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Unravelling the mechanism of stabilization and microstructure of oil-in-water emulsions by native cellulose microfibrils in primary plant cells dispersions

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KEYWORDS: microfibrillated cellulose, emulsion stabilization, Pickering emulsion,
flocculation, depletion interaction

ABSTRACT

It is long known that oil-in-water emulsions can be stable against coalescence in homogenized plant cell wall dispersions due to the presence of surface active biopolymers. When plant cell wall material is homogenized to the extent of deagglomeration of the cellulose microfibrils (CMFs), a much more complex dispersed system is obtained. Here we show that in such complex systems both surface active soluble polymers and individual CMFs are at the origin of this stabilization against coalescence, as they form a shell around the oil droplets providing Pickering-like stabilization. Individual CMFs and bundles of them in the presence of soluble biopolymers form a hybrid network in the continuous phase linking the droplets, creating a viscoelastic network that prevents the droplets from coalescing. Depletion induced attraction caused by soluble biopolymers and dispersed CMFs induces the formation of oil droplet clusters at low CMF concentrations leading to a highly heterogeneous distribution of oil droplets. This effect diminishes at high CMF concentrations at which the strong viscoelastic network arrests the droplets. These findings are important steps towards controlling complex dispersed systems comprising CMF – polymers mixtures with a second liquid or solid dispersed phase.

INTRODUCTION

Oil-in-water (o/w) emulsions are used throughout various consumer products, ranging from food products to skin creams and lotions. They are thermodynamically unstable, so they are usually prepared and stabilized using emulsifiers and stabilizers. Emulsifiers adsorb at the oil-water interface, reducing the interfacial tension of the system and creating steric barrier against coalescence, whereas stabilizers are dissolved in the aqueous phase, increasing the viscoelasticity and reducing the mobility of the oil droplets. Many substances meet the purpose of emulsifiers or stabilizers: surfactants, proteins, surface active particles etc. The latest trend now favors the use of natural and environmentally friendly products, especially in the food and

cosmetics industry, due to changing consumer demands and increased awareness of health impact and sustainability. The need for naturally and sustainably sourced products with minimal chemical processing has led to a search for new types of ingredients to replace traditional emulsifiers and stabilizers. Natural biopolymers derived from sustainable sources are an excellent substitute for petrochemical based emulsifiers as they can combine emulsifying and bulk stabilizing properties and are readily available in substantial amounts and are biodegradable.¹ Examples of biopolymers that have been successfully used to prepare and stabilize emulsions are plant proteins,²⁻⁴ hydrocolloids,⁵ modified starch particles,^{6,7} chitin nanocrystals,^{8,9} cellulose derived nanoparticles and cellulose microfibrils.^{10,11} Cellulosic microfibrils in particular have been receiving quite some attention these past few years, as it is the most abundant polysaccharide on Earth, and it has been used in food products to increase their dietary fiber content.¹²⁻¹⁵ These particles are extracted from plant waste materials, such as peels or pulp, and consist of soluble and insoluble materials from the cell walls such as polysaccharides, glycoproteins, and pectin.¹⁶ Multistep processing conditions involving alkaline extraction and bleaching can be used to obtain purified cellulose microfibril (CMF) systems¹⁷ and they have been shown to possess emulsifying properties at 10 wt % oil concentration by making Pickering emulsions.¹⁸ Milder processing conditions involving solvent extraction have also been used to extract cellulosic particles and prepare emulsions that are stable both against coalescence and creaming at oil concentrations lower than 15 vol %.^{19,20} From a sustainability and naturalness point of view, however, it is highly desirable to utilize CMFs from primary plant cell wall materials coming from byproducts and without further purification. The CMF found in primary plant cell wall materials can be found in wide class of bio-based materials, including fruits and vegetables.^{21,22} However, only very recently has the importance of deagglomeration of the CMFs in the emulsifying and stabilizing properties of

homogenized plant cell wall materials been demonstrated, hinting at the complex stabilization mechanism involving proteins, pectins and CMFs.²⁰

In this work, we use the CMF present in dispersions of citrus fiber as a model system to investigate the mechanism of stabilization of emulsions by a deagglomerated primary plant cell wall dispersions, and the influence of the particle concentration on the emulsion microstructure.

MATERIALS AND METHODS

Materials

Soybean oil was obtained from Sigma-Aldrich (Sigma Aldrich - S7381) and used as received (density = 0.9191 g/mL). Herbacel AQ+ type N from Herbafood Ingredients GmbH Germany (84-90 wt % dietary fiber, 4-9 wt % water, 2-5 wt % ash) is used as cellulose raw material originating from Citrus peels (Lot number: 30902065) and used as received. It contains around 60 wt % of cellulose, 3.4 wt % of hemicellulose, and 5 wt % of proteinaceous materials.²³

Preparation of emulsions

The citrus fiber powder was first suspended in deionized water and thoroughly mixed using a LM5-A Silverson laboratory mixer (Silverson, USA) with a 1 mm screen hole at 3500 rpm for 5 minutes and afterwards passed once through a high-pressure homogenizer (Microfluidizer M 110S (MF), Microfluidics Corp., USA) with a z-shape geometry (diameter 87 μ m) operating at a pressure of 1200 bar. Then soybean oil was added, and the mixture was once again mixed using the Silverson at 3500 rpm for 5 minutes and then microfluidized at 1200 bar. Samples were prepared with 0.5; 1.0; 1.5; 2.0 wt % plant cell wall dispersion in the water phase, hence concentrations in CMF are respectively 0.3; 0.6; 0.9; 1.2 wt%. Oil concentrations in the emulsions are 15; 30; 40; 50; 60 wt % soybean oil in total, all concentrations reported are according to these. All samples were stored in closed containers at 5 °C after preparation.

Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was performed to visualize the microstructure of the emulsions by location of the CMFs, oil droplets, and protein. For localization of the CMF and the proteinaceous materials, the fresh emulsions were stained with Direct yellow (Solophenyl Flavine 96 at 0.5 wt %) and a 0.05 wt % solution of Rhodamine B by adding a drop of the dyes to about 1g of the emulsion. A drop of the resulting mix was then placed on a cover slip and micrographs of the emulsions were acquired using a Leica TCS-SP5 and DMI6000 inverted microscope (Leica GmbH, Germany). Fluorescence from the samples was excited at 488 nm for Direct Yellow and 561 for Rhodamine. Emissions wavelengths are respectively 496-555 nm and 580-700 nm. A 63× oil-immersion objective was used to scan the images at approximately 30 µm below the cover slip. To image the oil droplets, fresh emulsions were stained by mixing around 1 g of the samples with a drop of 1 wt % Nile Blue-Nile red solution. Excitation wavelengths are 488 and 633 nm and detection at 520-602 nm and 661-786 nm.

Cryo-scanning electron microscopy

Cryo-scanning electron microscopy (cryo-SEM) to visualize the CMF structures at high resolution. A small volume of each sample (1 drop) was placed on a rivet-type holder and immersed in liquid nitrogen. Cryo-planing was done using a cryo-ultramicrotome (Leica Ultracut UCT EM FCS) with a section thickness of 100 nm and a speed of 60 mm/s with a glass knife. The last sections were reduced to 20 nm, at a speed of 2 mm/s using a diamond knife (Diatome histo cryo 8 mm from DiATOME PA, USA) at -110 °C. The rivet was mounted on a chamber Cryoprotective product (Gatan Inc., USA) and the temperature of the sample was increased for a brief time to -90 °C under vacuum to remove a thin layer of water by

sublimation. The sample was sputter-coated with platinum (20 to 120 s depending on the sample) and was imaged using a Zeiss Auriga field-emission SEM (Zeiss, Germany) at -125 °C and an acceleration voltage of 3 kV.

Rheology

Rheology was performed on a MRC301 rheometer (Anton Paar, Germany) at 20 °C using a sandblasted 40-mm parallel plate geometry with a 1 mm gap for viscous samples and a 27-mm Couette geometry for more liquid samples. Samples were carefully loaded using a plastic spoon in accordance with instrument guidelines. A time sweep was measured for 5 minutes, measuring G' and G'' at 0.1 % strain and a frequency of 1 Hz. For a determination of the yield stress of the plant cell wall dispersion, a shear rate was applied ranging from 0.001 to 10 s⁻¹ and back to 0.001 to measure the shear stress and viscosity response. The backwards shear stress curve was later fitted to the Herschel-Bulkley model to extract the yield stress. The Herschel-Bulkley model relates the shear stress to the shear rate according to $\tau = \tau_0 + k\dot{\gamma}^n$ with τ , the shear stress, τ_0 , the yield stress, k , the consistency index, n , the flow index and $\dot{\gamma}$, the shear rate. Every sample was measured in triplicate to ensure reproducibility.

Index of dispersion

Large micrographs of the emulsions containing at least 1000 droplets were taken using a confocal laser scanning microscope (CSLM) by imaging tile scans and stitching images together. The threshold was adjusted using ImageJ and the position of all droplets in the projection of the two-dimensional (2D) plane were analyzed with the “Analyze Particles” function. The quadrant method is then used to calculate the mean and standard deviation of the particle distribution.²⁴ Briefly, from CSLM micrographs, the 2D position of the droplets is analyzed, then the micrograph area is divided into small quadrats, and the number of droplets

falling into each quadrat is counted. The number of quadrats is taken to approximately equal to the number of droplets in the micrograph area. The number of droplets N in each quadrat is the counted, and the mean m and standard deviation s of the counts is evaluated. The index of dispersity which was chosen as a measure of the degree of flocculation is calculated as follows:

$$IoD = (N - 1) \frac{s^2}{m} \quad (1)$$

RESULTS AND DISCUSSION

Stability of the emulsions

Figure 1 shows the oil and CMF concentrations for which emulsions stable against coalescence and against creaming were obtained (green area). Emulsions made with low CMF concentrations tend to cream after preparation, the rate of creaming decreasing with increasing CMF concentration. Emulsification was not successful for very concentrated emulsions when the oil concentration exceeded 60 wt %: a coarse emulsion can be obtained after mixing the oil with the Silverson mixer but it phase separates after passing it through the microfluidizer. Preparation of concentrated emulsions has been reported using cellulose nanocrystals^{8,10} but reports of the use of purified cellulose microfibrils only use oil concentrations under 15 wt % and CMF concentrations under 1 wt %.^{18–20} To explain why it is possible to obtain concentrated emulsions using a plant cell wall dispersion containing deagglomerated CMFs we look first at the microstructure of said emulsions.

Figure 1. Apparent stability diagram of the emulsions stabilized by CMFs in dispersion of primary plant cells. The horizontal axis refers to CMF concentrations in the aqueous phase. The area with a red crossed hatch indicates concentrations for which emulsification was not successful, as the system phase separate. The area with a crossed hatch indicates concentrations for which emulsions tend to cream after preparation, but no coalescence was observed. The green area indicates concentrations at which stable emulsions both against coalescence and creaming were obtained.

Figure 2. Upper row: confocal images of an emulsion containing 1.2 wt % of CMF in the aqueous phase and 50 wt % of oil, showing the CMFs in green (a), the proteinaceous materials in magenta (b) and an overlay (c). Scale bar 10 μ m. Bottom row: Cryo-SEM images of the same emulsion at different magnifications where CMFs bundles (about 9 nm cross section diameter) can be seen directly on the oil-water interface and bigger ones (about 18 nm) form a network between other droplets. Scale bars (d) 200 nm, (e) 400 nm, (f) 800 nm.

Emulsions stained with Direct Yellow for the cellulosic particles and with Rhodamine B for the proteinaceous materials show that both cellulose and proteinaceous materials surround the oil droplets (Figures 2a and 2b). Cryo-SEM micrographs reveals that CMF bundles of about 9 nm in diameter are found sitting on the droplets forming a somewhat uniform layer, and they seem to cover the droplets to a high degree (Figures 2d and 2e). These small microfibrils appear to be in direct contact with the oil droplets. The nature of the particles that can adsorb at the oil-water interface can be determined by looking at the composition of the plant cell wall dispersion

used to prepare the emulsions. The dispersion contains mainly polysaccharides such as CMFs, hemicellulose but also some pectin and glycoproteins. Individual microfibrils of about 3-4 nm in diameter are bound into 10-25 nm bundles by the hemicellulose, pectins and proteins.²⁵ Native (not chemically modified) CMFs are not known to be intrinsically surface active but pectin is known to be able to lower the interfacial tension of oil in water emulsions.²⁶⁻²⁸ Hence the emulsifying property of the CMFs could be attributed to the proteinaceous fractions that are covalently bound to the polysaccharide chain,²⁹ possibly giving them ability to adsorb on the oil droplets.¹⁹ The confocal micrographs show that both proteins and CMFs can be found adsorbed on the oil droplets, it could be then inferred that the proteinaceous moieties on the microfibril backbone allows the CMFs to adsorb at the oil droplet interface. This synergetic action of proteins and microfibrils has also been observed in the case of protein-covered cellulose nanocrystals: they have been shown to improve the emulsification of stable high internal phase emulsions thanks to the amphiphilic moieties of the proteins on the nanocrystals.³⁰

CMFs and their bundles (those below 9 nm in width) form a uniform layer around the oil droplets, and the droplet surface is connected with multiple CMFs bundles that seem to bridge multiple droplets together. Studies on the ability of high aspect ratio microfibrils to stabilize emulsions have shown that particle size plays an important role on their ability to stabilize emulsions.^{19,31,32} Small particles nanorods stabilize emulsions by forming a dense layer around droplets as they can align around the droplets, leading to high coverage (80 to 100%).^{31,32} Longer and stiff particles on the other hand can only give partial coverage and stabilize emulsions by forming an interconnected network of droplets. On the cryo-SEM images we see both types of particles and both types of stabilization mechanisms: individual microfibrils covering the entire oil droplets and bundles of CMFs anchored to multiple droplets.

CMFs can run along the droplets, creating bridges between several droplets (Figures 2e and 3a) or form ball-like flocs in voids between the droplets as shown in Figure 3b. These

microfibril bundles and the ones that are not directly connected to any droplets contribute to the strengthening of the emulsions as shown by the rheological measurements (Figure 3c). Increasing the concentration of cellulosic fibrils increases drastically the viscoelastic modulus of the emulsions, especially for emulsions containing 50% of oil. This is due to the presence of flocs of CMFs and microfibrils bridging multiple droplets, Unadsorbed CMFs and their bundles create a viscoelastic network embedding the oil droplets, which is responsible for the absence of creaming observed at high CMF concentrations.

These results help clarify the mechanism of stabilization of emulsions stabilized by cellulose microfibrils from primary plant cell wall materials: the significant amount of CMF and in these materials help form a viscoelastic network that prevents creaming, while the protein from the extensins in the plant cells helps the adsorption of the CMF onto the oil droplets and the formation of a shell that helps prevent coalescence.

Figure 3. (a) Cryo-SEM image of an emulsion made with 50 wt % oil and in the aqueous phase 1.2 wt % CMF. CMFs run along the droplets and bridge multiple ones together. Scale bar: 1 μm . (b) Flocs of CMFs forming cotton ball-like particles can be seen between the droplets.

Scale 1 μm . (c) Storage modulus of the emulsions as a function of CMF concentration in the aqueous phase for different oil concentrations, measured at a frequency of 1 Hz and a strain of 0.1%. Lines are guides for the eye.

Microstructure of the emulsions

The presence of unadsorbed polymers in an emulsion can cause either depletion flocculation^{33,34} or bridging flocculation,^{5,35} depending on the nature of the polymer, their concentration and their interactions. This results in both cases in an inhomogeneous spatial distribution of the droplets. As can be seen on Figure 4, at very low CMF concentrations, droplets are randomly distributed, but clusters of droplets can be clearly seen as the concentration of CMFs increases. The inhomogeneity in the spatial distribution of the droplets was quantified using the quadrat method and a metric called the index of dispersion (IoD).²⁴ A high *IoD* value means that there is a large deviation from spatial uniformity, which can be used as a quantification of how clustered an emulsion is. Figure 4 shows that the index of dispersity is stagnant at low CMF concentrations, then increases sharply at 0.3 wt % CMF and then decreases as the CMF concentration increases. If we assume that depletion interaction is the main cause of the observed flocculation, then as the concentration in CMF increases, the concentration in unadsorbed polymer increases, which increases the strength of the depletion interaction, resulting in more flocculated droplets. However, at the same time, the viscosity of the continuous phase increases as the CMF concentration increases. When the yield stress of the continuous phase is too strong to allow the droplets to move, the droplets are less flocculated at higher CMF concentrations, as seen by the decrease in the IoD.³⁶

Figure 4. Top: Confocal images of the emulsions with 15 wt % oil, showing the apparent droplet clustering. Concentrations in CMFs are (a) 0.06 wt %, (b) 0.18 wt %, (c) 0.3 wt %, (d) 0.6 wt % and (e) 1.2 wt % in the aqueous phase. Scale bars 20 μm . Bottom: Graph displaying the index of dispersity (black square markers) as a function CMF concentration for an emulsion containing 15 wt % of oil, and the yield stress of the corresponding aqueous phase (blue round markers). Lines are guides for the eye.

The depletants are assumed to be the soluble polymers present in the plant cell wall dispersion, so to verify that depletion is truly at the origin of the observed flocculation, the plant cell wall dispersion was centrifuged at 15000 g for 2 hours and the supernatant was extracted, giving a stock solution that was diluted to different degrees. This stock solution contains only soluble polymers, including individual CMFs (Figure S2). Emulsions containing 10 wt % of oil were made by emulsifying with a benchtop mixer for 5 min at 3500 rpm, then imaged immediately. The supernatant contains surface active materials, which decrease the interfacial tension (Figure S3). The resulting emulsion is stable for about 30 minutes before it creams and coalesces.

Figure 5. Top: Optical micrographs of emulsions made with 10 wt % of oil and a stock solution of the supernatant at different concentrations: (a) 10%, (b) 30%, (c) 50%, (d) 70% and (e) 90%. Scale bar 100 μm . Bottom: (f) Separate interaction potentials normalized with the thermal energy $k_B T$ as a function of the interparticle distance, for $r = 40$ nm. (g) Total interactions potentials normalized by $k_B T$ for different radii of gyration, showing the influence of depletant size. Interaction potentials were calculated assuming $R = 5$ μm , $I = 5$ mM, $\kappa^{-1} = 3.6$ nm, $\varepsilon = 6.9 \times 10^{-10}$ F.m, $\Phi_0 = -51$ mV, $c = 3$ wt % and $T = 293$ K.

Influence of depletion interaction

As seen on Figure 5, clusters of droplets can be seen throughout the entire range of supernatant concentration. Let us make calculate the predicted interaction energy for this system. For such an emulsion, we take into consideration the Van der Waals interactions U_{vdw} , electrostatic interactions U_{el} , and depletion interactions U_d . The Van der Waals interaction potential in the limit of close approach is

$$U_{vdw} = -\frac{A_H R}{12h} \quad (2)$$

where R is the radius of the droplets, A_H is the Hamaker constant and h the distance between two droplets. In the case of oil droplets in an aqueous solution, A_H can be approximated to $4 \times 10^{-21} \text{J}$.³⁷ It must be mentioned that this simple approximation of the Van der Waals interaction does not take into account any short range interaction induced by the presence of CMF at the droplet interface.

The adsorbed polymers are highly charged, and they induce an electrostatic interaction between the oil droplets. For weakly overlapped double layers, using the non-linear superposition approximation, the electrostatic interaction is

$$U_e = \frac{64\pi I N_{av} k_B T R}{\kappa^2} \left(\tanh \left(\frac{e\Phi_0}{4k_B T} \right) \right)^2 e^{-\kappa h} \quad (3)$$

where ϵ ($6.9 \times 10^{-10} \text{ F.m}$) is the dielectric constant of the aqueous phase Φ is the surface potential of the oil droplets, and κ the inverse of the Debye length.³⁸ The surface potential Φ_0 used in this calculation was assumed to be similar to the measured zeta potential of diluted emulsion (-51 mV), as measured by a dynamic light scattering apparatus (Figure S5). The Debye length κ^{-1} is calculated as $\kappa^{-1} = \sqrt{\frac{\epsilon k_B T}{2e^2 I N_A}}$ with k_B the Boltzmann constant, T the temperature, ϕ_{oil} the volume fraction of oil, e ($1.6 \times 10^{-19} \text{ C}$) the charge of an electron, I the ionic strength and N_A ($6.02 \times 10^{23} \text{ mol}^{-1}$) the Avogadro number. The ionic strength of the aqueous phase (5 mM) is calculated from the conductivity $\sigma = 300 \text{ mS.cm}^{-1}$.³⁹

The presence of unadsorbed polymers in the supernatant give rise to a depletion interaction. For polymers that have a radius of gyration r , with a molecular weight M , at a concentration c , the depletion interaction potential is⁴⁰

$$U_{dep} = \begin{cases} -\frac{\pi c R_{av} T}{6M} (2R + 2r)^3 \left[1 - \frac{3(2R + h)}{2(2R + 2r)} + \frac{(2R + h)^3}{2(2R + 2r)^3} \right], & h < 2r \\ 0, & h \geq 2r \end{cases} \quad (4)$$

with R the radius of the droplets separated by a distance h , R_{av} the Avogadro number, and T the temperature.

The depletant concentration c is taken as 10 wt % of the concentration of all the soluble polymers present in the supernatant. Their molecular weight has been measured by light scattering using a Debye plot (Figure S4). This molecular weight can be used to get an approximate value of the hydrodynamic radius of the soluble polymers, with the empirical formulas such as the Mark-Houwink equation. Given a molecular weight of 214 kDa, the radius of gyration is between 18 and 38 nm for soluble polysaccharides.^{41,42}

Assuming the additivity of the interaction potentials, the total interaction is $U_{tot} = U_{vdw} + U_e + U_{dep}$. Figure 5 shows the potential curves predicted by those equations: the presence of depletants creates a well around 20 nm suggesting as strong flocculation of the droplets. The minimum in the potential curve gets deeper at higher depletant radii, as the strength of the depletion interaction force increases, confirming that the observed flocculation is due to depletion interaction. While it is not always mentioned nor discussed, the recent literature shows that depletion flocculation is prevalent in emulsion stabilized by primary plant cell materials. To name a few examples, CMF extracted from mangosteen rinds,^{18,43} orange pulp²⁰ and banana peels⁴⁴ yield emulsions with similar droplet sizes to our system, and droplets are also flocculated even at low CMF concentrations. This is likely due to the presence of soluble polymers from the plant cell wall material the CMF were extracted from: despite their very low concentration in the plant dispersion, they have a considerable influence on the resulting microstructure of the emulsions. Their concentration is determined by the washing steps and extraction procedures used to obtain the CMFs.

Influence of bridging flocculation

To check how the emulsion reacts to dilution, an emulsion stabilized by the plant cell wall dispersion was diluted to different degrees and optical micrographs of the resulting emulsions were imaged. Even when emulsions were highly diluted, oil droplets were still found in clusters as seen in Figure 6.

Figure 6. Optical micrographs of emulsions made from an emulsion containing 50 wt % of oil and 2 wt % of plant cell wall dispersion in the aqueous phase, then diluted with water. Dilution factors are (a) 5, (b) 10, (c) 20 and (d) 100. Flocculation of the oil droplets can be seen even at high dilution factors. Scale bar 100 μm .

Since depletion interaction forces between two droplet scales linearly with the depletant concentration, a dilution factor of more than 20 should render the depletion force negligible compared to electrostatic and Van der Waals forces so depletion induced flocculation should be suppressed. This suggests that depletion interaction is not the only mechanism that induces flocculation in our system. It has already been established earlier that CMF and their bundles

can connect multiple droplets together. Since bridging flocculation involves irreversible adsorption of polymers onto droplets,^{35,45} it is not sensitive to small dilution factors. Seeing that the clusters remain upon dilution, bridging could also play a role in the aggregation of droplets and their non-homogeneous spatial distribution. The major factors influencing bridging flocculation are the shape and length of the polymer and its affinity with the surface of the droplets.⁴⁶ Let us examine those factors for CMFs, which are thin, long, flexible rods. Longer polymer chains adsorb more easily on the surface of the droplets than shorter ones and linear polymers are more likely to induce bridging than coil polymers. Bridging by long bundles of CMF are indeed seen on the cryo-SEM images (Figure 3a and 3b). In addition, the oil droplets are covered with CMFs which means that additional CMF in the bulk can also adsorb on the surface via hydrogen bonding on the many hydroxyl moieties of the CMFs: the CMFs can adsorb anywhere on the oil droplets. The preparation of the emulsions involves mixing at very high shear rates in the microfluidizer, this is likely to promote the adsorption of CMF due to the intense mixing. Cryo-SEM images have shown that CMF bundles can bind multiple droplets together, creating droplet clusters (Figure 3a and 3b). We hence suggest that the flocculation observed is the result of depletion attraction mainly and bridging of the droplets to some extent.

CONCLUSIONS

This work clarifies the complex structural rearrangements and the mechanism of emulsion stabilization by plant cell wall dispersion containing CMFs. Both proteinaceous materials and CMFs adsorb at the surface of oil droplets, allowing the microfibrils to cover entirely the droplets and preventing them from coalescing. CMFs and bundles of microfibrils induce bridging between the droplets by anchoring at the surface of multiple droplets and as flocs in the continuous phase, creating a viscoelastic network that prevents the droplets from creaming. Unadsorbed soluble biopolymers are at the origin of attractive depletion interaction,

contributing to the flocculation of oil droplets observed at low CMF concentrations, along with bridging flocculation. As the CMF concentration increases, the yield stress created by the CMF network also increases and counteracts the depletion interaction, giving a more homogeneous spatial distribution of the droplets. The findings of this study are important for understanding other systems where emulsions contain CMFs from other types of deagglomerated primary cell wall materials.

ASSOCIATED CONTENT

The supporting information is available free of charge: Confocal images and cryo-SEM of the plant cell wall dispersion after deagglomeration, AFM image of the supernatant, interfacial tension between the supernatant and the oil, measurement of the molecular weight, and zeta potential. (PDF)

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Notes

The authors declare no competing financial interest.

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