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Estructuración de aceites mediante el uso de hidrocoloides para sustituir grasas plásticas en los alimentos

TESIS DOCTORAL

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HACEN CONSTAR QUE:

El trabajo de investigación "Estructuración de aceites mediante el uso de hidrocoloides para sustituir grasas plásticas en los alimentos", que presenta D. Santiago Martín Bascuas Véntola por la Universitat Politècnica de València, y que ha sido realizado bajo nuestra dirección en el Grupo de Microestructura y Química de Alimentos de la Universitat Politècnica de València, reúne las condiciones para optar al grado de Doctor.

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SE LES AMA

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Resumen

La presente Tesis doctoral plantea distintas estrategias para el diseño y desarrollo de oleogeles estables, con un perfil lipídico de alta calidad nutricional y la posterior incorporación de los oleogeles en la formulación de distintos alimentos como cremas untables de chocolate y panes dulces. Se pretende diseñar alimentos mediante el reemplazo de grasas sólidas, ricas en ácidos grasos saturados y *trans*, por aceites vegetales estructurados (oleogeles), que, por un lado, mantengan las propiedades texturales y organolépticas, y, por otro lado, presenten un perfil lipídico mejorado.

En una primera etapa, se desarrollaron oleogeles elaborados con 1% de hidroxipropilmetilcelulosa y 0,6% de goma xantana mediante el método *emulsion-template approach* empleando dos condiciones de secado diferentes: secado convencional en estufa a 80 °C durante 10 h 30 min y secado a vacío a 60 °C durante 24 h. Los aceites estructurados fueron aceite de oliva, lino, girasol y girasol alto oleico. La caracterización de la microestructura de los oleogeles se llevó a cabo mediante técnicas de criomicroscopía electrónica de barrido y microscopía óptica. La microestructura permitió apreciar oleogeles bien estructurados por una red polimérica formada por los hidrocoloides, cuando se utilizaron los aceites de oliva, girasol y girasol alto oleico. Los estudios sobre la estabilidad física y las propiedades reológicas corroboraron la formación de oleogeles de alta estabilidad física, a lo largo de 35 días de almacenamiento, y con un comportamiento de gel sólido. Se observó que tanto el grado de insaturación del aceite como las condiciones de secado afectaron a la estabilidad física y química del oleogel. De esta manera, se obtuvieron oleogeles poco estructurados y no homogéneos

al utilizar aceite con un alto grado de insaturación, como el aceite de lino, por secado convencional, mientras que no fue factible desarrollar oleogeles de lino con secado a vacío. Además, el secado en estufa convencional a 80 °C durante 10 h 30 min generó oleogeles de girasol y de girasol alto oleico con mayor estabilidad estructural y física que el secado a 60 °C durante 24 h. Los oleogeles de oliva y de girasol alto oleico producidos por secado convencional y los oleogeles de oliva y de girasol producidos por secado a vacío presentaron valores de estabilidad oxidativa primaria y secundaria dentro de los límites de aceptabilidad establecidos por el Codex Alimentarius, siendo candidatos idóneos para su incorporación en alimentos.

En una segunda etapa, se quiso conocer cómo influye la presencia de los oleogeles, cuando se emplean como sustitutos de grasas plásticas, en las propiedades de los alimentos. Para ello, se desarrollaron cremas de cacao untables y panes dulces. En las cremas se estudió la microestructura, textura, reología y atributos sensoriales. La reformulación de las cremas con un reemplazo total (100%) y parcial (50%) de grasa de coco por oleogeles de oliva o girasol obtenidos por secado a vacío permitió mantener sus propiedades estructurales. Concretamente, la sustitución parcial de grasa de coco por oleogel de girasol permitió obtener cremas con atributos sensoriales como "apariencia cremosa", "textura cremosa" y "sabor a cacao", por lo que podría ser una alternativa viable para reformular cremas untables con un perfil nutricional más saludable. Para evaluar la funcionalidad de los oleogeles como sustitutos de margarina en una matriz alimentaria, los oleogeles de oliva o girasol alto oleico obtenidos por

secado convencional y previamente caracterizados, se emplearon como ingredientes en la formulación de panes dulces elaborados al horno o al vapor. Se estudiaron las propiedades estructurales (apariencia del pan, textura y volumen específico) y la digestibilidad lipídica *in vitro* del producto final. La reformulación con oleogeles permitió obtener panes con unas características estructurales y apariencia similar a la de los panes elaborados con margarina. La realización de una prueba triangular mostró diferencias en algunos atributos, como la apariencia de la miga y el sabor, mientras que no se observaron diferencias para el atributo textura entre los panes elaborados con oleogeles y con margarina. No se encontraron diferencias en la cantidad de ácidos grasos liberados tras la digestión *in vitro* entre los panes dulces elaborados con oleogeles y con margarina. Sin embargo, la velocidad inicial de la digestibilidad lipídica fue diferente dependiendo del tipo de procesado, horneado o vaporización, empleado en la elaboración de los panes. El desarrollo de oleogeles a base de hidrocoloides además de ofrecer a la industria alimentaria una alternativa como sustituto de grasas plásticas, podría investigarse como estrategia para modular la digestión de lípidos y brindar beneficios para la salud.

Todas las estrategias abordadas en el transcurso de esta Tesis permitieron comprender y ahondar en los conocimientos que conducen a cómo reformular un alimento para mejorar su perfil lipídico sin comprometer sus características sensoriales y fisicoquímicas.

Resum

La present Tesi doctoral planteja diferents estratègies per al disseny i desenvolupament d'oleogels estables, amb un perfil lipídic d'alta qualitat nutricional, i la posterior incorporació dels oleogels en diferents aliments com cremes untables de xocolate i pans dolços. Es pretén dissenyar aliments mitjançant el reemplaçament de greixos sòlids, rics en àcids grassos saturats i *trans*, per olis vegetals estructurats (oleogels), que d'una banda, mantinguen les propietats texturals i organolèptiques, i d'altra banda, presenten un perfil lipídic millorat.

En una primera etapa, es van desenvolupar oleogels elaborats amb un 1% de hidroxipropilmetilcel·lulosa (HPMC "K4M"; 4000 cP) i un 0,6% de goma xantana (GX) mitjançant el mètode *emulsion-template approach* emprant dues condicions d'assecat diferents: assecat convencional en estufa a 80 °C durant 10 h 30 min i assecat a buit a 60 °C durant 24 h. Els olis estructurats van ser d'oliva, de lli, de gira-sol i de gira-sol alt oleic. La caracterització de la microestructura dels oleogels es va dur a terme mitjançant tècniques de criomicroscopía electrònica d'escombratge i microscòpia òptica. La microestructura va permetre apreciar oleogels ben estructurats per una xarxa polimèrica formada pels hidrocol·loides, quan es van utilitzar els olis d'oliva, gira-sol i gira-sol alt oleic. Els estudis sobre l'estabilitat física i les propietats reològiques van corroborar la formació d'oleogels d'alta estabilitat física, al llarg de 35 dies d'emmagatzematge, i amb un comportament de gel sòlid. Es va observar que tant el grau d'insaturació de l'oli com les condicions d'assecat van afectar l'estabilitat física i química del oleogel. D'aquesta manera, es van obtindre oleogels poc estructurats i no homogenis en utilitzar oli amb un alt grau d'insaturació, com l'oli de lli, per assecat

convencional, mentre que no va ser factible desenvolupar oleogels de lli amb assecat a buit. A més, l'assecat en estufa convencional a 80 °C durant 10 h 30 min va generar oleogels de gira-sol i de gira-sol alt oleic amb major estabilitat estructural i física que l'assecat a 60 °C durant 24 h. Els oleogels d'oliva i gira-sol alt oleic, produïts per assecat convencional i els oleogels d'oliva i gira-sol produïts per assecat a buit van presentar valors d'estabilitat oxidativa primària i secundària dins dels límits d'acceptabilitat establits pel Codex Alimentarius, sent candidats idonis per a la seua incorporació en aliments.

En una segona etapa, es va voler conéixer com es comporten els oleogels quan s'emprenen com a substituts de greixos plàstics en la formulació d'aliments. Per a això, es van desenvolupar cremes de cacau untables i pans dolços. En aquest treball, es va estudiar la microestructura, textura, reologia i atributs sensorials de les cremes untables. La reformulació de cremes de cacau amb un reemplaçament total (100%) i parcial (50%) de greix de coco per oleogels d'oliva o gira-sol obtinguts per assecat a buit, va permetre mantindre les propietats estructurals de les cremes untables. Concretament, la substitució parcial de greix de coco per oleogel de gira-sol va ser descrita amb atributs sensorials com a "aparença cremosa", "textura cremosa" i "sabor de cacau", la qual cosa podria ser una alternativa viable per a reformular cremes per a untar amb un perfil nutricional més saludable. Per a avaluar la funcionalitat dels oleogels com a substituts de margarina en una matriu alimentària, els oleogels d'oliva o gira-sol alt oleic obtinguts per assecat convencional i prèviament caracteritzats, es van emprar com a ingredients en la formulació de pans dolços cuinats al forn o al vapor. Es van estudiar

les propietats estructurals (aparença del pa, textura i volum específic), i la digestibilitat lipídica *in vitro* del producte final. La reformulació amb oleogels va permetre obtindre pans amb unes característiques estructurals i aparença similar a la dels pans elaborats amb margarina. La realització d'una prova triangular va mostrar diferències en alguns atributs, com l'aparença de la molla i el sabor, mentre que no es van observar diferències per a l'atribut textura, entre els pans elaborats amb oleogels i amb margarina. No es van trobar diferències en la quantitat d'àcids grassos alliberats després de la digestió *in vitro* entre els pans dolços elaborats amb oleogels i amb margarina. No obstant això, la velocitat inicial de la digestibilitat lipídica va ser diferent depenent de la mena de processament, enfornat o vaporatge, emprat en l'elaboració dels pans. El desenvolupament d'oleogels a base de hidrocol·loides, a més d´oferir a la indústria alimentària una alternativa com a substitut de greixos plàstics, podria investigar-se com a estratègia per modular la digestió de lípids i brindar beneficis per la salut.

Totes les estratègies abordades en el transcurs d'aquesta Tesi van permetre comprendre i aprofundir en els coneixements que condueixen a com reformular un aliment per a millorar el seu perfil lipídic sense comprometre les seues característiques sensorials i fisicoquímiques.

Abstract

The research of this doctoral thesis proposes different strategies for the design and development of stable oleogels, with high nutritional lipid profile and the subsequent incorporation of the oleogels in the formulation of different foods such as chocolate spreads and sweet breads. It aims to design foods by replacing solid fats, rich in saturated and *trans* fatty acids, with structured vegetable oils (oleogels), which, on the one hand, maintain the textural and organoleptic properties, and on the other hand, present an improved lipid profile.

In a first stage, oleogels made with 1% hydroxypropylmethylcellulose and 0.6% xanthan gum were developed by the emulsion-template approach using two different drying conditions: conventional drying in an oven at 80 ° C for 10 h 30 min and vacuum drying at 60 ° C for 24 h. The structured oils were olive, flaxseed, sunflower and high oleic sunflower oil. The characterization of the microstructure of the oleogels was carried out using Scanning Electron Cryomicroscopy and Light Microscopy techniques. The microstructure showed the oleogels structured by a polymeric network formed by hydrocolloids, when olive, sunflower or high oleic sunflower oils were used. Studies on physical stability and rheological properties corroborated the formation of oleogels with high physical stability, over 35 days of storage, and with a solid gel behaviour. Both the degree of unsaturation of the oil and the drying conditions affected the physical and chemical stability of the oleogel. In this way, unstructured non-homogeneous oleogels were obtained by using oil with a high degree of unsaturation, such as flaxseed oil, by conventional drying, while it was not feasible to develop flaxseed oleogels with vacuum

drying. Furthermore, drying in a conventional oven at 80 ° C for 10 h 30 min generated sunflower and high oleic sunflower oleogels with more structural and physical stability than drying at 60 ° C for 24 h. Olive and high oleic sunflower oleogels, produced by conventional drying, and olive and sunflower oleogels produced by vacuum drying, presented primary and secondary oxidative values within the acceptability limits established by the Codex Alimentarius, being these oleogels suitable candidates for their incorporation in food.

In a second stage, the behaviour of oleogels used as substitutes for plastic fats in food formulation was investigated. For this, spreadable cocoa creams and sweet breads were developed. The microstructure, texture, rheology and sensory attributes of spreadable creams were studied. The reformulation of cocoa creams with a total (100%) and partial (50%) replacement of coconut fat by olive or sunflower oleogels obtained by vacuum drying allowed to maintain the structural properties of spreadable creams. Specifically, the partial substitution of coconut fat for sunflower oleogel gave place to creams described with sensory attributes such as "creamy appearance", "creamy texture" and "cocoa flavor"; therefore, it could be a viable alternative to reformulate spreads with a healthier nutritional profile. To evaluate the functionality of oleogels as substitutes for margarine in a food matrix, the olive or sunflower oleogels obtained by conventional drying and previously characterized, were used as ingredients in the formulation of sweet breads made in the oven or steamed. The structural properties (appearance of bread, texture and specific volume), and the *in vitro* lipid digestibility of the final product

were studied. Replacement of margarine for oleogels produced breads with similar structural characteristics and appearance that those made with margarine. A triangular discriminatory test showed differences in some attributes, such as the appearance of the crumb and the flavor, while no differences were observed for the texture attribute, between the breads made with oleogels or with margarine. No differences were found in the amount of fatty acids released after *in vitro* digestion between sweet breads made with oleogels and margarine. However, the initial rate of lipid digestibility was different depending on the type of processing, baking or steaming, used in the preparation of the breads. In addition to offering the food industry an alternative as a substitute for plastic fats, the development of hydrocolloid-based oleogels could be investigated as a strategy to modulate lipid digestion and provide health benefits.

All the strategies addressed in this Thesis allowed to understand and deepen into the knowledge to reformulate foods with increased lipid profile without compromising their sensory and physicochemical characteristics.

Introducción

1. Nutrición y salud

El sobrepeso y la obesidad representan un problema de salud pública de proporciones epidémicas a nivel mundial. Según la Organización Mundial de la Salud (OMS) en 2016, el 13% de los adultos mayores de 18 años eran obesos y el 39% sufría sobrepeso (OMS, 2020). En cuanto a edades más tempranas, 38 millones de niños en el mundo, menores de 5 años sufrían sobrepeso o eran obesos en el año 2019 (OMS, 2020). En España, en el año 2019, el 40,6% de los niños de entre 6-9 años sufrían exceso de peso, según datos de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN), que aparecen en el estudio Alimentación, Actividad física, Desarrollo Infantil y Obesidad en España (ALADINO), sobre prevalencia de sobrepeso y obesidad infantil (AESAN, 2019).

Entre los principales factores que contribuyen a una mayor prevalencia de la obesidad se encuentran, los escasos niveles de actividad física y la ingesta de alimentos de alta densidad energética, ricos en grasas saturadas y azúcares añadidos (AESAN, 2019). En este contexto y para evitar el asentamiento de estos factores en las tendencias actuales de vida, se realizan numerosos esfuerzos a través del desarrollo de políticas en materia de salud pública. En 2004, la OMS en coordinación con los Estados Miembros aprobaron en la 57ª Asamblea Mundial de la Salud la estrategia mundial sobre régimen alimentario, actividad física y salud (WHO, 2004). En el año 2007, la Comisión Europea creó el *High Level Group on Nutrition and Physical Activity* con los Estados Miembros para facilitar una acción coordinada en materia de alimentación saludable (UE, 2007). España, alineada con las políticas marcadas por los organismos sanitarios

internacionales, impulsó la Estrategia NAOS (Nutrición, Actividad Física y Prevención de la Obesidad), que en 2011 fue consolidada a través de la Ley 17/2011, de 5 de julio, de seguridad alimentaria y nutrición (AESAN, 2005; Ley 17/2011, 2011). Entre las líneas estratégicas de actuación adoptadas en coordinación con las industrias alimentarias, se encuentra la reformulación y mejora de alimentos y bebidas, basada en la reducción del contenido de sal, grasas saturadas y/o azúcares añadidos (AESAN, 2020).

Las grasas y los aceites son parte indispensable de nuestra dieta; son fuente de energía, algunas son vitaminas o precursores de vitaminas, y actúan como disolvente de nutrientes tales como vitaminas, compuestos biológicamente activos y aromatizantes. Las grasas y los aceites son predominantemente mezclas de moléculas de triglicéridos. El triglicérido está constituido por tres ácidos grasos esterificados con una molécula de glicerol. Existen tres tipos de ácidos grasos; saturados (AGS), monoinsaturados (AGM) y poliinsaturados (AGP); los AGS tienen mayor punto de fusión que los insaturados. El contendido de ácidos grasos que conforman cada molécula de triglicérido determina la naturaleza del aceite o la grasa. En general, cuanto mayor es el contenido en ácidos grasos saturados, más sólida es la grasa (Davidovich-Pinhas et al., 2016).

La industria alimentaria tiende a utilizar diferentes fuentes de grasas sólidas, ya que juegan un importante papel al proporcionar textura, estabilidad, aroma y sabor a los alimentos. Alimentos como margarinas, cremas, chocolates, productos de panadería o productos cárnicos, están elaborados con grasas saturadas de origen animal

(mantequilla, grasa de sebo o manteca) u origen vegetal (aceite de palma o aceite de coco). Dada la importancia de las grasas con alto porcentaje de ácidos grasos saturados en la fabricación industrial, a lo largo de la historia, se han desarrollado diversos métodos para obtener grasas sólidas a partir de aceites líquidos. La hidrogenación ha sido una de las tecnologías más extendidas para convertir aceites líquidos en grasas sólidas, con la finalidad de minimizar costos, alcanzar las características deseadas (textura, apariencia y estabilidad) y la plasticidad requerida. En el caso de las grasas obtenidas por hidrogenación parcial, a la problemática de los ácidos grasos saturados se le suma la generación de ácidos grasos *trans* en importantes cantidades. En los últimos años se ha demostrado que los ácidos grasos *trans* provocan la reducción del colesterol transportado por lipoproteínas de alta densidad (HDL) y el incremento del colesterol transportado por lipoproteínas de baja densidad (LDL) (Fernández et al., 2011). Así mismo, también se ha demostrado que producen dislipidemia, arteriosclerosis acelerada, disfunción endotelial, aumento de la actividad inflamatoria y oxidativa (Martínez y Velasco, 2007). Por otra parte, los AGS, incrementan el colesterol transportado en las LDL y en las HDL, los AGP reducen ambas fracciones y los AGM disminuyen la fracción transportada en las LDL, sin modificar o incrementando la contenida en las HDL (Fernández et al., 2011).

Así pues, el consumo excesivo de algunos tipos de grasas saturadas y *trans* se ha asociado con un mayor riesgo de padecer enfermedades cardiovasculares, tales como obesidad, diabetes tipo 2 o resistencia a la insulina (EFSA, 2018; Estadella et al., 2013; Morenga

and Montez, 2017). A medida que han ido aumentando las evidencias científicas sobre los efectos perjudiciales de un consumo excesivo de grasas saturadas, distintas organizaciones internacionales han emitido recomendaciones encaminadas a limitar el consumo de este tipo de grasas. En 2003, la OMS en coordinación con la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) sugirió limitar el consumo de grasa total, grasas saturadas y grasas *trans* a un máximo del 30%, 10% y 1% de la ingesta calórica total, respectivamente (OMS/FAO, 2003). En 2015, la Administración de Medicamentos y Alimentos de los Estados Unidos de América (FDA) declaró que los aceites parcialmente hidrogenados empleados comúnmente en alimentos ultraprocesados, en cuyo proceso de producción se generan de manera artificial ácidos grasos *trans*, ya no eran generalmente considerados como seguros (GRAS) (FDA, 2015). En Europa, la Comisión Europea (UE) incluyó en el Reglamento nº 649 de 24 de abril de 2019 la limitación a un máximo de 2 g de grasa *trans* de origen no natural por cada 100 g de grasa en aquellos alimentos destinados al consumidor final (Reglamento 2019/649, 2019). En el año 2018, la OMS inició una campaña denominada "REPLACE" con el objetivo de hacer una extensa revisión de las fuentes dietéticas de grasas *trans* de producción industrial, promoviendo su remplazo por aceites más saludables ricos en ácidos grasos monoinsaturados y/o poliinsaturados, sensibilizar sobre los efectos de las grasas *trans* en la salud e implementar políticas y regulaciones (WHO, 2019).

Ante esta evidencia, está justificada la búsqueda de nuevas estrategias para reducir o eliminar el consumo de grasas saturadas y *trans*. La investigación en alimentación sigue realizando numerosos esfuerzos encaminados a diseñar alternativas más saludables que satisfagan los requisitos, cada vez más exigentes del consumidor, así como a dar respuesta a la demanda industrial. Diferentes fuentes de proteína, tales como el aislado de proteína de soja o la proteína de suero de leche (Paglarini et al., 2019; Yilsay, 2006), hidratos de carbono, tales como fibra de salvado de arroz, beta-glucanos, goma guar, inulina o polidextrosas (Brennan and Tudorica, 2008; Choi et al., 2008; Onacik-Gur et al., 2016) y sustitutos a base de grasa, tales como ésteres de ácidos grasos de sorbitano o a base de poliésteres de sacarosa (O'Sulliven, 2016), se han empleado para mimetizar la estructura y las propiedades sensoriales de la grasa saturada. Sin embargo, el reemplazo total o parcial de grasa saturada y/o grasa *trans* afecta negativamente a las propiedades sensoriales y mecánicas de los alimentos (Biguzzi et al., 2014). Conseguir reemplazar de forma adecuada la grasa sólida en la formulación de los alimentos es el objetivo prioritario de muchas investigaciones. Por todo ello, es necesario seguir explorando nuevas estrategias de reducción de los niveles de grasas sólidas convencionales empleados en la formulación de alimentos. En los últimos años, la estructuración de aceites vegetales ricos en ácidos grasos monoinsaturados y/o polinsaturados, sin modificar su composición química, ha ganado una importante popularidad como un novedoso enfoque para obtener grasas de propiedades similares a las grasas sólidas empleadas de forma tradicional en la industria.

2. Estructuración de aceites: Oleogeles

Los oleogeles son sistemas coloidales definidos como estructuras sólidas o semisólidas tipo gel, en las que el aceite está inmovilizado en una red tridimensional constituida por un agente o combinación de agentes estructurantes. El proceso de elaboración de oleogeles se conoce como oleogelificación. En la última década, este proceso, ha despertado un relativo interés científico e industrial al permitir obtener grasas de consistencia sólida con propiedades de textura, estabilidad física y química similares a las de las grasas sólidas saturadas convencionales, pero sin conferir los efectos negativos en la salud, consecuencia de la elevada cantidad de ácidos grasos saturados y/o *trans* presentes en su formulación (Stortz et al., 2012). Los oleogeles han ganado popularidad en la industria de cosméticos porque mejoran la estabilidad y liberación de compuestos bioactivos (Martinez et al., 2019), y en la industria farmacéutica por su uso en la encapsulación y/o liberación de compuestos farmacológicos (Bastiat and Leroux, 2009). En el área de la alimentación, el uso de oleogeles, se encuentra todavía en fases iniciales, pero ha despertado un significativo interés por su gran potencial (Puscas et al., 2020) como sustitutos de grasas plásticas industriales, al aportar una consistencia y firmeza específica y mantener el carácter insaturado de su composición (Pehlivanoğlu et al., 2018). Considerando todo ello, la aplicación de oleogeles ha atraído la atención de la comunidad científica. Se ha investigado la aplicación de oleogeles en diversos productos alimenticios, como sustitutos de grasas sólidas y para reducir la cantidad de grasas saturadas en helados, salchichas cocidas, galletas, pasteles, así como para la producción

de grasas de relleno de confitería comercial (Figura 1). Las distintas investigaciones han presentado avances prometedores en el desarrollo de nuevos ingredientes y de métodos innovadores de procesamiento, necesarios para la obtención de oleogeles.

En cuanto a los agentes gelificantes o estructurantes, estos deben preservar o mejorar las características estructurales y sensoriales de los aceites. Atendiendo al peso molecular, los agentes estructurantes se clasifican en bajo- y alto-peso molecular (LMOG y HMOG en inglés, respectivamente) (Hwang, 2020). Los LMOG son un grupo de moléculas anfifílicas de bajo peso molecular con capacidad de autoensamblarse o cristalizar formando una red tridimensional que estructura el aceite (Sagiri et al., 2014). En este grupo se distinguen entre otros, ceras (Botega et al., 2013), fitoesteroles (Bot and Agterof, 2006), o ácidos grasos (Rogers et al., 2008). Los HMOG son polímeros de alto peso molecular, como proteínas y polisacáridos, capaces de formar una red tridimensional a través de interacciones físicas como los enlaces de hidrógeno. Debido a la naturaleza polimérica, las propiedades estructurantes de estos polímeros dependerán en gran medida del peso molecular, la conformación y la concentración del polímero (Davidovich-Pinhas, 2019).

El concepto de usar un polímero como estructurante para gelificar aceites vegetales fue introducido por primera vez en la década de los noventa (Patel and Dewettinck, 2016). El primer polímero utilizado como agente gelificante fue la etilcelulosa, un derivado de celulosa, con la capacidad de inmovilizar el aceite de manera directa al tener naturaleza hidrofóbica (Laredo et al., 2011). Sin embargo, los oleogeles estructurados con

etilcelulosa presentan una baja estabilidad oxidativa debido a las altas temperaturas empleadas para inducir la gelificación de la etilcelulosa (> 135-140 °C) (Gravelle et al., 2012). La creación de nuevas estructuras alimentarias debido a las interacciones supramoleculares entre distintas fuentes o combinaciones de diferentes tipos de hidrocoloides, como son las proteínas y los polisacáridos, parecen ser los candidatos más prometedores para la estructuración de aceites y su uso en alimentos (Patel et al., 2015; de Vries et al., 2017).

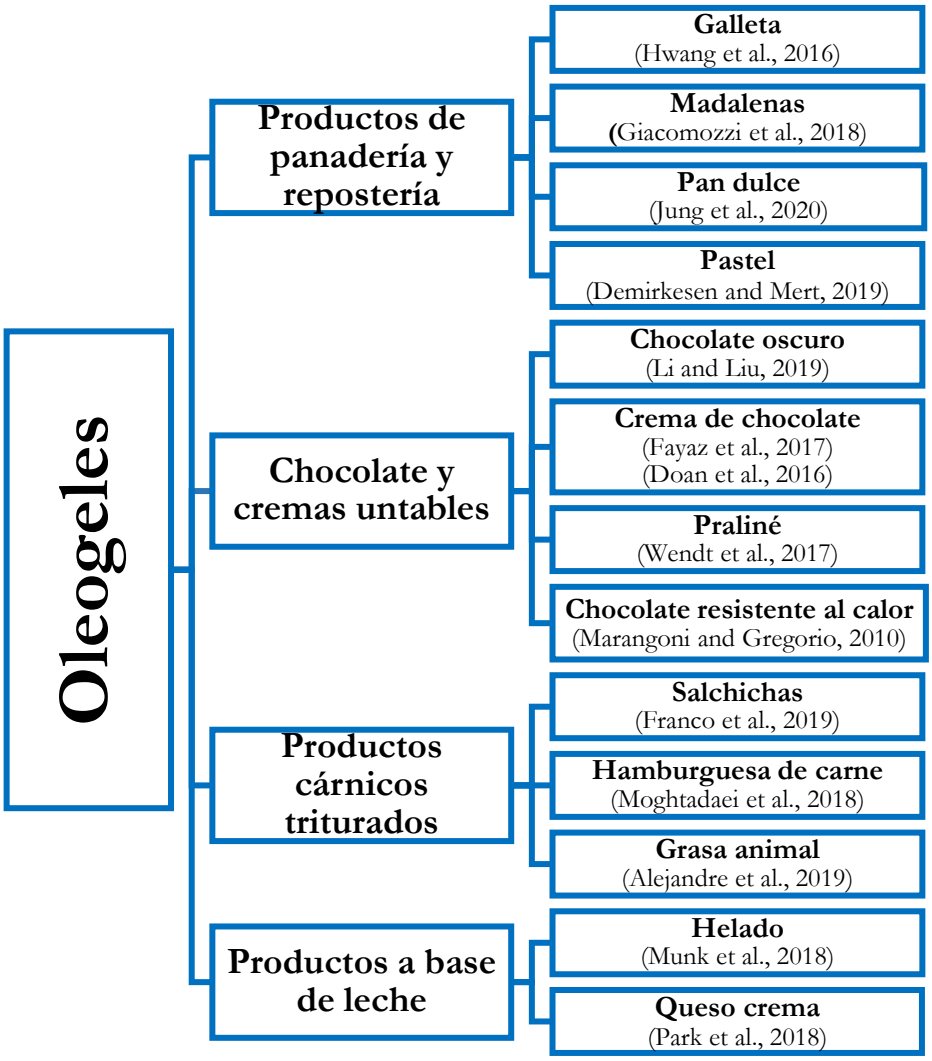

Figura 1. Aplicación de oleogeles en productos alimenticios.

Hidrocoloides como *agentes estructurantes*

Los hidrocoloides son macromoléculas de alto peso molecular ampliamente utilizados en la industria como aditivos alimentarios gracias a su baja toxicidad, alta biocompatibilidad, biodegradabilidad, y su estado generalmente reconocido como seguro (GRAS) (Abdolmaleki et al., 2019; Scholten, 2019). Comprenden fundamentalmente a polisacáridos y proteínas. Los polisacáridos se pueden extraer comúnmente de varias fuentes como algas (e.j. alginato y carragenato), animales (e.j. quitosano y condroitina), microorganismos (e.j. dextrano y goma xantana), y mayoritariamente plantas (e.j. celulosa y pectina) (Liu et al., 2008). Su funcionalidad viene determinada por su disposición molecular y por su composición química variable, lo que contribuye a una gran diversidad en estructura y propiedades físicas, biológicas y químicas. Debido al bajo coste de procesamiento, los polisacáridos vegetales se consideran una adecuada fuente natural para la estructuración de aceites de uso alimentario (Shao et al., 2020). La mayoría de los polisacáridos son hidrocoloides que no tienen tendencia a adsorberse en las interfases debido a su alto carácter hidrófilo; sin embargo, existen algunos polisacáridos, como los derivados de la celulosa, que tienen propiedades surfactantes. Los grupos hidroxilo (-OH) de la celulosa pueden reaccionar en presencia de reactivos dando lugar a derivados con propiedades útiles como los éteres de celulosa. La hidroxipropilmetilcelulosa (HPMC) es un biopolímero no iónico derivado de la celulosa, concretamente un éter de celulosa. Las propiedades surfactantes de la HPMC son dependientes del grado de sustitución de los grupos hidroxilos por los

grupos hidrofóbicos metoxilo (16,5-30%) y grupos hidrofílicos hidroxipropoxilo (4-32%) (Andueza et al., 2000). Sus propiedades anfifílicas permiten a la HPMC adsorberse en la interfaz de las gotas de grasa y actuar como emulsionante, convirtiendo a la HPMC en uno de los tensioactivos más empleados para la elaboración de oleogeles (Meng et al., 2018a; Oh et al., 2019; Patel et al., 2014a; Tanti et al., 2016a, 2016b).

Además, para la estructuración de aceites, la combinación de polisacáridos con propiedades surfactantes y polisacáridos no surfactantes permite mejorar la estabilidad de las estructuras oleosas. La adición de agentes espesantes como la goma xantana (GX), obtenida por la acción fermentativa de las bacterias *Xanthomonas campestris* y empleada en la industria alimentaria como agente espesante (E415), es favorable para la obtención de oleogeles estables, previniendo fenómenos de coalescencia de las gotas de aceite al aumentar la viscosidad de la fase acuosa y estabilizar la estructura tridimensional por enlaces puentes de hidrógeno intramolecular e intermolecular entre polisacáridos (Meng et al., 2018b). Patel et al. (2014a) estudiaron el papel de la GX en la elaboración de oleogeles, encontrando que la GX puede aumentar la viscosidad de la emulsión mejorando su estabilidad en combinación con metilcelulosa durante el proceso de secado. De hecho, observaron menor pérdida de aceite que en emulsiones estabilizadas usando solo derivados de celulosa.

Sin embargo, un inconveniente del uso de hidrocoloides para la elaboración de oleogeles es su naturaleza inherentemente hidrófila. Como consecuencia, no se pueden emplear de manera directa en la elaboración de oleogeles debido a su limitada dispersabilidad en

aceite. Para lograr la formación de la red polimérica requerida para la gelificación, es necesario primero prehidratar los hidrocoloides en la fase acuosa y los métodos utilizados se conocen con el nombre de métodos indirectos. Los métodos indirectos más utilizados incluyen, i) *foam-template approach*, que consiste en la liofilización de una espuma acuosa estabilizada por hidrocoloides, permitiendo la formación de una estructura porosa o criogel con la capacidad de absorber aceite, y ii) *emulsion-template approach*, donde la fase acuosa de una emulsión estabilizada por agentes estructurantes de tipo hidrocoloide es evaporada para obtener una estructura coloidal donde el aceite es atrapado físicamente dentro de la red tridimensional formada por los agentes estructurantes

El *foam-template approach* es un método que no emplea altas temperaturas, aditivos adicionales, productos químicos agresivos o agentes reticulantes. Fue introducido por primera vez por Patel et al. (2013). Sin embargo, la liofilización es una técnica costosa y requiere mucho tiempo para el secado de muestras (do Vale Morais et al., 2016).

El *emulsion-template approach* fue introducido por primera vez por Romoscanu y Mezzenga (2006), utilizando proteínas lácteas como agentes emulsionantes. El proceso se llevó a cabo mediante la reticulación, térmica o química con glutaraldehído, de las proteínas. Sin embargo, la complejidad del proceso y el uso de agentes químicos pueden limitar la aplicación específica para alimentos funcionales, farmacéuticos y formulaciones cosméticas (Chen and Yang, 2019). Posteriormente, este método fue abordado por Patel et al. (2014a) sin utilizar el procedimiento de reticulación y usando un método más

sencillo. En este método combinaron las propiedades surfactantes de la hidroxipropilmetilcelulosa o la metilcelulosa, con el poder espesante de la goma xantana, para elaborar emulsiones con un alto volumen de aceite de girasol (60%). Posteriormente, las emulsiones eran secadas para obtener oleogeles con un alto contenido en aceite (>97%).

3. Aplicación de oleogeles basados en hidrocoloides en la elaboración de alimentos

La estructuración de aceite líquido para formar un gel sólido u oleogel mediante el empleo de hidrocoloides, ha demostrado ser una tecnología prometedora para la obtención de sustitutos de grasas sólidas saturadas convencionales (Patel, 2018). Existen algunos trabajos acerca del uso de oleogeles a base de hidrocoloides en productos alimenticios. En este sentido, se ha estudiado el uso de oleogeles, elaborados con hidrocoloides, como alternativa a diferentes grasas sólidas convencionales en productos horneados, como pasteles (Luo et al., 2019; Mohanan et al., 2020; Patel et al., 2014b), galletas (Sholten and de Vries, 2018) y madalenas (Oh and Lee, 2017). Otros autores han evaluado la viabilidad de los oleogeles elaborados con hidrocoloides, como sustitutos de grasa animal en productos cárnicos, como empanadas de carne (Oh et al.,

2019) y salchichas (Sholten and de Vries, 2018), como agentes estabilizantes en productos cremosos, como cremas de cacahuete (Tanti et al., 2016a) y como sustitutos de grasa vegetal, en crema de helado (Tanti et al., 2016b). En general, estos estudios muestran que el reemplazo de grasas saturadas por oleogeles estrucurados con hidrocoloides confieren al producto final una textura y propiedades sensoriales comparables a las de los productos que contienen grasas sólidas saturadas. Sin embargo, los beneficios del uso de oleogeles se producen mayoritariamente cuando la sustitución es parcial. El reemplazo total de las grasas saturadas, todavía, es un desafío (Feichtinger and Sholten, 2020). Con todo ello, es interesante seguir estudiando la viabilidad de los hidrocoloides como agentes estructurantes de aceites vegetales comestibles y el desarrollo de oleogeles que permitan una sustitución total de las grasas plásticas en la formulación de los alimentos.

Las tendencias del consumidor se encaminan hacia la demanda de productos más saludables, naturales y de etiqueta limpia. La industria se encuentra con el desafío de rediseñar sus productos, ofreciendo alimentos más sostenibles, pero sin descuidar sus propiedades sensoriales. El desarrollo de oleogeles utilizando polisacáridos se beneficia de la probada capacidad de estructuración de los hidrocoloides y de su uso potencial en los productos alimenticios. La estructuración del aceite empleando hidrocoloides se presenta como una estrategia prometedora para la mejora del perfil lipídico de

alimentos, formulados de manera tradicional con grasas sólidas de alto contenido en ácidos grasos saturados y *trans*. Sin embargo, es necesario ampliar los conocimientos sobre la utilización de oleogeles a base de hidrocoloides en la formulación de productos alimenticios elucidando sus posibles aplicaciones alimentarias. Las cremas a base de cacao son uno de los alimentos básicos empleados en pastelería y confitería para la elaboración de diferentes productos. Forman parte del día a día de los consumidores como delicioso producto de confitería ya que es utilizado por la industria alimentaria como relleno de formulaciones como galletas y pasteles (Fayaz et al., 2017). Dependiendo de su aplicación, sus propiedades físicas varían ampliamente, presentando desde estructuras semisólidas a más extensibles y plásticas. Las cremas untables, generalmente, contienen entre un 30 y un 35% de grasa, aunque, esta cantidad puede alcanzar el 60% cuando se desean ciertas propiedades de untabilidad o adherencia (Espert et al., 2020). Generalmente, las cremas son fluidos no newtonianos, que se comportan como estructuras semisólidas que impiden la sedimentación de partículas sólidas y la separación de aceite, y a su vez fluyen de forma similar a un líquido cuando se aplican fuerzas de cizalla (Patel et al., 2014). Sus propiedades viscoelásticas vienen determinadas, especialmente, por la cantidad de grasa utilizada y su morfología, así como por la composición de los ingredientes en general. Las grasas utilizadas normalmente en este tipo de productos son sólidas, con un elevado contenido en ácidos grasos saturados, como la grasa de palma. Debido a que estas grasas adoptan una red cristalina, proporcionan textura, sensación en boca y sabor al producto (Marangoni et al., 2012). Sin embargo, como ya se ha comentado, existen importantes razones

nutricionales que apoyan la búsqueda de alternativas adecuadas para reemplazar la grasa saturada en este tipo de alimentos. Los oleogeles podrían ser potenciales sustitutos, al contener una gran cantidad de aceite vegetal (>90%) atrapado en una red tridimensional, y al presentar características y propiedades reológicas comparables a las de los materiales sólidos (Doan et al., 2016).

Otro alimento con alto atractivo para ser reformulado por su elevado consumo son los panes elaborados con grasa, como el pan brioche o pan de leche. La masa de pan es una dispersión coloidal acuosa, que comprende componentes hidrófobos (grasas y mantecas) e hidrófilos (harina y azúcar), que se entrecruzan y forman una estructura tridimensional que atrapa las burbujas de aire durante la cocción (Demirkesen and Mert, 2020). La industria del pan ha realizado numerosos esfuerzos para crear nuevos productos con diferente apariencia, forma, sabor y valor nutricional para satisfacer las demandas del consumidor (Martínez-Monzó et al., 2013). Los panes dulces, como el pan de leche, son una opción interesante establecida en la industria de la panificación. En este tipo de panes, los ingredientes lácteos aportan beneficios nutricionales y propiedades funcionales (sabor, color de la corteza, características de tostado, estructura y textura de la miga) (Graça et al., 2019). Sin embargo, el aporte nutricional, de este tipo de panes, se ve mermado ya que, en su formulación se emplean como ingredientes las grasas sólidas saturadas convencionales, y de efectos poco saludables. Aunque la grasa saturada juega un papel esencial al conferir aroma, sabor y textura, las recomendaciones dietéticas actuales respaldan la disminución de la ingesta de grasas saturadas y su

reemplazo por grasas insaturadas, incluidas grasas polinsaturadas y monoinsaturadas (Kris-Etherton and Krauss, 2020). Sin embargo, el uso de aceites líquidos en panificación puede tener un efecto negativo en la calidad estructural final del pan (Payret et al., 2011; Smith and Johansson, 2004). Los hidrocoloides han demostrado mejorar las características de la masa, la calidad y la estabilidad del producto final (Ferrero, 2017). Combinar la funcionalidad tecnológica de los hidrocoloides con el alto valor nutricional de aceites vegetales ricos en ácidos grasos insaturados a través del desarrollo de oleogeles, puede ser una opción prometedora para la elaboración de panes dulces saludables.

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Recent trends in oil structuring using hydrocolloids

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Abstract

Solid fats provide desirable functionality, texture, and palatability to foods; however, they are associated with adverse chronic health effects. Both consumer and regulatory authorities are increasing their demand for natural and healthy ingredients. Thus, in context of the high demand for healthy foods and the transition to more sustainable ingredients, oil structuring emerges as an outstanding strategy to substitute saturated and *trans* fats. Researchers have developed optimised indirect routes to structure oil with hydrocolloids resulting in gelled emulsions and oleogels. Novel healthy food structures based on polysaccharides, proteins, and their supramolecular interactions are suitable to create several types of healthy colloidal systems with unique sensory, texture, and stability properties.

Keywords: Oleogel, protein, polysaccharide, colloidal system, HIPE gels

1. Introduction

Oils and fats are important to our diet due to being an important energy source and solvent for key nutrients like vitamins and bioactive compounds. In foods, fats provide desirable functionality, texture, and palatability (Pehlivanoğlu et al., 2018). Fats had long been considered a bad influence on health, but, studies have shown that, principally, *trans* and some saturated fats are to blame (Liu et al., 2017; Zhu, Bo, & Liu, 2019). However, saturated fats have a technological role in foods because they are responsible for texture properties (Co & Marangoni, 2018). Solid-like lipids are preferred when processing some industrial products due to their specific features like better oxidative stability and solid lipid functionality. However, widely used methods, such as hydrogenisation, interesterification, and fractionation produce *trans* fatty acids which have severe adverse effects on coronary heart disease, blood lipoprotein profiles, diabetes, and cancer (Pehlivanoğlu et al., 2018). Academy researchers and research development experts have moved toward investigating ways for cheap and healthy production of solid fats with vegetable oil. Still, to replace saturated and *trans* fats in foods, maintaining their physicochemical and sensory properties is still a challenge (Iqbal, Xu, Huang, & Chen, 2019).

In the last decade, considerable progress in oil structuring technology has been made. Semisolid colloidal systems like oleogels incorporate a high percent of liquid oil within a structured network (composed by structurants) to give solid-like properties (Patel, Cludts, Sintang, Lesaffer, & Dewettinck, 2014). Moreover, highly concentrated gelled

emulsions such as high internal phase emulsions (HIPEs) have gained popularity in this scientific field. However, an oleogelation limitation is the available food-approved structurants suitable for gelling liquid oils; of which hydrocolloids appear to be the most promising candidate. Generally, the gelling concentrations of hydrocolloids are remarkably low because of their large molecular sizes, making them efficient structuring agents. Still, most food-approved polymers are fundamentally hydrophilic and cannot be dispersed easily in oil to achieve the necessary structure and network formation required for gelation (Patel, 2018; Pernetti, van Malssen, Flöter, & Bot, 2007).

Hydrocolloid-based oleogelation is possible using indirect methods, an innovative solution to structure oil (Martins, Vicente, Cunha, & Cerqueira, 2018). This group includes: i) The emulsion-template approach where the emulsion formation is followed by evaporation of water, giving tightly packed droplets. ii) A solvent exchange procedure replacing the internal aqueous phase with an intermediate solvent followed by liquid oil. iii) The physical sorption of oil in porous structures such as aerogels or cryogels (Nephomnyshy, Rosen-Kligvasser, & Davidovich-Pinhas, 2020). In addition, HIPEs are templates to create oil continuous gels using low-temperature-triggered gelation of closely packed water droplets (Patel and Dewettinck, 2015).

Several types of hydrocolloids can produce novel structures because of their supramolecular interactions. Proteins are amphiphilic molecules that can adsorb strongly at an oil-water interface, thus are highly effective emulsifiers. Still, the functional properties of proteins can be lost in acidic conditions, at high temperature,

high ionic strength, and in organic solvents, limiting their industrial uses (Akhtar & Ding, 2017). Polysaccharides are high-molecule-weight, hydrophilic, and biodegradable polymers regularly used as thickeners to affect the viscosity of aqueous phases to stabilise emulsions. However, many have poorer oil-water interfacial activity than proteins because of the lack of hydrophobic segments (Iqbal et al., 2019). The availability of many food-grade proteins and polysaccharides, and the versatility of the protein-polysaccharide interaction mechanisms, are fundamental to design suitable hydrogels that will later become oleogels (Anal, Shrestha, & Sadiq, 2019). These interactions have countless applications to improve the physical stability of the emulsion systems, and therefore in oleogels stabilisation.

Among indirect methods that require emulsification (such as the emulsion-template approach or HIPE gelation), a new category of food emulsifiers have increased interest from the oleogelation research field. Colloidal solid particles can form strong mechanical interfacial barriers via Pickering emulsions, anchoring at the interface as they can have an affinity for both oil and water (Berton-Carabin & Schroën, 2019). Interfacial layers containing Pickering particles give emulsions high physical stability, essential to develop stable gelled emulsions and oleogels (Lee, Tan, Ravanfar, & Abbaspourrad, 2019; Ma, Zou, McClements, & Liu, 2020).

In this review, the most recent insights of oleogelation using protein and polysaccharides are argued regarding the principal reasons that can influence the interactions and consequently the oleogelation. The promising hydrocolloid-based

indirect oil structuration strategies, the development and incorporation of HIPE gels, and using Pickering emulsions with micro- and nanoparticles are discussed. Furthermore, functional colloid formation, current applications, and potential applications of structuring hydrocolloids are discussed.

2. Polysaccharides and proteins as structurants

Oleogelation is achieved by combining a structuring agent (structurant or oleogelator) with liquid oil to confer solid-fat functionality. Oil structuring agents can be classified into two groups based on their molecular weight: low and high molecular weight oil gelators (Co & Marangoni, 2018). High molecular weight oil gelators are mainly proteins and polysaccharides, which can form three-dimensional polymeric networks through physical interactions. Such oleogels have viscoelastic properties which strongly depend on the polymer molecular weight, conformation, and concentration (Davidovich-Pinhas, 2019; Marangoni & Garti, 2011). These polymeric oleogelators can be proteins and polysaccharides or protein-polysaccharide complexes and conjugates (Co & Marangoni, 2018).

2.1. Polysaccharides

Using polysaccharides as structuring agents, stabilisers, or thickening agents in water-based systems is proven in the food industry. They are generally recognised as safe status. Most polysaccharides cannot adsorb at interfaces because of their strong

hydrophilic character, however, polysaccharides, like cellulose derivatives, can adsorb as they are surface-active. Because of the hydroxyl groups reactivity on cellulose, many derivatives can be synthesised displaying various physical and chemical characteristics. Ethyl-cellulose, methylcellulose, and hydroxypropylmethyl cellulose (HPMC) are cellulose ethers used in food and other industries. To date, ethyl-cellulose only has the capacity to directly gel liquid oil due to its lipophilic characteristics. Therefore, gelation of oil using polysaccharides is achieved using indirect methods (Davidovich-Pinhas, Barbut, & Marangoni, 2016). Adding methyl and hydroxypropyl groups to cellulose chains give a polymer with a high surface activity. Despite these hydrophobic groups in the cellulose derivative chain, the polymers in part keep the hydrophilic characteristic of cellulose (Meng, Qi, Guo, Wang, & Liu, 2018b).

Various unique polysaccharides are found in marine organisms like alginates, agar, and carrageenan. Seaweed polysaccharides are favoured among polysaccharides because of their biocompatibility, relatively low cost, and food-grade status. Carrageenan is a family of natural, water-soluble, sulphated galactans isolated from red seaweeds. Carrageenan gelation chains are linked through double helices using cation mediated aggregation to develop a cohesive network. Alginates, obtained from brown seaweeds, can interact with divalent ions forming cross-linking junctions leading to gelation. Hydrogels made of alginate have advantages like high oil retention, lipid oxidation inhibition, lipid digestion control, and target compound's controlled-release. Additional sources of carbohydrates are found in many bacteria species such as xanthan gum. Further,

combinations of this hydrocolloid with plant galactomannans like locust bean gum and guar gum give a synergistic viscosity increase (Davidovich-Pinhas, 2019; Lim et al., 2020).

In emulsion-based indirect technologies, combining surface-active and non-surface-active polysaccharides is mandatory to generate stable oleogels containing high liquid oil concentration (Patel, Cludts, et al., 2014). The surface-active polysaccharide can adsorb at the water-oil interface and acts as an emulsifying agent; whereas non-surface-active polysaccharides increase the viscosity of a continuous aqueous phase and act as a thickening agent preventing the coalescence of oil droplets. Addition of non-surface-active polysaccharides is mainly due to three factors: i) an increase in the bulk phase viscosity, ii) interaction with the surface-active component, forming complexes that show a higher interfacial activity, and iii) to anchor to the surface-active component and improve the interfacial stability, due to the cooperation between the two polysaccharides. To obtain the oleogel, selective evaporation of the aqueous phase is conducted, promoting the network formation which results in the entrapment of oil drops in the matrix formed by the combination of both polysaccharides (Meng et al., 2018b; Patel, 2018).

2.2. Proteins

Proteins used as oleogelators to structure oil is difficult, proteins do not easily make networks in oil or hydrophobic environments. Proteins are surface-active materials comprising flexible hydrophobic and hydrophilic moieties. Although proteins are

amphiphilic, their hydrophilic attributes limit their oil dispersibility. Therefore, an indirect method is necessary to include proteins in hydrophobic environments (Garti & Benichou, 2004; Scholten, 2019).

Proteins can form protein oleogels, and their properties can be modified by changing the proteins' interactions in the oleogels, also by changing the characteristics of the aqueous phase. This is achieved by reducing the electrostatic repulsion of charged proteins by altering their pH values close to their isoelectric point or by adding salts. Therefore, they come in to such close contact that attractive van der Waals forces dominate. Thus, the repulsive and attractive forces determine the network structure during aggregation (Scholten, 2018).

Alternative electrostatic mechanisms involve heating the protein inducing denaturation, exposing their hydrophobic groups and aggregation to form a three-dimensional network. Protein bonds are strong and when cooled or the aggregates are diluted they do not break. Even covalent bonds may form as disulphide bridges. Protein from different sources can provide disulphide bridges depending on their amino acid's chemical composition (Mehalebi, Nicolai, & Durand, 2008).

Protein-based oleogelation research has been focused on using whey protein. For these proteins, the network formation and how these proteins can be changed, together with how aggregates are made, is well known. However, other proteins may also be suitable for preparing small aggregates as initial building blocks for network formation

(Scholten, 2018). Water gelation hydrogels using proteins is common and include milk-derived proteins (de Vries, Wesseling, van der Linden, & Scholten, 2017), gelatin (Zhang et al., 2019), egg proteins (Tang, Yu, Lu, Fu, & Cai, 2019), and many plant-based proteins (Tavernier, Patel, Van der Meeren, & Dewettinck, 2017; Vélez-Erazo, Bosqui, Rabelo, Kurozawa, & Hubinger, 2020). However, the process of oil structuring is much more challenging.

Increasing global population has led to increased demand for protein which could cause a shortage of protein provision, and a serious future challenge. Current consumer trends have caused the food industry and scientific community to concentrate their efforts on developing oleogels using sustainable techno-functional ingredients sources, such as insect protein-based emulsifiers (Gould & Wolf, 2018) or protein extracts from microalgae species (Ebert, Grossmann, Hinrichs, & Weiss, 2019). These protein sources in oleogel formulations offer a promising innovation for functional fats.

The choice of proteins and suitable methods is determined by the rheological characteristics of the protein oleogels and intended food product. Creating oleogels by adding protein uses two main methods and the protein source used can vary. Using emulsion-template requires a protein that is surface-active. Adding polysaccharides can improve this characteristic by strengthening the interfacial network due to greater attractive interaction with the proteins. With the solvent exchange method, proteins must form aggregates when heating, thus all globular proteins can be used. However, the solvent exchange process gives more control these properties, yet needs an

intermediate solvent. Furthermore, disulphide bridges within the protein aggregates give higher stability, slowing their disintegration (de Vries, Lopez Gomez, Jansen, Van der Linden, & Scholten, 2017). These two indirect methods are used to make protein oleogels, however, the preparation has limits for commercial use. Before the proteins can be industrially applied as an efficient oil structuring agent, the limitations must be reduced (Scholten, 2019).

2.3. Protein-polysaccharide complexes and conjugates

Polysaccharides and proteins naturally interact to improve the texture, stability, and quality of food colloidal systems (emulsions, gels, dispersions, foams, and their mixed variants) (Anal et al., 2019; Semenova, 2017).

When these biopolymers are together in a system, their physicochemical properties and interactions are influenced by molar mass, concentration, pH, ionic strength, molecular conformation, charged density, polydispersity, temperature, solvent quality, and nature of interactions (Anal et al., 2019). Proteins and polysaccharide molecules are often connected by either non-covalent interactions (hydrogen bonding, steric exclusion, electrostatic, and hydrophobic) or covalent interaction (enzymatic cross-linking or formation of Maillard reaction products) (Dickinson, 2008).

Non-covalent interactions can be divided into two groups: unlike biopolymers are formed by attraction and repulsion (Fig. 1); these interactions in solution cause complex formations. Attractive electrostatic interactions occur when oppositely charged proteins

and polysaccharides come together in the mixed biopolymer system. This leads to soluble or insoluble complexes due to weak or strong interactions, respectively. When the pH is near the protein's isoelectric point, cationic protein's and anionic polysaccharide's interactions form somewhat stable soluble colloidal complexes (Wijaya, Patel, Setiowati, & Van der Meeren, 2017). In contrast, the binding of proteins and polysaccharides with the same amount of opposite charges gives an electrically neutralised insoluble complex and phase separation. Furthermore, the biopolymer-rich phase could aggregate forming a coacervate, depending on the strength of the attraction and nature of the polymers (McClements, 2006). When two polymers with the same charge are mixed, at low ionic strength, there is repulsive electrostatic interaction. Whereas when the biopolymers are at low concentration, they are co-soluble in a single phase. However, when the concentration is above a specific level there is thermodynamic incompatibility and segregate phase separation occurs. Therefore, a two-phase solution results, one rich in a type of biopolymer and depleted in the other, and vice versa (Anal et al., 2019; Rodriguez Patino & Pilosof, 2011).

In contrast, covalent interactions are specific and establish permanent bonds and irreversible interactions between proteins and polysaccharides, known as protein glycosylation. This is achieved through a chemical reaction between the protein's amino groups and the polysaccharide's carboxylic groups, resulting in a covalently bound amide (Wijaya et al., 2017). Dehydrating the complex under controlled humidity or by adding a cross-linker, the covalent interaction between proteins and polysaccharides can

be induced. Maillard-type conjugates are produced by dry-heating mixtures of two kinds of biopolymers, which improves the protein solubility, colloidal stability, and interfacial functionality (Anal et al., 2019).

The protein-polysaccharide complexes and conjugates show improved functional properties over proteins and polysaccharides alone. This enhancement can be attributed to the biopolymers being both present in the mixture, along with the complexes' structure (Benichou, Aserin, & Garti, 2002).

Protein-polysaccharide interaction mechanisms offer the prospect to design customised colloidal complexes. These combinations have great applications in improving oleogels stabilisation. Different protein-polysaccharide complexes have been formulated to develop hydrocolloids-based oleogels: soy protein-*κ*-carrageenan (Tavernier et al., 2017), sodium caseinate-xanthan gum, sodium caseinate-guar gum (Abdolmaleki, Alizadeh, Nayebzadeh, Hosseini, & Shahin, 2019), and gelatin-xanthan gum (Abdollahi, Goli, & Soltanizadeh, 2020). Other studies have used protein-polysaccharide conjugates to develop aerogels such as alginate-soy protein (Chen & Zhang, 2020).

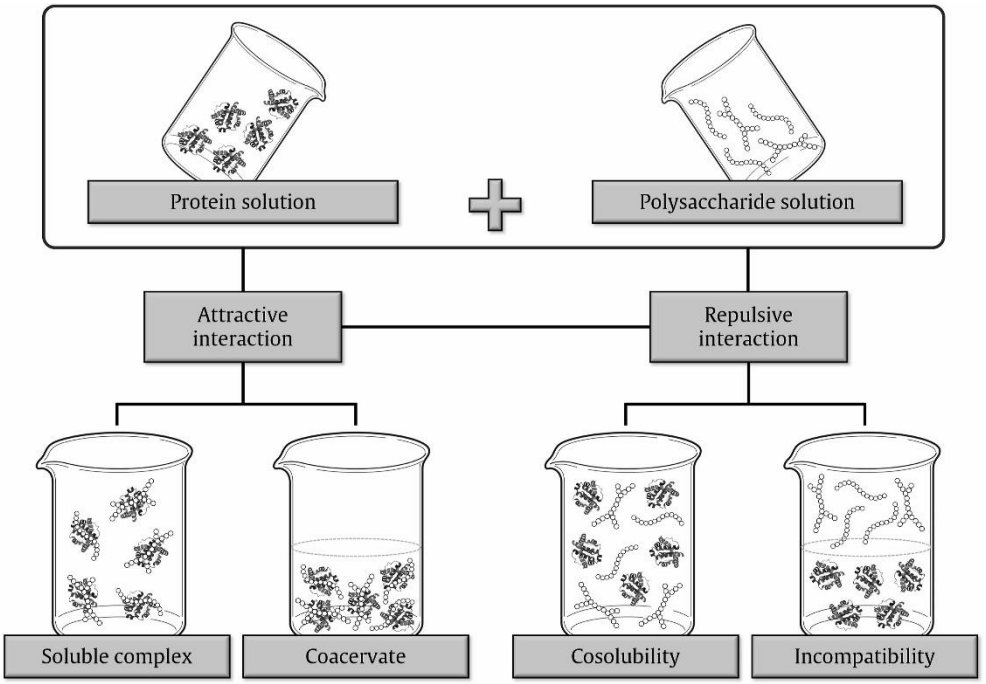

Figure 1. Schematic representation of the molecular structural arrangements involved when proteins and polysaccharides are mixed together (Adapted from McClements (2006)).

2.4. Protein-polysaccharide composite colloidal particles

Some indirect oleogelation methods require an emulsification process such as the emulsion-template approach and HIPE gelation. These methods offer new pathways to improve oleogels stability; one being particle-stabilised emulsions (Berton-Carabin & Schroën, 2015).

Dispersed colloidal particles were found to act as emulsion stabilisers, this concept has been formally recognised since the publication of Pickering (1907) (i.e. Pickering emulsion). The emerging research trends use natural biopolymer particles that act as

Pickering stabilisers, which are suitable for food, and can be scaled commercially (Rayner et al., 2014).

Natural biopolymers, like polysaccharides, are a large source of particulate material suitable for food use. Many studies have demonstrated that hydrophobic modified starches, cellulose-based, and chitin-based particles can act as Pickering stabilisers in food-grade emulsions (Dickinson, 2017; Murray, 2019). Additionally, proteins are exceptional natural building blocks for preparing micro- and nanoparticles because of their versatility in tenable conformations (Xiao, Li, & Huang, 2016). The main protein-based particles can be divided by their sources: animal origin (whey protein, bovine lactoglobulin, bovine serum albumin, α-lactalbumin, lactoferrin, and gelatin) and plant origin (prolamins like gliadin from wheat, soy protein, and pea protein). However, plant-based proteins are more relevant for renewable, sustainable, and eco-friendly reasons (Tavernier, Wijaya, Van der Meeren, Dewettinck, & Patel, 2016). Another interesting theoretical approach to produce Pickering emulsifiers uses protein-polysaccharide-based nanoparticles (Xiao et al., 2016).

Innovative opportunities for food emulsion stabilisation using biopolymer nanoparticles and microparticles are transpiring, with stabilisation and durability regarding coalescence, showing no significant changes after long storage periods. This is due to the effective steric structural barrier formed by adsorbed solid particles (Dickinson, 2013; Semenova, 2017). Recently, protein-polysaccharide composite particles are emerging as Pickering stabilisers to develop oleogels such as gliadin-

chitosan (Zhou et al., 2019), gliadin-arabic gum (Ma et al., 2020), or bovine serum albumin-cellulose nanocrystals (Liu, Zheng, Huang, Tang, & Ou, 2018). A noteworthy approach is using Pickering emulsions or Pickering HIPEs as templates to give structure to low-viscosity liquid oil forming soft solids and oleogels (Tavernier et al., 2016; Zeng et al., 2017).

3. Indirect methods to structuring liquid oils with hydrocolloids

The most common indirect methods include i) the emulsion-template approach, ii) a solvent exchange procedure, iii) physical sorption of oil into porous structures such as an aerogel or cryogel, and iv) HIPE gelation.

3.1. Emulsion-template approach

The emulsion-template is one of the most progressive multistep processes for oil structuring using indirect methods. This concept was first introduced by Romoscanu and Mezzenga (2006). They prepared a template emulsion with β-lactoglobulin proteins as the emulsifying agent. The process was accomplished by cross-linking adsorbed protein, either thermally or chemically using glutaraldehyde. However, to remove the several washing steps needed to allow protein adsorption at the oil interface, Patel, Cludts, et al. (2014) prepared oleogels by combining food polymers, used as templates, to create oil-based gels with high oil content, without using the cross-linking procedure.

This methodology involves removing the hydrophilic phase in the primary emulsion, then the exposed polymeric structure entraps the oil fraction, forming an oleogel. The polymer forms a hydrophilic solution, and is added to an oil phase with high energy homogenisation (e.g. Ultra-Turrax). After, once dehydration is complete, the exposed biopolymer structure functions as the building block for oleogelation. The oil can intake ≥97% of liquid oil with this technique, as previously demonstrated (Martins et al., 2018).

Several studies used the emulsion-template to develop edible oleogels, using combined surface-active and non-surface-active food polymers (Patel, 2020b, pp. 307–325). Combining the amphiphilic hydrocolloid with a thickening polysaccharide is favourable to stabilise O/W emulsions for preparing oleogels (Rodriguez Patino & Pilosof, 2011). Non-adsorbing hydrocolloids enhance the bulk phase viscosity by forming an extended network, and reinforce the network using adsorbed hydrocolloids molecules at the interfaces of oil droplets (Garti & Leser, 2001; Khouryieh, Puli, Williams, & Aramouni, 2015; Meng, Qi, Guo, Wang, & Liu, 2018a).

Two alternatives where emulsion droplets can be stabilised using protein-polysaccharide complexes exist (Dickinson, 2008). Different authors referred to these two cases as "bilayer emulsions" (method A) and "mixed emulsions" (method B); shown in Fig. 2. Method A comprises making a primary emulsion using protein as the only emulsifier, followed by adding a charged polysaccharide to the aqueous phase, producing a secondary emulsion; thus, giving droplets a protein-polysaccharide 'bilayer' surface coating. Method B requires a bulk aqueous solution of the protein-polysaccharide

complex, to be used as the emulsifying agent during a following homogenisation (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008). Depending on which method is used, emulsions with different textural properties and stability will be obtained.

Different protein-polysaccharide complexes were formulated to develop hydrocolloids-based oleogels using the emulsion-template approach: gelatin-xanthan gum (Patel et al., 2015), soy protein-κ-carrageenan (Tavernier et al., 2017), and sodium caseinate-xanthan gum/guar gum (Abdolmaleki et al., 2019). Other approaches used a combination of surface-active cellulose derivates, such as HPMC or methylcellulose, with the addition of other hydrocolloids, like thickening agents such as carboxymethyl cellulose, xanthan gum, sodium alginate, arabic gum, guar gum, or flaxseed gum (Bascuas, Hernando, Moraga, & Quiles, 2019; Martins et al., 2018; Meng et al., 2018a, 2018b). Another interesting potential application could alter the properties of the emulsion-template oleogel by combining hydrocolloid-based oleogelators (proteins and polysaccharides) with a low concentration of wax. Both alternative oil structuring options were explored by Tavernier, Doan, Van der Meeren, Heyman, and Dewettinck (2018) and Gao and Wu (2019), using soy protein-wax and starch-wax, respectively.

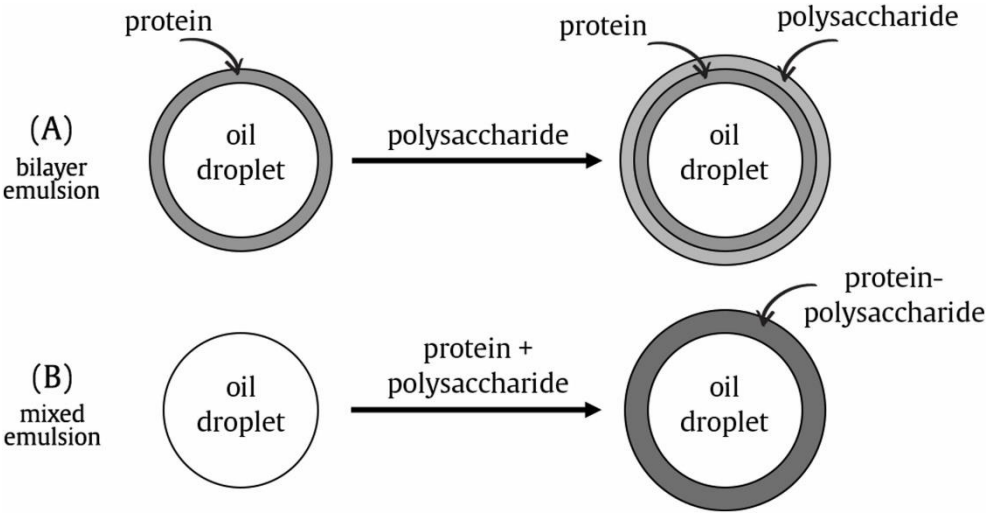

Figure 2. Illustration of two procedures for stabilisation of oil droplets using protein-polysaccharide complexes: (A) 'bilayer emulsion' preparation, with polysaccharide added after prior emulsification with a protein; (B) 'mixed emulsion' preparation, with both biopolymers present during emulsification (adapted from Dickinson (2008)).

3.2. Solvent exchange

Solvent exchange is an indirect method useable for oleogel development with protein aggregates and polysaccharides as building blocks. Introduced by de Vries, Hendriks, Van Der Linden, and Scholten (2015), the first step of gelation includes developing a hydrogel through producing a polymeric network in an aqueous medium. It has been shown that heat-set whey protein hydrogels can be macroscopic templates and by using the solvent exchange method, can create protein oleogels (de Vries et al., 2017). This method replaces the water in the internal areas of the heat-set protein matrix using an

intermediate organic solvent to replace the water content. Subsequently, this will be later replaced with the oil phase via a sequence of immersion steps, responsible for incorporating the oil within the polymeric network (Martins, Vicente, Pastrana, & Cerqueira, 2020).

Intermediate solvent alternatives are limited, however acetone and tetrahydrofuran can fit well (de Vries et al., 2015). The protein network structure remained and was not affected by different solvents. The features of the final protein oleogel are dependant of the protein-building block's properties and their interactions. Therefore, small protein aggregate building blocks result in a spreadable oleogel with plastic behaviour. Whereas a macroscopically large hydrogel leads to a firm oleogel with fracture properties. Because the protein-building blocks are made in an aqueous phase, it is possible to modify the properties of the protein oleogel by changing the setting conditions during the aggregation step (e.g. pH and ionic strength) (Marangoni & Garti, 2011; Scholten, 2018).

The solvent exchange path can be exploited to produce oleogels, even with non-protein hydrocolloids such as κ-carrageenan (Manzocco et al., 2017). The κ-carrageenan hydrogel is first converted into an alcohol-gel using solvent exchange, this is then subjected to supercritical CO2 drying to give a porous aerogel. This method could be used with other hydrocolloids to create aerogels which absorb a substantial amount of liquid oil (up to 80% capacity) (Patel, 2020a).

3.3. Physical sorption of oil into porous materials

A multitude of challenges have focused on oleogel-derived structures. The drying step is a shortcoming related to the process methods, which is need to remove the aqueous phase and expose the formed gel network. One must achieve oil structuring functionality previous to the hydrocolloid gelation in the water phase. After, the water should be removed, keeping the hydrocolloid structure, guaranteeing successful oil entrapment (Plazzotta, Calligaris, & Manzocco, 2019). A porous structure is produced in the drying process, and can be achieved by using supercritical CO2 forming aerogels or by freeze-drying, forming cryogels. Besides, aerogels and cryogels have been recognised as tools for bioactive compound microencapsulation in food because of their low density and high porosity properties (Comin, Temelli, & Saldaña, 2012; De Marco, Baldino, Cardea, & Reverchon, 2015).

3.3.1. Aerogels

Aerogels can be defined as a highly porous solid material with low density and high specific surface area. The definition of aerogel is still ambiguous due to its varied physicochemical properties, which depend on the production methods, like the drying procedure. Aerogels could be used with any highly porous solid material with low density, large inner surface area, and high porosity when the liquid in the three-dimensional network is replacing by gas during the preparation process (Zheng, Tian, Ye, Zhou, & Zhao, 2020).

The desire for ecological materials in aerogel fabrication has led focus to turn to biopolymers, believed to be promising precursors. The mechanical robustness and biodegradability of bio-aerogels have remarkable advantages. At present, bio-aerogel investigation is focused on polysaccharides (Obaidat, Alnaief, & Mashaqbeh, 2018; Zhao, Malfait, Guerrero-Alburquerque, Koebel, & Nyström, 2018) and proteins (Ahmadi, Madadlou, & Saboury, 2016; Kleemann, Selmer, Smirnova, & Kulozik, 2018). Some polysaccharides have been fabricated into aerogels successfully, showing excellent properties, including cellulose (Zhang, Zhai, & Turng, 2017), starch (Abhari, Madadlou, & Dini, 2017; De Marco, Riemma, & Iannone, 2019), chitosan (Obaidat, Tashtoush, Bayan, Al Bustami, & Alnaief, 2015), pectin (Tkalec, Knez, & Novak, 2015), and κ-carrageenan (Obaidat et al., 2018). The unique properties of polysaccharide aerogels include biodegradability, biocompatibility, sustainability, and renewability at relatively low cost make them ideal for food use.

As we mentioned in section 3.2, a two-step solvent exchange route to create aerogels then oleogels was exploited by Manzocco et al. (2017) using non-protein hydrocolloids like κ-carrageenan. The hydrogel contained different κ-carrageenan concentrations which were first converted into an alcohol-gel (maintained for 1 day in aqueous solutions of ethanol), followed by an alcohol extraction from the gel using a supercritical CO2 drying to obtain a porous aerogel structure (dried template) with larger polymer aggregates. The aerogel showed good capacity to absorb liquid oil (about 80%). However, the oleogels showed a remarkably high firmness and high-volume contraction

limiting their food applications. To overcome this issue, Plazzotta et al. (2019) studied the influence of a lettuce-filler addition, derived from fresh-cut lettuce processed in the hydrogel composition, on oleogel structure using κ-carrageenan hydrogel templates, with supercritical CO2 drying (solvent exchange approach) or freeze-drying (foam template approach).

New approaches could be used with other food-grade hydrocolloids with oil structuring functionality, such as cellulose, marine polysaccharides, or starch (Mikkonen, Parikka, Ghafar, & Tenkanen, 2013) to create oleogels with tailored physical properties using an aerogel template. Recently, Plazzotta, Calligaris, and Manzocco (2020) developed oleogels using a whey protein-based aerogel as a template with freeze-drying or supercritical CO2 drying techniques. When supercritical CO2 drying was applied, oleogels, with an oil content of 84.8%, presented a more stable structure and a more interesting plastic and semisolid texture than freeze-drying oleogels.

Oh, Lee, Lee, and Lee (2019) produced solid-like oleogels from canola oil with foam-structured HPMC and proved the HPMC oleogel viability as a substitute for animal fat in meat patties. The beef tallow substituted with HPMC oleogels enhanced the quality of the meat patties, by lowering the cooking volume loss and giving a softer texture. In addition, saturated fatty acid levels significantly reduced to 15% compared to the beef tallow sample (42%).

A novel and facile method was reported by Chen and Zhang (2020), using a protein-polysaccharide complex, by cross-linking the alginate-soy protein via the Maillard reaction. Real world applicability should be carefully assessed regarding the long times and dedicated equipment required for creating oleogels by the aerogel template approach. However, characteristics of the aerogels prepared from marine and plant-derivatives polysaccharides have potential for the food industry (Mikkonen et al., 2013).

3.3.2. Cryogels

Another method based on physical sorption of oil into porous material that achieves an oil structuring functionality may be conducted by freeze-drying, forming cryogels. A study by Patel, Schatteman, Lesaffer, and Dewettinck (2013) found, for the first time, an eco-friendly low-temperature system of making oleogels as a different way use a biopolymer to structure oil, referred to as foam template oleogels. They foamed and freeze-dried an HPMC solution to form a porous structure which absorbed a significant volume of oil. The freeze-drying process creates an open cell structure, and under compression oil is released from the oleogel. However, by applying shear to the oil-saturated foams this was prevented by dispersing the polymer sheets and trapping the oil. Because the HPMC foam template oleogels were obtained via shearing, they showed shear stability; thus, are a really good option for spread-like food products.

This relatively facile method using food-grade polymers has been used for practical food applications to decrease saturated fat content in meat patties (Oh et al., 2019), sandwich

cookie creams (Tanti, Barbut, & Marangoni, 2016a), muffins (Oh & Lee, 2018), and cakes (Patel & Dewettinck, 2015); they also extended the shelf stable period of peanut butter for over six months (Tanti, Barbut, & Marangoni, 2016b). Therefore, this can generate further research for adapting foam-templates to explore other water-soluble polymers with foaming properties. Recently, Abdollahi et al. (2020) optimised the biopolymers, gelatin and xanthan gum, concentrations to create an oleogel as a freeze-dried foam template. This structure demonstrated an oil binding capacity (>92%) with thixotropic behaviour and 60% structural recovery.

In solid foams, physical properties of cryogels can be defined by their pore size, shape distributions, and the organisation of the pores. Therefore, one must control the porous material's structure. Andrieux, Medina, Herbst, Berglund, and Stubenrauch (2019) recently tackled this issue using microfluidic-aided foam templating, allowing them to make monodisperse and highly ordered chitosan foams.

3.4. High internal phase emulsion (HIPE) gelation

Complex colloidal systems have become popular in multidisciplinary fields due to a variety of potential applications. This sub-category contains colloidal systems that have an unusual phase distribution such as HIPEs (Wijaya, Van der Meeren, Wijaya, & Patel, 2017). The HIPE's stability plays a key role in their use, when incorporated into products, the stability may affect the product's physicochemical properties and sensory attributes (Liu et al., 2019). HIPEs can form gel-like HIPEs that would have similar

properties to oleogels or could be used as the first step of emulsion-template method that requires a production of a stable emulsion to formulate a desirable oleogel (HIPE-template approach) (Fig. 3) (Patel, 2017). HIPE gels stabilised by protein-polysaccharide complexes/conjugates had better coalescence stability than gels stabilised with only proteins or polysaccharides. This was attributed to the greater resistance to rupture by the adsorbed composites (Wijaya, Van der Meeren et al., 2017). However, Pickering HIPE gels, a recent variation of HIPE constituents, are noteworthy as stabilisation is achieved through a layer of rigid particles surrounding each droplet. This leads to greater stability against coalescence, creaming, and Ostwald ripening (Zamani, Malchione, Selig, & Abbaspourrad, 2018) (Fig. 3).

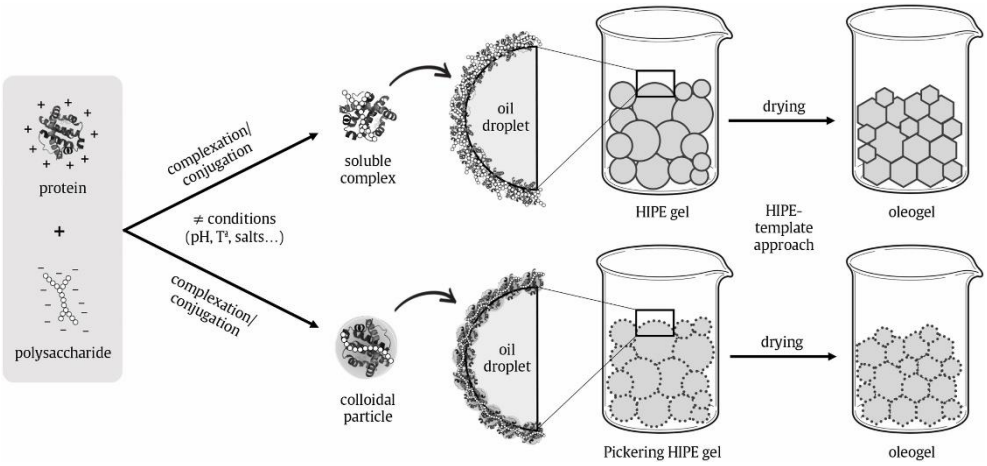

Figure 3. Schematic representation of stabilisation procedure of HIPE gels and oleogel formation by protein-polysaccharide soluble complex/conjugate and protein-polysaccharide composite colloidal particle.

3.4.1. HIPES gels

HIPEs are highly concentrated gelled emulsions with an internal phase volume fraction (Φ) exceeding 0.74 (Cameron & Sherrington, 1996, pp. 163–214). When the internal phase volume fraction is over 0.74, the dispersed droplets achieve their maximum packing density, leading to high viscoelastic flow behaviour. HIPEs' liquid droplets are often squeezed tightly together giving them a polyhedral geometry, and the highest geometric limit for packing of rigid spheres is exceeded (Fig. 3). HIPEs have a highly viscous or gelled soft solid texture (Zamani et al., 2018). Because of these rheological characteristics, HIPEs are popular in numerous applications, such as templates for porous materials (Hori, Sano, Suzuki, & Hanabusa, 2018), foams (Foudazi et al., 2020), oleofilms (Wijaya, Van Der Meeren, Dewettinck, & Patel, 2018), and as enhanced carriers of antioxidants (Wijaya et al., 2020).

To date, the modified emulsion-template approach developed by Patel, Rodriguez, et al. (2014) still suffers from long drying times, which can be unfavourable to the oil quality. To circumvent this, HIPEs were used as templates to decrease the overall water content (HIPE-template approach) (Wijaya et al., 2018, 2019). Furthermore, when using HIPE gels, the material that accumulates in the bulk phase decreases the aqueous content giving high oil volume polymer gels or gelled soft solids (Martins et al., 2020; Patel, 2018).

Having control over interfacial properties allows tuning of some gel properties. To achieve high oil content, the droplet surface is stretched to form plateau borders. To prevent droplet coalescence, improving the interface strength, appropriate interfacial proteins can be used. Proteins mostly give a rigid two-dimensional network at the interface, its viscoelastic behaviour with high elasticity make it notable (Nikiforidis & Scholten, 2015). However, high stiffness can stop large deformation, thus can stretch the interface, leading to the interfacial film failure (Van Aken, 2002). Therefore, interfacial layers should have a low dilatational modulus, to allow deformability of the surface, providing a barrier against droplet coalescence. Proteins are excellent emulsifiers as they are amphiphilic, still their stabilisation is improved when mixed with polysaccharides; especially when HIPE gels are formulated (Wijaya, Patel, et al., 2017).

Wijaya et al. (2019) showed how the pH and biopolymer ratio of a sodium caseinate and alginate mixture strongly affected the properties and stability of oleogels made with HIPE templates. Accordingly, in this mixture, the pH played a key role to obtain a highly stable HIPE. In addition, the protein to polysaccharide ratio was key to thickening the interface, functioning as a structural framework to entrap the oil phase. Therefore, firmer oleogels with less oil leakage, greater oxidative stability, and stronger gel properties were obtained at a higher polymer mixture ratio (sodium caseinate:alginate; 12:1) with a higher pH (7.0). Besides, Wijaya, Patel, Setiowati, and Van der Meeren (2017) showed that water continuous HIPE gels were successfully prepared, containing an internal phase of 82% and excellent stability, using aqueous

dispersions of whey protein isolate-low methoxyl pectin complexes with sunflower oil and homogenising.

Vélez-Erazo et al. (2020) prepared sunflower oil in HIPE using pea protein as an emulsifier, stabilised with multiple polysaccharides (carrageenan, xanthan gum, gum arabic, sodium alginate, pectin, gellan gum, locust bean gum, and tara gum) at a ratio of 4:1. Concerning HIPEs, only the pea protein formulated with xanthan gum and tara gum presented a creamy and homogeneous appearance, with minimal oil loss. Thus, this study provides information to elucidate the interaction behaviour of the pea protein and eight different polysaccharides in structured edible oils. Therefore, can be applicable as fat substitutes in numerous food formulations.

3.4.2. Pickering HIPES gels

Pickering stabilisers, include solid or semisolid particles, can form very stable HIPEs (Zamani et al., 2018) (Fig. 3). Evidence has rapidly accumulated over recent years to indicate gel-like HIPEs can be easily produced using different particles.

Regarding plant proteins, incorporating soy proteins in food is recurrent subject in food colloids, as they are beneficial to health (Tang, 2019). Soy β-conglycinin and soy glycinin, are two key storage globulins in soy protein isolates that could perform as excellent Pickering nanoparticles for stabilising HIPE gels. Hao, Peng, and Tang (2020) showed that untreated soy glycinin exhibited great potential to stabilise HIPE gels. The soy

glycinin glycated with soy soluble polysaccharides improved the emulsifying properties of proteins, the gel network stability formation, and the stability against heating or freeze-thawing because of HIPE gels.

Other protein-based particles have been used for HIPE gels stabilisation. Recently, Xu, Tang, Liu, and Liu (2018) stated that native ovalbumin is a remarkable Pickering stabiliser used in O/W HIPE gels, due to its strong intramolecular structural stability, ensuring its particulate nature when adsorbed at an interface. Further, HIPE gels show great coalescence stability upon storage or against heating. Moreover, Xu, Liu, and Tang (2019) demonstrated native soy β-conglycinin, with structural features like ovalbumin, is also effective as a Pickering nanostabiliser for HIPE gels. The findings are important because of their novel development, eco-friendly, and sustainable HIPEs for food.

Concerning animal protein, Li et al. (2020) confirmed several meat protein particles are excellent emulsifiers to stabilise olive oil in O/W HIPE gels. Stable HIPE gels could be produced at most pH range (pH 3–11) in the continuous phase. In addition, all formed HIPE gels exhibited exceptional stability over 60 days storage, heating, and freeze-thawing.

In contrast, different polysaccharides-derived particles have been used to stabilise HIPE gels. Zhu et al. (2020) prepared stable O/W Pickering HIPEs with chitin nanofibrils as the stabiliser, using a simple two-step strategy. The rod-like chitin nanofibrils gave great stability due to restricting coarsening, droplet breakage, and coalescence when forming

the emulsion. Chen et al. (2018) and Chang, Chen, Liu, and Wang (2020) developed Pickering HIPE gels using hydrophobic starch nanocrystals. These nanocrystals were modified by octenyl succinic anhydride (OSA) and were introduced as a stabiliser for gel-like Pickering emulsions. Among all commercial esterification reagents, OSA is the most common and effective to modify starch particles. OSA modification improves starch nanocrystals emulsion ability and offers its use to stabilise Pickering HIPEs (Chang et al., 2020).

Current trends in polysaccharide-based particles have seen use with insoluble fibre-based particles. Phoon & Jeyakumar, 2020 developed a novel approach to create oleogels using only cellulosic fibres, from nata de coco and citrus source, to trapped liquid oil in a three-dimensional structure. This study's main finding is that oleogels can be made without oiling off when compressed. Using the fibre source as a gelling agent shows great potential, thus opening the path for using food by-products, such as apple pomace and oat bran, with a high fibre content and bioactive compounds (Huc-Mathis, Journet, Fayolle, & Bosc, 2019).

Yang, Li, and Tang (2020) obtained nanoparticles from the insoluble soy polysaccharides of okara that performed as an outstanding Pickering stabiliser for HIPEs. Okara is a by-product of soybean products, which are rich in polysaccharides and proteins. The findings are of interest to develop novel stable HIPE gels for oil structuring able to incorporate high-added-value use of soybean processing by-products.

Ruan, Yang, Zeng, and Qi (2019) produced a mayonnaise replacer using citrus fibre (soluble and insoluble fibres) and corn peptides (particles) based HIPE gels, with 75% sunflower oil, which increased the dietary fibre in the emulsified food. The resulting HIPE gels showed good heat stability, freeze-thaw recovery, and minimal tribological properties; thus, provide more creaminess and smoothness sensory attributes. The citrus fibre-based HIPE gels could provide potential plant-based alternatives with enhanced oral sensation in food application (Yan et al., 2019).

Ma et al. (2020) prepared Pickering HIPE gels using a simple one-step process, blending a gliadin nanoparticle and gum arabic aqueous solution with corn oil (85%). The HIPE gel's apparent viscosity and storage modulus was higher than those stabilised with gliadin nanoparticles alone. Furthermore, the authors showed that, the HIPE gels, stabilised by gliadin nanoparticles-gum arabic, formed a dense three-dimensional network, comparatively stable to pH, ionic strength, and temperature changes.

Liu et al. (2018) developed O/W HIPE gels stabilised by bovine serum albumin-covered cellulose nanocrystals by mixing anionic cellulose nanocrystals aqueous solutions with cationic bovine serum albumin at pH 3.0. The surface modification with protein absorption could improve the emulsification performance of cellulose nanocrystals.

Huang et al. (2019) showed that chitosan-caseinophosphopeptides nanocomplexes are effective particulate stabilisers to develop O/W Pickering HIPE gels. A physical barrier was formed on the surface of the oil droplet by the nanocomplexes, and excess of

chitosan was dispersed in the continuous phase which formed a network, stabilising the HIPE gels. Furthermore, the Pickering HIPE gels stabilised by the positively charged chitosan-caseinophosphopeptides nanocomplexes were stable after 6 months storage.

Zeng et al. (2017) detailed using gliadin-chitosan hybrid particles as an emulsifier for HIPE gels development. Stable Pickering HIPE gels with internal phases up to 83% were prepared at low particle concentrations. Zhou et al. (2019) fabricated Pickering HIPE gels using gliadin-chitosan complex particles as a stabiliser, the dispersed phase volume fraction (90%) was one of the highest reported food-grade-particle-stabilised Pickering HIPE gels. The particle interfacial barrier and three-dimensional network formed by the complex particles in the continuous phase play key roles in stabilisation of HIPEs and help develop porous materials with a designed pore structure.

Conclusions

Under current demands for healthy, natural, and clean-label foods, plus a transition to more eco-friendly and sustainable ingredients, oil structuring using hydrocolloids has emerged as an outstanding strategy to substitute saturated and *trans* fats. Creation of oleogel systems using proteins and polysaccharides profit from hydrocolloid's proven structuring ability in food. However, hydrocolloids used as oil structuring agents raise a challenge due to their hydrophilic nature. Here, we have shown researchers have

developed and improved several optimised indirect routes to accomplish such tasks. However, these types of methods can increase the preparation time and complexity leading to a limited industrial application.

Evidence shows HIPEs are being used to form gel-like HIPEs that would have similar final properties as common oleogels. HIPE gels stabilised by protein-polysaccharide complexes and conjugates possess remarkable stability against coalescence, which is attributed to the resistance of the adsorbed complexes and confer unique sensory properties to the systems. Moreover, Pickering HIPE gels show stabilisation is achieved through a layer of rigid particles around each droplet giving extra stability against coalescence, creaming, and Ostwald ripening. Pickering emulsions are a notable item worthy of research in emulsion and colloidal sciences. This has inspired food scientists to use particles as a novel strategy for engineering functional food emulsion interfaces. Physical stability is a benefit, which can relate to the mechanism of stabilisation when compared to conventional emulsifiers. Additional chemical stability, nutritional quality, and controlled delivery benefits also have potential. Innovative food structures owing to oil structuring through hydrocolloids such as oleogels and Pickering HIPE gels are perfect to create several types of colloidal systems with unique and improved properties.

Despite the exponential number of scientific articles published in the field of oil structuring, there is a big gap between scientific research and industrial oleogel preparation. Future research in this field should focus on the development of efficient procedures, reducing time and complexity of the oleogel formation, specifically, at

industrial scale. Due to the difficulty of obtaining a good protein-polysaccharide complexation, multidisciplinary approach is expected to be key for future developments.

Moreover, there are some challenges that need to be addressed to enable application of oleogels in food formulations, such as the physical stability or the sensorial properties of the final food products. Development of reformulated products needs to move towards clean and green ingredients to produce healthy and eco-friendly foods, with good acceptability by current and future consumers.

Author statement

Pere Morell: Conceptualization, Writing – original draft, Isabel Hernando: Conceptualization, Writing- Reviewing and Editing, Funding acquisition. Amparo Quiles: Writing- Reviewing and Editing, Funding acquisition, Santiago Bascuas: Writing – original draft

Declaration of competing interest

The authors confirm there are not conflicts of interest.

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Objetivos

OBJETIVO GENERAL

El objetivo general de la presente Tesis doctoral es desarrollar oleogeles de alta estabilidad estructural y química, a partir de aceites vegetales, de elevado perfil nutricional, empleando como agentes estructurantes hidrocoloides. Las propiedades funcionales y sensoriales de los oleogeles desarrollados deben permitir sustituir, en la formulación de los alimentos, las grasas sólidas saturadas empleadas habitualmente en la industria.

OBJETIVOS PARCIALES

- Diseñar oleogeles de alto perfil lípídico y elevada estabilidad estructural y química, empleando aceites vegetales e hidrocoloides como estructurantes.

- Estudiar la influencia del grado de insaturación del aceite vegetal empleado en la formulación y de las condiciones de elaboración de los oleogeles estructurados con hidrocoloides, sobre su estabilidad estructural y química.

- Analizar la influencia del tipo de aceite empleado en la formulación y de las condiciones de elaboración de los oleogeles estructurados con hidrocoloides, sobre su estabilidad estructural y química, a lo largo del almacenamiento.

- Formular cremas untables empleando oleogeles elaborados con aceites vegetales e hidrocoloides, como sustitutos de grasa saturada. Estudiar el impacto del uso del oleogel sobre las propiedades estructurales y sensoriales.

- Formular panes dulces empleando oleogeles elaborados con aceites vegetales e hidrocoloides, como sustitutos de grasa saturada. Estudiar el impacto del uso del oleogel sobre las propiedades estructurales, sensoriales y sobre la digestibilidad *in vitro* de la fracción grasa de los panes dulces.

Estructura de la Tesis

La presente Tesis doctoral se enmarca dentro del proyecto del Ministerio de Ciencia, Innovación y Universidades titulado "Estructuración de aceites mediante la utilización de hidrocoloides como estrategia para sustituir grasas saturadas de alta plasticidad. Investigación reológica, estructural y sensorial" (RTI2018-099738-B-C22).

Reformular alimentos para que aporten un perfil nutricional saludable es uno de los retos de mayor interés para el control del sobrepeso y la mejora de la salud. Para ello, es necesario abordar esta problemática dando respuesta a las exigencias del consumidor y la industria. Las etiquetas limpias y el impacto medioambiental deben estar fuertemente cohesionados a la hora de formular alimentos más saludables y atractivos para el consumidor. El diseño y desarrollo de oleogeles mediante el empleo de hidrocoloides y aceites vegetales de alto perfil nutricional permitiría formular alimentos saludables, sin modificar demasiado sus propiedades funcionales y sensoriales. Sin embargo, existen pocos trabajos que estudien cómo actúan los oleogeles en un sistema alimentario real: cuáles son las interacciones con los otros componentes más frecuentes de los alimentos (proteínas, grasa, fibras o hidratos de carbono) y cómo modifican su reología, textura, estructura, estabilidad química y características sensoriales. En la introducción de esta Tesis, se pone en contexto la problemática actual y se adjunta un artículo de revisión abordando los conocimientos más recientes sobre el uso de hidrocoloides para la elaboración de oleogeles. Se indaga sobre las interacciones entre estos hidrocoloides y, en cómo pueden influir en la oleogelación. Se discuten las prometedoras estrategias de estructuración de aceite a base de hidrocoloides, el desarrollo de geles basados en

emulsiones altamente concentradas (High internal pase emulsions, *HIPE)* y el uso de emulsiones de Pickering. Además, se discuten la formación funcional de los sistemas coloidales, las aplicaciones alimentarias existentes y las aplicaciones potenciales de los hidrocoloides como agentes estructurantes. Este trabajo se publicó con el título "Recent trends in oil structuring using hydrocolloids" en la revista "Food Hydrocolloids".

En cuanto a los resultados, la Tesis aborda dos grandes bloques/capítulos: en el primero, se estudia como las características del tipo de aceite y las condiciones de secado influyen en la elaboración de oleogeles a base de hidrocoloides. El segundo bloque se centra en el diseño de dos alimentos, una crema untable y un pan dulce, en los que se reemplaza, total o parcialmente, las grasas saturadas que se emplean en la formulación de estos alimentos de forma tradicional, por distintos oleogeles. Una vez formulados estos alimentos con los oleogeles, se analiza cómo influye la presencia de los oleogeles en la microestructura, características físicoquímicas y percepción sensorial de los alimentos.

El primer capítulo se compone de dos trabajos. En el primer trabajo se planteó estudiar la influencia del tipo de aceite (oliva, girasol y lino), de las condiciones de secado empleadas (convencional y a vacío) en el método *emulsion-template* sobre las propiedades finales de los oleogeles elaborados con hidroxipropilmetilcelulosa y goma xantana. Se analizaron las propiedades microestructurales, reológicas y oxidativas de los oleogeles. Este trabajo se publicó con el título "Structure and stability of edible oleogels prepared

with different unsaturated oils and hydrocolloids" en la revista "International Journal of Food Science and Technology".

En el segundo trabajo se desarrollaron oleogeles de aceite de girasol y girasol alto oleico y se evaluó la influencia de dos condiciones de secado convencional y de la composición del aceite sobre la microestructura, propiedades reológicas y estabilidad oxidativa de los oleogeles a lo largo del almacenamiento. El trabajo se publicó con el título "Designing Hydrocolloid-Based Oleogels With High Physical, Chemical, and Structural Stability" en la revista "Frontiers in Sustainable Food Systems".

El segundo capítulo se compone de dos trabajos. En ellos se analiza la funcionalidad de los oleogeles, desarrollados y caracterizados en el primer bloque, en distintos sistemas alimentarios. En el primer trabajo de este segundo capítulo, publicado con el título "Structural and sensory studies on chocolate spreads with hydrocolloid-based oleogels as a fat alternative" en la revista "LWT - Food Science and Technology" se desarrollaron cremas untables de chocolate empleando diferentes oleogeles. Las cremas untables se diseñaron mediante el reemplazo parcial (50%) o total (100%) de la grasa saturada, concretamente grasa de coco empleada de forma habitual en estos productos, por oleogeles elaborados con aceite de oliva o girasol desarrollados en los trabajos anteriores de esta Tesis. Para conocer el impacto del uso de oleogeles en las propiedades finales de las cremas untables, se relacionó la microestructura, textura y reología de las cremas. A su vez se llevó a cabo un estudio sensorial mediante un "Free choice profile" para determinar los atributos descriptivos de las distintas cremas untables.

Finalmente, en el segundo trabajo del segundo capítulo se desarrollaron panes dulces formulados con diferentes oleogeles y procesados de dos formas diferentes, mediante horno y al vapor. Los panes se formularon mediante el reemplazo total de la grasa saturada, concretamente margarina empleada de forma habitual en este tipo de productos, por los oleogeles desarrollados y caracterizados en el primer capítulo de esta Tesis. Para conocer el impacto de la sustitución de la grasa saturada por los oleogeles se estudió la estructura de la miga, el volumen específico, la altura y textura, de los panes desarrollados. Además, se estudiaron las implicaciones de esta sustitución sobre la digestibilidad lipídica *in vitro* de los panes y se realizó una prueba triangular para determinar si existían diferencias entre los panes con margarina y los desarrollados con oleogeles. Este trabajo titulado "Use of oleogels to replace margarine in steamed and baked buns" ha sido enviado a la revista "Foods.

Las referencias de las publicaciones científicas derivadas de esta Tesis se presentan a lo largo de los capítulos en el siguiente orden:

Introducción:

Recent trends in oil structuring using hydrocolloids. Bascuas, S., Morell, P., Hernando, I., and Quiles, A. (2021). Food Hydrocolloids, 118 October, 106612. (DOI 10.1016/j.foodhyd.2021.106612).

Capítulo 1: Desarrollo de oleogeles de alto valor nutricional elaborados con hidrocoloides

Structure and stability of edible oleogels prepared with different unsaturated oils and hydrocolloids. Bascuas, S., Hernando, I., Moraga, G., and Quiles, A. (2020). International Journal of Food Science and Technology, 55(4), 1458–1467. (DOI: 10.1111/ijfs.14469).

Designing Hydrocolloid-Based Oleogels With High Physical, Chemical, and Structural Stability. Bascuas, S., Salvador, A., Hernando, I., and Quiles, A. (2020). Frontiers in Sustainable Food Systems, 4(7), 1–8. (DOI: 10.3389/fsufs.2020.00111).

Capítulo 2: Reformulación de alimentos mediante la incorporación de aceites de alto valor nutricional estructurados con hidrocoloides

Structural and sensory studies on chocolate spreads with hydrocolloid-based oleogels as a fat alternative. Bascuas, S., Espert, M., Llorca, E., Quiles, A., Salvador, A., and Hernando, I. (2021). Lwt, 135(9), 110228. (DOI: 10.1016/j.lwt.2020.110228).

Use of oleogels to replace margarine in steamed and baked buns. Bascuas, S., Morell, P., Quiles, A., Salvador, A., and Hernando, I (2021). Foods (en revisión)

Resultados y discusión

Capítulo 1

Desarrollo de oleogeles de alto valor nutricional elaborados con hidrocoloides

Structure and stability of edible oleogels prepared with different unsaturated oils and hydrocolloids

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Abstract

Edible oleogels, with three oil types (olive, sunflower and flaxseed), hydroxypropylmethylcellulose (HPMC) and xanthan gum (XG), as structuring agents, were developed using the emulsion-template approach, and subsequent drying of the emulsions using conventional or vacuum drying. Our results showed that for both drying methods, well-structured oleogels were obtained using olive and sunflower oils for the preparation. These oleogels showed oil losses <10% after 35 days of storage. However, unstructured non-homogeneous oleogels were obtained when using flaxseed oil and conventional drying, while it was not feasible to develop flaxseed oleogel with vacuum drying. Oleogels showed interesting rheological properties, including a high oleogel strength with an elastic modulus of the order 10^4 –10^5 Pa, weak dependence on frequency, and good thermostability. Moreover, high oxidative stability was obtained for olive oil oleogels, using both conventional and vacuum drying, and for sunflower oleogels using vacuum drying. Still, the initial oxidation rates of sunflower oleogels using conventional drying should be improved in future studies.

Keywords: Emulsion, hydroxypropylmethylcellulose, microstructure, oxidation, rheology, xanthan gum.

1. Introduction

Food products are regularly formulated with considerable amounts of solid fats that have a high content of saturated- and *trans*-fatty acids. Solid fats contribute to multiple functions in foods such as texture, flavour, firmness and functionality; desirable both for the consumer and the food industry (Co & Marangoni, 2012). However, there is a relationship between the consumption of solid fats and increased risk of cardiovascular disease, type 2 diabetes and ischaemic incidents (Estadella et al., 2013). In 2003, the World Health Organisation (WHO) and the United Nations Food and Agriculture Organization (FAO) recommended that saturated fat should not provide more than 10% of total caloric intake (Nishida et al., 2004). For this, industries give structure to vegetable (nonsaturated) oils, using methods based in partial hydrogenation, producing solid fats. Conversely, the Food and Drug Administration (FDA) announced to remove the consideration of GRAS (generally recognised as safe) for partially hydrogenated oils (FDA, 2015), as they are the primary dietary source of artificial *trans*-fat in processed foods. Current trends of consumers who demand healthier diets without compromising quality have made the food industry concentrate their efforts on reducing the levels of saturated- and *trans*-fats. Thus, research strategies to replace saturated- and *trans*-fats have been conducted in which solid fats used in the industry are replaced with a variety of lipid sources (Kanjilal et al., 2016), carbohydrates (Onacik-Gür et al., 2016) and proteins (Paglarini et al., 2019). Still, the total or partial replacement of the solid fat negatively affects the sensory and mechanical properties of the food (Biguzzi et al.,

2014). Therefore, the development of new solid fat substitutes with low saturated and high unsaturated fatty acids and *trans*-fat-free is the priority of many studies.

A promising alternative to replace saturated- and *trans*-fats is with the use of oleogels by structuring vegetable oils (Wassell et al., 2010). Edible oleogels are gelled systems where an oil continuous phase is immobilised in a three-dimensional network, with the assistance of an oleogelator or a combination of gelators. The process of formation for oleogels requires the use of gelling agents at low concentration, which have the ability to give structure to oils (>90%) (Abdolmaleki et al., 2019) and impart a solid-like material with the functionality (rheological, texturing, oil binding and stabilising properties, etc.) of solid fats but an improved nutritional profile (Stortz et al., 2012).

The hydrocolloid-based oleogelators are promising polymers used in food as methylcellulose (MC) (Patel et al., 2014a, 2014b) and hydroxypropylmethylcellulose (HPMC) (Patel & Dewettinck, 2015; Meng et al., 2018a). HPMC is an amphiphilic biopolymer derived from cellulose and is a stabilising additive (E464) in the food industry. Using HPMC as oleogelator is advantageous since it is inexpensive compared to more studied low molecular weight organogelators (Tavernier et al., 2018; Scholten, 2019) generally recognised as safe (GRAS) and has beneficial effects on health (Maki et al., 2009). However, there are limited studies considering the production of oleogels using HPMC, because of their hydrophilic nature. Hydrocolloids are unable to structure liquid oil because of their limited dispersibility in oil. Therefore, to disperse HPMC in oil, to achieve the required network formation for gelation, an indirect method is

necessary, like the emulsion-template approach, which was first reported by Romoscanu & Mezzenga (2006). They used β-lactoglobulin proteins as the stabiliser for water continuous emulsions, which were then used as templates to achieve protein-in-oil gels. Indirect physical gelation, using the emulsion-template method, is a multistep process; first hydrate the hydrocolloid, then prepare an oil-in-water emulsion (containing the hydrocolloid) followed by removal of the water, to obtain dried products driving the network formation. This results in the physical trapping of oil droplets in the polysaccharide matrix network (Patel et al., 2014a). The dried product must be homogenised to obtain the oleogel. The addition of other hydrocolloids, like thickening agents, such as xanthan gum (XG), could increase the stability of HPMC oleogels by enhancing the bulk phase viscosity of the aqueous continuous phase and prevent oil droplet coalescence (Meng et al., 2018a).

Oil type is a crucial factor when producing oleogels with high stability, rheological, textural and thermal properties like solid fats (Pehlivanoğlu et al., 2017). The oleogel should be made with nutritious vegetable oils rich in mono and polyunsaturated fatty acids, and with zero *trans*-fatty acids (Co & Marangoni, 2012). Although some vegetable oils have been used to prepare HPMC oleogels, sunflower (Patel et al., 2014a), soybean (Meng et al., 2018a) and rapeseed (Oh et al., 2019), no studies have been found comparing the effect of different types of oils (with different saturated/unsaturated fatty acid composition) on the structure and stability of oleogels with water-soluble polymers. The aim of this study is to compare and analyse the impact of unsaturated oil type used

in oleogel formulation, regarding the structural and stability properties, when HPMC and XG are used as gelling agents. The effect of vacuum drying is also studied as an alternative to conventional drying to improve the oxidative stability of the oleogels.

2. Materials and methods

2.1. Materials

Hydroxypropylmethylcellulose (HPMC; 4000 cP) was provided by Dow Chemical Company (Midland, MI, USA) and xanthan gum (XG; Satiaxine CX 931) by Cargill R & D (Vilvoorde, Belgium). Water (Bezoya, Segovia, Spain, with a calcium content 6.32 mg L^{-1}), oils; extra virgin olive oil (O) (Hacendado, Mercadona, Spain); refined sunflower oil (S) (Consum, Spain); and virgin flaxseed oil (F) (BIO CESTA, Spain) were purchased in supermarkets. The fatty acids compositions of oils (%, data provided by the supplier) were as follows: olive oil SFA: 14, MUFA: 78, PUFA: 8; sunflower oil SFA: 13, MUFA: 23, PUFA: 64; and flaxseed oil SFA: 11, MUFA: 18, PUFA: 71. Degree of oil unsaturation was measured using ISO 3961:2018 and the iodine values (g I_2 per 100 g oil) were 76.29 ± 2.01 for olive oil, 120.91 ± 6.08 for sunflower oil and 170.10 ± 7.98 for flaxseed oil.

2.2. Preparation of emulsions and oleogels

Based on the procedures described by Patel et al. (2014a) but with modifications we prepared emulsions and oleogels. HPMC (1 g) was dispersed in 38.4 g cold water using

a stirrer (Heidolph RZR 1, Schwabach, Germany) at 31 g for 30 min, with the resulting aqueous solution stored at 8°C overnight. Subsequently, 0.6 g of XG was added to the HPMC solution and stirred (Heidolph RZR 1) for 5 min, 60 g of oil was added and homogenised (Ultraturrax T18; IKA, Staufen, Germany) at 1230 g for 6 min. Three types of oil, O, S and F, were used in three emulsions formulations. The emulsions (EO, ES and EF) were dried, using two different drying conditions: conventional drying (C) in an oven (KB115; BINDER, Tuttlingen, Germany) at 80°C for 10 h 30 min, and vacuum drying (V) using a vacuum drying oven (Vaciotem-T, J.P. SELECTA, Spain) at 60°C/0.85 bar for 14 h. These were the minimum time points needed to reach constant dry weight at the indicated conditions. The dried products were homogenised at 728 g (Ultraturrax T18; IKA) to produce the oleogels. Five oleogels (OC, OV, SC, SV and FC) were produced in triplicate. The flaxseed oleogel could not be obtained when vacuum drying was used to dry the emulsions in the conditions established in this study.

2.3. Microstructure of emulsions and oleogels

Analysis was conducted with a Cryo-scanning electron microscopy (Cryo-SEM). The samples were frozen by immersion in slush nitrogen and transferred to a cryogenic unit (CT 15000 C; Oxford Instrument, Oxford, UK) connected to a scanning electron microscope JEOLJSM 5410 (JEOL, Tokyo, Japan). After fracturing, etching and being coated with gold, the samples were observed at 15 kV at a working distance of 15 mm. The droplet size of the emulsions and oleogels was determined using the software Image J (National Institutes of Health, Bethesda, MD, USA).

2.4. Oil loss of oleogels

Determination was made by the percentage of oil migration over 35 days at 20°C, using the method by Doan et al. (2016) with modifications. The weight of released oil was measured at time intervals 1, 2, 5, 7, 14, 21, 28 and 35 days. For this purpose, a funnel with a filter paper was positioned above an Erlenmeyer flask where the liquid oil from the oleogels dripped into. The weight of the funnel, the filter paper and the Erlenmeyer flask were measured (M1). Then, 10 g of oleogel was weighed (M3) and set into the funnel. Samples were removed at each time interval with a flat, small spatula. The weight of the funnel, the filter paper and the flask with the liquid oil released was measured again (M2). The results were expressed as g oil loss per 100 g oleogel, calculated using eqn 1 and were measured in triplicate for each sample.

$$Oil\ loss\ = \frac{M2-M1}{M3} \times 100 \qquad (1)$$

2.5. Oil viscosity and polarity

Viscosity was determined using a viscometer (Haake ViscoTester VT6R Plus, Thermo Scientific, Walthan, Ma, USA) equipped with spindle 1, at 60 rpm, at 25°C. The total polar components percentage (%TPC) was evaluated, using a Testo® 270 (Testo Inc., Sparta, NJ) at 45°C, as this equipment is designed to operate over the range 40 - 200°C.

2.6. Rheological measurements of emulsions and oleogels

Using a rotational rheometer (Kinexus Pro+, Malvern), equipped with a Peltier plate cartridge a series of tests were performed at 20°C with a parallel plate geometry (ϕ = 40 mm) and the geometry gap set at 1500 μm. For emulsion samples, amplitude sweeps (frequency = 1 Hz, stress = 1 - 500 Pa), frequency sweeps (stress = 10 Pa, frequency = 0.1 - 10 Hz), and flow measurements (shear rate 1 s-1 to 100 s-1) were conducted. For oleogels samples, amplitude sweeps (frequency = 1 Hz, stress = 1 - 1000 Pa), frequency sweeps (stress = 100 Pa, frequency = 0.1 - 10 Hz), and temperature sweeps (frequency = 1 Hz, stress = 100 Pa, temperature = 5 - 120°C) were conducted.

2.7. Oxidative stability of oleogels

Peroxide values (PV) and specific absorption in the visible ultraviolet (k_{232} and k_{270}) were used to study the oxidative stability of the oleogels during storage. The PV was analysed according to Cho and Lee, (2015) and k_{232} and k_{270} were determined according to ISO 3656:2011(ISO, 2011). All the samples were stored at 20°C for 35 days and were evaluated every 7 days.

2.8. Statistical analysis

The results were statistically analysed using the analysis of variance (ANOVA) with the least significant differences (LSD) calculated at a level of significance $p < 0.05$; the

statistical program Statgraphics Centurion XVI.II (StatPoint Technologies, Inc., Warrenton, VA, United States) was used.

3. Results and discussion

3.1. Microstructure of emulsions and oleogels

In the emulsions made with O and S (olive and sunflower oil, respectively) (Fig. 1a, b), the oil droplets with different sizes (ranging from 1 to 9 μm diameter) were distributed and trapped in a polymeric network with strong hydrocolloid-hydrocolloid interactions, probably stabilised by the addition of XG (xanthan gum), which increased the viscosity of the continuous phase and protected the oil droplets from coalescence (Patel et al., 2014a; Meng et al., 2018b). The interaction of XG with HPMC could also be increasing the thickness of the droplet surface layer (Meng et al., 2018b). In the F emulsion (flaxseed oil) (Fig. 1c), the hydrocolloid network had limited visibility with greater connections between the oil droplets, not properly separated by the hydrocolloid network.

The oleogels produced by convection drying of O (Fig. 1d) and S (Fig. 1e) emulsions were observed as well-structured with individual oil droplets separated by the hydrocolloid network. However, the flaxseed oleogel (Fig. 1f) presented oil droplets of greater size (up to 52 μm diameter) and irregular form and define edges between oil

droplets were lost in some areas. This indicates that during the conventional drying process, coalescence occurred between the flaxseed oil droplets. The polymeric network generated in flaxseed emulsions is evidently weaker, as the network has poor visibility in the emulsion; therefore, it is believed resists the drying process to a lesser extent, resulting in less homogeneous and unstructured oleogel (Fig. 1f). Oils with a low degree of unsaturation, such as olive and sunflower, form emulsions with strong polymeric networks stabilised by interactions between the structuring agents. This network resists the drying process resulting in oleogels with greater structural stability.

Vacuum drying produced oleogels from olive (Fig. 1g) and sunflower (Fig. 1h) oils, but not flaxseed. We believe that the expansion of the water vapour during vacuum drying led to a greater damage in the labile flaxseed network structure, where interactions between the polymer and the flaxseed oil occur, decreasing the interaction points between the hydrocolloids.

If the olive and sunflower oleogels obtained by both methods are compared, they all show a high degree of structuring. Thus, gel strength decreases when the interactions between polymer and oil are enhanced. Therefore, the nature of the oil and the gelling agent affects the formation process of physical polymer oleogels (Sawalha et al., 2012).

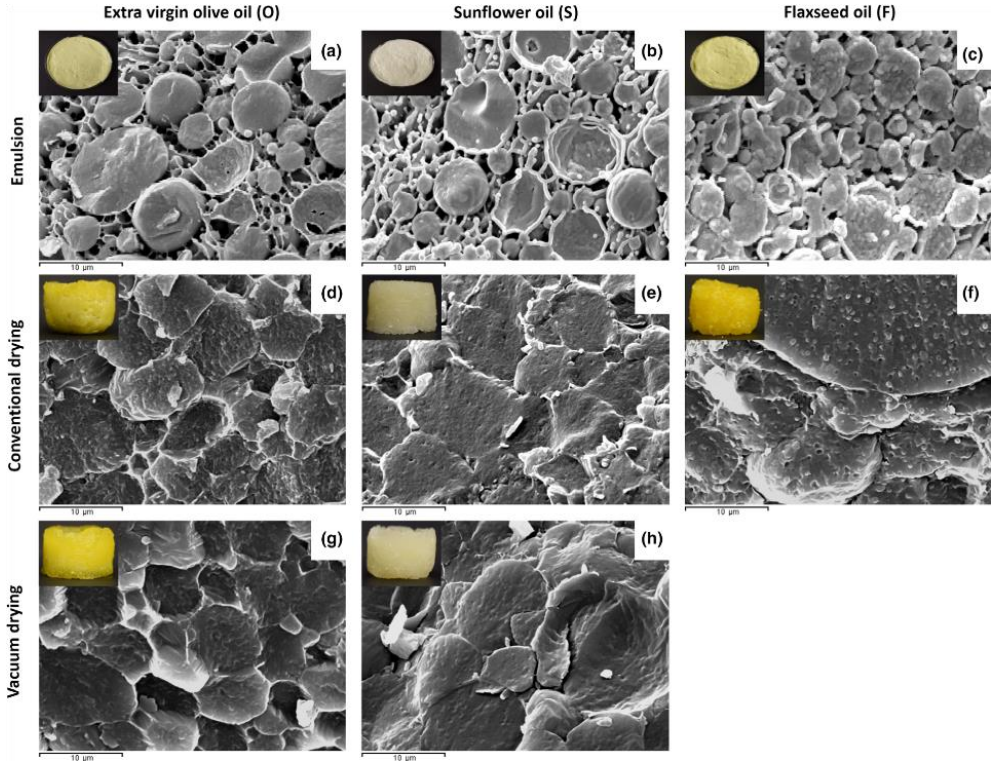

Figure 4. Cryo-scanning electron microscopy (Cryo-SEM) of emulsions (a-c), oleogels produced by conventional drying (d-f) and oleogels developed by vacuum drying (g, h). Images were taken at 3500 x. Pictures of oleogels are provided to present the concept more clearly.

3.2. Oil loss of oleogels

Fresh made oleogels had no oil loss; the greatest loss occurred during the first 24 h of storage (Table 1). This could be because of semi-crystallisation of the polymer network as suggested by Meng et al. (2018a, 2018b). These authors prepared oleogels with

different types of HPMC and different gums as arabic, guar or xanthan gum and obtained similar results for oil loss values.

For the oleogels made with olive oil (OC and OV) and sunflower oil (SC and SV), significant losses of oil were detected up to day 2 of storage, after values remained stable. Oleogel FC showed significant losses of oil up to day 21 of storage with stabilised values afterwards. No significant differences were found between oleogels made with the same type of oil depending on the drying treatment.

When oleogels, prepared with different oil but with the same drying treatment, are compared, no significant differences over total storage time are observed between those made with olive and sunflower oils. Concerning conventional drying, flaxseed oleogels (FC) presented significantly higher oil loss values over total storage time. This would be related to the structure of the oleogels shown in Fig. 1, since the freshly prepared olive and sunflower oleogels presented a better structure than those prepared with flaxseed.

Table 1. Oil loss (g oil loss/100 g oleogel) during storage at 20°C

Storage (day)	Conventional oven drying			Vacuum oven drying	
	OC	SC	FC	OV	SV
1	$9.14^{aA}(0.50)$	$9.68^{aA}(0.13)$	$11.49^{aB}(0.08)$	$9.36^{aA}(0.17)$	$9.80^{aA}(0.20)$
2	$9.56^{abA}(0.47)$	$10.06^{bA}(0.10)$	$11.88^{bB}(0.04)$	$10.15^{bA}(0.12)$	$10.06^{bcA}(0.17)$
5	$9.79^{bcA}(0.43)$	$10.12^{bA}(0.18)$	$12.47^{cB}(0.23)$	$10.35^{bcA}(0.02)$	$10.16^{bcA}(0.17)$
7	$9.89^{bcA}(0.44)$	$10.19^{bA}(0.19)$	$13.00^{dB}(0.06)$	$10.45^{cA}(0.02)$	$10.26^{cA}(0.19)$
14	$9.92^{cA}(0.47)$	10.22^{bA} (0.16)	$13.33^{eB}(0.01)$	$10.50^{cA}(0.07)$	$10.32^{cA}(0.19)$
21	$9.96^{cA}(0.49)$	$10.26^{bA}(0.15)$	$13.92^{fB}(0.05)$	$10.50^{cA}(0.07)$	$10.35^{cA}(0.19)$
28	$9.96^{cA}(0.49)$	$10.26^{bA}(0.15)$	$14.23^{fB}(0.02)$	$10.55^{cA}(0.02)$	$10.35^{cA}(0.19)$
35	$10.02^{cA}(0.40)$	$10.26^{bA}(0.15)$	$14.31^{fB}(0.11)$	$10.55^{cA}(0.02)$	$10.35^{cA}(0.19)$

Conventional (C) and vacuum (V) dried oleogels, prepared with extra virgin olive oil (OC and OV), sunflower oil (SC and SV) and flaxseed oil (FC). Values with different lowercase letters ($^{a, b... z}$) within the same column are significantly different ($p < 0.05$) according to the LSD multiple range test. Values with different capital letters ($^{A, B... Z}$) within the same row and the same drying treatment are significantly different ($p < 0.05$) according to the LSD multiple range test.

3.3. Oil viscosity and polarity

The apparent viscosity of the olive, sunflower and flaxseed oil used in this work were 95.67 ± 2.08, 76.33 ± 1.53 and 68.67 ± 3.51 mPa s, and their total polar components (%TPC) were 3%, 8% and 19%, respectively. Viscosity of the oils are inversely correlated with polarity (Kumar et al., 2013) and could be affecting the network formation and structure by modulating the network formation (Valoppi et al., 2017). Different correlations have been observed between the oil polarity and strength of the gel, depending on the gelling agent used. de Vries et al. (2017) reported that the gel

strength of the network, formed by proteins aggregates, was affected by the polarity of the oil, resulting in weaker oleogels when using a more polar oil. However, Gravelle et al. (2012) attributed the increase in mechanical strength of the oleogel to an increase in the polarity of the oils using ethylcellulose as gelling agent. We believe, because of its polarity, the linolenic fatty acids of flaxseed oil could interact with the HPMC-XG network, increasing the hydrocolloid-oil interactions, consequently forming a weak non-homogeneous structure of flaxseed oleogel (Fig. 1).

3.4. Rheological measurements of emulsions and oleogels

The dynamic viscoelastic properties of all the emulsions, the elastic modulus (G'), viscous modulus (G") and phase angle (δ) are shown in Fig. 2a. The limit of the linear viscoelastic region (LVR) in all the emulsions was ≈ 100 Pa (data not shown). In emulsions, G' values predominated G", showing greater elastic behaviour. A low G' and G" dependence on the frequency sweep and a phase angle of δ = 12°-15° suggested a system with a strong structure in all emulsions (Torres et al., 2007). Flaxseed emulsion (EF) presented a significantly higher G' value at 1 Hz ($G' = 1.41 \times 10^3 \pm 8.49 \times 10^1$ Pa) than EO ($G' = 1.15 \times 10^3 \pm 2.1 \times 10^2$ Pa) and ES ($G' = 1.06 \times 10^3 \pm 9.54 \times 10^1$ Pa), without significant differences between EO and ES. All the emulsions showed a strong shear thinning behaviour (Fig. 2b), with the apparent viscosity decreasing when the shear rate increased. The flaxseed emulsion presented a breakdown of the emulsion structure for the dynamic forces generated during shear, producing a marked decrease in the apparent viscosity. These results are in concordance with the microstructure of

emulsions, where the oil droplets of the flaxseed emulsion were observed homogeneously distributed and packed, but the network was not as visible, as in the other emulsions. This resulted in a weak structure when a shear rate was applied.

The oleogels had stronger mechanical strength, showing higher G' values than in emulsions (Fig. 2c) and G' ≫ G" over the 0.1-10 Hz of frequency sweep applied, indicating a marked solid-like behaviour. The network formation, after the water removal, is responsible of the different rheological behaviour. The limit of the linear viscoelastic region (LVR) in all the oleogels was ≈ 300 Pa (data not shown). All oleogels showed a weak dependence of G' on the frequency with a low phase angle $\delta \approx 5°$ indicating strong gel strength. Regarding the oil type and drying conditions, FC presented a significantly higher G' value at 1 Hz than OC ($G' = 8.86 \times 10^4 \pm 4.6 \times 10^3$ Pa), SC ($G' = 7.83 \times 10^4 \pm 4.95 \times 10^3$ Pa), OV ($G' = 9.78 \times 10^4 \pm 9.89 \times 10^3$ Pa) and SV ($G' = 6.88 \times 10^4 \pm 2.4 \times 10^3$ Pa), with no significant differences between OC, SC, OV and SV. Previously stated, during conventional drying, flaxseed oleogel presented a weak network structure (Fig. 1f) and a high oil loss (Table 1). The loss of the flaxseed oil during the rheological measurement could be the reason of a more compact network oleogel with higher G' value. Oleogels presented similar values to others prepared with 5-15% beeswax, propolis wax, a mixture of both (Fayaz et al., 2017), with a combination of 5% ethylcellulose (EC) and 5% surfactant (Gallego et al.,2013).

The elastic modulus (G') remained constant throughout the temperature sweep applied to the oleogels, up to temperatures of 120°C (Fig. 2d). The absence of a cross-over

point (G' = G") indicates that all the oleogels did not show a gel-sol transformation. Though the hydrogel systems produced by HPMC and oleogels based on EC and waxes are thermo-reversible (Chang & Zhang, 2011; Davidovich-Pinhas et al., 2015; Martins et al., 2017), the oleogels made with HPMC and XG did not have the same behaviour showing thermostable behaviour. Abdolmaleki et al. (2019) compared the formulation of oleogels based on sodium caseinate, xanthan gum, guar gum and drying method (freeze and oven drier) with an industrial shortening. For the industrial shortening, the oil binding capacity (OBC) and the storage modulus (Pa) were 99.5%, and 276 545 Pa ($\approx 10^5$), respectively. Our oleogels showed an OBC and storage modulus of the order of 90% and 10^4-10^5 Pa, respectively.

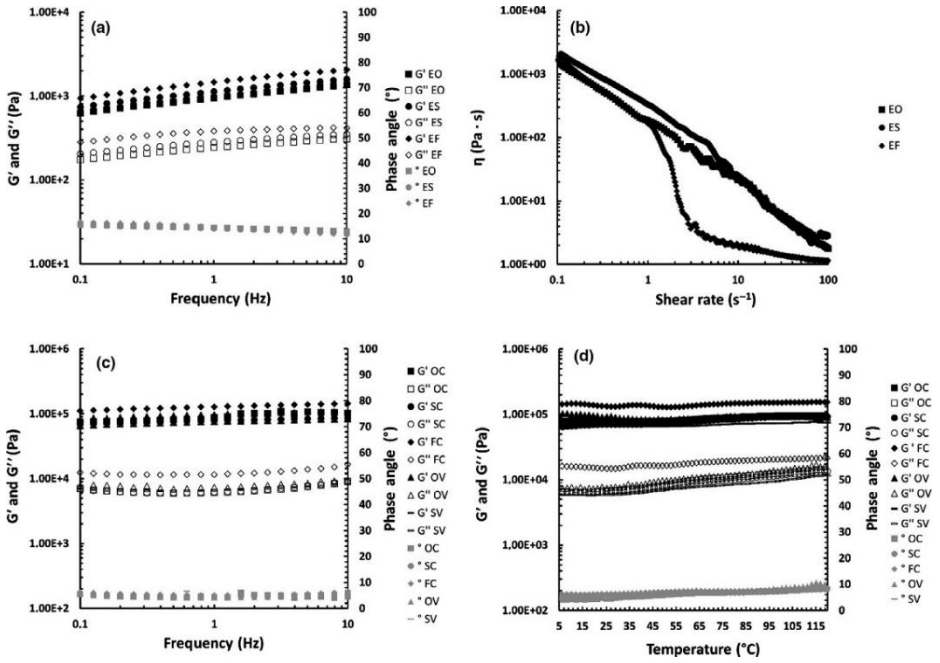

Figure 5. Frequency sweep (a) and flow measurement (b) for emulsions made with olive (EO), sunflower (ES) and flaxseed oil (EF). Frequency sweep (c) and temperature sweep (d) for oleogels developed with conventional (C) and vacuum (V) drying made with olive (OC and OV), sunflower (SC and SV) and flaxseed oil (FC).

3.5. Oxidative stability of oleogels

The Peroxide value (PV) of fresh oils is < 10 meq kg^{-1} (Codex Alimentarius, 2005). The acceptance limit in vegetable oils ranges between 15 and 20 meq kg^{-1}; actually, rancidity can be perceptible at low PV. The PV of the olive (O), sunflower (S), and, flaxseed oils (F) were 4.82 ± 0.14, 1.88 ± 0.12, and 5.08 ± 0.16 meq kg^{-1}, respectively.

Freshly made oleogels prepared by vacuum drying (OV and SV) had lower PV values than those prepared by conventional drying, with OV values like their correspondent oil. However, fresh OC, SC, and FC presented higher PV values than those of their oils (Table 2). All the oleogels had PV values below the limit of acceptance (< 20 meq kg^{-1}) after 35 days of storage, except conventionally dried flaxseed and sunflower oleogels (FC and SC). The oleogels prepared with olive oil, regardless of the treatment, showed significant increases in the PV values from day 14 of storage. Sunflower and flaxseed oleogels, prepared using conventional drying, showed significant increases of the PV values gradually throughout the storage period. Drying under vacuum minimised the primary oxidation of SC during the first week with increases in PV values detected from day 14.

When comparing the oleogels made with olive oil by convention and vacuum drying, no significant differences were found from day 14 until the end of storage, indicating an adequate oxidative stability. The degree of oxidation of SC (regarding PV values) was significantly more severe than SV, indicating that vacuum drying decreases the oxidation of the oil because of the extraction of air bubbles formed inside the gel.

If oleogels subjected to conventional drying are compared, OC presented values of PV significantly lower than the oleogels SC and FC during the entire storage period. This could be explained by the composition of fatty acids, since the high content of polyunsaturated fatty acids present in sunflower and flaxseed oil are more susceptible to autoxidation than the monounsaturated fatty acids present in olive oil (Lee et al.,

2007; Silalahi et al., 2017). Vacuum drying maintained similar PV values between the two types of oleogels (OV and SV) at days 14, 21, and 28, however, on day 35 of storage the OV had significantly lower values than SV.

Table 2. Peroxide value (meq kg^{-1}) during storage at 20°C

Storage (day)	Conventional oven drying			Vacuum oven drying	
	OC	SC	FC	OV	SV
0	6.13^{aA*}(0.16)	16.22^{aB*}(0.68)	16.56^{aB}(0.48)	4.81^{aB}(0.12)	3.07^{aA}(0.15)
7	6.35^{aA*}(0.08)	18.27^{bB*}(0.42)	19.33^{bC}(0.59)	4.95^{abB}(0.07)	3.60^{aA}(0.33)
14	7.31^{bcA}(1.09)	21.68^{cB*}(0.62)	22.75^{cC}(0.26)	6.21^{bcA}(0.57)	5.28^{bA}(0.52)
21	8.10^{cA}(0.27)	30.60^{dB*}(0.96)	33.11^{dC}(0.39)	7.57^{cA}(1.40)	6.20^{bA}(1.16)
28	10.64^{dA}(0.89)	34.71^{eB*}(0.94)	35.14^{eB}(1.21)	10.99^{dA}(0.89)	10.71^{cA}(0.82)
35	12.27^{eA}(0.75)	44.22^{fB*}(1.27)	46.34^{fC}(0.51)	13.09^{eA}(0.72)	18.38^{dB}(0.91)

Conventional (C) and vacuum (V) dried oleogels, prepared with extra virgin olive oil (OC and OV), sunflower oil (SC and SV), and flaxseed oil (FC). Values with different lowercase letters ($^{a, b... z}$) within the same columns are significantly different ($p < 0.05$) according to the LSD multiple range test. Values with different capital letters ($^{A, B... Z}$) within the same row and the same drying treatment are significantly different ($p < 0.05$) according to the LSD multiple range test. * means that there are differences ($p < 0.05$) between samples with the same oil type and different treatments.

The measurement of absorbance in the ultraviolet range (k_{232} and k_{270}) provides indications about the quality of an oil and the state of oil preservation. The k_{232} and k_{270}, are indicative, respectively, of conjugated trienes and the presence of carbonyl compounds (Malheiro et al., 2009). The k_{232} and k_{270} of the oils used in this study were 1.06 ± 0.05 and 0.05 ± 0.01 for olive oil, 3.40 ± 0.28 and 3.51 ± 0.14 for sunflower oil, and 2.4 ± 0.03 and 0.16 ± 0.01 for flaxseed oil, respectively. High initial values could be

for the refined sunflower oil's containing oil refining products that also absorb at 232 nm, and 270 nm (ISO 3656: 2011).

A gradual increase in k_{232} and k_{270} values throughout storage was detected for all the samples (Table 3) as previously observed by other authors. Bouaziz et al. (2008), and Borreani et al. (2017) observed a regular increase in k_{232} and k_{270} values as a function of storage for olive oils and for emulsions elaborated with HPMC and sunflower oil, respectively. Moreover, the values were significantly lower for vacuum dried oleogels than for conventionally dried, when comparing same type of oil. The use of vacuum drying allowed to reduce the drying temperature; thus oxidation was reduced. On the other hand, the water removing process adopted during vacuum drying could be decreasing the oxidation of the oleogel as reported by Meng et al. (2018c).

The oleogels made with olive oil presented the lowest values of k_{232} when they were prepared using either conventional or vacuum drying (OC or OV). Regarding k_{270} values, they were also lower for OV, however, when using conventional drying, FC had significantly lower k_{270} values.

Table 3. Oxidation spectrophotometric parameters k_{232} and k_{270} during storage at 20°C

	Conventional oven drying						Vacuum oven drying			
Storage (day)	**OC**		**SC**		**FC**		**OV**		**SV**	
	232 nm	**270 nm**	**232 nm**	**270 nm**	**232 nm**	**270 nm**	**232 nm**	**270 nm**	**232 nm**	**270 nm**
0	1.50^{abA*}(0.13)	0.88^{aB*}(0.10)	4.50^{aC*}(0.23)	5.33^{aC*}(0.15)	2.62^{aB}(0.19)	0.60^{aA}(0.07)	0.92^{aA}(0.04)	0.17^{aA}(0.07)	3.25^{aB}(0.03)	4.38^{aB}(0.09)
7	1.42^{aA*}(0.07)	1.11^{bB*}(0.13)	4.65^{abC*}(0.34)	5.52^{aC*}(0.34)	2.73^{aB}(0.23)	0.65^{aA}(0.07)	1.21^{bA}(0.11)	0.39^{bA}(0.10)	3.69^{bB}(0.23)	4.51^{aB}(0.13)
14	1.60^{abcA}(0.25)	1.03^{abB*}(0.07)	5.06^{bC*}(0.09)	5.50^{aC*}(0.19)	2.83^{aB}(0.12)	0.74^{aA}(0.10)	1.26^{bA}(0.13)	0.55^{bcA}(0.17)	4.27^{cB}(0.08)	4.76^{aB}(0.20)
21	1.75^{bcA*}(0.17)	1.04^{abA*}(0.15)	5.55^{cC*}(0.40)	5.62^{aB*}(0.19)	3.35^{bB}(0.26)	0.79^{abA}(0.19)	1.38^{bcA}(0.06)	0.66^{cdA}(0.01)	4.82^{dB}(0.07)	5.61^{bB}(0.24)
28	1.77^{bcA}(0.16)	1.14^{bA*}(0.11)	5.90^{cdC*}(0.15)	6.72^{bB*}(0.50)	3.46^{bB}(0.19)	0.96^{bcA}(0.12)	1.55^{cA}(0.25)	0.75^{dA}(0.05)	4.93^{dB}(0.03)	5.74^{bcB}(0.31)
35	1.91^{cA}(0.27)	1.39^{cA*}(0.06)	6.19^{dC*}(0.12)	7.16^{bB}(0.62)	3.61^{bB}(0.15)	1.08^{cA}(0.04)	1.61^{cA}(0.21)	0.78^{dA}(0.14)	5.25^{eB}(0.22)	6.12^{cB}(0.26)

Conventional (C) and vacuum (V) dried oleogels, prepared with extra virgin olive oil (OC and OV), sunflower oil (SC and SV), and flaxseed oil (FC). Values with different lowercase letters ($^{a, b... z}$) within the same columns are significantly different ($p < 0.05$) according to the LSD multiple range test. Values with different capital letters ($^{A, B... Z}$) within the same row and the same drying treatment are significantly different ($p < 0.05$) according to the LSD multiple range test. * means that there are differences ($p < 0.05$) between samples with the same oil type and different treatments.

Conclusions

Olive oil and sunflower oil oleogels using HPMC and XG as structuring agents were successfully produced using conventional and vacuum drying techniques. However, flaxseed oil oleogels, using vacuum drying, could not be produced as part of this study. Oleogels developed with flaxseed oil and conventional drying had a poorly organised structure with coalesced fat globules, resulting in oil loss during storage. The olive oil oleogel produced by conventional drying and the olive oil and sunflower oil oleogels produced by vacuum drying had primary and secondary oxidative stability values within the accepted limits. The evaluation of antioxidant incorporation in the oleogels, to minimise the deleterious effect of conventional drying temperature on sunflower oil quality, and the formulation of oleogels with oils with a high content in monounsaturated fatty acids would be interesting for future studies. This investigation provides a way to produce oleogels with potential applications in foods like bakery, meat and cream products.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

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

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Designing hydrocolloid-based oleogels with high physical, chemical, and structural stability tructure and stability of edible oleogels prepared with different unsaturated oils and hydrocolloids

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Abstract

Numerous studies conducted have shown a direct relationship between the high consumption of saturated and *trans*-fats and the risk of suffering from cardiovascular diseases, diabetes, and different cancers. Oleogels, with a suitable lipid profile of mono-poly-unsaturated fatty acids, and similar functionality to traditional solid fat, can be a healthy alternative in food formulation. The aim of this study is to develop edible oleogels with a healthy and stable lipid profile, using the emulsion-template approach and hydrocolloids as oleogelators. Oleogels were developed from sunflower oil and sunflower oil with a high content of monounsaturated acids, using hydroxypropylmethylcellulose (HPMC) and xanthan gum (XG) as oleogelators. The influence of two drying conditions (60°C for 24 h and 80°C for 10 h 30 min) along with the composition of the oil on the structural, physical, and oxidative stability of oleogels were studied. All oleogels presented a stable network and high physical stability with oil losses < 14% after 35 days of storage. Rheological properties showed that oleogels displayed a low frequency dependent and $G' > 10^5$ Pa related to solid gel-like behaviour. Oleogels made with sunflower oil rich in monounsaturated fatty acids resulted in higher oxidative stability, with those developed at drying temperatures of 80°C for 10 h 30 min having a greater structural and physical stability.

Keywords: Oleogelation, HPMC, xanthan gum, sunflower oil, rheological properties, peroxide value, light microscopy.

1. Introduction

Food products such as chocolate, ice cream, meat, butters, margarine, and bakery products, are formulated with considerable amounts of solid fats, rich in saturated and/or *trans*-fatty acids. Solid fats have a key role in improving quality attributes such as mouthfeel and texture. Several studies have reported the relationship between the negative cardiovascular effects and the increased consumption of saturated and *trans*-fatty acids (Mozaffarian and Clarke, 2009; Morenga and Montez, 2017). Therefore, authorities have regulated or provided some suggestions to limit consumption of many food products formulated with a large amount of saturated and/or *trans*-fats (Health Canada, 2012; Food and Drug Administration (FDA), 2015; European Union (EU), 2019). Thus, the food industry and food scientists show great interest to find new strategies and product formulations with a better nutritional profile, *trans*-fat free, low content in saturated fatty acids, and a high content in unsaturated fats (Moghtadaei et al., 2018; Pehlivanoglu et al., 2018; Luo et al., 2019).

Oleogels have gained popularity for their potential application in cosmetic and pharmaceutical industries (Vintiloiu and Leroux, 2008; Bastiat and Leroux, 2009) and with food processing (Singh et al., 2017). Oleogelation allows structuring high concentration liquid oil (>90%) into a "gel-like" system with viscoelastic properties (Rogers et al., 2009).

In many of these oleogels, gelation is achieved by using low molecular weight organogelators (LWOG) such as hydroxylated fatty acids (Rogers et al., 2008), waxes (Lim et al., 2017; Martins et al., 2017), and lecithin (Bodennec et al., 2016). Besides LWOG, there are structured systems where liquid oil is organized into a polymer network. Within polymer gelation, cellulose derivative, ethylcellulose (EC) is a non-aqueous gelator with the ability to produce oleogels using a direct approach (Laredo et al., 2011; Zetzl et al., 2012; Giacintucci et al., 2018). The most common limitations of the EC oleogels are the poor oxidative stability because of the high temperatures (>135-140°C) required to induce the polymer EC gelation (Gravelle et al., 2012). Therefore, using hydrocolloid-based oleogelators including different sources of proteins (Patel et al., 2015; de Vries et al., 2017) and polysaccharides like celluloses ethers, methylcellulose (MC) (Patel, et al., 2014a; Tanti et al., 2016a,b; Meng et al., 2018a), and hydroxypropylmethylcellulose (HPMC) (Patel et al., 2013; Oh and Lee, 2018; Oh et al., 2019; Bascuas et al., 2020), have attracted noticeable research attention. Hydrocolloids are widely used in food because of their commercial availability, large production, and low cost (Scholten, 2019; Abdolmaleki et al., 2020).

HPMC is a surface-active amphiphilic biopolymer and can be adsorbed to the oil droplet, protecting the oil droplets, thus, decreasing the amount of oil available for separation (Wollenweber et al., 2000; Li et al., 2013). Moreover, the addition of thickening agents, like XG, has shown an increase of the emulsion stability through bulk phase viscosity enhancement and interaction between the polysaccharides (Meng et al.,

2018b; Encina-Zelada et al., 2019). Since HPMC and XG have a predominantly hydrophilic characteristic, their dispersibilities are limited in non-polar solvents. To overcome this problem, HPMC and XG must first be hydrated in an aqueous solution. Foam-template and emulsion-template are the most indirect methods used in structuring edible oils with hydrocolloids. In the foam-template approach, a water soluble cellulose derivative is foamed and freeze-dried to create a porous structure and has been shown to absorb a large amount of oil (Patel et al., 2013). However, freeze-drying is an expensive and time-consuming technique (do Vale Morais et al., 2016). The emulsion-template approach, first prepared by Romoscanu and Mezzenga (2006) using proteins, comprises an indirect multi-step process. In this method, first, an oil-in-water emulsion is produced as a template stabilized by a combination of water soluble biopolymers. Second, the water phase is removed to drive the structure formation; finally, the dried product is homogenized to obtain an oleogel. In both methods, as the oil binding is purely physical, it is necessary to shear the oil-sorbed polymer obtain a strong gel (Patel et al., 2013; Oh et al., 2019).

Sunflower oil is one of the most attractive vegetable oils used by food industry and is one of the most ingested worldwide, with a domestic consumption of 18.07 million metric tons during 2018 - 2019 (United States Department of Agriculture (USDA), 2020). Because of its low cost and high overall acceptability, sunflower oil has been used to produce oleogels (Yang et al., 2017; Jiang et al., 2018; Okuro et al., 2018; Tavernier et al., 2018). However, the predominant unsaturated fatty acids present in sunflower

oils are susceptible to oxidation (Kozłowska and Gruczynska, 2018). Patel et al. (2014b) structured sunflower oil using MC and XG into solid-like oleogels using the emulsion-template approach; the drying of the emulsion in the oven (80°C for 32 h) gave oleogels with a poor oxidative stability. Developing strategies to improve the oil oxidative stability without influencing its nutritional and sensory properties, while maintaining the feasibility of use by food industry, represents an important advance in the quality of the oleogels made with hydrocolloids. Therefore, to improve the oxidative stability of oleogels, different strategies could optimize the processing conditions and the oil composition of the oleogel, favoring monounsaturated fatty acids (MUFA) with a longer oxidation induction period (Lee et al., 2007). It would be interesting to investigate the impact of using high MUFA oils and different processing conditions not only on the chemical stability, but also on the structural properties of oleogels based on water-soluble food polymers.

The objective of this work is to structure sunflower oil and sunflower oil with a high monounsaturated fatty acid content using the emulsion-template method, with HPMC and XG as oleogelators, to achieve oleogels with high structural, physical, and oxidative stability. For this, the influence of processing conditions and oil composition on the structure, physical, and chemical properties of oleogel will be compared and analyzed.

2. Materials and methods

2.1. Ingredients

Hydroxypropylmethylcellulose (HPMC "K4M"; 4000 cP) was provided by Dow Chemical Company (Midland, MI, United States) and xanthan gum (XG; Satiaxine CX 931) by Cargill R & D (Vilvoorde, Belgium). Water (Bezoya, Segovia, Spain, with a calcium content 6.32 mg L^{-1}, refined sunflower oil (fatty acids composition: SFA: 13, MUFA: 23, PUFA: 64, Consum, España), and high oleic sunflower oil (fatty acids composition: SFA: 10, MUFA: 65, PUFA: 25, Carrefour, España) were purchased in local supermarkets.

2.2. Oleogels preparation

Based on the procedures described by Patel et al. (2014a) with some modifications, we prepared oleogels using emulsion-template method, HPMC (1 g) was dispersed in 38.4g cold water and mixed using a stirrer (Heildolph RZR 1, Schwabach, Germany) at 1,010 rpm for 30 min, the resulting aqueous solution was stored at 8°C overnight. Subsequently, 0.6 g of XG was added to the HPMC solution and stirred (Heildolph RZR 1, Schwabach, Germany) for 5 min at 1,010 rpm, 60 g of oil was then added and homogenized (Ultraturrax T18, IKA, Germany) at 13,000 rpm for 6 min. The emulsions were spread on aluminum foil and dried in an oven (KB115, BINDER, Germany) using two different drying conditions: 80°C for 10 h 30 min, and 60°C for 24 h. These were the minimum times needed to reach constant dry weight (moisture:

2.31 ± 0.532% at 60°C; 1.59 ± 0.112% at 80°C) at the indicated conditions. The dried products were ground in a grinder (Moulinex A320R1, Paris, France) for 4 s to produce the oleogels. Four oleogels S60 (sunflower oil and drying at 60°C), SH60 (high oleic sunflower oil and drying at 60°C), S80 (sunflower oil and drying at 80°C), and SH80 (high oleic sunflower oil and drying at 80°C) were prepared in triplicate.

2.3. Microstructure of the oleogels

The microstructure of oleogels was studied by optical microscopy with a Nikon Eclipse 80i optical microscope (Nikon Co., Ltd., Tokyo, Japan) and incorporated camera (ExwaveHAD, model No. DXC-190, Sony Electronics Inc., Park Ridge, New Jersey, USA. UU.). The oleogels were cut with a cryostat (CM 1950, Leica) to obtain 20 μm thick sections that were placed on a glass slide. These sections were visualised by polarised light and by clear field microscopy using 2% Sudan as a staining agent to study the lipid fraction. The images were captured and stored at 1,280 x 1,024 pixels using the microscope software (NIS-Elements M, Version 4.0, Nikon, Tokyo, Japan).

2.4. Oil loss of oleogels

Determination was made by measuring the percentage of oil migration over 35 days at 20°C, using the method of Doan et al. (2016) with modifications. The weight of released oil was measured at intervals of 1, 7, 14, 21, 28, and 35 days. A funnel and filter paper was positioned above an Erlenmeyer flask collecting the dripping liquid oil from the oleogels. The weight of the funnel, the filter paper, and the Erlenmeyer flask were measured (M1). Then 10 g of oleogel was weighed (M3) and set into the funnel.

Samples were removed at each time interval with a flat, small spatula. The weight of the funnel, the filter paper, and the flask with the liquid oil released was measured again (M2). The results were expressed as g oil loss per 100 g oleogel, calculated using equation 1 and were measured in triplicate for each sample.

$$Oil\ loss\ = \frac{M2-M1}{M3} \times 100 \qquad (1)$$

The experimental data were fitted to a first-order equation using Solver software (Microsoft Excel):

$$OL = OLmax\ (1 - e^{-kt}) \qquad (2)$$

Where OLmax is the value of OL (oil loss) at sufficiently long (infinite) time, k is the kinetic constant, and t is the chosen time.

2.5. Oxidative Stability of Oleogels

Peroxide values (PV) and specific absorption in the visible ultraviolet (k_{232} and k_{270}) were used to study the oxidative stability of the oleogels during storage. The PV was analyzed using the acetic acid/chloroform solution method, according to Cho and Lee (2015), and k_{232} and k_{270} were determined according to ISO 3656:2011 (ISO, 2011), using a UV-VIS spectrophotometer (UV-VIS spectrophotometer, 1000, CECIL, UK). All the samples were stored at 20°C for 35 days and were evaluated every 7 days.

2.6. Rheological properties of oleogels

The rheological behavior of oleogels was evaluated using small amplitude oscillatory shear in a controlled stress rheometer [AR-G2, TA Instruments (Crawley, England)] with a Peltier heating system. A 20 mm diameter plate-plate sensor geometry with a serrated surface and a 1.5 mm gap was used. The oleogels rested for a 10 min equilibration time after reaching the measurement position.

Stress sweeps were conducted at a frequency of 1 Hz to measure the extent of the linear viscoelastic response. Frequency sweeps from 0.1 to 10 Hz at a stress wave amplitude (100 Pa) inside the linear region were performed. Storage modulus (G'), loss modulus (G"), and tan δ (G"/G') values were recorded. The testing temperature was always 20°C.

2.7. Statistical analysis

Results were statistically analyzed using analysis of variance (ANOVA) with the least significant differences (LSD) calculated at a level of significance $p < 0.05$. Statistical analyses were conducted using XLStat 2019 (Addinsoft, Barcelona, España).

3. Results and discussion

3.1. Oleogel microstructure

Figure 1 shows that the oleogel S60 constitutes a polymeric network that extends, forming branches that compartmentalize and trap fat globules (Figure 1A). However, accumulations of free and unstructured fat can also be seen (Figure 1E). This may be because of a coalescence phenomenon between fat globules, likely because the network formed by the structuring agents has not resisted the drying process and has not physically trapped all the fat globules (Figure 1E). Camino et al. (2009) and Wollenweber et al. (2000) studied the role of HPMC as a fat structuring agent; they found that this hydrocolloid could adsorb at the surface of fat globules, forming a viscoelastic multilayer structure because of a train loop tail conformation. In contrast, Patel et al. (2014a) studied the role of XG on the structure of oleogels, finding that XG can increase the viscosity of the emulsion by improving its stability in combination with MC during the drying process. In fact, they observed oil leakage on emulsions stabilized by using only cellulose derivates. Here, in the S80 oleogel, the hydrocolloids form a homogeneous network where most of the fat globules remain trapped (Figure 1F). In this oleogel structuring agents have a more homogeneous distribution and more structured fat (Figure 1B) than in the oil of S60 (Figure 1E) is observed. In oleogel SH60 the polymeric network formed by hydrocolloids is observed distributed throughout the oleogel (Figures 1C, G), surrounding the fat globules (Figure 1G).

However, unstructured fat and coalescence phenomena can also be observed. In oleogel SH80 hydrocolloids show a uniform and homogeneous network (Figure 1D) that surrounds and traps fat globules (Figure 1H). In SH80 the fat appears more structured than in the other oleogels, probably because the hydrocolloid network has a greater stability. Comparing the same type of oil oleogels undergone drying at 80°C, more stable polymeric networks exist, capable of retaining fat globules than those dried at 60°C. Probably, drying the emulsion at 60°C, which is slower than at 80°C, favors the attractive interactions between the xanthan gum helix (XG-XG), and weakens HPMC-XG interactions. This would lead to the formation of a weaker network when drying is carried out at 60°C if compared to 80°C (Carnali, 1992; Lapasin and Pricl, 1995). Monounsaturated sunflower oil seems to be retained more in the polymeric network than sunflower oil.

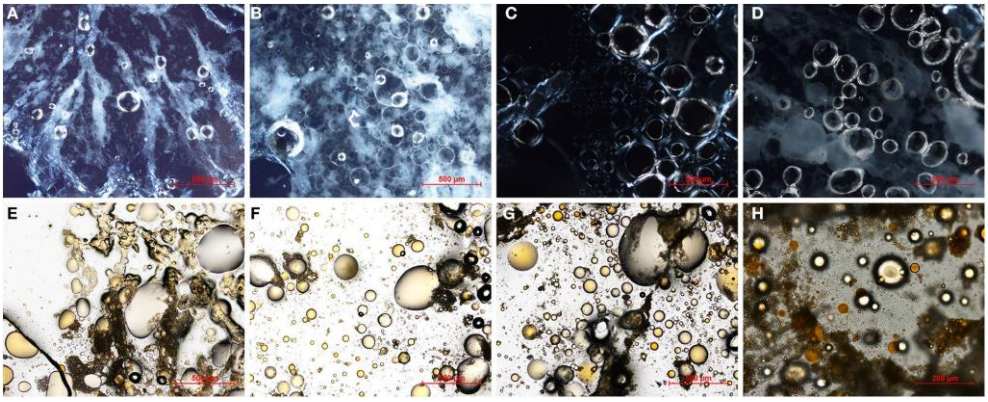

Figure 1. Optical microscopy micrographs of oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), and developed by conventional drying at 60°C (S60, SH60) and 80°C (S80, SH80), under (A-D): polarized light, (E-H): bright field with Sudan 2%.

3.2. Physical stability of Oleoges

The physical stability of oleogels is related to their ability to retain oil during storage. Figure 2 shows the proportion of oil loss at 1, 7, 14, 21, 28, and 35 days of storage at 20°C of all oleogels developed in this study. The experimental data were fitted to a first-order equation. Exponential decay kinetic model presented a R2 more than 0.99 for all cases (Table 1), evidencing the excellent fit between the formula and the experimental data.

Freshly made oleogels showed no oil losses, however the greatest loss of oil took place in all the oleogels studied during the first 24 h of storage. K values were significantly lower ($p < 0.05$) for S60 and SH60, indicating a slower loss of oil for these samples within the first 24h. However, OLmax values indicated that oleogels S80 and SH80 presented significantly lower ($p < 0.05$) amount of oil exuded over the whole storage, while S60 and SH60 oleogels had the highest values, without significant differences ($p < 0.05$) between them. Other authors (Meng et al., 2018c) also developed stable oleogels with soybean oil using the emulsion-template method, varying HPMC concentration (0.2-1%) and constant XG concentration (0.3%); however, the stability during storage was not studied. They suggested that the formation of semi-crystalline structure due to the hydrogen bonding within the chains of the polysaccharides, resulted in oleogels with high physical stability, especially from oleogel made with highest HPMC concentration. As explained before, a stronger network would be

obtained when drying at higher temperature; the strength of the network would be helping to prevent the oil release from the oleogel.

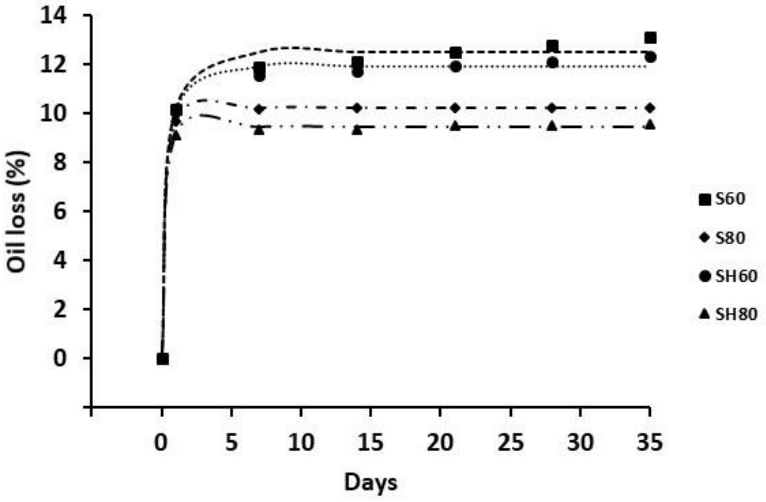

Figure 2. Oil loss curves for oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), and developed by conventional drying at 60°C (S60, SH60) and 80°C (S80, SH80).

Table 4. Kinetics parameters of the oil loss.

	S60	**S80**	**SH60**	**SH80**
OLmax (%)	12.49 ± 0.43 ^{B}	10.24 ± 0.19 ^{A}	11.94 ± 0.63 ^{B}	9.44 ± 0.42 ^{A}
K (days^{-1})	1.70 ± 0.05 ^{A}	2.91 ± 0.03 ^{B}	1.88 ± 0.47 ^{A}	3.41 ± 0.23 ^{B}
R^2	0.99	1.00	0.99	1.00

Oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), developed by drying at 60°C (S60 and SH60) and 80°C (S80 and SH80). Values with different capital letters (A, B... Z) within the same row are significantly different ($p < 0.05$) according to the LSD multiple range test.

3.3. Oxidative Stability

Table 2 shows the peroxide values (PV) of the oleogels stored for 35 days at 20°C. The upper limit for the PV of fresh oils is less than 15 - 20 meq kg^{-1} (Codex Alimentarius, 2001; Gómez-Alonso et al., 2004). PV > 20 correspond to very poor-quality fats and oils, which normally would have significant off flavours (O'Keefe and Pike, 2010). The PV of sunflower and high oleic sunflower oils used in this study were 3.24 ± 0.07 and 6.27 ± 0.29 meq kg^{-1}, respectively. These values indicate that both oils are suitable.

The oleogels S60, S80, SH60, and SH80 had initial PV of 12.86 ± 0.51, 16.22 ± 0.68, 7.78 ± 0.76, and 7.69 ± 0.07, respectively (Table 2). The process of making oleogels causes oxidative degradation of the oil, mainly in those formulated with sunflower oil. However, all the oleogels studied showed adequate initial PV, while oleogels made with high oleic sunflower oil (SH60 and SH80) presented significantly lower PV ($p < 0.05$) throughout storage, but with no significant differences ($p < 0.05$) between them. The oleogels made with sunflower oil (S60 and S80) had the highest PV, with S60 showing the highest PV throughout storage.

Regarding the behaviour of each oleogel, those made with sunflower oil (S60 and S80) showed a significant increase ($p < 0.05$) in PV throughout the entire storage, reaching values > 40 meq kg^{-1} at the end of the storage period. The oleogel made with high oleic sunflower oil (SH60), showed significant increases ($p < 0.05$) of PV on storage

days 14 and 28 and SH80 on days 14, 21, and 35, with both oleogels reaching PV around 15 meq kg^{-1} at the end of the storage period.

The specific UV extinction coefficient (k) at 232 nm and 270 nm is an estimator of fat deterioration. The k_{232} is normally considered an indicator of primary oxidation products, such as hydroperoxides and conjugated dienes. While k_{270} measures conjugated trienes (as secondary oxidation products), ketones, aldehydes, and primary oxidation products of linolenic acid (Maskan and Bağci, 2003; Tavakoli et al., 2017). The specific absorption values in the visible ultraviolet (k_{232} and k_{270}) of the oils used in the production of oleogels were 3.40 ± 0.28 and 3.51 ± 0.14 for sunflower oil, and 2.15 ± 0.02 and 0.78 ± 0.04 for high oleic sunflower. These values agree with Albi et al. (1997), who reported a k_{232} and k_{270} value for fresh sunflower oil of 4.70 and 3.15, and 2.32 and 0.83 for high oleic sunflower.

The oleogels S60, S80, SH60, and SH80 had initial values of k_{232} and k_{270} of 3.92 ± 0.50, 2.86 ± 0.01; 4.50 ± 0.23, 5.33 ± 0.15; 2.51 ± 0.05, 0.80 ± 0.08; and 2.20 ± 0.09, 0.86 ± 0.06; respectively (Table 3). The SH60 and SH80 oleogels showed significantly lower values of k_{232} and k_{270} ($p < 0.05$) throughout the storage period, without significant differences ($p < 0.05$) between them. While the S60 and S80 oleogels presented the highest values, with S60 showing the highest k_{232} values, and the lowest k_{270} values. In oleogels made with sunflower oil (S60 and S80) a significant increase ($p < 0.05$) of k_{232} values was observed throughout storage, while the k_{270} values remained stable while increasing at the end of storage, specifically on day 28.

In oleogels made with oleic-rich sunflower oil (SH60 and SH80), a significant increase in k_{232} values was observed every 14 days of storage, but from day 28 these values remained stable. The values of k_{270} remained stable in oleogel SH60 until day 7 of storage and after increased, mainly on day 14. In oleogel SH80 the values of k_{270} remained stable until day 21; on day 28 there was a significant increase ($p < 0.05$), but remained stable until day 35. The oleogels made with oleic-rich sunflower oil were more stable to oxidation than the oleogels made with sunflower oil. The high content in MUFA in SH60 and SH80 oleogels would be helping to delay oxidation, as MUFA have longer induction periods than PUFA, which are major components in S60 and S80 oleogels.

Table 2. Peroxide value (meq kg $^{-1}$) during storage at 20°C

Storage (days)	S60	S80	SH60	SH80
0	12.86 ± 0.51 ^{aB}	16.22 ± 0.68 ^{aC}	7.78 ± 0.76 ^{aA}	7.69 ± 0.07 ^{aA}
7	19.62 ± 1.64 ^{bB}	18.27 ± 0.42 ^{bB}	8.47 ± 0.62 ^{aA}	9.00 ± 0.52 ^{aA}
14	27.07 ± 0.56 ^{cC}	21.68 ± 0.62 ^{cB}	11.08 ± 0.17 ^{bA}	10.58 ± 0.18 ^{bA}
21	32.69 ± 1.43 ^{dC}	30.60 ± 0.96 ^{dB}	11.90 ± 0.14 ^{bA}	12.13 ± 1.26 ^{cA}
28	39.35 ± 0.95 ^{eC}	34.71 ± 0.94 ^{eB}	14.63 ± 0.32 ^{cA}	13.39 ± 1.29 ^{cdA}
35	46.81 ± 1.70 ^{fC}	44.22 ± 1.27 ^{fB}	15.49 ± 1.13 ^{cA}	14.66 ± 0.39 ^{dA}

Oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), developed by drying at 60°C (S60 and SH60) and 80°C (S80 and SH80). Values with different lowercase letters (a, b... z) within the same column are significantly different ($p < 0.05$) according to the LSD multiple range test. Values with different capital letters (A, B... Z) within the same row are significantly different ($p < 0.05$) according to the LSD multiple range test.

Table 3. Oxidation spectrophotometric parameters k_{232} and k_{270} during storage at 20°C

Storage (day)	S60		S80		SH60		SH80	
	232 nm	270 nm	232 nm	270 nm	232 nm	270 nm	232 nm	270 nm
0	3.92 ± 0.50^{aC}	2.86 ± 0.01^{aB}	4.50 ± 0.23^{aB}	5.33 ± 0.15^{aC}	2.51 ± 0.05^{Aa}	0.80 ± 0.08^{aA}	2.20 ± 0.09^{Aa}	0.86 ± 0.06^{aA}
7	4.74 ± 0.78^{bB}	2.69 ± 0.19^{aB}	4.65 ± 0.34^{abB}	5.52 ± 0.34^{aC}	2.63 ± 0.16^{aA}	0.83 ± 0.11^{abA}	2.29 ± 0.14^{aA}	1.00 ± 0.12^{abA}
14	$5.48 \pm 0.74b^{cB}$	3.06 ± 0.17^{abB}	5.06 ± 0.09^{bB}	5.50 ± 0.19^{aC}	3.20 ± 0.04^{bA}	1.29 ± 0.12^{dA}	2.74 ± 0.15^{bA}	0.90 ± 0.099^{aA}
21	5.77 ± 0.57^{cB}	3.05 ± 0.47^{abB}	5.55 ± 0.40^{Cb}	5.62 ± 0.19^{aC}	3.20 ± 0.22^{bA}	1.23 ± 0.16^{cdA}	3.01 ± 0.32^{bA}	0.97 ± 0.24^{aA}
28	6.67 ± 0.06^{dC}	3.46 ± 0.17^{bB}	5.90 ± 0.15^{cdB}	6.72 ± 0.50^{bC}	3.71 ± 0.24^{cA}	1.06 ± 0.12^{cA}	3.36 ± 0.08^{cA}	1.20 ± 0.09^{bA}
35	6.88 ± 0.28^{eC}	2.92 ± 0.15^{aB}	6.19 ± 0.12^{dB}	7.16 ± 0.62^{bC}	3.67 ± 0.40^{cA}	1.04 ± 0.16^{bcA}	3.37 ± 0.21^{cA}	1.22 ± 0.003^{bA}

Oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), developed by drying at 60°C (S60 and SH60) and 80°C (S80 and SH80). Values with different lowercase letters (a, b... z) within the same column are significantly different ($p < 0.05$) according to the LSD multiple range test. Values with different capital letters (A, B... Z) within the same row are significantly different ($p < 0.05$) according to the LSD multiple range test.

3.4. Rheology

To better understand the structural changes, the dynamic mechanical spectra were studied. The viscoelastic properties of the samples are shown in Figure 3. Over the entire frequency range studied (0.1-10 Hz) (Figure 3A), a low G' and G" dependence with frequency was observed, suggesting and all oleogels presented an elastic modulus (G') higher than viscous modulus (G"), indicating a typical behavior of solid gels (Guenet, 2016). Luo et al. (2019) obtained comparable low frequency dependent results from camellia-oil based oleogels structured with tea polyphenol-palmitate and varying citrus pectin concentration. Meng et al. (2018c) dried soybean emulsions in a vacuum drying oven at 90 °C and analyzed the frequency sweep of oleogels formulated with 0.2 – 1% of HPMC (400 and 1500 cP) and 0.3% XG, showing similar dependency on frequency, but a lower value of G', which may be for the viscosity of HPMC (4000 cP) and the higher XG concentration (0.6%) used in our study. The loss tangent (tan $\delta = G''/G'$) of oleogels, showed a similar trend, with a tan $\delta \approx 0.1$, confirming the existence of a strong internal network (Figure 3B). These results were corroborated analysing statistical differences at 1 Hz (Table 4) with no significant differences between oleogels for the dynamic modulus (G' and G") and tan δ values higher for the S80 and SH80 oleogels, indicating that these samples had lower viscoelastic behavior.

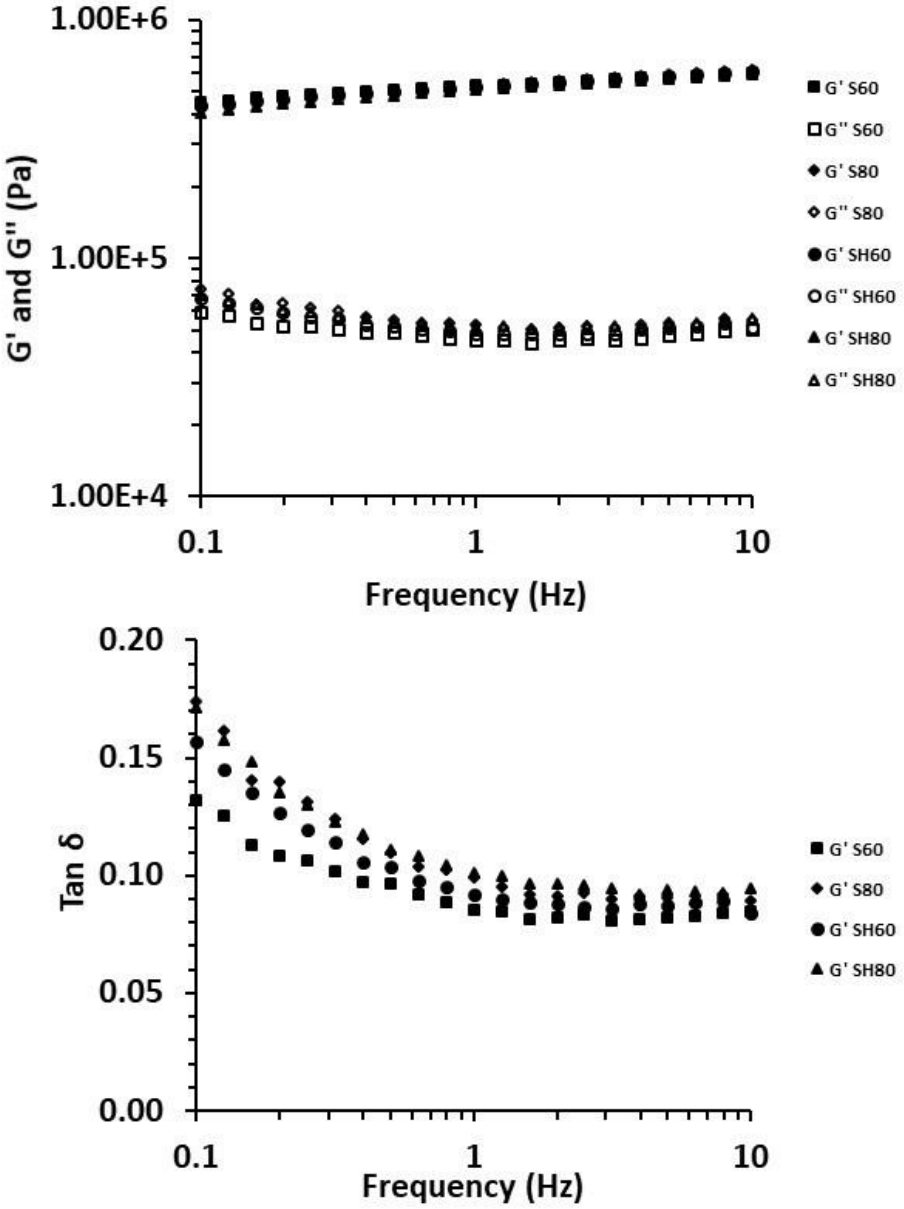

Figure 3. Oil loss curves for oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), and developed by conventional drying at 60°C (S60, SH60) and 80°C (S80, SH80).

Table 4. Viscoelastic rheological parameters (at 1 Hz) of oleogels.

	G' (Pa)	G" (Pa)	tan δ
S60	513,040 ± 25,686^{a}	48,291 ± 6,085^{a}	0.094 ± 0.010^{ab}
S80	604,043 ± 81,836^{a}	64,035 ± 15,816^{a}	0.105 ± 0.012^{b}
SH60	559,315 ± 37,206^{a}	48,100 ± 1,884^{a}	0.086 ± 0.005^{a}
SH80	545,941 ± 32,973^{a}	55,753 ± 3,706^{a}	0.102 ± 0.001^{b}

Oleogels prepared with sunflower (S) and high oleic sunflower oil (SH), developed by drying at 60°C (S60 and SH60) and 80°C (S80 and SH80). Values with different lowercase letters (a, b... z) within the same column are significantly different ($p < 0.05$) according to the LSD multiple range test.

Conclusions

It is possible to develop physically, chemically, and structurally stable oleogels using sunflower oil and sunflower oil with a high content monounsaturated fatty acids, using HPMC and XG as structuring agents with the emulsion-template method and the drying conditions (60°C for 24 h and 80°C for 10 h 30 min). The stability of oleogels during storage is influenced by the composition of the oil and the drying conditions of the oleogel process. However, oleogels made with sunflower oil high in monounsaturated fatty acids show better oxidative stability during storage than those made with sunflower oil, regardless of the drying conditions used. Furthermore, drying at 80°C for 10 h 30 min generates oleogels with greater structural and physical stability than drying at 60°C for 24 h, regardless of the oil composition of the oleogel. Therefore, the oxidative stability of the oleogel is greatly influenced by the type of oil, improving when the oil has a high content of monounsaturated fatty acids, still, the

drying conditions (time and temperature) have a marked influence on the structural and physical stability.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

SB: experimental work and writing the draft manuscript. AS: investigation and methodology. IH: writing–review & editing and funding acquisition. AQ: supervision and funding acquisition. All authors contributed to the article and have approved the final manuscript.

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Capítulo 2

Reformulación de alimentos mediante la incorporación de aceites de alto valor nutricional estructurados con hidrocoloides

Structural and sensory studies on chocolate spreads with hydrocolloid-based oleogels as a fat alternative

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Abstract

In this study, chocolate spreads were designed using oleogels with two oils (olive and sunflower), and hydroxypropylmethylcellulose (HPMC) and xanthan gum (XG), as structuring agents. Thus, the lipid profile of the spreads can be improved by totally or partially replacing the coconut butter used in their preparation. Structural behaviour was analysed using confocal laser scanning microscopy (CLSM), small amplitude oscillatory rheology, and a spreadability test. A Free Choice Profile analysis was performed by consumers to determine the sensorial attributes that described the chocolate spreads. The results showed that the oleogels conferred consistency to the spreads because of the network formed by HPMC and XG. However, while coconut butter replacement at 50% gave a similar structure to the control spread, 100% replacement resulted in less homogeneous spreads. This trend might be attributed to the chemical compatibility between the oleogel-coconut butter, which led to stronger systems. Sensory evaluation showed that chocolate spread replaced by sunflower oleogel at 50% presented sensory attributes like the control spread with "creamy appearance", "creamy texture", and "cocoa flavour". Therefore, using oleogels can be a viable and healthy alternative to replace the saturated fat present in chocolate spreads.

Keywords: HPMC; xanthan gum; CLSM; rheology; texture.

1. Introduction

Consumption of certain foods have a profound impact on health. To prevent a multitude of pathologies, such as obesity, cardiovascular disease, and cancer, it is necessary to follow healthy dietary guidelines. Currently, many foods contain solid fats, which have a high content of saturated and/or *trans* fatty acids; these fats are widely used in the food industry for their palatability, functionality, and texture (Co & Marangoni, 2012; Liu, Xu, & Guo, 2007; Pehlivanoglu et al., 2018). However, because of its influence on the sensory and functional properties of food (Pehlivanoglu et al., 2018), achieving the optimal substitution of solid fat is still a challenge (Lim, Inglett, & Lee, 2010). Previously, studies have partially or totally replaced solid fats in different foods with sources of lipids (Jacob & Leelavathi, 2007), carbohydrates (Gibis, Schuh, & Weiss, 2015; Rodríguez-García, Laguna, Puig, Salvador, & Hernando, 2013; Zahn, Pepke, & Rohm, 2010), and proteins (Paglarini, Martini, & Pollonio, 2019; Youssef, & Barbut, 2009).

Recently, the replacement of saturated and/or *trans* fats in food products has been studied making oleogels with high nutritional quality oils (Fayaz et al., 2017a; Pehlivanoglu et al., 2018). Edible oleogels are defined as a solid-like material with oil immobilised in a three-dimensional network with gelling capacity (Huang, Hallinan, & Maleky, 2018). Oleogelification is an emerging technology that allows semi-solid properties to be conferred to oils, without modifying their chemical characteristics (Lim,

Jeong, Oh, & Lee, 2017; Luo et al., 2019; Patel et al., 2014a). For this, gelling agents are required to obtain an oleogel with textural properties like solid fat (Co & Marangoni, 2012; O'Sullivan, Barbut & Marangoni, 2016). Hydroxypropylmethylcellulose (HPMC) is a biopolymer used as a stabilising additive in the food industry, because of its commercial availability and low cost (Abdolmaleki, Alizadeh, Nayebzadeh, Hosseini, & Shahin, 2019). Several studies confirm HPMC as an oleogelator (Bascuas, Hernando, Moraga, & Quiles, 2020; Meng, Qi, Guo, Wang, & Liu, 2018a; 2018b; Oh & Lee, 2018; Patel & Dewettinck, 2015; Tanti, Barbut, & Marangoni 2016a; 2016b) because it provides viscosity-enhancing properties (Zhao et al., 2009), giving oleogels with viscoelastic properties and high oil retention (Meng, Qi, Guo, Wang, & Liu, 2018c; Patel & Dewettinck, 2015). Besides, the addition of thickeners, such as xanthan gum (XG), increases the viscosity of the aqueous continuous phase of the emulsions, favouring the stability of the HPMC oleogels (Meng et al., 2018a; Patel et al., 2014a).

One industry that could benefit from the substitution of fat for oleogels is the spreads industry, where products are used in pastries and confectionery, and are mostly made of fat and sugar (Demirkesen, & Mert, 2019; Manzocco, Calligaris, Camerin, Pizzale, & Nicoli, 2014). The fat content of these spreads can reach up to 60% and largely determines the sensory, rheological, and textural properties, because of the network of fatty crystals (Espert, Salvador, Sanz, & Hernández, 2020; Miele, Di Monaco, Masi, & Cavella, 2015). Consequently, the substitution of solid fat for liquid oil can greatly affect the chocolate spread's performance and, therefore, the quality of the product (Fayaz et

al., 2017b). Our group has recently characterised the microstructure, rheological behaviour, and oxidative stability of oleogels prepared using HPMC and XG, indicating that sunflower or olive oleogels could have potential food applications (Bascuas et al., 2020). To date, very few studies exist regarding using oleogels in spreads. Fayaz et al. (2020b) and Patel, Rajarethinem, et al. (2014) studied the incorporation of wax- and shellac-based oleogels, respectively, in chocolate spreads but yet, no studies investigate using biopolymer-based oleogels in chocolate spreads.

The objective of this study is to evaluate the feasibility of using olive and sunflower oil oleogels, made with biopolymer oleogelators, to replace saturated fat in chocolate spreads by studying their structural properties (microstructure, rheology, and texture) and sensory attributes.

2. Materials and methods

2.1. Ingredients

Hydroxypropylmethylcellulose (HPMC; 4000 cP) was provided by Dow Chemical Company (Midland, MI, United States) and xanthan gum (XG; Satiaxine CX 931) by Cargill R & D (Vilvoorde, Belgium). Water (Bezoya, Segovia, Spain, with a calcium content 6.32 mg/l), olive oil (O) (Hacendado, Mercadona, Spain), refined sunflower oil (S) (Consum, Spain), sugar (Disem, Spain), skimmed powder milk (1 g/100 g fat) (Central Lechera Asturiana, Spain), and cocoa powder (Chocolates Valor S.A., Alicante,

Spain) were purchased in supermarkets. Coconut butter was supplied by Gracomsa (Catarroja, Valencia, Spain).

2.2. Oleogels preparation

The oleogels were prepared as described by Bascuas et al. (2020), and the emulsions were vacuum dried (Vaciotem-T, J.P. SELECTA, Spain) at 60°C and -0.85 bar for 14 h. The dried products were ground in a grinder (Moulinex A320R1, Paris, France) for 5 s to produce the oleogels. Two oleogels were produced using olive oil; olive oleogels and sunflower oil; sunflower oleogels.

2.3. Spreads preparation

Chocolate spreads were prepared using the proportions shown in Table 1. One control spread with coconut fat (C) and four spreads with partial or total fat coconut replacement, by the oleogel, were made. Thus, a spread with 50% replacement of coconut fat by olive oil (CO), a spread with 50% replacement of coconut fat by sunflower oil (CS), and two 100% replacements of coconut fat by olive oil (O) or sunflower (S) oleogel were obtained. The rest of the ingredients used were common in all the formulations.

A food processor (TM31 Thermomix, Vorwrek, Wuppertal, Germany) was used to mix the ingredients. First, sugar, skimmed powder milk, cocoa powder, and mineral water were mixed at 70°C for 6 min at speed 2. After cooling at room temperature, fat (coconut butter and/or oleogel) was added and mixed in the processor for 3 min at

speed 2. To achieve a suitable spread texture, the speed of the Thermomix was increased to 4 and mixed for 2 min, and to 5 for 1 min. After the spread was made, it was refrigerated at 5°C. All analyses were performed 24 h after the spreads were made.

Table 1. Composition of the studied chocolate spreads.

Ingredients	g / 100 g				
	C	**CO**	**CS**	**O**	**S**
Sugar	32.5	32.5	32.5	32.5	32.5
Oleogel	-	15	15	30	30
Coconut butter	30	15	15	-	-
Water	20	20	20	20	20
Skimmed powder milk	12.5	12.5	12.5	12.5	12.5
Cocoa poder	5	5	5	5	5

Control spread made with coconut fat (C); spread made with 50% coconut fat and 50% olive oleogel (CO); spread made with 50% coconut fat and 50% sunflower oleogel (CS); spread made with olive oleogel (O); spread made with sunflower oleogel (S).

2.4. Structural properties

2.4.1. Microstructure analysis

Confocal scanning laser microscopy (CLSM) was conducted using a ZEISS 780 microscope coupled to an Axio Observer Z1 inverted microscope (Carl Zeiss, Germany). To visualise the samples, the C-Apochromat 40X/1.2 W water immersion objective was used. The images were obtained and stored with a resolution of 1024 x 1024 pixels using the microscope software (ZEN). The stains used were Nile Red, Fluorescein (FITC), and Calcofluor White (Fluka, Sigma-Aldrich, Missouri, USA). The

Nile Red was used to detect fat, it was excited with the 561 laser line and was detected between 576 and 620 nm, the FITC stained protein and was excited with the 488 laser line and was detected between 499 and 525 nm, Calcofluor White stained polysaccharides and was excited with the diode line 405 and detected between 410 and 477 nm.

To observe and study the spread, a small amount of sample was placed on a slide, 20 µL of Nile Red solution was added and it was left to rest for 10 min. The same procedure was performed with FITC and Calcofluor White, and samples were covered with a glass coverslip.

2.4.2. Rheological measurements

To perform the rheology measurements, an AR-G2 controlled stress rheometer (TA Instruments®, New Castle, USA), coupled to a computer system (TA Instruments Universal Analysis 2000 Software) was used. A serrated plate-plate (40 mm) was used in all the experiments, with a gap of 1 mm. The tests were performed at 20°C in duplicate. Small amplitude oscillation sweeps (SAOS) were performed to analyse the viscoelastic properties. To determine the extent of the linear viscoelastic region (LVR) stress sweeps (from 0.1 to 200 Pa with a logarithmic distribution, 10 points per decade) were conducted at 1 Hz. Frequency sweeps were performed between 0.01 and 10 Hz within the linear region (at 5 Pa) with values of storage modulus (G'), loss modulus (G''), and tan δ recorded.

2.4.3. Texture measurements

A TA-XT plus Texture Analyzer equipped with the Texture Exponent software (Stable Microsystems, Godalming, UK) was used to determinate the texture properties of the samples.

Spreadability of samples was measured using a TTC Spreadability Rig (HDP/SR) attachment. Samples were filled into a female cone (90° angle), with special attention made to avoid bubbles formation, and were penetrated 22.5 mm using the corresponding male cone (90° angle) at a speed of 1 mm/s. Force expressed in N, as a measurement of firmness, and area under the curve (AUC; N s), as a measurement of spreadability, were recorded. Experiments were performed in duplicate.

2.5. Sensory analysis

The sensory analysis was conducted 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).

Twenty untrained consumers (60% women, 40% men), with ages between 25 and 50 years old, took part in a Free Choice Profile analysis. In the first session, the terms used by each consumer describing the differences among spreads were generated by a Repertory Grid Method (RGM). Three samples were presented and each consumer described the similarities and differences among samples in their own terms. Consumers

evaluated the appearance, taste, aroma and texture of the different spreads. In the second session, each consumer used their 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 served in white plastic cups labelled with random three-digit codes and served at room temperature in random order following a Williams design. They were asked to evaluate the appearance by observing the sample, the aroma and after eating a spoonful of the sample with a plastic spoon evaluate the taste and texture. Water was provided to clean the palate between samples.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was applied to study the effects of fat on the rheological and texture parameters studied. The least significant differences (LSD) were calculated using the Fisher's test, and the significance was determined at $p < 0.05$. A Generalized Procrustes Analysis (GPA) was applied to the Free Choice Profile data. XLSTAT statistical software (2010.5.02 (Addinsoft, Barcelona, Spain)) was used.

3. Results and discussion

3.1. Stuctural properties

3.1.1. Microstructure analysis

Fig. 1 shows the distribution of ingredients in the spreads studied using CLSM together with the pictures of the different spreads. Using staining agents Nile Red, FITC, and Calcofluor White, the components of the spreads observed were fat in red, protein in green, and polysaccharide in blue. In the images stained with Nile Red and FITC (left column), the control spread (C) (Fig. 1a) comprises a continuous phase of green colour, consisting mostly of protein, in which a second continuous phase of fat stained with Nile Red is distributed and diffused homogeneously. There is a part of the spread's fat that forms small globules; however, another part is homogeneously distributed and interacts with the components of the matrix. Distributed in the matrix, grey particles can be seen, possibly corresponding to undissolved sugar (Fig. 1a). In images stained with Nile Red and Calcofluor (Fig. 1f), there is a homogeneous distribution of fat, stained pink, merged with the remaining components making up the continuous phase. These images show also isolated blue particles, which probably correspond to undissolved cellulosic material of cocoa.

When the spreads are made with a mixture of oleogel and coconut fat (CO and CS), their appearance is similar, regardless of the oleogel used (Fig. 1b and g; 1c and h). Furthermore, their structure is more like the control spread than those made only with

oleogels (O and S) (Fig. 1d and i; 1e and j). In CO and CS spreads, the fat remains structured by the hydrocolloids used to make the oleogel: HPMC and XG. The presence of these polysaccharides is believed to partially prevent coalescence, because of the formation of a hard and thick interface layer limiting the mobility of the globules inside the matrix (Borreani et al., 2017; Meng et al., 2018a; Patel et al., 2014a). In addition, like the control spread, cocoa particles are distributed throughout the spread, along with possible HPMC segments, not adsorbed at the droplet surface. As observed previously by Meng et al. (2018c), in soybean oil oleogels structured by cellulose ethers, only few segments of the HPMC chain are adsorbed at the droplet surface because of its rigid backbone, with the hydrophilic segments stretching to the aqueous phase.

In spreads with 100% replaced coconut fat using olive and sunflower oleogels (O and S) (Fig. 1d and e), part of the fat is bound with the protein, constituting a continuous phase which contains sugar and cocoa particles distributed in the spread. However, large fat globules (black arrows in Fig. 1d and e) can also be observed. Therefore, a different fat distribution can be seen if compared to C, CO, and CS spreads, which show a more homogeneous fat distribution. These larger fat globules are observed in pink, trapped in the three-dimensional hydrocolloids network stained in blue by Calcofluor (Fig. 1i and j). This structure has been previously described by Espert et al. (2017) and Meng, Qi, Guo, Wang, and Liu (2018b), in emulsions and in oleogels prepared with polysaccharides, respectively. In Fig. 1i and j, incipient coalescence is observed, especially in the S spread (black arrows). This destabilisation phenomena can determine

many of the important properties of food such as appearance, shelf life, flavour profile, texture, and release characteristics (McClements, 2015). O and S spreads also show several undissolved particles stained in grey (red arrows), greater than in CO and CS spreads, likely corresponding to crystallised sugar and non-cellulose cocoa fibre. It can be speculated that the process of making the spreads had a different effect on their stability. In spreads O and S, the shear force used resulted in a less structured and homogeneous spread with several undissolved particles, while the spreads with a mixture of saturated fat and oleogel (CO and CS) gave a structure like the C spread, with a higher proportion of fused components. Other studies have shown that incorporating a little solid fat in oleogels improves their structure. Patel (2015) observed that incorporating palm stearin in oleogels made with HPMC allowed a full recovery of the structure after the shear force applied, stopped.

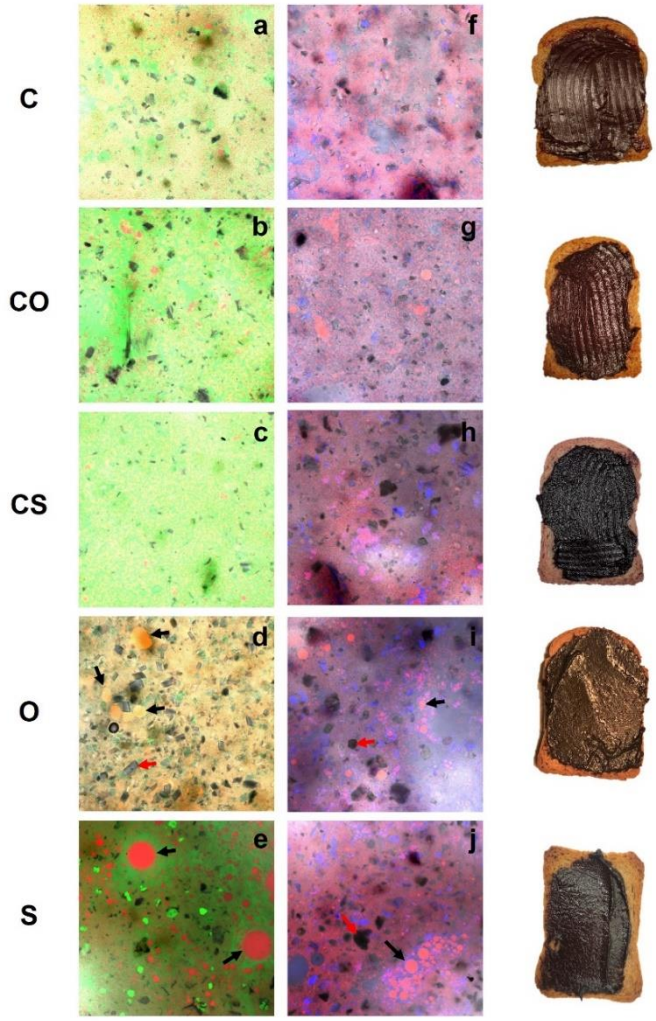

Figure 6. Images taken with a confocal scanning laser microscope (CLSM) of the different chocolate spreads. Staining with Nile Red and FITC (a–e); staining with Nile Red and Calcofluor (f–j). (a) and (f): Control spread made with coconut fat (C); (b) and (g): spread made with 50% coconut fat and 50% olive oleogel (CO); (c) and (h): spread made with 50% coconut fat and 50% sunflower oleogel (CS); (d) and (i): spread made with olive oleogel (O); (e) and (j): spread made with sunflower oleogel (S). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1.2. Rheological measurements

The mechanical spectra of the chocolate spreads (viscoelastic modules as a function of frequency) at 20°C are represented in Fig. 2. In all the spreads (Fig. 2a), the storage modulus (G') is greater than the loss modulus (G") and all samples have a similar frequency dependency. This gel-like behaviour was also found by Espert et al. (2020) and Espert, Wiking, Salvador, and Sanz, (2020) in spreads formulated with sunflower oil, and in spreads made with emulsions structured with hydrocolloids, such as methyl cellulose, HPMC, or XG. Furthermore, other filler spreads, formulated with oleogels using monoglycerides or waxes as structuring agents, also exhibited gel-like behaviour (Fayaz et al., 2017b; Patel et al., 2014b). Furthermore, Fig. 2b shows the variation of tan δ (G"/G') with frequency. This parameter shows the presence or absence of changes in the internal structure of the sample. As seen in Fig. 2b, spreads elaborated with total fat replacement by oleogels (spreads O and S) show higher tan δ values and a greater frequency dependence than the other spreads, thus having a lesser structured system. In contrast, spreads made with partial fat replacement (CS and CO), presented a tan δ profile like spread C, with lower values than O and S spreads, corresponding to systems with a more solid internal structure.

Table 2 shows the values of G', G", and tan δ at the frequency 1 Hz for each of the chocolate spreads. Spreads CO and CS presented significantly higher G' values than spreads C, O and S. Tan δ values for spreads O and S were the highest, indicating a

weaker internal structure, although, the difference in internal structure is much better appreciated in Fig 2. along the evolution of frequency.

Table 2. Rheological values (G', G", and tan δ) (mean ± standar deviation) of the different chocolate spreads at the frequency of 1 Hz.

	G'	G"	tan δ
C	8,396.47^{a} ± 1,507.43	2,467.045^{a} ± 333.80	0.30^{ab} ± 0.01
CO	14,507.25^{d} ± 693.60	3,985.795^{c} ± 37.70	0.27^{a} ± 0.01
CS	11,146.3^{c} ± 542.30	3,239.25^{b} ± 138.44	0.29^{a} ± 0.01
O	10,370.15^{bc} ± 46.17	3,295.63^{bc} ± 31.75	0.32^{ab} ± 0.01
S	9,194.455^{ab} ± 322.36	3,138.93^{ab} ± 576.31	0.34^{b} ± 0.05

Control spread made with coconut fat (C); spread made with 50% coconut fat and 50% olive oleogel (CO); spread made with 50% coconut fat and 50% sunflower oleogel (CS); spread made with olive oleogel (O); spread made with sunflower oleogel (S). Values with different lowercase letters (a, b, … z) within the same column are significantly different ($p < 0.05$) according to the LSD multiple range test.

These results agree with microstructural observations, where the spreads made with partial fat replacement presented a more homogeneous structure and were more similar to the control spread. Thus, a more organised structure may favour improved rheological properties (Pal, 1996). Fayaz et al. (2020b) reported good mechanical properties in chocolate spreads made with a mixture of palm fat and monoglyceride oleogels, attributed to the good chemical compatibility between the palm fat and the oleogel, and to a strengthening of the structure because of the formation of lamellar structures stabilised by hydrogen bonds.

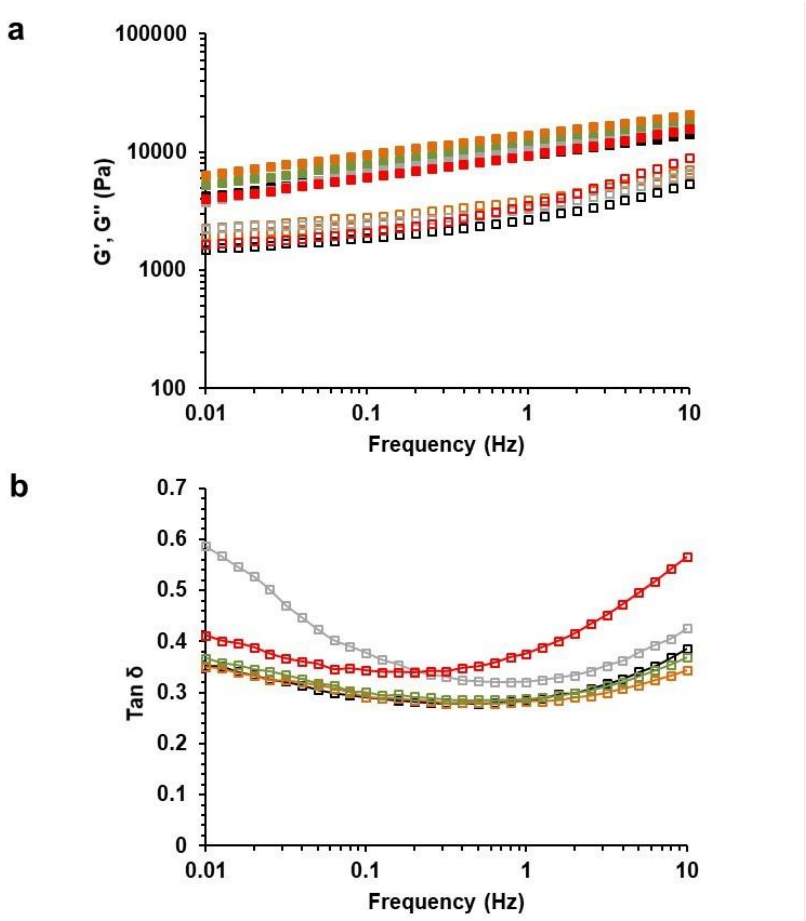

Figure 7. Frequency sweep (a) and variation of tan δ with frequency (b) of the different chocolate spreads. G': closed symbols; G'': empty symbols. Control spread made with coconut fat (■ C); spread made with 50% coconut fat and 50% olive oleogel (■ CO); spread made with 50% coconut fat and 50% sunflower oleogel (■ CS); spread made with olive oleogel (■ O); spread made with sunflower oleogel (■ S).

3.1.3. Texture measurements

The texture of spreads is one of the most influential factors for sensory acceptance. With increased firmness or maximum force of penetration, the sample is perceived as less wet or oily and has a lower spread, meaning it is more stable. Fig. 3 shows the profiles of the texture curves corresponding to the different spreads studied. Similar texture profiles can be seen between the samples, but with greater firmness seen in the CO and CS spreads, which were prepared with partial fat replacement with oleogel.

Table 3 shows the differences in the maximum peak force (N) and AUC (N s) values, calculated for each spread studied. The maximum force is related to the firmness of the samples and the AUC with the spreadability. As it can be seen, the CO and CS spreads presented significantly higher values both in firmness and AUC, which indicates they are samples with greater firmness, while being more spreadable than spreads C, O and S. However, no significant differences were found between the spread C and O or S. These results are consistent with the rheology results, where the CO and CS spreads exhibited a more solid/viscoelastic behaviour.

Nevertheless, this demonstrates the ability of oleogels formulated with HPMC and XG to impart a similar structure to spread C, formulated with a saturated fat, while improving the lipid profile of the fat.

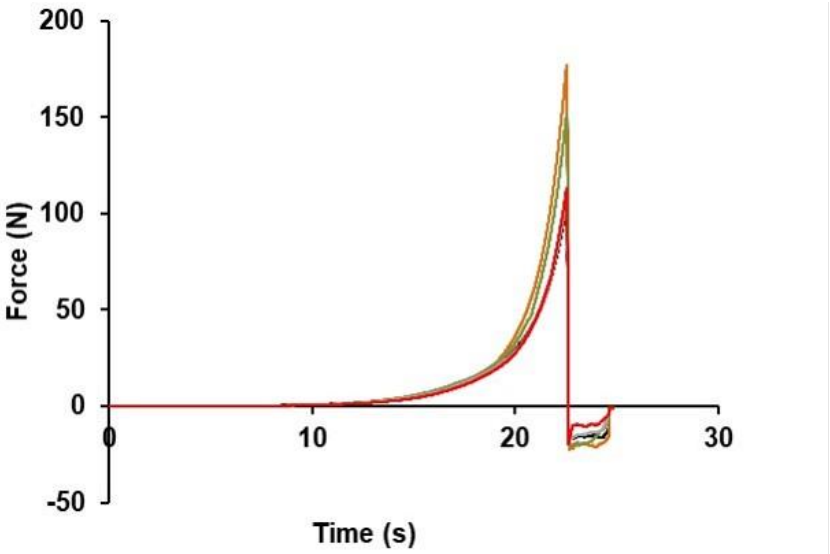

Figure 8. Texture profile of the different chocolate spreads. Control spread made with coconut fat (— C); spread made with 50% coconut fat and 50% olive oleogel (— CO); spread made with 50% coconut fat and 50% sunflower oleogel (— CS); spread made with olive oleogel (— O); spread made with sunflower oleogel (— S).

Table 3. Peak values of maximum force and areas under the curve (mean ± standar deviation) obtained by the spreadability test for the different chocolate spreads.

	Maximum Force (N)	**Area under the curve (N s)**
C	$107.01^{a} \pm 6.67$	$221.57^{a} \pm 7.16$
CO	$168.23^{b} \pm 12.59$	$291.38^{c} \pm 12.99$
CS	$140.71^{b} \pm 14.96$	$255.94^{b} \pm 22.86$
O	$104.88^{a} \pm 2.74$	$213.25^{a} \pm 5.27$
S	$109.34^{a} \pm 10.56$	$216.66^{a} \pm 2.90$

Control spread made with coconut fat (C); spread made with 50% coconut fat and 50% olive oleogel (CO); spread made with 50% coconut fat and 50% sunflower oleogel (CS); spread made with olive oleogel (O); spread made with sunflower oleogel (S). Values with different lowercase letters (a, b, … z) within the same column are significantly different ($p < 0.05$) according to the LSD multiple range test.

3.2. Sensory analysis

Free Choice Profile (FCP) analysis was performed to determine the attributes that describe the chocolate spreads. With this analysis, information on the spontaneous sensations that occur when the product is consumed is obtained; this information could have been lost using conventional descriptive analysis (Espert, Bresciani, Sanz, & Salvador, 2019; Varela & Ares, 2012). In a first session, consumers generated a series of sensory terms related to the appearance, taste, aroma, and texture of the samples. In a second session, the consumers evaluated the spreads using their own generated attributes to quantify their perception (Espert et al., 2019). A panel of 20 consumers was used, and 191 sensory descriptors were generated.

Following the analysis of Generalized Procrustes, a total variance of 78.51% was obtained that indicates the variability of the sensory attributes shown by the first two dimensions. In Fig. 4, all the descriptors used by each consumer in different colours can be observed. However, to better visualise the descriptors of each spread, Fig. 5 shows the distribution of the spreads obtained with a frequency of mention of the most representative attributes of each dimension.

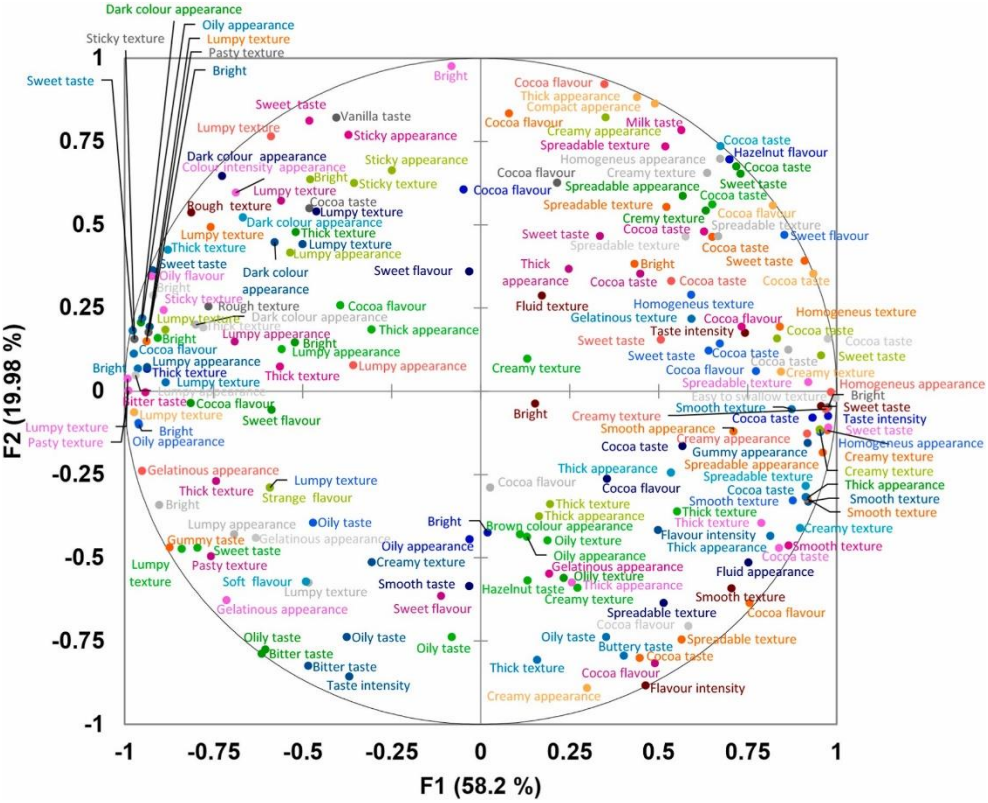

Figure 9. GPA mapping of Free Choice Profile data.

Dimension 1 represented 58.52% of the variance and was related to the terms of appearance and texture. In the positive side on the X axis (dimension 1), the terms "creamy appearance" and "bright appearance", "creamy texture" and "cocoa flavour" were related to the spreads C and CS. In contrast, attributes such as "lumpy appearance", "lumpy texture", and "thick texture" characterised the O and S spreads. These results are related to the images obtained by CLSM, where undissolved particles were observed in the O and S spreads, thus causing the "lumpy appearance" and "lumpy texture". Dimension 2 represented 19.98% of the variance. The negative side of the Y

axis showed attributes linked to texture such as "gummy texture" and "oily texture". Both parameters were related to spreads made with olive oil oleogels. However, on the positive side of the Y axis, desirable characteristics for spreads related to flavour as "cocoa taste" and "sweet taste" were described; these characteristics were related to spreads made with sunflower oil oleogels.

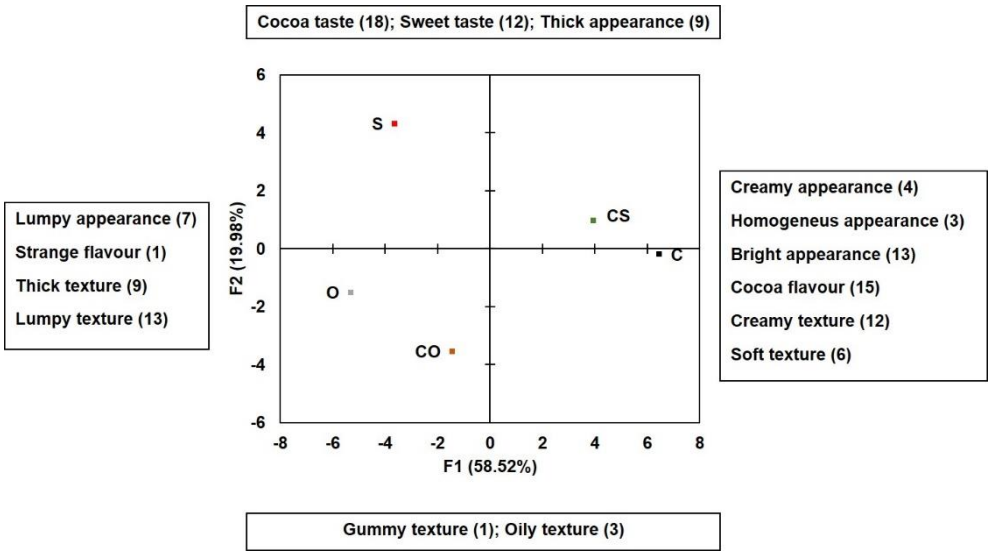

Figure 10. Representation of the samples in the two-dimensional GPA diagram where the main descriptors correlated with the first two dimensions of the average space are reflected. They are cited in the tables and the number of times each descriptor was mentioned. Control spread made with coconut fat (■ C); spread made with 50% coconut fat and 50% olive oleogel (■ CO); spread made with 50% coconut fat and 50% sunflower oleogel (■ CS) spread made with olive oleogel (■ O); spread made with sunflower oleogel (■ S).

Conclusions

This study shows that it is possible to replace the solid coconut fat in the formulation of chocolate spreads with oleogels made with sunflower or olive oil, structured with hydrocolloids (HPMC and XG), while maintaining the structural properties of traditional spreads. However, the partial replacement of coconut fat with oleogel favours the spreads characteristics, giving them a more homogeneous structure because of a fusion of their components. When sunflower oleogels are used together with coconut fat, the spread has sensory attributes like the control spread. Therefore, the partial substitution of fat by sunflower oleogel in a traditional spread can be a viable alternative for reformulating foods with a healthier nutritional profile.

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Credit authorship contribution statement

Santiago Bascuas: Investigation, Validation, Writing-original draft. María Espert: Investigation, Validation. Empar Llorca: Methodology, Investigation. Amparo Quiles: Methodology, Writing-review & editing, Funding acquisition. Ana Salvador: Methodology, Investigation, Writing - original draft. Isabel Hernando: Supervision, Writing -review & editing, Funding acquisition.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Use of oleogels to replace margarine in steamed and baked buns

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Foods (en revisión)

Abstract

Bakery products are usually formulated with solid fats, like margarines and shortenings, which contain high levels of saturated and *trans*-fatty acids and have negative effects on human health. In this study, hydroxypropyl methylcellulose (HPMC) and xanthan gum (XG) were used as oleogelators to prepare oleogels, using sunflower and olive oil, as substitutes for margarine in baked or steamed buns. The effect of oleogels on the physical properties of the buns was evaluated by analyzing the crumb structure, specific volume, height, and texture. In addition, a triangular discriminatory sensory test was conducted, and lipid digestibility was assessed through *in vitro* digestion studies. Replacement of margarine with oleogels produced steamed buns with no diferences in the crumb structure, volume, height, and texture; however, in baked buns, a less porous and harder structure was produced. No differences in texture were observed between the margarine buns and buns made with oleogels when the triangular test was conducted. The extent of lipolysis was not affected when margarine was replaced by oleogels in the baked and steamed buns. The results suggest that using oleogels instead of margarine in buns could represent an interesting strategy to prepare healthier bakery products

Keywords: Hydrocolloids; bakery products; oil structuring.

1. Introduction

Bread has been an important staple food in many aspects of humanity and civilization over the centuries. Commonly, dough as an aqueous colloidal dispersion comprises both hydrophobic (fats and shortenings) and hydrophilic (flour and sugar) components. During bread making, the components of dough form a three-dimensional structure, which traps air bubbles throughout baking [1].

The bread industry has made many efforts to create new products with different appearance, shape, flavor, and nutritional value to satisfy consumer demands (Martínez-Monzó et al., 2013). Sweet breads, such as buns, are an interesting choice established in the baking industry. Many food ingredients and bun-making processes are used to formulate a diversity of bun bread [2–4]. For example, incorporating dairy products is as-sociated with a variety of nutritional value and functional properties, such as texture, sensorial properties, and storage characteristics [5–7]. However, many bakery goods are formulated with solid fats, like margarines and shortenings, which contain high levels of saturated and *trans*-fatty acids [8,9]. The increased saturated fat and *trans*-fat intake has negative effects on chronic diseases, such as obesity, cardiovascular disease, and diabetes [10,11].

The replacement of margarines and shortenings in bakery food products is difficult to achieve without negatively affecting physical properties, because they play key roles tenderizing the crumb, retaining long-term softness, keeping quality, and extending the

shelf life [12,13]. However, bakery products seem suitable candidates for saturated and *trans*-fats replace or reduction, because of their high frequency of consumption, and would confer substantial health benefits [14,15].

Oleogels are an important technological advance in food science because of their versatility, easy processing, and affordability [16]. They can be a viable alternative to replace solid fats in buns, providing them with a healthier nutritional profile. There are only a few studies on hydrocolloids-based oleogels as a shortening alternative in bakery products, such as pea protein-xanthan gum (XG) foam-based oleogel in cakes (Mohanan et al., 2020), foam structured HPMC oleogels in muffins [17], and MC-XG oleogels in cakes [18]. Recently, the microstructure, rheological behavior, physical, and oxidative stability of oleogels prepared using HPMC and XG were characterized, resulting in oleogels with enhanced rheological properties and thermostability with low oil losses (<10%) after 35 days of storage [19,20]. These oleogels made with HPMC and XG can be a promising alternative to using solid fat in bakery products. Moreover, studies linking the role of oleogels made with HPMC and XG as structuring agents in the functionality and digestibility of buns have not been found.

The focus of this study is to compare the behavior and properties of buns elaborated with olive or sunflower oil oleogels, made with HPMC and XG as oleogelators, to replace margarine in steamed and baked buns. The results from the analysis of crumb structure, specific volume, and textural properties will provide an understanding of the structural aspects that underlie the incorporation of oleogels in bakery products.

Sensory and *in vitro* digestibility studies will also inform the use of oleogels for food formulation.

2. Materials and methods

2.1. Ingredients

Hydroxypropylmethylcellulose (HPMC 'K4M'; 4000 cP) was provided by Dow Chemical Company (Midland, MI, United States) and xanthan gum (XG; Satiaxine CX 931) by Cargill R&D (Vilvoorde, Belgium). Water (Bezoya, Segovia, Spain, with a calcium content 6.32 mg/L), olive oil (fatty acids composition: SFA:12, MUFA: 71, PUFA: 8, Coosur, España), high oleic sunflower oil (fatty acids composition: SFA: 10, MUFA: 65, PUFA: 25, Carrefour, España), wheat flour (Comercial Gallo S.A.U., Spain), fresh yeast (Lesaffre Ibé-rica, Spain), salt (Consum, Spain), sugar (Azucarera, Spain), and whole milk (3.6 g/100 mL fat) (Consum, Spain) were purchased in supermarkets. Margarine (SFA: 56-66) was supplied by Gracomsa (Catarroja, Valencia, Spain).

2.2. Oleogels preparation

Oleogels (olive and sunflower oil) were prepared following the procedure used by Bascuas et al. [20]. HPMC (2 g) was dispersed in 76.8 g cold water and mixed using a food processor (TM31 Thermomix, Vorwrek, Wuppertal, Germany). Subsequently, 1.2 g of XG was added to the resulting aqueous solution and mixed for 5 min at 300 rpm. Then, 120 g of oil was gradually added and homogenized in the processor for 5 min at

300 rpm. The emulsions were spread on a Teflon tray (42 cm x 35 cm x 0.13 mm, Pritogo, Germany) using a silicone pastry bag (Kurtzy, India) and plastic nozzle (diameter of 4.5 cm, Carrefour, TESCOMA, Spain). The emulsions were dried using forced convention air in an oven (KB115, BINDER, Germany) at 80 °C for 3 h 30 min. This was the minimum time needed to reach constant dry weight (moisture: 1.75 ± 0.51%) under the indicated conditions. The dried products were ground in a grinder (Moulinex A320R1, Paris, France) for 4 s to produce the oleogels.

2.3. Bun making

Six samples were formulated containing 53.5 g wheat flour, 26 g milk, 10 g fat (margarine or oleogel), 6 g sugar, 3.5 g fresh yeast, and 1 g salt. A food processor (TM31 Ther-momix, Vorwrek, Wuppertal, Germany) was used to mix the ingredients. First, sugar and milk were mixed at 37 °C for 2 min at 200 rpm. After, the fresh yeast was added and mixed in the processor for 5 s at 300 rpm. The wheat flour and salt were added and mixed for 15 s at 1100 rpm. Finally, the margarine, olive oil-oleogel, or sunflower oil-oleogel was added, and the kneading function was used at 500 rpm for 3 min to obtain dough C (margarine), dough O (olive oleogel), and dough S (sunflower oleogel), respectively. One hundred fifty grams of dough was spread on aluminum trays (0.5 L, Alibérico Food Packaging, Spain) and the dough was left to rise for 1 hour at 28ºC. The doughs were cooked using two conditions: baked (B) in an oven (Electrolux, model EOC3430DOX, Stockholm, Sweden) at 180 °C for 30 min, or steamed (S) in a Thermomix at 90 °C for 30 min using the Varoma (vapor) function. Six buns (BC, SC,

BO, SO, BS, and SS; first letter indicating type of cooking and second letter indicating use of margarine or oleogel) were kept covered at room temperature for 1 h and then analyzed.

2.4. Analysis of the crumb structure

The samples were cut into vertical slices of 15 mm thickness and scanned (with a resolution of 300 dpi) using a computer scanner Epson Perfection 1250 (Epson America Inc., Long Beach, CA, USA). Crumb cellular structure was analyzed using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The image was cropped to a 2 × 4 cm section, on which the analysis was performed. The image was split into color channels and the contrast was enhanced; the image was then binarised after a gray-scale threshold. The parameters calculated were air cell density (number of cells per field), air cell area (mm^2), cell circularity, and total air cell area

2.5. Specific volume and height

The specific volume of bread loaves was measured at room temperature using the rapeseed seed displacement method (AACC International Method 10–05.01) [21]. The maximum bread height was measured from the cross section of the image scanned using the software ImageJ.

2.6. Texture measurements

A TA-TX plus Texture Analyzer (Stable Micro Systems, Ltd., Godalming, UK) with the Texture Exponent Lite 32 software (version 6.1.4.0, Stable Micro Systems) was used to determine the texture properties of the samples. Measurements were performed in triplicate on eight cube samples (15 × 15 × 15 mm) taken from the central crumb of each bread.

Texture profile analysis (TPA) was performed with a 35 mm diameter aluminum plate (P/35) using a test speed of 1 mm/s with a strain of 40 % of the original cube height and a 5 s interval between the two compression cycles. The parameters obtained from the curves were hardness, springiness, cohesiveness, and chewiness.

2.7. Sensory analysis

A triangular discriminatory test was performed with a panel of 20 tasters to determine whether there were significant differences between two samples of buns. One triad was prepared per taster, who had a duplicate and a different sample. Each sample was coded with three random digits and was presented an equal number of times in each of the possible positions: BAA, AAB, ABA, ABB, BBA, and BAB in random order following a Williams design. Each taster evaluated the samples of the triad and marked the sample they considered different. Baked and steamed buns were analyzed separately, and the buns made with the different oleogels were presented against their respective control

buns. Results were analyzed following a unilateral hypothesis with a significance level of 5% [22].

2.8. *In vitro* digestion

In vitro digestion of buns was performed according to the procedures described by Diez-Sánchez et al. [23] with some modifications. Solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were elaborated according to the compositions described in the INFOGEST 2.0 protocol [24]. To mimic human physiological conditions, the analysis was conducted with a controlled temperature (37 °C) and agitation (150 rpm).

First, 7.5 g of each bun sample was ground using a hand blender (Ufesa, model BP4566, Barcelona, Spain) and then 6 mL of SSF + α-amylase (Sigma A3176), 27.5 µL of CaCl2, and 1.472 mL of distilled water were added and mixed by hand for 2 min to simulate mastication. Second, in the gastric stage, 24 mL of SGF + pepsin (Sigma P7000) and 12 µL of CaCl2 were added. The pH was adjusted to 3 using 1 M HCl, and the volume of distilled water necessary for a total volume of 30 mL was added. The mixture was incubated at 37 °C for 1 h under agitation. Third, for the intestinal stage, 12 mL of SIF + pancreatin (Sigma P1750, 4xUSP), 67.5 µL of CaCl2, 12 mL of SIF + bile salts (Sigma B8631) [25], and 12 mL of SIF + lipase (Sigma L3126; 2000U/mL) were added. The pH was adjusted to 7 using 1 M NaOH. The mixture was incubated at 37 °C for 2 h under agitation. The measurement was conducted in triplicate.

2.9. Free fatty acid release

The release of free fatty acids (FFA) of the samples were recorded during *in vitro* intestinal digestion using a pH-stat automatic titration unit (Mettler-Toledo DL 50, Greinfensee, Switzerland). This method is designed to simulate lipid digestion within the small intestine (where most lipid digestion normally occurs) and is based on measurements of the FFA released from lipids (usually triacylglycerols) after lipase addition [26]. pH was automatically monitored maintained at pH 7.0 by titrating appropriate amounts (mL) of NaOH solution (0.25 M). The volume of NaOH added to the sample was recorded and used to calculate the concentration of FFA by lipolysis using equation (1):

$$FFA\ (\%) = 100\ X \frac{V_{NaOH}\ X\ m_{NaOH}\ X\ M_{lipid}}{W_{lipid}\ X\ 2} \quad (1)$$

where V_{NaOH} is the volume of NaOH (L) added during the digestion process to neutralize the FFAs generated, m_{NaOH} is the molarity of the NaOH titrant, M_{lipid} is the average molecular weight of solid fat, and W_{lipid} is the total weight of oil in the digestion system.

The experimental data of FFA released were fitted with the empirical model following equation (2) [27]:

$$FFA\ (\%) = [(FFA)_{max}\ X\ t]/(B + t) \quad (2)$$

where % FFA and % FFA_{max} are the % FFA released at the time t and at the "pseudo-equilibrium," respectively, and B is the minimum time needed to reach half the lipolysis half time, that is % $FFA_{max}/2$.

The initial rate (K) of FFA release can be calculated using model (3):

$$K = (FFA)_{max} / B \quad (3)$$

2.10. Statistical analysis

Results were statistically analyzed using the analysis of variance (ANOVA) to study the effects of fat. The least significant differences (LSD) were calculated at a level of significance $P < 0.05$ using the statistical program Statgraphics Centurion XVI.II (StatPoint Technologies, Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1. Analysis of the crumb structure

The appearance of the bun slides is shown in Fig. 1. The baked buns (Fig. 1. BC, BO, and BS) have a hard and dark crust, whereas steamed bun (Fig. 1. SC, SO, and SS) present a thin white crust; both have a white and bright crumb. Comparing BC (Fig. 1. BC) with BO and BS (Fig. 1.BO and BS), BC seemed to present a more aerated and open structure, whereas BO and BS showed a more compact and denser crumb

structure, with a smaller number of cavities. However, these differences in the appearance could not be observed between the steamed samples (Fig 1.SC, SO, and SS).

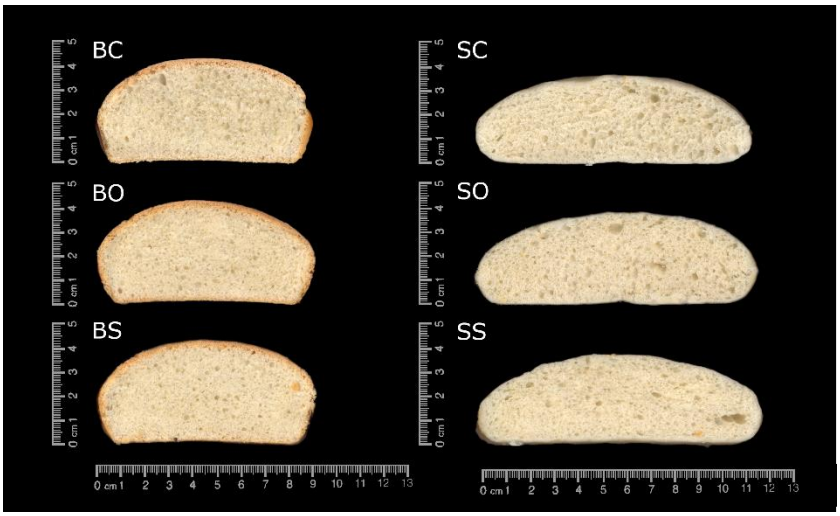

Figure 1. Cross section of breads. Baked control bun made with margarine (BC); Baked bun made with olive oleogel (BO); Baked bun made with sunflower oleogel (BS); Steamed control bun made with margarine (SC); Steamed bun made with olive oleogel (SO); Steamed bun made with sunflower oleogel (SS).

Table 1 and 2 show the crumb cell structure parameters of baked and steamed buns, respectively. BC had significantly higher values ($P < 0.05$) in the total cell area than BO and BS. Moreover, BC showed higher significant ($P < 0.05$) values than BS samples in cell circularity. No significant ($P > 0.05$) differences could be observed in the other cell structure characteristics (cell density and cell area). Regarding steamed buns (Table 2), no significant ($P > 0.05$) differences could be observed in the cell structure

characteristics (cell density, cell area, cell circularity, and total cell area) between BC, BO, and BS.

Solid fat plays a key role in the stabilization of air bubbles, forming a film in the interface air-matrix [28]. The formation of a structured network provided by oleogels appears very effective in air cell incorporation and stabilization in buns. This can explain the similar values of cell density and cell area observed when buns are formulated with oleogel or margarine. This demonstrates the ability of oleogels, formulated with HPMC and XG, to impart a similar structure like the control bun made with a saturated fat, while improving the lipid profile of the fat.

Table 1. Physical properties of baked buns.

		BC	**BO**	**BS**
Crumb cell Structure	Cell density	1235^{a} (113)	1045^{a} (140)	1064^{a} (100)
	Cell area (mm^2)	0.27^{a} (0.06)	0.33^{a} (0.06)	0.30^{a} (0.01)
	Cell circularity	0.85^{b} (0.02)	0.83^{ab} (0.01)	0.82^{a} (0.01)
	Total cell area (%)	45^{b} (3)	39.2^{b} (0.4)	36.6^{a} (0.7)
	Specific volume (cm^3/g)	2.7^{a} (0.1)	2.54^{a} (0.09)	2.7^{a} (0.02)
	Height (cm)	4.75^{a} (0.03)	4.7^{a} (0.1)	4.70^{a} (0.06)
Texture	Hardness (N)	0.9^{a} (0.1)	1.2^{b} (0.2)	1.1^{b} (0.3)
	Springiness	0.92^{b} (0.02)	0.90^{ab} (0.03)	0.8^{a} (0.2)
	Cohesiveness	0.74^{a} (0.02)	0.73^{a} (0.02)	0.74^{a} (0.06)
	Chewiness (N)	0.64^{a} (0.09)	0.8^{b} (0.1)	0.7^{ab} (0.2)

Control baked bun made with margarine (BC); baked bun made with olive oleogel (BO); baked bun made with sunflower oleogel (BS). Values with different lowercase letters (a, b... z) within the same row are significantly different ($P < 0.05$) according to th the LSD multiple range test.

Table 2. Physical properties of steamed buns

		SC	SO	SS
Crumb cell Structure	Cell density	1329[a] (87)	1101[a] (161)	1137[a] (105)
	Cell area (mm^2)	0.27[a] (0.06)	0.27[a] (0.06)	0.30[a] (0.01)
	Cell circularity	0.85[a] (0.01)	0.82[a] (0.02)	0.84[a] (0.01)
	Total cell area (%)	44[a] (4)	37[a] (6)	43[a] (2)
	Specific volume	2.50[a] (0.05)	2.7[a] (0.1)	2.6[a] (0.2)
	(cm^3/g)	3.9[a] (0.3)	4.0[a] (0.1)	3.8[a] (0.01)
	Height (cm)			
Texture	Hardness (N)	0.7[a] (0.1)	0.75[a] (0.08)	0.7[a] (0.1)
	Springiness	0.90[a] (0.03)	0.91[a] (0.03)	0.9[a] (0.1)
	Cohesiveness	0.72[a] (0.02)	0.72[a] (0.03)	0.74[a] (0.05)
	Chewiness (N)	0.48[a] (0.08)	0.49[a] (0.05)	0.5[a] (0.1)

Control steamed bun made with margarine (SC); steamed bun made with olive oleogel (SO); steamed bun made with sunflower oleogel (SS). Values with different lowercase letters (a, b... z) within the same row are significantly different ($P < 0.05$) according to the LSD multiple range test.

3.2. Specific volume

Specific volume is one of the most critical parameters to assess aerated baked goods' quality that strongly affects consumer acceptance [29]. Lipids have an important impact on dough volume [30]; hence, volume loss is a technological difficulty when replacing conventional fat in the baking industry with fat replacers.

Table 1 shows the effect of margarine and oleogels on the specific volume and height of baked buns. No significant differences ($P > 0.05$) were found in the specific volume and height between BC, BO, and BS. Using the hydrocolloids in the oleogels preparation would favor the formation of a gel network structure during baking,

improving the specific volume by expanding the gas cells without them collapsing, as explained by other studies [31,32]. Table 2 displays the specific volume and height of steamed buns. SO and SS showed no significant ($P > 0.05$) differences with SC. Other authors [33] also reported no significant differences in the specific volume of sweet bread replacing 75% of butter with oleogel prepared with candelilla wax.

Thus, the solid-like structure of the oleogel used in this study appears a viable alternative to resemble the functionality of conventional solid fats to maintain the quality standards of buns in terms of volume and height.

3.3. Texture

The textural properties of BC, BO, and BS are summarized in Table 1. BO and BS exhibited significantly higher hardness values ($P < 0.05$) than BC. As expected, these results are in line with the crumb structure results already analyzed. BC presented the highest total cell area values, which implies a more aerated structure that offers less resistance to compression. This relationship has been observed in cakes prepared with other fat replacers [34,35]. Moreover, Oh et al. [17] also reported higher hardness values in muffins elaborated with 100% replacement of shortening with HPMC oleogels when compared to control muffins. The authors attributed these differences to the lower values in the total porosity of the samples made with oleogels. Chewiness values presented a similar trend as hardness values; BO and BS had values higher than BC. Martínez-Cervera et al. [36] reported an increase in chewiness values when muffins were

made using cellulose emulsions instead of margarine. Regarding springiness, BC presented higher significant ($P < 0.05$) values than BS. The cohesiveness values showed no significant ($P > 0.05$) differences between BC, BO, and BS. These results agree with the specific volume analysis, where baked buns did not present significant ($P > 0.05$) differences.

As presented in Table 2, SO and SS exhibited no significant ($P > 0.05$) differences in texture properties compared to SC. These results are consistent with the appearance of in steamed bun slides, where the SO and SS buns exhibited a similar shape and aerated crumb structure, whereas the crumb cell structure showed no differences between the buns.

Texture parameters play a key role in the quality of leavened buns where the geometric and mechanical properties heavily depend on its cellular structure [37] Buns formulated using hydrocolloids-based oleogels lead to a well-developed porous crumb structure and could represent a suitable strategy to imitate the functionality of margarine in buns.

3.4. Sensory analysis

To know if there were differences between buns prepared with or without oleogel, a sensory discriminative test (triangle test) was performed; the triangle test is one of the most widely used analysis when products evaluated are sufficiently homogeneous [38]. The descriptors or attributes used in the triangle test in this study were the appearance of the crumb, texture, and taste. Twenty total triangle test responses require eleven

correct responses to be significant at the 95% confidence level according to ISO 4120:2004 [22]. The results indicated panelists could differentiate between buns made with margarine and buns made with oleogels, both baked and steamed.

Specifically, when baked buns were made using olive or high oleic sunflower oleogels, the panelists detected significant differences ($P < 0.05$) in the appearance of crumb and taste attributes when compared with the control buns. Furthermore, no significant ($P > 0.05$) differences were observed in texture by panelists. For steamed buns, a similar tendency was observed.

Texture is an essential sensory attribute that can determine the product quality and play a vital role regarding the acceptability of breads among consumers. In this study, the sensory results show full replacement of margarine using hydrocolloids-based oleogels did not affect the texture; this would be an important advantage of the oleogels, which could be used to obtain buns with high sensory quality in terms of texture.

3.5. Free fatty acid release

All samples mostly showed a fast release of FFA in the first 10 min of the intestinal digestion (Fig. 2), followed by a more gradual increase after the 10 min point, reaching a relatively constant final value. The saturation on the extent of lipolysis may be because the FFA and intermediate products have high surface activity and adsorb to the surface of oil droplets. At a sufficiently high concentration, they displace the lipase molecules from the oil-water interface, thus inhibiting the lipase activity [39].

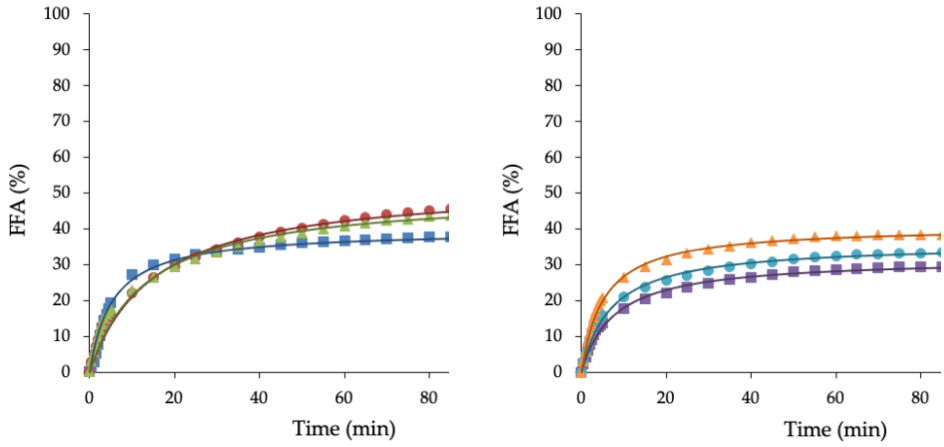

Figure 2. Free fatty acids (FFA) released during *in vitro* digestion of baked buns (a) and steamed buns (b). Control buns (squares), buns made with olive oleogel (circles), buns made with sunflower oleogel (triangles).

Table 3 and 4 describe the kinetic parameters of FFA released during the *in vitro* digestion process for the baked and steamed buns, respectively. The mathematical model presented an R2 over 0.98 for all cases, proving the excellent fit between the formula and the experimental data. The amount of FFA release during the digestion of buns made with olive or sunflower oleogels stabilized by HPMC and XG was around 35-48%. This result agrees with results obtained by other authors [40,41] who found that the amount of FFA released during the digestion of emulsions stabilized by HPMC was 44–51%. The HPMC interfacial activity and the lower destabilization under the digestion fluids plays a key role on lipolysis, decreasing the access of lipase to the interface [42]. Neither baked nor steamed buns made with oleogels (BO, BS, SO, and

SS), showed significant ($P < 0.05$) differences in FFA_{max} values compared with their respective buns made with margarine (BC and SC) (Table 3 and 4).

Buns made with oleogels showed higher FFA release profiles than margarine along digestion (Fig. 2) because i) margarine is formulated with emulsifiers, which inhibit the lipase activity more effectively than hydrocolloids [43], and ii) the presence of calcium ions from the digestion and the calcium presented in the food (from the milk used in the bun's making) increase the removal of long chain fatty acids from the oil-water interface by forming insoluble calcium soaps and thus allowing lipase access to the interface [44,45].

The initial digestion rate of the baked buns showed significant differences between BC and BS (Table 3) probably influenced by substituting saturated margarine fat, stabilized with surfactants, for sunflower oleogel rich in monounsaturated fatty acids and stabilized with hydrocolloids. The baked buns formulated with sunflower oleogels exhibited lower initial digestion rates, indicating than control buns were initially digested faster (Table 3). Commercial margarine was formulated with E-471 as a surfactant, a mixture of monoglycerides and diglycerides that are likely to contribute substrates for the action of intestinal lipase and generate additional FFAs and even serve as emulsifiers that boost the efficiency of lipolysis increasing the initial rate [46]. No significant ($P > 0.05$) differences were found between BO and the rest of the samples (Table 3). For steamed buns, the initial rate was higher ($P < 0.05$) in the SS buns than in SC buns

(Table 4). No significant (P > 0.05) differences were found between SO and the rest of the samples (Table 4).

Table 3. Kinetic parameters of lipid digestion in baked buns

	FFA_{max} (%)	**K (1/min)**	**R^2**
BC	38[a] (2)	6.5[b] (1.3)	0.99
BO	48[a] (3)	4.2[ab] (0.7)	0.99
BS	45[a] (7)	3.7[a] (0.1)	0.98

FFA_{max} (%): percentage of maximum free fatty acid released; K: initial rate of free fatty acid release. Control baked bun made with margarine fat (BC); baked bun made with olive oleogel (BO); baked bun made with sunflower oleogel (BS). Values with different lowercase letters (a, b... z) within the same column are significantly different ($P < 0.05$) according to the LSD multiple range test.

Table 4. Kinetic parameters of lipid digestion in steamed buns

	FFA_{max} (%)	**K (1/min)**	**R^2**
SC	31[a] (8)	3.8[b] (0.6)	0.99
SO	35.7[a] (0.4)	5.5[ab] (0.6)	0.99
SS	39[a] (4)	6.8[b] (1.4)	0.99

FFA_{max} (%): percentage of maximum free fatty acid released; K: initial rate of free fatty acid release Control steamed bun made with margarine fat (SC); steamed bun made with olive oleogel (SO); steamed bun made with sunflower oleogel (SS). Values with different lowercase letters (a, b... z) within the same column are significantly different ($P < 0.05$) according to the LSD multiple range test.

Conclusions

The replacement of margarine, a plastic fat traditionally used in manufactured baked products, with olive or sunflower oil oleogels structured with HPMC and XG, was effective in providing similar physical characteristics in baked or steamed buns.

Replacement of margarine for oleogels successfully produced steamed buns without differences in the crumb structure, volume, height, and texture. However, the baked buns made with oleogels had a less aerated crumb structure and were harder than the baked buns formulated with margarine. At the sensory level, no differences in texture were observed between the margarine buns and buns made with oleogels, both for baking and steaming conditions. Regarding lipid digestibility, the extent of digestion, as measured from the *in vitro* FFA release, was not affected by margarine replacement by oleogels. These results suggest that the reformulation of buns with oleogels as saturated fat replacers could help the food industry prepare healthy bakery products.

Author Contributions:

Santiago Bascuas: Investigation, Validation, and Writing-original draft. Pere Morell: Methodology, and Writing – original draft. Amparo Quiles: Methodology, Supervision, Writing-review and editing, Funding acquisition. Ana Salvador: Methodology, Investigation, and Writing - original draft. Isabel Hernando: Conceptualization, Supervision, Writing -review and editing, Funding acquisition.

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Conflicts of Interest

The authors declare no conflict of interest.

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Discusión general de los resultados

La Tesis plantea distintas estrategias para el desarrollo y diseño de oleogeles estables con un perfil lipídico de alta calidad nutricional, encaminados a reemplazar las grasas saturadas y *trans,* empleadas de forma habitual, por la industria, en la formulación de distintos alimentos, sin afectar a las propiedades del producto. Para el desarrollo de los oleogeles se emplearon distintos aceites vegetales e hidrocoloides como agentes estructurantes. El empleo de estos oleogeles en la formulación de alimentos, como grasa alternativa, puede permitir diseñar alimentos saludables y con ello, disminuir el consumo de grasas saturadas y eliminar el consumo de grasas *trans* en la alimentación habitual de los consumidores. Como matrices alimentarias para estudiar el diseño de alimentos saludables se eligieron cremas untables y panes dulces. En estos alimentos se reemplazó la grasa habitual, empleada en su formulación, por los oleogeles estructurados con hidrocoloides.

Desarrollo de oleogeles de alto valor nutricional elaborados con hidrocoloides

En un primer estudio, la hipótesis de partida fue que el grado de insaturación del aceite empleado en la formulación del oleogel y el tipo de secado empleado en el método *emulsion-template* para la obtención del oleogel, podrían tener un efecto directo sobre sus características finales. Se estudió, por una parte, el impacto del tipo de aceite (oliva virgen extra, girasol refinado y lino virgen) empleado en la formulación del oleogel y por otra parte del tipo de secado (convencional a 80 ºC, 10 h 30 y a vacío a 60 ºC, 24 h) empleado en el método de elaboración *emulsion-template*, sobre las propiedades estructurales, fisicoquímicas y la estabilidad de oleogeles estructurados con

hidroxipropilmetilcelulosa (HPMC) y goma xantana (GX). A partir del análisis por criomicroscopía electrónica de barrido se observó en las emulsiones, la presencia de una red tridimensional compacta debido a las interacciones polímero-polímero que limitó la movilidad de las gotas de aceite atrapadas físicamente, evitando fenómenos incipientes de coalescencia y la pérdida de la estructura. En consecuencia, fue factible obtener oleogeles a base de hidrocoloides que fueron estables durante 35 días de almacenamiento, empleando aceite de oliva o girasol tanto por secado convencional como a vacío. Sin embargo, los oleogeles desarrollados con lino mediante secado convencional resultaron tener una estructura poco organizada, con glóbulos de grasa en coalescencia, mientras que no fue viable obtener oleogeles de lino cuando las emulsiones fueron secadas a vacío. Estos resultados están en concordancia con la estabilidad física y los parámetros reológicos, que evidenciaron que los oleogeles de oliva o girasol, independientemente del tipo de secado, presentaron una alta estabilidad física y propiedades viscoelásticas, mientras que los oleogeles de lino sufrieron mayores pérdidas de aceite a lo largo del almacenamiento y mayor rotura durante la aplicación de fuerza de cizalla debido a la formación de una red débil y una pobre organización de la estructura. Tanto los oleogeles de aceite de oliva, obtenidos mediante secado convencional o a vacío, como los oleogeles de girasol secados a vacío presentaron una alta estabilidad oxidativa. Sin embargo, los valores de oxidación primaria y secundaria de los oleogeles de girasol obtenidos mediante secado convencional podrían ser susceptibles de mejora.

En el segundo trabajo, se quiso ahondar en el desarrollo y optimización de oleogeles de aceite de girasol, obtenidos por secado convencional. El aceite de girasol presenta un uso generalizado para la formulación de alimentos y el secado convencional puede ser más sencillo de instaurar en la industria que otros tipos de secado que son más caros o requieren mayor consumo energético. Se estableció la estabilidad oxidativa como un parámetro crítico en la caracterización de la funcionalidad de los oleogeles elaborados mediante el método *emulsion-template*. Para ello, en este trabajo se estudió la influencia de diferentes condiciones de secado convencional (60 °C, 24 h y 80 °C, 10 h 30 min) y de la composición en ácidos grasos del aceite de girasol sobre la estabilidad oxidativa, física y estructural de los oleogeles. Todos los oleogeles presentaron una estructura estable y alta estabilidad física con pérdidas de aceite menor de 14% tras 35 días de almacenamiento. Las propiedades reológicas mostraron que los oleogeles presentaron un comportamiento sólido similar a un gel. No obstante, independientemente del tipo de aceite de girasol empleado, cuando en las condiciones de elaboración se emplearon temperaturas más altas, se observó por microscopía óptica la formación de una red polimérica más fuerte y físicamente estable. Finalmente, los oleogeles elaborados con aceite de girasol alto oleico obtenidos por secado convencional a 80 ºC tuvieron una mayor estabilidad oxidativa, estructural y física, convirtiéndose en candidatos idóneos para su incorporación en matrices alimentarias como sustitutos de grasas sólidas convencionales.

En conclusión, para lograr un adecuado diseño de oleogeles estructurados con hidrocoloides y de elevado perfil nutricional, es importante comprender cómo afectan las condiciones de secado de las emulsiones y el tipo de aceite empleado en la elaboración de los oleogeles en la estructura, estabilidad física y química a lo largo del tiempo.

Reformulación de alimentos mediante la incorporación de aceites de alto valor nutricional estructurados con hidrocoloides

La aplicación de oleogeles en productos alimenticios debe permitir desarrollar alimentos saludables y seguir manteniendo sus expectativas hedónicas.

En un tercer trabajo, se desarrollaron cremas untables con un perfil lipídico saludable. Para ello, se reemplazó la grasa de coco, empleada de forma habitual en la formulación de las cremas, por oleogeles de oliva y girasol, obtenidos por secado a vacío, desarrollados y caracterizados en trabajos previos que forman parte de la Tesis. Se estudió la influencia de un reemplazo parcial (50%) y de un reemplazo total (100%) sobre la estructura, propiedades reológicas, texturales y sensoriales de las cremas untables. Los resultados experimentales demostraron que la presencia de los oleogeles confería consistencia a las cremas untables debido a la red formada por la HPMC y GX. La sustitución total de grasa de coco por oleogeles hizo que las cremas presentaran una distribución poco homogénea con grandes gotas de grasa, mientras que una sustitución parcial permitió obtener un sistema homogéneo y uniforme, debido a la compatibilidad química existente entre la grasa saturada y el oleogel con una mayor fusión de los

componentes. En consecuencia, las cremas elaboradas con 100% de oleogel de oliva o girasol presentaron menor estructuración y sensorialmente se percibieron como "grumosas" tanto en apariencia como en boca y "espesas", características que hicieron que fueran las peores valoradas. Cuando se sustituyó la grasa de coco en un 50% con oleogel, las cremas untables presentaron una mayor estructuración, y mayor untabilidad. Además, el estudio sensorial definió que los atributos sensoriales (apariencia, textura cremosa y sabor a cacao) de las cremas elaboradas con un 50% oleogel de girasol y 50% grasa de coco fue similar a la de las cremas formuladas totalmente con grasa saturada.

El cuarto y último trabajo de la Tesis doctoral se centró en valorar la viabilidad de aplicar los oleogeles de oliva y girasol desarrollados en trabajos anteriores, como sustitutos de margarina en la formulación de alimentos panificables. Concretamente, se seleccionaron panes dulces obtenidos por un proceso de horneado o al vapor. Estos alimentos tienen una alta aceptabilidad y frecuencia de consumo. Se estudió el efecto del reemplazo total de margarina, grasa empleada de forma habitual en la formulación de estos panes, por los oleogeles, sobre las propiedades estructurales y la digestibilidad lipídica *in vitro* de panes dulce sometidos a un proceso de horneado o al vapor. Adicionalmente se realizó una prueba sensorial triangular para detectar posibles diferencias en algunos atributos.

Los resultados mostraron que la sustitución de la margarina por oleogeles de aceite de oliva o de girasol estructurados con hidrocoloides, resultó eficaz para proporcionar características físicas similares a los panes dulces obtenidos por proceso de horneado o al vapor. En líneas generales, el análisis de imagen reveló que la elaboración de panes

dulces al vapor empleando oleogeles, parece ser muy eficaz en la incorporación y estabilización de las burbujas de aire. Esto puede explicar que no se encontraran diferencias en la altura, volumen específico y textura de la miga entre los panes con margarina y con oleogeles. Sin embargo, el reemplazo de margarina por oleogeles en la fabricación de panes dulces horneados, produjo una estructura menos porosa y más dura. Respecto a las propiedades sensoriales, no se observaron diferencias de textura entre los panes dulces con margarina y los elaborados con oleogeles, tanto en condiciones de horneado como al vapor. El grado de digestión de los lípidos, medido a partir de la liberación de FFA *in vitro*, no se vio afectado por el reemplazo de la margarina por oleogeles. Sin embargo, la velocidad inicial de la digestibilidad lipídica fue diferente dependiendo del tipo de procesado empleado en la elaboración de los panes. Este último resultado podría investigarse con más profundidad como estrategia para modular la digestión de lípidos con vistas a obtener alimentos más saludables.

Conclusiones

- El uso de los hidrocoloides hidroxipropilmetilcelulosa y goma xantana como agentes estructurantes de aceites líquidos vegetales mediante el método *emulsion-template* es una forma efectiva de obtener grasas sólidas de alto perfil nutricional

- Es factible elaborar oleogeles de aceite de oliva o aceite de girasol con buena estabilidad física y oxidativa, utilizando hidrocoloides como agentes estructurantes, tanto por secado convencional como a vacío.

- Los oleogeles elaborados con aceite de girasol alto oleico presentan una alta estabilidad oxidativa durante el almacenamiento. Además, el secado a 80 °C durante 10 h 30 min da lugar a oleogeles con mayor estabilidad estructural y física que el secado a 60 °C durante 24 h, independientemente del grado de insaturación del aceite usado para la elaboración del oleogel.

- La sustitución de un 50% de grasa saturada por oleogel de oliva o girasol es una alternativa prometedora en la formulación de cremas untables con un perfil nutricional saludable. Concretamente, el empleo de oleogeles de girasol proporciona a la crema una estructura homogénea y atributos sensoriales similares a los de las cremas elaboradas con 100% de grasa de coco.

- El uso de oleogeles de oliva o de girasol es una alternativa en la reformulación de panes dulces horneados o al vapor de mayor calidad nutricional. La aplicación de estos oleogeles en sustitución de la margarina permite desarrollar panes con características similares a las de los panes dulces tradicionales.

- La presente Tesis desarrolla estrategias aplicadas para el diseño de alimentos abarcando aspectos como la composición, estructura, características fisicoquímicas y propiedades sensoriales. Los oleogeles a base de hidrocoloides son una estrategia viable y prometedora para el diseño de alimentos más saludables manteniendo sus propiedades estructurales.