions as the quantitative-descriptive analysis. Three fried chicken nuggets were evaluated in each session, and

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166 sample order was randomized across assessors. Thirty samples from each batch were
167 analyzed.
168

169 *Triangle test*

170 A total of 30 subjects, including university students, academics and staff recruited at the
171 School of Veterinary Sciences (University of Extremadura, Cáceres, Spain) formed the
172 untrained consumer panel. Each fried chicken nugget from the different batches (C,
173 BFO, and MFO) was prepared for presentation by dividing the sample in four sections.
174 Subjects evaluated at once in the same plastic plate three samples from different batches
175 in each session, two from the same batch and one from another one, and they were
176 asked to identify the different sample. Order was randomised across subjects. Number
177 of judgments was 30 for each of the three possible combinations (C vs BFO, C vs MFO
178 and BFO vs MFO).
179

180 *Volatile compounds*

181 Volatile compounds were analysed by headspace solid phase microextraction (HS-
182 SPME) following the method described by Garcia-Esteban et al. [11] with some
183 modifications. Each chicken nugget was ground and 5 g were weighted into a 20 mL
184 glass flask sealed with an aluminium cap and a septum. Sealed vial was conditioned in a
185 thermostatized water bath at 37 ºC for 30 minutes. SPME was carried out by using a
186 cross-linked divinylbenzene/carboxen/polydimethylsiloxane fiber, 50/30 µm thick and 2
187 cm long (Supelco, Bellefonte, PA, USA), conditioned prior to use by heating in the
188 injection port of a gas chromatograph (GC) system under the conditions recommended
189 by the manufacturer (at 270 ºC for 1 h). The fiber was then inserted into the sample vial
190 through the septum and exposed to the headspace for 30 min at 37 ºC. The SPME fiber

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was desorbed and maintained in the injection port for 30 min. Analyses were performed using a Hewlett–Packard 6890 series II GC coupled to a mass selective (MS) detector (Hewlett-Packard HP 5973) (Wilmington, DE, USA). Volatiles were separated using a 5% phenylmethyl silicone (HP-5) bonded-phase fused silica capillary column (Hewlett–Packard, 50 m x 0.32 mm i.d., film thickness 1.05 µm), operating at 6 psi of column head pressure, resulting in a flow of 1.3 mL/min at 40 ºC. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 ºC, rose to 200 ºC at a rate of 5 ºC/min and then rose to 250 ºC at a rate of 20 ºC/min and held for 5 min. The transfer line to the mass spectrometer was maintained at 280 ºC. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of 1 scan/s over the m/z range of 30–550 u.m.a. nAlkanes (Sigma R-8769) were analysed under the same conditions to calculate the linear retention indices (LRI) for the volatile compounds. Compounds were identified by comparison with the mass spectrum and RI of commercial reference compounds (Sigma-Aldrich; Steinhein, Germany), by comparison of mass spectrum with mass spectral database (NIST and Wiley libraries) and by comparison of their RI with those available in the literature. Results from volatile analyses are provided in area units (AU).

Oxidation

Conjugated Dienes

The measurement of conjugated dienes (CD) was determined according to the procedure described by Juntachote et al. [12]. Frozen chicken nuggets were thawed and minced. Subsequently, 0.5 g were suspended in 5 ml of distilled water and homogenised to form smooth slurry. A 0.5 ml aliquot of this suspension was mixed with 5 ml of

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extracting solution (3:1 (v/v) hexane/isopropanol) for 1 min. After centrifugation at 3500 rpm for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of CD was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1} and the results were expressed as µmol per mg of sample.

Thiobarbiruric Acid Reactive Substances (TBARS)

TBARS content was measured by following the extraction method described by Salih et al. [13]. Each nugget was minced in a kitchen blender, and 2.5 g were homogenised for 2 min with 7.5 mL of 3.86% perchloric acid and 0.5 mL of butylatedhydroxytoluene. Tubes were kept in ice to avoid heat degradation. This homogenate was filtered and centrifuged (3 min, 3500 rpm). The supernatant (2 mL) was mixed with 2 mL of 97% 1,1,3,3- tetraethoxypropane (TEP). Immediately, the mixture was heated to 90 ºC for 30 min, cooled and centrifuged again (2 min, 3500 rpm). Absorbance was measured at 532 nm and 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The measurement at 600 nm is considered contamination and it was subtracted to the other measurement to obtain the final absorbance. The concentration of TBARS was calculated as malondialdehyde (MDA) from a standard curve, which was developed simultaneously with the samples using solutions of TEP (Merck, Schchardt, Germany). TBARs were expressed as mg MDA equivalents/kg sample.

Protein Carbonyls

Protein oxidation in chicken nuggets was followed by measuring the formation of protein carbonyls by converting them to 2,4 dinitrophenylhydrazones (DNPH), and the derivatives were measured spectrophotometrically according to method outlined by Oliver et al. (1987) [14] with slight modifications. Chicken nuggets were minced and then,

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1 g of meat was homogenised 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturrax homogeniser for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL eppendorf tubes. Proteins were precipitated by cold 10 % TCA (1 mL) and subsequently centrifuged for 5 min at 5000 rpm. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10 % TCA (1 mL) and washed twice with 1 mL ethanol/ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 5000 rpm to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using a BSA standard curve. The amount of carbonyls was expressed as nmol of carbonyl per milligram of protein using an absorption coefficient of 21.0 nM-1 x cm-1 at 370 nm for protein hydrazones.

Sampling replication and Statistical analysis

Three batches were prepared (n=3) for each chicken nugget formulation (C, BFO and MFO). For each physico-chemical analysis, 5 samples of chicken nuggets were analyzed in duplicate. The effect of type of enrichment (C, BFO or MFO) on oxidation parameters, volatile compounds and quantitative-descriptive were analysed using a one-way analysis of variance (ANOVA) according to the General Linear Model procedure. When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. Significant differences ($p < 0.05$) in the hedonic test were detected using a nonparametric analysis achieved using Friedman's Test. Significant differences in the triangle test were detected according to the table of

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266 minimum number of correct selections for a significant difference by Roessler et al. [15].
267 Statistical analysis was carried out using IBM SPSS Statistics v.19.

268 RESULTS AND DISCUSSION

269 *Sensory analysis*

270 The results of quantitative-descriptive analysis are shown in Figure 1. It is worth to
271 mention that none of the odour traits showed was affected by the effect of the addition
272 of fish oil. The fishy and rancid odour and flavour could be a risk for the acceptability
273 of food products enriched with fish oil, but it seems that either it was not produced or
274 the rest of ingredients and/or the cooking method masked it, as it were scored
275 considerably low by consumers. Only one of the traits grouped under texture (juiciness)
276 and two of the traits grouped under flavour (saltiness and meat flavour), showed
277 significant differences.

278 Differences in the scores for texture (Figure 1.b) and flavour (Figure 1.c) indicated there
279 was a more intense perception of juiciness and salty taste and lower of meat flavour in
280 BFO, whereas these traits remained the same in MFO compared to C. In deep-fat frying
281 of foods, the high temperatures of the oil leads to the appreciated textural characteristic
282 of fried products: dry and crispy crust, keeping a tender, moist and juicy core [7].

283 In all types of nuggets, the dominant flavour and taste were meat, oily, salty and spicy,
284 whereas rancid and fish flavours were scored very low and nearly not detected. The
285 difference in salty taste, which was scored higher in BFO nuggets, could be due to
286 interactions between bulk fish oil and salts in the dough. In a study made in emulsion
287 based foods it has been detected an increase in saltiness perception from oil-in water
288 emulsions with increasing oil phase volume and constant total amount of salt, and it was
289 hypothesised that the higher oil volume enhanced mixing with saliva, accelerating this

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way the transport of salt to the taste buds [16]. Moreover, BFO nuggets showed significant lower scores for meat flavour. Flavour is one of the most important sensory attributes for the overall acceptability of meat. However, despite the differences in the quantitative-descriptive test, the results of the acceptability test (Figure 2.a) indicate that overall acceptability of the nuggets was similar for all the batches. Thus, the enrichment caused no differences in the overall acceptability.

The results of the triangle test (Figure 2.b) showed that subjects were not able to differentiate between C and MFO nuggets, as only 19 out of the 48 judgments (39.6 %) correctly discerned between the samples. On the contrary, they perfectly discriminated ($p<0.001$) between BFO nuggets and the other batches (either C or MFO nuggets). In both cases, 29 out of the 48 judgments (60.4 %) correctly discerned between the samples. This is remarkable, since both types of chicken nuggets contained the same amount of added fish oil, with the only difference of way of addition (BFO and MFO). Therefore, this type of microcapsules appear to be useful in masking potential off-flavours arising from fish oil. From a commercial point of view, the fact that MFO nuggets are not discernible from C ones indicates that they seem to be more suitable for commercial purposes.

On the contrary, BFO nuggets were perfectly identified, which could be related to the previously discussed differences in sensory traits (higher perception of juiciness and salt taste and lower of meat flavour). Previous literature of food enriched with microencapsulated ω-3 PUFA report different results. In pan bread, Lu et al. found that the panelists distinguished the enriched products with 2.5% (w/w) of microcapsules added (approximately 0.2% w/w of EPA+DHA) [17], while De Conto et al. [18] using higher level of enrichment in the same food product (5% w/w of microcapsules and 0.6% w/w of EPA+DHA) concluded that the panelists did not distinguished the enriched products.

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3 315 It is remarkable to indicate that our study did not reached these enrichment levels, as the
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5 316 MFO nuggets were enriched with 5% (w/w) of microcapsules, which means 0.15% w/w
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7 317 EPA+DHA. However, in addition to the amount of microcapsules added and the
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9 318 enrichment of EPA+DHA obtained, other factors should be taken into account, such as
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11 319 formulation, manufacture process, technology process, cooking method and so on.
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15 320 *Volatile compounds*
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18 321 The profile of volatile compounds of fried chicken nuggets provides information about
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20 322 the formation of compounds related to oxidative deterioration and also about volatiles
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22 323 that could contribute to the aroma of the products with or without ω-3 enrichment [19].
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24 324 The GC-MS analysis allowed the identification of 68 different volatile compounds
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26 325 (Table 2) that were divided into 8 main groups: alcohols, aldehydes, aromatic
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28 326 hydrocarbons, aliphatic hydrocarbons, benzenes, furans, pyrazines, esters, ketones,
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30 327 terpenes and sulphur compounds. The most abundant groups in all chicken nuggets
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32 328 were aliphatic hydrocarbons, terpenes and aldehydes, followed far behind by aromatic
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34 329 hydrocarbons, ketones, benzenes and alcohols. The minor volatile families were furans,
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36 330 pyrazines, sulphur compounds and esters. This profile basically agrees with previous
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38 331 studies about deep-fried battered products [20]. Significant differences among different
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40 332 batches of nuggets for alcohols, benzenes, pyrazines, aliphatic hydrocarbons, ketones
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42 333 and terpenes were found.
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46 334 Of the 68 volatile compounds detected, 59 showed significant differences due to the
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48 335 addition of either bulk or encapsulated fish oil to the nuggets. The major compound in
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50 336 all samples was hexane, followed by 2-methylpentane, 3-methylpentane, methyl-
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52 337 cyclopentane, 3-methylbutanal, 2-methylbutanal, hexanal, 2-propanone, alpha-pinene,
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54 338 sabinene, 2-beta pinene, delta-3-carene and 1-limonene.
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3 339 Aliphatic hydrocarbons were similar in BFO and MFO, which showed higher values
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5 340 than C. However, various volatile lipid oxidation products have different partitioning
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7 341 coefficients in fat and water, which influence the volatility varies with polarity and is
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9 342 influenced by formulation of the products [21]. It is possible that the higher amount of fat
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11 343 in the enriched batches may have influenced the reaction pathways for the formation of
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13 344 volatile compounds and contribute to a higher total amount of aliphatic hydrocarbons
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15 345 with respect to that observed in the control batch. Hydrocarbons show high odour
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17 346 detection threshold, and thus, they mostly do not contribute significantly to flavour [22].
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19 347 As for the aromatic hydrocarbons, none is individually considered to be important in the
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21 348 overall flavour or odour of meat . While ω-3 PUFA lipid autoxidation is considered the
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23 349 main cause of fishy flavour, hydrocarbons, do not appear to contribute to fishy flavour
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25 350 or odour [23].
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27 351 Two ester compounds derived from acetic acid were detected in their ethyl form: acetic
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29 352 acid ethyl ester (only found in MFO) and acetic acid butyl ester (which show not
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31 353 differences between batches). The esters might be either synthesised from the free fatty
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33 354 acids and alcohols (esterification) or by transesterification reaction (alcoholysis) of the
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35 355 fatty acids of the triglycerides and ethanol [24]. They have been found in other cooked
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37 356 products enriched in ω-3 fatty acids [25]. However no implication in food quality has been
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39 357 described.
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41 358 Terpenes were higher in C than in both batches of enriched nuggets. However, this
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43 359 difference was not enough to produce differences in 'spicy' flavour between batchers
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45 360 appreciable by sensory assessors. Such difference in the amount of terpenes in the
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47 361 headspace could be related to the higher amount of fat in fish oil added nuggets:
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49 362 terpenes are non-polar compounds, and lipids could limit their release to the headspace
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51 363 [26]. A great variety terpenes were detected in the nugget samples, and most of them have
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3 364 been reported as volatile components and isolated in the headspace of several spiced
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5 365 foods being recognised odorants which are commonly used in the food industry as
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7 366 flavour and fragrance ingredients [27]. The major terpenes were delta-3-carene, 1-
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9 367 limonene, alpha-pinene and 2-beta-pinene. The main tendency for all terpenes was to be
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11 368 higher in C than in both types of enriched nuggets. Spices added as flavouring additives
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13 369 provide intense and desirable flavours, which could mask the rancid aroma notes from
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15 370 the lipid-derived volatiles. The contribution of these compounds to the overall aroma
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17 371 might have masked fish odour in our nuggets. Even though the concentration of most
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19 372 terpenes was lower in enriched nuggets than in C, assessors did not find differences in
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21 373 flavour notes referred to as 'spicy'. The attitude of consumers towards nuggets spicy
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23 374 aromatic notes seems to be positive because as mentioned above, good acceptability
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25 375 was recorded and spicy had relative good scores (around 5 out of 10). The mask effect
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27 376 of off-flavours of spices have been previously reported in other meat products enriched
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29 377 in ω-3 fatty acids, such as sausages [28].
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34 378 The highest levels of alcohols, ketones and benzenes were detected in BFO nuggets,
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36 379 while C and MFO ones showed similar values. In this type of products, the alcohols and
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38 380 ketones found are most likely formed through the oxidation of fatty acids [29]. So, it
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40 381 seems that in BFO nuggets, the fish oil added directly could have been less protected
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42 382 from oxidation than in MFO ones, leading to increasing levels of these groups of
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44 383 volatile compounds. Within the alcohols, ethanol and cyclopentanol were the most
45
46 384 abundant ones. The amount of ethanol was higher in BFO nuggets than in MFO and C
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48 385 ones while cyclopentanol was only detected in BFO. This could indicate higher lipid
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50 386 oxidation levels in BFO nuggets compared with the other batches. Nonetheless the
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52 387 contribution of alcohols to flavours of foods has been reported to be minor [25]. 2-
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54 388 propanone was the most relevant and abundant ketone, and was in higher concentration
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389 in BFO than in the other two batches. Ketones in foods have been implicated with off-
390 flavours referred to as “perfume” rancidity [30].

391 The detected aldehydes were saturated aldehydes (butanal, pentanal, hexanal, octanal
392 and nonanal), unsaturated aldehydes (2,4-hexadienal and 2,4-decadienal) and aldehydes
393 derived from Strecker degradation of amino acids (acetaldehyde, 2-methylpropanal, 3-
394 methylbutanal and 2-methylbutanal).

395 Saturated and unsaturated aldehydes have been extensively used as markers for lipid
396 oxidation in food [29]. In the present study, butanal and 2,4-hexadienal were only detected
397 in BFO samples. Hexanal, heptanal and nonanal were lower in MFO than in C and
398 BFO, which showed similar amounts. BFO showed the highest amounts of pentanal,
399 and octanal (which was not detected in MFO). The unsaturated aldehyde 2,4-decadienal,
400 was only detected in enriched batches with higher amounts in BFO than in MFO. These
401 compounds, especially hexanal, are often used as marker compounds for lipid oxidation
402 for ω-6 unsaturated fatty acids in meats [24]. In chicken meat, C18:1 and C18:2 are the
403 most abundant MUFA and PUFA, respectively. Thus, they can act as precursors of
404 these aldehydes in oxidative reactions. However, in fish products, the typical aldehyde
405 used as oxidation marker is propanal, as its formation from oxidation of ω-3 PUFA
406 seems to require a lower activation energy than that foe the formation of hexanal [31]. In
407 fact, in other meat products (sausages) enriched in ω-3, propanal concentration during
408 oxidation was higher than hexanal [6, 28]. Thus, it is remarkable that propanal was not
409 detected in any of the chicken nuggets of this study. However, as mentioned above,
410 some linear saturated aldehydes were higher in BFO nuggets. Moreover, the unsaturated
411 aldehyde 2,4-decadienal, which is also a key compound arising from the oxidation of
412 fish oil, was only detected in BFO samples. This points out to higher lipid oxidation

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413 levels in BFO nuggets as compared to MFO and C ones, which is remarkable, since the
414 amount of fish oil added to BFO and MFO samples was the same.

415 In addition to be used as oxidation markers, aldehydes, especially unsaturated ones, are
416 the main compounds responsible for characteristic off-flavours of oxidised fish oil. The
417 association between 'rancid' off-flavour that influences negatively the overall flavour of
418 different foodstuff, and the contents of aldehydes such as pentanal, hexanal, octanal and
419 nonanal, has been reported [19]. Some of them such as 2,4-decadienal impart flavours
420 which have been frequently described as 'cod-liver-oil-like' and 'painty' [32]. However,
421 neither fish nor rancid odours/flavours were detected in the sensory analysis in this
422 study. Formulation and cooking of the nuggets should be taken into account, so that it
423 might be that off-odours and flavours were simply masked by other volatiles generated
424 during frying, or directly coming from meat or spices.

425 Some aldehydes derived from Strecker degradation of amino acids also show an
426 influence on flavour, but in a positive way, being common components in cooked meat
427 flavour and contributing to desirable aroma notes, such as the typical 'toasted' of fried
428 products. In the chicken nuggets, acetaldehyde highest level was found in BFO,
429 followed by C and with the lowest detection in MFO. On the other hand, the highest
430 amount for the other Strecker aldehydes (2-methylpropanal, 2-methylbutanal and 3-
431 methylbutanal) was in MFO and the lowest in C, in all cases. Some authors studied the
432 addition of ω-3 fatty acids to amino acid and sugar mixtures, and observed that it
433 affected the kind and amount of most of these compounds [33]. This seems not to be the
434 case of fried nuggets enriched in ω-3 fatty acids either directly or microencapsulated,
435 which showed higher amounts of total Strecker derived aldehydes.

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The level of pyrazines found was different in the three batches, with higher amounts of these compounds in BFO followed by C and with the lowest amount in MFO. Pyrazines are products from Maillard reactions generated during meat cooking, which show a low odour threshold [34]. Surprisingly, it seems that pyrazines could have been promoted by enrichment with direct fish oil and decreased by enrichment with microencapsulated fish oil. In fact, different carbonyls from autoxidation of unsaturated lipids have been found to promote Maillard reactions [35]. Moreover, alkylpyrazines and thiols, which are also Maillard reaction products, were detected in all samples [36]. There was detected a significant effect of ω-3 enrichment in the amounts of 2,6-dimethyl-pyrazine, trimethyl-pyrazine and 3-ethyl-2-dimethyl-pyrazine. Trimethyl-pyrazine was not detected in MFO, while differences in the amounts of this compound in BFO and C were not significant.

As for furans, some authors have suggested that a few alkyl-furans could arise from PUFA oxidation, and that the reason of the low impact on overall flavour is their relatively high odour thresholds [37]. In that sense, 2-propyl-furan was in higher amount in BFO than in C and MFO, and 2-butyl-furan was higher in BFO than in C, not being detected in MFO. This again could indicate less oxidation of MFO.

To sum up, it seems that compounds related with specific oxidation of ω-3 PUFAs were not detected in neither type of enriched chicken nuggets (with bulk fish oil or with microencapsulated fish oil), whereas other volatiles related with oxidation of meat were numerous and showed high concentrations. This is in agreement with previous studies that have shown that it is more likely for ω-3 PUFAs to exert their impact on oxidation and sensory traits through accelerating the oxidation of other unsaturated fatty acids than through their own degradative products [37].

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Lipid and Protein oxidation

Stability oxidation was measured by TBARs and conjugated dienes as primary and secondary oxidation from lipid and protein oxidation was measured by DNPH analysis. As it is shown in Figure 3, the most remarkable finding concerning lipid oxidation (indicated by both primary and secondary oxidation products, conjugated dienes and TBARs, respectively) and protein oxidation (protein carbonyls) was the significantly higher amount in BFO as compared to C and MFO nuggets. As for MFO nuggets, higher amounts of TBARs than in C ones were detected, while conjugated dienes and protein carbonyls did not show significant differences. Therefore, it seems that bulk fish oil enrichment made the nuggets more prone to lipid and protein oxidation, and that encapsulation successfully controlled the development of such oxidative reactions. . Other authors have previously reported higher oxidative values in fermented sausages and yogurt enriched with bulk ω-3 oil in comparison with that enriched with microencapsulated ω-3 oil [6]

The protection of the microcapsules against oxidation could be a result of the combination of the inner protection provided by the microcapsule layers which act as a barrier that limits both the oxidation of the ω-3 fish oil [5], and probably also avoids the propagation of the oxidation radicals produced from the oxidation of these PUFA through the meat product. This propagation is probably lower in the microcapsules than in the bulk fish oil, which would avoid that the oxidation radicals could initiate a chain reaction in the nugget. However, the combination of the particular characteristics of the formulation of the microcapsules and of the product and their interactions should also influence the results of oxidation status [4, 6]. It should be noticed the agreement between the values of lipid and protein oxidation, which were higher in BFO in all cases. This has been previously reported in chicken meat, and it has been hypothesised that primary

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485 and secondary lipid oxidation products can promote protein oxidation, so after initiation
486 of the oxidation of lipids, the oxidation of proteins is also enhanced [38].

487 CONCLUSIONS

488 Enrichment of chicken nuggets with microencapsulated fish oil as source of ω-3 PUFA
489 does not affect neither sensory traits nor acceptability of the enriched meat products. On
490 the contrary enrichment with bulk fish oil affects negatively some sensory traits. In the
491 enriched chicken nuggets, it can be observed protection of both lipid and protein
492 oxidation provided by microencapsulation of the fish oil. Moreover, there was no
493 detected, specific oxidation of ω-3 PUFA. Thus, enrichment of pre-fried ready-to-heat
494 meat products with microencapsulated fish oil is suitable and gives as a result a ω-3
495 PUFA enriched product that could be commercially well accepted with advantage in
496 both lipid and protein oxidative protection respect to the enrichment with bulk fish oil.

497

498 ACKNOWLEDGMENT

499 Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo
500 Social Europeo are acknowledged for supporting the pre-doctoral grant (PD10026) and
501 support research groups. Biodymia Laboratory (University of Lyon) for making
502 possible the development of the work during a research stay. "Biomega Natural
503 Nutrients S.L." is also acknowledged for kindly providing the fish oil.

504

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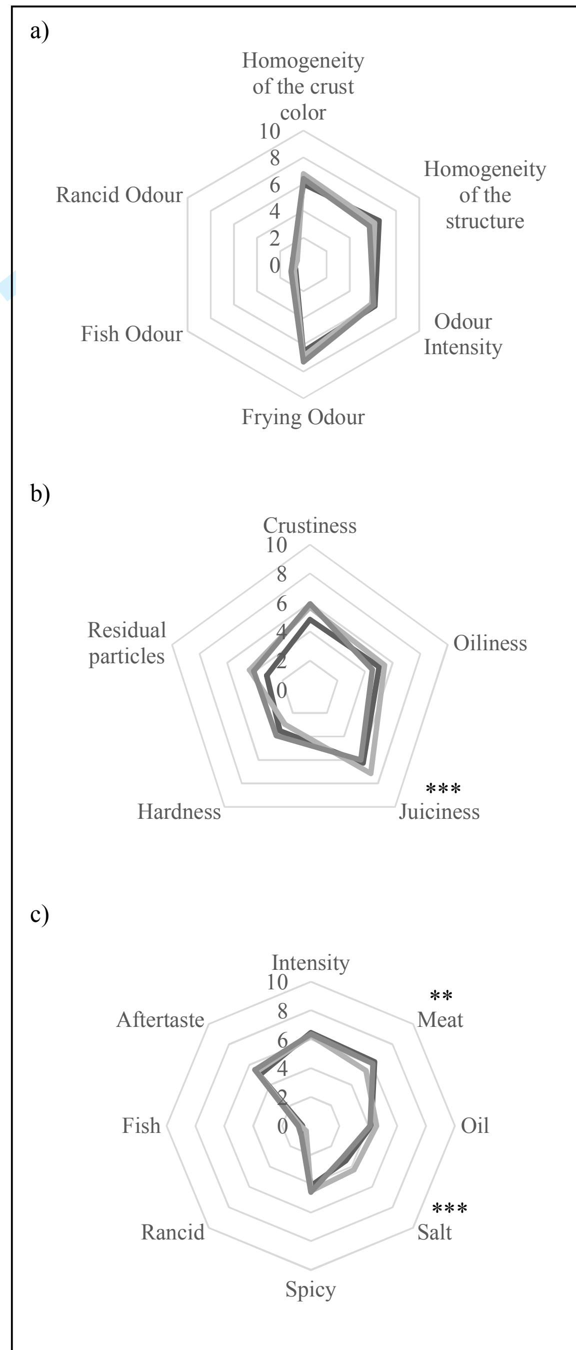
Figure 1. Quantitative-descriptive sensory analysis of deep-fried chicken nuggets (control in dark grey, enriched with bulk fish oil in light grey and enriched with fish oil microcapsules in medium grey): traits related with appearance and odour before eating (a), texture (b) and flavour (c).



** = p<0.01, *** = p<0.001.

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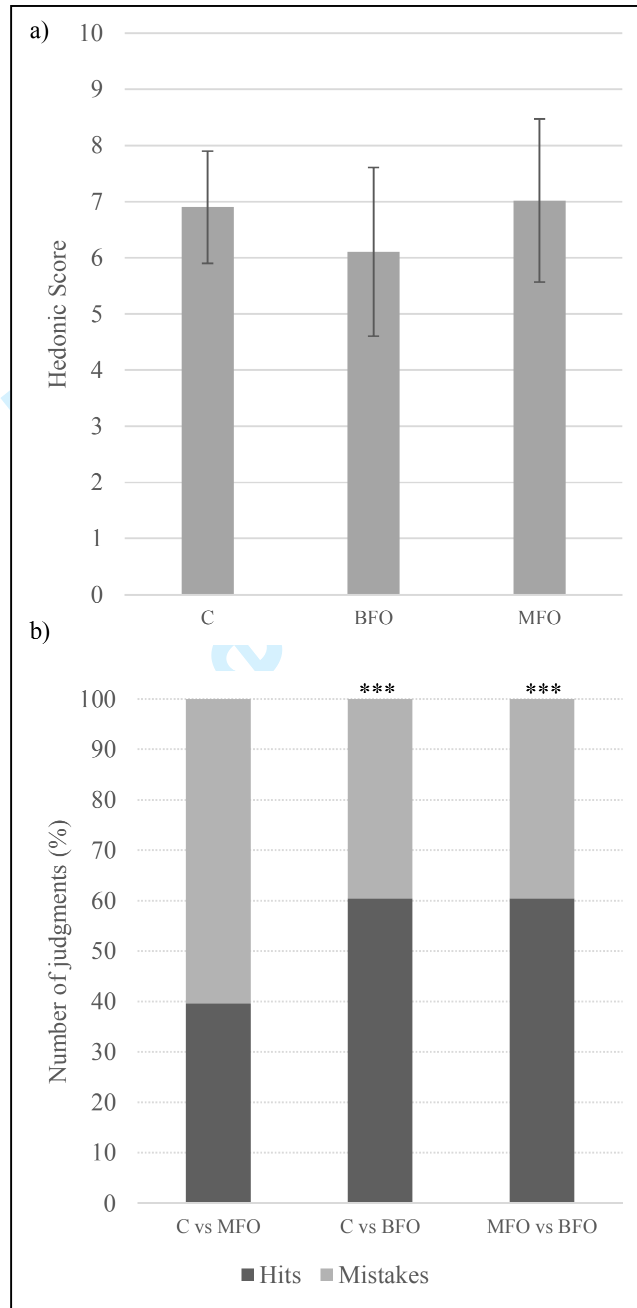
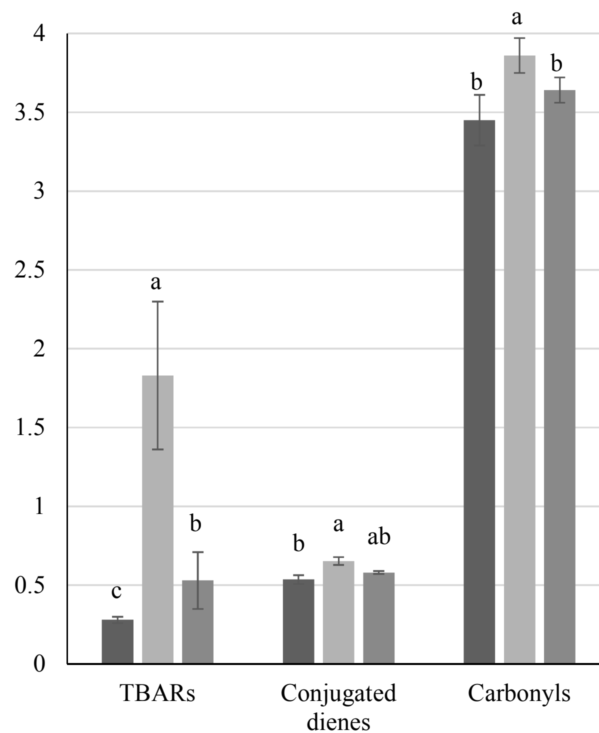
Figure 2. Acceptability (a) and triangle test (b) of the deep-fried chicken nuggets: control (C), enriched with bulk fish oil (BFO) and enriched with microcapsules of fish oil (MFO).



*** = $p<0.001$

Figure 3. Mean values and SD for TBARs (mg MDA equivalents/kg sample), conjugated dienes (μmol/mg sample), and protein carbonyls (nmol/mg protein) of the three batches of fried chicken nuggets: control (C, dark grey), enriched with bulk fish oil (BFO, light grey) and enriched with microcapsules of fish oil (MFO, medium grey).



Bars of the same determination with different letters significant differ ($p<0.05$).

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Table 1. Sensory attributes of fried chicken nuggets.

Sensory trait	Definition
Before eating	
Visual appearance	
Homogeneity of the crust colour	Homogeneity of the crust colour (less-much)
Homogeneity of the structure	Homogeneity of the structure (less-much)
Odour	
Intensity	Intensity of the general odour (odourless-very intense odour)
Frying	Intensity of the frying odour (odourless-very intense odour)
Fish	Intensity of the fish odour (odourless-very intense odour)
Rancid	Intensity of the rancid odour (odourless-very intense odour)
During eating	
Texture	
Crustiness	Feeling of crusty edge (not crusty-very crusty)
Oiliness	Sensation of oil content in the mouth (not oily-very oily)
Juiciness	Perception of juiciness during chewing (not juicy–very juicy)
Hardness	Firmness perception during chewing (very tender-very firm)
Taste and Flavour	
Intensity	Intensity of the general flavour (flavourless-very intense flavour)
Meat	Intensity of the meat flavour (less-much)
Oil	Intensity of the oil flavour (less-much)
Salt	Intensity of the salty taste (not salty-very salty)
Spicy	Intensity of the spicy flavour (not spicy-very spicy)
Rancid	Intensity of the rancid flavour (not rancid-very rancid)
Fish	Intensity of the fish flavour (less-much)
After eating	
Texture	
Residual particles	Sensation of particles in the mouth after swallowing (less-much)
Flavour	
Aftertaste	Persistence of flavour after swallowing (non persistent-very persistent flavour)

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Table 2. Volatile compounds (arbitrary area units x 10^{6}) in deep-fried control nuggets (C), bulk fish oil enriched nuggets (BFO) and microencapsulated fish oil enriched nuggets (MFO).

			Fried nuggets				
IK	ID	Compound	C	BFO	MFO	SEM	pE
		Alcohols	12.24^{b}	33.12^{a}	14.45^{b}	5.94	***
<500	C	Ethanol	3.19^{b}	19.54^{a}	14.45^{a}	3.47	***
681	A	Cyclopentanol	0.00^{b}	12.42^{a}	0.00^{b}	6.63	***
737	A	3-Methyl, 1-butanol	1.87^{a}	0.00^{b}	0.00^{b}	0.44	***
772	A	1-Pentanol	6.29^{a}	0.00^{b}	0.00^{b}	1.41	***
872	A	1-Pentanol, 4-methyl	0.89^{a}	0.00^{b}	0.00^{b}	0.20	***
1111	B	2,6-Dimethyl, 1,7-octadiene, 3-ol	0.00^{b}	1.16^{a}	0.00^{b}	0.26	***
		Aldehydes	114.31	144.91	146.89	8.29	ns
<500	C	Acetaldehyde	1.73^{b}	4.15^{a}	0.78^{c}	0.62	***
550	A	2-Methylpropanal	5.41^{b}	6.77^{b}	9.48^{a}	0.54	***
591	A	Butanal	0.00^{b}	0.12^{a}	0.00^{b}	0.08	ns
649	A	3-Methylbutanal	38.03^{b}	55.64^{a}	59.01^{a}	5.82	***
659	A	2-Methylbutanal	17.16^{c}	25.65^{b}	36.59^{a}	2.43	***
698	A	Pentanal	3.89^{b}	6.05^{a}	3.12^{b}	0.61	**
800	A	Hexanal	40.97^{a}	39.25^{a}	35.03^{b}	1.11	**
914	A	2,4-Hexadienal	0.00^{b}	0.80^{a}	0.00^{b}	0.17	***
901	A	Heptanal	2.25^{a}	1.87^{a}	1.07^{b}	0.18	**
1004	A	Octanal	0.99^{a}	0.69^{b}	0.00^{c}	0.14	***
1101	A	Nonanal	4.69^{a}	3.71^{a}	1.72^{b}	0.40	***
1325	A	2,4-Decadienal	0.00^{c}	0.21^{a}	0.11^{b}	0.48	***
		Aromatic hydrocarbons	40.23^{b}	48.91^{a}	37.67^{b}	2.25	**
		Benzenes	20.74^{ab}	25.63^{a}	18.52^{b}	1.22	*
772	B	Methyl-benzene	0.00^{c}	10.42^{a}	4.50^{b}	1.41	***
868	A	1,4-Dimethyl-benzene	0.27^{a}	0.00^{b}	0.00^{b}	0.06	***
876	A	1,3-Dimethyl-benzene	0.49^{a}	0.48^{a}	0.00^{b}	0.09	**
1004	A	1,2,4-Trimethyl-benzene	0.49^{a}	0.00^{b}	0.00^{b}	0.11	***
1032	A	1-methyl, 4(1-methylethyl)-benzene	16.92	12.67	12.42	0.94	ns
1096	A	3,5-Dimethyl-benzenemethanol	0.00^{b}	0.50^{a}	0.39^{a}	0.08	***
1558	B	4-methoxy-6-(2-pronenyl)-1,3-benzodioxole	2.57^{a}	1.55^{ab}	1.20^{b}	0.23	*
		Furans	15.18	17.25	15.05	1.31	ns
793	A	2-Propyl-furan	1.42^{b}	2.36^{a}	1.65^{b}	0.24	*
894	A	2-Butyl-furan	0.20^{b}	0.57^{a}	0.00^{c}	0.08	***
995	A	2-Pentyl-furan	13.56	14.32	13.40	1.22	ns
		Pyrazines	1.71^{b}	2.45^{a}	0.74^{c}	0.21	***
916	A	2,6-Dimethyl-pyrazine	0.53^{b}	0.89^{a}	0.43^{c}	0.10	**
1009	A	Trimethyl-pyrazine	0.81^{a}	1.12^{a}	0.00^{b}	0.17	***
1087	B	3-Ethyl, 2,-dimethyl-pyrazine	0.38^{ab}	0.45^{a}	0.31^{b}	0.02	*

n.d., not detected; IK, linear retention index; ID, method of identification: A, mass spectrum and IK identical to a reference compound; B, mass spectrum and IK in accordance with literature; C, tentative identification by mass spectrum.

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			Fried nuggets				
IK	ID	Compound	C	BFO	MFO	SEM	pE
		Aliphatic Hydrocarbons	253.54^{b}	392.19^{a}	354.49^{a}	19.47	**
500	A	Pentane	10.60^{b}	18.28^{a}	14.80^{ab}	1.04	**
555	A	2,3-Dimethylbutane	0.00^{c}	5.55^{a}	3.41^{b}	0.76	***
558	A	2-Methylpentane	20.23^{a}	48.36^{b}	38.55^{b}	3.75	***
577	A	3-Methylpentane	30.28^{a}	54.47^{b}	47.85^{b}	3.35	***
600	A	Hexane	148.49^{b}	214.41^{a}	206.12^{a}	10.78	*
700	A	Heptane	2.70	3.68	2.01	0.42	ns
965	A	2,3-Dimethyl, 2-hexene	0.24^{a}	0.00^{b}	0.00^{b}	0.05	***
1738	B	1,2-diol,1(2furanyl), 3-butene	7.04	5.92	7.50	0.41	ns
624	A	Methyl-cyclopentane	22.20^{b}	32.75^{a}	26.89^{a}	1.94	ns
703	A	1-Methyl-cyclohexene	0.77^{a}	1.85^{b}	1.55^{b}	0.14	***
1192	A	3,5,5-Trimethyl-Cyclohexene	4.70	1.02	1.35	0.54	***
1195	B	1-ol, 4-methyl-1(1-methylethyl)-3-cyclohexen	3.72	4.51	3.29	0.30	ns
1518	A	1,1-Dimethyl-cyclohexane	1.97	0.89	1.18	0.29	ns
1053	B	2(2,2-dichloro- 1,3-dimethylciclopropyl)- butane	0.57^{a}	0.48^{b}	0.00^{c}	0.08	***
		Esters	2.99	1.01	2.13	0.59	ns
613	A	Acetic acid ethyl ester	0.00^{b}	0.00^{b}	1.52^{a}	0.41	**
817	A	Acetic acid butyl ester	2.99	1.01	0.61	0.59	ns
		Ketones	19.77^{b}	35.12^{a}	22.36^{b}	2.17	***
501	A	2-Propanone	13.15^{b}	26.7^{3a}	15.14^{b}	2.02	**
809	A	Dihydro, 2-methyl-3(2H)-furanone	5.80^{b}	7.39^{a}	6.51^{ab}	0.22	**
890	A	2-Heptanone	0.83^{ab}	1.00^{a}	0.72^{b}	0.05	*
		Terpens	291.62^{a}	175.15^{b}	180.68^{b}	20.52	*
935	A	Thujene	9.10	5.26	6.62	0.97	ns
942	A	Alpha-pinene	40.60^{a}	22.62^{b}	25.91^{a}b	3.02	*
961	A	Camphene	1.19^{a}	0.00^{b}	0.00^{b}	0.26	***
983	A	Sabinene	24.99	15.68	14.09	2.68	ns
989	A	2-Beta-pinene	43.66^{a}	26.12^{b}	28.01^{b}	2.93	*
1015	A	1-Phellandrene	15.07^{a}	8.53^{b}	8.21^{b}	1.16	**
1022	A	Delta, 3-carene	83.67^{a}	47.34^{b}	50.28^{b}	6.06	*
1027	A	Alpha, terpinene	3.91^{a}	2.54^{b}	3.28^{ab}	0.23	*
1040	A	1-Limonene	55.06^{a}	37.62^{b}	35.20^{b}	3.29	*
1069	A	Gamma terpinene	4.63	3.32	4.24	0.28	ns
1079	A	Linalool	2.18^{ab}	2.55^{a}	1.59^{b}	0.14	**
1099	A	Alpha terpinolene	3.08^{a}	1.73^{b}	1.63^{b}	0.23	**
1459	A	Trans Caryophyllene	0.00	0.73	0.41	0.10	ns
		Sulfur Compounds	8.69	11.52	10.99	0.67	ns
<500	C	Methanethiol	1.47	0.98	1.19	0.13	ns
911	A	2-propen, 1-Thiol	7.22	10.54	9.80	0.74	ns

n.d., not detected; IK, linear retention index; ID, method of identification: A, mass spectrum and IK identical to a reference compound; B, mass spectrum and IK in accordance with literature; C, tentative identification by mass spectrum.

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Capítulo 2.3

Enrichment of chicken nuggets with microencapsulated omega-3 fish oil: effect of frozen storage time on oxidative stability and sensory quality

Food Bioprocess Technol (2015). DOI: 10.1007/s11947-015-1621-x

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Food Bioprocess Technol
DOI 10.1007/s11947-015-1621-x

ORIGINAL PAPER

Enrichment of Chicken Nuggets with Microencapsulated Omega-3 Fish Oil: Effect of Frozen Storage Time on Oxidative Stability and Sensory Quality

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Received: 24 June 2015 / Accepted: 5 October 2015
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Abstract This work studies for the first time the elaboration of frozen chicken nuggets enriched with microcapsules of omega-3 fatty acids using fish oil. Three types of chicken nuggets were prepared: control (C), enriched in bulk fish oil (BFO), and with added microencapsulated fish oil (MFO). Effect of length of frozen storage after pre-frying and before domestic frying was studied. The pre-fried nuggets were stored during 24 h at refrigeration temperature (0–2 °C) (T0) or during 1 month (T1M) or 3 months (T3M) in a domestic freezer at −18 °C before frying. Length of frozen storage after pre-frying and before domestic frying promoted lipid and protein oxidative reactions in omega-3-enriched nuggets. Microencapsulation showed a protective effect against lipid and protein oxidation, especially during the first month of storage. In MFO, sensory traits were not affected by enrichment. In BFO-T0, a higher juiciness and saltiness and a less intense meat flavor in comparison with C-T0 and MFO-T0 was found. Time of frozen storage did not influence the sensory quality of chicken nuggets enriched with omega-3. Microencapsulation seems to be a promising method for enrichment of pre-fried frozen meat products with fish oil, improving the oxidative shelf life and preserving the sensory quality characteristics of the enriched products.

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Keywords Microencapsulation · ω-3 · Chicken nuggets · Sensory characteristics · Oxidation

Introduction

Omega-3 polyunsaturated fatty acids (PUFA) have been widely investigated due to their effects on human health. Some of the advantages that have been related to their intake include the reduction in the risk of cardiovascular and chronic diseases and tumors (Sioen et al. 2006). The only food sources for long-chain omega-3 PUFA, such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are fish and seafood, in which presence in actual diets is not enough to reach omega-3 PUFA recommended intake (Sioen et al. 2006). Thus, a potential strategy to increase the consumption of EPA and DHA could be their inclusion in well-accepted products (Sioen et al. 2006). In that sense, numerous studies have been conducted applying different sources of omega-3 fatty acids to improve the lipid profile of various foods in order to make them healthier (Jimenez-Colmenero et al. 2001). Some alternatives to the usual direct addition of the source of EPA and DHA have been assayed, as gelled oil-in-water emulsions or konjac matrix gels, used as fat replacers (Salcedo-Sandoval et al. 2013; Poyato et al. 2014).

Whichever the source to be used, the main challenge of enrichment with omega-3 PUFA is their high susceptibility to oxidation (Jacobsen 1999). However, the way in which these bioactive compounds are added to the enriched product could influence the development of oxidative reactions and their adverse consequences. The breakdown of the ω-3 PUFA chain during oxidation implicates nutritional loss and detrimental effect on sensory, with an unacceptable rancidity and fishy off-flavor (Taneja and Zhu 2006). A possible tool to protect omega-3 PUFA from oxidation is microencapsulation,

Published online: 17 October 2015

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which limits the adverse food processing environment such as the contact with water and oxygen by keeping bioactive functional food components within protective matrix structures (Onwulata 2013). Fish oil is currently microencapsulated by different methods, spray drying being the most popular one (Gharsallaoui et al. 2007). Specifically, microencapsulation of fish oil by spray drying using multilayered emulsions of lecithin-chitosan combined with carbohydrates as coating material has been reported to have successful effects on protection from oxidation (Jiménez-Martín et al. 2015; Klinkesorn et al. 2005; Shaw et al. 2007).

Diet habits are changing in accordance with a lifestyle that is focused on time-saving, and consequently, there is a growing demand for "ready-to-heat" products, such us breaded pre-fried frozen ones (Miranda et al. 2010; Varela et al. 2008; Xiao et al. 2011). The popularity of these convenience products, among which chicken nuggets are the most popular (Barbut 2012), makes them a promising strategy to increase the intake of omega-3 fatty acids using enrichment methods. This would be of great interest for health-concerned consumers, since it would allow to reach the recommendations for omega-3 intake suggested by food authorities; in turn, it would constitute a potential opportunity for the food industry.

In relation to omega-3 microencapsulation, most studies have been focused on studying the microcapsule characteristics, whereas the use of these microcapsules for enrichment of food in ω-3 PUFA has rarely been considered, and only some bakery products (Santhanam et al. 2014; Gökmen et al. 2011; Umesha et al. 2015), beverages (Rubilar et al. 2012; Kolanowski et al. 2007; Ilyasoglu and El 2014), dairy products (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Tamjidi et al. 2012; Wan et al. 2011), and meat products (Josquin et al. 2012) have been studied, and no examples have been found in the scientific literature of pan-fried convenience meat products enriched with ω-3 microcapsules

Therefore, the objective of this study was to study for the first time a convenience frozen pan-fried meat product (chicken nuggets) enriched with fish oil omega-3 microcapsules, investigating the effect of time of frozen storage on the oxidative stability and sensory properties in comparison to bulk fish oil addition.

Material and Methods

Experimental Design

Three different types of chicken nuggets were produced: a control batch (C), a batch enriched with bulk fish oil (BFO), and a batch enriched with microencapsulated fish oil (MFO). Fish oil (kindly provided by Biomega Natural Nutrients S.L., Galicia, Spain) was used as source of ω-3 FA (6.9 % EPA, 24.6 % DHA).

Formulation and manufacture of the chicken nuggets of the control batch was made following the methodology optimized by Medina et al. (Medina et al. 2014) using chicken breast as the meat raw material. As for the enriched batches, formulation of the batter was modified by the addition of 0.5 % (*w*/*w*) of fish oil in the BFO batch and by the addition of 5 % (*w*/*w*) of multilayered microcapsules of fish oil optimized in a previous work (multilayered microcapsules prepared with lecithin-chitosan and maltodextrin containing 10 % of fish oil) (Jiménez-Martín et al. 2015). Thus, the enrichment level in EPA + DHA in BFO and MFO nuggets was 150 mg per 100 g (enough to exceed the minimum level required by the European Union legislation to label a food as "high in ω-3 fatty acids": at least 80 mg of the sum of EPA and DHA per 100 g and per 100 kcal) (EU 2010).

Chicken breast was purchased in a local market (Mercadona, Cáceres, Spain) and minced using a meat mincer (SEB IBERICA, Barcelona, Spain). Then, it was mixed and further minced with the ingredients in a domestic kitchen processor Thermomix (Vorwerk Wuppertal, Germany) according to each formulation. A commercial preparation (Procavi; ANVISA, Madrid, Spain) containing soya flour, salt, flavorings, soya protein, milk protein, dextrose, spices, pentasodium triphosphate, tetrasodium diphosphate, locust bean gum, guar gum, and sodium ascorbate (60 g/kg meat) was added. The obtained batter was stored at 0–2 °C during 24 h, and it was subsequently portioned to obtain each chicken nugget (4×4×1 cm, 25 g). Chicken nuggets were coated first with a commercial stick solution containing wheat starch, wheat corn, guar gum, and powdered turmeric (Avigum, ANVISA, Madrid, Spain) dissolved in cold water (25 g/L) and subsequently with breadcrumbs (35 g/kg nugget). Afterwards, all nuggets were immediately subjected to a pre-fried process in sunflower oil at 180 °C during 10 s and immediately removed from the fryer and placed on paper towel to remove excess of external oil.

Then, all the pre-fried nuggets were stored in a tray covered with a plastic wrap during 24 h at refrigeration temperature (0–2 °C). After this refrigeration period, part of the pre-fried nuggets of each batch were immediately deep-fried and analyzed (T0), while the rest were stored during 1 month (T1M) or 3 months (T3M) in a domestic freezer at −18 °C before deep frying. Nuggets were deep-fried at 180 °C in sunflower oil until the product reached 63 °C of internal temperature. Thus, nine groups of fried nuggets were obtained: C-T0, C-T1M, C-T3M, BFO-T0, BFO-T1M, BFO-T3M, MFO-T0, MFO-T1M, and MFO-T3M.

Temperature in the core of nuggets during frying was recorded using a thermometer probe (Testo 735-2, Lenzkirch, Germany). Nine nuggets were fried at the same time. The oil was replaced every four frying sessions. After each deep frying, nuggets were drained and placed on paper towel for removing external oil. Oxidation assays, volatile compounds,

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and sensory test were analyzed on the fried nuggets. For volatile compounds and oxidation assays, whole nugget, including meat and crust, was minced and analyzed by duplicate.

Methods

Sensory Analysis

All sessions were done in sensory panel rooms with the conditions specified in UNE-EN-ISO 8589:2010 Regulation (UNE-EN-ISO 2010). Panel rooms were equipped with white fluorescent lighting (220–230 V, 35 W). Nuggets were served hot on white plastic plates to panelists, marked with random three-digit codes. The panel sessions were held around 1–2 h before lunchtime. Salt-free crackers and a glass of water at room temperature were provided to each panelist to rinse between samples.

Quantitative Descriptive

Chicken nuggets were assessed by a trained panel of 15 members using a descriptive analysis method. The panelists were trained in four sessions for 1 h. In the first session, panelists tested chicken nuggets and were asked to express terms that describe their personal observations. In the second session, the redundant descriptive terms were eliminated and the samples were tested to include new attributes. In the third session, all the attributes selected were used in an unstructured scale of 0–10. At the fourth session, initially, the different attributes were evaluated individually by panelists in unknown representative samples, and then, panelists' responses were analyzed collectively. Data were gathered and analyzed by analysis of variance (ANOVA), and panelist deviations were assessed to determine where additional training was needed. Nineteen sensory traits of nuggets grouped under appearance, texture, odor, and flavor were assessed. Their definitions and extremes are explained in Table 1. Questions were presented to assessors in the normal perception order, as follows: visual analysis, texture, taste and flavor. Sensory traits of the samples of chicken nuggets were assessed by each panelist in a 10-cm unstructured line.

Hedonic Test

A hedonic test was carried out using an unstructured nine-point line scale with hedonic inscriptions at the lower extreme "disliked extremely" and upper extreme "liked extremely" of the scale. Thirty untrained consumers (students and staff of the university) volunteered to evaluate hedonic score of the chicken nuggets. The test took place in the tasting rooms, under the same conditions as the quantitative-descriptive analysis. Three fried chicken nuggets were evaluated in each session, and sample order was randomized across consumers. Thirty samples from each batch were analyzed.

Volatile Compounds

Volatile compounds were analyzed by headspace solid-phase microextraction (HS-SPME) following the method described by Garcia-Esteban et al. (Garcia-Esteban et al. 2004) with some modifications. Each chicken nugget was ground, and 5 g were weighted into a 20-mL glass flask sealed with an aluminum cap and a septum. Sealed vial was conditioned in a thermostatized water bath at 37 °C for 30 min. SPME was carried out by using a cross-linked divinylbenzene/carboxen/polydimethylsiloxane fiber, 50/30 μm thick and 2 cm long (Supelco, Bellefonte, PA, USA), conditioned prior to use by heating in the injection port of a gas chromatograph (GC) system under the conditions recommended by the manufacturer (at 270 °C for 1 h). The fiber was then inserted into the sample vial through the septum and exposed to the headspace for 30 min at 37 °C. The SPME fiber was desorbed and maintained in the injection port for 30 min. Analyses were performed using a Hewlett–Packard 6890 series II GC coupled to a mass selective (MS) detector (Hewlett–Packard HP 5973) (Wilmington, DE, USA). Volatiles were separated using a 5 % phenylmethyl silicone (HP-5) bonded-phase fused silica capillary column (Hewlett–Packard, 50 m×0.32 mm i.d., film thickness 1.05 μm), operating at 6 psi of column head pressure, resulting in a flow of 1.3 mL/min at 40 °C. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 °C, rose to 200 °C at a rate of 5 °C/min and then rose to 250 °C at a rate of 20 °C/min, and held for 5 min. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of one scan over the *m/z* range of 30–550 u.m.a. n-Alkanes (Sigma R-8769) were analyzed under the same conditions to calculate the linear retention indices (LRIs) for the volatile compounds. Compounds were identified by comparison with the mass spectrum and RI of commercial reference compounds (Sigma-Aldrich; Steinhein, Germany), by comparison of mass spectrum with mass spectral database (NIST and Wiley libraries) and by comparison of their RI with those available in the literature. Results from volatile analyses are provided in area units (AU).

Oxidation

Conjugated Dienes

The measurement of conjugated dienes (CDs) was determined according to the procedure described by (Juntachote et al. 2006). Frozen chicken nuggets were thawed and minced.

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Table 1 Sensory attributes of fried chicken nuggets

Sensory trait	Definition
Before eating	
Visual appearance	
Homogeneity of the crust color	Homogeneity of the crust color (less-much)
Homogeneity of the structure	Homogeneity of the structure (less-much)
Odor	
Intensity	Intensity of the general odor (odorless-very intense odor)
Frying	Intensity of the frying odor (odorless-very intense odor)
Fish	Intensity of the fish odor (odorless-very intense odor)
Rancid	Intensity of the rancid odor (odorless-very intense odor)
During eating	
Texture	
Crustiness	Feeling of crusty edge (not crusty-very crusty)
Oiliness	Sensation of oil content in the mouth (not oily-very oily)
Juiciness	Perception of juiciness during chewing (not juicy–very juicy)
Hardness	Firmness perception during chewing (very tender-very firm)
Taste and flavor	
Intensity	Intensity of the general flavor (flavorless-very intense flavor)
Meat	Intensity of the meat flavor (less-much)
Oil	Intensity of the oil flavor (less-much)
Salt	Intensity of the salty taste (not salty-very salty)
Spicy	Intensity of the spicy flavor (not spicy-very spicy)
Rancid	Intensity of the rancid flavor (not rancid-very rancid)
Fish	Intensity of the fish flavor (less-much)
After eating	
Texture	
Residual particles	Sensation of particles in the mouth after swallowing (less-much)
Flavor	
Aftertaste	Persistence of flavor after swallowing (non persistent-very persistent flavor)

Subsequently, 0.5 g were suspended in 5 mL of distilled water and homogenized to form smooth slurry. A 0.5 mL aliquot of this suspension was mixed with 5 mL of extracting solution (3:1 (*v*/*v*) hexane/isopropanol) for 1 min. After centrifugation at 3500 rpm for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of CD was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1}, and the results were expressed as micromoles per kilogram of sample.

Thiobarbituric Acid Reactive Substances

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

Protein Carbonyls

Protein oxidation in chicken nuggets was followed by measuring the formation of protein carbonyls by converting them to 2,4 dinitrophenylhydrazones (DNPH), and the derivatives were measured spectrophotometrically according to method outlined (Oliver et al. 1987) with slight modifications.

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Chicken nuggets were minced, and then, 1 g of meat was homogenized 1:10 (*w*/*v*) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturrax homogenizer for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL Eppendorf tubes. Proteins were precipitated by cold 10 % trichloroacetic acid (TCA) (1 mL) and subsequently centrifuged for 5 min at 5000 rpm. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2 % (*w*/*v*) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10 % TCA (1 mL) and washed twice with 1 mL ethanol/ethyl acetate (1:1, *v*/*v*) to remove excess of DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred, and centrifuged for 2 min at 5000 rpm to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using a BSA standard curve. The amount of carbonyls was expressed as nanomoles of carbonyl per milligram of protein using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones.

Statistical Analysis

Three batches were prepared ($n=3$) for each chicken nugget formulation (C, BFO, and MFO) and subjected to the different conditions of frozen storage. For each physicochemical analysis, five samples of chicken nuggets from each combination batch storage (C-T0, C-T1M, C-T3M, BFO-T0, BFO-T1M, BFO-T3M, MFO-T0, MFO-T1M, and MFO-T3M) were analyzed in duplicate.

The effect of enrichment (*pE*), frozen storage (*pS*), and their interaction (*pExS*) on oxidation parameters, volatile compounds, and quantitative-descriptive sensory data were analyzed using a two-way ANOVA with interaction. When a significant effect ($p<0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. Significant differences ($p<0.05$) in the hedonic test were detected using a nonparametric analysis achieved using Friedman's test. The statistics were run using the program IBM SPSS Statistics v.19.

Results and Discussion

Volatile Compounds

The GC-MS analysis allowed the identification of 88 different volatile compounds, 3-methylbutanal, 2-methylbutanal, hexanal, 2-methylpentane, 3-methylpentane, pentane, 2-propanone, and 2-pentylfuran being the major ones. This profile basically agrees with previous studies about deep-fried battered products (Özyurt et al. 2011).

Figure 1 shows the level of volatile compounds grouped in 11 main chemical families: terpenes, aldehydes, aliphatic hydrocarbons, ketones, alcohols, benzenes, furans, sulfur compounds, aromatic hydrocarbons, esters, and pyrazines. The most abundant families in all chicken nuggets in terms of area units (Fig. 1a) were aldehydes and terpenes, followed far behind by aliphatic hydrocarbons, ketones, alcohols, benzenes, and furans (Fig. 1b). The minor volatile groups (Fig. 1c), with just little amounts detected in all samples, were sulfur compounds, aromatic hydrocarbons, esters, and pyrazines. Enrichment caused significant differences (Table 3) between batches on total amount of volatile compounds and most of the chemical groups, excluding furans, sulfur compounds, and esters. The most remarked changes were those affecting alcohols, ketones, benzenes, and furans, which were detected in higher amounts in BFO than in C and MFO. In addition, the levels of aldehydes and pyrazines were also higher in BFO and C than in MFO. In meat products as chicken nuggets, the alcohols, ketones, furans, and aldehydes found are most likely formed through the oxidation of fatty acids (Shahidi et al. 1986; Jiang et al. 2011). Thus, the higher levels of these compounds in BFO as compared to the other batches could indicate a lower protection to oxidative processes of the fish oil when it is added directly to the batter during manufacture of the meat product.

Time of frozen storage significantly affected the levels of some chemical groups (terpenes, aldehydes, aliphatic hydrocarbons, ketones, furans, and esters) (Table 3) as well as the total amount of volatile compounds. However, this effect did not follow the same trend in C, BFO, and MFO batches. Thus, terpene content increased with frozen storage in C nuggets (C-TO>C-T1=C-T3), while no changes in the area units of this chemical family were found in BFO and MFO. Aldehyde content increased with frozen storage time in BFO and MFO nuggets (T0 < T1 < T3), whereas in C ones, C-T1 and C-T3 showed similar amount and higher than C-T0. As for total amount of volatile compounds (Fig. 1a), frozen storage had no influence on the total amount of volatile compounds in the C group. On the contrary, in enriched nuggets, frozen storage increased the total amount on volatile compounds in different ways. In BFO, the total amount of volatile compounds did not change in T1M with respect to T0 but increased around a 10 % in T3M with respect to T1M, whereas in MFO, it increased around a 50 % in T1M with respect to T0 and did not change in T3M with respect to T1M. Our results agree with the results of other studies that reported an increase in aldehydes, ketones, hydrocarbons, esters, and furans during frozen storage of meat products (Ladikos and Lougovois 1990). As for the differences found in the effect of frozen storage time among the batches of chicken nuggets, it could be related to the different type of enrichment in omega-3 PUFA. Some authors

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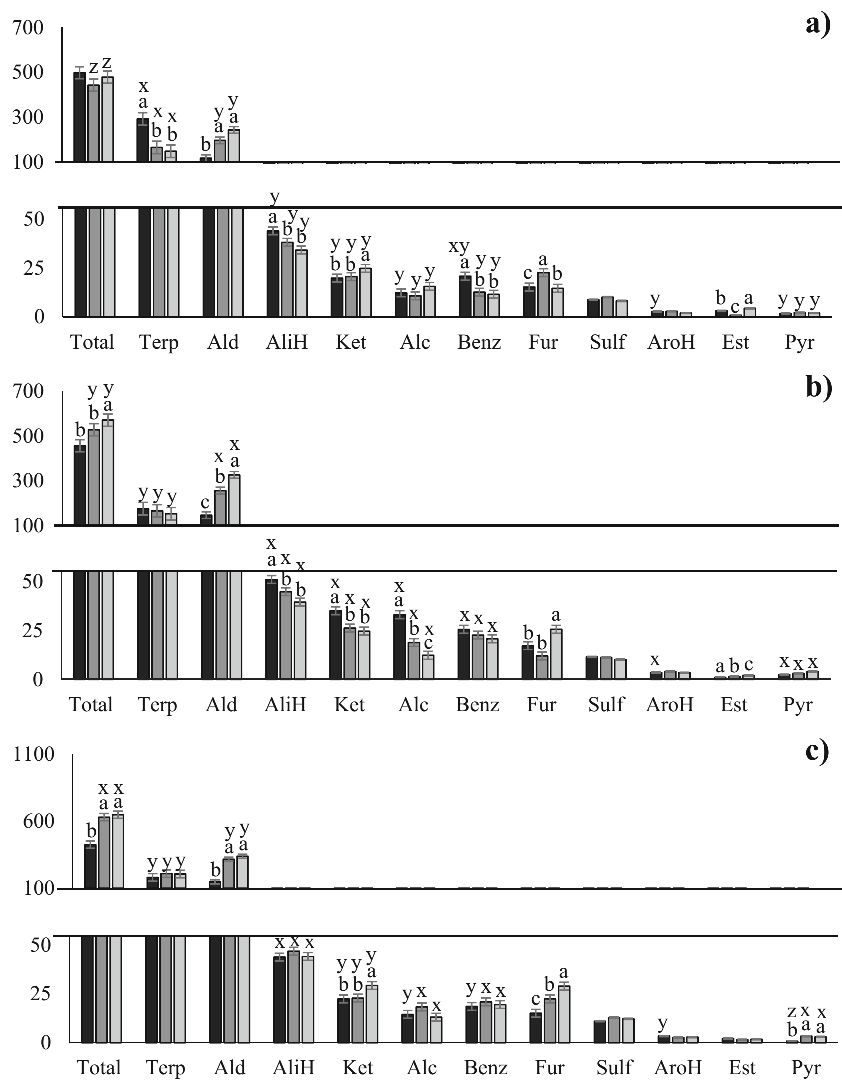

Fig. 1 Families of volatiles (in area units $\times 10^6$) in the control (**a**), enriched with bulk fish oil (**b**), and enriched with microencapsulated fish oil (**c**) fried chicken nuggets, at initial time (T0, *dark gray*) and after frozen storage for a month (T1M, *medium gray*) and 3 months (T3M, *light gray*). *Bars with different letters* (*a*, *b*, *c*) within the same formulation and family of volatile compounds show significant differences ($p<0.05$) due to frozen storage effect. *Bars with different letters* (*x*, *y*, *z*) among formulations (**a**, **b**, and **c**) indicate significant differences ($p<0.05$) due to enrichment effect. Five chicken nuggets of each of fried nugget batch ($n=3$) subjected to each storage condition (T0, T1M, and T3M) were analyzed in duplicate. *Total* total volatile compounds, *Terp* terpenes, *Ald* aldehydes, *AliH* aliphatic hydrocarbons, *Ket* ketones, *Alc* alcohols, *Benz* benzenes, *Fur* furans, *Sulf* sulfur compounds, *AroH* aromatic hydrocarbons, *Est* esters, *Pyr* pyrazines

have previously shown a higher total amount of volatiles as a consequence of a higher fat content in meat samples (Olsen et al. 2005).

From the 88 volatile compounds initially detected, 13 were selected (Table 2) as indicators of lipid oxidation (butanal, hexane, pentanal, hexanal, heptanal, 2,4-hexadienal, 2-pentylfuran, octanal, nonanal, and 2,4-decadienal) or Strecker degradation (2- and 3-methylpropanal and dimethyl disulfide) (Olsen et al. 2005; Estevez et al. 2005; Park et al. 2007; Perez-Palacios et al. 2013; Elmore et al. 1999; Soyer et al. 2010; Stadtman 1990; Xiong 2000; Huang et al. 2013). All 13 volatile compounds selected were found in the three batches C, BFO, and MFO, except for 2,4-hexadienal, which was only found in BFO nuggets, and 2-4-decadienal, not found in C samples. It is also worth noting the high content of hexane in the fried nuggets of this study, being the major volatile compound. Other authors have also shown large amount of this volatile compounds in coated fried products (Perez-Palacios et al. 2013), in which it is ascribed to lipid oxidation during the frying process (Özyurt et al. 2011).

Enrichment in fish oil and frozen storage caused differences on the levels of the selected volatile compounds, except for butanal and 2-pentylfuran, which were not affected by neither of these two factors, although they were affected by their interaction. In fact, interaction of frozen storage and temperature significantly affected all selected volatile compounds except for 2-methylbutanal and pentanal.

Overall, in C, BFO, and MFO nuggets, the length of frozen storage led to a significant increase in most volatile compounds from lipid oxidation and amino acid degradation, except for hexane that decreased. Nevertheless, the effect of frozen storage differed among nugget types. For example, the increase of hexanal, which has been widely used as oxidation marker in cooked meat and meat products (Park et al. 2007), was noticeable notable in enriched nuggets but not in C ones. A significant increment of heptanal, octanal, and

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Table 2 Selected volatile compounds (expressed as AU $\times 10^6$) related to oxidation in the control (C), enriched with bulk fish oil (BFO), and enriched with microencapsulated fish oil (MFO) fried chicken nuggets, at initial time (T0) and after frozen storage for a month (T1M) and 3 months (T3M)

		C			BFO			MFO					
IK	Compound	T0	T1M	T3M	T0	T1M	T3M	T0	T1M	T3M	pE	pS	pExS
591	Butanal	nd^{c}	14.56 ± 0.16^{b}	28.64 ± 3.56^{a}	0.12 ± 0.01^{b}	0.19 ± 0.05^{a}	0.23 ± 0.02^{a}	nd^{b}	0.15 ± 0.02^{a}	0.20 ± 0.03^{a}	ns	ns	*
600	Hexane	148.49 ± 46.93^{y}	171.24 ± 24.14	141.02 ± 16.26	$214.41\pm21.76^{a,x}$	202.99 ± 10.32^{b}	158.66 ± 6.13^{c}	$206.12\pm14.31^{a,x}$	186.27 ± 29.46^{b}	178.91 ± 11.76^{b}	*	***	***
649	3-Methylbutanal	$38.03\pm7.54^{c,y}$	$91.24\pm14.64^{b,z}$	$102.65\pm31.51^{a,y}$	$55.64\pm8.06^{c,x}$	$119.87\pm14.81^{b,y}$	$148.8\pm15.46^{a,x}$	$59.01\pm12.95^{b,x}$	$168.09\pm23.68^{a,x}$	$155.61\pm15.14^{a,x}$	***	***	*
659	2-Methylbutanal	$17.16\pm6.44^{b,z}$	$43.46\pm8.25^{a,y}$	$46.47\pm13.54^{a,y}$	$25.65\pm3.67^{b,y}$	$60.01\pm7.31^{a,x}$	$78.78\pm7.59^{a,x}$	$36.59\pm4.19^{b,x}$	$77.54\pm27.86^{a,x}$	$88.67\pm10.65^{a,x}$	***	***	ns
698	Pentanal	3.89 ± 1.65^{y}	4.65 ± 2.39	5.12 ± 1.95	$6.05\pm1.04^{b,x}$	5.25 ± 0.55^{b}	7.62 ± 0.67^{a}	$3.12\pm1.76^{c,y}$	5.56 ± 0.56^{b}	7.73 ± 0.59^{a}	**	***	ns
750	Dimethyl disulfide	nd^{b}	$nd^{b,z}$	$0.20\pm0.01^{a,y}$	nd^{b}	$0.26\pm0.06^{a,y}$	$0.31\pm0.15^{a,x}$	nd^{b}	$0.46\pm0.12^{a,x}$	$0.42\pm0.07^{a,x}$	***	***	***
800	Hexanal	$40.97\pm3.91^{a,x}$	32.98 ± 5.46^{b}	$41.25\pm3.83^{a,x}$	$39.25\pm3.46^{b,x}$	34.25 ± 3.47^{c}	$49.47\pm3.61^{a,y}$	$35.03\pm3.75^{b,y}$	31.13 ± 3.00^{b}	$44.60\pm2.69^{a,x}$	**	***	*
901	Heptanal	2.25 ± 0.41^{x}	1.99 ± 0.42^{x}	1.82 ± 0.35^{y}	$1.87\pm0.22^{b,x}$	$1.51\pm0.25^{c,x}$	$2.36\pm0.42^{a,x}$	$1.07\pm0.60^{b,y}$	$1.25\pm0.13^{b,y}$	$1.99\pm0.07^{a,y}$	**	**	***
914	2,4-Hexadienal	nd^{y}	nd^{y}	nd^{y}	$0.80\pm0.02^{b,x}$	$0.97\pm0.01^{b,x}$	$1.45\pm0.02^{a,x}$	nd^{y}	nd^{y}	nd^{y}	***	***	***
995	2-Pentylfuran	13.56 ± 3.95^{b}	9.16 ± 2.31^{c}	19.28 ± 2.07^{a}	14.32 ± 7.76	15.66 ± 1.15	16.79 ± 1.59	13.40 ± 1.30^{b}	14.34 ± 2.24^{b}	19.62 ± 1.13^{a}	ns	ns	***
1004	Octanal	$0.99\pm0.18^{a,x}$	0.50 ± 0.06^{b}	$0.61\pm0.19^{b,y}$	$0.69\pm0.17^{b,y}$	0.60 ± 0.19^{b}	$1.26\pm0.68^{a,x}$	$nd^{c,z}$	0.49 ± 0.14^{b}	$0.71\pm0.19^{a,y}$	**	**	***
1101	Nonanal	$4.69\pm1.48^{a,x}$	$3.89\pm1.03^{b,x}$	$3.14\pm0.69^{b,y}$	$3.71\pm0.32^{b,x}$	$2.33\pm0.42^{c,y}$	$4.33\pm0.90^{a,x}$	$1.72\pm0.59^{b,y}$	$1.68\pm0.56^{b,z}$	$2.86\pm0.83^{a,z}$	***	*	**
1325	2,4-Decadienal	nd^{z}	nd^{y}	nd^{z}	$0.21\pm0.03^{c,x}$	$5.71\pm2.85^{b,x}$	$7.80\pm0.90^{a,x}$	$0.11\pm0.01^{b,y}$	$nd^{c,y}$	$4.70\pm1.41^{a,y}$	***	***	***

Values in the columns with different letters (a, b, c) within the same formulation show significant differences due to frozen storage effect. Values in the columns with different letters (x, y, z) among formulations, indicate significant differences due to enrichment effect. Five chicken nuggets of each of fried nugget batch (n=3) subjected to each storage condition (T0, T1M, and T3M) were analyzed in duplicate, and values are expressed as mean±standard deviation

nd not detected, *ns* no significant differences, *IK* linear retention index (method of identification: mass spectrum and IK identical to a reference compound), *pE p* value of enrichment effect, *pS p* value of frozen storage effect, *pExS p* value of interaction (enrichment effect × frozen storage effect)

*$p<0.05$, **$p<0.01$, ***$p<0.001$

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nonanal in enriched nuggets was detected in BFO and MFO. This increment was more remarked in nuggets subjected to 3 months of frozen storage, especially in BFO batch. 2,4-Decadienal, an unsaturated aldehyde arising mainly from the oxidation of linoleic and arachidonic acids, was only detected in enriched products, showing a more marked increase in BFO than in MFO batches. In addition, 2,4-hexadienal was only detected in BFO samples. These facts indicate that omega-3-enriched nuggets tend to show increasing levels of compounds from polyunsaturated fatty acid oxidation throughout the frozen storage and that such an increase is quite dependent on the way of enrichment.

Volatile compounds as Strecker degradation markers, 2- and 3-methylbutanal, also reported an increase with time of frozen storage, which agrees with literature (Olsen et al. 2005). These compounds have an important implication in flavor of meat products and have been demonstrated to be originated from degradation of branched amino acids such as leucine and isoleucine (Elmore et al. 1999).

Dimethyl disulfide was not detected in T0 samples and was only detected after frozen storage, which indicates that during freezing and subsequent frozen storage, there are ongoing chemical changes that allows the further formation of this compound during the subsequent frying. In C nuggets, dimethyl disulfide only appeared after 3 months of frozen, while in enriched products, it was detected at the earlier storage time (T1M), but it did not increased with further storage length. Disulfides have been detected in frozen-stored meat, being implicated in the Strecker degradation of sulfur-containing amino acids (cysteine, methionine) via some free radical reactions and the formation of hydrogen sulfide (Huang et al. 2013).

Lipid and Protein Oxidation

CD, TBARs, and carbonyls were highly affected by enrichment, frozen storage, and their interaction ($p<0.001$) (Table 3). As expected, oxidation process in the chicken nuggets was reduced during frozen storage, but the reactions were not fully stopped, which agrees with literature dealing with oxidation during frozen storage (Huang et al. 2013). Results for lipid and protein oxidation are shown in Figs. 2 and 3, respectively.

In all batches, the levels of CD increased (Fig. 2a) from T0 to T1M and thereafter decreased in T3M. Since CD are primary lipid oxidation products, this result indicates the formation of CD in samples subjected to 1 month of frozen storage, while in samples frozen stored for 3 months, the decrease of CD seems to indicate that these compounds may have been subsequently transformed into other primary oxidation products that are produce later than CD, such as hydroperoxides. Thus, in all batches of nuggets frozen stored for 3 months before frying, it seems that rate of degradation of primary oxidation products into secondary oxidation products is faster than their rate of formation.

Botsoglou et al. (Botsoglou et al. 2014) studied oxidation rate during frozen storage in pork patties enriched with omega-3 PUFA and detected increased CDs after 2 months of storage, which increased further after 4 months but declined thereafter. This contrasts with our study, as decrease of CD in the chicken nuggets began earlier (after 1 month of frozen storage). However, the differences in enrichment and formulation of the product should be taken into account. Comparison of our data with similar studies is challenging, as literature dealing with frozen storage of products enriched with ω-3 PUFA is scarce and no records on effect of freezing or frozen storage on pre-fried enriched products with ω-3 PUFA are available.

As for TBARs (Fig. 2b), in general, there was a significant increase due to frozen storage time, although this effect was quite different among batches. Thus, in C nuggets, TBAR values highly increased with longer frozen storage time (T0 < T1M < T3M), while in enriched samples, TBAR values slightly increased only after 3 months of frozen storage. In addition, it is worth the higher TBAR values found in BFO in comparison to C and MFO batches at T0, T1M, and T3M. In the study of Botsoglou et al. (Botsoglou et al. 2014) previously mentioned, concentrations of TBARs in frozen pork patties enriched with omega-3 PUFA stored at −18 °C rapidly increased during 120 days of storage. However, the different composition of the meat and the specific formulation makes the comparison difficult.

Then, it seems that enriched batches (both BFO and MFO) had a more stable behavior related to secondary lipid oxidation during storage with fewer changes in rate of oxidation than C. However, in BFO, the results indicate an advanced stage of secondary lipid oxidation even in samples not subjected to frozen storage, which could be related to their formulation with the direct addition of fish oil. Thus, an increased concentration of PUFA in the batter of BFO batch could act as a prooxidant factor. An imbalance in the initial prooxidant-antioxidant equilibrium in the batter of this batch could have caused so lipid oxidation status at T0 that the steady state for secondary oxidation was reached faster. This steady state is the phase of oxidation process where formation and decomposition of oxidation products as well as interactions of oxidation compounds with other components are equivalent (Mielnik et al. 2002), and so, no changes in oxidation index are measured.

The results suggest that the lipid oxidation status in fish oil-enriched nuggets is influenced by time of frozen storage, although the type of addition seems to be a more marked effect, being the enrichment with microcapsules more effective against lipid oxidation than bulk fish oil addition.

The values of carbonyls (Fig. 3) in the nuggets of this study ranged from 3.71 to 6.31 nmol/mg protein, quite in

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Table 3 Statistic two-way ANOVA with interaction from results presented in Figs. 1, 2, 3, 4, and 5

	pE	*pS*	*pExS*
Volatile compounds (Fig. 1)			
Total	*	**	**
Terpenes	*	*	**
Aldehydes	*	***	ns
Aliphatic hydrocarbons	**	**	ns
Ketones	***	**	***
Alcohols	***	ns	ns
Benzenes	***	ns	*
Furans	ns	***	***
Sulfur compounds	ns	ns	ns
Aromatic hydrocarbons	**	ns	***
Esters	ns	*	ns
Pyrazines	***	ns	**
Lipid oxidation (Fig. 2)			
Conjugated dienes	***	***	***
TBARs	***	***	***
Protein oxidation (Fig. 3)			
Carbonyls	***	***	***
Sensory analysis			
Quantitative-descriptive assay (Fig. 4)			
Visual appearance			
Homogeneity of the crust color	ns	ns	ns
Homogeneity of the structure	ns	ns	ns
Odor			
Intensity	ns	ns	ns
Frying	ns	ns	ns
Fish	ns	ns	ns
Rancid	ns	ns	ns
Texture			
Crustiness	ns	ns	ns
Oiliness	ns	ns	ns
Juiciness	*	ns	ns
Hardness	ns	ns	ns
Residual particles	ns	ns	ns
Taste and flavor			
Intensity	ns	ns	ns
Meat	*	ns	ns
Oil	ns	ns	ns
Salt	*	ns	ns
Spicy	ns	ns	ns
Rancid	ns	ns	ns
Fish	ns	ns	ns
Aftertaste	ns	ns	ns
Hedonic test (Fig. 5)	ns	ns	ns

pE p value of enrichment effect, *pS p* value of frozen storage effect, *pExS p* value of interaction (enrichment effect × frozen storage effect), *ns* no significant differences

*$p<0.05$, **$p<0.01$, ***$p<0.001$

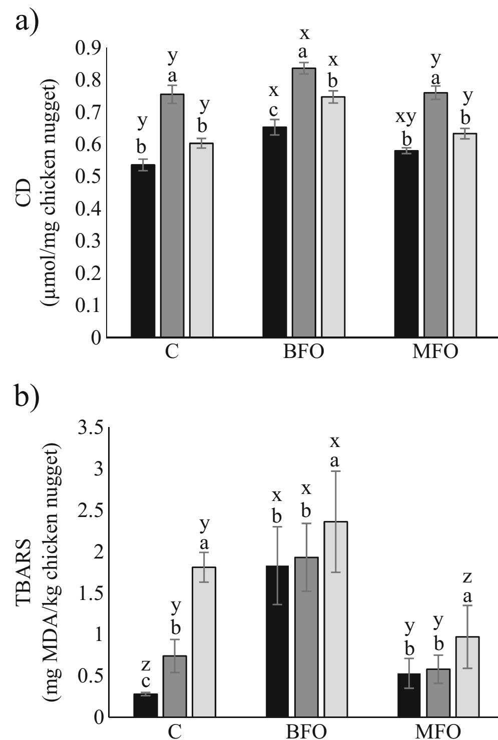

Fig. 2 Lipid oxidation, determined by CD (**a**) and TBARs (**b**) in the control (C), enriched with bulk fish oil (BFO), and enriched with microencapsulated fish oil (MFO) fried chicken nuggets, at initial time (T0, *dark gray*) and after frozen storage for a month (T1M, *medium gray*) and 3 months (T3M, *light gray*). *Bars with different letters* (*a*, *b*, *c*) within the same formulation show significant differences ($p<0.05$) due to frozen storage effect. *Bars with different letters* (*x*, *y*, *z*) among formulations indicate significant differences ($p<0.05$) due to enrichment effect. Five chicken nuggets of each of fried nugget batch ($n=3$) subjected to each storage condition (T0, T1M, and T3M) were analyzed in duplicate

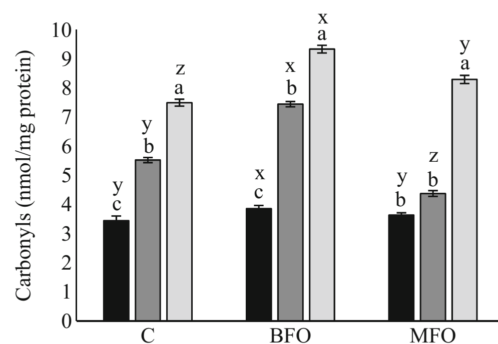

Fig. 3 Total protein carbonyls (DNPH method) in the control (C), enriched with bulk fish oil (BFO), and enriched with microencapsulated fish oil (MFO) fried chicken nuggets, at initial time (T0, *dark gray*) and after frozen storage for a month (T1M, *medium gray*) and 3 months (T3M, *light gray*). *Bars with different letters* (*a*, *b*, *c*) within the same formulation show significant differences ($p<0.05$) due to frozen storage effect. *Bars with different letters* (*x*, *y*, *z*) among formulations indicate significant differences ($p<0.05$) due to enrichment effect. Five chicken nuggets of each of fried nugget batch ($n=3$) subjected to each storage condition (T0, T1M, and T3M) were analyzed in duplicate

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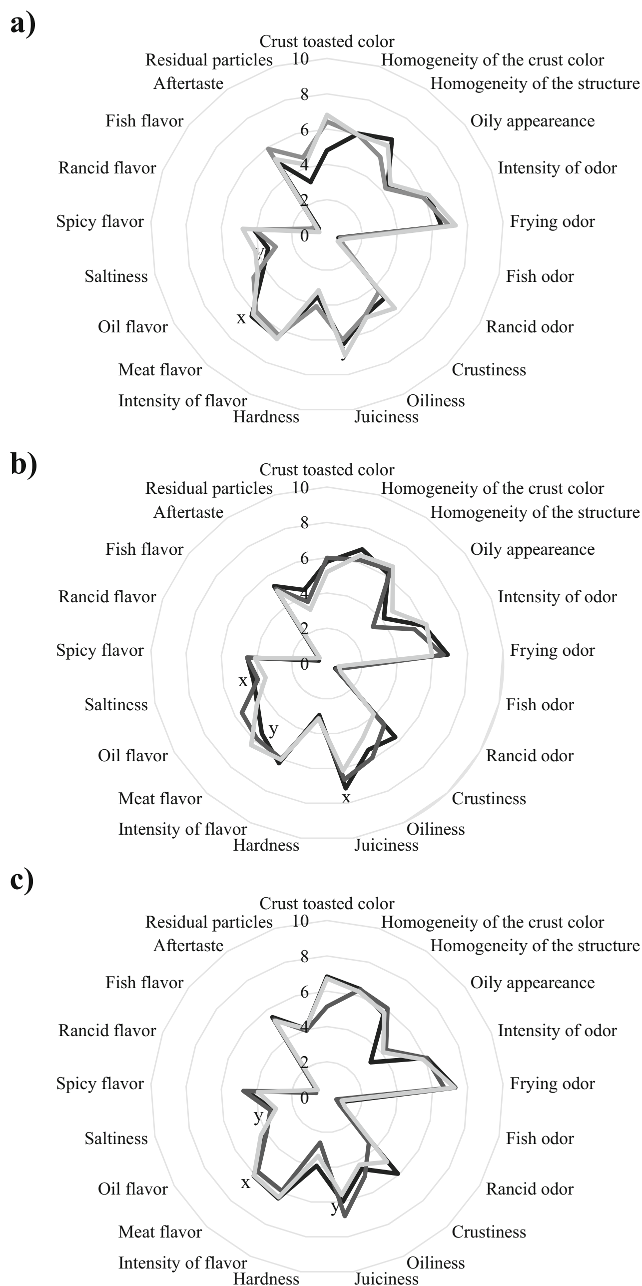

Fig. 4 Sensory quantitative-descriptive in the control (C) (**a**) fried chicken nuggets and the fried chicken nuggets enriched with bulk fish oil (BFO) (**b**) and microencapsulated fish oil (MFO) (**c**) at initial time (T0, *dark gray*) and after freezing storage for a month (T1M, *medium gray*) and 3 months (T3M, *light gray*). Three fried chicken nuggets from the different batches (n=3) of C, BFO, and MFO subjected to the different freezing storage conditions (T0, T1M, and T3M) were evaluated in each session, and sample order was randomized across assessors. Thirty samples from each batch were analyzed. *Different letters* within the same formulation (a, b, c) indicate significant differences (p<0.05) due to frozen storage effect. *Different letters* among formulations (**a**, **b**, and **c**) indicate significant differences (p<0.05) due to enrichment effect

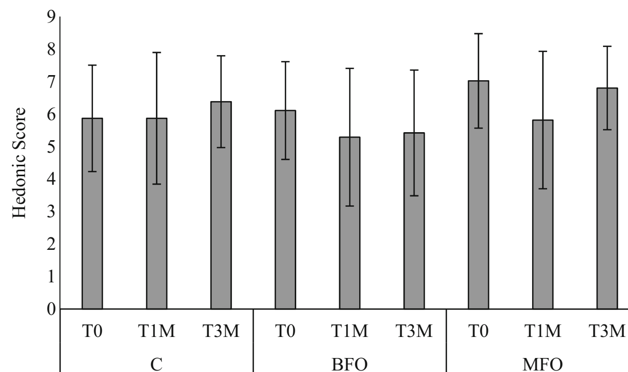

Fig. 5 Hedonic test of the control (C) fried chicken nuggets and the fried chicken nuggets enriched with bulk fish oil (BFO) and microencapsulated fish oil (MFO) at initial time (T0) and after frozen storage for a month (T1M) and 3 months (T3M). *Bars with different letters* (a, b, c) within the same formulation show significant differences (p<0.05) due to frozen storage effect. *Bars with different letters* (x, y, z) among formulations indicate significant differences (p<0.05) due to enrichment effect. Three fried chicken nuggets from the different batches (n=3) of C, BFO, and MFO subjected to the different storage conditions (T0, T1M, and T3M) were evaluated in each session, and sample order was randomized across assessors. Thirty samples from each batch were analyzed

concordance with results in other cooked products (Xiao et al. 2011). The effect of time of frozen storage was significant in the three batches of nuggets. In C and BFO nuggets, the amount of carbonyls successively increased with time of frozen storage (T0 < T1 < T3), while in MFO, this effect was less intense, as MFO-T0 and MFO-T1M showed similar carbonyl values, which experimented a significant increase in samples frozen stored during 3 months. In a study dealing with oxidation of long-term frozen n-3 fatty acid-enriched pork patties, the concentration of protein carbonyls also increased during frozen storage (Botsoglou et al. 2014). The carbonyl behavior also agrees with data of the oxidative deterioration of proteins previously found in frozen chicken and turkey meat, which showed increase of the carbonyls during 6 months of storage at −18 °C (Soyer et al. 2010). Similar increases in carbonyl groups have previously been reported during frozen storage of fish (Baron et al. 2007). This protection against protein oxidation in MFO nuggets could be related to the lesser extent of lipid oxidation in this bath, reducing the subsequent production of free radicals that could promote protein oxidation of the meat product. Also, the inner formulation of the microcapsules could have a role on protection from protein oxidation. Maltodextrin has been used as cryostabilizer to prevent alterations taking place in frozen-stored minced fatty fish and has shown to effectively prevent protein denaturation in horse mackerel surimi stored at −18 °C (Rodriguez-Herrera et al. 2006).

Sensory Analysis

Results and statistics for the quantitative-descriptive sensory analysis are shown in Fig. 4 and Table 3, respectively. There were no significant differences (p>0.05) for any sensory

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attribute as a function of length of frozen storage or the interaction frozen storage enrichment in any of the three formulations of nuggets. As for enrichment, only at T0 differences were detected between groups, with a more intense perception of juiciness and salty taste and lower of meat flavor in BFO, whereas these traits remained the same in MFO compared to C. As for samples at T1M and T3M, no differences were detected among different formulations. It was also noted that scores for most sensory attributes were similar in C, BFO, and MFO batches. This result is remarkable above all for fish and rancid odor and fish and rancid flavor, since these traits could be influenced due to the enrichment with of fish oil. It has been stated that trained sensory panels are able to detect off-odors at TBARS values of 1 to 2 mg MDA/kg sample (Barbut et al. 1990). As described previously, TBARS values in MFO were all below 1 mg MDA/kg sample (with the maximum value being 0.97 mg MDA/kg sample in MFO-T3M). In C, only C-T3M was above 1 (1.81 mg MDA/kg sample). On the contrary, in BFO, all samples were between the mentioned limits of 1–2 mg MDA/kg sample (1.83, 1.93, and 2.36 mg MDA/kg sample for BFO-T0, BFO-T1M, and BFO-T3M, respectively). The chicken nuggets in this study were formulated with a commercial preparation and a stick solution containing highly flavored ingredients such as spices (black pepper, powdered turmeric), which likely helped to mask odors or flavors associated with mild lipid oxidation. In fact, the mask effect of off-flavors of spices has previously been reported in other meat products enriched in ω-3 fatty acids, such as sausages (Salminen et al. 2013). When foods are fortified with oils high in omega-3 fatty acids, the concentration that is added is limited due to the impact these highly unsaturated oils have on the sensory attributes and palatability of the final product (Sell et al. 2015). Previous literature of food enriched with microencapsulated ω-3 PUFA is scarce and inconsistent. For example, the panelists scored bread-enriched products with 2.5 % (*w*/*w*) of microcapsules added (approximately 0.2 % *w*/*w* of EPA + DHA) with lower acceptability values (Henna Lu and Norziah 2011), while in other studies with bread with higher level of enrichment (5 % *w*/*w* of microcapsules and 0.6 % *w*/*w* of EPA + DHA), the panelists gave good hedonic scores to the enriched products (de Conto et al. 2012). Nevertheless, levels of EPA-DHA (0.15 % *w*/*w*) added in this study did not reached the enrichment levels used in those studies. Moreover, apart from the amount of EPA + DHA for enrichment, other factors, such as formulation, manufacture process, technology process, and cooking method, could also influence on the sensory quality.

Results and statistic on the hedonic test (Fig. 5 and Table 3) are quite in concordance with those of the quantitative-descriptive analysis, with no differences caused by any of the factors analyzed. Thus, no differences were detected for overall acceptability between chicken nuggets from different batches, neither for any type of studied frozen chicken nuggets stored for different times. All of them were scored with higher than 5 on a 0–9 cm scale, which could be considered as a good acceptability.

Conclusions

This work studies for the first time the elaboration of frozen chicken nuggets enriched with microcapsules of omega-3 fatty acids using fish oil. Length of frozen storage after pre-frying and before domestic frying promotes lipid and protein oxidative reactions in omega-3-enriched nuggets. This effect depends on the type of omega-3 addition, being the microencapsulation a protective method against lipid and protein oxidation, above all the first month of storage. Time of frozen storage does not influence on sensory quality of chicken nuggets enriched with omega-3. Microencapsulation seems to be a promising method for enrichment of pre-fried frozen meat products with fish oil, improving the oxidative shelf life and preserving the sensory quality characteristics of the enriched products.

Acknowledgments Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo for supporting the predoctoral grant (PD10026), equipment funding, and support to research groups. Biodymia Laboratory (University of Lyon) for making possible the development of the work during a research stay. "Biomega Natural Nutrients S.L." for kindly providing the fish oil.

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Capítulo 2.4

Chicken nuggets enriched with microencapsulated ω-3 fatty acids: changes in fatty acids composition during frozen storage

Enviado a Food and Bioprocess Technology

Diciembre 2015 (FABT-D-15-01294)

Food and Bioprocess Technology: An International Journal

Chicken nuggets enriched with microencapsulated ω-3 fatty acids: changes in fatty acids composition during frozen storage

--Manuscript Draft--

Manuscript Number:	FABT-D-15-01294
Full Title:	Chicken nuggets enriched with microencapsulated ω-3 fatty acids: changes in fatty acids composition during frozen storage
Article Type:	Original Research
Keywords:	microencapsulation; ω-3 fatty acids; chicken nuggets; fatty acid composition; physicochemical characteristics; frozen storage
Corresponding Author:	Estefania Jimenez-Martin Cáceres, SPAIN
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Funding Information:	Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo (PD10026) — Mrs. Estefania Jimenez-Martin Junta de Extremadura-Consejería de Economía, Comercio e Innovación and FEDER (GR15113) — Not applicable
Abstract:	This work study a convenience frozen pan-fried meat product (chicken nuggets) enriched with fish oil ω-3 microcapsules (MFO), investigating the effect of length of frozen storage (1 or 3 months) after pre-frying and before deep-frying on the fatty acid composition and physico-chemical characteristics in comparison to chicken nuggets enriched with bulk fish oil (BFO) addition and control ones. Type of ω-3 enrichment and frozen storage influenced color, moisture and water activity of fried chicken nuggets. The addition MFO and the frozen storage during 1 or 3 months led to darker fried chicken nuggets with lower moisture and water activity. Frozen storage seemed to promote fat uptake, and consequently increased the linoleic acid (C18:2 n-6) content in chicken nuggets during the deep-frying process in sunflower oil. However, the type of ω-3 enrichment did not influence the lipid content of these products. The ω-3 enrichment by addition of MFO led to higher eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) content in fried chicken nuggets compared to BFO addition, with no losses of these fatty acids during the frozen storage. Thus, it seems that microencapsulation provide these ω-3 PUFA stability and protection, being a good strategy for enrichment purposes.

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TITLE: Chicken nuggets enriched with microencapsulated ω-3 fatty acids: changes in fatty acids composition during frozen storage

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ABSTRACT

This work study a convenience frozen pan-fried meat product (chicken nuggets) enriched with fish oil ω-3 microcapsules (MFO), investigating the effect of length of frozen storage (1 or 3 months) after pre-frying and before deep-frying on the fatty acid composition and physico-chemical characteristics in comparison to chicken nuggets enriched with bulk fish oil (BFO) addition and control ones. Type of ω-3 enrichment and frozen storage influenced color, moisture and water activity of fried chicken nuggets. The addition MFO and the frozen storage during 1 or 3 months led to darker fried chicken nuggets with lower moisture and water activity. Frozen storage seemed to promote fat uptake, and consequently increased the linoleic acid (C18:2 n-6) content in chicken nuggets during the deep-frying process in sunflower oil. However, the type of ω-3 enrichment did not influence the lipid content of these products. The ω-3 enrichment by addition of MFO led to higher eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) content in fried chicken nuggets compared to BFO addition, with no losses of these fatty acids during the frozen storage. Thus, it seems that microencapsulation provide these ω-3 PUFA stability and protection, being a good strategy for enrichment purposes.

KEYWORDS: microencapsulation; ω-3 fatty acids; chicken nuggets; fatty acid composition; physicochemical characteristics; frozen storage

INTRODUCTION

Current lifestyle, with a rising lack of time for traditional cooking, is leading to the widespread consumption of ready-to eat and ready-to-cook products (Barbut 2012). Coated products are a good example of this type of food. They are very accepted, particularly among the youngers, due to their sensorial attributes, quick and easy preparation, and consequently, their consumption has increased in recent years (Varela et al. 2008). Among this type of widely consumed products, chicken nuggets are one of the most popular (Barbut 2012), which makes them be appropriate to act as carriers of functional food ingredients (Jiménez-Colmenero 2007).

Among fatty acids that can act as functional ingredients, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22: 6n-3) can be highlighted. EPA and DHA are abundant ω-3 polyunsaturated fatty acids (PUFA) in fish and seafood. However, the intake of these food sources are not enough to reach the recommended EPA and DHA daily intake by food health organizations (Sioen et al. 2006). In addition to be essential fatty acids, ω-3 PUFA have been associated to benefit effects for human health, such as reduction in the risk of cardiovascular and chronic diseases and tumors (Sioen et al. 2006). Addition of EPA and DHA in popular products, such as chicken nuggets, could be an option for health-concerned consumers to reach the recommended ω-3 PUFA intake, and also would be of great interest for food industry.

The major problem for enrichment of food with ω-3 PUFA is their high susceptibility to oxidation (Jacobsen 1999). Oxidation of the ω-3 PUFA implicates losing nutritional value and also has an effect on sensory quality, leading to rancidity and fishy off-flavor and tastes (Taneja and Zhu 2006). Moreover, processing and storage conditions of the products for ω-3 enrichment could also influence the oxidation reactions. Most ready-to-cook products are pre-fried and frozen stored, which can promote autooxidative and hydrolytic changes (Tomás and Aòón 1990). Thus, it results crucial developing methods for

enrichment that could protect added PUFA from the development of oxidative reactions, even during the processing and storage of the enriched products.

Microencapsulation appears as interesting protective strategy for enrichment in ω-3 PUFA. It limits the adverse effects of the food processing environment, such as the contact with water and oxygen, by keeping bioactive functional food components within protective matrix structures (Onwulata 2013). Among the methods used for microencapsulation, spray drying, which is the most popular one (Gharsallaoui et al. 2007), has been used for fish oil protection. Microencapsulation of fish oil by spray-drying using multilayered emulsions of lecithin-chitosan combined with carbohydrates as coating material has been reported to have successful effects on protection from oxidation (Jiménez-Martín et al. 2015a; Klinkesorn et al. 2005; Shaw et al. 2007; Jiménez-Martín et al. 2015b).

In relation to ω-3 microencapsulation, most studies have been focused on studying the microcapsule characteristics, whereas the application of these microcapsules for enrichment purposes has not been extensively considered. There are some studies dealing with enrichment of bakery products (Santhanam et al. 2014; Gökmen et al. 2011; Umesha et al.), beverages (Rubilar et al. 2012; Kolanowski et al. 2007; Ilyasoglu and El 2014) and dairy products (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Tamjidi et al. 2012; Wan et al. 2011) with microencapsulated ω-3. Only two studies have evaluated meat products with addition of microencapsulated ω-3, both of them used sausages as the meat product studied (Josquin et al. 2012; Pelser et al. 2007). However, there are no reports in the scientific literature of pan-fried convenience meat products enriched with ω-3 microcapsules. Therefore, the objective of this work was to study for the first time a convenience frozen pan-fried meat product (chicken nuggets) enriched with fish oil ω-3 microcapsules, investigating the effect of the length of frozen storage on the composition of fatty acids and physico-chemical characteristics in comparison to bulk fish oil addition.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

Three different types of nuggets were prepared: a control batch (C), a batch enriched with bulk fish oil (BFO) and a batch enriched with microencapsulated fish oil (MFO). Fish oil (kindly provided by Biomega Natural Nutrients S.L., Galicia, Spain) was used as source of ω-3 FA (6.9 % EPA, 24.6 % DHA).

Formulation and manufacture of the chicken nuggets of the control batch was made following the methodology optimized by Medina et al. (Medina et al. 2014) using chicken breast as the meat raw material. For the enriched batches, formulation of the batter was modified by the addition of 0.5% (w/w) of fish oil in the BFO batch and by the addition of 5 % (w/w) of multilayered MFO. Microencapsulation procedure was optimized in a previous work (multilayered microcapsules prepared with lecithin-chitosan and maltodextrin containing 10% of fish oil) (Jiménez-Martín et al. 2015a, b). Thus, the enrichment level in EPA+DHA in both BFO and MFO nuggets was 150 mg per 100 g (enough to exceed the minimum level required by the European Union (EU 2010) to label a food as “high in ω-3 fatty acids”: at least 80 mg of the sum of EPA and DHA per 100 g and per 100 kcal).

Chicken breast was purchased in a local market (Mercadona, Cáceres, Spain) and minced using a meat mincer (SEB IBERICA, Barcelona, Spain). Then, it was mixed and further minced with the ingredients in a domestic kitchen processor (Thermomix, Vorwerk Wuppertal, Germany), with the addition of fish oil or microencapsulated fish oil according to each formulation. A commercial preparation (Procavi; ANVISA, Madrid, Spain) containing soya flour, salt, flavorings, soya protein, milk protein, dextrose, spices, pentasodium triphosphate, tetrasodiumdiphosphate, locust bean gum, guar gum and sodium ascorbate (60 g/kg meat) was added. The obtained dough was stored at 0-2 ºC during 24h and it was subsequently portioned to obtain each chicken nugget (4 x 4 x 1 cm, 25 g) and coated, first with a commercial stick solution containing wheat starch, wheat corn, guar gum, wheat starch and powdered turmeric (Avigum, ANVISA, Madrid, Spain) dissolved in cold water (25 g/L), and subsequently with breadcrumbs (35 g/kg

nugget). Thereafter, all nuggets were subjected to a pre-fried process in sunflower oil during 10 sec at 180 ºC. Afterwards, nuggets were immediately removed from the fryer and placed on paper towel to remove the excess of external oil. Then, all the pre-fried nuggets were stored in a tray covered with a plastic wrap during 24h at refrigeration temperature (0-2 ºC). After this refrigeration period, part of the pre-fried nuggets of each batch was immediately deep-fried and analysed (T0). Deep-frying was performed in sunflower oil at 180 ºC, until the chicken nuggets reached 63 ºC of internal temperature. Temperature in the core of the nuggets during frying was recorded using a thermometer probe (Testo 735-2, Lenzkirch, Germany). The rest of nuggets were stored during 1 month (T1M) or 3 months (T3M) in a domestic freezer at -18ºC. At the end of each storage time, they were deep-fried under the same conditions described before. Thus, nine groups of fried nuggets were produced: C-T0, C-T1M, C-T3M, BFO-T0, BFO-T1M, BFO-T3M, MFO-T0, MFO-T1M and MFO-T3M. Nine nuggets were fried at the same time. The oil was replaced every four frying sessions. After each deep-frying, nuggets were drained, and placed on paper towel for removing external oil. Then, color, water activity and moisture were immediately analysed, and the rest of the sample were stored at -80 ºC until further analysis. Whole fried nugget (including meat and crust) was minced in a kitchen blender for being analyzed in terms of physico-chemical characteristics and fatty acid composition.

METHODS

Instrumental Color

Color was measured across the surface of the chicken nuggets. L* value (lightness), a* value (redness) and b* value (yellowness) were obtained using a Minolta Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) programmed to use the built-in internal illuminant D65. Means of readings on three locations on each sample were determined. Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Moisture

Moisture content was determined by drying the samples (5 g) at 102 °C until constant weight, according to the Association of Official Analytical Chemists (A.O.A.C., 2000) (reference 935.29).

Water activity

For the water activity, the system LabMaster-aw (NOVASINA AG, Switzerland) was used after calibration.

Fat content

Fat content was analyzed gravimetrically with chloroform/methanol (2:1, vol/vol), according to the method described by Pérez-Palacios et al. (2008).

Fatty acid profile

Fatty acid methyl esters (FAME) from extracted fat were prepared by trans-esterification in presence of sodium metal (0.1 N) and sulphuric acid in methanol (Sandler and Karo, 1992). FAMEs were analyzed by gas chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethylene glycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA, USA) (60 m x 0.32 mm i.d. x 0.25 lm film thickness). The GC oven program temperature was as follows: initial temperature of 180 ºC, which was increased at 5 ºC/min to 200 ºC, which was maintained during 40 min; thereafter temperature increased at 5 ºC/min to 250 ºC, and then kept during the final 21 min. The injector and detector temperatures were 250 ºC. The carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO, USA). Peak areas were measured and percentage of FAMEs was calculated, basing on the relative area percentages of the FAME analysed.

Sampling replication and Statistical analysis

Three batches were prepared (n=3) for each chicken nugget formulation (C, BFO and MFO) and subjected to the different conditions of freezing storage. For each physico-chemical analysis, 5 samples of

chicken nuggets from each combination batch-storage (C-T0, C-T1M, C-T3M, BFO-T0, BFO-T1M, BFO-T3M, MFO-T0, MFO-T1M, and MFO-T3M) were analyzed in duplicate.

The effects of enrichment (*pE*) and frozen storage (*pS*) on color, moisture, water activity, fat content and fatty acid profile were analysed using a two way ANOVA. When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. The statistical study was done with the program IBM SPSS Statistics v.19.

RESULTS AND DISCUSSION

Instrumental color, moisture and water activity

Table 1 shows color measurements of the C, BFO and MFO fried chicken nuggets at T0, T1M and T3M. Frozen storage affected a* coordinates (redness) but not L* nor b* ones. In BFO and MFO enriched nuggets a* value increased with the length of frozen storage. In C nuggets, this effect was not significant but it also indicated the same tendency. Accordingly, lamb nuggets also obtained higher a* values after 2 months of frozen storage (Medina et al. 2014).

As for the type of ω-3 enrichment, it significantly influenced L* and a* color coordinates. The value of L* at T1M and T3M was higher in C (55.91 and 51.37, respectively) and BFO (53.93 and 50.66) than in MFO (47.95 and 45.50), which was the batch with the lowest L* values. In agreement, in other studies there has also been observed a decrease in the lightness of breads with the increase in the concentration of added ω-3 microcapsules (de Conto et al. 2012), which these authors associated to the composition of its wall material. Wehrle et al. (Wehrle et al. 1999) incorporated fat to biscuits encapsulated with sodium caseinate and whey protein concentrate, obtaining darker color than in control biscuits following the same baking conditions. The values of a* at T0, T1M and T3M were higher in MFO (9.21, 11.81 and 14.10, respectively) than in BFO (5.87, 8.84 and 11.14, respectively) and C (6.68, 7.42 and 7.49, respectively). This agrees with the results obtained by Henna Lu et al. (2011), who analyzed breads with different added content of microencapsulated ω-3 and observed that a* value of bread crumbs was significantly increased with respect to control ones (Henna Lu and Norziah 2011). However, it is not specified the material used

for microencapsulation. Some authors have postulated that not only concentration and chemical state of meat pigments or physical properties of the meat affect the color of meat product, being an important factor the presence of non-meat products in the formulation (Sáyago-Ayerdi et al. 2009). Thus, in the chicken nuggets of the present work, the wall material used to prepare the microencapsulated fish oil could have also been responsible for the color changes.

The effect of frozen storage on moisture (Figure 1) and water activity (Figure 2) of the nuggets was only significant in the MFO nuggets. In these nuggets, moisture and water activity were lower in MFO at T1M (48.8 % and 0.851, respectively) or T3M (48.1 % and 0.849) in comparison to values of MFO nuggets at T0 (54.1 % and 0.864, respectively). No changes in moisture nor in water activity were detected in C and BFO due to frozen storage. This agrees with the results of a previous study on lamb nuggets, in which 2 months of frozen storage did not have any effect on moisture values (Medina et al. 2014). The influence of frozen storage only on MFO could then be associated to the particular composition of this batch, since the addition of microencapsulated fish oil, containing a significant amount of solids and osmotically active ingredients (i.e: maltodextrin), could most likely be the cause for the lower relative water content and the decreased water activity (Alabdulkarim et al. 2012).

In relation to the effect of the type of ω-3 enrichment, moisture content and water activity of MFO were much lower than those of BFO and C. Josquin et al. (2012) (Josquin et al. 2012) enriched sausages with pure fish oil and encapsulated fish oil, finding around 10% lower moisture content in sausages with encapsulated oil than in those with pure fish oil. This effect was most likely due to the addition of extra dry matter as a consequence of the incorporation of the microencapsulation material. Thus, in MFO batch, 5 % of microcapsules of fish oil were added, which contain around 4 % of moisture, with the rest of 96% of sample being dry matter (Jiménez-Martín et al. 2015b, a). Other authors have found the same effect, finding lower moisture and water activity in biscuits enriched with microencapsulated oils than in control ones (Umesha et al. ; Wehrle et al. 1999). However, De Conto et al. (de Conto et al. 2012) obtained the opposite result, with higher retention of water in bread crumb with increasing amounts of

microencapsulated ω-3, which they related to the ability of the hydrophilic compounds used as wall material to retain water during baking.

The lowest value of L* in MFO could be related to the lowest value of moisture in these chicken nuggets, which could have cause an increase of the browning during the progress of Maillard reaction. In fact, decrease in the value of lightness of the crust color in bread and pan-fried products is due to browning of the surface of the product, which is an indication of the progress of the Maillard reaction. Wehrle et al. (1999) (Wehrle et al. 1999) also detected this effect, finding that water activity and color development were positively correlated in biscuits enriched with encapsulated fat, with darker color (lower L*) and lower water activity than in control ones.

Fat content and fatty acid profile

Figure 3 shows the fat content in the different batches of chicken nuggets considered in this study. The behavior of the fat content of C, BFO and MFO chicken nuggets was similar, as there were not detected significant differences when there were compared the enriched chicken nuggets (BFO and MFO) with the C ones for each of the times of analysis. Fat content was affected by frozen storage (1 or 3 months). Thus, for all the formulations analyzed (C, BFO and MFO) it can be observed higher fat content in chicken nuggets at T1M and T3M than in T0. The same effect has also been reported in frozen chicken patties freezing stored during 2 months (Alabdulkarim et al. 2012). Some authors have hypothesized that meat matrix can be modified during the frozen storage (Librelotto et al. 2009). Thus, the nugget frame, more probably the crust, could be modified during the frozen storage period, which may favor the fat uptake during the deep-frying process. Such a fat increase could also be related to the loss of water during the frozen storage. However, as previously explained, the decrease in moisture and water activity from not frozen to frozen nuggets was only observed in MFO batch, but not in C or BFO ones.

The type of enrichment did not change fat content of chicken nuggets, as values were similar in the three batches of nuggets (C, BFO and MFO) for initial time (12.3, 11.3 and 12.0 g/100 g dry matter, respectively) and after 1 month (14.5, 14.8 and 15.6 g/100 g dry matter, respectively) or 3 months of

frozen storage (16.5, 15.3 and 15.4 g/100 g dry matter, respectively). Therefore, the amount of fish oil added (0.5 % w/w), either microencapsulated or not, was not high enough to cause changes in the total amount of fat of the chicken nuggets, being much more important the fat uptake during the deep-frying process. In fact, the fat uptake during deep-frying was 3.26 %, 3.59 % and 2.34 for C, BFO and MFO, respectively (data not shown).

The fatty acid profile of the chicken nuggets is presented in Table 2. General profile of fatty acids in all chicken nuggets of this study included around 50 % of PUFA, 33 % of MUFA and 17 % of SFA. This is a consequence of the predominance of linoleic acid (C18:2 n-6, around 50 %), oleic acid (C18:1 n-9, around 33 %), palmitic acid (C16:0, around 11 %) and stearic acid (C18:0, around 4 %). Thus, the accumulated amount of these predominant fatty acids comprised around 96% of the total amount of the FAMEs in each of the batches of chicken nuggets. The high content of linoleic acid (C18:2 n-6) is a clear consequence of the fatty acid profile of the vegetable oils (sunflower oil) used for pre-frying and frying process. Similar results have been found in other deep-fried coated products (Perez-Palacios et al. 2013) . Gibbs et al. (2013) (Gibbs et al. 2013) studied the fatty acid profile of processed chicken meat products and stated that the type of oil or fat added during processing was highly correlated to the proportions of major fatty acids in the products. Thus, these authors found that the use of sunflower oil during processing of chicken burgers and nuggets resulted in fatty acid profiles showing over 50% (w/w) of linoleic acid (C18:2 n-6) over the total fatty acids. It is important to remark that in raw chicken only around 20% (w/w) of the fatty acids is represented by linoleic acid (Gunstone et al. 2007).

Overall, the effect of frozen storage on the fatty acid profile led lowest amount of MUFA and SFA linked to highest amount of PUFA in T1M and T3M compared to T0, as a consequence of the higher fat uptake of T1M and T3M. However, the lowest amount of SFA in the chicken nuggets subjected to frozen storage only was significant in the C and MFO nuggets, while it can be observed the same tendency also in BFO nuggets. These differences in SFA were mainly due to the changes on C18:0 in the chicken nuggets after frozen storage.

The proportion of MUFA was significant lower at T0 than at T1M and T3M in C and BFO batches, due to changes in oleic acid (C18:1 n-9). MFO did not show significant differences due to frozen storage on MUFA. Other authors have reported a lowest amount in some MUFA in meat after frozen storage (Santos-Filho et al. 2005; Zymon et al. 2007). In our study, it is probable that the lower amount of MUFA and SFA were associated to the higher amount of PUFA, which is mainly due to the higher oil uptake of the chicken nuggets after frozen storage, previously mentioned in the discussion of the fat content. In fact, percentage of PUFA was higher in samples subjected to frozen storage for all the chicken nuggets formulations analyzed, mainly associated to the increase of linoleic acid (C18:2 n-6). Zymon et al. (Zymon et al. 2007) reported an increase of some PUFA, concretely of linoleic acid (C18:2 n-6), EPA (C20:5 n-3) and DHA (C22:6 n-3), in meat fat after 3 months of frozen storage. García-Arias et al. (García-Arias et al. 2003) reported that freezing and reheating produced an increase in linoleic acid (C18:2 n-6) in precooked sardine fillets. In the chicken nuggets analyzed in the present work, it seems that, as mentioned before for the fat content, frozen storage may have cause some changes in the matrix structure, causing that a higher sunflower oil uptake during the subsequent deep-frying, leading to an increase in linoleic acid (C18:2 n-6) compared to chicken nuggets not subjected to frozen storage.

Some authors (García-Arias et al. 2003) have reported a decrease of other PUFA in precooked sardine fillets after freezing and reheating, such as EPA (C20:5 n-3) and DHA (C22:6 n-3). However, in the present study, all the batches had the same behavior, as there were not significant differences on values of EPA (C20:5 n-3) and DHA (C22:6 n-3) comparing T0, T1M and T3M in C, BFO or MFO. This means that no significant loss of EPA (C20:5 n-3) and DHA (C22:6 n-3) were reported in the fried chicken nuggets enriched either with fish oil or microencapsulated fish oil during frozen storage after pre-frying.

However, the type of ω-3 enrichment also influenced the fatty acid profile of the chicken nuggets. In T0, MFO fried chicken nuggets had lower MUFA and higher PUFA than C and BFO, while no differences were detected for SFA among the different formulations. However, on the samples subjected to frozen storage (both T1M and T3M), MFO chicken nuggets had lower SFA and higher MUFA and PUFA than C

and BFO, with the only exception of MUFA at T1M, in which no differences were detected. It is remarkable that linoleic acid (C18:2 n-6) was higher in MFO than in C and BFO for all times of analysis. This could indicate a higher oil uptake during deep-frying in this batch of nuggets enriched with microcapsules compared to C and MFO. The higher oil uptake in MFO could be associated to the lower moisture content in comparison with the other batches. Indeed, some authors argue that fat uptake is inversely related to the total water volume (Perez-Palacios et al. 2013; Southern et al. 2000). In a study analyzing changes in fried food, it was concluded that in meat products the differences in stability of the fat fraction are related to differences in fat exchange and fat-matrix interaction during frying (Andersson et al. 2000). The different formulations could have also had an influence on the behavior during frozen storage of nuggets from different formulations.

The different enrichment methods also produced differences in the proportion of EPA (C20:5 n-3) and DHA (C22:6 n-3) of the resulting nuggets. For T0, T1M and T3M, the highest levels of EPA (C20:5 n-3) and DHA (C22:6 n-3) were found in MFO batch, followed by BFO, and the C batch showing the lowest proportions of these fatty acids. Thus, this points to the effectivity of the enrichment methods, as C were the chicken nuggets with the lowest level of EPA and DHA. Also, the results indicate more loss of EPA and DHA in BFO nuggets than in MFO ones, which points out to the stability and protection provided by the microencapsulation method. Accordingly, Wang et al. (2011) [31] observed higher EPA (C20:5 n-3) and DHA (C22:6 n-3) levels in baby food containing microencapsulated menhaden oil in comparison to baby food containing bulk menhaden oil or not enriched samples. Josquin et al. (2012) [14] also found an increase in long chain PUFA in Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with encapsulated fish oils.

In our study, it is worth to notice that for all times analyzed (T0, T1M and T3M) the PUFA/SFA ratio was lower in BFO (2.6, 2.7 and 2.8 g/100 g FAME, respectively) and in C (3.0, 2.9 and 3.3 g/100 g FAME, respectively) than in MFO (3.2, 4.0 and 3.6 g/100 g FAME, respectively). Also, for C and BFO at T1M no differences were detected in PUFA/SFA. Thus, it can be noticed that MFO attained the highest values

of PUFA/SFA in all samples of nuggets analyzed. This is in concordance with the results of Josquin et al. (2012) (Josquin et al. 2012), that enriched sausages with bulk fish oil or microencapsulated fish oil, obtaining higher PUFA/SFA ratio in the meat products enriched with 15 % or 30 % of microencapsulated fish oil than in meat products enriched with 15 % or 30 % of bulk fish oil. So, our results indicate a better profile of MFO chicken nuggets compared with BFO ones.

CONCLUSIONS

The type of ω-3 enrichment and frozen storage influence color, moisture and water activity of fried chicken nuggets, with the addition of microcapsules of fish oil and the frozen storage during 1 or 3 months leading to darker fried chicken nuggets with lower moisture.

Frozen storage seems to produce changes in the structure of the meat product which subsequently promote the fat uptake, and consequently increase the content of linoleic acid (C18:2 n-6), in the chicken nuggets during the deep-frying process in sunflower oil. However, the type of ω-3 enrichment does not influence the lipid content of these products.

The ω-3 enrichment by addition of microcapsules of fish oil allowed higher EPA (C20:5 n-3) and DHA (C22:6 n-3) content in fried chicken nuggets than bulk fish oil addition, with no losses of these fatty acids during the frozen storage. Thus, it seems that microencapsulation provide these ω-3 PUFA stability and protection, being a good strategy for enrichment purposes.

ACKNOWLEDGMENT

Estefanía Jiménez-Martín acknowledges Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) and Fondo Social Europeo for supporting the pre-doctoral grant (PD10026). The authors wish to acknowledge the funding received for this research from the Junta de Extremadura-Consejería de Economía, Comercio e Innovación and FEDER – economic support for research groups: GR15113. Biodymia Laboratory (University of Lyon) for making possible the development of the microcapsules during a research stay. "Biomega Natural Nutrients S.L." for kindly providing the fish oil. We also wish to thank the Animal Source Foodstuffs Innovation Service (SIPA) from the University of Extremadura, for their direct contribution and support.

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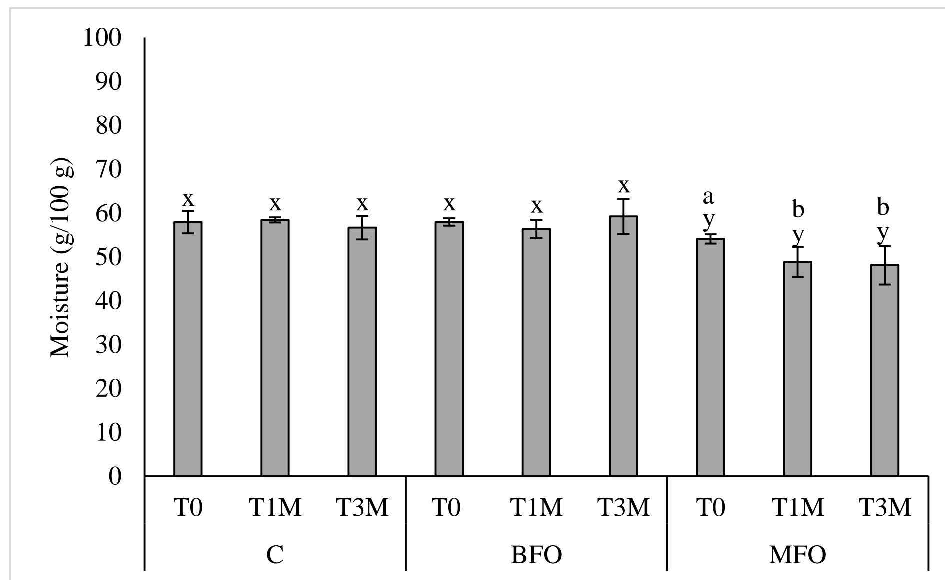
Figure 1. Moisture (g/100 g) of control (C), enriched with bulk fish oil (BFO) and enriched with microcapsules of fish oil (MFO) fried chicken nuggets without frozen storage (T0) and after frozen storage for a month (T1M) or three months (T3M). Values are expressed as mean and error bars indicate standard deviation.



Error bars indicate standard deviation. Columns with different letters (a, b, c) within the same batch (C, BFO and MFO) significant differ according to Tukey test ($p < 0.05$) due to effect of time of frozen storage. Columns with different letters (x, y, z) within the same time (T0, T1M and T3M) significant differ according to Tukey test ($p < 0.05$) due to effect of enrichment method.

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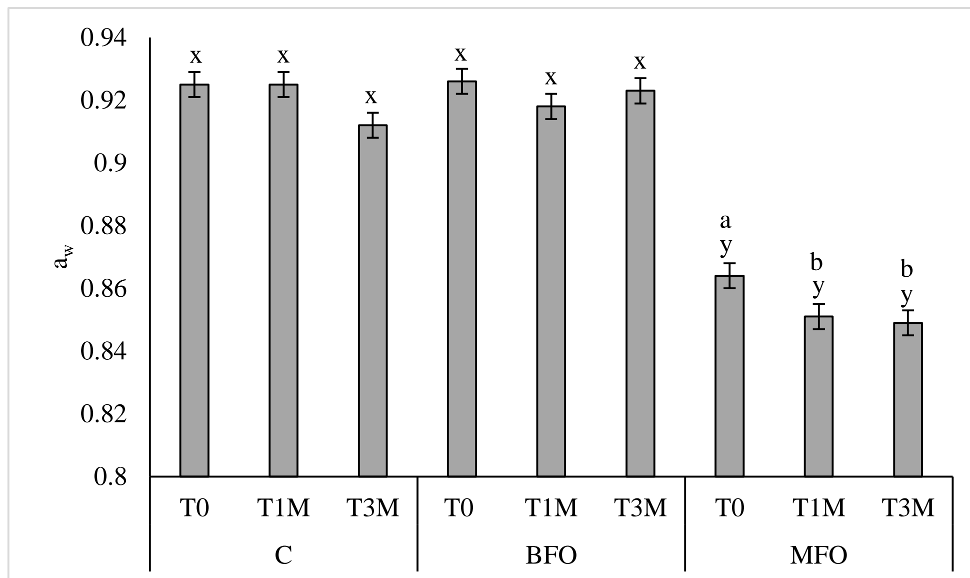
Figure 2. Water activity (a_w) of control (C), enriched with bulk fish oil (BFO) and enriched with microcapsules of fish oil (MFO) fried chicken nuggets without frozen storage (T0) and after frozen storage for a month (T1M) or three months (T3M). Values are expressed as mean and error bars indicate standard deviation.



Columns with different letters (a, b, c) within the same batch (C, BFO and MFO) significant differ according to Tukey test ($p < 0.05$) due to effect of time of frozen storage. Columns with different letters (x, y, z) within the same time (T0, T1M and T3M) significant differ according to Tukey test ($p < 0.05$) due to effect of enrichment method.

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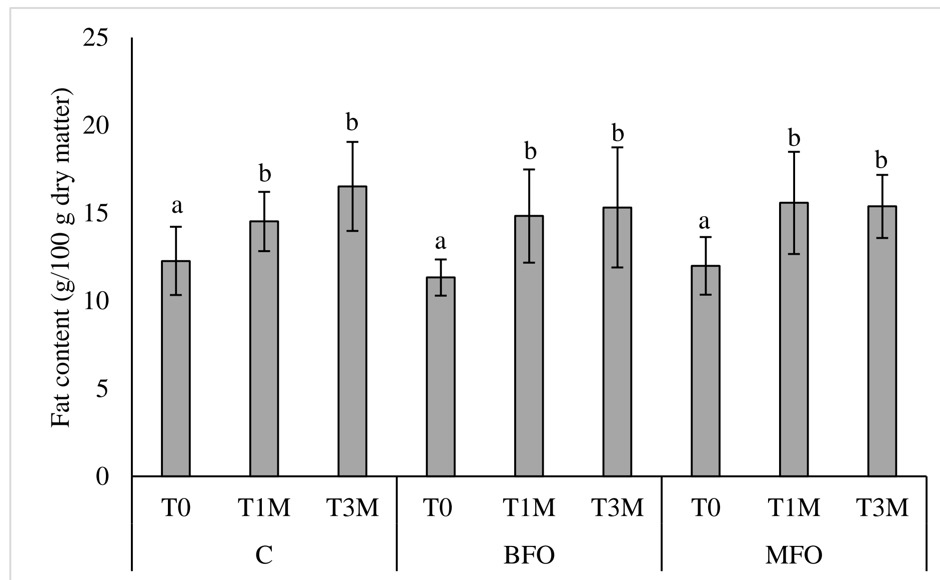
Figure 3. Fat content (g/100 g dry matter) of control (C), enriched with bulk fish oil (BFO) and enriched with microcapsules of fish oil (MFO) fried chicken nuggets without frozen storage (T0) and after frozen storage for a month (T1M) or three months (T3M). Values are expressed as mean and error bars indicate standard deviation.



Columns with different letters (a, b, c) within the same batch (C, BFO and MFO) significant differ according to Tukey test ($p < 0.05$) due to effect of time of frozen storage. Columns with different letters (x, y, z) within the same time (T0, T1M and T3M) significantly differ according to Tukey test ($p < 0.05$) due to effect of enrichment method.

Table 1 Click here to download Table Table 1.docx

Table 1. Instrumental color of control (C), enriched with bulk fish oil (BFO) and enriched with microcapsules of fish oil (MFO) fried chicken nuggets without frozen storage (T0) and after frozen storage for a month (T1M) or three months (T3M).

	C				BFO				MFO				T0	T1M	T3M
	T0	T1M	T3M	*p*S	T0	T1M	T3M	*pS*	T0	T1M	T3M	*pS*	*pE*	*pE*	*pE*
L*	56.3 ± 3.5	55.9± 2.9^{x}	51.4± 6.0^{x}	ns	54.7 ± 1.8	53.9± 2.0^{x}	50.7 ±1.8^{x}	ns	51.4 ± 3.4	47.9± 2.8^{y}	45.5± 3.6^{y}	ns	ns	*	*
a*	6.7± 1.3^{y}	7.4±1.3^{y}	7.9 ±1.8^{y}	ns	5.9 ± 0.5^{cy}	8.8 ±1.1^{by}	11.1± 1.9^{ay}	*	9.2 ± 1.1^{cx}	11.8± 1.1^{bx}	14.1 ±1.9^{ax}	*	**	**	**
b*	30.1 ± 2.8	32.6± 1.3	33.3± 5.1	ns	28.7 ± 1.6	31.2± 1.7	33.0± 1.8	ns	31.9 ± 1.5	32.4± 1.2	32.5± 1.7	ns	ns	ns	ns

ns = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Columns with different letters (a, b, c) within the same batch (C, BFO and MFO) significant differ according to Tukey test ($p < 0.05$) due to effect of time of frozen storage after pre-frying (*pS*). Rows with different letters (x, y, z) within the same time (T0, T1M and T3M) significant differ according to Tukey test ($p < 0.05$) due to effect of enrichment (*pE*).

Table 2

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Table 2. Fatty acid profile (g/100 g FAME) of control (C), enriched with bulk fish oil (BFO) and enriched with microcapsules of fish oil (MFO) fried chicken nuggets without frozen storage (T0) and after frozen storage for a month (T1M) or three months (T3M). Values are expressed as mean ± standard deviation.

	C				BFO				MFO				T0	T1M	T3M
	T-0	T-1M	T-3M	*p*S	T-0	T-1M	T-3M	*p*S	T-0	T-1M	T-3M	*p*S	pE	pE	pE
C14:0	0.33 ± 0.07	0.32 ± 0.06^{y}	0.28 ± 0.07	NS	0.43 ± 0.08	0.46 ± 0.05^{x}	0.42 ± 0.06	NS	0.34 ± 0.09	0.29 ± 0.03^{y}	0.32 ± 0.08	NS	NS	***	NS
C16:0	10.61 ± 0.74	11.06 ± 0.96^{y}	9.87 ± 0.88^{y}	NS	12.03 ±0.48	12.02 ± 0.45^{x}	11.65 ± 0.72^{x}	NS	10.31 ± 1.51^{a}	8.28 ± 0.47^{bz}	9.43 ± 1.31^{aby}	*	NS	***	**
C18:0	4.23 ± 0.18^{b}	5.07 ± 0.50^{ax}	4.55 ± 0.12^{abx}	**	4.68 ± 0.47	4.43 ± 0.19^{xy}	4.64 ± 0.22^{x}	NS	4.80 ± 0.17^{a}	4.16 ± 0.12^{by}	4.05 ± 0.13^{by}	***	NS	**	***
C20:0	0.20 ± 0.01	0.24 ± 0.05	0.20 ± 0.05	NS	0.20 ± 0.02	0.18 ± 0.01	0.20 ± 0.01	NS	0.20 ± 0.02	0.22 ± 0.02	0.19 ± 0.04	NS	NS	NS	NS
C21:0	0.05 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	NS	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	NS	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	NS	NS	NS	NS
C22:0	0.45 ± 0.05^{ax}	0.42 ± 0.05^{ax}	0.28 ± 0.03^{by}	***	0.36 ± 0.02^{y}	0.31 ± 0.05^{y}	0.38 ± 0.05^{x}	NS	0.35 ± 0.04^{by}	0.46 ± 0.06^{ax}	0.27 ± 0.03^{cy}	***	**	**	**
C24:0	0.56 ± 0.27	0.28 ± 0.04^{y}	0.41 ± 0.01^{x}	NS	0.48 ± 0.09^{a}	0.62 ± 0.11^{ax}	0.20 ± 0.02^{by}	***	0.25 ± 0.08^{a}	0.32 ± 0.09^{ay}	0.11 ± 0.03^{bz}	**	NS	***	***
SFA	16.43 ± 0.79^{ab}	17.45 ± 0.85^{ax}	15.63 ± 0.79^{bxy}	*	18.27 ± 0.74	18.08 ± 0.63^{x}	17.56 ± 0.95^{y}	NS	16.32 ± 1.59^{a}	13.79 ± 0.46^{by}	14.41 ± 0.85^{by}	**	NS	***	***
C14:1	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	NS	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	NS	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	NS	NS	NS	NS
C16:1 (ω-7)	0.69 ± 0.19^{y}	0.67 ± 0.05^{y}	0.59 ± 0.10^{y}	NS	0.98 ± 0.09^{x}	0.95 ± 0.07^{x}	0.83 ± 0.11^{x}	NS	0.66 ± 0.15^{y}	0.53 ± 0.06^{z}	0.58 ± 0.11^{y}	NS	**	***	**
C18:1 (ω-9)	33.67 ± 0.52^{ax}	31.42 ± 0.80^{b}	31.45 ± 0.51^{bx}	***	33.01 ± 0.92^{ax}	30.91 ± 0.39^{b}	31.71 ± 0.29^{bx}	***	30.95 ± 1.47^{y}	30.65 ± 0.13	29.97 ± 1.08^{y}	NS	**	NS	**
C20:1 (ω-9)	0.17 ± 0.01^{z}	0.18 ± 0.04^{y}	0.16 ± 0.02^{y}	NS	0.31 ± 0.04^{ax}	0.24 ± 0.03^{bx}	0.26 ± 0.02^{abx}	*	0.23 ± 0.04^{y}	0.23 ± 0.03^{x}	0.20 ± 0.03^{y}	NS	***	*	***
C22:1 (ω-9)	0.05 ± 0.02	0.04 ± 0.00	0.03 ± 0.00^{y}	NS	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.03^{xy}	NS	0.07 ± 0.03	0.05 ± 0.03	0.09 ± 0.02^{x}	NS	NS	NS	**
MUFA	34.60 ± 0.46^{ax}	32.34 ± 0.81^{b}	32.25 ± 0.45^{by}	***	34.35 ± 0.91^{ax}	32.16 ± 0.49^{b}	32.88 ± 0.35^{by}	***	31.93 ± 1.29^{y}	31.47 ± 0.19	30.86 ± 0.97^{x}	NS	***	NS	***
C18:2 (ω-6)	48.26 ± 0.68^{bxy}	49.38 ± 1.28^{ay}	51.32 ± 0.79^{ay}	***	46.11 ± 1.45^{by}	48.65 ± 1.12^{ay}	48.50 ± 1.30^{az}	*	50.46 ± 0.99^{bx}	53.63 ± 0.69^{ax}	53.68 ± 0.47^{ax}	***	***	***	***
C18:3 (ω-3)	0.35 ± 0.08	0.35 ± 0.03^{x}	0.34 ± 0.09	NS	0.37 ± 0.04^{a}	0.33 ± 0.03^{abx}	0.30 ± 0.02^{b}	*	0.28 ± 0.05	0.25 ± 0.04^{y}	0.25 ± 0.03	NS	NS	**	NS
C20:2 (ω-6)	0.06 ± 0.02^{y}	0.06 ± 0.02	0.08 ± 0.02	NS	0.09 ± 0.01^{x}	0.07 ± 0.01	0.07 ± 0.01	NS	0.08 ± 0.02^{xy}	0.07 ± 0.02	0.06 ± 0.01	NS	*	NS	NS
C20:4 (ω-6)	0.22 ± 0.13	0.33 ± 0.07	0.30 ± 0.09	NS	0.32 ± 0.04^{b}	0.27 ± 0.04^{a}	0.26 ± 0.03^{a}	*	0.27 ± 0.08	0.24 ± 0.07	0.22 ± 0.04	NS	NS	NS	NS
C20:3 (ω-3)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	NS	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	NS	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	NS	NS	NS	NS
C20:5 (ω-3)	0.02 ± 0.22^{y}	0.01 ± 0.00^{z}	0.01 ± 0.00^{z}	NS	0.02 ± 0.01^{y}	0.04 ± 0.02^{y}	0.05 ± 0.02^{y}	NS	0.08 ± 0.02^{x}	0.08 ± 0.02^{x}	0.08 ± 0.01^{x}	NS	***	***	***
C22:6 (ω-3)	0.04 ± 0.02^{z}	0.03 ± 0.00^{y}	0.02 ± 0.01^{z}	NS	0.44 ± 0.09^{y}	0.38 ± 0.06^{x}	0.35 ± 0.03^{y}	NS	0.56 ± 0.08^{x}	0.45 ± 0.10^{x}	0.42 ± 0.11^{x}	NS	***	***	***
PUFA	48.96 ± 0.60^{by}	50.21 ± 1.23^{by}	52.11 ± 1.58^{ay}	***	47.38 ± 1.49^{by}	49.76 ± 1.07^{ay}	49.56 ± 1.29^{az}	*	51.75 ± 0.86^{bx}	54.74 ± 0.48^{ax}	54.72 ± 0.57^{ax}	***	***	***	***
PUFA/SFA	2.99 ± 0.18^{y}	2.88 ± 0.24^{y}	3.34 ± 0.22^{y}	NS	2.60 ± 0.19^{z}	2.75 ± 0.15^{y}	2.83 ± 0.22^{z}	NS	3.20 ± 0.37^{bx}	3.97 ± 0.16^{ax}	3.66 ± 0.43^{ax}	***	**	***	***

ns = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Columns with different letters (a, b, c) within the same batch (C, BFO and MFO) significant differ according to Tukey test ($p < 0.05$) due to effect of time of frozen storage after pre-frying (*pS*). Columns with different letters (x, y, z) within the same time (T0, T1M and T3M) significantly differ according to Tukey test ($p < 0.05$) due to effect of enrichment (*pE*).

5. Discusión

SECCIÓN 1

En la primera parte de este trabajo de tesis doctoral se han elaborado tres tipos de emulsiones: dobles (DE), monocapa (MoE) y multicapa con diferente concentración de quitosano (MuE1 y MuE2) utilizando como fuente de ω-3 aceite de pescado rico en EPA y DHA. A partir de las emulsiones se han producido diferentes tipos de microcápsulas DM, MoM, MuM1 y MuM2, respectivamente, mediante el método de spray drying (secado por atomización).

En el caso de las emulsiones DE, el aceite de pescado se encuentra en la fase interna de una emulsión de aceite-en-agua-en-aceite-en-agua (O/W/O/W). En el caso de las emulsiones MoE, el aceite se encuentra rodeado por una combinación de una capa de lecitina y una matriz de maltodextrina. En el caso de las emulsiones MuE1 y MuE2, el aceite se encuentra cubierto por la combinación de una capa de lecitina y quitosano, y una matriz de maltodextrina.

Se encontró una mayor estabilidad en las emulsiones MuE, y sobre todo de MuE2, en comparación con las DE y con las MoE. Estas diferencias pueden relacionarse con la presencia y concentración de quitosano. El quitosano previene el contacto entre las capas de lecitina de las distintas gotas, y aumenta las fuerzas electrostáticas de repulsión y la viscosidad, lo que da lugar a una disminución en la movilidad de las gotas de la emulsión, evitando su agregación y manteniendo así la estabilidad [1-3]. Según esto, las diferencias en la estructura de la emulsión así como en el tamaño de sus gotas influyen directamente sobre su estabilidad. En este estudio, las membranas interfaciales estabilizadas por el polirricinoleato de poliglicerol, utilizado como agente emulsificante en DE, parecen ser menos rígidas que las estabilizadas por lecitina y quitosano en MuE. En cuanto al tamaño de las gotas, el “creaming index” aumenta con el cuadrado del radio de las partículas [4], por lo que la estabilidad de las emulsiones puede incrementarse reduciendo el tamaño de las partículas [5]. Esto explica que MuE2, con un menor tamaño de gotas que DE, obtuviera mejores resultados de estabilidad medida mediante “creaming index”, lo que indica mayor estabilidad física. Estas diferencias de estabilidad son un factor importante a tener en cuenta, ya que la mayor estabilidad de las emulsiones está relacionada con una mayor eficiencia de encapsulación [6].

En cuanto a las microcápsulas, la humedad fue significativamente menor en las microcápsulas obtenidas a partir de DE. En éste caso, la estructura de la emulsión y las propiedades de hidrofobicidad de su formulación podrían haber dado lugar a la menor cantidad de agua final en las DM. Por otro lado, los valores del contenido de humedad fueron similares en las microcápsulas preparadas a partir de emulsiones monocapa (MoM) y multicapa (MuM1 y MuM2), por lo que parece que la adición de quitosano a las emulsiones, así como su concentración, no tuvieron influencia en la retención de humedad durante el proceso de spray-drying. Estos resultados coinciden con los encontrados por otros autores, que han indicado que además del propio proceso de spray-drying, la humedad final en las microcápsulas también se relaciona con la composición de las emulsiones [7].

Las diferencia principal en la composición entre la emulsión doble y las monocapa y multicapa fue que, aunque la proporción total de aceite añadida en todas las emulsiones fue la misma (2.50% w/w de aceite total), en la emulsión doble el porcentaje de aceite de pescado fue menor (0.625 % w/w) que en las monocapa y multicapa (2.50% w/w). Esto es debido al hecho de que en la emulsión doble sólo parte del aceite total era aceite de pescado y el resto lo formaba aceite de oliva virgen extra. Estas diferencias en la composición entre las emulsiones dobles y multicapa, se reflejaron en el perfil de ácidos grasos de las microcápsulas.

Las diferencias entre DM y MuM2 estuvieron determinadas por la elevada cantidad de ácidos grasos monoinsaturados (AGMI) (66.89%) en DM, mientras que en MuM2 los ácidos grasos saturados (AGS) (41.74%) y poliinsaturados (AGPI) (41.30%) fueron los mayoritarios. La incorporación de aceite de oliva virgen extra en la emulsión utilizada para preparar DM dio lugar a una menor concentración de EPA y DHA y valores elevados de ácido oleico. Esto es similar a lo descrito por otros autores al incorporar aceite de maíz en microcápsulas de aceite de pescado [8]. En nuestro estudio encontramos una mayor proporción de EPA y DHA en las microcápsulas MuM2 (0.58 g/100 g de microcápsulas y 1.90 g/100 g de microcápsulas, respectivamente) que en las microcápsulas DM (0.12 g/100 g de microcápsulas y 0.42 g/100 g de microcápsulas, respectivamente). Este valor, denominado como “loading” o carga (g/100 g de microcápsulas de

ingrediente bioactivo), tiene importancia desde el punto de vista industrial, estando relacionado con la viabilidad económica del enriquecimiento, ya que una mayor carga en las microcápsulas hace que el enriquecimiento de los alimentos se pueda realizar con menor proporción de las mismas, haciendo más factible el proceso [9].

En cuanto al estudio del almacenamiento de las microcápsulas, la proporción de EPA y DHA que se conservó en las microcápsulas tras el almacenamiento a refrigeración y temperatura ambiente con respecto a su cantidad inicial fue mayor en MuM2 que en DM. Además, se conservó una mayor proporción de DHA que de EPA, tanto en MuM2 como en DM a ambas temperaturas de almacenamiento, siendo esta diferencia más marcada en el caso de MuM2. Esto podría deberse a que en MuM2 se observó mayor proporción de DHA efectivamente encapsulado en el interior de las microcápsulas en comparación con la proporción de EPA encapsulado, mientras que en DM los valores de proporción de EPA y DHA encapsulado fueron similares.

En cuanto al rendimiento de la microencapsulación, que indica la cantidad microcápsulas que se obtiene a partir de las emulsiones, no se detectaron diferencias entre las diferentes microcápsulas. Por ello, parece que la diferente estructura y composición de las microcápsulas (la estructura de emulsión multicapa o múltiple, así como la inclusión de quitosano y su concentración) no influyen en esta característica. Esto contrasta con lo encontrado por otros autores, que observaron una relación entre el rendimiento de la microencapsulación y la composición [10]. Sin embargo, también se ha discutido que las diferencias entre la naturaleza del aceite y el procedimiento de preparación pueden influir en las variaciones en el rendimiento de la microencapsulación [11].

Una mayor proporción de aceite encapsulado, es decir, una elevada eficiencia de la microencapsulación, se considera como una característica positiva [6]. Los resultados obtenidos para la eficiencia se corresponden con los comentados en el apartado anterior referentes a la estabilidad de las emulsiones, estando relacionada la mayor estabilidad de las emulsiones con una mayor eficiencia de la microencapsulación. Las emulsiones con el menor ratio aceite/sólidos, es decir

las multicapa MuE2 obtuvieron los mayores valores de eficiencia de encapsulación en las microcápsulas resultantes (MuM2), mientras que las emulsiones DE, con el mayor ratio aceite/sólidos dieron lugar a microcápsulas DM con menor valor de eficiencia. En otros estudios también se ha observado relación entre el ratio de aceite/sólidos y la eficiencia de la microencapsulación [12]. Los bajos contenidos de aceite en la emulsión en relación con los sólidos, es decir, ratios aceite/sólidos bajos, se relacionan con una rápida formación de una membrana superficial semipermeable alrededor de la superficie de las gotas que finalmente constituirá la pared de las microcápsulas durante el proceso de spray drying. La rápida formación de esta membrana evita la pérdida de aceite de las microcápsulas, lo que a su vez aumenta la eficiencia [13].

En el ensayo de oxidación acelerada, se detectaron diferencias significativas entre los niveles de oxidación de todas las muestras (aceite de pescado, emulsiones y microcápsulas), encontrándose las diferencias más marcadas a 60 ºC. En general, los menores niveles de oxidación se encontraron en las microcápsulas. También se encontraron diferencias en los niveles de oxidación en los tres tipos de microcápsulas, con los mayores valores de TBARS en MuM1 en la mayoría de los casos, seguido por MoM y teniendo MuM2 los valores más bajos, mostrando mejor estabilidad oxidativa tanto a 30 ºC como a 60 ºC. La estabilidad oxidativa de las microcápsulas parece incrementarse con el aumento de la concentración de quitosano y del número de capas de la emulsión. Es interesante destacar que hubo una relación estrecha entre la eficiencia de la microencapsulación y la oxidación lipídica. Este efecto ha sido previamente analizado en otros estudios [6] de donde se destaca la importancia de controlar los factores que influyen en la eficiencia de la microencapsulación para obtener una buena protección y vida útil del aceite microencapsulado.

En el ensayo de almacenamiento a dos temperaturas (4 ºC y 20 ºC) se detectaron diferencias significativas en los valores de TBARS entre microcápsulas, con mayores valores de TBARS para DM que para MuM2 tanto en el tiempo inicial como tras un mes de almacenamiento. Al igual que en el ensayo de almacenamiento de oxidación acelerada, el efecto significativo del tipo de microcápsula sobre los valores de TBARS podría relacionarse con las diferencias en la eficiencia de la microencapsulación. Algunos autores han

detectado mejor estabilidad oxidativa en microcápsulas con elevada eficiencia de la microencapsulación, y han relacionado este hecho con el aumento en la cantidad de material de pared [14]. Estos resultados coinciden con los obtenidos en este estudio, ya que se detectaron menores valores de TBARS en MuM2 que en DM. Así mismo, el ratio material de pared/aceite total fue mayor en MuM2 que en DM. El valor inicial de TBARS fue más elevado en DM que en MuM2, lo que podría ser debido a diferencias en la formulación de las emulsiones. Algunos autores han observado que la combinación de lecitina-quitosano utilizada en MuM2 produce un incremento del grosor de las capas que rodean las gotas de aceite. Esto a su vez previene el contacto con los prooxidantes y mantiene separadas las capas de lecitina de las distintas gotas, permitiendo mantener la estructura de la emulsión estable, y mejorando la estabilidad oxidativa [15]. Además, la maltodextrina, utilizada como material de recubrimiento en MuM2, es un polisacárido que tiene capacidad para formar emulsiones estables [16] y mejora la estabilidad del aceite encapsulado, proporcionando protección frente a la oxidación lipídica [17].

En este mismo ensayo, se monitorizaron los compuestos volátiles relacionados con la oxidación. En cuanto al almacenamiento, como era de esperar, el perfil de volátiles indicó menor oxidación en el momento inicial y a 4 ºC que en las muestras almacenadas a 20 ºC. En general, el perfil de compuestos volátiles de DM y MuM2 parece indicar que los procesos de oxidación producidos durante el almacenamiento de las microcápsulas en este estudio no fueron muy marcados. No obstante, se observó un efecto del tipo de microcápsulas sobre el perfil de compuestos volátiles provenientes de la oxidación lipídica, ya que la mayoría de los compuestos se detectaron en DM y no en MuM2, y los perfiles indicaron en general menor oxidación de las microcápsulas producidas mediante emulsiones multicapa. Esto concuerda con los resultados obtenidos para los TBARs y de nuevo indica que las ventajas de MuM2 frente a los otros tipos de microcápsulas estudiados.

En las imágenes de microscopía electrónica se apreciaron diferencias entre las distintas microcápsulas. En DM se observó mayor aglomeración de las partículas, detectando también algunas cápsulas rotas o dañadas y la presencia de poros o pequeños agujeros, mientras que no se observaron poros aparentes

en el caso de MoM, MuM1 y MuM2. La ausencia de poros en las microcápsulas asegura una mejor retención de los compuestos [18] y contribuye a su protección, ya que disminuye la difusión de oxígeno en las microcápsulas También se observó que las microcápsulas MuM2 fueron en general partículas esféricas con superficie lisa y homogénea, lo que se relaciona con una buena estabilidad. Estas diferencias probablemente hayan sido causadas por la diferente composición de las emulsiones. No obstante, es difícil establecer el efecto de cada variable específica de la estructura y composición de las emulsiones sobre la morfología de las microcápsulas [19].

Por todo ello, las microcápsulas multicapa producidas mediante spray-drying, y sobre todo las MuM2, parecen tener características que podrían relacionarse con una mejor estabilidad y protección frente a la oxidación del aceite de pescado como fuente de ácidos grasos ω-3, siendo una buena alternativa al almacenamiento de aceite de pescado y pudiendo ser utilizadas como método de enriquecimiento en alimentos.

SECCIÓN 2

Con el fin de evaluar la posibilidad de enriquecer productos cárnicos en EPA y DHA utilizando como fuente el aceite de pescado, en esta tesis se ha elegido como producto los nuggets de pollo. Se ha realizado un ensayo en el que se elaboraron tres tipos de nuggets de pollo: un lote control (Lote C), un lote enriquecido con aceite de pescado sin encapsular (Lote "Bulk Fish Oil" o BFO) y un lote enriquecido con microcápsulas de aceite de pescado (Lote "Microencapsulated Fish Oil" o MFO), evaluándose el efecto del tipo de enriquecimiento sobre las características físico-químicas y sensoriales del producto y también sobre los cambios que se producen durante la fritura y el almacenamiento a congelación durante 1 y 3 meses. Las microcápsulas utilizadas para el enriquecimiento de los nuggets MFO fueron las MuM2, ya que presentaron las mejores características morfológicas y de estabilidad.

En cuanto al **efecto del tipo de enriquecimiento** sobre la humedad y actividad de agua, en todos los nuggets (tanto pre-fritos, como en los fritos sin almacenamiento previo y en los fritos tras almacenamiento a congelación durante 1 o 3 meses) los valores fueron inferiores en los nuggets MFO que en los BFO y C. Este efecto es probable que sea consecuencia de la incorporación del material de microencapsulación. De hecho, en los nuggets MFO, se añadieron un 5 % de microcápsulas de aceite de pescado, cuyo contenido de humedad era aproximadamente un 4 %, y por tanto 96 % de materia seca [20, 21]. Josquin et al. [22] estudiaron salchichas enriquecidas con aceite de pescado encapsulado y sin encapsular, obteniendo resultados similares a los nuestros ya que presentaron menor humedad las salchichas con aceite encapsulado que aquellas con adición directa de aceite de pescado. Otros autores han detectado igualmente menor humedad y actividad de agua en galletas enriquecidas con aceites microencapsulados que en el lote control [23, 24].

El tipo de enriquecimiento influyó significantemente en los valores de las coordenadas L* y a* del color instrumental. El valor de L* fue mayor en C que en los nuggets enriquecidos, y además, dentro de los enriquecidos, MFO fueron los nuggets con menores valores de L*. En cuanto a los valores de a*, fueron mayores en MFO con respecto a C. Otros autores también han observado una disminución de L* y un aumento de a* con el aumento en la concentración de microcápsulas de ω-3 en pan [25], lo que asociaron a la composición del material utilizado para la microencapsulación. En esta tesis doctoral, el material utilizado para preparar las microcápsulas de aceite de pescado (lecitina, quitosano y maltodextrina) podrían haber sido los responsables de los cambios en los valores de color instrumental en los nuggets MFO.

El contenido en grasa de los todos los lotes de nuggets fue similar. Parece que la cantidad de aceite de pescado añadida (0.5 % w/w), tanto microencapsulada como sin microencapsular no fue suficientemente elevada como para producir cambios significativos en la cantidad total de grasa de los nuggets de pollo, siendo como era de esperar, más importante la absorción de aceite durante el proceso de fritura, como se discutirá más adelante.

El perfil general de ácidos grasos de los nuggets de pollo de este estudio fue similar, siendo el ácido graso mayoritario el ácido linoleico (C18:2 n-6, aproximadamente un 50 %), seguido por el ácido oleico (C18:1 n-9, aproximadamente un 33 %), el ácido palmítico (C16:0, aproximadamente 11 %) y el ácido esteárico (C18:0, aproximadamente 4 %). El elevado contenido de ácido linoleico (C18:2 n-6) se debe a que este es el ácido graso mayoritario del aceite de girasol utilizado durante los procesos de pre-fritura y fritura y el segundo ácido graso mayoritario en la carne de pollo. En otros estudios con productos empanados pre-fritos se han obtenido resultados similares [26].

El enriquecimiento con microcápsulas de aceite de pescado fue efectivo, observando mayor cantidad de EPA (C22:5 ω-3) y DHA (C22:6 ω-3) en MFO que en C. Sin embargo, en cuanto a los nuggets BFO, sólo se observó enriquecimiento en DHA (C22:6 ω-3), mientras que para el EPA (C22:5 ω-3) no se observaron diferencias con respecto a C. En cuanto al efecto del tipo de enriquecimiento, el mayor contenido en EPA (C22:5 ω-3) y DHA (C22:6 ω-3) observado en MFO con respecto a BFO parece indicar una mayor protección de estos AGPI ω-3 durante la elaboración y fritura de los nuggets de pollo cuando se añade a la masa MuM2 en lugar de añadirlo directamente el aceite sin encapsular. Josquin et al. (2012) [22] también observaron un incremento de AGPI ω-3 en salchichas enriquecidas con microcápsulas de aceite de pescado. Wang et al. (2011) [27] observaron mayor porcentaje de EPA y DHA en alimentos para bebé enriquecidos con microcápsulas de aceite de pescado en comparación con estos alimentos enriquecidos con adición directa de aceite.

En relación a parámetros de oxidación, se observó un nivel de oxidación lipídica (primaria y secundaria) y proteica mayor en los nuggets BFO que en los C y MFO. Entre estos dos últimos lotes, solo se observaron diferencias en los productos de oxidación secundaria, con menores cantidades en los nuggets MFO. Por lo tanto, los nuggets BFO fueron más susceptibles a la oxidación tanto lipídica como proteica, y la encapsulación controló con éxito el desarrollo de dichas reacciones. Otros autores han observado resultados similares en salchichas [22]. La protección de las microcápsulas frente a la oxidación puede ser el resultado de la propia protección proporcionada por las capas de las microcápsulas que actúan como una barrera que limita la oxidación del aceite de

pescado [21] y probablemente evita la propagación de los radicales producidos a partir de la oxidación de estos PUFA a través del producto cárnico. Las microcápsulas preparadas a partir de emulsiones multicapa de lecitina-quitosano se han relacionado previamente con una buena protección de aceite de pescado. Además de la protección ofrecida por las microcápsulas multicapa debido a la propia interfaz catiónica y el espesor de las capas, el quitosano incrementa la viscosidad en el interior de las microcápsulas, lo que produce una menor difusión de los prooxidantes [28]. La concentración de quitosano también tiene un papel importante, y se ha observado que puede considerarse que la estabilidad oxidativa de los ingredientes encapsulados se reduce cuando el quitosano se encuentra en exceso en el material de encapsulación [29]. Además, también se ha observado que algunos cambios que se producen en las microcápsulas podrían estar relacionados con una disminución del ratio de oxidación del aceite encapsulado. Por ejemplo, la cristalización de azúcares, podría disminuir la permeabilidad al oxígeno de las microcápsulas, y algunos productos de la reacción de Maillard pueden actuar como antioxidantes [30]. Por lo tanto, el menor nivel de oxidación observado en los nuggets MFO podría estar influido por productos de Maillard formados a partir del material de pared de las microcápsulas.

En cuanto a los compuestos volátiles relacionados con la oxidación, en BFO se observaron mayores cantidades de alcoholes y cetonas, que se han originado probablemente a través de la oxidación de los ácidos grasos [31], y de aldehídos saturados e insaturados, utilizados como marcadores de oxidación lipídica en alimentos [31], tales como butanal, 2,4-hexadienal, pentanal, octanal y 2,4-decadienal. Este último proviene específicamente de la oxidación de los ácidos grasos poliinsaturados de cadena larga (EPA y DHA). Esto indica un mayor nivel de oxidación de los nuggets BFO comparados con los nuggets C y los MFO. No obstante, los compuestos relacionados con la oxidación específica de los AGPI ω-3 no fueron detectados en ninguno de los grupos analizados, mientras que otros compuestos volátiles relacionados con la oxidación de la carne sí se detectaron en altas concentraciones. Esto concuerda con estudios anteriores que mostraron los ácidos grasos ω-3 no sólo ejercen su impacto sobre la oxidación y los atributos sensoriales a través de sus propios productos de

degradación, si no que también pueden acelerar la oxidación de otros ácidos grasos insaturados [32].

El enriquecimiento de los nuggets de pollo con microcápsulas de aceite de pescado no perjudicó los atributos sensoriales ni a la aceptabilidad de los productos cárnicos enriquecidos ya que no hubo diferencias significativas entre los nuggets MFO y los C. Sin embargo, la adición directa de aceite de pescado a los nuggets afectó a algunas características sensoriales. En concreto, los panelistas indicaron una mayor percepción de la jugosidad y sabor salado y menor intensidad del aroma cárnico en los nuggets BFO. No obstante, los aromas “rancios” o “a pescado” obtuvieron puntuaciones muy bajas en el análisis cuantitativo descriptivo en todos los lotes analizados, también en los nuggets BFO. La aceptabilidad de todos los nuggets fue buena y similar para todas las formulaciones. En cuanto a los resultados del test triangular, los panelistas no fueron capaces de diferenciar entre los nuggets C y los MFO. Sin embargo, fueron capaces de distinguir los nuggets BFO del resto de nuggets. Esto es importante, ya que indica que los nuggets MFO no sólo tienen buena aceptabilidad si no que los consumidores no serían capaces de distinguirlos de los nuggets C.

Además del efecto del tipo de enriquecimiento sobre las características físico-químicas y sensoriales de los nuggets, también se estudió su **influencia sobre las modificaciones físico químicas que se producen durante la fritura.**

En este estudio se observó para los nuggets MFO una mayor pérdida de agua y una menor absorción de aceite durante la fritura en comparación con los nuggets C y los BFO. Algunos autores han indicado que la absorción de grasa está inversamente relacionada con el volumen total de agua [26, 33].

En cuanto a los cambios de color observados como consecuencia de la fritura, se correspondieron con las modificaciones comunes observadas en productos empanados fritos, en los que se detecta un incremento del color marrón dorado. Por una parte, se produjo un aumento de los parámetros a* y b* en los tres tipos de nuggets, C, BFO y MFO. Sin embargo, en MFO se produjo además una disminución del valor de L* [34].

El contenido en grasa en los nuggets pre-fritos de los distintos nuggets C, BFO y MFO, fue similar. Lo mismo ocurrió tras la fritura, no observándose diferencias en la cantidad de grasa entre los tres tipos de nuggets. No obstante, sí que se observó un efecto de la fritura en cada uno de los tipos de nuggets, siendo este efecto diferente en los MFO en comparación con C y BFO. Por un lado, en C y BFO hubo diferencias entre los nuggets pre-fritos y fritos, mostrando mayor cantidad de grasa los fritos. Sin embargo, por otra parte, en MFO no hubo diferencias en la cantidad de grasa entre nuggets pre-fritos y fritos. Esto parece indicar menor absorción de grasa durante la fritura en los nuggets MFO en comparación con los C y BFO.

El menor porcentaje de DHA en las muestras pre-fritas en el caso de BFO en comparación con las muestras pre-fritas de MFO parece indicar que se produjo una pérdida de este ácido graso durante la elaboración y proceso de pre-fritura en los nuggets BFO. Además, posteriormente, durante la fritura, hubo pérdidas de EPA y DHA en los nuggets enriquecidos con BFO, mientras que en los MFO se perdió DHA pero no EPA. Henna and Norziah (2009) [35], también observaron pérdidas de EPA y DHA durante el horneado de panes enriquecidos con microcápsulas de AGPI ω-3. En nuestro estudio, los nuggets enriquecidos con microcápsulas conservaron mayor porcentaje de EPA y DHA tras la fritura que los enriquecidos con aceite.

La fritura produjo un aumento significativo de la oxidación de lípidos y proteínas en todos los tipos de nuggets. No obstante, tanto en los nuggets pre-fritos como en los fritos se observó mayor oxidación lipídica en BFO que en C y MFO, tanto en lo que respecta a dienos conjugados como a TBARs. Sin embargo, no se detectaron diferencias en la oxidación de proteínas entre los distintos tipos de nuggets ni en los nuggets pre-fritos ni en los fritos.

También se analizó si el **efecto del tiempo a congelación** (1 ó 3 meses) de los nuggets pre-fritos afecta de igual forma a las características físico-químicas y sensoriales de los nuggets fritos dependiendo del tipo de enriquecimiento.

El efecto del almacenamiento a congelación en la humedad y la actividad de agua fue sólo significativo en los nuggets MFO, encontrando valores más bajos en los nuggets sometidos a almacenamiento a congelación que en los no

congelados. No obstante, el tiempo de almacenamiento a congelación no fue significativo. La influencia del almacenamiento a congelación sólo en los nuggets MFO podría estar asociada con la adición de microcápsulas de aceite de pescado, que podría haber causado diferencias en la matriz del producto, dando lugar a pérdidas de agua durante el almacenamiento o aumentando las pérdidas de agua durante el proceso de fritura [36].

En cuanto al color instrumental, el almacenamiento a congelación afectó a la coordenada a* pero no a L* ni a b*. En los nuggets enriquecidos el efecto fue el mismo, ya que tanto en MFO como en BFO, el valor de a* aumentó sucesivamente con el almacenamiento a congelación, sin embargo en C no hubo diferencias significativas.

El almacenamiento a congelación produjo diferencias en el contenido de grasa en los nuggets fritos. Para todas las formulaciones analizadas se observó mayor contenido de grasa en los nuggets fritos que se habían sometido a congelación frente a los nuggets no congelados. Otros autores han observado este efecto en hamburguesas de pollo almacenadas a temperatura de congelación durante 2 meses [36]. Algunos autores han hipotetizado que la dienmatriz cárnica puede modificarse durante el almacenamiento a congelación [37]. En nuestro caso, el rebozado del nugget, podría haber sufrido modificaciones durante el periodo de almacenamiento a congelación, y esto ha podido provocar un incremento en la absorción de aceite durante la fritura. Este incremento en el porcentaje de grasa podría estar relacionado con pérdidas de agua del rebozado durante el almacenamiento a congelación.

En general, el efecto del almacenamiento a congelación sobre el perfil de ácidos grasos de los nuggets tras la fritura dio lugar a una disminución en la cantidad de AGMI y AGS como consecuencia de un aumento de los AGPI. Principalmente ácido linoleico (C18:2 n-6). Esto puede también relacionarse con el ya comentado aumento de la absorción de aceite durante la fritura en los nuggets sometidos a congelación.

En los nuggets sometidos a almacenamiento a congelación, los nuggets MFO tuvieron en general menor cantidad de AGS y mayor de AGMI y AGPI que los C y los BFO. Esto se relaciona con el hecho de que el ácido linoleico (C18:2 n-6)

fue mayor en los nuggets MFO que en los C y los BFO en todos los tiempos de análisis, debido a la mayor absorción de grasa durante la fritura de MFO tras el almacenamiento a congelación en comparación con C y BFO.

En relación al EPA y DHA, no se produjeron pérdidas de estos ácidos grasos durante el almacenamiento a congelación tras la pre-fritura.

Los procesos de oxidación en los nuggets de pollo no se detuvieron durante el almacenamiento a congelación por 1 o 3 meses, lo que coincide con otros autores [38]. No hubo diferencias en el efecto entre los tipos de nuggets, pues tanto en C como en BFO y MFO se produjo un incremento de los dienos conjugados almacenados 1 mes a congelación en comparación con los nuggets no congelados. Sin embargo, los nuggets almacenados durante 3 meses a congelación mostraron menores cantidades de dienos conjugados que los almacenados durante 1 mes. Esto podría indicar que en los nuggets almacenados durante 1 mes a temperatura de congelación se producen procesos de oxidación primaria que dan lugar a la formación de dienos conjugados, mientras que en las muestras almacenadas durante 3 meses, los menores valores de dienos conjugados puede deberse a la transformación de estos compuestos en otros productos que se producen en estados más avanzados de la oxidación, como los hidroperóxidos. De esta forma, en todos los nuggets almacenados a congelación durante 3 meses tras la pre-fritura, parece que la formación de productos secundarios de oxidación a partir de los primarios es es más rápida que la formación de estos últimos .

En cuanto al índice de TBARs, en general, hubo un incremento debido al tiempo de almacenamiento, aunque fue diferente entre las distintas formulaciones de nuggets. Se observaron mayores valores de TBARs en BFO que en C y MFO en ambos tiempos de almacenamiento a congelación. Según esto, la oxidación lipídica se ve afectada por el tiempo de almacenamiento a congelación, siendo el enriquecimiento con microcápsulas un método de protección efectivo frente a la oxidación lipídica.

En cuanto a la oxidación de proteínas, el almacenamiento a congelación incrementó el valor de carbonilos totales medidos por reacción con DNPH. En C y BFO, la cantidad de estos carbonilos aumentó sucesivamente con el tiempo de

almacenamiento a congelación, mientras que en MFO este efecto fue menos intenso. Este incremento de la oxidación proteica se ha observado previamente en otros productos cárnicos enriquecidos con AGPI ω-3 [39]. La protección frente a la oxidación proteica en MFO podría estar relacionada con la menor oxidación lipídica en estos nuggets, que podría haber reducido la consiguiente producción de radicales libres que pueden promover la oxidación proteica en los productos cárnicos [40].

En general, el almacenamiento a congelación produjo un incremento en la mayoría de los compuestos volátiles provenientes de oxidación lipídica y degradación de amino ácidos, siendo la excepción el hexano, que disminuyó con el almacenamiento. No obstante, este efecto fue diferente en los distintos tipos de nuggets. En comparación con C, el contenido de hexanal, heptanal, octanal, nonanal, 2,4-decadienal y 2,4-hexadienal fue superior en los nuggets enriquecidos, y sobre todo en el grupo BFO.

En relación a los atributos sensoriales, no hubo diferencias significativas en ningún atributo sensorial como consecuencia del almacenamiento a congelación o la interacción del almacenamiento a congelación y el enriquecimiento en ninguna de las formulaciones de nuggets. Además, no se detectaron aromas ni flavores relacionados con el enriquecimiento con aceite de pescado, ni con la rancidez. Algunos autores han indicado que un panel sensorial entrenado es capaz de detectar aromas relacionados con la oxidación en muestras con un nivel de TBARS entre 1-2 mg malonaldehído/kg de muestra [41]. No obstante, la formulación de los nuggets contenía una preparación comercial con especias, tales como pimienta negra y cúrcuma, lo que podría haber enmascarado aromas o flavores asociados con una ligera oxidación lipídica. En cuanto a los resultados del test hedónico, estuvieron en concordancia con los del análisis cuantitativo-descriptivo, ya que no se detectaron diferencias debidas al almacenamiento a congelación, obteniendo todos los nuggets buenos valores de aceptabilidad.

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6. Conclusiones/Conclusions

CONCLUSIONES

1. El tipo de emulsión (monocapa, multicapa, doble) y la composición (particularmente la concentración de quitosano) de las emulsiones influyen sobre las características físico-químicas y estabilidad oxidativa de las mismas y de sus correspondientes microcápsulas.
2. Las emulsiones multicapa son más estables que las emulsiones dobles y monocapa, presentando también mayor eficiencia de microencapsulación, dando lugar a microcápsulas con mejores características estructurales y con mayor cantidad de ácidos grasos encapsulados.
3. Las microcápsulas elaboradas a partir de emulsiones multicapa previenen la oxidación de los ácidos grasos encapsulados de forma más adecuada que las preparadas a partir de emulsiones monocapa o dobles, tanto a temperatura ambiente como cuando las microcápsulas se almacenan a refrigeración.
4. La microencapsulación mediante spray-drying de emulsiones multicapa de aceite de pescado con lecitina, maltodextrina y quitosano (1 % w/w) es una estrategia adecuada para obtener una fuente estable de EPA y DHA, y por lo tanto susceptible de ser utilizadas para el enriquecimiento de diferentes alimentos.
5. La adición de microcápsulas de aceite de pescado, como fuente de EPA y DHA, permite la producción de nuggets con mayor protección de los AGPI ω-3 y menor oxidación lipídica que cuando se adiciona directamente aceite de pescado a los nuggets de pollo.
6. Las características sensoriales y la aceptabilidad de los nuggets de pollo enriquecidos con AGPI ω-3 no se modifican mediante la adición de microcápsulas, mientras que el enriquecimiento con aceite de pescado sin encapsular influye en algunos atributos sensoriales.
7. El efecto del tipo de enriquecimiento influye sobre algunos de los cambios físico-químicos que ocurren durante el proceso de fritura, de tal manera que la adición de microcápsulas provoca menores pérdidas de agua, menor captación de grasa, menor oxidación lipídica y menos pérdida de AGPI ω-3 que el enriquecimiento con aceite sin microencapsular.

8. El efecto del tiempo de almacenamiento (1-3 meses) a congelación de los nuggets de pollo es diferente según el tipo de enriquecimiento utilizado, y afecta principalmente a los parámetros de oxidación lipídica, que aumentan con el almacenamiento, siendo este aumento mayor cuando se adiciona aceite de pescado sin encapsular que cuando se adicionan microcápsulas de aceite de pescado. Sin embargo, el contenido de ácidos grasos ω-3 y los atributos sensoriales no sufren modificaciones durante el almacenamiento.
9. La adición de microcápsulas de aceite de pescado, procedentes de emulsiones multicapa, a la masa de nuggets de pollo y su posterior pre-fritura, congelación hasta 3 meses y fritura final es una buena estrategia para elaborar productos cárnicos congelados pre-fritos enriquecidos en AGPI ω-3.

CONCLUSIONS

1. The type of emulsion (monolayer, multilayer, multiple) and composition (particularly the concentration of chitosan) of emulsions influence their physicochemical characteristics and oxidative stability and that of their corresponding microcapsules.
2. Multilayer emulsions are more stable than double and monolayer emulsions, having also higher microencapsulation efficiency, and better structural characteristics as well as higher amount of fatty acids encapsulated.
3. Microcapsules prepared from multilayer emulsions prevent the oxidation of encapsulated fatty acids in a more suitable way than those obtained from monolayer or double emulsions, when microcapsules are stored at refrigeration or ambient temperatures.
4. Microencapsulation by spray-drying from multilayer fish oil emulsions with lecithin, maltodextrin and chitosan (1 % w/w) is an appropriate strategy to obtain a stable source of ω-3 PUFA, and so, suitable to be used for enrichment of different types of food.
5. Addition of fish oil microcapsules, as source of EPA and DHA, allows the production of chicken nuggets with greater protection of ω-3 PUFAs and less lipid oxidation than direct addition of fish oil on chicken nuggets.
6. Sensory characteristics and acceptability of the chicken nuggets enriched ω-3 PUFA are not negatively modified by the addition of microcapsules of fish oil, while enrichment with non-encapsulated fish oil affects some sensory attributes.
7. The effect of the type of enrichment influences some of the physicochemical changes that occur during the frying process, so that the addition of microcapsules produces lower water loss, lower fat uptake, lower lipid oxidation and lower loss of ω-3 PUFAs than enrichment with non-encapsulated oil.
8. The effect of frozen storage time (1-3 months) of chicken nuggets is different depending of the type of enrichment used, and mainly affects the parameters of lipid oxidation, which increase with storage, this increase being higher when fish oil is added as non-encapsulated oil than when fish

oil microcapsules are added. However, the content of ω-3 fatty acids and sensory attributes remain with no modifications during storage.

9. The addition of fish oil microcapsules from multilayer emulsions to the batter of chicken nuggets and subsequent pre-frying, frozen storage up to 3 months and final frying is a good strategy to produce frozen pre-fried meat products enriched with ω-3 PUFA.

7. Anexo

Título: Microencapsulated fish oil as strategy for ω-3 enrichment pan-fried meat products: Oxidative status and organoleptic quality
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Congreso: 5th Euro-Global Summit and Expo on Food & Beverages
Lugar de celebración (Ciudad/País): Alicante/España   Año: 2015

Estefania Jimenez-Martin, J Food Process Technol 2015, 6:5
http://dx.doi.org/10.4172/2157-7110.S1.018

5th Euro-Global Summit and Expo on

Food & Beverages

June 16-18, 2015 Alicante, Spain

Microencapsulated fish oil as strategy for ω-3 enrichment pan-fried meat products: Oxidative status and organoleptic quality

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Enrichment of convenience pan-fried meat products with fish oil is a promising strategy to increase the intake of ω-3 fatty acids. Susceptibility to oxidation of ω-3 could influence quality of enriched food. So, the main challenge of enrichment of food in ω-3 is the control of the oxidation from manufacture to domestic cooking. Microencapsulation has been proved to protect ω-3 from oxidation. This work compares the effect of microencapsulated fish oil as method of ω-3 enrichment with the usual technique (direct addition of bulk fish oil) on some oxidative and sensory quality properties in convenience pan-fried meat products. Three batches of chicken nuggets were prepared: control (C), enriched with bulk fish oil (BFO) and enriched with multilayered fish oil microcapsules prepared with lecithin-chitosan emulsions (MFO). Lipid secondary oxidation products (TBARs) were higher in BFO (1.83 mg MDA/kg) than in MFO (0.53 mg MDA/kg) and C (0.28 mg MDA/kg). As for aldehyde volatile compounds selected as oxidation markers, 2, 4-decadienal was only detected in enriched products, with higher amounts in BFO (0.21 x10^7 AU) than in MFO (0.11 x10^7 AU). In addition, butanal was also detected in BFO but not in MFO. Panelists perceived higher juiciness and saltiness and lower meat flavor in BFO than in the other batches. No organoleptic differences were detected between MFO and C. Thus, enrichment of chicken nuggets in ω-3 with fish oil multilayered microcapsules can be successfully achieved maintaining quality characteristics.

Biography

Estefania Jimenez-Martin is a PhD student working at the University of Extremadura, Department of Food Science and Technology. She obtained her Veterinary degree from the University of Extremadura in 2017. Prior to beginning the PhD program, she received her Master's degree in Meat Science and Technology. Her master thesis versed on analyzing the effect of the supplementation of pig diets with conjugated linoleic acid on quality of pork meat. Her current work focuses on the use of microencapsulation by spray-drying as a strategy to protect ω-3 fatty acids in enriched products, preserving nutritional, technological, and organoleptic quality.

Notes:

Título: Estabilidad oxidativa de Nuggets de pollo enriquecidos con ácidos grasos ω-3 microencapsulados
Autor/es: T. Antequera, **E. Jiménez-Martín**, J. Ruiz, T. Pérez-Palacios
Congreso: VIII Congreso CYTA/CESIA
Lugar de celebración (Ciudad/País): Badajoz/España Año: 2015

ESTABILIDAD OXIDATIVA DE NUGGETS DE POLLO ENRIQUECIDOS CON ÁCIDOS GRASOS ω-3 MICROENCAPSULADOS

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El objetivo de este trabajo es analizar y comparar el efecto de la incorporación de ácidos grasos ω -3 sobre la oxidación de productos prefritos congelados (nuggets de pollo): Como fuente de ácidos grasos ω -3 se ha utilizado aceite de pescado sin microencapsular y microcápsulas de aceite de pescado producidas por spray-drying a partir emulsiones multilayer de lecitina-chitosan. La oxidación primaria (dienos conjugados) y secundaria (sustancias reactivas al ácido tiobarbitúrico, TBARs), así como los compuestos volátiles relacionados con la oxidación (hexanal y 2,4-decadienal) fueron menores en el lote con adición de microcápsulas que en el lote con adición de aceite sin microencapsular. La adición de microcápsulas de aceite de pescado a nuggets de pollo constituye una estrategia de enriquecimiento ventajosa con respecto a la adición aceite de pescado sin encapsular, obteniendo un producto cárnico con mayor estabilidad oxidativa.

Palabras clave – microencapsulación, nuggets, omega-3.

I. INTRODUCIÓN

El enriquecimiento en ácidos grasos ω -3 de productos de conveniencia prefritos y congelados constituye una interesante oportunidad de negocio para la industria, debido a la gran demanda y consumo actual de este tipo de alimentos, siendo los nuggets de pollo los más populares. Sin embargo los ácidos grasos ω -3, eicosapentaenoico (EPA) y docosahexaenoico (DHA), tienen una elevada susceptibilidad a la oxidación debido al elevado número de insaturaciones en su molécula [1]. Por lo que el principal reto es controlar la oxidación de EPA y DHA durante todos los pasos de la producción de estos productos cárnicos.
El objetivo de este trabajo fue evaluar la estabilidad oxidativa de nuggets de pollo enriquecidos en ω -3 microencapsulados mediante spray-drying.

II. MATERIALES Y MÉTODOS

Se prepararon 3 lotes de nuggets: un lote control (C), un lote enriquecido con aceite de pescado (A) y un lote enriquecido con microcápsulas producidas a partir de emulsiones multilayer de lecitina-chitosan [2] (M). Se realizó la fritura de todos los nuggets con aceite de girasol.
Se midieron los productos de oxidación primaria (dienos conjugados) [3] y secundaria (sustancias reactivas al ácido tiobarbitúrico o TBARs) [4], y se analizaron el hexanal y 2,4-decadienal como marcadores de oxidación [5].

III. RESULTADOS Y DISCUSIÓN

Se detectó mayor contenido en dienos conjugados en A (0.653 µmol/mg) que en C (0.536 µmol/mg) y M (0.580 µmol/mg) Los productos secundarios de oxidación (TBARs) también fueron significativamente mayores en A (1.83 mg MDA/kg) que en M (0.53 mg MDA/kg) y en C (0.28 mg MDA/kg). Los compuestos volátiles hexanal y 2,4-decadienal fueron seleccionados como marcadores de oxidación, no encontrando diferencias en las áreas de hexanal, mientras que el 2,4-decadienal se detectó sólo en los nuggets enriquecidos, con mayores áreas en A ($0.21x10^7$ UA) que en M ($0.11x10^7$ UA). Estos resultados muestran un mayor estatus oxidativo en A en comparación con M y C.

IV. CONCLUSIÓN

El enriquecimiento de nuggets de pollo con la incorporación de microcápsulas de aceite de pescado preparadas con emulsiones de lecitina-chitosan permite obtener un producto prefrito funcional enriquecido en ω -3 con mayor estabilidad oxidativa durante el proceso de fritura en comparación con la adición directa de aceite de pescado.

AGRADECIMIENTOS

Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) y Fondo Social Europeo por la concesión de la beca pre-doctoral PD10026, financiación de equipos y ayuda a grupos de investigación.

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Título: Microcápsulas de aceite de pescado como estrategia de enriquecimiento de productos pre-fritos en ácidos grasos ω-3

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Congreso: Maratón de Investigación Joven: I Congreso Multidisciplinar de Jóvenes Investigadores Extremeños

Lugar de celebración (Ciudad/País): Cáceres/España Año: 2015

TÍTULO: Microcápsulas de aceite de pescado producidas mediante spray-drying como estrategia de enriquecimiento de productos pre-fritos congelados en ácidos grasos ω-3

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RESUMEN

El enriquecimiento de productos de conveniencia pre-fritos y congelado añadiendo aceite de pescado constituye una interesante estrategia para aumentar la ingesta de ácidos grasos ω-3, debido a la popularidad y gran consumo actual de este tipo de alimentos. De entre ellos, los nuggets de pollo son los más populares, siendo ampliamente comercializados tanto en supermercados como en restaurantes de comida rápida. Sin embargo los ácidos grasos ω -3, eicosapentaenoico (EPA) y docosahexaenoico (DHA), tienen una elevada susceptibilidad a la oxidación debido al elevado número de insaturaciones en su molécula. Esta oxidación puede producir cambios físico-químicos y organolépticos con influencia en la calidad de los productos enriquecidos. Por ello, el principal reto del enriquecimiento de alimentos en ω-3 consiste en controlar la oxidación de EPA y DHA durante todos los pasos de la producción de estos productos cárnicos, desde la elaboración hasta el cocinado doméstico final. La microencapsulación ha demostrado ser una técnica eficaz para la protección de ω-3 frente a la oxidación. El objetivo de este trabajo es comparar la estabilidad oxidativa y la calidad sensorial de nuggets de pollo enriquecidos con microcápsulas de aceite de pescado como método de enriquecimiento en ω-3 con la técnica tradicional (adición directa de aceite de pescado). Se elaboraron 3 lotes de nuggets de pollo: control (C), enriquecido con aceite de pescado (AP) y enriquecido con microcápsulas de aceite de pescado (MAP). Estas microcápsulas fueron producidas mediante spray-drying a partir de emulsiones de lecitina-quitosán preparadas utilizando la técnica "layer by layer". Se analizaron los productos secundarios de oxidación lipídica (sustancias reactivas al ácido tiobarbitúrico o TBARs) siendo más elevados en AP (1.83 mg MDA/kg) que en MAP (0.53 mg MDA/kg) y en C (0.28 mg MDA/kg). Se analizaron los perfiles de compuestos volátiles, seleccionando como marcadores de oxidación algunos aldehídos volátiles. En cuanto al 2,4-decadienal, solo se detectó en productos enriquecidos, con mayores cantidades en AP (0.21 x107 AU) que en MAP (0.11 x107 AU). Además, también se detectó butanal en AP pero no en MAP. Los catadores percibieron mayor jugosidad y sabor salado y menor flavor a carne en AP en comparación con los otros dos lotes de nuggets. No se detectaron diferencias organolépticas entre MAP y C. El uso de microcápsulas "multilayered" de aceite de pescado en nuggets de pollo como estrategia de enriquecimiento en ω-3 permite mantener la estabilidad oxidativa y las características de calidad.

Currículum vitae

Estefanía Jiménez-Martín es Técnico de Apoyo a la Investigación y Estudiante de Doctorado del Departamento de Producción Animal y Ciencia de los Alimentos de la Universidad de Extremadura. Obtuvo la Licenciatura en Veterinaria en la Universidad de Extremadura en 2010. Previamente al inicio del programa de Doctorado, realizó un Máster en Ciencia y Tecnología de la Carne en esta misma Universidad. Su Trabajo Fin de Máster consistió en un estudio del efecto de la suplementación de dietas porcinas en ácido linoleico conjugado sobre parámetros de calidad de la carne de cerdo. Su Tesis Doctoral se centra en el uso de la microencapsulación mediante spray-drying como estrategia para la protección de ácidos grasos ω-3 en productos enriquecidos, preservando la calidad nutricional, tecnológica y organoléptica. Actualmente compagina la finalización de su tesis con el trabajo como Técnico de Apoyo a un proyecto de Investigación que estudia el uso de frutos de la dehesa extremeña como fuente de antioxidantes naturales para mejorar la vida comercial de la carne y productos cárnicos.

Título: Physicochemical and oxidative properties of chicken nuggets enriched with omega-3 fatty acids: comparison of the addition of bulk fish oil and spray dried-microencapsulated fish oil
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Congreso: 12th Euro Fed Lipid Congress
Lugar de celebración (Ciudad/País): Montpellier/Francia Año: 2014

Posters, Last Minute
LAMI-047

Physicochemical and Oxidative Properties of Chicken Nuggets Enriched with Omega-3 Fatty Acids: Comparison of the Addition of Bulk Fish Oil and Spray Dried-microencapsulated Fish Oil

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The aim of this work was to analyze and compare the effect of the incorporation of bulk fish oil and spray dried-microencapsulated fish oil as a source of omega-3 fatty acids on some physicochemical and oxidative properties of chicken nuggets. Three batches of nuggets were prepared: a control batch (C), a batch enriched with bulk fish oil (BFO) and a batch enriched with microencapsulated (multilayered microcapsules prepared with lecithin-chitosan emulsions) fish oil (MFO), all of them being deep-fried in sunflower oil. Moisture content, water activity, CIELAB color, lipid content, conjugated dienes, TBARs and volatile aldehydes were determined. Moisture content and water activity were lower in MFO (54.1% moisture and 0.86 a_w) than in C and BFO (both with 57.9% moisture and 0.92 a_w). Color measurement revealed differences between batches, the MFO nuggets showing lower lightness (L*) and higher redness (a*) and yellowness (b*). No differences were detected in lipid content between the different types of nuggets (11.3-12.2%). Lower conjugated dienes were detected in C (0.536 µmol/mg sample) than in both types of enriched nuggets, the values for MFO being lower (0.580 µmol/mg sample) than for BFO (0.653 µmol/mg sample). Lipid secondary oxidation products (TBARs) were significantly higher for BFO (1.83 mg MDA/kg) than for MFO (0.53 mg MDA/kg), which showed the same low oxidative status than C (0.28 mg MDA/kg). Hexanal and 2,4-decadienal were selected as volatile oxidation markers; no differences were found in hexanal areas, while 2,4-decadienal was only detected in the fish oil enriched nuggets, with higher areas in BFO ($0.21x10^7$ AU) than in MFO ($0.11x10^7$ AU). Multilayered microcapsules prepared with lecithin-chitosan emulsions provide a protective effect against lipid oxidation of fish oil during the frying process. Thus, the enrichment of chicken nuggets in fish oil with the incorporation of this type of microcapsules can be successfully achieved, obtaining a product with a good stability and oxidative characteristics.

Título: Microencapsulation of Omega-3 Fatty Acids from Monolayered and Multilayered Fish Oil Emulsions by Spray-Drying.
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Congreso: 19th International Symposium on Microencapsulation
Lugar de celebración (Ciudad/País): Pamplona/España Año: 2013

P92. Microencapsulation of Omega-3 fatty acids from monolayered and multilayered fish oil emulsions by spray-drying

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It is well known that long chain omega-3 fatty acids (ω-3), such as eicosapentaenoic and docosahexaenoic acids, have beneficial effects on human health[1]. However, except for some fatty fish, their content is scarce in most foods. Thus, addition of ω-3 to different food in order to increase their intake is becoming a common strategy. However, the high susceptibility to oxidation of ω-3 constitutes an important drawback for this practise. Alternatively, various ω-3 microencapsulation systems have been tested. This work aims to optimize the microencapsulation of ω-3 from different fish oil emulsions by spray drying and to study the oxidative stability of the obtained microcapsules. First, two type of emulsions, monolayered (Mo) and multilayered (Mu)[2] (containing 2.5% fish oil), were tested and the optimal concentrations of soybean lecithin and chitosan, which were used as wall materials, and maltodextrin, the drying matrix, were established for each type of emulsion, by means of particle size, stability, viscosity and pH. After microencapsulation, yield, efficiency and moisture of the microcapsules from Mo and Mu emulsion were calculated. In addition, the susceptibility to oxidation of Mo and Mu microcapsules in comparison to Mo and Mu emulsions and fish oil were evaluated by an assay of accelerated oxidation at different temperatures (4, 30 and 60° C) and times (0, 5 and 12 days).

Optimal concentrations of soybean lecithin, chitosan and maltodextrin were 0.75, 0.00 and 15.00 %, respectively for Mo emulsions, and 0.75, 0.25 and 15.00 %, respectively for Mu emulsion. Microcapsules from Mo and Mu emulsions did not show significant differences for yield (46.84 and 39.76 %, respectively), efficiency (49.09 and 40.09 %, respectively) or moisture (4.75 and 4.17 %, respectively). Mo and Mu microcapsules showed lower oxidative levels than the respective emulsions and the fish oil at 60° C for 12 days. In conclusion, this study shows the suitability of microencapsulating ω-3 by spray drying using both monolayered and multilayered fish oil emulsions, and demonstrates the protection effect of the microcapsules against lipid oxidation. Thus, the microencapsulation from fish emulsions by spray drying may constitute an appropriate tool for enriching food with ω-3 avoiding lipid oxidation problems.

[1]Shaw et al. (2007). J Agric. Food Chem. **55**(8): 3112-3119.

[2]Gharsallaoui et al. (2012). Food Chem. **132**: 1713–1720.

Título: Optimización de la microencapsulación de aceite de pescado como fuente de ácidos grasos omega-3.
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Congreso: III Jornadas Veterinarias para Estudiantes y II Jornadas de Ciencias de la Salud
Lugar de celebración (Ciudad/País): Cáceres/España Año: 2013

III Jornadas Veterinarias para Estudiantes y II Jornadas de Ciencias de la Salud
Cáceres, 11-13 Abril 2013

TA01. Optimización de la Microencapsulación de Aceite de Pescado como Fuente de Ácidos Grasos Omega-3

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Los ácidos grasos omega-3 (ω-3), y especialmente los de cadena larga como lo son el ácido eicosapentaenoico y el ácido docosahexaenoico, son conocidos por sus efectos beneficiosos sobre la salud humana. Sin embargo, a excepción de algunos pescados grasos, su contenido es escaso en la mayoría de los alimentos. Por ello, la adición de ω-3 a distintos alimentos para producir su enriquecimiento es una práctica muy común. Sin embargo, la elevada susceptibilidad a la oxidación de los ω-3 constituye un importante inconveniente para esta práctica. En los últimos años se han utilizado varios sistemas de microencapsulación como método para proteger los ω-3 de la oxidación. El presente trabajo tiene como objetivo optimizar la microencapsulación de ω-3 a partir de diferentes emulsiones de aceite de pescado mediante atomización (spray-drying) y estudiar la estabilidad oxidativa de las microcápsulas obtenidas. En primer lugar, se optimizaron las concentraciones de lecitina de soja y chitosán (materiales de pared capsular), y maltodextrina (matriz de secado), en función de la estabilidad, viscosidad y pH de las emulsiones. Tras la microencapsulación, se determinaron el rendimiento y la eficiencia de la microencapsulación y la humedad de las microcápsulas. Finalmente, se realizó un ensayo de oxidación acelerado a distintas temperaturas (4, 30 y 60ºC) y tiempos (0, 5 y 12 días). Las concentraciones óptimas de lecitina de soja, chitosán y maltodextrina fueron de 0,75, 0,00 y 15,00 %, respectivamente. El rendimiento de la microencapsulación (46,84 %), la eficiencia (49,09 %) y la humedad (4,75 %) fueron similares a los determinados por otros autores en microcápsulas similares. Se detectó menor nivel oxidativo en las microcápsulas en comparación con las emulsiones respectivas y el aceite de pescado a 60ºC tras 12 días. En conclusión, la microencapsulación mediante spray-drying puede constituir una buena estrategia para el enriquecimiento de alimentos en ω-3 evitando problemas de oxidación de lípidica.

E. Jiménez-Martín agradece al Gobierno de Extremadura (Consejería de Empleo, Empresa e Innovación) el Fondo Social Europeo su beca predoctoral (PD10026).

Exposición: ■ Oral □ Póster