FUNCIONALIDAD DE HIDROCOLOIDES EN LA DIGESTIBILIDAD DE EMULSIONES ACEITE/AGUA

TESIS DOCTORAL

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HACEN CONSTAR QUE:

el trabajo de investigación titulado "**Funcionalidad de hidrocoloides en la digestibilidad de emulsiones aceite/agua**" que presenta Dña. María Espert Tortajada por la Universidad de Valencia, ha sido realizado bajo nuestra dirección en el Departamento de Ciencias de los Alimentos del Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), y que reúne las condiciones para optar al grado de Doctor.

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RESUMEN

La presente tesis doctoral se centra en el estudio de las propiedades físicas y la digestibilidad lipídica de emulsiones alimentarias basadas en hidrocoloides, para valorar su aplicación como sustitutos de grasa en la reformulación de un producto final más saludable.

La obtención de alimentos más saludables se puede conseguir a través de diferentes vías; o bien disminuyendo la cantidad de grasa presente, o bien disminuyendo la digestibilidad de la grasa, considerándose también un aspecto clave la mejora del perfil lipídico. Teniendo estos aspectos en cuenta, se diseñaron y estudiaron emulsiones formuladas con diferentes tipos de grasa estabilizadas por diferentes tipos de matrices poliméricas, con la finalidad de evaluar sus propiedades estructurales, su digestibilidad y su funcionalidad en la aplicación en un producto final, en este caso cremas de relleno.

Se diseñaron emulsiones semisólidas aceite vegetal/agua estabilizadas con hidrocoloides de distinta naturaleza (éteres de celulosa y goma xantana). La caracterización reológica de las emulsiones mostró diferentes patrones de comportamiento dependiendo del tipo de hidrocoloide empleado. Este comportamiento estructural se relacionó con la resistencia a la digestión, es decir, con la digestibilidad de la grasa presente. La grasa liberada de la matriz tras la digestión es un indicador de la resistencia estructural; cuanto más resistente sea la estructura después de la digestión, mayor será la barrera que ejercerá contra un contacto físico apropiado entre el lípido emulsionado y las enzimas digestivas y, por tanto, menor será la generación de ácidos grasos libres. Comparando con una emulsión control

sin hidrocoloide, todos los hidrocoloides fueron efectivos en la reducción de la digestibilidad lipídica, aunque se encontraron diferencias según su composición. En el caso de la goma xantana, la reducción en la digestibilidad lipídica se potenció en presencia del emulsionante Tween 80.

Para valorar el efecto de la fase grasa en la emulsión y con la finalidad de aumentar la plasticidad de las emulsiones se elaboraron emulsiones con grasa con un mayor contenido en ácidos grasos saturados, sólida a temperatura ambiente, en concreto grasa de leche anhidra. Si bien estas emulsiones no suponen una mejora en cuanto al perfil lipídico de las grasas que contienen, sí que constituyen una ventaja por presentar un contenido de grasa (47%) notablemente inferior al que presenta la mantequilla o los shortening convencionales (80%). Se demostró que es posible formular emulsiones de éteres de celulosa con grasa sólida con características untables y reducido contenido en grasa. La calidad de la textura dependió del tipo de éter de celulosa empleado, que afectó la morfología de los cristales de grasa y, en consecuencia, las propiedades estructurales y térmicas. La estructura compacta original de la grasa se mejoró por la presencia de los hidrocoloides, y se demostró que estas emulsiones permitían obtener un producto estable y plástico reducido en grasa, más blando que la mantequilla y untable a temperatura de refrigeración y temperatura ambiente, pudiéndose emplear como un producto directamente untable o en aplicaciones alimentarias que requieren propiedades plásticas.

Para evaluar la funcionalidad de las emulsiones de baja digestibilidad en una matriz alimentaria, estas se aplicaron como sustituto de grasa en una crema de relleno. Se diseñaron cremas elaboradas con leche en polvo, agua,

azúcar, almidón y emulsiones formuladas con diferentes tipos de grasa (aceite de girasol y aceite de palma) y diferentes hidrocoloides (metilcelulosa, hidroxipropilmetilcelulosa y goma xantana). Se estudió el efecto de las distintas emulsiones en las propiedades estructurales y la digestibilidad del producto final. La digestión oral produjo una reducción de la consistencia en todas las muestras, asociada a la hidrólisis del almidón llevada a cabo por la α-amilasa presente en la saliva. Esta disminución fue mucho más pronunciada en la crema control sin hidrocoloide, ya que, en las cremas con emulsión, las redes de los hidrocoloides no se vieron afectadas por el efecto enzimático y continuaron proporcionando estabilidad al sistema. Tras la fase gástrica, en las cremas formuladas con las emulsiones de éteres de celulosa se observó una disminución de la consistencia, debida principalmente al nivel de dilución del sistema y no a las condiciones gástricas (pH ácido o presencia de enzimas). Sin embargo, en la crema con goma xantana, las condiciones la fase gástrica generaron, al igual que ocurría en la emulsión aislada, un aumento de la consistencia. La etapa de digestión intestinal afectó a la estructura de todos los sistemas, aunque de forma más notable en la crema control, en la cual la grasa fue digerida casi de forma total por la acción de la lipasa pancreática. La digestibilidad lipídica fue menor en las cremas con emulsiones basadas en hidrocoloides en comparación con la crema control. Como en el caso de las emulsiones, en las cremas la presencia del hidrocoloide actúa como una barrera física que confiere estabilidad estructural y dificulta la acción efectiva de las enzimas.

Adicionalmente, se realizó un estudio termorreológico de las distintas cremas para evaluar su aplicación industrial. Los resultados mostraron diferencias importantes en la viscosidad y en las propiedades viscoelásticas

a temperatura ambiente. La crema con emulsión elaborada con una metilcelulosa de alto grado de metoxilación (MX) fue diferente al resto de las cremas de celulosa, con un comportamiento similar al de la crema de control basada en almidón. Estas diferencias detectadas en la consistencia inicial se redujeron significativamente a velocidades de untado (velocidades de cizalla más altas). La evolución del módulo de almacenamiento (G') con la temperatura mostró diferentes temperaturas de gelificación dependiendo de la sustitución química de los éteres de celulosa. A 45 °C las cremas con metilcelulosa A4M y MX se encuentran gelificadas, mientras que las cremas con hidroxipropilmetilcelulosa gelificaron a mayor temperatura. A 70 °C todas las cremas con emulsión se encuentran gelificadas y presentaron características análogas tanto en el flujo como en el comportamiento viscoelástico. Por tanto, la sustitución química de los éteres de celulosa y la temperatura son factores clave a considerar para llevar a cabo la aplicación de las cremas estudiadas como alternativa a las grasas convencionales, teniendo en cuenta las características deseadas y el proceso de aplicación.

Finalmente se evaluó la aceptabilidad y los atributos sensoriales que mejor describían las cremas con cacao con las emulsiones formuladas con todos los hidrocoloides estudiados (metilcelulosas (MX y A4M), hidroxipropilmetilcelulosas (F4M y K4M) y goma xantana. Se evaluó la percepción del consumidor mediante un test de aceptabilidad en el que los catadores calificaron las muestras de acuerdo a la aceptabilidad general, la apariencia, el color, el sabor y la textura. La crema control fue la muestra que presentó las mejores puntuaciones, aunque la crema con emulsión de goma xantana obtuvo puntuaciones similares en apariencia, color y textura. Para determinar los atributos sensoriales que mejor se relacionaban con la

aceptabilidad, se realizó un cuestionario CATA (Marque todo lo que corresponda, “Check-All-That Apply”). Los consumidores marcaron los atributos que consideraron apropiados para describir cada muestra y se determinó la frecuencia de mención de cada uno de ellos. La crema control fue la que más gustó a los consumidores, aunque la crema formulada con la emulsión de goma xantana presentó una aceptabilidad cercana a esta. Ambas presentaron las mejores cualidades organolépticas, como el sabor dulce y la apariencia cremosa y suave. Aunque la crema con MX presentó atributos como el sabor dulce, también se consideró demasiado brillante y con textura grumosa. Por otro lado, las cremas elaboradas con A4M y K4M se caracterizaron por su textura grumosa y apariencia gomosa, y su sabor fue evaluado negativamente por el panel.

RESUM

La present tesi doctoral se centra en l'estudi de les propietats físiques i la digestibilitat lipídica d'emulsions alimentàries basades en hidrocol·loides, per a valorar-ne l'aplicació com a substituts de greix en la reformulació d'un producte final més saludable.

L'obtenció d'aliments més saludables es pot aconseguir a través de diferents vies; o bé disminuint la quantitat de greix present, o bé disminuint la digestibilitat del greix, considerant-se també un aspecte clau la millora del perfil lipídic. Tenint en compte aquests aspectes, es van dissenyar i es van estudiar emulsions formulades amb diferents tipus de greix estabilitzades per diferents tipus de matrius polimèriques, amb la finalitat d'avaluar les propietats estructurals, la seua digestibilitat i la seua funcionalitat en l'aplicació en un producte final, en aquest cas, cremes de farcit.

Es van dissenyar emulsions semisòlides d'oli vegetal/aigua estabilitzades amb hidrocol·loides de diferent naturalesa (èters de cel·lulosa i goma xantana). La caracterització reològica de les emulsions va mostrar diferents patrons de comportament depenent del tipus d'hidrocol·loide emprat. Aquest comportament estructural es va relacionar amb la resistència a la digestió, és a dir, amb la digestibilitat del greix present. El greix alliberat de la matriu després de la digestió és un indicador de la resistència estructural; com més resistent siga l'estructura després de la digestió, major serà la barrera que exercirà contra un contacte físic apropiat entre el lípid emulsionat i els enzims digestius i, per tant, menor serà la generació d'àcids grassos lliures. Si comparem amb una emulsió control sense

hidrocol·loide, tots els hidrocol·loides van ser efectius en la reducció de la digestibilitat lipídica, tot i que es van trobar diferències segons la seua composició. En el cas de la goma xantana, la reducció en la digestibilitat lipídica es va potenciar en presència de l'emulsionant Tween 80®.

Per a valorar l'efecte de la fase greixosa en l'emulsió i amb la finalitat d'augmentar la plasticitat de les emulsions, es van elaborar emulsions amb greixos amb un major contingut en àcids grassos saturats, que són sòlids a temperatura ambient, en concret greix de llet anhidra i greix de palma. Si bé aquestes emulsions no suposen una millora pel que fa al perfil lipídic dels greixos que utilitzen, sí que constitueixen un avantatge per presentar un contingut de greix (47%), que és notablement inferior al que presenta la mantega o els shortening convencionals (80%). Es va demostrar que és possible formular emulsions d'èters de cel·lulosa amb greix sòlid amb característiques untables i reduït contingut en greix. La qualitat de la textura va dependre del greix utilitzat, però també del tipus d'èter de cel·lulosa que va afectar la morfologia dels cristalls de greix i, en conseqüència les propietats estructurals i tèrmiques. L'estructura compacta original del greix es va millorar per la presència dels hidrocol·loides, i es va demostrar que aquestes emulsions permetien obtenir un producte estable i plàstic reduït en greix, més bla que la mantega i untuós a temperatura de refrigeració i temperatura ambient, podent-se emprar com un producte directament untable o en aplicacions alimentàries que requereixen propietats plàstiques.

Per a avaluar la funcionalitat de les emulsions de baixa digestibilitat estudiades en una matriu alimentària, es van aplicar com a substitut de greix en una crema de farcit. Es van dissenyar cremes elaborades amb llet en pols, aigua, sucre, midó i emulsions formulades amb diferents tipus de greix (oli de gira-sol i oli de palma) i diferents hidrocol·loides

(metilcel·lulosa, hidroxipropilmetilcel·lulosa i goma xantana). Es va estudiar l'efecte de les diferents emulsions en les propietats estructurals i la digestibilitat del producte final. La digestió oral va produir una reducció de la consistència en totes les mostres, associada a la hidròlisi del midó duta a terme per la α-amilasa present en la saliva. Aquesta disminució va ser molt més pronunciada en la crema control sense hidrocol·loide, ja que en les cremes amb emulsió, les xarxes dels hidrocol·loides no es van veure afectades per l'efecte enzimàtic i continuaren proporcionant estabilitat al sistema. Després de la fase gàstrica, en les cremes formulades amb les emulsions d'èters de cel·lulosa es va observar una disminució de la consistència, deguda principalment al nivell de dilució del sistema i no a les condicions gàstriques (pH àcid o presència d'enzims). No obstant això, en la crema amb goma xantana, les condicions gàstriques van generar, igual que ocorria en l'emulsió aïllada, un augment de la consistència. L'etapa de digestió intestinal va afectar l'estructura de tots els sistemes, encara que de forma més notable en la crema control, en la qual el greix va ser digerit quasi de forma total per l'acció de la lipasa pancreàtica. La digestibilitat lipídica va ser menor en les cremes amb emulsions basades en hidrocol·loides en comparació a la crema control. Com en el cas de les emulsions, la presència de l'hidrocol·loide en les cremes actua com una barrera física que confereix estabilitat estructural i dificulta l'acció efectiva dels enzims.

Per a avaluar l'aplicació industrial, es va fer un estudi termorreològic de les diferents cremes. Els resultats van mostrar diferències importants en la viscositat i en les propietats viscoelàstiques a temperatura ambient. La crema amb emulsió elaborada amb una metilcel·lulosa d'alt grau de metoxilació (MX) va ser diferent a la resta de les cremes de cel·lulosa, amb

un comportament similar al de la crema de control basada en midó. Aquestes diferències, detectades en la consistència inicial, es van reduir significativament a velocitats d'untabilitat (velocitats de cisalla més altes). L'evolució del mòdul d'emmagatzematge (G') amb la temperatura va mostrar diferents temperatures de gelificació depenent de la substitució química dels èters de cel·lulosa. A 45 °C les cremes amb metilcel·lulosa A4M i MX es troben gelificades, mentre que les cremes amb hidroxipropilmetilcel·lulosa van gelificar a major temperatura. A 70 °C totes les cremes amb emulsió es troben gelificades i van presentar característiques anàlogues tant en el flux com en el comportament viscoelàstic. Per tant, la substitució química dels èters de cel·lulosa i la temperatura són factors clau a considerar per a dur a terme l'aplicació de les cremes estudiades com a alternativa als greixos convencionals, tenint en compte les característiques desitjades i el procés d'aplicació.

Finalment es va avaluar l'acceptabilitat i els atributs sensorials que millor descrivien les cremes amb cacau amb les emulsions formulades amb tots els hidrocol·loides estudiats (metilcel·luloses (MX i A4M), hidroxipropilmetilcel·luloses (F4M i K4M) i goma xantana). Es va avaluar la percepció del consumidor mitjançant un test d'acceptabilitat en el qual els tastadors van qualificar les mostres d'acord a l'acceptabilitat general, l'aparença, el color, el sabor i la textura. La crema control va ser la mostra que va presentar les millors puntuacions, encara que la crema amb emulsió de goma xantana va obtenir puntuacions similars en aparença, color i textura. Per a determinar els atributs sensorials que millor es relacionaven amb l'acceptabilitat, es va realitzar un qüestionari CATA (Marque tot el que corresponga, "Check-All-That Apply"), Els consumidors van marcar els atributs que van considerar apropiats per a descriure cada mostra i es va determinar la freqüència de menció de cadascun. La crema control va ser la

que més va agradar als consumidors, tot i que la crema formulada amb l'emulsió de goma xantana va presentar una acceptabilitat pròxima a aquesta. Ambdues van presentar les millors qualitats organolèptiques, com el sabor dolç i l'aparença cremosa i suau. Encara que la crema amb MX va presentar atributs com el sabor dolç, també es va considerar massa brillant i amb textura grumosa. D'altra banda, las cremes elaborades amb A4M i K4M es van caracteritzar per la textura grumosa i aparença gomosa, i el seu sabor va ser avaluat negativament pel panel.

ABSTRACT

This doctoral thesis focuses on the study of the physical properties and fat digestibility of hydrocolloid based emulsions to assess their application as fat substitutes in the reformulation of a healthier foodstuffs.

Obtaining healthier foods can be achieved through different ways: by decreasing the amount of fat present or by decreasing the digestibility of this, although a lipid profile improvement is also considered as a key factor. Taking this into consideration, emulsions formulated with different types of fat stabilized by different types of polymer matrices were designed, in order to evaluate the structural properties, digestibility and the functionality in their application in a final product, in particular filling creams.

Semisolid vegetable oil in water emulsions stabilized with different types of hydrocolloids (cellulose ethers and xanthan gum) were designed. The rheological characterization of the emulsions showed different behaviour depending on the type of hydrocolloid used. This structural behavior was related to the resistance to digestion, that is, to the digestibility of the fat present. The fat released from the matrix after digestion is an indicator of structural resistance. More resistance will indicate a greater barrier exerted against a physical contact between the emulsified lipid and the digestive enzymes and, therefore, the generation of free fatty acids will be lower. Comparing with a control emulsion without hydrocolloid, all hydrocolloids were effective in reducing lipid digestibility, although differences were found according to their composition. In the case of xanthan gum, the reduction in lipid digestibility was enhanced with the presence of Tween 80 emulsifier.

To evaluate the effect of the fat phase in the emulsion and in order to increase the plasticity, emulsions were prepared using fats with a higher content of saturated fatty acids, which are solid at room temperature, in particular anhydrous milk fat. Although these emulsions do not improve the lipid profile, they are an advantage because they have a fat content (47%), which is significantly lower than butter or conventional shortenings (80 %). It was demonstrated that it is possible to formulate cellulose ether emulsions with solid fat with spreadable characteristics and reduced fat content. The texture quality depended on the fat used, but also on the type of cellulose ether, that affected the morphology of the fat crystals and, consequently, the structural and thermal properties. The original compact structure of the fat was improved by the presence of hydrocolloids, and it was demonstrated that these emulsions allowed to obtain a stable product and plastic reduced in fat, softer than butter and spreadable at refrigeration temperature and room temperature. These emulsions could be used as a directly spreadable product or in food applications that require plastic properties.

To evaluate the functionality of the low digestibility emulsions in a food matrix, these were applied as fat substitute in a filling cream. The creams were formulated with milk powder, water, sugar, starch and emulsions formulated with different types of fat (sunflower oil and palm oil) and different hydrocolloids (methylcellulose, hydroxypropylmethylcellulose and xanthan gum). The effect of the different emulsions on the structural properties and the digestibility of the final product was studied. The oral digestion produced a reduction of the consistency in all the samples, associated to the hydrolysis of the starch carried out by the α-amylase present in the saliva. This decrease was much more pronounced in the control cream without hydrocolloid, since in the creams with emulsion the

networks of the hydrocolloids were not affected by the enzymatic effect and these continued to provide stability to the system. After the gastric phase, creams formulated with cellulose ether emulsions showed a decrease in consistency, mainly due to the dilution level of the system and not to gastric conditions (acid pH or presence of enzymes). However, in the cream with xanthan gum, the gastric conditions generated an increase in the consistency, as occurs with the isolated emulsion. The stage of intestinal digestion affected the structure of all the systems, although more notably in the control cream, in which the fat was digested almost completely by the action of the pancreatic lipase. As in emulsions, in creams the presence of the hydrocolloid acts as a physical barrier that confers structural stability and hinders the effective action of enzymes.

To evaluate the industrial application, a thermoreological study of the different creams was carried out. The results showed important differences in viscosity and viscoelastic properties at room temperature. The cream with emulsion elaborated with a methylcellulose of high grade of methoxylation (MX) was different from the rest of the cellulose creams, with a behavior similar to the control cream based on starch. These differences detected in the initial consistency were significantly reduced at spreading speeds (higher shear rates). The evolution of the storage modulus (G') with the temperature showed different gelation temperatures depending on the chemical substitution of the cellulose ethers. At 45 °C the creams with methylcellulose A4M and MX are gelled, while the creams with hydroxypropyl methylcellulose gelled at a higher temperature. At 70 °C all the creams with emulsion are gelled and showed analogous characteristics both in flow and viscoelastic behavior. Therefore, the chemical substitution of cellulose ethers and temperature are key factors to consider in order to

carry out the application of the creams studied as an alternative to conventional fats, taking into account the desired characteristics and the application process.

Finally, the acceptability and sensorial attributes that described the cocoa creams with the emulsions formulated with all the hydrocolloids studied (methylcelluloses (MX and A4M), hydroxypropylmethylcelluloses (F4M and K4M) and xanthan gum were evaluated. The consumer's perception was evaluated through an acceptance test in which the tasters rated the samples according to general acceptability, appearance, color, taste and texture. The control cream was the sample that presented the best scores, although the emulsion cream of xanthan gum obtained similar scores in appearance, color and texture. To determine the sensory attributes that were best related to acceptability, a CATA questionnaire was carried out ("Check-All-That Apply"). Consumers marked the attributes that they considered appropriate to describe each sample and the frequency of mention of each of them were determined. The control cream was the one that most liked the consumers, although the cream formulated with the emulsion of xanthan gum showed an acceptability close to it. Both presented the best organoleptic qualities, such as sweet taste and creamy and smooth appearance. Although the MX cream presented attributes such as sweet taste, it was also considered too bright and with lumpy texture. On the other hand, creams made with A4M and K4M were characterized by their lumpy texture and gummy appearance, and their taste was evaluated negatively by the tasting panel.

INDICE

Introducción

1. GENERALIDADES DE LAS GRASAS

1.1 Consumo de grasas

El consumo excesivo de alimentos ricos en grasas en países desarrollados se ha relacionado con una mayor incidencia de enfermedades crónicas como la obesidad, las enfermedades coronarias, la diabetes, la hipertensión y el cáncer (Ballesteros-Vásquez, Valenzuela-Calvillo, Artalejo-Ochoa, & Robles-Sardin, 2012; Mozaffarian, 2009; Chajes, 2008). La Organización Mundial de la Salud (OMS) afirmó en 2015 que el 39% de la población mundial tenía sobrepeso y un 13% era obesa; además, las enfermedades cardiovasculares (ECV) fueron la causa principal de mortalidad por enfermedades no transmisibles (ENT) en 2016 (WHO, 2018). Es por ello que organizaciones internacionales como la OMS establecen unas recomendaciones respecto a la ingesta de grasa total, enfatizando en la reducción del consumo de grasas saturadas (AGS) y la eliminación de las grasas *trans* (AGT) (WHO, 2003; FAO/WHO ,2010; USDHHS y USDA, 2015), ya que numerosos estudios han mostrado que este tipo de ácidos grasos tienen un efecto adverso en la salud, incluso mayor al producido por las grasas saturadas (Ballesteros-Vásquez, Valenzuela-Calvillo, Artalejo-Ochoa, & Robles-Sardin, 2012). En consecuencia, existe la necesidad de desarrollar estrategias efectivas para la reformulación de alimentos reducidos en grasa y con perfil lipídico mejorado, manteniendo al mismo tiempo unas propiedades fisicoquímicas y sensoriales similares a las de su homólogo tradicional. Al elegir estos alimentos alternativos más saludables, los consumidores pueden cumplir más fácilmente con los patrones dietéticos y mantener una dieta baja en grasa.

Sin embargo, la reducción del contenido en grasa plantea diversos problemas, ya que los lípidos son responsables de gran parte de las características tecnológicas y sensoriales de los alimentos (Drewnowski, 1992).

1.2 Funcionalidad de las grasas

Las grasas son una forma concentrada de energía, ya que aportan 9 kcal/g, es decir, más del doble de la que aportan los hidratos de carbono y las proteínas. Mejoran la apariencia, la textura, el sabor y la palatabilidad de las matrices alimentarias (Drewnowski, 1992; Grigelmo-Miguel et al., 2001; Zoulias et al., 2002), además de contribuir a la retención de agua en los alimentos, al aumento de su volumen y al incremento de la saciedad durante la ingesta. Son también vehículo de moléculas aromáticas liposolubles que actúan como precursoras del desarrollo de aromas y sabores. Así pues, juegan un papel importante en las propiedades fisicoquímicas, la percepción sensorial, el valor nutricional y la respuesta biológica en muchos productos alimenticios (McClements, 2015).

Se pueden diferenciar dos tipos de grasa en función de su estado físico: las grasas plásticas (como por ejemplo la mantequilla), que tienen mayor proporción de ácidos grasos saturados (AGS) y son sólidas a temperatura ambiente; y los aceites, que tienen un alto contenido de ácidos grasos monoinsaturados y poliinsaturados (AGMI y AGPI) y son líquidos a temperatura ambiente. En función de la insaturación, las grasas pueden ser más o menos estables. Así pues, las grasas más saturadas presentan mayor resistencia a la oxidación que los aceites insaturados.

En la preparación de cierto tipo de productos como, por ejemplo, los de repostería, la plasticidad de la grasa puede considerarse un aspecto esencial,

es por eso que las grasas sólidas comúnmente empleadas en la industria alimentaria son la mantequilla y los "shortenings" industriales.

La mayor parte de los aceites vegetales no se pueden emplear directamente en alimentos que requieran una textura sólida o semisólida, a menos que se sean transformados a grasas más sólidas mediante procesos de saturación como la hidrogenación o la transesterificación, para obtener grasas con el punto de fusión y plasticidad deseados. Durante el proceso de hidrogenación de las grasas, se adiciona hidrógeno a los aceites vegetales. En estas condiciones, los dobles enlaces experimentan modificaciones estructurales; así, los AGMI y AGPI se transforman en AGS. La grasa que resulta es más sólida y con mayor vida útil, a diferencia de los aceites líquidos insaturados. Si la hidrogenación es parcial, durante este proceso se forma una nueva categoría de AG, los AG *trans* (AGT). En ocasiones se llegan a generar hasta un 40-60% de éstos, como en el caso de la producción de shortenings industriales (Ovesen, Leth, y Hansen, 1998; Stender, Dyerberg y Astrup, 2006).

Se han intentado diversas estrategias para reducir la proporción de grasa en los alimentos y/o reemplazar los aceites hidrogenados o grasas saturadas por grasas más saludables. Sin embargo, no es una tarea fácil, ya que muchas formulaciones requieren cierto porcentaje de sólidos en la grasa empleada para obtener las propiedades tecnológicas y sensoriales deseadas. Las cualidades relacionadas con la grasa disminuyen una vez que esta se reduce o se elimina del alimento original. Dependiendo del producto, esta reducción puede afectar aumentando o disminuyendo la viscosidad, la dureza, disminuyendo la estabilidad, alterando la percepción en boca, e incluso modificando el color (Peng y Yao, 2017; Drewnowski, 1992). Así pues, el reto científico-técnico que surge en la actualidad es rediseñar alimentos nutritivos y saludables sin descuidar las propiedades estructurales y

organolépticas, con el objetivo de obtener un alto nivel de aceptación del consumidor.

2. SUSTITUTOS DE GRASA

En las últimas décadas se han desarrollado diversos tipos de ingredientes y estrategias tecnológicas que simulan las características de la grasa para obtener alimentos reducidos en grasa y/o con un perfil lipídico mejorado (Akoh, 1998). Los sustitutos de grasa son ingredientes que imitan una o más de las funciones sensoriales y físicas de la grasa en los alimentos, y que aportan a su vez menos calorías que esta. Se pueden clasificar como sustitutos de la grasa y miméticos de la grasa, de acuerdo con su naturaleza química y sus funcionalidades. Los sustitutos de grasa normalmente tienen una estructura similar a los triacilglicéridos y se utilizan para sustituir grasas de forma individualizada, mientras que los miméticos de grasas muestran funcionalidades comparables a las de las grasas y no se usan necesariamente de forma aislada. En general, los sustitutos de grasa basados en lípidos son sustitutos de grasa, y los sustitutos de grasa basados en carbohidratos o proteínas son miméticos de grasas (Peng y Yao, 2017). Aunque las proteínas y los hidratos de carbono no pueden imitar las grasas a nivel molecular, son macromoléculas que proporcionan unas propiedades fisicoquímicas y sensoriales características, como el aporte de viscosidad, que puede dar lugar a un producto bajo en grasa con propiedades comparables a las de un producto rico en grasa.

Cada categoría de sustituto de grasa puede proporcionar unas cualidades diferentes, y pueden ser incorporados solos o en combinación. La elección del tipo de sustituto de grasa dependerá de la composición química de este y de la matriz alimentaria (Lucca & Tepper, 1994).

2.1 Sustitutos de grasa basados en hidratos de carbono

A diferencia de las opciones limitadas disponibles para los sustitutos de grasas basadas en lípidos y proteínas, los sustitutos basados en carbohidratos constituyen una gran familia de polisacáridos (Tabla 1). Se obtienen a partir de productos de origen vegetal e incluyen hidratos de carbono digeribles o fibra soluble (gomas, pectinas, almidones) y no digeribles o fibra insoluble (hemicelulosa, celulosa, lignina) (Phillips y Williams, 2009; Pasquel, 2001; Escudero Álvarez y González Sánchez, 2006). Estos polisacáridos y sus derivados químicos se utilizan principalmente por su capacidad de retener agua, actuar como estabilizadores, espesantes y proporcionar propiedades sensoriales que los hacen adecuados como sustitutos de la grasa (Saha y Bhattacharya, 2010). Además, su uso proporciona efectos beneficiosos a nivel fisiológico en el tracto intestinal y en el metabolismo de carbohidratos y lípidos (Gidley, 2013; Stark y Madar, 1994).

Tabla 1. Sustitutos de grasa basados en carbohidratos y sus funcionalidades (adaptación de Peng y Yao, 2017).

Tipos de sustituto de grasa		Energía (Kcal/g)	Aplicaciones	Funcionalidades
Almidones (tapioca, amaranto, trigo, avena, maíz, arroz, patata, guisante)	Nativos Modificados	1-4	Productos horneados, margarinas, glaseados, rellenos, productos lácteos para untar, aderezos para ensaladas, emulsiones, salsas	Gelificante, mejora de la capacidad de retención de agua, engrosamiento, modificación de la textura, estabilizante
Maltodextrina (de maíz, trigo, tapioca, patata)		4	Aderezos para ensaladas, cremas, untables, glaseados, rellenos, mantecas, productos horneados, salsas, postres congelados, carne procesada	Aporte de viscosidad y unión/control del agua, lo que contribuye a la suavidad de la sensación en la boca
Polidextrosa (sintetizada)		1	Productos horneados, goma de mascar, dulces, aderezos para ensaladas, productos congelados, productos gelificantes, untables, salsas	Mejora la suavidad y la sensación en la boca relacionada con la grasa, retiene la humedad, modifica la textura y actúa como agente de carga

Goma	Guar (semillas leguminosas)	0	Productos horneados	Retención de humedad, retardo del enranciamiento
	De algarrobo			
	Xantana (*Xanthomonas campestris*)			
	Carragenano (alga marina roja)		Aderezos para ensaladas	Aumento de la viscosidad, mejorando la textura y la sensación en boca
	Pectinas (pulpa de fruta y cáscara, pulpa de remolacha azucarera)		Salsas	Espesante, proporcionando textura y sensación en boca
Fibra	β-glucano (de avena y cebada)	1-4	Productos horneados y otros alimentos	Aporta cuerpo y textura
	Inulina (alcachofas y tubérculos de achicoria)	1.5	Postres helados, productos de panadería y confitería	Aporta cuerpo, consistencia y sensación en boca

	Fibra de cereal (cáscara y salvado de la planta; por ejemplo, trigo, avena, soja, guisantes, maíz y arroz)		0	Queso, hamburguesas, productos horneados	Aporta consistencia, suavidad y sensación en la boca, proporcionando humedad
	Derivados celulosa (plantas)	Celulosa microcristalina y celulosa en polvo		Productos lácteos, postres congelados, salsas, productos fritos y horneados, y aderezos para ensaladas	Emulsiones y espumas estabilizadoras, control de la sinéresis, aporte de consistencia y sensación en boca; aporte de brillo y opacidad a los alimentos
		Metilcelulosa (MC), carboximetil-celulosa (CMC) e hidroxipropil-metilcelulosa (HPMC)		Mezcla de salsas secas, postres congelados, salsas, productos horneados, aderezos para ensaladas	Mejora el vertido y las cualidades de "cuchara". Imparte cremosidad y lubricidad, retención de aire y humedad

2.2 Desarrollo de sistemas sustitutos de grasa saturada

El uso de grasa insaturada en la reformulación de un alimento permite una mejora en el perfil lipídico del producto final, pero el bajo porcentaje de ácidos grasos saturados en su composición no puede lograr la estructura semisólida asociada a la grasa convencional. Una forma de obtener las características deseables es la incorporación de esta grasa líquida en una emulsión aceite-agua.

Una emulsión es un sistema formado por dos líquidos inmiscibles entre sí (normalmente agua y aceite) con uno de los líquidos disperso en forma de pequeñas gotas esféricas en el otro. Constituye una fase dispersa o interna (líquido en forma de pequeñas gotas) contenida en una fase continua o externa (que rodea las gotículas formando glóbulos) (McClements, 1999). Según la distribución de las dos fases se pueden clasificar en:

- Emulsión aceite en agua (oil-in-water (O/W)), en el que las gotas de grasa están dispersas en la fase acuosa (como la leche o la mayonesa).
- Emulsión agua en aceite (water-in-oil ó W/O)), en el que las gotas de agua se dispersan en una fase oleosa (como la mantequilla o la margarina).
- Emulsión múltiple o polifásica (O/W/O ó W/O/W), en la que la fase dispersa (interna) contiene otra fase dispersa en ella.

El proceso por el que dos líquidos pasan a formar una emulsión se denomina homogenización, que se suele dividir en dos fases para lograr una mayor estabilidad. En la primera fase se formaría la emulsión inicial, y en la segunda se reduciría el tamaño de las partículas. En general, las emulsiones son

sistemas termodinámicamente inestables que pueden sufrir una separación de fases a través de diversos mecanismos (floculación, separación gravitacional, coalescencia y maduración de Ostwald) (Piorkowski & McClements, 2013). Sin embargo, es posible formar emulsiones cinéticamente más estables mediante la adición de un hidrocoloide antes de la homogenización (McClements, 1999).

El término "hidrocoloide" se usa comúnmente para describir una gama de polisacáridos y proteínas solubles en agua que son ampliamente utilizados actualmente en la industria para realizar una serie de funciones, entre las que se incluyen el aumento de la consistencia y la gelificación de soluciones acuosas, espumas, emulsiones y dispersiones, la inhibición de la formación de hielo y de cristales de azúcar o la liberación controlada de sabores (Phillips y Williams, 2009).

La mayoría de los hidrocoloides pueden actuar como agentes estabilizantes de emulsiones de aceite en agua, pero solo unos pocos pueden actuar como agentes emulsionantes (Dickinson, 2009). Los emulsionantes son moléculas anfifílicas (tienen regiones polares y apolares) que se adsorben en la superficie de las gotas formando una membrana que previene la agregación de estas. No obstante, algunos de los polisacáridos sin capacidad emulsionante, al ser modificados física, química o enzimáticamente, pueden derivar en sustancias que sí sean eficaces como emulsionantes (Piorkowski y McClements, 2013).

Los emulsionantes polisacarídicos más ampliamente utilizados en aplicaciones alimentarias son goma arábiga, almidones modificados, celulosas modificadas, algunos tipos de pectina y algunos galactomananos (Dickinson, 2003; Garti y Reichman, 1993). Otros polisacáridos, considerados únicamente espesantes, aumentan la viscosidad de la fase continua de la emulsión al retardar el movimiento de las gotas, consiguiendo

así una mayor estabilidad. Así pues, todos los mencionados estarían incluidos en el término "estabilizante". La función de estas moléculas es actuar como agente de estructuración, espesamiento o gelificación en medio acuoso, modificando la estructura de la fase continua. Así pues, puede obtenerse un alimento sólido/semisólido empleando una pequeña proporción de aceite, atrapado en una red de gel.
Generalmente se emplean polisacáridos o proteínas en emulsiones aceite-agua, o cristales de grasa en emulsiones agua-aceite (Mc Clements, 2015).

Hasta ahora se han explorado muchos enfoques diferentes de estructuración del aceite, que pueden impartir propiedades sólidas similares a las de la grasa convencional.
El uso de emulsiones compuestas por aceite de girasol o de oliva y agua estabilizadas por un éter de celulosa ha sido reconocido como una buena estrategia para reemplazar las grasas saturadas en una formulación de galletas con un perfil lipídico más saludable (sin grasas *trans* y bajo en grasas saturadas), manteniendo unas propiedades reológicas y una aceptación sensorial similares al producto convencional, y permitiendo una reducción de grasa total del 33% (Sanz, Salvador, Fiszman y Laguna, 2011; Tarancón, Salvador, Sanz, 2013; Tarancón, Fiszman, Salvador, y Tárrega, 2013).
Otros mecanismos estructurantes alternativos que han recibido atención en los últimos años incluyen la ingeniería de cristales de grasa, los sistemas basados en fitosteroles, los organogeles (oleogeles e hidrogeles) y la aplicación de ceras para formar estructuras "lipo-coloidales" (Wasell et al, 2010; Rogers, 2009).
En el caso de la gelificación con aceite, aunque se han identificado varios estructurantes, todavía existe la necesidad de encontrar un oleogelificante de calidad alimentaria que sea económico, eficiente a baja concentración,

tolerante a las condiciones de procesamiento y compatible con la matriz del producto (Patel y Dewettinck, 2016).

Entre los oleogelificantes de grado alimenticio explorados hasta ahora, las ceras naturales se acercan más a las características deseadas. Sin embargo, dado que solo están aprobados como aditivos indirectos, existen problemas regulatorios que deben abordarse. Además, debido a la susceptibilidad de las ceras a los cambios post-cristalización (agregación cristalina), los oleogeles a base de cera pueden no ser estables durante períodos largos de almacenamiento. Polímeros como derivados de la celulosa, algunas proteínas y otros polisacáridos hidrófilos también podrían considerarse estructurantes ideales; sin embargo, pasos previos de procesamiento requeridos para la oleogelación, como tratamiento a alta temperatura, liofilización, eliminación de solventes, etc., hacen que los oleogeles poliméricos sean menos factibles. Así pues, la gelificación de aceites vegetales mediante el uso de biopolímeros supone actualmente un reto para la industria, debido a la elevada hidrofilia de estos aditivos y la insolubilidad en aceites o disolventes apolares que presentan. Hasta el momento, la etilcelulosa ha sido el único el único polímero eficaz para conseguir este propósito (Gravelle, Barbut y Marangoni, 2012).

A diferencia de los oleogeles, los sistemas bifásicos estructurados (emulsiones) ofrecen más flexibilidad en términos de la elección de los aditivos aprobados (hidrocoloides) que pueden ser empleados. Además de disminuir el contenido de grasa saturada, estos sistemas también contribuyen a la reducción general de grasa (calorías) (Patel & Dewettinck, 2016).

3. EMULSIONES NO DIGERIBLES

Además de ofrecer interesantes posibilidades en cuanto a la reducción de grasa y calorías, las emulsiones tienen capacidad para atrapar ciertas sustancias y/o producir también una liberación controlada de estas, mejorando la estabilidad y eficacia de los ingredientes funcionales. Este hecho ha sido de gran valor para su aplicación en farmacología, cosmética o alimentos funcionales. Así, componentes como minerales, fibras, ácidos grasos o vitaminas, pueden ser incorporados en una emulsión para desarrollar productos con efectos beneficiosos (Jimenez-Colmenero, 2013). Determinar el comportamiento y la resistencia de estas sustancias en el tracto gastrointestinal permitirá el diseño de alimentos con propiedades funcionales, como por ejemplo la reducción de la biodisponibilidad de la grasa o el incremento de la saciedad.

3.1 Digestibilidad de emulsiones

Después de la ingestión, los lípidos emulsionados experimentan una compleja serie de cambios físicos y químicos a medida que pasan a través de la boca, el estómago, el intestino delgado y el intestino grueso, que afectan a su capacidad para ser digeridos y/o absorbidos. La composición, estructura y propiedades de las gotas de una emulsión pueden verse afectadas durante los procesos de masticación, digestión y absorción, debido a las fuerzas mecánicas, a la presencia de enzimas y sales biliares, y a cambios de pH y/o temperatura (Figura 1) (McClements & Li, 2010; Mc Clements, 2015b). Así pues, la estructura de las emulsiones y su estabilidad desempeñan un papel importante en la digestión y absorción de los lípidos (Golding y Wooster, 2010).

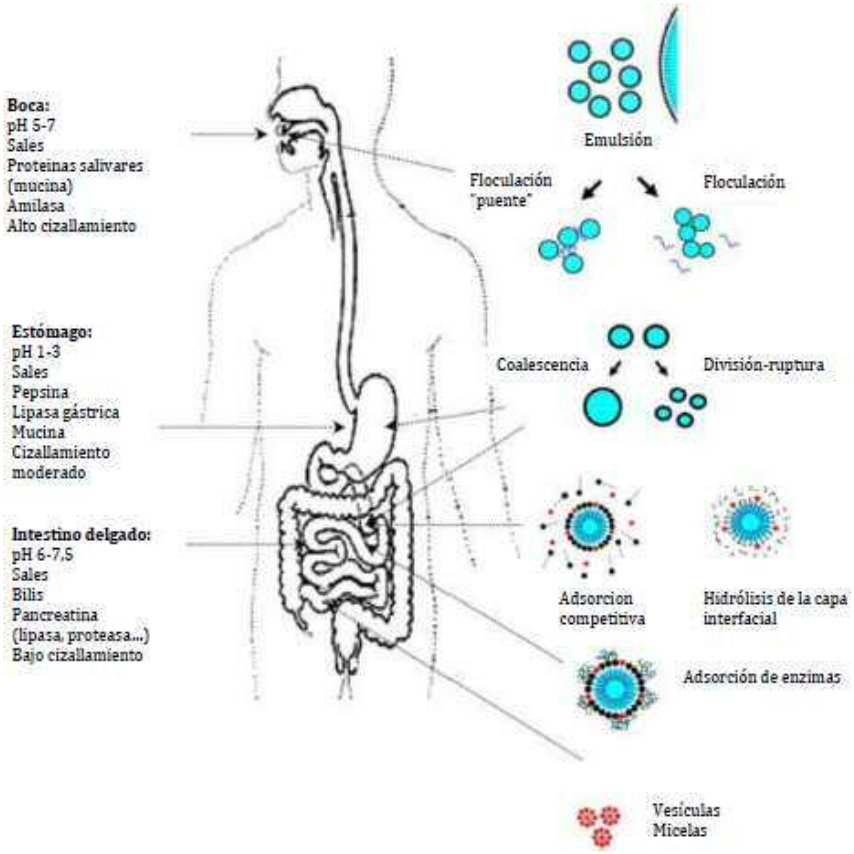

Figura 1. Representación de los posibles cambios en las emulsiones a medida que son digeridas (Singh, Ye y Horne, 2009).

Tras la ingestión se produce la digestión oral, con un tiempo relativamente corto (de 5 a 20 segundos) (Sanz y Luyten, 2007). Los glóbulos de grasa de la emulsión juegan un papel importante en esta etapa de la digestión, ya que contribuyen a la lubricación, el recubrimiento oral, la percepción de textura y la liberación de sabor (Chung, Smith, Degner y McClements, 2016; McClements, 2015a). Aunque en esta fase apenas se produce digestión lipídica (McClements, Decker y Park, 2009), la emulsión puede sufrir

cambios en su estructura debido a la acción de la α-amilasa, la mucina y las fuerzas mecánicas durante la masticación (Mao y Miao, 2015).

Durante la digestión en el estómago, las emulsiones se someten a diversos cambios estructurales en función de su composición. Tras la etapa oral, el bolo puede permanecer en el estómago durante un período que varía desde unos pocos minutos hasta unas pocas horas, dependiendo de la cantidad y la estructura física de éste. En este transcurso de tiempo, el bolo es expuesto a fluidos gástricos ácidos (pH 1-3) que contienen enzimas proteolíticas y lipolíticas, así como a los movimientos peristálticos.

El alimento parcialmente digerido ("quimo") accede al intestino delgado, donde el pH asciende hasta un valor de 7. Los fluidos intestinales contienen enzimas digestivas (lipasas, proteasas, amilasa) y surfactantes biológicos (sales biliares y fosfolípidos) que degradan todavía más los nutrientes en una forma que puede ser fácilmente absorbida por el organismo. Por lo tanto, estos tendrán una gran influencia sobre la estructura de la emulsión y la digestión de la grasa contenida en ella. En la etapa intestinal es en la que se lleva a cabo la mayor parte digestión lipídica (70-90%) (Armand et al., 1996), debido a la acción de la lipasa pancreática. Para que esta enzima pueda ejercer su acción se debe adsorber previamente a la superficie de las gotas de grasa; es por ello que las características de la interfaz entre la grasa y la fase continua acuosa son determinantes en la digestión lipídica.

No menos importante es desatacar que los diferentes componentes de un alimento ingerido provocan diferentes respuestas biológicas que regulan el hambre, la saciedad o el placer. Alterando el tipo o la concentración de

alguno de sus componentes mayoritarios, como la grasa, estas respuestas fisiológicas se verán modificadas (McClements, 2015b).

Los ensayos de digestión *in vitro* simulan las condiciones fisiológicas de la digestión *in vivo* y son herramientas útiles para estudiar y comprender los cambios y las interacciones de nutrientes, fármacos y otros compuestos, así como la bioaccesibilidad de éstos. La técnica es ampliamente utilizada en campos como la nutrición, la farmacología y la química de los alimentos. A diferencia de los ensayos en humanos, los métodos *in vitro* tienen la ventaja de ser más rápidos, menos costosos y sin restricciones éticas.

Estos métodos de digestión suelen incluir la fase oral, gástrica y del intestino delgado, e intentan imitar las condiciones fisiológicas *in vivo*, teniendo en cuenta la presencia de enzimas digestivas y sus concentraciones, el pH, el tiempo de digestión y las concentraciones de electrolitos, entre otros factores. Aunque existen algunos modelos más precisos y sofisticados como los dinámicos, la mayoría de los modelos reportados en la literatura son estáticos, con proporciones constantes de enzimas, electrolitos y sales biliares en cada etapa de la digestión. Estos modelos se han utilizado para abordar aspectos como la digestibilidad y la bioaccesibilidad (es decir, la cantidad de un compuesto que se libera de una matriz y se considera que está disponible para la absorción a través de la pared intestinal) de muchos compuestos (Minekus et al., 2014).

A lo largo del tiempo ha proliferado una gran multitud de metodología de digestión *in vitro*. Recientemente se ha establecido un protocolo conjunto de condiciones estándar para ser utilizado en un modelo de digestión *in vitro* estático, armonizando así los procedimientos a seguir, los parámetros clave y las condiciones específicas para estudiar un sustrato en concreto y para medir un valor específico (Minekus et al., 2014; Dupont et al., 2011).

Aunque los experimentos *in vitro* proporcionan información útil sobre los posibles mecanismos fisicoquímicos que ocurren durante la ingestión, digestión y absorción de lípidos, el sistema digestivo humano es extremadamente complejo y, por lo tanto, es difícil de imitar por completo en el laboratorio. En consecuencia, los estudios *in vivo* con animales y/o humanos son necesarios para comprender mejor las bases fisicoquímicas y fisiológicas de la digestión y absorción de los lípidos. No obstante, existen muchas limitaciones asociadas con la realización de estudios en humanos, como las regulaciones estrictas, los altos costes, las variaciones entre sujetos y las dificultades para controlar la dieta y el estilo de vida durante la fase de estudio. Por lo tanto, los animales son los que se usan comúnmente para estudiar los factores que influyen en la digestión y absorción de los componentes de la dieta. Sin embargo, el tracto digestivo de los animales a menudo es apreciablemente diferente del tracto digestivo humano, por lo que los estudios en animales solo pueden proporcionar información limitada (McClements, Decker y Park, 2008).

Estudios demuestran que un modelo de digestión *in vitro* es una herramienta predictiva útil para los estudios de alimentación *in vivo* (Li, Kim, Park y McClements, 2012). Dado que es extremadamente difícil imitar con precisión los complejos procesos fisiológicos del tracto digestivo humano, generalmente es recomendable combinar estudios *in vitro* con estudios *in vivo* usando animales y humanos (cuando sea posible). Además, es importante correlacionar la información obtenida mediante las dos metodologías empleadas, para garantizar la efectividad del método *in vitro*. Por ejemplo, se podría medir la tasa de la digestión lipídica usando un método *in vitro* y correlacionarla con los resultados de un método *in vivo* (por ejemplo, estudios de alimentación en humanos) (McClements y Li, 2010). Esto permitiría el desarrollo de estrategias efectivas para el diseño

de alimentos que pueden aumentar o disminuir la biodisponibilidad de los alimentos.

3.2. Bioaccesibilidad de las grasas

El término bioaccesibilidad ha sido definido como la fracción de los componentes lipófilos que se libera de la matriz alimentaria en los jugos del tracto gastrointestinal, e influirá directamente en la fracción lipídica que terminará en la circulación sistémica (biodisponibilidad). En consecuencia, es importante medir la concentración de un componente lipídico tras la digestión. Uno de los parámetros más importantes para medir la bioaccesibilidad en un modelo de digestión *in vitro* es la velocidad y el grado de digestión de los lípidos sometidos a la acción de la lipasa pancreática, es decir, la conversión de triacilgliceroles y diacilgliceroles en monoacilgliceroles y ácidos grasos libres. Uno de los métodos más comúnmente utilizados para medir la digestión de los lípidos es la determinación de la cantidad de ácidos grasos libres producidos durante la digestión intestinal mediante una titulación con una solución alcalina (McClements y Li, 2010; Mun, Decker y McClements, 2007).

Muchos trabajos realizados para la determinación de la digestibilidad de la grasa simulan sólo la etapa intestinal, sin tener en cuenta la fase gástrica y la fase oral. En consecuencia, puede haber diferencias apreciables en el comportamiento de las emulsiones, ya que durante la etapa gástrica se producen importantes cambios que influirán en su posterior digestión y absorción en el intestino delgado. Para diseñar alimentos con emulsiones a medida capaces de controlar/resistir el proceso de la digestión, es necesario conocer la influencia de todas las etapas de la digestión sobre la estructura

del sistema. Por lo tanto, sería útil utilizar un modelo de digestión *in vitro* completo para poder imitar realmente el proceso digestivo.

3.3. Funcionalidad de los hidrocoloides en la digestibilidad de las emulsiones

El diseño de emulsiones con el objetivo de inhibir o reducir la digestión de las grasas se está considerando como una estrategia efectiva de reducir el aporte de grasa en la dieta, de controlar la saciedad y de promover un aporte energético más estable. No obstante, si bien existen estudios en los que se diseñan emulsiones capaces de influir en la digestión de las grasas, éstos están centrados en emulsiones modelo. Sin embargo, el estudio de la digestibilidad de emulsiones en matrices complejas, como son los alimentos, es un tema escasamente estudiado.

La presencia de partículas no grasas y el tipo y cantidad de grasa en una emulsión juegan un papel importante en las propiedades fisicoquímicas, sensoriales y nutricionales de los alimentos, determinando la textura, sensación en la boca, la estabilidad del sabor, la apariencia y la respuesta biológica en productos basados en emulsiones (Chung, Smith, Degner, y McClements, 2016). Además del uso de aceites vegetales estructurados para mejorar las propiedades físicas de la grasa, también sería interesante que la estructura de la emulsión estabilizada pudiera resistir durante el proceso de digestión, lo que influiría en la digestibilidad lipídica.

Las propiedades iniciales de las emulsiones de aceite-agua influyen directamente en el grado de digestión de los lípidos presentes (Mun et al., 2006, 2007). Se considera que el principal factor que controla la tasa de lipólisis es el área superficial de la gota, disponible para la adsorción de la

lipasa pancreática, que se rige por la inestabilidad de la emulsión (Golding et al., 2011). El tipo de grasa presente también es determinante en su grado de digestibilidad. Bonnaire y col. (2008) demostraron que la velocidad de conversión de triglicéridos a ácidos grasos libres fue mayor en una emulsión con aceite líquido que con gotas sólidas. Otra variable que también influye en la lipólisis es el tamaño inicial de las partículas de la matriz; los glóbulos de grasa pequeños, pero cubiertos con agentes tensioactivos en un medio viscoso, son hidrolizados de forma más lenta que los glóbulos de grasa grandes cubiertos con surfactantes más débilmente unidos al glóbulo y en un medio menos viscoso (McClements y Decker, 2009). Seimon et al. (2009) concluyeron que el efecto de las emulsiones de grasa sobre la motilidad gastrointestinal, la liberación de hormonas, el apetito y la ingesta de energía están relacionados con el tamaño de sus gotas.

Si la capa interfacial que rodea las gotas no es suficientemente fuerte, estas pueden coalescer en el tracto grastrointestinal, lo que conduciría a un aumento del tamaño de partícula (van Aken et al., 2005). Estos procesos de desestabilización se podrían modular mediante la adición de sustancias que modifiquen las propiedades interfaciales, como los emulsionantes. La incorporación de emulsionantes con alta capacidad surfactante dificulta que otras sustancias con propiedades superficiales presentes en la digestión (bilis, lipasa pancreática) se adhieran a las gotas de grasa, reduciéndose así su digestión; por lo tanto, la presencia de hidrocoloides en la fase continua de la emulsión podría ejercer una influencia determinante en la lipólisis de las grasas.

Las condiciones ácidas del estómago pueden causar la desestabilización de fases en algunas emulsiones. Las estabilizadas por proteínas tienden a flocular durante esta etapa de la digestión (Sarkar et al., 2009); las

estabilizadas por surfactantes no iónicos tienden a permanecer estables (Marciani et al., 2006, 2007); y las estabilizadas con surfactantes iónicos pueden sufrir coalescencia (Golding et al., 2011). Keogh et al. (2011) demostraron que la coalescencia de una emulsión en el estómago deriva en una disminución en la absorción de los triglicéridos

La adición de polisacáridos a emulsiones estabilizadas por proteínas aumenta la estabilidad física de las emulsiones en condiciones gástricas (Xu et al., 2014). Una amplia variedad de hidrocoloides se han relacionado con un aumento de la saciedad, como consecuencia de su espesamiento, gelificación y/o propiedades no digeribles. Este potencial se ha encontrado en polisacáridos tales como pectinas, goma guar, goma arábiga o β-glucanos. Estas fibras solubles reducen la emulsificación de los lípidos, proporcionando una disminución de la lipólisis (Pasquier et al., 1996). Además, influyen notablemente en las concentraciones de colesterol en sangre, en la respuesta glucémica y en las respuestas hormonales intestinales a corto plazo, modificando la respuesta postprandial (Dikeman y Fahey, 2006; Juvonen et al., 2009; Mudgil y Barak, 2013; Zhang and Vardhanabhuti, 2014).

En emulsiones aceite/agua el estabilizante presente en la fase continua constituye la primera barrera que entrará en contacto con los fluidos intestinales, por lo que se espera que ejerza un papel determinante en el control de la digestión. Se ha demostrado que la incorporación de hidrocoloides en la fase continua de emulsiones es una herramienta efectiva para reducir la lipólisis grasa (Beysseriat, Decker, & McClements, 2006; Gidley, 2013; Pasquier et al., 1996; Qin, Yang, Gao, Yao y McClements, 2017). Sin embargo, existe información contradictoria sobre la efectividad de los hidrocoloides para este fin.

Malinauskyte et al. (2014) estudiaron el impacto de la carboximetilcelulosa (CMC) en la digestión de los lípidos y en las propiedades fisicoquímicas de las emulsiones estabilizadas con proteína de suero durante la digestión. La red espesa formada por la CMC en la fase continua limita la interacción de las gotas de grasa con los fluidos intestinales, lo que ralentiza la velocidad de la digestión de los lípidos. También se encontró efectividad en la metilcelulosa, el quitosano y la pectina (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, y McClements, 2014), así como en gelatina, caseína coloidal y almidón (Wooster et al., 2014).

Se ha estudiado la capacidad de los éteres de celulosa para competir por la interfaz aceite/agua con las sales biliares; estos se unen a las sales biliares, disminuyendo así la digestión y posterior absorción de los lípidos (Torcello-Gómez, 2014). Helgason y col. (2008) evidenciaron la eficacia del quitosano en la reducción de la digestibilidad de la grasa de una emulsión sometida a la digestión, evidenciando factores como la formación de agregados insolubles de esta emulsión en el intestino. La unión del hidrocoloide a las sales biliares se traduce en una reducción de la reabsorción de estas a través de la circulación enterohepática, lo que producirá una reducción del colesterol plasmático (Zarras y Vogl, 1999).

Qiu, Zhao, Decker y Mc Clements (2015) estudiaron la influencia de la goma de xantana y la pectina en la digestibilidad de los lípidos de las emulsiones de aceite de pescado estabilizadas por proteínas de trigo. En este caso, sorprendentemente, los polisacáridos promovieron el proceso de digestión de los lípidos. Este hecho se asoció a su capacidad de alterar el estado de agregación de las gotas de aceite, aumentando así la cantidad de fase lipídica expuesta a la lipasa.

En definitiva, se puede concluir que la presencia de hidrocoloides ejerce una influencia determinante en la digestión de los lípidos, por distintas vías de actuación. No obstante, a pesar de los estudios existentes, no existe una tendencia clara en la digestibilidad lipídica y su relación con el tipo de hidrocoloide presente en la emulsión, así como el tipo de aceite empleado. Por tanto, se necesita más investigación en este sentido. Además, los estudios existentes se centran en emulsiones modelo, cuyas características no se asemejan a sistemas reales, es decir, emulsiones de consistencia semisólida de consumo habitual que presenten un importante contenido graso (entre el 30 y el 80%), como mayonesas, salsas, o cremas de relleno. Los cambios estructurales en estas matrices más complejas en las que se producen interacciones entre todos sus componentes son determinantes en el grado de digestibilidad lipídica y las propiedades físicas.

4. CARACTERIZACIÓN DE LA ESTRUCTURA DE LAS EMULSIONES

Las propiedades fisicoquímicas de las emulsiones (propiedades ópticas, reología o estabilidad) dependen en gran medida de las propiedades fisicoquímicas de las diferentes fases (aceite, agua y la fase interfacial), así como de las interacciones que se producen entre ellas (Mc Clements, 2016). En los últimos años se han puesto a disposición muchas técnicas analíticas mejoradas para determinar las propiedades moleculares, interfaciales, coloidales, fisicoquímicas y biológicas de las emulsiones. Estas técnicas proporcionan también información sobre los cambios estructurales que se producen durante el proceso de la digestión. Su aplicación ha llevado a considerables avances en investigación, al desarrollo de nuevos productos y al control de calidad dentro de la industria alimentaria.

En términos alimentarios, la reología es el estudio de la deformación y el flujo de las materias primas, los productos intermedios y los productos finales de la industria alimentaria. Los productos alimenticios son un material estructurado muy complejo que se compone de agua, proteínas, carbohidratos, grasas y una importante cantidad de fibras. Todos estos constituyentes influyen significativamente en el flujo y el comportamiento estructural de los alimentos y, por lo tanto, en sus propiedades reológicas (Ahmed, Ptaszek, & Basu, 2016).

En concreto, las propiedades reológicas aportan información sobre la estabilidad física de las emulsiones. Los parámetros que determinan la reología de las emulsiones son: (i) la reología de fase continua; (ii) la naturaleza de las partículas, tales como su distribución de tamaño, deformación, viscosidad interna y concentración; y (iii) la naturaleza de las interacciones partícula-partícula (Barnes, 1994). Las características reológicas de una emulsión se pueden modular dependiendo del polisacárido empleado como estabilizante de fases. Estos modificadores de la reología pueden controlar la consistencia de las emulsiones y pueden adsorberse en la interfase, creando una barrera electrostática o estérica entre las gotas (Vianna-Filho, Petkowicz y Silveira, 2013).

Existen diversos métodos instrumentales para determinar las propiedades reológicas de las emulsiones alimentarias (McClements, 2007; Tadros, 2004). Estos métodos varían de acuerdo con el tipo de muestras que pueden analizar (líquidos, sólidos, materiales viscoelásticos); el tipo de deformación que aplican a la muestra (cizalla, compresión o una combinación de ambas); y la propiedad que miden (viscosidad, módulo elástico). Los instrumentos más comúnmente utilizados para caracterizar las propiedades reológicas de las emulsiones alimentarias son dispositivos de cizallamiento (reómetros de cizallamiento dinámico) para analizar emulsiones fluidas y viscoelásticas; y

dispositivos de compresión para ensayar emulsiones plásticas o de tipo sólido. La reología de las emulsiones de fluidos se puede caracterizar en términos de la dependencia de su viscosidad aparente con el esfuerzo de cizalla. La reología de las emulsiones viscoelásticas y sólidas se puede caracterizar en términos de módulos dinámicos (G' y G") (McClements, 2007). Los resultados pueden tratarse utilizando modelos matemáticos apropiados.

Muchas de las propiedades más importantes de los productos alimenticios basados en emulsiones están determinadas por el tamaño de las gotas que contienen; por ejemplo, la vida útil, la apariencia, la textura, las propiedades de liberación, el perfil de sabor o la estabilidad (McClements, 2015). Se sabe que el tamaño de partícula es una variable muy importante en las propiedades reológicas de una emulsión, y dependerá del tipo y nivel de estabilizante utilizado y también del tipo y nivel de agitación al que sea sometida la emulsión (Barnes, 1994).
Existe una amplia variedad de instrumentos analíticos que se suelen emplear para medir la distribución del tamaño de partícula de las emulsiones, proporcionando mediciones rápidas y precisas. Estos difieren considerablemente de acuerdo con los principios físicos sobre los que operan, por ejemplo, dispersión de luz, velocidad de partículas en un campo, dispersión o absorción de ondas ultrasónicas, conteo de partículas, medición de difusión molecular restringida, etc. (McClements, 2007).

Por otro lado, la microscopía nos proporciona información sobre sistemas estructuralmente complejos en forma de "imágenes", que son relativamente fáciles de observar a través del ojo humano. Esta técnica permite estudiar aspectos como el tamaño de las gotas, flóculos, cristales de grasa o presencia

de aire, por ejemplo, en emulsiones alimentarias. Se han desarrollado diversos sistemas para llevar a cabo estas observaciones, pero la microscopía óptica y la electrónica son las más empleadas actualmente (McClements, 2007).

En microscopía óptica, el contraste natural entre los principales componentes de las emulsiones alimentarias suele ser bastante pobre, ya que los índices de refracción son bastante similares; esto hace que sea difícil distinguirlos de manera fiable utilizando técnicas convencionales. Por este motivo, se han desarrollado diversos enfoques para mejorar el contraste entre los diferentes componentes con el fin de mejorar la calidad general de la imagen y proporcionar información sobre la estructura de cada componente. Existen varios tipos de tintes que se unen a componentes particulares dentro de una emulsión (por ejemplo, a proteínas, polisacáridos o lípidos), o que se dividen preferentemente en fase oleosa o acuosa (Murphy, 2001), para poder estudiar las características morfológicas y estructurales mediante la observación de los diferentes colores. Sin embargo, estos tintes deben usarse con precaución porque su aplicación puede alterar las estructuras; además, a menudo son difíciles de incorporar en emulsiones semisólidas.

Uno de los avances más importantes en las técnicas de microscopía en los últimos años es la microscopía confocal de barrido láser (LSCM). Esta técnica permite obtener imágenes tridimensionales de muestras que se pueden usar para determinar la ubicación espacial de las gotas en una emulsión, obteniendo una información detallada sobre las microestructuras de glóbulos. Además, se pueden utilizar sondas fluorescentes para marcar moléculas o fases específicas.

Existe también la posibilidad de observar el contraste entre los diferentes componentes de la muestra sin utilizar tintes, utilizando la microscopía de

contraste de fase o contraste diferencial (DIC) (Murphy, 2001). Esta técnica es particularmente útil para controlar las transiciones de fase de la grasa y para determinar la morfología de los cristales de grasa en las gotas de la emulsión.

Uno de los principales inconvenientes de las técnicas de microscopía en general es la posibilidad de que la preparación de muestras altere las propiedades estructurales del sistema. Por lo tanto, debe llevarse a cabo con sumo cuidado y reproducibilidad. Otra de las desventajas de la microscopía es que las observaciones suelen ser lentas y subjetivas, y a menudo es necesario analizar un gran número de regiones diferentes dentro de una misma muestra para obtener datos fiables. La mayor ventaja de la microscopía es que los microscopios modernos están conectados a ordenadores, en los que a través de programas específicos se pueden analizar rápidamente imágenes obtenidas.

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Objetivos

El objetivo general de la presente tesis doctoral fue desarrollar emulsiones basadas en hidrocoloides, que sean resistentes a la digestión lipídica y aptas como sustitutos de grasa, para formular alimentos que aporten menor grasa al organismo y que posean una óptima calidad fisicoquímica y sensorial.
Para la consecución de este objetivo general se establecieron los siguientes objetivos específicos:

- Investigar los cambios reológicos, de textura y de tamaño de partícula producidos en cada etapa de la digestión *in vitro* de emulsiones elaboradas con derivados de celulosa y aceite vegetal, y determinar su relación con la digestibilidad de la grasa.

- Evaluar el efecto de la sustitución química de los éteres de celulosa en la estructura de la emulsión y relacionarla con la digestibilidad lipídica.

- Estudiar el comportamiento de la goma xantana como estabilizante de una emulsión aceite/agua e investigar los cambios reológicos y los cambios macro y micro-estructurales producidos en cada etapa de la digestión *in vitro.*

- Investigar el efecto del tipo de grasa en las propiedades estructurales y la digestibilidad de una emulsión aceite/agua elaborada con hidrocoloides.

- Aplicar las emulsiones desarrolladas en la formulación de cremas de relleno y determinar los cambios estructurales producidos en esta nueva matriz y su aceptación sensorial.

- Estudiar la digestibilidad lipídica de las cremas de relleno y su relación con la digestibilidad de las emulsiones después de la digestión *in vitro*.

Estructura de la tesis

La presente tesis doctoral se enmarca dentro del proyecto del Ministerio de Economía y Competitividad titulado "*Funcionalidad de los hidrocoloides en la reducción de la digestibilidad lipidica in vitro de emulsiones alimentarias: reología, estructura y percepción sensorial" (AGL2015-68923-C2-1-R)*. La tesis se estructura en tres bloques o capítulos. El primero se centra principalmente en el efecto del tipo de hidrocoloide, el segundo en el tipo de grasa y el tercero recoge los resultados de la aplicación en cremas de relleno. A continuación se citan los artículos de investigación generados en la temática de los tres capítulos, y seguidamente se presentan los contenidos íntegros de las publicaciones. Seguidamente se presenta el apartado de resumen y discusión, en el que se comentan y discuten los aspectos más destacados de la investigación. Por último, se enumeran las conclusiones de la tesis doctoral.

CAPÍTULO 1

Espert, M., Salvador, A., & Sanz, T. (2016). *In vitro* digestibility of highly concentrated methylcellulose O/W emulsions: rheological and structural changes. *Food & function*, *7*(9), 3933-3942.

Espert, M., Borreani, J., Hernando, I., Quiles, A., Salvador, A., & Sanz, T. (2017). Relationship between cellulose chemical substitution, structure and fat digestion in o/w emulsions. *Food Hydrocolloids*, *69*, 76-85.

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CAPÍTULO 2

Espert, M., Wiking, L., Salvador, A., & Sanz, T. Effect of cellulose ethers on physical properties of milk fat emulsions: Designing reduced-fat spreads. Enviado a *Journal of Dairy Sciencie.*

Espert, M., Constantinescu, L., Sanz, T., & Salvador, A. (2019). Effect of xanthan gum on palm oil *in vitro* digestion. Application in starch-based filling creams. *Food Hydrocolloids*, 86, 87-94.

CAPÍTULO 3

Espert, M., Borreani, J., Hernando, I., Quiles, A., Sanz, T., and Salvador, A. Structural changes of filling creams after *in vitro* digestion. Application of hydrocolloid based emulsions as a fat replacer. Enviado a *LWT-Food Science and Technology*.

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Espert, M., Bresciani, A., Sanz, T., & Salvador, A. (2019). Functionality of low digestibility emulsions in cocoa creams. Structural changes during in vitro digestion and sensory perception. *Journal of Functional Foods*, 54, 146-153.

Capítulo 1

Efecto del tipo de hidrocoloide en la digestibilidad de emulsiones aceite/agua

In vitro digestibility of highly concentrated methylcellulose O/W emulsions: rheological and structural changes

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ABSTRACT

The changes in structure during the digestion of highly concentrated methyl cellulose (MC) O/W emulsions and of hydrated MC were investigated. The effect of human saliva and in vitro stomach digestion was attributed to a dilution effect, rather than to pH or pepsin activity. After in vitro intestine incubation, a decrease in viscoelasticity and an increase in fat globule size were observed. The fat released after the digestion of the MC emulsion was 49.8% of the initial fat, indicating the existence of a big physical impediment. In comparison with an O/W whey protein emulsion with fat content equal to the fat released during the MC emulsion digestion, a 12% reduction in free fatty acid formation was found, which indicates that the decrease in fat bioaccessibility in the MC emulsion should be attributed not only to a physical effect against fat release but also to a further impediment related to the fat digestion process. Fat released quantification informs about the physical retention of fat in the emulsion matrix structure. Enzymes may not act if fat is not released and solubilized. Free fatty acid quantification is the real indicator of fat digestion, but contrary to the total fat released, it is affected by a wide variety of enzymatic factors, which should be considered for the correct comparison of systems of different properties, for example systems where the amount of fat release during the digestion may be different or initially unknown.

1. INTRODUCTION

The design of colloid delivery systems to control the rate and extent of lipid digestion within the gastro-intestinal tract has been the subject of extensive attention lately. Inhibiting or slowing down lipid digestion is considered to be an effective means with which to reduce appetite and promote satiety, leading to a reduction in obesity and a more balanced energy intake.[1,2]

After ingestion, the emulsions undergo a complex series of physical and chemical changes as they pass through the mouth, stomach and small and large intestines (mechanical strength, presence of enzymes, changes in pH, etc.),[3,4] which affect their capacity to be ingested. When designing low absorption emulsions, it is vital to bear these parameters in mind so as to achieve a level of structural stability which limits the enzymatic attack.

More attention has been paid to the effect of the interfacial layer surrounding the lipid droplets on the emulsion structuring/ breakdown during digestion than anything else. Depending on the physicochemical properties of the interfacial layer, the lipid droplets may break up or coalesce as the emulsion passes through the mouth into the stomach and then the intestines, while at the same time altering the surface area of lipid exposed to enzymes. Emulsions stabilised by non-ionic surfactants tend to remain stable during the transit through the stomach because of the highly stable nature of the emulsion. [5,6] Emulsions stabilised by proteins tend to flocculate[7] while those stabilised by ionic surfactants can undergo coalescence.8 The effect of HPMC, β-lactoglobulin and soy protein as emulsifiers in the control of lipid digestion was studied by Bellesi, Martinez, Pizones Ruiz-Henestrosa & Pilosof.9 Soy protein was found to be resistant to digestion as HPMC, which is a non-digestible emulsifier. Likewise, the

physico-chemical composition of fat and the size and distribution of fat globules noticeably regulate lipase activity.[10–12]

Polysaccharides can act as emulsion stabilisers by increasing the viscosity or gel strength of the continuous phase, as well as by inducing the flocculation of emulsion droplets through bridging or depletion mechanisms, depending on the adsorbing properties of the polysaccharides.[13] The role of emulsion stabilizers is gaining increasing importance in the area of lipid digestion control. It has been shown that the presence of certain hydrocolloids potentially influences lipid digestión control.[11,14,15] It has even been demonstrated that emulsions which remain stable in the stomach and/or have a delayed digestion in the small intestine may stimulate the release of intestinal hormones that induce a sensation of

satiety and, therefore, reduce the quantity of foodstuffs ingested.[16] The impact of carboxymethylcellulose (CMC) on lipid digestion and the physicochemical properties of whey protein-stabilised emulsions during digestion was studied by Malinauskytė et al.[13] The thickening network formed in the continuous phase by CMC limits the interaction of fat droplets with gastrointestinal fluids, slowing down the rate of lipid

digestion.13 Methylcellulose, chitosan and pectin were also found to be effective at reducing lipid digestibility in a 2% corn oil in water emulsion stabilized by Tween 80. This behaviour was attributed to the ability of the polysaccharides to induce droplet flocculation due to their interaction with molecular species.[17] Qiu et al.[15] studied the influence of xanthan gum and pectin on the lipid digestibility of fish oil emulsions stabilized by wheat proteins. In this case, surprisingly, the polysaccharides were found to promote the lipid digestión process. The increase in the lipid digestion rate in the presence of dietary fibres was attributed to their ability to alter the

aggregation state of the oil droplets, thereby increasing the amount of lipid phase exposed to the lipase. Other mechanisms associated with the presence of dietary fibres, such as binding to calcium ions, bile salts, free fatty acids, and lipase, were not able to explain the observed increase in lipid digestion.[15]

Highly fat-concentrated (50% fat) oil in water (O/W) cellulose ether emulsions have been recently studied due to the fact that they may act as healthy substitutes of conventional sources of solid fat in the diet, such as butter or margarine. Biscuits, in which conventional shortening was totally replaced by the cellulose ether emulsion, exhibited good sensory acceptability, having 33% less fat and no trans-fatty acids.[18,19] This oil in water cellulose emulsion also showed a reversible thermal gelation ability,[20,21] which makes it a suitable option for applications where thermal stability is required, such as in cream fillers for bakery products. In these emulsions, the cellulose ether exhibits surface active properties and confers stability to the continuous phase due to the thickening effect.

This study focuses on the structural changes during the in vitro mouth, stomach and small intestine digestion of a highly concentrated methylcellulose O/W emulsion and their relationship with lipid digestion. The emulsion's rheological properties, microstructure and droplet size distribution are investigated. The total fat release and the free fatty acids generated after the end of the digestion were calculated.

Likewise, in addition to the behaviour of the emulsion, the effect of in vitro digestion of an aqueous solution of methylcellulose is researched in isolation. It is estimated that the behaviour of the hydrated hydrocolloid will be a determining factor in the structural changes undergone by the emulsion, as it constitutes the first barrier to come into contact with the digestion solutions. The research into the structural changes undergone

during the in vitro digestion in the mouth, stomach and intestine will allow a better understanding of the factors affecting fat release and fat digestibility. This will permit the rational design of fat emulsions which have low digestibility and that may substitute conventional fats.

2. MATERIAL AND METHODS

2.1 Materials

Methylcellulose (A4 M type: 30.0% methoxyl, viscosity of 4000 mPa s at 2% aqueous solution at 20 °C, measured by The Dow Chemical Company following reference methods ASTM D1347 and ASTM D2363) was supplied by The Dow Chemical Company. Sunflower oil "Koipe Sol" was purchased from Deoleo S.A. (Madrid, Spain). Whey protein was supplied by Best Protein (Barcelona, Spain).

Hydrochloric acid (6 N), ammonia solution (25%) and ethanol (96%) were purchased from Scharlab S.L. (Spain) and sodium hydroxide (0.1 N) was provided by Panreac Química S.L.U. (Spain). Phenolphthalein solution, pepsin from porcine gastric mucosa (P7000), bile extract porcine (B8631) and pancreatin from porcine pancreas (P1750) were supplied by Sigma-Aldrich Chemical Company (St Louis, MO).

2.2 Emulsion reparation

Oil–water–cellulose ether emulsions were composed of sunflower oil (51%), methylcellulose (2%) and water (47%). The cellulose ether was first dispersed in the oil using a Heidolph stirrer at the lowest speed for five minutes. The mixture was then hydrated by gradually adding water at 10 °C

while continuing to stir. The 200 g mixture contained in a 600 ml beaker (10 cm diameter) was then homogenized with an IKA T18 basic (Ultra-Turrax) with the dispersion tool S18N-19G (stator diameter 19 mm and rotor diameter 12.7 mm) at 6500 (1 min^{-1}) for 15 s and subsequently at 24 000 (1 min^{-1}) for 30 s.

2.3 Methylcellulose water dilution

Two hundred grams of a solution of methylcellulose (2% w/w) were prepared according to the hot/cold technique (The Dow Chemical Company). The powder was previously dispersed by gentle mixing with 1/3 of the total water at 80 °C for approximately 3 min (Heidolph stirrer at speed 3). Subsequently the beaker with the dispersed MC was quickly transferred to a refrigerated water bath at 10 °C and the rest of the water was added at 1 °C and stirred continuously for 10 min, allowing a correct MC hydration.

2.4 *In vitro* digestion

An *in vitro* digestion model that simulated the mouth, stomach and small intestine was used.

2.4.1 Mouth phase

25 g of emulsion sample were gently mixed for 5 s with 0.5 ml of fresh stimulated human saliva (corresponding to a ratio of saliva/emulsion of 1 ml saliva per 50 g emulsion) inside a water bath at 37 °C.[22,23] The ratio of human saliva/emulsion was selected according to the saliva flow data provided by Humphrey & Williamson,[24] considering a short retention time of the emulsion in the mouth. The stimulated human saliva was obtained as

described by Engelen et al.[25] The mouth of the donor was rinsed three times with water; subsequently, saliva stimulation was performed by chewing a 5 cm × 5 cm sheet of tasteless parafilm (Parafilm American National Can, Greenwich, CT, USA). Informed consent was obtained from the donor. Saliva was always employed within 1 h and was stored at 4 °C.

2.4.2 Gastric phase

The sample from the mouth phase was mixed with 1.5 ml of a simulated gastric fluid (SGF), 6 ml of distilled water and 100 µl of 6 N HCl so that a final pH of 2.0 was obtained. The ratio of gastric volume/emulsion weight was 1/3. This ratio was selected so as to be able to evaluate the changes in the rheological properties associated with the digestion process. The SGF consisted of a pepsin solution containing 1.6 g of pepsin in 10 ml of 0.1 N HCl. The mixture was incubated at 37 °C for 1 hour with continuous agitation in a shaking water bath (speed 70 U min^{-1}).

2.4.3 Small intestine phase

After the gastric phase, 2 ml of simulated intestinal fluid (SIF) was added and the pH was adjusted to 7 with NH3 (25% w/w). The ratio of intestine volume/emulsion weight was 1/2.5. This ratio was selected so as to be able to evaluate the changes in the rheological properties associated with the digestion process, as a higher dilution in the system would have reduced the measurement sensitivity. The SIF consisted of 0.1 g of pancreatin, 0.625 g of bile extract and 0.21 g of ammonium hydrogen carbonate in 25 ml Milli-Q water. The pH of the SIF was adjusted to 7 with NH3 (25% w/w). The final oil concentration in the small intestine phase was 35.2%. However, it should be considered that not all the initial oil in the emulsion sample will be bioaccessible or available for digestion. In this article the amount of oil

available for digestion is referred to as “released fat” and is considered to be the oil isolated in the supernatant after centrifugation of the small intestine digesta (see section 2.9). The oil that remains in the pellet after centrifugation is considered not to be available for digestion.
The mixture was incubated for two additional hours in the shaking water bath under the same conditions as those described in the gastric phase.

2.4.4 Effect of water dilution
Samples were also incubated in the stomach and the small intestine model but only with the addition of distilled water at the dilution level of the stomach and small intestine. The incubation process (time, temperature and shaking conditions) was the same as that in the samples with enzymes.

2.5 Rheological behaviour

The rheological behavior was evaluated by small amplitude oscillatory shear using a controlled stress rheometer (AR-G2, TA Instruments (Crawley, England)) with a Peltier heating system. A 40 mm diameter plate–plate sensor geometry with a serrated surface and a 1 mm gap was employed. In every case, the sample was protected with vaseline oil (Panreac, Barcelona, Spain) in order to prevent the sample from drying as a result of either the time or temperature used.
Stress sweeps were carried out at a frequency of 1 Hz to measure the extent of the linear viscoelastic response. Frequency sweeps from 10 to 0.01 Hz at a stress wave amplitude inside the linear region were performed. In some sample data, values at lower frequencies (from 0.1 to 0.01) are not shown due to a lack of sensitivity during measurements. Storage modulus (G′), loss

modulus (G″) and loss tangent (tan δ = G″/G′) values were recorded. The test temperature was always 37 °C.

The tests were carried out in fresh systems (emulsion and hydrated cellulose) and after incubation in each of the in vitro digestion steps (oral, gastric and intestinal), with and without enzymes.

2.6 Extrusion properties

The extrusion properties of the samples were determined using a TA-XT plus texture analyzer equipped with the Texture Exponent software (Stable Microsystems, Godalming, UK). A back extrusion assay was carried out using a bucket of 5 cm diameter and 7.5 cm height and a compression probe of 4.9 cm diameter. The distance force was 15 mm, the compression rate 1 mm s^{-1}, and the trigger force 10 g. From the force time profiles obtained, the area under the curve and the maximum force achieved were recorded.

2.7 Particle size measurements

The measurements were taken with a particle size analyzer by laser diffraction (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The particle size calculations are based on Mie theory or Mie scattering theory and were performed with the software provided with equipment (Mastersizer 2000 V5.40). Refractive indexes of 1.330 and 1.472 were used for the aqueous phase and the fat phase respectively. The volumeweighted mean particle diameter D[4,3] was calculated.

2.8. Microstructure

The microstructure of the emulsions was evaluated using optical microscopy (Nikon Eclipse 90i, Kanagawa, Japan). A small aliquot of each sample was placed on a microscope slide and observed using a magnification of 20×. The emulsions were observed after 24 h of preparation and after digestion in in vitro models of the mouth, stomach and intestine.

2.9. Amount of fat released after in vitro digestión

Before fat can be digested, one necessary step is its reléase from the initial matrix and solubilisation. In order to determine the real amount of fat that will be available for digestion, the amount of fat released from the emulsion after centrifugation was calculated. It is necessary to consider that fat release from the hydrocolloid/emulsion structure is a first necessary requirement for the correct action of digestive enzymes.
After small intestine in vitro digestion the sample was mixed with 15 ml ethanol and centrifuged (10 minutes, 10 000 rpm) (Sorvall® RC-5B Refrigerated Superspeed centrifuge). The total supernatant was quantified and the mixture of water and ethanol was evaporated in a boiling water bath. After evaporation, the container was dried in an oven at 100 °C for 30 minutes to completely eliminate residual water or ethanol. The remaining liquid is considered to be the amount of fat released. The amount of fat that remains in the pellet after centrifugation will not be bioaccessible fat.

2.10. Free fatty acid (FFA) content

Fat digestion was determined by measuring the amount of FFA before and at the end of the in vitro digestion. FFAs were determined in the MC emulsion and in an O/W whey protein (2% w/w) emulsion, considered as the control.

The percentage of oil in the control emulsion (25% w/w) was selected according to the total fat released from the MC emulsion (section 2.9), so the total amount of fat available for the digestive enzymes was kept constant.
After incubating in the intestine model, 15 ml of etanol were added to the digestion mixture (6.25 g) in order to stop the enzyme action of pancreatic lipase. The sample mixed with ethanol was centrifuged for 10 minutes at 10 000 rpm using a centrifuge (Sorvall® RC-5B Refrigerated Superspeed centrifuge). The total supernatant was quantified and the free fatty acids were determined in 10 ml of the supernatant by titration with 0.05 M NaOH and phenolphthalein as an indicator to end point (pink color). A ph-stat automatic titration unit was not employed due to the inhomogeneous consistency of the emulsion digested sample, as many of the FFAs released will not be soluble enough to be detected.[26]
A standard curve was prepared using oleic acid (0, 50, 100, 150, 200 and 250 mM), and this was used to calculate the free fatty acid concentration of the samples. The results are expressed as "g oleic acid per g fat" and in the MC emulsion also as "g oleic acid per g fat released". The determination of free fatty acids was performed on the emulsion sample and in a control consisting of a 2% whey protein O/W emulsion taken as an example of the digestible emulsifier, without a hydrocolloid barrier.

2.11 Statistical analysis

For each test, three replicates were performed with samples prepared on different days. An analysis of variance (ANOVA) was applied to study the differences between the samples. The least significant differences were calculated by Tukey's test and the significance at $P < 0.05$ was determined.

These analyses were performed using the XLSTAT 2009.4.03 statistical software (Addinsoft, Barcelona, Spain).

3. RESULTS AND DISCUSSION

3.1 Hydrated cellulose ether

3.1.1 Linear viscoelastic properties.

Before the evaluation of the changes in the viscoelastic properties of the cellulose ether emulsion during digestion, the behaviour of the solely hydrated methylcellulose ether was investigated. In the emulsion the hydrated cellulose constitutes the continuous phase of the emulsion and the oil in the dispersed phase. Therefore, hydrated cellulose is the first barrier that will come into contact with digestion fluids during the digestion of emulsions. For this reason, it is expected that the changes in the solely hydrated cellulose ether would be closely related to the emulsion stability during digestion and may help us to understand the general behaviour of the emulsion.

A study of the linear viscoelastic properties of the fresh and the digested samples was carried out by applying small amplitude oscillatory shear. These small forces do not simulate the peristaltic movement along the digestive tract but are considered an effective tool for the purpose of evaluating the resulting inner structure after incubation in the shaking water bath.

The effect of mouth, stomach and small intestine in vitro digestion on the linear viscoelastic properties of hydrated cellulose at 37 °C is shown in Fig. 1. The spectra reveal the very low elasticity of hydrated methylcellulose at this temperature. In the fresh sample, the terminal zone of the mechanical

spectra was observed, with the values of G″ being higher than those of G′ in the available frequency window, implying the predominance of the viscous properties of the simple (Fig. 1A).

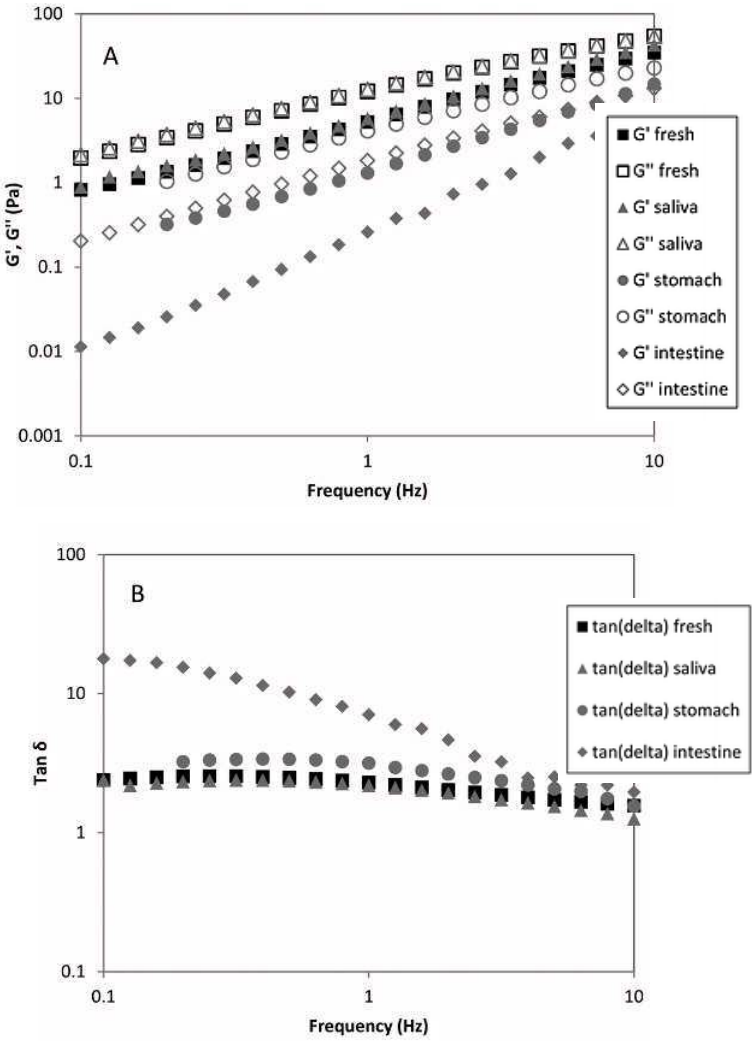

Figure 1. A (G' and G'') and B (tan δ) as a function of frequency of the fresh hydrated methylcellulose ether and after in vitro digestion at 37ºC.

It is interesting to compare these mechanical spectra obtained at 37 °C with previously reported ones measured at 15 °C.[27] In comparison with 15 °C, at 37 °C the distance between G′ and ″ is shorter and the slope of the increase in G′ and G″ with frequency is also gentler. These differences between the shape of the mechanical spectra at 15 °C and 37 °C are explained by the thermal changes associated with methylcellulose ether. As previously reported,27 the increase in temperature reduced the differences in G″ and G′ until the gelation temperature was reached (around 49 °C) and a crossover of G″ and G′ occurs. This explains the higher viscoelasticity at 37 °C in comparison with the previous results at 15 °C.

Mixing the hydrated hydrocolloid with human saliva did not affect the characteristic shape of the mechanical spectra or the values of the viscoelastic functions, implying that saliva did not affect the fresh methylcellulose structure. After digestion in the stomach, only a mild increase in tan δ (lower viscoelasticity) was observed (Fig. 1B), although a significant decrease in the values of G′ and G″ was observed. Finally, the intestine digestion induced a greater decrease in the viscoelastic functions with a significant increase in tan δ. In order to establish whether the observed changes in the viscoelastic properties are associated merely with a dilution effect or more specifically with the enzymatic effect and the changes in pH, the hydrated methylcellulose dispersions were also incubated only in the presence of water, at the corresponding dilution level. The effect of water dilution is shown in Table 1, where the values of G′, G″, and tan δ at 1 Hz of the digestion systems and water diluted systems are shown. The dilution of water at the level of the stomach showed a similar profile to the stomach incubation with no significant differences in tan δ, indicating that the observed changes after stomach incubation could be mainly associated with the dilution effect. The slight effect of the acidic pH

on the methylcellulose structure was expected to be due to its non-ionic nature.

Table 1. Viscoelastic rheological parameters of the fresh hydrated methylcellulose ether and after *in vitro* digestion.

Sample	G' (Pa)	G'' (Pa)	tan δ
Fresh	5.6a	12.4a	2.2c
Saliva	5.6a	11.9a	2.1c
Stomach	1.0b	3.7b	3.9bc
Stomach dilution	1.1b	3.5b	3.3bc
Intestine	0.3b	2.1b	6.5a
Intestine dilution	0.5b	2.6b	5.3ab

[abcd] Means in the same column without a common letter differ ($P < 0.05$) according to the Tukey test.

In contrast, dilution at the level of the intestine did not affect tan δ values while intestine digestion did, indicating that, in this case, the observed changes in the structure can be associated with the effect of the bile salts and the pancreatic enzymes.

3.1.2 Extrusion properties

The effect of greater forces on the structure was studied by applying a back extrusion test. The extrusion forces versus time were recorded in the fresh samples and after digestion in the mouth and stomach models, also analysing the isolated effect of water dilution.

The area under the curve and mean force values are shown (Table 2). After stomach incubation, the values of force and area were significantly lower than in both the fresh sample and the saliva sample. No significant differences were found between the stomach sample and the diluted sample, which, similarly to the viscoelastic results, indicates that the decrease in the

force values should be attributed to water dilution more than to the structural change associated with stomach conditions (acid pH and pepsin). Although saliva incubation only produced a small decrease in force values, the differences found were significant.

Table 2. Extrusion parameters of the fresh hydrated methylcellulose ether and after in vitro digestion.

Sample	Are under the curve (N mm)	Maximum force (N)
Fresh	7.4a	0.5a
Saliva	5.5b	0.4b
Stomach	3.0c	0.2c
Stomach dilution	2.4c	0.2c

[abcd] Means in the same column without a common letter differ ($P < 0.05$) according to the Tukey test.

3.2 Methylcellulose O/W emulsion

The visual appearance of the emulsions after incubation in different in vitro models is shown in Fig. 2.

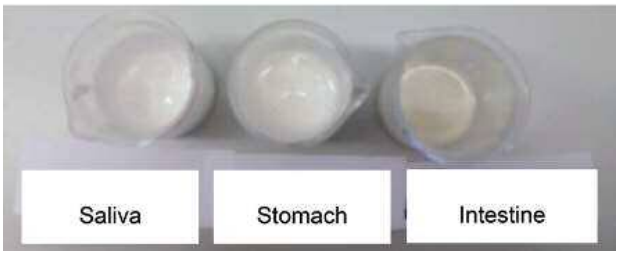

Figure 2. Visual appearance of the fresh methylcellulose O/W emulsion and after *in vitro* digestion at 37 °C.

No phase separation can be distinguished in the emulsion and, after the different phases of digestion, the structure adopted by the emulsion is homogeneous.

3.2.1. Linear viscoelastic properties

The effects of saliva, stomach and intestine digestion on the emulsion viscoelastic properties are shown in Fig. 3.

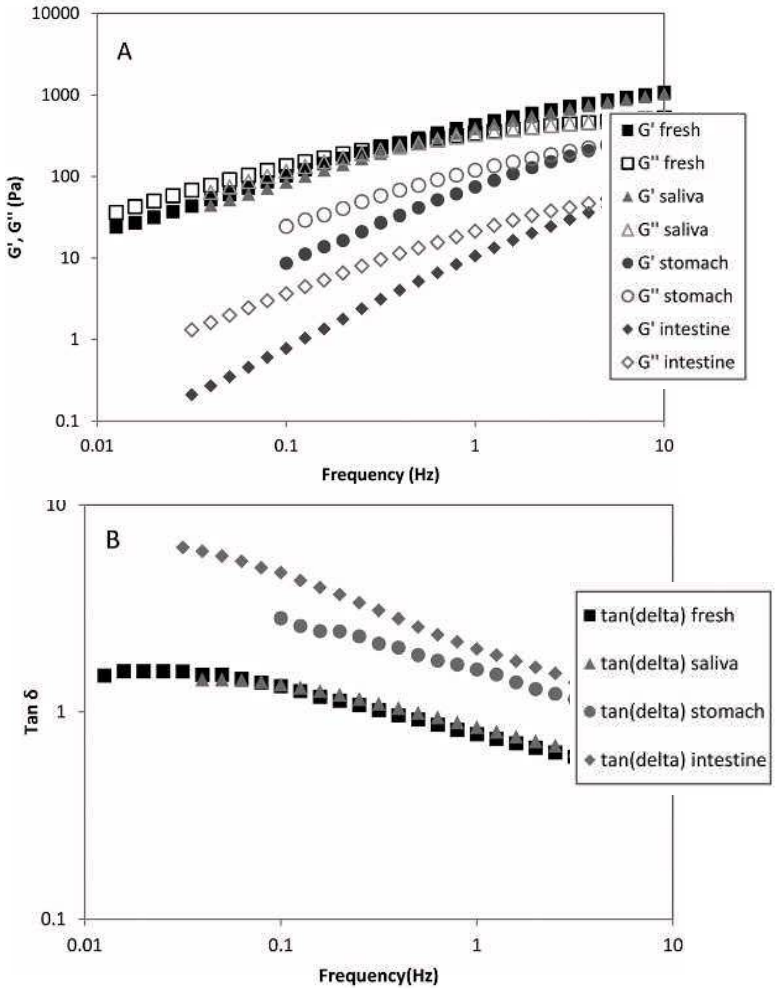

Figure 3. (A) G' and G'' and (B) tan δ as a function of frequency of the fresh methylcellulose O/W emulsion and after *in vitro* digestion at 37 °C.

The fresh emulsion was highly dependent on frequency. In comparison with the hydrated hydrocolloid at the same temperature, the emulsion is characterized by a higher elasticity. The values of the viscoelastic function in the emulsions were more than one order of magnitude higher than those in the hydrated hydrocolloid (Fig. 3A). In the fresh emulsion, the end of the plateau zone was observed in the available frequency window with the crossover between G′ and G″, the values of G″ being higher than those of G′ at lower frequencies. Similarly to the behaviour found in the hydrated methylcellulose, the behaviour of the undigested emulsion at 37 °C differs slightly from the behaviour of the emulsion at 20 °C, as previously described.21 At both temperatures, the crossover of G′ and G″ was observed in the available frequency window, but the distance between G′ and G″ was shorter in the emulsion at 37 °C, which is associated with the decrease in viscoelasticity occurring in this temperatura range.21 Mixing with saliva did not exert any effect on the emulsion viscoelastic properties, and the values of tan δ (Figure 3B) and G′ and G″ (Fig. 3A) were unaltered. After incubation in the stomach, a change in viscoelasticity occurred: the values of G′ and G″ fell and the crossover point between G′ and G″ moved towards higher frequencies, implying a decrease in viscoelasticity. After digestion in the intestine, the system's viscoelasticity continues to decrease: the G′ and G″ values fell, tan δ rose and the crossover point shifted towards higher frequencies.

The effect of water dilution was also studied. The increase in water dilution led to a progressive decrease in the values of G′ and G″ and moved the crossover towards higher frequencies. The comparison of the mechanical spectra of the water dilution and the stomach digestion reveals very similar spectra, indicating that the observed effect could simply be associated with dilution. Values of G′, G″ and tan δ are shown in Table 3. After digestion in

the intestine, the values of G′ and G″ were slightly lower than those corresponding to the dilution, although the differences were not significant.

Table 3. Viscoelastic rheological parameters of the fresh methylcellulose O/W emulsion and after *in vitro* digestion.

Sample	G' (Pa)	G'' (Pa)	tan δ
Fresh	418.1a	319.0a	0.8c
Saliva	389.1a	322.7a	0.8bc
Stomach	82.7b	126.9b	1.5a
Stomach dilution	89.9b	133.5b	1.5ab
Intestine	10.6c	20.1c	1.9a
Intestine dilution	31.2c	55.1c	1.8a

[abcd]Means in the same column without a common letter differ (P < 0.05) according to the Tukey test.

3.2.2 Extrusion properties

The area under the curve and medium force values are shown in Table 4.

Table 4. Extrusion parameters of the fresh methylcellulose O/W emulsion and after *in vitro* digestion.

Sample	Are under the curve (N mm)	Maximum force (N)
Fresh	107.4a	8.0a
Saliva	83.2b	6.3b
Stomach	18.3c	1.4c
Stomach dilution	14.9c	1.1d

[abcd]Means in the same column without a common letter differ (P < 0.05) according to the Tukey test.

Similarly to the viscoelastic results, the extrusion profile (data not shown) revealed the greater consistency of the methylcellulose emulsions in comparison with the hydrated methylcellulose (mean extrusion force

around 0.5 N for the hydrated cellulose and around 8 N for the emulsions). After digestion in the stomach, the extrusion force decreased significantly and no differences were found between the stomach digestion samples and the water diluted samples; similarly to the linear viscoelastic results, this implies that the effect of stomach digestión should be mainly associated with the dilution effect, rather than with the specific effect of the stomach conditions.

3.2.3. Emulsion microstructure

The microstructures of both the fresh emulsion and the emulsion after digestion in in vitro models of the mouth, stomach and intestine are shown in Fig. 4.

The initial microstructure corresponds to the existence of a dense matrix composed of fat globules that are immersed in the continuous phase of the emulsion, made up of water and hydrated cellulose.

No significant changes in the microstructure can be appreciated after it is mixed with saliva. After incubation in the stomach, it can be seen that the cellulose still retains its emulsifying effect on the acidity of the stomach and no flocculation or coalescence phenomena can be observed. As a result of the existing dilution, the fat droplets in the emulsion can be seen to be farther apart after incubation in the stomach. Finally, the microstructure reveals that there has been a significant increase in the size of the fat globules after incubation in the intestine. These results indicate that the bile salts and pancreatic lipase have been to some extent capable of accessing the interface of the fat globules and displacing the methylcellulose of the interfacial surface.

Figure 4. Microstructure of the fresh methylcellulose O/W emulsion and the emulsion after *in vitro* digestion at 37 °C.

Li, Hu & McClements[28] found an increase in the mean particle diameter when bile was added to the emulsions. Mc Clements[29] and Mun et al.[10] associated the phenomenon of coalescence with fat digestion in the following manner: the formation of free fatty acids and monoacylglycerides on the surface of the droplets during lipase digestion boosts coalescence as the surfactant effect of these substances is not strong enough to stabilise the oil emulsions in water when coalescence occurs. The degree towhich this happens will depend on both the ability of the lipase to come into contact with the

emulsified lipids and the composition and properties of the interfacial films that surround the water droplets.

3.2.4. Particle size distribution

The changes in the oil droplet size distribution during mouth, stomach and intestine digestion are shown in Fig. 5.

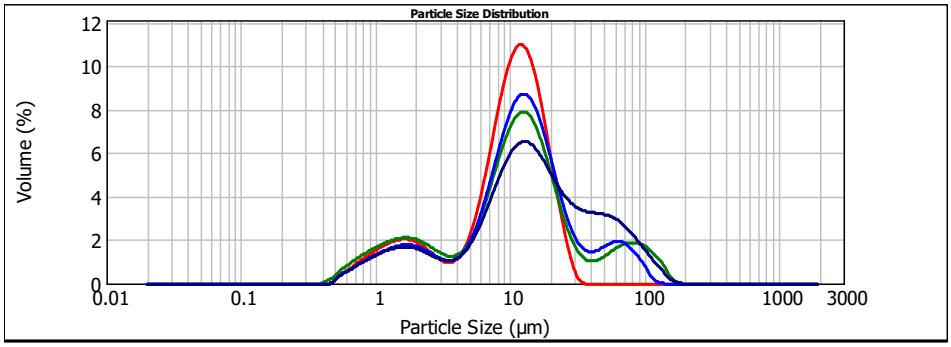

Figure 5. Particle size distribution of the fresh methylcellulose O/W emulsion (red) and the emulsion after in vitro digestion at 37 °C (saliva: green; stomach: blue; intestine: black).

Prior to digestion, the emulsion exhibited a bimodal distribution with a minority population of a smaller size, around 1 µm, and a majority, larger-sized population (around 10 µm).

The digestion with saliva leads to the appearance of a small tail on the right-hand side and a slight reduction in the size of the majority population. This means that the presence of saliva does have an effect on the emulsion's structure that could be associated with a gentle displacement of the interface cellulose caused by the saliva's glycoproteins (mucines), which would lead to slight coagulation or coalescence. Digestion in the stomach produced no noticeable changes compared with in-mouth digestion. Lastly, after

digestion in the intestine there was a widening of the curve and a clear displacement to the right, which coincides with a growth in the size of the fat globules as observed in the microstructural analysis.

The values of the average droplet diameter D[4,3] are shown in Table 5. A slight, but significant, change can be observed during digestion. This change in the size distribution of the particles reflects the fact that, although only to a limited extent, the bile salts and pancreatic lipase do gain access to the dispersed phase of the emulsion, leading to coagulation or coalescence phenomena.

Table 5. D[4,3] values of the fresh methylcellulose O/W emulsion and after *in vitro* digestion.

Sample	D[4,3] (μm)
Fresh	10.5d
Saliva	20.1b
Stomach	17.4c
Intestine	23.9a

[abcd]Means in the same column without a common letter differ ($P < 0.05$) according to Tukey's test.

3.2.5. Total fat released after emulsion digestion

Two main requirements are needed for the fat in the cellulose emulsion to be digested. Firstly, the fat should be released from the hydrocolloid matrix and solubilized; secondly, the lipase must be in close contact with the fat surface. Undoubtedly if the digestive fluids have limited access to the oil phase, fat digestion will be reduced.

The amount of fat which becomes released from the initial emulsion structure was calculated quantifying the amount of fat present in the total supernatant after emulsion centrifugation. It is considered that the fat remaining in the pellet after emulsion centrifugation will not be available for digestion. A photo of the appearance of the digested emulsion is shown in Fig. 6; the pellet and the supernatant are clearly observed.

In a fresh, non-digested MC emulsion, the amount of fat quantified in the supernatant was very low (3.76%). After being digested in the intestine model, 49.8 (±6.3)% of the fat was quantified in the supernatant (Fig. 6), implying that 50.2% of the initial fat present in the emulsion will not be released from the semisolid structure.

Figure 6. Appearance of the digested intestine sample after centrifugation.

This result indicates that independently of a possible additional inhibition of methylcellulose during the fat digestion process, there exists a first effective physical barrier that limits fat release to the aqueous phase. Therefore, the use of highly concentrated O/W methylcellulose emulsions is an effective physical strategy to reduce fat bioaccessibility.

It should be kept in mind that the total fat released will include non-digested fat and digested fat products, so it is not an index of fat digestion. Fat released is an index of the available fat to be solubilized and subsequently digested.

3.2.6. Free fatty acids generated after emulsion digestion

In addition to fat released quantification, the content of free fatty acids at the end of the small intestine digestion was quantified, measured as oleic acid. Free fatty acids are the product of fat digestion, so they are a real indicator of the amount of fat which has been digested. However, contrary to total fat released, free fatty acid generation is the result of an enzymatic reaction, which is influenced by a wide variety of factors. It is well known that lipid digestibility is influenced by a great variety of factors, which makes the quantitative comparison of systems of a different nature inappropriate. Li et al.[28] studied some of the factors that affect emulsion lipid digestion. These authors found that the rate and extent of lipid digestion increase as the lipase concentration rises, decreasing the bile extract, droplet size and droplet concentration.

Special care has to be taken in employing free fatty acids as an indicator of fat digestion or fat release. In systems where the amount of fat available for digestion may be different (amount and particle size) or initially unknown, it is necessary to guarantee that the enzyme concentration and other reaction factors such as bile content are suitable. This is of extreme importance, especially in highly concentrated oil systems, where if a high release of fat occurs, the amount of lipase in the in vitro system may not be enough to hydrolyse all the fat in the system, or an inactivation of the enzyme may occur due to an increase in the reaction products.

In this work the free fatty acids generated after the digestion of the cellulose ether emulsion were compared with a whey protein stabilized emulsion (without hydrocolloid), taking as an example a digestible emulsion with no hydrocolloid barrier. The oil content in the control emulsion was selected considering the total fat released after the digestion of the cellulose ether emulsion (section 3.2.5). In this way the amount of fat available for digestion will be the same in both systems, making simpler a comparison of the results. The amount of free fatty acid (g oleic acid per g fat) was very low in every system before digestion: 0.0018 (cellulose ether emulsion) and 0.0031 (whey protein emulsion), corresponding to the very small amount of free fatty acids present in the fresh oil before digestion. After digestion in the intestine, the values of free fatty acids expressed as g oleic acid per g total fat were 0.105 (±0.033) g g^{-1} in the MC emulsion and 0.236 (±0.017) g g^{-1} in the control emulsion, which implies a total reduction of 55.51%. If the results on the MC emulsion are expressed relative to fat released (instead of relative to total fat), the origin of the differences (physical or associated with digestion) can be isolated. The value of g oleic acid per g fat released was 0.207 (0.073) g g^{-1}, which implies a 12.28% reduction associated solely with the oil digestion process.

Undoubtedly, a more realistic approach will be obtained performing in vivo analyses. Also, the evaluation of the effect of specific conditions on the in vitro digestion process and the use of more realistic in vitro models, such as the one proposed by Minekus et al.,[30] will be a future line of research. Somehow, with the results obtained in this paper we can affirm that the cellulose emulsion tested in this study is a promising structure with which to protect fat from being digested and deserves further investigation.

4. CONCLUSIONS

This study represents an important contribution regarding the role of methylcellulose as a physical barrier in the control of the digestion of highly concentrated oil/water emulsions. The results reveal that the methylcellulose emulsion structure is highly resistant during digestion, reducing by 49.8% the release of the initial fat due to a physical effect, therefore preventing an effective action of the enzymes.

In comparison with a whey protein control emulsion with an equal amount of fat available for digestion, a 12% reduction in the free fatty acid content was also found, which supposes a 55% reduction in free fatty acids per gram of initial fat.

The effect of human saliva and in vitro stomach digestion on the emulsion's rheological properties and microstructure was attributed to a dilution effect, rather than to pH or pepsin activity. After *in vitro* intestine incubation, a decrease in viscoelasticity and an increase in fat globule size were observed, indicating that the intestine digestive fluids were able to come into contact, at least partially, with the oil phase of the emulsion. Special care should be taken in the correct interpretation of free fatty acid generation as an indicator of digestion in highly concentrated emulsions and in the establishment of the relationships between free fatty acid generation and total fat release from a specific matrix structure. If the amount of fat released is very high, fat digestion could not be completed.

A definitive answer about the real effectivity of methylcellulose emulsions in reducing fat bioaccessibility will be obtained performing in vivo studies.

In this study it is concluded that the highly concentrated methylcellulose emulsion studied is a good candidate to perform in vivo studies.

A further point of interest of this study is the application of these emulsions in the development of food with low fat bioaccessibility, which will help in obesity control and treatment of fat related illnesses.

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Relationship between cellulose chemical substitution, structure and fat digestion in o/w emulsions.

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ABSTRACT

The effect of cellulose ether chemical substitution on the reduction of fat digestion in an o/w emulsion was investigated. Emulsions containing 47% sunflower oil and water were prepared with two types of hydroxypropyl methylcellulose (HPMC) and two types of methyl cellulose (MC), with different hydroxypropyl and methoxyl content. The changes in the emulsion structure were evaluated after mouth, stomach and small intestine *in vitro* digestion by Confocal laser microscopy and by small amplitude oscillatory shear (viscoelastic properties). The total amount of fat present in the supernatant after digesta centrifugation, serving as an indicator of fat bioaccessibility, and the free fatty acids, serving as an indicator of fat digestion, were determined at the end of the digestion. A relationship was found between cellulose ether chemical substitution, initial structure, structural changes during digestion, fat bioaccessibility and fat digestion. All the cellulose ether emulsions showed a lower level of fat digestion in comparison with a whey protein emulsion, the cellulose ether containing the highest amount of methoxyl being the most effective. The rise in the methoxyl content increases the emulsion viscoelastic properties before and after digestion and reduces fat bioaccessibility and the generation of free fatty acids. The decrease in the fat digestibility of the cellulose ether emulsions was mainly associated with a physical effect, which limits the emulsification of appropriate fats by bile salts, and the subsequent lipase digestion effect.

Keywords: Cellulose ethers, emulsion, fat digestion, rheology, viscoelasticity, microstructure, fat replacer.

1. INTRODUCTION

Fat is an essential ingredient in the human diet; however, fat overconsumption is directly associated with overweight and obesity, which causes illnesses such as insulin resistance, dyslipidemia, pulmonary dysfunction, hypertension diabetes, among others. In addition, obese people may be subjected to unfair treatment in terms of employment opportunities, health-care facilities and educational positions and they may also be stigmatized by the media (Madadlou, Rakhsi, & Abbaspourrad, 2016).

A recent strategy for the purposes of controlling excessive fat intake is to decrease fat bioaccessibility through the employment of specifically designed emulsions.

The structure and stability of the emulsions play an important role in the digestion and absorption of the lipids (Golding & Wooster, 2010; McClements, Decker, & Park, 2009a; McClements, Decker, Park, & Weiss, 2009b). The initial properties of the oil-in-water emulsions affect both the rate and degree of lipid digestion (Armand et al., 1992; 1999; Mun, Decker, Park, Weiss, & McClements, 2006; Mun, Decker, & McClements, 2007). The flocculation and coalescence stability of the emulsions is heavily dependent on the nature of the emulsifiers. The use of emulsifiers with a great surfactant capacity can impede other substances with superficial properties that are present in the gastrointestinal tract (bile, lipase) from adhering to the interface of the fat droplets; therefore, the right modification to the oil-water interface can be used to inhibit lipid digestion. In addition, both the size and distribution of the fat globules affect the lipase activity (Mun et al., 2007). The rheological properties of the continuous phase are also of great importance. For example, fat globules which are small in size but covered with tightly bonded surfactants in a viscous medium are hydrolysed by the

lipase more slowly than the bigger ones covered with surfactants that are more loosely bonded to the globule's surface in a less viscous medium (McClements & Decker, 2009).

The presence of hydrocolloids in the continuous phase of the emulsion is also of significant influence. The effect of gelatin**,** colloidal casein and starch dispersion on an oil and water emulsion has been studied, using 20% oil and sodium caseinate and monoglyceride as surfactants (Wooster et al., 2014). The incorporation of low methoxyl pectin into caseinate emulsions with 2% corn oil increased the rate of digestion, which was attributed to the pectin exerting a suppressive effect on the flocculation process. Incorporating high levels of pectin in lactoferrin and Tween 80 emulsions, on the other hand, decreased the digestion rate; this was in all likelihood due to the calcium fixation and the rise in viscosity that restricts lipase access to the surface of the fat (Zhang, Zhang, Xhang, Decker, & Mc Clements, 2015). The impact of carboxymethyl cellulose (CMC) on both lipid digestion and on the physicochemical properties of whey protein-stabilised emulsions during digestion was studied by Malinauskyte et al. (2014). The thickening network formed in the continuous phase by CMC limits the interaction of fat droplets with gastrointestinal fluids, slowing down the rate of lipid digestion. Methylcellulose, chitosan and pectin were also found to be effective at reducing lipid digestibility in a 2% corn oil-in-water emulsion stabilized by Tween 80. This behaviour was attributed to the ability of the polysaccharides to induce droplet flocculation due to their interaction with molecular species. Qiu, Zhao, Decker, & Mc Clements (2015) studied the influence of xanthan gum and pectin on the lipid digestibility of fish oil emulsions stabilized by wheat proteins. In this case, surprisingly, the polysaccharides were found to promote the lipid digestion process. The

increase in the lipid digestion rate in the presence of dietary fibres was attributed to their ability to alter the aggregation state of the oil droplets, thereby increasing the amount of lipid phase exposed to the lipase. Other mechanisms associated with the presence of dietary fibres, such as binding to calcium ions, bile salts, free fatty acids and lipase, were not able to explain the observed increase in lipid digestion (Qiu et al., 2015).

The ethers of cellulose methyl cellulose (MC) and hydroxypropyl methylcellulose (HPMC) are non-ionic cellulose derivatives with methyl and hydroxypropyl groups added to the anhydroglucose backbone. Despite the fact that methyl and hydroxypropyl moieties are hydrophobic groups, the polymer retains enough hydrophilicity to be highly water soluble. The introduction of these hydrophobic groups provides the polymer with surface activity and unique hydration-dehydration characteristics (Sarkar, 1979). Hydroxypropyl groups are more hydrophilic than methyl groups. HPMC and MC have been found to be suitable emulsifiers/thickeners of emulsions o/w with a high fat content (47% fat). These emulsions are considered low fat alternatives to conventional fat sources, such as butter and shortenings, having a lower fat content, fewer saturated fatty acids and no trans fatty acids. They are composed of a vegetable oil (for example, sunflower oil or olive oil), cellulose ether and water (Sanz, Falomir, & Salvador, 2015).

In a previous study (Espert, Salvador, & Sanz, 2016), a MC stabilized emulsion was found to be effective at reducing fat digestion. It was speculated that the lower fat digestibility might be associated with the physical barrier exerted by the undigested continuous phase (hydrated cellulose ether), which impedes fat release and the appropriate contact between fat and the digestive fluids, reducing the effectivity of the fat digestion process.

In this study, the application of different cellulose ethers as a means of reducing emulsion fat digestibility is further investigated. Cellulose ethers with different substitution types are investigated and compared. The objective is to identify the effect of methoxyl and hydroxypropyl methylcellulose substitution on the structure of emulsions before and after *in vitro* digestion (mouth, stomach and small intestine), and to investigate the existing relationship between cellulose ether substitution type, structure (small amplitude oscillatory shear and confocal laser scanning microscopy), amount of fat released after digestion and the degree of lipid digestion. Two types of methylcellulose and two types of hydroxypropyl methylcellulose are employed. The rheological properties of the solely hydrated cellulose ethers after digestion are also investigated, as one of the hypotheses is that their structural changes will be related to the emulsion's structural changes during digestion.

2. MATERIALS AND METHODS

2.1 Materials and reagents

Oil-water-cellulose ether emulsions were prepared with sunflower oil with high levels of oleic acid (Carrefour, Madrid, Spain), water and four different cellulose ethers with thermogelling ability supplied by The Dow Chemical Co: two hydroxypropyl methyl celluloses ("K4M" (HPMC(M/HP:2.9)), "F4M" (HPMC(M/HP:4.3)) and two methyl celluloses ("A4M" (MC(30%M)) and "MX" (MC(>30%M)). The four types have different degrees of methoxyl content. Their percentage of chemical substitution is: HPMC(M/HP:2.9) (22.5% methoxyl, 7.7% hydroxypropyl), HPMC(M/HP:4.3) (29% methoxyl,

6.8% hydroxypropyl), MC(30%M) (30% methoxyl) and MC(>30%M) (methoxyl> 30%).

HPMC(M/HP:2.9), HPMC(M/HP:4.3) and MC(30%M) have approximately the same molecular weight (MW) and a viscosity of 4000 mPa s (2 % aqueous solution at 20 °C measured by The Dow Chemical Company following reference methods ASTM D1347 and ASTM D2363), type MC(>30%M) has a higher MW with a viscosity of 50000 mPa s (2 % aqueous solution at 20 °C measured by The Dow Chemical Company following reference methods ASTM D1347 and ASTM D2363). Whey protein was provided from Best Protein (Barcelona, Spain).

Hydrochloric acid (6 N), ammonia solution (25%), ethanol (96%), calcium chloride anhydrous, di-potassium hydrogen phosphate trihydrate and di-sodium hydrogen phosphate dihydrate were purchased from Scharlab S.L. (Spain) and sodium hydroxide (0.1 N), sodium carbonate anhydrous, sodium chloride, sodium hydrogen carbonate, potassium chloride pure and calcium chloride 2-hydrate were provided by Panreac Química S.L.U. (Spain). Sodium phosphate monobasic dehydrate was provided by Acros Organics (Belgium). Phenolphthalein solution, α-amylase from porcine pancreas (A3176-1MU), mucin from porcine stomach (M2378), pepsin from porcine gastric mucosa (P7000), bile extract porcine (B8631) and pancreatin from porcine pancreas (P1750) were supplied by Sigma-Aldrich Chemical Company (St Louis, MO).

Simulated Saliva Fluid (SSF) was composed of 5.2g of $NaHCO_3$, 1.37g of $K_2HO_4P \cdot 3\ H_2O$, 0.88g of NaCl, 0.48g of KCl and 0.44g of $CaCl_2 \cdot 2H_2O$, dissolved in 1L of bidestillated water. 8.70g of α-amylase and 2.16g of mucin were added to this solution.

Simulated Gastric Fluid (SGF) was prepared by dissolving 3.10g of NaCl, 0.11g of $CaCl_2$, 1.10g of KCl and 5.68ml of Na_2CO_3 (1M) in 1L of bidestillated

water. The solution was adjusted to pH 2. 0.15g of pepsin was dissolved in 1L of SGF.

Simulated Intestinal Fluid (SIF) was composed of an electrolyte solution and bile and pancreatin solutions. The electrolyte solution was prepared by dissolving 1.25g of NaCl, 0.15g of KCl and 0.055g of $CaCl_2$ in 1L of destilled water. Phosphate buffer solution was prepared (103.5mg $NaH_2PO_4 \cdot 2H_2O$ and 44.5mg $Na_2HPO_4 \cdot 2H_2O$ in 100ml of destilled water) setting the pH to 7.0 if necessary to prepare bile and pancreatin freshly suspensions.

2.2 Emulsion preparation

The different cellulose o/w emulsions were prepared using the following proportions: sunflower oil 47% (w/w), cellulose ether 2% (w/w) and water 51% (w/w), for a total final mass of 200g. The cellulose ether was first dispersed in the oil using a Heidolph stirrer at the lowest speed for five minutes. The mixture was then hydrated by gradually adding the water at 1ºC while continuing to stir for 30 s. A water temperature of 1 ºC was selected according to the specific hydration requirement of the cellulose with the highest methoxyl content (MC(>30%M)), and was also used for the other cellulose types. Stirring continued using a homogenizer (Ultraturrax T18, IKA, Germany) at 6500 (1 min−1) for 15 s and subsequently at 17500 (1 min−1) for 60 s until the emulsion was obtained.

2.3 Cellulose ether water dilution

Two hundred grams of a solution of the different cellulose ethers (2% w/w) was prepared according to the hot/cold technique (The Dow Chemical

Company). The powder was previously dispersed by gentle mixing with 1/3 of the total water at 80ºC for approximately 3 min (Heidolph stirrer at speed 3). Subsequently, the beaker with the dispersed cellulose ether was quickly transferred to a refrigerated water bath at 10ºC and the rest of the water was added at 1ºC and stirred continuously for 10 min, allowing a correct cellulose ether hydration.

2.4 *In vitro* digestion

An *in vitro* digestion model that simulated the mouth, stomach and small intestine was used. The digestion process in the gastrointestinal tract in humans was simulated through an *in vitro* digestion model which was a modified version of that described by Qiu et al. (2015) and López-Pena et al. (2016).

2.4.1. Mouth phase

0.5 mL of Simulated Saliva Fluid (SSF) was added to 25 g of the emulsion to obtain a final ratio of 1:45 (v/v). The blend was gently mixed for 5 seconds in a water bath at 37°C, to mimic the time the food material spends within the mouth prior to swallowing. The ratio saliva/emulsion was selected according to the saliva flow data provided by Humphrey & Williamson (2001), considering a short retention time of the emulsion in the mouth.

2.4.2. Gastric phase

The “bolus” sample from the mouth phase was mixed with 8 mL of Simulated Gastric Fluid (SGF) to obtain a final enzyme-sample ratio of 1:250 (v/v). The pH of the mixture was adjusted to 2.0 and incubated for 1 hour under continuous agitation in a shaking water bath at 37ºC (speed 70 U/min).

2.4.3. Small intestinal phase

A total volume of 10mL of Simulated Intestinal Fluid (SIF) was added to the digested sample mix. Firstly, 5.3 mL of bile extract (46.87mg/mL) solution dissolved in phosphate buffer and 2 mL of electrolyte solution was added to the sample, and the pH was adjusted to 7.0 using NH_3 (25% w/w). Then, 2.67mL of pancreatin dissolved in phosphate buffer was added to the mix (1:14 (v/v) ratio). The resulting mixture was incubated for two additional hours in the shaking water bath under the same conditions as described in the gastric phase.

2.5. Rheological behavior

The rheological behaviour was evaluated by small amplitude oscillatory shear in a controlled stress rheometer (AR-G2, TA Instruments (Crawley, England)) with a Peltier heating system. A 40 mm diameter plate–plate sensor geometry with a serrated surface and a 1 mm gap was employed. In every case, the sample was protected with vaseline oil (Panreac, Barcelona, Spain) in order to prevent the sample from drying, as a result of either the time or temperature used. The samples were allowed to rest for a 10 min equilibration time after reaching the measurement position, as equilibration time.

Stress sweeps were carried out at a frequency of 1 Hz to measure the extent of the linear viscoelastic response. Frequency sweeps from 10 to 0.01 Hz at a stress wave amplitude inside the linear region were performed. Storage modulus (G′), loss modulus (G″) and loss tangent (tan δ = G″/G′) values were recorded. Test temperature was always 37ºC.

The tests were carried out in the fresh systems (emulsion and hydrated cellulose) and after incubation in each of the *in vitro* digestion steps (oral, gastric and intestinal), with and without enzymes.
In the hydrated cellulose, temperature sweeps were also carried out at 1Hz, 1ºC/min, in the linear viscoelastic region (strain 1.00E-3) from 20ºC to 37ºC, followed by a time sweep at 37ºC for 20 minutes.

2.6. Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy was carried out according to Rodríguez-García, Laguna, Puig, Salvador, & Hernando (2013). A Nikon confocal microscope C1 unit that was fitted on a Nikon Eclipse E800 V-PS100E microscope (Nikon, Tokyo, Japan) was used. An argon laser line (488 nm) was employed as the light source to excite fluorescent dyes, Rhodamine B and Nile Red. Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) was solubilised in distilled water at 0.2%. This dye was used to stain proteins and carbohydrates. Nile Red (Fluka, Sigma-Aldrich, Missouri, USA) was solubilized in PEG 200 at 0.1 g/L. This dye was used to stain fat. The detection wavelengths were 515 nm and 570 nm for Nile Red and Rhodamine B, respectively. A 60X/1.0/oil/Plan Apo VC Nikon objective lens was used. Twenty microliters of the sample were placed in the central microscope slide. Rhodamine B solution and Nile Red solution were added and the cover slide was carefully positioned to exclude air pockets. The observations were performed 10 min after the diffusion of the dyes into the sample. The images were observed and stored at 1024X1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

The droplet size of the emulsions was determined from the CLSM images. The diameter of at least 95 droplets was measured with the software NIS-Elements F, Version 4.0 (Nikon, Tokyo, Japan).

2.7. Amount of fat released after *in vitro* digestion

Before fat can be digested, one necessary step is the release from the initial matrix and solubilization. In order to determine the real amount of fat that will be available for digestion (bioaccessible), the amount of fat released after centrifugation both from the cellulose ether emulsions and from a whey protein (2% w/w) emulsion containing the same oil content 47% (w/w), considered as control, was calculated. It is necessary to consider that the release of fat from the hydrocolloid/emulsion structure is the first requirement if the digestive enzymes are to act correctly.

After small intestine *in vitro* digestion, the digesta was mixed with 15 mL ethanol and centrifuged (10 minutes, 10.000rpm) (Sorvall® RC-5B Refrigerated Superspeed centrifuge). The total supernatant was quantified and the mixture of water and ethanol was evaporated in a boiling water bath. After evaporation, the container was dried in an oven at 100°C for 30 minutes to completely eliminate residual water or ethanol. The remaining liquid is considered to be the amount of fat released. The amount of fat that remains in the pellet after centrifugation will not be bioaccessible fat.

2.8. Free fatty acid (FFA) content

Fat digestion was determined by measuring the amount of FFA before and at the end of the *in vitro* digestion. FFA were determined in the cellulose ether emulsions and in a whey protein (2% w/w) emulsion, containing 25%

oil (w/w), considered as control. The 25% oil concentration in the control emulsion (without cellulose) was selected on the basics of the fat released results, as the amount of fat released was significantly higher in the control than in the cellulose emulsion. After incubating in the intestine model, 15 mL of ethanol were added to the digestion mixture (6.25g) in order to stop the enzyme action of pancreatic lipase. The sample mixed with ethanol was centrifuged for 10 min at 10,000 rpm in a Sorvall® RC-5B Refrigerated Superspeed centrifuge. The total supernatant was quantified and the free fatty acids were determined in 10 mL of supernatant by titration with 0.05M NaOH and phenolphthalein as an indicator to end point (colored pink).
A standard curve was prepared using oleic acid (0, 50, 100, 150, 200 and 250 mM), and this was used to calculate the free fatty acid concentration of the samples. The results are expressed as "g oleic acid/g fat" and as “g oleic acid/g fat released”.

2.9. Statistical analysis

For each test, three replicates were performed with samples prepared on different days. An analysis of variance (ANOVA) was applied to study the differences between the samples. The least significant differences were calculated by the Tukey test and the significance at $p < 0.05$ was determined. These analyses were performed using XLSTAT 2009.4.03 statistical software (Addinsoft, Barcelona, Spain).

3. RESULTS AND DISCUSSION

3.1. Linear viscoelastic properties

3.1.1. Hydrated cellulose ethers

This work focuses on the investigation into the structural changes during the *in vitro* digestion of o/w emulsions prepared with different types of cellulose ethers. The cellulose ethers will act as emulsifiers and/or thickeners of the aqueous continuous phase, this ability being dependent on the specific cellulose chemical substitution.

As a first step, the properties of the solely hydrated cellulose ethers during digestion were analysed, as they are expected to be of help in the understanding of the mechanisms governing the emulsion behaviour.

The effect of mouth, stomach and small intestine *in vitro* digestion on the frequency dependence at 37°C before and after each digestion step is shown in Figure 1. A big difference was found between the type MC(>30%M) fresh methylcellulose and all the other fresh cellulose ether types. In type MC(>30%M) cellulose, the plateau zone was visualized in the available frequency window, with values of G' higher than G''. In the hydroxypropyl methylcellulose types (HPMC(M/HP:4.3) and HPMC(M/HP:2.9)) and the methylcellulose, type MC(30%M), the terminal zone of the mechanical spectra was visualized, denoting their lower viscoelasticity in comparison to type MC(>30%M).

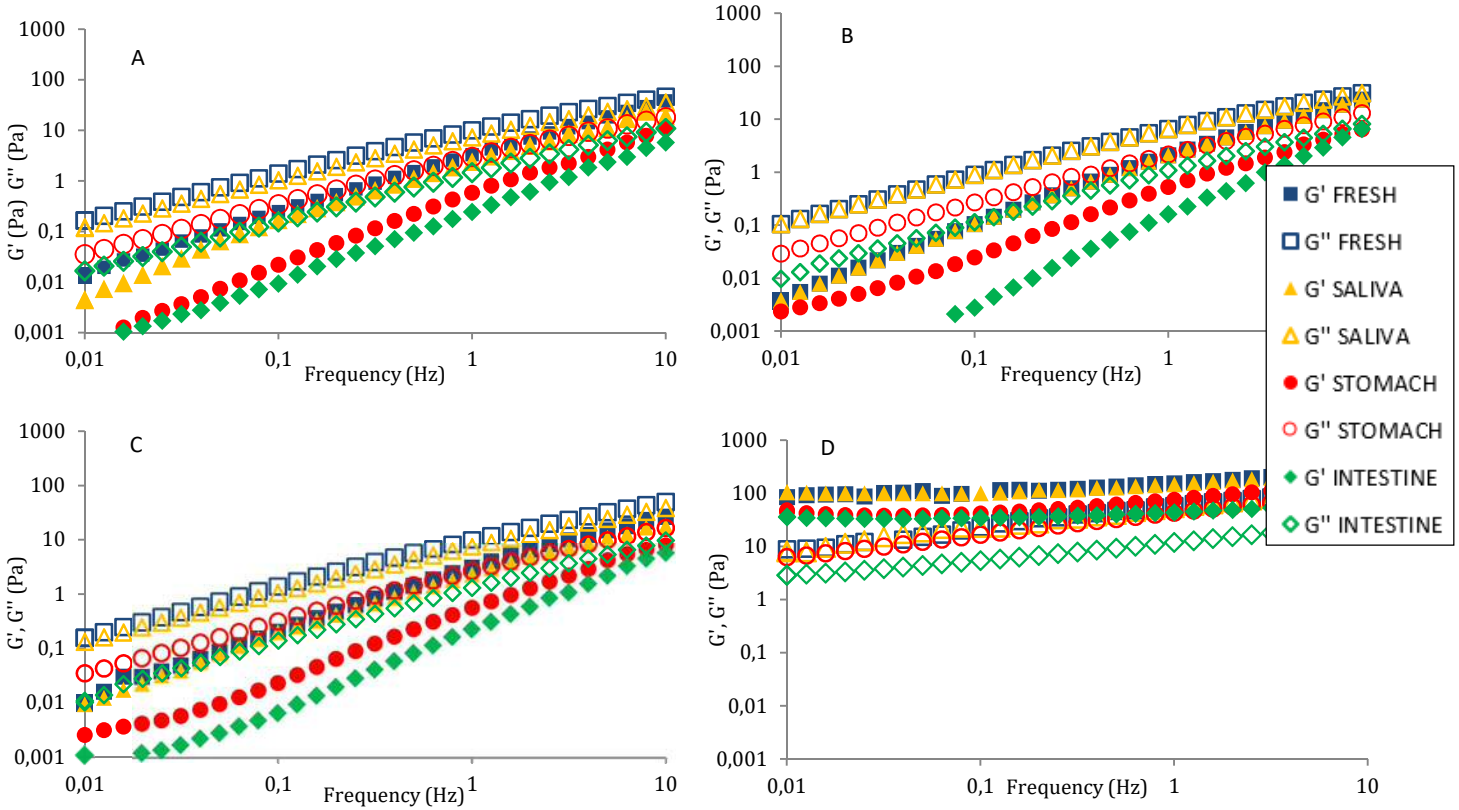

Figure 1. G' and G'' as a function of frequency of the fresh hydrated cellulose ethers and after *in vitro* digestion at 37 °C (A: MC(30%M), B: HPMC(M/HP:4.3), C: HPMC(M/HP:2.9), D: MC(>30%M)).

The mean values of G', G'' and tan δ at 1 Hz corresponding to the fresh and digested cellulose ethers are shown in Table 1.

The viscoelastic behaviour is related to the degree of methoxyl content. The highest values of G' and G'' and the highest viscoelasticity (the lowest tan δ value) was found in type MC(>30%M) methylcellulose, which contains the highest methoxyl content. However, the main hypothesis justifying such a large difference between the viscoelastic behaviour of the type MC(>30%M) methylcellulose and all the other celluloses is the fact that, at 37°C, the sol gel transition associated with thermogelling must have already occurred in this cellulose type. This lower gelation temperature will be associated with the higher methyl content, which increases the hydrophobicity of the system, reducing the gelation temperature.

Methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) ethers possess the unique property of reversible thermogelation. In solution form these polymers are completely hydrated and there is little polymer–polymer interaction other than simple entanglement. Upon heating, these structures distort and break to expose the hydrophobic regions, inducing the formation of aggregates. When lower critical solution temperature (LCST) is reached, sufficient dehydration occurs to promote polymer–polymer interactions instead of polymer–solvent interactions. As a consequence, these cellulose ether solutions start to gel. Thus, gelation is a manifestation of the hydrophobic effect. The specific temperature at which bulk thermal gelation occurs (incipient gel temperature) (IGT) and the strength of the gel formed depends on the type and degree of substitution of the cellulose, molecular weight and concentration and presence of electrolytes (Nishinari, Hofmann, Moritaka, Kohyama, & Nishinari, 1997; Sarkar, 1979).

To investigate whether the greater viscoelasticity of the fresh MC (>30%M) cellulose ethers could be associated with gelation, the changes in G' and G''

upon increasing the temperature from 20 to 37°C were analysed. In Figure 2, the evolution of G' and G'' versus temperature at 1 Hz for cellulose types MC(>30%M), MC(30% M) and HPMC(M/HP:4.3) (as an example of hydroxypropyl methylcellulose) is shown. In type MC(>30%M), the values of G' were equal to G'' in this temperature range. In types HPMC(M/HP:4.3) and MC(30%M), the values of G' and G'' and the viscoelasticity were lower than for type MC(>30%M) and a predominance of G'' versus G' is observed. In consequence, the predominance of G' versus G'' found in the mechanical spectra (Figure 1) of MC(>30%M) cellulose cannot be explained.

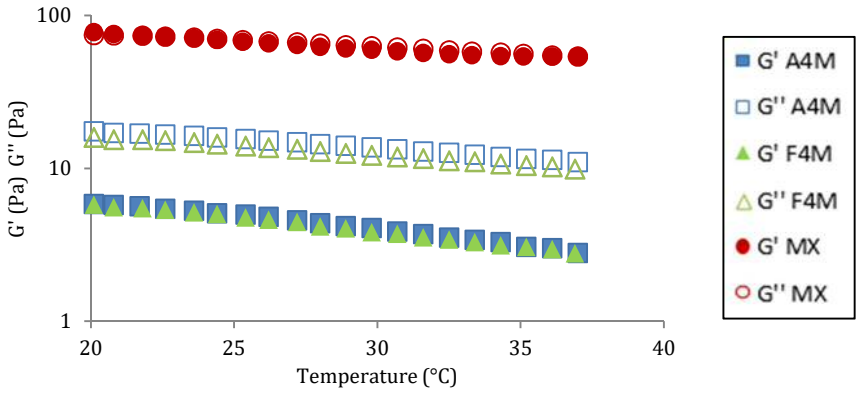

Figure 2. G' and G'' versus temperature in the linear viscoelastic region of the fresh hydrated cellulose ethers at 1Hz.

To obtain further information, the evolution of G' and G'' at 37°C over time just after the application of the temperature sweep was also evaluated (Figure 3). Figure 3 shows that a change in structure occurs at 37°C over time in cellulose MC (>30%M). In type MC (>30%M), an increase in the elastic modulus G' over time was observed, revealing an increase in viscoelasticity and the fact that 37°C is the temperature at which gelation starts. In types MC (30%M) and HPMC (M/HP:4.3), the stability of the

viscoelastic functions G' and G'' is found. This result confirms the hypothesis that the greater viscoelasticity shown by MC (>30%M) can be associated with the fact that gelation is already under way at this lower temperature, because of the higher degree of hydrophobicity in the system.

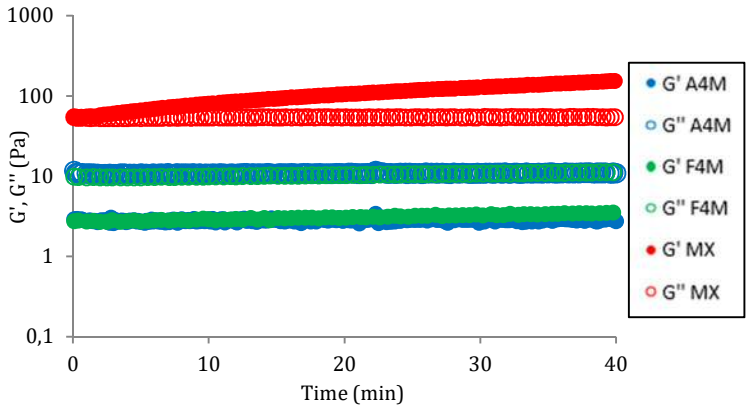

Figure 3. G' and G'' versus time in the linear viscoelastic region of the fresh hydrated cellulose ethers at 1Hz.

It should be emphasized that the correct measurement of the frequency dependence of type MC(>30%M) at 37°C should consider the instability of the polymer at this temperature; therefore, appropriate equilibration time is required in the measurement position of the rheometer, otherwise the structure of the system will not be stable during the frequency sweep. This increase in the viscoelastic properties of type MC (>30%M) at 37°C should be considered an interesting point to take into consideration for further new food applications, for example as a satiety enhancer. An appropriately designed emulsion for the treatment of obesity should not only decrease lipid digestibility/absorbability in the upper intestine but may also promote a feeling of fullness in the consumer, leading to a reduction in the size of the portions. A delayed emptying of the stomach and a prolonged transit of the

food through the small intestine would mean that the obese would reduce their food intake (Maljaars, Peters, Mela, & Masclee, 2008).

During digestion, the behaviour of the different cellulose ethers was very similar (Figure 1 and Table 1).

Table 1. G'. G'' and tan δ for each hydrated cellulose ethers as a function of digestion step.

Cellulose	Digestion step	G' (Pa)	G'' (Pa)	tan δ
MC(30%M)	Fresh	2.9a	9.6a	3.3a
	Saliva	2.4a	7.5b	3.1a
	Stomach	0.6b	3.0c	5.1ab
	Stomach dilution	0.4b	2.3cd	5.5ab
	Intestine	0.2b	1.5de	6.2ab
	Intestine dilution	0.1b	0.8e	7.8b
HPMC(M/HP:4.3)	Fresh	1.9a	5.1a	3.2bc
	Saliva	1.8a	5.2a	2.8c
	Stomach	0.4b	1.9ab	4.5b
	Stomach dilution	0.3b	1.0b	3.7bc
	Intestine	0.2b	1.4b	6.4a
	Intestine dilution	0.1b	0.7b	6.7a
HPMC(M/HP:2.9)	Fresh	2.5a	8.4a	3.4c
	Saliva	2.1a	7.0a	3.4c
	Stomach	0.3b	1.7b	5.2ac
	Stomach dilution	0.4b	2.3b	5.2ac
	Intestine	0.2b	1.2b	6.2ab
	Intestine dilution	0.1b	0.9b	7.2b
MC(>30%M)	Fresh	151.1ab	55.6a	0.4c
	Saliva	143.9ab	51.2ab	0.4c
	Stomach	60.4ab	36.9ab	0.6b
	Stomach dilution	27.4b	23.3ab	0.9a
	Intestine	40.6ab	10.8b	0.3c
	Intestine dilution	188.7a	58.5a	0.3c

[abc]Means with different letter in columns for each parameter and each cellulose type indicate significant differences among the samples ($p < 0.05$) according to the Tukey test.

Mixing with saliva did not affect the viscoelastic properties in any of the cellulose types. After stomach incubation, whereas a significant decrease was found in the values of G' and G'', tan δ was observed to increase, implying the sample was more viscous in character. To differentiate whether the effect found after stomach incubation is associated with the specific stomach conditions (pH, electrolytes, enzymes) or only with the effect of dilution, samples were incubated in the stomach conditions (time, temperature, shaking), but only in the presence of water. In general, no significant differences were found between the viscoelastic parameters of the stomach samples and the water diluted samples, which implies that the changes in the viscoelastic properties occurring in the cellulose ethers are not specific to the stomach environment but associated with a dilution effect. After intestine incubation, a slight decrease, not enough to be significant, was found in the viscoelastic functions, whereas tan δ was seen to increase. Similarly to the stomach, the effects found in the intestine were mainly associated with a dilution effect, rather than with a specific effect of the intestinal fluids in the structure of the hydrated cellulose ether.

3.1.2. Emulsions

The effect of incubation in the mouth, stomach and intestine models on the frequency-dependence of the viscoelastic functions is shown in Figure 4. Similarly to the results found in the case of hydrated cellulose ethers, the MC(>30%M) demonstrated completely different viscoelastic behaviour. The viscoelastic properties of the fresh MC(30%M), HPMC(M/HP:4.3) and HPMC(M/HP:2.9) showed a marked dependence on frequency. The end of the plateau zones of the mechanical spectra were visualized, with a cross over between G' and G'' occurring in the available frequency window.

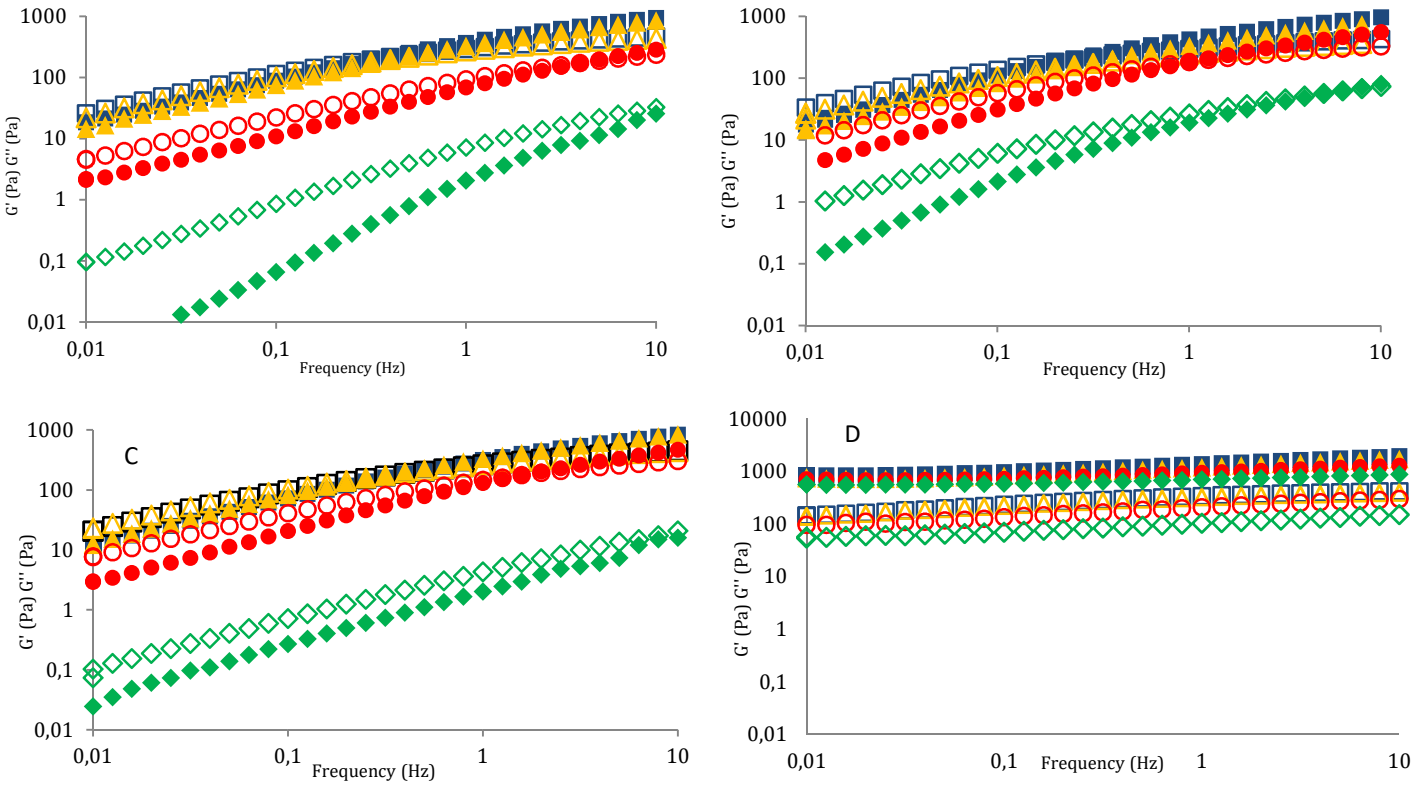

Figure 4. G' and G'' as a function of frequency of the fresh cellulose o/w emulsions and after in vitro digestion at 37 °C (A: MC(30%M), B: HPMC(M/HP:4.3), C: HPMC(M/HP:2.9), D: MC(>30%M)).

These mechanical spectra were defined in previous studies (Sanz et al, 2015 and Espert et al, 2016). Saliva only produced a slight decrease in the values of both G' and G'', and did not significantly affect tan δ values at 1 Hz in any of the cellulose emulsions studied (Table 2).

Table 2. G, G'' and tanδ for each cellulose emulsion as a function of digestion step.

Cellulose	Digestion step	G' (Pa)	G'' (Pa)	tan δ
MC(30%M)	Fresh	418.2a	295.9a	0.7b
	Saliva	368.6a	293.5a	0.8b
	Stomach	42.4b	65.5bc	1.6ab
	Stomach dilution	76.5b	103.7b	1.5ab
	Intestine	3.3b	7.0c	2.3a
	Intestine dilution	8.4b	19.5bc	2.3a
HPMC(M/HP:4.3)	Fresh	412.6a	293.9a	0.7c
	Saliva	318.6b	232.4ab	0.7c
	Stomach	183.1c	181.1bc	1.0bc
	Stomach dilution	134.0c	144.9c	1.1b
	Intestine	13.5d	19.5d	1.5a
	Intestine dilution	99.0cd	119.0c	1.2ab
HPMC(M/HP:2.9)	Fresh	320.5a	261.2a	0.8b
	Saliva	323.8a	259.8a	0.8b
	Stomach	138.1b	155.2b	1.1b
	Stomach dilution	82.3bc	104.5b	1.3b
	Intestine	3.1c	7.8c	2.4a
	Intestine dilution	53.2bc	70.6bc	1.4b
MC(>30%M)	Fresh	1834.3a	339.8a	0.2ab
	Saliva	1368.5a	368.6a	0.3a
	Stomach	832.0a	194.2b	0.2ab
	Stomach dilution	957.9a	228.0b	0.2ab
	Intestine	817.6a	127.0b	0.2b
	Intestine dilution	1392.0a	210.8b	0.2b

[abc]Means with different letter in columns for each parameter and each cellulose type indicate significant differences among the samples ($p < 0.05$) according to the Tukey test.

After incubation in the stomach, although a significant decrease was found in the values of G' and G'' in emulsions MC(30%M), HPMC(M/HP:4.3) and HPMC(M/HP:2.9), the tan δ values were observed to increase. The shape of the mechanical spectra also changed after stomach incubation with a displacement of the cross over point towards higher frequencies, revealing a decrease in viscoelasticity, and the range of frequencies wherein G'' predominates over G' grows. In comparison, a further decrease in viscoelasticity was found after intestine incubation (a decrease in the moduli, and a displacement of the cross over point to higher frequencies).

When incubated in the stomach and intestine models in the presence of water only, there were no significant differences if compared with the standard incubation using MC(30%M), HPMC(M/HP:4.3) and MC(>30%M) emulsions, revealing that the observed change must be mainly associated with the contribution of water dilution, more than with a specific effect of pH or enzymes. It was type HPMC(M/HP:2.9) which was the most affected by intestine incubation, with a significant increase in tan δ values in comparison to the water dilution, implying that the structure of the HPMC(M/HP:2.9) emulsion was specifically affected by the intestine fluids (Figure 5). The MC(>30%M) emulsion demonstrated different viscoelastic properties, both in terms of the behaviour of the fresh sample, and of the changes during digestion. The changes observed in the MC(>30%M) emulsion during digestion were smaller than in the other cellulose emulsions. The fresh MC(>30%M) emulsion followed a viscoelastic behaviour which was characterized by values of G' higher than G'' in the entire available frequency window, with practically no frequency dependence. Although incubation in the mouth, stomach and intestine produced a mild, progressive decrease in the values of G' and G'', no significant differences in the viscoelastic functions were found.

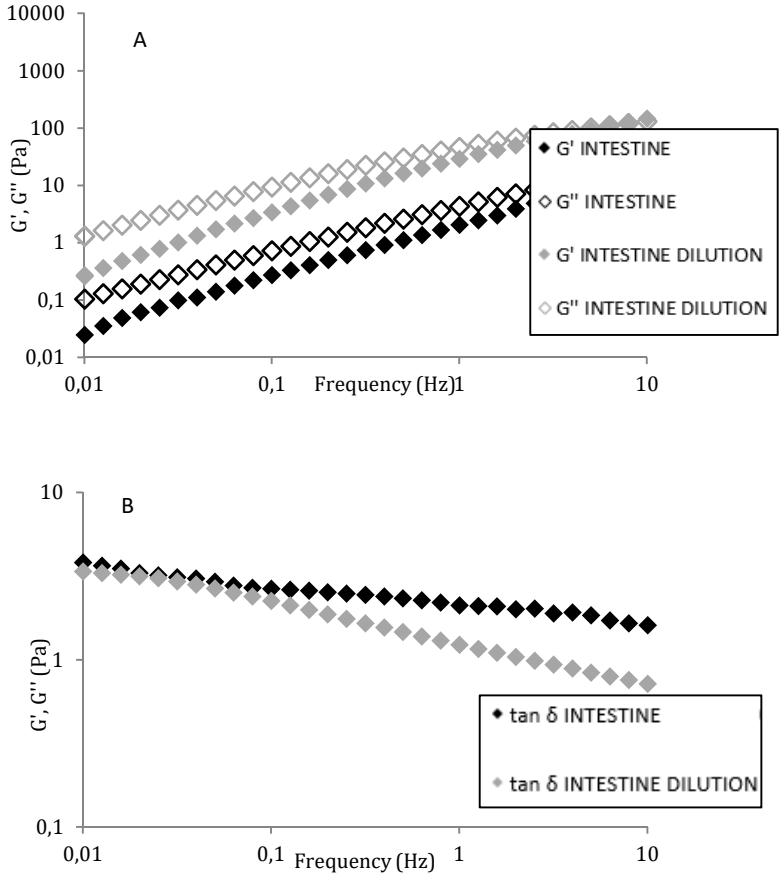

Figure 5. G', G'' (A) and tan δ (B) as a function of frequency of HPMC(M/HP:2.9) o/w emulsion after the intestine step versus the water-diluted sample (at 37ºC).

3.2. Confocal Laser Scanning Microscopy of the emulsions

The emulsions made with cellulose were generally compact and stable in appearance, in all likelihood due to the formation of a network of hydrated cellulose in the aqueous phase which covers the fat globules (Lin, Wang, & Xu, 2003). This network prevents possible flocculation and coalescence phenomena thanks to the more limited mobility of the globules inside the

matrix (Aranberri, Binks, Clint, & Fletcher, 2006). The original emulsions formulated with the hydroxypropyl methylcelluloses, HPMC(M/HP:4.3) and HPMC(M/HP:2.9) (Figure 6, A and E), made up of globules which are semi-rounded in appearance and of differing sizes (from 2.14 to 21.34 μm), looked compact and thick. The mean diameters of F4M and K4M emulsions were 9.84 and 9.35 μm, respectively. No flocculation or coalescence phenomena could be observed.

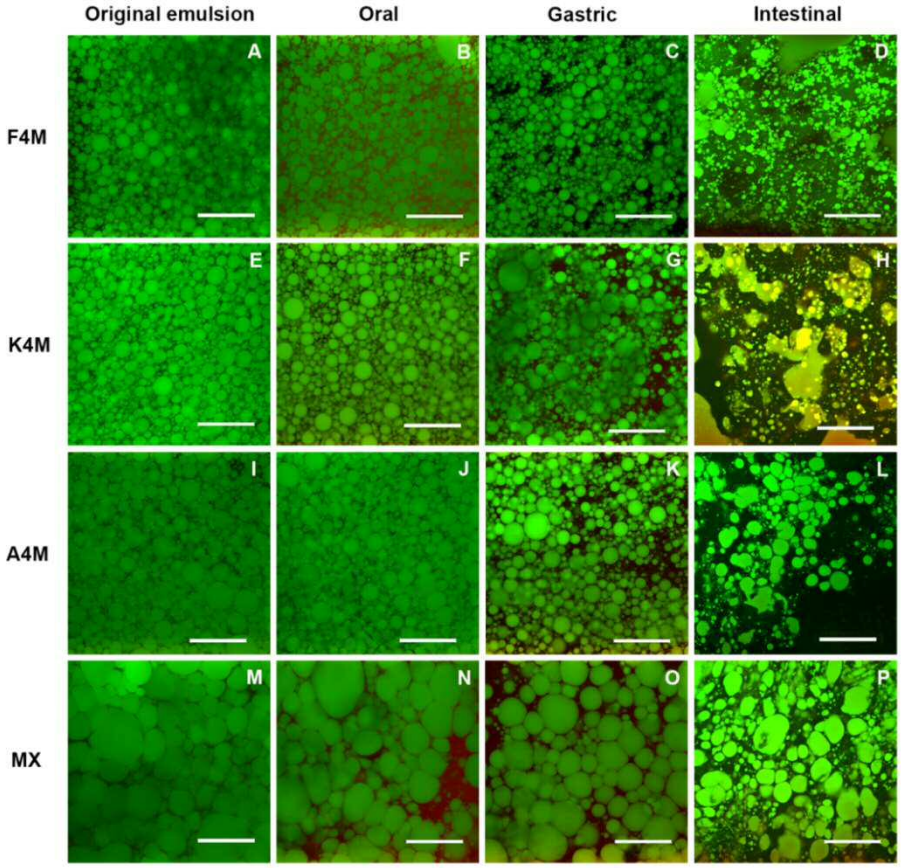

Figure 6. Confocal laser scanner microscopy (CLSM) of stained fresh cellulose o/w emulsions and after in vitro digestion at 37 °C with Rhodamine B and Nile Red (fat in green. Magnification 60x.

The *in vitro* mouth digestion phase (Figure 6, B and F) did not seem to affect the structure of these emulsions as they were very similar in appearance to the undigested or original one (Figure 6, A and E). The mean diameters of F4M and K4M emulsions at the mouth digestion phase were 8.67 and 9.55 μm, respectively. Neither did the *in vitro* gastric digestion phase (Figure 6, C and G) affect either the size (8.62 μm for F4M and 9.00 μm for K4M) or the shape of the globules which were still semi-rounded; it did, however, influence the overall appearance of the emulsion which lost density and became more fluid due to the addition of the digestion fluids. The *in vitro* intestine digestion phase (Figure 6, D and H) had a marked effect on the two emulsions, HPMC(M/HP:4.3) and HPMC(M/HP:2.9); the globules became significantly smaller (3.28 and 2.51 μm, respectively) if compared with the original emulsions that are to say, undigested. In accordance, Bellesi, Martinez, Ruiz-Henestrosa, & Pilosof (2016) observed that HPMC-coated droplets only underwent a slight change in size throughout gastric digestion, which remained almost constant, but sizeable changes when passing from gastric to intestinal conditions, where the oil droplets became smaller. Furthermore, flocculation and coalescence phenomena were observed, which gave rise to large, irregular structures; these were more significant in the case of emulsion HPMC(M/HP:2.9). Some authors (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014; López-Pena, et al., 2016) also observed an appreciable aggregation of lipid droplets after the intestinal phase leading to an increase in mean particle size, which suggests that the droplets had become coalesced or flocculated. Droplet aggregation may have occurred because of the presence of bile salts and mineral ions in the simulated intestinal fluid (Bellesi et al., 2016; Li & McClements, 2010). Therefore, these results indicate that bile salts are able to displace some of the cellulose ether molecules from the droplet surface,

facilitating the accessibility of lipase to the lipid core (Mun et al., 2007), thereby resulting in emulsion destabilization (Singh & Sarkar, 2011). Mineral ions may also have promoted droplet flocculation through charge neutralization and bridging effects (Li & McClements, 2010). The fact that the droplets were flocculated or coalesced in the emulsions might alter their rate of lipid digestion. Firstly, droplet flocculation reduces the surface area of lipids available for lipase adsorption (Bellesi et al., 2016; López-Pena et al., 2016). Secondly, it would be more difficult for lipase molecules to penetrate through a floc and reach the lipid droplets in the interior (Espinal-Ruiz et al., 2014; Li & McClements, 2010). Therefore, what the micrographs show is that, in all likelihood, the HPMC(M/HP:4.3) and HPMC(M/HP:2.9) emulsions have been digested to a similar degree. Furthermore, emulsified fat globules may still be seen in both cases, which would indicate that neither of the emulsions has been fully digested.

The original emulsions formulated using methylcellulose, MC(30%M) y MC(>30%M) (Figure 6, I and M), were more compact and denser in appearance than those formulated using hydroxypropyl methylcellulose (HPMC(M/HP:4.3) and HPMC(M/HP:2.9)) (Figure 6, A and E). This can be attributed to the fact that there is a greater presence of hydrophobic substituent groups in the methylcellulose compared with the hydroxypropyl methylcellulose. This favours intermolecular interactions between the hydrophobic groups and leads to the emulsion looking denser (Sanz et al., 2015). The MC (30%M) emulsion (Figure 6 I) had differently sized globules (from 2.18 to 31.18 μm), polyhedric in shape, whereas the MC(>30%M) emulsion was made up of globules which were generally bigger in size (17.74 μm) than those in the other emulsions (10.41 μm in MC(30%M), 9.84 μm in HPMC(M/HP:4.3) and 9.35 μm in HPMC(M/HP:2.9)) and were also polyhedric in shape (Figure 6 M). As in the case of the HPMC(M/HP:4.3) and

HPMC(M/HP:2.9) emulsions, the *in vitro* mouth digestion phase (Figure 6, J and N) did not seem to affect the structure of the MC(30%M) and MC(>30%M) emulsions, since they were similar in appearance to the undigested ones. Moreover, the droplet size remained almost unchanged (10.38 and 16.07 μm in A4M and MX emulsions at mouth level). The *in vitro* gastric digestion phase, however, (Figure 6, K and O) did modify the appearance of the globules as their structure was less polyhedric. The *in vitro* intestine digestion (Figure 6, L and P) markedly affected the structure of both emulsions, as has previously been seen in the case of the emulsions formulated using hydroxypropyl methylcellulose. The MC(30%M) and MC(>30%M) digested emulsions were of irregular appearance and different from the rest of the emulsions. Not only was the structure of the fat in these two emulsions different in shape and size, there being both small round globules and large polyhedric ones, but there were also major flocculation and/or coalescence phenomena. In the case of the MC(30%M) emulsion (Figure 6 L), the globules were seen to be generally smaller (4.94 μm) than in the MC(>30%M) (6.42 μm), which could point to the fact that the former emulsion was more digested than the latter. Nevertheless, as occurred with the emulsions formulated using hydroxypropyl methylcellulose, the MC(30%M) and MC(>30%M) emulsions still partly preserved a compact appearance prior to digestion because the globules remained embedded within the emulsions.

If the emulsions are compared and the results analysed, it can be seen that it is the emulsions containing methylcellulose which have been digested the least, particularly the MC(>30%M). The reason for this is probably twofold: it is due both to the original dense appearance of these emulsions, made up of large, closely bonded globules which means that there is a limited surface area upon which the enzymes may act and also to the fact that they preserve

this very density after *in vitro* digestion. As mentioned before, this is especially so in the case of the MC(>30%M) emulsion, which has the largest globules both before and after digestion. Espinal-Ruiz et al. (2014) observed that methylcellulose promoted depletion flocculation when it is present in sufficiently high concentrations and, therefore, this highly flocculated appearance resulted in appreciably reduced digestion rates. So, according to the results, it is important to take into account the size of the lipid droplets reaching the small intestine. As described previously, the initial size of the lipid droplets affects their digestion rate because the surface area of lipid exposed to the surrounding aqueous environment is inversely related to the mean droplet diameter. Emulsions with smaller droplets have a bigger specific surface area, which provides more sites for lipase molecules to bind to and, therefore, the release rate of free fatty acids (FFA) was appreciably higher (Li & McClements, 2010).

3.3. Fat release and free fatty acid generation after intestine digestion

In this study, the quantification of the fat released from the structure is considered a method by which to simulate the amount of fat which will be accessible to bile and the digestive enzymes during *in vitro* digestion (Espert et al., 2016). It is to be expected that the higher fat that is released, the greater is the possibility that the digestive fluids can exert their functionality, leading to a higher degree of hydrolysis of the triglycerides into monoglycerides and free fatty acids.

Table 3 shows the amount of fat released after emulsion centrifugation. When compared with all the cellulose ether emulsions, the whey protein emulsion showed the highest percentage of fat released (87.8%).

Table 3. Fat released after intestine *in vitro* digestion of the whey protein and the cellulose emulsions. Oil concentration in all the emulsions was 47%.

Emulsion type	Released fat (g)	Released fat/ initial fat (%)
Whey protein (control)	2.57a	87.80a
HPMC(M/HP:4.3)	2.01ab	68.61ab
HPMC(M/HP:2.9)	1.94ab	66.13ab
MC(30%M)	1.40bc	47.61bc
MC(>30%M)	0.31c	10.50c

[abc]Means with different letter in columns for each parameter indicate significant differences among the samples ($p < 0.05$) according to the Tukey test.

Significant differences were found among the difference cellulose ether emulsions. The two HPMC types studied showed a significantly higher amount of fat released than the MC(>30%M) type. No differences were found between HPMC types HPMC(M/HP:4.3) and HPMC(M/HP:2.9). Although no significant differences were found between MC(30%M) and MC(>30%M) types, the MC(>30%M) emulsion was the one that released the lowest amount of fat (only 10.5%). This result is associated with its highest hydrophobicity and viscoelasticity. After digestion the changes in the structure of emulsion MC(>30%M) remained little, reducing the accessibility of the enzymes and other digestive fluids to the fat.

In Table 4 the amount of oleic acid values generated in the different cellulose emulsions and in a whey protein control with 25% oil content are shown. The percentage of oil in the control sample (25%) differed from the one employed in the cellulose ether emulsions (47%) because of their big differences in fat released. As the percentage of fat released was significantly higher in the control without cellulose, the initial amount of oil was reduced, so the real amount of fat which becomes available for the digestion enzymes

will be closer to the fat released in the cellulose emulsion. When considering the amount of oleic acid generated after the emulsion digestion, differences were also found between the whey protein and the cellulose ether emulsions (Table 4). The amount of oleic acid (in relation to the total initial fat) present in the emulsion was significantly higher ($p<0.05$) in the whey protein emulsion than in all the cellulose ether emulsions. The lowest amount was found in the MC(>30%M) emulsion, which indicates that this type of cellulose is the most effective in terms of reducing fat digestion. No significant differences ($p>0.05$) were found among the cellulose types HPMC(M/HP:4.3), HPMC(M/HP:2.9) and MC(30%M).

Table 4. Oleic acid values after in vitro digestion of the cellulose emulsions (47% oil) and the whey protein with 25% oil.

Emulsion type	Oleic acid (g)	Oleic acid/initial fat	Oleic acid/released fat
Whey protein (control)	0.69a	0.44a	0.51a
HPMC(M/HP:4.3)	0.40b	0.14b	0.20a
HPMC(M/HP:2.9)	0.38b	0.13b	0.20a
MC(30%M)	0.30b	0.10b	0.22a
MC(>30%M)	0.14c	0.05c	0.44b

[abc]Means with different letter in columns for each parameter indicate significant differences among the samples ($p < 0.05$) according to the Tukey test.

If the results are expressed as g of oleic acid in relation to g of fat released, the differences associated with a mere physical effect are eliminated (Espert et al., 2016). In this case, the MC(>30%M) emulsion showed higher values than the other cellulose emulsions, which indicates that the big inhibition of fat digestion in this system can be mainly related to the physical effect. The

other cellulose types also show lower values than the whey protein emulsion.

Fat constitutes the dispersed phase in the emulsions and it is, therefore, involved inside the continuous phase structure. Fat digestion requires, as a first step, the "destructuration" or weakening of the continuous outer phase, so the activity of the digestive fluids (bile and enzymes) can be effective. In this study, it is speculated that the resistance of the continuous phase to digestion is associated with a decrease in the amount of fat that the digestive fluids have access to. Lipase is water soluble and can only work on the surface of fat globules, for which reason emulsification is a necessary prelude to its efficient activity. Bile acids play a critical role in lipid assimilation by promoting emulsification. On exposure to a large triglyceride aggregate, the hydrophobic portion of bile acids intercalate into the lipid, with the hydrophilic domains remaining on the surface.

4. CONCLUSIONS

Cellulose chemical substitution affects the emulsion structure, structural changes during digestion and fat bioaccessibility. Although all of the cellulose ethers were found to be effective at reducing emulsion fat bioaccessibility, differences were found. The highest methoxyl content (type MC(>30%M)) was associated with the highest viscoelasticity (before and after digestion), and the lowest fat bioaccessibility. The highest viscoelasticiy of type MC(>30%M) was mainly associated with its greater hydrophobicity, which reduces the gelling temperature, so that the system gellified during incubation at 37ºC. The fact that the largest oil droplets are found in the initial MC(>30%M) emulsion and maintained during digestion, can also be related to the decrease in fat digestibility.

The total fat released from the emulsion structure after digestion is an indicator of the degree of structural resistance, and is positively related to the fat digestion process. It is speculated that the more resistant the structure is after digestion, the higher the barrier that will be exerted against an appropiate physical contact between the emulsified lipid and the digestive enzymes, with a subsequent decrease in fatty acid generation.
A physical barrier is recognized as being the main reason for the fact that the lowest fat digestion is to be found mainly in type MC (>30%M), the effectivity of the barrier being dependent on the cellulose ether substitution type: the physical effect was more marked for type MC(>30%M) (a higher methoxyl content) and lower for type HPMC(M/HP:2.9) (a lower methoxyl content).
Animal testing will be required to validate the obtained *in vitro* results.
Future research will also focus on using the cellulose emulsions as substitutes for conventional fat in different food applications.

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Rheological and microstructural behaviour of xanthan gum and xanthan gum-Tween 80 emulsions during *in vitro* digestion.

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ABSTRACT

The rheological, texture and microstructure properties of xanthan gum (XG) water solutions and XG emulsions, with and without Tween 80, were investigated and related to the emulsion fat lipolysis during *in vitro* digestion. The effect of digestion on the rheological properties of the XG differed between the water and the emulsion systems. The viscoelasticity of XG aqueous dispersion decreased after stomach digestion, while an increase in viscoelasticity was found in the XG emulsion without Tween 80. The viscoelasticity results were explained by the microstructure observations, where fat coagulation in the stomach in vitro model (pH: 2.5 and pepsin) is observed in the absence of Tween 80, associated with the weaknesses of the XG structure at acidic pH. In all the systems, a decrease in the viscoelastic properties is observed after intestine digestion. In comparison to control emulsions without XG, the XG emulsions partially resist the digestion process, decreasing the quantity of free fatty acids (FFA). In the presence of Tween 80, the decrease in fat digestion was enhanced, the XG-Tween 80emulsion being the one with the lowest fat digestibility. These results may have important implications for the design of satiating food and food with low fat digestibility.

Key words: Microstructure, Non-digestible emulsions, Rheology, Xanthan gum

1. INTRODUCTION

The design of non-digestible emulsions has gained increasing importance among the rational strategies with which to control excessive dietary fat intake. It has been shown that the rate of fat digestion depends on certain factors, such as the size of emulsion droplets, the degree of mastication, the interfacial composition, the presence of certain food components or the type of emulsifier used. Mun, Decker, & McClements (2007) analyzed in vitro digestibility by comparing the influence of different emulsifiers. Tween 20 provided a greater ability to prevent the action of lipase in the emulsified lipids than the other emulsifiers.

In addition to the type of emulsifier, the rheological and structural properties of the continuous phase have also been related to the emulsion digestion properties. The effect of employing gelatin, colloidal casein and a starch dispersion in a 20% oil emulsion with sodium caseinate and monoglycerides as surfactants was studied by Wooster et al. (2014). The incorporation of carboximethylcellulose in whey protein emulsions reduces the access of lipase to the fat surface, which has been associated with an increase in viscosity (Malinauskyte et al., 2014). Low methoxyl pectin increased fat digestion in 2% corn oil caseinate emulsions, which was attributed to a suppression of fat flocculation by pectin. On the contrary, the incorporation of high levels of pectin in emulsions with lactoferrine and Tween 80 decreases fat digestion, probably due to calcium fixation and the increase in viscosity, which reduces lipase effectivity (Zhang, Zhang, Zhang, Decker, & McClements, 2015). Cellulose ethers have also been found to be an effective tool for the purposes of reducing fat digestion in highly concentrated o/w emulsions (Espert et al., 2017). Emulsions stabilized by protein-xanthan gum mixtures were more effective for decreasing the

digestion rate than whey protein emulsions, as shown by the stability analysis and free fatty acids release rates during *in vitro* digestion. According to the authors, the xanthan gum formed coatings around the interfacial proteins that inhibited the adsorption of protease and lipase, on the other hand xanthan gum altered the mixing and mass transfer processes due to changes in rheology of the mixtures of emulsions and simulated gastrointestinal fluids (Sun, et al., 2018).

A delayed emptying of the stomach and a prolonged transit of the food through the small intestine has been associated with the control of food overconsumption (Maljaars, Peters, Mela, & Masclee, 2008). A variety of hydrocolloids have been linked with the sensation of satiety, as a consequence of their thickening, gelling and/or non-digestible properties. The acid self-structuring of anionic polysaccharides is regarded as one of the most efficient strategies with which to promote the control of gastric structuring and retard stomach emptying, leading to an enhanced satiety response. Intragastric structural potential has been found in anionic polysaccharides, such as sodium alginate, low methoxyl pectins and low acyl gellan gum. In vitro intragastric gelation of a mixed solution of whey protein isolate and pectin has been found (Zhang & Vardhanabhuti, 2014). Methylcellulose with a higher methoxyl content has also been found to increase its viscoelastic properties during incubation at 37°C, as a consequence of thermal gelation (Espert et al., 2017).

Emulsion gelation within the stomach is considered a promising method of preventing emulsion destabilization. In addition, the emulsions that gel intragastrically are anticipated to be highly potent at slowing down the emptying of the stomach, thereby providing an increased sense of fullness (Madadlou, Rakhshi, & Abbaspourrad, 2016).

Emulsifiers have differing emulsion-forming abilities due to the differences in how well they adsorb to surfaces, reduce interfacial tensions and prevent droplet aggregation (Ozturk & McClements, 2016). Most polysaccharides are highly hydrophilic molecules that not possess surface activity, and are, therefore, not good emulsifiers. Xanthan gum is a stiff high molecular weight anionic polysaccharide produced by the microorganism Xanthomonas campestris (García-Ochoa, Santos, Casas, & Gómez, 2000; Moschakis, Murray, & Dickinson, 2005). Xanthan gum is a representative example of hydrophilic polysaccharide with no surface-active properties; however, it brings about an increase in the viscosity of the aqueous phase, hindering the movement of the drops and, therefore, stabilizing the emulsions. Xanthan gum solutions exhibit pseudoplastic behaviour, even at very low concentrations, being a suitable thickener for many foodstuffs.

One way to provide surface activity is through the use of surfactants like polysorbates. Polyoxyethylene (20) sorbitan monooleate (Tween 80) is a low molecular weight non-ionic surfactant. By generating short-range hydration/protrusion forces, non-ionic surfactants may prevent emulsion droplets from coming into direct contact with each other and, hence, can suppress coalescence. Non-ionic surfactants are not influenced by the presence of electrolytes due to the fact that the dimensions of the non-ionic chains are only slightly influenced by electrolyte concentration. Moreover, steric repulsion is fairly insensitive to pH. Emulsifiers are surface-active substances that can be adsorbed at an oil-water interface and prevent the dispersed-phase droplets in emulsions from aggregating. At concentrations higher than the critical micellar concentration (CMC), Tween 80 contributes to a dense adsorption layer, preventing lipase from coming into direct contact with the lipids (Yao et al., 2013). Mun et al. (2007) investigated the

intestinal digestion of sodium caseinate, whey protein isolate (WPI) and Tween 20 emulsions. They concluded that the stability of the emulsions to droplet flocculation and coalescence during hydrolysis was dependent on the emulsifier type, with the WPI emulsions being the least stable and the Tween 20 emulsions the most stable.

This study aims to better understand the functionality of xanthan gum during in vitro digestion and to evaluate its suitability for either new food applications, such as non-digestible emulsions, or food to increase the sensation of fullness. Three different xanthan gum matrices were studied: a water solution of xanthan gum, a xanthan gum oil water emulsion (without emulsifier) and a Tween 80 o/w emulsion stabilized by xanthan gum. The viscoelastic and textural properties and the microstructure were evaluated after *in vitro* mouth, stomach and small intestine simulation. Free fatty acids were quantified at the end of digestion, as a means of measuring fat digestion.

2. MATERIALS AND METHODS

2.1 Emulsion preparation

2.1.1 Xanthan gum and xanthan gum-Tween 80 emulsions

Four different systems were prepared. These consisted of: sunflower oil ("Koipe Sol", Deoleo S.A., Spain) (47% w/w), xanthan gum (Cargill, France) (2% w/w) with and without Tween 80 (Sigma-Aldrich Chemical Company) as emulsifier (5%), and water up to 100% (w/w). The XG was first dispersed in the water at room temperature using a Heidolph stirrer at 300-500 rpm for 20 minutes. Then, the oil was gradually added while continuing to stir,

increasing the speed up to 1800 rpm. Stirring was maintained for an additional 15 minutes. The 200 g mixture contained in a 500 ml beaker was then homogenized using an IKA T18 basic (Ultra-Turrax) with the dispersion tool S18N-19G (stator diameter 19 mm and rotor diameter 12.7 mm) at 6500 (1/min) for 30 s, and subsequently at 24000 (1/min) for 60 s. Despite not having emulsifier, the XG emulsions were physically stable at room temperature and they had a weak gel-based structure, which was associated to the high level of XG employed (2%).

Emulsions containing emulsifier followed the same preparation process. In this case, Tween 80 was added before the oil, maintaining the stirring for 5 minutes, and following the same subsequent steps.

The emulsions were stored at room temperature for 24 hours before the measurements.

2.1.2 Control emulsions for the analysis of free fatty acids

To evaluate the effect of XG in fat digestibility, two control emulsions without XG were employed : a whey protein (WP) o/w emulsion and a Tween 80 o/w emulsion. The WP was composed of WP (2%) (w/w), oil (25%) (w/w) and water up to 100%. The Tween 80 emulsion was composed of Tween 80 (5%) (w/w), oil (25%) (w/w) and water up to 100%.

The control emulsions were prepared by dissolving whey protein powder (Best Protein, Barcelona, Spain) or Tween 80 in water at room temperature, stirring at 300 rpm (5-10 minutes) and subsequently adding the sunflower oil slowly. Finally, the mixtures were homogenized under the same conditions as the model emulsions.

2.2 Xanthan gum water solution

A xanthan gum water solution (2% w/w) was also prepared. The XG powder was hydrated by gently mixing with water at room temperature for approximately 20 min (Heidolph stirrer) at 800 rpm, increasing the stirring speed continuously up to 1800 rpm to permit a correct XG hydration.

2.3 *In vitro* digestion model

A three-step *in vitro* digestion model mimicking oral, gastric and small intestinal digestion previously described by Borreani et al. (2017) and Espert et al. (2017) were carried out, maintaining the detailed enzymatic, time and temperature conditions.

Samples were also incubated with only the addition of distilled water, simulating the level of dilution of the stomach and small intestine model. The incubation conditions were the same as those in the digested samples with enzymes.

2.4 Rheological measurements

Small amplitude oscillatory tests were carried at 37°C using a controlled stress rheometer (AR-G2, TA Instruments) with a Peltier heating system. A serrated plate-plate (40 mm diameter) was employed with 1 mm gap. Vaseline oil was applied to prevent dehydration. The linear viscoelastic region was determined by performing a stress sweep at 1 Hz. Then, frequency sweeps were performed from 10 to 0.01 Hz at a stress wave amplitude within the linear region. The dependence of G' and G'' versus frequency was adjusted to a power law equation:

$$G' = K' * f^{n'}$$

$$G'' = K'' * f^{n''}$$

Where, n' and n'' are the power law indexes related to the slope of the straight lines in the log-log scale; and K' and K'' corresponds to the values of G' and G'' at 1 Hz.

The tests were carried out in the fresh systems (xanthan gum water solutions, xanthan gum-oil systems, and xanthan gum-Tween 80 O/W emulsions) and after incubation in each of the *in vitro* digestion steps (oral, gastric and intestinal), with and without enzymes (effect of water dilution).

2.5 Extrusion properties

The extrusion properties were evaluated using a TA-XT plus Texture Analyzer equipped with the Texture Exponent software (Stable Microsystems, Godalming, UK).

A bucket (5 cm in diameter and 7.5 cm in height) was filled with the samples (50 g) and a back-extrusion test was conducted using a compression probe of 4.9 cm diameter. The distance force was 15 mm, the compression rate 1 mm s^{-1}, and the trigger force 10 g. From the force-time profiles obtained, the area under the curve and the maximum force achieved were recorded.

2.6 Microstructure

The microstructure of the emulsions was examined by optical microscopy (Nikon Eclipse 90i, Kanagawa, Japan). A 20x objective was used to observe an aliquot of each sample previously placed on a microscope slide. The

systems were observed before and after digestion in the *in vitro* models (mouth, stomach and intestine).

2.7 Free fatty acid release

The release of FFA from the emulsified lipids was determined at the end of in vitro digestion by using a titration method previously described by Mun, Decker, & McClements (2007), with some modifications (Espert et al., 2017). Two controls were used without hydrocolloid barrier: a whey protein emulsion and a Tween 80 emulsion (see section 2.1.2).

2.8 Statistical analysis

The data obtained were statistically analyzed by means of an analysis of variance (ANOVA) using Tukey's test in order to establish significant differences between the samples ($P< 0.05$). Calculations were carried out using the XLSTAT statistical software (2009.4.03, Microsoft Excel®, Barcelona, Spain). The assays were performed in triplicate with samples prepared on different days.

3. RESULTS AND DISCUSSION

3.1 Effect of *in vitro* digestion on the linear viscoelastic properties

The *in vitro* simulation of the different steps of the digestion process (oral, gastric and intestinal) in the mechanical spectra of the xanthan gum water solution, xanthan gum o/w emulsion and xanthan gum-Tween 80 o/w emulsion is shown in Figure 1.

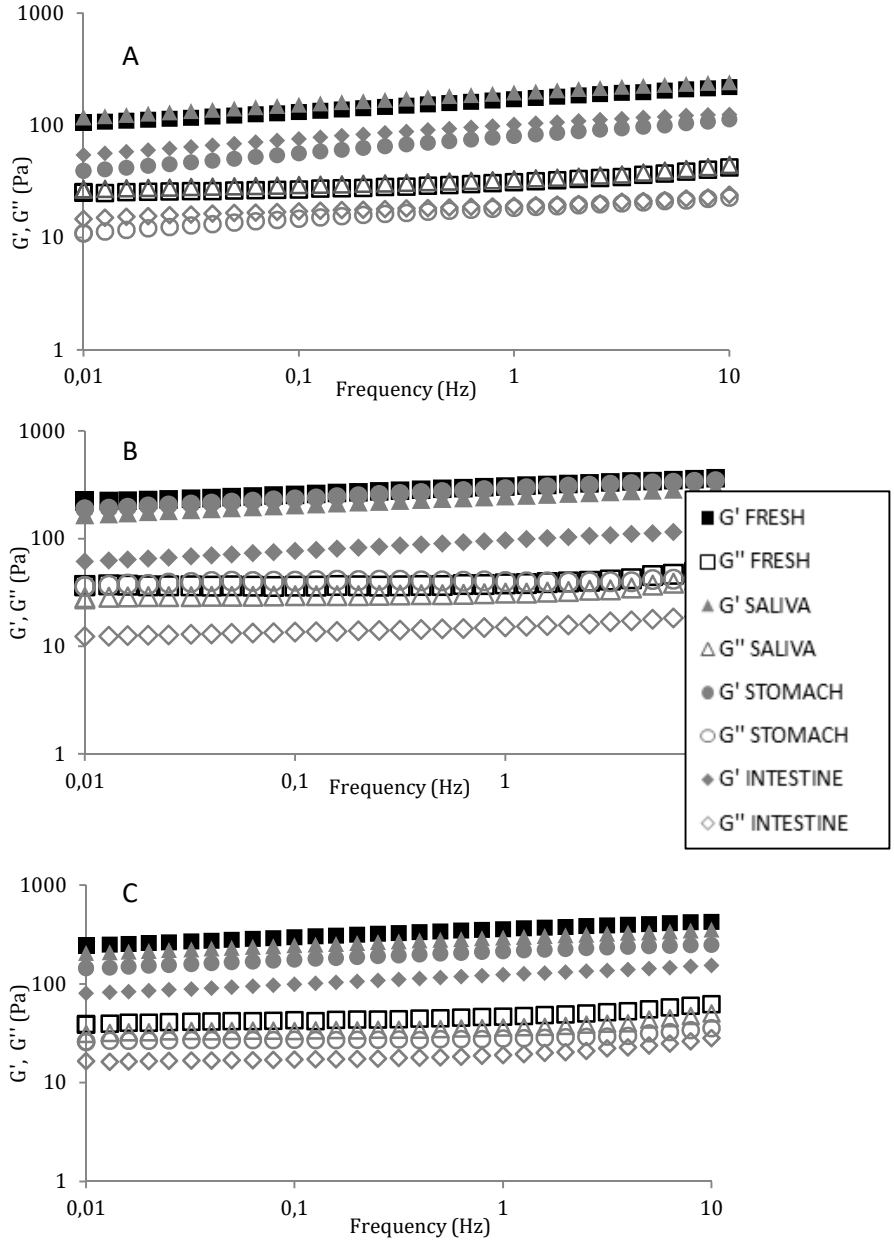

Figure 1. G′ and G′′ as a function of frequency of the xanthan gum water solution (A), the xanthan gum o/w emulsion (B) and the xanthan gum Tween 80 o/w emulsion (C) during *in vitro* digestion.

3.1.1 Xhantan gum water solution

The evolution of G' and G'' versus frequency for the fresh xanthan gum water solution reveals the plateau area of the mechanical spectra with values of G' higher than those of G''. Xanthan gum produces highly viscous solutions, even at low concentrations (Born, Langendorff, & Boulenguer, 2002; Galindo, 1994; García-Ochoa et al., 2000; McNeely, & Kang, 1973) because of its high molecular weight and its secondary structure (Born et al., 2002; Katzbauer, 1998; Rinaudo, 2001).

Mixing xanthan gum water solution with saliva produces no effect on the shape or on the values of G' or G'' (Figure 1 A). This agrees with previous results, which found no impact of saliva addition on the large or small deformation shear rheological responses in xanthan gum (Choi, Mitchell, Gaddipati, Hill, & Wolf, 2014). However, these authors found an effect of saliva on xanthan gum in filament thinning experiments: the addition of saliva significantly increased filament break-up time in xanthan, whereas it had little effect on the break-up time of the CMC filaments (Choi, Mitchell, Gaddipati, Hill, & Wolf, 2014).

After incubation in the stomach, a significant decrease is observed in both G' and G'', and an increase in tan δ (Table 1), in comparison to the saliva sample, which implies decreased viscoelasticity. To differentiate the effect of the specific conditions of the stomach (pH and enzymes) on the dilution effect, the stomach sample was compared with a stomach water dilution sample (incubation with the same dilution factor and conditions, but at neutral pH and without the addition of pepsin). The stomach sample showed significantly lower G' and G'' values and lower viscoelasticity (tg δ: G''/G') than the stomach dilution sample, which revealed a specific effect of the stomach condition on the xanthan gum structure. Due to the anionic nature

of xanthan gum, the effect could be related to the decrease in the electrostatic repulsion at acidic pH, creating a more flexible chain.

Table 1. Viscoelastic parameters of the xanthan gum (XG) water solution and XG o/w emulsions before and after *in vitro* digestion.

Sample	XG WATER SOLUTION			XG O/W EMULSION			XG + TWEEN O/W EMULSION		
	G'	G''	tgδ	G'	G''	tgδ	G'	G''	tgδ
FRESH	159.85ab	30.57a	0.19abc	305.50a	37.40ab	0.12c	333.40a	42.00a	0.13c
SALIVA	193.10a	32.44a	0.17c	244.80a	30.50abc	0.13b	297.60a	37.20ab	0.13c
STOMACH	81.40c	18.10b	0.22a	297.00a	39.30a	0.13b	230.20b	30.40b	0.13c
STOMACH DILUTION	111.55bc	20.46b	0.18bc	164.20b	26.50bc	0.16b	204.20b	31.20b	0.15b
INTESTINE	102.05c	19.22b	0.19abc	82.40c	13.20d	0.16b	122.70c	18.30c	0.15b
INTESTINE DILUTION	105.91c	21.35b	0.20ab	104.40bc	18.4cd	0.18a	111.70c	19.60c	0.18a

[abcd] Within the same column, values with the same letter are not statistically different according to the Tukey test ($p > 0.05$).

Finally, after intestinal incubation (neutral pH), the values of G' and G'' increase once again until they are higher than after stomach incubation, despite the greater dilution in the intestinal system. This implies that, at neutral pH, the electrostatic repulsion increases again leading to the expansion of the xanthan gum chains and the increase in viscoelasticity. In the intestinal phase, no differences were found among the intestine sample and the intestine dilution sample.

The dependency of G' and G'' versus frequency was adjusted to a power law equation. The values of the parameters K', n' (corresponding to G' curves) and K'', m' (corresponding to G'' curves) for the different samples are shown in Table 2. As observed in the mechanical spectra, the most remarkable effect on the XG water solution systems is the lower value of k' in the stomach than in the stomach water dilution, which implies that the XG water solution is specifically affected at the stomach level by the enzymes and the acidic pH.

Table 2. Influence of the different incubation steps on the power law parameters.

		$G' = G'_1 * f^n$		$G'' = G''_1 * f^m$	
		G'_1 (Pa s^n)	n	G''_1 (Pa s^n)	m
XG WATER SOLUTION	FRESH	129ab (16)	0.12b (0.01)	28ab (1)	0.08a (0.01)
	SALIVA	158a (3)	0.11b (0.00)	30ab (1)	0.06a (0.00)
	STOMACH	63c (4)	0.145a (0.01)	15c (0)	0.09a (0.00)
	STOMACH WATER DILUTION	111abc (24)	0.11b (0.00)	22bc (4)	0.06a (0.01)
	INTESTINE	80bc (0)	0.13ab (0.00)	17c (0)	0.06a (0.01)
	INTESTINE WATER DILUTION	74c (11)	0.13ab (0.00)	17c (2)	0.09a (0.00)
XG O/W EMULSION	FRESH	261a (6)	0.08b (0.00)	37[a] (1)	0.04b (0.00)
	SALIVA	210a (3)	0.08b (0.00)	30ab (1)	0.04b (0.01)
	STOMACH	250[a] (39)	0.09ab (0.01)	39a (8)	0.01c (0.01)
	STOMACH WATER DILUTION	135b (9)	0.10ab (0.00)	25abc (3)	0.05ab (0.01)
	INTESTINE	78b (5)	0.10a (0.00)	14c (0)	0.06a (0.00)
	INTESTINE WATER DILUTION	85b (5)	0.11a (0.00)	17bc (1)	0.06a (0.00)
XG + TWEEN O/W EMULSION	FRESH	284a (32)	0.08d (0.00)	40a (6)	0.05ab (0)
	SALIVA	258ab (48)	0.08d (0.00)	36a (7)	0.05ab (0)
	STOMACH	198abc (22)	0.08cd (0.00)	29ab (2)	0.03b (0)
	STOMACH WATER DILUTION	168bcd (16)	0.09b (0.00)	30ab (4)	0.06a (0)
	INTESTINE	106cd (3)	0.09bc (0.00)	18b (0)	0.06a (0)
	INTESTINE WATER DILUTION	93d (4)	0.11a (0.00)	18b (0)	0.07a (0)

[abcd] For each sample, values in the same column with the same letter are not statistically different ($p<0.05$) according to the Tukey test.

The n' value, which reflects the dependence of G' versus frequency, was significantly higher in the stomach in comparison with the stomach water dilution, revealing a greater frequency dependence (steeper slope). An

increase in the frequency dependence of G' indicates a decrease in the degree of structure of the system.

3.1.2 Xhantan gum emulsion

During digestion, the xanthan gum emulsion behaved completely differently from the xanthan gum water systems (Figure 1 B). The effect of saliva on the behaviour of the fresh samples was similar in both the water system and the o/w emulsion. The difference between both systems is related to the effect caused by stomach incubation. Contrary to the xanthan water dilution, no appreciable decrease in the values of G' was observed after stomach incubation in the case of the xanthan o/w emulsion when compared to the fresh samples, despite the greater dilution of the stomach samples. The comparison between the stomach sample and the stomach water dilution sample is shown in Table 1. Although no significant differences in G' were found between the fresh sample and the stomach sample, there were significant differences found between the stomach and the stomach water dilution sample. These results imply that the xanthan gum emulsion suffered a structural change at stomach level, which caused an increase in the viscoelastic properties, contrary to what happened in the xanthan gum water solution. The structural changes that explain the increase in viscoelasticity in the emulsions at stomach level are later discussed in the microstructure section. After intestinal incubation, a decrease in the moduli is observed. In this case, no significant differences were found between the intestine sample and the intestine diluted sample. The same conclusions are obtained from the parameters, K', n', K'' and m' (Table 2). The parameter K', indicative of the consistency of the sample associated with G', was significantly higher in the stomach than in the stomach dilution sample, this

implies that, at that level, an increase in consistency associated with the stomach enzymes and the low pH is observed.

3.1.3 Xanthan gum Tween 80 emulsion

The behaviour of the xanthan gum Tween 80 o/w emulsion when digested is shown in Figure 1 C. In this case, no significant differences were found between the G' and G'' values of the stomach sample and the stomach water dilution sample. Nor were anysignificant differences found between the K' and n' parameters (Table 2). Therefore, in the presence of Tween 80, there was no increase in viscoelasticity in the gastric phase. After intestinal incubation, a decrease in the moduli was observed.

3.2 Effect of *in vitro* digestion on textural properties

The extrusion force versus time corresponding to the fresh, saliva, stomach and stomach water dilution samples are shown in Figure 2 A (xanthan gum water solution), B (xanthan gum o/w emulsion) and C (xanthan gum-Tween 80 o/w emulsion). The texture properties after intestinal incubation were not studied due to the liquid consistency of the samples, which makes measuring impossible.

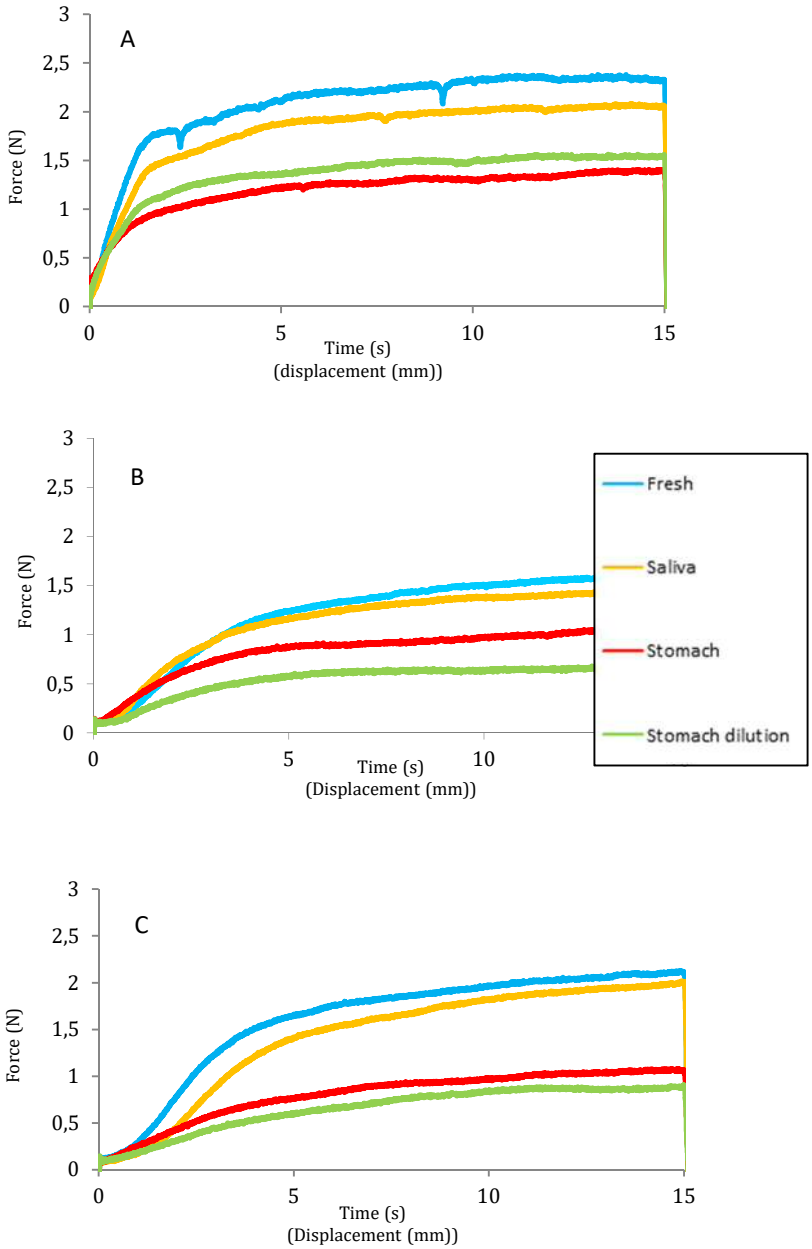

Figure 2. Effect of *in vitro* digestion in the extrusion profiles: A) xanthan gum water solution, B) xanthan gum o/w emulsion and C) xanthan gum-Tween80 o/w emulsion.

The mean values of the area under the curve and maximum force are shown in Table 3.

Table 3. Extrusion parameters of the xanthan gum (XG) water solutions and XG o/w emulsions before and after *in vitro* digestion.

Sample	XG WATER SOLUTION		XG EMULSION		XG + TWEEN EMULSION	
	AUC (N mm)	Maximum force (N)	AUC (N mm)	Maximum force (N)	AUC (N mm)	Maximum force (N)
FRESH	28.78a (2.56)	2.15a (0.16)	17.93a (0.85)	1.19a (0.10)	23.06a (1.28)	1.53a (0.08)
SALIVA	26.59ab (1.61)	2.02a (0.11)	17.04ab (0.29)	1.13ab (0.05)	21.04ab (0.95)	1.40ab (0.06)
STOMACH	17.55c (1.48)	1.27b (0.14)	12.75c (0.93)	0.85c (0.20)	11.77c (1.33)	0.78c (0.09)
STOMACH WATER DILUTION	20.31bc (3.33)	1.47b (0.25)	8.50d (0.80)	0.56d (0.03)	10.90c (1.04)	0.72c (0.07)

[abcd] Within the same column, values with the same letter are not statistically different according to the Tukey test ($p > 0.05$).

Values in parentheses are standard deviations.

AUC (Area Under the Curve).

Correlations between the texture results and the previously discussed rheological results were found. Accordingly, the xanthan water solution exhibited the greatest decrease in force after stomach incubation, the force of the stomach water dilution sample being greater than that of the stomach sample. In the xanthan emulsion on the other hand, a significantly greater extrusion force was found in the stomach sample than in the diluted one. This behaviour of the xanthan emulsion in the stomach is not observed in the presence of Tween (no significant differences were found between the stomach and the stomach water dilution samples), which may be explained

by the fact that the Tween emulsifying properties are maintained after stomach digestion, as later explained in the microstructural section. The increase in consistency of the xanthan gum emulsion (without Tween) at stomach level may be considered an interesting point for applications where what is desired is precisely an increase in consistency at stomach level: for example, for the purposes of delaying gastric emptying or in the design of food for satiety control.

3.3 Microstructure during *in vitro* digestion

The microstructure of the samples was analysed with the aim of better understanding the rheological and textural properties.

The microstructure during digestion is shown in Figure 3. In the fresh xanthan gum emulsions (no emulsifier), the fat globules are very big in size (around 60 and 80μm, respectively), dispersed in a xanthan gum water-based continuous phase. The fat globules are expected to be large, as xanthan gum is not an emulsifier, and only acts as an emulsion stabilizer by thickening the continuous phase. The incorporation of the Tween emulsifier into the system leads to a marked reduction in the fat globule size (fat globules from 5 to 20 μm).

The incorporation of saliva does not produce a big change in the microstructure in any of the systems. After stomach incubation, coalescence is observed in the xanthan gum emulsion without emulsifier (fat globules higher than 100 μm can be observed). This change in the fat microstructure after stomach incubation explains the increase observed in the viscoelastic properties and the textural parameters, as fat coalescence is associated with an increase in both consistency and viscoelasticity.

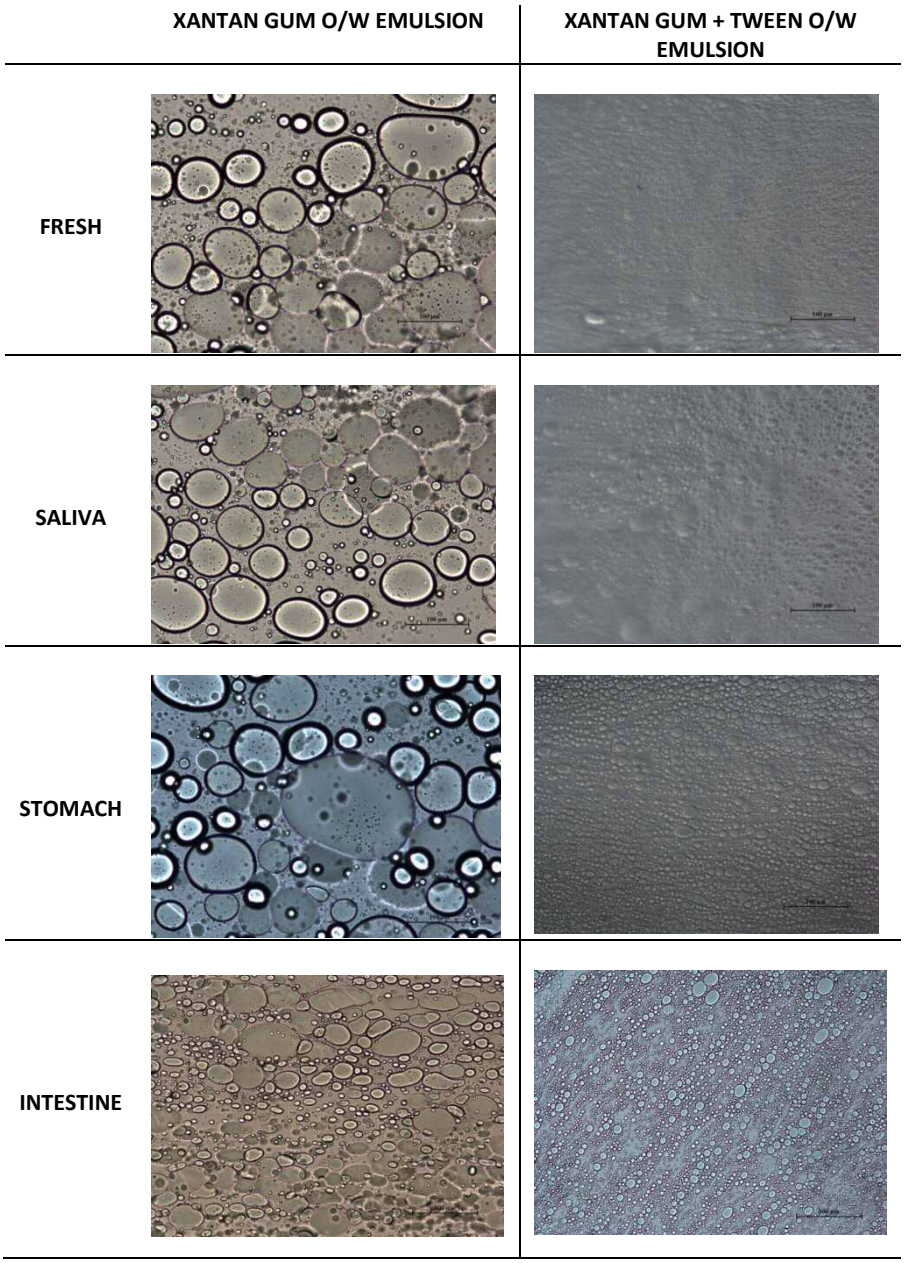

Figure 3. Microstructure of the fresh xanthan gum o/w emulsions and after *in vitro* digestion at 37 °C.

This phenomenon could be linked to the sensitivity of the xanthan gum structure to the stomach conditions. The weaknesses of the xanthan gel matrix at acidic pH will favour physical contact among the fat globules, which are only stabilized in the system due to the physical support of the xanthan matrix structure. In the presence of the Tween emulsifier, this increase in the size of the fat globules after stomach digestion is not observed, only the appearance of a small degree of fat flocculation is observed; in this case, fat globules are emulsified by the presence of Tween, which is not affected in the acidic conditions of the stomach, due to their resistant behaviour. Non-ionic surfactants and phospholipids provide more protection in emulsions against lipase-induced destabilization in the presence of bile salts than proteins. The accessibility of pancreatic lipase in emulsions stabilized by surfactants (e.g. Tween 20 and lecithin) is much more limited than in emulsions stabilized by casein and whey protein (Chang & Mc Clements, 2016; Van Aken et al., 2011; Mun, Decker, & McClements, 2007).

After intestinal incubation, there is an observed decrease in the size of the fat globules in the xanthan gum emulsion, in comparison with the stomach microstructure, which is associated with the emulsifying effect of the bile salts present in the intestinal digestion fluid. Xanthan gum does not have surface active properties; therefore, the fat globule surface is freely available for the bile salts to conduct their physiological emulsifying function. On the contrary, in the presence of Tween as emulsifier, no appreciable change in the microstructure of the fat globules can be visualized, revealing the barrier effect caused by both xanthan gum in the continuous phase and also by Tween in the oil interface against the action of the digestive fluids.

3.4 Free fatty acids after *in vitro* intestine digestion

Two controls were employed to evaluate the effect of both xanthan gum and Tween-80 on fat digestion: a whey protein o/w control emulsion and a Tween-80 o/w control emulsion (Table 4).

Table 4. Oleic acid values after *in vitro* digestion.

	g oleic acid / g fat
WHEY PROTEIN CONTROL EMULSION	0.32a (0.03)
XANTHAN GUM O/W EMULSION	0.11cd (0.02)
TWEEN CONTROL EMULSION	0.15b (0.01)
XANTHAN GUM + TWEEN O/W EMULSION	0.05e (0.01)

[abcde] Within the same column, values with the same letter are not statistically different according to the Tukey test ($p > 0.05$).
Values in parentheses are standard deviations.

The whey protein emulsion exhibited by far the greatest generation of free fatty acid, followed by the Tween control emulsion. The effectivity of Tween in comparison to protein-based emulsifiers against fat digestion has been previously described (Chang & Mc Clements, 2016; Van Aken et al., 2011; Mun, Decker, & McClements, 2007).

In the xanthan emulsions, in both the absence and presence of Tween as emulsifier, a significantly less free fatty acid was generated, in comparison to the controls without xanthan gum (65.6% reduction in comparison to whey protein and 12.5% reduction in comparison to Tween o/w emulsion).

This reduction increases significantly in the system where xanthan gum is combined with Tween (84.4% reduction in comparison to whey protein emulsion and 66.6% in comparison to Tween o/w emulsion). The presence of xanthan gum at 2% concentration in the continuous phase, acts as a physical barrier, enhancing the barrier effect of Tween at the oil interface.

4. CONCLUSIONS

Xanthan gum at 2% concentration was effective at reducing fat lipolysis in o/w emulsions with 47% fat content. The order of fat lipolysis, from the highest to the lowest, was: whey protein emulsion, Tween-80 emulsion, xanthan gum emulsion and xanthan gum-Tween-80 emulsion (lowest lipolysis).

The viscoelastic properties of a water solution of xanthan gum decrease after stomach incubation, due to the fact that there is less electrostatic repulsion at acidic pH, creating more flexible chains. In this regard, the xanthan gum water solution should not be the first option when an increase in consistency at stomach level is desired. On the contrary, in the XG emulsion, an increase in viscoelasticity is observed at stomach level, due to the fat coalescence and coagulation induced by the weakness of the supporting XG structure. This increase in viscoelasticity at stomach level is not observed if Tween 80 is employed as emulsifier, as Tween 80 functionality is stable at stomach level and no changes in the fat microstructure are observed.

This study opens up new applications for the use of XG in emulsions. In the presence of the Tween 80 emulsifier, the barrier against fat digestion is enhanced. On the other hand, in the absence of an emulsifier, an increase in the consistency of the xanthan gum emulsion occurs at stomach level, which

may be of interest for applications requiring the generation of a feeling of fullness or a delay in gastric emptying.

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Capítulo 2

Efecto del tipo de grasa en el desarrollo de emulsiones con alto contenido en grasa

Effect of cellulose ethers on physical properties of milk fat emulsions: designing reduce-fat spreads.

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ABSTRACT

The design of reduced-fat spreads as a healthy alternative has received high interest lately. Low fat cellulose ether emulsions prepared using anhydrous milk fat (AMF) were compared with butter and pure AMF. The emulsions were composed of 47% AMF, water, and 2% cellulose ether. Three types of cellulose ethers were investigated: two methylcelluloses (A4M and MX) and one hydroxypropyl methylcellulose (F4M)). Structural behavior was analyzed by penetration test and small amplitude oscillatory rheology at 5°C and 20°C, and linked to the microstructure observation by confocal laser scanning microscopy. Melting behavior was examined by thermal analysis using differential scanning calorimetry. Cellulose ether type affected the crystal network formed and consequently the textural and rheological properties of the emulsions. The original compact structure of the AMF was softened by the presence of all cellulose types. All the AMF based emulsions showed a physical appearance similar to butter but with a softer consistency and spreadable at refrigeration and room temperature. The cellulose type MX provided the lowest viscoelasticity at refrigerated temperature. The emulsions could be used as a direct spreadable food or in applications that require plastic properties.

Keywords: reduced-fat spread, emulsion, cellulose ether, anhydrous milk fat.

1. INTRODUCTION

In recent years, the consumer trend is based on demanding healthier foods, but retaining their technological and sensory characteristics. Butter, margarine and shortenings are fat sources widely used in the food industry due to the ability to provide specific structures and excellent sensory properties. Their high levels of fat (80%) (Keogh, 2006) bring out the need for fat reduction alternatives, although large reductions in fat content alter the properties of food products (Bayarri, Taylor, & Joanne, 2006; Mela, 1990; Drewnowski, 1992). One of the challenges of food manufacturers is to produce alternative low fat sources able to mimic the structural properties and sensory properties provided by conventional fat. Fat reduction and fat replacement are very complicated in foodstuffs which depend on the unique functional properties of such fats, such as the specific melting profiles in different applications, and the plasticity of laminating shortenings used in puff pastries.

Understanding the physical properties of fat is crucial to develop new fat sources and new products. Additives are used for the influence they have on crystallization, surface gloss, temperature stability, rheology, polymorphic stability, etc. (Smith, K.W., Bhaggan, K., Talbot, G., van Malssen, K.F., 2011). Addition of non-fat components modify the physical behavior of the fat, especially the crystallization, which affect attributes such as spreadability, hardness, and mouthfeel (Weyland & Hartel, 2008; Marangoni & Narine, 2002).

Anhydrous Milk Fat (AMF) is a solid fat with functional properties such as plasticity, flavor and mouthfeel. It possess a wide diversity of fatty acids (FA) and triglyceride composition, including a broad thermal range of melting transitions (from -40°C to 40°C). It has been proven that the addition of

other components on a solid fat and the processing conditions have important effects on final physicochemical and structural properties. The structural properties of AMF during temperature fluctuations (Buldo, Andersen, & Wiking, 2013) and the effect of processing conditions of AMF with different melting fractions (Herrera & Hartel (2000a,b)) were studied. Martini, Carelli, and Lee (2008) showed that the addition of waxes on AMF affect the crystallization behavior, modifying the texture and mouthfeel of system. The stability of o/w emulsion composed of AMF and soya bean oil stabilized by whey protein isolate was studied by Tippetts & Martini (2009); they showed that maintaining constant the amount of emulsifier, the lipid droplet size and the amount of fat directly affect the stability of the emulsion. Hydrocolloids have been widely employed in the development of low fat food. Addition of a polysaccharide in a fat emulsion contributes to replace some characteristics normally provided by the fat (McClements, 2015), and may also provide nutritional benefits associated with consumption of dietary fiber (Mudgil, D., & Barak, S., 2013). Recently oil/water cellulose ether emulsions have been claimed to be low fat alternative suitable to replace conventional fat in specific food products, such for example biscuits (Tarancón, P., Salvador, A., & Sanz, T., 2013). The cellulose ethers act as emulsifier and stabilizer of the liquid oil and an emulsion with semisolid consistency suitable to replace conventional fat is obtained (Espert, Salvador & Sanz, 2016; Espert et al., 2017) The limitation of this o/w emulsions made from liquid oils is that they do not have the plastic properties and spreadability properties necessary in the production of many fat products.

Therefore, the aim of the present work is to study the texture, rheological, thermal and microstructure properties of reduced fat cellulose ether emulsions using anhydrous milk as fat source solid fat at room temperature. The obtained emulsions are expected to enlarge the possible applications of

cellulose ether emulsions as fat substitutes in the food industry. The obtained properties are compared with pure AMF and commercial butter.

2. MATERIALS AND METHODS

2.1 Raw material

The emulsions were prepared with AMF, drinking water and three different cellulose ethers. AMF was supplied by ARLA Foods (Denmark) and cellulose ethers were provided by The Dow Chemical Co (Germany). Two type of methylcellulose with different degrees of methoxyl content (METHOCELTM A4M and MX) and one type of hydroxypropyl methylcellulose (METHOCELTM F4M) were employed. Butter (Lurpak) was supplied by Arla Foods (Denmark).

2.2 Emulsion preparation

The different emulsions were composed of AMF (47% (w/w)), cellulose ether (2% (w/w)) and water (51% (w/w)). The fat was melted at 65°C and there after keept at 40°C to begin the preparation process. The cellulose ether was first dispersed in the fat using a stirrer (IKA Eurostar 20D) at low-medium speed for five minutes. The mixture was then hydrated by gradually adding water at 20 °C while the stirring speed was increased to 300 rpm. The 200 g mixture was then homogenized using an IKA T25 basic Ultra-Turrax with the dispersion tool S18N-19G, at 8000 rpm for 1 minute and subsequently at 9500 rpm for 2 minutes. Finally, the product was cooled and stored at 5 °C.

2.3 Rheological behavior

The rheological behavior was evaluated by oscillatory shear experiments performed on a controlled stress rheometer (AR-G2, TA Instruments (New Castle, DE, USA)) with a Peltier heating system, using 40 mm parallel plate geometry. Strain sweeps test were carried out from 2.00E-4% to 100% at a constant frequency of 1 Hz to measure the linear viscoelastic region. Frequency sweeps from 0.01 to 100 Hz at a stress wave amplitude inside the linear region were performed. Storage modulus (G′), loss modulus (G″) and loss tangent (tan δ =G″/G′) values were represented as a function of the angular frequency (rad/s). All rheological tests were performed at 5°C and 20°C.

2.4 Texture measurements

Evaluation of the emulsions firmness was carried out with a texture analyser (FTC TMS-Touch, Food Technology Corporation, USA) with a 25N load cell. A penetration test was carried out at a speed of 0.5mm/s and a distance of 8mm, using a spherical probe (1/4'' Sph. Stainless P/0.25S). Samples were placed in 2 cm beakers and were analyzed at 5°C and 20°C, after 15 days of storage.

2.5 Microstructure analysis

The microstructure of the emulsions was analysed by confocal laser scanning microscopy (CLSM) (Nikon C2, Nikon Instrument Inc, Tokyo, Japan) using 0.01% Nile Red solution to stain fat. Sample was place in a glass slide after the solvent evaporated and was carefully covered by slide. The

argon laser beam was set at 568 nm excitation. A 20x objective lens was used to acquire the images. Measurements were carried out at room temperature (20 ± 1°C).

2.6 Analysis of thermal behavior

The thermal behavior of all emulsions was analyzed by Differential Scanning Calorimetry (DSC, Q2000 TA Instruments, UK). Of each sample, 10-15 mg was placed into a sealed aluminum pan. An empty sealed pan was used as reference. The samples were equilibrated at 5°C and the temperature was increased to 75°C at a rate of 5°C/min. The analysis was conducted after 3 and 15 days of storage (5°C).

2.7 Statistical Analysis

The data obtained by texture, rheology and DSC were statistically analyzed by Analysis of variance (ANOVA) using XLSTAT statistical software (version 2010.5.02, Microsoft Excel®, Barcelona, Spain). Tukey test was used to assess the differences in mean values ($p < 0.05$). Three replicates were performed with samples prepared on different days.

3. RESULTS AND DISCUSSION

3.1 Large deformation properties: texture measurements

The maximum penetration force obtained from the force/time profiles for the penetration test of the AMF cellulose ether spreads, pure AMF and butter, at 5°C and 20°C are shown in Table 1.

Table 1. Maximum force values of AMF based emulsions.

Sample	Max. Force	
	5°C	20°C
AMF	34.28a	3.15a
BUTTER	12.41b	2.86a
AMF + F4M	12.14b	1.66b
AMF + A4M	11.31bc	0.84bc
AMF + MX	1.02c	0.37c

[abcd] Values in the same column with the same letter are not statistically different ($P<0.05$) according to Tukey's test.

As expected, the maximum penetration force was higher at 5°C than at 20°C for all the systems. At 5°C, the AMF showed the highest hardness and irregularities in the force-distance curves, which reflect a brittle structure, as previously described by Buldo, Andersen, & Wiking (2013). The force values of butter were lower than for the pure AMF but similar to F4M and A4M spreads at 5°C. The MX spread showed significantly the lowest force values, indicating a softer structure.

At room temperature (20°C), AMF continued showing the highest hardness, however, the differences with butter became significantly lower than at 5°C. At 20°C, all the hydrocolloid-based spreads showed lower hardness than butter, being the F4M spread the closest to butter. At this temperature the texture profile of both A4M and MX were very similar. Therefore, incorporation of AMF as the fat phase of cellulose ethers emulsions provides a spread product softer than AMF, with a texture profile similar to butter, but with a 30% lower fat.

3.2 Linear viscoelastic properties

The viscoelastic behavior of the AMF cellulose ether spreads in comparison to pure AMF and butter at 5°C and 20°C are shown in Figure 1 a and b, respectively. Tan δ as function of frequency is also shown in Figure 2.

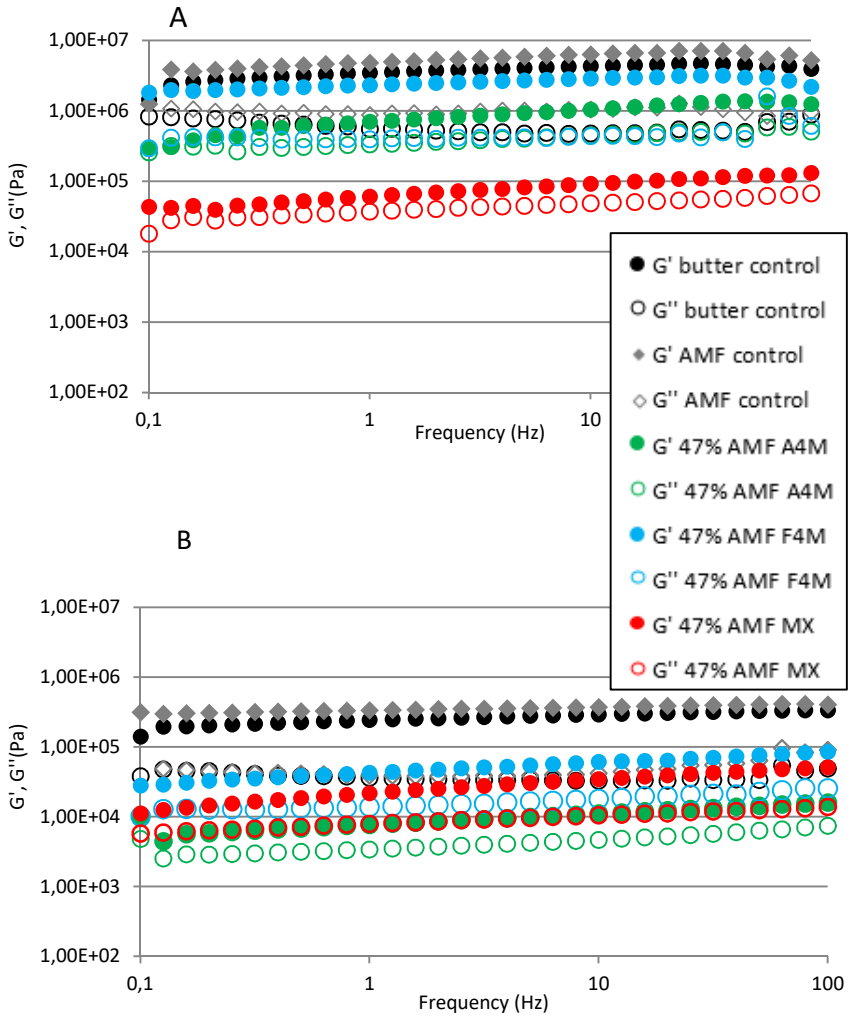

Figure 1. G' and G'' as function of frequency of 47% AMF emulsions and AMF and butter controls at 5°C (A) and 20°C (B).

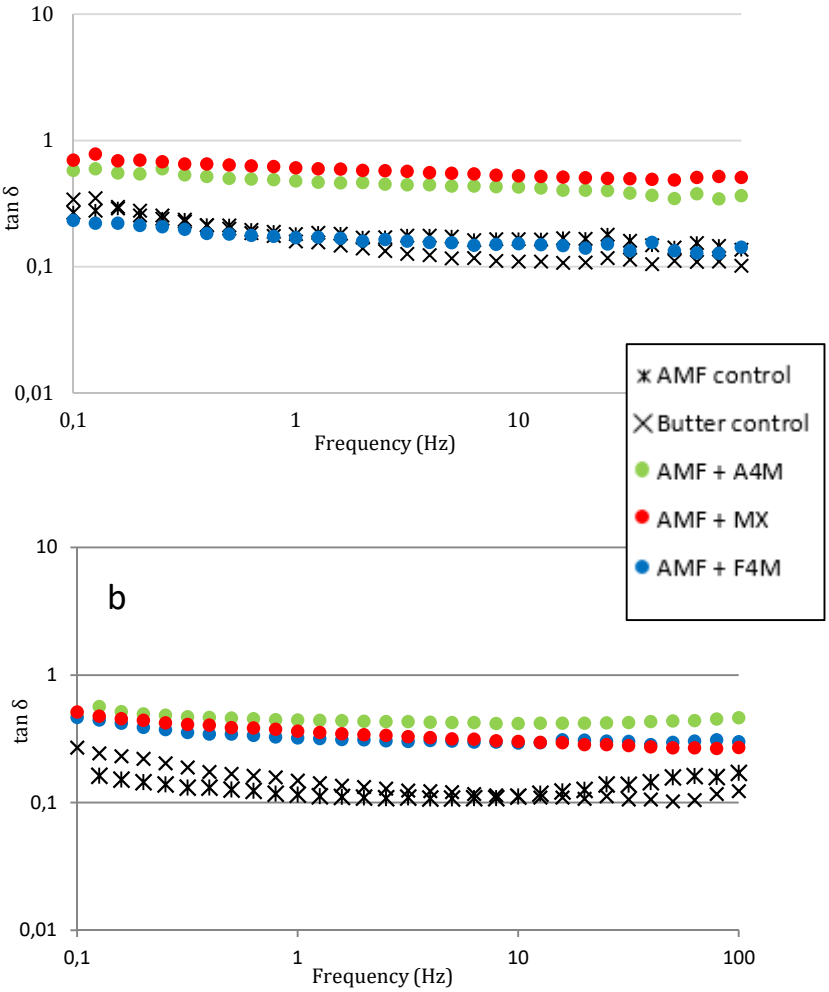

Figure 2. Tan δ as function of frequency of 47% AMF emulsions and AMF and butter controls at 5°C (a) and 20°C (b).

In addition, mean values of G', G'' and tan δ values at 1Hz are shown in Table 2. A viscoelastic behavior with a predominance of the elastic or solid component (G') versus the viscous or liquid component (G'') was found in the available frequency window. The plateaus area of the mechanical spectra was visualized in all the systems, with values of G' always higher than G'', and

low frequency dependence. The highest G' values, reflecting a harder structure, were found in AMF, followed very closely to butter and F4M spread, being the extend of the differences dependent on temperature.

Table 2. Viscoelastic rheological parameters of AMF emulsions and controls at 5°C and 20°C.

	5°C			20°C		
Sample	G'	G''	tan δ	G'	G''	tan δ
AMF control	4855000^{a}	882200^{a}	0.18^{b}	338900^{a}	39220^{a}	0.11^{d}
Butter control	3485000^{b}	554500^{b}	0.16^{b}	244500^{b}	36360^{a}	0.15^{cd}
AMF + F4M	2202000^{c}	399100^{bc}	0.18^{b}	67910^{c}	19535^{b}	0.29^{bc}
AMF + A4M	668050^{d}	267620^{c}	0.40^{ab}	29750^{d}	12305^{c}	0.41^{a}
AMF + MX	57815^{e}	35610^{d}	0.62^{a}	33950^{d}	11825^{c}	0.35^{ab}

^{abcd} Means with different letters within the same column are significantly different ($p<0.05$) according to Tukey's test.

In all the systems the increase in temperature from 5 to 20°C produced a significant decrease in the viscoelastic functions G' and G'', being the extent of the difference a function of the type of system. At 5°C, F4M spread showed the highest value of G' and the closest behavior to butter and AMF, on the other hand, MX emulsion presented the lowest values of G' and G'', and the lowest viscoelasticity (tan δ closer to 1).

The increase in temperature up to 20°C reduced the values of G' and G'' in all the systems, although values of G' continue to be higher than G'' in all the frequency sweep studied. The lowest effect of the increase in temperature was found in the MX emulsion, where G' and G'' decreased at 20°C but not so noticeable. In fact, in the MX emulsion the predominancy of G' versus G''

(degree of viscoelasticity) increase at 20°C: tan d (G''/G') decrease from 0.62 at 5°C to 0.35 at 20°C, indicating a higher predominancy of the solid properties at 20°C than at 5°C. On the other hand, the differences among F4M emulsion and butter and AMF became higher at 20°C, as the F4M decrease its viscoelasticity at 20°C (tan δ was 0.18 at 5°C and 0.29 at 20°C) while the viscoelasticity of butter was not affected. Despite the lowest effect of temperature in the MX emulsion and A4M emulsion, the F4M continue showing the highest values of G' and G'' and the highest viscoelasticity of all the cellulose types, and the closest behavior to butter, but the extent of the differences with A4M and MX emulsions became lower at 20°C than at 5°C.

3.3 Microstructure analysis

To a better understanding of the structural properties, a microstructural analysis of the systems was studied. Confocal images of butter, AMF, and the different cellulose ether spreads are shown in Figure 3. The fat composition determines the amount of fat crystals, as well as the shape and the aggregation of the individual crystals into a network. Whether the fat is present as a continuous mass (like in anhydrous milk fat or butter oils) or in numerous small globules (e.g., as in milk or cream) has a considerable influence on its crystallization behavior (Huppertz & Kelly, 2006).

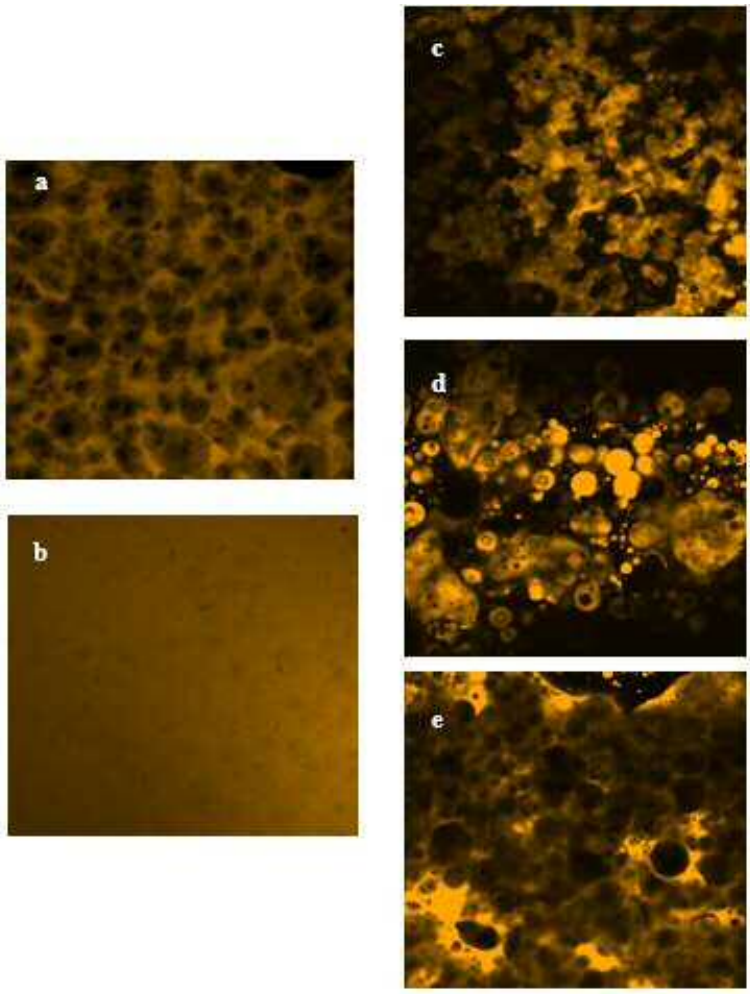

Figure 3. Confocal laser scanning microscopy images of the different systems: AMF (a), butter (b), AMF + A4M emulsion (c), AMF + MX emulsion (d) and AMF + F4M emulsion (e).

Pure AMF shows a dense network made up from small spherulite crystals surrounded with liquid oil. For the most part, crystals are well-defined single entities (Kaufman et al., 2012), although it is visible the formation of crystals aggregates that establish connections between each other giving the system certain hardness. Butter shows a different microstructure characterized by

a discontinuous structure of fat globules that consist of an outer crystalline layer composed of high melting fat, enclosing liquid oil inside (Heertje, 1993). It is generally assumed that the presence of fat globules in the microstructure of butter decreases the hardness of the product, as the continuous fat crystal network is interrupted by the globules (Buldo, Andersen y Wiking, 2013). The water droplets show the crystalline nature of interface in margarine, which is composed of a continuous network of fat crystals aggregates. As it is known, the presence of emulsifiers also affects the structure of the crystal network and the emulsion stability (Juriaanse & Heertje, 1988; Ghosh & Rousseau, 2011).

In the cellulose spreads a lower amount of fat is observed embedded in a continuous network formed by the hydrated hydrocolloid (black area). Differences in the microstructure were found depending on the cellulose ether type. In the AMF-A4M spread (Figure 7d) the discontinuous fat phase is mainly composed of liquid fat that remained uncrystallized, and of a minor proportion of diffuse crystals (black shadow), smaller in size than the crystals of the AMF control. In the AMF-MX spread (Figure 7e), liquid fat was uniformly distributed in spheroidal globules and low level of crystallization is observed and the observed crystals are the smallest in size. This microstructure explains the lowest firmness and the lowest values of the viscoelastic functions found in the MX spreads, as smaller crystals form weaker links and these result in a decrease in viscoelasticity (Herrera & Hartel, 2000). AMF-F4M spread (Figure 7f) shows a different microstructure, which reflects is harder structure in comparison to the other cellulose spreads. Fat appears uniformly distributed in the background, forming a continuous phase. Apparently a partial phase inversion may have occurred. More dense crystalline structure was observed. Crystal shape is affected by the presence of the cellulose ether network, producing larger

crystals compared with AMF control. Also large aggregates are present in the structure; this could explain the highest hardness values, since the hardness of a spread depends on the amount of fat crystals (Juriaanse & Heertje, 1988).

3.4 Thermal behavior

Structural properties of plastic fats are strongly affected by the thermal properties of the crystal network formed by the fat. In particular, the melting point is one of the factors which big influence in the functional behavior of fat-based products. Melting characteristics have been related to flavor release and consumer perception. Keogh (2006) suggested that the melting point of fat used for spreads should not exceed mouth temperature, as an unpleasant waxy effect starts to develop above this temperature.

The melting behavior of the different systems monitored by DSC is shown in Figure 4. The medium melting fraction (MMF) (from 10°C to 19°C) and the high melting fraction (HMF) (above 20°C) typical of AMF are observed in all AMF spreads. The melting parameters are summarized in Table 3. In the MMF no significant differences were found in the onset temperature among the different samples, although the peak temperature in this fraction was higher in all the cellulose spreads in comparison to AMF control, which indicates a delay in melting temperature. The main difference was observed in the enthalpy values (ΔH), which was significantly higher in the pure AMF.

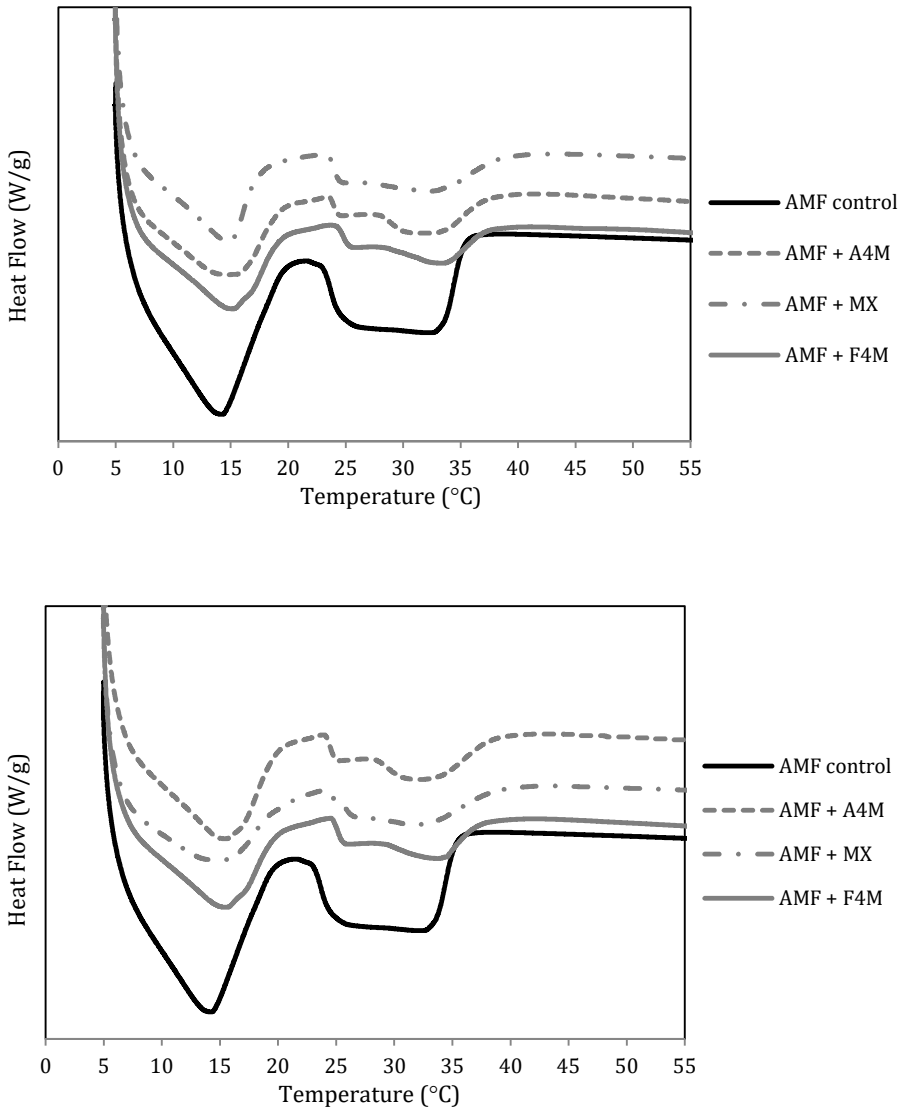

Figure 4. Differential scanning calorimetry melting curves of AMF spreads at 3 days (a) and 15 days (b) of storage at 5°C.

Table 3. Thermal parameters of the different AMF based spreads and controls at 3 days.

		Start °C	Onset °C	Maximum °C	Stop °C	Area J/g
MMF	**AMF control**	9.25a	9.31a	14.37b	20.81a	12.94a
	A4M	8.90ab	9.16a	15.07ab	20.03a	7.61b
	MX	8.11b	9.13a	15.02ab	19.11a	7.76b
	F4M	8.70ab	9.25a	15.33a	19.64a	8.53b
HMF	**AMF Control**	20.55b	22.96b	32.57ab	36.52c	19.10a
	A4M	22.48ab	23.88ab	32.28b	39.00b	7.53bc
	MX	22.72ab	23.89ab	32.60ab	40.75a	8.44b
	F4M	24.02a	24.35a	33.32a	39.94a	6.958c

[abcd] For each melting fraction, values in the same column with the same letter are not statistically different (P<0.05) according to Tukey's test.

Either the presence of the hydrocolloid-water network and/or the lower proportion of fat in the cellulose spreads reduced the original compact structure of AMF, leading to a lower area of the MMF peak. The AMF-F4M spread showed higher area values than the other cellulose spreads, which can be attributed to the higher degree of crystalline structure as visualized in the microstructure analysis in this type of cellulose spread.

The storage conditions did not reveal a high impact on the fat crystal network in any of the AMF systems. Similar exothermic peaks were found in all spreads, indicating no significant changes in fat melting behavior through storage time (Table 4).

Table 4. Thermal parameters of the different AMF based spreads at 15 days.

		Start °C	Onset °C	Maximum °C	Stop °C	Area J/g
MMF	**AMF control**	9.25a	9.31a	14.37b	20.81a	12.94a
	A4M	8.74a	8.94a	15.25ab	19.97a	9.58ab
	MX	8.71a	8.72a	16.45ab	21.61a	7.98b
	F4M	9.40a	12.25a	16.89a	21.69a	9.26b
HMF	**AMF control**	20.55b	22.96a	32.57a	36.52a	19.10a
	A4M	23.20a	24.22a	32.40a	39.12a	8.48b
	MX	22.90a	23.94a	32.70a	39.41a	7.11b
	F4M	23.63a	24.76a	33.83a	38.71a	7.07b

[ab] For each melting fraction, values in the same column with the same letter are not statistically different ($P<0.05$) according to Tukey's test.

4. CONCLUSIONS

This study demonstrated that it is possible to obtain reduced-fat spreads based on cellulose ethers and AMF. The quality of the spreads depended on the type of cellulose ether, since the structure of continuous phase impacted significantly on the morphology of the fat crystals and consequently on the structural and thermal properties. The original compact structure of AMF was reduced in the cellulose ether emulsions, either by the presence of the hydrocolloid network or by the lower proportion of fat, providing softer and spreadable systems. Moreover, it was observed that the presence of

cellulose caused a delay in the melting temperature of the fat, and the storage conditions did not reveal a high impact on the crystal network.

These emulsions allow the development of a reduced-fat product with a similar appearance to butter but softer and spreadable at refrigeration (5°C) and room temperature (20°C) and could have a great potential as reduced-fat spreadable products and in the manufacturing of more complex foods that require a fat source with plastic properties.

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Effect of xanthan gum on palm oil *in vitro* digestion. Application in starch-based filling creams.

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ABSTRACT

The purpose of this study was to investigate the influence of xanthan gum (XG) on the structural changes of palm oil during *in vitro* digestion and to evaluate the application of the XG-palm fat system in filling creams. The textural, rheological and microstructural properties were studied in three systems: a XG-palm fat system, a filling cream with the XG-palm fat system, and a control filling cream with palm oil but without xanthan gum. Free fatty acids were measured at the end of the *in vitro* digestion as indicators of fat digestion and related with the structural changes.

The incorporation of palm oil inside the xanthan gum matrix significantly reduces fat digestion. In comparison to the control cream, the microstructure reveals the presence of undigested fat when XG is present at the end of intestine *in vitro* incubation, while the presence of fat was significantly reduced in the control cream. After stomach incubation, an increase in the extrusion force and in the elastic and viscous modulus was observed in the xanthan gum systems, this was despite the greater water dilution, which was associated with an increase in fat globule size. In the control cream, a decrease in viscoelasticity is observed after stomach incubation.

The results of the present research may have applications in the design of low-fat food and in applications where stomach content structuring is desired.

Key words: *in vitro* digestion, rheology, food structure, structuring/destructuring, palm fat

1. INTRODUCTION

In the last decades consumption of high lipid content food stuff has increased in developed countries. This has led directly to a greater prevalence of both obesity and also several afflictions, such as diabetes or cardiovascular illnesses and certain types of cancer (Bray, Paeratakul, & Popkin, 2004; Chajès et al., 2008; Mozaffarian, Aro, & Willett, 2009). The challenge faced nowadays is that of producing nutritional, healthy foodstuffs without neglecting the organoleptic properties, for the purposes of obtaining a high level of consumer acceptance. The reduction of the lipid content poses many problems, as lipids are responsible for several technological and sensorial characteristics of the foodstuffs (Drewnowski, 1992).

Solid fat, rich in saturated fatty acids, cannot be directly replaced by liquid fat without it producing a negative effect on the quality of the final product due to perceptible changes in color, flavor, texture and stability, among other things. This is why there is still a great number of products made using high quantities of saturated and even *trans* fats on the market. Palm oil or fat is one of the most widely used in the Food Industry because of its physicochemical properties. The fact that it is made up of saturated and unsaturated fatty acids (nearly 50/50) makes it ideal for the elaboration of shortenings, ice creams, food for frying, among many others (Mba, Dumont, & Ngadi, 2015). Despite the fact that it contains beneficial compounds, such as vitamin E, carotenoids and sterols (Edem, 2002; Mba, Dumont, & Ngadi, 2015), numerous studies link it with several cardiovascular illnesses due to its high content in saturated fatty acids (Fattore & Fanelli, 2013; Mancini et al., 2015; Sun et al., 2015). However, studies such as that by de Lucci et al., refute this hypothesis and conclude that palm oil produces effects similar to

those generated by olive oil on human plasma lipids, due to its oleic acid content. (Lucci et al., 2016).

Several fat substitutes have been developed for the purposes of reducing fat consumption or improving the lipid profile, with physical, chemical and sensorial properties similar to those of fats, but little or no calorie content (ADA, 2005). The fat substitutes are classified according to whether they are based on carbohydrates, proteins or fats. Choosing the fat substitute will depend on the chemical composition and on the food matrix in which it is to be used.

The development of emulsion with low fat digestibility is a recent strategy in the topic of low fat food. The incorporation of hydrocolloids in the continuous phase of emulsions has been found an effective tool to reduce fat lipolysis (Beysseriat, Decker, & McClements, 2006; Gidley, 2013; Pasquier et al, 1996a; Qin, Yang, Gao, Yao, & McClements, 2017). However, there exists some contradictory information regarding the effectivity of hydrocolloids to reduce fat digestion.

Malinauskyte et al. (2014) studied the impact of carboxymethyl cellulose (CMC) on both lipid digestion and on the physicochemical properties of whey protein-stabilised emulsions during digestion. The thickening network formed in the continuous phase by CMC limits the interaction of fat droplets with gastrointestinal fluids, slowing down the rate of lipid digestion. Methylcellulose, chitosan and pectin were also found to be effective (Espinal-Ruiz, M., Parada-Alfonso, F., Restrepo-Sánchez, L.P., Narváez-Cuenca, C.E., & McClements, D.J., 2014), as well as gelatin, colloidal casein and starch dispersion (Wooster et al., 2014). Methylcellulose and hydroxypropilmethylcellulose reduce fat lipolysis in 47% oil emulsion (Espert et al, 2017).

Low methoxyl pectin incorporation into caseinate-stabilized emulsions with 2% corn oil increased the rate of digestion, which was attributed to the pectin exerting a suppressive effect on the flocculation process. However, incorporating high levels of pectin in lactoferrin and Tween 80 emulsion, decreased the digestion rate, possibly due to the calcium fixation and the rise in viscosity that restricts lipase access to the surface of the fat (Zhang, Zhang, Xhang, Decker, & Mc Clements, 2015).

Qiu, Zhao, Decker, & Mc Clements (2015) studied the influence of xanthan gum and pectin on the lipid digestibility of fish oil emulsions stabilized by wheat proteins. In this case, surprisingly, the polysaccharides promoted the lipid digestion process. This fact was associated to their ability to alter the aggregation state of the oil droplets, thereby increasing the amount of lipid phase exposed to the lipase.

Xanthan gum, widely used because of its thickening properties in aqueous solutions (Garcıa-Ochoa, Santos, Casas, Gomez, 2000; Palaniraj & Jayaraman, 2011), possess pseudoplastic behavior that enhances sensory qualities (flavor release, mouth feel) in food products, and the thermal and pH stability of this gum is superior to many other water-soluble polysaccharides (Katzbauer, 1998). It has recently been shown that the use of sunflower oil emulsions estabilised with xanthan gum allows for both the substitution of conventional fat and the reduction in lipid bioaccessibility. It has also been seen that the presence of xanthan gum during gastric digestion (pH 2) increases the viscosity of the system, producing a possible satiating effect (Espert, Salvador, & Sanz, 2018).

The primary objective of this study was to assess the structure of a palm oil-water system stabilised with xanthan gum after the *in vitro* digestion processes and to evaluate the digestibility of the fat. The second one was to

gauge the application of this system as a substitute for conventional fat in food products, such as filling creams. For this purpose, the structural changes during *in vitro* digestion in a filling cream with the palm oil xanthan system and in a control filling were studied and related to fat digestibility.

2. MATERIALS AND METHODS

2.1 Materials and reagents

Xanthan gum-palm oil system was prepared with commercial palm oil (KECOM TDS, Guinea, Africa), drinking water and xanthan gum (Cargill France SAS). Filling creams were composed of sugar (Disem, Spain), skimmed powdered milk (1% fat) (Central Lechera Asturiana, Spain), starch (CTex 06205, Cargill BV, Netherlands), drinking water and the XG-palm oil system. For the control cream, the XG-palm oil system was replaced by palm oil.

Simulated Saliva Fluid (SSF) was composed of 5.2g of $NaHCO_3$, 1.37g of $K_2HO_4P \cdot 3\ H_2O$, 0.88g of NaCl, 0.48g of KCl and 0.44g of $CaCl_2 \cdot 2H_2O$, dissolved in 1L of bidestillated water. 8.70g of α-amylase from porcine pancreas (A3176-1MU) and 2.16g of mucin from porcine stomach (M2378) (Sigma-Aldrich Chemical Company) were added to this solution (Mishellany-Dutour et al., 2011).

Simulated Gastric Fluid (SGF) was prepared according to a previous study (Sanz, Handschin, Nuessli, & Conde-Petit, 2007) with some modifications. 3.10g of NaCl, 0.11g of $CaCl_2$, 1.10g of KCl and 5.68ml of Na_2CO_3 (1M) were dissolved in 1L of bidestillated water. The solution was adjusted to pH 2. 0.15g of pepsin from porcine gastric mucosa (P7000) (Sigma-Aldrich Chemical Company) was dissolved in 1L of SGF.

Simulated Intestinal Fluid (SIF) was composed of an electrolyte solution and bile and pancreatin solutions. The electrolyte solution was prepared by dissolving 1.25g of NaCl, 0.15g of KCl and 0.055g of $CaCl_2$ in 1L of distilled water. Phosphate buffer solution was prepared (103.5mg $NaH_2PO_4 \cdot 2H_2O$ and 44.5mg $Na_2HPO_4 \cdot 2H_2O$ in 100ml of distilled water), setting the pH to 7.0 if necessary to prepare bile (bile extract B8631) and pancreatin (P1750) (Sigma-Aldrich Chemical Company) suspensions. (Sanz et al., 2007).

2.2 Preparation of XG-palm oil system

XG-palm oil system was prepared in the following proportions: water 102g, palm oil 94g and xanthan gum 4g, for a total final mass of 200g. The XG was first dispersed in the water at room temperature using a Heidolph stirrer at 300-500 rpm for 10-12 minutes. Then, palm oil previously heated at 45°C, was gradually added while continuing to stir, increasing the speed up to 1800 rpm. Stirring was maintained until a homogeneous mixture was obtained (10-15 minutes). The total mixture (200g) was homogenized using an IKA T18 basic (Ultra-Turrax) with the dispersion tool S18N-19G (stator diameter 19 mm and rotor diameter 12.7 mm) at 6500 (1 min^{-1}) for 60 s, 13500 (1 min^{-1}) for 60 s and subsequently at 17500 (1 min^{-1}) for 60 s. Finally, it was stored at 5°C for 24 hours before the measurements.

2.3 Filling cream preparation

One filling cream with XG-palm oil system and one control cream without XG were prepared. The ingredients used for the preparation of the different cream formulations are shown in Table 1.

Table 1. List of ingredients of the filling creams.

Ingredients (g/400 g cream)	Control	Cream with XG-palm oil system
Sugar	20	20
Milk powder	8	8
Starch	16	9
Water	262	163
XG-palm oil system	-	200
Palm oil	94	-

The preparation of the filling creams was performed in a TM31 Thermomix (Thermomix, Vorwrek, Wuppertal, Germany) and was carried out in two stages. In the first step, sugar, milk powder, starch and water were mixed at speed 2 for 6 minutes at 90°C and then the mixture was allowed to cool to 30ºC. In the second stage, the palm oil previously heated at 55ºC fat (control cream) or the XG-palm oil system was added by mixing at speed 2 for 5 minutes. The resulting creams were stored at 5ºC for 24 hours before the measurements.

2.4 *In vitro* digestion

An *in vitro* digestion model, consisting of oral, gastric and intestinal phases previously described by Borreani et al. (2017) and Espert et al. (2017), was used to simulate the digestion process in the gastrointestinal tract in humans.

- Oral phase: 0.5 mL of Simulated Saliva Fluid (SSF) was added to 25 g of sample and mixed for 5 s in a water bath at 37°C.

- Gastric phase: The "bolus" sample from the oral phase was mixed with 8 mL of Simulated Gastric Fluid (SGF) to obtain a final enzyme-sample ratio of 1:250 (v/v). The pH was adjusted to 2.0 with HCl (6 N) (Scharlab S.L., Spain) and the mix was maintained at 37ºC in a water bath with continuous stirring (60 min^{-1}) for 1 hour.
- Small intestinal phase: After gastric step, 5.3 mL of bile extract (46.87mg/mL) solution dissolved in phosphate buffer and 2 mL of electrolyte solution were added to the sample, and the pH was adjusted to 7.0 using NH3 (25% w/w) (Scharlab S.L., Spain). Then, 2.67mL of pancreatin dissolved in phosphate buffer was added to the mix (1:14 (v/v) ratio) and it was incubated for two hours with continuous agitation (60 min^{-1}) at 37ºC.

2.5 Texture analysis

A TA-XT plus texture analyzer equipped with the Texture Exponent software (Stable Microsystems, Godalming, UK) was used to evaluate the texture of the samples. A back extrusion assay was carried out using an A/BE back extrusion cell (40 mm diameter). 50g of sample were placed into an extrusion cylinder (50 mm diameter and 75 mm height) and the extrusion cycle was applied. The distance force was 15 mm, the compression rate 1 mm s^{-1}, and the trigger force 10g. From the force time profiles obtained, the area under the curve (N s) and the maximum force achieved were recorded.

2.6 Linear viscoelastic properties

Linear viscoelastic properties were studied using a controlled stress rheometer (AR-G2, TA Instruments (Crawley, England)) with a Peltier

heating system. A 40 mm roughened parallel plate and a gap of 1 mm were used. Samples were protected with vaseline oil (Panreac, Barcelona, Spain) in order to prevent them from drying during measurements.

Stress sweep tests were performed at 1 Hz to determine the linear viscoelastic region (LVR). Frequency sweeps were performed from 10 to 0.01 Hz at a stress wave amplitude inside the linear region. Storage modulus (G′), loss modulus (G′′) and loss tangent ($\tan\delta = G''/G'$) values were recorded. The tests were carried out in the fresh emulsions and after each digestion stage at a temperature of 37° C.

2.7 Microstructure analysis

The microstructure of the systems was evaluated using light microscopy (Nikon Eclipse 90i, Kanagawa, Japan). A small aliquot of each sample was placed on a glass slide and observed at 20X magnification. The images were captured from fresh samples and after digestion in *in vitro* models of the mouth, stomach and intestine.

2.8 Free Fatty acid (FFA) content

The amount of FFA was determined after total *in vitro* digestion by the previously described titration method (Mun, Decker, & McClements, 2007), with some modifications. After intestinal incubation, 15mL of ethanol (96%) (Scharlab S.L., Spain) was added to the digesta (6.25g of fresh sample with the digestion fluids) and was centrifuged for 10 min at 10.000 rpm (Sorvall® RC-5B Refrigerated Superspeed centrifuge). The total supernatant was quantified and the free fatty acids were determined in 10 ml of the supernatant by titration with 0.05 M NaOH (Panreac Química, Spain) and

phenolphthalein (Sigma-Aldrich Chemical Company (St Louis, MO)) as an indicator to end point (pink color). The FFA concentration of the samples was calculated from a standard curve, previously prepared using oleic acid (0, 50, 100, 150, 200 and 250 mM). The total amount of FFA were calculated considering the total volume of supernatant present in each sample. The results are expressed as "g oleic acid/g fat".

2.9 Statistical analysis

For each test, three replicates were performed with samples prepared on different days. An analysis of variance (ANOVA) was performed using XLSTAT statistical software (version 2009.4.03, Addinsoft, Barcelona, Spain). Tukey's test was used to determine the least significant differences ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Texture analysis

Figure 1 shows the effect of oral and gastric digestion on the extrusion profiles of the palm oil-XG system, the control cream and the cream prepared with the palm oil-XG system. To evaluate the effect associated with water dilution, the profiles corresponding to the water-diluted systems are also shown. Table 2 shows the mean values of the areas under the curve.

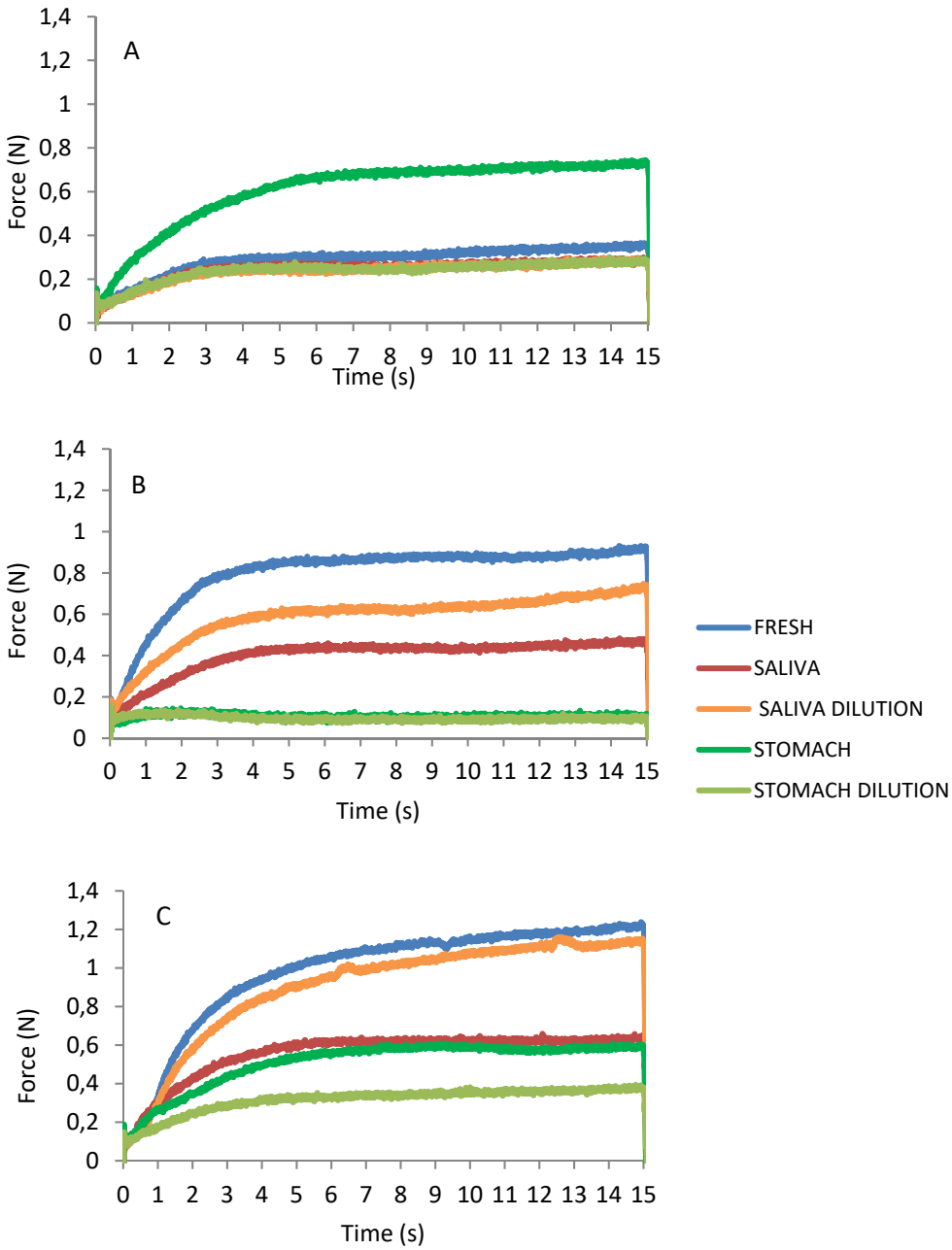

Figure 1. Effect of *in vitro* digestion on the extrusion profiles. A: XG-palm oil system; B: Filling cream control; C: Filling cream with XG-palm oil system.

The cream with the XG-palm oil emulsion showed the highest extrusion force and AUC, implying the highest consistency.

Table 2. Area under the curve (AUC) of force versus time values during extrusion.

	DIGESTION STEP	AREA (N·s)
XG-palm oil system	Fresh	$4.29^{B} \pm 0.45$
	Saliva	$4.86^{B} \pm 1.15$
	Saliva dilution	$4.56^{B} \pm 1.43$
	Stomach	$8.82^{A} \pm 0.58$
	Stomach dilution	$3.64^{B} \pm 0.20$
Control cream	Fresh	$10.54^{A} \pm 0.93$
	Saliva	$5.01^{B} \pm 1.58$
	Saliva dilution	$8.72^{A} \pm 0.10$
	Stomach	$1.70^{C} \pm 0.09$
	Stomach dilution	$1.43^{C} \pm 0.02$
Cream with XG-palm oil system	Fresh	$15.30^{A} \pm 1.27$
	Saliva	$8.76^{B} \pm 1.07$
	Saliva dilution	$13.40^{A} \pm 0.60$
	Stomach	$7.71^{B} \pm 0.98$
	Stomach dilution	$4.59^{C} \pm 0.12$

^{ABC} Means in the same column without a common letter differ ($P < 0.05$) according to Tukey's test.

The effect of saliva (oral digestion) was different in the creams than in the XG-palm oil system. In the latter system, no significant differences were found as regards the AUC of the fresh, saliva and saliva-diluted samples. On

the contrary, in both types of creams (with and without xanthan), the addition of saliva produced a significant decrease in the extrusion force, the values of AUC being lower for the saliva than for the corresponding water dilution systems. This structure breakdown in the creams upon incubation with saliva is associated with the presence of starch in the formulation of both creams (Robyt, 2008; Sanz and Luyten, 2006). The saliva structure breakdown in starch-based systems is recognized as a relevant fact for oral processing and for sensory perception (Lapis, Penner, Balto, & Lim, 2017; Prinz, Janssen, & de Wijk, 2007).

After stomach incubation, the significant increase in the extrusion force in the XG- palm oil system is noteworthy. This phenomenon has also been observed in xanthan gum sunflower oil systems and is associated with the weaknesses of the anionic XG matrix in the acidic stomach conditions, which produce fat coalescence and the subsequence increase in the consistency of the system (Espert et al., 2018).

With regard to the creams' behaviour after stomach incubation, a significant decrease in AUC is observed in the control cream (no xanthan gum), but no significant differences were found between the stomach sample and the water-diluted sample. On the other hand, the creams containing the XG-palm oil system exhibit no decrease in the AUC after stomach incubation. No significant differences were found between the saliva and stomach samples, the AUC corresponding to the water-diluted sample being significantly lower. This phenomenon indicates that the increase in consistency observed in the XG-palm oil system after stomach incubation is also evident in the cream matrix. This increase in consistency in the presence of xanthan gum at the level of the stomach may be an interesting strategy for the possible design of food to increase satiety as, in principle, an increase in the stomach

consistency is a good means of promoting satiety signals (Juvonen et al., 2009; Schroeder, Marquart, & Gallaher, 2013).

3.2 Linear viscoelastic properties

To better understand the structural changes that took place, the mechanical spectra were studied. The effect of digestion on the viscoelastic properties of the samples is shown in Figure 2.

The plateau zone of the mechanical spectra was visualized, with values of G' always higher than G'' and only a mild dependence on frequency in the available frequency window. This shape of the mechanical spectra was maintained in all the samples during digestion, with the exception of the control cream, which became fluid after intestine incubation. The XG-palm oil system showed the highest values of G' and G'' (Table 3).

Mixing with saliva produced a significant decrease in G' and G'' in the two types of creams (control and with XG-palm oil system), although in the cream with XG-palm oil system no significant difference in viscoelasticity (tan δ values) was observed, implying a weaker effect of saliva in comparison with the control cream.

Similarly to the results found in the texture analysis, the presence of xanthan gum affected the behaviour upon stomach digestion. In the XG-palm oil system, the stomach sample showed no significant decrease in comparison with the saliva sample, in spite of the higher dilution level of the stomach.

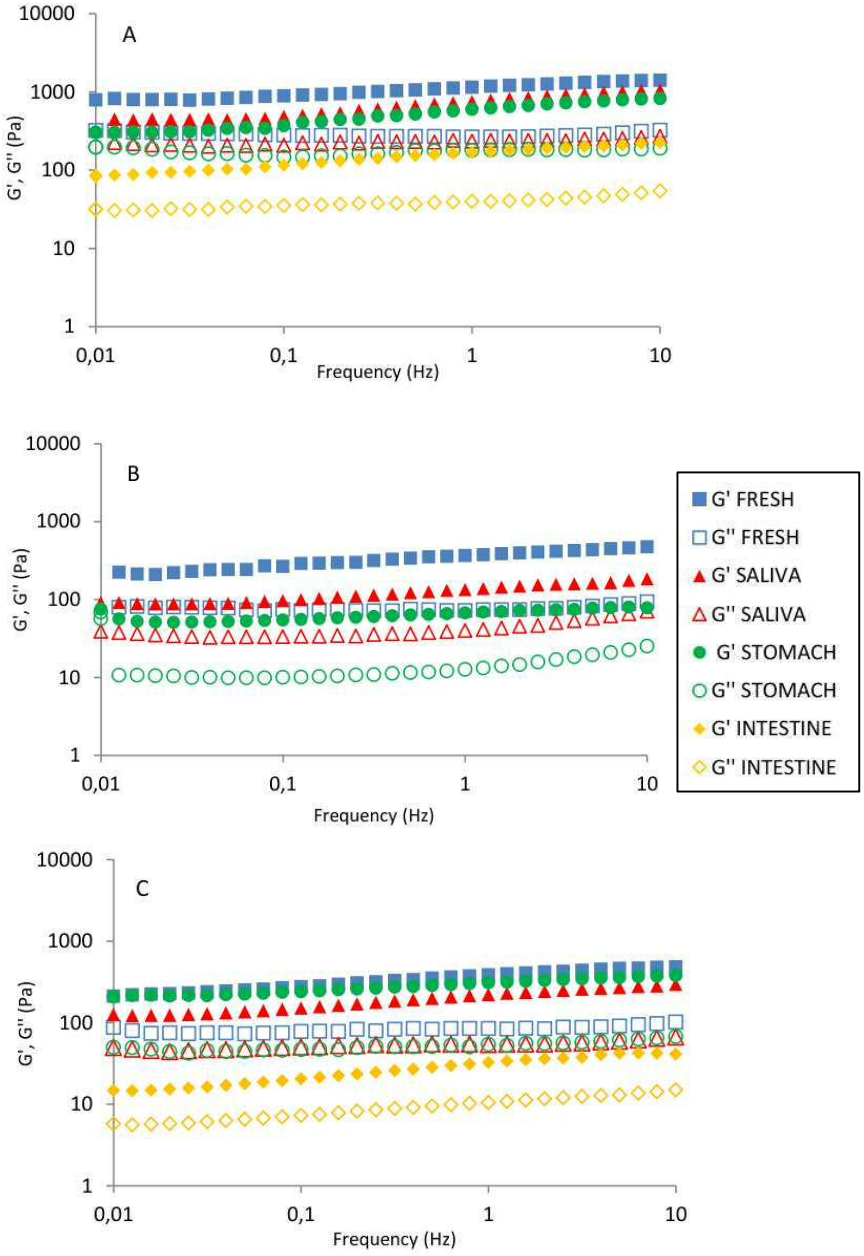

Figure 2. G' and G'' as a function of frequency of the fresh samples and after *in vitro* digestion at 37 °C (A: XG-oil system; B: Filling cream control; C: Filling cream with XG-oil system).

Table 3. Viscoelastic rheological parameters at 1Hz of the different systems, before and after *in vitro* digestion.

	Digestion step	G' (Pa)	G'' (Pa)	tan δ
XG-palm oil system	Fresh	1051,95 ^{A} ± 142.91	233,75^{A} ± 47.59	0,27^{A} ± 0.05
	Saliva	643.15^{B} ± 155.06	184.10^{A,B} ± 71.56	0.28^{A} ± 0.04
	Stomach	519.07^{B} ± 80.00	123.40^{A,B} ± 48.52	0.23^{A} ± 0.06
	Intestine	163.83^{C} ± 6.29	37.31^{B} ± 2.43	0.23^{A} ± 0.01
Control cream	Fresh	349.20^{A} ± 20.39	69.57^{A} ± 4.40	0.20^{B} 0.00
	Saliva	91.25^{B} ± 60.46	28.12^{B} ± 16.38	0.32^{A} ± 0.03
	Stomach	63.25^{B} ± 12.56	11.97^{B} ± 2.37	0.19^{B} ± 0.00
Cream with XG-palm oil system	Fresh	374.40^{A} ± 10.25	83.08^{A} ± 3.66	0.22^{B} ± 0.00
	Saliva	169.43^{C} ± 30.21	39.75^{C} ± 7.82	0.23^{B} ± 0.00
	Stomach	315.87^{B} ± 9.85	54.78^{B} ± 0.63	0.17^{C} ± 0.00
	Intestine	34.31^{D} ± 4.79	10.94^{D} ± 1.08	0.32^{A} ± 0.01

^{ABCD} Means in the same column without a common letter differ (P < 0.05) according to Tukey's test.

In the Xanthan cream, values of G' significantly increased and tan δ decreased (increase in viscoelasticity) after stomach incubation. As explained before, this increase in the consistency of the system is expected to be associated with fat coalescence and coagulation due to the weakness of the xanthan gum matrix in the acidic conditions, which favours the instability of the fat globules. In the case of the control cream, stomach incubation significantly decreased G' and viscoelasticity.

After intestine incubation, a decrease in both the viscoelastic moduli and the viscoelasticy was found in every system. In the control cream, the

viscoelastic properties were not measured due to the fluid behaviour of the sample.

3.3 Microstructure analysis

Light micrographs corresponding to the XG-palm oil system and the two types of creams are shown in Figure 3.

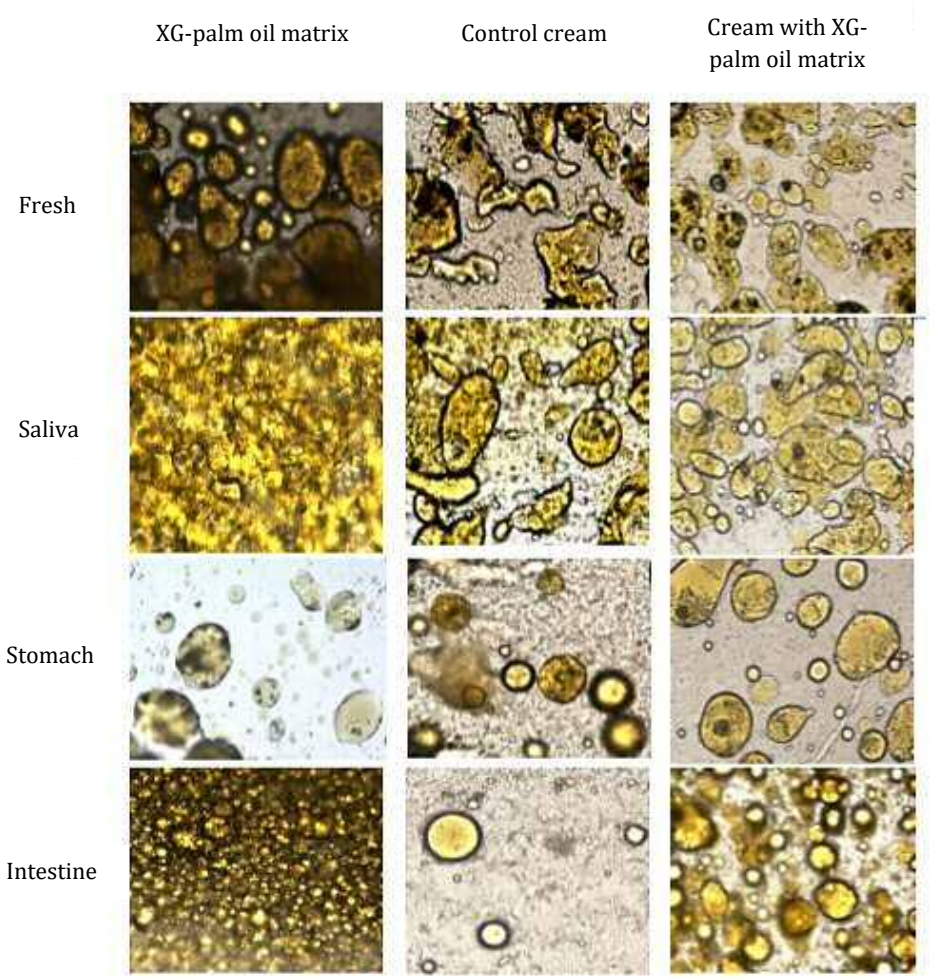

Figure 3. Microstructure of the different systems before and after *in vitro* digestion.

The microstructure of the fresh XG-palm oil system corresponds to an o/w emulsion of mainly fat crystals, spheroid in shape, embedded in a continuous phase composed of the hydrated xanthan gum. The microstructure is more complex in the creams and it is composed of gelatinized starch granules, liquid fat and fat crystals embedded in a milk and sugar water dilution (control cream) or in a milk, sugar and xanthan gum one (XG-palm oil cream). The appearance of fat crystals was noticeably more limited in the creams in comparison to the XG-palm oil system, which was attributable to the fat melting while the cream was being prepared. The fat microstructure in both creams was also found to be different. In the control cream, the fat was mainly dispersed as it was not emulsified before its incorporation into the cream, while in the XG –palm oil cream, fat globules are observed.

After saliva incubation at 37ºC, most of the fat crystals melt. This phenomenon is particularly evident in the XG-palm oil system, which contains the highest fat proportion. In the creams, saliva induces starch structural breakdown and fat flocculation associated with the mucin present in saliva is observed (van Aken, Vingerhoeds, & de Hoog, 2005), particularly so in the control cream.

After stomach incubation, the control cream exhibits smaller fat globules, while a general increase in the fat globule size is observed in the XG-palm oil cream. This growth in size is in accordance with the increase in the viscoelastic properties found after stomach incubation when in the presence of xanthan gum, and it is associated with a weakness of the xanthan gum network under the acidic conditions. The carboxylic groups of XG are not ionized at acidic pH, and the electrostatic interactions and the hydrocolloid network become weak. The presence of other gums in an emulsion also increased the droplet size in stomach conditions (Pasquier et al., 1996b).

After intestine incubation, every system experiences important changes in the microstructure. In the control cream, the fat almost disappears and only some remaining fat globules may be visualized. The existing fat is expected to have been digested by the combined action of bile and the lipase activity. In both the XG-palm oil cream and the XG-palm oil system, the fat globules are observed to be smaller, which is associated with the surfactant effect of bile salts; however, an extensive amount of fat globules is observed, implying that the fat digestion process is still not completed. The role played by the xanthan gum network prevents the fat digestion process from functioning effectively (Sasaki & Kohyama, 2012; Espert et al., 2018).

3.4 FFA release during *in vitro* digestion

The amount of free fatty acids released after intestine *in vitro* digestion, expressed as oleic acid, is shown in Table 4.

Table 4. Oleic acid values generated in the different systems after *in vitro* digestion

	g oleic acid/g fat
XG-palm oil system	$0.06^{B} \pm 0.02$
Control cream	$0.25^{A} \pm 0.02$
Cream with XG-palm oil system	$0.08^{B} \pm 0.01$

^{AB} Means in the same column without a common letter differ ($P < 0.05$) according to Tukey's test.

The amount of oleic acid is an indicator of the free fatty acids generated in the system as a consequence of triglyceride digestion by lipase activity. In the digestion process, bile salts adsorb on to the lipid droplet surface facilitating the emulsification of the lipids, then pancreatic lipase adsorbs on to the surface of the emulsified lipids and breaks the fat into free fatty acids (Mc Clements, Decker, & Park, 2007).

The amount of oleic acid was significantly lower in the presence of xanthan gum. Despite both creams having the same initial amount of palm oil, free fatty acid generation in the XG cream was significantly lower than in the control cream. A reduction of 68% in free fatty acid content was found in the XG cream in comparison to the control cream. The incorporation of the palm oil by means of the XG-palm oil system represents a barrier to fat digestion. Bile salts and enzymes have limited access to the oil phase and, therefore, lipase does not act efficiently on the surface of the fat. This FFA reduction agrees with the hypothesis shown by Pasquier et al. (1996b), which indicates that the presence of soluble fibers such as guar gums could alter the emulsification of dietary lipids and, subsequently, lower the extent of fat lipolysis.

Data obtained coincides with the microstructural images, where an almost complete disappearance of the fat globules was found in the control cream; in the cream with the XG-palm oil system, however, a considerable amount of fat globules can still be observed after intestine digestion.

4. CONCLUSIONS

The incorporation of palm oil into a cream filling as a XG-palm oil system was investigated. XG–palm oil systems were developed and applied in the formulation of a low-fat cream filling. The texture, rheology and microstructure were studied and related with the fat digestion process.

Systems with xanthan gum showed a significant increase in viscoelastic properties during stomach digestion, which was mainly associated with the acidic pH conditions. This increase in the consistency of the systems may have an application when some type of food structuring is required during

digestion; for example, for the purposes of developing food with satiating properties, among other applications.

Moreover, there was less lipid digestion observed in the presence of XG. This is an indicator of the degree of structural resistance provided by the hydrocolloid network in the continuous phase.

In conclusion, the application of XG in a fat matrix is very valuable when designing foods with novel functionalities. Firstly, the greater viscosity of the system in the stomach is expected to slow down gastric emptying, increasing the perception of satiety. Secondly, the decrease in fat bioaccessibility makes the product an interesting option when a fat reduction is desired. Future studies will be focused on the analysis of the XG-palm oil cream sensory properties.

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Capítulo 3

Aplicación de emulsiones de baja digestibilidad lipídica al desarrollo de cremas untables

Structural changes of filling creams after *in vitro* digestion. Application of hydrocolloid based emulsions as a fat replacer.

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Enviado a *LWT - Food Science and Technology*

ABSTRACT

The objective of this study was to evaluate the application of hydrocolloid-based o/w emulsions as a substitute for conventional fat in foodstuffs, such as filling creams. Emulsions containing 47% of sunflower oil stabilized by different hydrocolloids (hydroxypropyl methylcellulose HHPMC, methylcellulose MC and xanthan gum XG at 2%, were used to prepare filling creams with 23.5% final fat content. The changes in the structure of the systems before and after *in vitro* digestion were studied by textural analysis and confocal and light microscopy. Lipid digestibility was evaluated after the small intestine *in vitro* digestion phase and the relationship between structural changes and fat digestibility evaluated. The textural characterization concluded that the presence of XG led to an increase in consistency in the stomach digestion phase, in comparison with cellulose ethers. Fewer free fatty acids (FFA) were generated in creams that contained the hydrocolloid-based emulsions if compared with a hydrocolloid-free control sample. Therefore, these o/w emulsions stabilized with HHPMC, MC or XG could be applied in the development of both food with low fat digestibility and satiating food, which will help in obesity control and the treatment of fat related illnesses.

Key words: hydrocolloids, structure, lipid digestibility, emulsions.

1. INTRODUCTION

In today's society where consumers increasingly value healthy, low-fat foods, interest in the design, formulation and development of foods that are

able to contribute fewer calories to the body is growing. However, reducing fat consumption is not an easy task, due to the fact that fat provides a wide variety of desirable food properties, affecting the texture, mouthfeel, flavors and functionality of the final product (Co & Marangoni, 2012; Jones, 1996). Consequently, there is a need to develop effective strategies with which to reformulate high quality reduced-fat foods with properties similar to those found in traditional full-fat products, while attempting to preserve the attributes so desired by the consumer.

The most commonly used fats in pastries and confectionery food are butter, margarine or hydrogenated oils (Talbot, 2009; Van den Bremt, Van hoed, Müllendorff, & Arnaut, 2012). The fat content of the creams can reach up to 60% and has a great effect on its sensory, rheological and textural properties (Miele, Di Monaco, Masi, & Cavella, 2015; Young & Wassell, 2008). As regards hydrogenated vegetable oils, they have been used in a wide variety of confectionery and pastry food in recent years. However, hydrogenated oils are the primary dietary source for *trans* fats and there is scientific evidence showing that the consumption of *trans* fatty acids is associated with coronary heart disease and diabetes (Mozaffarian, Katan, Ascherio, Stampfer, & Willett, 2006). An additional problem is that some alternatives to *trans* fats may lead to higher levels of saturated fatty acids.

One option to reduce the fat content in filling creams is to use food emulsions. The use of vegetable fat in these emulsions improves the lipid profile in the final product, but the low percentage of saturated fat in its composition cannot achieve the semi-solid structure associated with solid fat. One way to obtain the desirable characteristics is by incorporating hydrocolloids as stabilizers in an oil-water emulsion. Some hydrocolloid polymers are used in food systems for their physicochemical properties. They possess the ability to retain water, to act as stabilizers, to thicken and

to provide sensory properties, all of which make them suitable as fat substitutes (Saha, & Bhattacharya, 2010).

The presence of non-fat particles and the type and amount of fat in an emulsion play an important role in the physicochemical, sensory and nutritional properties, determining the texture, mouthfeel, flavour stability, appearance and biological response in emulsion-based products (Chung, Smith, Degner, & McClements, 2016). However, besides using vegetable oils emulsified by hydrocolloids to improve the plasticity of fat (semi-solid character), it would also be interesting for the structure of the stabilized emulsion to be able to resist throughout the digestion process, thus influencing lipid digestion (Bonnaire et al., 2008; Qiu, Zhao, Decker, & Mc Clements, 2015). Recently, cellulose ether-based o/w emulsions have been recognized as representing a good strategy by which to replace saturated fats and reduce the fat digestibility (Tarancón, Salvador, & Sanz, 2013; Espert et al., 2017; Espert, Salvador, & Sanz, 2018).

Therefore, the objective of this study is to evaluate the physical properties and fat lipolysis of filling creams composed of o/w hydrocolloid-based emulsions as fat replacers. For this purpose, the structural changes of filling creams during *in vitro* digestion and their relationship with fat digestibility are evaluated.

2. MATERIALS AND METHODS

2.1 Materials

Filling creams were composed of sugar (Disem, Spain), skimmed powder milk (1% fat) (Central Lechera Asturiana, Spain), starch (CTex 06205, Cargill BV, Netherlands), mineral water and o/w emulsion. For the control cream,

the o/w emulsion was replaced by sunflower oil (Koipe Sol, Deoleo S.A., Madrid, Spain). Three different emulsions were prepared with sunflower oil, water and three different hydrocolloids (two types of cellulose ethers and xanthan gum (XG)). Cellulose ethers with thermogelling ability were supplied by The Dow Chemical Co (METHOCEL™ K4M and A4M, from now on referred to as HHPMC (high hydroxypropyl methylcellulose) and MC (methylcellulose), respectively) with differing degrees of methoxyl content. Their percentage of chemical substitution is: HHPMC, 22.5%; methoxyl, 7.7%; hydroxypropyl and MC, 30%. methoxyl. Both celluloses have approximately the same molecular weight (MW) and a viscosity of 4000 mPa s (2 % aqueous solution at 20 °C measured by The Dow Chemical Company following reference methods ASTM D1347 and ASTM D2363). Xanthan gum (XG) (Satiaxane CX911), with a viscosity of 1500 mPa s in a 1% aqueous solution (+1% KCl), measured by Cargill France SAS, was supplied by Cargill.

2.2. Emulsion preparation

The emulsions were prepared using the following proportions: water 51% (w/w), sunflower oil 47% (w/w) and hydrocolloid 2% (w/w), for a total final mass of 200 g.

To prepare the cellulose o/w emulsion, cellulose ether was first dispersed in the oil using a Heidolph stirrer at the lowest speed (300 rpm) for five minutes. The mixture was then hydrated by gradually adding the water at 10ºC while continuing to stir for 30 s. Stirring continued using a homogenizer (Ultraturrax T18, IKA, Germany) with the S18N-19G dispersion tool at 6500 (1 min−1) for 15 s, and subsequently at 17500 (1 min−1) for 60 s until the emulsion was obtained.

In XG o/w emulsion, the XG was first dispersed in the water at room temperature using a Heidolph stirrer at 300-500 rpm for 10-12 min. Then the oil was gradually added while continuing to stir, increasing the speed up to 1800 rpm. Stirring was maintained for an additional 15 min. The 200g mixture was then homogenized using an IKA T18 Ultra-Turrax with the S18N-19G dispersion tool at 6500 (1 min−1) for 60 s, 13500 (1 min−1) for 60 s and subsequently at 17500 (1 min−1) for 60 s.
The emulsions were stored at room temperature for 24 h before the cream preparation.

2.3. Cream preparation

The different cream formulations are shown in Table 1.

Table 1. Ingredients of filling cream formulations.

Ingredients (g/400 g cream)	**Control**	**MC/HHPMC/XG**
Sugar	20	20
Milk powder	8	8
Starch	16	9
Water	262	163
Emulsion	-	200
Sunflower oil	94	-

The preparation of the cream was performed in a TM31 Thermomix (Thermomix, Vorwrek, Wuppertal, Germany) and was carried out in two stages. In the first step, sugar, milk powder, starch and water were mixed at speed 2 for 6 min at 90°C and then the mixture was allowed to cool to room temperature. In the second stage, the fat (sunflower oil (in control cream) or

o/w emulsion (in emulsion creams)) was gradually added by mixing at speed 2 for 5 min. Once the cream was made, it was stored at 5ºC for 24 h before the measurements.

2.4. *In vitro* digestion model

The digestion process was simulated using an *in vitro* digestion protocol previously reported by Borreani et al. (2017) and Espert et al. (2017). To consider the effect of water dilution, samples were also incubated in mouth and stomach models, but only with the addition of distilled water. The incubation process (time, temperature and shaking conditions) was the same as that in the samples with enzymes.

2.5. Extrusion test

The extrusion properties of filling creams were evaluated according to Espert et al. (2017) using a TA-XT plus Texture Analyzer. A back extrusion test was conducted using an A/BE back extrusion rig cell (40 mm diameter). From the force time profiles obtained, the area under the curve (AUC) (N s) was recorded as a measure of consistency.

2.6. Microstructure

The different creams were observed at 40x magnification for confocal laser scanning microscopy (CLSM) according to Borreani, Llorca, Quiles, & Hernando (2017) and at 10x magnification for light microscopy (LM) (Borreani, Llorca, Larrea, & Hernando, 2016).

2.7. Free fatty acid (FFA) measurement

The extent of lipid digestion was determined by measuring the free fatty acids (FFA) produced after small intestine digestion according to a method reported by Espert et al. (2017) adapted from a previously described titration method (Mun, Decker, & McClements, 2007).

2.8. Statistical analysis

The analyses were performed on triplicate samples prepared on different days, and results were expressed as the mean and standard deviations. Analysis of variance (ANOVA) was performed on the data using XLSTAT statistical software (version 2010.5.02, Addinsoft, Barcelona, Spain) and Tukey's test was used to assess the differences in mean values ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Textural properties

Figure 1 shows the extrusion profiles corresponding to the different creams before and after *in vitro* digestion steps and Table 2 shows the AUC values calculated from these curves. As can be seen, all the creams prepared with hydrocolloid-based emulsions showed a significantly greater consistency than the control cream, with the XG cream presenting significantly the highest force values (Table 2), which was associated with the greater thickening ability of XG in comparison to the cellulose ethers and starch. After the oral step, significantly lower consistency values were found in all the samples when they were incubated with SSF if compared to those

incubated with water at the same dilution factor, this is because the α-amylase of saliva acts by hydrolyzing the starch causing the consequent loss of consistency, as previously observed by Sanz, Handschin, Nuessli, & Conde-Petit (2007) in custards. In the samples incubated with water (water saliva dilution), the control and XG creams were found to have no significant differences when compared with the fresh creams, and only a slight decrease was found in the cellulose creams when also compared with the fresh samples.

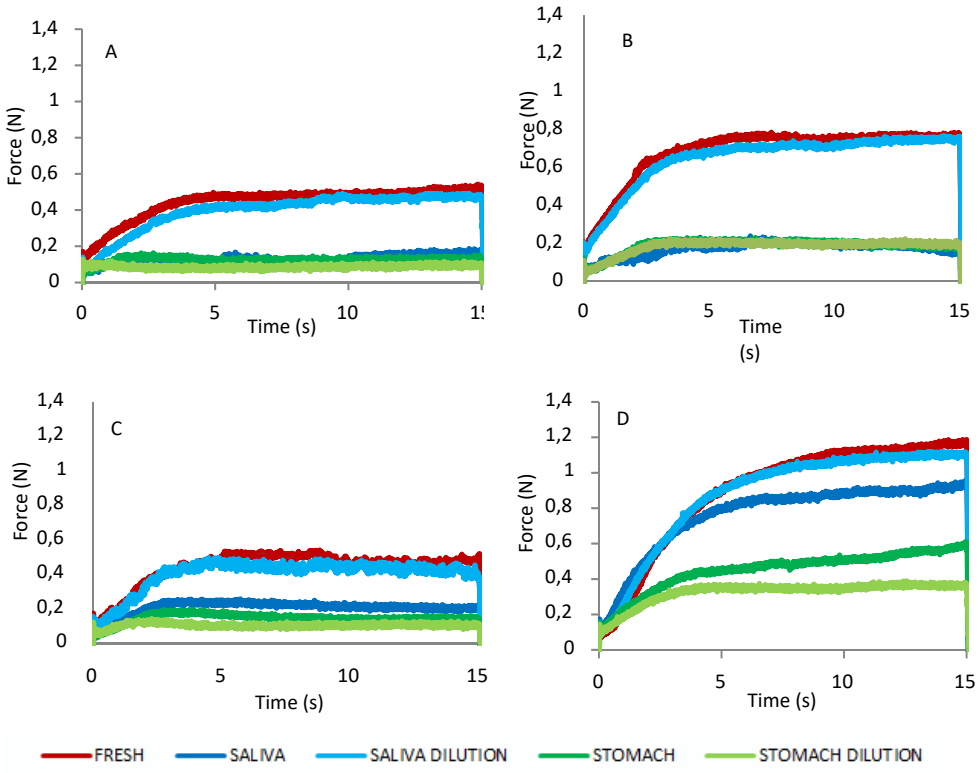

Figure 1. Effect of *in vitro* digestion on the extrusion profiles. A: control cream; B: MC cream; C: HHPMC cream; D: XG cream.

The fresh XG cream exhibited significantly higher force values than the other fresh creams and after saliva addition, as well as after saliva water dilution incubation. MC cream was the second most consistent sample, followed by HHPMC and control creams, with there being no significant differences between the latter two.

Table 2. Area under the curve (AUC) (N.s) of the creams, before and after *in vitro* digestion.

Digestion step	CONTROL	MC	HHPMC	XANTHAN GUM
Fresh	$7.29^{aD}\pm0.05$	$10.57^{aB}\pm0.08$	$8.59^{aC}\pm0.11$	$13.25^{aA}\pm 0.17$
Simulated Saliva Fluid	$3.47^{bC}\pm0.57$	$5.56^{cB}\pm0.20$	$3.17^{cC}\pm0.38$	$11.26^{bA}\pm0.24$
Saliva Water Dilution	$6.63^{aC}\pm0.06$	$8.96^{bB}\pm0.63$	$6.88^{bC}\pm0.41$	$13.02^{aA}\pm0.28$
Simulated Gastric Fluid	$1.84^{cC}\pm0.12$	$2.58^{dB}\pm0.35$	$2.20^{dBC}\pm0.14$	$6.32^{cA}\pm0.30$
Stomach Water Dilution	$1.31^{cC}\pm0.01$	$2.66^{dB}\pm0.21$	$1.55^{dC}\pm0.11$	$4.72^{dA}\pm0.26$

abcd Different letters in the same column and for the same cream type denote values with statistically significant differences ($p<0.05$) according to the Tukey test.
ABCD Different letters in the same row and for the same digestion step denote values with statistically significant differences ($p<0.05$) according to the Tukey test.

Gastric digestion originated a decrease in the consistency of all the samples if compared to fresh and oral digested ones. However, the reduction in AUC values in these samples was mainly due to the dilution effect and not due to enzyme action, as no significant differences were found between the incubated sample with simulated gastric fluids and the incubated sample with stomach water dilution, except for the sample prepared with XG. Therefore, the presence of enzymes and gastric conditions had the same effect as that caused by water in control and cellulose samples. Nevertheless, the behaviour in the stomach phase was different in the cream with XG, where the stomach conditions exerted about a smaller decrease in consistency compared to the stomach water dilution sample. The cream prepared with XG showed significantly higher force values after gastric

incubation than after the stomach water dilution, which indicates an increase in consistency associated with the specific stomach conditions. Espert, Salvador, & Sanz (2018) also observed this increase in force values in XG-oil in water emulsion in stomach conditions (acid pH), which was attributed to the anionic nature of XG which produces fat coalescence and, therefore, an increase in the consistency of the sample. Sasaki & Kohyama (2012) concluded that the presence of XG in starch suspensions exerted a suppressive effect on starch digestibility.

3.2. Microstructure of filling creams during in vitro digestion

The microstructure of the creams was observed using CLSM and LM as they passed through each phase of the *in vitro* digestion model (Figures 2 and 3). The creams exhibited a continuous red matrix consisting mainly of starch (arrows in Figures 2 A, E, I and 3 M) milk protein and carbohydrates, where the fat globules may be observed in green (Figure 2). Fresh control cream (Figures 2 A and 3 A) contained large, rounded lipid globules of differing sizes. In creams made with celluloses (Figures 2 E, I, and 3 E, I), fat globules of differing sizes were rounded, but smaller than in the control cream. In all likelihood, this suggests that the fat globules in creams elaborated with cellulose emulsions were stabilized due to the formation of a hydrated cellulose network present in the aqueous phase. This network immobilizes the fat globules of the dispersed phase (Li, Wang, & Xu, 2003), preventing flocculation and coalescence phenomena due to the decreased mobility of globules within the matrix (Aranberri, Binks, Clint, & Fletcher, 2006; Piorkowski, & McClements, 2014). Visually, it could be seen that fat globules in creams with HHPMC (Figures 2 E and 3 E) were smaller than those in creams with MC (Figures 2 I and 3 I), possibly because of the presence of

hydrophobic substituent groups in the MC (Espert et al., 2017). In the case of XG cream (Figures 2 M and 3 M), although some fat globules appeared to be very big, different sizes can be distinguished. In general, these globules were less rounded than in the other creams (control, HHPMC and MC creams). This is as expected, as xanthan gum is not an emulsifier, and only acts as an emulsion stabilizer by thickening the continuous phase.

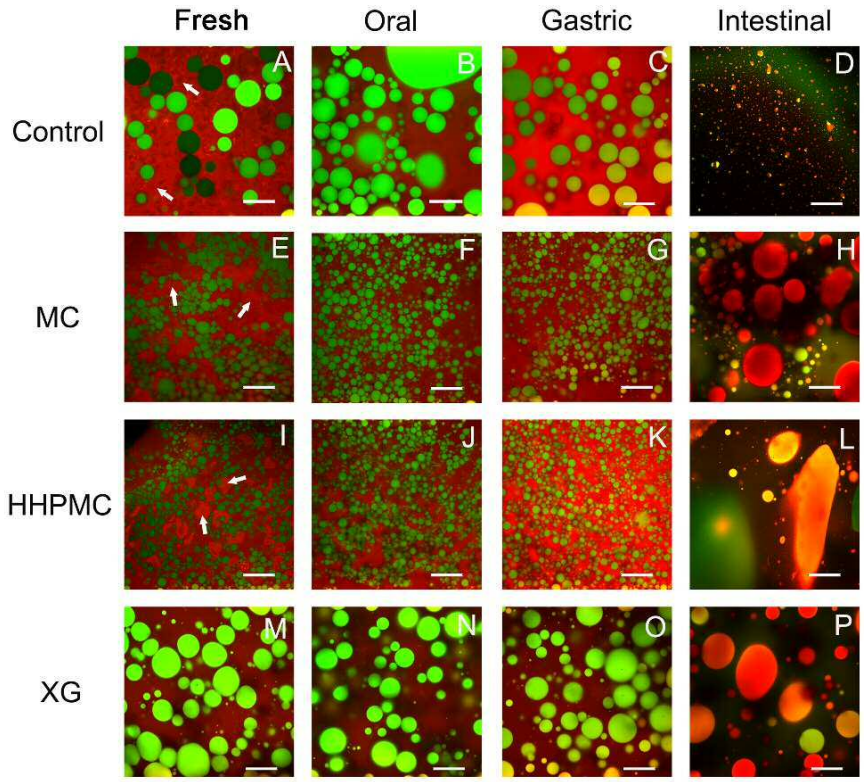

Figure 2. Confocal micrographs of the different creams before and during *in vitro* digestion. White arrows point to starch granules. Magnification 40x. Nile Red and Rhodamine B staining. The scale bars measure 60 μm.

After oral incubation, the continuous phase was observed to be more homogeneous in all the creams (Figures 2 and 3); starch granules (pointed

to by arrows in Figures 2 A, E, I and 3 M) are not observed in the oral digested samples due to starch hydrolysis by the α-amylase, as previously observed in other studies (Robyt, 2008; Sanz, & Luyten, 2006). In this phase, fat globule flocculation and coalescence were observed in the control cream (Figures 2 B and 3 B). This phenomenon is not observed in cellulose creams or xanthan gum creams (Figures 2 F, J, N and 3 F, J, N), because the hydrocolloid networks continue providing stabilizing capacity, as they are not affected by the enzymatic effect of saliva.

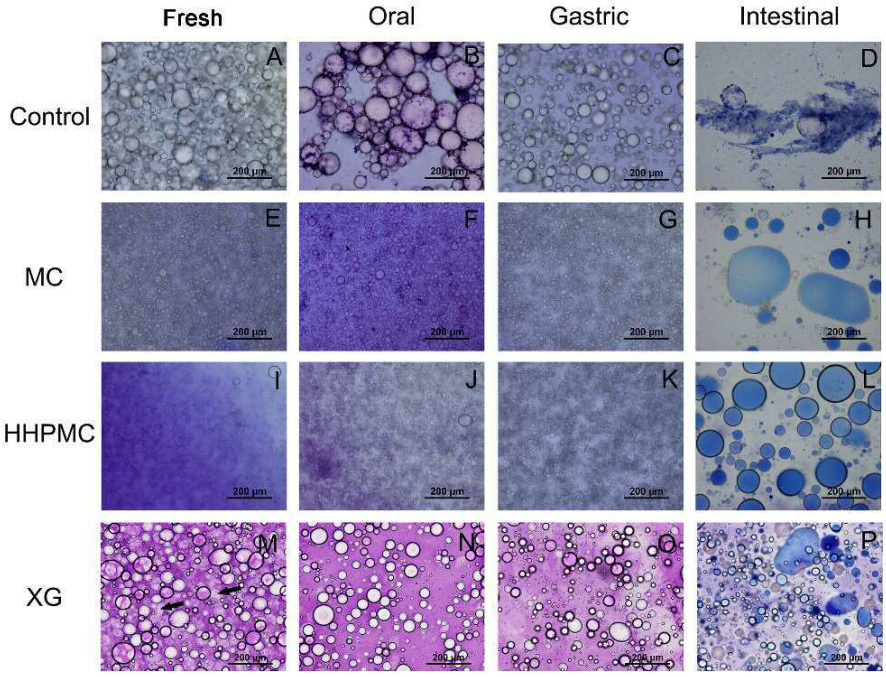

Figure 3. Light microscopy micrographs of the different creams before and during *in vitro* digestion. Black arrows point to starch granules. Magnification 10x. Toluidine blue staining.

After the gastric digestion phase, the control cream (Figures 2 C and 3 C) exhibited smaller fat globules, while in cellulose creams (Figures 2 G, K and 3 G, K) and in xanthan creams (Figures 2 O and 3 O), the fat globules were

similar in shape and size to the previous digestion steps. However, a lower density of fat globules can be observed, attributable to the diluting effect of the digestion fluids.

The intestine digestion step markedly affected the structure of all the systems, although it was more noticeable in the control cream (Figures 2 D and 3 D), in which the fat was mostly digested by the action of pancreatic lipase. In all the hydrocolloid creams, a significant increase in both the size of the fat globules and droplet coalescence was observed. At this level of digestion, some of the bile salts are able to displace the hydrocolloid molecules, causing a destabilizing effect (Li, Hu, & McClements, 2011). It has been reported that the hydrolysis of triglycerides in the small intestine is related to the available surface area, and an increase in droplet size is associated with a reduced surface area and a reduction in the rate of lipid hydrolysis (Lairon, 1007; Schneeman, & Gallaher, 2001). Even though the cellulose creams showed a clear change in their microstructure after *in vitro* digestion, the fat globules were not fully digested in either cream with cellulose. The creams elaborated with xanthan gum were not completely digested in the intestinal step, either. Similarly to both cellulose creams, an marked increase in both the size of the fat globules and droplet coalescence was observed in the xanthan cream. In all the hydrocolloid creams, the effect of intestine digestion is reflected by an increase in globule size and the appearance of coalescence due to changes in fat stability, implying an effect of the digestive fluids. However, fat globules are still visualized, implying that both celluloses and xanthan gum continue to exert a stabilizing effect.

3.3. FFA release after *in vitro* digestion

The extent of lipid digestion is dependent on the fat content, the physicochemical processes that occur during the digestion phases, the lipid

droplet concentration and the droplet size, among other conditions (McClements, Decker, & Park, 2009; Li, & McClements, 2010; Li, Hu, & McClements, 2011). The FFA generated after *in vitro* digestion were used to quantify the extent of lipid hydrolysis. Table 3 shows the oleic acid values generated in the different filling creams during *in vitro* digestion.

Table 3. Oleic acid values after intestine *in vitro* digestion of the filling creams.

Cream type	Oleic acid (g)	Oleic acid/initial fat
CONTROL	0.261a	0.168a
MC	0.198b	0.127b
HHPMC	0.201b	0.129b
XANTHAN GUM	0.133c	0.085c

[abc] Within the same column, values with the same letter are not statistically different according to the Tukey test (p> 0.05).

The extent of lipid digestion was found to decrease in creams with hydrocolloid-based emulsions (XG>MC=HHPMC) if compared to the control cream with non-emulsified oil, being significantly in the case of the XG sample. These results were similar to those found in the cellulose/xanthan gum oil/water emulsions (Espert et al., 2017; Espert, Salvador, & Sanz, 2018), where it is explained that the main mechanism of the hydrocolloid is acting as a physical barrier, preventing an effective action of the enzymes and conferring structural stability on digestion. Lairon (1997), Schneeman and Gallaher (2001) and Lairon, Play, & Jourdheuil-Rahmani (2007) also investigate the use of gums and cellulose ethers for the purposes of reducing lipid digestibility. They attributed this behaviour to different mechanisms, such as direct interaction between dietary fiber-enzyme, the formation of a protective coating by the fiber around the lipid droplet, the binding of the bile salts or an increase in the viscosity.

4. CONCLUSIONS

O/w emulsions, stabilized by cellulose ethers or xanthan gum, were applied in a filling cream formulation and investigated. The type of hydrocolloid influenced the consistency of the creams, the structural changes during *in vitro* digestion and the lipid digestibility. The creams prepared with MC, HHPMC or XG o/w emulsion were more resistant to *in vitro* digestion than the control cream, exhibiting a significant decrease in fat digestibility. Moreover, in XG creams, stomach conditions brought about an increase in consistency, which could result in other applications other than a decrease in fat digestion, such as an increase in the satiating capacity. These results suggest that the vegetable oil/ water emulsions, stabilized with either cellulose ethers or xanthan gum, can be applied and considered as a low saturated fat, a *trans* free alternative to conventional fats, also reducing the oil bioaccessibility during *in vitro* digestion. Furthermore, the use of a XG system could also be applied in the formulation of foodstuffs that promote satiety. The information provided in this study should prove useful for the purposes of designing reduced fat products with improved physical and functional characteristics.

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Thermorheological properties of reduced-fat cocoa creams based on cellulose ethers emulsions

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Enviado a *Food Hydrocolloids.*

ABSTRACT

Flow behaviour and viscoelastic properties of cocoa filling creams have been studied. Creams were composed of cocoa, starch, sugar, skimmed milk powder and cellulose ether based o/w emulsions as fat replacer. They were compared with a starch based cream with the same fat content, considered as a control. Two types of methylcelluloses, MC, and two types of hydroxypropyl methylcelluloses, HPMC, with different chemical substitution degrees were employed. Results showed important differences in zero shear viscosity and shear thinning character at room temperature, due to the different internal structure revealed by the viscoelastic moduli spectra. Temperature sweeps of storage modulus showed different temperature gelation depending on the chemical substitution in cellulose ethers. Rheological behaviour for MC creams at 45 ^{o}C was similar than HPMC creams behaviour at 70 $^{\circ}$C, what means all creams had analogous characteristics once the cellulose ethers are gelled.

Keywords: flow behaviour, viscoelasticity, emulsions, cellulose ether, cocoa cream

1. INTRODUCTION

Filling creams are a major component in many pastry and confectionary products. Their physical properties vary extensively from semisolid to more spreadable and plastic, depending on their specific application. The most common use is to insert a cream into the product by injection into a dough, or use it laminated between two doughs. Typical baked fillings usually contain 30-35% fat, although this amount can reach up to 60% when certain properties of spreadability or adhesion are desired (Abboud, 1999; Miele et al., 2015). The fats normally used in this type of products are saturated fats (Idris and Samsuddin, 1993). In short, there exist a wide variety of cream recipes, but fat and sugar are major ingredients in all of them and usually they also contain starch.

The amount of fat used and its characteristics, as well as the composition of ingredients in general, will have a significant influence on the structural properties of the final product. Rheological properties are among the most important physical properties defining the structure of a food system and they contribute to the characterization and control of the texture when different formulations are used (Liu et al., 2007). The modification of any of physical factors, such as stress applied or temperature, as well as any change in the ingredients proportion of the system (elimination or incorporation of new ingredients) will influence the rheological behaviour (Lončarević et al., 2016; Marcotte et al., 2001; Meza et al., 2018; Pichler et al., 2012), and thus have a key role in industrial food manufacturing processes. Moreover, due to the correlation between rheological parameters and sensory evaluation of food, rheology can be used as a tool to establish new formulations or improve existing ones.

Generally, this type of creams shows shear thinning flow behaviour and viscoelastic properties characteristic of weak gels (Tárrega et al., 2005). Nevertheless, the presence of hydrocolloids and the changes in application temperature can influence these characteristics.

The intake of full-fat products is associated with obesity and other related diseases (Bray and Popkin, 1998; Chajès et al., 2008; Mozaffarian, 2016; Sun et al., 2007). In addition to healthier foodstuffs, the consumers demand products with similar physical and sensory properties to those of the conventional product, which has led the industry to develop strategies to obtain optimal structural characteristics.

Regarding to fat content, the interest has been focused in: general fat reduction, improvement of lipid profile (saturated fat reduction) and *trans* fatty acid elimination. Nevertheless, fat reduction is not an easy task for food manufacturers, due to the wide variety of important functionalities exerted by fat, both in structure and sensory properties as well as in technological aspects (Drewnowski, 1992; Drewnowski and Almiron-Roig, 2009; Zoulias et al., 2002).

A wide number of strategies have been employed to mimic fat functionality in food items. It is recognized that hydrocolloids are widely used as texture modifiers with the purpose of improving the rheology of food systems (Saha and Bhattacharya, 2010) since they can compensate the loss of structure in reduced-fat food (Meyer et al., 2011; Peng and Yao, 2017). They are used mainly as thickeners and stabilizers in a variety of foods (ADA, 2005; Lucca and Tepper, 1994) and could offer similar eating pleasure as high-foods, without providing excessive energy (Drewnowski, 1992). In recent years, structuring liquid oils through the formulation of oleogeles or structured biphasic systems (emulsions) by using biopolymers has shown potential as an alternative to conventional fats (Jiménez-Colmenero, 2013; Wang et al.,

2016). One way to confer structure to sunflower oil is to incorporate them in a cellulose ether emulsion. These cellulose ether based emulsions present a semisolid consistency and had been successfully employed as replacer of conventional solid fat in certain food applications (Tarancón et al., 2013). The advantages of this system are lower fat content than conventional fat, a fat profile rich in unsaturated fatty acids, no *trans* fatty acids and lower fat digestibility (Espert et al., 2017).

The cellulose ethers methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) possess surface activity and unique hydration characteristics (Sanz et al., 2005) which means that they have a wide range of applications. The thermorheological behaviour of methylcellulose solutions has been previously studied (Chen et al., 2008; Li, 2002; Sanz et al., 2005) and also the influence of methylcellulose and hydroxypropyl methylcellulose in the rheological behaviour of oil-in-water emulsions (Sanz et al., 2015). However, rheological properties have not been studied in more complex systems which incorporate other ingredients in the formulation.

In this study, reduced-fat cocoa filling creams containing vegetable oil / water emulsion as fat replacer are developed. The emulsions were prepared using cellulose ethers with different chemical substitution as a structuring agent. The objective was to investigate the rheological behaviour of the cocoa filling creams for different temperatures, compared with a starch base cream with the same fat content that will be considered as a control. The obtained conclusions will be helpful to determine the suitability of each emulsion according to the required cream properties and the specific application.

2. MATERIALS AND METHODS

2.1 Preparation of emulsions

Four types of emulsions were prepared using different types of cellulose ethers supplied by The Dow Chemical Co. (USA): methylcelluloses A4M and MX, and hydroxypropyl methylcelluloses F4M and K4M. The percentage of chemical substitution is: A4M (30% methoxyl), MX (methoxyl >30%), F4M (29% methoxyl and 6.8% hydroxypropyl) and K4M (22.5% methoxyl, 7.7% hydroxypropyl). All of them possess the same molecular weight, except for the MX that has higher molecular weight (The Dow Chemical Company, 2002).

The emulsions were composed of 47% (w/w) sunflower oil (Koipe Sol, Deoleo S.A, Madrid, Spain), 51% (w/w) water and the different hydrocolloid (2% w/w). After preparation they were stored at room temperature for 24 hours before the measurements.

The cellulose ether was first dispersed in the oil using a Heidolph stirrer at the lowest speed for five minutes. Then, water previously cooled to 1 ° C was gradually added while continuing to stir. The 200 g mixture was homogenized using an IKA T18 basic homogenizer (Ultra-Turrax, IKA, Germany) (S18N-19G dispersion tool) for 15 seconds at speed 1 (6500rpm) and 60 seconds at speed 4 (17500 rpm) until the emulsion was obtained.

2.2 Preparation of creams

Cocoa creams were prepared using the proportions shown in Table 1.

Table 1. Composition of the studied cocoa creams.

Ingredients	% (w/w)	
	Control	**A4M/F4M/K4M/MX**
Starch	4	2.25
Cocoa powder	2.5	2.5
Skimmed milk powder	5	5
Sugar	10	10
Water	55	30.25
Emulsion	-	50
Sunflower oil	23.5	-

A food processor (TM31 Thermomix, Vorwrek, Wuppertal, Germany) was used to mix the ingredients. At first, starch (CTex 06205, Cargill BV, Netherlands), sugar (Disem, Spain), skimmed powder milk (1% fat) (Central Lechera Asturiana, Spain), cocoa powder (Chocolates Valor S.A., Alicante, Spain) and mineral water were mixed at 90°C for 6 minutes at speed 2 in order to enable starch gelatinization. After that, mixture was allowed to cool at room temperature. Then, the hydrocolloid based emulsion or sunflower oil (in case of control cream) were added by mixing in the processor for 6 minutes at speed 2 without temperature selection to obtain the filling cream. Control cream and cellulose based creams present the same proportion of fat (23.5%). All the creams were stored at 5°C for 24 hours before the measurements.

2.3 Rheological characterization

Rheological measurements were carried out with a controlled stress rheometer (AR-G2, TA Instruments, Crawley, England) equipped with a Peltier system for temperature control. A serrated plate–plate (40 mm) was used in all the experiments, applying a gap of 1 mm. Exposed surfaces of the

samples were covered with Vaseline oil (Panreac, Spain) to avoid dehydration.

Small amplitude oscillation sweeps (SAOS) were performed in order to analyse the viscoelastic properties. To determine the extent of the linear viscoelastic region (LVR) stress sweeps were carried out at 1 Hz.

Temperature sweeps were performed from 20°C to 80°C at a heating rate of 1°C/min in order to observe the effect of heating in the cream structure. Storage moduli at a frequency of 1 Hz within the LVR (0.15 % strain) were recorded as a function of temperature.

Flow curves and frequency sweeps were carried out at 20 ºC and also repeated in fresh samples previously heated until 45 °C and 70°C (1°C/min heating rate from room temperature).

Frequency sweeps were performed between 0.01 and 10 Hz within the linear region (at 0.5 and 1 Pa, depending on the characteristic of the cream) and both the values of storage modulus (G′), loss modulus (G″) and tan δ were recorded.

Stepped flow curves were performed in control stress mode in the range of 1 to1000 Pa with a logarithmic distribution of 10 points per decade, setting a time of 30 seconds per point.

In order to ensure reproducibility, all the measurements were repeated at least three times, with samples prepared in different days and performed 24 h after formulation of the different creams. A fresh sample was loaded for each measurement. In measurements at 20ºC samples were allowed to rest for 15 minutes (to stabilise and temper the sample) before the measurements.

Experimental values of flow curves and oscillatory frequency sweeps were fitted to mathematical expressions using the program KaleidaGraph 4.0 (Synergy Software).

2.4 Statistical analysis

All the rheological data for triplicate creams measurements were subjected to analysis of variance (ANOVA). To determine the significant differences between the samples, the Tukey test was used (XLStat 2010 (Addinsoft, Barcelona, Spain)). A significance level of $p < 0.05$ was applied.

3. RESULTS AND DISCUSSION

All the cocoa creams studied showed a clear shear thinning behaviour, as viscosity decreased when increasing shear rate. This can be seen in Figure 1A where representative flow curves of the different creams at 20°C have been plotted. Differences of even six orders of magnitude are performed in the shear rate range measured. Moreover, a constant plateau for very low shear rates appeared in all cases. This behaviour fits well to simplified Carreau model (Tarrega et al, 2012, Mezgder, 2014)

$$\eta = \frac{\eta_0}{\left(1+\left(\frac{\dot{\gamma}}{\dot{\gamma}_c}\right)^2\right)^s} \qquad (1)$$

where η_0 (Pa s) is the zero shear viscosity, corresponding to the constant viscosity of the plateau for very low viscosity, γ_c is the critical shear rate, related to the bend between the plateau and the falling of the flow curve in the range of shear thinning behaviour, and s (dimensionless) gives information about the shear thinning character of the sample, as it corresponds to the slope of the straight line in the log-log plot in the second

region (Mezdger, 2014). The fits correspond to the continuous lines in Figure 1.

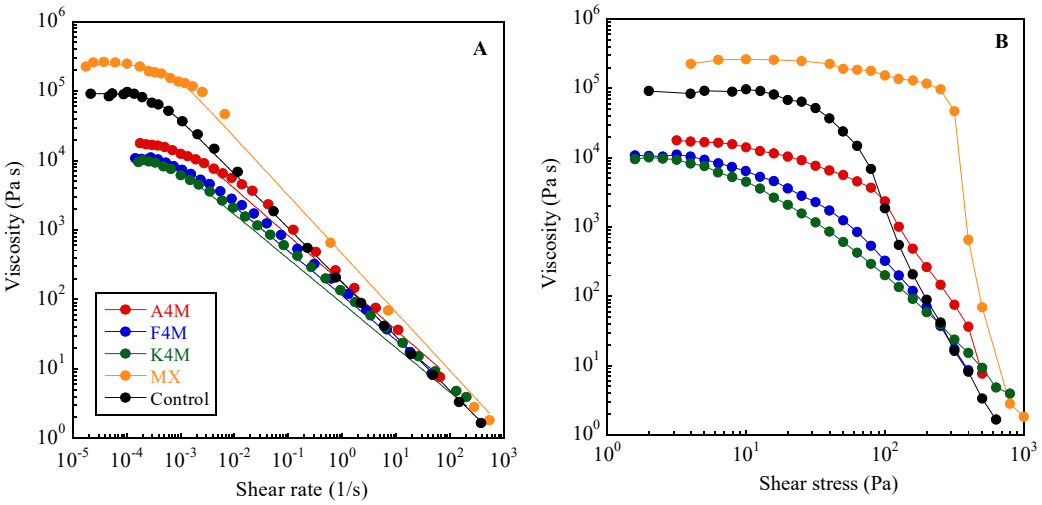

Figure 1. Flow curves, viscosity as a function of shear rate (A) and shear stress (B), for the different cocoa creams studied at 20ºC.

Mean values of the parameters obtained when fitting the triplicated flow curves are shown in Table 2 ($r<0.995$). ANOVA had been performed and significant differences are indicated with letters in superscript.

It is clear than both creams containing HPMC emulsions presented similar flow behaviour, and they were not really different from the cream containing the methylcellulose A4M. No significant differences are shown in Table 2. However, the MX cream was quite different for the other three tested, as the zero shear viscosity was even one order of magnitude higher and it had a stronger shear thinning behaviour (highest s index). An intermediate flow behaviour corresponded to the control starch based cream.

Table 2. Mean values of the parameters obtained from flow curves fits to Carreau model (1) and viscosity values for 100 s^{-1} corresponding to the different creams measured at 20 °C.

Sample	η_0 (Pa s)	$\dot{\gamma}_c$ (1/s)	s	η_{100} (Pa s)
MX	253497^{a}	0.000464^{bc}	0.42^{a}	7.63^{a}
A4M	15201^{c}	0.001053^{a}	0.33^{c}	7.79^{a}
F4M	11093^{c}	0.000873^{ab}	0.32^{cd}	6.30^{b}
K4M	9864^{c}	0.000625^{abc}	0.30^{d}	5.78^{b}
Control	88266^{b}	0.000301^{c}	0.39^{b}	4.59^{c}

^{abcd} Different letters in each column indicate significant differences between the means ($p < 0.05$) according to the Tukey test.

The zero shear viscosity can be related with consistency of the semisolid product in the recipient. These differences are clear in the photographs in Figure 2 were the three levels of consistency in spoon are seen.

Figure 2. Consistency at the recipient of the control cocoa cream and two creams formulated with cellulose ether emulsions.

CONTROL CREAM

MX CREAM

F4M CREAM

However, since the creams presented different shear thinning behaviour, as s index indicated, the differences in viscosity for high shear rates (100 s^{-1}) were reduced (Table 2). The behaviour of all the creams for spreadable

velocities was not so important, and the differences between the two MC disappeared.

These differences are also shown when visualizing viscosity as a function of shear stress (Figure 1B). The range of shear stresses at which viscosity is constant is higher for control and MX cream than for the other three creams studied. It stands out the MX abrupt fall of viscosity occurred at about 300 Pa.

In order to study viscoelastic behaviour of the creams, small amplitude oscillatory sweeps in linear viscoelastic region (previously determined) were performed. The mechanical spectra obtained (viscoelastic moduli as a function of frequency) for 20 ˚C are plotted in Figure 3.

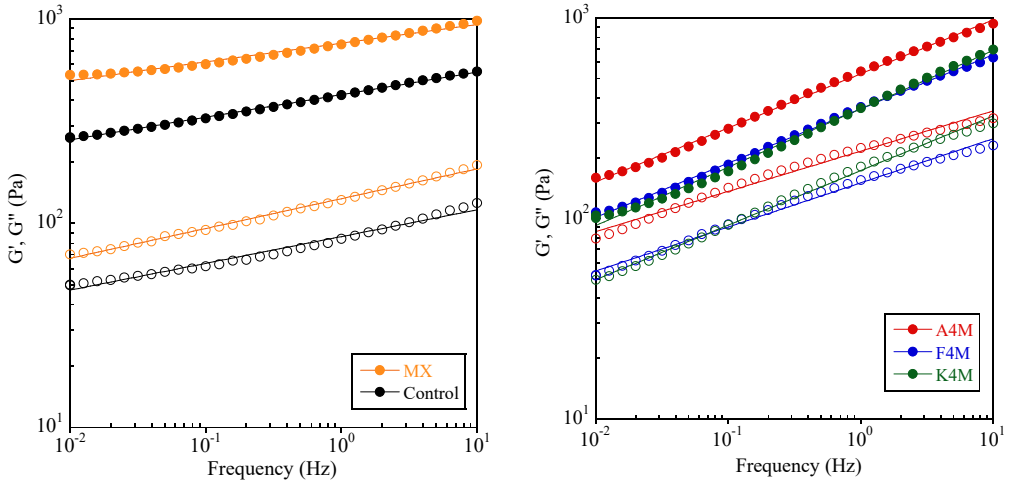

Figure 3. Storage modulus (G': filled symbols) and loss modulus (G": open symbols) as a function of oscillation frequency for the different cocoa creams studied at 20 ºC.

Since both elastic modulus or storage modulus (G') and loss modulus or viscous modulus (G") are straight lines in the log-log plot, they were fitted to power law equations which are widely used (Sanz et al, 2017).

$$G' = G_1' \, \nu^{m'} \qquad (2)$$

$$G'' = G_1'' \, \nu^{m''} \qquad (3)$$

where G'_1 and G''_1 correspond to the viscoelastic moduli at 1 Hz and the dimensionless parameters m' and m" give information about the frequency dependence. All the spectra were satisfactorily fitted (r>0.995) to equations (2) and (3) and the mean values of the parameters obtained for the three replicates measured are shown in Table 3, where loss tangent for 1 Hz have also been included.

Table 3. Mean values of the parameters obtained from power equations (2,3) fits applied to mechanical spectra and tan δ (G"/G') corresponding to 1Hz, for the different creams at 20 °C.

Sample	G_1' (Pa)	m'	G_1'' (Pa)	m''	tan δ at 1 Hz
MX	742.7^{a}	0.090^{b}	130.1^{d}	0.154^{c}	0.174^{b}
A4M	524.5^{b}	0.268^{a}	215.8^{a}	0.200^{bc}	0.418^{a}
F4M	350.8^{d}	0.270^{a}	150.1^{c}	0.220^{ab}	0.428^{a}
K4M	350.9^{d}	0.292^{a}	172.7^{b}	0.273^{a}	0.514^{a}
Control	426.3^{c}	0.114^{b}	87.9^{e}	0.136^{c}	0.201^{b}

^{abcde} Different letters in each column indicate significant differences between the means ($p < 0.05$) according to the Tukey test.

The predominance of the storage modulus (G') over the loss modulus (G") in the whole range of frequencies studied indicates that all creams presented a gel-like behaviour, with an internal three-dimensional network (Mezger, 2014; Sanz et al., 2015, 2017; Tárrega et al., 2012).

The results showed two groups of creams with different viscoelastic behaviour, as it is observed in Figure 3.

The spectra of MX creams turned to be quite similar to the control starch based cream. Both creams have the same frequency dependence (as reveals

ANOVA in Table 3), although MX creams were firmer and with a stronger elastic character (lower values of tan δ= G"/G'). On the other hand, the rest of the cocoa creams had different dynamic spectra, with higher frequency dependence of both moduli (m' were about twice the corresponding to the previous group) and higher values of the loss tangent (G"/G').

These results indicated that the second group presented a weaker internal structure and are in accordance with the flow behaviour previously analysed. MX creams were significantly different from the other MC cream and the stronger internal structure lead to a higher consistence at rest (η_0), a stronger shear thinning behaviour and a greater value for the stress necessary to decrease viscosity (resistance to flow). However, the methoxyl substitution in A4M cream did not make them too different from the hydroxypropyl methylcellulose creams, only increased slightly the elastic moduli and the viscosity.

Once characterized the different creams at room temperature, evolution of their viscoelastic behaviour with temperature was analysed. The values of storage modulus measured at 1Hz within the LVR in the range 20 to 80˚C are presented in Figure 4. Three different kind of behaviour are clearly observed, regarding to the control cream, the MC and the HPMC creams.

As previously observed by Sanz et al. (2015), the increase in methoxyl content in cellulose ether reduced the temperature required to observe an increase in elasticity, so creams with methylcellulose emulsion (MX and A4M) showed a lower gelation temperature. At low temperatures cellulose ethers have a great capacity of water retention, but after heating a release of water will occur forming strong bonds with internal chains, resulting in very stable gel structures. The higher molecular weight and the higher methoxyl substitution increase cellulose hydrophobicity, resulting in greater intermolecular association since the hydrophobic groups are closer. On the

contrary, the presence of hydroxypropyl substitutions produced an increase in the gelation temperature, which depended on the substitution degree. The increase in G' values, related to cellulose ether gelation, took place at the highest temperature for the K4M cream.

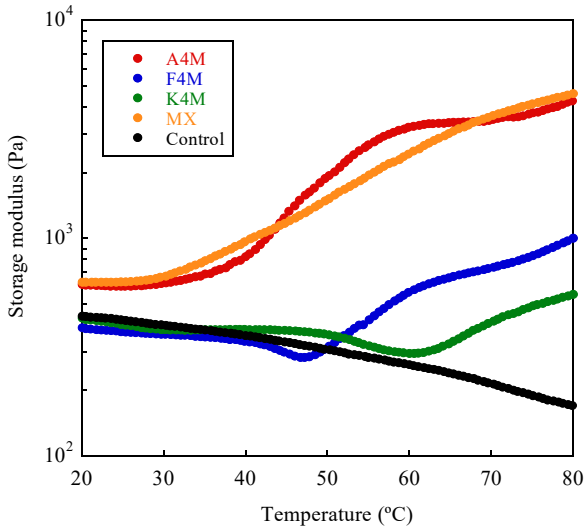

Figure 4. Temperature sweeps (1ºC/min) for the storage modulus (G') corresponding to the cocoa creams studied.

Taking these trends into account, flow curves and SAOS tests were performed at 45 and 70 degrees Celsius in order to compare the expected different behaviours of both kinds of cellulose ethers. All the flow curves were fitted to Carreau simplified model (1) and the mechanical spectra were fitted to power law equations (2, 3). The mean values of the replicates are shown in Table 4, where the letters in superscripts correspond to the ANOVA analysis performed considering temperature influence in each sample. Values corresponding to 20 ˚C have been included again for comparative purposes.

Table 4. Mean values of the parameters obtained from flow curves fits to Carreau model (1) and potential equations (2,3) fits for mechanical spectra for the different creams, at the three temperatures studied.

			Flow curves			Mechanical spectra			
	Sample	T (°C)	η_0 (Pa s)	$\dot{\gamma}_c$ (1/s)	s	G'(Pa)	m'	G''(Pa)	m''
MC emulsions	MX	20	253496^{b}	0.0005^{b}	0.423^{b}	743^{a}	0.090^{a}	130^{b}	0.154^{a}
		45	419620^{a}	0.0009^{a}	0.480^{a}	1568^{b}	0.021^{b}	123^{b}	0.005^{b}
	A4M	20	16323^{b}	0.0011^{a}	0.330^{b}	524^{b}	0.268^{a}	216^{a}	0.201^{a}
		45	405533^{a}	0.0013^{a}	0.483^{a}	1394^{a}	0.063^{b}	148^{a}	0.105^{b}
HPMC emulsions	F4M	20	11093^{b}	0.0009^{a}	0.320^{c}	351^{b}	0.271^{a}	150^{a}	0.220^{a}
		45	43992^{b}	0.0004^{a}	0.383^{b}	377^{b}	0.186^{b}	134^{a}	0.254^{a}
		70	180066^{a}	0.0007^{a}	0.453^{a}	819^{a}	0.100^{c}	130^{a}	0.105^{b}
	K4M	20	9863^{b}	0.0006^{a}	0.307^{c}	351^{b}	0.293^{a}	173^{a}	0.273^{a}
		45	15508^{b}	0.0004^{a}	0.343^{b}	380^{b}	0.267^{a}	168^{a}	0.285^{a}
		70	104547^{a}	0.0007^{a}	0.433^{a}	592^{a}	0.070^{b}	90^{b}	0.067^{b}
Starch based	Control	20	94260^{a}	0.0003^{a}	0.390^{a}	426^{a}	0.114^{a}	88^{a}	0.136^{a}
		45	88806^{a}	0.0002^{a}	0.400^{a}	322^{a}	0.119^{a}	68^{a}	0.187^{a}
		70	86730^{a}	0.0002^{a}	0.410^{a}	306^{a}	0.086^{a}	51^{a}	0.209^{a}

^{abcd} For each sample, values in the same column with the same letter are not statistically different ($P<0.05$) according to Tukey's test.

Figure 5 includes the flow curves and loss tangent as a function of frequency for MC creams while the analogous results for HPMC creams are plotted in Figure 6.

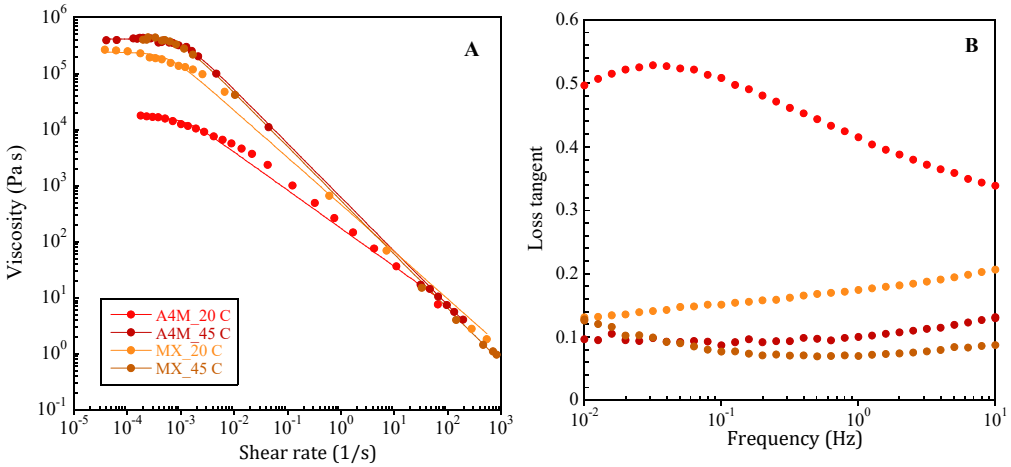

Figure 5. Rheological properties of cocoa creams containing methylcelluloses (A4M y MX) at 20°C and 45°C. **A**: Flow curves. **B**: Loss tangent (G"/G') as a function of frequency.

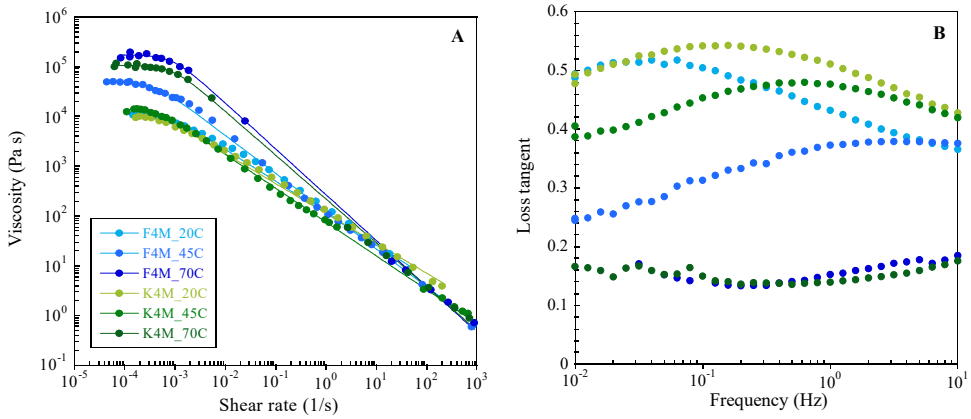

Figure 6. Rheological properties of cocoa creams containing hydroxipropilcelluloses (F4M and K4M) at 20°C, 45°C and 70°C. **A**: Flow curves. **B**: Loss tangent (G"/G') as a function of frequency.

It is interesting to point out that the differences showed at room temperature between the two creams containing methylcellulloses disappeared at 45 degrees when both methylcelluloses are completely gelled. At 45 ˚C the loss tangent of both creams were about 0.1 (corresponding to loss angles of approx. 5 degrees) and almost not frequency dependent, what indicates the existence of a strong internal network. A4M cream highly increased viscosity approaching MX values and the mechanical spectra became similar to the corresponding to MX cream. The temperature increase also had influence in MX creams, enhancing their viscosity and firmness, but the effect was not so significant.

On the other hand, results for both HPMC (Table 4) showed that these celluloses were not significantly affected by temperature when changing from 20 to 45 Celsius degrees.

In Figure 5 it is observed that F4M creams were a bit more viscous than the creams at room temperature, but K4M creams did not present changes neither in viscosity nor viscoelasticity. These creams containing HPMC emulsions needed higher temperatures to gelify. At 70 ˚C the changes in viscosity and structure are clear, as zero shear viscosity increased one order of magnitude and tangent values dropped to 0.15 (loss angles of 10 degrees approx.). The differences detected at 45 degrees disappeared, as creams containing both HPMC emulsion had the same rheological behaviour when they are gelled.

Regarding to the control starch based creams, parameters in Table 4 shows that they did not present significant changes with temperature in both flow

and viscoelastic behaviour, only a slight decrease in viscosity and in dynamic moduli was produced.

When comparing all the cocoa creams for high temperatures, it stands out that MC creams became the most viscous ones and also the creams with a strongest internal network. When HPMC were gelled the cocoa creams behaviour approached the control cream rheological behaviour.

Moreover, the shear thinning behaviour increased when the celluloses gelify, so the viscosity drop with shear rate was more abrupt. As a consequence, the viscosity values for high velocities are similar to the ones corresponding to room temperature, although the consistency at the recipient was much greater.

CONCLUSIONS

A thermorheological characterization of reduced-fat cocoa creams was carried out. Temperature sweeps of storage modulus showed different temperature gelation depending on the chemical substitution in cellulose ethers.

Significant differences existed in zero shear viscosity and shear thinning character at room temperature, due to the different internal structure revealed by the viscoelastic moduli spectra. MX cream was clearly different to the rest of the cellulose creams, with a similar behaviour to the control starch based cream. The large differences detected in consistency at the recipient were reduced for viscosity at spreadable velocities.

At 45 ˚C, when the gelation was already produced in methylcelluloses, behaviour of both creams containing A4M and MX emulsions became quite similar and differences with HPMC creams were clear. However, once HPMC

were also gelled at 70 ˚C all the creams had analogous characteristics both in flow and viscoelastic behaviour.

The chemical substitution of cellulose ethers and temperature are both key factors to consider for establishing the most suitable formulation, especially when heating processes are applied. The studied creams could be an alternative to conventional full fat products and they may provide different options according to the desired characteristics and the application process.

ACKNOWLEDGEMENTS

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Functionality of low digestibility emulsions in cocoa creams. Structural changes during *in vitro* digestion and sensory perception.

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ABSTRACT

The objective of this work was to evaluate the application of low digestibility oil/water emulsions as fat source in a cocoa cream. Emulsions were composed by water, sunflower oil and cellulose ethers or xanthan gum. Back extrusion assays were measured before and after in vitro digestion and free fatty acids release were measured to evaluate the fat digestibility. Finally consumer acceptability was carried out to determine the degree of liking of each system. The results revealed that all the emulsions confer a suitable consistency to the creams and the structure provided by the hydrocolloids was resistant to digestion, reducing the fat digestibility. However, after gastric digestion only cream with xanthan gum showed a significant increase in consistency what it could be related with an increase in satiety. Regarding the sensory characteristics, the cream elaborated with xanthan gum was rated close to the control cream that received the highest scores.

Keywords: filling cream, hydrocolloids, free fatty acids, texture, acceptability.

1. INTRODUCTION

In the last decades, the increasing of a large number of diseases directly linked to fast food has driven the industry to focus on the design and formulation of food products with reduced fat and/or calories content. The contribution of fat in the flavour, texture, appearance and mouthfeel of foodstuffs has been confirmed by several studies (Drewnowski, 1992, 1987; Sandrou & Arvanitoyannis, 2000). Hence, removing or reducing fat adversely affects some of the characteristics reducing the quality of the final product. The main challenge is the manufacture of products with high lipid content, such as pastry and confectionery products. This type of foodstuffs contains a high percentage of saturated fats and/or trans fats, which gives them unique textural properties. Lipid content in cocoa creams can be more than 60% and provide a significant effect in organoleptic and physicochemical properties. In order to reduce calories content, fat could be reduced/replaced by a system that can replicate the texture, flavour and palatability of the full-fat counterpart. There are several systems that can be used as fat replacers, including protein-based fat mimetics, carbohydrate-based fat mimetics and fat based replacers (Lucca & Tepper, 1994; Sandrou & Arvanitoyannis, 2000). Most of the low-fat products reformulated in recent years contain carbohydrate-based fat mimetics such as inulin (Tárrega & Costell, 2006; Krystyjan, Gumul, Ziobro, & Sikora, 2015), starch (Laguna, Varela, Salvador, Sanz, & Fiszman, 2012), cellulose (Nsor-Atindana, Chen, Goff, Zhong, Sharif, & Li, 2017) and gums (Ranalli, Andrés, & Califano, 2017; Rather, Masoodi, Akhter, Gani, Wani, & Malik, 2015) that are widely used as thickeners, stabilizers and emulsifiers to compensate the loss of desirable textural attributes when fat is reduced or removed (Mudgil & Barak, 2013).

The incorporation of a polysaccharide (e.g cellulose ether) in the continuous phase of oil/water emulsion allows using vegetable oil in reformulated products. The semi-solid consistency was suitable for mimicking the textural and rheological properties of fat, conferring good sensory acceptability (Martínez-Cervera, Salvador & Sanz, 2015; Tarancón, Fiszman, Salvador, & Tárrega, 2013). An important attribute of emulsion-based food products is the behaviour within the mouth after ingestion that will determine the perceived mouthfeel (McClements, 2015). People like the taste of fat-containing foods. More viscous stimuli are generally perceived as rich in fat content. So, this feeling can be created through the use of stabilizers or thickeners that enhance the perceived creaminess of the reformulated product (Drewnoski, 1990). Nevertheless, depending on fat content and the type of thickener used, the aroma release and taste perceived may change (Wendin et al., 1997). Some studies showed that the use of thickeners results in an increase in texture and a decrease in aroma release and taste, but depending on the type and concentration of hydrocolloid (Arancibia, Castro, Jublot, Costell & Bayarri, 2015; Hollowood, Linforth, &Taylor, 2002). Moreover, some studies have shown that the presence of cellulose ethers or xantan gum in the continuous phase of oil/water emulsion makes more difficult for the digestive fluids to come into contact with the emulsified fat, reducing lipolysis (Espert at al., 2017; Espert, Salvador, & Sanz, 2018).

The objective of this work was to study the effect of low-digestible vegetable oil/water emulsion as a fat source on the structural and sensory properties of cocoa cream, considering a starch base cocoa cream with the same fat content as a control. The emulsions were prepared using cellulose ethers with different chemical substitution (methyl and hydroxypropyl methylcelluloses and xanthan gum as structuring agents. Lipid digestibility

was also determined after in vitro digestion to evaluate the relationship between structural changes and fat digestibility in this new matrix.

2. MATERIALS AND METHODS

2.1. Emulsion preparation

Different o/w emulsions were prepared using different hydrocolloids as stabilizers: xanthan gum (XG) (Cargill, France) and three types of cellulose ethers (METHOCELTM A4M, METHOCELTM MX and METHOCELTM K4M) (The Dow Chemical Company, Midland,Michigan, USA). These celluloses present different chemical substitution: A4M and MX are methylcelluloses (30.0% methoxyl and >30.0% methoxyl respectively) and K4M is a hydroxylpropyl methylcellulose (22.5% methoxyl, 7.7% hydroxypropyl). A4M and K4M have approximately the same molecular weight (MW) and a viscosity of 4000 mPa s (measured at 20 °C following ASTM D1347 and ASTM D2363 reference methods (The Dow Chemical Company)). MX has a higher MW and a viscosity of 50,000 mPa s at 20 °C (measured following the same methods).
Emulsions were prepared using the following proportions: 47% (w/w) sunflower oil (Koipe Sol, Deoleo S.A., Spain) 2% (w/w) hydrocolloid and 51% (w/w) water. The total final mass was 200 g.

2.1.1 Cellulose ether based emulsion

At first, cellulose ether was dispersed in sunflower oil using a Heidolph stirrer (Heidolph Instruments GmbH & Co. KG) at 280 min-1 for five minutes. Then the mixture was hydrated by gradually adding of water at 1°C while

continuous stirring. A water temperature of 1 °C was selected according to the specific hydration requirement of MX cellulose (the highest methoxyl content) and then it was also used for the other cellulose types. Finally the emulsion was homogenized using an Ultra-turrax T18 homogenizer (IKA, Germany) at 6500 rpm for 15 seconds and at 17500 rpm for 60 seconds.

2.1.2 Xanthan gum based emulsion

The XG was dispersed in the water at room temperature (22ºC) water using a Heidolph stirrer at 300-500 rpm for 10 minutes. Then, sunflower oil was gradually added increasing the speed up to 1800 rpm. Stirring continued using a homegenizer (Ultra-turrax) at 6500 rpm for 60 seconds, subsequently at 13500 rpm for 60 seconds and at last 17500 rpm for 60 seconds.

2.2. Creams preparation

Emulsion based cocoa creams were composed of water (30.25%), sugar (Disem, Spain) (10%), skimmed milk powder (Central Lechera Asturiana, Spain) (5%), cocoa powder (Chocolates Valor S.A., Alicante, Spain) (2.5%), starch (CTex 06205, Cargill BV, Netherlands) (2.25%) and emulsion (50%). A food processor (TM31 Thermomix, Vorwrek, Wuppertal, Germany) was used to mix the ingredients. At first, starch, sugar, milk powder, cocoa powder and mineral water were mixed at 90°C for 6 minutes at speed 2 in order to enable starch gelatinization. After that, mixture was allowed to cool at room temperature. Then, the hydrocolloid-based emulsion was added by mixing in the processor for 6 minutes at speed 2 without temperature selection to obtain the filling cream.

Control cream was formulated with the same ingredients, but instead of emulsion sunflower oil (23.5%) was added and the amount of water and starch were increased to 55% and 4% respectively. Control cream was prepared in the same way, but after cooling of the first step, the sunflower oil was added gradually to the mixture at room temperature.
All the creams contained the same proportion of fat (23.5%). They were stored at 5°C for 24 hours before the measurements.

2.3. *In vitro* digestion

2.3.1. Composition of digestive fluids

Simulated Saliva Fluid (SSF) was prepared according to the method described by Mishellany-Dutour et al. (2011), with some modifications. SSF was composed of 5.2g of NaHCO3, 1.37g K2HO4P·3 H2O, 0.88g NaCl, 0.48g KCl and 0.44g CaCl2·2H2O, dissolved in 1L of bi-distilled water. 0.70g of α-amylase from porcine pancreas (A3176-1MU, Sigma-Aldrich) and 2.16g of mucin from porcine stomach (M2378, Sigma-Aldrich were added to this solution.
Simulated Gastric Fluid (SGF) was prepared according to a previous study (Sanz, Handschin, Nuessli & Conde Petit, 2007) with some modifications. 3.10g NaCl, 0.11g CaCl2, 1.10g KCl and 5.68ml Na2CO3 (1M) were dissolved in 1L of bi-distilled water. The solution was adjusted to pH 2. 0.15g of pepsin from porcine gastric mucosa (P7000, Sigma-Aldrich) was dissolved in 1L of SGF.
Simulated Intestinal Fluid (SIF) was composed of an electrolyte solution and bile and pancreatin solutions. The electrolyte solution was prepared by dissolving 1.25g NaCl, 0.15g KCl and 0.055g CaCl2 in 1L of distilled water.

Phosphate buffer solution was prepared (103.5mg $NaH_2PO_4 \cdot 2H_2O$ and 44.5mg $Na_2HPO_4 \cdot 2H_2O$ in 100ml of distilled water) (pH 7) to prepare bile (B8631, Sigma-Aldrich) and pancreatin (P1750 (lipase activity 8 USP units/mg), Sigma-Aldrich) freshly suspensions (Sanz et al., 2007).

2.3.2. In vitro digestion model

To simulate different digestion phases, an in vitro digestion model to simulated oral, gastric and small intestine digestion previously described was used (Borreani et al., 2016; Espert et al., 2017).

Oral phase: 50g of sample were mixed for 5 s with 1 ml of Simulated Saliva Fluid (SSF) (0.7 mg/mL α-amylase) in a shaking water bath (Raypa®, Barcelona, Spain) (60 rpm) at 37°C. The ratio saliva/sample was selected considering the data provided by Humphrey & Williamson (2001).

Gastric phase: the "bolus" sample from the oral phase was mixed with 16 mL of Simulated Gastric Fluid (SGF) (0.15 mg/mL) to obtain a final enzyme-sample ratio of 1:250 (v/v). The pH of the mixture was adjusted to 2.0 using 6M HCl (Scharlab S.L., Spain) and incubated for 1 h under continuous agitation (60 rpm) at 37ºC.

Small intestine phase: After gastric step, 10.6 mL of bile extract (46.87mg/mL) solution and 4 mL of electrolyte solution was added to the sample, and the pH was adjusted to 7.0 using NaOH (0.1N). Then, 5.34 mL of pancreatin solution was added to the mix (1:14 (v/v) ratio pancreatin/oil). The resulting mixture was incubated for two hours under continuous agitation at 37ºC.

In order to compare the effects caused by the volume of dilution with the effects caused by the presence of enzymes and pH changes, oral and gastric water dilution incubation was also carried out in which only distilled water

was added. The incubation process (time, temperature and shaking conditions) and the dilution factor were the same as that in the samples with enzymes.

2.4. Fat digestibility

To determinate the digestibility of fat, the amount of Free Fatty Acids (FFA) released at the end of in vitro digestion were calculated. A pH-stat automatic titration (Mettler Toledo, Spain) was used to monitor automatically the pH at intestinal pre-set value (pH 7.0) by titration of NaOH 0.1N solution (Panreac Química S.L.U., Spain). The volume of NaOH added to neutralize the samples was recorded. A standard curve was prepared using oleic acid (0, 50, 100, 150, 200 and 250 mM) and was used to calculate free fatty acid concentration of the samples ("g oleic acid/g fat").

2.5. Textural properties

TA-XT plus Texture Analyzer equipped with the Texture Exponent software (Stable Microsystems, Godalming, UK) was used to determinate the extrusion properties of the samples. A back-extrusion test was carried out, using a bucket of 50 mm diameter and 75 mm height and a compression probe of 49 mm diameter. The distance force was 15mm, the compression rate 1 mm s-1, and the trigger force 10g. From the force time profiles obtained the area under the curve (AUC; N*s) as a measure of consistency were recorded. Measurements were performed in triplicate.

2.6. Sensory analysis

The sensory analysis was carried out in a sensory room equipped with individual booths designed in accordance with ISO 8589:2007 (ISO, 2007), under artificial daylight and controlled temperature (22°C).

2.6.1. Free Choice Profile

A total of 20 untrained consumers (60% women, 40% men), with ages ranging from 25 to 50 years old, took part in a Free Choice Profile (FCP) analysis. In the first session, the terms used by each consumer describing the differences among creams were generated by Repertory Grid Method (RGM). The samples were presented in triads and each consumer described the similarities and differences among samples within each triad in their own terms. This method was repeated until all samples were tested. Consumers evaluated the appearance, taste, aroma and texture of the different creams. Each consumer evaluated his own list of terms by rating the intensity for each sample using a 10 cm unstructured line scale with the anchors "Not perceived" and "Intense". The samples were labelled with random three-digit codes and served at room temperature. Water was provided to clean the palate between samples.

2.6.2. Liking test and CATA questionnaire

A sensory analysis of cocoa creams was carried out by 82 untrained consumers (69% women, 31% men) recruited among the students and employees of the Institute for Agrochemistry and Food Technology (IATA-CSIC). They were asked to taste the five samples of creams (control, A4M,

F4M, MX and XG) and rate their overall acceptability and liking of their appearance, colour, taste and texture on a 9-point hedonic scale from 1 = "dislike extremely" to 9 = "like extremely". After that, the consumers were asked to answer a Check-All-That Apply (CATA) questionnaire. The terms included were previously generated in a session with 20 consumers by using the Repertory Grid method (Table 3). They were first given the most different samples and then they were asked to choose and write down the most appropriate attributes with which to describe the characteristics of the samples. At the end of the session, a consensus on the list of sensory attributes was reached (Stone & Sidel, 2004). The CATA questionnaire included seventeen sensory terms. Each consumer was asked to check the terms that he/she considered appropriate for describing the cream sample. The five cocoa creams samples were served at 20ºC identified with random three-digit codes and were presented monadically following a Williams design. Data acquisition and analysis was performed by Compusense Cloud version 8.8.6642.32014 (Ontario, Canada).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was applied to study the effects of digestion on the different instrumental and sensorial parameters studied. The least significant differences were calculated by the Tukey test and the significance at $p < 0.05$ was determined.

A Generalized Procrustes Analysis (GPA) was applied to the Free Choice Profile data.

The non-parametric Cochran's test analysis of variance was performed for each descriptor to evaluate whether the CATA question was able to detect differences in the consumer perception of the cocoa creams. A descriptor

was no longer considered when Cochran's test found that the differences between samples were not significant. The variability in the frequencies of mention of significant attributes was analysed by using a Correspondence Analysis (CA) and a Multiple Factor Analysis (MFA) was performed on the frequency of mention of the CATA question to assess the relationship between CATA question responses and acceptability scores. Every calculation was carried out using XLSTAT statistical software (2010.5.02 (Addinsoft, Barcelona, Spain)).

3. RESULTS AND DISCUSSION

3.1. Textural properties

The texture of emulsion-based products is one of the most important factors that influence their overall sensory acceptance. The texture profiles of creams before and after in vitro digestion, as measured by extrusion tests, are shown in Figure 1. Fresh samples have the highest consistency in all creams, as its structure has not been altered by the effect of any digestion step. The use of polysaccharides contributes viscosity to the system, depending on the chemical composition of them. The area under the curve (AUC) values of fresh cocoa creams showed significant differences ($p<0.0001$) depending on the composition of the hydrocolloid used (Table 1).

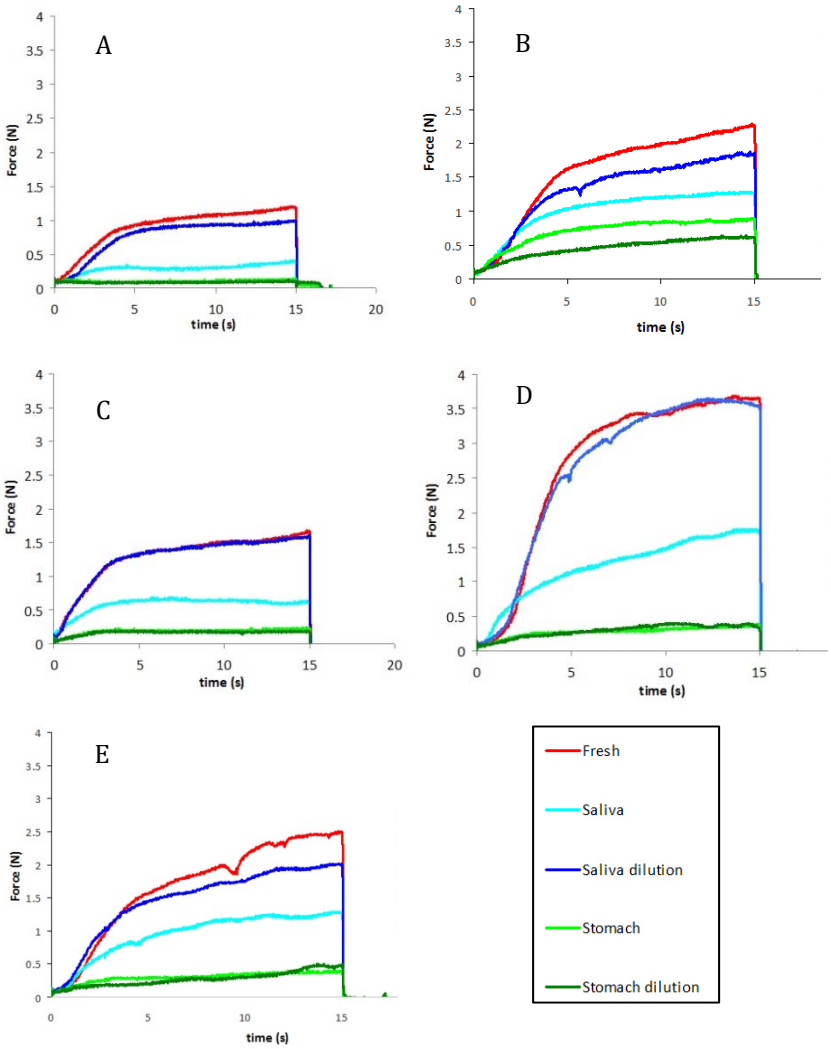

Figure 1. Back extrusion curves of cocoa creams (A: control; B: xanthan gum; C: K4M, D: A4M; E MX).

The creams with A4M showed the highest value of AUC (42.78a), followed by MX methylcellulose and xanthan gum (24.73b and 23.15b respectively).

Hydroxypropyl methylcellulose showed lower values (19.54bc), although the control cream presented the lowest AUC value (15.20c).

Table 1. Area under the curve (AUC) calculated from the extrusion curves of the different cocoa creams.

COCOA CREAMS	DIGESTION PHASE	AREA (N x mm)
Control	Fresh	15.20a (2.22)
	Saliva	4.10b (0.96)
	Saliva Dilution	11.35a (1.95)
	Stomach	1.90c (0.46)
	Stomach Dilution	1.23c (0.09)
Xanthan gum	Fresh	23.15a (2.40)
	Saliva	15.34b (0.48)
	Saliva Dilution	21.78a (3.67)
	Stomach	10.60c (0.76)
	Stomach Dilution	6.00d (0.53)
K4M	Fresh	19.54a (1.83)
	Saliva	8.74b (2.03)
	Saliva Dilution	19.02a (1.21)
	Stomach	2.87c (0.31)
	Stomach Dilution	2.86c (0.77)
A4M	Fresh	42.78a (2.54)
	Saliva	18.37b (2.28)
	Saliva Dilution	38.66a (0.85)
	Stomach	4.30c (0.54)
	Stomach Dilution	6.39c (1.74)
MX	Fresh	24.73a (3.76)
	Saliva	14.59b (3.65)
	Saliva Dilution	23.28a (1.81)
	Stomach	5.87c (1.29)
	Stomach Dilution	4.55c (0.47)

[a,b,c]Means with different letter in columns for each parameter and each cellulose type indicate significant differences among the sample ($p<0.05$) according to Tukey test.

After oral phase, a significant decrease in consistency was observed as compared to the consistency of fresh samples. This fact is due to the presence of α-amylase enzyme in SSF that promotes the enzymatic

degradation of the starch, causing a loss of consistency (de Wijk, Prinz, & Janssen, 2006; Sanz, Handschin, Nuessli, & Conde-Petit, 2007). This decrease in consistency is more evident in control cream, suggesting that the hydrocolloids provide consistency at the system and, in addition, it is known that the presence of hydrocolloid has a suppressive effect on starch digestibility (Sasaki & Kohyama, 2012). Samples without saliva enzymes (SSF) (saliva dilution samples) exhibit a higher consistency than the corresponding ones with SSF. They showed consistency values close to the fresh samples, showing no significant differences.

After in vitro stomach incubation, the extrusion profile of control cream (Figure 1A), MX cream (Figure 1E), A4M cream (Figure 1D), and K4M cream (Figure 1C) did not show significant differences with respect to water stomach dilution (without pepsin and initial pH). These results indicate that the change in consistency in this phase should be attributed to the dilution effect more than to the stomach conditions. However, contrary to cellulose ethers based creams, gastric digestion of XG cream showed a significant increase in AUC compared to its corresponding water dilution (Figure 1B, Table 1). This increase could be related to the behavior of the xanthan gum matrix in the acid pH of the stomach, where its viscous consistency is maintained. Moreover, the XG network weakens and there is more contact between the fat globules, which produces fat coalescence and therefore an increase in the consistency of the system. This behaviour has been also found in xanthan gum emulsions (Espert, Salvador, & Sanz, 2018). Several studies confirm that viscous fibres have been associated with a decrease stomach emptying and slower transit time through the small intestine, and have also been shown to influence blood glucose and cholesterol levels (Dickeman & Fahey, 2006; Mälkki, 2001). Insoluble fibres, such as cellulose, are mostly associated with large bowel function, although both types of fibre enhance

postprandial sensations of satiety and to decrease hunger feelings (Juvonen et al., 2009; Howarth, Saltzman, & Roberts, 2001; Gustafsson, Asp, Hagander & Nyman, 1995). It is important to note that control cream showed the least resistance to back-extrusion test after in vitro digestion. This could be related to the fact that in control cream liquid fat is not emulsified and there is no hydrocolloid network, which provides consistency and cohesiveness to the system. Therefore, the use of xanthan gum emulsion as a fat replacer in cocoa cream make this product interesting in the design of satiating foodstuffs due to its increase in consistency in stomach phase.

3.2. Fat digestibility

Free Fatty Acids (FFA) are the product of fat digestion, so they are an indicator of the amount of fat which has been digested. The extent of lipid digestion varies depending on the enzymatic activity and a great number of physicochemical factors (Golding, Wooster, Day, Xu, Lundin, Keogh, & Clifton, 2011; Li, Hu & McClements, 2011; McClements, Decker & Park, 2007). Significant differences in free fatty acid generation were found between cocoa control cream (without hydrocolloid emulsion) and cocoa creams based on hydrocolloids emulsion (Table 2). It can be shown that creams based on hydrocolloid emulsions required less NaOH volume to neutralize any FFA produced by digestion, which indicates that these samples had a lower fat digestibility, so less oleic acid concentration was generated. Besides, and increase in the size of the fat globules and in droplet coalescence was observed in all hydrocolloid creams (data not shown).

Table 2. Quantity of NaOH required to neutralize FFA released and oleic acid values after in vitro digestion.

COCOA CREAMS	ml NaOH	g oleic acid/g fat
Control	5.720a (0.696)	0.132a (0.016)
Xanthan gum	3.905b (0.600)	0.082b (0.004)
K4M	3.778b (0.728)	0.079b (0.010)
A4M	3.500b (0.196)	0.078b (0.003)
MX	3.144b (0.881)	0.063b (0.017)

[a,b]Means with different letter indicate significant differences among the sample ($p<0.05$) according to Tukey test. Values between parentheses are standard deviations.

The sample with the lowest fat digestibility was MX cocoa cream, although no significant differences were found among the creams prepared with the different hydrocolloid emulsions. Schneeman, & Gallaher (2001) found that the hydrolysis of triglycerides in the small intestine is related to the available surface area, and an increase in droplet size is associated with a reduced surface area and a reduction in the rate of lipid hydrolysis. On the other hand, it is already known that the presence of fibres potentially influence on lipid digestion, making the access of bile salts and digestive enzymes to the oil phase difficult. Similar results using the same shear speed (60 rpm) were obtained by Hur, Lim, Park, & Joo (2009) and Mugdil & Barak (2013). They found that the molecular and physicochemical differences of the different polysaccharides can be expected to cause significant alterations in their effectiveness at reducing lipid digestion by interfering with the various physiological processes. Pasquier et al. (1996) showed that some viscous

fibres reduce the lipid emulsification, lowering of the extent of fat lipolysis. Espinal-Ruiz et al. (2014) found a noticeable decrease in lipid digestion with the presence of methyl cellulose.

In conclusion, the results found evidence that hydrocolloid barrier could prevent the accessibility of the enzyme to the lipid phase, reducing the extent of lipid digestion. So it has been demonstrated that the application of this type of emulsions are feasible to obtain a cream with low digestibility.

3.3. Sensory analysis

3.3.1. Free Choice Analysis

Free Choice Profile (FCP) analysis was performed to determine the attributes that describe the cocoa creams. This analysis provides information about the spontaneous sensations that occur when the product is consumed (Varela and Ares, 2012; González-Tomás & Costell, 2006). The consumers generated different terms, subdivided into appearance, taste, aroma and texture attributes. The results from the FCP analysis are shown in Figure 2, that shows the two dimensions of Generalized Procrustes Analysis (GPA) graph. In this figure the most mentioned attributes and their frequency mention are summarized. The total amount of variance explained by the two dimensions was 73.46%. Dimension 1 accounted for 52.66% of the variance and was mainly related to appearance and texture terms. On the left side of the plot, lumpy appearance, lumpy texture and thick texture were placed which characterized creams elaborated with cellulose emulsions (MX, A4M and K4M). However, on the right side of the plot, terms as creamy appearance, homogeneus and bright appearance and creamy and

soft texture were related to control cream and cream elaborated with XG emulsion.

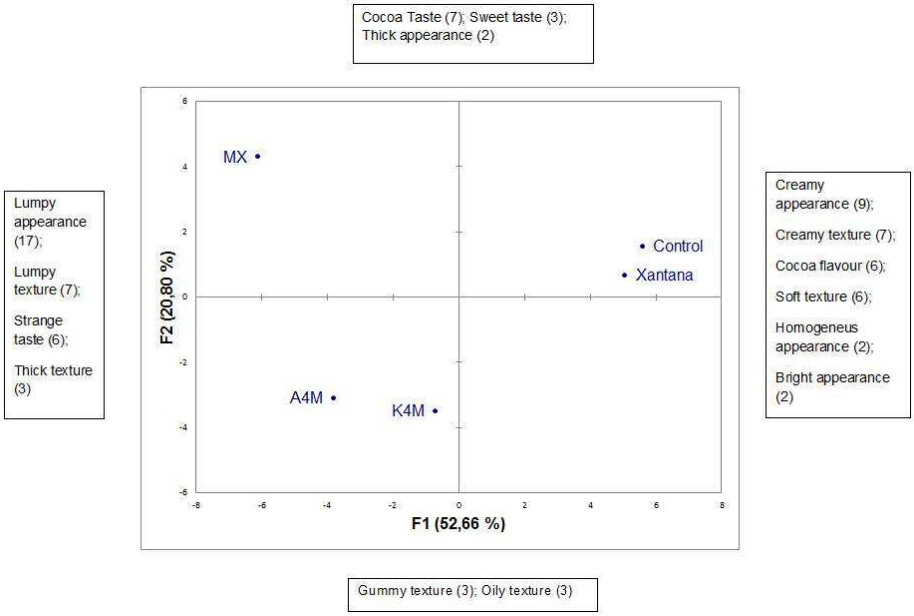

Figure 2. Two dimensions GPA plot of the differences among creams. The main descriptors correlated with the first two dimensions of the average space are listed on the boxes and the number of times that the descriptor was mentioned.

Dimension 2 accounted for 20.80% of the variance and was mainly related to taste and texture terms. The A4M and K4M creams were placed in the negative part of the Y axis, and are related to gummy and oily texture, while control cream, XG cream and MX emulsion appeared in the positive part of the Y axis, and were related to sweet and cocoa taste.

Therefore, in this study attributes related with appearance and texture perceived in mouth are obtained. In conclusion, of all creams studied,

xanthan gum cream was the one that was related to sensory attributes similar to the control cream.

3.3.2. Acceptability test and CATA questions

The results of the liking of the different creams are shown in Figure 3. Cocoa control cream was the sample that presented the highest liking scores, although cream elaborated with xanthan gum emulsion obtained similar scores in appearance, colour and texture.

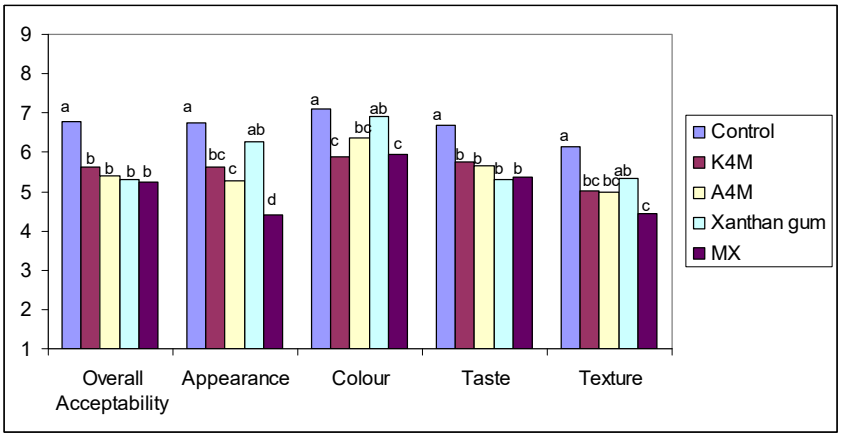

Figure 3. Acceptability scores of cocoa creams studied.

Fat is a well-known enhancer of creaminess sensations, due to its lubricating and coating properties, and also is associated to enhanced flavour perception (Wijk, van Gemert, Terpstra, & Wilkinson, 2003). Although all creams have the same fat content, the fact that the control cream presents the oil in free form (not emulsified) could probably affect the different mouth perception, regarding texture and taste and therefore can affect the cream liking.

In order to determine the specific attributes that are related to the liking scores, a CATA test was made. The CATA questionnaire is a technique that is increasingly being applied in food research. It consists of multiple-choice lists of words or phrases from which consumers select those they consider appropriate for describing the sample they have tasted (Smyth et al., 2006). In this study, 17 sensory attributes were selected. A non-parametric Cochran's test was used to study the significant differences in the frequencies of the 17 attributes used to describe the creams (Table 3).

Table 3. Frequency of selection of CATA terms and p value of Cochran's Q test for differences among cacao filling creams.

Attributes	p (Cochran test)	Frequency of mention				
		Control	Xanthan gum	K4M	A4M	MX
Lumpy	< 0.0001	31	21	31	34	51
Creamy	< 0.0001	36	28	25	15	11
Strange aroma	0.604*	5	7	5	3	3
Cocoa aroma	0.064*	27	26	26	28	16
Thick	< 0.0001	10	29	37	40	27
Bright	< 0.0001	41	30	32	9	9
Tasteless	0.000	3	22	13	14	19
Gummy	0.007	4	18	14	18	10
Cocoa taste	0.013	44	27	34	36	32
Hazelnut taste	0.000	67	49	50	48	46
Spreadable	< 0.0001	39	36	30	17	13
Sweet taste	0.023	37	25	29	24	24
Soft texture	< 0.0001	38	33	22	17	11
Pasty taste	0.001	14	23	33	36	26
Sandy taste	< 0.0001	37	26	45	32	50
Strange taste	0.055*	6	18	11	11	10
Bitter taste	0.924*	5	4	5	4	6

*Attributes that do not present significant differences with Cochran's test.

As can be seen, frequencies of 13 of the 17 attributes studied presented significant differences that indicates that these terms could be used to

describe perceived differences in the creams studied. No significant differences in strange aroma, cocoa aroma, strange taste and bitter taste were found. After that, a Correspondence Analysis (CA) was performed with the frequency of mention of the 13 attributes that exhibited significant differences. The first and second dimensions of the CA represent 66.55% and 22.82% of the total variability, respectively (Figure 4A); it could also be observed how the cellulose creams was placed in the negative part of the first dimension. Terms as "lumpy" and "sandy" were associated with the MX cream, and terms as "pasty", "thick", "tasteless" and "gummy" were associated with A4M and K4M creams. However, the positive part of the first dimension was related to control and xanthan gum creams described with attributes as "sweet taste", "hazelnut taste", "bright", "creamy", "soft texture" and "spreadable". Lastly, a Multifactorial Analysis (MFA) (Figure 4B) was made to know which sensory attributes were associated with acceptability, and the layout of the samples is similar to samples shown in Figure 4A. The first and second dimensions explain 61.82% and 25.06% of the total variability, respectively. The control cream was the one that the consumers liked the most and was perceived as the highest in cocoa taste, sweet taste and hazelnut taste, although the xanthan gum cream sample also approaches acceptability values that are linked to the attributes of creamy, soft texture, bright and spreadable.

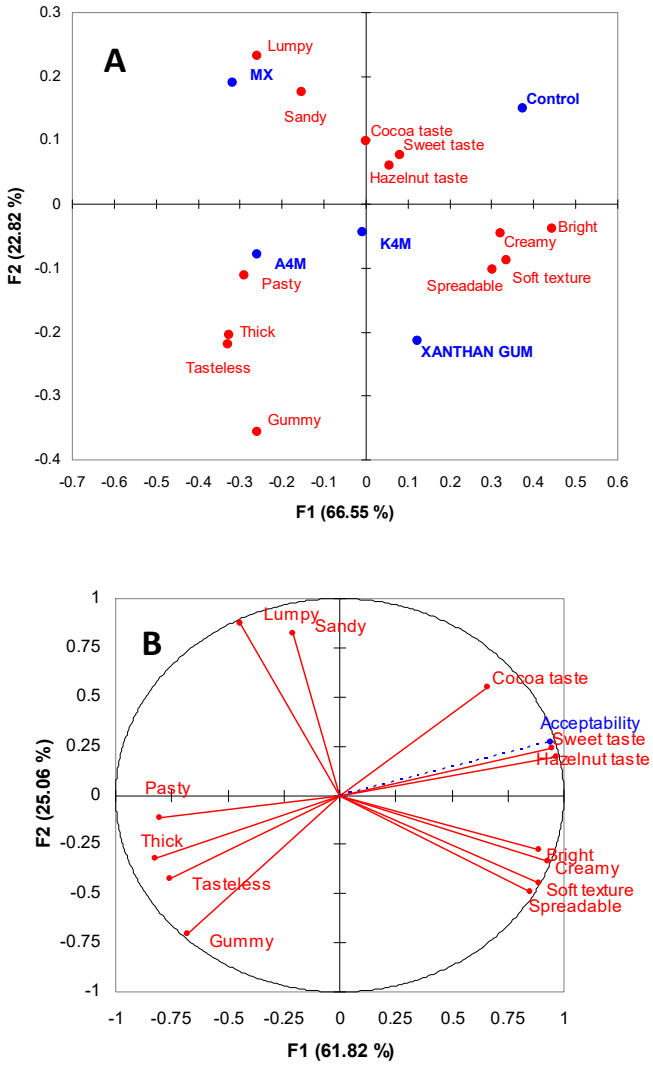

Figure 4. Representation of the sensory terms and cream samples: (A) Correspondence Analysis performed on data from the CATA question and (B) Multifactor Analysis using acceptability scores and CATA data for consumers of cocoa creams.

The samples with the lowest liking ratings, however, were the cellulose creams due to the fact that they are lumpy, gummy, sandy, pasty, thick and tasteless. These results are similar to the results obtained with Free Choice Profile so when a large number of consumers is not available, it is possible to obtain a description of the samples from the sensory point of view using the Free Choice Profile technique. However, in liking test a large number of consumers still are need.

Therefore, considering the results obtained in the sensory analysis, it could be concluded that the cocoa cream made with the xanthan gum emulsion presented sensory attributes close to the control cream.

4. CONCLUSIONS

The results highlighted a relationship between the type of hydrocolloid used and the structural characteristics of cocoa creams. A4M cream was the cream related with thick and lumpy. In vitro digestion of the creams formulated with the emulsions caused a decrease in the consistency, except xanthan gum cream, which showed an increase in consistency after gastric digestion that could be related to a satiety perception. All the studied creams decreased the extent on lipid digestion after in vitro incubation, compared to control cream. However, in sensory analysis, only the cream elaborated with xanthan gum was related to positive attributes for texture, flavour an overall liking, close to the control cream, that was the most acceptable cream. It could be concluded that the reformulation of a cocoa cream with hydrocolloid-based emulsion is a good option to obtain food with improved lipid profile and low bioaccesibility. Note that the properties provided by xanthan gum cream make this product interesting in food design of satiating foodstuffs; it has the same sensory properties as a traditional cocoa cream

with the advantage that increases the consistency in stomach phase and furthermore, provides a reduction in the lipid digestibility.

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Resumen y discusión de los resultados

La presente tesis pretende diseñar emulsiones basadas en hidrocoloides, que sean resistentes a la digestión lipídica y aptas como sustitutos de grasa, para formular alimentos que aporten menor cantidad de grasa al organismo. Con tal fin, se evalúan los cambios estructurales de las emulsiones durante la digestión *in vitro* y se relacionan con la digestibilidad lipídica. En una última parte, las emulsiones se aplican en la formulación de una crema de relleno, evaluándose la influencia en la estructura, las propiedades sensoriales y la digestibilidad lipídica.

Los resultados de la tesis se estructuran en torno a tres bloques principales: el efecto del tipo de hidrocoloide, el efecto del tipo de grasa, y la aplicación en cremas de relleno.

Efecto del tipo de hidrocoloide en la digestibilidad de emulsiones aceite/agua.

El primer hidrocoloide estudiado fue un éter de celulosa con capacidad de termogelificar, en concreto una metilcelulosa. Se estudiaron los cambios estructurales producidos en una emulsión de aceite/agua compuesta por 47% de aceite de girasol, 51% de agua y 2% de metilcelulosa tipo A4M (30% metoxil sustitución). Se investigaron las propiedades reológicas, las propiedades de extrusión, la microestructura y la distribución del tamaño de partícula de la emulsión antes y después de cada etapa de la digestión *in vitro*. Finalmente se determinó la liberación de grasa total y los ácidos grasos libres generados tras la digestión intestinal.

En la emulsión, la celulosa hidratada constituye la fase continua y el aceite la fase dispersa. Por lo tanto, la celulosa hidratada es la primera barrera que establecerá contacto con los fluidos digestivos durante la digestión de las

emulsiones. Por esta razón, se evaluó adicionalmente el efecto de la digestión *in vitro* en una solución acuosa de metilcelulosa, ya que se esperaba que los cambios estructurales sufridos por la solución acuosa fueran determinantes en el comportamiento general de la emulsión.

En comparación con la solución acuosa de celulosa, la emulsión se caracterizó por una mayor elasticidad. En la emulsión, el espectro mecánico a 37°C mostró una alta dependencia de los módulos viscoelásticos frente a la frecuencia. A frecuencia bajas existe un predominio de las propiedades viscosas frente a las elásticas (los valores del módulo de viscosidad (G'') fueron mayores que los del módulo de elasticidad (G')), visualizándose a frecuencias más altas el cruce entre G' y G'' y el inicio de la zona plateau. La mezcla con saliva no influyó en las propiedades viscoelásticas (los valores de G 'y G'' no se alteraron); tampoco se observaron cambios en la microestructura tras esta fase de digestión. La incubación en el estómago sí provocó una disminución de la viscoelasticidad, aunque en la microestructura no se observaron fenómenos de desestabilización de la emulsión, lo que indica que la metilcelulosa todavía posee un efecto emulsionante bajo las condiciones gástricas. El efecto de la saliva y la digestión gástrica sobre las propiedades reológicas y la microestructura de la emulsión se atribuyó a un efecto de dilución, más que al pH o la presencia de enzimas, de acuerdo a la comparación con controles incubados únicamente en una dilución acuosa, sin la adición de enzimas, ni cambios en el pH.

Tras la fase intestinal la viscoelasticidad continuó disminuyendo. Se observó un aumento en el tamaño de los glóbulos de grasa, lo que indicó que las sales biliares y la lipasa pancreática fueron, al menos parcialmente, capaces de

acceder a la interfaz de los glóbulos de grasa y desplazar la metilcelulosa de la superficie interfacial.

Respecto a la valoración del efecto de la MC en la digestión de las grasas, se determinaron los ácidos grasos libres como indicativo de la digestión de la grasa, pero también el porcentaje total de grasa liberada de la matriz de la emulsión tras la digestión. Se considera que para que las sales biliares y la lipasa puedan ejercer su efecto tienen que ser capaces de poder tener acceso físico a la grasa. La cuantificación de la grasa liberada confiere información de la cantidad de grasa que está bioaccesible para ser digerida. Una menor cantidad de grasa total liberada indicará un mayor impedimento físico al proceso de digestión y se asociará a una digestión menor.

Después de la digestión, se cuantificó un 49.8% de grasa liberada de la matriz, lo que implica que un 50.2% de la grasa inicial de la emulsión no se liberó de la estructura semisólida. Por lo tanto, se puede afirmar que la metilcelulosa representa una primera barrera física que dificulta la acción efectiva de los fluidos digestivos. En comparación con una emulsión control con proteína de suero, la emulsión con metilcelulosa presentó una reducción del 55% en ácidos grasos libres por gramo de grasa inicial tras la digestión intestinal.

Todos estos resultados concluyeron que la estructura de la emulsión de metilcelulosa es altamente resistente a la digestión y su aplicación sería de gran interés en el desarrollo de alimentos con baja digestibilidad lipídica.

Como consecuencia de los buenos resultados presentados por la metilcelulosa, en un segundo estudio se planteó evaluar la influencia del tipo de sustitución química del éter de celulosa en la estructura y digestibilidad de las emulsiones. Se emplearon cuatro tipos de celulosa con diferente sustitución química: dos metilcelulosas (MX y A4M) y dos hidroxipropil

metilcelulosas (K4M y F4M). Todas poseen diferente grado de metoxil e hidroxipropil sustitución: MX (>30% metoxilo), A4M (30% metoxilo), K4M (22.5% metoxilo, 7.7 hidroxipropilo), y F4M (29% metoxilo, 6.8% hidroxipropilo), y además también poseen diferente capacidad de termogelificar.

El comportamiento viscoelástico de la emulsión se relacionó con el grado de metoxil sustitución. La emulsión con el tipo de metilcelulosa con mayor porcentaje de metoxil sustitución, MX, presentó valores significativamente más altos de G' y G" y mayor viscoelasticidad (menor valor de tan δ). La hipótesis principal que justifica esa notable diferencia entre el comportamiento viscoelástico de MX y las demás celulosas es el hecho de que, a 37 ° C, la MX ya comienza a gelificar. Esta temperatura de gelificación más baja se asocia con su mayor contenido de grupos metilo, que aumenta la hidrofobicidad del sistema. El aumento en las propiedades viscoelásticas de la MX a la temperatura de digestión (37° C) es un aspecto a tener en cuenta no solo para reducir la digestión de la grasa, sino también para otras aplicaciones como, por ejemplo, potenciador de la saciedad, que se traduciría en una reducción en la ingesta total.

Respecto al efecto de la digestión, la adición de saliva solo produjo una ligera disminución en la viscoelasticidad, sin afectar significativamente a la estructura de ninguna de las emulsiones estudiadas. Después de la incubación en el estómago se produjo una disminución en los valores de G' y G" en las emulsiones de A4M, F4M y K4M, aunque este descenso de los módulos viscoelásticos fue más significativo después de la fase intestinal. La incubación en la etapa gástrica e intestinal de las emulsiones únicamente en presencia de agua (sin enzimas ni cambios de pH), no mostró diferencias significativas, en comparación con la incubación estándar de las emulsiones de A4M, F4M y MX. Este hecho revela que la disminución en la consistencia

de las emulsiones debe asociarse principalmente a un efecto de dilución, más que a un efecto específico de pH o enzimas. La emulsión K4M fue la que presentó la mayor disminución en la viscoelasticidad en comparación con la dilución con agua, lo que demostró una menor resistencia estructural a la incubación intestinal.

La emulsión con MX mostró diferentes propiedades viscoelásticas, tanto en fresco como tras cada etapa de la digestión. Los cambios observados en esta emulsión durante la digestión fueron menores que en las otras emulsiones formuladas con los otros tipos de éter de celulosa. Aunque la incubación en la boca, el estómago y el intestino produjo una disminución leve y progresiva en los valores de G 'y G ", no se encontraron diferencias significativas en las funciones viscoelásticas entre las diferentes etapas de la digestión.

En cuanto a la microscopía, se observó que las emulsiones formuladas con metilcelulosa (MX y A4M), fueron más compactas y de apariencia más densa que las formuladas usando hidroxipropil metilcelulosa (K4M y F4M); esto se podría atribuir al hecho de que hay una mayor presencia de grupos hidrófobos en la metilcelulosa en comparación con la hidroxipropil metilcelulosa, lo que favorece las interacciones intermoleculares entre estos grupos, haciendo que la emulsión posea mayor consistencia. Las fases oral y gástrica de la digestión *in vitro* no parecieron afectar al tamaño y la forma de los glóbulos de las emulsiones. Las emulsiones con metilcelulosa fueron las más resistentes a la digestión intestinal, particularmente la MX. Esta etapa de la digestión tuvo un notable efecto sobre las dos emulsiones con hidroxipropil metilcelulosa (K4M y F4M); los glóbulos se volvieron significativamente más pequeños en comparación con las emulsiones en fresco, es decir, sin digerir. Se observaron fenómenos de floculación y coalescencia, con formación de globulos de grasa de mayor tamaño e irregulares; estos cambios fueron más significativos en el caso de la

emulsión K4M. Los fenómenos de floculación y coalescencia se podrían asociar al desplazamiento por parte de las sales biliares, de las moléculas de éter de celulosa de la superficie de las gotas, provocando la desestabilización de la emulsión y facilitando el acceso de la lipasa al núcleo lipídico. Sin embargo, todavía se pueden observar glóbulos de grasa emulsionados en ambos casos, lo que indicaría que ninguna de las emulsiones se ha digerido por completo.

Los resultados correspondientes a la liberación de grasa total y la cuantificación de los ácidos grasos libres tras la digestión intestinal se correlacionaron con la resistencia estructural de las distintas emulsiones. Las emulsiones formuladas con HPMC (K4M y F4M) liberaron una mayor cantidad de grasa total tras la digestión, mientras que la emulsión con metilcelulosa MX fue la que menor cantidad de grasa liberó (solo 10.5%). Así mismo, la emulsión con MX presentó la menor liberación de ácidos grasos libres. Este resultado se asocia a la mayor hidrofobicidad de la celulosa MX que proporciona una mayor viscoelasticidad, y resistencia estructural durante la digestión, lo que limita la accesibilidad de los fluidos y enzimas de digestión a la grasa, y su digestión.

Por lo tanto, el grado de metoxil e hidroxipropil sustitución de los éteres de celulosa tuvo un efecto significativo en las propiedades estructurales de la emulsión antes y después del proceso de digestión, que se relacionó con la digestibilidad de la grasa presente en la emulsión.

Como tercer objetivo se planteó la utilización de un hidrocoloide de diferente naturaleza química, la goma xantana. A diferencia de los éteres de celulosa, la goma xantana no posee propiedades surfactantes, se utiliza en emulsiones por su efecto estabilizador de la fase continua. Se investigó el comportamiento de emulsiones con goma xantana (2%) en presencia y

ausencia del emulsionante polisorbato 80 (Tween 80), un surfactante no iónico de bajo peso molecular, que se caracteriza por su elevada eficacia. Así mismo se estudió el comportamiento durante la digestión de una disolución de goma xantana. Los resultados en la disolución acuosa de goma xantana mostraron que la adición de saliva no afectó la viscoelasticidad. Por su parte la incubación en el estómago produjo una disminución significativa en G 'y G'', lo que implica una disminución en el comportamiento viscoelástico, que se asoció al carácter aniónico de la goma xantana, que reduce su capacidad como espesante a pH ácido.

Sin embargo, esta disminución en la viscoelasticidad tras la fase gástrica no se observó en el caso de emulsiones de goma xantana sin agente emulsionante. A pesar de presentar una mayor dilución tras la etapa gástrica no se observó disminución en la viscoelasticidad en comparación con las muestras frescas.

En comparación con la incubación gástrica en presencia de agua (sin fluidos gástricos), la emulsión sometida a las condiciones del estómago presentó una viscoelasticidad significativamente superior, que implica que la emulsión de goma xantana sufrió un cambio estructural en esta etapa de la digestión que causó un aumento en las propiedades viscoelásticas. Sin embargo, en presencia de emulsionante Tween 80 no se apreció este aumento de la consistencia (no se encontraron diferencias significativas entre las muestras incubadas con agua y la incubación convencional). El aumento de la consistencia de la emulsión con goma xantana a nivel del estómago puede considerarse una propiedad interesante para aplicaciones en las que se busca retrasar el vaciado gástrico y/o el aumentarde la saciedad.

Después de la incubación del intestino, se observó una disminución de la viscoelasticidad en las emulsiones con y sin emulsionante.

Para una mejor comprensión de los resultados reológicos, se evaluó la microestructura mediante microscopía óptica. Las emulsiones formuladas únicamente con goma xantana, sin emulsionante, mostraron glóbulos de grasa de tamaño muy grande dispersos en una fase contínua formada por la goma hidratada. Este resultado era previsible, ya que la funcionalidad de la goma xantana se limita a estabilizar el sistema aportando consistencia a la fase continua, sin presentar efecto emulsionante. La incorporación del emulsionante Tween 80 produjo una marcada reducción en el tamaño de las gotas de grasa. La adición de saliva no produjo un gran cambio en la microestructura de ninguno de los sistemas.

La observación de la microestructura tras la etapa gástrica explica los resultados viscoelásticos. En las emulsiones de xantana sin emulsionante, tras la etapa gástrica se observaron fenómenos de coalescencia y floculación de la grasa, hecho que explica el aumento observado en las propiedades viscoelásticas y los parámetros de textura, ya que la floculación de las grasas se asocia con un aumento de la consistencia. Este fenómeno podría estar relacionado con la sensibilidad de la estructura de la goma xantana a las condiciones ácidas del estómago; la desestabilización de la matriz de la red de xantana a pH ácido favorece el contacto físico entre los glóbulos de grasa En presencia del emulsionante Tween 80, la emulsión no se vio afectada en las condiciones ácidas del estómago, y los glóbulos de grasa permanecieron estables, debido a la estabilidad del polisorbato y de su acción emulgente en las condiciones del estómago, que hace posible que la estructura de la emulsión no se altere a pesar del debilitamiento de la red de xantana.

Después de la incubación del intestino, se observó una disminución en el tamaño de los glóbulos de grasa en la emulsión con goma xantana en ausencia de Tween 80. Al no haber presente un agente emulsionante, la superficie de la gota queda completamente expuesta y libre para que las

sales biliares lleven a cabo su acción emulsionante. En presencia de Tween 80, no se vio ningún cambio apreciable en la microestructura de los glóbulos, lo que pone de manifiesto el mayor efecto barrera causado por el emulsionante contra la acción de los fluidos digestivos. Este hecho se confirmó al determinar la liberación de ácidos grasos tras la digestión; aunque la presencia de goma xantana provocó una reducción significativa en la generación de ácidos grasos libres en comparación con el control, la presencia del polisorbato disminuyó significativamente la digestibilidad lipídica.

Este estudio abre nuevas aplicaciones para el uso de goma xantana en emulsiones. En primer lugar, la combinanción goma xantana-Tween proporcionó la estructura más efectiva para reducir la accesibilidad de la lipasa pancreática, creando una barrera contra la digestión lipídica. Por otro lado, en ausencia de un emulsionante, destaca el aumento en la consistencia de la emulsión de goma xantana a nivel gástrico, lo que puede ser de interés para aplicaciones que requieran la generación de una sensación de saciedad.

Efecto del tipo de grasa en el desarrollo de emulsiones con alto contenido en grasa.

Otro de los objetivos planteados en esta tesis doctoral fue evaluar los cambios producidos en las propiedades físicas de las emulsiones basadas en hidrocoloides en función del tipo de grasa. Las emulsiones con aceite vegetal líquido, si bien son apropiadas para reemplazar la grasa en determinados alimentos, como galletas, magdalenas, no son adecuadas en aplicaciones en las que se requiere que la grasa presente elevada plasticidad, como por

ejemplo en la elaboración de hojaldres. Con la finalidad de aumentar la plasticidad de las emulsiones se elaboraron emulsiones con grasa con un mayor contenido en ácidos grasos saturados, sólidas a temperatura ambiente: grasa de leche anhidra. Si bien estas emulsiones no suponen una mejora en cuanto al perfil lipídico de las grasas que utilizan, sí que constituyen una ventaja por presentar un contenido de grasa (47%), que es notablemente inferior al que presenta la mantequilla o los shortening convencionales (80%).

Se evaluaron las propiedades físicas de las emulsiones formuladas con grasa de leche (47%) y éteres de celulosa (2%) con distinta sustitución química (dos metilcelulosas (MX y A4M) y una hidroxipropil metilcelulosa (F4M)).

Tanto a temperatura ambiente (20°C) como a temperatura de refrigeración (5°C) la presencia de celulosa provocó una disminución en la fuerza de penetración y los valores de viscoelasticidad respecto al control de grasa de partida (grasa de leche anhidra). Los valores de dureza de las emulsiones de F4M y A4M fueron similares a la mantequilla comercial, y la emulsión de MX mostró valores significativamente más bajos, lo que indica que posee una estructura más suave. A temperatura ambiente el control de grasa de leche continuó mostrando la mayor dureza, y todas las emulsiones estudiadas mostraron menor dureza que la mantequilla, siendo la de F4M la más cercana a esta. Por lo tanto, la incorporación de grasa de leche como fase grasa de las emulsiones de éteres de celulosa proporcionó un producto untable más suave que la grasa de leche inicial, con un perfil de textura similar al de la mantequilla, pero con un 30% menos de grasa.

Las propiedades reológicas mostraron en todos los sistemas valores de G' más altos que G'' y baja dependencia con la frecuencia. A 5 ° C, la emulsión con F4M mostró el mayor valor de G', mostrando un comportamiento más cercano a la mantequilla y a la grasa de leche; por otro lado, al igual que en

los valores de penetración, la emulsión con MX presentó valores más bajos de viscoelasticidad. A temperatura ambiente, la emulsión de F4M continuó mostrando los valores más altos de G' y G'', pero las diferencias con respecto a la A4M y la MX fueron menos evidentes a 20°C que las mostradas a 5°C.

Para una mejor comprensión de las propiedades estructurales, se llevó a cabo el estudio de la microestructura de cada uno de los sistemas. La composición de la grasa determina la cantidad de cristales de grasa, así como la forma y la agregación de los cristales individuales en una red. Así pues, los controles de grasa de leche anhidra y mantequilla mostraron una microestructura diferente. La presencia del hidrocoloide modificó la estructura de la red de la emulsión; se observó una menor cantidad de grasa, y esta apareció incrustada en una red contínua formada por el hidrocoloide hidratado. En la emulsión de grasa de leche-MX la grasa líquida se distribuyó uniformemente en los glóbulos esferoidales, observándose un bajo nivel de cristalización, con cristales de pequeño tamaño. Esta microestructura explica la menor firmeza y los valores más bajos de las funciones viscoelásticas que se encuentran en este sistema, ya que los cristales más pequeños forman enlaces más débiles y esto da lugar a una disminución de la viscoelasticidad. Sin embargo, en la emulsión de grasa de leche-F4M se observó una estructura cristalina más densa, con cristales más grandes en comparación con el control de grasa de leche pura. Esto podría explicar los valores más altos de dureza en este sistema.

Las propiedades estructurales de las grasas plásticas se ven fuertemente afectadas por las propiedades térmicas de la red de cristal formada por la grasa. Así pues, como último paso, se planteó el estudio del comportamiento de fusión de los diferentes sistemas. La fracción de fusión media (de 10° C a 19° C) y la fracción de fusión alta (por encima de 20° C), típicas del perfil de fusión de la grasa de leche, se observaron en todos los sistemas formulados

con este tipo de grasa. La presencia de celulosa provocó un retraso en la temperatura de fusión de la grasa. En las emulsiones con celulosa se redujo la estructura compacta original de la grasa de leche, ya sea por la presencia de la red hidrocoloide-agua y/o la menor proporción de grasa, lo que llevó a un área más baja del pico de la fracción mínima. La emulsión con F4M mostró valores de área más altos que las otras celulosas, lo que puede atribuirse al mayor grado de cristalización observado en el análisis de la microestructura en este tipo de emulsión.

Las condiciones de almacenamiento no revelaron un alto impacto en la red de cristales en ninguno de los sistemas, lo que indica que no hubo cambios significativos en el comportamiento de fusión de la grasa tras los 15 dias de almacenamiento.

En un segundo estudio se evaluó la influencia de la goma xantana como estabilizante de la matriz. Se desarrolló una emulsión aceite/agua con un 47% de aceite de palma y un 2% de goma xantana, y se determinaron las propiedades macro y microestructurales. Se evaluó también la resistencia estructural durante la digestión *in vitro*, relacionándola con la digestibilidad de la fase grasa de la emulsión.

Al someter la emulsión a la digestión oral, no se produjeron diferencias respecto a la textura y el comportamiento viscoelástico de la muestra en fresco. Tras la incubación gástrica se produjo un incremento significativo en la fuerza de extrusión. Este incremento de consistencia a pH ácido también se produjo en las emulsiones de goma xantana y aceite de girasol anteriormente estudiadas. Así mismo, tras la digestión intestinal, el comportamiento fue similar al observado con aceite de girasol, observándose una notable disminución de las propiedades viscoelásticas, indicando una pérdida de consistencia del sistema.

La caracterización microestructural ayudó a la interpretación del grado de estructuración-desestructuración del sistema en cada etapa digestiva. Tras la etapa oral, los cristales de grasa fundieron, debido al efecto de la temperatura de incubación. En la digestión gástrica se observó un incremento del tamaño del glóbulo de grasa y coalescencia entre ellos, que confirma el aumento de la consistencia y de las propiedades viscoelásticas producido en presencia de la goma. Este cambio se asocia a la debilidad de la red de goma xantana y de las interacciones electrostáticas bajo las condiciones ácidas del estómago. Tras la incubación intestinal se observó una disminución en el tamaño de los glóbulos de grasa, propiciado por el efecto surfactante de las sales biliares. Sin embargo, se observaron todavía gran cantidad de glóbulos de grasa, lo que implica que el proceso de digestión de la grasa no había sido total, ni tampoco la desestructuración de la matriz. Este hecho se confirmó mediante la determinación de los ácidos grasos liberados tras la digestión intestinal como indicador de la acción lipolítica ejercida por la lipasa pancreática. Se observó que la presencia de la goma xantana redujo notablemente la cantidad de ácidos grasos generados tras la digestión de los triglicéridos de la emulsión. Al igual que se observó en las emulsiones de aceite de girasol, la incorporación de este hidrocoloide en la matriz de aceite de palma-agua representó una barrera frente a la digestión de las grasas; las sales biliares y las enzimas tuvieron acceso limitado a la fase oleosa y, por lo tanto, no actuaron de manera eficiente en la superficie de la grasa.

Aplicación de emulsiones de baja digestibilidad lipídica al desarrollo de cremas untables.

La última parte de la tesis doctoral se centró en valorar la viabilidad de la aplicación en un alimento de las emulsiones sustitutas de grasa y baja digestibilidad desarrolladas.

El alimento seleccionado fue una crema de relleno dulce, por su alto contenido en grasa, su alta aceptabilidad y nivel de consumo y por ser una matriz con una composición y forma de preparación relativamente simple y versátil. Se estudió el efecto de las distintas emulsiones en las propiedades estructurales, la digestibilidad y la percepción sensorial de diferentes formulaciones de crema.

En un primer trabajo se comparó el efecto de la utilización de metilcelulosa tipo A4M, hidroxipropilmetilcelulosa tipo K4M y goma xantana en emulsiones de aceite de girasol. Los ingredientes de la crema fueron: emulsión sustituta de grasa (50%), azúcar (5%), leche en polvo (2%), almidón (2.25%) y agua. Como control se utilizó una crema compuesta por azúcar (5%), leche en polvo (2%), almidón (4%), aceite de girasol (23.5%) y agua. Todas las cremas presentaron un 23.5% de grasa en su composición.

Los resultados de textura mostraron que las cremas con emulsión a base de hidrocoloide proporcionaron una mayor consistencia en comparación con la crema control, siendo la de goma xantana la que mayores valores de fuerza presentó (goma xantana>A4M>K4M>control), lo que se asoció con la mayor capacidad espesante de este hidrocoloide en comparación con los éteres de celulosa y el almidón. Como se observó en el análisis de la microestructura, las cremas formuladas con celulosas presentaron glóbulos de grasa más pequeños que la crema control. Esto sugiere que los glóbulos de grasa en cremas elaboradas con las emulsiones de celulosa se estabilizaron debido a

la formación de una red de hidrocoloide hidratado presente en la fase acuosa, que inmovilizó los glóbulos grasos de la fase dispersa evitando los fenómenos de floculación y coalescencia. Se pudo observar que los glóbulos de grasa en cremas con hidroxipropilmetilcelulosa fueron más pequeños que los de cremas con metilcelulosa, posiblemente debido al menor número de grupos sustituyentes hidrófobos en su composición. Sin embargo, en el caso de la crema con goma xantana, los glóbulos fueron de mayor tamaño y menos circulares, lo que se asocia a que la goma xantana no es un emulsionante, y solo actúa como estabilizante del sistema al espesar la fase continua.

Después de la digestión oral se encontraron valores más bajos de consistencia en todas las muestras, aunque esta disminución no fue tan significativa en presencia de goma xantana. La pérdida de consistencia del sistema se asoció a la presencia de α-amilasa, que hidrolizó el almidón presente en la formulación. En la crema control se pudieron observar fenómenos de floculación y coalescencia de los glóbulos de grasa, algo que no se produjo en las cremas con celulosa o goma xantana; las redes de los hidrocoloides continúaron proporcionando estabilidad al sistema, y no se vieron afectadas por el efecto enzimático de la saliva. Tras la digestión gástrica, la reducción en los valores de textura en las cremas con celulosa y la crema control se debió principalmente al efecto de dilución más que a la acción enzimática o al cambio de pH, ya que no se encontraron diferencias significativas entre la muestra incubada con fluidos gástricos simulados y la muestra incubada con agua. Sin embargo, el comportamiento en la fase del estómago fue diferente en la crema con goma xantana; esta mostró valores de fuerza significativamente más altos después de la incubación gástrica que después de la dilución de agua en el estómago, lo que indica un aumento en la consistencia asociada con las condiciones específicas de esta etapa (pH 2

y presencia de enzimas). Este comportamiento estaría relacionado, como en el caso de la emulsión, al debilitamiento de la red de xantana a pH ácido. Destacar que este aumento de consistencia de las cremas con emulsión de xantana a nivel del estómago podría ser interesante en su aplicación como alimento para controlar la saciedad, ya que, un aumento de la consistencia en el estómago es uno de los factores que se asocia con un retraso en el vaciado gástrico, y por tanto, a una prolongación de la sensación de plenitud, y a un retraso en la sensación de hambre.

La etapa de digestión intestinal afectó significativamente a la estructura de todos los sistemas, aunque este efecto fue más notable en la crema control, en la cual la grasa fue digerida casi de forma total por la acción de la lipasa pancreática. En todas las cremas con hidrocoloides, el efecto de la digestión del intestino produce un aumento del tamaño de los glóbulos y la aparición de coalescencia debido a cambios en la estabilidad de la grasa, lo que implica cierto efecto de los fluidos digestivos. Sin embargo, los glóbulos de grasa fueron todavía visibles en estos sistemas, lo que implica que tanto las celulosas como la goma xantana continúan ejerciendo un efecto estabilizador tras someterse a todas las etapas de la digestión. La determinación de los ácidos grasos liberados tras la digestión confirmó este hecho, ya que la digestibilidad lipídica fue menor en las cremas con emulsiones basadas en hidrocoloides (goma xantana < metilcelulosa = hidroxipropilmetilcelulosa), en comparación con la crema control. Este resultado se atribuyó al comportamiento del hidrocoloide, que puede responder a diferentes mecanismos tales como la interacción directa fibra-enzima, la formación de un revestimiento protector alrededor de la gota de grasa, la unión de las sales biliares o el aumento de la viscosidad. Como en el caso de las emulsiones, en las cremas la presencia del hidrocoloide actua

como una barrera física que confiere estabilidad estructural y dificulta la acción efectiva de las enzimas.

La estabilidad, la apariencia y la aplicación de los alimentos reformulados dependen frecuentemente de las características reológicas que poseen. Por eso, como segundo paso, se planteó investigar el comportamiento reológico de diferentes cremas de relleno formuladas con las emulsiones. En concreto, para este estudio se seleccionaron emulsiones formuladas con distintos éteres de celulosa y se identificó la funcionalidad proporcionada por cada uno de ellos y las interacciones entre la emulsión y la matriz del producto cremoso. En este caso, en la formulación de las cremas se incorporó cacao en polvo. Los hidrocoloides utilizados para preparar las emulsiones de baja digestibilidad fueron dos tipos de metilcelulosa (MC) (MX y A4M) y dos tipos de hidroxipropilmetilcelulosa (HPMC) (F4M y K4M). Se analizaron las propiedades termorreológicas de cada sistema determinando el comportamiento de flujo y las propiedades viscoelásticas a diferentes temperaturas (20°C, 45°C y 70°C).

Respecto a las propiedades de flujo, a temperatura ambiente, todas las cremas estudiadas presentaron un claro comportamiento pseudoplástico, ya que la viscosidad disminuyó al aumentar la velocidad de cizalla aplicada. La crema MC (MX) fue claramente diferente al resto de las cremas de celulosa, con un comportamiento similar al de la crema de control. La viscosidad cero (η_0) (viscosidad inicial) en la crema control y la crema con MX fue un orden de magnitud superior al grupo de cremas que contenían A4M, F4M y K4M, lo que significa que los dos grupos tuvieron una consistencia apreciablemente diferente en el recipiente. Sin embargo, debido a su diferente carácter pseudoplástico, la viscosidad de todas las cremas fue similar a velocidades de untado (> 100 s^{-1}).

Las ensayos de viscoelasticidad mostraron que todas las cremas tuvieron un comportamiento elástico predominante (G'>G''), característico de los sistemas tipo gel. La crema control y la MX fueron menos dependientes de la frecuencia que las otras cremas (A4M, F4M y K4M), y presentaron además una mayor distancia entre los módulos G' y G'', lo que indicó la existencia de una red tridimensional interna más fuerte en estos sistemas. Estos resultados coincidieron con el comportamiento observado en el flujo, ya que el grupo que se corresponde con una estructura interna más fuerte es el que presentó también una viscosidad más alta en reposo y un mayor comportamiento pseudoplástico (control y MX).

Dado que este tipo de cremas pueden someterse a altas temperaturas durante su procesado tecnológico en la industria alimentaria, se estudiaron sus propiedades reológicas a diferentes temperaturas. La crema control no varió su comportamiento reológico al variar la temperatura; esto es debido a que esta no contiene emulsión, es decir, no presenta ningún hidrocoloide que pueda modificar su estructura. El cambio más significativo se produjo en la crema con A4M, en la que a 45°C ya se observó un aumento de la viscosidad, además de un significativo aumento del módulo G' (componente sólida), lo que reveló que a esta temperatura la celulosa había gelificado, formando un sistema más estructurado. Esto posiblemente se traduzca en una dificultad a la hora del manejo de estas cremas en procesos donde se tenga en cuenta la temperatura. Un proceso similar ocurrió con la crema con MX, aunque de forma menos significativa.

Sin embargo, en el caso de las cremas con hidroxipropilmetilcelulosas (F4M y K4M), el aumento de viscosidad y de los valores de los módulos dinámicos se produjo a temperaturas más altas (70°C). Estos resultados son coherentes con la evolución del módulo elástico con la temperatura; todas las celulosas estudiadas (MC y HPMC) poseen capacidad termogelificante, pero la

presencia de grupos hidroxipropil produce un aumento en la temperatura de gelificación, dependiendo del grado de sustitución. La crema con K4M fue la que necesitó una mayor temperatura para mostrar un aumento en los valores de viscoelasticidad y los módulos dinámicos y la que menor consistencia presentó, ya que el mayor grado de hidroxipropil sustitución le confirió un aumento en la temperatura de gelificación y una estructura más débil que las demás. Así pues, la sustitución química del éter de celulosa afectó tanto la gelificación térmica como el comportamiento reológico de las cremas de cacao formuladas con emulsiones de celulosa.

Finalmente, se evaluó la percepción sensorial de las cremas de cacao formuladas con las diferentes emulsiones. Para este estudio se seleccionaron todos los hidrocoloides utilizados a lo largo de la presente tesis; dos metilcelulosas (MX y A4M), dos hidroxipropilmetilcelulosas (F4M y K4M) y la goma xantana.

Inicialmente se realizó un análisis de Perfil de Libre Elección ("*Free Choice Profile*" (FCP)) para determinar los atributos que describen las cremas de cacao. Los 20 consumidores reclutados generaron 189 términos, respecto a la apariencia, el sabor, la textura y el aroma. El Análisis de Procrustes Generalizados (GPA) de dos dimensiones aplicado para analizar los datos mostró las diferencias entre las muestras. La crema control, la crema con goma xantana y la crema con MX se relacionaron con los términos "dulce" y "sabor a cacao". Respecto a la apariencia y la textura, los atributos "apariencia cremosa", o "textura cremosa" caracterizaron a la crema control y la crema elaborada con emulsión de goma xantana. Sin embargo, las cremas elaboradas con emulsiones de éteres de celulosa (MX, A4M y K4M) se asociaron con atributos como "apariencia grumosa", "textura grumosa" y "sabor extraño".

A continuación, se evaluó la percepción del consumidor mediante un test de aceptabilidad realizado por 82 catadores no entrenados, que calificaron las muestras de acuerdo a la aceptabilidad general, la apariencia, el color, el sabor y la textura, en una escala hedónica de 9 puntos (de 1="No me gusta extremadamente" a 9 = "Me gusta extremadamente"). La crema control fue la muestra que presentó las mejores puntuaciones, aunque la crema con emulsión de goma xantana obtuvo puntuaciones similares en apariencia, color y textura.

Para determinar los atributos específicos relacionados con las puntuaciones de aceptabilidad, se realizó un cuestionario CATA (Marque todo lo que corresponda, "*Check-All-That Apply*"). Esta técnica, cada vez más empleada, consiste en una lista de selección múltiple de palabras o frases entre las cuales los consumidores seleccionan aquellas que consideran apropiadas para describir la muestra que han probado. En este estudio se seleccionaron 17 atributos sensoriales, y de estos, 13 presentaron diferencias significativas que indicaron que estos términos podrían usarse para describir las diferencias percibidas en las cremas estudiadas. Los consumidores marcaron los atributos que consideraron apropiados para describir cada muestra y se determinó la frecuencia de mención de cada uno. La variabilidad en las frecuencias de mención de atributos significativos se analizó mediante un Análisis de Correspondencia (CA) en el que se observó que la crema control y la crema con goma xantana se relacionaron con atributos como "sabor cacao", "sabor dulce", "brillante", "cremosa", "textura suave" y "untable". En cambio, términos como "gomoso" y "arenoso" se asociaron con la crema MX; y las cremas con A4M y K4M se asociaron con atributos como "pastoso", "espeso", "sin sabor" y "gomoso". Con el fin de comprender qué atributos sensoriales se asociaron con la aceptabilidad, la frecuencia de mención obtenida al utilizar las preguntas CATA se estudió en

combinación con los datos de aceptabilidad general del consumidor mediante un Análisis Multifactorial (MFA). La crema de control fue la que más gustó a los consumidores, que percibieron en ella atributos como el sabor a cacao y sabor dulce, aunque la muestra de crema de goma xantana también se aproximó a los valores de aceptabilidad vinculados a los atributos de textura cremosa y suave, brillante y untable. En cambio, las muestras con los índices de aceptabilidad más bajos fueron las cremas de celulosa, ya que se percibieron como como "grumosa", "gomosa", "arenosa", "pastosa", "espesa" y "sin sabor".

Por lo tanto, considerando los resultados obtenidos, se concluyó que la crema de cacao formulada con la emulsión de goma xantana presentó atributos sensoriales cercanos a la crema control. Ambas presentaron las mejores cualidades organolépticas, como el sabor dulce y la apariencia cremosa. La crema con MX también presentó atributos sensoriales como el sabor dulce, pero durante el análisis sensorial se consideró demasiado brillante y con textura grumosa. Las cremas con A4M y K4M se caracterizaron por su textura grumosa y apariencia gomosa, y su sabor fue evaluado negativamente por el panel.

Conclusiones

Las principales conclusiones que se extraen de la presente tesis son las siguientes:

- La incorporación de éteres de celulosa o goma xantana como emulsionantes y/o estabilizantes en emulsiones aceite/agua es una forma efectiva de reducir la digestibilidad de la grasa tras la digestión *in vitro*.

- El principal factor que se ha asociado a la reducción de la digestibilidad lipídica es un impedimento físico ocasionado por la red de los hidrocoloides presente en la fase continua de las emulsiones y que limita la correcta función de los jugos digestivos.

- Una mayor resistencia estructural durante la digestión *in vitro* se asoció a una mayor viscoelasticidad y a una menor bioaccesibilidad y digestibilidad de la grasa.

- La sustitución química de los éteres de celulosa afectó a la estructura de la emulsión, a los cambios estructurales durante la digestión y a la bioaccesibilidad de la grasa. El mayor contenido de metoxilo se asoció con una menor disminución de la viscoelasticidad durante la digestión, reflejo de una mayor resistencia estructural y a una menor digestibilidad de la grasa. Por otra parte, la celulosa con menor contenido de metoxilo presentó una mayor desestructuración y una mayor digestibilidad.

- El efecto reductor de la digestibilidad producido por la goma xantana se potenció en presencia del polisorbato como emulsionante. No obstante, en ausencia de este emulsionante, la emulsión de goma xantana sufre un aumento de consistencia a nivel gástrico, que es una propiedad interesante para aplicaciones alternativas como aumento de la saciedad.

- El uso de grasas de diferente naturaleza y estado físico permitió obtener emulsiones con distintas propiedades macro y microestructurales, que proporcionaron diferentes patrones de estructuración/desestructuración durante la digestión *in vitro*.

- El uso de éteres de celulosa en el diseño de sistemas grasa/agua partiendo de grasa sólida/semisólida presenta un gran interés en la formulación de grasas plásticas y untables con menor cantidad de grasa y similares a la mantequilla o la margarina convencionales.

- El uso de emulsiones estabilizadas con los hidrocoloides estudiados presentó una alternativa en la reformulación de una crema de relleno convencional baja en grasa total y baja en grasas saturadas. La aplicación de estas emulsiones dio lugar a cremas con diferente consistencia y cremosidad, que presentaron además mayor resistencia estructural a la digestión *in vitro*, y por consiguiente una disminución significativa en la digestibilidad de la grasa.

- Debido a la capacidad de termogelificar de los éteres de celulosa empleados, las propiedades viscoelasticas de las cremas de cacao formuladas con las emulsiones variaron en función de la

temperatura. Las cremas con metilcelulosa presentaron menor temperatura de gelificación que las cremas con hidroxipropil metilcelulosa. Por tanto, la gelificación térmica de las cremas elaboradas con las emulsiones de celulosa es un aspecto fundamental a considerar en procesos industriales que incluyan cambios térmicos.

- La crema con goma xantana fue la que presentó propiedades sensoriales más cercanas al producto convencional, y una mayor aceptabilidad, con la ventaja añadida de que aumenta la consistencia en la fase gástrica, proporcionando un retraso en el vaciamiento gástrico, y por tanto a una prolongación de la sensación de plenitud.

- La combinación de técnicas macroestructurales, microestructurales y sensoriales ha demostrado ser una herramienta útil en la evaluación de las propiedades físicas y funcionales de las emulsiones y del producto reformulado.

- Los resultados se consideran prometedores, y convierten a las emulsiones estudiadas como candidatas para realizar futuros estudios más sofisticados como la utilización de experimentación animal o la realización de ensayos clínicos en humanos, que permitan validar los resultados obtenidos en la metodología *in vitro*.

Anexo

Presentación de los artículos publicados

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In vitro digestibility of highly concentrated methylcellulose O/W emulsions: rheological and structural changes

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The changes in structure during the digestion of highly concentrated methyl cellulose (MC) O/W emulsions and of hydrated MC were investigated. The effect of human saliva and *in vitro* stomach digestion was attributed to a dilution effect, rather than to pH or pepsin activity. After *in vitro* intestine incubation, a decrease in viscoelasticity and an increase in fat globule size were observed. The fat released after the digestion of the MC emulsion was 49.8% of the initial fat, indicating the existence of a big physical impediment. In comparison with an O/W whey protein emulsion with fat content equal to the fat released during the MC emulsion digestion, a 12% reduction in free fatty acid formation was found, which indicates that the decrease in fat bioaccessibility in the MC emulsion should be attributed not only to a physical effect against fat release but also to a further impediment related to the fat digestion process. Fat released quantification informs about the physical retention of fat in the emulsion matrix structure. Enzymes may not act if fat is not released and solubilized. Free fatty acid quantification is the real indicator of fat digestion, but contrary to the total fat released, it is affected by a wide variety of enzymatic factors, which should be considered for the correct comparison of systems of different properties, for example systems where the amount of fat release during the digestion may be different or initially unknown.

1. Introduction

The design of colloid delivery systems to control the rate and extent of lipid digestion within the gastro-intestinal tract has been the subject of extensive attention lately. Inhibiting or slowing down lipid digestion is considered to be an effective means with which to reduce appetite and promote satiety, leading to a reduction in obesity and a more balanced energy intake.[1,2]

After ingestion, the emulsions undergo a complex series of physical and chemical changes as they pass through the mouth, stomach and small and large intestines (mechanical strength, presence of enzymes, changes in pH, *etc.*),[3,4] which affect their capacity to be ingested. When designing low absorption emulsions, it is vital to bear these parameters in mind so as to achieve a level of structural stability which limits the enzymatic attack.

More attention has been paid to the effect of the interfacial layer surrounding the lipid droplets on the emulsion structuring/breakdown during digestion than anything else. Depending on the physicochemical properties of the interfacial layer, the lipid droplets may break up or coalesce as the emulsion passes through the mouth into the stomach and then the intestines, while at the same time altering the surface area of lipid exposed to enzymes. Emulsions stabilised by non-ionic surfactants tend to remain stable during the transit through the stomach because of the highly stable nature of the emulsion.[5,6] Emulsions stabilised by proteins tend to flocculate[7] while those stabilised by ionic surfactants can undergo coalescence.[8] The effect of HPMC, β-lactoglobulin and soy protein as emulsifiers in the control of lipid digestion was studied by Bellesi, Martinez, Pizones Ruiz-Henestrosa & Pilosof.[9] Soy protein was found to be resistant to digestion as HPMC, which is a non-digestible emulsifier. Likewise, the physico-chemical composition of fat and the size and distribution of fat globules noticeably regulate lipase activity.[10–12]

Polysaccharides can act as emulsion stabilisers by increasing the viscosity or gel strength of the continuous phase, as well as by inducing the flocculation of emulsion droplets through bridging or depletion mechanisms, depending on the adsorbing properties of the polysaccharides.[13] The role of emulsion stabilizers is gaining increasing importance in the area of lipid digestion control. It has been shown that the presence of certain hydrocolloids potentially influences lipid digestion control.[11,14,15] It has even been demonstrated that emulsions which remain stable in the stomach and/or have a

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Relationship between cellulose chemical substitution, structure and fat digestion in o/w emulsions

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ABSTRACT

The effect of cellulose ether chemical substitution on the reduction of fat digestion in an o/w emulsion was investigated. Emulsions containing 47% sunflower oil and water were prepared with two types of hydroxypropyl methylcellulose (HPMC) and two types of methyl cellulose (MC), with different hydroxypropyl and methoxyl content. The changes in the emulsion structure were evaluated after mouth, stomach and small intestine *in vitro* digestion by Confocal laser microscopy and by small amplitude oscillatory shear (viscoelastic properties). The total amount of fat present in the supernatant after digesta centrifugation, serving as an indicator of fat bioaccessibility, and the free fatty acids, serving as an indicator of fat digestion, were determined at the end of the digestion. A relationship was found between cellulose ether chemical substitution, initial structure, structural changes during digestion, fat bioaccessibility and fat digestion. All the cellulose ether emulsions showed a lower level of fat digestion in comparison with a whey protein emulsion, the cellulose ether containing the highest amount of methoxyl being the most effective. The rise in the methoxyl content increases the emulsion viscoelastic properties before and after digestion and reduces fat bioaccessibility and the generation of free fatty acids. The decrease in the fat digestibility of the cellulose ether emulsions was mainly associated with a physical effect, which limits the emulsification of appropriate fats by bile salts, and the subsequent lipase digestion effect.

1. Introduction

Fat is an essential ingredient in the human diet; however, fat overconsumption is directly associated with overweight and obesity, which causes illnesses such as insulin resistance, dyslipidemia, pulmonary dysfunction, hypertension diabetes, among others. In addition, obese people may be subjected to unfair treatment in terms of employment opportunities, health-care facilities and educational positions and they may also be stigmatized by the media (Madadlou, Rakhsi, & Abbaspourrad, 2016).

A recent strategy for the purposes of controlling excessive fat intake is to decrease fat bioaccessibility through the employment of specifically designed emulsions.

The structure and stability of the emulsions play an important role in the digestion and absorption of the lipids (Golding & Wooster, 2010; McClements, Decker, & Park, 2009; McClements, Decker, Park, & Weiss, 2009). The initial properties of the oil-in-water emulsions affect both the rate and degree of lipid digestion (Armand et al., 1999, 1992; Mun, Decker, & McClements, 2007; Mun, Decker, Park, Weiss, & McClements, 2006). The flocculation and coalescence stability of the emulsions is heavily dependent on the nature of the emulsifiers. The use of emulsifiers with a great surfactant capacity can impede other substances with superficial properties that are present in the gastrointestinal tract (bile, lipase) from adhering to the interface of the fat droplets; therefore, the right modification to the oil-water interface can be used to inhibit lipid digestion. In addition, both the size and distribution of the fat globules affect the lipase activity (Mun et al., 2007). The rheological properties of the continuous phase are also of great importance. For example, fat globules which are small in size but covered with tightly bonded surfactants in a viscous medium are hydrolysed by the lipase more slowly than the bigger ones covered with surfactants that are more loosely bonded to the globule's surface in a less viscous medium (McClements & Decker, 2009).

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Effect of xanthan gum on palm oil *in vitro* digestion. Application in starch-based filling creams

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ABSTRACT

The purpose of this study was to investigate the influence of xanthan gum (XG) on the structural changes of palm oil during *in vitro* digestion and to evaluate the application of the XG-palm fat system in filling creams. The textural, rheological and microstructural properties were studied in three systems: a XG-palm fat system, a filling cream with the XG-palm fat system, and a control filling cream with palm oil but without xanthan gum. Free fatty acids were measured at the end of the *in vitro* digestion as indicators of fat digestion and related with the structural changes.

The incorporation of palm oil inside the xanthan gum matrix significantly reduces fat digestion. In comparison to the control cream, the microstructure reveals the presence of undigested fat when XG is present at the end of intestine *in vitro* incubation, while the presence of fat was significantly reduced in the control cream. After stomach incubation, an increase in the extrusion force and in the elastic and viscous modulus was observed in the xanthan gum systems, this was despite the greater water dilution, which was associated with an increase in fat globule size. In the control cream, a decrease in viscoelasticity is observed after stomach incubation.

The results of the present research may have applications in the design of low-fat food and in applications where stomach content structuring is desired.

1. Introduction

In the last decades consumption of high lipid content food stuff has increased in developed countries. This has led directly to a greater prevalence of both obesity and also several afflictions, such as diabetes or cardiovascular illnesses and certain types of cancer (Bray, Paeratakul, & Popkin, 2004; Chajès et al., 2008; Mozaffarian, Aro, & Willett, 2009). The challenge faced nowadays is that of producing nutritional, healthy foodstuffs without neglecting the organoleptic properties, for the purposes of obtaining a high level of consumer acceptance. The reduction of the lipid content poses many problems, as lipids are responsible for several technological and sensorial characteristics of the foodstuffs (Drewnowski, 1992).

Solid fat, rich in saturated fatty acids, cannot be directly replaced by liquid fat without it producing a negative effect on the quality of the final product due to perceptible changes in color, flavor, texture and stability, among other things. This is why there is still a great number of products made using high quantities of saturated and even *trans* fats on the market. Palm oil or fat is one of the most widely used in the Food Industry because of its physicochemical properties. The fact that it is made up of saturated and unsaturated fatty acids (nearly 50/50) makes it ideal for the elaboration of shortenings, ice creams, food for frying, among many others (Mba, Dumont, & Ngadi, 2015). Despite the fact that it contains beneficial compounds, such as vitamin E, carotenoids and sterols (Edem, 2002; Mba et al., 2015), numerous studies link it with several cardiovascular illnesses due to its high content in saturated fatty acids (Fattore & Fanelli, 2013; Mancini et al., 2015; Sun et al., 2015). However, studies such as that by de Lucci et al., refute this hypothesis and conclude that palm oil produces effects similar to those generated by olive oil on human plasma lipids, due to its oleic acid content. (Lucci et al., 2016).

Several fat substitutes have been developed for the purposes of reducing fat consumption or improving the lipid profile, with physical, chemical and sensorial properties similar to those of fats, but little or no calorie content (ADA, 2005). The fat substitutes are classified according to whether they are based on carbohydrates,

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Functionality of low digestibility emulsions in cocoa creams. Structural changes during *in vitro* digestion and sensory perception

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ABSTRACT

The objective of this work was to evaluate the application of low digestibility oil/water emulsions as fat source in a cocoa cream. Emulsions were composed by water, sunflower oil and cellulose ethers or xanthan gum. Back extrusion assays were measured before and after *in vitro* digestion and free fatty acids release were measured to evaluate the fat digestibility. Finally consumer acceptability was carried out to determine the degree of liking of each system. The results revealed that all the emulsions confer a suitable consistency to the creams and the structure provided by the hydrocolloids was resistant to digestion, reducing the fat digestibility. However, after gastric digestion only cream with xanthan gum showed a significant increase in consistency what it could be related with an increase in satiety. Regarding the sensory characteristics, the cream elaborated with xanthan gum was rated close to the control cream that received the highest scores.

1. Introduction

In the last decades, the increasing of a large number of diseases directly linked to fast food has driven the industry to focus on the design and formulation of food products with reduced fat and/or calories content. The contribution of fat in the flavour, texture, appearance and mouthfeel of foodstuffs has been confirmed by several studies (Drewnowski, 1992, 1987; Sandrou & Arvanitoyannis, 2000). Hence, removing or reducing fat adversely affects some of the characteristics reducing the quality of the final product. The main challenge is the manufacture of products with high lipid content, such as pastry and confectionery products. This type of foodstuffs contains a high percentage of saturated fats and/or *trans* fats, which gives them unique textural properties. Lipid content in cocoa creams can be more than 60% and provide a significant effect in organoleptic and physicochemical properties. In order to reduce calories content, fat could be reduced/replaced by a system that can replicate the texture, flavour and palatability of the full-fat counterpart. There are several systems that can be used as fat replacers, including protein-based fat mimetics, carbohydrate-based fat mimetics and fat based replacers (Lucca & Tepper, 1994; Sandrou & Arvanitoyannis, 2000). Most of the low-fat products reformulated in recent years contain carbohydrate-based fat mimetics such as inulin (Tárrega & Costell, 2006; Krystyjan, Gumul, Ziobro, & Sikora, 2015), starch (Laguna, Varela, Salvador, Sanz, & Fiszman, 2012), cellulose (Nsor-Atindana et al., 2017) and gums (Ranalli, Andrés, & Califano, 2017; Rather, Masoodi, Akhter, Rather, Amin, 2017) that are widely used as thickeners, stabilizers and emulsifiers to compensate the loss of desirable textural attributes when fat is reduced or removed (Mudgil & Barak, 2013).

The incorporation of a polysaccharide (e.g cellulose ether) in the continuous phase of oil/water emulsion allows using vegetable oil in reformulated products. The semi-solid consistency was suitable for mimicking the textural and rheological properties of fat, conferring good sensory acceptability (Martínez-Cervera, Salvador, & Sanz, 2015; Tarancón, Fiszman, Salvador, & Tárrega, 2013). An important attribute of emulsion-based food products is the behaviour within the mouth after ingestion that will determine the perceived mouthfeel (McClements, 2015). People like the taste of fat-containing foods. More viscous stimuli are generally perceived as rich in fat content. So, this feeling can be created through the use of stabilizers or thickeners that enhance the perceived creaminess of the reformulated product (Drewnowski, 1990). Nevertheless, depending on fat content and the type of thickener used, the aroma release and taste perceived may change (Wendin et al., 1997). Some studies showed that the use of thickeners results in an increase in texture and a decrease in aroma release and taste, but depending on the type and concentration of hydrocolloid (Arancibia, Castro, Jublot, Costell, & Bayarri, 2015; Hollowood, Linforth, and Taylor, 2002). Moreover, some studies have

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