

Natural particles can armor emulsions against lipid oxidation and coalescence

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ABSTRACT

Traditional functional ingredients, such as conventional emulsifiers (surfactants, animal-derived proteins), and synthetic antioxidants may become obsolete in the development of clean-label, plant-based, sustainable food emulsions. Previously, we showed that tailor-made antioxidant-loaded particles can yield both physically and oxidatively stable emulsions, and we expected that natural particles with related properties could also show these beneficial effects. Here, we investigated Pickering emulsions prepared with natural plant particulate materials. Particles that showed weak aggregation in acidic aqueous media, indicating a relatively hydrophobic surface, were able to physically stabilize oil-in-water emulsions, through either Pickering stabilization (powders of matcha tea, spinach leaves, and spirulina cake), or an increase in viscosity (pineapple fibers). Matcha tea and spinach leaf particle-stabilized emulsions were highly stable to lipid oxidation, as compared to emulsions stabilized by conventional emulsifiers. Taking this dual particle functionality as a starting point for emulsion design is, in our view, essential to achieve clean-label food emulsions.

1. Introduction

Many food products are dispersions of oil droplets in water known as oil-in-water (O/W) emulsions. Recent trends in the food sector area, such as the consumers' demand for clean-label, plant-based, sustainable and healthy products, have led to changes in formulation requirements (Abdullah et al., 2020; Berton-Carabin & Schroën, 2019). For example, key functional ingredients such as conventional emulsifiers (surfactants (Kralova & Sjöblom, 2017) and animal-derived proteins (Day, 2013)) that provide physical stability to the emulsions, and synthetic antioxidants such as ethylenediaminetetraacetic acid (EDTA) and butylated hydroxyanisole (BHA) (Shahidi & Zhong, 2010) that ensure their oxidative stability, need to be reconsidered, which is a big challenge.

Pickering particles have become popular for biocompatible applications over the last decade, and could potentially mitigate both issues (Berton-Carabin & Schroën, 2015). When wetted by both oil and water, such particles can anchor at the interface and form an excellent physical barrier to droplet coalescence. Particles that are preferentially wetted by

water will tend to stabilize oil-in-water (O/W) emulsions, whereas those preferentially wetted by oil form water-in-oil (W/O) emulsions. For large enough particles (typically, above 10 nm), the adsorption energy ($\Delta G_a(J)$), defined as: $\Delta G_a = \pi r^2 \gamma_{ow}(1 - \cos\theta)^2$ (Eq. 1), where r (m) is the particle radius, γ_{ow} ($N m^{-1}$), the interfacial tension between the two immiscible fluids and θ ($^\circ$) the three phase contact angle, exceeds thermal energy by orders of magnitude, resulting in irreversible adsorption (Chevalier & Bolzinger, 2013).

Substantial work has been done on the formulation rules and physical characterization of Pickering emulsions, for a large part on inorganic particles, such as silicon dioxide and other silica-based (i.e., thermally processed or chemically modified silica) particles (Eskandar, 2007). Such particles are commercially available in various sizes (nanometers to microns) and hydrophobicity, and are allowed as food ingredients (EFSA, 2009). Yet, their size makes them questionable with respect to digestive and metabolic fate, and their synthetic origin limits their attractivity in terms of sustainability and clean-label potential. Minimally processed biobased particles can be interesting options for

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future food applications.

Several biobased Pickering particles have been developed over the past years such as modified starch granules (Rayner et al., 2012), cellulose fibers, cellulose nanocrystals (Kalashnikova, 2011), fat particles (Schröder et al., 2017), chitin nanocrystals (Tzoumaki et al., 2011), flavonoid particles (Luo et al., 2011), and protein-based. Still, most of these particles require chemical modifications and/or additional processing steps such as heating, hydrophobic chain grafting, or antisolvent precipitation, or are produced via a bottom-up approach, i.e., are assembled from individual molecules into the desired particle, which requires complex processing (Schröder et al., 2017).

In order to achieve a clean label alternative, it is thus important to identify biobased particles that can be obtained through mild processing and have appropriate properties to allow strong nesting at the oil–water interface (Huc-mathis et al., 2019). Many traditional and home-made food products are stabilized by natural endogenous particles, such as mustard particles in mayonnaise and dressings, fat crystals in margarine and spreads, or casein micelles in dairy products, but generalizing this toward the food industrial era is still a challenge.

Apart from the physical stability of food emulsions, lipid oxidation in such systems has become a renewed concern due to the recommendations for high amounts of omega-3 polyunsaturated fatty acids (PUFAs) in combination with fewer additives, such as synthetic antioxidants. This means that the strategies to prevent lipid oxidation have to be based on increasing the effectiveness of natural antioxidants. A way to do so is by positioning them at the oil–water interface, where lipid oxidation is initiated (Berton-Carabin, Ropers, & Genot, 2014; Laguerre, Bily, Roller, & Birtic, 2017). In previous work, we prepared Pickering emulsions stabilized by colloidal lipid particles that contained the lipophilic antioxidant α -tocopherol, and showed for the first time that both the physical and oxidative stability of emulsions could be greatly improved (Schröder, Laguerre, Sprakel, Schroën, & Berton-Carabin, 2020).

Given the composition and abundance of available plant-based natural particles, it can be safely assumed that some of them have intrinsic antioxidant properties, such as cocoa particles that contain polyphenols (Gould et al., 2013). Besides, several authors have speculated that the formation of a thick interfacial layer by Pickering particles would have a protective effect against lipid oxidation, by decreasing the permeability of free radicals and oxygen to the oil phase (Kargar, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012), although others could not find such a physical barrier effect (Schröder, Sprakel, Boerkamp, Schroën, & Berton-Carabin, 2019).

In the present work, we investigated the physical and oxidative stability of emulsions prepared with natural particles from different sources (raw powders of matcha tea, spinach leaves, spirulina cake, pineapple fibers, and rosemary cake and powdered extracts of turmeric and red radish), and compared it with the stability of emulsions stabilized with conventional emulsifiers: a polysorbate (Tween 60), often used as a model surfactant, and egg yolk, an application-relevant emulsifier as it is commonly used in mayonnaise and salad dressings (Norm, 2015). We first report on the physical characteristics of the particle dispersions, after which we discuss their emulsification behavior and potential ability to counteract lipid oxidation.

2. Materials & methods

2.1. Materials

Sodium acetate trihydrate, sodium chloride, potassium sorbate and polyethylene glycol sorbitan monostearate (Tween 60) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Egg yolk powder was ordered from MyProtein (Chicago, Manchester). Glacial acetic acid ($C_2H_4O_2$) was purchased from VWR Chemicals (Leicestershire, England). Methanol and chloroform were obtained from Actua-All Chemicals (Oss, The Netherlands). Deuterated chloroform and dimethylsulfoxide ($CDCl_3$ and $DMSO-d_6$) were purchased from Euriso-

top (Saint-Aubin, France). Sunflower oil was purchased in a local supermarket and stripped using alumina powder (MP EcoChromet ALUMINA N, Activity: Super I, Biomedicals, Eschwege, Germany) to remove polar impurities and tocopherols. Powdered extracts of turmeric and red radish, and raw powders of matcha tea, pineapple fibers, rosemary cake, spirulina cake, and spinach leaves were supplied by NATUREX (Avignon, France). An overview of the chemical composition of the particles is presented in Supporting information, Tables S1, S2 and S3. Ultrapure water (18.2 M Ω) was prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA) and was used for all the experiments.

2.2. Methods

2.2.1. Preparation of particle dispersions

Each dispersion was prepared by mixing the powdered material (i.e., matcha tea, spinach leaves, spirulina cake, pineapple fibers, rosemary cake, egg yolk and extracts of turmeric and red radish) or liquid (Tween 60) (1 or 5% w/w) in acetic acid buffer (50 mM, pH 4.5). The dispersion was magnetically stirred overnight at 4 °C and either used as such (non-washed particles), or centrifuged at 10,000 \times g for 20 min at 20 °C. The supernatant, which contained most of the soluble components, was collected and the pellet was resuspended in the same amount of acetic acid buffer. The centrifugation/resuspension procedure was repeated four times to finally yield a washed particle fraction.

2.2.1.1. Preparation of emulsions. Stripped sunflower oil (10% w/w) was added to an aqueous phase containing 5% w/w particles or 1% Tween 60, or the supernatant (obtained after the first washing step), or washed particles obtained from a 5% w/w particle dispersion in acetic acid buffer (50 mM, pH 4.5). A coarse emulsion was prepared using a rotor–stator homogenizer (Ultra-turrax IKA T18 basic, Staufen, Germany) at 11,000 rpm for 1 min, and was then processed through a lab-scale colloid mill with gap width of 0.32 mm (IKA Magic Lab, Staufen, Germany) operating for 1 min at 15,000 rpm. The emulsions were stored at either 4 or 25 °C.

2.2.1.2. Physical characterization of particle dispersions and emulsions.

To visualize the morphology of the powders in dry state, a scanning electron microscope (Phenom G2 Pure, Eindhoven, The Netherlands) operating at 5 kV was used. The samples were fixed with double-sided adhesive conductive carbon tabs (JEOL Europe BV, Nieuw-Vennep, The Netherlands) on 12.7 mm aluminum pin-type stub mounts (JEOL Europe BV, Nieuw-Vennep, The Netherlands).

The aggregation behavior of particles in aqueous media and the microstructure of emulsions were evaluated by light microscopy using a Carl Zeiss AxioScope A1 microscopy equipped with a camera (AxioCam Mrc5).

The particle size in aqueous dispersion, and the emulsion droplet size, were measured by static light scattering using a Mastersizer 3000 (Malvern Instruments Ltd.; Worcestershire, UK). The following optical properties were used: refractive indices of 1.45 (particles), 1.465 (stripped sunflower oil) and 1.330 (ultrapure water), with an absorption index of 0.01.

The interfacial tension between stripped sunflower oil and the supernatant (obtained after the first washing step) of 1% w/w particle dispersions (filtered using a glass-fiber (1 μ m) syringe filter (Acrodisc glass fiber, Sigma Aldrich, Steinheim, Germany)) was measured with a drop tensiometer (Tracker, Teclis, Longessaigne, France) used in rising drop configuration (i.e., a drop of stripped sunflower oil was formed on the tip of a needle (curved G18, internal diameter 0.84 mm, length 10 cm) immersed in a cuvette filled with the supernatant). Interfacial tension measurements were conducted for 3.5 h at room temperature, keeping the area of the droplet constant (30 mm²) throughout the experiment. The interfacial tension was determined by analyzing the

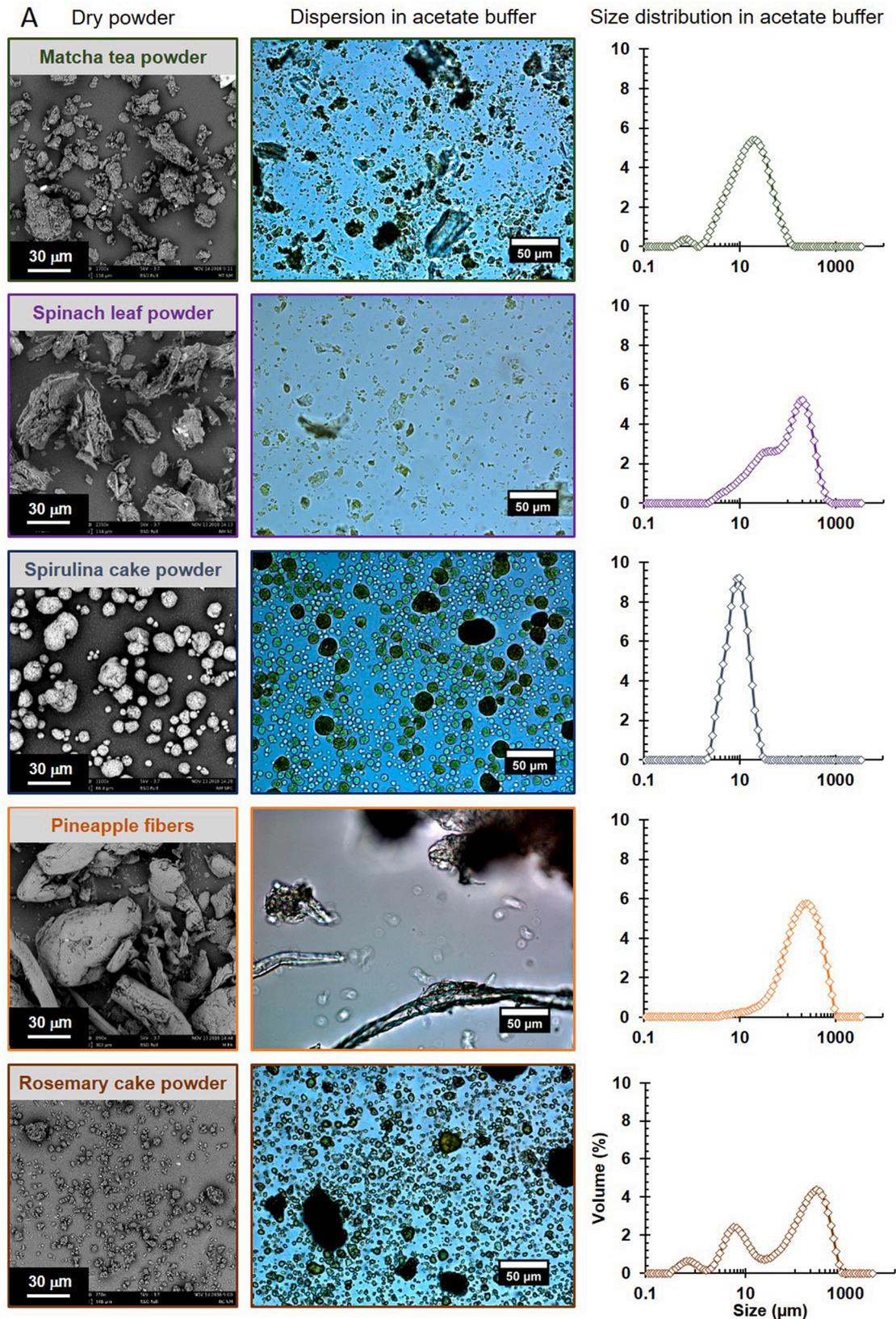


Fig. 1. A. Characterization of raw powders of matcha tea, spinach leaf, spirulina cake, pineapple fibers, and rosemary cake: Left: SEM images. Middle: light microscopy images of 1% w/w dispersions in acetate buffer (50 mM, pH 4.5). Right: particle size distribution (PSD) of 1% w/w dispersions in acetate buffer (50 mM, pH 4.5) measured by static light scattering, with on the x-axis the size in μm and on the y-axis the volume-based frequency. B. Characterization of turmeric and red radish powder: Left: SEM images. Right: light microscopy images of 1% w/w dispersions in acetate buffer (50 mM, pH 4.5) or oil. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

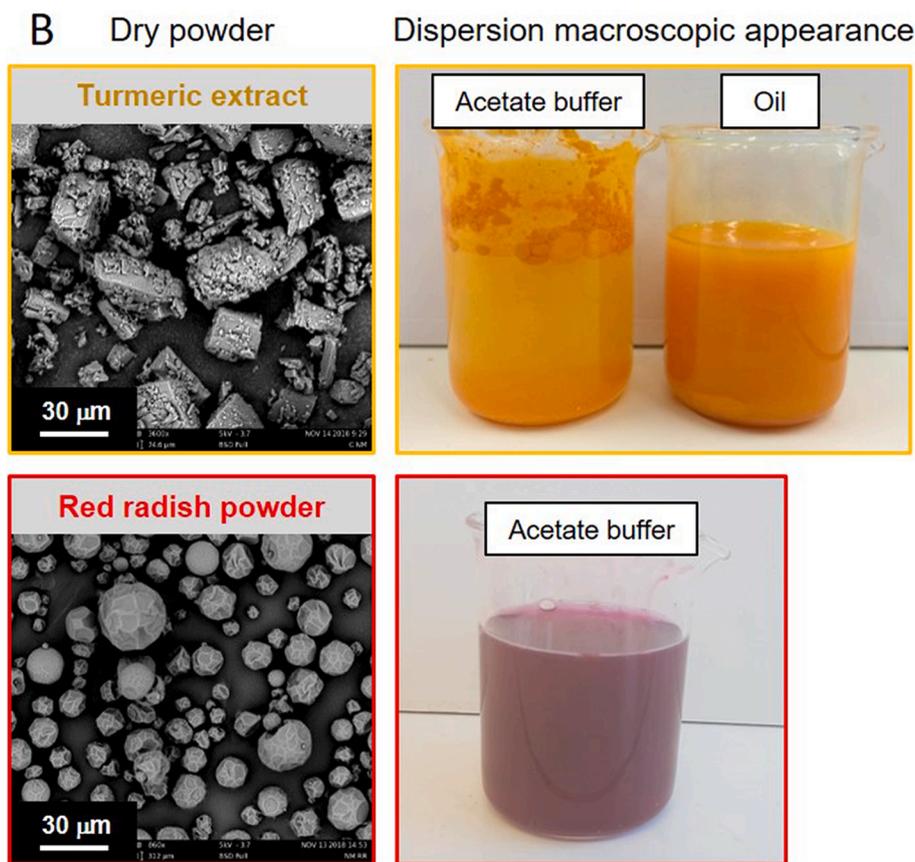


Fig. 1. (continued).

profile of the oil droplet using the Laplace equation.

2.2.1.3. Quantification of lipid oxidation products. Aliquots of emulsion (2 g) were put in 15-mL polypropylene tubes and incubated in a climate chamber at 25 °C, in the dark, without agitation. At selected time points, the oil was extracted by mixing 2 g emulsion with 4 mL chloroform, 3 mL methanol and 1 mL saturated sodium chloride solution, followed by centrifugation at 2000 xg for 8 min at 4 °C. The chloroform layer containing the extracted oil was collected by cautiously punching a hole at the bottom of the centrifugation tube, and chloroform was then evaporated under nitrogen flow at 25 °C. A total of 150 μL oil was collected, to which 450 μL 5:1 CDCl₃/DMSO-*d*₆ were added. Lipid oxidation products (hydroperoxides, conjugated dienes, and aldehydes) were quantified using nuclear magnetic resonance (NMR) on a Bruker Avance HD 700 MHz NMR spectrometer (Bruker BioSpin, Fällanden, Switzerland) equipped with a 5 mm BBI-probe at 295 K. For each sample, both a single pulse and band selective experiment were recorded, following Merks, Hong, Ermacora, and Van Duynhoven (2018). The data were processed with the Bruker TopSpin 4.0 software. From the single pulse experiment, the peaks from the conjugated dienes at δ 6.5 ppm and the glycerol backbone at δ 4.4 ppm were used for the quantification. In the band selective pulse, the region between δ 13.0 and 8.0 ppm was selectively excited. Here, the hydroperoxide signals resonated between δ 11.2 and 10.7 ppm and the aldehydes between δ 9.8 and 9.4 ppm. The calculations, including a factor that accounts for intensity loss during the selective pulse, are described in Merks et al. (2018). In some cases, it was necessary to subtract a background signal that originated from the particles from the overall signal. Data were obtained in independent duplicate experiments for 1-month storage.

2.2.2. Experimental design

SEM and interfacial tension measurements were performed as

dependent duplicates. For all other measurements, at least two dispersions or emulsions were prepared independently, and samples were analyzed in duplicate. The compositional analysis data in the supplementary information reports single measurements performed by the particles' supplier.

3. Results and discussion

3.1. Physical characterization of the particles

The matcha tea, spinach leaf, and rosemary cake particles have an irregular structure with high porosity (Fig. 1A). Pineapple fibers contained elongated and more irregular structures, with low porosity. Red radish (Fig. 1B) and spirulina cake (Fig. 1A) contained roughly spherical elements, with the latter showing small pores. Lastly, turmeric particles were polygonal and compact (Fig. 1B). The aspect ratio of particles is known to influence interfacial behavior: anisotropic particles can cover a higher fraction of the interface, and may induce jamming through lateral particle–particle interactions, resulting in strong mechanical properties of the interface (Madihala et al., 2009).

The dispersibility and aggregation behavior of the different powdered materials was investigated in acetate buffer. Powders of matcha tea, spirulina cake, spinach leaf and pineapple fibers seemingly dispersed well in acetate buffer, but when observed by light microscopy, they showed moderate aggregation (Fig. 1A). Additionally, pineapple fibers exhibited fast sedimentation, and eventually increased the viscosity considerably. Rosemary cake powder was macroscopically dispersible in acetate buffer, but light microscopy revealed strong particle aggregation, whereas turmeric powder (Fig. 1B) was not dispersible in this buffer. Conversely, both powders disperse well in sunflower oil (see Fig. 1B for turmeric powder). The red radish powder fully dissolved in buffer; no particles were detected by light microscopy, nor by static

light scattering (Fig. 1B), making it a particle-free control sample for the present study.

Binks et al. (2007) observed that Pickering O/W emulsions were most stable when prepared with slightly aggregating particles (Binks, Rodrigues, & Frith, 2007), as this is a typical behavior of moderately hydrophobic particles. When relating that with the behavior of our particles, it could be expected that powders of matcha tea, spirulina cake, spinach leaves and pineapple fibers have the highest potential to act as Pickering stabilizers for O/W emulsions. On the contrary, due to their hampered solubility in buffer, rosemary cake and turmeric powders are expected to be more suitable to stabilize W/O emulsions, whereas red radish powder cannot be considered a Pickering stabilizer.

We investigated the particle size distribution of the buffer-dispersible particles and found that spirulina cake and matcha tea particles were the smallest, with d_{32} of 9 and 11 μm , respectively (Fig. 1A). The average size of pineapple fibers and spinach leaf particles was much larger (d_{32} of 107 and 40 μm , respectively), with the latter showing a broad size distribution. Given the poor dispersibility of rosemary cake particles in acetate buffer, it is not surprising that this particle dispersion showed a highly polydisperse size distribution with peaks at ~ 0.5 , 5 and 200 μm , and d_{32} of 9 μm .

Pickering particles are usually substantially smaller than the targeted emulsion droplet size (Schulman & Leja, 1954); when at least one order of magnitude smaller, they generally provide good surface coverage, and are able to achieve effective Pickering stabilization. Still, for several food-grade particles, it has been reported that they could stabilize oil droplets of similar size, that is compared to the particle size in dispersion measured prior to emulsion preparation (Gould et al., 2013; Kurukji et al., 2013). This may be explained by two reasons: first, only the finest particle fraction may stabilize the emulsion, as found for cocoa particles (Gould et al., 2013); or second, the homogenization process applied to make the emulsion may disrupt or de-aggregate the particles (Kurukji et al., 2013). To assess the latter potential effect, we measured the particle size distribution of the particle dispersion after passage through the colloid mill homogenizer in the same conditions as used to produce emulsions. For rosemary cake particles, the size decreased considerably leading to a large fraction of particles between 0.5 and 5 μm (Supplementary information, Figure S4). The particle size of the other materials was not substantially affected.

From the equation defining the desorption energy of a Pickering particle (Eq. (1)), the interfacial tension of the oil–water interface, and the particle three-phase contact angle determine the energy involved in removing particles from an interface, that is, in conjunction with their size. Since our dispersions may contain surface-active components that could decrease the interfacial tension, or affect the contact angle by

adsorbing to the particles (Binks & Rodrigues, 2007), we measured the interfacial tension between oil and the supernatant obtained after centrifugation of 1% w/w dispersions of powders in acetate buffer.

The interfacial tension between stripped sunflower oil and acetic acid buffer was around 30 mN m^{-1} and was stable over the timescale of the experiment (Fig. 2). The soluble components in the spirulina cake supernatant were able to decrease the interfacial tension the fastest, and reached the lowest value ($\sim 10 \text{ mN m}^{-1}$), probably due to the presence of proteins (composition in Supplementary information, Table S2), particularly phycocyanins (Benedetti et al., 2004). The matcha tea powder supernatant was also able to decrease the interfacial tension to 10 mN m^{-1} , probably because of the proteins present, and that also holds for spinach leaf soluble components that were able to decrease the interfacial tension to 13.5 mN m^{-1} , which may be due to the presence of thylakoid fragments that are rich in proteins (Tenorio et al., 2017). The red radish powder was completely soluble and decreased the interfacial tension rapidly, to reach a final value of 14.5 mN m^{-1} . Pineapple fiber and rosemary cake supernatants had lower surface activity (final recorded value of 17 mN m^{-1}), and the aqueous supernatant of the turmeric powder was not able to decrease the interfacial tension.

As mentioned, soluble components originating from our biobased materials may adsorb at the surface of the particles, therewith changing their wettability (Binks et al., 2007; Cui et al., 2012). The involved interactions are generally of electrostatic or hydrophobic origin (Berton-Carabin & Schroën, 2015; Santini, Guzmán, Ferrari, & Liggieri, 2014), and for silica particles and cationic surfactants this interaction has been reported to lead to more hydrophobic particles, and to a synergistic stabilization of emulsions (Binks et al., 2007). Surface-active components may also compete with particles and have even been suggested to desorb particles from the interface (Vashisth et al., 2010), although we expect this to be only possible if the resulting contact angle is close to 0 or 180°.

3.2. Emulsion physical stability

We prepared 10% w/w O/W emulsions with 5% w/w particles dispersed in the aqueous phase, and measured droplet size in time to evaluate physical stability. Powders of matcha tea, spinach leaves, spirulina cake and pineapple fibers were able to physically stabilize O/W emulsions for at least 3 months (Fig. 3). In the measured droplet size distribution, non-adsorbed particles also contribute to the scattered light signal; therefore, light microscopy images were analyzed to estimate the actual droplet size. Matcha tea powder- and spirulina cake powder-stabilized droplets were around 10 μm , and showed network formation in the continuous phase, most likely due to particle bridging.

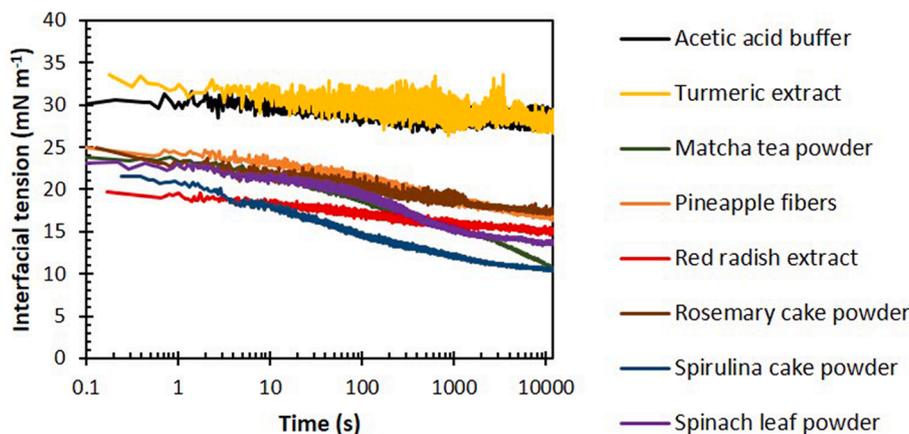


Fig. 2. Interfacial tension over time at the stripped sunflower oil-acetic acid buffer interface, with the aqueous phase containing the soluble components of a 1% w/w dispersion of turmeric, red radish, matcha tea, pineapple fibers, rosemary cake, spirulina cake, or spinach leaf powders. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

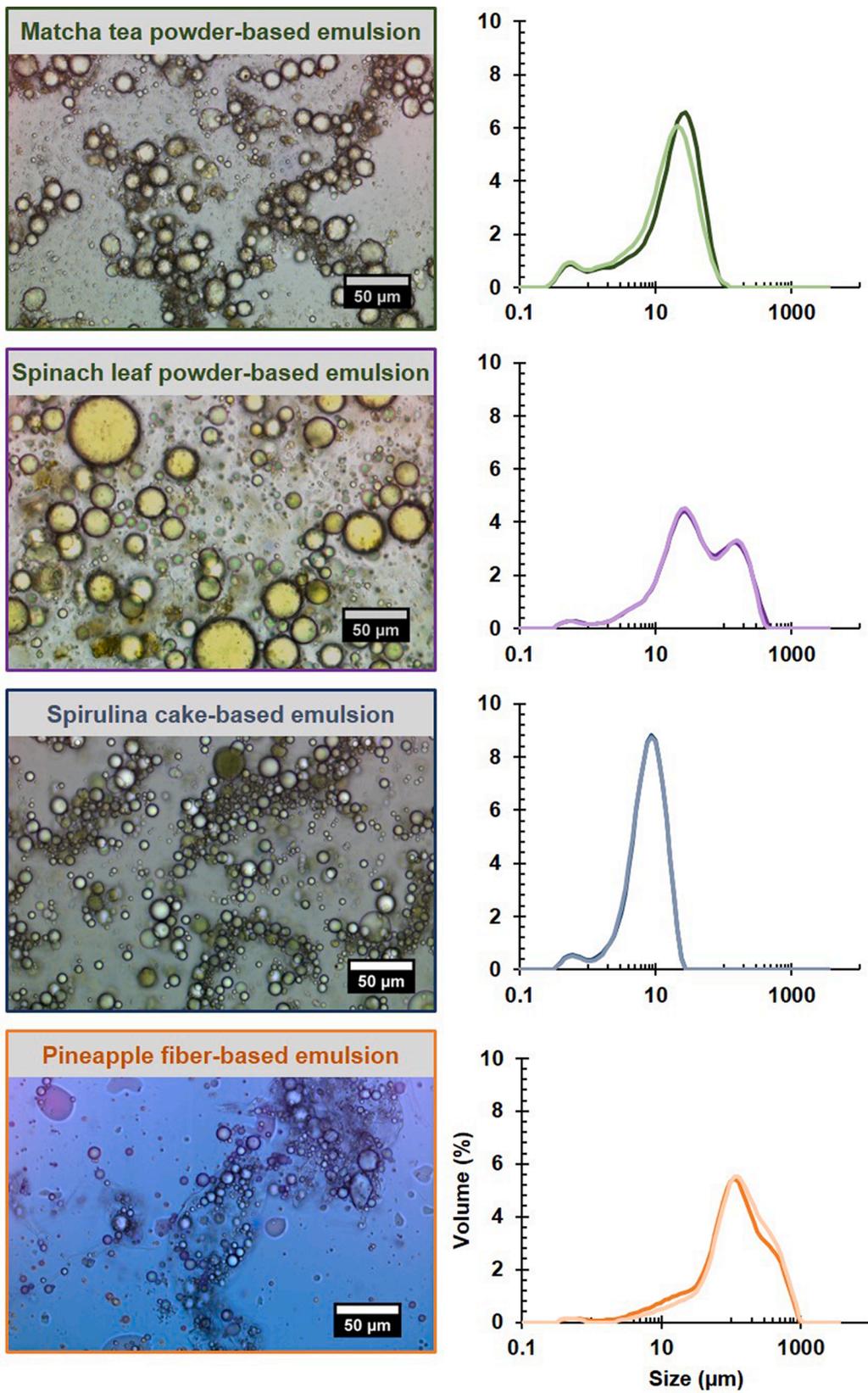


Fig. 3. Physical characterization of 10% w/w O/W emulsions stabilized by 5% w/w powders of matcha tea, spinach leaves, spirulina cake, or pineapple fibers: Left: light microscopy images of fresh samples. Right: Droplet size distribution (freshly prepared (dark colored line), or after 3 months storage at 4 °C (light colored line)).

Spinach leaf powder formed larger oil droplets of around 25–50 μm , and showed less bridging. Emulsions stabilized by pineapple fibers were highly viscous due to swelling and fiber network formation in the continuous phase. The high viscosity was probably instrumental in reducing the droplet size to $\sim 10 \mu\text{m}$ (Walstra, 2004). The red radish dispersion was not able to form stable emulsions (Supplementary information, Fig. S5).

Emulsions prepared with rosemary cake powder phase inverted upon homogenization, probably due to the hydrophobicity of these particles. To investigate their emulsification potential for water-in-oil (W/O) emulsions, as well as that of the turmeric particles, we homogenized a sunflower oil phase containing the dispersed particles with an aqueous phase using a rotor stator homogenizer, and stable W/O emulsions were obtained (Fig. 4), with turmeric particles visible at the droplet surface by optical microscopy (Fig. 4B). We did not further investigate the emulsifying properties of these particles, as the rest of the study focused on O/W emulsions.

To further investigate the stabilization mechanism of the most promising materials (powders of matcha tea, spinach leaves, spirulina cake and pineapple fibers), we prepared O/W emulsions with only the supernatant (soluble fraction), or the re-suspended pellet (washed particles) obtained after centrifugation of the particle suspension. The supernatant of the spirulina cake powder suspension, which was the most surface-active (Fig. 2), and of the pineapple fiber suspension, led to emulsion droplets of around 5 μm (d_{32}) that destabilized due to flocculation and coalescence within one week (Fig. 5E and 5G). This may have occurred because of a lack of steric and/or electrostatic repulsion, i.e., the droplets were not covered or charged enough. In emulsions formed with the soluble components of matcha tea and spinach leaf powders, immediate coalescence took place, as illustrated by their large droplets and polydisperse size distribution (Fig. 5A and C), showing that these components are not effective emulsifiers.

By contrast, washed particles from matcha tea, spinach leaf and spirulina cake powders were able to form physically stable droplets for at least 4 weeks. Matcha tea and spinach leaf particles are thus able to do so through a true Pickering stabilization mechanism. For spirulina cake particles, soluble components such as proteins (see Supplementary information Table S2) may have contributed to the overall emulsion stability, for example through co-adsorption, or facilitation of droplet breakup. In emulsions stabilized by washed pineapple fibers, visible oiling-off took place within one week. Pineapple fibers were not able to stabilize emulsions through a Pickering stabilization mechanism, but probably the soluble components in this material adsorbed at the interface, and the fibers increased the viscosity of the continuous phase, therewith enhancing physical stability.

3.3. Oxidative stability of emulsions

We incubated matcha tea powder-, spinach leaf powder-, spirulina cake powder- and pineapple fiber-stabilized emulsions (non-washed dispersions) at 25 $^{\circ}\text{C}$, and measured the formation of primary lipid oxidation products (conjugated dienes, Fig. 6A, and total hydroperoxides (Supplementary information, Fig. S6)) and secondary oxidation products (aldehydes, Fig. 6B). We also compared their oxidative stability to that of reference emulsions stabilized by egg yolk or Tween 60 (droplet size distributions and microscopy images of these reference emulsions are given in Supplementary information, Fig. S7). All emulsions remained physically stable over the time of incubation. Interestingly, matcha tea powder- and spinach leaf powder-stabilized emulsions remained oxidatively stable for 6 weeks with hardly any primary and secondary oxidation products detected. Egg yolk-stabilized emulsions were oxidatively stable for 2 weeks, but after that showed quick formation of conjugated dienes, and moderate formation of aldehydes. The oxidative stability of the other three emulsions was less good and ranked as follows: spirulina cake powder > Tween 60 > pineapple fibers, with the latter showing formation of both primary and secondary oxidation products as soon as incubation started.

Different factors may contribute to the susceptibility or resistance of emulsions to lipid oxidation, and for this the composition of the particles, and the properties of the components present in the continuous phase and at the interface need to be considered. In the following paragraphs we start by discussing lipid oxidation in the particle-stabilized emulsions, then in the reference emulsions, after which all effects are putatively brought together.

Matcha tea is known to contain exceptionally high concentrations of antioxidants (as also reported in Supplementary material, Table S1), particularly catechins of which epigallocatechin gallate is the most abundant, and the flavonoid quercetin (Ozgen et al., 2016). Both components are free radical-scavenging antioxidants and able to interrupt the lipid oxidation chain reaction, which can explain the high oxidative stability of this emulsion, especially when located at the interface (Berton-Carabin et al., 2014; Schröder et al., 2020). Spinach leaf powder contains 40-fold lower contents of phenolics compared to matcha tea powder (Supplementary material, Table S1). Yet, it contains oxalic acid (Supplementary material, Table S3), which has been reported to chelate metal ions such as iron (Suter et al., 1988), thereby inactivating this transition metal with regard to its pro-oxidant effect. Understanding what causes the effect of spirulina cake particles on oxidation is more elusive. Although spirulina cake is an extraction residue, and thus supposedly exhausted from phycocyanin, it may still contain residual amounts of this protein that is able to scavenge free radicals. In this emulsion, fast formation of conjugated diene hydroperoxides took place, but hydroperoxide decomposition was rather slow, which could indicate that the iron generally present in spirulina is chelated (Gorji et al.,

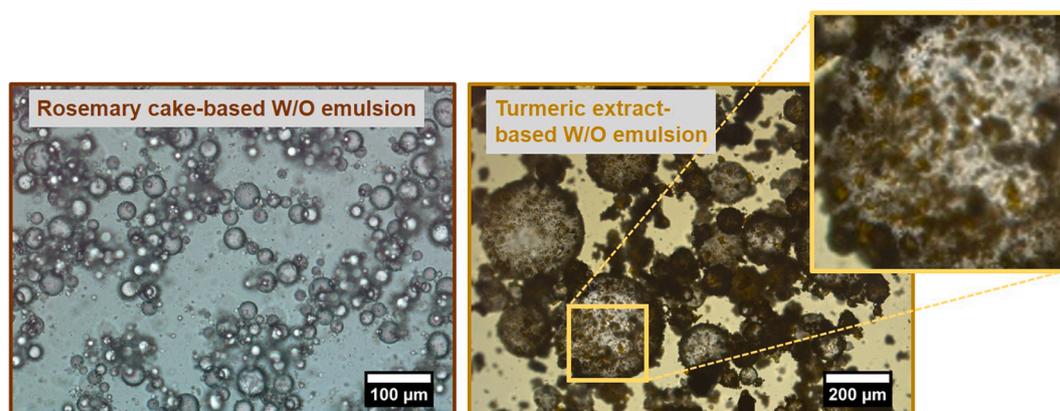


Fig. 4. Light microscopy images of 10% w/w W/O emulsions stabilized by 5% w/w rosemary cake powder (left) or turmeric powder (right).

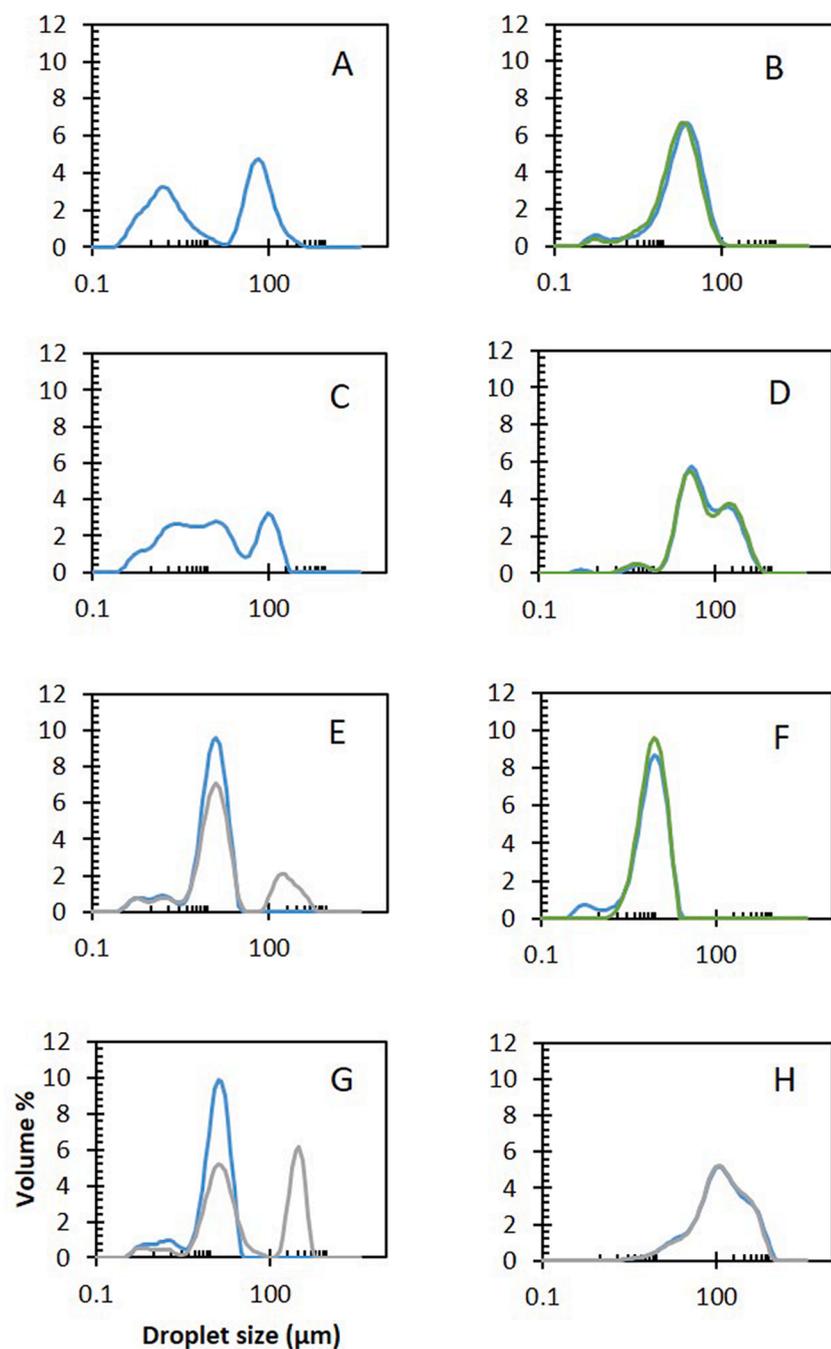


Fig. 5. Droplet size distributions of emulsions stabilized by the supernatant obtained after the first washing step (A, C, E, G), or by washed particle dispersions obtained from a 5% w/w particle dispersion (B, D, F, H) of matcha tea powder (A, B), spinach leaf powder (C, D), spirulina cake powder (E, F) and pineapple fibers (G, H), at t_0 (blue), and after 1 week (grey) or 4 weeks (green) storage. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2016). In fact, iron has a strong catalytic effect for the decomposition of hydroperoxides, leading to alkoxy radicals and later on secondary lipid oxidation products (Schaich et al., 2013). Pineapple generally contains considerable amounts of vitamin C that can scavenge free radicals and regenerate hydrogen-donating antioxidants (Shahidi & Zhong, 2010), while it is also able to reduce ferric iron to ferrous iron, which is a more active oxidation catalyst, and could thus explain the fast oxidation in the corresponding emulsion. On the other hand, it is questionable if the pineapple fibers used (again, an extraction residue) still contained significant amounts of vitamin C.

Egg yolk is especially rich in the protein phosvitin that has strong metal binding ability at pH 6.5 (Castellani et al., 2004). However, at pH 4.5 as used in our study and typical for dressing-like food emulsions including mayonnaise, the binding constant is lower, which may allow iron to be available for lipid oxidation (Merkx, Delić, et al., 2018).

Phosvitin is also surface-active, and will therefore locate at the oil–water interface where it may release iron, thus promoting lipid oxidation, which may be prevented in food emulsions by a strong chelator such as EDTA. To the best of our knowledge, Tween 60 is not known for specific antioxidant activity. Therefore, no active inhibition of oxidation of the corresponding emulsion could be expected.

To summarize, as lipid oxidation is initiated at the oil–water interface in most situations (Berton-Carabin et al., 2014; Laguerre et al., 2017, 2020), localizing antioxidants at this specific locus using natural Pickering particles appears as an efficient strategy to promote oxidative stability. Most natural antioxidants are highly lipophilic or hydrophobic, and therefore locate in the oil droplet, or in the aqueous phase, where their efficiency is far from optimal. The hierarchical emulsion design that stems from the use of Pickering particles can be an interesting way to boost the efficiency of natural antioxidants, as we previously also

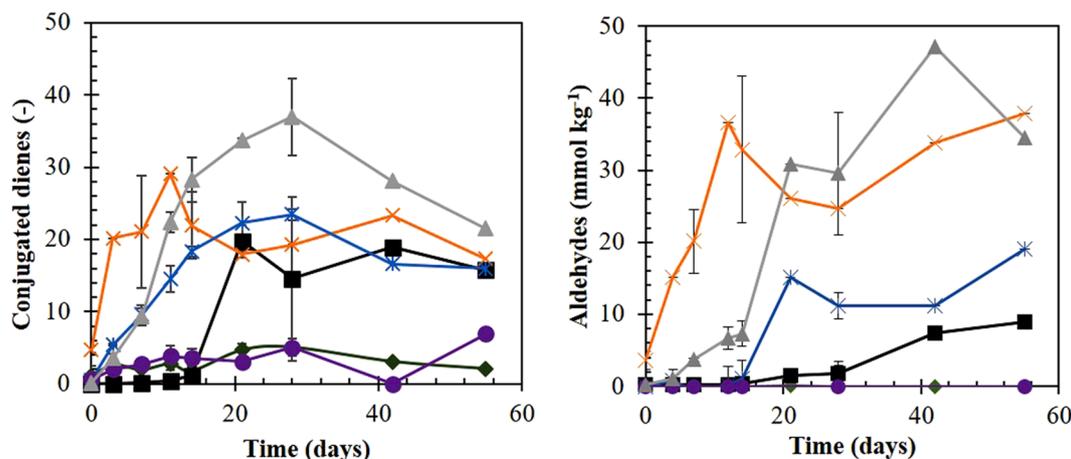


Fig. 6. Formation of conjugated dienes (left) and aldehydes (right) in O/W emulsions (10% w/w stripped sunflower oil) stabilized with matcha tea powder (green), spinach leaf powder (purple), spirulina cake powder (blue), pineapple fibers (orange), egg yolk (black) or Tween 60 (grey), stored at 25 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demonstrated for purposely built particles (Schröder, Sprakel, Boerkamp, Schroën, & Claire, 2019; Schröder et al., 2020). Some of the biobased Pickering particles used in the present study contain endogenous chain-breaking antioxidants or chelators embedded in their natural matrix, which are probably still available for hydrogen atom-donating or metal-chelating activities. In either case, interfacial localization would be optimal.

Here, we provide a proof-of-principle that it is possible to create a dual stabilizing functionality that leads to emulsions naturally armored against oxidation and physical destabilization, while complying with clean-label requirements. This new approach, which holds great potential for a wide range of applications, is inspired by the way in which emulsions were traditionally prepared before the advent of the food industry and the massive use of synthetic emulsifiers. Perhaps ironically, this retrospective can provide the key to formulating tomorrow's food emulsions.

4. Conclusions

We have investigated the physical and oxidative stability of Pickering emulsions stabilized by natural particulate materials, i.e., powders of matcha tea, spinach leaves, spirulina cake, pineapple fibers, and rosemary cake, and powdered extracts of red radish and turmeric. Particles that showed weak aggregation in aqueous media (powders of matcha tea, spinach leaves, spirulina cake and pineapple fibers) were able to physically stabilize oil-in-water (O/W) emulsions, through either a Pickering stabilization mechanism (for the former three), or an increase of the viscosity of the aqueous phase. Additionally, matcha tea powder- and spinach leaf powder-stabilized emulsions were also highly stable to lipid oxidation. This is probably due to accumulation at the droplet surface of chain-breaking antioxidants and/or chelators embedded in the particles' matrix, yet still available for reacting or interacting with pro-oxidative catalysts. This suggests that natural Pickering particles have a great potential to mitigate usual instability issues related to emulsions, while fully complying with clean-label strategies.

CRediT authorship contribution statement

Anja Schröder: Conceptualization, data curation, formal analysis, investigation, methodology, writing - original draft. **Mickael Laguerre, Karim Schroen and Claire C. Berton:** Writing - review & editing, conceptualization, methodology, supervision. **Mathieu Tenon:** Data curation, methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.129003>.

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