Borreani, JAA.; Espert, M.; Salvador Alcaraz, A.; Sanz, MT.; Quiles Chuliá, MD.; Hernando Hernando, MI. (2017). Oil-in-water emulsions stabilised by cellulose ethers: stability, structure and in vitro digestion. Food & Function. 8(4):1547-1557. doi:10.1039/c7fo00159b

Food & Function

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: J. Borreani, M. Espert, A. Salvador, T. Sanz, A. Quiles and I. Hernando, *Food Funct.*, 2017, DOI: 10.1039/C7FO00159B.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

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DOI: 10.1039/C7FO00159B

Oil-in-water emulsions stabilised by cellulose ethers: stability, structure and *in vitro* digestion

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DOI: 10.1039/C7FO00159B

Abstract

The effect of cellulose ethers in oil-in-water emulsions on stability during storage and on texture, microstructure and lipid digestibility during *in vitro* gastrointestinal digestion was investigated. All the cellulose ether emulsions showed good physical and oxidative stability during storage. In particular, the methylcellulose with high methoxyl substituents (HMC) made it possible to obtain emulsions with high consistency which remained almost unchanged during gastric digestion, and thus could enhance fullness and satiety perceptions at gastric level. Moreover, the HMC emulsion slowed down lipid digestion to a greater extent than a conventional protein emulsion or the emulsions stabilised by the other cellulose ethers. Therefore, HMC emulsions could be used in weight management to increase satiation capacity and decrease lipid digestion.

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1. Introduction

Fat-rich diets have been associated with high incidences of obesity and a higher risk of coronary heart disease, diabetes, and certain forms of cancer.[1] A potential strategy for combating these chronic diseases is to develop healthier foods by reducing the amount of fat.[1,2] However, the development of fat-reduced products is challenging because fats have a major impact on the physicochemical, sensory, and nutritional properties of foods.[1] Since this makes it difficult to formulate foods whilst maintaining consumer satisfaction, another solution is to develop functional foods by using strategies associated with controlling lipid digestion in the gastrointestinal human tract in order to reduce the bioavailability of the fat.[3,4] This solution requires a good understanding of the relationship between food structure and its behaviour before and during digestion.[5]

A large part of the lipids in processed foods is consumed in the form of oil-in-water (o/w) emulsions, in which the lipids are embedded in the form of droplets in an aqueous medium in order to enhance their stability and organoleptic quality. Emulsions are thermodynamically unstable systems, but they can be physically stabilised by emulsifiers to avoid immediate separation into oil and aqueous phases.[6] Proteins and polysaccharides have been widely used as emulsifiers, stabilisers, thickening or gelling agents in the food industry, to form physically stable emulsions and to control microstructure, texture, flavour and shelf life.[7–9]

Besides their physical instability, o/w emulsions also suffer oxidative deterioration (such as lipid oxidation) during storage.[7] This is directly associated with negative effects on taste, appearance, texture and shelf life and also leads to the formation of off-flavours (rancidity) and toxic compounds.[10] Various factors can influence the rate of lipid oxidation in emulsion-based foods, such as droplet size, composition of the interfacial layer, colloid structures in the aqueous phase, the presence of antioxidants and pro-oxidants (transition metals), etc.[6,10]

Nevertheless, in whatever form they are consumed, lipids are emulsified in the mouth, the stomach, and/or the small intestine due to: i) the mechanical

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stresses they experience, ii) the presence of various endogenous and dietary surface-active and stabilising components[4] and iii) lipid digestion being an interfacial process[3] in which gastric and pancreatic lipases have to bind to the o/w interface, via complexation with co-lipase which adsorbs onto bile salts in the case of pancreatic lipase.[11] Hence, the substrate for dietary fat digestion is usually lipid droplets coated by a complex layer of surface-active material.[4] In general, in a state of lipase abundancy in the duodenum[12] the human body has an excess capacity for fat digestion, so the rate and extent of fat digestion are controlled by the ability of lipase to bind to emulsion interfaces. This ability is controlled in turn by the lipid droplet's characteristics (its surface area and the composition of the lipid itself) and interfacial composition (e.g. the presence of bile salts and the nature of the interfacial layer).[13] Thus, in order to obtain healthier foods, the choice of emulsifiers, particle size and fatty acid composition are major factors to be taken into account when processing food with the aim of delaying or limiting lipid digestion and absorption.[3]

Moreover, several studies have shown that adding polysaccharides in the form of fibre can increase viscosity and induce the formation of gels in the stomach, and these properties can slow down gastric emptying and concurrently increase gastric volume/stomach distension,[14] which is positively and linearly correlated with postprandial fullness.[15] Therefore, controlling the emulsion structure and thus its digestion behaviour could make it possible to obtain emulsions with enhanced satiating capacity and lower lipid digestion which could be used in weight management.

Cellulose ethers are non-ionic dietary fibres that differ principally in molecular weight, viscosity and degree of substitution. In recent studies, the effect of different hydroxypropyl methylcelluloses (HPMCs) on lipid digestion of o/w emulsions has been investigated.[2,16] Torcello-Gómez and Foster[2] found similar results of lipolysis curves regardless the molecular weight, substitution pattern or initial concentration in the bulk of the HPMCs. However, Pizones Ruiz-Henestrosa *et al.*[16] attributed the slight difference in lipolysis extent between two types of HPMC to the molecular events occurring at the interface upon bile salts adsorption, due to their different methyl/hydroxyl ratio. Therefore, there is no a clear trend on the lipid digestion and its relation to the molecular weight or degree and type of substitution of the cellulose ethers used as emulsifiers in o/w

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emulsions and thus further investigation is needed. In this regard, two types of hydroxypropyl methylcellulose (HPMC) and two types of methylcellulose (MC) are used in this study as emulsifiers in o/w emulsions in order to design new emulsions with satiation capacity and low lipid digestion. These new emulsions are prepared with high fat content, thus they can be used as fat replacers of conventional sources of solid fat in the diet, such as butter or shortening.

The first aim of this study, therefore, was to study the physical and oxidative stability of o/w emulsions stabilised by cellulose ethers and the second aim was to study their microstructure, texture and lipid digestion (free fatty acid release – FFA) during *in vitro* gastrointestinal digestion.

2. Material and methods

2.1. Emulsion ingredients

Oil-in-water emulsions were prepared with commercial sunflower oil (Koipe Sol, Deoleo S.A., Córdoba, Spain), drinking water (Bezoya, Calidad Pascual S.A.U., Burgos, Spain) and four different cellulose ethers with thermo-gelling ability (METHOCEL™ K4M, F4M, A4M and MX, from now on referred to as HHPMC, HPMC, MC and HMC, respectively), supplied by The Dow Chemical Company. HHPMC and HPMC are hydroxypropyl methylcelluloses. HHPMC (high hydroxypropyl methylcellulose) has a higher percentage of hydroxypropyl (7.7% hydroxypropyl, 22.5% methoxyl) than HPMC (hydroxypropyl methylcellulose) (6.8% hydroxypropyl, 29% methoxyl). MC and HMC are methylcelluloses. MC (methylcellulose) has less methoxyl substitution (30% methoxyl) than HMC (high methylcellulose) (methoxyl >30%). HHPMC, HPMC and MC have approximately the same viscosity (4000 mPa·s, measured at 2% aqueous solution at 20 °C by The Dow Chemical Company following reference methods ASTM D1347 and ASTM D2363) while HMC has a higher viscosity (50000 mPa·s, measured in the same way).

2.2. Emulsion preparation

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The emulsions were prepared according to Sanz *et al.*[17] with some modifications. Each cellulose ether (2% w/w) was dispersed in the oil (47% w/w) using a Heidolph stirrer (Heidolph RZR 1, Schwabach, Germany) at 283 rpm for 5 min. The mixture was then hydrated by gradually adding water at 1 °C while continuing to stir. A water temperature of 1 °C was selected in accordance with the specific hydration requirement of HMC and was employed for the other emulsifiers as well. Stirring continued using a homogenizer (Ultraturrax T18, IKA, Germany) at 6500 rpm for 15 s and subsequently at 17500 rpm for 60 s. Sorbic acid (0.1% w/w) was added as an antimicrobial agent to prevent microbial growth in the emulsions during storage (30 days at 4 °C).

A control emulsion with calcium caseinate (CaCN) (Fonterra Co-operative Group Ltd, Palmerston North, New Zealand) was also prepared for the *in vitro* digestion study. The CaCN powder (4.5% w/w) was slowly dispersed in the oil and then hydrated by gradually adding water, as previously described for the cellulose ether emulsions. In order to form an emulsion with similar oil droplet size to that of the cellulose ether emulsions, the homogenization conditions were also modified slightly: the first homogenizer speed (6500 rpm) was maintained for 30 s and the second (17500 rpm) for 120 s.

2.3. Physical stability

Physical stability was examined according to Goyal *et al.*[18] with few modifications. Immediately after preparation, approximately 20 g of sample were transferred into glass tubes (internal diameter 27 mm, height 100 mm), which were sealed with a plastic cap and stored at a low temperature (4 °C) for a period of 30 days. Digital photographs (Olympus E-510, Tokyo, Japan) of the samples were taken every 10 days. This physical stability investigation was performed in duplicate.

2.4. Oxidative stability

Fresh emulsions were placed in glass beakers, covered with aluminium foil and stored at a low temperature (4 °C) for 30 days. Before oxidative stability

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determination, lipid extraction was carried out according to Timm-Heinrich *et al.*[19] with some modifications. The samples (approximately 20 g) were deep-frozen (-70 °C) for 24 h in a conical centrifuge tube and thawed before centrifugation at 10765 rpm for 10 min.

The primary lipid oxidation products were measured by the peroxide value method (PV) according to Hornero-Méndez *et al.*[20] In addition, formation of the secondary products was measured by the specific extinction value method (K270) according to ISO 3656.[21] Three replications were performed every 10 days during the storage time.

2.5. *In vitro* digestion model

An *in vitro* gastrointestinal tract model consisting of oral, gastric and intestinal phases was used to simulate the biological fate of ingested samples, following Morell *et al.*[22], Sanz *et al.*[23] and Qiu *et al.*[24] with some modifications.

To simulate oral digestion, 15 g of fresh emulsion sample were gently mixed for 5 s with 0.33 mL of fresh artificial saliva (pH 6.8, 62 mM $NaHCO_3$, 6 mM $K_2HPO_4 \cdot 3H_2O$, 15 mM NaCl, 6.4 mM KCl, 3 mM $CaCl_2$, mucin type II from porcine stomach (M2378, Sigma-Aldrich), α-amylase type VI-B from porcine pancreas (A3176, Sigma-Aldrich)).

To simulate gastric digestion, the sample obtained after the oral phase (bolus) was mixed with 4.8 mL of pre-incubated (37 °C; 5 min) simulated gastric fluid (pH 2, 53 mM NaCl, 1 mM $CaCl_2$, 14.8 mM KCl, 5.7 mM Na_2CO_3). The pH was adjusted to 2.0 with HCl and 0.7 mg of pepsin (P7125, pepsin from porcine gastric mucosa, ≥ 400 units/mg protein, Sigma-Aldrich) was added. The mix was maintained at 37 °C with continuous stirring for 60 min.

To simulate intestinal digestion, 3.18 mL of bile extract (B8631, Sigma-Aldrich) solution (46.87 mg/mL phosphate buffer pH 7) and 1.2 mL of electrolyte mixture (pH 7, 21 mM NaCl, 2 mM KCl, 0.5 mM $CaCl_2$) were added and the pH was increased to pH 7 with NaOH 1 M. After this, 0.5 g of pancreatin from porcine pancreas (P3292, Sigma-Aldrich) and 2.8 g of lipase from porcine pancreas (L3126, type II, 100-500 units/mg protein, Sigma-Aldrich) dissolved in

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1.62 mL of phosphate buffer (pH 7) were added and the mix was maintained at 37 °C and pH 7 with continuous stirring for 120 min.

2.6. Texture analysis

Emulsion texture measurement was carried out with a TA.XT.plus Texture Analyser (Stable Microsystems, Godalming, UK) using a 30 kg load cell. A back extrusion test was conducted using an A/BE-D40 back extrusion cell (40 mm diameter). The samples (50 g) were placed into an extrusion cylinder (50 mm internal diameter and 75 mm height) and one cycle was applied (speed: 1 $mm \cdot s^{-1}$; distance: 15 mm). The area under the curve (N·s) after reaching the maximum force was recorded from the force-time profiles. The texture analysis was performed in triplicate.

2.7. Free fatty acid release

The extent of lipolysis was measured through the amount of free fatty acids (FFA) released during the intestinal phase. The pH of the mixture was monitored and the volume of NaOH 0.5 M used to neutralize the FFA released through lipid digestion was recorded using a pH-stat (Mettler-Toledo DL 50, Greinfensee, Switzerland). The amount of FFA released was calculated as the percentage of FFA (% FFA) released during the digestion time as described by Li and McClements.[25] The measurement was carried out in duplicate.

2.8. Microstructure analysis

A Nikon ECLIPSE 80i (Nikon Co., Ltd., Tokyo, Japan) light microscope was used as described by Borreani *et al.*[26] An aliquot of each formulation was placed on a glass slide and observed at 20x magnification. A camera (ExWaveHAD, model no. DXC-190, Sony Electronics Inc, Park Ridge, New Jersey, USA) was attached to the microscope and connected to the video entry port of a computer. The images were captured and stored at 1280 x 1024 pixels using the microscope software (NIS-Elements F, Version 4.0, Nikon, Tokyo,

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Japan). The software interfaced directly with the microscope, enabling image recording control. The images were taken from fresh samples and at the end of each digestion phase. Toluidine blue (0.2%) was used to stain the proteins and celluloses.

A Nikon confocal microscope C1 unit that was fitted on a Nikon Eclipse E800 V-PS100E microscope (Nikon, Tokyo, Japan) was used. An aliquot of each formulation was placed on a glass slide and Nile Red (0.2%) and Rhodamine B (0.01%) solutions were added to stain fat and proteins and/or carbohydrates, respectively. Observations were performed 10 min after diffusion of the dyes into the sample at 60x magnification. Images were observed and stored with 1024×1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

The droplet size of the fresh and intestinal-digested emulsions was determined from CLSM images. The diameter of 180 droplets from each sample was measured with the microscope software (NIS-Elements F, Version 4.0, Nikon, Tokyo, Japan).

2.9. Statistical analysis

Analysis of variance (ANOVA) was performed on the data using XLSTAT statistical software (version 2014.5.02, Microsoft Excel®, Barcelona, Spain). Fisher's Least Significant Difference (*LSD*) test was used to assess the differences in mean values ($P < 0.05$).

3. Results and discussion

3.1. Physical stability

Phase separation was investigated to assess the stability of o/w emulsions during the storage time (Fig. 1). None of the cellulose ether emulsions exhibited phase separation during the 30-day storage period. The excellent physical stability of the emulsions containing cellulose ethers was probably due to the ability of these polysaccharides to increase the viscosity of the continuous

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phase, which decreased droplet collisions, thus decreasing flocculation and coalescence and therefore reducing the creaming rate.[8] In the same way, Karlberg *et al.*[27] reported that the viscosity of the continuous phase and the adsorption of the hydrophobically modified cellulose at the o/w interface are the key factors for the stabilization mechanism of the emulsion.

3.2. Oxidative stability

The peroxide value (PV) of the emulsions over time is shown in Fig. 2A. In general, a continuous rising trend in PV was found throughout the storage time. However, the increase in PV seemed to differ according to the cellulose ether used to stabilise the emulsion. Specifically, the PV increased during storage in the following order: HPMC < MC < HHPMC < HMC. Therefore, the HPMC emulsion seemed to be the most oxidative-stable of the emulsions, as the PV increased slightly but significantly ($P < 0.05$) between day 0 (5.4 meq/kg) and day 10 (6.6 meq/kg), then remained almost constant ($P > 0.05$). This could mean that a smaller fraction of lipids was susceptible to oxidation due to the good protection afforded by HMPC in this emulsion. In general, the oxidation stability provided by these cellulose ethers could be due to their adsorption ability on the o/w interface, acting as a physical barrier and thus separating the lipid substrates from the pro-oxidants present in the aqueous phase.[28] In addition, the amount of unadsorbed celluloses present in the continuous phase of the emulsions could enhance viscosity, resulting in slow diffusion of pro-oxidants and hence a decreased lipid oxidation rate, as observed by Khouryieh *et al.*[8] in whey protein-stabilised o/w emulsions with xanthan-locust bean gum mixtures. In this regard, as the HMC emulsion exhibited the highest viscosity (visual observations and textural results in section 3.3), it could be expected to be the most stable emulsion. However, the HMC emulsion exhibited a significant ($P < 0.05$) increase in PV during the storage time (from 5.0 meq/kg at day 0 to 11.5 meq/kg at day 30), so it was the least oxidative-stable emulsion. This could be because some air bubbles formed inside the gel (Fig. 1) during the preparation of the HMC emulsion. The presence of these bubbles, and thus the presence of oxygen, could have promoted the formation of hydroperoxides.

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Moreover, some authors have found a positive correlation between oil droplet size and lipid oxidation.[29–31] In this regard, the higher PV in HMC emulsion than in the other ones could be associated with its larger oil droplet size (see fresh emulsions mean diameters in microstructure section). Nonetheless, other works have shown no effect of droplet size on lipid oxidation[32,33] or an inverse correlation between droplet diameter and lipid oxidation.[34,35] Therefore, no consistent results are found in literature and thus there is no a clear trend on the lipid oxidation and its relation to the particle size measurements.[31] In conclusion, the good protection against oxidation afforded by cellulose ethers in o/w emulsions could be mainly due to their ability to separate the lipid substrate from the pro-oxidants (physical barrier on the interface) and their high capacity to thicken the aqueous phase (high bulk viscosity), which would result in slow diffusion of pro-oxidants.

As a consequence of hydroperoxide degradation, secondary oxidation products such as conjugated triens, aldehydes and ketones are formed and can be measured using the specific extinction coefficient at 270 nm (K270) (Fig. 2B). High initial values could be due to the refined sunflower oil's containing oil refining products that also absorb at 270 nm. In general, all the emulsions showed a slight change in K270 values over the storage time. Therefore, few secondary oxidation products were expected to be formed. The HPMC emulsion exhibited a significant ($P < 0.05$) increase in the K270 coefficient, from 5.46 (day 0) to 6.45 (day 20). This could show that hydroperoxides formed during those 10 days degraded into few secondary oxidative products. Although the HMC emulsion exhibited a sharp increase in PV during storage, no significant ($P > 0.05$) changes were observed in its K270 values.

In conclusion, in general, cellulose ethers provide good oxidative stability for o/w emulsions.

3.3. Texture analysis

The area under the curve (AUC) was taken as representative of the extrusion force profiles in relation to time, indicating the consistency of the samples (Table 1). On the one hand, the results showed that all the samples behaved in

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the same way, as the AUC values of each cellulose ether emulsion decreased during the digestion phases as follows: fresh emulsion > after oral digestion > after gastric digestion. Specifically, the initial AUC values (fresh emulsions) exhibited a slight decrease after the oral phase but an accentuated decrease after the gastric phase. The reduction in AUC values for all the emulsions during the digestion phases was mainly due to the dilution effect of adding simulated oral and gastric fluids, because approximately the same results were obtained on carrying out the same test with water instead of simulated fluids (data not shown). Espert *et al.*[36] also observed that the decrease in force values in highly concentrated methylcellulose o/w emulsions should be attributed to water dilution rather than stomach conditions (acid pH and pepsin activity). Moreover, Espinal-Ruiz *et al.*[1] noted that the viscosity of all the emulsions they analysed (o/w emulsions stabilised by Tween-80 mixed with methylcellulose, chitosan or pectin) was relatively low under simulated gastric and intestinal conditions. They suggested that this could be attributed to the progressive dilution that occurs after passage through each stage of the gastrointestinal model. The emulsions stabilised with HPMC, HHPMC and MC exhibited similar AUC values in each phase, and therefore possessed a similar consistency. Although HPMC and MC emulsions were significantly different ($P < 0.05$) before digestion (fresh emulsions), they did not exhibit significant differences ($P > 0.05$) after the oral and gastric phases. The HMC emulsion showed significantly higher AUC values ($P < 0.05$) compared to the other emulsions in all the phases. Hence, the HMC emulsion presented the highest resistance to extrusion, as it was the most consistent in all phases.

These results could offer an initial approach to weight management, because simply increasing the viscosity of foods and beverages increases subsequent satiety responses.[37] The intake of food or fluid distends the stomach and triggers mechanoreceptors and vagal afferents, which regulate satiation and satiety,[15] as the postprandial gastric volumes are linearly associated with perceptions of fullness and satiety.[38] As a consequence of larger gastric volumes, gastric emptying is delayed.[39] However, it must be taken into account that the intestine also plays a dominant role in satiation and satiety. The digestion and absorption of the nutrients influence gastrointestinal processes related with satiation and satiety. Therefore, the HMC emulsion, which exhibited

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the highest consistency at gastric level, may be expected to slow down gastric emptying and concurrently increase gastric distension to a higher extent than the other cellulose emulsions and thus to increase fullness and satiety perceptions. Hence, this could be a good way to combat excess weight and obesity.

Table 1. Area under the curve (AUC) values (N·s) of the cellulose ether emulsions before (fresh emulsion) and after oral and gastric *in vitro* digestion.

Sample	Fresh emulsion	Oral phase	Gastric phase
HPMC	24.69 ^{a} (1.20)	21.53 ^{a} (0.99)	5.03 ^{ab} (0.47)
HHPMC	28.01 ^{ab} (0.42)	17.44 ^{b} (1.25)	4.46 ^{b} (0.83)
MC	31.52 ^{b} (2.68)	23.82 ^{a} (1.73)	6.27 ^{a} (0.29)
HMC	69.79 ^{c} (4.94)	53.89 ^{c} (1.27)	21.31 ^{c} (1.50)

Values in parentheses are standard deviations. Different superscript letters in the same column denote values with statistically significant differences ($P < 0.05$) according to the LSD multiple range test.
HPMC: hydroxypropyl methylcellulose emulsion; HHPMC: high hydroxypropyl methylcellulose emulsion; MC: methylcellulose emulsion; HMC: high methylcellulose emulsion

3.4. FFA release during *in vitro* digestion

The free fatty acids (FFA) released during digestion of the cellulose ether emulsions were compared with a CaCN emulsion in order to check the effectiveness of cellulose ethers in decreasing fat digestibility. Fig. 3 shows the profiles of the FFA released from the different emulsions over the digestion time. As a general trend, there was a relatively rapid release of FFA during the first 10 min, after which the rate of lipid digestion decreased, reaching an almost constant value at the longer times. The slower rate of FFA release could be associated with an accumulation of lipolysis products at the droplet surface, which could compete with the lipase molecules for adsorption at the interface, reducing the lipase activity.[4,11] The CaCN emulsion had the highest digestion rate and extent of FFA release (approximately 50%). Conversely, the cellulose ether emulsions seemed to stabilise at 40 min and therefore showed an

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appreciable decrease in the extent of lipid digestion compared to the CaCN emulsion. The relatively slower initial digestion rate of the cellulose ether emulsions might have been due to the higher time taken for the surface-active components in the bile extract or lipase to adsorb to the droplet surfaces and displace the initial emulsifier layer.[24] Among the emulsions stabilised with different types of cellulose ethers, the HHPMC, HPMC and MC emulsions behaved in the same way. They exhibited the same FFA release profile and small differences in the extent of lipid digestion (25-30% FFA released). This agrees with Pizones Ruiz-Henestrosa *et al.*[16], who found that the amount of FFA released during the digestion of two emulsions stabilised by two different HPMCs (different molecular weight and hydrophobicity) was similar (45-50%). Moreover, in another study, all the emulsions stabilised with different types of HPMC had very similar digestion profiles regardless of their molecular weight or methoxyl content.[2] In contrast, in the present study the HMC emulsion was the least-digested one, exhibiting a very slow increase in FFA release and reaching approximately 20% of FFA released. The results obtained suggest that the lipase was able to access the emulsified lipid more readily in the CaCN-coated droplets than in the cellulose ether-coated droplets, with the HMC emulsion being the least accessible. Similarly, the lipid hydrolysis experiments of Mun *et al*.[4] suggested that the initial caseinate layer surrounding the droplets did not prevent the formation of free fatty acids in the emulsions. In addition, some authors have found non-ionic surfactants (such as different celluloses ethers and polysorbate 20 (Tween20) emulsions) to be more resistant to lipid digestion than protein or other polysaccharide-stabilised emulsions.[1,4,11] Moreover, different types of HPMC-stabilised emulsions have been reported as being more resistant to lipid digestion than a Tween20 emulsion.[2]

The low release of FFA from cellulose ether emulsions in the present study might have been due to a number of possible reasons. Firstly, cellulose ethers may have been able to form interfaces that were more resistant to displacement by bile salts, which may make it difficult for lipase to access the interface required for lipid digestion.[2,24] Secondly, greater interactions between the cellulose and bile salts might take place, hindering the access of bile salts to the o/w interface.[2] Thirdly, the high consistency of cellulose emulsions at the end of the gastric phase (especially that of the HMC emulsion) may have been able to

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alter mass transport, inhibiting the ability of lipase to reach the lipid droplet surfaces.[1] Therefore, these results, together with those of several other researchers, demonstrate that lipase activity, and hence lipid digestion, could depend on the nature of the emulsifier, among other physicochemical factors.

The present results could offer a second approach to weight management: reducing lipid digestion and thus, possibly, lipid absorption. Several gastrointestinal processes affect satiation and satiety. They include gastric distension and gastric emptying, as previously mentioned, but also digestion and absorption, which are influenced by the physicochemical properties of the nutrients present in a meal.[40] When fat is emptied from the stomach into the small bowel, the presence of fatty acids is sensed by the small intestinal mucosa, which leads to secretion of gut peptides such as cholecystokinin (CCK) and peptide YY (PYY), the two important satiety hormones.[12] In turn, these hormones lead to a delay in gastric emptying,[41] influencing hunger and food intake.[42] Therefore, although reducing lipid digestion in order to reduce lipid absorption could be a good strategy for combating chronic diseases associated with overweight and obesity, it is important to digest a relatively small part of the lipids in order to influence satiety as well and avoid possible digestive problems. In this regard, the possibility of controlling the structure and lipid digestion of novel emulsions in order to control the appetite (increasing the feeling of satiation and satiety, which might lead to lower total calorie consumption) and nutrient delivery is of considerable interest.[1,16]

3.5. Microstructure analysis

LM and CLSM were used to observe the initial microstructure of the cellulose and caseinate emulsions and follow the microstructural changes that took place during gastrointestinal digestion (Fig. 4 and 5A). The fresh emulsions had a heterogeneous distribution of oil droplets size (Fig. 5B). The mean diameter of the oil droplets were 9.4 ± 4.1 μm for CaCN emulsion, 10.3 ± 3.1 μm for HPMC emulsion, 9.2 ± 2.9 μm for HHPMC emulsion, and 10.5 ± 4.6 μm for MC emulsion. Therefore, these emulsions exhibited a similar mean droplet diameter. On the contrary, the HMC emulsion showed several large oval oil

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droplets (Fig. 4 and 5A) with a mean diameter of 16.4 ± 5.6 μm and a droplet size distribution with higher values than those of the other emulsions (Fig. 5B), as well as some air spaces among the oil droplets. These results were in accordance with the visual aspect observed in Fig. 1 (shown in section 3.1), where some holes could be observed in the HMC emulsion but none were visible in the other emulsions.

Although the consistency values decreased after the oral phase (see results section 3.3), no dilution effect in the cellulose ether emulsions was appreciable in the micrographs. This effect could be due to water holding capacity of the cellulose ethers. However, this effect was noticeable in the CaCN emulsion, where the oil droplets seemed to be more dispersed and the protein network formed by the CaCN could be clearly seen in purple.

After the gastric phase (Fig. 4), the purple-stained protein network of the CaCN emulsion disappeared, due to pepsin digestion, and several oil droplets therefore appeared flocculated, forming a large floc (around 267 μm). Mun *et al.*[4] also observed many clustered droplets rather than large individual droplets in a caseinate emulsion, indicating that it appeared to be more prone to droplet flocculation than coalescence. One the one hand, this fact could be due to the drop of the pH from the oral phase (pH 6.8) to the gastric phase (pH 2) that could destabilise CaCN, because CaCN reaches its isoelectric point (around pH 4.6) and could aggregate and precipitate. On the other hand, the proteolysis of the interfacial layer promotes the formation of oil droplet aggregates as it causes a gradual loss in the superficial charge of the droplets and reduces the thickness of the interfacial layer.[11] In the micrographs of the cellulose ether emulsions after the gastric phase (Fig. 4), the dilution effect was more visible than after the oral phase, the size of the oil droplets remained almost unchanged (Fig. 4) and flocculation mechanisms were absent. Bellesi *et al*.[11] found that after few minutes of gastric digestion, HPMC-coated droplets showed a slight change in particle size distribution, which remained almost constant for the rest of the gastric digestion time. The authors explained that this was because of the lower number of ionizable groups reported for the HPMC compared to the proteins (soy and whey proteins) and because the pepsin had no effect on fats and carbohydrates. Moreover, Gallier *et al.*[43] observed that a non-ionic surfactant was not affected by the drop in pH in the stomach and thus

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Tween-oil emulsions remained stable under gastric conditions. The results corroborate the fact that cellulose ether emulsions are more resistant under gastric conditions than protein emulsions.

After the intestinal phase (Fig. 4 and 5A), the micrographs showed that large changes had occurred in all the emulsions, especially in the CaCN emulsion. Very small oil droplets (with a mean diameter of 2.2 ± 0.9 μm for CaCN emulsion, 3.1 ± 1.0 μm for HPMC emulsion, 2.7 ± 1.1 μm for HHPMC emulsion, 2.3 ± 0.6 μm for MC emulsion, and 4.5 ± 2.8 μm for HMC emulsion) and the formation of new kinds of aggregates were observed, which were very large (around 130-140 μm) in the case of the CaCN emulsion (Fig. 4). However, the HMC emulsion exhibited smaller changes (Fig. 4 and 5A), as several oil droplets with different sizes were still observed and aggregates did not seem to have been formed. The oil droplet size distribution of the HMC emulsion (Fig. 5B) showed a wide peak around 3-4 μm near the peaks of the other emulsions, but also another peak around 8 μm and a tail around 10-14 μm. These results matched those obtained for the percentage of FFA release. On the one hand, the CaCN emulsion was the one with highest values of FFA release, indicating that it was the most-digested sample, and this emulsion showed the smallest oil droplets (with a mean diameter of 2.2 ± 0.9 μm at the end of the intestinal phase). On the other hand, the HMC emulsion was the least-digested (it showed lower %FFA values) likely due to the largest oil droplets (with a mean diameter of 4.5 ± 2.8 μm) exhibited after *in vitro* gastrointestinal digestion. Bellesi *et al.*[11] observed that irrespective of the composition/structure of the emulsions, the initial surface area determined the initial rate of lipolysis. Therefore, the lower release of FFA from the HMC emulsion could be due to a wide variety of reasons. Firstly, its higher droplet size compared to the other cellulose ether emulsions. Hence, this emulsifier provided a smaller initial interfacial area for the lipase to attach to, with the possibility of hydrolysing lipids at a lower rate and to a smaller extent, as Torcello-Gómez and Foster[2] observed in different HPMC emulsions. In this regard, the inhibition of lipid digestion could be expected to increase as the flocs size rose and as the packing of droplets and polymers within the flocs grew, since these factors would reduce the ability of lipase molecules to diffuse rapidly through the whole of the flocs.[1] Nevertheless, although the CaCN emulsion could present a

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smaller initial interfacial area due to the formation of flocs at the gastric phase (Fig. 4), it was the most-digested sample. Secondly, the highest bulk viscosity of the HMC emulsion, which implies a physical impediment for the lipase to reach the interface. Thirdly, the possible thermal gelation of the continuous aqueous phase at 37 °C,[17] which could make even more difficult the access of the enzyme to the substrate.

Pizones Ruiz-Henestrosa *et al.*[16] concluded that the difference in the rate and extent of lipolysis found in their results could mainly be attributed to the molecular events occurring at the interface upon bile salt adsorption, rather than to differences in the molecular weight/viscosity or the size/surface area available for the action of lipase/colipase. Hydrophobic interactions have been postulated to take place between cellulose ethers and bile salts[44] and both methyl and hydroxypropyl groups can bind or "sequester" bile salts.[16] Specifically, the hydrophobic faces of bile salt molecules adsorb to the hydrophobic portions of cellulose ethers.[45] In the case of methylcelluloses (which only have methyl group substituents), although bile salts would adsorb to the methyl groups, other methyl groups would be still available for hydrophobic association for cellulose molecule self-assembly.[45] In the case of hydroxypropyl methylcelluloses (HPMCs), the adsorption of bile salts onto the larger hydroxypropyl groups would hinder the hydrophobic association to a larger extent due to steric effects and because hydroxypropyl groups are more "difficult" to incorporate within ordered structures than methylcelluloses.[44] Therefore, in the case of HPMCs the lower methyl group content and the presence of more polar and larger hydroxypropyl groups that inhibit intermolecular association leads to the formation of a more untangled system than with MC and explains why HPMCs would be more affected by bile salts.[44,45] In this context, bile salts interacting with the hydrophobic groups of the cellulose backbone would impart a negative charge that would increase the repulsion between the cellulose molecules, thus decreasing their tendency to aggregate or self-assemble. As the self-assembly or aggregation tendency of HPMCs was more hindered by bile salts than that of the other cellulose ethers, as described above, this would provoke more untangling of the cellulose molecules at the interface, making more sites available for lipase adsorption and resulting in more extensive lipolysis.[16] Therefore, a thicker adsorbed

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interfacial layer formed by cellulose ethers and/or an interfacial arrangement with more entanglements, which could be the case of HMC emulsion due to higher methyl substitution, and thus stronger hydrophobic interactions, could possibly be less susceptible to disruption by intestinal components (mainly bile salts as described above).[2] This could be why the HMC emulsion exhibited the lowest percentage of FFA release, besides its larger initial droplet size and its higher consistency. Consequently, according to the findings of the present study, the physical barrier effect of the cellulose ethers on the droplet interfaces, the increased viscosity in the continuous phase, the molecular events occurring at the interface as well as the droplet size could have a great impact on lipid digestibility.

4. Conclusions

This study has demonstrated that the use of cellulose ethers provided good physical and oxidative stability to o/w emulsions. This seemed to be due to the ability of the cellulose ethers to form a physical barrier on the interface, allowing the lipid substrate to be separated from the pro-oxidants, as well as to a thickened aqueous phase that would slow down the diffusion of these pro-oxidants.

Also, the use of these cellulose ethers with thermo-gelling ability, specifically HMC, made it possible to obtain o/w emulsions with high consistency even during gastric digestion, which could slow down gastric emptying and increase gastric distension, thus increasing fullness and satiety perceptions.

Moreover, this study has shown that cellulose ethers, in particular HMC, delay lipid digestion of o/w emulsions compared to a conventional food emulsifier (calcium caseinate).

Therefore, considering the results as a whole, it was concluded that controlling the structure of emulsions and their digestion behaviour could achieve emulsions that enhance satiation capacity and decrease lipid digestion, which could be used in weight management.

Acknowledgements

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The authors are grateful to the Spanish Ministry of the Economy and Competitiveness for financial support (AGL2015-68923-C2 (MINECO/FEDER)) and gratefully acknowledge the financial support of EU FEDER funds. They would also like to thank Mary Georgina Hardinge for assistance in correcting the English manuscript.

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Figures captions

Fig. 1 Effect of emulsifier type and storage time on physical stability. HPMC: hydroxypropyl methylcellulose emulsion; HHPMC: high hydroxypropyl methylcellulose emulsion; MC: methylcellulose emulsion; HMC: high methylcellulose emulsion.

Fig. 2 Lipid oxidation rate of o/w emulsions stabilised with different cellulose ethers. **A)** Peroxide value (PV) and **B)** specific extinction coefficient at 270 nm (K270) during a storage period of 30 days. The error bars represent standard deviations. Different lowercase letters on the bars indicate significant differences ($P < 0.05$) during the storage period within each emulsion. 0; 10; 20 and 30 denote storage time in days. HPMC: hydroxypropyl methylcellulose emulsion; HHPMC: high hydroxypropyl methylcellulose emulsion; MC: methylcellulose emulsion; HMC: high methylcellulose emulsion.

Fig. 3 Free fatty acid (FFA) released under simulated *in vitro* intestinal conditions from emulsions stabilised by protein (CaCN ×) and by different cellulose ethers (HHPMC ●, MC ■, HPMC ♦ and HMC ▲). The error bars represent standard deviations.

Fig. 4 Light microscopy micrographs of emulsions stabilised by different emulsifiers before (fresh emulsion) and after oral, gastric and intestinal digestion phases. Magnification 20x. The scale bars measure 100 μm. White and black arrows show cellulose ethers and oil droplets respectively. CaCN: calcium caseinate emulsion; HPMC: hydroxypropyl methylcellulose emulsion; HHPMC: high hydroxypropyl methylcellulose emulsion; MC: methylcellulose emulsion; HMC: high methylcellulose emulsion.

Fig. 5 A) Confocal micrographs of emulsions stabilised by different emulsifiers before (fresh emulsion) and after intestinal digestion phase. Magnification 60x. The scale bars measure 60 μm. **B)** Droplet size distribution of emulsions before (fresh) and after intestinal digestion phase. CaCN: calcium caseinate emulsion;

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738 HPMC: hydroxypropyl methylcellulose emulsion; HHPMC: high hydroxypropyl
739 methylcellulose emulsion; MC: methylcellulose emulsion; HMC: high
740 methylcellulose emulsion.
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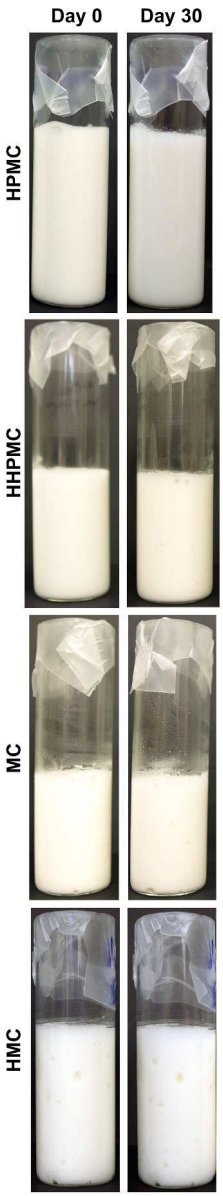

Figure 1

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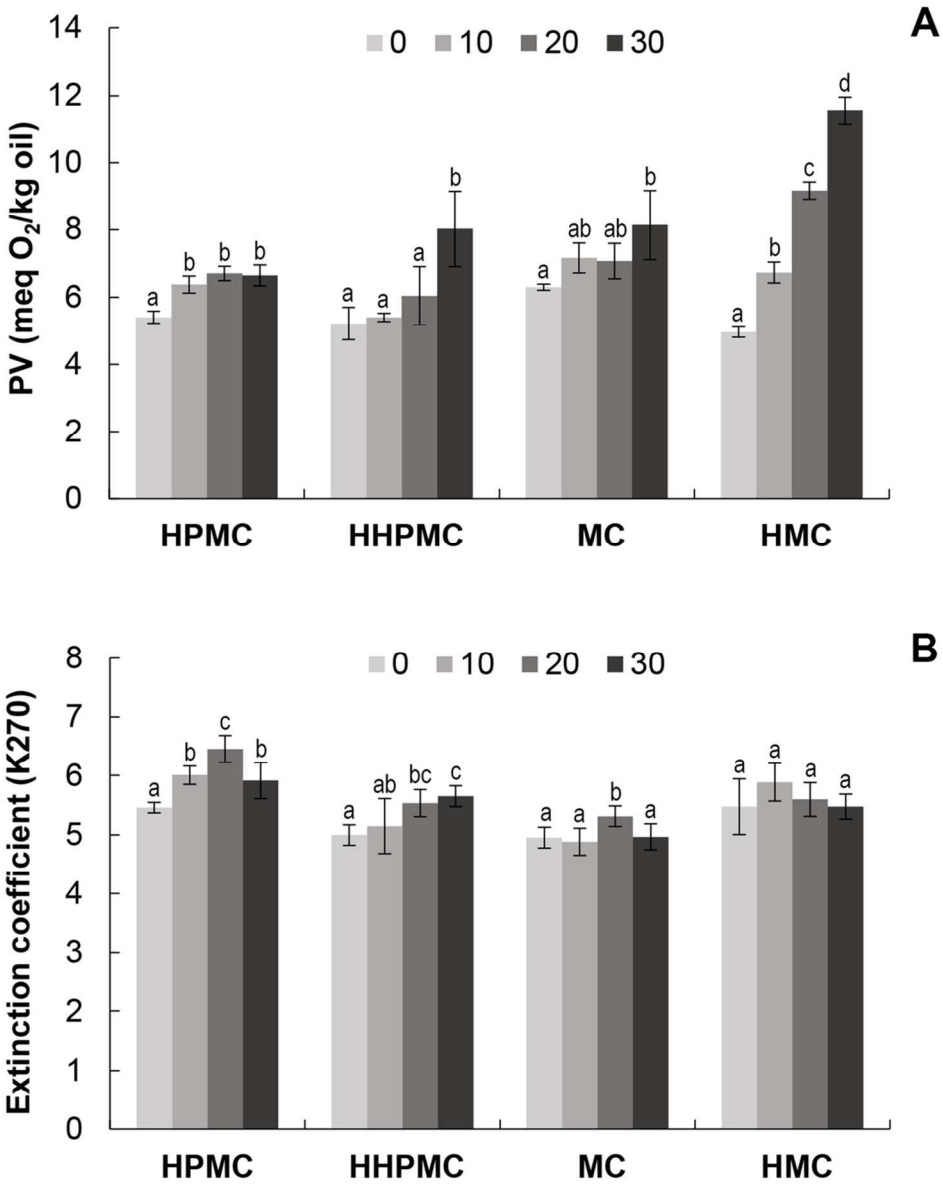

Figure 2

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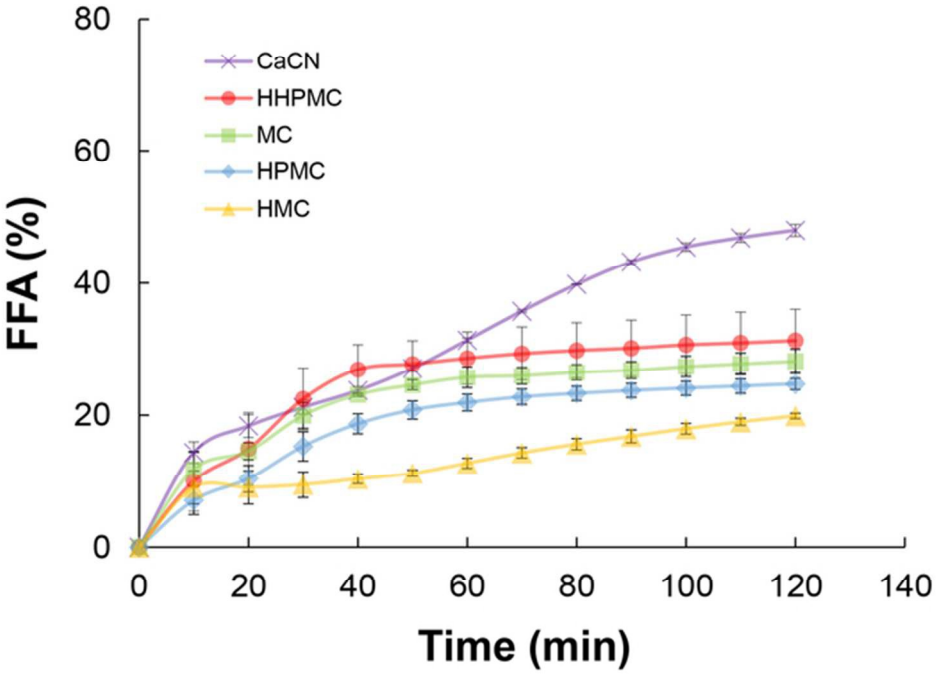

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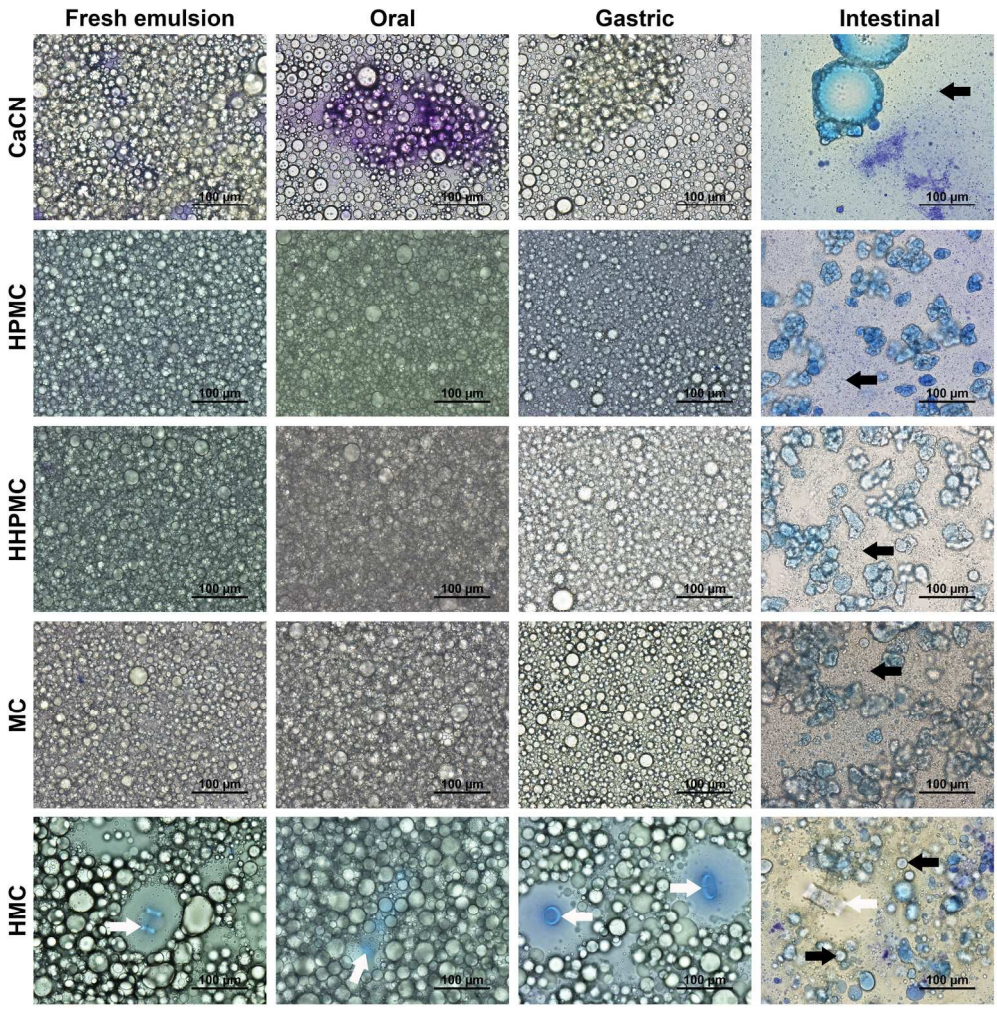

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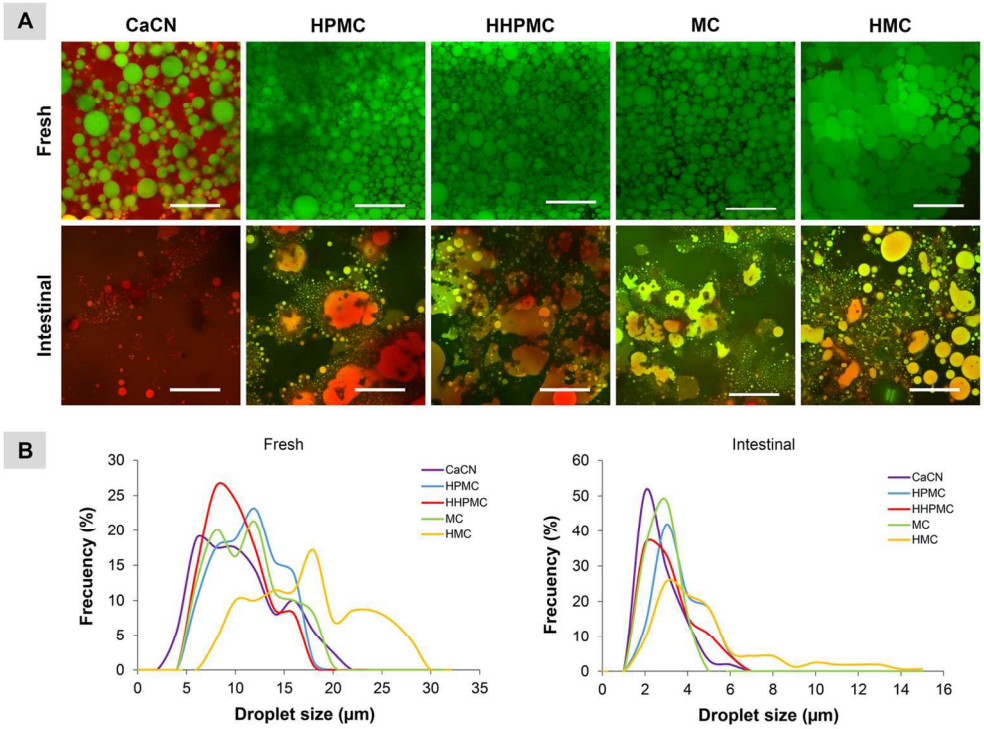

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Cellulose ether emulsions have good physical and oxidative stability and can delay *in vitro* lipid digestion.
HMC emulsions inhibit lipolysis more than others and could enhance gastric fullness and satiety