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Pickering Emulsions Containing Cellulose Microfibers Produced by Mechanical Treatments as Stabilizer in the Food Industry

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Received: 19 December 2018; Accepted: 18 January 2019; Published: 21 January 2019

Featured Application: Cellulose microfibers can be used as stabilizer in sunflower oil-in-water Pickering emulsions, replacing trans and saturated fats in food.

Abstract: Pickering emulsions are emulsions stabilized by solid particles, which generally provide a more stable system than traditional surfactants. Among various solid stabilizers, bio-based particles from renewable resources, such as micro- and nanofibrillated cellulose, may open up new opportunities for the future of Pickering emulsions owing to their properties of nanosize, biodegradability, biocompatibility, and renewability. The aim of this research was to obtain oil-in water (O/W) Pickering emulsions using cellulose microfibers (CMF) produced from cotton cellulose linters by mechanical treatment through a high-pressure homogenizer. The O/W Pickering emulsions were prepared with different O/W ratios by mixing edible oil (sunflower oil) with water containing CMF at concentrations of up to 1.0 wt%. The apparent viscosity of the separated emulsion phase was measured. Results showed the feasibility of using low concentration of CMF for preparing and stabilizing Pickering emulsions, with the apparent viscosity of the emulsion phase increasing 60–90 times with respect to the sunflower oil, for a shear rate of 1 s^{-1}. In addition, theoretical nutrition facts of the emulsions were calculated and compared with other fats used in foods, showing that they can be a promising low-calorie product containing dietary fiber, replacing trans and saturated fats in foods.

Keywords: cellulose microfibers; Pickering emulsions; sunflower oil; rheology; emulsification index; food applications

1. Introduction

Pickering emulsions, which contain solid particles as stabilizer, are nowadays used for different applications. Several emulsifiers have been used. such as lecithin and monooleates [1], mono- and diglyceride of fatty acids [2], polymer particles [3], chitin nanocrystals [4], magnetic nanoparticles [5], starch [6,7], oligosaccharides as α-cyclodextrin [8], and several products from cellulose.

Cellulose products have also been used to prepare Pickering emulsions as milled cellulose [9]; cellulose nanofibers (CNF) chemically modified using lauroyl chloride [10]; bacterial cellulose [11]; hydroxypropyl microcellulose [12]; cellulose microfibers (CMF) treated with essential oils [13]; chemically unmodified cellulose nano/microcrystals using different alkanes as the oil phase [14–17]; and chemically modified cellulose, such as sulfated [10], carboxylated [18], or grafted cellulose

nanocrystals [19]. To obtain these products, mechanical treatments, such as homogenization, are used. In most cases, some mechanical, biological, or chemical pretreatment is necessary to reduce the energy consumption in the mechanical process [20,21]. Several reviews have been published for cellulose product characteristics, their properties, or applications [22–25]. However, not all pretreatments are acceptable in the food industry due to the toxicity of some chemical agents used.

In the food industry, Pickering emulsions using cellulose products as emulsifiers include cellulose nanocrystals chemically modified with octenyl succinic anhydride [26] or ethyl lauroyl arginate [27] and CMF modified with hydrophilic polymers (cellulose esters/ethers) or pretreated with enzymatical or carboxymethylation pretreatment as emulsifier/stabilizer in whipped toppings, sauces, foams, or bakery products [28,29]. In this way, cellulose is not digested by human beings, although it can be partially broken down into smaller components for absorption into the bloodstream and is considered as dietary fiber [30].

To the best of our knowledge, there have been few studies related to the use of chemically unmodified CMF in food-grade Pickering emulsions that can substitute unhealthy fats in food products. Winuprasith and Suphantharika [31] studied the stability and the rheology of soy oil-in-water emulsions with low oil content (10%) using chemically unmodified CMF produced from mangosteen rind. Bai et al. [32] studied the synergy of cellulose nanocrystals and CNF without any modification to stabilize edible oil-in-water emulsions with 10% sunflower oil. This paper is focused on the use of chemically unmodified CMF as a stabilizer in emulsions with high oil content, above 20%, and the influence of CMF doses on the stability and rheology of these emulsions.

Food manufacturers use the partial hydrogenation of unsaturated fatty acids (UFA) to produce saturated fatty acids (SFA) with more desirable properties, such as increased hardness, greater stability, higher resistance to oxidation, and longer shelf life of vegetable oils. In this process, UFA (with cis configuration in nature) converts some of the cis double bonds into trans bonds by an isomerization reaction, creating trans fatty acids (TFA). TFA are less healthy than SFA and UFA as they decrease HDL cholesterol and increase LDL cholesterol in the blood, increasing the probability of heart disease and sudden cardiac death. As a result, governments and international organizations are making an effort to warn people about TFA and are developing laws to limit the amount of TFA in foods by replacing SFA and TFA with UFA [2,33–35]. On the other hand, many organizations have pointed out the importance of meeting future societal needs by promoting the conservation and care of population health through healthy eating [36]. Some alternatives to hydrogenation of UFA have been explored in industries, such as interesterification or fractionation. However, these techniques have drawbacks, including concerns about the health effects of interesterification and the residual products produced by the fractionation process [37,38]. In this scenario, Pickering emulsions using CMF as stabilizer with an unsaturated oil phase could be used as a potentially healthy alternative to the hydrogenation of UFA, increasing the product hardness.

The aim of this research was to obtain Pickering emulsions using chemically unmodified CMF obtained from cotton fibers as the stabilizer and sunflower oil as the healthy fat. The present work studied the stability and rheology of oil-in water (O/W) emulsions using different CMF dosages and high oil content above 20%. Afterward, theoretical nutrition facts of the emulsions were calculated and compared with other fats used in foods in order to study the feasibility of their use as food-grade Pickering emulsions. In this way, a healthy and low-calorie product for the food industry could be produced, replacing SFA and TFA with healthy fats such as UFA.

2. Materials and Methods

The raw materials used to prepare O/W emulsions were refined sunflower oil (Jaen, Spain), purchased from a local supermarket, and high purity cellulose powder, obtained from cotton linters (Sigma-Aldrich, St. Louis, MO, USA), to produce the CMF. Chemically unmodified CMF were obtained by soaking in water for at least 24 h before high-pressure homogenization. The homogenization

process was carried out at 600 bar and 10 passes using a laboratory homogenizer PANDA PLUS 2000 manufactured by GEA Niro Soavy (Parma, Italy). Chemicals were not used in this process.

CMF were characterized by determining the consistency, carboxylic groups, nanofibrillation degree, transmittance, cationic demand, polymerization degree, and CMF diameter distribution according to Balea et al. [39].

The amount of carboxylic groups in the oxidized fibers was determined by conductimetric titration before homogenization. A pulp sample containing 0.15 g of dry CMF was added to 5 mL of 0.01 M NaCl, and deionized water was added to a total volume of 55 mL. pH was adjusted to 2.5–2.8 by adding 0.1 M HCl to protonate all carboxylate groups. 0.05 M NaOH was added to the sample in 0.2 mL increments, and the conductivity was recorded after each addition.

The amount of carboxylic groups was calculated from the curve of conductivity vs. NaOH (meq) added [39,40].

Nanofibrillation degree was determined by centrifugation of 0.1% of dry CMF suspension at 4500× *g* for 30 min. The nanofibrillated fraction was isolated in the supernatant, and the yield was calculated as the relationship between dry solids in the supernatant and total dry solids [39,41].

Transmittance readings of 0.1 wt% CMF diluted suspensions were performed at 800 nm on a Cary 50 Conc UV-visible spectrophotometer (Varian Australia PTI LTD, Victoria, Australia), and cationic demand measurements were taken by colloidal titration of 0.1 wt% CMF suspensions with 0.001 N-polyDADMAC on a Mütek PCD04 particle charge detector (BTG Instruments GmbH, Herrsching, Germany).

Polymerization degree (PD) was calculated from the limiting viscosity number (intrinsic viscosity) of the CMF suspension, which was determined by the ISO 5351 standard (2010) according to Equation (1) [39,42,43]:

$$\eta = K \cdot PD^{a} \quad (1)$$

where **η** is the intrinsic viscosity (mL/g). **K** and **a** have different values according to PD; when PD < 950, K = 0.42 mL/g and a = 1 [42].

Finally, CNF was characterized by scanning electron microscopy (SEM) with a JEOL JSM 6335F microscope (JEOL, Tokyo, Japan). Microscopic analyses were carried out in the National Centre of Electronic Microscopy at the Complutense University of Madrid. To prepare the CMF samples for SEM, a drop of diluted suspension (0.001 wt% and 0.005 wt%) was dried on a carbon tape at room temperature and covered with a thin layer of Au to avoid superficial charge accumulation. Image J software was used as image processing program, and diameter distribution of the CMF suspension was evaluated by measuring the diameter of the fibers located in diagonal line of the images.

The O/W **Pickering emulsions** were prepared with O/W ratios from 20/80 to 70/30 (wt%). CMF were added in the aqueous phase with a total concentration of 0.25, 0.5, 0.75, and 1.0 wt%. Emulsions were carried out by mixing sunflower oil and aqueous phase with a high-speed overhead stirrer Heidolph RZR 2051 (Heidolph Instruments, Schwabach, Germany) at 2000 rpm for 60 min. The O/W emulsion samples were prepared in duplicate and stored at 25 °C.

Stability over time of the emulsions was assessed by measuring the heights of water, oil, and emulsion phases for 48 h in a 100 mL graduated cylinder at ambient temperature. The emulsifying capacity of CMF and the stability of the emulsions were expressed by the emulsification index (EI). The EI was calculated with a margin of error of ± 0.3% using Equation (2) [7,44]:

$$EI\ (\%) = V_{emulsion}/V_{total} \cdot 100 \quad (2)$$

where the volume of the emulsion phase formed after 48 h is $V_{emulsion}$, and the total volume of the sample is V_{total}.

Shear stress (τ) of O/W emulsions was measured using a Brookfield DV-II + Pro viscosimeter (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). For each shear rate (γ), τ was calculated. From these values, rheograms were evaluated at ambient temperature and fitted following a three-parameter rheological model known as Herschel–Bulkley model, as given in Equation (3).

Apparent viscosity (μ_a) of emulsions was calculated with the parameters of Herschel–Bulkley model, as given in Equation (4):

$$\tau = \tau_0 + k \cdot \gamma^n \quad (3)$$

$$\mu_a = \tau_0/\gamma + k \cdot \gamma^{(n-1)} \quad (4)$$

where τ_0 is the yield stress, k the consistency index, and n the flow index.

Gravitational stability after 7 days was studied according to Mikulcová et al. [13] by centrifugation of 10 mL of the emulsion phase in a Hettich Universal 320 for 2 min at 500 rpm in order to measure the **stability toward centrifugation** (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) by visual inspection of the different phases and microscope analysis of the oil drops.

Emulsion phase droplets were observed before and after centrifugation using a Zeiss Axio Lab.A1 optical microscope and a color microscope camera Zeiss AxioCam ERc 5s (Carl Zeiss Microscopy GmbH, Göttingen, Germany). Prior to observation, emulsions were placed onto a glass microscope slide and viewed under 10× magnification.

3. Results and Discussion

3.1. CMF Characterization

Table 1 lists the properties of CMF used in this study. Figure 1 shows a CMF image by SEM analysis in which the microfibers can be observed. The diameter distribution of CMF was measured, obtaining an average diameter of 105 nm and a median of 90 nm. These results indicate that the cellulose structure is microfibrillated with a low content of nanofibers, as expected, in CMF obtained without pretreatment [39,45,46].

Table 1. Characteristics of cellulose microfibers (CMF).

Parameter	Units	Value
Consistency	(%)	3.67 ± 0.02
Carboxylic groups	(mmol COOH/g)	0.1 ± 0.03
Nanofibrillation degree	(%)	6.1 ± 0.5
Transmittance 800 nm	(%)	3.8 ± 0.2
Cationic demand	(μeq/g)	19.0 ± 1.0
Polymerization degree	(monomeric units)	399 ± 11

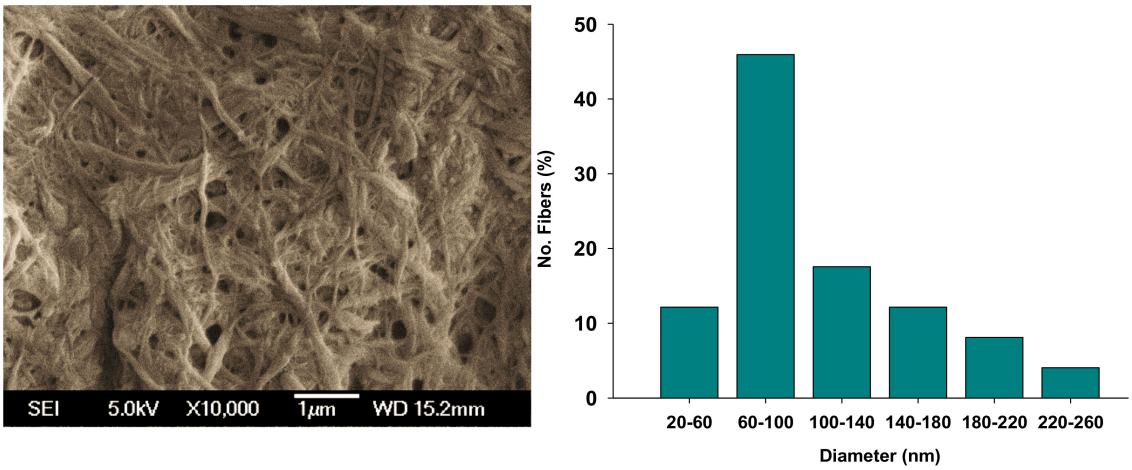


Figure 1. CMF characterization. (**a**) SEM image and (**b**) CMF diameter distribution.

3.2. Stability over Time

The EI values of sunflower O/W emulsions with different dosages of CMF as emulsifier were studied to measure their stability over time (Figure 2). All measurements were carried out in duplicate, and the average error between the replicates was always under 5%.

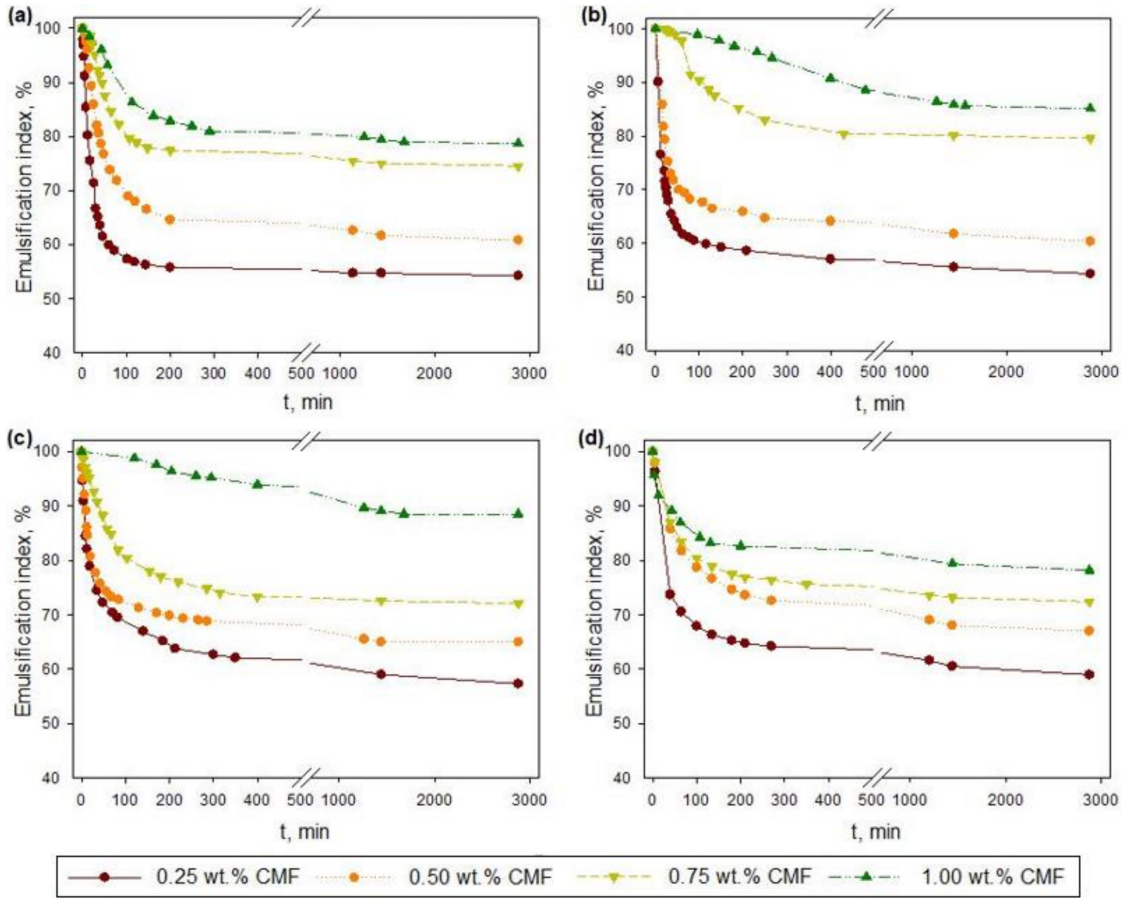


Figure 2. Effect of CMF dose on the emulsification index for sunflower oil-in-water (O/W) emulsions: (**a**) 40/60, (**b**) 50/50, (**c**) 60/40, and (**d**) 70/30.

The evolution of EI was evaluated for 48 h using O/W ratios of 40/60, 50/50, 60/40, and 70/30. At all O/W ratios studied, the emulsions were formed using only CMF, without the use of any additional surfactant or other stabilizers. Results showed that the emulsion phase formed after CMF addition remained stable after 24 h, maintaining the EI values practically constant with time. In all cases, the emulsion phase decreased less than 3% between the first and the second day. One of the main reasons for the efficiently stabilized O/W emulsions in presence of CMF was the amphiphilic surface nature of the microfibers, which originated from the hydrophobic face and hydrophilic edge of cellulose chains, facilitating CMF assembly in the O/W interface [3,5,11,14,47,48]. Furthermore, the CMF formed a densely packed network, which acted as a mechanical steric barrier around the emulsion droplets, preventing their coalescence and stabilizing the emulsion phase over time [31,49,50], as shown in Figure 3. Additionally, higher doses of CMF created a more entangled network that increased the EI in all O/W ratios studied.

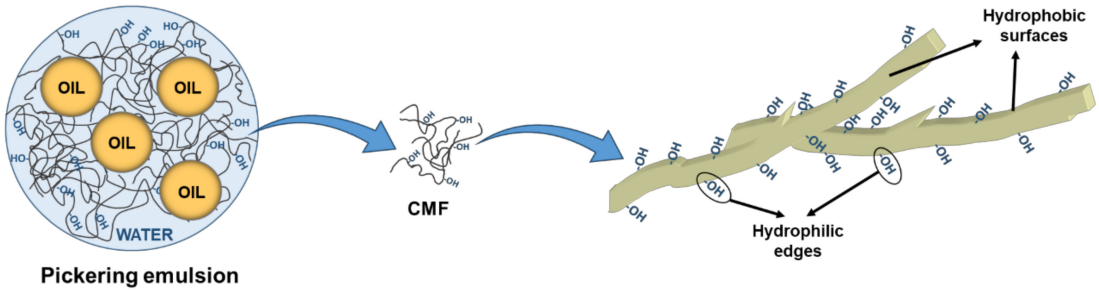


Figure 3. Role of CMF in Pickering emulsions.

Figure 4 shows emulsion, water, and oil phase distribution after 48 h. The addition of CMF was required in all O/W to form the emulsion phase. At the lowest O/W ratio studied (40/60), the addition of CMF formed a thick creaming layer with a clear aqueous phase below. Increased addition of CMF reduced the aqueous phase and raised the emulsion phase, as expected, by forming an extended network that stabilized the emulsion. These results are according to the study by Mikulcová et al. [13], which used CMF modified with essential oils with antimicrobial properties. On the other hand, an oil phase appeared on the top of the graduated cylinder during the stabilization of the samples when the O/W ratio increased. This was due to the CMF being incapable of enclosing all the oil drops, producing a decrease in the encapsulation efficiency [2]. However, for an O/W ratio, the increasing addition of CMF significantly decreased the oil phase, indicating that a high amount of oil droplets were stabilized by CMF in the emulsion phase. The highest EI was observed in samples with 0.75 wt% and 1.00 wt% of CMF (C and D samples, respectively). Among the samples with 0.75 wt% CMF (C samples), the maximum EI (79.5%) was achieved with a 50/50 O/W ratio. Meanwhile, for samples with 1.00 wt% CMF (D samples), the maximum EI (88.5%) was achieved with a 60/40 O/W ratio. In the case of 70/30 O/W emulsions, the addition of 1.00 wt% CMF was not enough to stabilize all oil droplets. Therefore, higher amount of CMF would be required to increase the EI value.

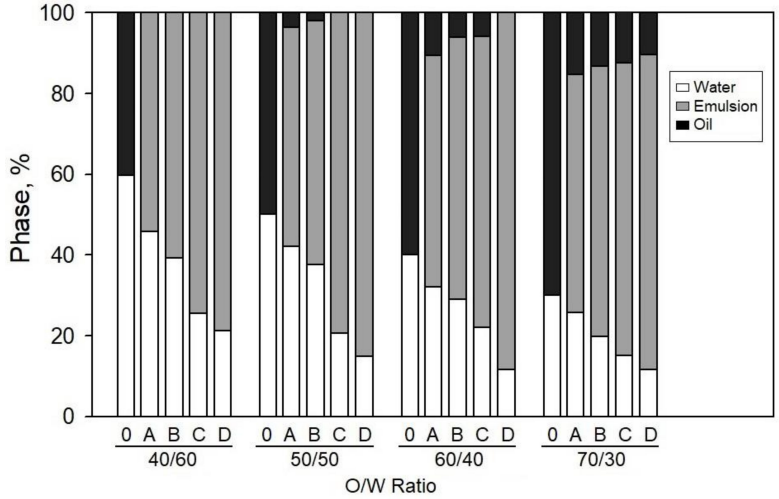


Figure 4. Stabilization over time results of different O/W emulsions after 48 h. (CMF dosages: 0: 0 wt%, A: 0.25 wt%, B: 0.50 wt%, C: 0.75 wt%, D: 1.00 wt%).

Figure 5 shows an example of the graduated cylinders after 48 h at several O/W ratios and the same CMF dose (0.75 wt% CMF, Figure 4, samples C). It can be seen that the different phases were clearly separated. Microscope images of the emulsion phase (Figure 5a,b) clearly showed the two phases formed, corresponding to emulsion and water phases, at low O/W ratios. Optical microscope images showed that the oil and water phases were completely separated due to CMF enclosing the sunflower oil drops, avoiding their coalescence. Figure 5c,d show the emulsions with higher O/W ratios. It can be seen that an additional oil phase was formed on the top of the graduated cylinder.

This CMF dose did not allow the oil excess to be enclosed, so a part of the oil was mixed with water outside the drops. For this reason, sunflower oil went up on the top of the cylinder due to its immiscibility. Some researchers have studied the size of oil droplets formed in the emulsion phase depending on the O/W ratio with milled cellulose [9] and carboxylated cellulose nanocrystals [18]. In both cases, the droplets size slightly increased with the O/W ratio. However, in our case, the effect of drop size could be clearly observed; the droplets shape varied from big and irregular to small and spherical shape when the EI increased. These differences might be due to the different nature and concentration of the cellulose emulsifiers studied in each case. In addition, the droplets had a more uniform size distribution, and there were more of them when the EI was maximum. In this instance, 0.75 wt% CMF could stabilize a 50/50 O/W emulsion without oil phase separation (Figure 5b), while an increase in O/W content produced the coalescence of oil drops.

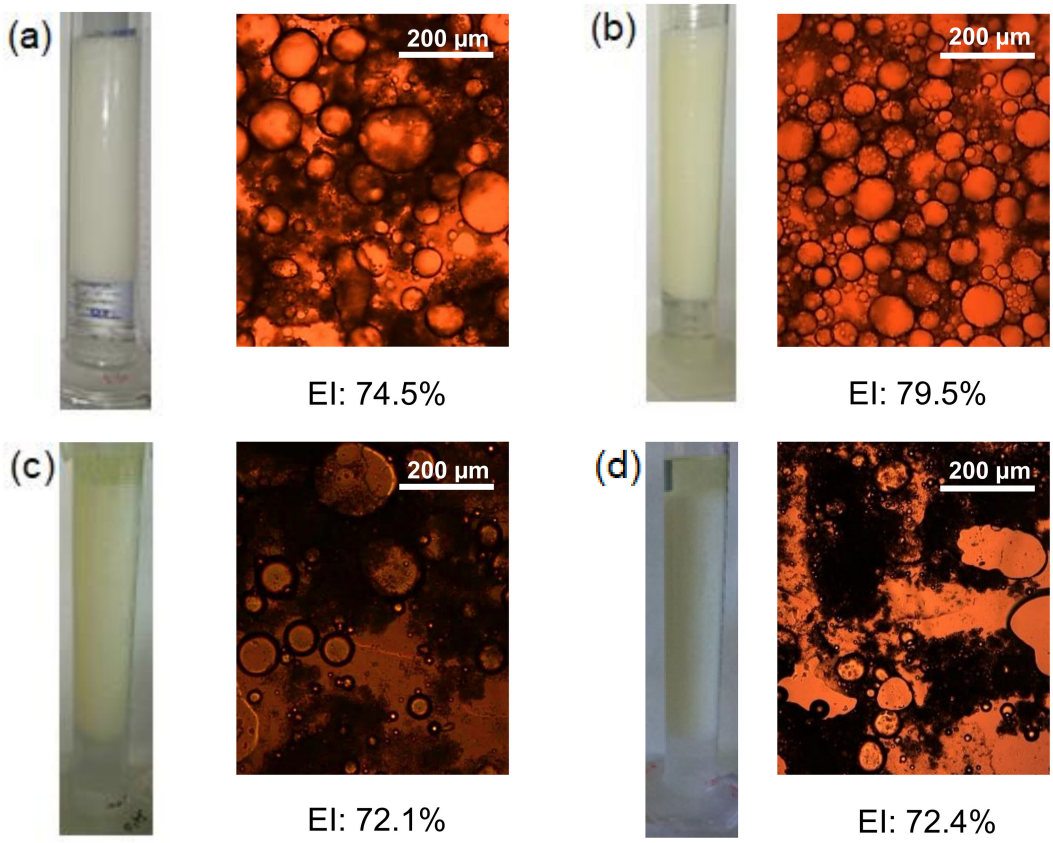


Figure 5. Microscope images of emulsion phases and photographs of the graduated cylinder after 48 h with 0.75 wt% CMF using different O/W ratios: (**a**) 40/60, (**b**) 50/50, (**c**) 60/40, (**d**) 70/30.

3.3. Rheological Measurements

Figure 6 shows the apparent viscosity of the emulsion phase for different shear rates and CMF doses. As observed with other emulsifiers, such as monoglycerides [2], an increase in shear rate decreased the μ_a of the emulsion, following the Herschel–Bulkley model. This behavior of the emulsions was contrary to water or sunflower oil, both Newtonian fluids with viscosities of 0.001 and 0.1 Pa·s, respectively. The highest μ_a was achieved in 50/50 O/W ratio with 0.75 wt% CMF (Figure 6b) and in 60/40 O/W ratio with 1.00 wt% CMF dose (Figure 6c). These results were according to the stability over time; the emulsions with the highest μ_a had the maximum EI value and a uniform size distribution of emulsion droplets.

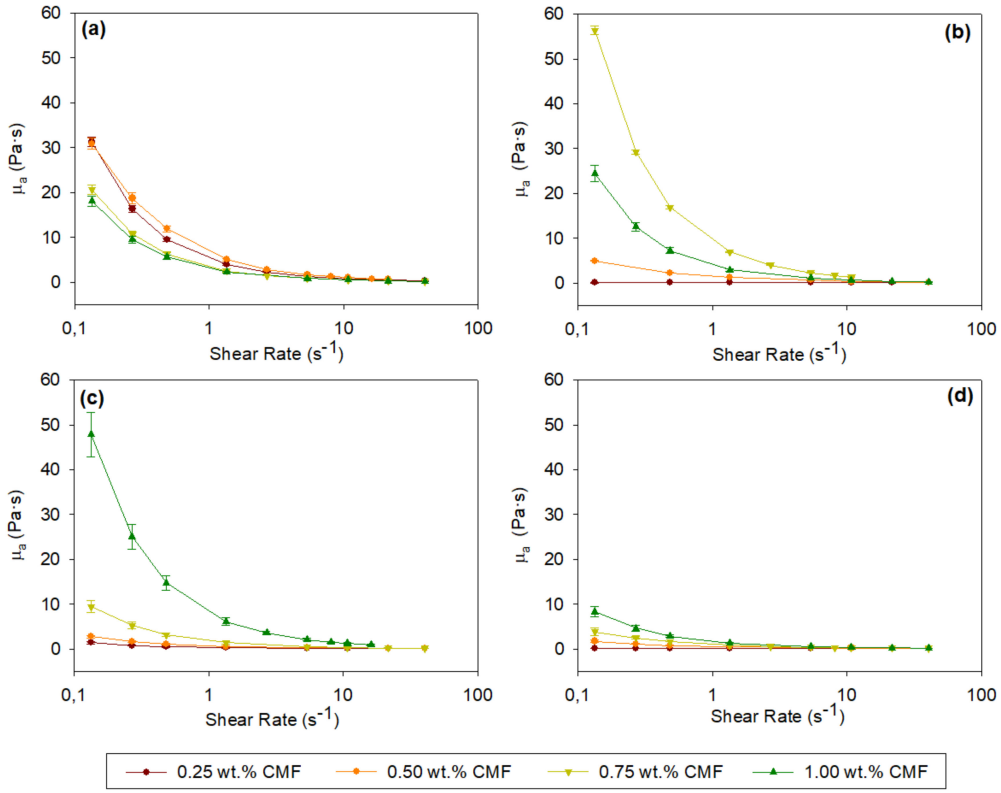


Figure 6. Effect of CMF dose on apparent viscosity for emulsions with different O/W ratio: (**a**) 40/60, (**b**) 50/50, (**c**) 60/40, and (**d**) 70/30.

The relationship between the oil content in the emulsion phase (OCEP) and the μ_a was also studied (Figure 7). The OCEP was calculated according to Equation (5) in which all CMF are in the emulsion phase, and the water and oil phases are formed only by water or oil, respectively.

$$\text{OCEP (\%)} = [\text{Oil ratio (\%)} - \text{Oil phase (\%)}] \,/\, [\text{EI (\%)}] \cdot 100 \quad (5)$$

where the oil ratio represents the initial oil content to prepare the emulsion, and the oil phase represents the amount of oil on the top of the graduated cylinder after 48 h.

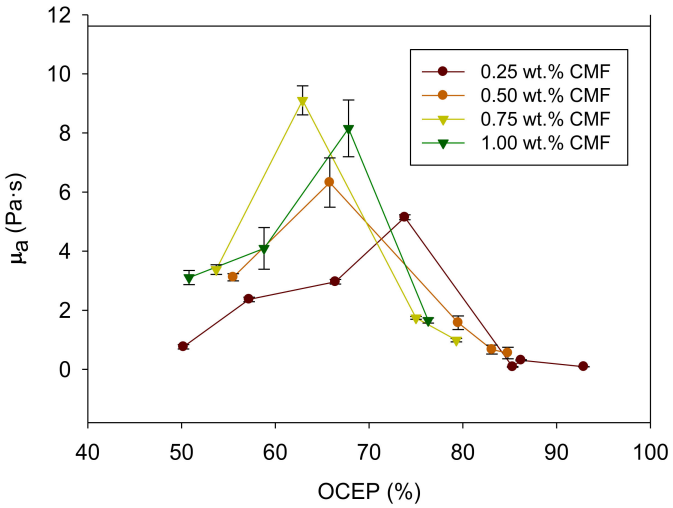


Figure 7. Effect of oil content in the emulsion phase (OCEP) on apparent viscosity for different CMF doses using 1 s^{-1} as shear stress.

Figure 7 shows that the μ_a was quite similar for the same oil amount in the emulsion from 0.5 wt% CMF dose onward, showing there was no dependence on the CMF dose. The OCEP for the samples with the highest μ_a values for each CMF dose from 0.5 wt% onward was between 62% and 68%. However, when 0.25 wt% of CMF were added, the μ_a was lower than in the other doses.

Table 2 summarizes the properties of the emulsions with the highest μ_a for each CMF dose. It can be seen that, from 0.5 wt% CMF dose onward, when the μ_a was maximum, the OCEP was around 65%, while the initial O/W ratio increased. In these cases, the amount of emulsion (EI) increased when the O/W ratio increased. In addition, the CMF required to enclose the oil drops was higher. Therefore, the characteristics of the final emulsions in the three cases were quite similar regarding oil, water, and CMF content, but the EI was higher or lower. The ideal emulsion would be an emulsion with around 65/35 O/W ratio and a CMF dose slightly higher than 1.00 wt% to obtain a high μ_a and reach a 100% EI value.

Table 2. Summary of emulsions with the highest μ_a for each CMF dose.

CMF Dose (%)	μ_a Max (Pa·s)	OCEP (%)	EI (%)	O/W Ratio
0.25	5.1	73.8	54.2	40/60
0.50	6.3	65.8	60.8	40/60
0.75	9.1	62.9	79.5	50/50
1.00	8.2	67.8	88.5	60/40

3.4. Stability toward Centrifugation

The emulsion phase of the samples with the highest viscosities for each CMF dose (0.50, 0.75, and 1.00 wt%) and OCEP between 62% and 68% was observed by optical microscope before and after centrifugation at 500 rpm for 2 min. In addition, emulsions with higher OCEP (75%–80%) and lower OCEP (52%–59%) with respect to the ones that had the maximum μ_a were also studied (Figure 8). According to these results, a stabilized emulsion could be defined as the one which maintained the number, the size, and the shape of oil droplets after centrifugation without the coalescence of droplets and a mixture of oil and water phases.

As can be observed in Figure 8, for emulsions with a CMF dose of 0.50 wt% before centrifugation, the emulsion with the highest μ_a (40/60 O/W ratio) and around 65% OCEP had droplets with a small size and CMF (in black) distributed around them. However, 30/70 and 50/50 O/W emulsion droplets were more separated and had bigger size and irregular shape. In this case, CMF stacks could be observed in aqueous phase. After centrifugation, the emulsion with the highest μ_a, around 6.3 Pa·s, maintained the size and shape of droplets with a homogeneous CMF distribution and without phase separation in the test tube, showing this emulsion to be quite stable toward centrifugation. However, although 52.9% OCEP emulsion contained a similar size of droplets, it had a more irregular shape of droplets, making the continuous fluid phase clearer. A small water phase on the base of the test tube and a small amount of oil phase on the top could be seen. Low viscosity emulsion with 79.5% OCEP emulsion had a big size and an irregular shape of droplets after centrifugation. Drop numbers decreased because they passed to the continuous liquid phase, and the test tube showed phase separation. These facts indicate that the emulsion with the highest viscosity (6.3 Pa.s) was more stable than emulsions with lower viscosity.

Emulsions with 0.75 wt% and 1.00 wt% CMF showed the same results as emulsions with 0.50 wt% CMF dose. As in the last case, before centrifugation, the emulsions with the highest μ_a and around 65% OCEP showed a high number of small droplets and a regular shape, with CMF homogeneously distributed among them. After centrifugation, the emulsions did not present phase separation in the test tubes, and the droplets diameters were maintained with values between 3 and 100 µm (0.75 wt% CMF) and from 3 to 60 µm (1.00 wt% CMF). These emulsions were considered stable. On the other hand, emulsions with an OCEP lower or higher than 65% showed heterogeneous CMF dispersions before and after centrifugation, with a high deformation of the droplets after centrifugation and a

mixture of oil and water phases. These emulsions were considered unstable. Among these unstable emulsions, 50/50 O/W emulsion with 1.00 wt% CMF had an OCEP more close to 65% (58.8%); the emulsion had more homogeneous droplets and could be considered as a half-stable emulsion.

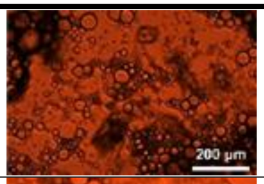
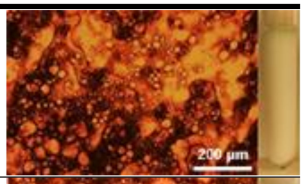
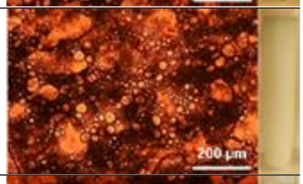
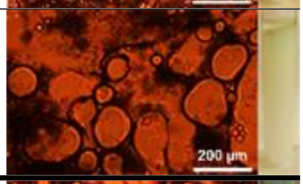
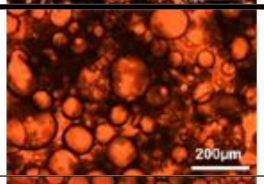
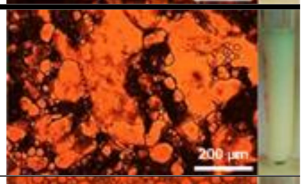
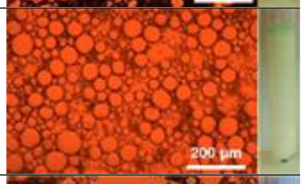
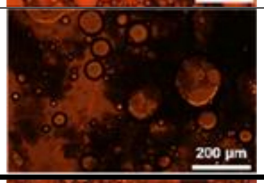
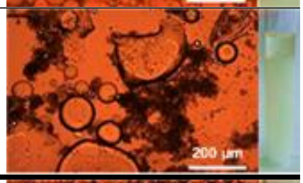
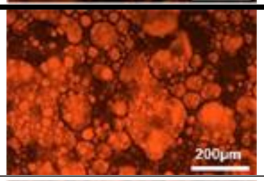
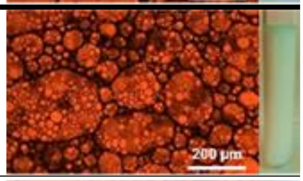
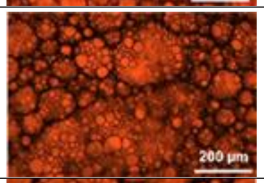
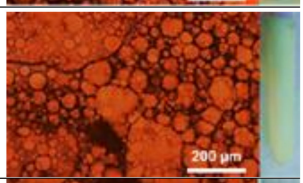
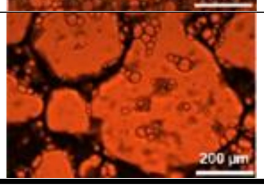
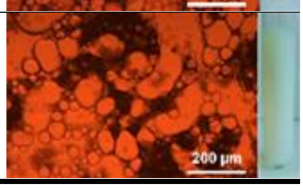
CNF (wt%)	**O/W** (μ_a; $\gamma = 1s^{-1}$) OCEP	**Before Centrifugation** (10x)	**After Centrifugation** (10x)
0.50	**30/70** (3.1 Pa·s) 52.9%		
	40/60 (6.3 Pa·s) 65.8%		
	50/50 (1.6 Pa·s) 79.5%		
0.75	**40/60** (3.4 Pa·s) 53.7%		
	50/50 (9.1 Pa·s) 62.9%		
	60/40 (1.7 Pa·s) 75.0%		
1.00	**50/50** (4.1 Pa·s) 58.8%		
	60/40 (8.2 Pa·s) 67.8%		
	70/30 (1.7 Pa·s) 76.3%		

Figure 8. Microscope images and test tubes for several CMF doses.

In general, centrifugation did not affect the number and the shape of droplets when emulsions had around 65% OCEP and higher μ_a, with stable emulsions and no dependence on the CMF dose. In addition, droplets size had an order of magnitude of 10^{-5} m, independent of CMF dose, stability, or OCEP. This finding is in agreement with the work by Lu et al. [9], which examined droplet size of Pickering emulsions with milled cellulose. The droplets size for stabilized emulsions in that study had the same order of magnitude as in our study. The same order of magnitude and a similar nondependence of droplet size distribution on the amount of CNF and oil content was also observed by Gestranius et al. [14]. Other publications are also in agreement with the use of cellulose in O/W emulsions using CNF chemically treated with lauroyl chloride [10] and CMF with essential oils that have antimicrobial functions [12]. Other stabilizers, such as quinoa starch granules, had the same ability to stabilize oil drops in food-grade Pickering emulsions [6] or CNC with a food-grade surfactant [27] to stabilize sunflower oil-in-water emulsions. In this last case, emulsions with a low surfactant addition (<0.03 wt% ethyl lauroyl arginate (LAE)) had the same droplets structure as stabilized emulsions, with around 65% OCEP and similar size distribution. When LAE concentration increased from 0.05 wt% to 0.2 wt%, thick droplet boundaries could be distinguished in the scarce droplets of the emulsion, just as in our emulsions with high OCEP between 75%–80%. Only the droplets with thick boundaries resisted the emulsion preparation.

3.5. Application of Pickering Emulsions as an Alternative to Trans and Saturated Fats in the Food Industry

In order to study the feasibility of using Pickering emulsions with CMF in the food industry from a nutritional point of view, theoretical nutrition facts were calculated, taking as reference a recipe of a standard cookie containing 20% sugar and one-third traditional fats, butter (T1), and sunflower oil (T2) as ingredients. The values were compared to those obtained if the fat was substituted with two formulations of Pickering emulsions with CMF (E1 and E2). These emulsions would contain the same O/W ratio (50/50) but with different CMF doses (0.50 wt% and 0.75 wt%). Emulsion with 0.50 wt% CMF (E1) would have similar oil content as butter and sunflower oil of the standard cookie. The theoretical ingredients of the food products and the theoretical results of the nutrition facts for each case are shown in Table 3. As can be seen, Pickering emulsion with 0.75 wt% CMF (E2) would have less oil content, obtaining a low-calorie product.

Table 3. Nutrition facts (per 100 g product).

Food Product	T1	T2	E1	E2
Emulsion ingredients	Butter	Sunflower oil (+ water)	Pickering emulsionsusing CMF as stabilizer	
O/W, %/%	82/18	82/18	79.5 [1]/20.5	62.9 [1]/37.1
CMF, wt%	0	0	0.50/(0.83 [2])	0.75/(0.94 [2])
Emulsion, g	21.5			
Flour, g	30.4			
Brown sugar, g	13.0			
Energy (kcal)	544.6	542.8	533.9	478.6
Total Fat (g)	31.0	31.0	30.1	23.9
SFA (g)	20.9	4.1	4.0	3.2
MUFA (g)	7.9	7.4	7.2	5.7
PUFA (g)	1.5	19.6	19.0	15.1
TFA (g)	0.7	-	-	-
Carbohydrates (g)	60.8	60.2	60.2	60.2
Proteins (g)	5.6	5.4	5.4	5.4
Dietary fiber (g)	0	0	0.31	0.34

[1] Values corresponding to OCEP. [2] Values corresponding to the percentages of CMF in the emulsion phase.

The theoretical nutrition facts demonstrate the advantages of a food product prepared with a Pickering emulsion using CMF as stabilizer compared to the use of butter and sunflower oil as ingredients. Both Pickering emulsions substitute SFA with monounsaturated fats (MUFA) and polyunsaturated fats (PUFA), obtaining a more healthy final product. The reduction in SFA using E1 and E2 (in relation to butter) is 81 and 85 %, respectively, with a slight intake of 0.3% of dietary fiber in both cases. In addition, E2 Pickering emulsion can be considered as a low-calorie product, with a 23% reduction in total fat compared to butter and sunflower oil as ingredients. Therefore, Pickering emulsion with CMF as stabilizer can be used as a promising ingredient in the food industry, substituting trans and saturated fats in some foods. However, a more detailed research should be carried out to study the effect of this new ingredient on the organoleptic properties of the food, such as taste, texture, and crumble.

4. Conclusions

In our study, O/W Pickering emulsions with CMF maintained the stability of the emulsion phase for 24 h. One of the main reasons for the efficiently stabilized O/W emulsions was the amphiphilic surface nature of the microfibers. The unmodified CMF formed an expanded and strong network that enclosed oil in small droplets, avoiding the coalescence in optimal conditions. Pickering emulsions with more than 0.50 wt% CMF had a maximum μ_a with an OCEP around 65%, increasing up to 90 times the μ_a of the emulsion phase with respect to the sunflower oil, for a shear rate of 1 s^{-1}. Lower O/W ratios showed that the oil and water phases were completely separated, and CMF enclosed the entire oil phase in drops without an oil phase on the top of the emulsion. Higher O/W ratios did not allow the same amount of CMF to enclose the oil, therefore a part of the drops was broken and mixed with water, and an oil phase was formed on the top of the emulsion. An increase in CMF dose helped to increase the strength of the network. Microscope images showed the stability of emulsions before and after centrifugation. High apparent viscosity emulsions had regular shape, small size, and a large number of drops with a homogenous CMF distribution. Finally, we found Pickering emulsions can be used in the food industry, replacing less unhealthy fats, such as butter. An emulsion with 50/50 O/W and 0.75 wt% CMF can obtain a low-calorie emulsion with a reduction in SFA and total fats of 85% and 23%, respectively, compared with butter. Therefore, O/W Pickering emulsions can be considered as an alternative to trans and saturated fats in the food industry.

Author Contributions: Conceptualization, A.B. (Ana Balea), M.C.M., A.B. (Angeles Blanco) and C.N.; Formal analysis, J.L.S.-S.; Funding acquisition, C.N.; Investigation, J.L.S.-S. and A.B. (Ana Balea); Project administration, C.N.; Supervision, M.C.M. and A.B. (Angeles Blanco); Writing—original draft, J.L.S.-S.; Writing—review & editing, A.B. (Ana Balea), M.C.M., and A.B. (Angeles Blanco).

Funding: This research was funded by the Economy and Competitiveness Ministry of Spain, project CTQ2017-85654-C2-2-R, and by a grant from Universidad Complutense de Madrid and Banco de Santander to J.L. Sanchez-Salvador, grant number CT17/17.

Acknowledgments: The authors wish to thank the Economy and Competitiveness Ministry of Spain for their support of the project CTQ2017-85654-C2-2-R as well as the support of Universidad Complutense de Madrid and Banco de Santander for the grant to J.L. Sanchez-Salvador (CT17/17).

Conflicts of Interest: The authors declare no conflict of interest.

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