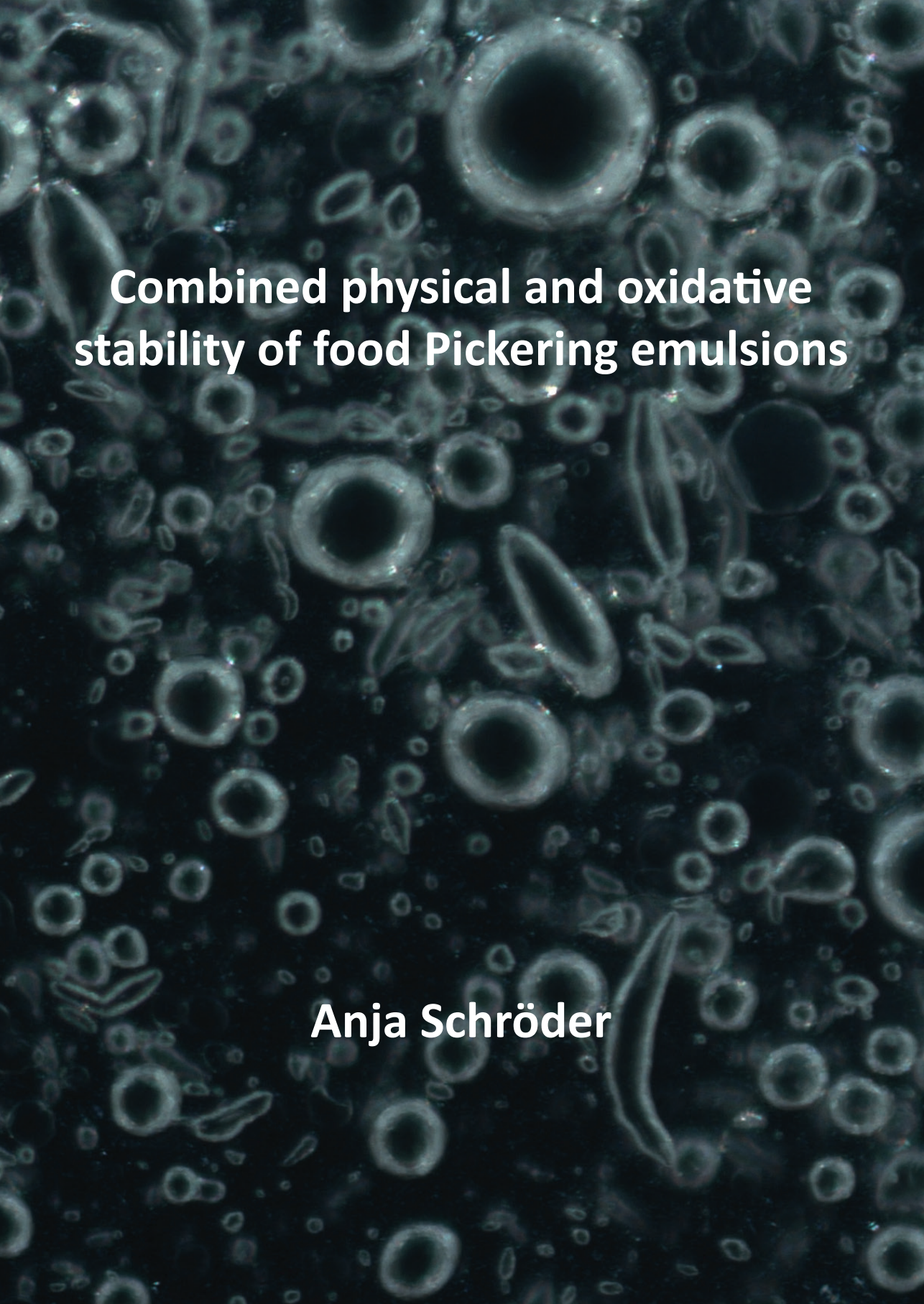

Combined physical and oxidative stability of food Pickering emulsions

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Anja Schröder

Thesis

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Contents

Chapter 1

General introduction

1.1. Food emulsions

Many products from the food, pharmaceutical, cosmetic, agrochemical, and various other fields are dispersions of immiscible liquids, typically oil and water, i.e., are emulsions. Emulsions can be either naturally present as in raw milk, but they are often purposely manufactured from different components as done for skin creams, paints, salad dressings and many more food products (Figure 1.1.). In such food products, water droplets can be present in a continuous oil phase (water-in-oil (W/O) emulsions) e.g., margarine, but in most cases oil droplets are present in a continuous aqueous phase (oil-in-water (O/W) emulsions) e.g., milk and mayonnaise.

Figure 1.1. Examples of food emulsion products: (from left to right) margarine, milk and salad dressing.

1.1.1. Emulsion stability

Emulsions may be subjected to destabilization, both from a physical or from a chemical perspective (Figure 1.2. and elaborated in the respective sections), of which the latter is especially relevant for emulsions that contain polyunsaturated fatty acids (PUFAs).

1.1.1.1. Physical stability

Emulsions are, in essence, thermodynamically unstable systems due to the molecular incompatibility between oil and water. Phase separation in emulsions is driven by the free energy of the system (ΔG, J) defined as (Equation 1.1.):

$$\Delta G = \gamma \Delta A \qquad \text{(Eq. 1.1.)}$$

where γ is the interfacial tension (N m^{-1}) between oil and water, and ΔA is the total interfacial area (m^2) in the system (McClements, 2005). The system strives to minimize its free energy, and this can be achieved by either a reduction in interfacial area ultimately leading to phase separation to minimize the contact area, or by a reduction in interfacial tension.

The average droplet size in food emulsions generally varies from ~100 nm to 100 µm, resulting in an interfacial area of up to several m² per gram of dispersed material (Berton-Carabin, Sagis, & Schroën, 2018), and typical interfacial tensions between commercial vegetable oils and water of ~20 mN m^{-1} (Coupland, 2015), resulting in a driving force for phase separation which can thus be great.

Furthermore, due to the density difference between both phases, there is a driving force for droplet creaming or sedimentation. Such a gravitational separation of droplets from the continuous phase is governed by different parameters that are captured in the Stokes equation (Equation 1.2.), which applies to dilute emulsions.

$$v = \frac{2\,(\rho_d - \rho_c)\,g\,r^2}{9\,\eta} \qquad \text{(Eq. 1.2.)}$$

where v is the velocity (m s^{-1}) at which the droplets cream or sediment, ρ_d and ρ_c are the densities (kg m^{-3}) of the dispersed and continuous phase, respectively, g is the gravitational acceleration (m s^{-2}), r is the radius of the droplet (m) and η is the viscosity of the continuous phase (kg m^{-1} s^{-1}). From this equation it is clear that the size of the droplets is of great importance for ensuring emulsions with sufficient physically stability. An increase in viscosity of the continuous phase can be achieved using thickeners such as xanthan gum and alginates (Tadros, 2009). In turn, this also slows down physical destabilization mechanisms such as droplet flocculation and coalescence (McClements, 2005), of which the latter is irreversible, and ultimately leads to complete demixing (oiling off).

Emulsifiers are generally used to provide kinetic stability, that is, to slow down thermodynamically favorable phase separation. They decrease the interfacial tension between oil and water, which favors droplet breakup during emulsification, decreases the total free energy of the system, and prevents flocculation and coalescence via steric and/or electrostatic repulsion between droplets. Conventional food emulsifiers include low molecular weight components, generally called surfactants, and amphiphilic biopolymers, which are mostly proteins. Low molecular weight components are small surface-active molecules constituted of a lipophilic tail and a hydrophilic head group, which can be very efficient in emulsion stabilization (Berton-Carabin, Ropers, & Genot, 2014). Their position is not fixed to the interface due to their small size; they have lateral mobility at the interface, and can even leave it by exchanging with components present in the bulk. A number of food-grade surfactants are synthetic molecules, and therefore they can be expensive, do not comply with the clean-label trend, and sometimes give undesirable taste (Birch &

Ogunmoyela, 1980; Dickinson, 1993). Biopolymers such as proteins are amphiphilic because they have lipophilic and hydrophilic domains distributed along their chain, which leads to conformational changes after adsorption. They form thicker layers, which generally give more steric repulsion between droplets, compared to surfactants, although surfactants with large head groups, such as polysorbates, also give steric repulsion. Dairy proteins are widely used for this purpose, and some of them have also shown to form covalent intermolecular bonds, resulting in a strong viscoelastic network at the interface.

Currently, plant proteins also attract a lot of attention as more sustainable alternatives for dairy-based components. They are generally rather cheap, abundantly available, and already well studied in some cases such as soy proteins. The big question is, however, whether they can also be used to make physically stable emulsions. The general tendency is that this question should be answered in a negative way.

From the above it is clear that emulsions stabilized with traditional emulsifiers can be subject to various instability phenomena, and may be criticized because of the use of components that do not comply with clean-label strategies. This shows that alternative approaches are relevant for food applications.

1.1.1.2. Oxidative stability

Although the use of oils and fats containing PUFAs has beneficial health effects in relation to, amongst others, cardiovascular diseases, their use makes food emulsions prone to chemical destabilization during processing, storage and end-use. This is due to oxidative reactions, of which lipid oxidation leads to the formation of a broad range of compounds that have noticeable rancid off-flavors at low detection thresholds, and to the degradation of compounds of nutritional interest (e.g., vitamins and the PUFAs themselves). Besides, potentially toxic compounds can be formed (El-beltagi & Mohamed, 2013).

The lipid oxidation reaction has been investigated for decades and three stages are distinguished: initiation, propagation and termination (Schaich, 2005). Initiation consists of the abstraction of a hydrogen atom from an unsaturated fatty acid, resulting in alkyl radical formation (Figure 1.2.). During the propagation stage, the alkyl radical rapidly reacts with triplet oxygen, forming a peroxyl radical. This peroxyl radical is unstable, and can abstract a hydrogen atom from another unsaturated fatty acid, leading to the formation of a hydroperoxide, and of a new alkyl radical (i.e., propagating the reaction). In the termination

stage, radicals may recombine into non-radical compounds, and concomitantly, hydroperoxides may undergo decomposition into a broad range of compounds that are mostly responsible for off-flavors (Falkeborg, Berton-Carabin, & Cheong, 2016).

It is commonly assumed that lipid oxidation in emulsions is controlled, at least partly, by the chemical composition and structural organization of the oil-water interface (Berton-Carabin, Ropers, & Genot, 2014). In fact, lipid oxidation is presumably initiated at the interface, the region where unsaturated lipids come into close contact with aqueous phase pro-oxidants (e.g., metal ions and reactive oxygen species).

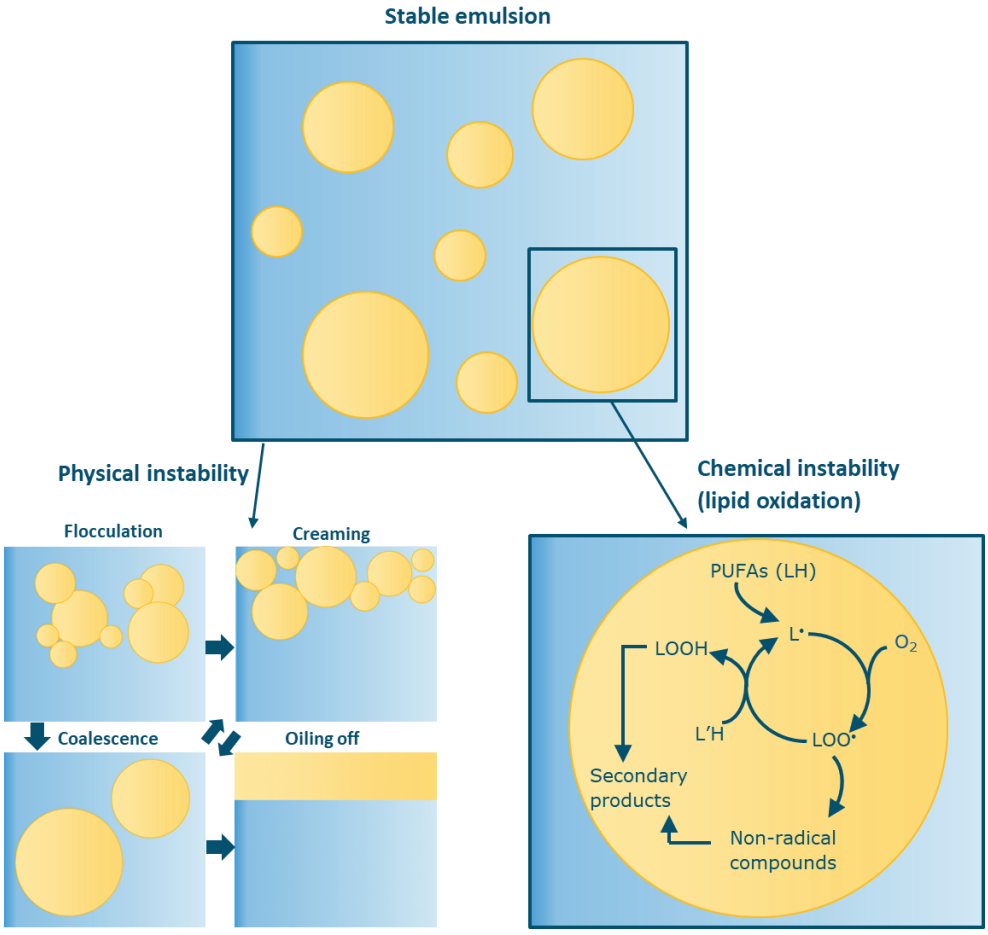

Figure 1.2. Schematic representation of the main physical and chemical destabilization (lipid oxidation) mechanisms in O/W emulsions: LH = unsaturated fatty acid, L• = alkyl radical, LOO• = peroxyl radical and LOOH = hydroperoxide.

Several characteristics of the oil-water interface have been described to influence lipid oxidation, such as the emulsifier itself and its electrostatic charge, which depends on the properties of the aqueous phase (pH, ionic strength). A negatively charged interface may attract positively charged pro-oxidant metal ions resulting in relatively fast oxidation, whereas a negatively charged interface is hypothesized to repel them (Mancuso, McClements, & Decker, 1999). This relation has been suggested for low molecular weight surfactants, but failed to be generalized to protein-covered interfaces that may differ in their metal chelating and free radical scavenging properties (Berton, Ropers, Bertrand, Viau, & Genot, 2012), and thus antioxidant properties. Additionally, lipid oxidation products (e.g., hydroperoxides) are more surface-active than the triglycerides they originate from (Abousalham, Fotiadu, Buono, & Verger, 2000; Nuchi, Hernandez, McClements, & Decker, 2002) and accumulate at the oil-water interface, where they may further decompose.

Although, in general, it is assumed that the thickness, density, and structural homogeneity of the interface influence lipid oxidation, results from literature are contradictory, which hampers establishing a concise emulsion formulation strategy that does not require the use of synthetic antioxidants. In the food industry large amounts of these components, e.g., butylated hydroxytoluene, a free radical scavenger (BHA, E320), and ethylenediaminetetraacetic acid (EDTA, E385), a metal chelator (McClements & Decker, 2000) are used to keep products chemically stable. The latter is known to be very efficient in products such as mayonnaises, that contain phosvitin from egg and associated iron (Samaraweera, Zhang, Lee, & Ahn, 2011). Alternatively, natural antioxidants such as tocopherols and rosemary extracts have also been considered, but they are unfortunately not optimally effective because they preferentially locate in the bulk oil or water phases due to their chemical nature, but not at the oil-water interface where lipid oxidation is initiated.

It is known that the efficiency of natural antioxidants can be enhanced by tuning their hydrophobicity such that they have affinity for the interface (Laguerre et al., 2013; Laguerre et al., 2015; Lomova, Sukhorukov, & Antipina, 2010; Yuji et al., 2007). This has been confirmed by Laguerre et al. (2010) who chemically modified hydrophilic phenolic antioxidants through lipophilization, and found that antioxidant efficiency increased with the length of the alkyl chain grafted, until an optimum beyond which further increase led to a collapse in antioxidant efficiency (Laguerre et al., 2013, 2010). This non-monotonic

dependency, referred to as the cut-off effect, is associated with the propensity of medium chain-grafted molecules to position at the oil-water interface (Laguerre et al., 2009, 2011). Large scale implementation of these so-called phenolipids is, however, hampered by their cost, and the fact that they are no longer natural molecules (Ghorbani Gorji, Smyth, Sharma, & Fitzgerald, 2016).

From a chemical point of view it is clear that alternative strategies to increase natural antioxidant efficiency in food systems are very relevant, and as mentioned previously, this would also be the case for improvement of physical stability. In this thesis, we explore food emulsions with a new and controlled microstructure, using particles that either have or allow incorporation of natural antioxidants, and that thus nest in the interface to achieve high physical stability, and may also influence oxidative stability.

1.2. Pickering emulsions

In recent years, interest has been on the rise regarding the use of particles as emulsifiers in food products (Berton-Carabin & Schroën, 2015). These so-called Pickering particles are known to provide high stability against coalescence as compared to conventional surfactant- or protein-stabilized emulsions; however, putting these particles in an interface, making particles with the right properties, and in sufficient quantities is far from trivial.

1.2.1. Physical stability aspects

Particle-stabilized emulsions have already been known since the beginning of the twentieth century (Pickering, 1907; Ramsden, 1903). They have also been unintentionally used in food products such as mayonnaise and margarine, in which mustard seeds and fat crystals act as Pickering particles (Fox, 1983), but mostly they remained unexplored for a long time (Rayner, Timgren, Sjöö, & Dejmek, 2012). In the last decade, attention for Pickering stabilization has peaked due to the potentiality to make emulsions with high physical stability (Berton-Carabin & Schroën, 2015) and the high demand for clean-label products (Linke & Drusch, 2018).

The mechanism through which solid particles stabilize emulsions is fundamentally different compared to conventional emulsifiers; their tendency to adhere at interfaces can be understood from the perspective of wetting, rather than amphiphilicity (Finkle, Draper, & Hildebrand, 1923); the energy involved is related to the size of the particle, the interfacial tension of oil-water interface, and the contact angle of the particle at the interface, which

determines the position of the particle at the interface (Figure 1.3). For spherical particles, the desorption energy (ΔG_a, J) is expressed by Equation 1.3:

$$\Delta G_a = \pi r^2 \gamma_{ow} (1 - \cos\theta)^2 \quad \text{(Eq. 1.3.)}$$

with r the particle radius (m), γ_{ow} the oil-water interfacial tension (N m^{-1}), and θ the contact angle. The adsorption energy of the particle at the interface may exceed the thermal energy (indicated by the dashed line) by orders of magnitude, ensuring their irreversible anchoring (Figure 1.4.).

Other particle attributes that influence Pickering stabilization include particle shape, surface charge and inter-particle interactions (Binks, 2002; Tcholakova, Denkov, & Lips, 2008b). Substantial work has been performed on model systems (i.e., with silica particles), and it was often necessary to chemically modify the particle surface to ensure good anchoring at the interface. In principle, from these investigations, guidelines for the design of food Pickering emulsion may be derived.

To make acceptable food Pickering emulsions, it is key to find food-grade particles with the right size and contact angle, that can be produced at large scale; and ideally, without chemical modification. Most of the food-related particles that have been suggested for Pickering stabilization of O/W emulsions are protein- or carbohydrate-based, e.g., protein aggregates and hydrophobized starch granules, that often are produced via solvent-induced precipitation or heating (Tan et al., 2014), and derivatization with octenyl succinic anhydride (Timgren, Rayner, Dejmek, & Marku, 2013), respectively, and therefore do not comply with the requirements pointed out earlier.

In this thesis, we first chose to explore lipid-based particles for the Pickering stabilization of O/W emulsions. Such particles have already been recognized as stabilizers for W/O emulsions, due to their intrinsic hydrophobic nature (Rousseau, 2013). To make lipid-based particles effective for Pickering stabilization of O/W emulsions, surface-active hydrophilic molecules such as proteins or surfactants have been used in the present work. We first built particles via a bottom-up approach to have high control over their composition and properties, and generate a proof of concept for their efficacy as physical stabilizers. Furthermore, we have used natural plant-based particles, using existing structures that only require size reduction, thereby obtained through a top-down approach.

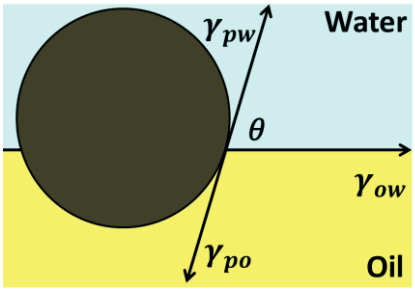

Figure 1.3. Single colloidal solid particle at the oil-water interface with its positioning determined by its three-phase contact angle.

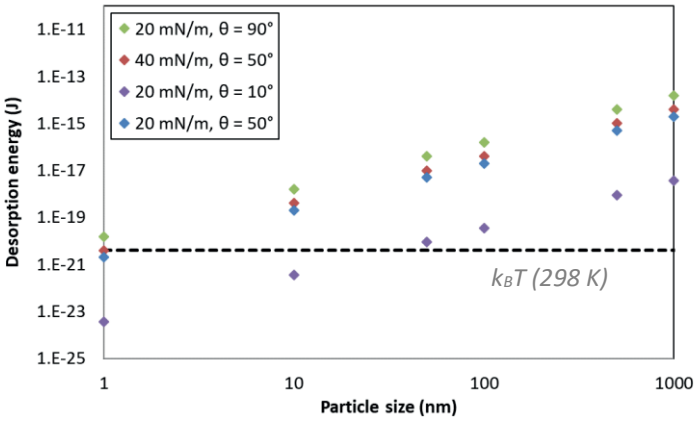

Figure 1.4. Plot illustrating the desorption energy of a single colloidal solid particle at the oil-water interface with a three-phase contact angle of 10°, 50° or 90° as function of particle radius, for oil-water interfacial tensions of 20 and 40 mN m^{-1}. The dashed line represents thermal energy at 298 K (1 k_BT). Source: Berton-Carabin & Schroën (2015).

1.2.2. Oxidative stability aspects

Lipid oxidation in emulsions stabilized by Pickering particles has so far only been discussed in a few papers (Kargar, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012; Kargar, Spyropoulos, & Norton, 2011; Pan, Tikekar, Wang, Avena-Bustillos, & Nitin, 2015; Xiao, Li, & Huang, 2015; Zeng et al., 2017). It has been reported that the amount of primary lipid oxidation products (i.e., hydroperoxides) in emulsions stabilized by silica particles was lower compared to Tween 20-stabilized emulsions, but higher compared to sodium caseinate-stabilized emulsions (Kargar et al., 2011b). Whether this protective effect of silica particles was a physical effect only, i.e., by decreasing the interface permeability to aqueous phase

pro-oxidants, is questionable (Zhao, Elias, & Coupland, 2015) given the size of these particles, and therefore the interfacial gaps existing between them.

1.3. Problem definition and research aim

In food emulsion formulation, the amount of PUFAs has increased over the past decades, which leads to products that are prone to lipid oxidation. Currently, mostly synthetic antioxidants are used to mitigate this issue, but they are becoming more and more a topic of hot debate amongst consumers, and therefore industry has been pushed to minimize their use (Berton-Carabin & Schroën, 2019). From what is described earlier, we hypothesize that Pickering particles could lead to an increase in both the physical and oxidative stability of emulsions. Finding appropriate and efficient strategies to make food emulsions with such a dual stability is the core of this thesis. We consider particles that contain antioxidants that are naturally present, or to which antioxidants are added on purpose, and that simultaneously enhance physical stability of the emulsion (Figure 1.5.).

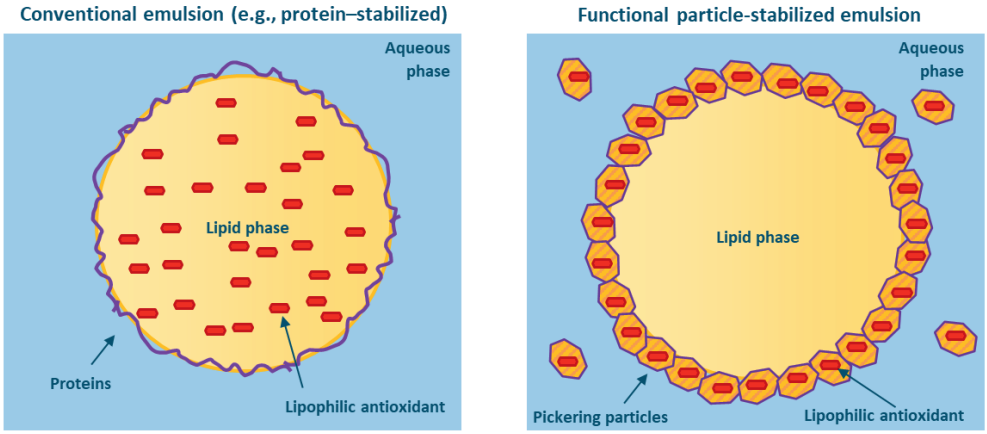

Figure 1.5. Left: Conventional protein-stabilized emulsion with antioxidants either in the bulk oil or water phase. Right: Proposed Pickering emulsion system with active food-grade particles adsorbed at the oil-water interface where they boost antioxidant efficiency.

1.4. Outline of the thesis

Chapter 2 provides detailed information about Pickering emulsions in general, and their possible use in food applications, including the delivery of food bioactive compounds. This chapter also highlights relevant regulations regarding health and safety. In **Chapter 3**, we introduce a new type of lipid-based particles, referred to as colloidal lipid particles (CLPs), which we use as Pickering stabilizers. We investigate how the solid–liquid fat ratio affects

particle nanostructure and morphology, and their stabilization performance. **Chapter 4** revolves around the coalescence stability of CLP-stabilized emulsions, investigated using a microfluidic coalescence chamber, highlighting the effect of the particle coverage at the droplet surface. Lipid oxidation in these CLP-stabilized emulsions is investigated in **Chapter 5**, and compared with that of emulsions stabilized with conventional emulsifiers. In **Chapter 6**, we investigate the use of CLPs as a reservoir for α-tocopherol, a model lipophilic antioxidant, and focus on the morphology and crystalline structure of CLPs in relation to the chemical stability of α-tocopherol. In **Chapter 7** we use α-tocopherol-containing CLPs as Pickering stabilizers for O/W emulsions and compare their oxidative stability with that of an emulsion with the exact same structure, but with α-tocopherol in the core of the oil droplets. This leads to a proof of principle: the two main instability issues (i.e., physical and chemical) in emulsions can be mitigated through one single approach. **Chapter 8** focusses on the physical and chemical stability of emulsions stabilized by antioxidant-containing Pickering particles from several natural sources that were produced via a top-down approach. With this, we show that our proof of principle can be extended to natural materials with intrinsic antioxidant properties. A general discussion of the results of this thesis is presented in **Chapter 9**, in which we propose strategies that can be used to make food emulsions both physically and oxidatively stable. We also put our findings into a wider perspective, with links to prospective industrial applications.

1.5. References

Abousalham, A., Fotiadu, F., Buono, G., & Verger, R. (2000). Surface properties of unsaturated non-oxidized and oxidized free fatty acids spread as monomolecular films at an argon/water interface. Chemistry and Physics of Lipids, 104(1), 93–99.

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Comprehensive Reviews in Food Science and Food Safety, 13(5), 945–977.

Berton-Carabin, C. C., Sagis, L., & Schroën, K. (2018). Formation, structure, and functionality of interfacial layers in food emulsions. Annual Review of Food Science and Technology, 9(1), 551–587.

Berton-Carabin, C. C., & Schroën, K. (2015). Pickering emulsions for food applications: background, trends, and challenges. Annual Review of Food Science and Technology, 6, 263–297.

Berton-Carabin, C. C., & Schroën, K. (2019). Towards new food emulsions: Designing the interface and beyond. Current Opinion in Food Science, 27, 74-81..

Berton, C., Ropers, M., Bertrand, D., Viau, M., & Genot, C. (2012). Oxidative stability of oil-in-water emulsions stabilised with protein or surfactant emulsifiers in various oxidation conditions. Food Chemistry, 131(4), 1360–1369.

Binks. (2002). Particle as surfactants - Similarities and differences. Current Opinion in Colloid & Interface Science, 7, 21–41.

Birch, G. G., & Ogunmoyela, G. (1980). Effect of surfactants on the taste and flavor of drinking chocolate. Journal of Food Science, 45(4), 981–984.

Coupland, J. N. (2015). An introduction to the physical chemistry of food. In Choice Reviews Online (Vol. 52).

Dickinson, E. (1993). Towards more natural emulsifiers. Trends in Food Science and Technology, 4(10), 330–334.

El-beltagi, H. S., & Mohamed, H. I. (2013). Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. 41(1), 44–57.

Falkeborg, M., Berton-Carabin, C. C., & Cheong, L.-Z. (2016). Ionic liquids in the synthesis of antioxidant targeted compounds. In Ionic Liquids in Lipid Processing and Analysis.

Finkle, P., Draper, H. D., & Hildebrand, J. H. (1923). The theory of emulsification. Journal of the American Chemical Society, 45(12), 2780–2788.

Fox, P. F. (Ed.). (1983). Developments in dairy chemistry—2: Lipids (1st ed.). Springer Netherlands.

Ghorbani Gorji, S., Smyth, H. E., Sharma, M., & Fitzgerald, M. (2016). Lipid oxidation in mayonnaise and the role of natural antioxidants: A review. Trends in Food Science and Technology, 56, 88–102.

Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., & Norton, I. T. (2012). Investigation into the potential ability of Pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 366(1), 209–215.

Kargar, M., Spyropoulos, F., & Norton, I. T. (2011). The effect of interfacial microstructure on the lipid oxidation stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 357(2), 527–533.

Laguerre, M., Bayrasy, C., Lecomte, J., Chabi, B., Decker, E. A., Wrutniak-Cabello, C., … Villeneuve, P. (2013). How to boost antioxidants by lipophilization? Biochimie, 95(1), 20–26.

Laguerre, M., Bayrasy, C., Panya, A., Weiss, J., McClements, D. J., Lecomte, J., … Villeneuve, P. (2015). What makes good antioxidants in lipid-based systems? The next theories beyond the polar paradox. Critical Reviews in Food Science and Nutrition, 55(2), 183–201.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M. C., Baréa, B., Weiss, J., … Villeneuve, P. (2009). Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. Journal of Agricultural and Food Chemistry, 57(23), 11335–11342.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M. C., Baréa, B., Weiss, J., … Villeneuve, P. (2010). Relationship between hydrophobicity and antioxidant ability of "phenolipids" in emulsion: A parabolic effect of the chain length of rosmarinate esters. Journal of Agricultural and Food Chemistry, 58(5), 2869–2876.

Laguerre, M., Wrutniak-Cabello, C., Chabi, B., López Giraldo, L. J., Lecomte, J., Villeneuve, P., & Cabello, G. (2011). Does hydrophobicity always enhance antioxidant drugs? A cut-off effect of the chain length of functionalized chlorogenate esters on ROS-overexpressing fibroblasts. Journal of Pharmacy and Pharmacology, 63(4), 531–540.

Linke, C., & Drusch, S. (2018). Pickering emulsions in foods - opportunities and limitations. Critical Reviews in Food Science and Nutrition, 58(12), 1971–1985.

Lomova, M. V, Sukhorukov, G. B., & Antipina, M. N. (2010). Antioxidant coating of micronsize droplets for prevention of lipid peroxidation in oil-in-water emulsion. ACS Applied Materials & Interfaces, 2(12), 3669–3676.

Mancuso, J. R., McClements, D. J., & Decker, E. A. (1999). The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 47(10), 4112–4116.

McClements, D. J. (2005). Food emulsions principles, practices, and techniques - Second Edition. CRC Press: Boca Raton, FL.

McClements, D.J., & Decker, E. A. (2000). Lipid Oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. Journal of Food Science, 65(8), 1270–1282.

Nuchi, C. D., Hernandez, P., McClements, J. J., & Decker, E. a. (2002). Ability of lipid hydroperoxides to partition into surfactant micelles and alter lipid oxidation rates in emulsions. Journal of Agricultural and Food Chemistry, 50, 5445–5449.

Pan, Y., Tikekar, R. V., Wang, M. S., Avena-Bustillos, R. J., & Nitin, N. (2015). Effect of barrier properties of zein colloidal particles and oil-in-water emulsions on oxidative stability of encapsulated bioactive compounds. Food Hydrocolloids, 43, 82–90.

Pickering, S. U. (1907). Emulsions. Journal of the Chemical Society, 91, 2001–2021.

Ramsden, W. (1903). Separation of solids in the surface-layers of solutions and "suspensions" (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation). Proceedings of the Royal Society of London, 72, 156–164.

Rayner, M., Timgren, A., Sjöö, M., & Dejmek, P. (2012). Quinoa starch granules: A candidate for stabilising food-grade Pickering emulsions. Journal of the Science of Food and Agriculture, 92(9), 1841–1847.

Rousseau, D. (2013). Trends in structuring edible emulsions with Pickering fat crystals. Current Opinion in Colloid and Interface Science, 18(4), 283–291.

Samaraweera, H., Zhang, W., Lee, E. J., & Ahn, D. U. (2011). Egg yolk phosvitin and functional phosphopeptides--review. Journal of Food Science, 76(7), R143-50.

Schaich, K. M. (2005). Lipid oxidation: theoretical aspects. In Baileys Ind Oil Fat Prod 6th Ed (Vol. 1).

Tadros, T. F. (2009). Emulsion Science and Technology : A General Introduction. Wiley-VCH: Weinheim.

Tan, Y., Xu, K., Niu, C., Liu, C., Li, Y., Wang, P., & Binks, B. P. (2014). Triglyceride-water emulsions stabilised by starch-based nanoparticles. Food Hydrocolloids, 36, 70–75.

Tcholakova, S., Denkov, N. D., & Lips, A. (2008). Comparison of solid particles, globular proteins and surfactants as emulsifiers. Physical Chemistry Chemical Physics, 10(12), 1608–1627.

Timgren, A., Rayner, M., Dejmek, P., & Marku, D. (2013). Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride. Food Science & Nutrition, 1(2), 157-171.

Yuji, H., Weiss, J., Villeneuve, P., Giraldo, L. J. L., Figueroa-Espinoza, M. C., & Decker, E. a. (2007). Ability of surface-active antioxidants to inhibit lipid oxidation in oil-in-water emulsion. Journal of Agricultural and Food Chemistry, 55, 11052–11056.

Xiao, J., Li, C., & Huang, Q. (2015). Kafirin Nanoparticle-Stabilized Pickering Emulsions as Oral Delivery Vehicles: Physicochemical Stability and in Vitro Digestion Profile. *Journal of Agricultural and Food Chemistry*, *63*(47), 10263–10270.

Zeng, T., Wu, Z. ling, Zhu, J. Y., Yin, S. W., Tang, C. H., Wu, L. Y., & Yang, X. Q. (2017). Development of antioxidant Pickering high internal phase emulsions (HIPEs) stabilized by protein/polysaccharide hybrid particles as potential alternative for PHOs. *Food Chemistry*, *231*, 122–130.

Zhao, Y., Elias, R. J., & Coupland, J. N. (2015). Effect of food structure on the distribution and reactivity of small molecules. Current Opinion in Food Science, 4, 19–24.

Chapter 2

Pickering emulsions

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Summary

Pickering emulsions are liquid dispersions that are physically stabilized by colloidal solid particles. The surface of suitable particles should be partially wetted by both oil and water, and the relative affinity of the particles for both liquids determines whether an oil-in-water (o/w) or water-in-oil (w/o) emulsion is formed. Even though most of the research on Pickering emulsions has been conducted on model, non-food systems, the interest in food-grade Pickering emulsions has increased sconsiderably, i.e. for the delivery of bioactive components in foods. This chapter first explains the mechanisms through which particles physically stabilize o/w emulsions. Then, the composition of food-grade systems that have proved suitable for Pickering stabilization is discussed. The feasibility of using Pickering emulsions for delivery of food active compounds is also reviewed, including the behavior of these systems during in vitro and in vivo digestion. Finally, the regulations for health and safety regarding the use of submicron particles in foods are discussed, as this could be a key factor in the development of Pickering emulsions for real food applications.

2.1. Introduction

At the beginning of the twentieth century, Ramsden and Pickering independently discovered that particles could be used as efficient interface stabilizers (Pickering, 1907; Ramsden, 1903). Ramsden's work focused on the adsorption of organic soft solid particles called "proteids" (e.g., albumin) at air-water interfaces (Chevalier and Bolzinger, 2013). Despite the absence of experimental proof, Ramsden claimed that solid particles were able to adsorb at the oil-water interface (Ramsden, 1903), which was confirmed 4 years later by Pickering who published a paper on the formation of paraffin oil-in-water (o/w) emulsions stabilized by interfacial adsorbed solid particles (Pickering, 1907). Although Pickering acknowledged Ramsden's work, the term "Pickering particle" is now commonly used for colloidal solid particles that have the ability to physically stabilize emulsions, termed Pickering emulsions.

Interestingly, it was later recognized that Pickering stabilization was part of earlier patents by William Haynes (Haynes, 1860) and the Bessel brothers (Bessel, 1877), with the latter patent illustrating the adsorption of graphite flakes to bubbles (Hubbard, 2004). In Pickering's time, it was immediately evident that particle stabilization had advantages (specifically in terms of stability) compared to surfactant stabilization. Despite this, Pickering emulsions remained relatively unexplored in research, while particle stabilization has been used unintentionally in food products such as mayonnaise, table spreads, and whipped cream (Douaire et al., 2014a). Only in the last two decades has research attention to Pickering stabilization increased, for example, in the fields of soft matter and physics targeting assembly of colloidal particles into supracolloidal structures (e.g., colloidosomes) (Dinsmore et al., 2002).

Most of the research on Pickering emulsions has been conducted on model systems, based on synthetic, inorganic materials, yet the generated knowledge indicates many potential applications for food, cosmetics, and pharmaceuticals. Food-grade particles are currently gaining interest, as evidenced by the growing number of related scientific publications in the last few years (Berton-Carabin and Schroën, 2015). Improving the physical stability of emulsions is the primary target in Pickering emulsion research. Additionally, enhanced functionality (e.g., novel texture, targeted delivery in the gastrointestinal tract) and improved chemical stability have been mentioned (Berton-Carabin and Schroën, 2015). Moreover, having a surfactant-free label, Pickering emulsions may be an attractive

alternative for cosmetic and pharmaceutical applications where surfactants often show adverse effects (e.g., irritancy and hemolytic action) (Chevalier and Bolzinger, 2013).

Particles can be used for stabilizing both o/w and w/o emulsions, irrespective of droplet size, as long as the particle surface can be partially wetted by both liquids (Finkle et al., 1923). Other important particle characteristics include size (from a few nanometers up to several micrometers), shape (e.g., spherical, rod or disk), and surface charge (Pawlik and Norton, 2014), all of which will be discussed in this chapter. Besides, it is challenging to find biobased, food-grade particles with good emulsifying properties. Thus, it is often necessary to fine-tune surface chemistry to ensure good anchoring at the interface, but this is difficult due to limited food-compatible options (Monteillet, 2015) or processing conditions (e.g., emulsification) that may be too harsh to maintain particle integrity (Chevalier and Bolzinger, 2013). It is clear that these considerations need to be addressed before Pickering food emulsions can be formulated.

As with any new technology, the potential safety risks must be examined. There are concerns regarding the use of nano-sized solid particles in foods, especially when they are purposely manufactured (Bleeker et al., 2012). At first glance, this may seem to limit the development of Pickering emulsions and particles, but recent advances are promising and include innovative usage of natural ingredients such as fat, carbohydrate or protein (Rayner et al., 2014).

2.2. Formation and stability of Pickering emulsions

Emulsion formation requires mechanical energy to break up the dispersed phase, so that small droplets of one liquid become dispersed throughout the other liquid phase (Walstra, 1993). To prevent immediate phase separation after formation, emulsifiers have to be used. Conventional emulsifiers (surfactants and biopolymers) are surface-active compounds due to their amphiphilic character (i.e. they possess hydrophilic and hydrophobic moieties). This allows them to decrease the interfacial tension between the dispersed and continuous phases and subsequently facilitate droplet break-up (Dickinson, 2013; Genot et al., 2013; Rayner, 2015). Solid particles are generally not amphiphilic and their adsorption at the interface is not spontaneous (Rousseau, 2013). Still, particles lower the free energy of the system by reducing the liquid-liquid contact area (Bott, 2014; Kaewsaneha et al., 2013).

Although the formation and stabilization of emulsions by solid particles are fundamentally different from conventional emulsifiers, many basic rules can be extrapolated, as extensively reviewed by Binks (2002) and Tcholakova et al. (2008). For example, the quantification of the hydrophilic-lipophilic balance (HLB) for surfactants can be compared to the wettability via the three-phase contact angle for particles. Particle characteristics mainly determine Pickering emulsion formation and stability, with partial wetting by both liquid phases being the most important prerequisite for solid particle attachment at the interface, which is influenced by the particle surface composition. Other important characteristics to consider for Pickering stabilization include size, shape, and surface charge (Bott, 2014; Dickinson, 2012; Finkle et al., 1923; Hunter et al., 2008). All these aspects are discussed in this chapter and are related to both physical and chemical stability of emulsions.

2.2.1. Particle characteristics

2.2.1.1. Particle wettability

The tendency of a particle to adhere at an interface can be understood from the perspective of wetting, rather than amphiphilicity. The wettability of a particle determines its position at the interface and can be characterized through the three-phase contact angle (Figure 2.1.) that is expressed for the most polar liquid. The contact angle is a result of the balance of the surface free energy of the particle at the water-oil, particle-water, and the particle-oil interfaces, as expressed by Young's equation (Equation 2.1.):

$$\cos\theta = (\gamma_{po} - \gamma_{pw})/\gamma_{ow} \qquad \text{(Eq. 2.1.)}$$

where γ_{po}, γ_{pw}, and γ_{ow} are the surface free energies (that can be interpreted as interfacial tensions) of particle-oil, particle-water and oil-water interfaces, respectively.

Finkle et al. (1923) first described the relationship between the wettability of colloidal particles and their ability to stabilize either o/w or w/o emulsions. Particles with a contact angle ranging from $0 \leq \theta < 90°$ have a greater affinity for water and will generate o/w emulsions, while particles with a contact angle ranging from $90° < \theta \leq 180°$ will form w/o emulsions. Simply, particles will make the interface bend towards the phase for which their affinity is lower, as illustrated in Figure 2.1. Particles that have the same affinity for oil and

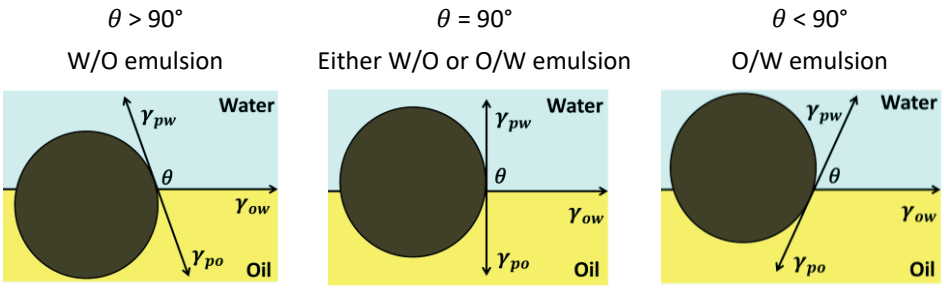

Figure 2.1. Location of a colloidal solid particle at the oil-water interface, as determined by the contact angle, θ, measured through the aqueous phase.

water have a contact angle of θ = 90°, and should in theory be able to stabilize o/w as well as w/o emulsions.

Surface modification of particles (i.e. by either chemical grafting of organic molecules or adsorption of different molecules) can be used to tailor the wettability (Bott, 2014), which ensures appropriate anchoring of the particles at the interface (Monteillet et al., 2014). Examples to modify the wettability of particles for food applications are the derivatization of starch granules with octenyl succinic acid (OSA) (Rayner et al., 2012) and the *in situ* adsorption of surfactants to the particle surface (Binks et al., 2017). However, chemical modifications are limited for clean-label purposes.

In summary, particle wettability ensures appropriate anchoring of Pickering particles at the interface, and concomitantly largely determines their stabilization efficiency.

2.2.1.2. Particle size

Besides appropriate wettability, the size of particles plays an important role, since the adsorption energy is proportional to the contacting area (Figure 2.2.), which will be explained in detail later (Equation 2.6). In general, it is recommended to use colloidal particles that are substantially smaller (at least one order of magnitude) than the emulsion droplets in order to form structured interface layers (Dickinson, 2012; Gould et al., 2013). Nevertheless, some food-grade Pickering emulsions have been claimed to be formed with similar size of oil droplets and Pickering particles (Kurukji et al., although it should be noted that the authors measured particle size prior to emulsification and did not consider possible particle disruption during homogenization nor exclusive stabilization by the smallest particles (Gould et al., 2013).

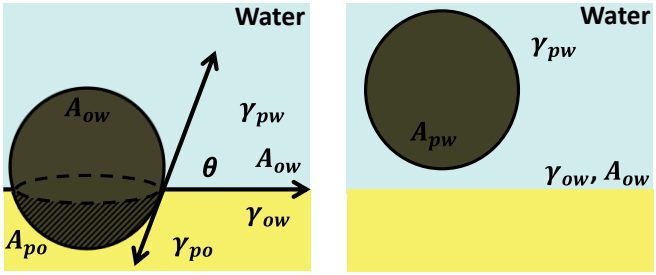

Figure 2.2. Schematic representation of a Pickering particle adsorbed at the oil-water interface, with its position defined by the contact angle, ϑ, and surface free energy as described in Equation 2.3 (situation a, left). For situation b, the surface free energy is defined in Equation 2.5; the difference in free energy between situation a and b is the desorption energy.

2.2.1.3. Particle shape

The stabilization performance of particles is mostly studied using model particles (i.e. monodispersed, solid, spherical), while food-grade particles are often much more complex (i.e. polydispersed, deformable, and non-spherical). Therefore, it is important to chart shape anisotropy effects on interfacial packing and orientation, as well as on capillary interactions (see section 2.2.2.2). The aspect ratio (the length to width ratio) of particles with similar surface chemical characteristics has been related to emulsification efficiency. Higher aspect ratio particles (>4.6) that sufficiently wet both liquid phases are efficient in Pickering stabilization while low aspect ratio particles are in some cases incapable of forming emulsions (Lou et al., 2016; Madivala et al., 2009). This has been linked to an increase in the capillary attractive forces with increasing particle aspect ratio (Dugyala et al., 2013).

When comparing cubic and peanut-shaped particles, unique interfacial packings and orientations were found due to particle anisotropy (de Folter et al., 2014), which was confirmed through computer simulations (Cheng and Wang, 2013). Furthermore, it has been found that rods have a 31.5% higher desorption energy compared to spherical particles and disks 57.0%, all at 90° contact angle (Horozov and Binks, 2006). Besides, complex anisotropic particles (microbowl particles with holes on their surface) can be used to stabilize supracolloidal systems, such as non-spherical droplets and double emulsions (Nonomura et al., 2011).

Both surface coverage and contact area may be enhanced through the use of deformable particles that can adapt their shape to the interface after adsorption (e.g., flattening). Such deformation may occur depending on the molecular interactions between particles, particles and the interface, and the elastic properties of the particles (Mehrabian et al., 2016). For example, microgel particles have been shown to adopt a core corona (also called fried egg) shape at the oil-water interface, which can be influenced by emulsification energy (Destribats et al., 2011, 2013). High shear rates led to strong flattening of microgel particles at oil-water interfaces whereas low shear rates led to dense monolayers where microgels are laterally compressed (Destribats et al., 2013; Monteillet et al., 2014; Schmitt and Ravaine, 2013).

In summary, non-spherical particles are often an asset in terms of emulsion stability, not only through better nesting (i.e. more interfacial coverage), but also through the formation of a viscoelastic interfacial network (e.g., jamming as a result of capillary interactions) (de Folter et al., 2014; Dugyala et al., 2013).

2.2.2. Physical stability of emulsions

Emulsions are thermodynamically unstable systems, due to an imbalance of molecular forces at the interface, expressed by the interfacial tension, γ_{ow}. In other words, there is excess free energy (ΔG) associated with the interfacial area between two liquid phases, defined as:

$$\Delta G = \gamma_{ow} * \Delta A_{ow} \qquad \text{(Eq. 2.2.)}$$

with γ_{ow} representing the interfacial tension between oil and water, and ΔA_{ow} the total interfacial area between oil and water.

As stated before, conventional emulsifiers reduce the interfacial energy by lowering γ_{ow} and provide steric and electrostatic repulsion between droplets. In contrast, solid particles reduce ΔA_{ow} (Kaewsaneha et al., 2013) and ensure the physical stability of emulsions through different mechanisms: interactions between particles and the dispersed and continuous phases lead to high desorption energy; attractive interactions between particles at the interface (resulting from capillary forces) induce jamming and hence the formation of a strong interfacial shell; and attractive interactions between particles in the continuous phase induce network formation which may set the whole emulsion structure and induce a yield stress (Pawar et al., 2011).

2.2.2.1. Desorption energy

The main mechanism by which colloidal particles stabilize emulsions relates to their interaction energy with the interface (Whitby et al., 2011), the attachment/detachment energy being the difference between the free energy of an adsorbed solid colloidal particle and the free energy of the particle in solution (Rayner et al., 2014; Young, 1805). The free energy (G_a) of the adsorbed particle (Situation a, Figure 2.2.) is defined by:

$$G_a = \gamma_{ow} * A_{ow(a)} + \gamma_{pw} * A_{pw(a)} + \gamma_{po} * A_{po(a)} \quad \text{(Eq. 2.3.)}$$

where γ_{po}, γ_{pw}, and γ_{ow} are the particle-oil, particle-water and oil-water interfacial tensions, $A_{ow(a)}$ is the area of the oil-water interface, and $A_{po(a)}$ and $A_{pw(a)}$ are the surface areas of the particle in contact with the water and oil phases, respectively. The total surface area of the particle equals:

$$A_{pw(a)} + A_{po(a)} = A_p \quad \text{(Eq. 2.4.)}$$

in which A_{pw} and A_{po} are the surface areas of the particle in contact with either water or oil, respectively. When present in the water phase (Situation b, Figure 2.2.), the surface free energy of the particle (G_w) is given by:

$$G_w = \gamma_{ow} * A_{ow(b)} + \gamma_{pw} * A_{pw(b)} \quad \text{(Eq. 2.5.)}$$

where $A_{ow(b)}$ represents the oil-water interfacial area of a dispersed droplet without particles being adsorbed. The difference in free energy between Situations a and b (ΔG_{dw}) is the free energy of detachment of a colloidal particle from the interface into the water phase (Equation 2.6).

$$\Delta G_{dw} = G_w - G_a = \gamma_{ow} * A_{ow} + \gamma_{pw} * A_{pw} - \left(\gamma_{ow} * A_{ow} + \gamma_{pw} * A_{pw} + \gamma_{po} * A_{po}\right) \quad \text{(Eq. 2.6.)}$$

Assuming that particles are spherical, the detachment energy can be expressed by:

$$\Delta G_{dw} = \pi r^2 \gamma_{ow} (1 - \cos\theta)^2 \quad \text{(Eq. 2.7.)}$$

The desorption energy of particles relates directly to Pickering emulsion stability and depends strongly on the particle size and contact angle, as is seen from Equation 2.6 and Figure 2.3., in which various particle properties are compared (Berton-Carabin and Schroën, 2015). This energy is generally several thousands of k_BT (with k_B the Boltzmann constant and T the absolute temperature) for colloidal particles of 10–1000 nm, which is much larger than the energy involved in Brownian motion (dashed line in Figure 2.3). Thus, the free energy required for desorption of particles with appreciable wettability is much greater than the thermal energy, which makes it highly unlikely that these particles will be released, and accordingly they can be considered as irreversibly adsorbed.

As a result of this, particles form a steric barrier that can efficiently prevent droplet coalescence. If the interface is insufficiently covered, emulsion droplets may coalesce, decreasing the total emulsion droplet surface area, until the surface coverage is high enough to provide stability. Because of the high particle desorption energy, the binary fusion of two droplets into one spherical droplet can be arrested at an intermediate state. The shape relaxation of such an anisotropic droplet is retarded by interfacial (or in some cases internal) droplet elasticity (e.g., due to jamming of particles) that offsets the Laplace pressure driving force (Pawar et al., 2011, 2012). This arrested coalescence is a special feature of Pickering emulsions, which can be used to design complex anisotropic colloidal structures.

The high-energy barrier also implies that adsorption of colloidal particles is not a spontaneous process, and mechanical action is needed, especially for large particles, to bring them into the interface. These effects are strongest for particles with a contact angle of 90°, resulting in a cos ϑ of zero, that have the highest desorption energy ΔG_{dw}, as illustrated in Figure 2.3 (Berton-Carabin and Schroën, 2015; Rayner et al., 2014; Yusoff and Murray, 2011). From this figure, it is also clear that surfactants (or very small particles) have a desorption energy that is in the same order of magnitude as the thermal energy (<1 nm; less than 10 k_BT), which facilitates rapid adsorption and desorption from the interface within short time-scales (which also explains the relative instability of these systems).

As mentioned, particle size, shape, and wettability largely determine the stability of Pickering emulsions. Nevertheless, changes in environmental conditions may affect the physicochemical characteristics of the particles; for example, particles can undergo chemical modifications (changes in surface chemistry) in response to changes in pH or salt concentration (Tang et al., 2015), resulting in changes in electrostatic and steric interactions and subsequent alteration of particle interaction with the surroundings (Tavernier et al., 2016). This also affects contact angle and hence desorption energy, resulting in stimuli-dependent emulsion stability. These effects occur with both organic (Zhang et al., 2014) and inorganic particles (de Folter et al., 2012), and can be used in controlled-release systems. For example, food applications could utilize pH-responsive systems to release bioactive compounds when exposed to various physiological pH levels. Alternatively, thermo-responsive systems can also be considered, which utilize a change in physical state of the particle, such as particle disintegration as a result of melting (Tang et al., 2015). In addition,

the dense interfacial layer and particle network of Pickering emulsions may be shear sensitive and disruption can compromise stability by inducing coalescence. Such shear-induced coalescence can be used in applications where the system only needs to be stable under quiescent conditions (Whitby et al., 2011). On the other hand, the heat or shear sensitivity of particles, or the very restricted pH or salt range in which particles perform well, can be a limitation for food processing and their applicability in a wide range of applications.

In summary, the desorption energy of Pickering particles is generally much higher than thermal energy, resulting in irreversible adsorption, but strongly depends on particle size, shape, and wettability. In addition, changes in environmental conditions that influence particle wettability, thermal, and shear triggers can alter emulsion stability which may be used to induce controlled release.

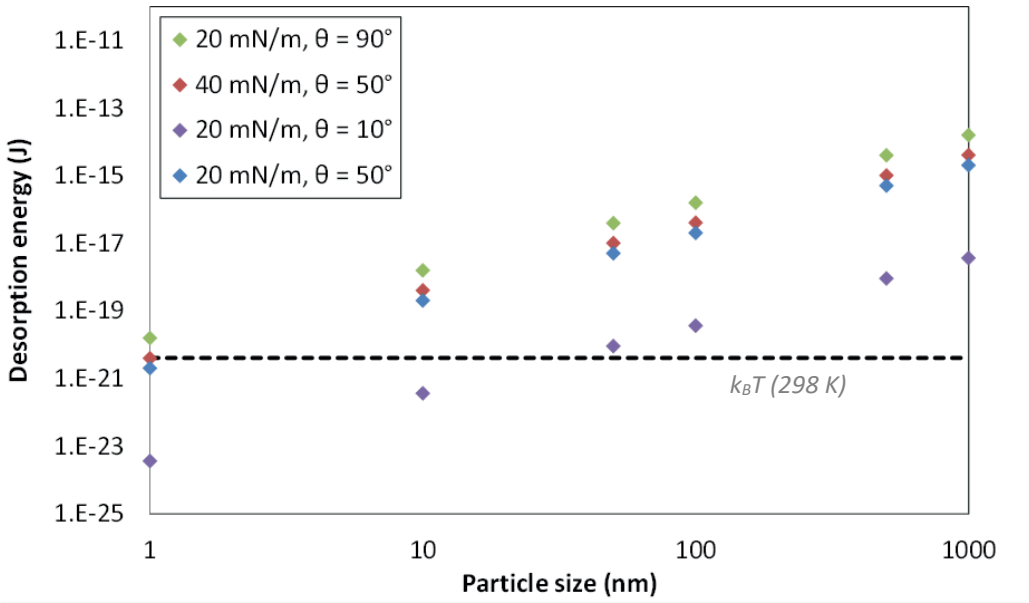

Figure 2.3. Plot illustrating the desorption energy of a single colloidal solid particle with contact angle 10°, 50° or 90° as function of particle radius, for oil-water interfacial tension of 20 mN m^{-1} and 40 mN m^{-1}. The dashed line represents thermal energy at 298 K (1 kBT). Source: Berton-Carabin et al. (2014). Reproduced with permission of John Wiley & Sons.

2.2.2.2. Capillary forces (particle-particle interactions)

Aside from particle attachment, lateral capillary forces also contribute to Pickering stabilization (Figure 2.4.). Capillary forces, which are attractive, appear when perturbations caused by the contact between particles and the interface overlap. The larger the perturbations induced by the particles, the stronger the capillary interaction between them. Such interfacial deformations can have different origins. First of all, particles larger than 10 µm can deform the interface and hence induce capillary forces by their weight (buoyancy force). As most Pickering particles are smaller than 10 µm buoyancy forces are generally not relevant for particle-stabilized emulsions (Kralchevsky and Nagayama, 2000; Lucassen, 1992). The second way to induce interfacial deformations is by particle confinement in the liquid film, which is related to particle wetting properties (i.e. to the position of the contact line and the magnitude of the contact angle), this even being operative for very small particles, as long as they are significantly larger than the solvent molecules.

Kralchevsky et al. discussed the dependence of capillary attraction forces on particle radius, interparticle distance, surface tension, and capillary charge, the latter being a measure to characterize the magnitude of the interfacial deformation (depending on, for example, particle charge), which can be both positive and negative (Kralchevsky and Nagayama, 2000; Zeng et al., 2012). Non-spherical particles require a non-uniform deformation of the interface near the particles, which, together with the higher desorption energy of such particles, explains the higher emulsion stability obtained with plate and rod-like particles compared to spherical particles (Madivala et al., 2009). Lateral capillary attractive forces may result in a cohesive interfacial two-dimensional network (Hunter et al., 2008; Levine and Bowen, 1993) with considerably higher surface shear and dilatational moduli than interfaces stabilized by conventional emulsifiers, resulting in remarkable physical stability (Sagis and Scholten, 2014).

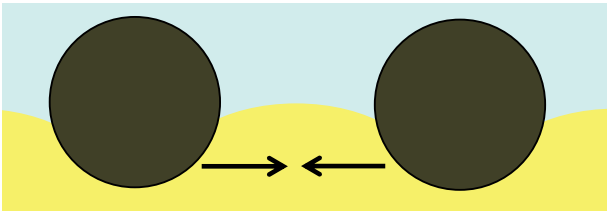

Figure 2.4. Interfacial deformation and associated capillary attractive force between solid particles at an oil-water interface.

2.2.2.3 Network formation

Classically, an interfacial layer may be represented as two dense monolayers separating adjoining droplets, as illustrated in Figure 2.5.A (Dickinson, 2010). However, severe bridging can occur when particle contact angle is significantly smaller than 90°; a single particle can adsorb at two droplet interfaces at the same time (Figure 2.5.B). It should be noted that there is a difference between bridging in Pickering emulsions and polymer-stabilized emulsions, where bridging only occurs when insufficient emulsifier is present, whereas in Pickering emulsions bridging can occur independent of particle concentration (Monteillet et al., 2014).

Besides an ordered interfacial network, particles can also form a disordered network. The particles need to be in a state of (weak) aggregation and can contribute to physical emulsion stability through the formation of a viscoelastic network for which rather large amounts of particles are needed. The network supplies the emulsions with a yield stress, which can arrest droplet motion and as a result, creaming and coalescence may be prevented (Figure 2.5.C) (Dickinson, 2010; Rayner et al., 2014). The related emulsion stability depends strongly on the ratio of particles/interfacial area and the dispersed phase volume fraction; dilution or shearing may be detrimental to the network, leading to emulsion destabilization (Whitby et al., 2011), and as a result, the rheological properties of Pickering emulsions may largely vary. The yield stress and emulsion viscosity are largely determined by three-dimensional network formation (Benjamins et al., 2009).

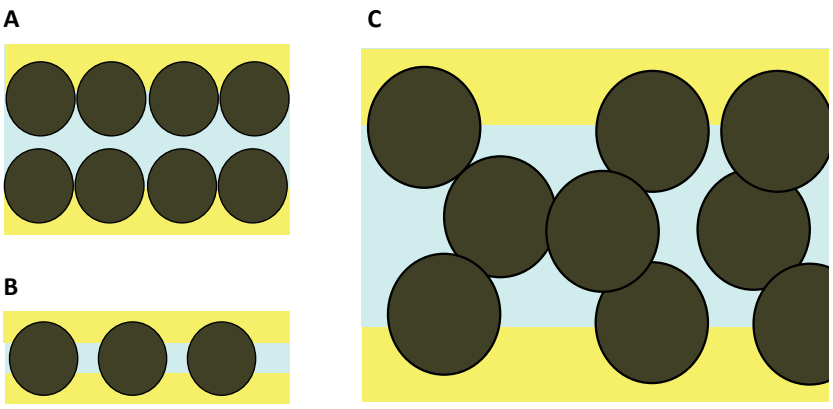

Figure 2.5. Schematic representation of particles at the surface of two neighboring emulsion droplets: (A) two dense particle monolayers separating two adjoining droplets; (B) single particles adsorbed at two distinct adjoining droplets; (C) low-density network of aggregated Pickering particles.

When considering a practical Pickering emulsion made with polydispersed particles, it is expected that the finest particles are at the oil-water interface, meaning less energy is needed to put them there, while the larger particles are in the continuous phase (Gould et al., 2013), where they may contribute to stability by network formation. At low particle concentrations, emulsion droplet size will depend on the availability of particles, including aggregated ones, while at high concentrations the droplet size is most affected by homogenization pressure (Arditty et al., 2003).

In summary, the structural arrangement of Pickering particles can differ (i.e. monolayers, bridges, or aggregated structures) and may influence emulsion physical stability.

2.2.3. Chemical aspects related to emulsion stability

Chemical degradation (e.g., oxidation of unsaturated fatty acids) influences the nutritional and sensory characteristics of food products. In addition, emulsion physical stability can be affected because the interface plays an instrumental role in lipid oxidation in emulsions. The interface is the region where unsaturated fatty acids meet water-soluble reactants, including oxygen and reactive pro-oxidants in the aqueous phase (Berton-Carabin et al., 2014; McClements and Decker, 2000). The potential of Pickering emulsions to protect emulsified oil against chemical degradation is still largely unexplored, but some authors have speculated that, in this respect, a closely packed particle layer may have better barrier properties than a protein or surfactant emulsifier layer.

Kargar et al. (2011) found that the formation of primary lipid oxidation products (i.e. hydroperoxides) was lower in emulsions stabilized by silica particles compared to Tween 20-stabilized emulsions, but not as low as found for sodium caseinate-stabilized emulsions. When comparing different Pickering particles, the same group found that microcrystalline cellulose particles were more effective against oxidation compared to modified starch particles, and they attributed this to the higher oxygen-scavenging ability of microcrystalline cellulose particles (negative charge and thicker interfacial film) compared to modified starch particles (Kargar et al., 2012).

In another study, zein/chitosan complexes containing curcumin were found to be more effective in inhibiting lipid oxidation than single zein or chitosan as a result of steric hindrance of particles and the antioxidant properties of curcumin (Wang et al., 2015). Antioxidant activity was also claimed for polyphenol-beta-lactoglobulin nanocomplexes

(Von Staszewski et al., 2014), and kafirin nanoparticles at interfaces, in relation to specific amino acids in the kafirin chain (Xiao et al., 2015). Besides, it was mentioned that the use of silica aggregates led to enhanced chemical stability of curcumin compared to single interfacial silica nanoparticles (Zhao et al., 2014) which was related to the thickness of the interfacial layer (Zhao et al., 2015).

From the above, it is clear that various claims have been made, and explanations are given, leading us to believe that particle stabilization may positively contribute to enhanced chemical stability. However, a comparison with regular emulsifiers is mostly missing in these studies, which makes it hard to draw concrete conclusions.

2.3. Pickering emulsions for food applications

Food delivers energy and essential nutrients that are critical for human health, and ideally, our diet should contain all components, including vitamins and minerals, and bioactive phytochemicals such as polyphenols, curcuminoids, carotenoids or phytosterols, that play important roles in reducing health risks (e.g., cancers, cardiovascular diseases, diabetes, etc.) (Kris-Etherton et al., 2002). A major food application for Pickering emulsions is bioactive delivery, and for this, functional and technical requirements such as loading capacity, efficiency, ingredient compatibility, and physicochemical stability need to be met. The starting point is to use food-grade ingredients, and to ensure food matrix compatibility, followed by scale-up and economic feasibility (McClements and Li, 2010).

To date, most research performed on Pickering emulsions has been conducted with inorganic particles based on silica or latex, which are used as model Pickering stabilizers for non-food-grade emulsions. Silica particles are commercially available with a wide range of characteristics: well-defined particle sizes from nanometer scale to micrometer scale, promising surface areas, and hydrophobicity, which can be modified by chemical treatment (e.g., with alkylsilane), coated or substituted with functional groups (Berton-Carabin and Schroën, 2015; Destribats et al., 2014; Ngai and Bon, 2014). In actual food and pharma products, this knowledge could be applied for delivery systems for active ingredients or to develop non-Newtonian textures (e.g,. thixotropy) while simultaneously making use of the improved physical and chemical stability of Pickering emulsions (Chen et al., 2011). Besides, surfactant-containing products may show adverse effects in the body, such as irritancy and hemolytic action (Laredj-Bourezg et al., 2013), while Pickering emulsions offer a surfactant-free alternative.

The following section describes how food-grade Pickering emulsions can be prepared with a strong focus on the food-grade particles used in literature.

2.3.1. Composition of food emulsions

Food products are complex, multicomponent systems, which very likely contain compounds that are surface-active, which may be either advantageous or disadvantageous to particle stabilization (Berton-Carabin and Schroën, 2015). Surface-active compounds may be added both before or after emulsification, which may affect the emulsion differently.

Hu et al. (2015) studied the effect of *in situ* adsorption of cationic surfactants on anionic cellulose nanocrystal stabilized Pickering emulsions. They found that surfactants adsorbed with their head group onto the particle surface at low concentrations, and in an aggregated morphology above the critical micelle concentration, leading to either hydrophobic or more hydrophilic particles. The change in particle wettability was directly correlated to emulsion stability and could even be used to induce phase inversion (Nesterenko et al., 2014).

Furthermore, Lacava et al. (2014) showed that the stability and structure of particle-containing emulsions strongly depend on the type of surfactant. Strong surfactants such as sodium dodecylsulfate (SDS) fully blocked the oil-water interface for particles, whereas surfactants with intermediate interfacial activity (e.g., Span 20) formed synergistic mixtures with the particles at the interface.

Proteins can also interact synergistically with particles for interfacial stabilization (Pichot et al., 2010); smaller emulsion droplets were formed when sodium caseinate was combined with silica particles, due to the enhanced shear viscosity of the interfacial layer (Murray et al., 2011). In addition, it has been proven that the method of preparation substantially affects emulsion formation and stability (Binks et al., 2007; Eskandar et al., 2007); for example, droplet size and interfacial packing vary depending on the phase in which the particles are initially dispersed, and the order in which the phases are emulsified (Eskandar et al., 2007).

To summarize, some studies have been performed on the synergies between particles and molecular emulsifiers for emulsion formation and stabilization, and various effects were reported, although it should be mentioned that the outcomes are still highly unpredictable. This also has to do with the fact that particles diffuse much slower than surfactants

(Eskandar et al., 2007), so both equilibrium emulsifier-particle interactions and kinetic effects contribute to the final emulsion's properties.

2.3.2. Composition of Pickering particles

A limited amount of particles have been suggested to adsorb at liquid interfaces and stabilize food emulsions, including inorganic particles and biobased particles (protein-, carbohydrate- or lipid-based) (Berton-Carabin and Schroën, 2015). In the following sections, all these particles are reviewed.

2.3.2.1. Inorganic particles

Silica, alumina, clay, and other materials have been investigated as Pickering particles. Silica (SiO_2) is most studied as it can be synthesized with reasonable monodispersity in size ranging from approximate 5 to a few hundred nanometers via, for example, Stöber synthesis (Ngai and Bon, 2014), for which water, ethanol, and tetraethylorthosilicate (TEOS) are used with ammonia as a catalyst for the hydrolysis of TEOS. Silica is formed through condensation of silanol groups:

$$Si(OC_2H_5)_4 + 2H_2O \rightarrow SiO_2 + 4C_2H_5OH \quad \text{(Eq. 2.8)}$$

The characteristics of the particles can be tuned through the reaction conditions: pH, temperature, reaction time and amount of water, and after-treatment. Silica particles are mostly hydrophilic and, through chemical or thermal treatment, their surface hydrophobicity (i.e. wettability) can easily be modified, which is imperative for Pickering stabilization. White et al. (2011) showed that a thermal treatment below 170 °C can be used to remove water from the surface, whereas at >180 °C, silanol groups condense to siloxane bridges, both leading to increased hydrophobicity. Besides, chemical treatments can be used, leading to substitution of silanol groups by, for example, alkylsilane.

2.3.2.2. Protein particles

Proteins are natural amphiphilic polymers, which can be obtained from several plant (e.g., soy) and animal sources (e.g., milk and meat). Proteins, protein micelles, and protein aggregates are generally not considered solid particles as they unfold, disassociate or rearrange upon adsorption. Nevertheless, protein particles have successfully been used for Pickering stabilization. For example, submicron-sized beta-lactoglobulin particles or microgels have been produced using a thermal process far from the isoelectric point (Santipanichwong et al., 2008). Destribats et al. (2014) produced whey protein microgels as Pickering stabilizers by sonication, and zein-based solid particles were also produced by

solvent-induced precipitation (Chen and Zhong, 2015; Pan et al., 2015). Also, soy and hydrophobin-based solid particles have been used as Pickering stabilizers in food (Liu and Tang, 2013).

2.3.2.3. Polysaccharide particles

Polysaccharides are natural polymeric carbohydrates mostly found as structural building blocks (e.g., cellulose or chitin) or storage polysaccharides (e.g., starch and glycogen). Starch granules are mostly used as thickeners and gelling agents, but recently also as Pickering stabilizers. Native starch granules can be found in a size range from several nanometers (rice: <5 nm) to micrometers (quinoa: 500 nm to 3 µm) (Ngai and Bon, 2014), and are highly hydrophilic but not water soluble as they are partly crystallized. Yet physical (e.g., thermal or mechanical treatments) and chemical treatments (e.g., by cross-linking, substitution or conversion reactions) can be used to tailor particle wettability. The most widely used method is by substituting hydroxyl groups using alkenyl succinyl anhydrides such as oxtenyl succinic acid (OSA), which has been approved for food use up to 3% with the level of free OSA not exceeding 0.3%. This method was also applied to quinoa starch granules (Rayner et al., 2012), rendering optimal emulsifying properties at 2–3% OSA (Rayner et al., 2012). Furthermore, polycaprolactone (PLC) grafting to starch granules has been suggested (Habibi and Dufresne, 2008), along with a physical, thermal treatment (Rayner et al., 2012) and freeze-milling to reduce polydispersity (Luo et al., 2011).

Other carbohydrate-based particles that are used for Pickering stabilization can be found in Berton-Carabin and Schroën (2015) and include, amongst others, microcrystalline cellulose particles (Kargar et al., 2012; Kovačević et al., 2014; Wen et al., 2014; Hu et al., 2015), cocoa particles (Gould et al., 2013), and chitin nanocrystals (Tzoumaki et al., 2013).

2.3.2.4. Lipid particles

Lipids are a large and diverse group of naturally occurring organic compounds that include fats, waxes, sterols, fat-soluble vitamins, phospholipids, glycerides, and glycolipids. The most widely used lipid-based particles for Pickering stabilization are fat crystals. Since triacylglycerols may crystallize into different polymorphs, they offer versatility in wetting properties. Fat crystals are predominantly hydrophobic, thus favoring stabilization of w/o emulsions albeit through different mechanisms (i.e. by surface templated crystallization, adsorption of solid particles at the interface, or shearing-induced agglomeration) (Rousseau, 2013). Lipid particles may also be mixed with more hydrophilic compounds or

contain several hydrophilic groups to make them applicable to o/w emulsions, and solid lipid nanoplatelets, flavonoid, and phytosterol particles have been successfully used (see also section 2.5) (Gupta and Rousseau, 2012; Liu and Tang, 2014b; Luo et al., 2012). The lipid interior of some lipid particles allows incorporation of lipophilic compounds, which makes them potentially useful as delivery systems.

2.3.2.5. Janus particles

The particles described so far have a relatively homogeneous surface composition, but particles with mesoscale chemical anisotropy can also be used to stabilize emulsions. These particles have two sides and are known as “Janus” particles (named after the Roman god with two faces), with different surface chemistry, and can be produced with high precision (Kumar et al., 2013).

It has repeatedly been shown that Janus particles with average contact angles approaching either 0° or 180° remain strongly attached at the interface Binks, 2002; Bott, 2014; Kumar et al., 2013), and show strong attractive interactions when in the interface due to the distinct hydrophobic and hydrophilic parts (Figure 2.6.A), resulting in cohesive films. Janus particles with dumbbell, plate-like, and ellipsoid shapes have been produced (Janus boundary; see Figure 2.6.), and showed a distinct formation of closely packed interfacial films and decrease in interfacial tension as they arrange differently at the interface (Ruhland et al., 2011). The synthesis of such particles when starting from two materials is rather complex, due to material incompatibility (Kumar et al., 2013); however, modification of homogeneous particles after adsorption at the interface may also be considered (Kaewsaneha et al., 2013). It is expected that production of food-compatible Janus particles can be achieved in the near future, using protein- or carbohydrate-based polymers (Ruhland et al., 2011).

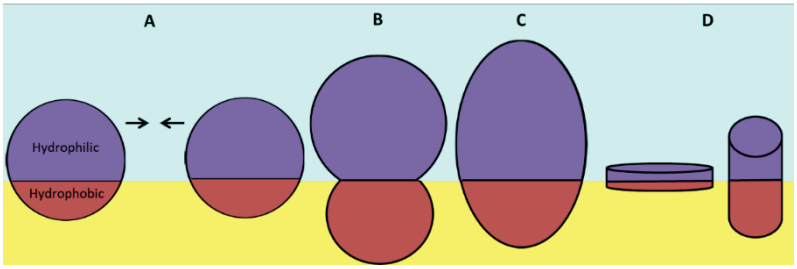

Figure 2.6. Schematic illustration of Janus particles with several shapes and clear Janus boundaries: (A) spherical-shaped particles showing the attractive capillary interaction arising from undulations in the Janus boundary, (B) dumbbell shaped, (C) ellipsoid shaped, and (D) cylinder shaped.

2.4. Characterization of Pickering emulsions

To achieve food-grade Pickering emulsions, bulk physicochemical properties, colloidal properties of droplets and/or particles, and physical stability of the final emulsion during shelf-life need to be assessed. Since mostly non-food-grade particles have been tested in Pickering emulsion research, the existing knowledge base needs to be extended to the study of food-grade particles. Several specific analytical techniques and methodologies have been used for this purpose such as contact angle, interfacial rheology and surface coverage measurements, and microscopy techniques, as discussed below in greater detail. For more general characterization techniques, we refer the interested reader to other chapters.

2.4.1. Contact angle measurements

Dual wettability of particles, as reflected in the three-phase contact angle θ, plays a crucial role in the formation and stability of Pickering emulsions as it determines the particle position in the interface, and related to that the energy needed to remove the particle from the interface. The contact angle can be measured in several ways; drop shape analysis using the Young–Laplace equation is one of the simpler methods that needs to be applied to a larger solid model surface (e.g., consisting of compressed particles), which makes it sometimes challenging to reflect the wettability of the actual particles, especially for composite ones. Besides, an advancing or receding contact line may lead to different contact angle values, and that is a point of attention (Hu et al., 2015). To determine contact angles in situ, the three-phase contact angle of colloidal particles needs to be measured, for which the gel trapping technique (GTT) was proposed by Paunov (2003). The particles are spread onto an oil-water interface, after which the water phase is gelled with a non-

adsorbing polysaccharide (gellan) at lower temperature. The oil is removed and replaced by a polysilicon elastomer (mostly polydimethylsiloxane), which is peeled off after polymerization, and imaged with a scanning electron microscope so that the imprint of the particles in the polymerized elastomer can be analyzed. Although this technique works relatively well, it should be mentioned that GTT is an indirect method that involves many steps that may have an impact on the obtained contact angle (Paunov, 2003), and the method is limited to particles of 500 nm and larger (Horozov et al., 2008). The contact angle of particles as small as 10 nm can be measured by freeze-fracture shadow-casting cryo-scanning electron microscopy (FreSCa cryo-SEM) (Binks et al., 2013). For this, particle-stabilized interfaces are rapidly frozen, coated with a thin metal layer at an oblique angle, and fractured. Due to the coating, a shadow is created behind each of the particles from which the height of the particle can be calculated and hence the contact angle. The method is limited to contact angles below 150° in order to obtain a clear shadowing effect (Geisel, 2015), and may be susceptible to particle deformation that will influence the measured values (Geisel et al., 2014). For more information on contact angle measurement of micro- and nanoparticles at liquid interfaces, see the recently published review by Zanini and Isa (2016).

2.4.2. Microscopy

The microstructure of Pickering emulsions is linked to functionality, sensory attributes, and stability of the system, and to visualize this, various microscopic techniques are used that help elucidate various levels of detail, as discussed below.

2.4.2.1. Confocal laser scanning microscopy (CLSM)

In CLSM, a spatial pinhole is placed at the confocal plan to eliminate out-of-focus light, which increases optical resolution and contrast and makes it possible to study Pickering emulsions at different depths of the sample (Nie et al., 2008; Tan et al., 2012a), thereby allowing three-dimensional imaging of the degree of coverage and shape of the droplet. CLSM can be used for (co)-localization: one or several dyes may be incorporated in the emulsion to stain the different phases (i.e. oil phase, water phase, and the colloidal particles), and an overall picture of the emulsion structure can be created, including the location of the particles ranging from monolayer to network in the continuous phase. To quantify mobility of particles in the interface, fluorescence recovery after photo-bleaching

(FRAP) can be used. For Pickering particles, no recovery of the fluorescence label is expected, which is linked to absence of mobility in the interfacial layer (Dickinson, 2010).

2.4.2.2. High-resolution microscopy

An even greater level of detail can be investigated by high-resolution microscopy, including atomic force microscopy (AFM) and electron microscopy. In AFM, the resolution limit can be as low as 2 nm in non-contact mode, and information is generated through the action of a mechanical probe that measures interfacial forces between a (soft) surface and the AFM nano-scaled probe. Tan et al., (2012b) used AFM to probe the mechanical properties of clay-stabilized emulsion droplets to develop structure-function correlations at different pH, and under compression with a colloidal probe (Tan et al., 2012). The major advantage of AFM is that it does not require sophisticated sample preparation (Shah et al., 2015).

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are direct methods used to visualize nanoparticles. As electron beams have much shorter wavelengths compared to visible light, much higher resolutions can be reached, which is a major advantage of this technique. This allows visualization of morphological details of nano-sized structures, and also the three-dimensional structure of the particle may be elucidated (Binks and Kirkland, 2002; Sihler et al., 2015; Yan et al., 2015). However, it should be noted that samples need to be viewed in vacuum, which may make sample preparation procedures time-consuming. In general, samples of hydrated materials such as emulsions are subjected to quick freezing and possibly staining to increase the electron optical contrast, and this may alter the sample, leading to artefacts that further complicate interpretation (Yan et al., 2015).

2.4.2.3. Super resolution microscopy: iPAINT

In the last decade, super resolution microscopy has emerged as an attractive, non-invasive, three-dimensional imaging technique to study relatively simple interfaces with nanometer resolution. The technique relies on the switching of fluorescent markers between a dark and a bright fluorescent state that is achieved by an external trigger, for example UV light (photo-activation process – PALM) (Betzig et al., 2006), chemical reactions (stochastic blinking – STORM) (Huang et al., 2008), or change in hydrophobicity (accumulation of binding probes – PAINT) (Chakraborty et al., 2016) and DNA-PAINT (Jungmann et al., 2010). The common ground among these techniques is the covalent labeling of the surface of the object by switchable dyes, or alternatively by specific ligand/receptors pairs.

Unfortunately, several surfaces of major interest in soft matter cannot be investigated; neither of the labeling methods works due to their very nature (e.g., emulsions and ice crystals). To overcome this, interface point accumulation for imaging in nanoscale topography (iPAINT) was developed, which is based on the reversible adsorption of polymer chains carrying a photo-activatable moiety that specifically labels the object of interest. The adsorption of such a polymer has been proven to occur at any interface, irrespectively of the surface chemistry (Aloi et al., 2016) or presence of charges (Olijve and Voets, 2016). Analyzing the super resolution images showing the particles and fluid interface, the three-phase contact angle can be determined using geometrical relations (Sabapathy et al., 2015). Although still new, iPAINT shows clear promise as a tool to investigate the shape and contact angle of particles at liquid interfaces, *in situ* and in a non-invasive way.

2.4.3. Surface coverage and interfacial rheology

The stability of interfaces stabilized by particles is co-determined by the surface coverage and resulting rheological properties. A distinct feature of Pickering emulsions is the strong and irreversible adsorption of colloidal particles, that can be used to study the interfacial coverage based on droplet size, as proposed by Destribats et al. (2011). If the interface is insufficiently covered, coalescence may take place to reduce the total amount of interfacial area, and increase surface coverage up to the point that the surface coverage is high enough to halt coalescence (Pawar et al., 2011). By using different concentrations of particles, the average droplet size will change, from which the interfacial particle coverage can be directly deduced (Equation 2.9.) (Destribats et al., 2014):

$$\frac{1}{D} = \frac{n\pi d^2}{24\,CV_d} \qquad \text{(Eq. 2.9.)}$$

where D is the droplet size, n is the total number of particles, d is the hydrodynamic diameter of the particles, V_d is the oil volume, and C is a parameter characterizing the particle packing that can be obtained directly from the $1/D$ curve. The droplet and/or particle size can be measured with widely available dynamic light scattering and laser diffraction techniques.

At high interfacial coverage, colloidal particles can orient and interact in different ways at the interface, for example forming two-dimensional particle gels, a glass phase or crystalline structures that have high shear and dilatational moduli compared to protein or surfactant stabilized interfaces. These mechanical properties can be quantified through interface dilatational rheological measurements under isotropic compression or extension, using

tensiometry on either a flat surface of a Langmuir trough or a single droplet in a droplet volume tensiometer. In a Langmuir trough, the interface is deformed by moving a barrier, and in a droplet volume tensiometer, a droplet is oscillated by pumping fluid in and out of the droplet (Sagis and Scholten, 2014); in both cases, interfacial tension is measured as a function of time from which the previously mentioned moduli can be derived. Alternatively, Lissajous plots can be constructed to extract insights regarding dynamic interfacial behavior (Berton-Carabin et al., 2016). It is challenging to position particles at (droplet) surfaces (Henneke, 2011; Ruhland et al., 2011), as most particles do not adsorb spontaneously. Despite this, several methods have been shown to be suitable. For example, a micro-syringe has been used to deposit nanoparticles in a spreading solvent at the interface in desired numbers (Fernandez-Rodriguez et al., 2014; Monteux et al., 2007).

2.4.4. Microfluidics

Although mostly used in combination with emulsifiers, some work on microfluidics has been done with particles. It has been reported that microfluidic channels are well suited to control particle load at the interface by varying particle concentration and the continuous phase flow rate (Nie et al., 2008). The dimensions of the microfluidic channel determine the size of the droplets and the extent of deformation (Guido and Preziosi, 2010), which may lead to buckling and crumpling, and other droplet shape changes. Buckling and crumpling are caused by deformation of a droplet with a solid-like or jammed interfacial layer, and droplet deformation was found to increase with increasing capillary number for amine-modified silica particles (Mulligan and Rothstein, 2011), which are interesting starting points for the formation of microcapsules for delivery of active compounds (Lee and Weitz, 2008). A number of microfluidics-based systems have been developed to study emulsion stability (coalescence cell and micro-centrifuge), and will provide added benefits when investigating the stability of Pickering emulsions (Krebs et al., 2012, 2013).

2.4.5. Sensory aspects of Pickering emulsions

The ultimate test for a food Pickering emulsion is sensory analysis, which could determine the acceptability of the emulsion consistency or of the product as a whole. The minimum particle size that can be detected by the palate is approximately 25 µm (Kilcast and Clegg, 2002). Considering this, Pickering emulsions are expected to have a unique structure, and potentially a unique sensory profile, as particle stabilization not only allows the presence of stable, bigger sized droplets, but also thicker interfacial films and three-dimensional

structures in the continuous phase. Thus, development of Pickering emulsion food products can provide opportunities to formulate a wider range of textures or consistencies compared to conventional emulsions. Despite this, to the best of our knowledge, no results have yet been published on the sensory perception of Pickering emulsions.

In principle, sensory tests available for conventional emulsions can also be used to characterize Pickering emulsions, and some sensory attributes may be deduced from knowledge of conventional emulsions. For example viscosity and partial coalescence during oral processing are related to creaminess (Benjamins et al., 2009), and although Pickering emulsions may be very viscous, partial coalescence is expected to take place to a lesser extent due to the high stability of these emulsions, thereby counteracting the expected increase in creaminess. In addition, the distinct interfacial structure formed by particles influences flavor release kinetics as well, which will also influence aroma and taste perception (Chung et al., 2015; Pawlik and Norton, 2014). When applied in pharma products, for example, sensory attributes of Pickering emulsions can be characterized through the standard tests used in this field, such as visual appearance, feel of cream, skinfeel during and after application, wateriness, thickness, and stickiness (Marku et al., 2012).

2.5. Pickering emulsions as carriers for active compounds

Pickering particles have been used to produce emulsions with good physical stability (Dai et al., 2005; Kargar et al., 2011 Tarimala and Dai, 2004). This makes them interesting candidates for delivery systems. Non-food Pickering emulsions have been developed with interfaces that adapt to dynamic environmental changes (Destribats et al., 2014; Richtering, 2012; Schmitt and Ravaine, 2013). For food, this implies that strategic release of active compounds could be achieved (e.g., induced by pH changes in the gastrointestinal tract) and is within reach (Berton-Carabin and Schroën, 2015). In the next sections, we will give an overview of developments in the food field that may ultimately lead to Pickering-based controlled-release systems.

2.5.1. Inclusion of active ingredients in Pickering emulsions

Oil-in-water emulsions are among the most relevant and versatile delivery systems for food applications. In general, active compounds are most frequently incorporated in the oil phase of an emulsion but in particle-stabilized emulsions, they can also be formulated in

the particles or at the interface, depending on composition and solubility. Lipophilic compounds are most suitable for incorporation in the dispersed lipid phase or in particles, if they have high solubility, while amphiphilic compounds are found at the interface, depending on particle hydrophobicity. If double emulsions are considered, hydrophilic compounds can also be encapsulated.

Curcumin, a hydrophobic polyphenol that shows oxidative instability, is one of the best studied bioactives in Pickering emulsions (Salem et al., 2014). Silica particle-stabilized o/w emulsions have been used as controlled-release vehicles for curcumin, leading to >80% retention after gastric digestion and >60% release of curcumin within 2 hours of intestinal digestion (Tikekar et al., 2013). However, no comparisons were made with conventional emulsifiers, so it is difficult to conclude if the Pickering emulsion enhanced performance compared to other emulsion systems.

The authors also independently tested the effects of bile salts on Pickering emulsion stability and found that destabilization during digestion was likely driven by the combination of bile and calcium salts with digestive enzymes. This is potentially caused by partial removal of the Pickering particles from the interface while exposed to digestive conditions. Considering the high desorption energy of Pickering particles, further studies are needed to elucidate if interfacial particles are truly displaced during digestion, or if lipase and bile salts adsorb on top of existing particles at the interface (Tzoumaki et al., 2013).

Starch granule-stabilized Pickering emulsions have also been studied for stabilizing curcumin (Wang et al., 2014) and exhibited higher oxidative stability (stored at room temperature) compared to Tween 20-stabilized emulsions. As expected, the rate of curcumin release increased as a function of amylase concentration over 1 hour. Overall, these results suggest that Pickering emulsions can provide high chemical stability during storage and can be designed to release curcumin under certain environmental conditions.

Carotenoids are hydrophobic terpenoids that have high susceptibility to light, heat, and oxidation (Khachik et al., 1992), and can potentially be stabilized in Pickering emulsions. Currently, the most common carotenoid-loaded Pickering emulsions reported in literature are stabilized with protein particles and report gel-like properties as a driver for stability. Freeze-dried pea protein particles have been used to stabilize Pickering emulsions (pH = 3)

for beta-carotene delivery at oil fractions 0.3 or 0.6, the latter of which was found to limit lipid hydrolysis and carotenoid bioavailability due to an increased viscosity and gel-like emulsion structure (Shao and Tang, 2016). Although the authors did not directly compare Pickering emulsions with conventional emulsions, beta-carotene bioaccessibility (for both oil fractions) was higher than in bulk oil. Similarly, the presence of a gel-like emulsion structure was reported for soy protein particle-stabilized emulsions following heat treatment (Liu and Tang, 2013, 2014a).

In another study, beta-carotene-loaded soy protein Pickering emulsions exhibited gel-like properties but had similar beta-carotene release during digestion compared to control whey- and sodium caseinate-stabilized emulsions (Liu and Tang, 2016). While none of the emulsions showed dramatic beta-carotene degradation, these Pickering particles could be considered genuine alternatives to classic emulsifiers for carotenoid delivery.

Pickering emulsions show an ability to stabilize and facilitate the release of lipophilic bioactives. Studies suggest that particle-stabilized interfaces allow for good physicochemical stability during storage and targeted release in the gastrointestinal (GI) tract (Liu and Tang, 2016; Shao and Tang, 2016). Ideally, comparable conventional emulsions should be used as controls to determine whether enhanced stabilization is due to Pickering stabilization. Aside from digestibility and bioaccessibility (the solubilization and potential intestinal uptake) of active ingredients, bioavailability (intestinal epithelial uptake and transport) should also be considered for future *in vitro* studies. The bioavailability (and eventual physiological utilization) of a bioactive depends heavily on intestinal uptake (e.g., passive or active transport), intestinal solubility, and metabolism (among other factors), which have not yet been extensively studied for food Pickering emulsions.

There are also examples in the field of food safety. Pickering emulsions containing *Artemisia argyi* oil, an antimicrobial agent, demonstrated >83% bacterial inhibition rates of *Staphylococcus aureus* and *Escherichia coli* after 60 days of storage at 37 °C (Hu et al., 2013), although there was no direct comparison with free oil. Bi et al. (2011) found that chemically stable nisin had prolonged antibacterial activity against *Listeria monocytogenes* in a carbohydrate-stabilized Pickering emulsion. Following 40 days of storage, the Pickering emulsion inhibited bacterial growth, while Tween 20-stabilized interfaces and free nisin were not capable of doing so. Although these results show the potential of Pickering emulsions for food safety, further investigation of the interactions between Pickering

particles and active ingredients is needed to better target bacteria for future food applications.

2.5.2. Inclusion of active ingredients in Pickering particles

Solid bioactive ingredients, such as flavonoids, may be used directly as Pickering particles (Luo et al., 2011). Flavonoids are a class of secondary plant metabolites commonly found in fruits, vegetables, and food products (chocolate and red wine). Their consumption has been associated with anti-inflammatory and antioxidant functionalities both *in vitro* (Henson et al., 2008; Vinson et al., 1995) and *in vivo* (Illek et al., 1998; Wu et al., 2015). Flavonoids can also form complexes (Wieca et al., 2013) or be used in combination with biopolymers to stabilize novel Pickering particles. The flavonoid rutin was found to improve oxidative stability of whey protein-stabilized sunflower o/w emulsions stored at 50 °C (Atarés et al., 2012) and improved stability against coalescence, which is possibly due to rutin particles coadsorbing or replacing whey protein at the interface. Although the non-spherical rutin particles form a densely adsorbed layer at the interface, it is unclear if the protective effect is due to the formed layer, the antioxidant capacity of the bioactive, or a combination of both.

Some proteins, such as lactoferrin, have been described as having additional health benefits in the medical field (Crouch et al., 1992; Damiens et al., 1999). Lactoferrin-based Pickering systems, in combination with alginate and carrageenan, demonstrated improved emulsion stability against proteolysis during in vitro gastric digestion compared to conventional emulsifiers (Shimoni et al., 2013). However, further study would be needed to assess if the lactoferrin exhibits a biological benefit when used in this manner.

Furthermore, phytosterol particles have been reported to be surface-active at the water-hexadecane interface (Cercaci et al., 2007), albeit potentially producing toxic byproducts when oxidized (Ryan et al., 2005). When coated with whey protein, these particles were found to be capable of stabilizing a gel-like emulsion (Liu and Tang, 2014b). Clearly, chemical stability and digestibility need to be confirmed, but it is obvious that these particles hold promise for Pickering stabilization.

It should be noted that the interface is a critical site for chemical reactions to take place (e.g., oxidation) (Berton-Carabin et al., 2014; Genot et al., 2013), and if a bioactive compound is used, chemical stability and functionality should be considered. Additionally,

if a bioactive compound is used in combination with a biopolymer as a Pickering particle (e.g., protein-polyphenol or protein-carotenoid complex), the chemical stability and potential bioavailability could be altered. For example, in bulk systems, carotenoids are chemically stable but less bioavailable when bound to proteins (Britfon, 1995; van het Hof et al., 2000), but show enhanced functionality for cancer therapy when coupled with a carrot pectic polysaccharide (Natarajmurthy et al., 2016). Similarly, protein-phenolic interactions can result in lower antioxidant capacity compared to that of the individual protein and polyphenol (Arts et al., 2002; Ozdal et al., 2013). The dosage chosen should be such that toxicity levels are not reached.

Nushtaeva (2016) reported that mustard and cinnamon can be used safely as Pickering particles, but nutmeg required concentrations much higher than the recommended acceptable concentration (>0.1%). Conversely, the amount of particles that can be used in a formulation may not be high enough to yield a measurable biological effect, or may lead to undesired organoleptic changes. For example, polyphenol-protein binding can promote hydrophobicity and cross-linking, potentially enhancing functionality but also causing precipitation and astringency (Rodriguez et al., 2003). It is good to keep these effects in mind, together with digestibility and biological fate *in vivo*, since food Pickering emulsions are still in the early stages of development.

2.6. *In vitro* and *in vivo* digestion of Pickering emulsions

In recent years, Pickering emulsions have gained interest as controlled-release systems for the GI tract and delivery systems for the skin (Chevalier et al., 2015). In order to be effective in the GI tract, food Pickering emulsions need to be protected against conditions in the human digestive tract that has evolved towards highly efficient digestion, while still allowing release of the active component at the target location. This may be accomplished by, for example, slowing down lipolysis to deliver substrates into the ileum, thereby enhancing natural gut-brain signaling pathways of satiety that are normally induced by meal intake (Corstens et al., 2017).

A first impression on the potential mechanisms of digestion can be obtained through *in vitro* studies, but a test in humans is needed due to the complexity of the GI tract. To the best of our knowledge, Pickering emulsion systems have not been tested in human clinical trials, but the first animal studies have been reported by the research group of Clive Prestidge, from the University of South Australia. This group used dried Pickering-like systems and

compared seven orally dosed formulations (n = 5 rats) against a control (intravenously dosed indomethacin). They found that these Pickering-templated lipid microcapsules showed improved dissolution kinetics as a result of improved lipolysis kinetics, which enhanced the bioavailability of the poorly soluble drug compared to aqueous solutions or conventional o/w emulsions (Simovic et al., 2009, 2010).

As described in the previous section, only very limited *in vivo* work has been carried out, but more *in vitro* studies have been done, as summarized in Table 2.1. For Pickering emulsions stabilized by lactoferrin particles, similar oral behavior occurred compared to emulsions stabilized by native lactoferrin (Shimoni et al., 2013). This similarity is most probably caused by destabilization in the stomach, and could be circumvented by the use of alginate or carrageenan as additional layers. The authors found improved oral stability and reduced gastric proteolysis, which make these emulsion systems suitable as a delivery system (Shimoni et al., 2013). Pickering emulsions stabilized by kafirin (a globular storage protein from sorghum) showed a better stability in an acid environment compared to a basic environment, but had no resistance against gastric proteolysis (Xiao et al., 2015), and the same was found for emulsions stabilized by whey protein microgels (Sarkar et al., 2016). This implies that gastric proteolysis removed the barrier of protein-based Pickering particles (Sarkar et al., 2016).

When looking at more complex Pickering emulsions, kafirin Pickering double emulsions did not provide additional protection against simulated gastric and intestinal fluid (Xiao et al., 2017a), but susceptibility to gastric digestion was improved by incorporating kafirin Pickering emulsions in calcium-alginate hydrogels (Xiao et al., 2017b). Alternatively, a silica-based Pickering emulsion was found to survive passage through the stomach, and released the lipophilic drug curcumin at 60% during the intestinal phase as a result of emulsion destabilization (Tikekar et al., 2013).

For targeting the small intestine, chitin nanocrystal-stabilized emulsions showed promising results, as they reduced intestinal lipolysis compared to emulsions containing adsorbed whey proteins or caseinate (Tzoumaki et al., 2013). In addition, emulsions stabilized by particles made of *Ginkgo biloba* extracts or their flavonoid glycosides fraction showed a lower rate and extent of intestinal lipolysis compared to conventional Tween 20-stabilized emulsions (Yang et al., 2014), which may be related to the strong irreversible adsorption of the particles.

In addition to the resistance of interfacial structure, the release kinetics from Pickering emulsions have also been related to gel formation, either of the protein-gel layer, as discussed before (Sarkar et al., 2016), or of the whole system (Liu and Tang, 2016; Shao and Tang, 2016). For example, at higher oil fraction of 60%, a 2–3-fold lower intestinal release of the lipophilic model drug beta-carotene was measured compared to 30% oil emulsions (Shao and Tang, 2016), which was confirmed with heat-treated glycinin particles that are known to form a gel-like Pickering emulsion (Liu and Tang, 2016). It should be mentioned that these authors focused on intestinal digestion only, while glycinin most likely undergoes proteolysis in the stomach and will be degraded there (Sarkar et al., 2016; Xiao et al., 2015).

In conclusion, there may be an effect of Pickering emulsions on intestinal digestibility but currently, the effect cannot be clearly attributed to either the Pickering stabilization itself or the difference in material used as reference in standard emulsions.

2.7. Pickering emulsions: Rules and regulations for health and safety

Nanomaterials have unique physicochemical characteristics (such as quantum size, shape, charge, area to volume ratio, surface roughness) and biological properties, which differ in toxic manifestations compared to the base material, and need to be well characterized to ensure appropriate usage (Bleeker et al., 2013). Unfortunately, legislation trails compared to technological development. Hence, it takes a relatively long time before the risks of nanomaterials are officially defined.

A recommendation of the European Commission in 2011 defines nanomaterials as “natural, incidental or manufactured materials containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm (Bleeker et al., 2012; EU, 2011a). It also states that: “In specific cases, where warranted by concerns for the environment, health, safety or competitiveness (e.g., by other compounds), the number size distribution threshold of 50% may be replaced by a threshold between 1% and 50%.” This definition covers all kinds of particles independent of shape, charge, ratio of surface area to volume or other physical or chemical properties. Although Pickering particles are often considered as nanomaterials, many of them do not belong to this group, based on size (Figure 2.7.).

Table 2.1. Overview of studies on in vitro and in vivo digestion of Pickering emulsions.

	The system	Food active compound	*In vitro* conditions*	*In vivo* study	Findings	Publication
Protein-based particles	0.2% LF-NP (bare and A- or CR- coated; 0.2-1.6 µm); 2% olive oil (1-10 µm; o/w)	n.a.	I (2h, pH=7.00, 5 mM PB, 5 mg mL^{-1} BS, 590 UmL^{-1} L, 20 mM $CaCl_2$, 0.5% oil, pH stat)	n.a.	LF-NP-emulsion comparable lipolysis to native LF; A-coated NP reduced lipolysis by 14% and Cr-coated NP increased by 10%	(Meshulam & Lesmes, 2013)
	0.2% LF-NP (<0.1 µm); 2% olive oil (4-6 µm; o/w)	n.a.	O (pH 6.8, artificial saliva containing salts, amylase, mucin) G (2h, pH 1.7, NaCl, HCl, pepsin, SDS-PAGE)	n.a.	LF-NP-emulsion similar behavior to native LF, but when LF-NP-emulsion was covered by A or Cr, improved oral stability and reduced gastric proteolysis	(Shimoni *et al.*, 2013)
	0.3-2% gelatin particles (0.2 µm); 80% oil (10-40 µm; o/w)	β-carotene	Membrane-free model using hexane and a Tween80 solution - spectrophotometer	n.a.	Higher particle concentration delayed release compared to lower concentration	(H. Tan, Sun, Lin, Mu, & Ngai, 2014)
	1% kafirin NP; 20-80% oil (49 ± 17 µm; o/w, except w/o with 80% oil)	Curcumin	G(1h, pH 1.5, pepsin) I(2h, pH 7.5, 50 mM Tris maleate, 150 mM NaCl, 5 mM $CaCl_2$, 20 mM BS, 5 mM phosphatidylcholine, 10 mg mL^{-1} L, ~1% oil, HPLC)	n.a.	Kafirin NP-stabilized emulsion retarded lipid oxidation compared to Tween80- emulsion. Better acid than basic stability, but not resistant against gastric digestion due to proteolysis. Kafirin NP-stabilized emulsion slight delay in lipolysis compared to Tween80-emulsion	(Xiao *et al.*, 2015)
	6% pea protein NP; 20-60% soy oil (3-22 µm; o/w)	β-carotene	G (30min, pH 1.2, NaCl, pepsin) I (2h, pH 7.5, 10 mM PB, 10 mg mL^{-1} BS, 2.4 mg mL^{-1} L, ~0.8-2.4% oil, absorbance measurement))	n.a.	All emulsions showed coalesced oil in the gastric phase. At higher oil fraction (gel-like system) slightly less β-carotene degradation, and in 60% oil emulsions about 2-3 fold lower intestinal release than in 30% oil emulsions	(Shao & Tang, 2016b)

	1% WP microgels (0.3 µm); 20% sunflower oil (43 µm; o/w) emulsion heat treated or not	n.a.	G (2h, pH 2.0, pepsin, salt range) I (3h, pH 6.8, salt range, 0.23 mg mL^{-1} BS, 125 mg mL^{-1} pancreatin (or 260 U mL^{-1} pure lipase) , ~5% oil, pH stat)	n.a.	Gastric proteolysis removed barrier effect of microgels When looking at lipase digestion only, heat treatment delayed intestinal digestion compared to non-heated emulsions, due to formation of a fused interfacial network, but no difference was found when pancreatin was used due to degradation of that interfacial network	(Sarkar *et al.*, 2016)
	1-6% soy glycinin heated NP; 20-60% soy oil (2-10 µm; o/w)	β-carotene	I (2h, pH 7.5, 10 mM PB, 120 mM NaCl, 10 mg mL^{-1} BS, 2.4 mg mL^{-1} L, ~0.8-2.4% oil, absorbance measurement))	n.a.	Gel-like emulsions showed slower release (not defined by %protein or oil fraction)	(Liu & Tang, 2016)
Polysaccharide-based particles	0.2-6% OSA starch particles (1-2 µm); 13-33% MCT oil (20-50 µm; o/w)	n.a.	I (15-18 min, pH 7, BS, 1 mg mL^{-1} L, pH stat)	n.a.	OSA starch particle-stabilized emulsion lower lipase activity (up to 68% decrease) compared to emulsions stabilized with unheated starch	(Timgren, Rayner, Sjöö, & Dejmek, 2011)
	0.03-0.5% ChNP (0.24*0.02 µm); 10% corn oil (6-50 µm; o/w)	n.a.	I (1h, pH 7.0 PB, 5 mM $CaCl_2$, 5 mg mL^{-1} BS, 1.6 mg mL^{-1} L, 0.5% oil, pH stat)	n.a.	ChNP- stabilized emulsions lower rate and extent of lipolysis compared to protein (WPI or sodium caseinate); hardly effect of [ChNP]	(Tzoumaki *et al.*, 2013)
	0.1% Ginkgo biloba extracts (GBE) and flavonoid glycoside (FA) particles; 10% soybean oil (0.8-1 µm; o/w)	n.a.	I (10 min, pH 8.1, complex salt solution, 2.4 mg mL^{-1} L, 19 mg mL^{-1} BS, 0.8% oil, pH stat)	n.a.	FA- and GBE-particle-stabilized emulsions much lower lipolysis compared to surfactant-stabilized emulsions	(Yang *et al.*, 2014)
Silica particles	0.5-5% hydrophobised SiNP; 5% olive oil (4-8 µm; o/w)	n.a.	I (2h, pH=7.00, 10 mM PB, 5 mg mL^{-1} BS, 590 U mL^{-1} L, 20 mM $CaCl_2$, 0.5% oil, pH stat)	n.a.	Higher separation velocity in saliva (2-5% Si). Pickering emulsion lower extent of lipolysis compared to β-lg-emulsions (50% versus 60% after 2h), but no difference in initial lipolysis rate	(Ruiz-Rodriguez, Meshulam, & Lesmes, 2014)

4% SiNP (8 nm); 5% canola oil (0.2 - 1.3 µm, o/w)	Curcumin	G (2h, pH 1.2, NaCl, HCl, pepsin); I (3h, pH 7.5, 50 mM PB, 60 mM$CaCl_2$, 25 mg mL^{-1} BS, 10 mg mL^{-1} L, ~1% oil, absorbance measurement) Cell model biocompatibility	n.a.	In the gastric phase, only ~25% curcumin was released, and in intestinal phase ~60% more. Cells that were exposed to Pickering emulsions had only 88% viability compared to cells that had not been exposed	(Tikekar *et al*., 2013)
lipid-SiNP hybrids; 50-70% oil in dry microcapsule (8-13 µm; o/w)	Coumarin	I (1h, pH 7.50, 50 mM Trizma maleate, 150 mM NaCl, 5mM $CaCl_2$, 6.25 mM BS, 100 TBU mL^{-1} L, 1% lipid, pH stat)	n.a.	Immediate drug release from all microcapsules , and complete release; controllable lipolysis higher than pure lipid, resulting in high solubilisation of the drug	(Lim, Tan, Simovic, & Prestidge, 2011)
lipid-SiNP (0.1-1 µm) hybrids; 6-43 % lipid in dry state	n.a.	I (1h, pH 7.50, 50 mM Trizma maleate, 150 mM NaCl, 5 mM $CaCl_2$, 6.25 mM BS, 1000 TBU mL^{-1} L, 1% oil, pH stat)	n.a.	Hybrids made with attractive nanoparticle-droplet interactions delayed lipolysis, while with repulsive interactions enhanced lipolysis	(Tan, Colliat-dangus, Whitby, & Prestidge, 2014)
lipid (with lecithin or oleylamine)- SiNP (5-50%, hydrophilic) hybrids; 50-85% oil in dry microcapsule	Indo-methacin	I (1h, pH 7.5, 50 mM TRIS maleate, 150 mM NaCl, 5 mM $CaCl_2$, 5mM BS, 1000 TBU mL^{-1} L, 0.5% lipid, HPLC)	7 orally dosed formulations (n=5 rats) vs IV, blood samples HPLC	Lipid-silica hybrids improved the *in vitro* lipolysis and drug dissolution kinetics compared to o/w emulsion or pure drug. In addition, the i*n vivo* bioavailability was improved	(Simovic *et al*., 2009)
lipid-SiNP hybrids; 60-70% oil in dry microcapsule	Indo-methacin	I (1h, pH 7.5, 50 mM TRIS maleate, 150 mM NaCl, 5mM $CaCl_2$, 5 mM BS, 1000 TBU mL^{-1} L, 0.5% lipid, HPLC)	7 orally dosed formulations (n=5 rats) vs IV, blood samples HPLC	Complete adsorption (fasted state); Lipid-silica hybrids higher bioavailability compared to aqueous solution or conventional o/w-emulsion	(Simovic *et al*., 2010)

*All at 37 °C. A, alginate; beta-lg, beta-lactoglobulin; BS, bile salts; C, colon phase; CCNC, carboxylated cellulose nanocrystals; Ch, chitin; CO, cross-over; CR, carageenan; DB, double blind; G, gastric phase; HPLC, high-performance liquid chromatography; I, intestinal phase; IV, intravenous; L, lipase; LF, lactoferrin; NP, nanoparticle; O, oral phase; OSA, octenyl succinate anhydride; PB, phosphate buffer; PC, placebo controlled; Si, silica; WP, whey protein. *Not conclusively considered as Pickering systems.*

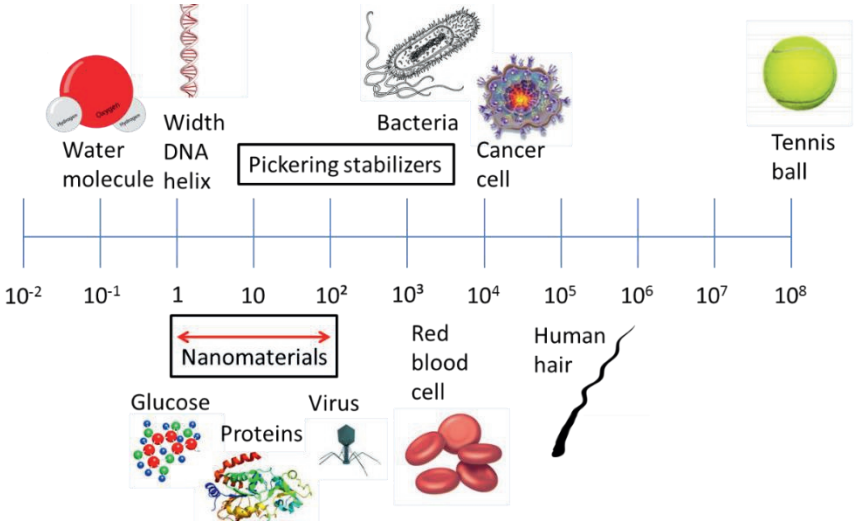

Figure 2.7. Size of objects (mainly of biological origin) on a nanometer scale to represent the size of Pickering stabilizers with respect to the definition of nanomaterials.

The main concern regarding Pickering emulsions is related to the small size of Pickering particles as size may alter their biological fate of absorption, distribution, metabolism, and excretion (ADME) *in vivo* (McClements and Rao, 2011). An illustrative example of such a particle is asbestos. Nanoparticles can have altered persistence in the human body, compared to dissolved molecules or larger materials, thereby increasing their potential toxicity. They may induce oxyradical generation, which leads to oxidative cellular damage, accumulation in organs, or cytotoxicity. EU Regulation No. 1169/2011 on the provision of food information to consumers states that: "all ingredients present in the form of engineered nanomaterials shall be clearly indicated in the list of ingredients" and the names of such ingredients shall be followed by the word "nano" in brackets (Bleeker et al., 2012; EU, 2011b). If the ingredient is added as a nano-sized material that changes to a non-nano-sized material (e.g., by dissolution), the name of the ingredient does not have to be followed by "nano." In addition, if nanomaterials fall in the category "ingredients for which an omission from the list of ingredients is allowed," labeling is also not required.

There is concern not only regarding the risks of nanoparticle use in food, but also regarding consumer acceptability. This was nicely stated by Dickinson (2010), who wrote that "it is a key challenge for the food industry to produce nanoparticles and microparticles that are both effective and also acceptable for use in food products on a commercial scale."

Chau et al. (2007) and Alkilany and Murphy (2010) reviewed major health concerns regarding nano-sized materials, including potential inhalation, cellular endocytosis, and blood–brain barrier penetration for particles smaller than 20 (Oberdörster et al., 2004), 30 (Conner and Schmid, 2003), and 12 nm in diameter (Oberdörster et al., 2004; Sarin et al., 2008; Sonavane et al., 2008), respectively. Biodegradable nanoparticles are generally considered safe because they have a low risk of accumulation (compared to inorganic materials), as do many "naturally" occurring nano-sized food components that have been proven safe by hundreds of years of consumption. For example, casein micelles in milk reportedly span a wide range of nano sizes from approximate 55 to 115 nm (de Kruif and Huppertz, 2012), yet have been safely consumed in commercially pasteurized milk since the late 1800s.

Inorganic materials and, specifically, small positively charged particles are suspected of causing serious health issues, and these particles are not typically used in food formulations. However, despite the fact that inorganic particles have greater toxicity risks, some have already been applied in food or cosmetics. For example, titanium dioxide is widely applied as a colorant and UF filter in concentrations up to 25% w/w (EU, 2016).

Silicon dioxide and silicates have been reviewed by the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). In a technical report from the Select Committee on Generally Recognized As Safe (GRAS) substances, the FDA concluded that there is no evidence in the available literature that suggests reasonable grounds to suspect a hazard to the public when the various silicates (including silicon dioxide) are used at specified or reasonable levels expected in food (Lewis and Harrison, 2009). Silica is used in food formulations as a thickening agent (hygroscopic applications) and flowing agent in powdered foods. Silicon dioxide can be used as a direct ingredient in food and as a component of food packaging materials, at levels in accordance with good manufacturing practices, and silica can also be used indirectly in the manufacture of adhesives, coatings, defoaming agents, greases and lubricants, paper and paperboard and polymers that are then used as components of food packaging materials. Although the exposure level for silicon dioxide in food products is up to 1500 mg per day (EFSA, 2009), it seems advisable to develop biobased, food-grade particles for Pickering stabilization in food applications as these carry a lower toxicity risk.

The biological fate, bioavailability, and potential toxicity of nano-emulsions (size range 1–100 nm) was reviewed by McClements and Rao (2011) and was described as depending on composition and physicochemical properties. No evidence was found that edible nanoparticles fabricated from components that are normally digested within the GI tract pass the GI system intact and cause discomfort or harm. However, quantifying nanoparticles in complex materials such as food is difficult, and low numbers of nanoparticles have been studied, which makes it difficult to draw conclusions. Raynes et al. (2014) reviewed the use of protein nanostructures in foods and recognized that some are toxic and associated with disease, such as protein amyloid fibrils, but no general statement can be made on toxicity of nano-sized proteins.

In conclusion, Pickering emulsions for commercial food applications need to be developed according to the regulations on human exposure to nanomaterials (Aschberger et al., 2015; Berton-Carabin and Schroën, 2015); similar to conventional chemical agents, new products can only be launched when proven safe. The risks connected to Pickering particles in foods are mostly low, but the European regulation makes classification of the various particles difficult, since it only uses size as a distinguishing factor. Given the rapid development in the field of applications for nanomaterials, it would be advisable to extend legislation, to give clarity not only to manufacturers but also consumers.

2.8. Conclusions and future directions

In this chapter, we reviewed the mechanisms through which particles physically stabilize emulsions, with a main focus on food-grade particles that could potentially be applied in delivery systems for bioactive components. Substantial work has been aimed at understanding model, non-food Pickering emulsions, yet the knowledge generated indicates many potential applications and benefits for food, cosmetic, and pharmaceutical applications, by using biobased ingredients such as fat, carbohydrates, and proteins. Particle stabilization may positively contribute to enhanced chemical stability of bioactive components, making Pickering emulsions efficient delivery systems. However, in-depth studies on the fate of Pickering emulsions in digestive conditions are still lacking. Moreover, the regulations regarding health and safety of biobased particles are in development. Existing toxicity concerns are primarily associated with particle size, dose, and biopersistance (e.g., inorganic versus organic), and have to be considered before such particles can be applied in food.

2.9. References

Alkilany, A. M., & Murphy, C. J. (2010). Toxicity and cellular uptake of gold nanoparticles: What we have learned so far? Journal of Nanoparticle Research, 12(7), 2313–2333.

Aloi, A., Vilanova, N., Albertazzi, L., & Voets, I. K. (2016). iPAINT: a general approach tailored to image the topology of interfaces with nanometer resolution. Nanoscale, 8, 8712–8716.

Arditty, S., Whitby, C. P., Binks, B. P., Schmitt, V., & Leal-Calderon, F. (2003). Some general features of limited coalescence in solid-stabilized emulsions. European Physical Journal E, 11(3), 273–281.

Arts, M. J. T. J., Haenen, G. R. M. M., Wilms, L. C., Beetstra, S. A. J. N., Heijnen, C. G. M., Voss, H.-P., & Bast, A. (2002). Interactions between Flavonoids and Proteins: Effect on the Total Antioxidant Capacity. Journal of Agricultural and Food Chemistry, 50(5), 1184–1187.

Aschberger, K., Gottardo, S., Amenta, V., Arena, M., Moniz, F. B., Bouwmeester, H., … Peters, R. (2015). Nanomaterials in Food - Current and Future Applications and Regulatory Aspects. Journal of Physics: Conference Series, 617, 012032.

Atarés, L., Marshall, L. J., Akhtar, M., & Murray, B. S. (2012). Structure and oxidative stability of oil in water emulsions as affected by rutin and homogenization procedure. Food Chemistry, 134(3), 1418–1424.

Benjamins, J., Vingerhoeds, M. H., Zoet, F. D., de Hoog, E. H. A., & van Aken, G. A. (2009). Partial coalescence as a tool to control sensory perception of emulsions. Food Hydrocolloids, 23(1), 102–115.

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Comprehensive Reviews in Food Science and Food Safety, 13(5), 945–977.

Berton-Carabin, C. C., Schröder, A., Rovalino, A., Schröen, K., & Sagis, L. (2016). Protein and lipid oxidation affect the viscoelasticity of whey protein layers at the oil-water interface. European Journal of Lipid Science and Technology. European Journal of Lipid Science and Technology, 118, 1630–1643.

Berton-Carabin, C. C., & Schroën, K. (2015). Pickering emulsions for food applications: Background, trends, and challenges. Annual Review of Food Science and Technology, 6(1), 263–297.

Bessel, G. (1877). Berlin Patent 42.

Betzig, E., Patterson, G. H., Sougrat, R., Lindwasser, O. W., Bonifacino, J. S., Davidson, M. W., … Hesss, H. F. (2016). Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. 313(5793), 1642–1645.

Binks. (2002). Particle as surfactants - Similarities and differences. Current Opinion in Colloid & Interface Science, 7, 21–41.

Binks, B. P., & Kirkland, M. (2002). Interfacial structure of solid-stabilised emulsions studied by scanning electron microscopy. Physical Chemistry Chemical Physics, 4(15), 3727–3733.

Binks, B.P. (2002). Particles as surfactants—similarities and differences. Current Opinion in Colloid & Interface Science, 7(1–2), 21–41.

Binks, B.P., Muijlwijk, K., Koman, H., & Poortinga, A. T. (2017). Food-grade Pickering stabilisation of foams by in situ hydrophobisation of calcium carbonate particles. Food Hydrocolloids, 63, 585-592.

Binks, Bernard P., Desforges, A., & Duff, D. G. (2007). Synergistic stabilization of emulsions by a mixture of surface-active nanoparticles and surfactant. Langmuir, 23(3), 1098–1106.

Binks, P. B., Isa, L., & Tyowua, A. T. (2013). Direct measurement of contact angles of silica particles in relation to double inversion of pickering emulsions. Langmuir, 29(16), 4923–4927.

Bleeker, Cassee, Geertsma, Jong, D., Heugens, Koers-Jacquemijns, … Environment, D. N. I. for P. H. and the. (2012). Interpretation and implications of the European Commission Recommendation on the definition of nanomaterial. RIVM Letter Report 601358001/2012.

Bleeker, E. A. J., de Jong, W. H., Geertsma, R. E., Groenewold, M., Heugens, E. H. W., Koers-Jacquemijns, M., … Oomen, A. G. (2013). Considerations on the EU definition of a nanomaterial: Science to support policy making. Regulatory Toxicology and Pharmacology, 65(1), 119–125.

Bott, R. (2014). Wettability of solid particles in relation to particle-stabilised foams and emulsions. Igarss 2014, (1), 1–5.

Britfon, G. (1995). Structure and properties of carotenoids in relation to function. The FASEB Journal, 9(15), 1551–1558.

Cercaci, L., Rodriguez-Estrada, M. T., Lercker, G., & Decker, E. A. (2007). Phytosterol oxidation in oil-in-water emulsions and bulk oil. Food Chemistry, 102(1), 161–167.

Chakraborty, K., Veetil, A. T., Jaffrey, S. R., & Krishnan, Y. (2016). Nucleic Acid – Based Nanodevices in Biological Imaging. Annual Review of Biochemistry, 85, 349–376.

Chau, C. F., Wu, S. H., & Yen, G. C. (2007). The development of regulations for food nanotechnology. Trends in Food Science and Technology, 18(5), 269–280.

Chen, H., & Zhong, Q. (2015). A novel method of preparing stable zein nanoparticle dispersions for encapsulation of peppermint oil. Food Hydrocolloids, 43, 593–602.

Chen, J., Vogel, R., Werner, S., Heinrich, G., Clausse, D., & Dutschk, V. (2011). Influence of the particle type on the rheological behavior of Pickering emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 382(1–3), 238–245.

Cheng, T. Le, & Wang, Y. U. (2013). Shape-anisotropic particles at curved fluid interfaces and role of Laplace pressure: A computational study. Journal of Colloid and Interface Science, 402, 267–278.

Chevalier, Y., & Bolzinger, M. A. (2013). Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 439, 23–34.

Chevalier, Y., Bolzinger, M., & Briancon, S. (2015). Pickering Emulsions for Controlled Drug Delivery to the Skin. In Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement (eds N. Dragicevic and H. Maibach), Springer, Berlin.

Chung, C., Smith, G., Degner, B., & McClements, D. J. (2015). Reduced fat food emulsions: Physicochemical, sensory, and biological aspects. Critical Reviews in Food Science and Nutrition, 38(1), 1–138.

Conner, S. D., & Schmid, S. L. (2003). Regulated portals of entry into the cell. Nature, 422(March), 37–43.

Corstens, M. N., Berton-Carabin, C.C., de Vries, R., Troost, F.J., Masclee, A. and Schroën, K. (2017) Food-grade micro-encapsulation systems that may induce satiety via delayed lipolysis: a review. Critical Reviews in Food Science and Nutrition, 57, 2218–2244.

Crouch, S. P., Slater, K. J., & Fletcher, J. (1992). Regulation of cytokine release from mononuclear cells by the iron-binding protein lactoferrin. Blood, 80(1), 235–240.

Dai, L. L., Sharma, R., & Wu, C. (2005). Self-assembled structure of nanoparticles at a liquid-liquid interface. Langmuir : The ACS Journal of Surfaces and Colloids, 21(7), 2641–2643.

Damiens, E., El Yazidi, I., Mazurier, J., Duthille, I., Spik, G., & Boilly-Marer, Y. (1999). Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. Journal of Cellular Biochemistry, 74(3), 486–498.

de Folter, J. W. J., Hutter, E. M., Castillo, S. I. R., Klop, K. E., Philipse, A. P., & Kegel, W. K. (2014). Particle shape anisotropy in pickering emulsions: Cubes and peanuts. Langmuir, 30(4), 955–964.

de Folter, J. W. J., van Ruijven, M. W. M., & Velikov, K. P. (2012). Oil-in-water Pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein. Soft Matter, 8(25), 6807–6815.

De Kruif, C. G. K., & Huppertz, T. (2012). Casein micelles: Size distribution in milks from individual cows. Journal of Agricultural and Food Chemistry, 60(18), 4649–4655.

Destribats, M., Lapeyre, V., Wolfs, M., Sellier, E., Leal-Calderon, F., Ravaine, V., & Schmitt, V. (2011). Soft microgels as Pickering emulsion stabilisers: role of particle deformability. Soft Matter, 7(17), 7689–7698.

Destribats, M., Rouvet, M., Gehin-Delval, C., Schmitt, C., & Binks, B. P. (2014). Emulsions stabilised by whey protein microgel particles : towards food-grade Pickering emulsions. Soft Matter, 10, 6941–6954.

Destribats, M., Wolfs, M., Pinaud, F., Lapeyre, V., Sellier, E., Schmitt, V., & Ravaine, V. (2013). Pickering emulsions stabilized by soft microgels: Influence of the emulsification process on particle interfacial organization and emulsion properties. Langmuir, 29(40), 12367–12374.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. Current Opinion in Colloid and Interface Science, 15(1–2), 40–49.

Dickinson, E. (2012). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. Trends in Food Science and Technology, 24(1), 4–12.

Dickinson, E. (2013). Stabilising emulsion-based colloidal structures with mixed food ingredients. Journal of the Science of Food and Agriculture, Vol. 93, pp. 710–721.

Dinsmore, A. D., Hsu, M. F., Nikolaides, M. G., Marquez, M., Bausch, A. R., Weitz, D. A., … Gordillo, J. M. (2002). Colloidosomes: selectively permeable capsules composed of colloidal particles. Science (New York, N.Y.), 298(5595), 1006–1009.

Douaire, M., Di Bari, V., Norton, J. E., Sullo, A., Lillford, P., & Norton, I. T. (2014). Fat crystallisation at oil-water interfaces. Advances in Colloid and Interface Science, 203, 1–10.

Dugyala, V. R., Daware, S. V., & Basavaraj, M. G. (2013). Shape anisotropic colloids: synthesis, packing behavior, evaporation driven assembly, and their application in emulsion stabilization. Soft Matter, 9(29), 6711.

EFSA. (2009). Scientific opinion of the panel on food additives and nutrient sources added to food on calcium silicate, silicon dioxide and silicic acid gel added for nutritional purposes to food supplements following a request from the European Commission. The EFSA Journal, 1132, 1–24.

Eskandar, N. G., Simovic, S., & Prestidge, C. A. (2007). Synergistic effect of silica nanoparticles and charged surfactants in the formation and stability of submicron oil-in-water emulsions. Physical Chemistry Chemical Physics : PCCP, 9, 6426–6434.

EU. (2011a). Commision recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). (June 2010), 2010–2012.

EU. (2011b). Regulation (EU) No 1169/2011 of the European parliament and of the council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, an. Official Journal of the European Union, (1169), 18–63.

EU. (2016). Commission Regulation (EU) 2016/1143 of 14.07.2016 amending Annex VI to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic (Titanium Dioxide).

Fernandez-Rodriguez, M. A., Song, Y., Rodríguez-Valverde, M. Á., Chen, S., Cabrerizo-Vilchez, M. A., & Hidalgo-Alvarez, R. (2014). Comparison of the interfacial activity between homogeneous and Janus gold nanoparticles by pendant drop tensiometry. Langmuir, 30(7), 1799–1804.

Finkle, P., Draper, H. D., & Hildebrand, J. H. (1923). The theory of emulsification. Journal of the American Chemical Society, 45(12), 2780–2788.

Geisel, K. A. (2015). Microgels at Oil-Water Interfaces : Deformation , Assembly and Compression, 177.

Geisel, K., Richtering, W., & Isa, L. (2014). Highly Ordered 2D Microgel Arrays: Compression versus Self-Assembly. Soft Matter, 10, 7968.

Genot, C., Berton, C., & Ropers, M.-H. (2013). The role of the interfacial layer and emulsifying proteins in the oxidation in oil-in-water emulsions. Lipid Oxidation: Challenges in Food Systems (eds A. Logan, U. Nienaber and X. Pan), AOCS Press, Urbana.

Gould, J., Vieira, J., & Wolf, B. (2013). Cocoa particles for food emulsion stabilisation. Food & Function, 4(9), 1369–1375.

Guido, S., & Preziosi, V. (2010). Droplet deformation under confined Poiseuille flow. Advances in Colloid and Interface Science, 161(1–2), 89–101.

Gupta, R., & Rousseau, D. (2012). Surface-active solid lipid nanoparticles as Pickering stabilizers for oil-in-water emulsions. Food & Function, 3(3), 302.

Habibi, Y., & Dufresne, A. (2008). Highly filled bionanocomposites from functionalized polysaccharide nanocrystals. Biomacromolecules, 9(7), 1974–1980.

Haynes, W. (1860). Berlin Patent 488.

Henneke, D. (2011). Adsorption kinetics of alkanethiol-capped gold nanoparticles at the hexane – water interface. 6579–6589.

Henson, S., Masakure, O., & Cranfield, J. (2008). The propensity for consumers to offset health risks through the use of functional foods and nutraceuticals: The case of lycopene. Food Quality and Preference, 19(4), 395–406.

Hof, K. H. Van, Boer, B. C. J. De, Tijburg, L. B. M., Lucius, B. R. H. M., Zijp, I., West, C. E., … Weststrate, J. A. (2000). Human nutrition and metabolism carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after Fo. (August 1999), 1189–1196.

Horozov, T. S., & Binks, B. P. (2006). Particle-stabilized emulsions: A bilayer or a bridging monolayer? Angewandte Chemie - International Edition, 45(5), 773–776.

Hu, Y., Yang, Y., Ning, Y., Wang, C., & Tong, Z. (2013). Facile preparation of artemisia argyi oil-loaded antibacterial microcapsules by hydroxyapatite-stabilized pickering emulsion templating. Colloids and Surfaces B: Biointerfaces, 112, 96–102.

Hu, Z., Ballinger, S., Pelton, R., & Cranston, E. D. (2015). Surfactant-enhanced cellulose nanocrystal Pickering emulsions. Journal of Colloid and Interface Science, 439, 139–148.

Huang, B., Wang, W., Bates, M., Zhuang, X., Huang, B., Wang, W., … Zhuang, X. (2016). Three-Dimensional Super-Resolution Imaging by Stochastic Optical Reconstruction Microscopy. 319(5864), 810–813.

Hubbard, A. (2004). Colloidal Science of Flotation: By Anh V. Nguyen and Hans Joachim Schulze. Marcel Dekker, New York, 2004, 850 pp (book review). Journal of Colloid and Interface Science, 273, 343.

Hunter, T., Puch, R., Franks, G., & Jameson, G. (2008). The role of particles in stabilising foams and emulsions. Advances in Colloid and Interface Science, 137(2), 57–81.

Illek, B., Fischer, H., Akiyama, T., Ogawara, H., Alton, E. W. F. W., Currie, A. D., … Frizzell, R. A. (1998). Flavonoids stimulate Cl conductance of human airway epithelium in vitro and in vivo. The American Journal of Physiology, 275(5 Pt 1), L902-10.

Jungmann, R., Steinhauer, C., Scheible, M., Kuzyk, A., Tinnefeld, P., & Simmel, F. C. (2010). Super-resolution microscopy by fluorescence imaging of transient binding on DNA origami. Nano letters, 4756–4761.

Kaewsaneha, C., Tangboriboonrat, P., Polpanich, D., Eissa, M., & Elaissari, A. (2013). Preparation of Janus colloidal particles via Pickering emulsion: An overview. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 439, 35–42.

Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., & Norton, I. T. (2012). Investigation into the potential ability of Pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 366(1), 209–215.

Kargar, M., Spyropoulos, F., & Norton, I. T. (2011). The effect of interfacial microstructure on the lipid oxidation stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 357(2), 527–533.

Khachik, F., Goli, M. B., Beecher, G. R., Holden, J., Lusby, W. R., Tenorio, M. D., & Barrera, M. R. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. Journal of Agricultural and Food Chemistry, 40(3), 390–398.

Kilcast, D., & Clegg, S. (2002). Sensory perception of creaminess and its relationship with food structure. Food Quality and Preference, 13, 609–623.

Kovačević, A. B., Müller, R. H., Savić, S. D., Vuleta, G. M., & Keck, C. M. (2014). Solid lipid nanoparticles (SLN) stabilized with polyhydroxy surfactants: Preparation, characterization and physical stability investigation. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 444, 15–25.

Kralchevsky, P. A., & Nagayama, K. (2000). Capillary interactions between particles bound to interfaces, liquid films and biomembranes. Advances in Colloid and Interface Science, 85(2), 145–192.

Krebs, T., Ershov, D., Schroen, C. G. P. H., & Boom, R. M. (2013). Coalescence and compression in centrifuged emulsions studied with in situ optical microscopy. Soft Matter, 9, 4026.

Krebs, Thomas, Schroën, K., & Boom, R. (2012). Coalescence dynamics of surfactant-stabilized emulsions studied with microfluidics. Soft Matter, 8(41), 10650–10657.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation, 106(21), 2747–2757.

Kumar, A., Park, B. J., Tu, F., & Lee, D. (2013). Amphiphilic Janus particles at fluid interfaces. Soft Matter, 9(29), 6604–7202.

Kurukji, D., Pichot, R., Spyropoulos, F., & Norton, I. T. (2013). Interfacial behaviour of sodium stearoyllactylate (SSL) as an oil-in-water pickering emulsion stabiliser. Journal of Colloid and Interface Science, 409, 88–97.

Laredj-Bourezg, F., Bolzinger, M. A., Pelletier, J., Rovere, M. R., Smatti, B., & Chevalier, Y. (2013). Pickering emulsions stabilised by biodegradable particles offer a double level of controlled delivery of hydrophobic drugs. In Advances in Dermatological Sciences (pp. 157–168).

Lee, D., & Weitz, D. A. (2008). Double emulsion-templated nanoparticle colloidosomes with selective permeability. Advanced Materials, 20(18), 3498–3503.

Levine, S., & Bowen, B. D. (1993). Capillary interaction of spherical particles adsorbed on the surface of an oil/water droplet stabilized by the particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 70(1), 33–45.

Lewis, & Harrison. (2009). Generally recognized as safe determination for silicon dioxide when added directly and/or indirectly to human food.

Lim, L. H., Tan, A., Simovic, S., & Prestidge, C. a. (2011). Silica-lipid hybrid microcapsules: influence of lipid and emulsifier type on in vitro performance. International Journal of Pharmaceutics, 409(1–2), 297–306.

Liu, F., & Tang, C.-H. (2013). Soy protein nanoparticle aggregates as pickering stabilizers for oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 61(37), 8888–8898.

Liu, F., & Tang, C.-H. (2014a). Emulsifying properties of soy protein nanoparticles: Influence of the protein concentration and/or emulsification process. Journal of Agricultural and Food Chemistry, 62(12), 2644–2654.

Liu, F., & Tang, C.-H. (2014b). Phytosterol colloidal particles as Pickering stabilizers for emulsions. Journal of Agricultural and Food Chemistry, 62(22), 5133–5141.

Liu, F., & Tang, C.-H. (2016). Soy Glycinin as Food-grade Pickering Stabilizers: Part. III. Fabrication of gel-like emulsions and their potential as sustained-release delivery systems for β-carotene. Food Hydrocolloids, 56, 434–444.

Lou, F., Ye, L., Kong, M., Yang, Q., Li, G., & Huang, Y. (2016). Pickering emulsions stabilized by shape-controlled silica microrods. RSC Adv., 6(29), 24195–24202.

Lucassen, J. (1992). Capillary forces between solid particles in fluid interfaces. Colloids and Surfaces, 65(2–3), 131–137.

Luo, Z., Murray, B. S., Ross, A. L., Povey, M. J. W., Morgan, M. R. a, & Day, A. J. (2012). Effects of pH on the ability of flavonoids to act as Pickering emulsion stabilizers. Colloids and Surfaces B: Biointerfaces, 92, 84–90.

Luo, Z., Murray, B. S., Yusoff, A., Morgan, M. R. a, Povey, M. J. W., & Day, A. J. (2011). Particle-stabilizing effects of flavonoids at the oil-water interface. Journal of Agricultural and Food Chemistry, 59(6), 2636–2645.

Madivala, B., Vandebril, S., Fransaer, J., & Vermant, J. (2009). Exploiting particle shape in solid stabilized emulsions. Soft Matter, 5(8), 1717.

Marku, D., Wahlgren, M., Rayner, M., Sjöö, M., & Timgren, A. (2012). Characterization of starch Pickering emulsions for potential applications in topical formulations. International Journal of Pharmaceutics, 428(1–2), 1–7.

McClements, D.J., & Decker, E. A. (2000). Super-resolution microscopy by fluorescence imaging of transient binding on DNA origams. Nano letters, 65(8), 1270–1282.

McClements, David Julian, & Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Advances in Colloid and Interface Science, 159(2), 213–228.

McClements, David Julian, & Rao, J. (2011). Food-grade nanoemulsions: Formulation, fabrication, properties, performance, biological fate, and potential toxicity. Critical Reviews in Food Science and Nutrition, 51(4), 285–330.

Mehrabian, H., Harting, J., & Snoeijer, J. H. (2015). Soft particles at a fluid interface. Soft Matter, 1–17.

Meshulam, D., & Lesmes, U. (2013). Responsiveness of emulsions stabilized by lactoferrin nano-particles to simulated intestinal conditions. Food & Function, 5(1), 65–73.

Monteillet, H. (2015). Complex Coacervates and Microgels for Emulsions.

Monteillet, H., Workamp, M., Appel, J., Kleijn, J. M., Leermakers, F. A. M., & Sprakel, J. (2014). Ultrastrong anchoring yet barrier-free adsorption of composite microgels at liquid interfaces. Advanced Materials Interfaces, 1, 1300121.

Monteux, C., Kirkwood, J., Xu, H., Jung, E., & Fuller, G. G. (2007). Determining the mechanical response of particle-laden fluid interfaces using surface pressure isotherms and bulk pressure measurements of droplets. Physical Chemistry Chemical Physics, 9, 6344– 6350.

Mulligan, M. K., & Rothstein, J. P. (2011). Deformation and breakup of micro- and nanoparticle stabilized droplets in microfluidic extensional flows. Langmuir, 27(16), 9760–9768.

Murray, B. S., Durga, K., Yusoff, A., & Stoyanov, S. D. (2011). Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. Food Hydrocolloids, 25(4), 627–638.

Natarajmurthy, S. H., Askari, M., Pullabhatla, S., & Dharmesh, S. M. (2016). Basic nutritional investigation A novel b-carotene–associated carrot (Daucus carota L.) pectic polysaccharide. Nutrition, 32(7–8), 818–826.

Nesterenko, A., Drelich, A., Lu, H., Clausse, D., & Pezron, I. (2014). Influence of a mixed particle/surfactant emulsifier system on water-in-oil emulsion stability. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 457(1), 49–57.

Ngai, T., & Bon, S. A. F. (Eds.). (2014). Particle-Stabilized Emulsions and Colloids : Formation and Applications. Royal Society of Chemistry, London.

Nie, Z., Jai, I. P., Li, W., Bon, S. A. F., & Kumacheva, E. (2008). An "inside-out" microfluidic approach to monodisperse emulsions stabilized by solid particles. Journal of the American Chemical Society, 130(49), 16508–16509.

Nonomura, Y., Kobayashi, N., & Nakagawa, N. (2011). Multiple Pickering Emulsions Stabilized by Microbowls. Langmuir, 27(8), 4557–4562.

Nushtaeva, A. V. (2016). Natural food-grade solid particles for emulsion stabilization. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 504, 449–457.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., & Cox, C. (2004). Translocation of Inhaled Ultrafine Particles to the Brain. Inhalation Toxicology, 16(6–7), 437–445.

Olijve, L. L. C., & Voets, I. K. (2016). Morphological evolution of complex coacervate core micelles revealed by iPAINT microscopy. 6–11.

Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein–phenolic interactions and associated changes. FRIN, 51(2), 954–970.

Pan, Y., Tikekar, R. V., & Nitin, N. (2013). Effect of antioxidant properties of lecithin emulsifier on oxidative stability of encapsulated bioactive compounds. International Journal of Pharmaceutics, 450(1–2), 129–137.

Pan, Y., Tikekar, R. V., Wang, M. S., Avena-Bustillos, R. J., & Nitin, N. (2015). Effect of barrier properties of zein colloidal particles and oil-in-water emulsions on oxidative stability of encapsulated bioactive compounds. Food Hydrocolloids, 43, 82–90.

Paunov, V. N. (2003). Novel Method for Determining the Three-Phase Contact Angle of Colloid Particles Adsorbed at Air - Water and Oil - Water Interfaces, (13), 7970–7976.

Pawar, A. B., Caggioni, M., Ergun, R., Hartel, R. W., & Spicer, P. T. (2011). Arrested coalescence in Pickering emulsions. Soft Matter, 7(17), 7710.

Pawar, A. B., Caggioni, M., Hartel, R. W., & Spicer, P. T. (2012). Arrested coalescence of viscoelastic droplets with internal microstructure. Faraday Discussions, 158, 341.

Pawlik, A. K., & Norton, I. T. (2014). Bridging benchtop research and industrial processed foods: Structuring of model food emulsions. Food Structure, 1(1), 24–38.

Pichot, R., Spyropoulos, F., & Norton, I. T. (2010). O/W emulsions stabilised by both low molecular weight surfactants and colloidal particles: The effect of surfactant type and concentration. Journal of Colloid and Interface Science, 352(1), 128–135.

Pickering, S. U. (1907). Emulsions. Journal of the Chemical Society, 91, 2001–2021.

Ramsden, W. (1903). Separation of solids in the surface-layers of solutions and "suspensions" (observations on surface-membranes, bubbles, emulsions, and Mechanical Coagulation). Proceedings of the Royal Society of London, 72, 156–164.

Rayner, M. (2015). Current status on novel ways for stabilizing food dispersions by oleosins, particles and microgels. Current Opinion in Food Science, 3, 94–109.

Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P., & Wahlgren, M. (2014). Biomass-based particles for the formulation of Pickering type emulsions in food and topical applications. Colloids and Surfaces A, 458, 48–62.

Rayner, M., Sjöö, M., Timgren, A., & Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of Pickering emulsions. Faraday Discussions, 158, 139.

Richtering, W. (2012). Responsive emulsions stabilized by stimuli-sensitive microgels: emulsions with special non-Pickering properties. Langmuir : The ACS Journal of Surfaces and Colloids, 28(50), 17218–17229.

Rodriguez, M. S., Albertengo, L. A., Vitale, I., & Agullo, E. (2003). Relationship Between Astringency and Chitosan-Saliva Solutions Turbidity at Different pH. Journal of Food Science, 68(2), 665–667.

Rousseau, D. (2013). Trends in structuring edible emulsions with Pickering fat crystals. Current Opinion in Colloid and Interface Science, 18(4), 283–291.

Ruhland, T. M., Walther, A., & Axel, H. E. M. (2011). Janus Cylinders at Liquid À Liquid Interfaces. 9807–9814.

Ruiz-Rodriguez, P. E., Meshulam, D., & Lesmes, U. (2014). Characterization of Pickering O/W Emulsions Stabilized by Silica Nanoparticles and Their Responsiveness to In vitro Digestion Conditions. Food Biophysics, 9, 406–415.

Ryan, E., Chopra, J., Mccarthy, F., Maguire, A. R., & O 'brien, N. M. (2005). Qualitative and quantitative comparison of the cytotoxic and apoptotic potential of phytosterol oxidation products with their corresponding cholesterol oxidation products. British Journal of Nutrition, 94(3), 443–451.

Sabapathy, M., Kollabattula, V., Basavaraj, M. G., & Mani, E. (2015). Visualization of the equilibrium position of colloidal particles at fl uid – water interfaces by deposition of nanoparticles †. Nanoscale, 7, 13868–13876.

Sagis, L. M. C., & Scholten, E. (2014). Complex interfaces in food: Structure and mechanical properties. Trends in Food Science and Technology, 37(1), 59–71.

Salem, M., Rohani, S., & Gillies, E. R. (2014). Curcumin, a promising anti-cancer therapeutic: a review of its chemical properties, bioactivity and approaches to cancer cell delivery. Rsc Advances, 21(4), 10815–10829.

Santipanichwong, R., Suphantharika, M., Weiss, J., & McClements, D. J. (2008). Core-shell biopolymer nanoparticles produced by electrostatic deposition of beet pectin onto heat-denatured a-lactoglobulin aggregates. Journal of Food Science, 73(6).

Sarin, H., Kanevsky, A. S., Wu, H., Brimacombe, K. R., Fung, S. H., Sousa, A. a, … Hall, M. D. (2008). Effective transvascular delivery of nanoparticles across the blood-brain tumor barrier into malignant glioma cells. Journal of Translational Medicine, 6(October 2008), 80.

Sarkar, A., Murray, B., Holmes, M., Ettelaie, R., Abdalla, A., & Yang, X. (2016). In vitro digestion of Pickering emulsions stabilized by soft whey protein microgel particles: influence of thermal treatment. Soft Matter, 12(15), 3558–3569.

Schmitt, V., & Ravaine, V. (2013). Surface compaction versus stretching in Pickering emulsions stabilised by microgels. Current Opinion in Colloid and Interface Science, 18(6), 532–541.

Shah, R., Eldridge, D., Palombo, E., & Harding, I. (2015). Lipid nanoparticles: Production, characterization and stability. Springer International Publishing, Berlin.

Shao, Y., & Tang, C.-H. (2016a). Gel-like pea protein Pickering emulsions at pH3.0 as a potential intestine-targeted and sustained-release delivery system for β-carotene. Food Research International, 79, 64–72.

Shao, Y., & Tang, C. H. (2016b). Gel-like pea protein Pickering emulsions at pH3.0 as a potential intestine-targeted and sustained-release delivery system for β-carotene. Food Research International, 79, 64–72.

Shimoni, G., Shani Levi, C., Levi Tal, S., & Lesmes, U. (2013). Emulsions stabilization by lactoferrin nano-particles under in vitro digestion conditions. Food Hydrocolloids, 33(2), 264–272.

Sihler, S., Schrade, A., Cao, Z., & Ziener, U. (2015). Inverse Pickering emulsions with droplet sizes below 500 nm. Langmuir, 31(38), 10392–10401.

Simovic, S., Heard, P., Hui, H., Song, Y., Peddie, F., Davey, A. K., … Prestidge, C. A. (2009). Dry Hybrid Lipid - Silica Microcapsules Engineered from Submicron Lipid Droplets and Nanoparticles as a Novel Delivery System for Poorly Soluble Drugs. Molecular Pharmaceutics, 6(3), 861–872.

Simovic, S., Hui, H., Song, Y., Davey, A. K., Rades, T., & Prestidge, C. a. (2010). An oral delivery system for indomethicin engineered from cationic lipid emulsions and silica nanoparticles. Journal of Controlled Release : Official Journal of the Controlled Release Society, 143(3), 367–373.

Sonavane, G., Tomoda, K., & Makino, K. (2008). Biodistribution of colloidal gold nanoparticles after intravenous administration: Effect of particle size. Colloids and Surfaces B: Biointerfaces, 66(2), 274–280.

Tan, A., Colliat-dangus, P., Whitby, C. P., & Prestidge, C. A. (2014). Controlling the enzymatic digestion of lipids using hybrid nanostructured materials. Applied Materials & Interfaces, 6, 15363–15371.

Tan, H., Sun, G., Lin, W., Mu, C., & Ngai, T. (2014). Gelatin particle-stabilized high internal phase emulsions as nutraceutical containers. ACS Applied Materials & Interfaces, 6(16), 13977–13984.

Tan, Y., Xu, K., Liu, C., Li, Y., Lu, C., & Wang, P. (2012a). Fabrication of starch-based nanospheres to stabilize pickering emulsion. Carbohydrate Polymers, 88(4), 1358–1363.

Tan, S.-Y., Tabor, R. F., Ong, L., Stevens, G. W., & Dagastine, R. R. (2012b). Nano-mechanical properties of clay-armoured emulsion droplets. Soft Matter, 8(11), 3112.

Tang, J., Quinlan, P. J., & Tam, K. C. (2015). Stimuli-responsive Pickering emulsions: recent advances and potential applications. Soft Matter, 11(18), 3512–3529.

Tarimala, S., & Dai, L. L. (2004). Structure of microparticles in solid-stabilized emulsions. Langmuir, 20(9), 3492–3494.

Tavernier, I., Wijaya, W., Van der Meeren, P., Dewettinck, K., & Patel, A. R. (2016). Food-grade particles for emulsion stabilization. Trends in Food Science and Technology, 50, 159–174.

Tcholakova, S., Denkov, N. D., & Lips, A. (2008). Comparison of solid particles, globular proteins and surfactants as emulsifiers. Physical Chemistry Chemical Physics : PCCP, 10(12), 1608–1627.

Tikekar, R. V., Pan, Y., & Nitin, N. (2013). Fate of curcumin encapsulated in silica nanoparticle stabilized Pickering emulsion during storage and simulated digestion. Food Research International, 51(1), 370–377.

Timgren, A., Rayner, M., Sjöö, M., & Dejmek, P. (2011). Starch particles for food based Pickering emulsions. Procedia Food Science, 1, 95–103.

Tzoumaki, M. V, Moschakis, T., Scholten, E., & Biliaderis, C. G. (2013). In vitro lipid digestion of chitin nanocrystal stabilized o/w emulsions. Food & Function, 4(1), 121–129.

Vinson, J. A., Dabbagh, Y. A., Serry, M. M., & Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. J. Agric. Food Chem, 43, 2800–2802.

Von Staszewski, M., Pizones Ruiz-Henestrosa, V. M., & Pilosof, A. M. R. (2014). Green tea polyphenols-β-lactoglobulin nanocomplexes: Interfacial behavior, emulsification and oxidation stability of fish oil. Food Hydrocolloids, 35, 505–511.

Walstra, P. (1993). Principles of emulsion formation. Chemical Engineering Science, 48(2), 333–349.

Wang, L. J., Hu, Y. Q., Yin, S. W., Yang, X. Q., Lai, F. R., & Wang, S. Q. (2015). Fabrication and characterization of antioxidant pickering emulsions stabilized by zein/chitosan complex particles (ZCPs). Journal of Agricultural and Food Chemistry, 63(9), 2514–2524.

Wang, M. S., Chaudhari, A., Pan, Y., Young, S., & Nitin, N. (2014). Controlled release of natural polyphenols in oral cavity using starch Pickering emulsion. Materials Research Society Symposia Proceedings, 1688.

Wen, C., Yuan, Q., Liang, H., & Vriesekoop, F. (2014). Preparation and stabilization of d-limonene Pickering emulsions by cellulose nanocrystals. Carbohydrate Polymers, 112, 695–700.

Whitby, C. P., Fischer, F. E., Fornasiero, D., & Ralston, J. (2011). Shear-induced coalescence of oil-in-water Pickering emulsions. Journal of Colloid and Interface Science, 361(1), 170–177.

White, K. A., Schofield, A. B., Wormald, P., Tavacoli, J. W., Binks, B. P., & Clegg, P. S. (2011). Inversion of particle-stabilized emulsions of partially miscible liquids by mild drying of modified silica particles. Journal of Colloid and Interface Science, 359(1), 126–135.

Wieca, M., Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Czy, J. (2013). The influence of protein-flavonoid interactions on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. Food Chemistry, 141(1), 451–458.

Wu, P., Ma, G., Li, N., Deng, Q., Yin, Y., & Huang, R. (2014). Investigation of in vitro and in vivo antioxidant activities of flavonoids rich extract from the berries of Rhodomyrtus tomentosa(Ait.) Hassk.

Xiao, J., Li, C., & Huang, Q. (2015). Kafirin nanoparticle-stabilized Pickering emulsions as oral delivery vehicles: physicochemical stability and in vitro digestion profile. Journal of Agricultural and Food Chemistry, 63(47), 10263–10270.

Xiao, J., Lu, X., & Huang, Q. (2017). Double emulsion derived from ka fi rin nanoparticles stabilized Pickering emulsion : Fabrication , microstructure , stability and in vitro digestion profile. Food Hydrocolloids, 62, 230–238.

Xiao, J., Shi, C., Li, Y., Pan, Y., & Huang, Q. (2017). Food Hydrocolloids Pickering emulsions immobilized within hydrogel matrix with enhanced resistance against harsh processing conditions and sequential digestion. Food Hydrocolloids, 62, 35–42.

Yan, H., Zhao, B., Long, Y., Zheng, L., Tung, C. H., & Song, K. (2015). New pickering emulsions stabilized by silica nanowires. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 482, 639–646.

Yang, D., Wang, X.-Y., Ji, C.-M., Lee, K.-T., Shin, J.-A., Lee, E.-S., & Hong, S.-T. (2014). Influence of Ginkgo biloba extracts and of their flavonoid glycosides fraction on the in vitro digestibility of emulsion systems. Food Hydrocolloids, 42, 196–203.

Young, T. (1805). An Essay on the Cohesion of Fluids. Philosophical Transactions of the Royal Society of London, 95, 65–87.

Yusoff, A., & Murray, B. S. (2011). Modified starch granules as particle-stabilizers of oil-in-water emulsions. Food Hydrocolloids, 25(1), 42–55.

Zanini, M., & Isa, L. (2016). Particle contact angles at fluid interfaces: pushing the boundary beyond hard uniform spherical colloids. Journal of Physics: Condensed Matter, 28(31), 313002.

Zeng, C., Brau, F., Davidovitch, B., & Dinsmore, A. D. (2012). Capillary interactions among spherical particles at curved liquid interfaces. Soft Matter, 8(33), 8582.

Zhang, J., Li, R., Jiang, F.-L., Zhou, B., Luo, Q.-Y., Yu, Q.-L.-Y., … Wang, Y.-L. (2014). An electrochemical and surface plasmon resonance study of adsorption actions of DNA by Escherichia coli. Colloids and Surfaces. B, Biointerfaces, 117C, 68–74.

Zhao, Y., Guan, Y., Pan, Y., Nitin, N., & Tikekar, R. V. (2015). Improved oxidative barrier properties of emulsions stabilized by silica-polymer microparticles for enhanced stability of encapsulants. Food Research International, 74, 269–274.

Zhao, Y., Pan, Y., Nitin, N., & Tikekar, R. V. (2014). Enhanced stability of curcumin in colloidosomes stabilized by silica aggregates. LWT - Food Science and Technology, 58(2), 667–671.

Chapter 3

Tailored microstructure of colloidal lipid particles for Pickering emulsions with tunable properties

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Abstract

Sub-micron colloidal lipid particles (CLPs) can successfully be used as Pickering stabilizers in oil-in-water (O/W) emulsions, leading to enhanced stability compared to conventional emulsifier-stabilized emulsions. Varying the lipid solid-liquid ratio leads to particles with distinct nanostructure and morphology, resulting in tunable emulsion stabilization performance. Our CLPs are produced by hot high pressure homogenization of high melting point fats in water, and subsequent cooling to induce lipid crystallization. Lath-like tripalmitin and palm stearin CLPs form jammed, cohesive interfacial layers that prevent relaxation of emulsion droplets, and form a three-dimensional network in the continuous aqueous phase. CLPs consisting of a mixture of solid tripalmitin and liquid tricaprylin are polycrystalline platelet-like particles that form O/W emulsions with spherical and bridged droplets covered by a thin particle layer. Our results present a versatile approach to interfacial design that also opens up new perspectives for development of novel delivery systems for active ingredients.

3.1. Introduction

Particle-stabilized emulsions, better known as Pickering (Pickering, 1907) or Ramsden (Ramsden, 1903) emulsions, are known for their enhanced stability against coalescence and Ostwald ripening as compared to conventional surfactant- or polymer-stabilized emulsions. The use of colloidal particles as emulsion stabilizers has seen a surge of interest, for example in the food, pharmaceutical and cosmetic industry (Berton-Carabin & Schroën, 2015a; Frelichowska, Bolzinger, Pelletier, Valour, & Chevalier, 2009; Marku et al., 2012; Rayner et al., 2014; Schröder, Corstens, Ho, Schroën, & Berton-Carabin, 2018), in separation processes (Monteillet et al., 2014) and in the fabrication of new emulsion-templated materials, such as colloidosomes (Kotula & Anna, 2012; Thompson, Williams, & Armes, 2014). Over the past decades a variety of organic and inorganic particles, such as inorganic silica and clay particles and polymeric latex colloids have been used for those purposes (Binks & Kirkland, 2002; Binks & Lumsdon, 2001; Horozov & Binks, 2006; Zang, Stocco, Langevin, Wei, & Binks, 2009). Their effectiveness as Pickering stabilizers depends strongly on their wettability, size, shape, inter-particle interactions and concentration (Binks, 2002; Tcholakova et al., 2008a).

The particle wettability, as characterized by the three-phase contact angle, determines the position of the particles in the interface and their adsorption energy (Leal-Calderon & Schmitt, 2008). Moreover, it determines the preferred emulsion type: O/W emulsions are more effectively stabilized by particles that are preferentially wetted by water, whereas water-in-oil (W/O) emulsions are most effectively formed by relatively hydrophobic particles, which are mostly wetted by the oil (Aveyard, Binks, & Clint, 2003; Dickinson, 2010b; Finkle et al., 1923). As colloidal particles anchor in the interface, they provide an excellent mechanical barrier to droplet coalescence. The remarkable stability of Pickering emulsions results from the strong attachment of particles in the interface, quantified by the adsorption energy ΔG_a:

$$\Delta G_a = \pi r^2 \gamma_{ow} (1 - \cos\theta)^2 \qquad \text{(Eq. 3.1.)}$$

where r is the particle radius, γ_{ow}, the interfacial tension between the two immiscible fluids and θ the three phase contact angle. Even for small particles, adsorption energies invariably exceed the thermal energy by orders-of-magnitude, ensuring their irreversible anchoring at liquid interfaces. Due to their practically irreversible adsorption, they can also create high interfacial pressures that can prevent mass transfer across the interface in a process known as Ostwald ripening. In addition, inter-particle interactions are key to control the stability of Pickering emulsions: in the continuous phase, such interactions lead to the formation of a

three-dimensional network of aggregated particles that contributes to emulsion stability (Lam, Velikov, & Velev, 2014); at the interface, attractive inter-particle interactions also occur due to capillary forces (Lucassen, 1992) resulting in cohesive (jammed) interfacial networks. Model particles can be produced with high monodispersity, in various sizes, and can be made perfectly spherical and truly solid.

However, it is challenging to produce particles not only suitable for Pickering stabilization of O/W emulsions but which consist of sustainable biobased materials, are available in sufficient quantify and whose production enables industrial application at scales significantly larger than those typically explored in laboratory studies. Protein- or carbohydrate-based particles have been suggested for Pickering stabilization (Destribats, Rouvet, et al., 2014a; Liu & Tang, 2013; Rayner, Sjöö, et al., 2012), possibly after surface modification to ensure appropriate wettability (Dokića, Krstonošićb, & Nikolića, 2012; Timgren et al., 2013; Yusoff & Murray, 2011) and thereby strong anchoring of particles at the interface.

A few examples report on the stabilization of W/O emulsions (Pawlik, Kurukji, Norton, & Spyropoulos, 2016a) or bubbles (Brun, Delample, Harte, Lecomte, & Leal-Calderon, 2015) with lipid-based particles such as fat crystals (Ghosh & Rousseau, 2011). It is even possible to modify the surface properties of fat particles, *e.g.,* through introducing polar groups or mixing with hydrophilic molecules (*e.g.,* proteins (Pawlik et al., 2016a)) to make them adequate for O/W emulsion stabilization (Gupta & Rousseau, 2012; Kurukji et al., 2013; Liu & Tang, 2014b; Luo et al., 2011; Ye, Zhu, & Singh, 2013). For instance, water-insoluble phytosterol crystals (Liu & Tang, 2014b), solid lipid nanoplatelets from glyceryl stearyl citrate (Gupta & Rousseau, 2012) and casein-coated hexadecane droplets (Ye et al., 2013) have been successfully used in Pickering emulsions. Despite these examples, which illustrate the feasibility of a biobased and sustainable route to producing Pickering emulsions, no clear design rules that link the properties of biobased particles to emulsion stability have been established.

In this paper, we explore the use of colloidal lipid particles (CLPs) in the tailored design of biobased Pickering emulsions. The choice of the lipid allows tailoring of the particle microstructure, such as its internal crystallinity; we show how this affects the particle morphology and its ability to stabilize Pickering emulsions. We systematically explore these effects to elucidate the involved mechanisms of Pickering stabilization.

3.2. Experimental section

3.2.1 Materials

Tripalmitin (#T8127, purity > 99%), tricaprylin (#91040, purity > 90%), Tween 40 (#P1504), sodium phosphate monobasic (#S9638), sodium phosphate dibasic (#S9763), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiOC18(3)) (#468495), ammonium thiocyanate (#1.01213) and methylene chloride (#270997) were purchased from Sigma-Aldrich. Palm stearin (palmitic acid, 82 %; oleic acid, 9 %; stearic acid, 5 %) was supplied by ADM (Saint Laurent Bangy, France) (composition can be found in Supporting information, Figure S3.2.). Cobalt nitrate hexahydrate was purchased from ACROS Organics. Sunflower oil was obtained from a local supermarket, and stripped with alumina powder (MP EcoChrome™ ALUMINA N, Activity: Super I, Biomedicals) to remove impurities and tocopherols (Berton, Genot, & Ropers, 2011). Ultrapure water obtained from a Milli-Q system (Millipore Corporation, Billerica, Massachusetts, USA) was used throughout. All other chemicals used were of analytical grade.

3.2.2. Preparation and characterization of CLP dispersions

Briefly, we melted the lipid phase (5% w/w tripalmitin, palm stearin or tripalmitin/tricaprylin (4:1 w/w)) at 80 °C in a water bath and added it to an aqueous phase (95% w/w) containing 2% w/w Tween 40 in 10 mM phosphate buffer at pH 7.0 at the same temperature. A coarse emulsion was prepared by high speed stirring at 11,000 rpm for one min using a preheated rotor stator homogenizer (Ultra-turrax IKA T18 basic, Germany). This coarse emulsion was then homogenized at 800 bar for 5 cycles through a high pressure homogenizer (Microfluidizer® Processor MF 110Y with Y-shaped interaction chamber (F12Y; min. internal channel: 75 µm), Microfluidics, Newton, Massachusetts, USA) operated at 80 °C to obtain submicron-sized melted fat droplets. It was then left to cool to 20 °C over the course of ~ 6 h by turning off the water bath, during which the lipid crystallized. The resulting dispersions of colloidal lipid particles were stored at 4 °C (Figure 3.1., first step).

The particle size distribution and average diameters were measured by static light scattering (Malvern Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). The following optical properties were used: refractive indices of 1.540 (solid lipid) and 1.330 (water) with an absorption index of 0.01.

The hydrodynamic diameter (z-average) and the polydispersity index (PI) of the CLPs were determined by dynamic light scattering (Zetasizer Nano, Malvern, Worcestershire, UK) at 25 °C. Typically, the particle dispersions needed to be diluted 100 fold in 10 mM phosphate buffer at pH 7 to prevent multiple scattering.

Transmission electron microscopy (TEM) was performed on CLPs which were deposited onto a freshly glow discharged carbonized copper grid (200 mesh) from a 0.5% w/w CLP dispersion. The excess solvent was blotted using standard filter paper; the particles were stained using a 3% w/w ammonium molybdate solution in water at pH 6.8. Samples were imaged using a JEOL JEM1011 TEM (Peabody, USA) operating at 80 kV in combination with a 2K x 2K SIS Veleta camera. To create a tomogram, images were taken at every degree ranging from -60 to 60 in a JEOl 2100 TEM operated at 200 kV. Images were aligned and a tomogram was reconstructed using IMOD software, and visualized using Chimera.

The melting and crystallization behavior of the high melting point lipids either in the bulk phase or in the form of CLPs was investigated by differential scanning calorimetry (DSC) (Pyris Diamond DSC, Perkin-Elmer, DSC 8000, Norwalk, USA). Approximately 5 mg of bulk lipid material was placed in a stainless steel pan, after which it was sealed hermetically. An empty pan was used as reference. Both pans were heated from -10 °C to 75 °C at 1 °C min^{-1}, cooled down to -10 °C at 1 °C min^{-1}, after which the pans were held at -10 °C for 1 min, followed by two identical heating/cooling cycles. The CLP dispersions were diluted in phosphate buffer (10 mM, pH 7) to 0.125% w/w lipid prior to analysis, and were loaded into a VP-DSC (Microcal, Northampton, MA) in which the heat flux was measured relative to phosphate buffer. Each aliquot was heated from 5 °C to 70 °C at 1 °C min^{-1}, cooled down to 5 °C at 1 °C min^{-1}, followed by two identical heating/cooling cycles.

The non-adsorbed Tween 40 concentration in the aqueous phase of CLP dispersions was quantified by spectrophotometric analysis (Khossravi, Kao, Mrsny, & Sweeney, 2002). Freshly prepared (non-crystallized) CLP dispersions, cooled CLP dispersions, and a Tween 40 solution with known concentration were centrifuged using ultrafiltration-centrifugation units (Amicon cells, cut-off 100 kDa, Sigma Aldrich, Saint Louis, MO, USA) at 4000 g for 30 min at 40 °C (non-crystallized dispersions) or 4 °C (cooled dispersions and Tween 40 solution). The permeate (i.e., the aqueous phase of the dispersion) was recovered and 1 mL was added to 1 mL methanol. The solution was evaporated to dryness under nitrogen at 85 °C, then cooled down. Ammonium cobalt thiocyanate (ACTC) reagent was prepared by

dissolving 4.35 g of ammonium thiocyanate and 0.70 g of cobalt nitrate hexahydrate in 25 mL of a 10 mM phosphate buffer at pH 7.0 (Khossravi et al., 2002). Then 0.4 mL of ACTC reagent and 0.8 mL of methylene chloride were added to the dried samples. Subsequently, the samples were mixed and centrifuged at 2000 g for 15 min. The absorbance of the bottom methylene chloride layer was measured at 620 nm. A calibration curve was generated with Tween 40 solutions with known concentrations ranging from 0.005 to 0.04% w/w.

3.2.3. Preparation and characterization of O/W emulsions

Sunflower oil, stripped from surface-active impurities, was dispersed using CLP dispersions as the stabilizer to prepare O/W emulsions. This was done either by high pressure homogenization or manually. For the high pressure homogenization procedure, a primary emulsion was first formed by high speed stirring at 7000 rpm for 1 min using a rotor stator homogenizer (Ultra-turrax IKA T18 basic, Germany). This primary emulsion was passed through a high pressure homogenizer (Microfluidizer® Processor MF 110Y with Y-shaped interaction chamber (F12Y; min. internal channel: 75 µm), Microfluidics, Newton, Massachusetts, USA) at 400 bar for 5 cycles. Manual emulsion preparation implied forcefully shaking the emulsion by hand for 10 seconds in 15 mL centrifuge tubes (see Figure 3.1. for an overview). Emulsions were prepared with aqueous dispersions containing 1% w/w CLPs and 10% w/w oil, or with 5% w/w CLPs and 50% w/w oil, and stored at 4 °C.

The droplet size was measured by static light scattering (Malvern Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK) as described previously. The following optical properties were used: refractive indices of 1.465 (sunflower oil) and 1.330 (water) with an absorption index of 0.01.

The microstructure of the emulsions stabilized by CLPs was evaluated by light microscopy using a Carl Zeiss Axioscope A1 optical microscope equipped with a camera (AxioCam Mrc5) and polarization filters.

Confocal microscopy images of O/W emulsions stabilized by CLPs were obtained on a Zeiss Axiovert 200M with an LSM 5 exciter using a 100x oil immersion objective (Göttingen, Germany). For visualization of the CLPs the high melting point lipid was dyed by incorporation of 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) (0.5 mg/g), dissolved in the lipid prior to particle formation. The fluorescent dye was excited with the 488 nm line

of an argon laser and the fluorescence light was collected with a bandpass filter between 490-505 nm. Fluorescence recovery after photobleaching (FRAP) was used to study the mobility of the CLPs within the interfacial layer. The fluorescence of the interfacial layer was recorded with 50 images, and immediately after the area was photobleached by localized exposure to intense laser illumination; after this, 800 confocal fluorescence images were recorded. From this, the fluorescence recovery was deduced, using the background signal as a reference.

All measurements were repeated three times on samples that were prepared at least in duplicate in independent experiments. Results are presented as the average over these experiments including their standard deviation.

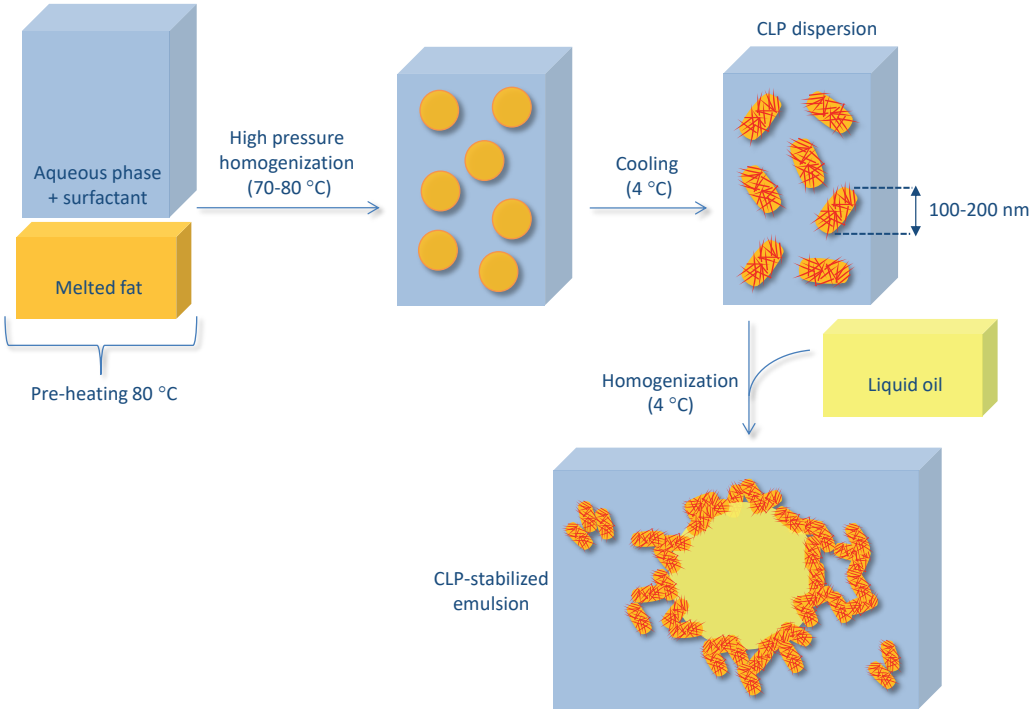


Figure 3.1. Schematic overview of the production process of CLP dispersions, and CLP-stabilized emulsions.

3.3. Results & discussion

To explore the use of CLPs with different design as Pickering stabilizers for O/W emulsions, we applied a hierarchical approach, which started with preparing CLPs by hot high pressure homogenization of high melting point fat (tripalmitin, tripalmitin/tricaprylin (4:1 w/w) or palm stearin) in an aqueous phase containing Tween 40. When no or a low surfactant concentration (< 2% w/w) was used, extensive aggregation and gelling took place when the system was allowed to cool down (Supporting information, Figure S3.1.). In order to prevent this, 2% w/w Tween 40 was chosen as a suitable concentration. Tween 40 is a food-grade surfactant and mainly contains palmitic acid (C16:0) as the hydrophobic tail, which is also the main fatty acid in tripalmitin and palm stearin. Therefore, Tween 40 hydrophobic tails can tightly align with alkyl chains of the fat phase and induce surfactant-templated crystallization, therewith mediating crystallization (Salminen, Gömmel, Leuenberger, & Weiss, 2016). After fat crystallization, CLP aqueous dispersions were homogenized with low melting point oil to make Pickering emulsions (Figure 3.1.). We first report on the physical characterization of CLPs, after which their ability to physically stabilize emulsions is discussed.

3.3.1. Particle characterization

The mean diameter of all three CLPs as determined by static light scattering was approximately 120 nm, with a fairly unimodal size distribution (Figure 3.2.A-C), which is not affected by fat crystallization. When using dynamic light scattering, we found that the hydrodynamic diameter of CLPs made of tripalmitin/tricaprylin (4:1 w/w) was slightly smaller than that of CLPs made of tripalmitin or palm stearin (~130 nm, vs. ~160 nm), which can be attributed to the lower viscosity of tricaprylin that facilitates droplet break-up (Supporting information, Table S3.3.).

While light scattering techniques allow us to measure the average diameter of the CLPs, they do not give insight into their morphology and structural organization; for that we used DSC melting thermograms and TEM. We were able to produce particles with distinct differences in morphology depending on their lipid composition (Figure 3.2.D-F). Tripalmitin CLPs had a lath-like morphology with a length of ~250 nm and a width of ~70 nm. Particles composed of a tripalmitin/tricaprylin mixture (4:1, w/w) existed as individual yet connected crystalline structures, also called crystallites or grains, and consisted of cubic or plate-like structures of ~70 to 300 nm in size (Supporting information, Figure S3.4.A). Palm stearin

CLPs showed a lath-like morphology comparable to that found for tripalmitin, but were slightly shorter with a smaller aspect ratio (length ~100 to 250 nm, and width ~70 to 100 nm). The sizes obtained by TEM and light scattering are in good agreement. In addition, DSC melting thermograms of CLPs showed a sequence of melting peaks corresponding to a complex layered structure within the particles, which melted in multiple identifiable events from the particle surface (Bunjes, Koch, & Westesen, 2000; Coupland, 2002; Unruh, Bunjes, Westesen, & Koch, 2001) (Figure 3.3.A-C). Such a physical organization was corroborated by the TEM images and reconstructed 3D tomogram (Supporting information S3.4.B and Figure S3.5.) that clearly showed a layered structure within the tripalmitin/tricaprylin-based CLPs.

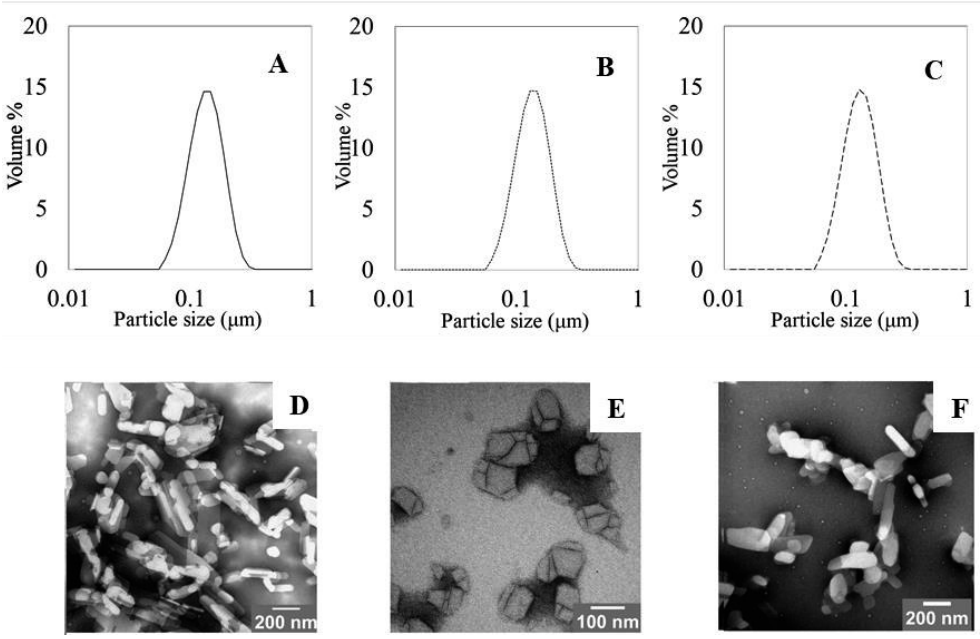


Figure 3.2. Characterization of tripalmitin (left), tripalmitin/tricaprylin (4:1 w/w) (middle) and palm stearin (right) CLP dispersions. Upper row: particle size distributions (specifics in the supporting information); Bottom row: TEM images.

All three CLPs were non-spherical, and have clear morphological differences related to the crystalline microstructure within the particles. To further investigate the crystal polymorphism we studied the cooling thermograms from DSC experiments, which showed that the three systems had different crystallization behavior, both as bulk fat phases and

dispersed as particles (Figure 3.3.D-F). For all three CLPs, the DSC profiles did not change over the tested storage period (2 months) (data not shown), indicating that the crystalline polymorph was at least metastable. Bulk tripalmitin showed a small exothermic peak at 43 °C followed by a main exothermic peak at 41 °C, most probably corresponding to the formation of β' and β-subcell crystals (see Supporting information, Figure S3.6.) (Helgason, Awad, Kristbergsson, McClements, & Weiss, 2009a; Rønholt, Mortensen, & Knudsen, 2013; Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013; Sato, 2001), while the corresponding CLPs showed two exothermic peaks, at ~24 and 29 °C, which are also expected to correspond to the needle or platelet shaped β' and β-subcell crystals (Helgason, Awad, Kristbergsson, McClements, & Weiss, 2009b). The bulk mixture of tripalmitin and tricaprylin (4:1 w/w) showed a broad crystallization peak with three maximums (at ~35, 36 and 37.5 °C) corresponding to several structural forms within the crystalline matrix, while the analogous CLPs show two broad exothermic peaks at ~16 and 25 °C, most probably corresponding to β' and β-subcell crystals. Bulk palm stearin showed two exothermic peaks at ~36 and 38 °C, which may suggest again that β' and β polymorphisms coexisted, while CLPs show β' and β-subcell crystallization peaks at ~21 and 24 °C, respectively.

These results showed that crystalline domains were formed within all particles, present in two polymorphic forms that also existed in the bulk. Interestingly, we noted a strong super-cooling effect within the nanoparticles, as the crystallization temperature of the CLPs was shifted by ~13 to 20 °C as compared to the bulk lipid, which is in accordance with previous studies (Bunjes, Westesen, & Koch, 1996; Westesen & Bunjes, 1995). This may be due to two effects: first, in bulk fat, nuclei formation is heterogeneous, due to the presence of impurities which may act as crystal templates (Chen, McClements, & Decker, 2011; Yu, Lin, Yu, & Liu, 2015), but when fat is emulsified into submicron-sized droplets, the probability that an impurity is present is dramatically decreased, and consequently crystallization in the CLPs is predominantly a result of homogeneous nucleation, which requires super-cooling. Secondly, the large curvature induced Laplace pressure in the sub-micron sized droplets decreases the nucleation rate and accordingly the crystallization temperature (Li, Donadio, & Galli, 2013). The crystallization peaks of CLPs are also broader compared to those of bulk lipids, especially in the case of the tripalmitin/tricaprylin system. This can be attributed to a decrease in crystalline order within the particles (Kovačević et al., 2014) due to confinement, which especially occurs for particles made of triglyceride blends.

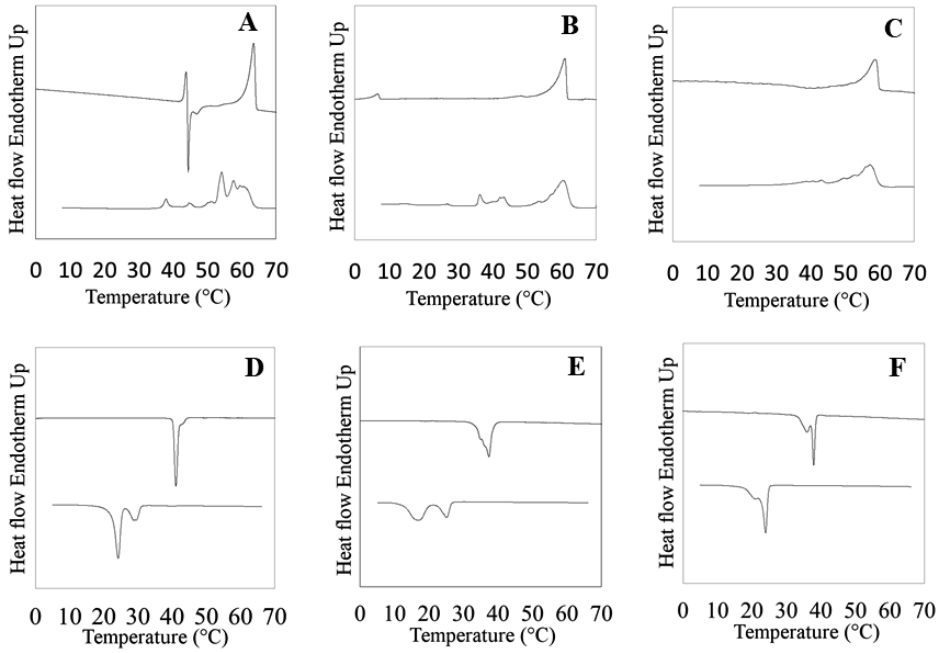


Figure 3.3. Characterization of tripalmitin (A, D), tripalmitin/tricaprylin (4:1 w/w) (B, E) and palm stearin (C, F) CLP dispersions. DSC thermograms of bulk material (top line) and CLPs (bottom line). Top row: Melting thermograms; Bottom row: Crystallization thermograms.

These results illustrate how the morphology of submicron-sized particles can be tailored by changing the composition of the lipid phase. Pure compounds, such as tripalmitin, form almost perfect needle or platelet shaped β' and β crystals, which results in long lath-like structures that may protrude through the particle surface (Douaire et al., 2014b; Leal-Calderon & Schmitt, 2008). In palm stearin CLPs (82% palmitic acid, 9% oleic acid, 5% stearic acid), some crystallographic mismatches occurred, eventually leading to shorter and wider colloidal structures composed mainly of β' sub-cell crystals (Yu et al., 2015). In CLPs made of mixed tripalmitin and tricaprylin (4:1 w/w), liquid and crystalline domains coexisted within the particles, resulting in a complex organization in the form of a layered nanostructure.

3.3.2. Formation and characterization of CLP-stabilized emulsions

To explore their efficacy in stabilizing emulsions, we started by emulsifying 10% w/w sunflower oil in an aqueous dispersion containing 1% w/w CLPs. All emulsions showed a

distinctive birefringent polarization ring at the droplet surface in polarized light microscopy images, indicating not only that CLPs adsorbed but that they maintained their crystallinity at the oil-water interface (Figure 3.4.A-F). The Pickering emulsions prepared with cubic tripalmitin/tricaprylin (4:1 w/w) CLPs appeared homogeneous at the macroscopic scale. Microscopically, a thin interfacial layer was formed (Figure 3.4.B&E); the oil droplets had a spherical shape and showed bridging (indicated by arrows). Bridging is known to occur in Pickering emulsion systems when the particles are largely hydrophilic, and occurs independent of particle concentration (Dickinson, 2010b). These data suggest that these particles adsorbed in a particulate monolayer.

By contrast, emulsions prepared with tripalmitin or palm stearin CLPs macroscopically separated into a phase containing a three-dimensional aggregated network of particles, entrapping many oil droplets, and an aqueous phase with little or no oil droplets (Figure 3.4.A&D, 3.4.C&F). In these systems, we observed that a considerable fraction of the droplets adopted and maintained a non-spherical shape. This suggests that CLPs strongly adsorbed at the interface and formed a jammed, cohesive interfacial network that prevented surface-tension driven shape relaxation of emulsion droplets (Tan, Zhang, Wang, Xu, & Sun, 2011).

The emulsions stabilized by tripalmitin CLPs were further characterized by CLSM (Figure 3.5.A-C), which revealed an aggregated tripalmitin CLP network fully covering the droplets, with a three dimensional CLP network in the continuous phase, that may enhance the physical stability of the emulsion (Dong et al., 2014). Fluorescence recovery after photobleaching (FRAP) experiments (Supporting information, Figure S3.7.) confirmed that the interfacial film was immobile, suggesting that the tripalmitin CLPs were irreversibly adsorbed and aggregated at the interface.

Our two-step emulsification process consisting of sequential particle preparation followed by liquid oil emulsification results in the formation of liquid oil droplets surrounded by a shell of crystallized CLPs, which can thus be described as Pickering emulsions. For comparison purposes, the same starting materials were processed through a one-step emulsification process, where tripalmitin (1% w/w), sunflower oil (10% w/w) and an aqueous phase containing 2% w/w Tween 40 were directly manually homogenized. In that case, tripalmitin separated from the emulsion upon fat crystallization (*i.e.,* big fat clumps were formed). No three-dimensional aggregated network was observed in the continuous

phase that could enhance emulsion physical stability (Supporting information, Figure S3.9.). Furthermore, fast coalescence and oiling-off took place. This clearly shows the added value and necessity of the two-step emulsification process for the formation of CLP-stabilized Pickering emulsions.

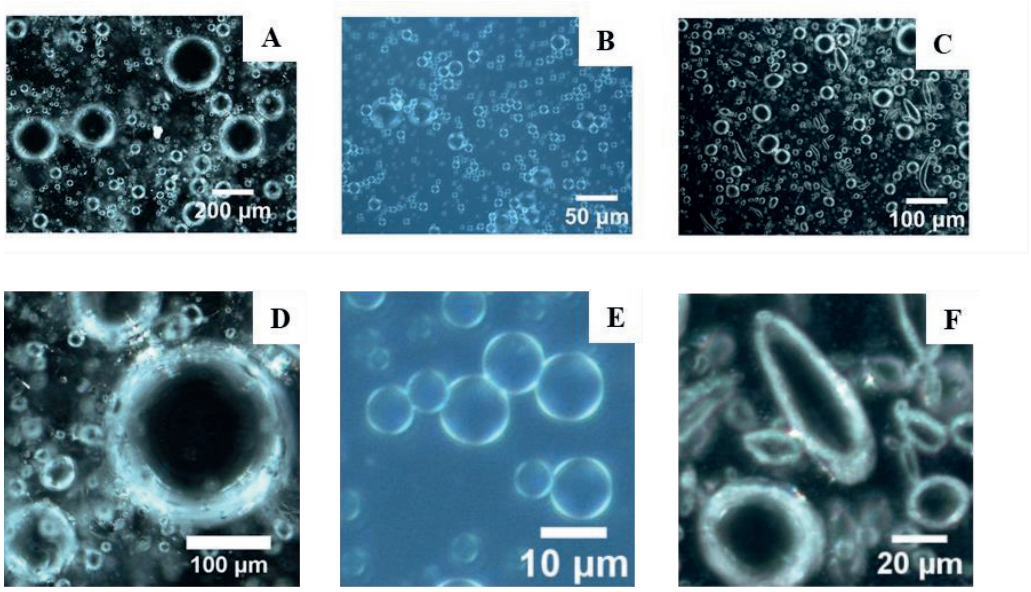


Figure 3.4. Polarized light images of emulsions produced by simple shaking with (A, D) tripalmitin, (B, E) tripalmitin/tricaprylin (4:1 w/w) (bridging between droplet is indicated by arrows) or (C, F) palm stearin CLPs.

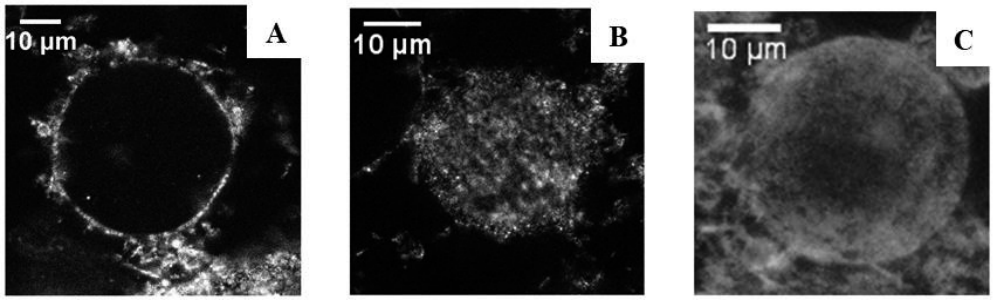


Figure 3.5. CLSM images of emulsions stabilized by tripalmitin CLPs dyed by incorporation of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate: (A) cross section, (B) surface and (C) 3D z-stack.

We can speculate on how the microstructural properties of the three types of CLPs affected their emulsion stabilization efficiency. During CLP solidification, the sub-micron sized fat droplets were converted into non-spherical particles, accompanied by a considerable increase in specific surface area, especially for lath-like CLPs. In order to make a quantitative estimation of this increase, the average surface area of spherical tripalmitin droplets (~120 nm) was compared to the average surface area of lath-like tripalmitin particles as imaged by TEM (*i.e.,* with length of ~ 220 nm and width of ~70 nm), assuming the volume remained constant. Accordingly, an increase of about 50% of surface area was found. In addition, the concentration of Tween 40 in the aqueous phase of the tripalmitin CLP dispersions was quantified before and after fat crystallization. Non-crystallized and crystallized CLP dispersions showed a non-adsorbed Tween 40 concentration of 0.61 ± 0.03% w/w and 0.51 ± 0.04% w/w, respectively. Thus the amount of Tween 40 adsorbed at the surface of CLPs increased only by ~7% upon crystallization, corresponding to surface coverages of 5.1 and 3.7 mg/m² for non-crystallized and crystallized CLPs. This shows that crystallization of CLPs resulted in a decrease in the interfacial Tween 40 concentration, which probably led to an increased hydrophobicity of the particles.

Tripalmitin/tricaprylin (4:1 w/w) CLPs were less subjected to deformation upon crystallization, and thus possibly more hydrophilic than tripalmitin and palm stearin CLPs. The higher hydrophilicity of tripalmitin/tricaprylin (4:1 w/w) CLPs could explain their ability to bridge interfaces of neighboring droplets, whereas the hydrophobic inter-particle interactions of tripalmitin and palm stearin CLPs could explain particle aggregation and network formation at the interface, and in the continuous phase (Westesen & Siekmann, 1997). This could, in turn, have facilitated the formation of a jammed interfacial structure that prevents relaxation of emulsion droplets (as reflected in the non-spherical shape of some emulsion droplets).

Another effect contributing to increasing particle hydrophobicity upon crystallization is the fact that fat crystals can also protrude throughout thin surfactant interfacial films (Douaire et al., 2014a; Fredrick, Walstra, & Dewettinck, 2010; Leal-Calderon & Schmitt, 2008; Whitby & Wanless, 2016). This may again promote hydrophobic inter-particle interactions. It is expected that the large needle or platelet shaped crystals in tripalmitin and palm stearin CLPs are able to penetrate the surface of neighboring particles, resulting in jammed, thick and strong interfacial films, and the formation of a solid network in the continuous phase

of the emulsion. Conversely, the smaller crystals in tripalmitin/tricaprylin (4:1 w/w) CLPs can hardly induce jamming or network formation. Such fat particle-particle bridging is well known to occur when particles are covered with thin surfactant layers (Graca, Bongaerts, Stokes, & Granick, 2007), and this effect can actually be induced on purpose, such as in ice cream processing where surfactants are added to weaken the surface of protein covered fat droplets (Fuller, Considine, Golding, Matia-Merino, & MacGibbon, 2015; Fuller et al., 2014).

Finally, it should be pointed out that the fact that our CLPs are non-spherical can be an asset for emulsion stabilization: it has been reported that the detachment energy of rod and disk shaped particles (at contact angles of 90°) is about 32 and 58 % higher compared to spherical particles (Binks & Horozov, 2006; Tavernier, Wijaya, Van der Meeren, Dewettinck, & Patel, 2016b). The needle, lath or platelet shape of our CLPs is thus certainly an asset, and their degree of anisotropy can be conveniently tuned by the fat composition, opening perspectives for tailored design routes.

The fact that CLP-stabilized emulsions could be made in a reproducible manner by simple hand shaking (i.e., very low energy input) gave evidence that we had particles with remarkable ability to act as Pickering stabilizers, albeit that the emulsion droplets where large. To investigate whether Pickering emulsions with smaller droplet sizes could also be produced, we applied harsher emulsification conditions (high pressure homogenization) using 50% w/w oil and 5% w/w tripalmitin CLPs as stabilizers. The resulting emulsion was macroscopically homogeneous and viscous, and this may indicate that an interconnected network was formed, that stabilized the emulsion physically. The droplet size distribution (Figure 3.6.) shows two distinct peaks corresponding to the individual CLPs (~0.1 µm), and to emulsion droplets ranging from ~0.3 – 6.0 µm in size. Size did not change over more than 6 weeks, indicating that no coalescence or phase separation took place. Polarized light microscopy confirmed the presence of small oil droplets with adsorbed tripalmitin CLPs at the interface (Supporting information, Figure S3.8.).

Maybe even more importantly, this indicated that our CLP particles could be used in combination with high pressure homogenization. Many biobased particles cannot withstand high shear homogenization methods, therefore most of the previous studies on Pickering emulsions stabilized by biobased particles involve large emulsions droplets (at least a few tens of microns). When a small emulsion droplet size is targeted, alternatives to

true Pickering stabilization have been tested: for example nanostructured colloidosomes were produced by electrostatic deposition, where negatively charged solid lipid particles were adsorbed onto positively charged whey protein-stabilized micron-sized oil droplets (Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2016). It is clear that our approach is much more versatile as we do not have constrains regarding either the charge of our particles, the pH of the system, or the emulsification method used.

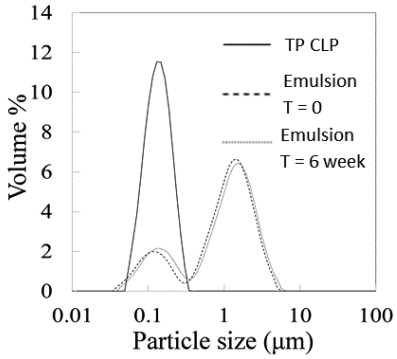


Figure 3.6. Particle size distribution of an emulsion prepared with an aqueous dispersion containing 5% w/w tripalmitin CLPs and 50% w/w oil produced by high pressure homogenization, fresh or after 6 weeks storage (the solid black line is the particle size distribution for the CLP dispersions).

3.4. Conclusions

In this chapter, we successfully developed CLPs as interface stabilizers for O/W emulsions. We show that particles could be modified by changing the composition of the lipid phase, resulting in particles with distinct morphology and crystalline structure. This influenced their performance as emulsion stabilizers, e.g., by tuning their wettability and affecting inter-particle interactions.

The interfacial structure of O/W emulsions stabilized by lath-like tripalmitin or palm stearin CLPs appeared as a jammed, fully covered, cohesive interfacial network that prevented relaxation of emulsion droplets into a spherical shape. By contrast, CLPs consisting of a mixture of tripalmitin and tricaprylin appeared as polycrystalline cubic or plate like structures with clear grain boundaries. They formed O/W emulsions with spherical and bridged droplets covered by a thin CLP layer.

The nanostructure of CLPs greatly influenced particle adsorption and emulsion structure, and could lead to emulsions with remarkable stability. CLPs are a promising novel type of Pickering stabilizers for O/W emulsions, due to their simplicity of preparation, tunable structure and properties, effectiveness in emulsion stabilization, and resistance against homogenization conditions.

3.5. Supporting information

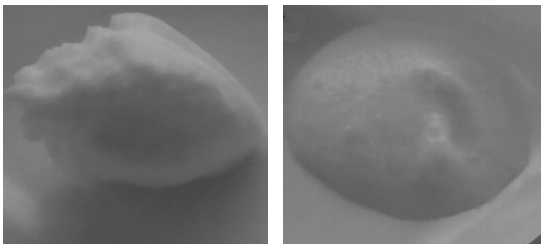


Figure S3.1. Tripalmitin CLP dispersions produced with 0.5% w/w (left) and 1% w/w (right) Tween40 in the aqueous phase.

Table S3.2. Fatty acid composition of palm stearin.

Component name	Percentage average
C14:0	1.16
C16:0	82.18
C18:0	5.12
C18:1	9.03
C18:2	1.83
C18:3	0.02
C20:0	0.32
C22:0	0.25
C22:1	0.04
C24:0	0.05

Table S3.3. Hydrodynamic diameter (z-average) measured by dynamic light scattering, and, D[4,3], D[3,2], D10, D50 and D90 measured by static light scattering of CLPs composed of tripalmitin, tripalmitin/tricaprylin 4:1, palm stearin.

Type of lipid	z-average (µm)	PdI	D[4,3] A (µm)	D[3,2] (µm)	D10 (µm)	D50 (µm)	D90 (µm)
Tripalmitin	0.162 ± 0.005	0.112 ± 0.02	0.130 ± 0.004	0.118 ± 0.003	0.082 ± 0.002	0.124 ± 0.004	0.184 ± 0.005
Tripalmitin/ tricaprylin (4:1)	0.130 ± 0.011	0.148 ± 0.02	0.131 ± 0.003	0.117 ± 0.001	0.080 ± 0.004	0.124 ± 0.002	0.191 ± 0.010
Palm stearin	0.158 ± 0.007	0.199 ± 0.02	0.133 ± 0.002	0.119 ± 0.002	0.081 ± 0.005	0.127 ± 0.000	0.195 ± 0.010

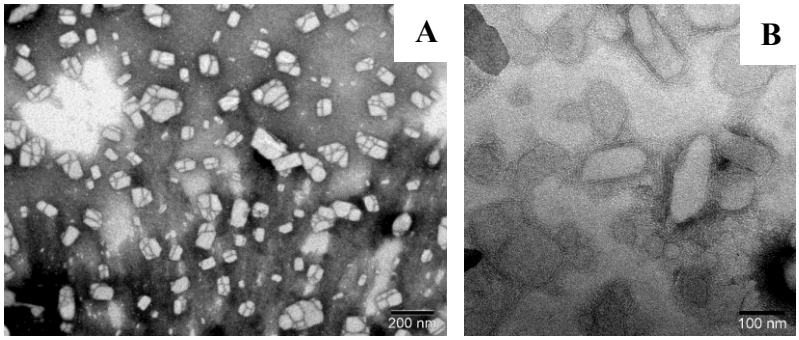


Figure S3.4. TEM images of tripalmitin/tricaprylin (4:1 w/w) CLPs.

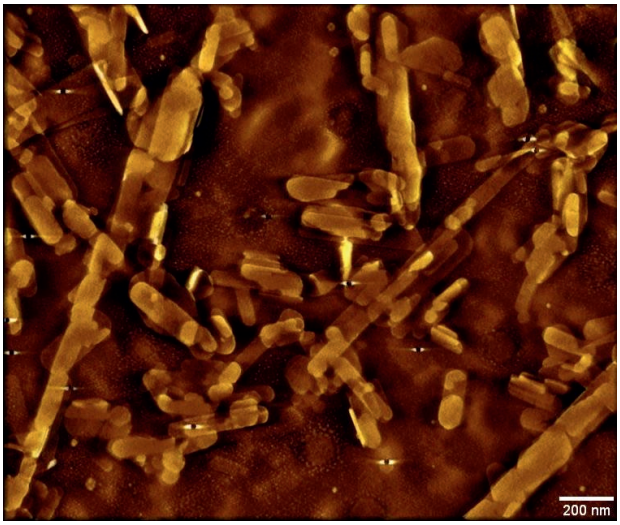


Figure S3.5. TEM 3D volume model from the tomogram of tripalmitin CLPs.

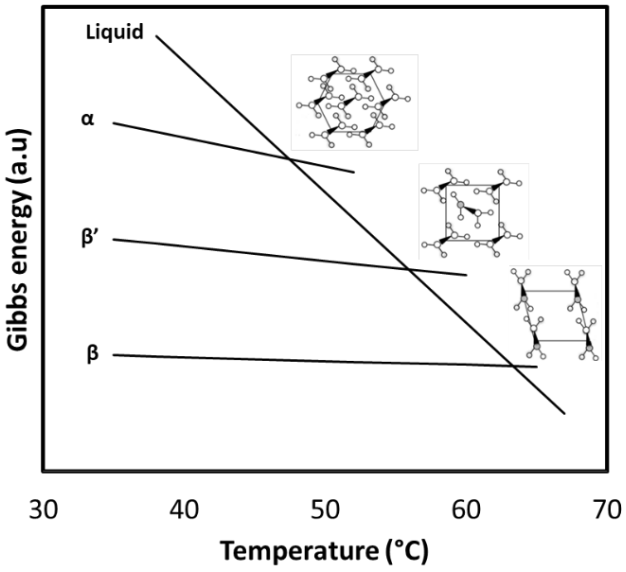


Figure S3.6. Structure models and Gibbs energy (G)–temperature relationship of three polymorphs of tripalmitin. Addapted from Sato, 2001 and Rønholt, Mortensen, & Knudsen, 2013.

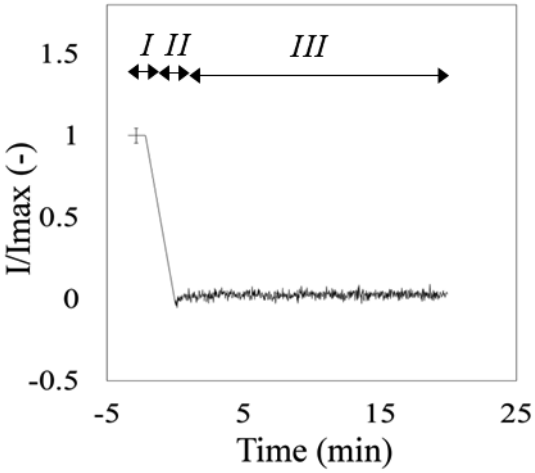


Figure S3.7. Fluorescence recovery after photobleaching curve of an emulsion stabilized by tripalmitin CLPs, showing the normalized fluorescence intensity before bleaching (I), during bleaching (II) and during recovery (III).

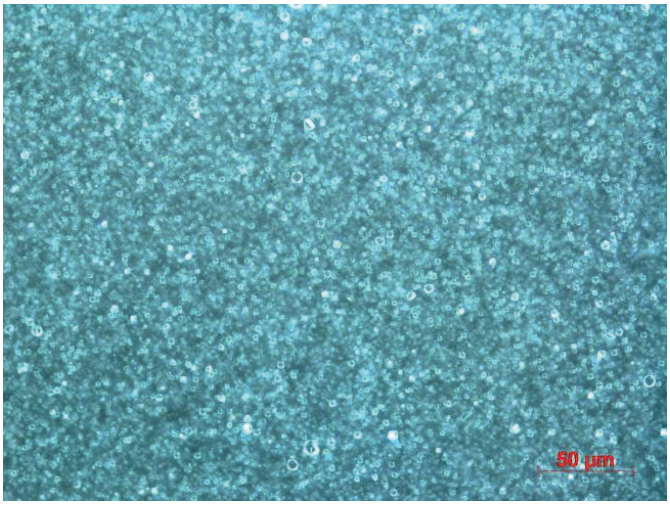


Figure S3.8. Polarized light image of an O/W emulsion produced by high pressure homogenization with 50% w/w oil and 4.5% w/w tripalmitin CLPs.

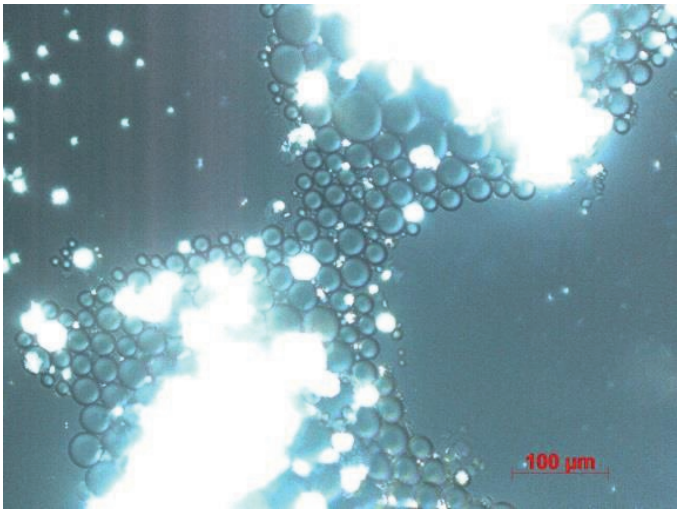


Figure S3.9. Polarized light image of an O/W emulsion produced wth a one-step emulsification process, where tripalmitin (1% w/w), sunflower oil (10% w/w) and an aqueous phase containing 2% w/w Tween 40 were directly manually homogenized.

3.6. References

Aveyard, R., Binks, B. P., & Clint, J. H. (2003). Emulsions stabilised solely by colloidal particles. Advances in Colloid and Interface Science, 100–102(SUPPL.), 503–546.

Berton-Carabin, C. C., & Schroën, K. (2015). Pickering emulsions for food applications: background, trends, and challenges. Annual Review of Food Science and Technology, 6, 263–297.

Berton, C., Genot, C., & Ropers, M.-H. (2011). Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. Journal of Colloid and Interface Science, 354(2), 739–748.

Binks, B., & Horozov, T. (2006). Colloidal particles at liquid interfaces: an introduction. In Colloidal Particles at Liquid Interfaces, ….

Binks, B. P. (2002). Particles as surfactants—similarities and differences. Current Opinion in Colloid & Interface Science, 7(1–2), 21–41.

Binks, B. P., & Kirkland, M. (2002). Interfacial structure of solid-stabilised emulsions studied by scanning electron microscopy. Physical Chemistry Chemical Physics, 4(15), 3727–3733.

Binks, B. P., & Lumsdon, S. O. (2001). Pickering emulsions stabilized by monodisperse latex particles: Effects of particle size. Langmuir, 17(15), 4540–4547.

Brun, M., Delample, M., Harte, E., Lecomte, S., & Leal-Calderon, F. (2015). Stabilization of air bubbles in oil by surfactant crystals: A route to produce air-in-oil foams and air-in-oil-in-water emulsions. Food Research International, 67, 366–375.

Bunjes, H., Koch, M. H. J., & Westesen, K. (2000). Effect of particle size on colloidal solid triglycerides. Langmuir, 16, 5234–5241.

Bunjes, H., Westesen, K., & Koch, M. H. J. (1996). Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. International Journal of Pharmaceutics, 129(1–2), 159–173.

Chen, B., McClements, D. J., & Decker, E. A. (2011). Minor components in food oils: A critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. Critical Reviews in Food Science and Nutrition, 51(January 2014), 901–916.

Coupland, J. N. (2002). Crystallization in emulsions. Current Opinion in Colloid and Interface Science, 7(5–6), 445–450.

Destribats, M., Rouvet, M., Gehin-Delval, C., Schmitt, C., & Binks, B. P. (2014). Emulsions stabilised by whey protein microgel particles : towards food-grade Pickering emulsions. Soft Matter, 10, 6941–6954.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. Current Opinion in Colloid and Interface Science, 15(1–2), 40–49.

Dokića, L., Krstonošićb, V., & Nikolića, I. (2012). Physicochemical characteristics and stability of oil-in-water emulsions stabilized by OSA starch. Food Hydrocolloids, 29(1) 185-192.

Dong, J., Worthen, A. J., Foster, L. M., Chen, Y., Cornell, K. A., Bryant, S. L., … Johnston, K. P. (2014). Modified montmorillonite clay microparticles for stable oil-in-seawater emulsions. ACS Applied Materials and Interfaces, 6(14), 11502–11513.

Douaire, M., Di Bari, V., Norton, J. E., Sullo, A., Lillford, P., & Norton, I. T. (2014a). Fat crystallisation at oil-water interfaces. Advances in Colloid and Interface Science, 203, 1–10.

Finkle, P., Draper, H. D., & Hildebrand, J. H. (1923). The theory of emulsification. Journal of the American Chemical Society, 45(12), 2780–2788.

Fredrick, E., Walstra, P., & Dewettinck, K. (2010). Factors governing partial coalescence in oil-in-water emulsions. Advances in Colloid and Interface Science, 153(1–2), 30–42.

Frelichowska, J., Bolzinger, M.-A., Pelletier, J., Valour, J.-P., & Chevalier, Y. (2009). Topical delivery of lipophilic drugs from o/w Pickering emulsions. International Journal of Pharmaceutics, 371(1–2), 56–63.

Fuller, G. T., Considine, T., Golding, M., Matia-Merino, L., & MacGibbon, A. (2015). Aggregation behavior of partially crystalline oil-in-water emulsions: Part II - Effect of solid fat content and interfacial film composition on quiescent and shear stability. Food Hydrocolloids, 51, 23–32.

Fuller, G. T., Considine, T., Golding, M., Matia-Merino, L., MacGibbon, A., & Gillies, G. (2014). Aggregation behavior of partially crystalline oil-in-water emulsions: Part I - Characterization under steady shear. Food Hydrocolloids, 43, 521–528.

Ghosh, S., & Rousseau, D. (2011). Fat crystals and water-in-oil emulsion stability. Current Opinion in Colloid & Interface Science, 16(5), 421–431.

Graca, M., Bongaerts, J. H. H., Stokes, J. R., & Granick, S. (2007). Friction and adsorption of aqueous polyoxyethylene (Tween) surfactants at hydrophobic surfaces. 315, 662–670.

Gupta, R., & Rousseau, D. (2012). Surface-active solid lipid nanoparticles as Pickering stabilizers for oil-in-water emulsions. Food & Function, 3(3), 302.

Helgason, T., Awad, T. S., Kristbergsson, K., McClements, D. J., & Weiss, J. (2009a). Effect of surfactant surface coverage on formation of solid lipid nanoparticles (SLN). Journal of Colloid and Interface Science, 334(1), 75–81.

Horozov, T. S., & Binks, B. P. (2006). Particle-stabilized emulsions: A bilayer or a bridging monolayer? Angewandte Chemie - International Edition, 45(5), 773–776.

Khossravi, M., Kao, Y., Mrsny, R. J., & Sweeney, T. D. (2002). Analysis methods of polysorbate 20 : A new method to assess the stability of p ... 19(May), 634–639.

Kotula, A. P., & Anna, S. L. (2012). Probing timescales for colloidal particle adsorption using slug bubbles in rectangular microchannels. Soft Matter, 8(41), 10759.

Kovačević, A. B., Müller, R. H., Savić, S. D., Vuleta, G. M., & Keck, C. M. (2014). Solid lipid nanoparticles (SLN) stabilized with polyhydroxy surfactants: Preparation, characterization and physical stability investigation. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 444, 15–25.

Kurukji, D., Pichot, R., Spyropoulos, F., & Norton, I. T. (2013). Interfacial behaviour of sodium stearoyllactylate (SSL) as an oil-in-water pickering emulsion stabiliser. Journal of Colloid and Interface Science, 409, 88–97.

Lam, S., Velikov, K. P., & Velev, O. D. (2014). Pickering stabilization of foams and emulsions with particles of biological origin. Current Opinion in Colloid and Interface Science, 19(5), 490–500.

Leal-Calderon, F., & Schmitt, V. (2008). Solid-stabilized emulsions. Current Opinion in Colloid and Interface Science, 13(4), 217–227.

Li, T., Donadio, D., & Galli, G. (2013). Ice nucleation at the nanoscale probes no man's land of water. Nature Communications, 4(May), 1–6.

Liu, F., & Tang, C.-H. (2013). Soy protein nanoparticle aggregates as pickering stabilizers for oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 61(37), 8888–8898.

Liu, F., & Tang, C.-H. (2014). Phytosterol colloidal particles as Pickering stabilizers for emulsions. Journal of Agricultural and Food Chemistry, 62(22), 5133–5141.

Lucassen, J. (1992). Capillary forces between solid particles in fluid interfaces. Colloids and Surfaces, 65(2–3), 131–137.

Luo, Z., Murray, B. S., Yusoff, A., Morgan, M. R. a, Povey, M. J. W., & Day, A. J. (2011). Particle-stabilizing effects of flavonoids at the oil-water interface. Journal of Agricultural and Food Chemistry, 59(6), 2636–2645.

Marku, D., Wahlgren, M., Rayner, M., Sjöö, M., & Timgren, A. (2012). Characterization of starch Pickering emulsions for potential applications in topical formulations. International Journal of Pharmaceutics, 428(1–2), 1–7.

Monteillet, H., Workamp, M., Li, X., Schuur, B., Kleijn, J. M., Leermakers, F. A. M., & Sprakel, J. (2014). Multi-responsive ionic liquid emulsions stabilized by microgels. Chemical Communications, 50(81), 12197–12200.

Pawlik, A., Kurukji, D., Norton, I., & Spyropoulos, F. (2016). Food-grade Pickering emulsions stabilised with solid lipid particles. Food Funct., 2712–2721.

Pickering, S. (1907). Emulsions. Journal of the Chemical Society, Transactions, translated 2001-2021.

Ramsden, W. (1903). Separation of Solids in the Surface-layers of Solutions and "suspensions" (observations on Surface-membranes, Bubbles, Emulsions, and Mechanical Coagulation). Proceedings of the Royal Society of London, 72, 156–164.

Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P., & Wahlgren, M. (2014). Biomass-based particles for the formulation of Pickering type emulsions in food and topical applications. Colloids and Surfaces A, 458, 48–62.

Rayner, M., Sjöö, M., Timgren, A., & Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of Pickering emulsions. Faraday Discussions, 158, 139.

Rønholt, S., Mortensen, K., & Knudsen, J. C. (2013). The effective factors on the structure of butter and other milk fat-based products. Comprehensive Reviews in Food Science and Food Safety, 12(5), 468–482.

Salminen, H., Gömmel, C., Leuenberger, B. H., & Weiss, J. (2016). Influence of encapsulated functional lipids on crystal structure and chemical stability in solid lipid nanoparticles : Towards bioactive-based design of delivery systems. Food Chemistry, 190, 928–937.

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2013). Formation of solid shell nanoparticles with liquid ω-3 fatty acid core. Food Chemistry, 141(3), 2934–2943.

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2016). Formation of nanostructured colloidosomes using electrostatic deposition of solid lipid nanoparticles onto an oil droplet interface. Food Research International, 79, 11–18.

Sato, K. (2001). Crystallization behaviour of fats and lipids — a review. Chemical Engineering Science, 56(7), 2255–2265.

Schröder, A., Corstens, M., Ho, K., Schroën, K., & Berton-Carabin, C. (2018). Pickering emulsions for delivery of bioactive compounds in foods. In S. Roohinejad, R. Greiner, I. Oey, & J. Wen (Eds.), Emulsion-based systems for delivery of food active compounds: Formation, application, health and safety. John Wiley & Sons.

Tan, J., Zhang, M., Wang, J., Xu, J., & Sun, D. (2011). Journal of Colloid and Interface Science Temperature induced formation of particle coated non-spherical droplets. Journal of Colloid And Interface Science, 359(1), 171–178.

Tavernier, I., Wijaya, W., Van der Meeren, P., Dewettinck, K., & Patel, A. R. (2016). Food-grade particles for emulsion stabilization. Trends in Food Science and Technology, 50, 159–174.

Tcholakova, S., Denkov, N. D., & Lips, A. (2008). Comparison of solid particles, globular proteins and surfactants as emulsifiers. Physical Chemistry Chemical Physics : PCCP, 10(12), 1608–1627.

Thompson, K. L., Williams, M., & Armes, S. P. (2014). Colloidosomes: Synthesis, properties and applications. Journal of Colloid and Interface Science, 447, 217–228.

Timgren, A., Rayner, M., Dejmek, P., & Marku, D. (2013). Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride.

Unruh, T., Bunjes, H., Westesen, K., & Koch, M. H. J. (2001). Investigations on the melting behaviour of triglyceride nanoparticles. Colloid and Polymer Science, 279(4), 398–403.

Westesen, K., & Bunjes, H. (1995). Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? International Journal of Pharmaceutics, 115(1), 129–131.

Westesen, K., & Siekmann, B. (1997). Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. International Journal of Pharmaceutics, 151, 35–45.

Whitby, C. P., & Wanless, E. J. (2016). Controlling pickering emulsion destabilisation: A route to fabricating new materials by phase inversion. Materials, 9(8).

Ye, A., Zhu, X., & Singh, H. (2013). Oil-in-water emulsion system stabilized by protein-coated nanoemulsion droplets. Langmuir, 29(47), 14403–14410.

Yu, R., Lin, N., Yu, W., & Liu, X. Y. (2015). Crystal networks in supramolecular gels: formation kinetics and mesoscopic engineering principles. CrystEngComm, 17(42), 7986–8010.

Yusoff, A., & Murray, B. S. (2011). Modified starch granules as particle-stabilizers of oil-in-water emulsions. Food Hydrocolloids, 25(1), 42–55.

Zang, D., Stocco, A., Langevin, D., Wei, B., & Binks, B. P. (2009). An ellipsometry study of silica nanoparticle layers at the water surface. Physical Chemistry Chemical Physics, 11(41), 9522–9529.

Chapter 4

Coalescence stability of Pickering emulsions produced with lipid particles: A microfluidic study

This chapter has been published as Schröder, A., Sprakel, J., Schroën, K., Spaen, J.N., and Berton-Carabin, C. C. (2018). Coalescence stability of Pickering emulsions produced with lipid particles: A microfluidic study. Journal of Food Engineering. Soft Matter. 234, 63-72.

Abstract

In the quest to find approaches to prepare food-grade Pickering emulsions, we studied the formation and stability to coalescence of colloidal lipid particle (CLP)-stabilized emulsions within a cross-flow microfluidic device. We show that the particles can either stabilize or destabilize the emulsions depending on the particle adsorption rate versus droplet formation rate, and on the resulting surface coverage when the droplet is formed. At low surface coverage, when droplet formation is significantly faster than adsorption, CLPs have a destabilizing effect as incomplete surface coverage leads to droplet-droplet bridging. At high surface coverage, the dense particle layer results in an effective barrier against droplet coalescence, resulting in physically stable emulsions. The observed non-monotonic dependency of emulsion droplet stability on surface coverage of CLP-stabilized emulsions is in stark contrast to what is observed for conventional surfactant-stabilized emulsions, and thus should be taken into account for the rational design of Pickering emulsions.

4.1. Introduction

In recent years, biocompatible and food-grade particles have raised a lot of interest for the application as emulsion stabilizers (Rayner et al., 2014). Moreover, these days there has been considerable empirical evidence that suitable Pickering particles exist in nature, can be purposely manufactured, or even have already been used unintentionally such as in mayonnaise (Binks, 2007; Firoozmand & Rousseau, 2016; Gould et al., 2013; Luo et al., 2011; A. Pawlik, Kurukji, Norton, & Spyropoulos, 2016b). For oil-in-water (O/W) emulsions, most reported particles are based on proteins (de Folter et al., 2012; Liu & Tang, 2013) or polysaccharides (Mikulcová, Bordes, & Kašpárková, 2016; Rayner, Sjöö, et al., 2012; Timgren et al., 2013; Yusoff & Murray, 2011).

Recently, we have shown that colloidal lipid particles (CLPs) can be used as Pickering stabilizers in O/W emulsions, leading to remarkable physical stability compared to conventional emulsifiers (Schröder, Sprakel, Schroën, & Berton-Carabin, 2017). CLPs are especially promising as they are simple to prepare, have a tunable microstructure, and can be used in combination with high pressure homogenization to target for a small emulsion droplet size, and this entails the interest to gain knowledge on how such particles form and stabilize emulsions (Chevalier & Bolzinger, 2013a; Fouilloux, Malloggi, Daillant, & Thill, 2016; Monteux et al., 2007).

The fact that solid particles can adsorb to liquid-liquid interfaces and confer excellent stability to emulsions (Aveyard et al., 2003; McGorty, Fung, Kaz, & Manoharan, 2010), has been known since the beginning of the 20th century from the pioneering work of Ramsden (Ramsden, 1903) and Pickering (Pickering, 1907), and is the main feature that makes solid particles af interest as Pickering stabilizers for biocompatible applications. This remarkable physical stability results from a change in the total interfacial energy upon particle adsorption, leading to a considerable decrease in free energy (ΔG_a):

$$\Delta G_a = \pi r^2 \gamma_{ow} (1-\cos\theta)^2 \qquad \text{(Eq. 4.1.)}$$

where *r* is the particle radius, γ_{ow} the interfacial tension between the two immiscible fluids and θ the three phase contact angle (Aveyard et al., 2003; Leal-Calderon & Schmitt, 2008). Providing that the particles can be wetted by both oil and water (i.e., θ is substantially different from 0° or 180°), the decrease in free energy typically exceeds the thermal energy by orders of magnitude, hence particle adsorption may be considered irreversible (Monteillet et al., 2014), as long as no additional components adsorb onto the particles or

at the liquid/liquid interface. Nevertheless, particle wettability is only one of many fundamental attributes to control the properties of particle-stabilized emulsions and to exploit the use of Pickering particles. Besides, particle size (distribution), shape, microstructure, concentration and inter particle interactions are crucial particle properties, though many properties are inter-related (Binks, 2002; Destribats et al., 2011; Dugyala et al., 2013).

Pickering particles should not only be able to adsorb, but the oil-water interface needs to be sufficiently covered by particles to form an effective barrier against droplet coalescence, i.e., to prevent the droplets to come in close contact with each other resulting and to merge into bigger droplets (Fouilloux et al., 2016; M. Pan, Lyu, & Tang, 2017). Often it is assumed that for such a barrier either monolayer or multilayer coverage is required, depending on particle characteristics (*e.g.,* size, shape, charge etc.) and conditions (pH, salt concentration etc.), although particular cases where the surface coverage is extremely low have also been reported (Figure 4.1.A) (Gautier et al., 2007). Interfacial coverage depends on electrostatic interactions , *i.e.,* when particles are neutral or slightly charged they pack more tightly compared to highly charged particles with a long-range dipolar moment (Deshmukh, van den Ende, Stuart, Mugele, & Duits, 2015; Destribats, Laurichesse, Tanner, & Leal-calderon, 2014). Furthermore, particles may form bridging monolayers, by embedding themselves within the interfaces of two droplets, thus keeping droplets at finite distance, while stabilizing the liquid film between droplets (Figure 4.1.B). The droplets may then even be stable to coalescence when the entire interfacial layer is closely enough packed (Horozov & Binks, 2006; Nagarkar & Velankar, 2012). As mentioned before, particles may also form a three dimensional network in the continuous phase, which enhances emulsion physical stability (Figure 4.1.C) (Firoozmand & Rousseau, 2016; Horozov & Binks, 2006).

A number of general aspects regarding the mechanisms of Pickering stabilization are not yet fully understood, for example, the dynamic adsorption behavior of colloidal particles at fluid interfaces (Garbin, Crocker, & Stebe, 2012). Adsorption of particles at fluid interfaces can either occur spontaneously, or significant energy barriers can be present as was mainly deduced from self-assembly studies on static flat interfaces. From those experiments it has been evidenced that electrostatic interactions between colloidal particles and the interface play an important role in particle adsorption: it is experimentally determined that bare air-water or oil-water interfaces are negatively charged - even though the origin of this negative

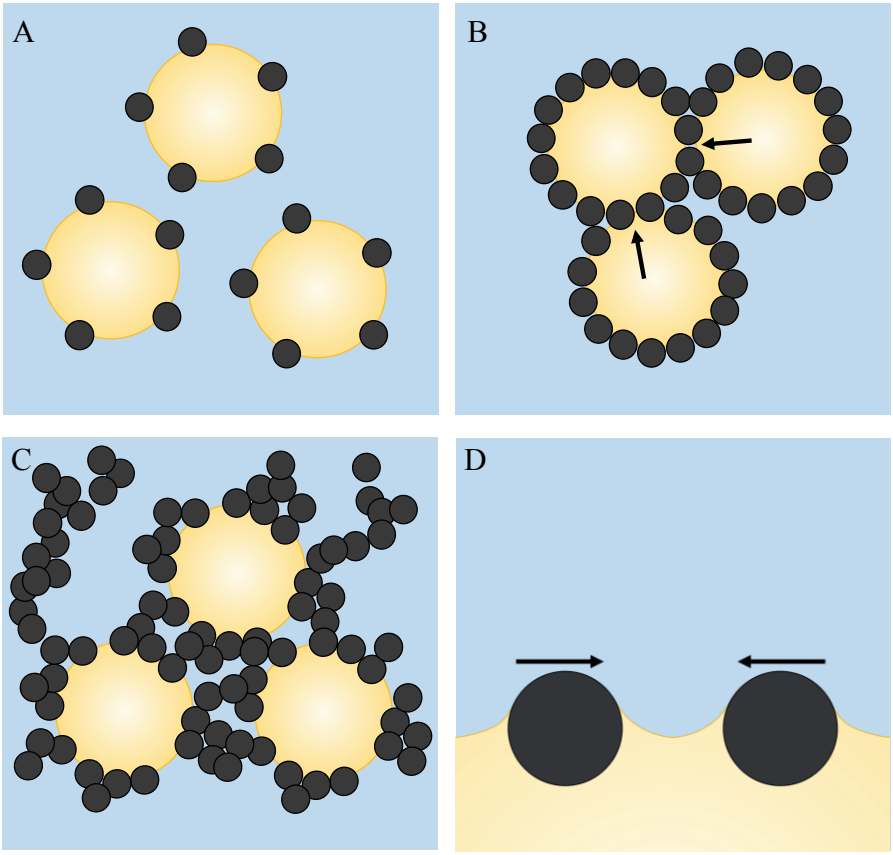


Figure 4.1. Schematic representation of Pickering particles at the surface and/or in the continuous phase of O/W emulsions: (A) Low surface coverage, (B) Monolayer surface coverage showing bridging (indicated by arrows), (C) Multilayer coverage and a 3D network of aggregated particles in the continuous phase, (D) Particle-particle interactions at the interface (capillary forces).

charge is still in debate - (Beattie & Gray-Weale, 2012; Roger & Cabane, 2012), and depending on the ionic strength negatively charged particles would adsorb only slowly, or not at all, while positively charged particles may adsorb readily (Deshmukh et al., 2015).

Additionally, particle adsorption requires particles to breach the oil-water interface, a process in which the liquid film that separates the colloidal particle and the fluid interface breaks (Kaz, Mcgorty, Mani, Brenner, & Manoharan, 2011). For this to occur, significant energy input is needed, which implies that particle adsorption is non-spontaneous (Tcholakova et al., 2008a). Other colloidal particle interactions at interfaces have also been

investigated; e.g., monodisperse spherical particles can penetrate and anchor in the interface without deforming it, whereas non-spherical particles have to deform the interface to adopt a constant contact angle resulting in capillary forces between the particles (Figure 4.1.D) (McGorty et al., 2010).

It is clear that particle adsorption rate, and, related to that surface coverage is critical to successful formation of a stable emulsion (Chevalier & Bolzinger, 2013a). Until now, adsorption rates were mainly investigated using model, inorganic particles (*i.e.,* monodispersed, solid, spherical) and in the absence of mechanical energy. However, the gained knowledge from these model systems has limited applicability for less well-defined particles, as would apply for practical applications in biocompatible systems (Kutuzov et al., 2007; Tcholakova et al., 2008a). Suitable particles are often highly polydisperse, non-spherical, and inhomogeneous in composition and structure.

In the present paper, we aim to gain understanding on how CLPs form and stabilize O/W emulsions. For this we use a microfluidic device (T-junction), which enables us to produce monodisperse O/W emulsions, to monitor the extent of droplet coalescence, and contributes to a better understanding of emulsification processes by decoupling droplet stability measurements from droplet formation (Krebs, Schroën, et al., 2012; Pan et al., 2017; Schroen, Bliznyuk, Muijlwijk, Sahin, & Berton-Carabin, 2015). The findings contribute to a better understanding of the dynamic conditions in which CLPs and presumably also other biocompatible and food-grade particles can be used as efficient Pickering stabilizers at for processing relevant length- and time-scales.

4.2. Materials & methods

4.2.1. Materials

Tripalmitin (#T8127, purity >99 %), sodium phosphate monobasic (#S9638), sodium phosphate dibasic (#S9763) and LUDOX® TM-40 colloidal silica (#420786) are purchased from Sigma-Aldrich (Saint Louis, MO, USA). Sodium caseinate is supplied by DMV International (#41610, spray dried, protein content 91.0%). Sunflower oil is obtained from a local supermarket, and stripped with alumina powder (MP EcoChrome™ ALUMINA N, Activity: Super I, Biomedicals) to remove impurities and tocopherols (Berton, Genot, & Ropers, 2011). Ultrapure water (18 MΩ) for all experiments is prepared using a Milli-Q

system (Millipore Corporation, Billerica, MA, USA). All other chemicals or solvents are of analytical grade.

4.2.2. Methods

4.2.2.1. Preparation of CLP dispersions

In brief, we heat an aqueous phase (95% w/w) containing 1% w/w sodium caseinate in phosphate buffer (10 mM, pH 7.0) at 80 °C in a water bath and add it to melted tripalmitin (5% w/w). A coarse emulsion is prepared by high speed stirring at 11,000 rpm for 1 min using a preheated rotor stator homogenizer (Ultra-turrax IKA T18 basic, Germany). We then repeatedly homogenize this coarse emulsion (Microfluidizer® Processor MF 110Y with Y-shaped interaction chamber (F12Y; minimum internal dimension: 75 µm), Microfluidics, Newton, Massachusetts, USA) at 800 bar (5 cycles) and 80 °C to obtain submicron-sized melted fat droplets, which are left to cool down to 20 °C over the course of ~ 6 h by turning off the water bath, allowing for the lipid phase to crystallize. All in all, we end with NaCas-coated CLPs in dispersion.

Excess sodium caseinate in the aqueous phase of the dispersion is removed by diafiltration at 2 bar and room temperature (Amicon® stirred cell, Merck Millipore, Darmstadt, Germany equipped with polyethersulfone membranes of 0.03 µm pore size from Sterlitech Corporation, US). Fresh buffer is added regularly to maintain the starting volume; the resulting CLP dispersion is stored at 4 °C.

Before use, we filter the CLP dispersion with 5-µm PVDF syringe filters (Millex SV, Millipore Corporation, Bedford, MA) to remove any agglomerates that may be formed after homogenization, and thus prevent clogging of the microfluidic circuit.

4.2.2.2. Preparation of O/W emulsions by high pressure homogenization

Sunflower oil (10% w/w), stripped from surface-active impurities, is mixed with a 1% w/w CLP dispersion to make a CLP-stabilized Pickering emulsion. First we prepare a coarse emulsion by high speed stirring at 7,000 rpm for 1 min using a rotor stator homogenizer (Ultra-turrax IKA T18 basic, Germany). This coarse emulsion is then repeatedly homogenized at 400 bar (5 cycles, to obtain small particles) using the previously described high pressure homogenizer, now cooled with ice water. In between each cycle, the sample is kept on ice water for 1 min to keep the emulsion at ~4 °C.

4.2.2.3. Particle size measurements

The particle and emulsion droplet size distribution and average diameters are measured by static light scattering (SLS) (Malvern Mastersizer 3000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). The following optical properties are used: refractive indices of 1.540 (solid tripalmitin), 1.465 (sunflower oil) and 1.330 (water) with an absorption index of 0.01.

4.2.2.4. Transmission electron microscopy

Transmission electron microscopy (TEM) is performed on CLP dispersions and CLP-stabilized emulsions (both diluted 10 fold using 10 mM phosphate buffer pH 7) deposited onto a freshly glown discharged carbonized copper grid (200 mesh). The excess solvent is blotted using standard filter paper; the particles are stained using an aqueous 1% w/w phosphotungstic acid solution. Images are recorded on a JEOL JEM1011 transmission electron microscope (Peabody, USA) operating at 80 kV in combination with a 2K x 2K SIS Veleta camera.

4.2.2.5. Differential scanning calorimetry (DSC)

The melting and crystallization behavior of the CLPs and bulk tripalmitin is investigated using a differential scanning calorimeter (Discovery Series DSC 250, TA Instruments, Zellik, Belgium). Approximately 25 µg of CLP dispersion or 2.5 µg of tripalmitin is placed in a Tzero pan and closed with a Tzero hermetic lid. An empty pan is used as a reference. Both pans are heated from 5 °C to 80 °C at 1 °C min^{-1}, then cooled down to 5 °C at 1 °C min^{-1} followed by two identical heating/cooling cycles. The thermograms are evaluated using TRIOS software. Two independent measurements are taken for each sample.

4.2.2.6. ζ-potential

The *ζ*-potential of CLPs is determined by measuring their electrophoretic mobility via laser Doppler velocimetry using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The CLPs are diluted ~100 times in a 10 mM phosphate buffer pH 7.0 to prevent multiple scattering. The measurements are performed at 25 °C and the results are expressed as the average from three independent samples.

4.2.2.7. Particle surface coverage

A 2-mL aliquot of CLP suspension is placed in an eppendorf tube and is centrifuged for 3 h at 20,000×g (Centrifuge 5424, Eppendorf Hamburg, Germany) to separate the aqueous phase containing non-adsorbed proteins from the CLPs. The aqueous phase is collected by

cautiously boring a hole in the tube and is centrifuged again for 3 h at 20,000×g to remove the remaining CLPs. We determine the protein content in the aqueous phase with the bicinchoninic acid (BCA) assay (Mallia et al., 1985) and we estimate the surface coverage based on the mean diameter of the CLPs ($d_{3,2}$) by calculating the difference between the total amount of protein used to prepare the CLP dispersion, and that of the aqueous phase after centrifugation. The measurements are expressed as the average of two independent samples.

4.2.2.8. Microfluidic experiment

Custom-designed borosilicate-glass microfluidic chips are purchased from Micronit Microtechnologies B.V. (The Netherlands). The chip consists of three channels: the T-junction, the meandering channel and the collision channel (Figure 4.2.). All three channels have a uniform depth of 45 µm. The T-junction has a width of 100 µm and the width and length of meandering channel and collision channel are 100 µm x 25.6 mm and 0.5 mm x 30.0 mm, respectively. The chip contains three inlets: one for the continuous phase (flow rate q_c), one for the dispersed phase (flow rate q_d), and one that remains unused. At the end of the collision channel, the chip contains one outlet (flow rate q_t).

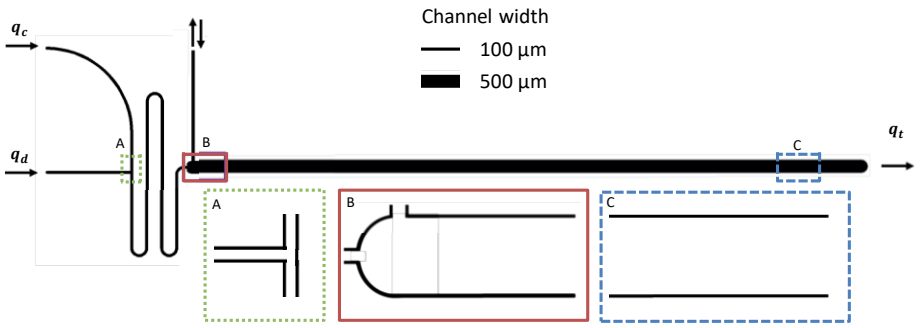


Figure 4.2. Layout of the microfluidic circuit with qc and qd denoting the flow rates of the continuous and dispersed phases at the respective inlets, and qt denoting the total flow rate at the outlet of the chip. The regions where images are recorded are indicated by rectangles: (A) T-junction, (B) inlet collision channel and (C) outlet collision channel.

We place the microfluidic chip in a chip holder (Micronit Microtechnologies B.V., The Netherlands), and connect the inlet and outlet sections of the chip to glass capillaries (ID

150, Polymicro Technologies) (Krebs, Schroën, et al., 2012; Muijlwijk, Hinderink, Ershov, Berton-Carabin, & Schroën, 2016). The dispersed phase capillary is connected to a glass syringe of 1 mL (SGE, Australia) of which the flow rate (1–3 µL min^{-1}) is controlled with a syringe pump (NE-300, Prosense, The Netherlands). The continuous phase capillary is connected to a plastic centrifuge (Falcon) tube of 15 mL of which the flow rate (30-110 µL min^{-1}) is controlled with an OBI1 MKII pressure & flow controller connected to a flow sensor (MFS 4, Elveflow, France).

Before use, we clean the microfluidic chip thoroughly to hydrophilize it. Briefly, we sonicate the chip for 10 min in a 5% v/v detergent solution (Decon 90, Decon Laboratories Ltd.), extensively rinsed with water, sonicate it in water for another 10 min, and dry it afterwards with pressurized air. We then bake out the chip in an oven at 500 °C for at least 2 h, and subsequently put it in an oxygen plasma cleaner (Zepto B Plasma Cleaner, Diener Electronic GmbH) for 10 min.

Oil droplets are formed at the T-junction where the continuous aqueous phase and dispersed phase come together. The formed droplets pass through the meandering channel and flow into the collision channel where they meet, possibly leading to coalescence. Droplet formation in the T-junction, and droplet interactions in the collision chamber is monitored with a high-speed camera (MotionPro Y4-S2, IDT, USA) connected to an inverted light microscope (Axiovert 200 MAT, Carl Zeiss, The Netherlands). During each experiment 3 movies of 500 images each are recorded at each spot in the chip (indicated by colored rectangles in Figure 4.2.) at frame rates of either 7000 frames sec^{-1} at the T-junction and inlet of the collision channel, or 30 frames sec^{-1} at the outlet of the collision channel. The recorded images are processed with ImageJ using a custom-written script (Krebs, Schroën, et al., 2012). The measurements are expressed as the average of two independent samples.

The mean number of coalescence events ($N_{coalescence}$) during the experiment is obtained by taking the initial mean droplet area (A_i) and the mean droplet area at the end of the collision channel (A_f) from all images according to Equation 4.2.

$$N_{Coalescence} = \frac{A_f}{A_i} - 1 \qquad \text{(Eq. 4.2.)}$$

The residence time inside the coalescence channel is calculated from the length of the coalescence channel, and the mean droplet velocity (Muijlwijk et al., 2017).

Emulsion microstructure and physical stability outside the microfluidic chip is evaluated by light microscopy using a Carl Zeiss Axioscope A1 optical microscope equipped with a camera (AxioCam Mrc5) and polarization filters to visualize crystal structures. For this, the formed emulsion is recovered at the outlet of the microfluidic chip, and small volumes are put on a microscopy slide and covered with a cover slip.

4.3 Results & discussion

4.3.1. Particle characterization

Our CLPs are produced by hot high pressure homogenization of the high melting point fat tripalmitin in an aqueous phase containing 1% w/w sodium caseinate (NaCas), and subsequent cooling. The particle size distribution of crystallized CLPs is unimodal with a mean particle diameter ($d_{3,2}$) of approximately 170 nm (Figure 4.3.A). TEM (Figure 4.3.B&C) reveals an almost spherical particle morphology, and particle sizes in the range of ~100-350 nm, which is in good agreement with the data shown in Figure 4.3.A.

The melting and crystallization properties of CLPs, which are of great importance during processing, provides information on particle microstructure and amongst others influences particle performance as emulsion stabilizers, is examined by DSC (Figure 4.3.D) and compared to those of bulk tripalmitin (Figure 4.3.E) (Schröder et al., 2017). The melting thermogram of the CLPs shows a small exothermic peak at ~42 °C, which may indicate that the crystals reorganize and recrystallize in a more stable crystalline form. This small exothermic peak is followed by a broad and much bigger endothermic melting peak with a maximum at ~62 °C that consists of a series of distinct smaller peaks indicative of several structured layers melting in multiple events (Zafeiri, Norton, Smith, Norton, & Spyropoulos, 2017).

The melting enthalpy of tripalmitin CLPs is lower than that of bulk tripalmitin (121 vs. 203 J g^{-1} of fat). This difference may point to a relatively lower degree of crystallinity in CLPs, and indicates that liquid domains or liquid crystal domains coexist in the crystalline particle matrix (Choi, Aditya, & Ko, 2014). Alternatively bulk tripalmitin may crystallize in different polymorphic forms with higher melting enthalpies compared to CLPs (Kellens, Meeussen, Riekel, & Reynaers, 1990), or polymorphic transitions take place during melting (Bunjes et al., 1996).

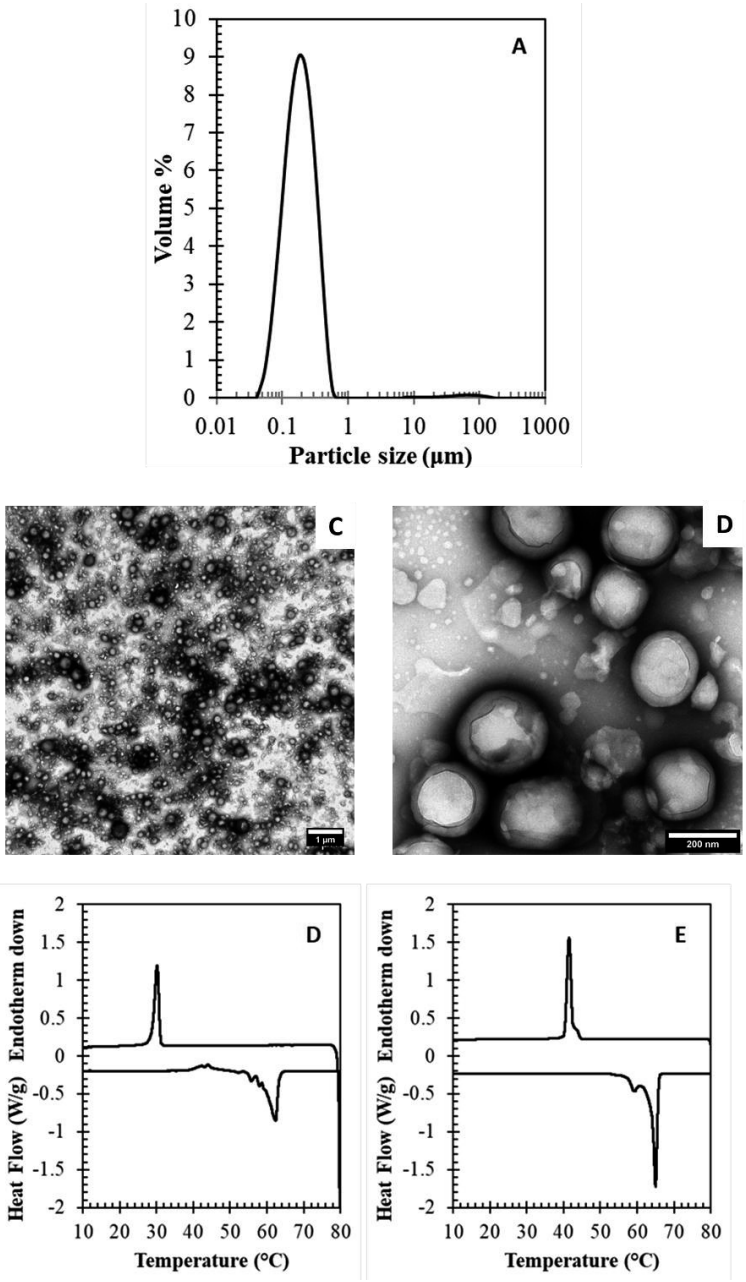


Figure 4.3. Characterization of a NaCas-coated CLPs. (A) Particle size distribution via static light scattering, (B&C) TEM micrographs of the CLPs, (D) DSC melting (bottom line) and crystallization (top line) thermogram of the CLP dispersion, and (E) DSC melting (bottom line) and crystallization (top line) thermogram of bulk tripalmitin.

The CLPs have one crystallization peak at 30 °C indicating that the tripalmitin crystallizes in one form, and that there is a strong supercooling (~12 °C compared to bulk tripalmitin). The probability that an impurity is present in submicron-sized droplets is very low, resulting in homogeneous nucleation instead of heterogeneous nucleation that occurs in the bulk that crystallizes much more readily (Bunjes et al., 1996). In addition, the small size of the lipid particles induces a large Laplace pressure that decreases the nucleation rate, and hence the crystallization temperature (Li et al., 2013). Besides differences in crystallization temperature, also the crystallization enthalpy is different for emulsified and bulk tripalmitin (107 vs. 126 J g^{-1}), which points to a lower degree of crystallinity for CLPs.

Since it is established that the surface properties of the particles play a large role in their ability and efficacy of stabilizing liquid interfaces, we also measure the effective surface charge through measurement of the particle electrophoretic mobility. We find that the CLPs exhibit a ζ-potential of -37 mV due to the presence of ionizable groups in NaCas at the surface of the particle, which induces some repulsion between the particles. The measured protein surface coverage of CLPs is 3.7 mg m^{-2}. This protein surface coverage corresponds to saturated monolayer to multilayer coverage and a film thickness of about 6-10 nm (Graham & Phillips, 1979a, 1979b), which makes the particle hydrophilic (θ < 90°), and may reduce jamming or network formation of the CLPs by fat crystals that protrude throughout the interfacial film (Douaire et al., 2014a).

4.3.2. Pickering emulsion characterization

We prepare O/W emulsions using high pressure homogenization (droplet size ~1 µm, which does not change over one week) to further evaluate the physical properties of our NaCas-coated CLPs and to determine if they could successfully be used as Pickering stabilizers. The emulsion shows a distinctive ring at the droplet surface in polarized light microscopy images (Figure 4.4.A&B), which indicates that the NaCas-coated CLPs are present at the oil-water interface, and retain their crystallinity.

The wettability of our CLPs can be qualitatively estimated from the three phase contact angle, θ, at the oil-water interface as visualized by TEM (Figure 4.3.C). θ is clearly < 90° for these hydrophilic particles as they mostly appear as laying onto the oil droplet surface, with a large part in the continuous phase (see arrows on Figure 4.4.C). From these observations,

we estimate that the corresponding desorption energy (Equation 4.1.) must be at least 10^5 k_bT (k_b is the Boltzmann constant and T is the absolute temperature) per particle, which makes thermally-activated desorption practically impossible, and the particles are thus very suitable to stabilize O/W emulsions.

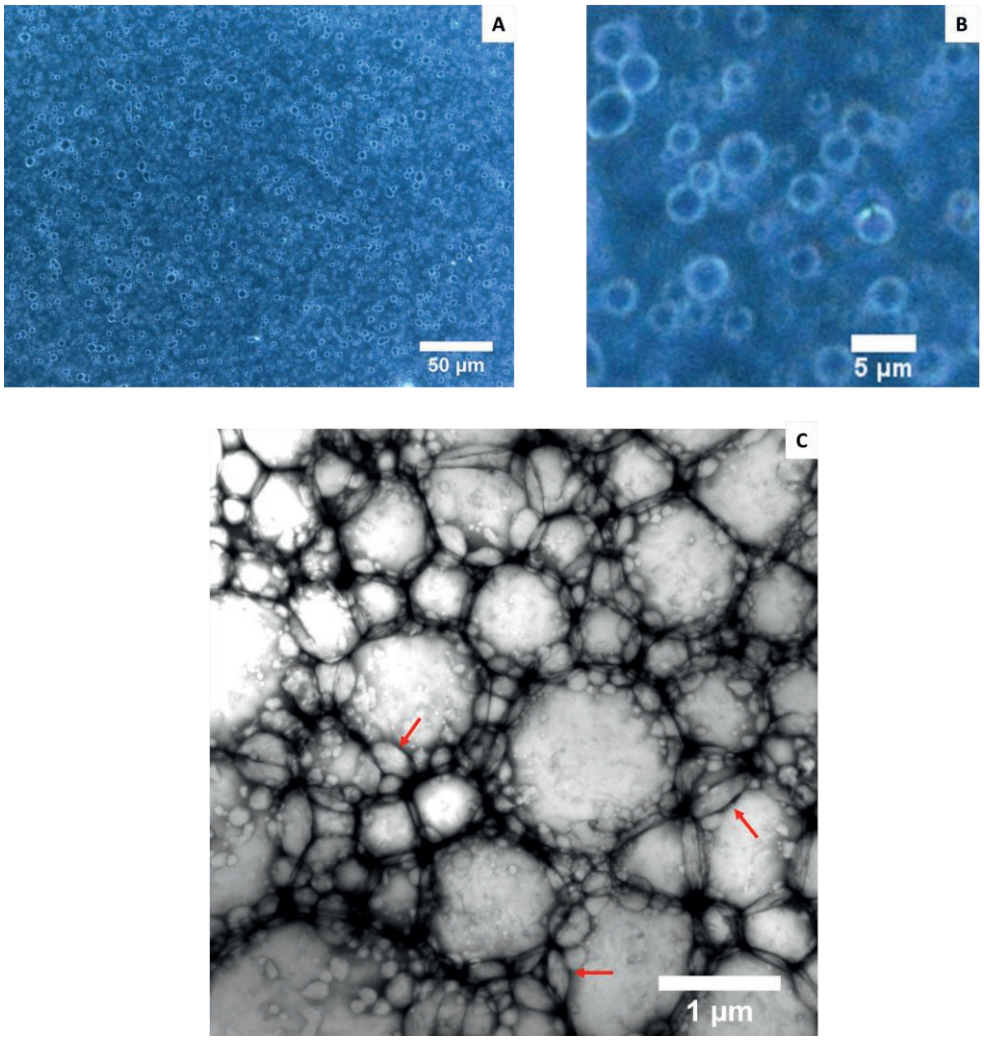


*Figure 4.4. Emulsion containing 1% w/w CLPs and 10% w/w sunflower oil produced by high pressure homogenization (400 bar, 5 passes) (**A, B**) Polarized light images (**C**) TEM micrograph. Arrows are pointing to particles that are clearly positioned with their largest part in the contineous phase.*

4.3.3. Emulsion formation and stability inside the microfluidic chip

In order to understand how CLPs form and stabilize O/W emulsions, we prepare O/W emulsions in a microfluidic T-junction using sunflower oil, stripped from surface-active impurities, and a 5% w/w CLP dispersion as the aqueous phase. As a reference, we prepare an emulsion with stripped sunflower oil and buffer, without CLPs. We vary the continuous phase flow rate (q_c, 50 -110 µL min^{-1}) and keep the dispersed phase flow rate (q_d, 1 µL min^{-1}) constant. This results in droplet formation times ranging from 0.6-3 ms and adsorption times from 6-135 ms, which are comparable to conditions used for emulsification processes carried out at industrial scale (Muijlwijk et al., 2017). The number of coalescence events is determined from the initial droplet size and the mean droplet size at the end of the collision channel (Figure 4.5.), following the procedure of Krebs, Schroën, et al., 2012.

4.3.4. Coalescence stability of emulsions stabilized with NaCas-coated CLPs

For emulsions formed with sunflower oil and buffer almost no coalescence events take place at all measured continuous phase flow rates as the residence time in the collision channel (varying from ~0.9 to 2.0 sec) is too short to destabilize the emulsion. However, this does not imply the emulsion is physically stable (see section: Emulsion stability outside the collision channel). The emulsions formed with CLPs show a coalescence dependency on continuous phase flow rate going from too high to be analyzed at $q_c < 50$ µL min^{-1}, to almost 0 between 50 and 70 µL min^{-1} (Figure 4.5.). Surprisingly, it thus appears that the presence of the particles can enhance coalescence as compared to systems in which no interfacial stabilizer is present. Hence, CLPs can have an active destabilizing effect on emulsion droplets and even induce coalescence. We can speculate regarding several causes for this unexpected effect; for example, it could be due to a depletion attraction between the fluid interfaces mediated by the particles in the continuous phase, or could also be the result of surface coverage effects.

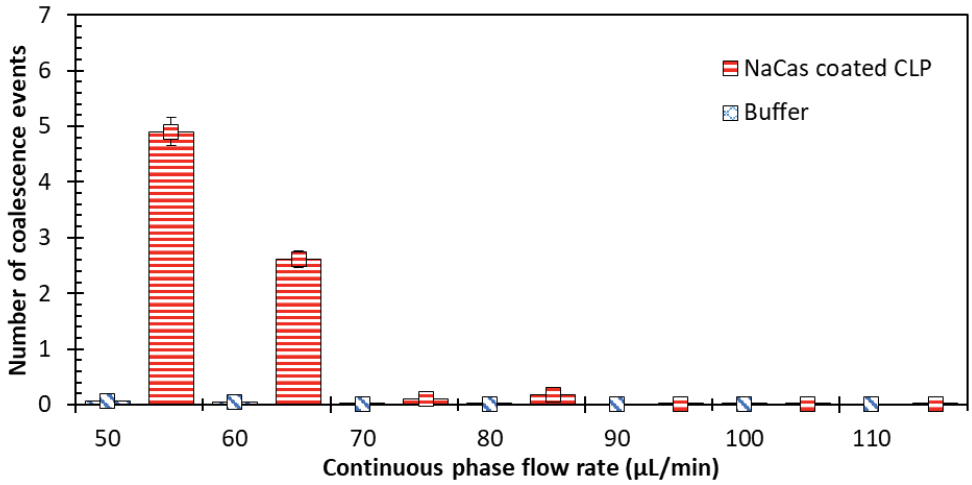


Figure 4.5. Number of coalescence events as function of continuous phase flow rate for sunflower O/W emulsions formed with either buffer or a NaCas-coated CLPs dispersion (5% w/w) as the continuous phase. The dispersed phase flow rate was 1 µL min^{-1}

Depletion attraction arises when small colloidal particles (in this case, the CLPs) are introduced to a suspension of larger ones (the emulsion droplets) due to the exclusion of the centre-of-mass of the small particles from a depletion zone surrounding the larger particles. For undeformable large particles, the attractive strength can be estimated as (Crocker, Matteo, Dinsmore, & Yodh, 1999):

$$\frac{U}{k_B T} = \frac{3}{2}\frac{a_e}{a_p}\Phi_s \qquad \text{(Eq. 4.3.)}$$

where $\frac{U}{k_B T}$ is the attraction strength in units of thermal energy k_BT, a_e and a_p are the radii of the emulsion droplets and the CLPs, respectively, and Φ_s is the volume fraction of the small objects (Laar, Klooster, Schroën, & Sprakel, 2016).

To test if depletion interactions can be responsible for the destabilizing effects we observe, we prepare O/W emulsions in the T-junction using sunflower oil, and add hydrophilic silica nanoparticles (Ludox TM-40, a_s~ 7 nm) as depletants at concentrations ranging from 0.4 to 40% w/w, at a continuous flow rate of 50 µL min^{-1}. These silica nanoparticles do not adsorb at the oil-water interface, but induce depletion attractions, whose magnitude is estimated between 22 to 2200 k_BT using Equation 4.3., which is about 1 to 100 times the attraction forces exerted by the CLPs at the concentrations we use (at 5% w/w in the aqueous phase). With these reference experiments we observe no coalescence at all tested silica particle

concentrations, such that we can rigorously exclude CLP-induced depletion attractions as the cause of emulsion destabilization in the presence of CLPs (Supporting information, Figure S4.1.).

A second explanation for emulsion destabilization by CLPs may be droplet-droplet bridging, due to a particle simultaneously adsorbing at the surface of two neighboring droplets (Monteillet et al., 2014), which is only possible when the surface of the oil droplets is incompletely covered. In O/W emulsions, bridging can occur when particles have dual wettability yet are largely hydrophilic, i.e., the three phase contact angle is considerably smaller than 90°, which is deduced for our CLPs based on TEM images (Figure 4.4.C).

Although bridging may occur at various surface coverage of the oil-water interface by particles, it is favored at low surface coverage, with the particles preferentially accumulating in the bridging region (Nagarkar & Velankar, 2012), leaving most of the droplet surface (the non-bridging region) nearly particle-free. This particle free surface can be subjected to disturbances, allowing the thin region, next to the bridging layer to rupture and subsequently the droplets to coalesce. Disturbances are strongly promoted under flow conditions, such as in our microfluidic setup, and do normally not occur at substantial rate under quiescent conditions (Nagarkar & Velankar, 2012). Hence, particles can actively destabilize emulsions due to bridging in circumstances of low surface coverage and in flow as coalescence proceeds when droplets are held in close proximity and insufficiently covered (Nagarkar & Velankar, 2012). Only when droplets become completely covered with (bridged) particles, the droplet surface may become immobile and coalescence is suppressed.

The CLPs have a high desorption energy which makes adsorption practically irreversible, but for them to breach the liquid film that separates particle from interface, in order to anchor into the oil-water interface an energy barrier must be overcome, resulting from both thermodynamic effects, such as a finite critical disjoining pressure, and hydrodynamic effects such as substantial lubrication forces. Overcoming this energy barrier can be facilitated by the application of mechanical forces, such as hydrodynamic forces that the particles experience in the flow field of the microfluidic T-junction. To explain the kinetics of particle adsorption, we presume that in the absence of any mechanical forcing, particle adsorption is thermally-activated following an Eyring process:

$$k_{ad} = \omega_0 * exp^{\frac{-E_a}{RT}} \quad \text{(Eq. 4.4.)}$$

in which k_{ad} is the adsorption rate, and ω the attempt frequency, typically set by the diffusion rate of particle towards the interface. In a fluid flow field, a particle experiences a drag force *F* exerted by the continuous phase, approximated by Stokes law as $F = 6\pi\eta r v$, in which η is the fluid viscosity, *r* the particle radius and *v* the flow velocity of the fluid relative to the particle (Dong, Zheng, Zhang, & Lin, 2014; Laar et al., 2016). We propose that these hydrodynamic forces exponentially enhance the adsorption rate in a Kramers process (Kramers, 1940):

$$k_{ad} = \omega_0 * exp^{\frac{-E_a + F\delta}{RT}} \quad \text{(Eq. 4.5.)}$$

where δ is a characteristic activation length, which is related to the thickness of the interfacial film that needs to break for particle adsorption. This would predict a significant enhancement of particle adsorption under the effect of fluid flow forces, thereby leading to exponentially increasing particle adsorption rates that ultimately may prevent coalescence. We also see an exponential decrease in coalescence events with increasing continuous phase flow rate, which lets us conclude that indeed at low adsorption rates and hence low surface coverage the CLPs destabilize the emulsion due to bridging, while at high adsorption rates, surface coverage is sufficiently high to prevent coalescence.

We note that with increasing q_c (50-110 µL min^{-1}), the droplet volume fraction in the collision channel decreases from ~2 to ~1 vol%, which may have reduced droplet collision probability. Furthermore, droplet size decreases from 96 to 57 µm (Supporting information, Figure S4.2.). These effects of droplet volume fraction and size leads to a decrease in the amount of oil-water interface (0.25 vs 0.19 m² per 100 mL emulsion for a q_c of 50 or 110 µL min^{-1}, respectively) (Supporting information, Figure S4.2.). Additionally, the amount of available particles increases slightly due to the change in oil fraction (4.90 - 4.95 % w/w per 100 mL; Supporting information, Figure S4.2.). This leads us to conclude that in our experiments the amount of particles available per interfacial area varies from 20 to 26 gram of particles per m^2. Although this difference is small, and particles are in large excess in all cases, it may contribute to droplet stability, and is investigated further.

4.3.5. Effect of particle concentration and continuous phase flow rate

We dilute our CLP dispersion (0.005% w/w to 5% w/w) and monitor the number of droplet coalescence events at continuous phase flow rates above the previously determined threshold for coalescence (q_c of 90, 100 and 110 µL min^{-1}, Figure 4.6.). In general, we

observe that droplet stability increases with increasing particle concentration and q_c. Emulsions formed with either buffer or a 5% w/w CLP dispersion do not show coalescence events. This dependency on concentration confirms the lack of a substantial role of depletion forces for emulsion destabilization. Interestingly, at q_c of 100 and 110 µL min^{-1}, emulsions formed with 0.05% w/w CLPs in the aqueous phase show more coalescence events than the ones formed with 0.005% w/w CLPs, which may reveal a non-monotonic dependency of particle concentration on emulsion stability. At very low particle concentration, the droplets do not coalesce because of a low bridging probability, while at intermediate concentration, instability occurs due to a greater droplet-droplet bridging probability. At even higher particle concentrations, droplet stability increases due to the formation of a protective particle layer around the emulsion droplets. In order to shed more light on emulsion physical stability vs surface coverage, we investigate samples collected from the coalescence chamber, of which the results are shown next.

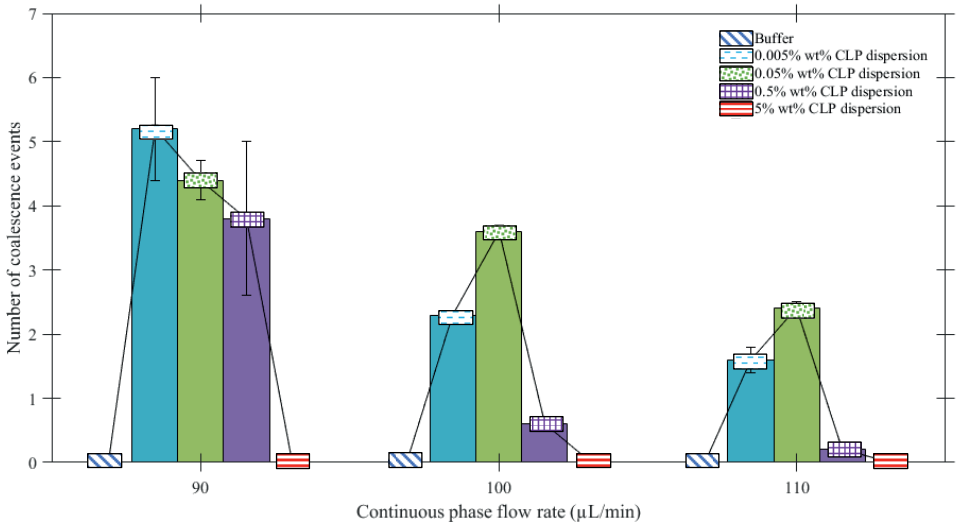


Figure 4.6. Number of coalescence events as a function of the continuous phase flow rate (µL min^{-1}) for sunflower O/W emulsions formed with various concentrations of NaCas-coated CLPs in the continuous phase. The dispersed phase flow rate was 1 µL min^{-1}. No coalescence events are observed for phosphate buffer and the 5% w/w CLP dispersion.

4.3.6. Emulsion stability outside the collision channel

The morphology and stability of droplets formed with 5% w/w CLPs is also studied by polarized light microscopy, after collecting samples from the microfluidic chip, which allows us to probe stability at a different time scale. Emulsions formed with buffer at any q_c or with CLPs at $q_c \leq 70$ µL min^{-1} are highly unstable, show (extensive) coalescence, and no bright birefringent polarization ring around the droplets, which is indicative of the absence of particles at the interface (Table 4.7.; Supporting information, Table S4.3.). At $q_c > 70$ µL min^{-1}, the droplets formed with CLPs are physically stable (they do not coalesce on the microscopic slide), and a birefringent polarization ring is clearly seen, indicating that CLPs adsorb at the oil-water interface. In addition, the polarization ring becomes brighter with increasing q_c, indicating increasing amounts of CLPs adsorb at the interface, while substantial amounts of CLPs remain in the continuous phase as is clear from the continuous phase polarization.

We thus show that the physical stability of CLP-stabilized emulsions strongly depends on the particle adsorption rate. CLPs destabilize emulsion droplets by bridging at insufficient surface coverage, while at high coverage coalescence inside the coalescence channel is prevented, as well as outside the coalescence channel. Contrary to surfactants that adsorb rapidly and spontaneously, a certain amount of energy is needed for particles to anchor in the interface. If this is successfully achieved, this leads to emulsions that are exceptionally stable, and that do not even show coalescence dependency as function of the approach velocity of colliding droplets, unlike surfactant stabilized emulsions (Krebs, Schroen, & Boom, 2012).

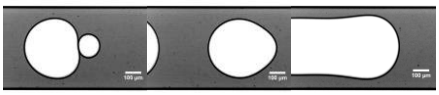
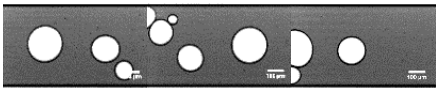
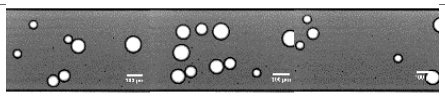
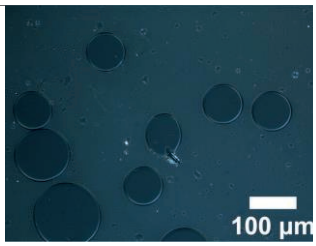
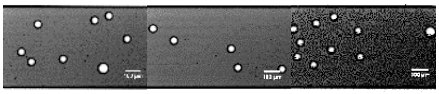
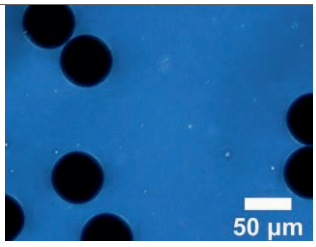
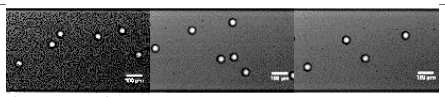
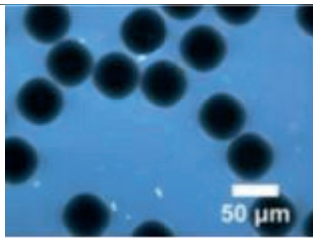
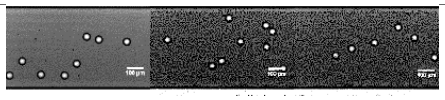
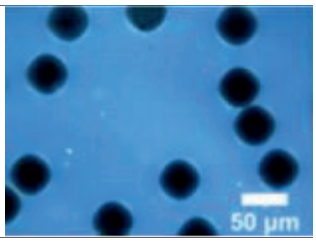
Continuous flow rate (μL min^{-1})	Images at the outlet of the collision channel	Polarized light microscopy images of emulsions outside the microfluidic chip
40		Highly unstable
50		Highly unstable
70		
80		
90		
110		

Table 4.7. Light microscopy images of sunflower O/W emulsions (5% w/w NaCas-coated CLP of which flow rate indicated at the left) at the end of the coalescence channel, and polarized images after collection from microfluidic set-up (right).

4.4. Conclusions

The coalescence stability of CLP-stabilized emulsions is investigated using a microfluidic T-junction. The CLPs have an almost spherical morphology and a unimodal size distribution with a mean particle diameter of ~170 nm. The three phase contact angle at the oil-water interface is < 90°, which makes the particles suitable to stabilize O/W emulsions.

Depending on the continuous phase flow rate, particles obtain sufficient kinetic energy to anchor into the interface. We show that the tendency of emulsion droplets to coalesce strongly depends on particle coverage. CLPs have a destabilizing effect on the emulsion droplets at low surface coverage, probably as a result of bridging. At higher surface coverage, droplet coalescence could be prevented, and emulsions are physically stable when particles are prominently present in the interface. The observed non-monotonic dependency of emulsion droplet stability on surface coverage is distinctly different from the classic behavior found for surfactant-stabilized emulsions, and this understanding is of great importance for the formulation of Pickering emulsions.

4.5. Supporting information

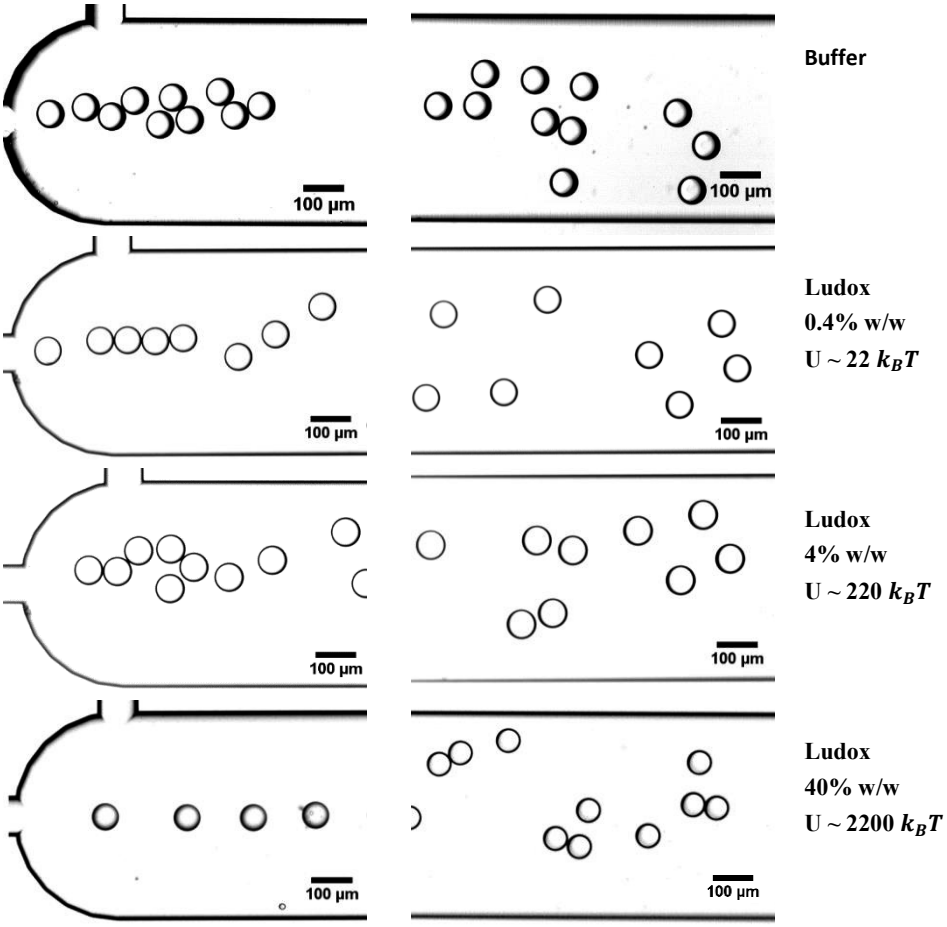


Figure S4.1. Microscopy images of stripped sunflower oil-in-water emulsions formed in a microfluidic device with phosphate buffer, or 0.4, 4.0 or 40% w/w Ludox particles in the continuous phase, at the inlet or outlet of the collision channel.

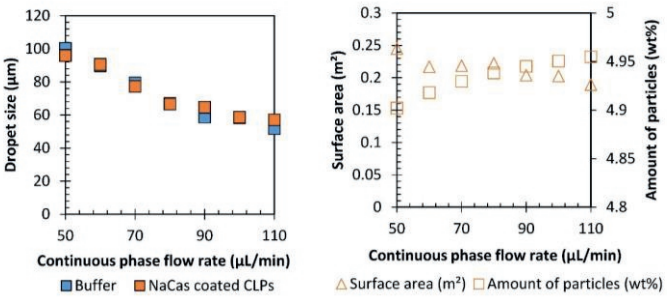
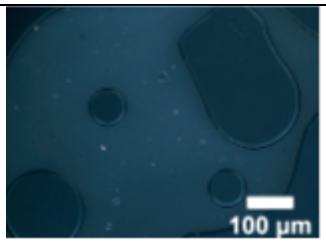
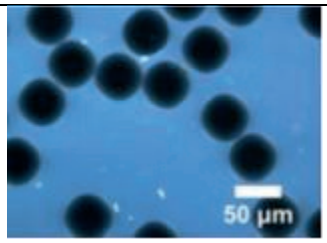
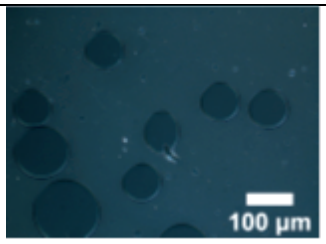
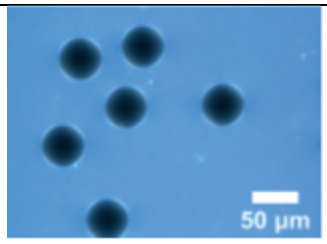
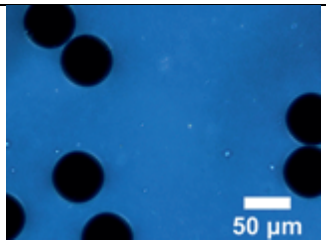
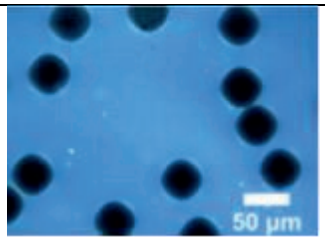


Figure S4.2.(Right) Droplet size as function of the continuous phase flow rate for emulsions formed with (Blue) buffer and (Red) NaCas-coated CLPs (5 wt.% in the continuous phase). (Left) (Triangles) Total interfacial area (m2/100 mL emulsion) and (Squares) mass concentration of NaCas-coated CLPs in the continuous phase for emulsions formed with 5% w/w CLPs in the continuous phase at different continuous phase flow rates. The dispersed phase flow rate was 1 µL min^{-1}. The dispersed phase flow rate was 1 µL min^{-1}.

Table S4.3. Polarized light microscopy images of sunflower oil-in-water emulsions (5% w/w NaCas-coated CLP of which flow rate indicated at the left) after collection from microfluidic set-up.

Continuous flow rate (µL min^{-1})	**Polarized light microscopy images of emulsions outside the microfluidic chip**		
40	Highly unstable		
50	Highly unstable		
60		**90**	
70		**100**	
80		**110**	

4.6. References

Aveyard, R., Binks, B. P., & Clint, J. H. (2003). Emulsions stabilised solely by colloidal particles. Advances in Colloid and Interface Science, 100–102(SUPPL.), 503–546.

Beattie, J. K., & Gray-Weale, A. (2012). Oil/water interface charged by hydroxide ions and deprotonated fatty acids: A comment. Angewandte Chemie - International Edition, 51(52), 12941–12942.

Berton, C., Genot, C., & Ropers, M.-H. (2011). Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. Journal of Colloid and Interface Science, 354(2), 739–748.

Binks. (2002). Particle as surfactants - Similarities and differences. Current Opinion in Colloid & Interface Science, 7, 21–41.

Binks, B. P. (2007). Colloidal particles at liquid interfaces. Physical Chemistry Chemical Physics, 9(48), 6298–6299.

Bunjes, H., Westesen, K., & Koch, M. H. J. (1996). Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. International Journal of Pharmaceutics, 129(1–2), 159–173.

Chevalier, Y., & Bolzinger, M. A. (2013). Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 439, 23–34.

Choi, K.-O., Aditya, N. P., & Ko, S. (2014). Effect of aqueous pH and electrolyte concentration on structure, stability and flow behavior of non-ionic surfactant based solid lipid nanoparticles. Food Chemistry, 147, 239–244.

Crocker, J. C., Matteo, J. A., Dinsmore, A. D., & Yodh, A. G. (1999). Entropic attraction and repulsion in binary colloids probed with a line optical tweezer. Physical Review Letters, 82(21), 4352–4355.

de Folter, J. W. J., van Ruijven, M. W. M., & Velikov, K. P. (2012). Oil-in-water Pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein. Soft Matter, 8(25), 6807–6815.

Deshmukh, O. S., van den Ende, D., Stuart, M. C., Mugele, F., & Duits, M. H. G. (2015). Hard and soft colloids at fluid interfaces: Adsorption, interactions, assembly & rheology. Advances in Colloid and Interface Science, 222, 215–227.

Destribats, M., Lapeyre, V., Wolfs, M., Sellier, E., Leal-Calderon, F., Ravaine, V., & Schmitt, V. (2011). Soft microgels as Pickering emulsion stabilisers: role of particle deformability. Soft Matter, 7(17), 7689–7698.

Destribats, M., Laurichesse, E., Tanner, H., & Leal-calderon, F. (2014). Pickering Emulsions : What Are the Main Parameters Determining the Emulsion Type and Interfacial Properties ? Langmuir, 30, 9313-9326.

Dong, S., Zheng, L., Zhang, X., & Lin, P. (2014). Improved drag force model and its application in simulating nanofluid flow. Microfluidics and Nanofluidics, 17(2), 253–261.

Douaire, M., Di Bari, V., Norton, J. E., Sullo, A., Lillford, P., & Norton, I. T. (2014). Fat crystallisation at oil-water interfaces. Advances in Colloid and Interface Science, 203, 1–10.

Dugyala, V. R., Daware, S. V., & Basavaraj, M. G. (2013). Shape anisotropic colloids: synthesis, packing behavior, evaporation driven assembly, and their application in emulsion stabilization. Soft Matter, 9(29), 6711.

Firoozmand, H., & Rousseau, D. (2016). Microbial cells as colloidal particles : Pickering oil-in-water emulsions stabilized by bacteria and yeast. FRIN, 81, 66–73.

Fouilloux, S., Malloggi, F., Daillant, J., & Thill, A. (2016). Aging mechanism in model Pickering emulsion. Soft Matter, 12(3), 900–904.

Garbin, V., Crocker, J. C., & Stebe, K. J. (2012). Nanoparticles at fluid interfaces: Exploiting capping ligands to control adsorption, stability and dynamics. Journal of Colloid and Interface Science, 387(1), 1–11.

Gautier, F., Destribats, M., Perrier-Cornet, R., Dechézelles, J., Giermanska, J., Héroguez, V., … V. Schmitt. (2007). Pickering emulsions with stimulable particles: from highly- to weakly-covered interfaces. Physical Chemistry Chemical Physics : PCCP, 9(48), 6455–6462.

Gould, J., Vieira, J., & Wolf, B. (2013). Cocoa particles for food emulsion stabilisation. Food & Function, 4(9), 1369–1375.

Graham, D. E., & Phillips, M. C. (1979a). Proteins at liquid interfaces II. Adsorption isotherms. Journal of Colloid and Interface Science, 70(3), 415–426.

Graham, D. E., & Phillips, M. C. (1979b). Proteins at liquid interfaces III. Molecular structures of adsorbed films. Journal of Colloid and Interface Science, 70(3), 427–439.

Horozov, T. S., & Binks, B. P. (2006). Particle-stabilized emulsions: A bilayer or a bridging monolayer? Angewandte Chemie - International Edition, 45(5), 773–776.

Kaz, D. M., Mcgorty, R., Mani, M., Brenner, M. P., & Manoharan, V. N. (2011). Physical ageing of the contact line on colloidal particles at liquid interfaces. Nature Materials, 11(2), 138–142.

Kellens, M., Meeussen, W., Riekel, C., & Reynaers, H. (1990). Time resolved x-ray diffraction studies of the polymorphic behaviour of tripalmitin using synchrotron radiation. Chemistry and Physics of Lipids, 52(2), 79–98.

Kramers, H. A. (1940). Brownian motion in a field of force and the diffusion model. Physica, (4), 284–304.

Krebs, T., Schroen, K., & Boom, R. (2012). A microfluidic method to study demulsification kinetics. Lab on a Chip, 12(207890), 1060–1070.

Krebs, T., Schroën, K., & Boom, R. (2012). Coalescence dynamics of surfactant-stabilized emulsions studied with microfluidics. Soft Matter, 8(41), 10650–10657.

Kutuzov, S., He, J., Tangirala, R., Emrick, T., Russell, T. P., & Böker, A. (2007). On the kinetics of nanoparticle self-assembly at liquid/liquid interfaces. Physical Chemistry Chemical Physics, 9, 6351–6358.

Laar, T. Van De, Klooster, S., Schroën, K., & Sprakel, J. (2016). Transition-state theory predicts clogging at the microscale. Nature Publishing Group, (April), 1–8.

Leal-Calderon, F., & Schmitt, V. (2008). Solid-stabilized emulsions. Current Opinion in Colloid and Interface Science, 13(4), 217–227.

Li, T., Donadio, D., & Galli, G. (2013). Ice nucleation at the nanoscale probes no man's land of water. Nature Communications, 4(May), 1–6.

Liu, F., & Tang, C.-H. (2013). Soy protein nanoparticle aggregates as pickering stabilizers for oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 61(37), 8888–8898.

Luo, Z., Murray, B. S., Yusoff, A., Morgan, M. R. a, Povey, M. J. W., & Day, A. J. (2011). Particle-stabilizing effects of flavonoids at the oil-water interface. Journal of Agricultural and Food Chemistry, 59(6), 2636–2645.

Mallia, A. K., Frovenzano, M. D., Fujimoto, E. K., Olson, B. J., Klenk, D. C., & Company, P. C. (1985). Measurement of Protein Using Bicinchoninic Acid. 85, 76–85.

McGorty, R., Fung, J., Kaz, D., & Manoharan, V. N. (2010). Colloidal self-assembly at an interface. Materials Today, 13(6), 34–42.

Mikulcová, V., Bordes, R., & Kašpárková, V. (2016). On the preparation and antibacterial activity of emulsions stabilized with nanocellulose particles. Food Hydrocolloids, 61, 780–792.

Monteillet, H., Workamp, M., Appel, J., Kleijn, J. M., Leermakers, F. a. M., & Sprakel, J. (2014). Ultrastrong anchoring yet barrier-free adsorption of composite microgels at liquid interfaces. Advanced Materials Interfaces, 1, 1300121.

Monteux, C., Kirkwood, J., Xu, H., Jung, E., & Fuller, G. G. (2007). Determining the mechanical response of particle-laden fluid interfaces using surface pressure isotherms and bulk pressure measurements of droplets. Physical Chemistry Chemical Physics, 9, 6344– 6350.

Muijlwijk, K., Colijn, I., Harsono, H., Krebs, T., Berton-Carabin, C., & Schroën, K. (2017). Coalescence of protein-stabilised emulsions studied with microfluidics. Food Hydrocolloids, 70, 96–104.

Muijlwijk, K., Hinderink, E., Ershov, D., Berton-Carabin, C., & Schroën, K. (2016). Interfacial tension measured at high expansion rates and within milliseconds using microfluidics. Journal of Colloid and Interface Science, 470, 71–79.

Nagarkar, S. P., & Velankar, S. S. (2012). Morphology and rheology of ternary fluid–fluid–solid systems. Soft Matter, 8(32), 8464.

Pan, M., Lyu, F., & Tang, S. K. Y. (2017). Methods to coalesce fluorinated Pickering emulsions. Anal. Methods, 4622–4629.

Pawlik, A., Kurukji, D., Norton, I., & Spyropoulos, F. (2016). Food-grade Pickering emulsions stabilised with solid lipid particles. Food Funct.

Pickering, S. U. (1907). Emulsions. Journal of the Chemical Society, 91, 2001–2021.

Ramsden, W. (1903). Separation of Solids in the Surface-layers of Solutions and "suspensions" (observations on Surface-membranes, Bubbles, Emulsions, and Mechanical Coagulation). Proceedings of the Royal Society of London, 72, 156–164.

Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P., & Wahlgren, M. (2014). Biomass-based particles for the formulation of Pickering type emulsions in food and topical applications. Colloids and Surfaces A, 458, 48–62.

Rayner, M., Sjöö, M., Timgren, A., & Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of Pickering emulsions. Faraday Discussions, 158, 139.

Roger, K., & Cabane, B. (2012). Why are hydrophobic/water interfaces negatively charged? Angewandte Chemie - International Edition, 51(23), 5625–5628.

Schröder, A., Sprakel, J., Schroën, K., & Berton-Carabin, C. (2017). Tailored microstructure of colloidal lipid particles for Pickering emulsions with tunable properties. Soft Matter, 3190–3198.

Schroen, K., Bliznyuk, O., Muijlwijk, K., Sahin, S., & Berton-Carabin, C. C. (2015). Microfluidic emulsification devices: From micrometer insights to large-scale food emulsion production. Current Opinion in Food Science, 3, 33–40.

Tcholakova, S., Denkov, N. D., & Lips, A. (2008). Comparison of solid particles, globular proteins and surfactants as emulsifiers. Physical Chemistry Chemical Physics : PCCP, 10(12), 1608–1627.

Timgren, A., Rayner, M., Dejmek, P., & Marku, D. (2013). Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride.

Yusoff, A., & Murray, B. S. (2011). Modified starch granules as particle-stabilizers of oil-in-water emulsions. Food Hydrocolloids, 25(1), 42–55.

Zafeiri, I., Norton, J. E., Smith, P., Norton, I. T., & Spyropoulos, F. (2017). The role of surface active species in the fabrication and functionality of edible solid lipid particles. Journal of Colloid and Interface Science.

5

Can we prevent lipid oxidation in emulsions by using fat-based Pickering particles?

This chapter is published as Schröder, A., Sprakel, Boerkamp, W., Schroën, K. and Berton-Carabin, C. C. (2019). Can we prevent lipid oxidation in emulsions by using fat-based Pickering particles? Food Research International. 120, 352-363.

Abstract:

Interest has recently been rising in the development of food-compatible Pickering emulsions, i.e., particle-stabilized emulsions, and various biobased particles have been demonstrated as useful for such a purpose. Most of the related work has focused on the physical stability of the emulsions, but whether such particles can be advantageous in terms of chemical stability, and in particular, with regard to lipid oxidation, is largely unexplored. Recently, we found that colloidal lipid particles (CLPs) are efficient Pickering stabilizers, and the objective of the present study was to investigate the oxidative stability of emulsions containing these particles. Three types of sunflower oil-in-water (O/W) emulsions were considered: Pickering emulsions stabilized with colloidal lipid particles (CLPs) made of high melting point (HMP) fat (tripalmitin or palm stearin), adsorbed onto the liquid oil droplets; and, as references, two conventional sodium caseinate-stabilized emulsions, of which one contained only liquid oil, and the other liquid oil mixed with HMP fat as the core of the emulsion droplets.

In the presence of iron, the latter oxidized faster than conventional liquid oil and Pickering emulsions, resulting in 2- to 3-fold higher amounts of primary and secondary lipid oxidation products. This may be due to intra-droplet HMP fat pushing oxidizable lipids towards the oil-water interface, which would promote lipid oxidation. This shows that the localization of solid fat in O/W emulsions affects lipid oxidation. We also found that CLP-stabilized Pickering emulsions had similar oxidation rates as conventional sodium caseinate-stabilized emulsions containing only liquid oil. This suggests that the potential of such Pickering particles to prevent lipid oxidation is limited. This could be because diffusion of small pro-oxidant molecules is not hindered by Pickering particles, as they cannot form an interfacial barrier that is structurally homogeneous at such a small scale.

5.1. Introduction

In many food products, lipids are dispersed in an aqueous phase, i.e., are oil-in-water (O/W) emulsions. If the lipid phase contains unsaturated fatty acids, lipid oxidation can occur, which decreases the nutritional and sensory quality of the food product (El-Beltagi & Mohamed, 2013; Schaich, Shahidi, Zhongy, & Eskin, 2013).

It is well established that lipid oxidation in multiphase systems, such as emulsions, is affected by many compositional factors. This is, at least in part, explained by the chemical reactivity of some ingredients (e.g., antioxidants, transition metals, proteins) (Chen et al., 2011; Laguerre, Bily, Roller, & Birtic, 2017; D.J. McClements & Decker, 2000a). A few factors have clearly been identified as protecting lipids against oxidation in emulsion systems. In particular, high concentrations of non-adsorbed proteins greatly prevent oxidation, which is due to their ability to chelate metal ions, or to scavenge free radicals (Berton, Genot, & Biopolym, 2011; Berton, Genot, & Ropers, 2011; Faraji, McClements, & Decker, 2004; Koh, 2002; Osborn & Akoh, 2004).

Furthermore, it is commonly accepted that lipid oxidation is initiated at the oil-water interface, the region where unsaturated lipids and aqueous pro-oxidants, such as metal ions, come into close contact (Berton-Carabin et al., 2014c). Therefore, lipid oxidation depends to a large extent on the composition of the oil-water interface (Berton, Ropers, Viau, & Genot, 2011; Genot; Berton, Claire; Ropers, 2013). For instance, the location of antioxidants at the oil-water interface promotes oxidative stability, since these compounds are more efficient when located near the initiation reaction site (Laguerre et al., 2015; Yuji et al., 2007). For many other factors, the effects on lipid oxidation are controversial and very much system-dependent (e.g., type of emulsifier, pH of the continuous phase, minor hydrophilic or lipophilic components). It is often mentioned that the structure of the oil-water interface, and in particular, its thickness (Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2017) and structural homogeneity (Genot et al., 2013) can have a protective effect on lipid oxidation in emulsions; however, experimental evidence is scarce .

Recently, strong interest has emerged regarding the development of Pickering emulsions for food applications (Berton-Carabin & Schroën, 2015). Pickering emulsions are particle-stabilized emulsions, and are known for their high physical stability compared to conventional emulsifier-stabilized emulsions. The particles strongly attach to the interface,

exceeding the thermal energy of desorption by orders-of-magnitude, unlike regular emulsifiers for which these two energies are much closer, facilitating desorption and possible emulsion destabilization.

Although the state-of-the-art research in this field has historically been established with inorganic particles, such as silica particles, substantial work over the past decade has demonstrated that biobased particles (e.g., starch granules, insoluble protein particles) can also be useful for the physical stabilization of emulsions (Berton-Carabin & Schroën, 2015). Yet, whether Pickering particle layers can affect lipid oxidation is largely unexplored; only a few studies on this topic have been reported (Kargar et al., 2012; Kargar, Spyropoulos, & Norton, 2011a; Kargar et al., 2011b; Xiao et al., 2015; T. Zeng et al., 2017; Yuan Zhao et al., 2015). Some authors speculate that a closely packed particle layer may have better barrier properties than a protein or surfactant emulsifier layer. Kargar et al. (2011a) found that emulsions stabilized by silica particles were more oxidatively stable than Tween 20-stabilized emulsions, but less oxidatively stable than sodium caseinate-stabilized emulsions (Kargar et al., 2011a). In another study, the same authors found that lipid oxidation was lower in emulsions stabilized by microcrystalline cellulose particles compared to emulsions stabilized by modified starch particles, which was explained by the free radical scavenging properties of the microcrystalline cellulose particles, and their ability to form a thick interfacial layer (Kargar et al., 2012).

Recently, we have developed a new type of food-grade particle-stabilized emulsions, using colloidal lipid particles (CLPs) as Pickering stabilizers (Schröder, Sprakel, Schroën, & Berton-Carabin, 2017; Schröder, Sprakel, Schroën, Spaen, & Berton-Carabin, 2018). Our CLPs are made of edible high melting point (HMP) lipids, and of a small amount of emulsifier. They can form a thick and mechanically resistant layer at the surface of oil droplets. In the present work, we investigate what the effect of such layers could be on lipid oxidation, and in particular, whether they protect the dispersed oil phase against oxidation.

To study how such particles affect lipid oxidation in emulsions, we studied lipid oxidation in three types of emulsions: Pickering emulsions stabilized by sodium caseinate-coated CLPs; and, for comparison purposes, two types of conventional sodium caseinate-stabilized O/W emulsions, of which one contained only liquid oil, and the other liquid oil mixed with HMP fat, to have the same overall composition as that of the Pickering emulsion (Figure 5.1.). This research allowed us to link the physical structure of emulsions, i.e., the spatial

distribution of the different components within the droplet and at its interface, to the extent of lipid oxidation.

5.2. Materials & methods

5.2.1. Materials

Tripalmitin (#T8127, purity >99 %), sodium phosphate monobasic (#S9638), sodium phosphate dibasic (#S9763), sodium chloride (#S7653), iron(II) sulfate heptahydrate (#F8633), ethylenediaminetetraacetic acid disodium salt dihydrate (#E6635), *para*-anisidine (#A88255), and acetic acid (#45726) were purchased from Sigma-Aldrich. n-Hexane (#808023502) was obtained from Actu-All Chemicals (Oss, the Netherlands). 2-Propanol was purchased from Merck (Darmstadt, Germany). Sodium caseinate was supplied by DMV International (#41610, spray dried, protein content 91.0%). Sunflower oil was obtained from a local supermarket, and was stripped with alumina powder (MP EcoChrome™ ALUMINA N, Activity: Super I, Biomedicals) to remove impurities and tocopherols (Berton, Genot, & Ropers, 2011). Palm stearin (palmitic acid, 82%; oleic acid, 9%; stearic acid, 5%) was supplied by ADM (Saint Laurent Bangy, France) (composition can be found in Supplementary material (Table S1). Ultrapure water (18.2 MΩ) was used for all experiments, and prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA). All other chemicals or solvents were of analytical grade.

5.2.2. Purification of tripalmitin

Tripalmitin was purified by three recrystallization steps using ethanol. Briefly, tripalmitin was dissolved in ethanol at 60-70 °C while stirring for 15 min, and left to cool down to room temperature to allow recrystallization, after which ethanol was removed in a vacuum oven, which was repeated two more times.

5.2.3. Preparation of CLP dispersions

An aqueous phase (95% w/w) containing 1% w/w sodium caseinate in phosphate buffer (10 mM, pH 7.0) was heated at 80 °C in a water bath and added to melted tripalmitin or palm stearin (5% w/w). Then, a coarse emulsion was prepared by high speed stirring at 11,000 rpm for one min using a preheated rotor-stator homogenizer (Ultra-turrax IKA T18 basic, Germany). The obtained coarse emulsion was then homogenized (Microfluidizer® Processor MF 110Y with Y-shaped interaction chamber (F12Y; minimum internal dimension: 75 µm),

Microfluidics, Newton, Massachusetts, USA) at 800 bar and 80 °C (5 cycles) to obtain submicron-sized melted fat droplets, which were left to cool down to 20 °C over the course of ~ 6 h, allowing for the lipid phase to crystallize. The resulting dispersion of CLPs was stored at 4 °C (Schröder et al., 2018).

5.2.4. Preparation of O/W emulsions

We prepared three types of emulsions: Pickering emulsions, stabilized by CLPs; and two types of conventional sodium caseinate-stabilized O/W emulsions, of which one contained only liquid oil, and the other liquid oil mixed with HMP fat. All emulsions contained the same amount of sodium caseinate, and two of them contained HMP fat in the same amounts, but they differ in their structural organization (Figure 5.1., Table 5.2.). To prepare a conventional sodium caseinate-stabilized liquid core emulsion, stripped sunflower oil (10% w/w) was mixed with phosphate buffer (10 mM, pH 7.0, 47.75% w/w) and 1% w/w sodium caseinate in phosphate buffer (10 mM, pH 7.0, 45% w/w). For the conventional sodium caseinate-stabilized emulsion containing HMP fat (i.e., solid core emulsion), stripped sunflower oil (10% w/w) was mixed with melted tripalmitin (2.25% w/w), phosphate buffer (10 mM, pH 7.0, 45% w/w) and 1% w/w sodium caseinate in phosphate buffer (10 mM, pH 7.0, 45% w/w) at 80 °C. For the CLP-stabilized Pickering emulsion, stripped sunflower oil (10% w/w) was mixed with phosphate buffer (10 mM, pH 7.0, 45% w/w) and a 5% w/w CLP dispersion (45% w/w) (Table 5.2.).

We first prepared coarse emulsions by high speed stirring at 7,000 rpm for one min using a rotor-stator homogenizer (Ultra-turrax IKA T18 basic, Germany). The obtained coarse emulsions were then either homogenized at 400 bar (5 cycles) using the previously described high pressure homogenizer, or processed through a lab scale colloid mill with gap width of 0.32 mm (IKA Magic Lab, Staufen, Germany) operating for one min at 15,000 rpm. For the conventional emulsion containing HMP fat a temperature of 80 °C was used, whereas for the conventional liquid oil and Pickering emulsions this was 0 °C. Preliminary trials showed that the homogenization temperature did not affect the oxidative fate of the emulsions (Supporting information, Figure S5.2.). The resulting emulsions were stored at 4 °C until incubation (for a period which was always less than 24 h).

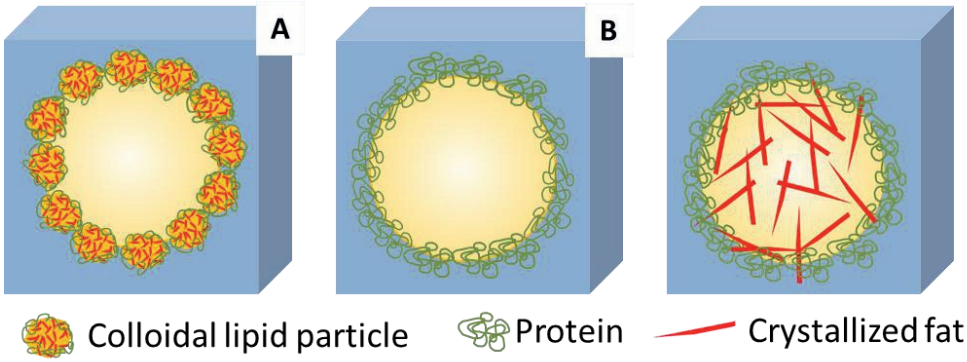


Figure 5.1. Graphical representation of a sodium caseinate-coated CLP-stabilized Pickering emulsion (A), a conventional sodium caseinate-stabilized emulsion with liquid oil droplets (B) and a conventional sodium caseinate-stabilized emulsion containing liquid oil mixed with crystallized high melting point fat (C).

5.2.5. Physical characterization of CLPs and O/W emulsions

The CLP and emulsion droplet size was measured by static light scattering (Malvern Mastersizer 3000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). The following optical properties were used: refractive indices of 1.540 (solid tripalmitin), 1.465 (sunflower oil) and 1.330 (water) with an absorption index of 0.01.

The droplet surface charge was evaluated by the measurement of ζ-potential with a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, UK) at 25 °C. Prior to the measurement, particles and emulsions were diluted ~1000 fold in phosphate buffer (10 mM, pH 7.0) to prevent multiple scattering.

Melting and crystallization were investigated for bulk fat, CLPs and O/W emulsions using a differential scanning calorimeter (Discovery Series DSC 250, TA Instruments, Zellik, Belgium). Fat, CLP dispersions or emulsions were placed in an aluminum pan closed with a hermetic lid, and heated from -10 °C to 80 °C at 1 °C min^{-1}, then cooled down to -10 °C at the same rate followed by two identical heating/cooling cycles. An empty pan was used as a reference. The thermograms were evaluated using TRIOS software (v4.1.1.33073, TA instruments, Zellik, Belgium).

Table 5.2. Composition of tripalmitin or palm stearin CLP-stabilized Pickering emulsions and conventional sodium caseinate-stabilized emulsions containing sunflower oil, or sunflower oil mixed with tripalmitin or palm stearin.

Emulsion	**Pickering**		**Conventional NaCas-stabilized**		
	Tripalmitin CLP emulsion	Palm stearin CLP emulsion	Liquid core emulsion	Tripalmitin partly solid core emulsion	Palm stearin partly solid core emulsion
Sunflower oil [w/w %]	10.00	10.00	10.00	10.00	10.00
Tripalmitin [w/w %]	2.25	0.00	0.00	2.25	0.00
Palm stearin [w/w %]	0.00	2.25	0.00	0.00	2.25
1 w/w % NaCas sol. [w/w %]	42.75	42.75	42.75	42.75	42.75
Buffer [w/w %]	45.00	45.00	47.25	45.00	45.00

The protein surface coverage of CLPs was determined by calculating the difference between the total amount of proteins used to prepare the CLPs, and the residual protein concentration in the aqueous phase of the CLP dispersion, and was based on the mean diameter of the CLPs ($d_{3,2}$). The concentration of non-adsorbed protein in CLP dispersions and in emulsions was quantified by determining the protein content in the aqueous phase with the bicinchoninic acid (BCA) assay. We separated the aqueous phase of the dispersions or of the emulsions by centrifuging a 2-mL aliquot for 3 h at 20,000×g. The aqueous phase was collected by cautiously boring a hole in the tube, and centrifuging again for 3 h at 20,000×g to remove any remaining CLPs or oil droplets.

Transmission electron microscopy (TEM) was performed on CLP dispersions and on emulsions containing HMP fat (i.e., CLP-stabilized Pickering emulsions, or conventional emulsions with HMP fat in the droplet core; both were diluted 10-fold using 10 mM phosphate buffer pH 7.0). The samples were deposited onto a freshly glown discharged carbonized copper grid (400 mesh). The excess solvent was blotted using standard filter paper. The CLPs and emulsion droplets were stained with an aqueous 1% w/w phosphotungstic acid solution. Images were recorded on a JEOL JEM 1400 plus transmission electron microscope (Peabody, USA) operating at 120 kV in combination with JEOL CCD camera Ruby (8 M pixel).

The microstructure of the emulsions was also evaluated by light microscopy using a Carl Zeiss Axioscope A1 microscope equipped with a camera (AxioCam Mrc5) and polarization filters.

5.2.6. Incubation of O/W emulsions

An oxidation initiator system consisting of an equimolar mixture of $FeSO_4$ and EDTA was prepared by separately dissolving $FeSO_4$ and EDTA (8.4 or 84 mM) in ultrapure water. Equivalent volumes of each solution were then mixed, and the iron-EDTA complex was allowed to form under moderate stirring for 1 h in the dark (Berton, Ropers, et al., 2011). Aliquots of emulsion (2 g) were distributed in 15-mL polypropylene centrifuge tubes. The oxidation initiator solution (100 µL) was added to the emulsions to obtain a final concentration of 200 µM or 2 mM of both iron and EDTA. The tubes were rotated in the dark at 2 rpm at 25 °C for 72 h (SB3 rotator, Stuart, Staffordshire, UK).

5.2.7. Measurements for lipid oxidation

5.2.7.1. Conjugated diene (CD) hydroperoxides

The procedure for quantification of CD hydroperoxides, which are primary oxidation products, was adapted from Corongiu & Banni (1994). In short, the incubated emulsions were diluted 4000 fold in 2-propanol, in sequential dilution steps. The final solutions were centrifuged at 20,000×g for one min, and the absorbance of the supernatant was measured at 233 nm with a UV-visible spectrophotometer. The reference cell contained 2-propanol and phosphate buffer (10 mM, pH 7.0) in the same proportions as in the final dilution of the samples. Results were expressed in mmol of equivalent hydroperoxides per kg of oil (mmol eq HP kg^{-1} oil) with 27,000 M^{-1} cm^{-1} as the molar extinction coefficient of CD hydroperoxides at 233 nm.

5.2.7.2. Aldehydes

The *para*-anisidine value (*p*AV), a measure of total aldehydes, was used as a marker of secondary lipid oxidation products (AOCS, 1998). In short, one mL of saturated sodium chloride solution and 5 mL of hexane/isopropanol (1/1, v/v) were added per aliquot of incubated emulsion (2.1 mL). The obtained mixtures were vortexed followed by centrifugation at 2,000×g for 8 min at 4 °C. The upper hexane layer (> 2 mL) was collected and placed on ice for 3 min, followed by centrifugation at 20,000×g for 1 min. The absorbance of the supernatant was measured at 350 nm with pure hexane as a blank (*Ab*). In an centrifugation vial, 1 mL of the supernatant was mixed with 0.2 mL of 2.5 g L^{-1} *para*-anisidine in acetic acid solution. After exactly 10 min the absorbance was measured at 350 nm, using 1 mL pure hexane mixed with 0.2 mL of 2.5 g L^{-1} *para*-anisidine in acetic acid solution, also incubated for 10 min, as a blank (*As*). The *para*-anisidine value (arbitrary units) was determined as follows (Equation 5.1.):

$$pAV = \frac{(1.2As - Ab)}{m} \qquad \text{(Eq. 5.1.)}$$

Where *m* is the concentration of oil in the supernatant (g mL^{-1}).

5.2.7.3. Physical stability of emulsions

The size distribution of emulsion droplets incubated under oxidative conditions was monitored with static light scattering, as explained in the physical characterization section; it was checked immediately after homogenization and after 72 h of incubation.

5.2.8. Experimental design

For each measurement, whether related to size, ζ-potential, DSC, surface coverage, or oxidation (CD hydroperoxides and *p*AV)), at least two emulsions were prepared independently and samples were analyzed in duplicate. Additionally, two independent samples were incubated per time point, and analyzed in duplicate for CD hydroperoxide, and in single for *p*AV.

Statistical analysis of variance (F-test and T-test) was carried out on the experimental lipid oxidation values at the end of the incubation period. Differences at $p < 0.05$ were considered to be significant.

5.3. Results & discussion

5.3.1. Physical characterization of colloidal lipid particles (CLPs)

We produced CLPs by hot high pressure homogenization of tripalmitin or palm stearin in an aqueous phase containing 1% w/w sodium caseinate, and subsequent cooling to allow for lipid crystallization. The particle size distribution of both tripalmitin and palm stearin CLPs was fairly unimodal with a mean particle diameter ($d_{3,2}$) around 150 nm (Figure 5.3.A). TEM images show a nearly spherical morphology for tripalmitin CLPs and an irregular shape for palm stearin CLPs, with particle sizes ranging from ~100-350 nm (Figure 5.3.B and C), which is in good agreement with the particle size distributions.

Tripalmitin and palm stearin CLPs had similar ζ-potentials of -36 ± 2 mV, due to the presence of ionizable groups in sodium caseinate at the surface of the particle. The calculated protein surface coverage was 3.7 mg m^{-2} and 3.5 mg m^{-2} for tripalmitin and palm stearin CLPs, respectively, which, according to Graham & Phillips (1979a,b), slightly exceeds monolayer coverage and corresponds to a film thickness of about 6-10 nm. It should be pointed out that the protein layer gives a hydrophilic character to the particles (Graham & Phillips, 1979b, 1979a; Schröder et al., 2018).

The DSC melting thermogram of tripalmitin CLPs shows an exothermic melting peak at ~42 °C, which is indicative of crystals reorganizing and recrystallizing in a more stable form (Figure 5.3.D). The endothermic melting peak at ~62 °C is very broad, and consists of a sequence of peaks corresponding to multiple melting events. The thermogram of palm stearin CLPs shows a broad melting peak with a maximum at ~58 °C (Figure 5.3.E). The

crystallization temperatures of 30 °C and 19 °C for tripalmitin and palm stearin CLPs (Figure 5.2.D&E, respectively) are much lower than those of bulk tripalmitin and palm stearin, which are 41 °C and 39 °C, respectively (Supporting information, Figure S5.3.A and S5.3.C). This shows that there is a strong supercooling effect when the HMP fats are emulsified as CLPs, which was expected due to the small particle size (Schröder et al., 2017).

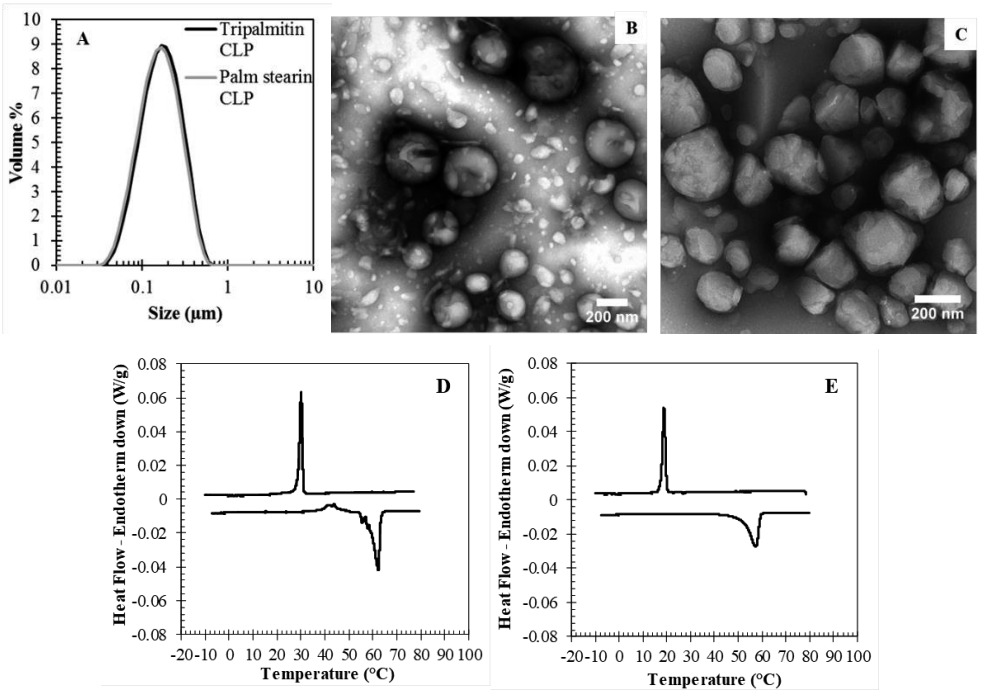


Figure 5.3. Characterization of tripalmitin (A, B & D) and palm stearin (A, C & E) CLPs. Particle size distribution (A); TEM images (B & C); and DSC thermograms (D & E).

5.3.2. Physical characterization of emulsions

We prepared five different emulsions: two sunflower O/W Pickering emulsions stabilized by either tripalmitin or palm stearin CLPs; and, for comparison purposes, three conventional O/W emulsions stabilized by sodium caseinate, of which one contained only liquid sunflower oil, and the other two liquid sunflower oil mixed with HMP fat (either tripalmitin or palm stearin) to obtain droplets with a partially crystallized core. Thus, all emulsions contained the same amount of sodium caseinate, and four of them the same amount of HMP fat (Pickering and partially crystallized core emulsions), but they differed in their

structural organization (Table 5.2., Figure 5.1.), whereas the emulsion with fully liquid oil droplets was used as a standard reference.

All conventional emulsions showed a comparable mean droplet size ($d_{3,2}$) of 0.3-0.4 µm, although the solid core tripalmitin emulsions showed a broader size distribution compared to the other two conventional emulsions. The droplet size distributions of the Pickering emulsions showed two distinct peaks, corresponding to non-adsorbed CLPs (~0.2 µm) and to emulsion droplets ranging from ~0.3-3 µm, with a maximum at ~1 µm (Figure 5.4.A).

All emulsions exhibited a comparable ζ-potential of around -40 to -42 mV (Figure 5.4.B), due to the presence of ionizable groups in the proteins. For the conventional emulsions, the surface coverage of sodium caseinate was between 1.3 and 1.5 mg m^{-2}, which is similar to reported values for monolayers (Graham & Phillips, 1979b).

We visualized the morphology of the Pickering emulsions and of the conventional solid core emulsions by TEM (Figure 5.4.C – F). This analysis first confirmed that CLPs adsorb at the oil-water interface in Pickering emulsions (Figure 5.4.E & F). Solid core emulsions with palm stearin showed deformed droplets with clear facets between them, whereas the ones with tripalmitin were less deformed (Figure 5.4.C & D), which is probably because these droplets have a more solid structure.

While TEM gave us information about the emulsions' structure and morphology, it did not give insight into the physical state of the fat inside the emulsions; for that we used DSC. We found that tripalmitin and palm stearin in the solid core emulsions crystallized at 5-18 °C and 5-15 °C, and melted at 35-54 °C and 35-51 °C, respectively (Figure 5.5.A & B), which are much lower values compared to bulk fats (Supporting information, Figure S5.3.A & S5.3.C) due to melting point depression (i.e., due to mixing with lower melting point triglycerides) (Gunstone, Harwood & Dijkstra, 2007; Lovegren, Gray, & Fuege, 1976). For the Pickering emulsions prepared with tripalmitin or palm stearin CLPs, the thermograms showed two distinct crystallization peaks (Figure 5.5.C & D). The first peak (highest temperature) probably corresponds to non-adsorbed CLPs, which show a similar crystallization temperature in dispersion, as discussed earlier. The second peak (lowest temperature) occurs at the crystallization temperature of solid core emulsions, which we interpret as being related to CLPs in contact or blended with the sunflower oil at the interface of the droplet.

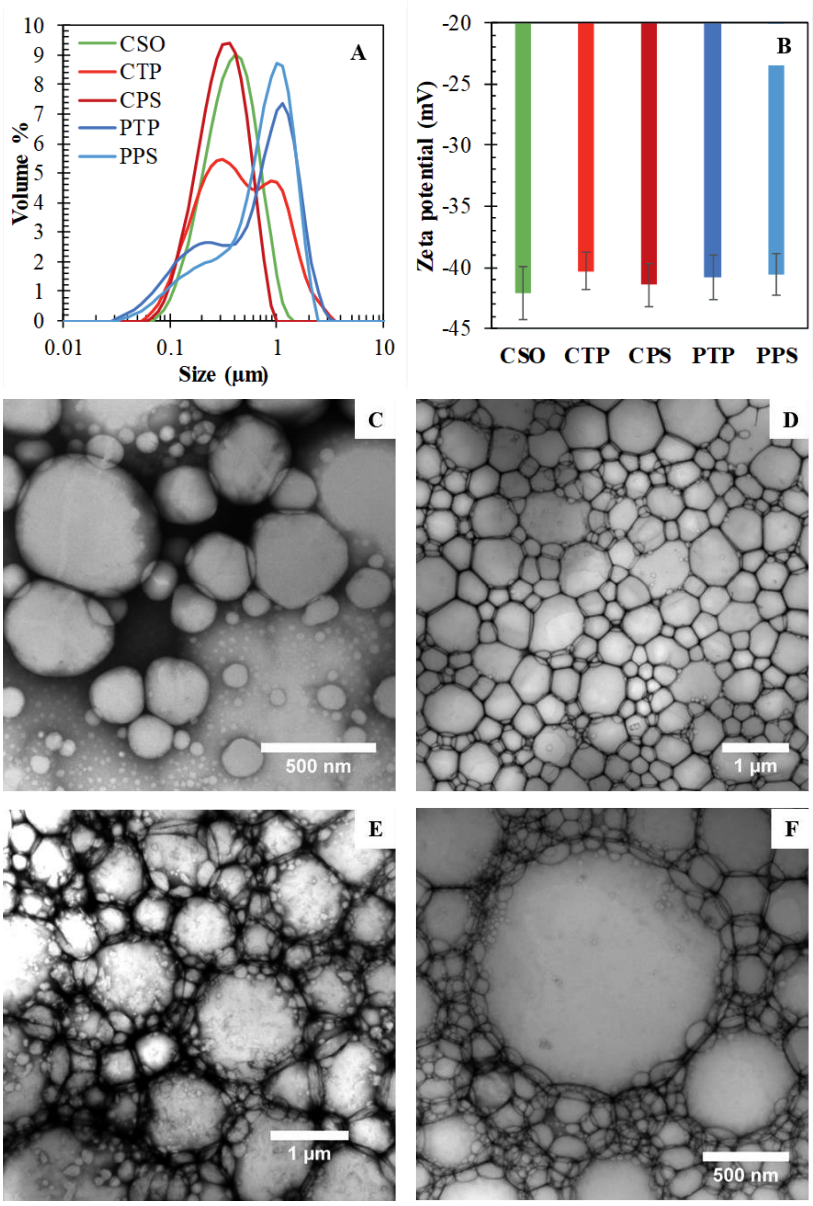


Figure 5.4. Characterization of conventional and Pickering O/W emulsions by droplet size distribution (A); ζ-potential (B); TEM imaging (C-F) of solid core emulsions with tripalmitin (C) and palm stearin (D); and Pickering emulsions with particles prepared with tripalmitin (E) and palm stearin (F). The emulsions coded with C are the conventional emulsions, the ones with P the Pickering emulsions, and SO, TP and PS indicate sunflower oil, tripalmitin and palm stearin, respectively.

We also produced emulsions by colloid mill homogenization, to obtain larger droplet sizes (Figure 5.6.A) that allow for visualization of the physical structure of emulsions by light microscopy. We observed spherical and non-aggregated droplets for conventional sunflower oil emulsions (Figure 5.6.B) and for those containing tripalmitin (Figure 5.6.C) when using regular light. When using polarization filters, clear differences were noticed: tripalmitin was not homogeneously distributed within the emulsion droplets, but formed crystallized islands of various dimensions inside and at the interface of the oil droplets (Figure 5.6.D and E). For the Pickering emulsions, we clearly saw a thick birefringent polarization ring at the droplet surface, indicating that the CLPs were adsorbed at the interface (Figure 5.6.F).

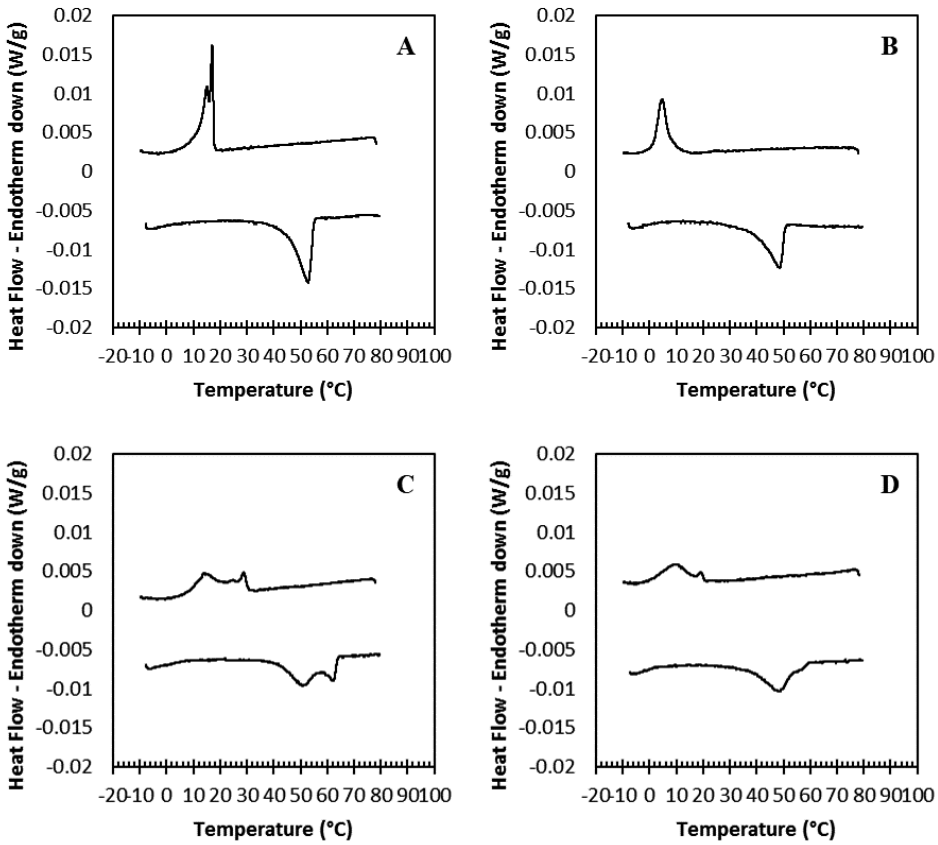


Figure 5.5. DSC thermograms of the solid core emulsions with tripalmitin (A), and palm stearin (B); and of the Pickering emulsions with particles prepared with tripalmitin (C) and palm stearin (D).

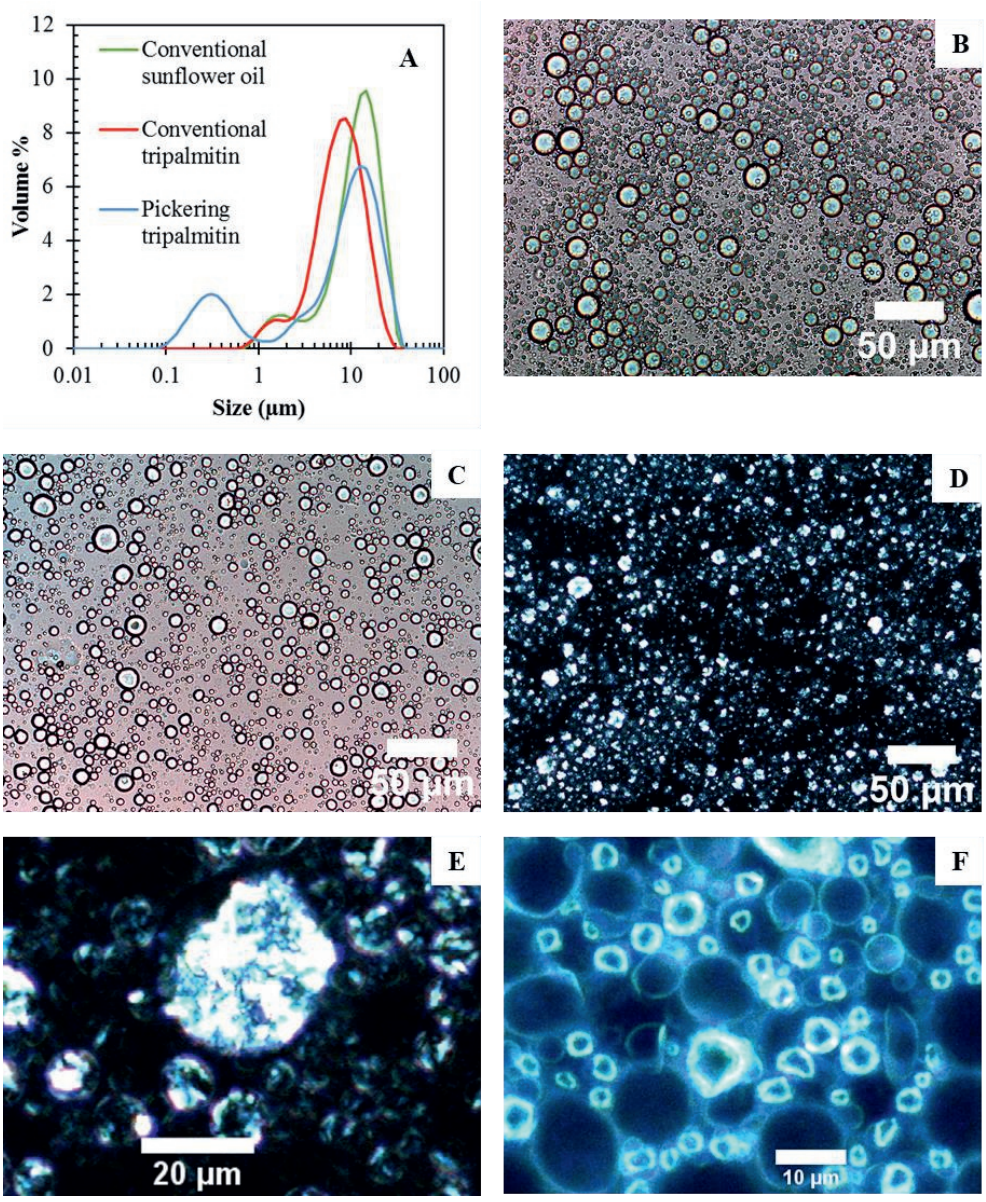


Figure 5.6. Characterization of coarse emulsions prepared with a lab scale colloid mill: droplet size distribution (A); light microscopy images of conventional sunflower oil (B), and solid core tripalmitin emulsions (C), and polarized light images of the conventional tripalmitin emulsion (D&E), and the Pickering emulsion prepared with tripalmitin particles (F).

5.3.3. Oxidative stability of O/W emulsions: Formation of primary and secondary lipid oxidation products

Measurements of conjugated diene (CD) hydroperoxides and total aldehydes (*p*AV) were performed to characterize the oxidative stability of emulsions, whereas physical stability was monitored through the droplet size at the beginning and the end of the incubation period.

First, we incubated three emulsions with 2 mM $FeSO_4$/EDTA at 25 °C: the conventional sunflower oil emulsion, the solid core tripalmitin emulsion, and the tripalmitin CLP-stabilized Pickering emulsion. The emulsions containing tripalmitin showed some aggregation, but no irreversible physical destabilization, whereas the conventional sunflower oil emulsion showed coalescence and visible oiling off (Supporting information, Figure S5.4.) when incubated in oxidative conditions.

We observed an immediate and rapid increase in CD hydroperoxide formation in all emulsions within the first 30-40 h (Figure 5.7.A). After 72 hours, the CD hydroperoxide concentration was the highest in the solid core tripalmitin emulsion (300 mmol kg^{-1} oil), followed by the Pickering emulsion (225 mmol kg^{-1} oil), and the lowest in the conventional sunflower oil emulsion (125 mmol kg^{-1} oil), with significant differences between the values ($p < 0.05$). We also found a rapid formation of aldehydes in all emulsions within the first few hours of incubation (Figure 5.7.B); after 72 h of incubation, the corresponding *p*AV were 18, 14 and 8, with significant differences between the values ($p < 0.05$). It is important to mention that the low values found for the conventional sunflower oil emulsion may be a result of the physical instability in this emulsion. The observed increase in droplet size, due to coalescence over the time-scale of the incubation period, implies that the amount of oil-water interfacial area decreased, which can slow down lipid oxidation (Berton, Ropers, et al., 2011).

To limit the physical instability of emulsions during the incubation period, we incubated the same emulsions with a lower oxidation initiator concentration (200 µM) at 25 °C. These conditions were also applied for the incubation of the emulsions containing palm stearin. The Pickering emulsions and the conventional emulsions containing HMP fat remained physically stable, and the conventional emulsions with only sunflower oil showed a slight increase in droplet size (Figure 5.8.A - C), albeit much less than found with the higher

concentration of oxidation initiator. The formation of lipid oxidation products is shown in Figure 5.7.C & D for the emulsions containing tripalmitin, and in Figure 5.7.E & F for the emulsions containing palm stearin. For both conventional solid core emulsions, a rapid increase in the CD hydroperoxide concentration was observed immediately after the start of incubation, followed by a levelling off towards the end of the incubation period, during which aldehydes started to form, as shown by the increase in *p*AV. Final CD hydroperoxide concentrations, and *p*AV for both solid core emulsions were about 250 mmol kg^{-1} oil and 6-7, respectively. Lipid oxidation in the Pickering emulsions and in the conventional sunflower oil emulsion proceeded slower and to a significantly lower extent than in the solid core emulsions; the sunflower oil emulsion, and palm stearin Pickering emulsion even showed a lag phase in aldehyde formation in the first ~20 h of incubation. At the end of the incubation period, CD hydroperoxide concentrations and *p*AV of about 125 mmol kg^{-1} oil and 2-4 were measured in both emulsions that behaved rather similarly.

Conventional solid core emulsions with palm stearin oxidized slightly faster than their tripalmitin counterparts (not significant). Two factors may explain this: the difference in physical state of the HMP fat, or the chemical composition. Palm stearin emulsions showed a lower onset crystallization temperature and lower crystallization enthalpy, hence the solid fat content in these emulsions was expected to be lower. Based on literature, a higher solid fat content could favor oxidation, as it increases the local concentration of unsaturated triglycerides at the interface where they are expected to interact with pro-oxidants (Calligaris, Sovrano, Manzocco, & Nicoli, 2006; Calligaris, Manzocco, & Nicoli, 2006; Okuda, McClements, & Decker, 2005; Waraho, McClements, & Decker, 2011). If this would also be the case for our solid core tripalmitin emulsion, they should theoretically oxidize faster than solid core palm stearin emulsions, which is not what we found. From a chemical composition point of view, palm stearin contains some unsaturated fatty acids (approximately 9.0% 18:1 and 1.8% 18:2), which could have favored lipid oxidation; this seems to us the most likely explanation for the present findings.

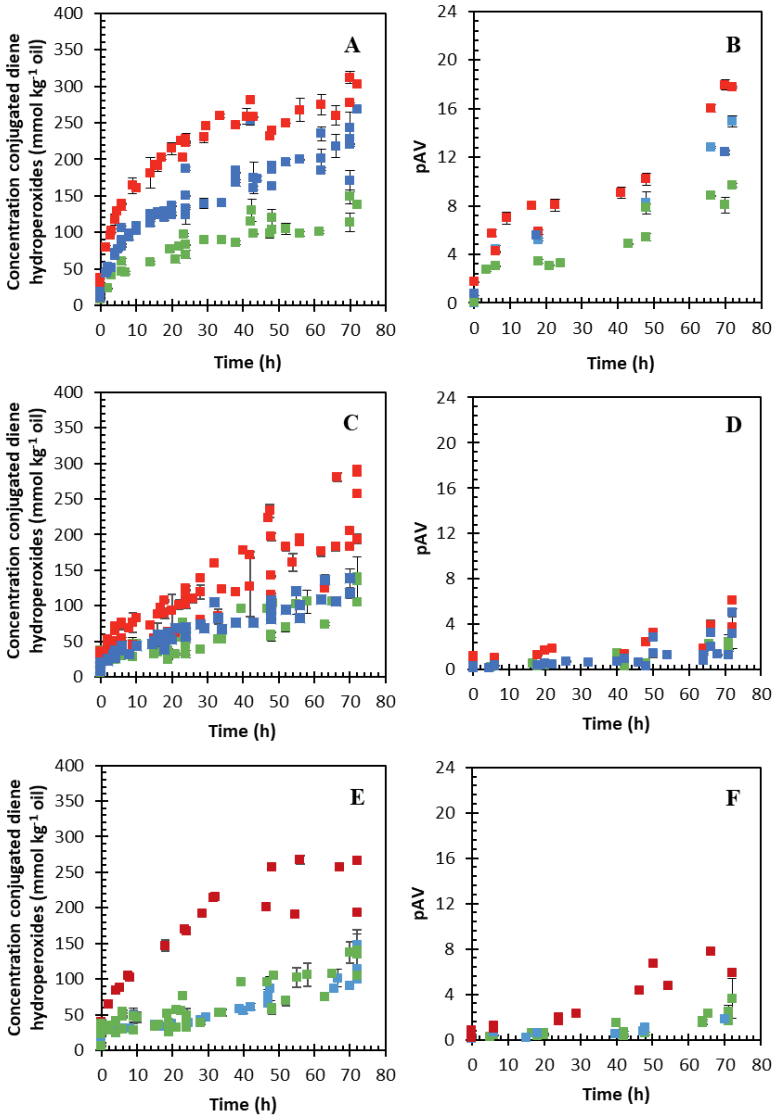


Figure 5.7. CD hydroperoxide concentration and pAV of conventional sunflower oil (green), solid core tripalmitin emulsion (red), and tripalmitin CLP-stabilized Pickering emulsion (blue) when incubated with 2 mM $FeSO_4$/EDTA (A and B) or 200 µM $FeSO_4$/EDTA (C & D), and of conventional sunflower oil (green), solid core palm stearin emulsion (dark red), and palm stearin CLP-stabilized Pickering emulsion (dark blue) when incubated with 200 µM $FeSO_4$/EDTA (E & F) at 25 °C.

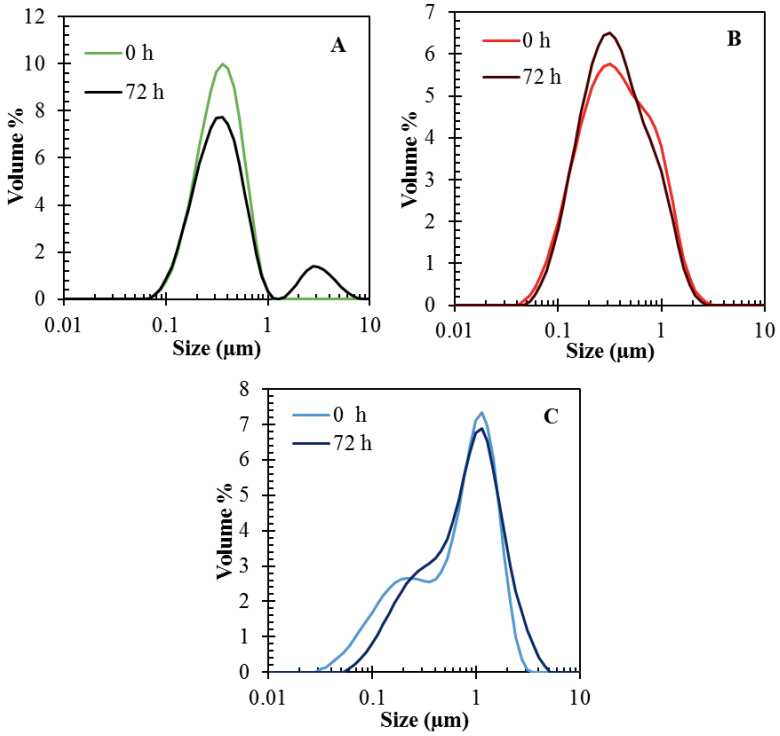


Figure 5.8. Particle size distribution of fresh emulsions and after 72 h of incubation with 200 µM $FeSO_4$/EDTA at 25 °C: Conventional sunflower oil (A), and tripalmitin (B) emulsion; and Pickering emulsion with tripalmitin particles (C).

To exclude that the fast oxidation of solid core emulsions was caused by pro-oxidant impurities in the HMP fat, we purified tripalmitin (particle size distribution and DSC thermogram in Supporting information, Figure S5.5.A & B), and measured the lipid oxidation markers during incubation with $FeSO_4$/EDTA 2 mM or 200 µM (Supporting information, Figure S5.5C-F). Although purification resulted in sharper size distribution of the particles, and slight differences in CLP crystalline structure, this did not significantly influence the extent of oxidation, which suggests that the involved oxidation pathways cannot be ascribed to the presence of minor components or impurities in the HMP fat itself.

5.3.4. Comparison with interpretations from literature

5.3.4.1. Solid core emulsions

The results obtained with conventional sodium caseinate-stabilized emulsions, either with liquid or partially solid core, show the importance of the structural organization of emulsions on lipid oxidation. The HMP fat in our conventional solid core emulsion droplets crystallized as inhomogeneously distributed intra-droplet clusters (Figure 5.6.). This probably increased the amount of oxidizable lipids close to the interface, where they are more prone to interact with aqueous pro-oxidants (and in particular, iron ions), resulting in fast oxidation.

Okuda et al. (2005) studied SDS-stabilized octadecane/methyl linolenate-based emulsions and found higher metal-catalyzed oxidation when methyl linolenate phase-separated from solid octadecane, compared to a system with liquid octadecane. Electron paramagnetic resonance has also been used to illustrate that lipid crystallization in sub-micron oil droplets could push lipophilic spin probes towards the droplet surface, making them more prone to reaction with hydrophilic components (Berton-Carabin, Coupland, & Elias, 2013). It seems therefore that partial or full intra-droplet lipid crystallization generally hampers the chemical stability of labile lipophilic molecules.

Alternatively, when lipid crystallization is templated at the interface, a solid lipid shell can be formed at the droplet surface, leading to opposite effects with regard to the location and reactivity of lipophilic molecules. For instance, Salminen et al. (2017) showed that HMP lecithin-induced crystallization of tristearin at the interface formed a solid interfacial shell that inhibited oxidation of fish oil, compared to emulsions containing low melting point lecithin, in which tristearin crystallization was random.

All these results show that the location of oxidizable fat in O/W emulsions influences their chemical stability. In some cases this is a positive effect as found for SLNs or nanostructured lipid carriers that have been reported to provide protection to encapsulated materials (Mehrad, Ravanfar, Licker, Regenstein, & Abbaspourrad, 2018; Wen, J. , Chen, G. and Chen, 2018), but it is clear that this is not necessarily the case. Especially for triglycerides with a narrow melting range, expulsion of oxidizable compounds is likely to occur, therewith promoting oxidation (Qian, Decker, Xiao, & McClements, 2013).

5.3.4.2. Pickering emulsions

A few previous studies have proposed that an interfacial particle layer could act as a physical barrier separating aqueous pro-oxidant species from oxidizable lipids (Kargar et al., 2012, 2011b, 2011a; Zhao et al., 2015). For example, Zhao et al. (2015) showed that the use of silica particle aggregates led to enhanced chemical stability of curcumin compared to a single layer of particles, but this may be alternatively explained as enhanced ability to quench oxidative species.

Our CLP-stabilized Pickering emulsions showed similar lipid oxidation rates to those measured in conventional sodium caseinate-stabilized sunflower oil emulsions (Figure 5.7.C-F), which suggests that the physical barrier effect is, at most, limited. It could be argued that the surface of the oil droplet is not fully covered and that, at the scale of very small molecules such as metal ions or reactive oxygen species, it cannot be expected that a particle layer can keep them from reaching the interface; therefore a physical barrier effect seems highly unlikely given the currently available particles and fabrication processes. It may be worth mentioning that protein-stabilized emulsions have often been reported to have a good oxidative stability because of the intrinsic antioxidant activity of proteins (Berton-Carabin et al., 2014), which could also have contributed to the stability of the conventional emulsions studied here. However, this effect, if present at all, was certainly limited due to the low protein concentration used. Besides, all the emulsions studied in the present work had the same total protein concentration.

It should be pointed out that Pickering particles can be modified to achieve a certain functionality. For example, Timgren et al. (2011) showed that gelatinization of starch Pickering particles adsorbed onto lipid emulsion droplets effectively reduced lipolysis compared to emulsions stabilized by intact starch granules. Their work shows that making the oil-water interfacial layer structurally more homogeneous made it less permeable at the level of enzymes such as lipase (i.e., the nanometer range; Timgren et al., 2011). At an even smaller scale, relevant to lipid oxidation, Zeng et al. (2017) showed that modification of Pickering particles with inclusion of an antioxidant (proanthocyanidin) did reduce oxidation. It seems thus that many options can still be considered to improve the ability of Pickering particles to promote the chemical stability of emulsions.

5.4. Conclusions

We have investigated the ability of HMP fat-based Pickering particles to protect emulsions against lipid oxidation. To unambiguously assess their potential in that respect, we have compared lipid oxidation in emulsions with the same overall composition but with different structural organization (i.e., HMP fat present either at the oil droplet surface as Pickering particles, or mixed within the oil droplet core), and found that partial fat crystallization within the emulsion droplets promotes lipid oxidation. This is probably because the HMP fat crystallized as inhomogeneously distributed intra-droplet clusters, that pushed oxidizable lipids to the oil-water interface, where they were more accessible to aqueous phase pro-oxidants, and thus more prone to oxidation. Pickering emulsions showed similar lipid oxidation rates compared to sodium caseinate-stabilized emulsions, which suggests that CLPs were not able to enhance the chemical stability of emulsions compared to conventional emulsifiers through a physical barrier effect. Presumably, pro-oxidant molecules in the aqueous phase of the emulsions can still come into direct contact with oxidizable lipids at the interface. Yet, for a fixed lipid composition with a fraction of HMP fat, a CLP-based Pickering emulsion structure has advantages in terms of physico-chemical stability. This work illustrates that the location of solid fat is of great influence on the chemical stability of O/W emulsions, and thus needs to be carefully considered and controlled for emulsion design.

5.5. Supporting information

Table S5.1. Fatty acid composition of palm stearin (n=2)

Component name	Percentage average
C14:0	$1.16^{\pm 0.03}$
C16:0	$82.18^{\pm 0.13}$
C18:0	$5.12^{\pm 0.05}$
C18:1	$9.03^{\pm 0.07}$
C18:2	$1.83^{\pm 0.01}$
C18:3	$0.02^{\pm 0.00}$
C20:0	$0.32^{\pm 0.01}$
C22:0	$0.25^{\pm 0.01}$
C22:1	$0.04^{\pm 0.01}$
C24:0	$0.05^{\pm 0.00}$

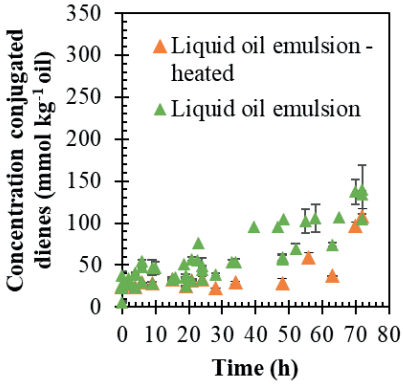


Figure S5.2. CD hydroperoxide concentration in sunflower oil emulsions made with high or low homogenization temperature, incubated with 200 µM $FeSO_4$/EDTA.

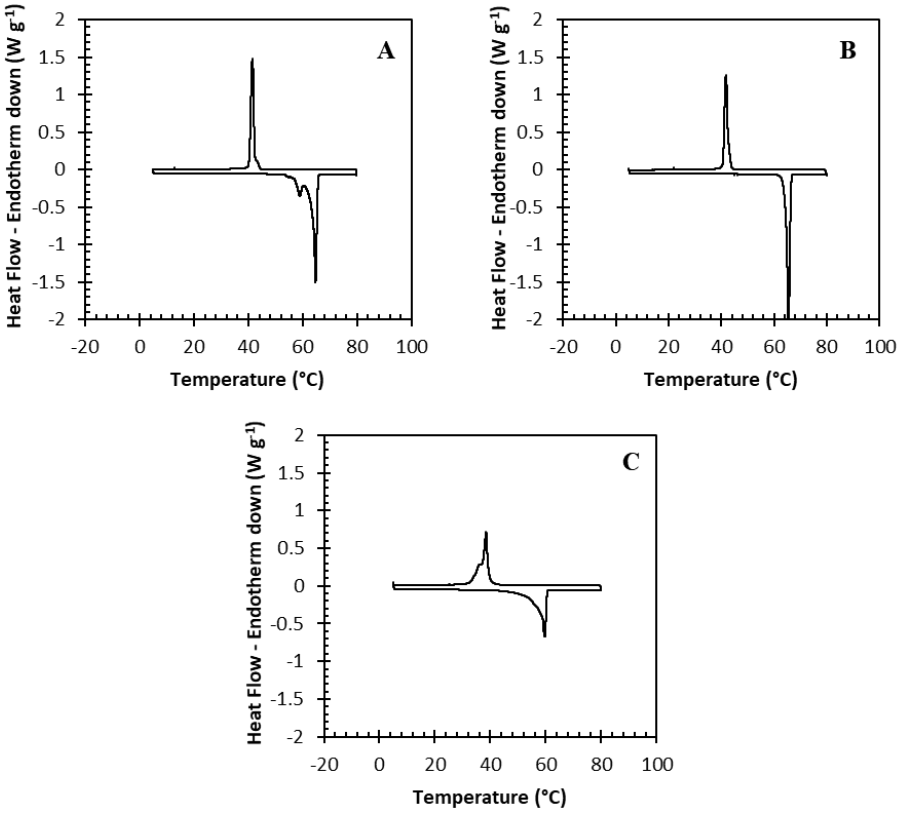


Figure S5.3. DSC thermograms of bulk tripalmitin (A); purified bulk tripalmitin (B); and bulk palm stearin (C).

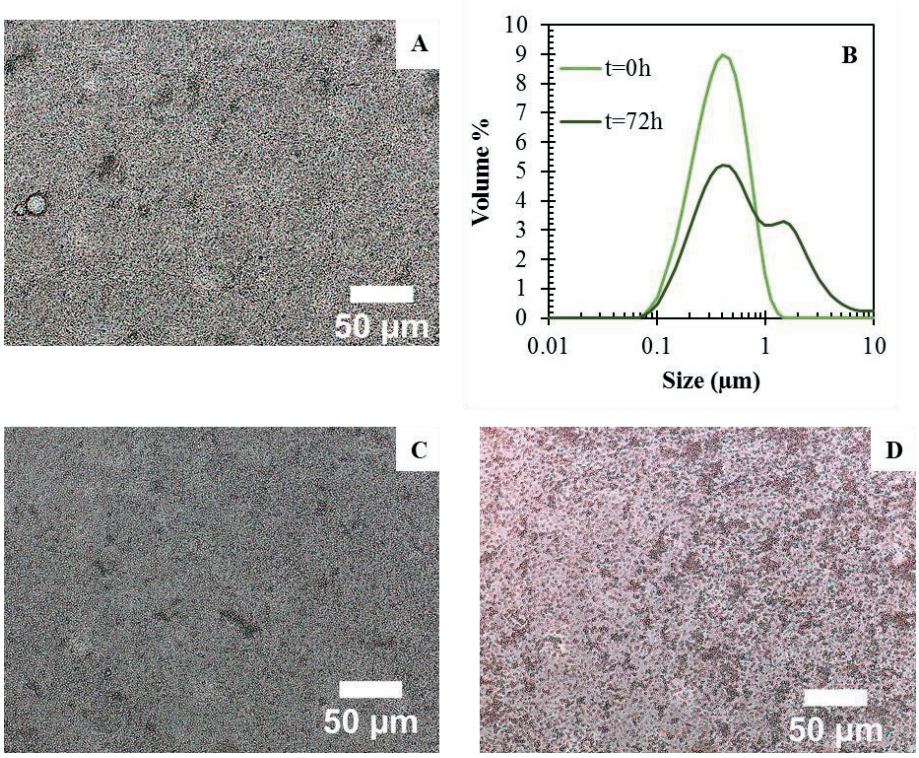


Figure S5.4. Emulsion stability after 72 h of incubation with $FeSO_4$/EDTA, 1/1, M/M, 2 mM: Light microscopy image (A) and particle size distribution of conventional sunflower oil emulsion (B), conventional emulsion containing tripalmitin (C) and Pickering emulsion stabilized by tripalmitin CLPs (D).

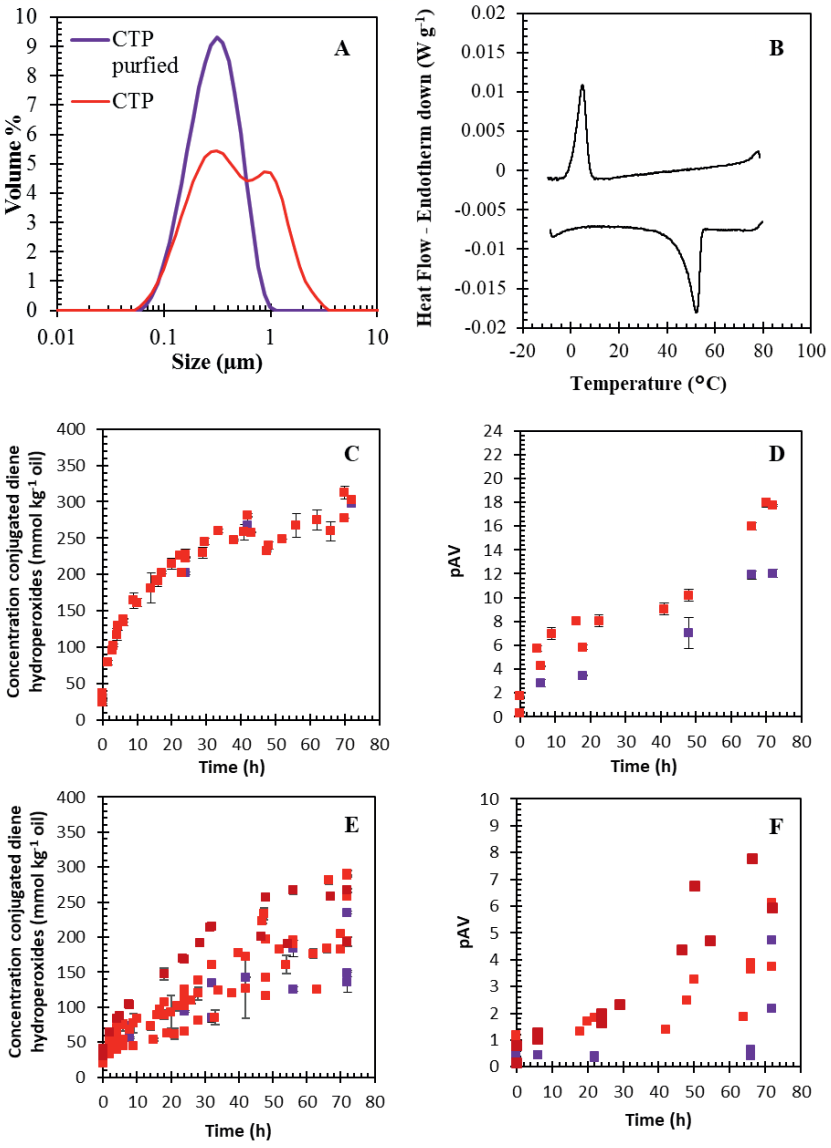


Figure S5.5. Characterization of conventional emulsions containing tripalmitin (CTP. red), purified tripalmitin (CTP purified, purple) or palm stearin (dark red) in the core of the oil droplets: Particle size distribution (A) and DSC thermogram of CTP purified emulsion (B). CD hydroperoxide concentration (C and E) and pAV (D and F) incubated with 2 mM $FeSO_4$/EDTA(C and D) or 200 µM $FeSO_4$/EDTA (E and F) at 25 °C.

5.6. References

AOCS. (1998). p-Anisidine value - Official method CD 18-90. In Official Methods and Recommended Practices of the American Oil Chemists. AOCS Press, Champaign (USA).

Berton-Carabin, C. C., Coupland, J. N., & Elias, R. J. (2013). Effect of the lipophilicity of model ingredients on their location and reactivity in emulsions and solid lipid nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 431, 9–17.

Berton-Carabin, C. C., Ropers, M. H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Comprehensive Reviews in Food Science and Food Safety, 13(5), 945–977.

Berton-Carabin, C. C., & Schroën, K. (2015). Pickering emulsions for food applications: background, trends, and challenges. Annual Review of Food Science and Technology, 6, 263–297.

Berton, C., Genot, C., & Biopolym, U. R. (2011). Contribution of the Interfacial Layer to the Protection of Emulsified Lipids against Oxidation. 5052–5061.

Berton, C., Genot, C., & Ropers, M.-H. (2011). Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. Journal of Colloid and Interface Science, 354(2), 739–748.

Berton, C., Ropers, M. H., Viau, M., & Genot, C. (2011). Contribution of the interfacial layer to the protection of emulsified lipids against oxidation. Journal of Agricultural and Food Chemistry, 59(9), 5052–5061.

Calligaris, S, Sovrano, S., Manzocco, L., & Nicoli, M. C. (2006). Influence of crystallization on the oxidative stability of extra virgin olive oil . Journal of Agricultural and Food Chemistry, 54, 535–539.

Calligaris, Sonia, Manzocco, L., & Nicoli, M. C. (2006). Modelling the temperature dependence of oxidation rate in water-in-oil emulsions stored at sub-zero temperatures. Food Chemistry, 101(3), 1019–1024.

Chen, B., McClements, D. J., & Decker, E. A. (2011). Minor components in food oils: A critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. Critical Reviews in Food Science and Nutrition, 51(January 2014), 901–916.

Corongiu, F. P., & Banni, S. (1994). Detection of conjugated dienes by second derivative ultraviolet spectrophotometry. Methods in Enzymology, 233, 303–310.

El-beltagi, H. S., & Mohamed, H. I. (2013). Reactive Oxygen Species , Lipid Peroxidation and Antioxidative Defense Mechanism. 41(1), 44–57.

Faraji, H., McClements, D. J., & Decker, E. A. (2004). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 52(14), 4558–4564.

Genot, C., Berton, C., & Ropers, M.-H. (2013). The role of the interfacial layer and emulsifying proteins in the oxidation in oil-in-water emulsions. In X. P. A. Logan, U. Nienaber (Ed.), Lipid Oxidation: Challenges in Food Systems. Urbana, IL: AOCS Press.

Graham, D. E., & Phillips, M. C. (1979a). Proteins at liquid interfaces II. Adsorption isotherms. Journal of Colloid and Interface Science, 70(3), 415–426.

Graham, D. E., & Phillips, M. C. (1979b). Proteins at liquid interfaces III. Molecular structures of adsorbed films. Journal of Colloid and Interface Science, 70(3), 427–439.

Gunstone, F. D., Harwood, J. L., & Dijkstra, A. J. (2007). The Lipid Handbook. CRC Press.

Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., & Norton, I. T. (2012). Investigation into the potential ability of Pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 366(1), 209–215.

Kargar, M., Spyropoulos, F., & Norton, I. T. (2011a). Microstructural design to reduce lipid oxidation in oil-in- water emulsions. Italian Oral Surgery, 1, 104–108.

Kargar, M., Spyropoulos, F., & Norton, I. T. (2011b). The effect of interfacial microstructure on the lipid oxidation stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 357(2), 527–533.

Koh, C. A. C. A. (2002). Effect of Emulsifier on Oxidation Properties of Fish Oil-Based Structured Lipid Emulsions. Journal of Agricultural and Food Chemistry, 2957–2961.

Laguerre, M., Bayrasy, C., Panya, A., Weiss, J., McClements, D. J., Lecomte, J., … Villeneuve, P. (2015). What makes good antioxidants in lipid-based systems? The next theories beyond the polar paradox. Critical Reviews in Food Science and Nutrition, 55(2), 183–201.

Laguerre, M., Bily, A., Roller, M., & Birtic, S. (2017). Mass transport phenomena in lipid oxidation and antioxidation. Annual Review of Food Science and Technology, 8(1), annurev-food-030216-025812.

Lovegren, N. V., Gray, M. S., & Fuege, R. O. (1976). Effect of liquid fat on melting point and polymorphic behaviour of cocoa butter and a cocoa butter fraction. Journal of the American Oil Chemists' Society, 53, 108–112.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. Journal of Food Science, 65(8), 1270–1282.

Mehrad, B., Ravanfar, R., Licker, J., Regenstein, J. M., & Abbaspourrad, A. (2018). Enhancing the physicochemical stability of β-carotene solid lipid nanoparticle (SLNP) using whey protein isolate. Food Research International, 105(December 2017), 962–969.

Okuda, S., McClements, D. J., & Decker, E. A. (2005). Impact of Lipid Physical State on the Oxidation of Methyl Linolenate in Oil-in-Water Emulsions. Journal of Agricultural and Food Chemistry, 53(24), 9624–9628.

Osborn, H. T., & Akoh, C. C. (2004). Effect of emulsifier type , droplet size , and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. 84, 451–456.

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2013). Impact of lipid nanoparticle physical state on particle aggregation and β-carotene degradation: Potential limitations of solid lipid nanoparticles. Food Research International, 52(1), 342–349.

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2017). Tuning of shell thickness of solid lipid particles impacts the chemical stability of encapsulated Omega-3 fish oil. Journal of Colloid and Interface Science, 490, 207–216.

Schaich, K. M., Shahidi, F., Zhongy, Y., & Eskin, N. A. M. (2013). Lipid oxidation. In Biochemistry of Foods (Third Edit, pp. 419–478).

Schröder, A., Sprakel, J., Schroën, K., & Berton-Carabin, C. (2017). Tailored microstructure of colloidal lipid particles for Pickering emulsions with tunable properties. Soft Matter, 3190–3198.

Schröder, A., Sprakel, J., Schroën, K., Spaen, J., & Berton-Carabin, C. C. (2018). Coalescence stability of Pickering emulsions produced with lipid particles: A microfluidic study. Journal of Food Engineering, 234, 63–72.

Timgren, A., Rayner, M., Sjöö, M., & Dejmek, P. (2011). Starch particles for food based Pickering emulsions. Procedia Food Science, 1, 95–103.

Waraho, T., McClements, D. J., & Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. Trends in Food Science & Technology, 22(1), 3–13.

Wen, J. , Chen, G. and Chen, S. (2018). Nanostructured lipid carriers. In I. O. and J. W. S. Roohinejad, R. Greiner (Ed.), Emulsion-based Systems for Delivery of Food Active Compounds.

Xiao, J., Li, C., & Huang, Q. (2015). Kafirin nanoparticle-stabilized Pickering emulsions as oral delivery vehicles: physicochemical stability and in vitro digestion profile. Journal of Agricultural and Food Chemistry, 63(47), 10263–10270.

Yuji, H., Weiss, J., Villeneuve, P., Giraldo, L. J. L., Figueroa-Espinoza, M. C., & Decker, E. a. (2007). Ability of surface-active antioxidants to inhibit lipid oxidation in oil-in-water emulsion. Journal of Agricultural and Food Chemistry, 55, 11052–11056.

Zeng, T., Wu, Z. ling, Zhu, J. Y., Yin, S. W., Tang, C. H., Wu, L. Y., & Yang, X. Q. (2017). Development of antioxidant Pickering high internal phase emulsions (HIPEs) stabilized by protein/polysaccharide hybrid particles as potential alternative for PHOs. Food Chemistry, 231, 122–130.

Zhao, Y., Guan, Y., Pan, Y., Nitin, N., & Tikekar, R. V. (2015). Improved oxidative barrier properties of emulsions stabilized by silica-polymer microparticles for enhanced stability of encapsulants. Food Research International, 74, 269–274.

Chapter 6

Chemical stability of α-tocopherol in colloidal lipid particles with various morphologies

This chapter has been submitted as Schröder, A., Sprakel, J., Schroën, K. and Berton-Carabin, C. C. Chemical stability of α-tocopherol in colloidal lipid particles with various morphologies.

Abstract

Colloidal lipid particles (CLPs) are promising encapsulation systems for lipophilic bioactives, such as oil-soluble antioxidants that are applied in food and pharmaceutical formulations. Currently, there is no clear consensus regarding the relation between particle structure and the chemical stability of such bioactives. Using α-tocopherol as a model antioxidant, we showed that emulsifier type (Tween 20 or 40, or sodium caseinate) and lipid composition (tripalmitin, tricaprylin, or combinations thereof) modulated particle morphology and antioxidant stability.

The emulsifier affected particle shape, with the polysorbates facilitating tripalmitin crystallization into highly ordered lath-like particles, and sodium caseinate resulting in less ordered spherical particles. The fastest degradation of α-tocopherol was observed in tripalmitin-based CLPs, which may be attributed to its expulsion to the particle surface induced by lipid crystallization. This effect was stronger in CLPs stabilized by Tween 40, which may act as a template for crystallization.

This work not only shows how the architecture of CLPs can be controlled through the type of lipid and emulsifier used, but also gives evidence that lipid crystallization does not necessarily protect entrapped lipophilic bioactives, which is an important clue for encapsulation system design.

6.1. Introduction

The interest in enriching food and pharmaceutical products with lipophilic bioactives (for example, vitamins, flavors, pigments, antioxidants) is growing rapidly, and often involves encapsulation to protect these bioactives against chemical degradation, to enhance their solubility, activity or absorption, and to control their delivery (Qian, Decker, Xiao, & McClements, 2013; Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013a). Submicron and partly solid lipid droplets, that may be referred to as colloidal lipid particles (CLPs) have emerged as potential encapsulation systems for lipophilic bioactive compounds, and have been extensively studied lately (Jenning & Gohla, 2001; Oehlke, Behsnilian, Mayer-miebach, Weidler, & Greiner, 2017; Salminen, Helgason, et al., 2014; Trombino et al., 2009; Yadav, Khatak, Vir, & Sara, 2013; zur Mühlen, Schwarz, & Mehnert, 1998).

It may be useful to clarify the related terminology: the term “solid lipid nanoparticle” (SLN) has been extensively used, but is not always appropriate, because (i) the lipid phase may not be fully solid, and (ii) the prefix “nano” applies to “natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate of which, for 50% or more of the particles in the number size distribution, one or more external dimensions is between 1 nm-100 nm” (Bleeker et al., 2012). This classification does not hold for all particles termed SLNs (Bleeker et al., 2012; Mcclements, 2012). This is why “solid lipid particle” (SLP) should be preferred (McClements & Li, 2010); or, if the lipid phase is not fully crystallized, “(nano)structured lipid carrier” may be more accurate (Dan, 2016), or “colloidal lipid particle”, which is more generic, and will be used in the present work.

CLP dispersions can be manufactured easily at large scale using high pressure homogenization, which also allows elevated temperatures when using high melting point (HMP) fats (Aditya & Ko, 2015). Biocompatible lipids can be used to make a lipid matrix with minimal toxicity, and relatively high encapsulation efficiency for lipophilic components compared to e.g., liposomes (Mukherjee, Ray, & Thakur, 2009; Salminen et al., 2013a; Suresh, Manjunath, Venkateswarlu, & Satyanarayana, 2007; Thrandur et al., 2009). Lipid materials that are partly or fully crystallized have been suggested to protect labile lipophilic molecules against chemical degradation (often, oxidation) by preventing accumulation at the interface (Helgason, Awad, Kristbergsson, McClements, & Weiss, 2009a; Salminen, Gömmel, Leuenberger, & Weiss, 2016), and by limiting diffusion of molecules involved in the oxidative reaction throughout the lipid phase (McClements & Li, 2010). A solid lipid

matrix has also been suggested to increase control over bioactive release e.g., under digestive conditions (Dan, 2014, 2016; McClements & Li, 2010), to improve absorption into the lymph and blood (Bargoni et al., 1998; Miglietta, Cavalli, Bocca, Gabriel, & Rosa Gasco, 2000), and to delay lipid digestion (McClements & Li, 2010).

The design of CLPs entrapping a lipophilic bioactive brings along challenges since the functionality depends on the lipid matrix structure (Bunjes, Westesen, & Koch, 1996). Lipids with a sufficiently high melting point may crystallize directly after CLP preparation in a polymorphic form that will depend on the lipid purity, presence of other ingredients (in particular, emulsifiers), and cooling rate. CLPs may also recrystallize during storage into more stable polymorphic forms. Lipids with a high purity can form highly ordered crystalline structures, which can result in the expulsion of the encapsulated lipophilic compound from the crystalline matrix (Salminen et al., 2016). This is especially the case when CLPs are manufactured with HMP alkanes or triacylglycerols with a narrow melting range (Qian et al., 2013; Tikekar & Nitin, 2011; Yucel, Elias, & Coupland, 2012). This may lead to enhanced chemical degradation of the encapsulated compound by aqueous phase reactants (Berton-Carabin, Coupland, & Elias, 2013; Yucel et al., 2012), although in some applications slow release (i.e., a certain degree of expulsion) may be desirable, e.g., when the activity of an antioxidant should be maintained for prolonged periods (Oehlke et al., 2017).

To counteract such an often undesirable expulsion and to increase the lipophilic compound-loading capacity, the use of blended lipids has been proposed (Dan, 2014, 2016). This leads to a lipid phase with a broader melting range compared to a pure lipid, resulting in a more disordered crystalline structure that is less prone to polymorphic transitions (Qian et al., 2013). Alternatively, fat crystallization can be influenced by the molecular structure of emulsifier used to stabilize the CLPs (Arima, Ueji, Ueno, Ogawa, & Sato, 2007; Helgason, Awad, Kristbergsson, McClements, & Weiss, 2009b; Salminen, Helgason, et al., 2014). For instance, surfactants with a high melting point alkyl chain can induce surface-templated crystallization of the lipid phase, promoting crystal growth from the interface therewith initiating particle structure and morphology (Rousseau, 2013; Salminen, Aulbach, Leuenberger, Tedeschi, & Weiss, 2014; Schröder, Sprakel, Schroën, & Berton-Carabin, 2017), which promoted the stability of labile lipophilic molecules (Salminen et al., 2013a, 2014).

We selected α-tocopherol (vitamin E), as model lipophilic bioactive to be encapsulated in CLPs. Being a naturally occurring chain-breaking antioxidant, it is relevant to a broad range of food and biobased applications, and poses challenges due to its chemical instability and poor solubility in water (Dima, Dima, & Iordachescu, 2015; Oehlke et al., 2017; Saez, Souza, & Mansur, 2018; Trombino et al., 2009). A few studies have attempted at encapsulating α-tocopherol in related systems; Dingler et al. (1999) showed that SLNs protected it better against chemical degradation than an oil-in-water emulsion (Dingler, Blum, Niehus, Müller, & Gohla, 1999). Oehlke et al. (2017) found that tocopherol-containing SLNs had good physical stability and showed a gradual release of tocopherol, which is important for systems in which long term radical scavenging activity is desired (Oehlke et al., 2017). For oil-in-water emulsions, it was found that up to 95% of tocopherol was located in the emulsifier layer rather than the core of the droplets (Sánchez-Paz et al., 2008). Although these findings are very relevant, general guidelines that link the choice of lipids and emulsifiers to CLP structure, and to bioactive stability, are not available yet.

In the present work, we therefore systematically investigated the effect of lipid (tripalmitin, tricaprylin, or combinations thereof) and emulsifier (Tween 20 or Tween 40, i.e., surfactants with alkyl chains of low or high melting point, respectively; or sodium caseinate, i.e., a disordered protein) on CLP morphology, crystalline structure, and the stability of α-tocopherol. Understanding these aspects, and how they are linked, can be used as the starting point for the rational design of CLPs as delivery systems for lipophilic bioactives such as oil-soluble antioxidants.

6.2. Materials & methods

6.2.1. Materials

Tripalmitin (#T8127, purity 99%), tricaprylin (#91040, purity 90%), Tween 20 (#P1379), Tween 40 (#P1504), sodium phosphate monobasic (#S9638), sodium phosphate dibasic (#S9763), sodium chloride (#S9888, purity 99%), iron(II) sulfate heptahydrate (#F8633), ethylenediaminetetraacetic acid disodium salt dihydrate (#E6635), and α-tocopherol (#T3251) were purchased from Sigma-Aldrich (Saint Louis, USA). Methanol (#813012802), chloroform (#803010802) and hexane (#808023502) were obtained from Actu-All Chemicals (Oss, the Netherlands). Sodium caseinate (#41610, spray dried, protein content 91.0%) was supplied by DMV International (Veghel, the Netherlands). Ultrapure water (18

MΩ) was prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA) and was used for all the experiments. All other chemicals used were of analytical grade. The chemicals were used without further purification.

6.2.2. Methods

6.2.2.1. Preparation of colloidal lipid particles (CLPs)

We heated an aqueous phase (95% w/w) containing 1% w/w sodium caseinate or 2% w/w Tween 20 or 40 in phosphate buffer (10 mM, pH 7.0) to 80 °C in a water bath and added it to a melted fat phase (5% w/w) (tripalmitin, tricaprylin, or tripalmitin mixed with tricaprylin in a mass ratio 4:1) preheated at the same temperature, which had previously been spiked with 100 µL methanolic solution of α-tocopherol (200 mg mL^{-1}). Final α-tocopherol concentration was 4 mg g^{-1} of fat. A coarse emulsion was prepared by high speed stirring the mixture at 11,000 rpm for one min using a preheated rotor-stator homogenizer (Ultra-turrax IKA T18 basic, Germany). We then homogenized this coarse emulsion using a high pressure homogenizer (Microfluidizer® Processor MF 110Y equipped with a Y-shaped interaction chamber (F12Y; minimum internal dimension: 75 µm); Microfluidics, Newton, Massachusetts, USA) at 800 bar (5 cycles) and 80 °C to obtain submicron-sized droplets, which were left to cool at refrigerated temperature (4 °C) over ~2 h, inducing crystallization, except for the CLPs made with pure tricaprylin as the oil phase, which were fully liquid at the temperatures used in this work.

6.2.2.2. Characterization of CLPs

The particle size distribution and average diameters ($d_{3,2}$) were determined by static light scattering (Malvern Mastersizer 3000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). The following optical properties were used: refractive indices of 1.540 (lipid phase) and 1.330 (water) with an absorption index of 0.01. Particle size distributions of CLPs after 14 days incubation under oxidative conditions are reported in Supporting information, Figure S6.1.

Particle surface charge was evaluated by ζ-potential measured with a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, UK) at 25 °C. Emulsions were diluted ~500 times in phosphate buffer (10 mM, pH 7.0) to prevent multiple scattering.

Transmission electron microscopy (TEM) was performed on CLPs (dispersions were diluted ~100 fold) deposited onto a freshly glow discharged carbonized copper grid (200 mesh). The excess solvent was blotted using standard filter paper. The particles were stained with a 1% w/w phosphotungstic acid solution (PTA). Images were recorded on a JEOL JEM 1400 plus transmission electron microscope (Peabody, USA) operating at 120 kV in combination with a JEOL CCD camera Ruby (8 M pixel).

The melting and crystallization behavior of CLPs was investigated using a differential scanning calorimeter (Discovery Series DSC 250, TA Instruments, Zellik, Belgium). CLP dispersion (~25 mg) was placed in a T zero pan closed with a hermetic lid, and was heated from -10 °C to 80 °C at 1 °C min^{-1}, then cooled down to -10 °C at 1 °C min^{-1} followed by two identical heating/cooling cycles. An empty pan was used as a reference. The thermograms were evaluated using the TRIOS software; melting and crystallization enthalpies are reported in Supporting information, Table S6.2.

6.2.2.3. Incubation of CLP dispersions

An oxidation initiator system consisting of an equimolar mixture of $FeSO_4$ and EDTA was prepared by mixing equivalent volumes of separately dissolved $FeSO_4$ and EDTA (8.4 mM) in phosphate buffer under moderate stirring in the dark for one hour (Berton, Ropers, Viau, & Genot, 2011). Aliquots of CLP dispersion (2 g) were distributed in 15-mL polypropylene centrifuge tubes, and the oxidation initiator solution (100 µL) was added to obtain a final concentration of 200 µM of both iron and EDTA. The tubes were rotated in the dark at 2 rpm at 25 °C for 72 h (SB3 rotator, Stuart, Staffordshire, UK).

6.2.2.4. Extraction of *α*-tocopherol

α-Tocopherol was extracted from our CLPs by adding 4 mL of chloroform, 3 mL of methanol and 1 mL of saturated sodium chloride solution to 2 mL CLP dispersion in a 15-mL polypropylene centrifuge tube, which was vortexed followed by centrifugation at 2000×g for 8 min. The clear chloroform phase was then collected by cautiously boring a hole in the bottom of the centrifugation tube.

6.2.2.5. Quantification of *α*-tocopherol by HPLC analysis

The obtained extracts were analyzed on a UltiMate 3000 liquid chromatography system (Thermo Scientific, Sunnyvale, CA, USA) using a carotenoid C30 reversed phase column, 3 µm, 150 x 4.6 mm (YMC, Dinslaken, Germany). Extracts were eluted at 1 mL min^{-1} at 30 °C

using a mobile phase with a linear gradient going from 81% methanol, 14% methyl t-butyl ether (MTBE) and 4% ultrapure water to 74% methanol, 22% methyl t-butyl ether and 4% ultrapure water within 8 min, and going back to its initial composition in 2 min. α-Tocopherol was detected with a UV-VIS detector at 292 nm (Dionex™ UltiMate™ 3000 Variable Wavelength Detector), and contents were calculated using a calibration curve that was linear in the range from 5 μg mL^{-1} to 5000 μg mL^{-1}. When studying the chemical degradation of α-tocopherol during incubation of CLP dispersions, the results were expressed as normalized α-tocopherol amount (%), taking as a reference the α-tocopherol concentration in the respective CLP suspension just after production.

6.2.2.6. Experimental design

All CLPs were prepared and characterized as at least independent duplicates. Size and ζ-potential measurements were performed in triplicate, and extractions and HPLC analyses were performed in duplicate. All results are reported as the mean and standard deviation of all measurements.

6.3. Results & discussion

6.3.1. Physical characterization of CLPs

CLPs were produced by high pressure homogenization of an aqueous phase containing Tween 20, Tween 40, or sodium caseinate with either melted tripalmitin, or tripalmitin mixed with tricaprylin in a mass ratio 4:1, or tricaprylin, followed by cooling. The particle size distribution of all CLPs just after preparation was unimodal, with a mean diameter ($d_{3,2}$) of 90-110 nm for surfactant-stabilized CLPs, or 120-150 nm for sodium caseinate-stabilized CLPs (Figure 6.1.A-C). The particle size was thus slightly dependent on the type of emulsifier, which can be explained by the fact that surfactants lower the interfacial tension more compared to proteins, which facilitates droplet break-up during homogenization (Bos & van Vliet, 2001). For a given emulsifier, increasing the liquid fat content slightly decreased the droplet size, which can be attributed to the lower viscosity of these oil phases, which, again, facilitates droplet break-up (Walstra, 1993). Fat composition also affected the surface charge of CLPs, with more negative ζ-potential for tripalmitin particles (Figure 6.1.D). This could be caused by the crystallinity of the fat in the CLPs, promoting ion binding at the particle surface (Freitas & Müller, 1998). CLPs stabilized by Tween 20 and Tween 40 showed a lower net ζ-potential (-5 to -15 mV) compared to sodium caseinate-stabilized CLPs (-30 to

-35 mV): polysorbate surfactants are non-ionic, whereas sodium caseinate contains ionizable groups and has an isoelectric point around 4.6 (Braga, Menossi, & Cunha, 2006), making it strongly negatively charged at neutral pH.

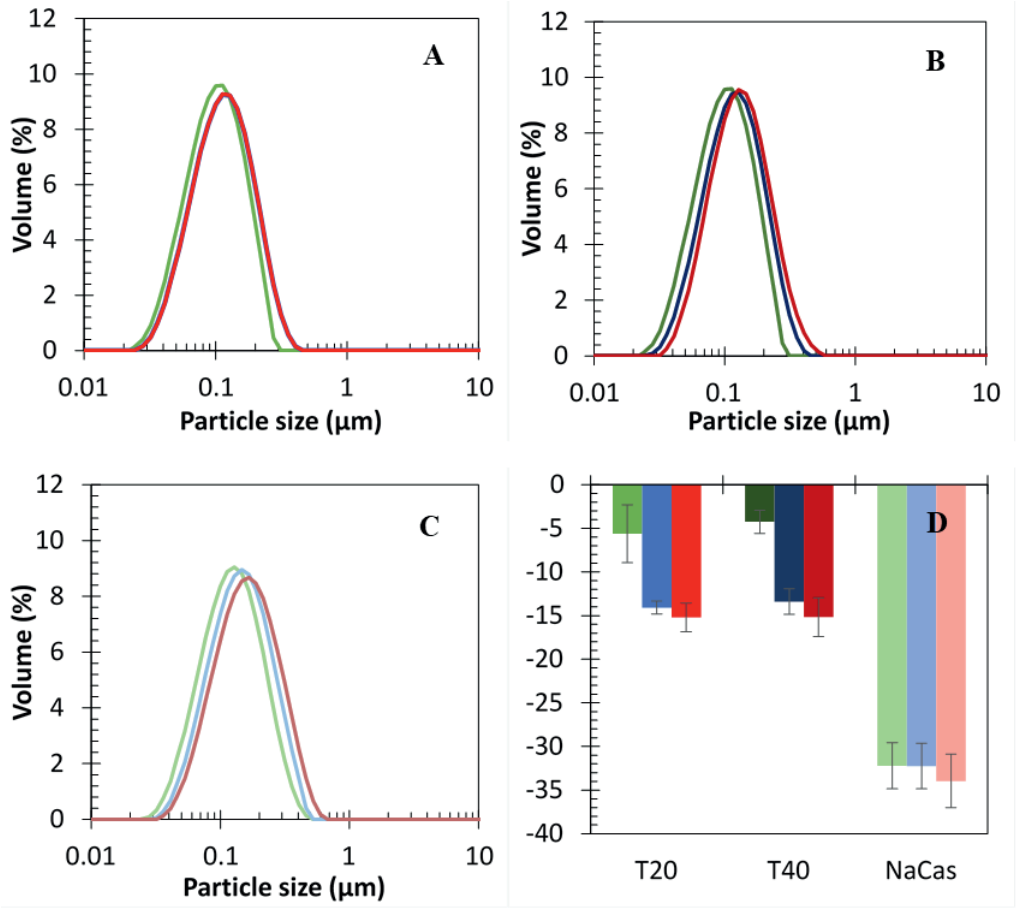


Figure 6.1. Particle size distributions of Tween 20- (A), Tween 40- (B) and sodium caseinate- (C) stabilized CLPs containing tripalmitin (red), tripalmitin:tricaprylin 4:1 (blue), or tricaprylin (green), and their ζ-potential (D). On panel D, T20, T40 and NaCas stand for Tween 20, Tween 40 and sodium caseinate, respectively.

We visualized the CLPs with transmission electron microscopy, and found clear morphological differences (Figure 6.2.). Pure tripalmitin-based CLPs stabilized with Tween 20 or Tween 40 had a lath-like morphology with a high aspect ratio. When they contained a fraction of tricaprylin, more platelet-like particles with a much lower aspect ratio were

obtained. Conversely, pure tripalmitin-based CLPs stabilized with sodium caseinate were nearly spherical. When these sodium caseinate-stabilized CLPs contained a fraction of tricaprylin, their morphology also showed a low aspect ratio, but were more irregular, compared to tripalmitin-based ones. The morphological differences of the particles may be related to their crystalline microstructure, which was further investigated by differential scanning calorimetry (Figure 6.3.).

DSC melting thermograms of all pure tripalmitin-based CLPs showed a sequence of melting peaks that point to a complex layered structure, which would melt in multiple identifiable events from the interface (Bunjes, Koch, & Westesen, 2000; Coupland, 2002; Schröder et al., 2017). The melting thermograms of sodium caseinate-stabilized CLPs also showed a small exothermic peak that indicates crystal reorganization and recrystallization into a more stable polymorphic form (Awad, Helgason, Weiss, Decker, & Mcclements, 2009; Schröder, Sprakel, Schroën, Spaen, & Berton-Carabin, 2018). The cooling thermograms of pure tripalmitin-based CLPs showed a crystallization onset at about 23, 29 and 32 °C for Tween 20-, Tween 40- and sodium caseinate-stabilized CLPs, respectively (Figure 6.3. A, C & E), which was much lower than for bulk tripalmitin (about 43 °C), indicative of a strong supercooling effect in all CLPs (Abramov, Ruppik, & Schuchmann, 2016; Schröder et al., 2018; Westesen & Bunjes, 1995).

The cooling thermograms of Tween 20-stabilized tripalmitin CLPs showed a main crystallization peak with a small shoulder, which probably corresponds to a dominant β-subcell fraction and a small fraction of β'-subcell crystals (Helgason et al., 2009b; Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013b; Schröder et al., 2017). Tween 40-stabilized tripalmitin CLPs showed two distinct crystallization peaks, of which the main one corresponds to β-subcell crystals that were formed in the core of the CLP, and the other one most probably to α-subcell crystals that were formed by Tween 40-induced surfactant-templated crystallization (Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2017; Thrandur et al., 2009; Yadav et al., 2013), as reported for high melting lecithin-induced crystallization (Salminen et al., 2013b). Tween 40 primarily contains palmitic acid (C16:0) as alkyl chain, which can align with alike alkyl chains of tripalmitin at the interface, promoting crystallization at the interface. Conversely, sodium caseinate-stabilized tripalmitin CLPs showed a single crystallization peak, indicating that the tripalmitin crystallized into one polymorphic form, most likely corresponding to α-subcell crystals (Awad et al., 2009).

The melting thermograms of CLPs made with tripalmitin-tricaprylin blends showed a sequence of melting peaks indicative of multiple melting events, although these events were less distinct (Figure 6.3.B, D and E), and showed lower enthalpies than in pure tripalmitin particles (Supporting information, Table S6.2.). Tween 20-stabilized CLPs showed one crystallization peak most likely corresponding to β-subcell crystals, whereas for Tween 40-stabilized CLPs α-subcell crystallization was found corresponding to surfactant-templated interfacial crystallization, followed by two overlapping β'and β-subcell crystallization peaks. Lastly, sodium caseinate-stabilized CLPs showed one main α-subcell crystallization peak followed by a small β'or β-subcell crystallization peak (Helgason et al., 2009b; Salminen et al., 2013b). In general, the CLPs made with tripalmitin-tricaprylin blends showed lower crystallization enthalpy and melting temperatures compared to tripalmitin CLPs, which can be attributed to less ordered crystals (Gunstone, Harwood, & Dijkstra, 2007; Salminen, Aulbach, et al., 2014).

6.3.2. Chemical stability of α-tocopherol

The amounts of α-tocopherol recovered in the CLPs immediately after production were between 77.0 and 90.1%, with no significant differences between the particles (Supporting information, Table S6.3.). These initial losses can be caused by the high temperature, pressure and presence of oxygen during homogenization. To further study the chemical stability of α-tocopherol upon storage of the CLP suspensions in accelerated ageing conditions, we incubated the samples with 200 μM $FeSO_4$/EDTA at 25 °C, and measured the concentration of α-tocopherol in time.

The chemical stability of α-tocopherol was considerably lower in tripalmitin-based CLPs than in pure tricaprylin-based ones (Figure 6.4.). After 14 days storage, pure tripalmitin CLPs showed remaining amounts of ~85, 60 and 80 % when stabilized by Tween 20, Tween 40 or sodium caseinate, respectively, compared to ~95 % in tricaprylin CLPs. Increasing the fraction of liquid tricaprylin in the CLPs increased the chemical stability of α-tocopherol when using Tween 40 and sodium caseinate as emulsifiers, but not when using Tween 20. With regard to the effect of the emulsifier type, the chemical stability of α-tocopherol was the lowest for Tween 40-stabilized CLPs, compared to Tween 20- or sodium caseinate-stabilized CLPs.

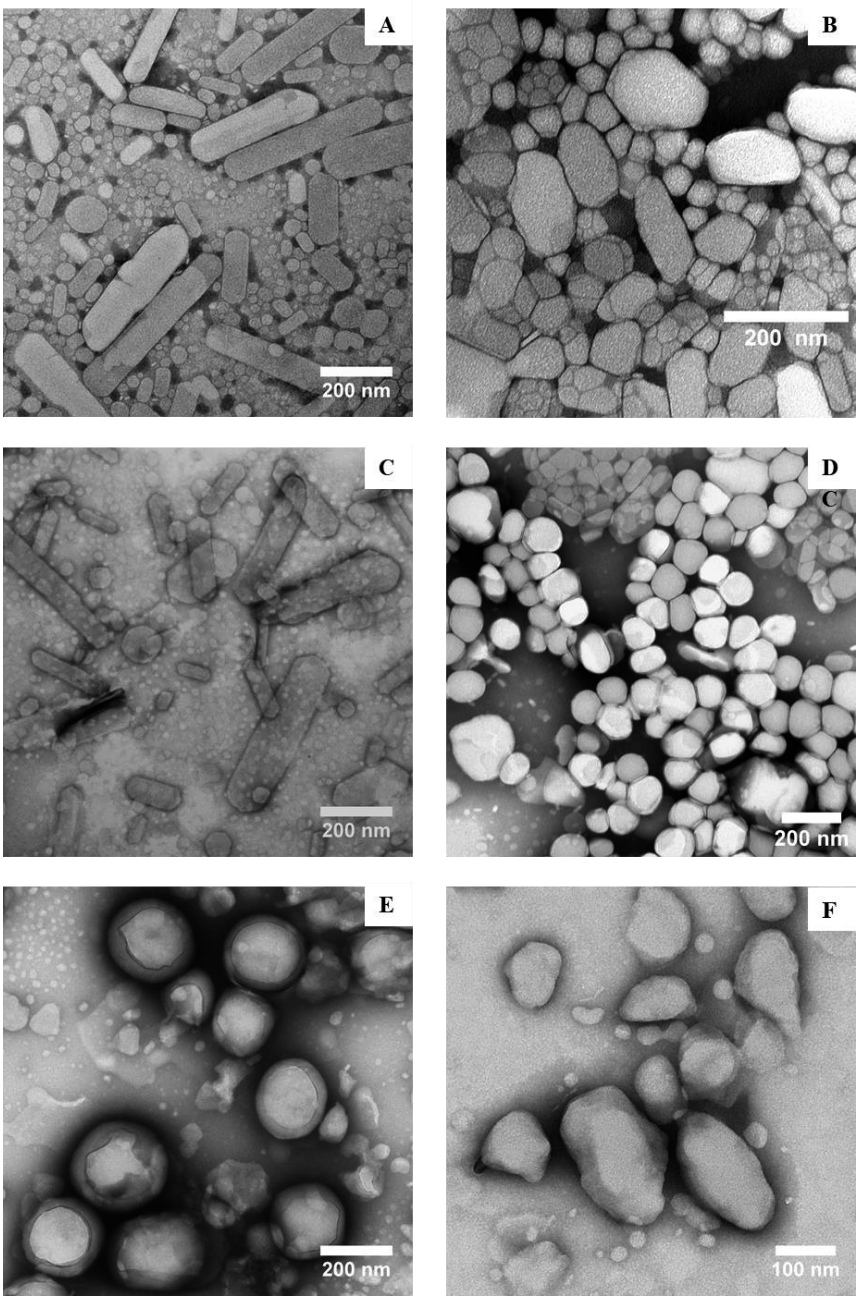


Figure 6.2. TEM images of CLPs made of tripalmitin (left), or tripalmitin/tricaprylin 4:1 w/w blend (right), stabilized by Tween 20 (A & B), Tween 40 (C & D) or sodium caseinate (E & F).

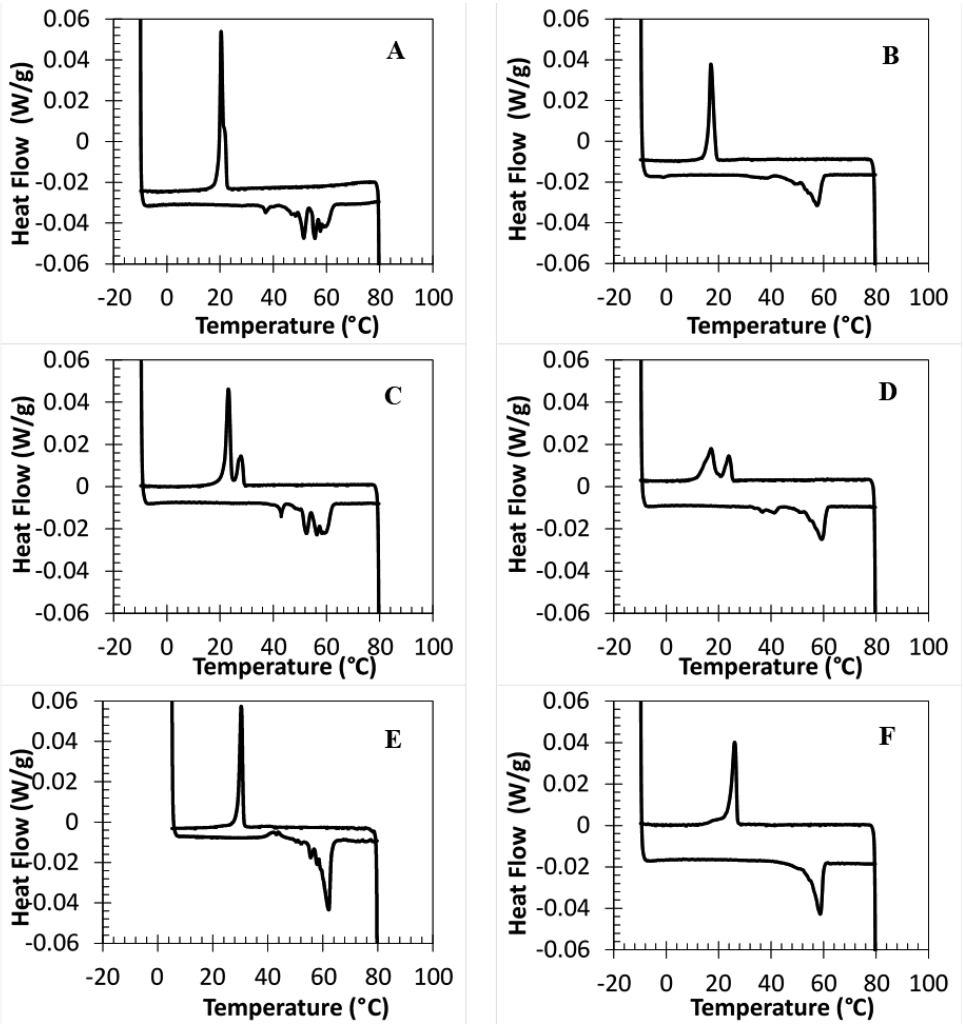


Figure 6.3. DSC crystallization and melting thermograms of CLPs made of tripalmitin (left) or tripalmitin/tricaprylin 4:1 w/w blend (right), and stabilized by Tween 20 (A & B), Tween 40 (C & D) or sodium caseinate (E & F).

We found a high α-tocopherol stability in tricaprylin CLPs irrespective of the emulsifier used. Tripalmitin or tricaprylin-tripalmitin blends resulted in crystallized CLPs with various morphologies, in which the chemical stability of α-tocopherol was lower compared to that in tricaprylin-based CLPs. Although immobilization of lipophilic bioactives within a solid lipid matrix has sometimes been suggested as protective (Jenning, Schäfer-Korting, & Gohla, 2000; Relkin, Yung, Kalnin, & Ollivon, 2008; Yadav et al., 2013), the location of the bioactive

and the structure of the solid lipid matrix also need to be taken into account (Salminen et al., 2013b; Tikekar & Nitin, 2011). For example Berton-Carabin et al. (2013) demonstrated that the location and mobility of small lipophilic molecules were largely dependent on their structure and on the physical state of lipids in emulsion systems, which could substantially affect their chemical stability. For molecules structurally close to α-tocopherol, fat crystallization promoted their localization and immobilization at the interface, but did not change their chemical reactivity with aqueous phase reactants (Berton-Carabin et al., 2013).

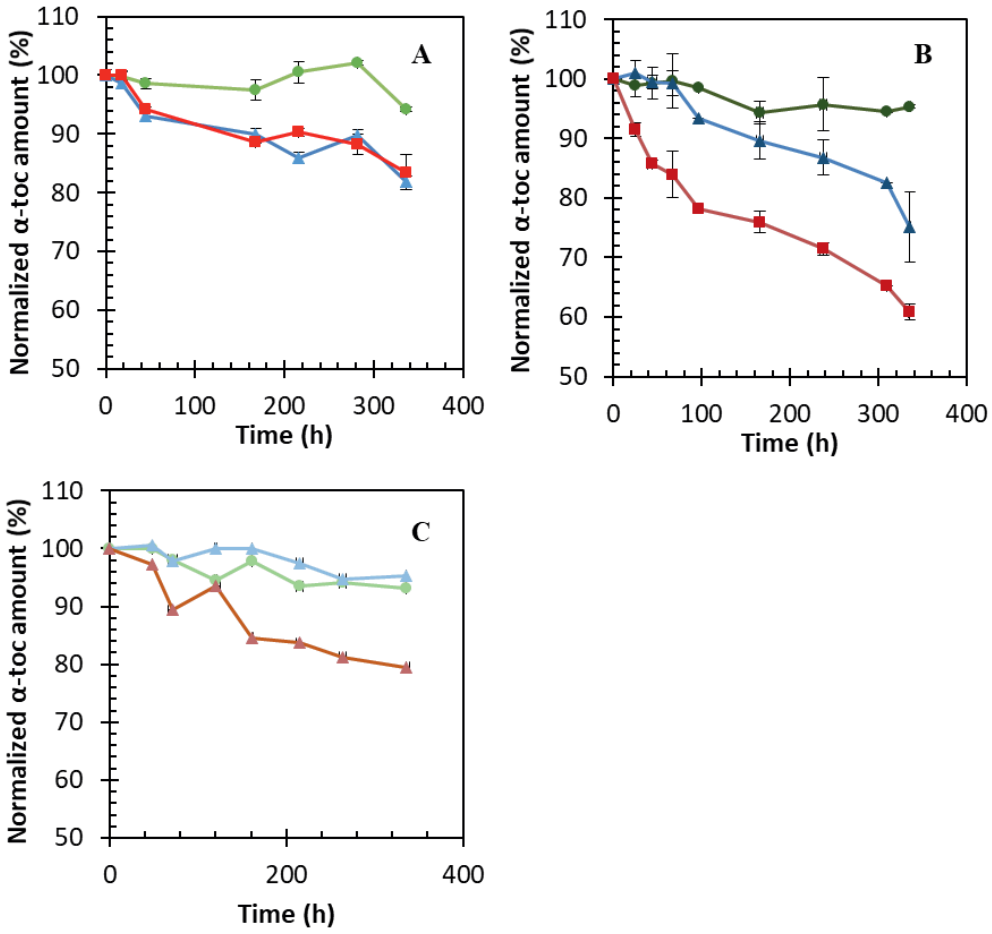


Figure 6.4. Chemical stability of α-tocopherol, expressed as normalized α-tocopherol amount (%), in CLPs stabilized by Tween 20 (A), Tween 40 (B) or sodium caseinate (NaCas) (C), and prepared with tripalmitin (TP100, red), tripalmitin:tricaprylin 4:1 blend (TP80, blue), or tricaprylin (TP0, green), during storage under oxidative conditions.

Physical exclusion of the bioactive compound can take place during crystallization, resulting in closed compartments loaded with the compound, which leads to an enhanced chemical stability when in the core of the droplet (Dan, 2016; Relkin et al., 2008; Salminen et al., 2016; Thrandur et al., 2009). If these compartments are close to the particle surface, degradation may be promoted, since they may come into contact with aqueous pro-oxidant species (Dan, 2016; Qian et al., 2013), and leach out due to partitioning. Jenning & Gohla (2001) hypothesized that bioactives with a melting point lower than that of the lipids used for encapsulation may be pushed toward the particle surface due to lipid crystallization in the lipid core prior to the bioactive. This could have been the case in our research, resulting in lower chemical stability of α-tocopherol in solid or semi-solid CLPs, compared to liquid ones.

In literature, it was suggested that high melting point surfactants form a shell by surface templating that limits diffusion of pro-oxidants or oxygen to the oxidizable components (Salminen et al., 2016; Thrandur et al., 2009). Such a shell let tripalmitin crystallize in a loosely packed crystal form (α or β'-subcell), in which lipophilic compounds can be encapsulated better than in tightly packed β-subcell crystals (Salminen et al., 2017; Thrandur et al., 2009; Yadav et al., 2013). Therefore, it was expected that Tween 40 would limit α-tocopherol degradation more compared to Tween 20 or sodium caseinate, but the opposite was observed. We expect that highly ordered β-subcell crystals were formed in the core of Tween 40-stabilized tripalmitin CLPs, as revealed by DSC, which resulted in needle-shaped CLPs with a high surface area. α-Tocopherol molecules could have been pushed out from the particle core by the growing crystals, and thus present close to the particle surface, where they would be prone to chemical degradation by aqueous phase pro-oxidants (Yucel et al., 2012).

Tween 40-stabilized CLPs with mixed tripalmitin-tricaprylin contained less β-subcell crystals compared to pure tripalmitin-based CLPs, had less surface area, and could contain some liquid lipid patches in the core of the CLP (Saez et al., 2018). All these effects may have kept α-tocopherol more buried within the particles, leading to higher chemical stability.

Tween 20-stabilized CLPs contained mostly β-subcell crystals, which are expected to induce migration of α-tocopherol from the core to the surface of the CLPs, where it would eventually have been degraded. Compared to Tween 40, less α-tocopherol could have been initially present at the interface, leading to slower degradation. Sodium caseinate-stabilized

CLPs were able to inhibit degradation of α-tocopherol to a larger extent compared to both surfactant-stabilized CLPs; for the tripalmitin-tricaprylin blend, the stability was even as good as for tricaprylin. Although sodium caseinate is known to be a metal chelator that effectively prevent lipids from oxidation when present in large excess in the aqueous phase (Berton et al., 2011; Faraji, McClements, & Decker, 2004), this seems an unlikely explanation for our results, as only low concentrations of excess proteins were present (< 1 g L^{-1}). More likely, the less ordered crystalline structure compared to Tween 20 and Tween 40 CLPs allows for α-tocopherol immobilization, or its inclusion in liquid patches within the core of the CLPs. This would lead to slow release of α-tocopherol, which is important for products in which antioxidant activity needs to be maintained for an extended period.

6.4 Conclusions

We investigated colloidal lipid particles (CLPs) made with tripalmitin, tricaprylin, or a blend thereof, as encapsulation systems for α-tocopherol. We showed that emulsifier type and lipid composition modulate the morphology and crystalline structure of the particles, which consequently affected the chemical stability of α-tocopherol. Tween 20 and Tween 40 allowed tripalmitin to crystallize in highly ordered structures with lath-like morphology, from which α-tocopherol was probably expulsed. In Tween 40-stabilized CLPs, α-tocopherol probably accumulated at the surface of the particles, where it was prone to chemical degradation by aqueous pro-oxidants. Sodium caseinate tripalmitin CLPs crystallized in a nearly spherical shape with less ordered crystalline structure in which the α-tocopherol stability was high. When a lipid blend was used, the chemical stability of α-tocopherol increased in most cases, possibly due to the formation of liquid patches that would keep the bioactive buried within the particle core. Tricaprylin CLPs had highest chemical stability of α-tocopherol irrespective of the emulsifier used, showing that lipid crystallization does not necessarily protect lipophilic bioactives. From these results it is clear that for efficient encapsulation of lipophilic bioactives, the particle architecture plays an important role; to tailor this, both the lipid, and the emulsifier need to be considered.

6.5. Supporting information

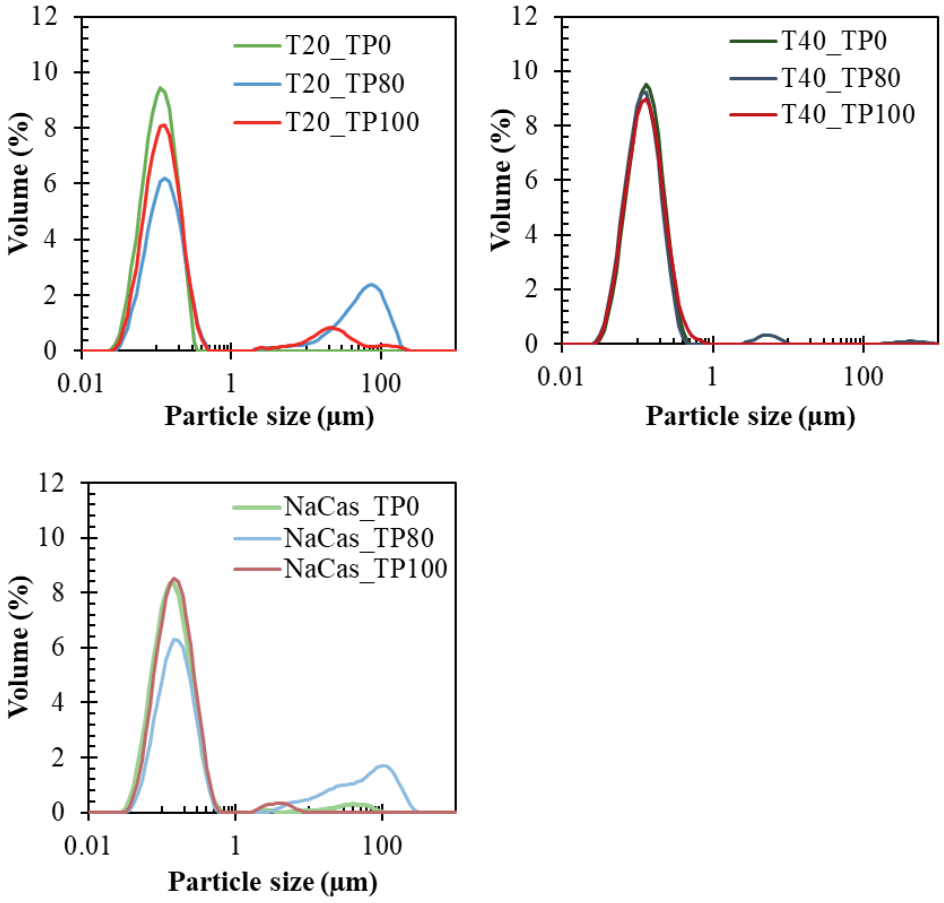


Figure S6.1. Particle size distributions of Tween 20 (A), Tween 40 (B) and sodium caseinate (C) – stabilized CLPs prepared with tripalmitin (TP100, red), tripalmitin:tricaprylin 4:1 blend (TP80, blue), or tricaprylin (TP0, green) after 14 days in oxidative conditions.

Table S6.2. Crystallization and melting enthalpy (J g^{-1} fat) of CLPs stabilized by Tween 20, Tween 40 or sodium caseinate, containing tripalmitin (TP100), or tripalmitin:tricaprylin 4:1 blend (TP80).

	TP100 Crystallization	TP100 Melting	TP80 Crystallization	TP80 Melting
Tween20	156.45±4.74	164.77±9.57	156.38±6.51	165.80±5.98
Tween40	159.33±3.98	162.896±9.03	145.72±9.52	141.21±11.41
NaCas	118.80±14.15	159.15±13.25	150.30±10.13	176.88±1.98

Table S6.3. Normalized α-tocopherol amount (%) in freshly prepared CLPs stabilized by Tween 20, Tween 40, or sodium caseinate (NaCas), containing tripalmitin (TP100), tripalmitin:tricaprylin 4:1 blend (TP80), or tricaprylin (TP0).

Lipid phase / Emulsifier	TP100	TP80	TP0
Tween20	82.6±1.0	84.1±0.2	85.5±2.7
Tween40	80.5±2.5	77.0±4.5	85.5±3.4
NaCas	85.3±9.6	90.1±8.7	86.5±6.9

6.6. References

Abramov, S., Ruppik, P., & Schuchmann, H. P. (2016). Crystallization in emulsions : A thermo-optical method to determine single crystallization events in droplet clusters. Processes, 4(3), 25.

Aditya, N. P., & Ko, S. (2015). Solid lipid nanoparticles (SLNs): Delivery vehicles for food bioactives. RSC Advances, 5(39), 30902–30911.

Arima, S., Ueji, T., Ueno, S., Ogawa, A., & Sato, K. (2007). Retardation of crystallization-induced destabilization of PMF-in-water emulsion with emulsifier additives. Colloids and Surfaces B: Biointerfaces, 55(1), 98–106.

Awad, T. S., Helgason, T., Weiss, J., Decker, E. A., & Mcclements, D. J. (2009). Effect of Omega-3 Fatty Acids on Crystallization , Polymorphic Transformation and Stability of Tripalmitin Solid Lipid Nanoparticle Suspensions & Crystal growth & design, 9(8) 3405-3411.

Bargoni, A., Cavalli, R., Caputo, O., Fundarò, A., Gasco, M. R., & Zara, G. P. (1998). Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. Pharmaceutical Research, Vol. 15, pp. 745–750.

Berton-Carabin, C. C., Coupland, J. N., & Elias, R. J. (2013). Effect of the lipophilicity of model ingredients on their location and reactivity in emulsions and solid lipid nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 431, 9–17.

Berton, C., Ropers, M. H., Viau, M., & Genot, C. (2011). Contribution of the interfacial layer to the protection of emulsified lipids against oxidation. Journal of Agricultural and Food Chemistry, 59(9), 5052–5061.

Bleeker, Cassee, Geertsma, Jong, D., Heugens, Koers-Jacquemijns, … Environment, D. N. I. for P. H. and the. (2012). Interpretation and implications of the European Commission Recommendation on the definition of nanomaterial. RIVM Letter Report 601358001/2012.

Bos, M. a, & van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: a review. Advances in Colloid and Interface Science, 91(3), 437–471.

Braga, A. L. M., Menossi, M., & Cunha, R. L. (2006). The effect of the glucono-δ-lactone/caseinate ratio on sodium caseinate gelation. International Dairy Journal, 16(5), 389–398.

Bunjes, H., Koch, M. H. J., & Westesen, K. (2000). Effect of particle size on colloidal solid triglycerides. Langmuir, 16, 5234–5241.

Bunjes, H., Westesen, K., & Koch, M. H. J. (1996). Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. International Journal of Pharmaceutics, 129(1–2), 159–173.

Coupland, J. N. (2002). Crystallization in emulsions. Current Opinion in Colloid and Interface Science, 7(5–6), 445–450.

Dan, N. (2014). Nanostructured lipid carriers: Effect of solid phase fraction and distribution on the release of encapsulated materials. Langmuir, 30(46), 13809–13814.

Dan, N. (2016). Compound release from nanostructured lipid carriers (NLCs). Journal of Food Engineering, 171, 37–43.

Dima, S., Dima, C., & Iordachescu, G. (2015). Encapsulation of functional lipophilic food and drug biocomponents. Food Engineering Reviews, 7(4), 417–438.

Dingler, A., Blum, R. P., Niehus, H., Müller, R. H., & Gohla, S. (1999). Solid lipid nanoparticles (SLN(TM)/Lipopearls(TM)) - A pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. Journal of Microencapsulation, 16(6), 751–767.

Faraji, H., McClements, D. J., & Decker, E. A. (2004). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 52(14), 4558–4564.

Freitas, C., & Müller, R. H. (1998). Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN TM) dispersions. 168, 221–229.

Gunstone, F. D., Harwood, J. L., & Dijkstra, A. J. (2007). The Lipid Handbook. CRC Press.

Helgason, T., Awad, T. S., Kristbergsson, K., McClements, D. J., & Weiss, J. (2009a). Effect of surfactant surface coverage on formation of solid lipid nanoparticles (SLN). Journal of Colloid and Interface Science, 334(1), 75–81.

Helgason, T., Awad, T. S., Kristbergsson, K., McClements, D. J., & Weiss, J. (2009b). Effect of surfactant surface coverage on formation of solid lipid nanoparticles (SLN). Journal of Colloid and Interface Science, 334(1), 75–81.

Jenning, V., & Gohla, S. H. (2001). Encapsulation of retinoids in solid lipid nanoparticles (SLN®). Journal of Microencapsulation, 18(2), 149–158.

Jenning, V., Schäfer-Korting, M., & Gohla, S. (2000). Vitamin A-loaded solid-lipid-nanoparticlesfor topical use: drug release properties. Journal of Controlled Release, 66, 115–126.

Mcclements, D. J. (2012). Nanoemulsions versus microemulsions : terminology , differences , and similarities. 1719–1729.

McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Advances in Colloid and Interface Science, 159(2), 213–228.

Miglietta, A., Cavalli, R., Bocca, C., Gabriel, L., & Rosa Gasco, M. (2000). Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. International Journal of Pharmaceutics, 210(1–2), 61–67.

Mukherjee, S., Ray, S., & Thakur, R. S. (2009). Solid lipid nanoparticles : A modern formulation approach in drug delivery system. Indian Journal of Pharmaceutical Sciences.

Oehlke, K., Behsnilian, D., Mayer-miebach, E., Weidler, P. G., & Greiner, R. (2017). Edible solid lipid nanoparticles (SLN) as carrier system for antioxidants of different lipophilicity. Plos One, 1–18.

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2013). Impact of lipid nanoparticle physical state on particle aggregation and β-carotene degradation: Potential limitations of solid lipid nanoparticles. Food Research International, 52(1), 342–349.

Relkin, P., Yung, J. M., Kalnin, D., & Ollivon, M. (2008). Structural behaviour of lipid droplets in protein-stabilized nano-emulsions and stability of α-tocopherol. Food Biophysics, 3(2), 163–168.

Rousseau, D. (2013). Trends in structuring edible emulsions with Pickering fat crystals. Current Opinion in Colloid and Interface Science, 18(4), 283–291.

Saez, V., Souza, I. D. L., & Mansur, C. R. E. (2018). Lipid nanoparticles (SLN & NLC) for delivery of vitamin E: a comprehensive review. International Journal of Cosmetic Science, 40(2), 103–116.

Salminen, H., Aulbach, S., Leuenberger, B. H., Tedeschi, C., & Weiss, J. (2014). Influence of surfactant composition on physical and oxidative stability of Quillaja saponin-stabilized lipid particles with encapsulated ω-3 fish oil. Colloids and Surfaces B: Biointerfaces, 122, 46–55.

Salminen, H., Gömmel, C., Leuenberger, B. H., & Weiss, J. (2016). Influence of encapsulated functional lipids on crystal structure and chemical stability in solid lipid nanoparticles: Towards bioactive-based design of delivery systems. Food Chemistry, 190, 928–937.

Salminen, H., Helgason, T., Aulbach, S., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2014). Influence of co-surfactants on crystallization and stability of solid lipid nanoparticles. Journal of Colloid and Interface Science, 426, 256–263.

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2013a). Formation of solid shell nanoparticles with liquid Omega-3 fatty acid core. Food Chemistry, 141(3), 2934–2943.

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2013b). Formation of solid shell nanoparticles with liquid ω-3 fatty acid core. Food Chemistry, 141(3), 2934–2943.

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2017). Tuning of shell thickness of solid lipid particles impacts the chemical stability of encapsulated Omega-3 fish oil. Journal of Colloid and Interface Science, 490, 207–216.

Sánchez-Paz, V., Pastoriza-Gallego, M. J., Losada-Barreiro, S., Bravo-Díaz, C., Gunaseelan, K., & Romsted, L. S. (2008). Quantitative determination of α-tocopherol distribution in a tributyrin/Brij 30/water model food emulsion. Journal of Colloid and Interface Science, 320(1), 1–8.

Schröder, A., Sprakel, J., Schroën, K., & Berton-Carabin, C. (2017). Tailored microstructure of colloidal lipid particles for Pickering emulsions with tunable properties. Soft Matter, 3190–3198.

Schröder, A., Sprakel, J., Schroën, K., Spaen, J., & Berton-Carabin, C. C. (2018). Coalescence stability of Pickering emulsions produced with lipid particles: A microfluidic study. Journal of Food Engineering, 234, 63–72.

Suresh, G., Manjunath, K., Venkateswarlu, V., & Satyanarayana, V. (2007). Preparation, characterization, and in vitro and in vivo evaluation of lovastatin solid lipid nanoparticles. AAPS PharmSciTech, 8(1), 24.

Thrandur, H., Awad, T. S., Kristberg, K., Eric, A. D., David, J. M. a, & Jochen, W. (2009). Impact of surfactant properties on oxidative stability of b-carotene encapsulated within solid lipid nanoparticles. Journal of Agricultural and Food Chemistry, 57, 8033–8040.

Tikekar, R. V., & Nitin, N. (2011). Effect of physical state (solid vs. liquid) of lipid core on the rate of transport of oxygen and free radicals in solid lipid nanoparticles and emulsion. Soft Matter, 7(18), 8149–8157.

Trombino, S., Cassano, R., Muzzalupo, R., Pingitore, A., Cione, E., & Picci, N. (2009). Stearyl ferulate-based solid lipid nanoparticles for the encapsulation and stabilization of beta-carotene and alpha-tocopherol. Colloids and Surfaces. B, Biointerfaces, 72(2), 181–187.

Walstra, P. (1993). Principles of emulsion formation. Chemical Engineering Science, 48(2), 333–349.

Westesen, K., & Bunjes, H. (1995). Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? International Journal of Pharmaceutics, 115(1), 129–131.

Yadav, N., Khatak, S., Vir, U., & Sara, S. (2013). Solid lipid nanoparticles - a review. International Journal of Applied Pharmaceutics, 5(2), 8–18.

Yucel, U., Elias, R. J., & Coupland, J. N. (2012). Solute distribution and stability in emulsion-based delivery systems: An EPR study. Journal of Colloid and Interface Science, 377(1), 105–113.

zur Mühlen, A., Schwarz, C., & Mehnert, W. (1998). Solid lipid nanoparticles (SLN) for controlled drug delivery--drug release and release mechanism. European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V, 45(2), 149–155.

Chapter 7

Pickering particles as interfacial reservoirs of antioxidants

This chapter has been submitted as Schröder, A., Laguerre, M., Sprakel, J., Schroën, K. and Berton-Carabin, C. C. Pickering particles as interfacial reservoirs of antioxidants.

Abstract:

Emulsions are common structures for the encapsulation of delicate bioactive molecules, both in biological systems and in manufactured products. Protecting functional molecules from chemical oxidation is crucial in these systems. Nature excels at doing so by placing antioxidants at the oil-water interface, where oxidative reactions primarily occur and delicate building blocks need to be protected. Inspired by Nature's protective strategies, we propose a novel approach to boost antioxidant activity in designer emulsions by employing surface-anchored particles that act both as physical emulsion stabilizers as well as interfacial reservoirs of antioxidants. We show how these interfacial reservoirs largely enhance the oxidative stability of emulsions, confirming how interfacial localization is crucial to their efficiency. This approach offers new possibilities for the formulation of protective encapsulation strategies in a wide variety of applications.

7.1. Introduction

Lipid droplets are omnipresent in living organisms, such as bacteria, yeasts, plants, animals, and in many man-made materials such as pharmaceuticals, cosmetic, food, or agrochemical products. They have been referred to as oil bodies, fat bodies, oil droplets, adiposomes, lipoproteins, or oil-in-water (O/W) emulsions (Murphy, 2001). All these systems share a common fate when they contain unsaturated lipids or other chemically labile molecules: they are susceptible to oxidation. The degradation of the substrate molecules, as well as the formation of lipid oxidation products, are harmful to the quality of the products, their functionality and even their safety (Frankel, 1980; Gilbert & Colton, 1999; McClements & Decker, 2000).

Lipid oxidation in multiphase systems occurs primarily at the oil-water interface, where the oxidation-sensitive molecules come into contact with pro-oxidants, such as metal ions or reactive-oxygen species. Thus, to protect delicate biomolecules, Nature has engineered its emulsions to localize antioxidants at the oil-water interface (Sagalowicz, Michel, Blank, Schafer, & Leser, 2017). In oil bodies, storage containers for oils in plants, antioxidants, such as α-tocopherol, accumulate at the interface. This results in remarkable oxidative stability (Fisk, White, Lad, & Gray, 2008). Also in the animal kingdom, similar strategies are found. The protective effect of ascorbate in preventing the oxidation of low density lipoproteins relies on its capability to reactivate α-tocopherol radicals at the droplet surface, where the chromanol ring of α-tocopherol faces the aqueous ascorbate-containing medium (Esterbauer, Gebicki, Puhl, & Jürgens, 1992). Accumulating antioxidants at the interface thus appears to be an excellent approach to inhibit lipid oxidation (Berton-Carabin, Ropers, & Genot, 2014). At this locus, antioxidants can counteract the initiating radicals — those formed during the initiation step — before they begin propagating oxidation to other lipid molecules (Burton & Ingold, 2002).

Surprisingly, this natural protective strategy, has to date not been translated to the domain of man-made emulsions, despite their ubiquitous use in a variety of industries (Sagalowicz et al., 2017). Also here, the effective prevention of oxidation reactions is crucial to ensure product safety, functionality and shelf-life. For example, the vast majority of newly discovered pharmaceutical compounds are hydrophobic and prone to oxidation; when oxidized, they lose their therapeutic activity or can even form potentially toxic by-products. It is thus essential to design effective strategies for the formulation of these bioactives into

useful drugs. Similarly, oxidation of lipophilic molecules in food or cosmetic emulsions is an issue, as it damages the products' quality and leads to product waste. The most common approach to prevent lipid oxidation in manufactured emulsions involves the use of large amounts of synthetic antioxidants, such as butylated hydroxytoluene (BHA) dissolved in the core of the oil droplets, or ethylenediaminetetraacetic acid (EDTA) in the bulk aqueous phase (Laguerre, Lecomte, & Villeneuve, 2007; McClements & Decker, 2000). These compounds are under scrutiny for their potential toxicity. By contrast, natural antioxidants, such as tocopherols and rosemary extracts meet the demand of consumers towards naturalness and clean-labels, and may be equally efficient when their activity is optimized.

This raises the question if the activity of natural antioxidants can be boosted by taking inspiration from Nature and ensuring their localization at the oil-water interface. This hypothesis has been confirmed by Laguerre et al., who chemically modified hydrophilic phenolic antioxidants through lipophilization, and found that antioxidant efficiency increased with the length of the alkyl chain grafted, until an optimum beyond which further lengthening led to a collapse in antioxidant efficiency (Laguerre et al., 2013). This non-monotonic dependency, referred to as the cut-off effect, is associated to the interfacial positioning of antioxidants grafted with an alkyl chain of intermediate length (Laguerre et al., 2009, 2011). Large-scale implementation of these so-called phenolipids is, however, hampered by their cost, and the fact that they are no longer natural molecules. Alternative strategies are therefore needed to boost the activity of natural antioxidants in emulsion systems.

We design a novel strategy to prevent lipid oxidation in emulsions using colloidal lipid particles (CLPs), a new type of Pickering stabilizers (Schröder, Sprakel, Schroën, & Berton-Carabin, 2017) as interfacial reservoirs of natural antioxidants. CLPs are promising stabilizers that are simple to prepare from biosourced building blocks, provide superior physical stability to the emulsions as compared to conventional emulsifiers, and are compatible with a range of emulsification methods (Schröder, Sprakel, Schroën, & Berton-Carabin, 2017; Schröder, Sprakel, Schroën, Spaen, & Berton-Carabin, 2018). CLPs are prepared with biobased high melting point lipids, and thus possess a solid or semi-solid lipid interior which can be loaded with lipophilic antioxidants, such as α-tocopherol. In this way, antioxidants are forced to reside at the interface, where their efficiency to prevent lipid oxidation is maximized. Hence, through the controlled hierarchical structuration of emulsions, we

expect to achieve a dual functionality, where the Pickering particles act not only as physical stabilizers, but also as interface-anchored reservoirs of antioxidants. To test our hypothesis, we evaluated lipid oxidation in two CLP-stabilized Pickering emulsions, which both had the exact same composition, but different physical location of the lipophilic antioxidant α-tocopherol: either in the CLPs (concept emulsion) or in the core of the oil droplets (control emulsion) (Figure 7.1.A & B). This bio-inspired approach to the protection and encapsulation of labile lipid molecules offers an order-of-magnitude improvement over current protective strategies and will enable the use of even the most delicate molecules, for which formulations have to date remained elusive due to their weak oxidative stability'.

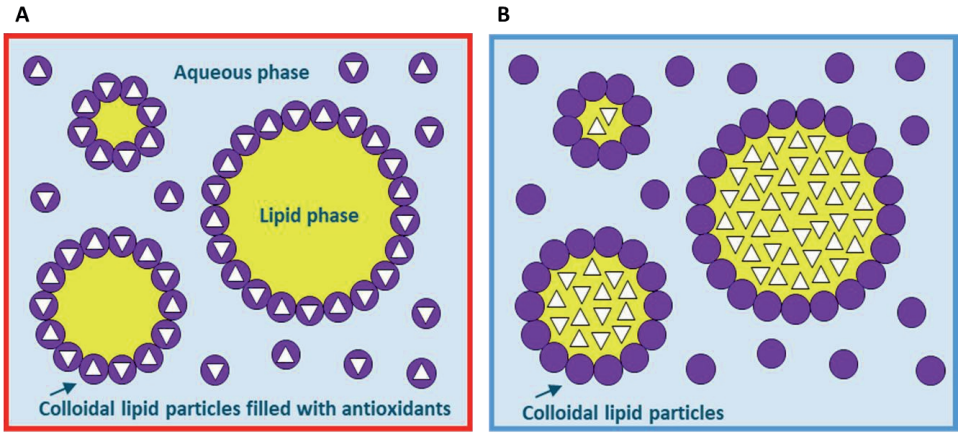


Figure 7.1. Schematic illustration of CLP-stabilized Pickering O/W emulsions with α-tocopherol incorporated (A) in the particles (concept emulsion) or (B) in the liquid PUFA oil droplets (control emulsion).

7.2. Results & discussion

First, we prepare CLPs, both with and without the natural antioxidant α-tocopherol, by high-pressure homogenization of molten tripalmitin, a model biocompatible high melting point fat, in an aqueous sodium caseinate solution, and subsequent cooling. Please note that all ingredients used here can be derived from natural sources and are inherently biocompatible and food-grade. The resulting CLPs have a unimodal size distribution with average diameter of 145 nm and a negative surface charge, characterized by a ζ-potential of -36 mV. The inclusion of the antioxidant does not affect the particle properties. They exhibit an almost spherical morphology, containing a complex internal structure of layered crystals with

crystallization and melting temperatures around 30 and 60 °C, respectively (Supporting information, Figure S7.1.).

We then produce CLP-stabilized Pickering emulsions by high pressure homogenization of an aqueous CLP suspension and sunflower oil, a food-grade oil rich in polyunsaturated fatty acids (PUFAs), which is stripped from endogenous antioxidants prior to use. The antioxidant α-tocopherol is either added in the CLPs or in the sunflower oil. We find that the CLPs are excellent stabilizers of the O/W emulsion against coalescence. A fraction of the particles adsorb at the oil-water interface, and the rest remains suspended in the continuous phase. This results in emulsion droplets ranging from 0.3 to 3 µm, with an average diameter around 1 µm (Supporting information, Figure S7.2.). The ζ- potential of the emulsion is identical to that of the CLP suspension.

We compare two CLP-stabilized Pickering emulsions of the exact same chemical composition, but with a different physical location of the lipophilic antioxidant α-tocopherol: either in the CLPs (concept emulsion) or in the core of the oil droplets (control emulsion). To study lipid oxidation, we accelerate the chemical reaction using a conventional iron-catalyzed oxidation system at 25 °C, and monitor the formation of oxidation products using spectrophotometry. Over 14 days of incubation, the accumulation of both primary (conjugated diene (CD) hydroperoxides (LOOH)) and secondary (aldehydes, measured by the *para*-anisidine value (*p*AV)) oxidation products is largely higher in the control emulsion containing α-tocopherol in the oil droplets, than in the concept emulsion containing the antioxidant in the CLPs (Figure 7.1.C & D). Indeed, our Nature-inspired strategy to trap antioxidants at the locus of oxidation, largely boosts the oxidative stability of the emulsion.

Moreover, the fact that lipid oxidation products hardly form in our concept Pickering emulsion indicates that the autocatalytic pattern of the formation of lipid radicals (propagation) does not take place within the time-scale of the experiment. The specific emulsion architecture we propose therefore confers the natural antioxidant with an initiation-breaking role.

To dig deeper into the chemical role of α-tocopherol, we monitor its loss during incubation of the emulsions. Emulsions containing α-tocopherol in CLPs show a higher α-tocopherol amount at t_0 compared to emulsions containing α-tocopherol in the oil droplets (157 vs.

127 µmol kg^{-1}). This implies that in addition to providing boosted antioxidant activity in the emulsion, CLP encapsulation also protects the antioxidant itself against degradation during emulsion processing (Figure 7.1.E). Tocopherol can theoretically be consumed in many different ways: through exposure to high temperature such as in our homogenization procedure; or in the presence of iron, reactive oxygen species, or lipid radicals. To distinguish between these potential causes, we prepare the exact same Pickering emulsions (concept and control) with medium chain triglycerides, a model saturated liquid oil that cannot oxidize, instead of stripped sunflower oil. When monitoring the α-tocopherol level during incubation, we find that it is perfectly stable in both emulsions (Supporting information, Figure S7.3.). Thus, the pro-oxidant ferrous iron itself is not able to degrade α-tocopherol in O/W emulsions that do not contain an oxidizable lipid phase. From this follows that in the control PUFA-rich oil emulsions, α-tocopherol is degraded during homogenization when present in the core of the droplets because it is in direct contact with lipid oxidation products. The CLPs protect the antioxidant from this contact, thereby offering additional stability.

During incubation, α-tocopherol is also degraded faster and to a larger extent in the control emulsion (Figure 7.1.E), showing that its chemical stability aligns with that of the PUFAs. This is remarkable because chain-breaking antioxidants are traditionally considered sacrificial molecules that oxidize instead of unsaturated lipids; then, once most of the antioxidant has been consumed, the autocatalytic propagation of lipid oxidation may start (Kamal-Eldin, 2003; Labuza & Dugan, 1971).

We hypothesize that the use of Pickering particles as interfacial reservoirs of antioxidants confers α-tocopherol with the ability to scavenge the first radicals generated during initiation, whereas when present in the core of the droplets, α-tocopherol acts during the propagation of the radical chain reaction. Accordingly, a small amount of interface-anchored α-tocopherol should be more efficient than many of the same molecules located within the oil droplets. This logically raises the question of how much the α-tocopherol concentration can be reduced in the concept emulsion to obtain a protection equivalent to that attained in the control emulsion with 200 µmol kg^{-1} of α-tocopherol. Therefore, we reduced the initial α-tocopherol concentration from 200 µmol kg^{-1} to 100 then 50 µmol kg^{-1} (2 to 4 times lower) in the concept emulsion. Interestingly, at least four to two times less antioxidants were required to inhibit the production of CD-LOOH and aldehyde

(respectively) as effectively as 200 µmol kg^{-1} of α-tocopherol in the control emulsion (Supporting information, Figure S7.4.), which further substantiates the added value of the interfacial localization of antioxidants to boost their activity.

Thus, we establish for the first time the proof-of-concept that loading Pickering particles with natural antioxidants is an efficient strategy to boost the activity of the latter. This demonstrates that the hierarchical construction of the emulsion is key, as both the concept and control emulsions have the exact same composition. This raises two crucial questions on the mechanisms by which this enhancement works. First, as our emulsions are not at a thermodynamic equilibrium, the localization of the lipophilic bioactive may change within time-scales relevant to the incubation period. Second, since only a fraction of CLPs is actually located at the surface of the oil droplets, whereas the rest remains in excess in the continuous phase, one may wonder what the role of the particles in suspension is and if they play a role in the boosting effect. To investigate the dynamic aspects relative to the encapsulation of a lipophilic antioxidant in the CLPs, we select a fluorescently labelled probe with comparable lipophilicity, 25-NBD cholesterol, and use it to prepare concept and control Pickering emulsions as described previously, except that we apply lower shear homogenization to obtain large oil droplets that allow for visualization by light microscopy and confocal laser scanning microscopy (CLSM).

In the concept Pickering emulsions immediately after homogenization, the fluorescent probe is present both in CLPs at the interface and the continuous phase, and not within the liquid oil droplets (Figure 7.1.F). However, in time, we observe progressive diffusion of the probe towards the core of the oil droplets. This reveals that the location of the fluorescent probe, and thus, by analogy, that of α-tocopherol in the concept Pickering emulsions, evolves within time scales relevant to the incubation period. Interestingly, polarized light microscopy (Figure 7.1.G & H) and differential scanning calorimetry (DSC) (Supporting information, Figure S7.5.) show that the CLPs, even when adsorbed at the oil-water interface, remain intact and crystalline over the entire experiment. This rules out that diffusion of the probe would be caused by solubilization of the CLPs in the liquid oil droplets in time, which is in agreement with the long-term physical stability of those emulsions demonstrated in earlier work (Schröder, Sprakel, Boerkamp, Schroën, & Berton-Carabin, 2019). This dynamic transfer of lipophilic compounds implies that α-tocopherol is not retained permanently at the interface and is slowly released to the oil droplet environment.

The CLPs may behave as transient antioxidant reservoirs, which boost the activity of antioxidants by imparting them with a certain residence time at the interface.

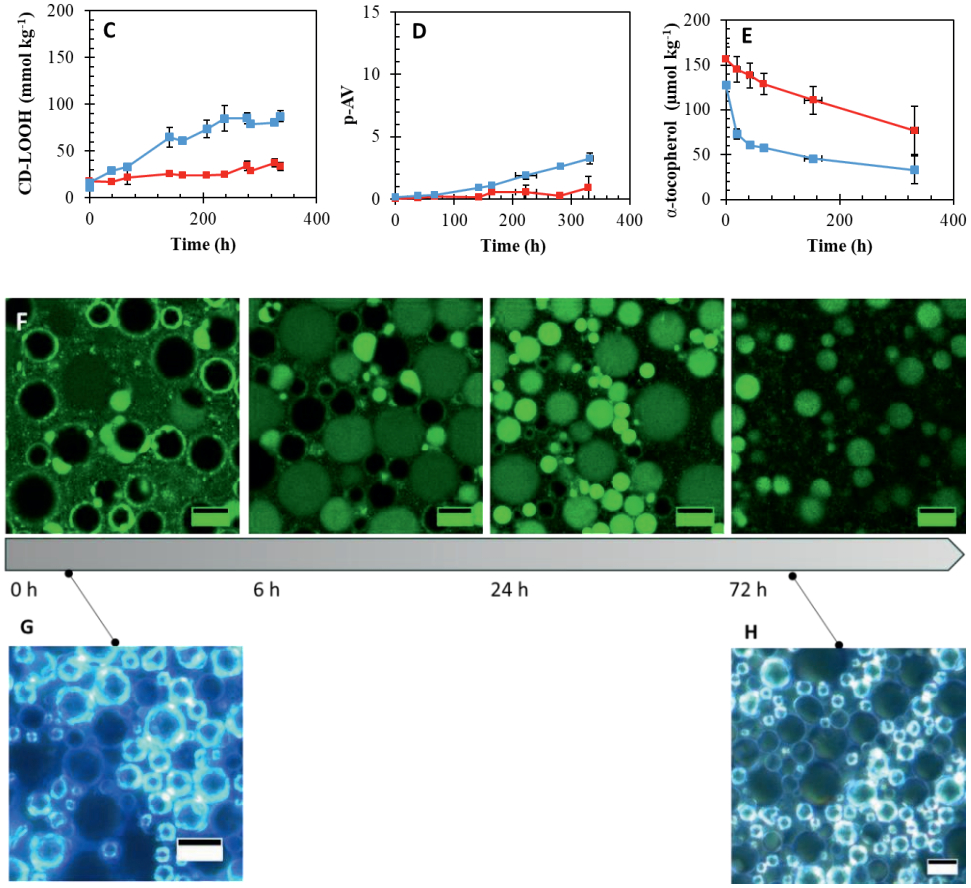


Figure 7.1 continued. CD hydroperoxide concentration (mmol kg^{-1} oil) (C), pAV (D) and α-tocopherol concentration (μmol kg^{-1} emulsion) (E) during incubation of the concept and control emulsions (red and blue lines, respectively) containing 200 μmol kg^{-1} α-tocopherol. CLSM images of the concept Pickering emulsion with the fluorescent analogue initially added in CLPs, taken at different time points (F). Polarized light microscopy images of the same concept Pickering emulsion, taken at t_0 and after 72 h incubation (G and H, respectively). The scale bar on all images is 10 μm.

To evaluate the possible contribution of continuous phase CLPs to the high oxidative stability in the concept Pickering emulsions, we then prepare a simple sodium caseinate-stabilized emulsion, and add antioxidant containing CLPs to this emulsion, post

homogenization (Figure 7.2.A) to design a system in which the CLPs are exclusively located in the aqueous phase. When incubating this emulsion, and in its counterpart emulsion with α-tocopherol initially present in the oil droplet core (Figure 7.2.B), we find similar lipid oxidation and α-tocopherol degradation rates (Figure 7.2.C-E). Thus, the encapsulation of α-tocopherol in CLPs added to the continuous phase of a protein-stabilized emulsion does not improve the antioxidant activity, which contrasts the antioxidant boosting effect found in our concept Pickering emulsion. This is in line with the explanation that this latter effect is unequivocally due to the interfacial accumulation of antioxidants conveyed by the Pickering particles.

We then replace α-tocopherol by the fluorescent probe in similar emulsions. In the freshly prepared emulsions, a continuous green background surrounding black droplets shows that 25-NBD cholesterol is present only in the CLPs in the aqueous phase (Figure 7.2.F). Over incubation, the probe is rapidly transferred to the liquid oil droplets, which was not necessarily expected. Both CLPs and oil droplets are negatively charged, which should thus prevent prolonged contacts between both structures. In addition, no alteration of the DSC profile of the CLPs is detected, further indicating that no physical interactions between the high melting fat of the CLPs and the liquid oil of the droplets take place (Supporting information, Figure S7.6.). It thus seems that in such a simple protein-stabilized emulsion, the diffusion of a lipophilic molecule initially loaded in the CLPs towards the liquid oil droplets does not require a prolonged contact and occurs very fast. Using the analogy of the probe's behavior, the antioxidant performance of α-tocopherol regardless of its initial location in these emulsions (Figure 7.2.D & E) is probably explained by the fact that it rapidly diffuses in the oil droplets, thus erasing the initial construction difference between the two emulsions.

To decipher the respective contribution of the adsorbed and non-adsorbed CLPs to the overall antioxidant activity, while sticking to the original interface structure, we finally design a Pickering emulsion stabilized by α-tocopherol-free CLPs, to which α-tocopherol-loaded CLPs are added post homogenization, hence located in the continuous phase (Figure 7.3.A). Interestingly, lipid oxidation proceeds significantly faster in Pickering emulsions containing α-tocopherol exclusively in the continuous phase CLPs, compared to the control emulsion with the antioxidant in the oil droplets (Figure 7.3.C & D).

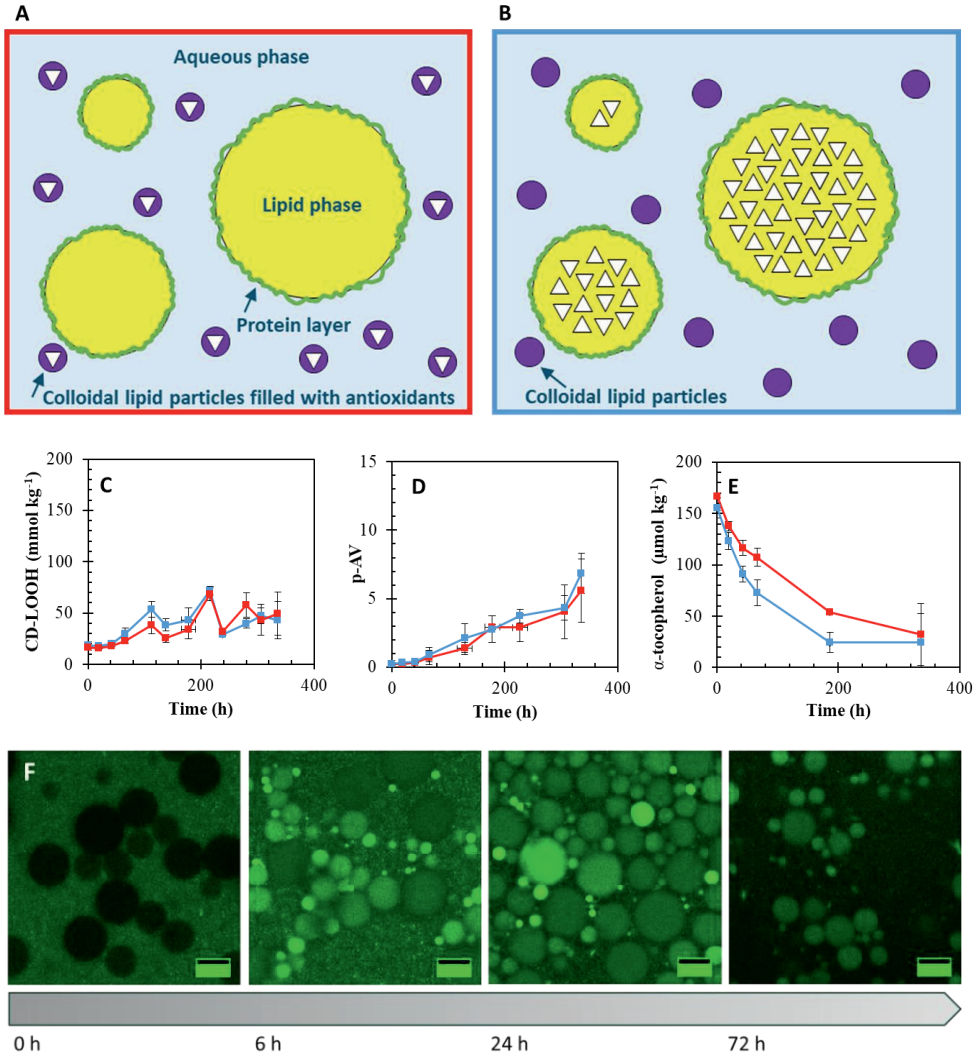


Figure 7.2. Schematic illustration of conventional sodium caseinate-stabilized emulsions containing CLPs added post-homogenization with α-tocopherol incorporated (A) in the particles or (B) in the liquid PUFA oil droplets. CD hydroperoxide concentration (mmol kg^{-1} oil) (C), pAV (D) and α-tocopherol concentration (μmol kg^{-1} emulsion) (E) during incubation of both emulsions (red and blue lines, respectively) initially containing 200 μmol kg^{-1} α-tocopherol. CLSM images of the conventional emulsion with added CLPs with the fluorescent analogue initially added in CLPs, taken at different time points (F). The scale bar on all images is 10 μm.

This can be explained by the physical segregation of the PUFAs in the oil droplets and the α-tocopherol in the water phase, precluding this latter component to reduce PUFA-derived free radicals *in situ*. Along with the fast PUFA oxidation, the degradation of α-tocopherol also proceeds very fast when it is located in the continuous phase CLPs (Figure 7.3.E). This implies that it was reached by lipid oxidation products able to rapidly escape from oxidizing droplets —presumably LOOH micelles—, which constitutes one of the first experimental evidences for such a scenario (Laguerre, Bily, Roller, & Birtic, 2017).

As previous, emulsions with similar construction principle are also prepared with 25-NBD cholesterol. The diffusion of the fluorescent probe from the aqueous phase CLPs to the emulsion droplet core during incubation does occur, yet much slower compared to what we observed in the protein-stabilized emulsion (Figure 7.3.F). The CLP-based interfacial layer can act as a physical barrier that slows down the diffusion of the aqueous CLPs' components to the oil droplets. This suggests that the CLP-based layer may have a unilateral, or at least variable, permeability to different colloids depending on their size: CLPs and the components that they carry cannot rapidly be transferred, while LOOH micelles can. Importantly, this last data set confirms that the interfacial localization of our model antioxidant is essential to yield the boosting effect for antioxidant activity evidenced in our concept Pickering emulsions.

Inspired by Nature's approach to protect oxidation-sensitive lipophilic bioactives, we have proposed a hierarchical structure for designer emulsions in which the activity of natural antioxidants is boosted substantially. The use of antioxidant-loaded Pickering particles results in a dual functionality, providing both excellent physical and chemical stability to biocompatible and food-grade emulsions. These interfacial reservoirs increase the residence time of antioxidants at the interface during crucial stages in the chemical oxidation reaction cascade, scavenging the very first lipid radicals generated during the initiation step before the irreversible autoxidation chain reaction propagates. Accordingly, our proposed interfacial design can turn an archetypal chain-breaking antioxidant into a new type of initiation-breaking antioxidant. Such a shift in the antioxidant mode of action may augment the potential of oxidation inhibitors to an unprecedented level, and consequently lead to a drastic reduction of the antioxidant content needed to achieve protection of emulsions toward oxidative degradation. Beyond the potential of this concept for a broad range of applications, from biocompatible emulsion design to synthetic biology,

this work yields findings that could be pivotal in the field of lipid oxidation in discontinuous systems, such as the ability of lipid oxidation products to be transferred via the aqueous phase in the absence of any added surfactant.

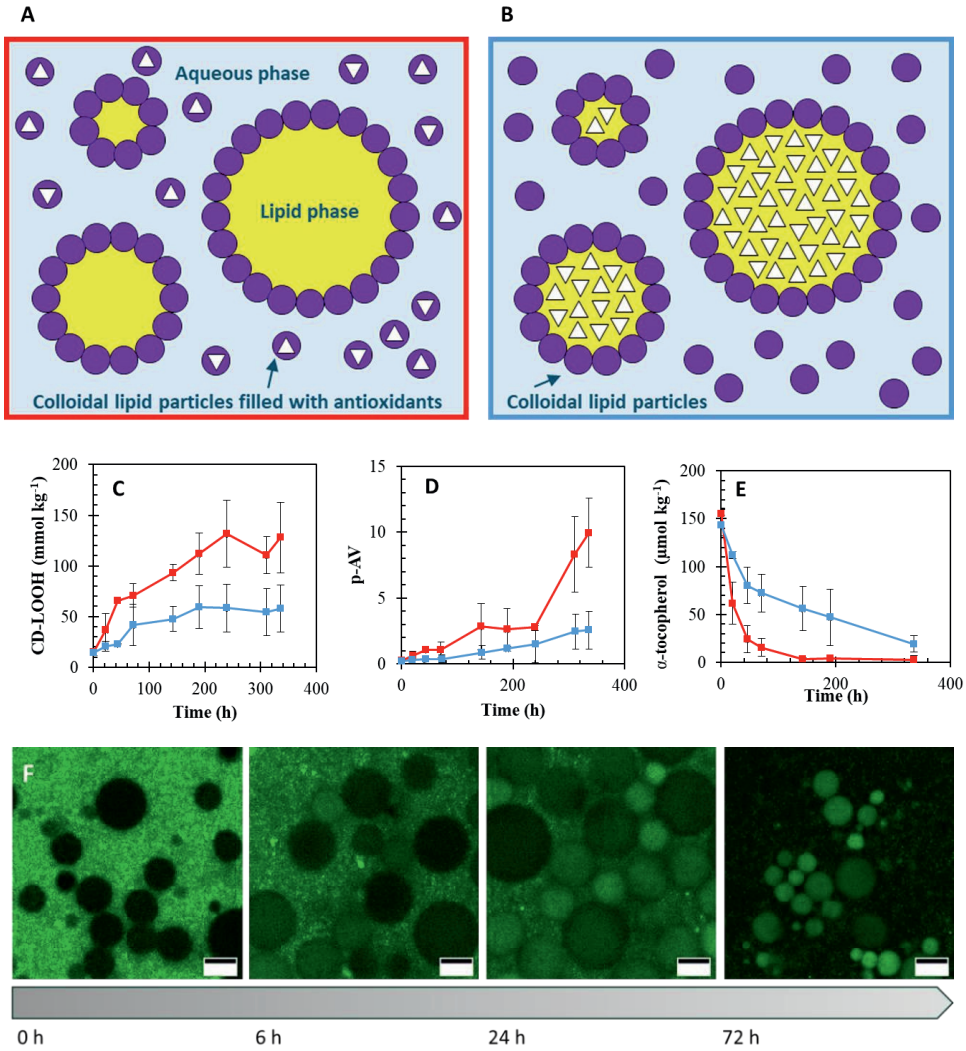


Figure 7.3. Schematic illustration of Pickering emulsions with extra CLPs added post-homogenization, with α-tocopherol incorporated (A) in the added CLPs or (B) in the liquid PUFA oil droplets. CD hydroperoxide concentration (mmol kg^{-1} oil) (C), pAV (D) and α-tocopherol concentration (μmol kg^{-1} emulsion) (E) during incubation of both emulsions (red and blue lines, respectively) containing 200 μmol kg^{-1} α-tocopherol. CLSM images of the Pickering emulsion with added CLPs with the fluorescent analogue initially added in CLPs, taken at different time points (F). The scale bar on all images is 10 μm.

7.3. Experimental procedures

CLPs were prepared according to Schröder et al., (2019). When CLPs were loaded with α-tocopherol, the molten lipid phase was added through a methanolic solution of α-tocopherol prior to mixing with the aqueous phase, such that the final concentration in the CLP suspension was 446 µmol kg^{-1}.

Pickering emulsions were prepared by mixing sunflower oil (10 % w/w), preliminary stripped from surface-active impurities and endogenous antioxidants, with phosphate buffer (10 mM, pH 7.0, 45 % w/w) and a 5 % w/w CLP dispersion (with α-tocopherol in the particles) (45 % w/w). The mixture was processed by high-speed stirring at 7,000 rpm for one min, and the obtained coarse emulsion was then homogenized (Microfluidizer® Processor MF 110Y with Y-shaped interaction chamber (F12Y; minimum internal dimension: 75 µm), Microfluidics, Newton, Massachusetts, USA) at 400 bar (5 cycles) at 0 °C. The resulting Pickering emulsions were stored at 4 °C. In control Pickering emulsions (i.e., with α-tocopherol in the liquid oil droplet core), α-tocopherol was incorporated in the sunflower oil prior to homogenization via a methanolic solution as mentioned previously, such that the final concentration in the Pickering emulsion was 200 µmol kg^{-1}, i.e., similar to that in the concept emulsion.

Conventional emulsions were prepared by homogenizing stripped sunflower oil (20% w/w) with a 2% w/w sodium caseinate solution (80% w/w) with the same procedure. After this, 50% w/w of this emulsion was mixed with 45% w/w CLP dispersion, and 5% w/w buffer.

Pickering emulsions with added CLPs were produced by mixing 66.7% w/w of a Pickering emulsion (containing 15% w/w sunflower oil, 45% w/w CLP dispersion, and 40% w/w buffer) with or without α-tocopherol in the core of the oil droplets, with 33.3% w/w of CLP dispersion containing no or 300 µmol kg^{-1} α-tocopherol.

Characterization of particle dispersions and emulsions (particle size distribution, ζ-potential, transmission electron microscopy, light microscopy and differential scanning calorimetry) was done as described in Schröder et al., (2019).

To obtain larger droplet sizes that allow for visualization by confocal laser scanning microscopy (CLSM), the coarse (premixed) emulsions were processed through a lab scale colloid mill homogenizer with gap width of 0.32 mm (IKA Magic Lab, Staufen, Germany)

operating for one min at 15,000 rpm at 0 °C. CSLM images of O/W emulsions stabilized by CLPs were obtained with a Nikon C-2 CLSM with a 60x oil immersion objective. For visualization, the CLPs or emulsion droplets were dyed by incorporation of 0.001% w/w of 25-NBD cholesterol in the emulsions. The fluorescent dye was excited with the 488-nm line of an argon laser and the fluorescence light was collected with a bandpass filter between 490–505 nm.

A metal-catalyzed oxidation system consisting of an equimolar mixture of $FeSO_4$ and EDTA was prepared by separately dissolving $FeSO_4$ and EDTA (12 mM) in ultrapure water. Equivalent volumes of each solution were mixed, and the iron-EDTA complex was allowed to form under moderate stirring for 1 h in the dark (Berton, Ropers, Viau, & Genot, 2011). Aliquots of emulsion (2 g) were distributed in 15-mL polypropylene centrifugation tubes. The catalyst (100 µL) was added to the emulsions to obtain a final concentration of 200 µM of both iron and EDTA. The tubes were rotated in the dark at 2 rpm at 25 °C for 0 to 336 h.

Quantification of conjugated diene hydroperoxides and total aldehydes (*para*-anisidine value (pAV)) was performed according to Schröder et al., (2019).

α-Tocopherol was extracted from CLPs dispersions or emulsions. First, 4 mL chloroform, 3 mL methanol and 1 mL saturated sodium chloride solution were added to 2 mL of CLP dispersion or emulsion in a 15-mL polypropylene centrifuge tube, which were vortexed followed by centrifugation at 2000×g for 8 min. The clear chloroform phase was then collected by cautiously boring a hole in the bottom of the centrifuge tube.

Extracts were analyzed on a UltiMate 3000 liquid chromatography system (Thermo Scientific, Sunnyvale, CA, USA) using a C30 reversed phase column, 3 µm, 150 x 4.6 mm (YMC, Dinslaken, Germany). Extracts were eluted at 1 mL min^{-1} at 30 °C using a mobile phase with a linear gradient going from 81% methanol, 14% methyl t-butyl ether (MTBE) and 4% water to 74% methanol, 22% methyl t-butyl ether and 4% water in 8 min, and going back to its initial composition in 2 min. α-Tocopherol was detected with a UV-VIS detector at 292 nm (Dionex™ UltiMate™ 3000 Variable Wavelength Detector), and contents were calculated using a calibration curve that was linear in the range from 5 µg mL^{-1} to 5000 µg mL^{-1}.

7.4. Supporting information

We produce two types of colloidal lipid particles (CLPs) by hot high pressure homogenization of the high melting point fat tripalmitin in an aqueous phase containing 1% w/w sodium caseinate, and subsequent cooling; one containing α-tocopherol and one not. Their size is determined by static light scattering (A), their melting and crystallization behavior by differential scanning calorimetry (DSC) (B), and their morphology by transmission electron microscopy (TEM) (C).

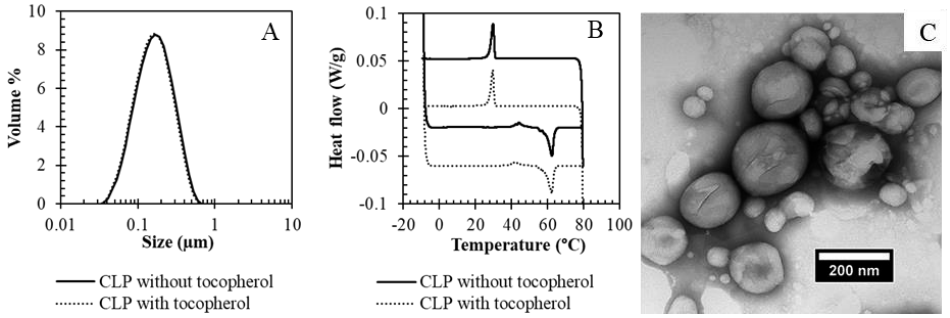


Figure S7.1. Characterization of tripalmitin CLPs with or without α-tocopherol. Particle size distribution (A), DSC melting and crystallization thermograms (B), and TEM image of CLP with α-tocopherol (C).

We produce two CLP-stabilized Pickering emulsions, which both have the exact same composition, but a different physical location of the lipophilic antioxidant α-tocopherol: either in the CLPs (concept emulsion) or in the core of the oil droplets (control emulsion), and determine their droplet size by static light scattering (A), their melting and crystallization behavior by DSC (B), and their morphology by TEM (C).

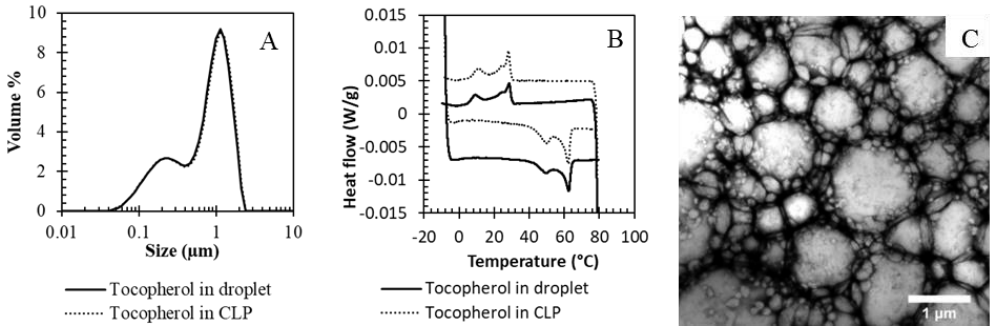


Figure S7.2. Characterization of Pickering emulsions with α-tocopherol either in the CLPs (concept emulsion) or in the core of the oil droplets (control emulsion). Droplet size distribution (A), DSC melting and crystallization thermogram (B) and TEM image of concept emulsion (C).

We prepare our concept and control Pickering emulsions with medium chain triglycerides, a model saturated oil that cannot oxidize, and monitor the α-tocopherol concentration during incubation. We find that the α-tocopherol is perfectly stable in both emulsions, although an iron-catalyzed oxidation system is used, similar as for the PUFA oil-based emulsions.

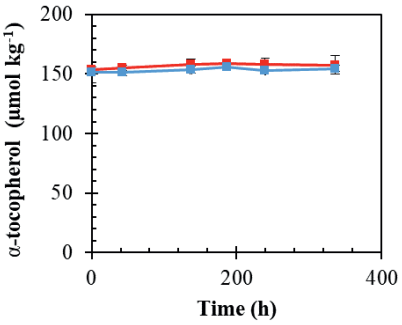


Figure S7.3. Stability of α-tocopherol during incubation of Pickering emulsions containing MCT oil with α-tocopherol either in the CLPs (concept emulsion; red) or in the core of the oil droplets (control emulsion; blue). Both emulsions contain 200 µmol kg^{-1} α-tocopherol and are incubated with 200 µM $FeSO_4$/EDTA as an oxidation catalyst.

We prepare our concept and control Pickering emulsions with 200 µmol kg^{-1} α-tocopherol, and compare their oxidative stability and α-tocopherol degradation to concept emulsions containing a reduced amount of α-tocopherol (100 and 50 µmol kg^{-1}).

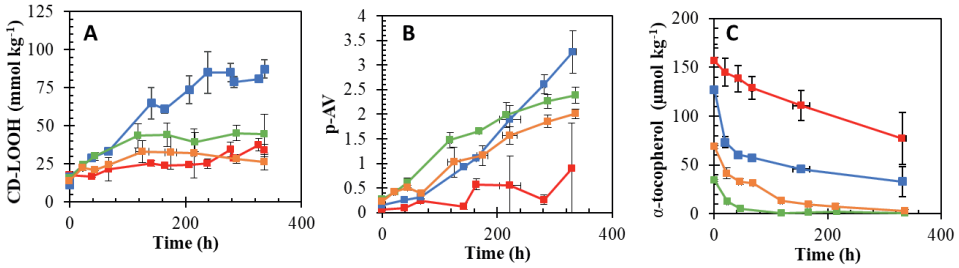


Figure S7.4. CD hydroperoxide concentration (mmol kg^{-1} oil) (A), pAV (B) and α-tocopherol concentration (µmol kg^{-1} emulsion) (C) during incubation of the concept and control emulsions (red and blue lines, respectively) containing 200 µmol kg^{-1} α-tocopherol, and similar measurements for concept emulsions with reduced α-tocopherol amounts (100 and 50 µmol kg^{-1}, orange and green, respectively).

We compare the melting and crystallization thermograms of concept emulsions at t_0 and $t_{336\ h}$, to ensure their physical stability.

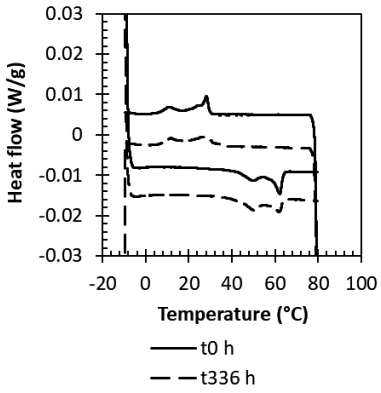


Figure S7.5. DSC melting and crystallization thermograms of concept Pickering emulsion at t_0 and $t_{336\ h}$.

We compare the melting and crystallization thermograms of CLP dispersions to conventional sodium caseinate-stabilized emulsion containing added CLPs in the aqueous phase to assess physical interactions between CLPs and liquid oil in conventional emulsions.

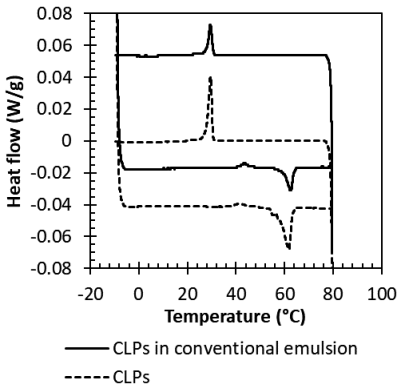


Figure S7.6. DSC melting and crystallization thermograms of a conventional sodium caseinate-stabilized emulsion containing added CLPs in the aqueous phase (solid line), and a CLP dispersion (dashed line).

7.5. References

Berton-Carabin, C. C., Ropers, M. H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Comprehensive Reviews in Food Science and Food Safety, 13(5), 945–977.

Berton, C., Ropers, M. H., Viau, M., & Genot, C. (2011). Contribution of the interfacial layer to the protection of emulsified lipids against oxidation. Journal of Agricultural and Food Chemistry, 59(9), 5052–5061.

Esterbauer, H., Gebicki, J., Puhl, H., & Jürgens, G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radical Biology and Medicine, 13(4), 341–390.

Fisk, I. D., White, D. A., Lad, M., & Gray, D. A. (2008). Oxidative stability of sunflower oil bodies. European Journal of Lipid Science and Technology, 110(10), 962–968.

Frankel, E. N. (1980). Lipid oxidation. Progress in Lipid Research, 19(1–2), 1–22.

Gilbert, D. L., & Colton, C. A. (1999). Reactive oxygen species in biological systems: An interdisciplinary approach (1st ed.). Springer US.

Kamal-Eldin, A. (2003). Lipid oxidation pathways. CRC Press.

Labuza, T. P., & Dugan, L. R. (1971). Kinetics of lipid oxidation in foods. C R C Critical Reviews in Food Technology, 2(3), 355–405.

Laguerre, M., Lecomte, J., & Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Progress in Lipid Research, 46(5), 244–282.

Laguerre, Mickaël, Bayrasy, C., Lecomte, J., Chabi, B., Decker, E. A., Wrutniak-Cabello, C., … Villeneuve, P. (2013). How to boost antioxidants by lipophilization? Biochimie, 95(1), 20–26.

Laguerre, Mickaël, Bily, A., Roller, M., & Birtic, S. (2017). Mass transport phenomena in lipid oxidation and antioxidation. Annual Review of Food Science and Technology, 8(1), annurev-food-030216-025812.

Laguerre, Mickaël, López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M. C., Baréa, B., Weiss, J., … Villeneuve, P. (2009). Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. Journal of Agricultural and Food Chemistry, 57(23), 11335–11342.

Laguerre, Mickaël, Wrutniak-Cabello, C., Chabi, B., López Giraldo, L. J., Lecomte, J., Villeneuve, P., & Cabello, G. (2011). Does hydrophobicity always enhance antioxidant drugs? A cut-off effect of the chain length of functionalized chlorogenate esters on ROS-overexpressing fibroblasts. Journal of Pharmacy and Pharmacology, 63(4), 531–540.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. 65(8), 1270–1282.

Murphy, D. J. (2001). The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Progress in Lipid Research, 40(5), 325–438.

Sagalowicz, L., Michel, M., Blank, I., Schafer, O., & Leser, M. E. (2017). Self-assembly in food — A concept for structure formation inspired by Nature. Current Opinion in Colloid and Interface Science, 28, 87–95.

Schröder, A., Sprakel, J., Schroën, K., & Berton-Carabin, C. (2017). Tailored Microstructure of Colloidal Lipid Particles for Pickering Emulsions with Tunable Properties. Soft Matter, 3190–3198.

Schröder, A, Sprakel, J., Schroën, K., Spaen, J., & Berton-Carabin, C. C. (2018). Coalescence stability of Pickering emulsions produced with lipid particles: A microfluidic study. Journal of Food Engineering, 234, 63–72.

Schröder, Anja;, Sprakel, J., Boerkamp, W., Schroën, K., & Claire C., B.-C. (2019). Can we prevent lipid oxidation in emulsions by using fat-based Pickering particles? Food Research International, 120, 352–363.

Burton, W. G., & U. Ingold, K. (2002). Vitamin E: application of the principles of physical organic chemistry to the exploration of its structure and function. Accounts of Chemical Research, 19(7), 194–201.

Chapter 8

Emulsions naturally armored against oxidation and coalescence using biobased particles

Abstract

Developing clean-label, plant-based, and sustainable food emulsions has gained large interest lately. This takes traditional emulsion design away from key functional ingredients, such as conventional emulsifiers (surfactants, animal-derived proteins), and synthetic antioxidants. 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 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 leaf, and spirulina cakes), or increasing the viscosity of the aqueous phase (pineapple fibers). Matcha tea and spinach leaf particle-stabilized emulsions were highly stable to lipid oxidation, as compared to reference emulsions stabilized by conventional emulsifiers. Such bi-functional Pickering particles thus seem promising for clean-label food emulsion formulation.

8.1. Introduction

Many food products are dispersions of oil droplets in water, i.e., are O/W emulsions (McClements, 2005). 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 (Berton-Carabin & Schroën, 2019). For example, the key functional ingredients, such as conventional emulsifiers (surfactants (Kralova & Sjöblom, 2017) and animal-derived proteins (Day, 2013)) that provide physical stability to the emulsions (Dickinson, 1993), 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 (McClements & Decker, 2018).

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 (Binks & Lumsdon, 2000; Finkle, Draper, & Hildebrand, 1923). For large enough particles (typically, above 10 nanometers), the adsorption energy, defined as: $\Delta G_a = \pi r^2 \gamma_{ow}(1 - \cos\theta)^2$, exceeds thermal energy by orders of magnitude, resulting in irreversible adsorption (Chevalier & Bolzinger, 2013; Schröder, Sprakel, Schroën, & Berton-Carabin, 2017; Zembyla, Murray, Radford, & Sarkar, 2019).

Substantial work has already been done on Pickering emulsions, and most of it on inorganic particles, such as silicon dioxide and other silica-based (i.e., thermally processed or chemically modified silica) particles (Binks & Kirkland, 2002; Eskandar, Simovic, & Prestidge, 2007; Midmore, 1998). Such particles are commercially available with various sizes (nanometers to microns) and hydrophobicity, and are allowed as food ingredients (EFSA, 2009). Yet, their size makes them suspect with respect to digestive fate, and their synthetic origin makes them less attractive in terms of sustainability and clean-label potential. Hence biobased particles, that can be minimally processed, are an interesting option for future food applications.

Several biobased Pickering particles have been developed over the past years such as modified starch (Rayner, Timgren, Sjöö, & Dejmek, 2012), cellulose fibers (Ougiya, Watanabe, Morinaga, & Yoshinaga, 1997), fat particles (Schröder et al., 2017), chitin nanocrystals (Perrin, Bizot, Cathala, & Capron, 2014), flavonoid particles (Luo et al., 2011), and protein-based particles (de Folter, van Ruijven, & Velikov, 2012). 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 may require complex processing (Schröder et al., 2017). It is thus important to find biobased particles that can be made through mild processing and have appropriate properties to allow irreversible nesting in the interface. In fact, many traditional and home-made food products are empirically stabilized by particles that are naturally present, such as mustard particles in mayonnaise and dressings (Rayner et al., 2014), fat crystals in margarine and spreads, or casein micelles in dairy products (Dickinson, 2010), but generalizing this purposely into the industrial food 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 healthy 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 dramatically improved (Schröder, Laguerre, Sprakel, Schroën, & Berton-Carabin, submitted).

Given the composition of natural particles, we could presume that some of them have intrinsic antioxidant properties, such as cocoa particles that typically contain polyphenols (Gould, Vieira, & Wolf, 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; Kargar, Spyropoulos, & Norton,

2011), although others reported contradictory effects (Schröder, Sprakel, Boerkamp, Schroën, & Berton-Carabin., 2019).

In the present work, we studied the physical and oxidative stability of emulsions prepared with natural particles from different sources (raw powders of matcha tea, spinach leaf, spirulina cakes, pineapple fibers, and rosemary cakes and extracts of turmeric and red radish), in comparison with conventional emulsions. 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.

8.2. Materials & methods

8.2.1. Materials

Sodium acetate trihydrate, sodium chloride and potassium sorbate were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Acetic acid glacial ($C_2H_4O_2$) was purchased from VWR Chemicals (Leicestershire, England). Methanol and chloroform were obtained from Actu-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) to remove polar impurities and tocopherols. Extracts of turmeric and red radish, and raw powders of matcha tea, pineapple fibers, rosemary cakes, spirulina cakes, and spinach leaf were supplied by NATUREX (Avignon, France). A chemical characterization is presented in Supporting information, Tables S8.1., S8.2. & S8.3. Ultrapure water (18.2 MΩ) was prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA) and was used for all the experiments.

8.2.2. Methods

8.2.2.1. Preparation of particle dispersions

A particle dispersion was prepared by mixing the powders or extracts (1 or 5% w/w) in acetic acid buffer (50 mM, pH 4.5). This dispersion was magnetically stirred overnight at 4 °C and used as such, or centrifuged at 10,000xg for 20 min at 20 °C. The supernatant, which contained most of the soluble components, was collected and filtered using a glass-fiber (1 µm) syringe filter (Acrodisc glass fiber, Sigma Aldrich, Steinheim, Germany) 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.

8.2.2.2. Preparation of emulsions

Stripped sunflower oil (10% w/w) was added to an aqueous phase containing 5% w/w particles, or the supernatant, 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, 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 °C or 25 °C.

8.2.2.3. Characterization of particle dispersions and emulsions

To visualize the morphology of the powders or extracts, 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, the Netherlands) on 12.7 mm aluminum pin-type stub mounts (JEOL Europe BV, 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 dispersion, and the emulsion droplet sizes, were measured by static light scattering using a Mastersizer 3000 (Malvern Instruments Ltd.; Worcestheshire, 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 of 1% w/w particle dispersions 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 mm^2) throughout the experiment. The interfacial tension was determined by analyzing the profile of the oil droplet using the Laplace equation.

8.2.2.4. 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 2,000xg 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 $CDCl_3$/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, Switerland) equipped with a 5 mm BBI-probe at 295 K. For each sample, both a single pulse and band selective experiment were recorded, following Merkx et al. (2018). The data were processed with the Bruker TopSpin 4.0 software. From the single pulse experiment, the peaks from the conjugated E,Z-Ln-OOH at δ 6.5 ppm and the glycerol backbone at δ 4.4 pm 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 Merkx et al. (2018). In some cases, it was necessary to subtract a background signal that originated from the particles from the overall signal.

8.3. Results & discussion

8.3.1. Physical characterization of particles

We first investigated the morphology of the biobased particles in dry state by scanning electron microscopy (SEM), which revealed that matcha tea, spinach leaf, and rosemary cake particles have an irregular structure with high porosity (Figure 8.1.). Pineapple fibers contained elongated and more irregular structures, with low porosity. Red radish and spirulina cake powders contained roughly spherical elements, with the latter showing small pores. Lastly, turmeric extract particles appeared as polygonal, compact structures. 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 (de Folter et

al., 2014; Günther, Frijters, & Harting, 2014; Madivala, Vandebril, Fransaer, & Vermant, 2009).

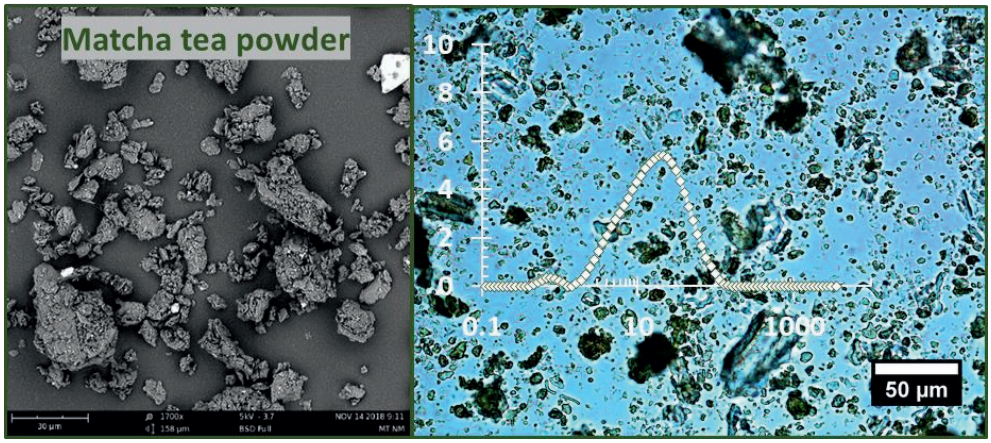
The dispersibility and aggregation behavior of the different powdered particulate materials was investigated in acetate buffer. Powders of matcha tea, spirulina cakes, spinach leaf and pineapple fibers seemed to disperse well in acetate buffer, but when observed by light microscopy, they showed moderate aggregation (Figure 8.1.). Additionally, pineapple fibers exhibited fast sedimentation, and increased the viscosity of the continuous phase considerably. Rosemary cake powder was dispersible in acetate buffer, but light microscopy revealed strong aggregation, whereas turmeric extract was not dispersible; both powders could be dispersed well in sunflower oil (Figure 8.1.). Red radish extract fully dissolved in buffer; no particles were detected by light microscopy, nor by static light scattering (Figure 8.1.).

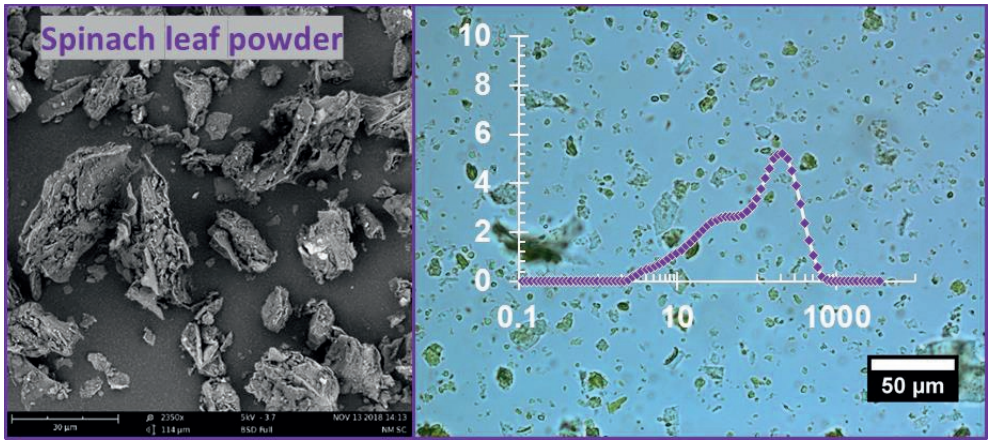
Binks et al. (2007) observed that emulsions were most stable when prepared with slightly aggregated particles (Binks, Rodrigues, & Frith, 2007). When comparing that with the behavior of our particles, it could be expected that powders of matcha tea, spirulina cakes, spinach leaf and pineapple fibers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

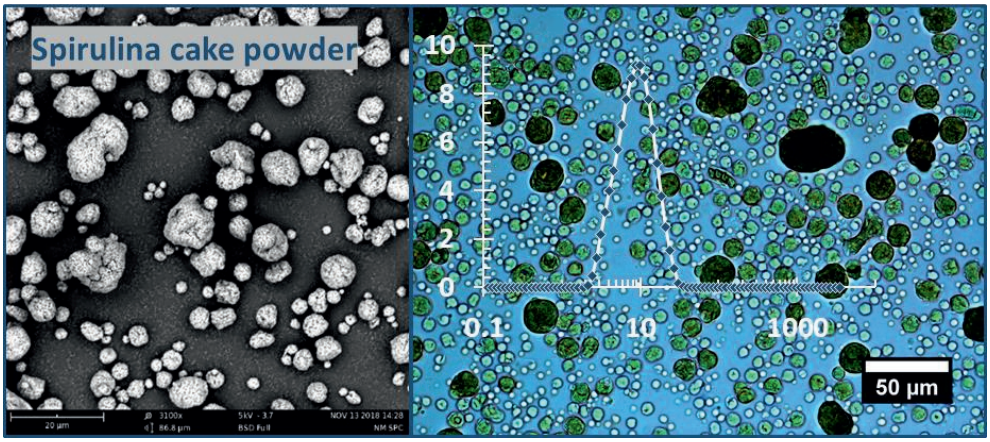
We investigated the particle size distribution of the buffer-dispersible particles, and found that spirulina cake and matcha tea particles were the smallest, with d_{32} of 9 and 11 µm, respectively (Figure 8.1.). The average size of pineapple fibers and spinach leaf particles was much larger (d_{32} of 107 and 40 µm, respectively), with the latter showing a wide size distribution. Given the poor dispersibility of rosemary cake particles in acetate buffer, it was 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 ideally substantially smaller than the targeted emulsion droplet size (Schulman & Leja, 1954); when at least one order of magnitude smaller, they generally provide better surface coverage, and are able to achieve effective Pickering stabilization (Binks & Lumsdon, 2001; Dickinson, 2012; Garti, Aserin, Tiunova, & Binyamin, 1999). 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 prior to emulsion preparation (Gould et al., 2013; Kurukji, Pichot, Spyropoulos, & Norton, 2013). This may be explained by two reasons: first, only the finest particle fraction may have stabilized the emulsion, as found for cocoa particles (Gould et al., 2013); or second, the homogenization process to make the emulsion may have disrupted or de-aggregated the particles (Kurukji et al., 2013). To assess the latter potential effect, we measured the particle size distribution after homogenization of the particle dispersion 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 (Supporting information, Figure S8.4.). The particle size of the other materials was not largely affected.






Spirulina cake powder
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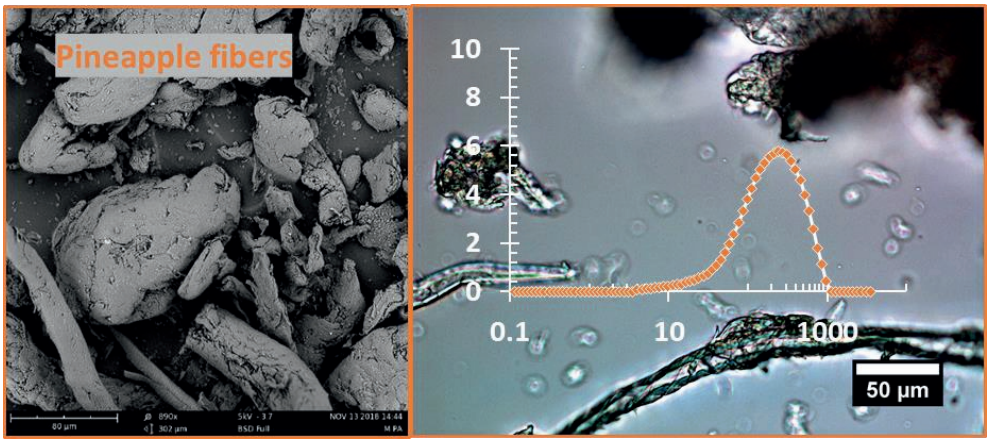
8
6
4
2
0
0.1
10
1000
50 µm


Pineapple fibers
10
8
6
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0.1
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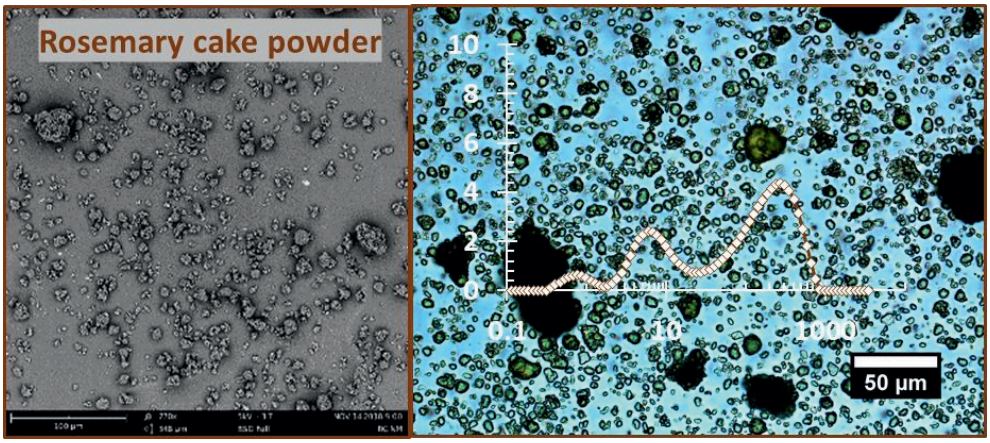
50 µm


Rosemary cake powder
10
8
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2
0
50 µm

Figure 8.1. Characterization of raw powders of matcha tea, spinach leaf, spirulina cakes, pineapple fibers, and rosemary cakes, and extracts of turmeric and red radish: Left: SEM images. Right: first 4 entries, 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 (%), superimposed over light microscopy images (dispersions). Last two entries are photos of (i) turmeric extract in acetate buffer (left) and stripped sunflower oil (dispersible, right); and (ii) red radish extract in acetate buffer.

From the equation defining the desorption energy of a Pickering particle, as given earlier, 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 would

directly lower the interfacial tension, or affect the contact angle by adsorbing to the particles (Binks et al., 2007; Cui, Cui, Zhu, & Binks, 2012), we first measured the dynamic interfacial tension of the supernatant obtained after centrifugation of 1% w/w dispersions of powders or extracts 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 time-scale of the experiment (Figure 8.2.). The soluble components in the spirulina cake supernatant were able to decrease the interfacial tension the fastest, and reached the lowest value (~10 mN m^{-1}), most probably due the presence of proteins (composition in Supporting information Table S8.2.), particularly phycocyanins (Benedetti et al., 2004). The matcha tea supernatant was also able to decrease the interfacial tension to 10 mN m^{-1}, most 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}, most likely through thylakoid fragments that are rich in proteins (Tenorio, De Jong, Nikiforidis, Boom, & Van Der Goot, 2017). Red radish was completely soluble and decreased the interfacial tension rapidly, to reach a final value of 14.5 mN m^{-1}, compared to which pineapple fiber and rosemary cake supernatants had lower surface activity (17 mN m^{-1}), and turmeric extract was not able to decrease the interfacial tension.

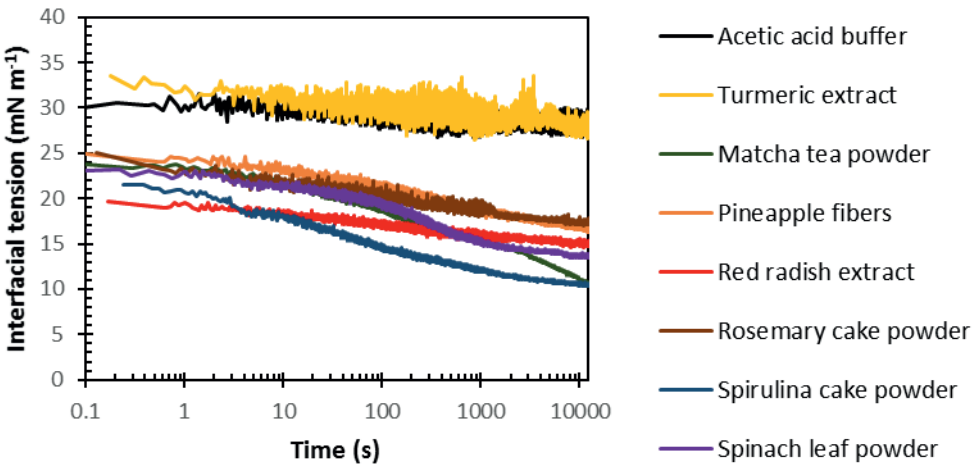

Figure 8.2. Dynamic interfacial tension at the stripped sunflower oil-acetic acid buffer interface, with the aqueous phase containing the soluble components of a 1% w/w dispersion of turmeric and red radish extracts, and powders of matcha tea, pineapple fibers, rosemary cakes, spirulina cakes, or spinach leaf.

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 a synergistic stabilization (Binks et al., 2007). Surface-active components may also compete with particles and have even been suggested to desorb particles from the interface (Vashisth, Whitby, Fornasiero, & Ralston, 2010), although we expect this to only be possible if the resulting contact angle is close to 0 or 180° (Dickinson, 2012).

8.3.2. 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 leaf, spirulina cake and pineapple fibers were able to physically stabilize O/W emulsions for at least 3 months (Figure 8.3.). In the measured droplet size distribution, also non-adsorbed particles play a role; 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. 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 very instrumental in reducing the droplet size to ~10 µm (Walstra, 2004). The red radish dispersion was not able to form stable emulsions (Supporting information, Figure S8.5.).

Emulsions prepared with rosemary cake powder phase inverted upon homogenization, most probably due to the hydrophobicity of these particles. To investigate the emulsification potential of these, and turmeric particles, we homogenized a sunflower oil phase containing the dispersed particles with an aqueous phase using the same homogenizer, and visually stable W/O emulsions were obtained (Figure 8.4.), with turmeric extract particles visible at the droplet surface (Figure 8.4.B).

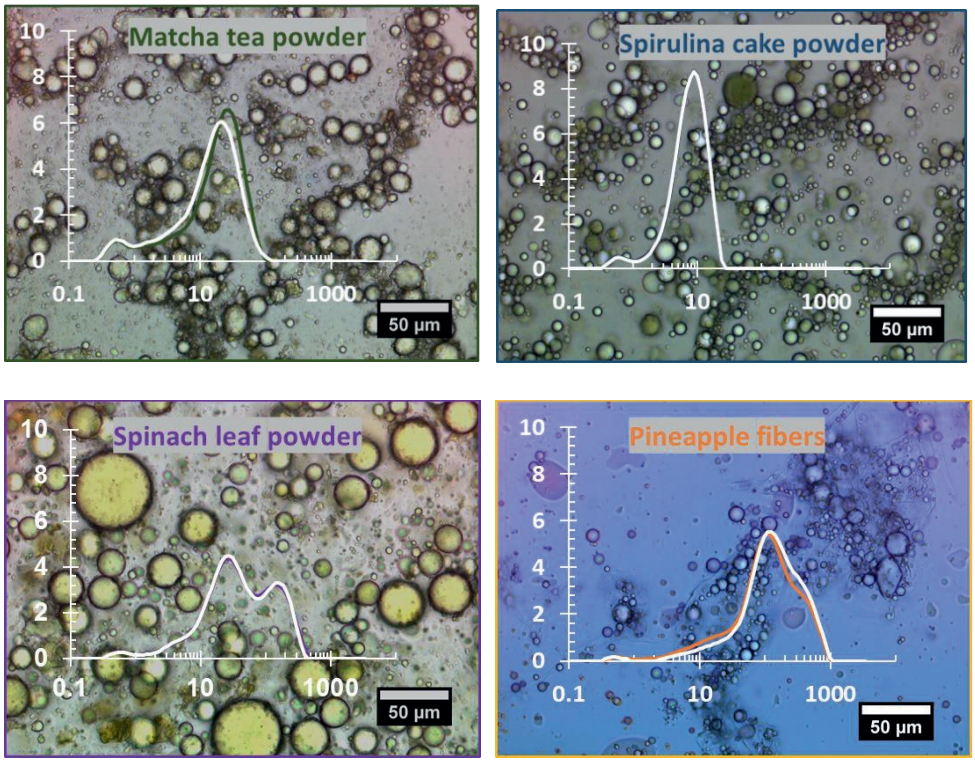

Figure 8.3. Physical characterization of 10% w/w O/W emulsions stabilized by 5% w/w powders of matcha tea, spinach leaf, spirulina cakes, or pineapple fibers: Droplet size distribution (freshly prepared (colored line), or after 3 months storage at 4 °C (white line)) superimposed over light microscopy images of fresh samples.

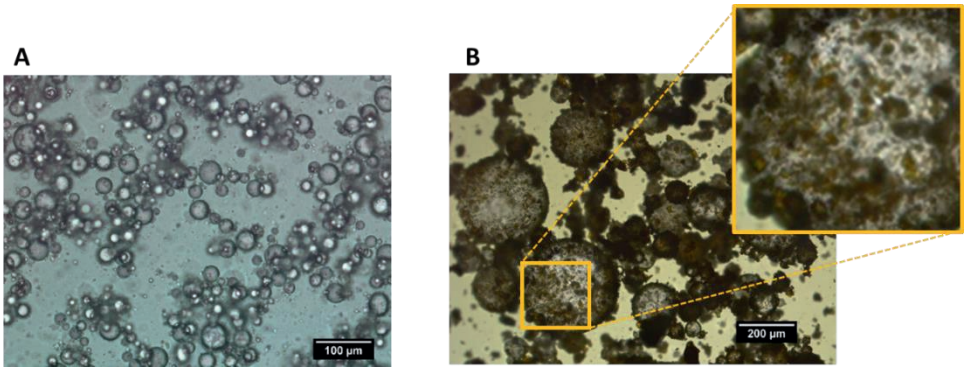

Figure 8.4. Light microscopy images of 10% w/w W/O emulsions stabilized by 5% w/w rosemary cake powder (A) or turmeric extract (B).

To further investigate the stabilization mechanism of the most promising materials (powders of matcha tea, spinach leaf, spirulina cakes and pineapple fibers), we prepared emulsions with only the supernatant (soluble fraction), or the re-suspended pellet (washed particles) obtained after centrifugation of the particle suspension. The supernatants of the spirulina cake powder suspension, which was the most surface-active (Figure 8.2.), and of the pineapple fiber suspension, led to emulsion droplets of around 5 µm (d_{32}) that destabilized due to flocculation and coalescence within 1 week (Figure 8.5.). This may have occurred due to a lack of steric and/or electrostatic repulsion, i.e., the droplets were not covered or charged enough (McClements, 2005). 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 (Figure 8.5.), showing that these components are not suitable as emulsifiers.

Washed particles form matcha tea, spinach leaf and spirulina cakes 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 Supporting information, Table S8.2.) 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 stability, which may have been further increased by network formation.

8.3.3. 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 °C, and measured the formation of primary lipid oxidation products (conjugated E,Z-Ln-OOH, Figure 8.6.A, and total hydroperoxides (Supporting information, Figure S8.6.)) and secondary oxidation products (aldehydes, Figure 8.6.B) and 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 Supporting information, Figure S8.7.).

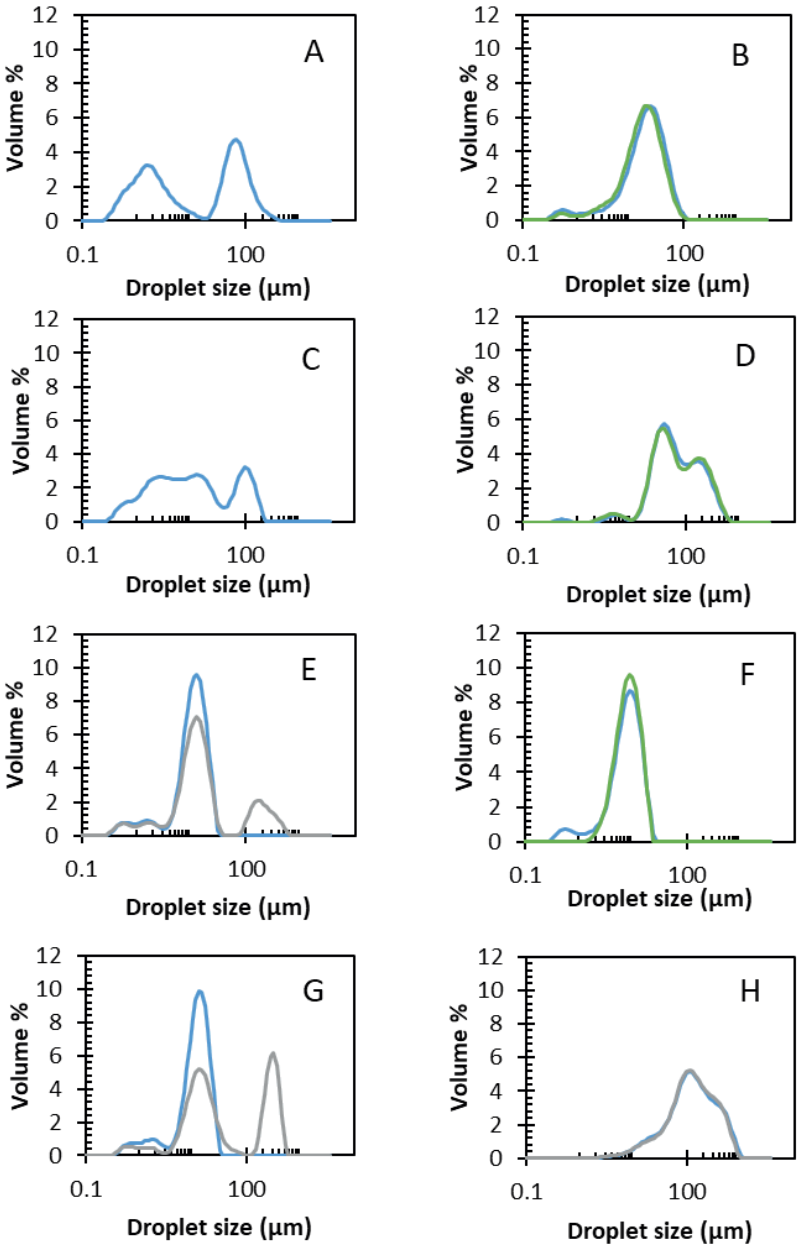

Figure 8.5. Droplet size distributions of emulsions stabilized by the supernatant (A, C, E, G) of particle dispersions, or by washed particle dispersions (B, D, F, H) of matcha tea powder (A, B), spinach leaf powder (C, D), spirulina cake powder (E, F) and pineapple fibers (G, H), at t_0 (blue), and after 1 week (grey) or 4 weeks (green) storage.

All emulsions remained physically stable over the time-scale of the incubation period. 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 E,Z-Ln-OOH, 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. We start by discussing on the particle-stabilized emulsions, and in the following section, the reference emulsions are addressed, after which all effects are putatively brought together.

Matcha tea is known to contain exceptionally high concentrations of antioxidants (as also reported in Supporting information, Table S8.1.), particularly catechins of which epigallocatechin gallate is the most abundant, and the flavonoid quercetin (Ozgen, Kilinc, & Selamoğlu, 2016). Both components are free radical-scavenging antioxidants and able to interrupt the lipid oxidation chain reaction, which could explain the high oxidative stability of this emulsion, especially when located at the interface (Berton-Carabin et al., 2014; Schröder et al., submitted.). Spinach leaf powder contains 40-fold lower amounts of polyphenols compared to matcha tea powder (Supplementary information, Table S8.1.). Yet, it contains oxalic acid (Supporting information, Table S8.3.) (Zheng, Yang, Pu, & Zhang, 2009), which has been reported to chelate metal ions such as iron (Suter, Siffert, Sulzberger, & Stumm, 1988), thereby inactivating this transition metal with regard to its pro-oxidant effect. Understanding what causes the effect of spirulina powder on oxidation is more elusive. Although spirulina cake powder 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 (Belay, Kato, & Ota, 1996; Patel, Mishra, & Ghosh, 2006). 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 (Ghorbani Gorji, Smyth, Sharma, & Fitzgerald, 2016). In fact, iron has a strong catalytic effect for the decomposition of hydroperoxides, leading to alkoxyl

radicals and later on secondary lipid oxidation products (Schaich, Shahidi, Zhongy, & Eskin, 2013). Pineapple generally contains considerable amounts of vitamin C that can scavenge free radicals (Uluata, McClements, & Decker, 2015) 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 (Ghorbani Gorji et al., 2016), 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 (Nimalaratne & Wu, 2015) at pH 6.5 (Castellani, Guérin-Dubiard, David-Briand, & Anton, 2004). However, at pH 4.5 - the typical pH of dressing-like food emulsions, including mayonnaise, which notably prevents the growth of pathogens - the binding constant is lower, which may allow iron to be available for lipid oxidation (McClements, 2005; Merkx, Delić, Wierenga, Hennebelle, & van Duynhoven, 2018). Phosvitin is also surface-active, and will therefore locate at the oil-water interface where it may release iron, leading to fast lipid oxidation, which may be prevented in food emulsions such as mayonnaise by the use of EDTA, a strong chelator (Ghorbani Gorji et al., 2016). To the best of our knowledge, Tween 60 is not known for any specific antioxidant activity. Therefore, no inhibition of oxidation of the corresponding emulsion could be expected (Pérez-Rosés, Risco, Vila, Peñalver, & Cañigueral, 2015). In order to deconvolute these various effects, one would need to measure free radical scavenging properties as well as chelation of ferrous iron of the different emulsifiers, which would be a study by itself.

To summarize, as lipid oxidation is probably initiated at the oil-water interface (Berton-Carabin et al., 2014; Laguerre et al., 2009), 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 (Schröder et al., 2019; Schröder et al., submitted), as we previously also demonstrated for purposely built particles. Some of the biobased Pickering particles used in the present study contain endogenous antioxidants or chelators embedded in their natural matrix. These antioxidants

are probably still available for hydrogen atom-donating or metal-chelating activities, which can be boosted by an interfacial localization. Here, we provide a proof-of-principle that it is possible to create a dual stabilizing functionality that leads to naturally armored emulsions against oxidation and physical damage, 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.

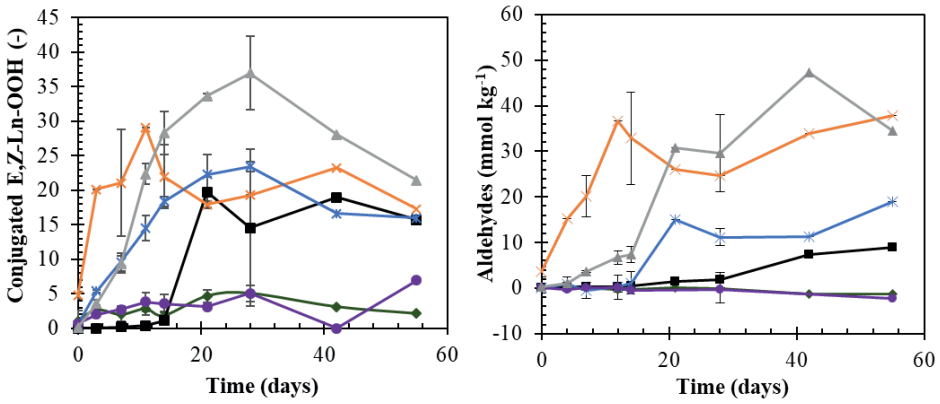

Figure 8.6. Formation of conjugated E.Z-Ln-OOH (left) and aldehydes (right) in O/W emulsions (10% w/w stripped sunflower oil) stabilized with matcha tea powder (green), spinach leaf powder (purple), spirulina cake powder (blue), pineapple fibers (orange), egg yolk (black) or Tween 60 (grey), stored at 25 ℃.

8.4. Conclusions

We have investigated the physical and oxidative stability of Pickering emulsions stabilized by natural particulate materials, i.e., powders of matcha tea, spinach leaf, spirulina cakes, pineapple fibers, and rosemary cakes, and extracts of red radish and turmeric. Particles that showed weak aggregation in aqueous media (powders of matcha tea, spinach leaf, spirulina cakes 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 an accumulation at the droplet surface of antioxidants and/or chelators embedded in the particles matrix but 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.

8.5. Supporting information

Table S8.1. Particle composition.

Polyphenol content with asterix determined by HPLC, others by Folin-Ciocalteu method.

	Content %									
Samples	**Malto-dextrins**	**Free sugars**	**Total Sugars**	**Free glucose**	**Total glucose**	**Starch**	**Ash**	**Poly-phenols**	**Organic acids**	**Anions**
Curcumin powder	ND	ND	0.19	ND	ND	ND	ND	92.75*	ND	0.01
Rosemary cake powder	ND	2.20	4.17	0.25	ND	ND	1.58	10.95	0.88	0.05
Red radish powder	44.06	0.52	94.01	0.52	77.1	24.10	ND	1.62	1.36	0.25
Spirulina cake powder	ND	0.20	5.79	0.20	ND	ND	17.79	0.36	ND	5.71
Matcha tea powder	ND	4.95	15.70	0.73	ND	ND	4.70	21.00	1.56	0.33
Pineapple fibers	ND	0.88	31.06	ND	ND	ND	1.09	1.45	0.80	0.02
Spinach leaf powder	ND	5.22	17.75	0.79	5.14	3.87	15.00	1.11	6.38	1.38

Table S8.2. Particle composition (continued).

	Content %						
Samples	**Proteins**	**Total nitrogen**	**Cellulose**	**Neutral detergent Fibers**	**Fibers**	**Lignin**	**Hemi-cellulose**
Curcumin powder	<0.50	<0.08	<2.00	2.20	<0.50	<0.50	2.20
Rosemary cake powder	1.20	0.18	14.60	34.20	17.70	16.80	16.50
Red radish powder	0.90	0.14	< 2.00	3.80	1.30	< 0.50	2.50
Spirulina cake powder	58.10	9.30	< 2.00	1.20	0.60	< 0.50	< 0.60
Matcha tea powder	22.00	3.52	4.60	27.60	16.40	8.50	11.20
Pineapple fibers	1.60	0.26	28.60	76.20	35.60	9.60	40.60
Spinach leaf powder	28.00	4.48	6.70	15.10	8.40	1.30	6.70

Table S8.3. Particle composition (continued).

	Content %						
Samples	**Quinic**	**Malic**	**Oxalic**	**Citric**	**Chloride**	**Phosphate**	**Sulfate**
Curcumin powder	ND	ND	ND	ND	ND	ND	0.01
Rosemary cake powder	0.23	0.14	0.05	0.46	0.05	ND	ND
Red radish powder	ND	ND	ND	1.36	0.25	ND	ND
Spirulina cake powder	ND	ND	ND	ND	2.15	3.24	0.32
Matcha tea powder	0.57	0.29	0.51	0.19	0.05	0.12	0.16
Pineapple fibers	ND	0.08	0.72	ND	0.02	ND	ND
Spinach leaf powder	ND	1.24	4.75	0.39	0.41	0.48	0.49

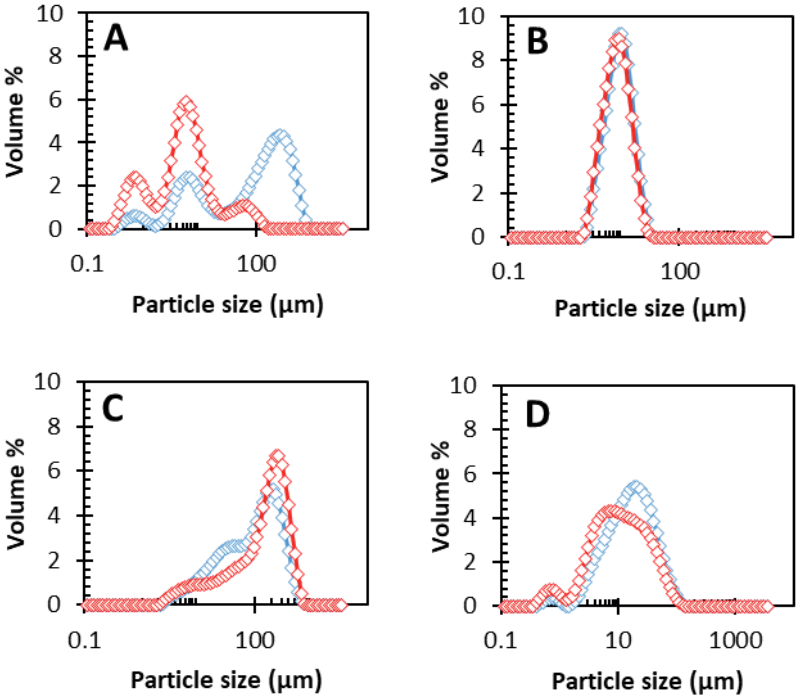

Figure S8.4. Particle size distribution of 1% w/w rosemary cake powder, spirulina cake powder, spinach leaf powder, and matcha tea powder dispersions in acetate buffer (50 mM, pH 4.5) measured by static light scattering, before (blue) and after homogenization (red).

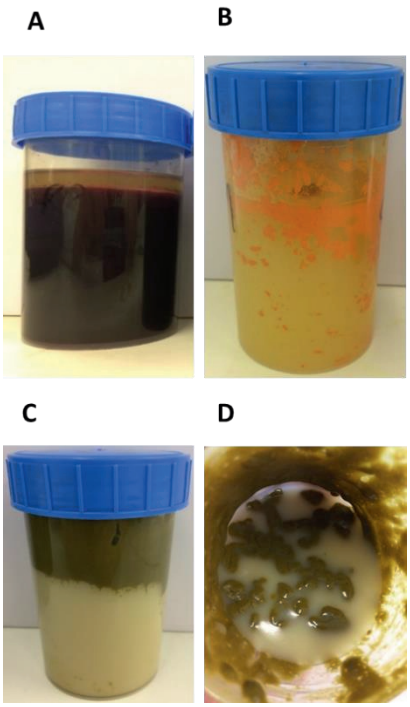

Figure S8.5. Photo of emulsions stabilized by red radish extract (A), turmeric extract (B) and rosemary cake powder (C & D).

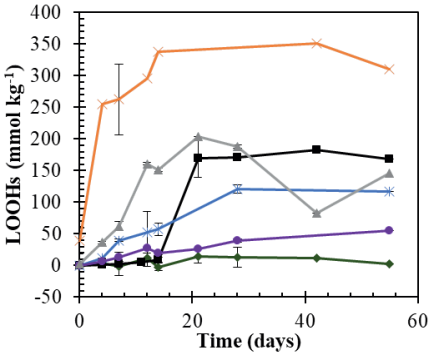

Figure S8.6. Formation of hydroperoxides (LOOHs) in O/W emulsions (10% w/w stripped sunflower oil) stabilized with matcha tea powder (green), spinach leaf powder (purple), spirulina cake powder (blue), pineapple fibers (orange), egg yolk (black) or Tween 60 (grey), stored at 25 °C.

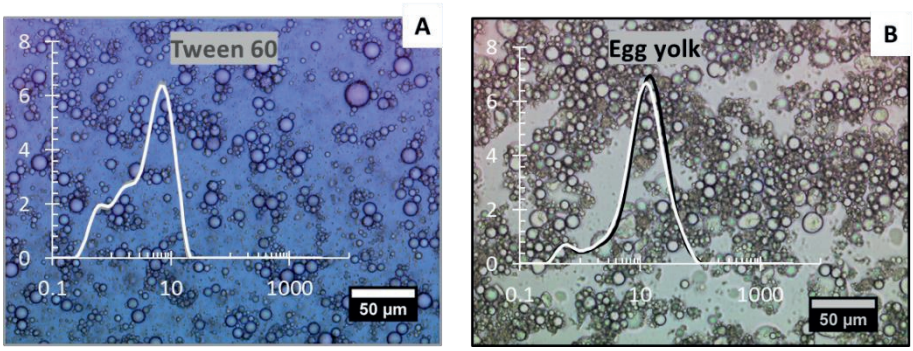

Figure S8.7. Physical characterization of O/W emulsions stabilized by 1% w/w Tween 60 and 5% w/w egg yolk: Droplet size distribution (freshly prepared (colored line), or after 3 months storage at 4 °C (white line)) superimposed over light microscopy images of the fresh samples.

8.6. References

Belay, A., Kato, T., & Ota, Y. (1996). Spirulina (Arthrospira): Potential application as an animal feed supplement. Journal of Applied Phycology, 8(4–5), 303–311.

Benedetti, S., Benvenuti, F., Pagliarani, S., Francogli, S., Scoglio, S., & Canestrari, F. (2004). Antioxidant properties of a novel phycocyanin extract from the blue-green alga Aphanizomenon flos-aquae. Life Sciences, 75(19), 2353–2362.

Berton-Carabin, C. C., Ropers, M. H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Comprehensive Reviews in Food Science and Food Safety, 13(5), 945–977.

Berton-Carabin, C. C., & Schroën, K. (2015). Pickering emulsions for food applications: background, trends, and challenges. Annual Review of Food Science and Technology, 6, 263–297.

Berton-Carabin, C., & Schroën, K. (2019). Towards new food emulsions: Designing the interface and beyond. Current Opinion in Food Science.

Binks, B. P., & Kirkland, M. (2002). Interfacial structure of solid-stabilised emulsions studied by scanning electron microscopy. Physical Chemistry Chemical Physics, 4(15), 3727–3733.

Binks, B. P., & Lumsdon, S. O. (2000). Influence of particle wettability on the type and stability of surfactant-free emulsions. Langmuir, 16(23), 8622–8631.

Binks, B. P., & Lumsdon, S. O. (2001). Pickering emulsions stabilized by monodisperse latex particles: Effects of particle size. Langmuir, 17(15), 4540–4547.

Binks, Bernard P., Rodrigues, J. A., & Frith, W. J. (2007). Synergistic interaction in emulsions stabilized by a mixture of silica nanoparticles and cationic surfactant. Langmuir, 23(7), 3626–3636.

Castellani, O., Guérin-Dubiard, C., David-Briand, E., & Anton, M. (2004). Influence of physicochemical conditions and technological treatments on the iron binding capacity of egg yolk phosvitin. Food Chemistry, 85(4), 569–577.

Chevalier, Y., & Bolzinger, M. A. (2013). Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 439, 23–34.

Cui, Z. G., Cui, C. F., Zhu, Y., & Binks, B. P. (2012). Multiple phase inversion of emulsions stabilized by in situ surface activation of CaCO 3 nanoparticles via adsorption of fatty acids. Langmuir, 28(1), 314–320.

Day, L. (2013). Proteins from land plants - Potential resources for human nutrition and food security. Trends in Food Science and Technology, 32(1), 25–42.

de Folter, J. W. J., Hutter, E. M., Castillo, S. I. R., Klop, K. E., Philipse, A. P., & Kegel, W. K. (2014). Particle shape anisotropy in pickering emulsions: Cubes and peanuts. Langmuir, 30(4), 955–964.

de Folter, J. W. J., van Ruijven, M. W. M., & Velikov, K. P. (2012). Oil-in-water Pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein. Soft Matter, 8(25), 6807.

Dickinson, E. (1993). Towards more natural emulsifiers. Trends in Food Science and Technology, 4(10), 330–334.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. Current Opinion in Colloid and Interface Science, 15(1–2), 40–49.

Dickinson, E. (2012). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. Trends in Food Science and Technology, 24(1), 4–12.

EFSA. (2009). Scientific opinion of the panel on food additives and nutrient sources added to food on calcium silicate, silicon dioxide and silicic acid gel added for nutritional purposes to food supplements following a request from the European Commission. The EFSA Journal, 1132, 1–24.

Eskandar, N. G., Simovic, S., & Prestidge, C. A. (2007). Synergistic effect of silica nanoparticles and charged surfactants in the formation and stability of submicron oil-in-water emulsions. Physical Chemistry Chemical Physics : PCCP, 9, 6426–6434.

Finkle, P., Draper, H. D., & Hildebrand, J. H. (1923). The theory of emulsification. Journal of the American Chemical Society, 45(12), 2780–2788.

Garti, N., Aserin, a., Tiunova, I., & Binyamin, H. (1999). Double emulsions of water-in-oil-in-water stabilized by α-form fat microcrystals. Part 1: Selection of emulsifiers and fat microcrystalline particles. Journal of the American Oil Chemists' Society, 76(3), 383–389.

Ghorbani Gorji, S., Smyth, H. E., Sharma, M., & Fitzgerald, M. (2016). Lipid oxidation in mayonnaise and the role of natural antioxidants: A review. Trends in Food Science and Technology, Vol. 56.

Gould, J., Vieira, J., & Wolf, B. (2013). Cocoa particles for food emulsion stabilisation. Food & Function, 4, 1369–1375.

Günther, F., Frijters, S., & Harting, J. (2014). Timescales of emulsion formation caused by anisotropic particles. Soft Matter, 10(27), 4977–4989.

Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., & Norton, I. T. (2012). Investigation into the potential ability of Pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. Journal of Colloid and Interface Science, 366(1), 209–215.

Kargar, M., Spyropoulos, F., & Norton, I. T. (2011). Microstructural design to reduce lipid oxidation in oil-in-water emulsions. Italian Oral Surgery, 1, 104–108.

Kralova, I., & Sjöblom, J. (2017). Surfactants used in food industry : A Review. Journal of Disperison Science and Technology, 2691(May).

Kurukji, D., Pichot, R., Spyropoulos, F., & Norton, I. T. (2013). Interfacial behaviour of sodium stearoyllactylate (SSL) as an oil-in-water pickering emulsion stabiliser. Journal of Colloid and Interface Science, 409, 88–97.

Laguerre, M., Bily, A., Roller, M., & Birtic, S. (2017). Mass transport phenomena in lipid oxidation and antioxidation. Annual Review of Food Science and Technology, 8(1), annurev-food-030216-025812.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M. C., Baréa, B., Weiss, J., … Villeneuve, P. (2009). Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. Journal of Agricultural and Food Chemistry, 57(23), 11335–11342.

Luo, Z., Murray, B. S., Yusoff, A., Morgan, M. R. a, Povey, M. J. W., & Day, A. J. (2011). Particle-stabilizing effects of flavonoids at the oil-water interface. Journal of Agricultural and Food Chemistry, 59(6), 2636–2645.

Madivala, B., Vandebril, S., Fransaer, J., & Vermant, J. (2009). Exploiting particle shape in solid stabilized emulsions. Soft Matter, 5(8), 1717.

McClements, D. J. (2005). Food emulsions principles, practices, and techniques - Second Edition. CRC Press: Boca Raton, FL.

McClements, D. Julian. (2005). Interfacial properties and their characterization 5.1. (Chapter 3). (Taylor and Francis).

McClements, David Julian, & Decker, E. (2018). Interfacial antioxidants: A review of natural and synthetic emulsifiers and coemulsifiers that can inhibit lipid oxidation. Journal of Agricultural and Food Chemistry, 66(1), 20–25.

Merkx, D. W. H., Delić, F., Wierenga, P. A., Hennebelle, M., & van Duynhoven, J. P. M. (2018). 31P NMR assessment of the phosvitin-iron complex in mayonnaise. Magnetic Resonance in Chemistry, (September), 1–8.

Merkx, D. W. H., Hong, G. T. S., Ermacora, A., & Van Duynhoven, J. P. M. (2018). Rapid Quantitative Profiling of Lipid Oxidation Products in a Food Emulsion by1H NMR. Analytical Chemistry, 90(7), 4863–4870.

Midmore, B. R. (1998). Synergy between silica and polyoxyethylene surfactants in the formation of O/W emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 145(1–3), 133–143.

Nimalaratne, C., & Wu, J. (2015). Hen egg as an antioxidant food commodity: A review. Nutrients, 7(10), 8274–8293.

Ougiya, H., Watanabe, K., Morinaga, Y., & Yoshinaga, F. (1997). Emulsion-stabilizing effect of bacterial cellulose. Bioscience, Biotechnology, and Biochemistry, 61(9), 1541–1545.

Ozgen, S., Kilinc, O. K., & Selamoğlu, Z. (2016). Antioxidant activity of quercetin: A mechanistic review. Turkish Journal of Agriculture - Food Science and Technology, 4(12), 1134.

Patel, A., Mishra, S., & Ghosh, P. K. (2006). Antioxidant potential of C-phycocyanin isolated from cyanobacterial species Lyngbya, Phormidium and Spirulina spp. Indian Journal of Biochemistry and Biophysics, 43(1), 25–31.

Pérez-Rosés, R., Risco, E., Vila, R., Peñalver, P., & Cañigueral, S. (2015). Antioxidant activity of Tween-20 and Tween-80 evaluated through different in-vitro tests. Journal of Pharmacy and Pharmacology, 67(5), 666–672.

Perrin, E., Bizot, H., Cathala, B., & Capron, I. (2014). Chitin nanocrystals for pickering high internal phase emulsions. Biomacromolecules, 15(10), 3766–3771.

Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P., & Wahlgren, M. (2014). Biomass-based particles for the formulation of Pickering type emulsions in food and topical applications. Colloids and Surfaces A, 458, 48–62.

Rayner, M., Timgren, A., Sjöö, M., & Dejmek, P. (2012). Quinoa starch granules: A candidate for stabilising food-grade Pickering emulsions. Journal of the Science of Food and Agriculture, 92(9), 1841–1847.

Santini, E., Guzmán, E., Ferrari, M., & Liggieri, L. (2014). Emulsions stabilized by the interaction of silica nanoparticles and palmitic acid at the water-hexane interface. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 460, 333–341.

Schaich, K. M., Shahidi, F., Zhongy, Y., & Eskin, N. A. M. (2013). Lipid oxidation. In Biochemistry of Foods (Third Edit, pp. 419–478).

Schröder, A. J. ., Laguerre, M. ., Sprakel, J. H. B. ., Birtic, S. ., Schroen, C. G. P. H. ., & Berton-Carabin, C. (2019). Emulsion comprising antioxidant particles. PCT/EP2019/067780.

Schröder, A., Sprakel, J., Schroën, K., & Berton-Carabin, C. (2017). Tailored microstructure of colloidal lipid particles for Pickering emulsions with tunable properties. Soft Matter, 3190–3198.

Schröder, Anja;, Sprakel, J., Boerkamp, W., Schroën, K., & Claire C., B.-C. (2019). Can we prevent lipid oxidation in emulsions by using fat-based Pickering particles? Food Research International, 120, 352–363.

Schröder, Anja, Laguerre, M., Sprakel, J., Schroën, K., & Berton-Carabin, C. C. (n.d.). Pickering particles as interfacial reservoirs of antioxidants. Submitted.

Schulman, J. H., & Leja, J. (1954). Control of contact angles at the oil- water-solid interfaces. Emulsions stabilized by solid particles (BaS. Transactions of the Faraday Society, 50(598), 598–605.

Shahidi, F., & Zhong, Y. (2010a). Lipid oxidation and improving the oxidative stability. Chemical Society Reviews, 39(11), 4067–4079.

Suter, D., Siffert, C., Sulzberger, B., & Stumm, W. (1988). Catalytic dissoluion of iron(III)(hydr)oxides by oxalic acid in the presence of Fe(II). Naturwissenschaften, 75(11), 571–573.

Tenorio, A. T., De Jong, E. W. M., Nikiforidis, C. V., Boom, R. M., & Van Der Goot, A. J. (2017). Interfacial properties and emulsification performance of thylakoid membrane fragments. Soft Matter, 13(3), 608–618.

Uluata, S., McClements, D. J., & Decker, E. A. (2015). How the multiple antioxidant properties of ascorbic acid affect lipid oxidation in oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 63(6), 1819–1824.

Vashisth, C., Whitby, C. P., Fornasiero, D., & Ralston, J. (2010). Interfacial displacement of nanoparticles by surfactant molecules in emulsions. Journal of Colloid And Interface Science, 349(2), 537–543.

Walstra, P. (2004). Physical Chemistry of Foods. In European Journal of Pharmaceutics and Biopharmaceutics (Vol. 57).

Zembyla, M., Murray, B. S., Radford, S. J., & Sarkar, A. (2019). Water-in-oil Pickering emulsions stabilized by an interfacial complex of water-insoluble polyphenol crystals and protein. Journal of Colloid and Interface Science, 548, 88–99.

Zheng, Y., Yang, C., Pu, W., & Zhang, J. (2009). Determination of oxalic acid in spinach with carbon nanotubes-modified electrode. Food Chemistry, 114(4), 1523–1528.

Chapter 9

General discussion

9.1. Introduction

The objective of this project was to develop food emulsions with a new and controlled architecture directed at achieving a specific functionality. In these emulsions, food-grade Pickering particles exerted a double role: they acted as physical stabilizers through their position at the oil-water interface, and they were used as a reservoir for antioxidant molecules. This allowed antioxidants locating close to the oil-water interface, hence affecting lipid oxidation at the position where it is initiated, which in turn drastically enhanced antioxidant activity. The major emphasis of the project was to understand the link between the physicochemical properties of such Pickering particles (e.g., their behavior at the oil-water interface, and their potential to act as antioxidant reservoirs), and the performance of the final emulsions. The results may be translated into formulation guidelines for the rational design of food products containing high levels of unsaturated lipids and minimal amounts of antioxidants.

9.2. Main findings and conclusions

In this thesis, we first summarized the information available in literature on Pickering emulsions (**Chapter 2**). Based on this, we decided that both building particles from scratch, and making use of particles present in nature would be viable routes to investigate the dual functionality that we want to create. First, we explored the use of lipid-based Pickering particles for the stabilization of O/W emulsions. These particles were built via a so-called bottom-up approach; i.e., assembled from individual lipid, emulsifier and antioxidant molecules into the desired colloidal structure (Figure 9.1.), to obtain control over composition and properties. We generated a proof of concept of the ability of these particles to both physically stabilize, and mitigate lipid oxidation, in emulsions (more details are given in section 9.2.1. and 9.3.3.). Next, we considered biobased particles obtained via a so-called top-down approach; i.e., natural materials broken into building blocks of interest (Figure 9.1.), as Pickering stabilizers in O/W emulsions. More details are given in section 9.2.2., and also for these particles, in some cases dual functionality could be proven.

9.2.1. Lipid-based particles

In **Chapter 3**, we successfully developed a new type of lipid-based particles, referred to as colloidal lipid particles (CLPs). The choice of the lipid material altered the particles' microstructure, such as their internal crystallinity, and their morphology. For surfactant-based CLPs, a higher solid fat content led to an increased aspect ratio of the particles.

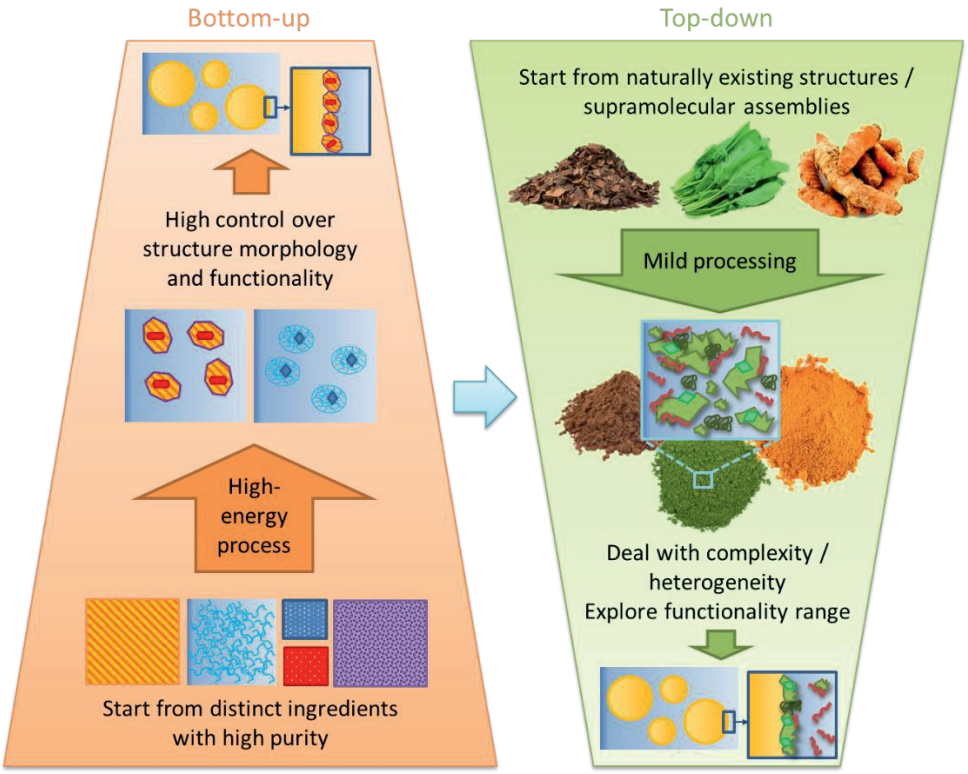

Figure 9.1. Schematic illustration of a bottom-up, and top-down production of Pickering particles.

This increased their hydrophobicity and thus affected their performance as emulsion stabilizers, e.g., it resulted in a jammed, cohesive interfacial network. Protein-covered CLPs were particularly resilient to subsequent emulsification processes, we thus used them further in **Chapter 4** in which we studied the formation of CLP-stabilized oil droplets in a microfluidic device and their stability to short-term coalescence. From this, is became clear that there is a non-monotonic effect of the particle concentration on emulsion stability. At low surface coverage, CLPs had a destabilizing effect due to droplet-droplet bridging and subsequent coalescence, whereas at higher surface coverage, particles formed an effective barrier against droplet coalescence, resulting in physically stable emulsions.

As a next step, lipid oxidation was investigated in Pickering emulsions stabilized by protein-based CLPs in **Chapter 5**. As such, these Pickering emulsions do not have a higher oxidative

stability compared to conventional sodium caseinate-stabilized emulsions, for similar composition of the oil droplets, but they do have a better physical stability. Besides, when HMP fat is an integral part of emulsion formulation, it is advantageous to add this as Pickering particles, instead of applying it in the core of the droplet. In **Chapter 6**, we added antioxidants to CLPs that serve as a reservoir (for α-tocopherol). The chemical stability of α-tocopherol depended on the fat used; crystallization probably led to preferential presence of α-tocopherol at the particle surface, which was further promoted by emulsifiers that actively induce lipid crystallization. This negatively influenced the chemical stability of α-tocopherol. In **Chapter 7**, these α-tocopherol-containing CLPs were used as Pickering stabilizers for O/W emulsions, leading to substantially better oxidative stability compared to that of a control emulsion with the exact same structure and composition, but wherein the antioxidant was present in the core of the oil droplet. Most importantly, this confirmed that the interfacial localization of antioxidants is crucial to prevent lipid oxidation in emulsions. Through this, a proof of concept was reached: the two main instability issues (i.e., physical and chemical instability) of emulsions can be mitigated through one approach.

9.2.2. Natural materials

After establishing the proof of concept with the CLPs in **Chapter 7**, we considered various natural particles prepared via a top-down approach as Pickering stabilizers for O/W emulsions in **Chapter 8**. We found that matcha tea powder and spinach leaf powder were promising emulsion stabilizers that induced both physical and oxidative stability. This showed that the double functionality that we achieved using purposely built particles (CLPs) can also be achieved using certain naturally occurring particles.

9.3. Advantages and limitations of Pickering emulsions for real applications

9.3.1. Technological challenges

Interest in Pickering emulsions for the food, pharmaceutical or cosmetic industry has grown tremendously in recent years (Berton-Carabin & Schroën, 2015a). Next we discuss the potential advantages and limitations such emulsions systems could have for real applications.

The main selling point for Pickering emulsions is their high stability against coalescence due the formation of a steric and mechanically strong interfacial barrier formed by irreversibly adsorbed particles (Pawar et al., 2011). Even Pickering emulsions with large droplets sizes,

i.e., 100 µm or larger stay physically stable for several months (Timgren et al., 2013). One of the main criticisms of using Pickering particles is the fact that generally much more materials is needed to cover the interface as compared to conventional emulsifiers. In theory, starting from 150-nm CLPs a typical surface coverage would be 56 mg m^{-2} (assuming 60% surface coverage), whereas for proteins typically 1-3 mg m^{-2} would be needed. Still, we found that a 2.5% w/w CLP-stabilized emulsion was much more resistant against coalescence than a 1% w/w sodium caseinate-stabilized emulsion (Schröder, Sprakel, Boerkamp, Schroën, & Berton-Carabin., 2019) of the same droplet size; the difference is clearly not that great as theoretically expected. In fact, most particles do not adsorb, which may increase the viscosity of the continuous phase, or even lead to formation of a particle network (Bijsterbosch, Bos, Dickinson, Van Opheusden, & Walstra, 1995; Binks, 2002). This reduces the need for thickening agents to prevent droplet creaming, and if a particle network is formed, this will also reduce creaming completely.

Other reasons to consider Pickering particles are the replacement of low molecular weight surfactants that may cause undesired physiological responses, such as a damaged enzyme activity, may accumulate in the human body (Yuan et al., 2014) and do not meet the clean-label trend (Chevalier & Bolzinger, 2013b; Frelichowska et al., 2009), or dairy proteins that are not sustainable (Tang, 2017), and the possibility to make products that are otherwise difficult to produce, such as liquid marbles (Aussillous & Quéré, 2001; McHale & Newton, 2011), or ultra-stable foams (Arriaga et al., 2012).

Despite the fact that Pickering particles have many advantages, also some challenges need to be met before Pickering particles can be applied in the food market. Given the complexity of foods that vary in pH, electrolyte concentration, and in which components interact (Dickinson, 1993; Berton-Carabin & Schroën, 2015a), it is difficult to predict how particles behave (Anjali & Basavaraj, 2018; Binks, Rodrigues, & Frith, 2007). The interaction energy of a particle in the interface is a function of the interfacial tension, the contact angle, and size of the particle, and these three factors can be influenced by the food composition. For example, pH-dependent surface groups have a large effect on particle wettability (and possibly on particle size if they can swell), and thus on emulsion stability. This effect is co-determined by the presence of electrolytes that may screen charges on the particles' surface, thus making them less hydrophilic. Other compounds, such as surfactants, may adsorb at the particle surface and change their wettability (Binks, Rodrigues, et al., 2007),

or adsorb at the oil-water interface, lowering the interfacial tension; both effects may lead to desorption of particles (Vashisth, Whitby, Fornasiero, & Ralston, 2010).

It would be ideal to have commercially available particles that are resistant to such variations, leading to a broad operational window with regard to system composition. However, this ideal has not been reached yet; production of most particles is complex, energy-intensive, unsustainable, and expensive, because of the need for e.g. complexation or heating (Firoozmand & Rousseau, 2016). We feel that true Pickering stabilizers for food products will rely on renewable resources that can be mildly processed.

Next to that, for these novel stabilizers, scaling relations that link particle properties and processing conditions to product properties are not available. This includes, for example, the homogenization conditions that can be detrimental to the particles, or lead to aggregation or partially coalesced droplets (Whitby & Krebsz, 2014; Whitby et al., 2011). Still, sufficient energy input needs to be applied to allow particle adsorption, as it is not spontaneous (as elaborated on in **Chapter 2 and 4**). This was a challenge for the CLP-stabilized emulsions; the Tween 40-coated CLPs tended to aggregate upon emulsification, probably due to their hydrophobic character.

Particles need to be selected based on their contact angle (as indicated earlier); however, it is difficult to determine contact angles, especially for small particles, composite particles (Al-Shehri, Horozov, & Paunov, 2014; Binks & Rodrigues, 2007), and extreme contact angles, either high or low. The gel trapping technique has been used to measure contact angles, but is rather laborious (Paunov, 2003); alternatively the Washburn method, which is based on capillary rise of liquids can be applied (Galet, Patry, & Dodds, 2010) although for this method the preparation of the packed powder is of imminent importance. In **Chapter 9**, we approached the hydrophobicity of natural particles from their aggregation behavior in aqueous suspensions, which we expect to be linked to their potential to stabilize emulsions. Water-dispersible particles that have a tendency to aggregate are expected to have hydrophobic surface properties next to hydrophilic properties, and are therefore anticipated to be suitable as Pickering stabilizers for O/W emulsions.

Besides the production as such, also shelf-life considerations are important. Many Pickering particles, including our CLPs, are sensitive to high temperatures, i.e., they melt or get degraded upon heating. This means that regular thermal pasteurization cannot be applied,

and alternative pasteurization methods such as high pressure processing or pulsed electric fields would need to be tested. In line with this, drying of such emulsions by conventional methods may pose problems due to the temperatures involved, which may be mitigated by using reduced pressure, or freeze drying. It is expected that the natural particles that we describe are less sensitive to thermal changes.

9.3.2. Acceptability of Pickering particles in foods

The main concern about using small particles is their biological fate after ingestion (Borel & Sabliov, 2014). Nanomaterials, i.e., materials where more than 50% of particles in unbound or aggregated state are within the range of 1 – 100 nm in their number size distribution, are currently subject to debate (Bleeker et al., 2012). They also need to be indicated in the list of ingredients as such, which does not make them clean-label ingredients (Bleeker et al., 2012). The CLPs that we used, and actually many other Pickering particles, do not fall in the nano-materials category, although often referred to as such in literature.

Clean-labeling, which is an expanding business, also relates to particle composition, origin, processing, and health aspects. It is thus important that Pickering particles are made from biobased, i.e., natural, renewable, and food-grade sources, that can be treated mildly to obtain the particles without using complex, energy intensive methods, that may bring ingredients perceived as unhealthy into the product. As mentioned earlier, these ideal particles are not readily available yet, but it is clear that there is a lot of room to develop this emerging market. Our CLPs contain saturated, palm-based fats, which are associated to strong environmental impact, and health issues such as cardiovascular diseases. In this sense, it would be good to replace palm oil with more sustainable waxes, or even oleogels. The natural materials used in **Chapter 8** are a great step toward more ideal Pickering stabilizers regarding sustainability, health and clean-label aspects.

9.3.3. Lipid oxidation in Pickering emulsions

In **Chapter 7**, we showed that emulsions containing α-tocopherol in Pickering particles, resisted oxidation better than a control emulsion with the same composition and structure, but with antioxidant in the core of the oil droplets. We found that α-tocopherol breaks down, and concomitant formation of lipid oxidation products takes place, which may hint at a different mechanism than mostly assumed (Labuza & Dugan, 1971). Traditionally, antioxidants are thought as sacrificial molecules that oxidize instead of the unsaturated

lipids, and therewith prevent lipid oxidation. From our work it is clear that the interfacial localization of the antioxidant is crucial; the CLPs behaved as an antioxidant reservoir at the interface, where probably the very first lipid radicals were scavenged during the initiation step, therewith preventing rapid propagation of the radical chain reaction.

By applying the antioxidant loaded particles, the total antioxidant concentration could be reduced with a factor 2-4 compared to emulsions with antioxidant dissolved in the oil droplets, while not compromising the oxidative stability. We further investigated our concept by varying the CLP composition. We prepared CLPs consisting of tripalmitin:tricaprylin 4:1 (w/w), instead of only tripalmitin, and measured lipid oxidation, breakdown of α-tocopherol, and dynamic aspects of the antioxidant using a fluorescent analog. The addition of tricaprylin (liquid oil) to the CLPs, resulted in faster diffusion of the fluorescent analog into the core of the droplet as compared to tripalmitin CLPs (ratio of black to green droplets; Figure 9.2.D & E). We also found slightly faster oxidation (Figure 9.2. A – C), which is most probably related to the shorter residence time of α-tocopherol at the interface. From this, we learned that the efficiency of antioxidants can be boosted, by choosing higher melting point components for the particles, for which natural waxes such as candelilla wax (Hwang, Fhaner, Winkler-Moser, & Liu, 2018) could be an option. Further, in oleogels that contain a 3D-network (Zulim Botega, Marangoni, Smith, & Goff, 2013; Dassanayake, Kodali, & Ueno, 2011) lipophilic antioxidants could be physically or chemically trapped, therewith increasing residence time at the interface. Besides, whey protein microgels (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014; Schmitt, Bovay, Vuilliomenet, Rouvet, & Bovetto, 2011) or hydrophobized alginate microgels (Paques, Van der Linden, Van Rijn, & Sagis, 2013; Nan et al., 2014; Zhang, Sun, Fan, Li, & He, 2018) may serve as reservoirs for hydrophilic antioxidants.

It is good to point out that the results in Figure 9.2. are a result of two kinetic phenomena, that of oxidation that occurs immediately due to the presence of the ferrous iron catalyst, and that of antioxidant diffusion. In an emulsion without catalyst, the diffusion of tocopherol will not be affected, but the oxidation rate will be lower, and to translate our findings to practical oxidative stability, these time scales would need to be linked.

To show the versatility of our approach, we encapsulated carnosic acid (found in rosemary), a highly reactive oxygen species (ROS) scavenging lipophilic antioxidant in sodium caseinate-coated tripalmitin CLPs, and measured lipid oxidation in Pickering emulsions, and the

control emulsion with carnosic acid incorporated in the oil droplets. In both emulsions, lipid oxidation was largely prevented at comparable amounts as used for α-tocopherol (0.27 mmol kg^{-1}), showing that the antioxidant activity of carnosic acid is higher than of α-tocopherol (Figure 9.3.A & B) (Huang, Frankel, Schwarz, Aeschbach, & German, 1996), due to its' hyper-stoichiometry and the presence of two phenolic hydroxyl groups (Zhang et al., 2010; McPhail, Hartley, Gardner, & Duthie, 2003). In emulsions containing lower amounts of carnosic acid (0.07 mmol kg^{-1}), lipid oxidation was prevented more efficiently when the antioxidant was present in the CLPs (Figure 9.3.C & D); the emulsion architecture clearly contributes to oxidative stability as found for α-tocopherol, which indicates that this concept is generic, and applicable to a broad range of chemically active compounds.

To conclude, we have demonstrated a proof of concept to boost the efficiency of natural antioxidants in O/W emulsions by using antioxidant-loaded colloidal lipid particles as emulsions stabilizers. This system is versatile in design, and we expect that it could be further tailored to the product in which it is going to be applied, i.e., by increasing the residence time of antioxidants at the interface. Ultimately, this concept can be used to rationally identify sustainable Pickering particles produced via a top-down approach, that naturally contain antioxidants.

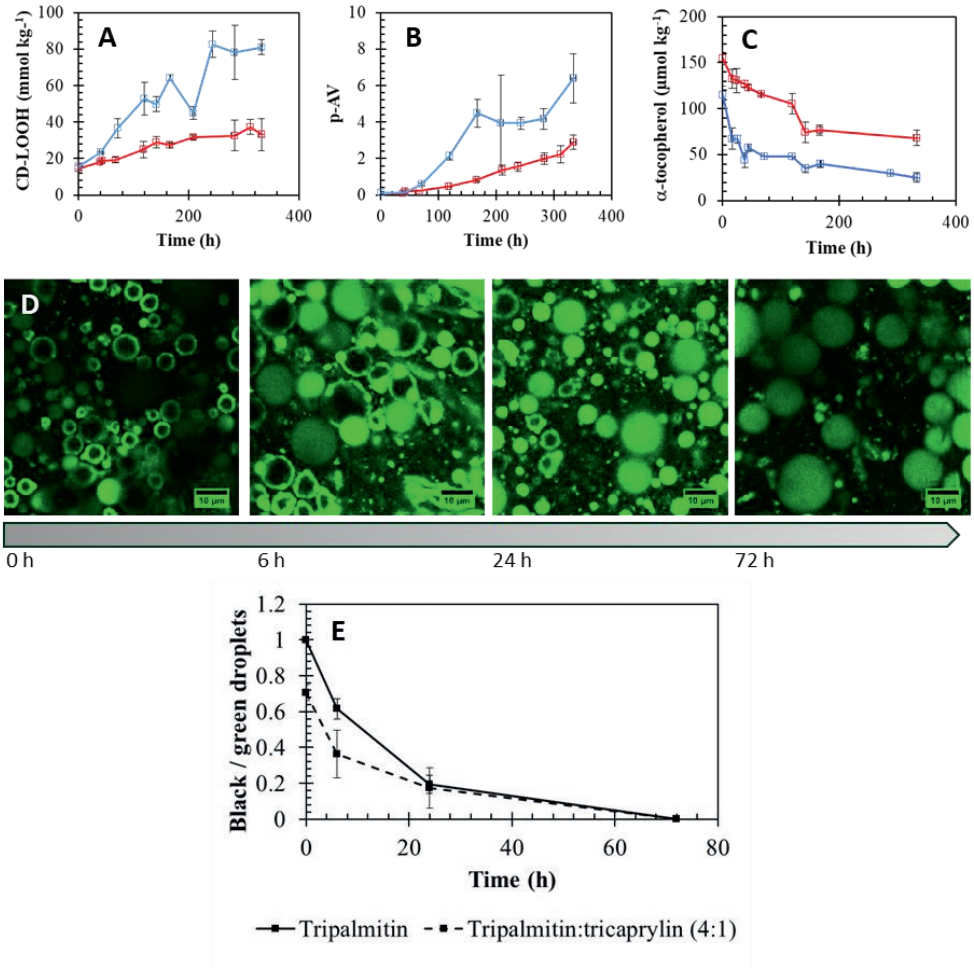

Figure 9.2. (A) CD hydroperoxide concentration (mmol kg-1 oil), (B) pAV and (C) α-tocopherol degradation (μmol kg-1 emulsion) during incubation of Pickering emulsions prepared with stripped sunflower oil and tripalmitin:tricapylin 4:1 (w/w) CLPs, with α-tocopherol incorporated in the particles or in the liquid PUFA oil droplets (red and blue lines, respectively); (D) CLSM images of Pickering emulsions with the fluorescent analogue initially in CLPs, taken at different time points, and (E) ratio of green to black droplets in tripalmitin and tripalmitin:tricaprylin 4:1 (w/w) CLP-stabilized emulsions containing the fluorescent analogue at different time points.

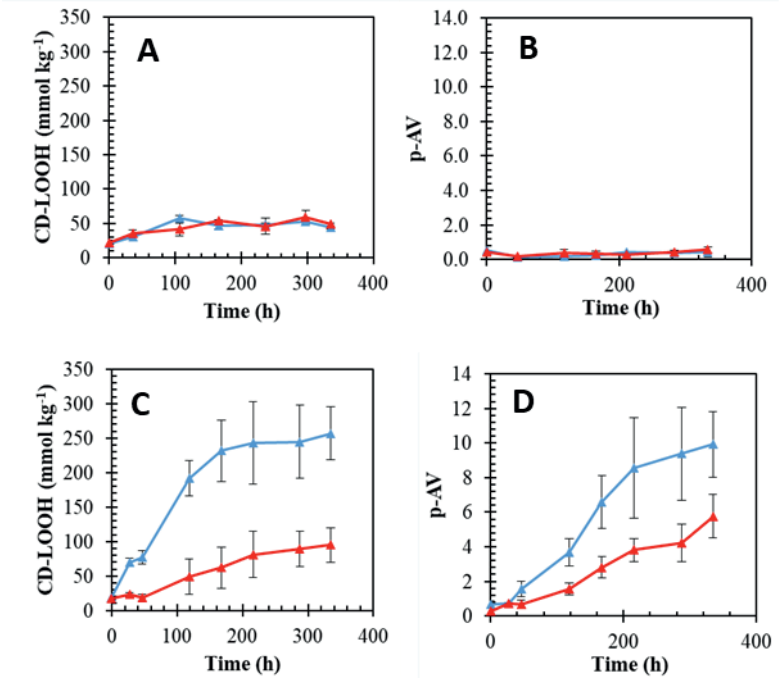

Figure 9.3. (A, C) CD hydroperoxide concentration and (B, D) pAV during incubation of Pickering O/W emulsions prepared with stripped sunflower oil, containing (A, B) 0.27 mmol kg^{-1} or (C, D) 0.07 mmol kg^{-1} carnosic acid, incorporated either in the CLPs (concept emulsion) or in oil droplets (control emulsion; red and blue lines, respectively).

9.4. References

Al-Shehri, H., Horozov, T. S., & Paunov, V. N. (2014). Adsorption of carboxylic modified latex particles at liquid interfaces studied by the gel trapping technique. Soft Matter, 10, 6433–6441.

Anjali, T. G., & Basavaraj, M. G. (2018). Influence of pH and salt concentration on Pickering emulsions stabilized by colloidal peanuts [Research-article]. Langmuir, 34(44), 13312–13321.

Arriaga, L. R., Drenckhan, W., Salonen, A., Rodrigues, J. A., Íñiguez-Palomares, R., Rio, E., & Langevin, D. (2012). On the long-term stability of foams stabilised by mixtures of nano-particles and oppositely charged short chain surfactants. Soft Matter, 8(43), 11085–11097.

Aussillous, P., & Quéré, D. (2001). Liquid marbles. Nature, 411(June), 924–928.

Berton-Carabin, C. C., & Schroën, K. (2015). Pickering emulsions for food applications: background, trends, and challenges. Annual Review of Food Science and Technology, 6, 263–297.

Bijsterbosch, B. H., Bos, M. T. A., Dickinson, E., Van Opheusden, J. H. J., & Walstra, P. (1995). Brownian dynamics simulation of particle gel formation: From argon to yoghurt. Faraday Discussions, 101, 51–64.

Binks. (2002). Particle as surfactants - Similarities and differences. Current Opinion in Colloid & Interface Science, 7, 21–41.

Binks, B. P., & Rodrigues, J. A. (2007). Enhanced stabilization of emulsions due to surfactant-induced nanoparticle flocculation. Langmuir, 23(14), 7436–7439.

Binks, B. P., Rodrigues, J. A., & Frith, W. J. (2007). Synergistic interaction in emulsions stabilized by a mixture of silica nanoparticles and cationic surfactant. Langmuir, 23(7), 3626–3636.

Bleeker, Cassee, Geertsma, Jong, D., Heugens, Koers-Jacquemijns, … Environment, D. N. I. for P. H. and the. (2012). Interpretation and implications of the European Commission Recommendation on the definition of nanomaterial. RIVM Letter Report 601358001/2012.

Borel, T., & Sabliov, C. M. (2014). Nanodelivery of bioactive components for food applications: Types of delivery systems, properties, and their effect on ADME profiles and toxicity of nanoparticles. Annual Review of Food Science and Technology, 5(1), 197–213.

Chevalier, Y., & Bolzinger, M. A. (2013). Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 439, 23–34.

Dassanayake, L. S. K., Kodali, D. R., & Ueno, S. (2011). Formation of oleogels based on edible lipid materials. Current Opinion in Colloid and Interface Science, 16(5), 432–439.

Destribats, M., Rouvet, M., Gehin-Delval, C., Schmitt, C., & Binks, B. P. (2014). Emulsions stabilised by whey protein microgel particles: towards food-grade Pickering emulsions. Soft Matter, 10(36), 6941–6954.

Dickinson, E. (1993). Towards more natural emulsifiers. Trends in Food Science and Technology, 4(10), 330–334.

Firoozmand, H., & Rousseau, D. (2016). Microbial cells as colloidal particles : Pickering oil-in-water emulsions stabilized by bacteria and yeast. FRIN, 81, 66–73.

Frelichowska, J., Bolzinger, M.-A., Pelletier, J., Valour, J.-P., & Chevalier, Y. (2009). Topical delivery of lipophilic drugs from o/w Pickering emulsions. International Journal of Pharmaceutics, 371(1–2), 56–63.

Galet, L., Patry, S., & Dodds, J. (2010). Determination of the wettability of powders by the Washburn capillary rise method with bed preparation by a centrifugal packing technique. Journal of Colloid and Interface Science, 346(2), 470–475.

Huang, S. W., Frankel, E. N., Schwarz, K., Aeschbach, R., & German, J. B. (1996). Antioxidant activity of carnosic acid and methyl carnosate in bulk oils and oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 44(10), 2951–2956.

Hwang, H. S., Fhaner, M., Winkler-Moser, J. K., & Liu, S. X. (2018). Oxidation of fish oil oleogels formed by natural waxes in comparison with bulk oil. European Journal of Lipid Science and Technology, 120(5).

Labuza, T. P., & Dugan, L. R. (1971). Kinetics of lipid oxidation in foods. C R C Critical Reviews in Food Technology, Vol. 2, pp. 355–405.

McHale, G., & Newton, M. I. (2011). Liquid marbles: Principles and applications. Soft Matter, 7(12), 5473–5481.

McPhail, D. B., Hartley, R. C., Gardner, P. T., & Duthie, G. G. (2003). Kinetic and stoichiometric assessment of the antioxidant activity of flavonoids by electron spin resonance spectroscopy. Journal of Agricultural and Food Chemistry, 51(6), 1684–1690.

Nan, F., Wu, J., Qi, F., Liu, Y., Ngai, T., & Ma, G. (2014). Uniform chitosan-coated alginate particles as emulsifiers for preparation of stable Pickering emulsions with stimulus dependence. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 456(1).

Paques, J. P., Van der Linden, E., Van Rijn, C. J. M., & Sagis, L. M. C. (2013). Alginate submicron beads prepared through w/o emulsification and gelation with CaCl2 nanoparticles. Food Hydrocolloids, 31(2), 428–434.

Paunov, V. N. (2003). Novel Method for Determining the Three-Phase Contact Angle of Colloid Particles Adsorbed at Air - Water and Oil - Water Interfaces. Langmuir, 19(13), 7970–7976.

Pawar, A. B., Caggioni, M., Ergun, R., Hartel, R. W., & Spicer, P. T. (2011). Arrested coalescence in Pickering emulsions. Soft Matter, 7(17), 7710.

Schmitt, C., Bovay, C., Vuilliomenet, A. M., Rouvet, M., & Bovetto, L. (2011). Influence of protein and mineral composition on the formation of whey protein heat-induced microgels. Food Hydrocolloids, 25(4), 558–567.

Schröder, A., Sprakel, J., Boerkamp, W., Schroën, K., & Claire C., B.-C. (2019). Can we prevent lipid oxidation in emulsions by using fat-based Pickering particles? Food Research International, 120, 352–363.

Tang, C.-H. (2017). Emulsifying properties of soy proteins: A critical review with emphasis on the role of conformational flexibility. Critical Reviews in Food Science and Nutrition, 57(12), 2636–2679.

Timgren, A., Rayner, M., Dejmek, P., & Marku, D. (2013). Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride. Food Science & Nutrition 2013; 1(2): 157– 171.

Vashisth, C., Whitby, C. P., Fornasiero, D., & Ralston, J. (2010). Interfacial displacement of nanoparticles by surfactant molecules in emulsions. Journal of Colloid And Interface Science, 349(2), 537–543.

Whitby, C. P., Fischer, F. E., Fornasiero, D., & Ralston, J. (2011). Shear-induced coalescence of oil-in-water Pickering emulsions. Journal of Colloid and Interface Science, 361(1), 170–177.

Whitby, C. P., & Krebsz, M. (2014). Coalescence in concentrated Pickering emulsions under shear. Soft Matter, 10(27), 4848–4854.

Yuan, C. L., Xu, Z. Z., Fan, M. X., Liu, H. Y., Xie, Y. H., & Zhu, T. (2014). Study on characteristics and harm of surfactants. Journal of Chemical and Pharmaceutical Research, 6(7), 2233–2237.

Zhang, W., Sun, X., Fan, X., Li, M., & He, G. (2018). Pickering emulsions stabilized by hydrophobically modified alginate nanoparticles: Preparation and pH-responsive performance in vitro. Journal of Dispersion Science and Technology, 39(3), 367–374.

Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., & Liu, F. (2010). Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. Food Chemistry, 118(3), 656–662.

Zulim Botega, D. C., Marangoni, A. G., Smith, A. K., & Goff, H. D. (2013). The potential application of rice bran wax oleogel to replace solid fat and enhance unsaturated fat content in ice cream. Journal of Food Science, 78(9).

Summary

English and Dutch summary

Many food products contain lipid droplets dispersed in an aqueous phase (e.g., milk, mayonnaise), thus are oil-in-water (O/W) emulsions. Food emulsions may be subjected to destabilization, both from a physical and a chemical perspective. Physical destabilization is generally prevented by the use of conventional emulsifiers such as surfactants and proteins. Chemical destabilization, in particular lipid oxidation, is a major concern in food products, especially when healthy polyunsaturated fatty acids are present, and this degradation is usually mitigated by the use of synthetic antioxidants, often in large amounts.

The use of alternative ingredients for the formulation of food emulsions has been emerging, for example solid particles (so-called Pickering particles, that are very popular nowadays) that irreversibly adsorb to the interface and therewith provide high physical stability; or natural antioxidants such as tocopherols and rosemary extracts, which are attractive in the current clean-label trend to prevent lipid oxidation. The efficiency of these natural antioxidants is unfortunately often not optimal, which can be explained by their tendency to locate into the oil or water phase, whereas lipid oxidation is initiated at the oil-water interface, and thus is the place where antioxidants should be located to optimally exert their protective effect.

The objective of this project was to develop food emulsions with a new and controlled architecture directed at yielding both excellent physical and oxidative stability. In these emulsions the oil droplets were covered by food-grade Pickering particles that exert a double role: they act as physical stabilizers, and as a reservoir for antioxidant molecules located close to the oil-water interface, therewith preventing the first lipid oxidation events, which is expected to drastically enhance antioxidant activity.

The first part of this thesis focused on the preparation and characterization of a new food-grade lipid-based Pickering particles, referred to as colloidal lipid particles (CLPs). We prepared both surfactant-covered and protein-covered CLPs, and found that the type of emulsifier largely determined their morphology: protein-covered CLPs were roughly spherical, whereas surfactant-covered CLPs looked more lath-like (**Chapters 3 and 6**). We also showed that the lipid material alters the crystal polymorphism and subsequent CLP structure, which consequently influenced their performance as emulsion stabilizers (**Chapter 3**). For instance, surfactant-covered CLPs containing only high melting point lipids showed highly ordered crystalline structures, and formed jammed, cohesive interfacial layers once adsorbed onto oil droplets, whereas the ones containing a fraction of low

melting point lipids showed less ordered crystalline structures and formed thin and bridged layers.

Since protein-covered CLPs were particularly resilient to subsequent emulsification processes, these particles were used to study the formation of emulsion droplets in a microfluidic device and their stability to short-term coalescence (**Chapter 4**). We found a non-monotonic dependency of the droplet stability on the particle concentration: at low surface coverage, CLPs had a destabilizing effect as incompletely covered surfaces led to droplet-droplet bridging and subsequent coalescence, whereas at higher surface coverage, particles formed an effective barrier against droplet coalescence, resulting in physically stable emulsions over the time scales probed.

As a next step, we investigated lipid oxidation in Pickering emulsions stabilized by protein-based CLPs that did not contain antioxidants (**Chapter 5**). We showed that these Pickering emulsions had a similar oxidative stability as conventional protein-stabilized emulsions for a similar composition of the oil droplets. Yet, when in both emulsions the same amount of solid lipids was present (either as stabilizing CLPs, or within the oil droplet core), a Pickering emulsion had a higher physicochemical stability. This shows that the location of crystallizable lipids influences lipid oxidation in O/W emulsions, and thus needs to be carefully considered in emulsion design.

CLPs that did contain the lipophilic antioxidant α-tocopherol are presented in **Chapter 6**. The chemical stability of α-tocopherol was negatively influenced by lipid crystallization that probably promoted the localization of α-tocopherol close to the particle surface, which was further enhanced by emulsifiers that actively induce lipid crystallization. When applied as Pickering stabilizers in O/W emulsions (**Chapter 7**), lipid oxidation was reduced compared to control emulsions with the same composition and structure, but where the antioxidant was present in the core of the oil droplets. This confirmed that the interfacial localization of the antioxidant is crucial to prevent lipid oxidation in emulsions, and that the two main instability issues (i.e., physical and chemical instability) of emulsions can be mitigated through one single approach.

After establishing the proof of concept with the CLPs, we used biobased particles (that may contain antioxidants) from various natural sources to stabilize O/W emulsions (**Chapter 8**). Emulsions stabilized by matcha tea powder or spinach leaf powder were both highly

physically and oxidatively stable, which shows that the double functionality that we achieved using purposely built particles (CLPs) can also be achieved with naturally occurring particles.

In the general discussion of the thesis (**Chapter 9**) we describe that the dual functionality of CLPs can also be reached using other food components, which makes this approach a generic one. We expect that the system could be further improved, for example, by increasing the residence time of antioxidants at the interface. To do so, we probably need to link the time scale at which the relevant oxidation events occur with those during which the antioxidant actually resides at the interface. Follow-up research on entrapment of antioxidants within particles is needed to reach long residence times at the interface while not compromising the ability of antioxidants to exert their chemical activity. To conclude: through our approach the highly-stable food emulsions of the future may come within reach.

Veel levensmiddelen bevatten oliedruppels gedispergeerd in een water fase (bijvoorbeeld melk en mayonaise), en zijn dus olie-in-water emulsies. Levensmiddelenemulsies kunnen onderhevig zijn aan destabilisatie, zowel fysisch en chemisch gezien. Fysische instabiliteit wordt over het algemeen voorkomen door gebruik te maken van emulgatoren, zoals oppervlakte-actieve stoffen of eiwitten. Chemische instabiliteit, in het bijzonder vetoxidatie, is een zorg voor levensmiddelen met gezonde meervoudig onverzadigde vetzuren. Dit wordt meestal tegengegaan door het gebruik van (grote hoeveelheden) synthetische antioxidanten.

Er is tegenwoordig een groeiende vraag naar levensmiddelenemulsies waarin alternatieve ingrediënten worden gebruikt. Vaste deeltjes (Pickering-deeltjes) zijn populair omdat ze in tegenstelling tot de conventionele emulgatoren zich irreversibel in een grensvlak kunnen nestelen, en daardoor een hoge fysische stabiliteit verschaffen. Verder zijn natuurlijke antioxidanten zoals tocoferolen en rozemarijn-extracten aantrekkelijk om vetoxidatie te voorkomen vanuit een clean-label perspectief. De efficiëntie van deze natuurlijke antioxidanten is helaas vaak niet optimaal, omdat ze de neiging hebben om zich in de bulk olie of water fase te positioneren, terwijl vetoxidatie geïnitieerd wordt op het olie-water-grensvlak wat dus de aangewezen plek zou moeten zijn om antioxidanten zo goed mogelijk hun werk te laten doen.

Het doel van dit project was om levensmiddelenemulsies met een nieuwe en gecontroleerde architectuur te ontwikkelen, gericht op het verschaffen van zowel de fysische en oxidatieve stabiliteit. In deze emulsies werden de oliedruppels bedekt met eetbare Pickering-deeltjes die een dubbele rol vervullen: ze dienen als fysische stabilisatoren en worden gebruikt als antioxidant reservoirs. Antioxidanten worden daardoor dicht bij het olie-water-grensvlak geplaatst, waardoor de eerste vetoxidatiereacties kunnen worden voorkomen wat naar verwachting hun effectiviteit drastisch zal verbeteren.

Het eerste deel van dit proefschrift richtte zich op het produceren en karakteriseren van een nieuw soort eetbare Pickering-deeltjes (**Hoofdstuk 3 en 6**) die we colloïdale vetdeeltjes (CVDs) noemen. We hebben zowel CVDs bedekt met oppervlakte-actieve stoffen als met eiwitten gemaakt en vonden dat de stof op het grensvlak grotendeels de morfologie van de CVDs bepaalt: deeltjes bedekt met oppervlakte-actieve stoffen waren langgerekt, en deeltjes bedekt met eiwit waren bolvormig. We hebben ook aangetoond dat het soort vet

de interne kristalliniteit van CVDs kan veranderen en hierdoor hun gedrag als emulgatoren (**Hoofdstuk 3**). Zo vormden CVDs die bedekt zijn met oppervlakte-actieve stoffen en bestaan uit vetten met een hoog smeltpunt geordende kristallijne structuren en dikke lagen in het grensvlak. CVDs die voor een deel vet bevatten met een laag smeltpunt vormden een minder geordende kristallijne structuur en dunnere lagen.

Omdat het makkelijker was om emulsies te maken met eiwitbedekte CVDs, zijn deze deeltjes gebruikt om de vorming van emulsiedruppels te bestuderen in een microfluidisch kanaal, en hun korte termijn coalescentiestabiliteit te onderzoeken (**Hoofdstuk 4**). We vonden een niet-monotone verband tussen druppelstabiliteit en oppervlaktebedekking: bij lage bedekking hadden CVDs een destabiliserend effect door brugvorming wat leidde tot coalescentie, terwijl bij hoge bezetting de deeltjes een effectieve barrière vormden die coalescentie kon voorkomen, resulterend in een fysisch stabiele emulsie gedurende de tijd van het experiment.

Als volgende stap onderzochten we vetoxidatie in emulsies gestabiliseerd met eiwitbedekte CVDs die geen antioxidanten bevatten (**Hoofdstuk 5**). We hebben aangetoond dat deze Pickering-emulsies een vergelijkbare oxidatieve stabiliteit vertoonden als eiwitgestabiliseerde emulsies bij gelijke samenstelling van de oliedruppels. Echter, wanneer in beide emulsies dezelfde hoeveelheid vast vet aanwezig was (hetzij als stabiliserende CVDs of in de kern van de oliedruppel) dan had de Pickering-emulsie een hogere fysische en chemische stabiliteit. Dit toont aan dat de positie van kristalliseerbaar vet een groot effect heeft op de chemische emulsiestabiliteit. Dit effect moet zorgvuldig worden overwogen in het ontwerp van nieuwe emulsies.

CVDs die de lipofiele antioxidant α-tocoferol bevatten worden gepresenteerd in **Hoofdstuk 6**. De chemische stabiliteit van α-tocoferol werd negatief beïnvloed door vetkristallisatie, wat leidde tot ophoping van α-tocoferol dicht bij het oppervlak van het deeltje en wat verder werd bevorderd door emulgatoren die vetkristallisatie kunnen induceren. Wanneer deze α-tocoferol bevattende CVDs werden toegepast als Pickering-stabilisatoren in olie-in-water emulsies (**Hoofdstuk 7**), werd de vetoxidatie verlaagd ten opzichte van emulsies met dezelfde samenstelling en structuur, maar waarin α-tocoferol in de kern van de oliedruppels aanwezig was. Dit bevestigt dat de positionering van α-tocoferol in het grensvlak cruciaal is om vetoxidatie in emulsies te voorkomen, en dat de twee voornaamste instabiliteiten in

emulsies (fysisch en chemisch) onder controle kunnen worden gehouden middels één aanpak.

Na het leveren van dit bewijs met CVDs, gebruikten we biobased deeltjes (die antioxidanten kunnen bevatten) uit verschillend natuurlijke bronnen om olie-in-water emulsies te stabiliseren (**Hoofdstuk 8**). Emulsies die gestabiliseerd werden door matcha-thee of spinaziebladdeeltjes waren zowel fysisch als oxidatief zeer stabiel. Dit toon aan dat de dubbele functionaliteit die we hadden bereikt met de door ons ontworpen CVDs ook kan worden bereikt met deeltjes die voorkomen in de natuur.

In de discussie van dit proefschrift (**Hoofdstuk 9**) bespreken we dat de dubbele functionaliteit van CVDs ook kan worden bereikt wanneer andere ingrediënten worden gebruikt, wat onze aanpak tot een generieke maakt. We verwachten dan ook dat dit systeem verder kan worden verbeterd door, bijvoorbeeld, het verhogen van de verblijftijd van de antioxidanten aan het grensvlak. Om dit mogelijk te maken moeten we waarschijnlijk de tijdschalen waarop relevante oxidatiereacties plaatsvinden koppelen aan de verblijftijd van de antioxidant in het grensvlak. Vervolgonderzoek aan encapsulatie van antioxidanten in deeltjes is nodig om langere verblijftijden aan het grensvlak te bereiken zonder een compromis te moeten sluiten betreffende de chemische activiteit van de antioxidant. Om af te sluiten: middels onze aanpak kunnen de zeer stabiele levensmiddelenemulsies van de toekomst binnen bereik komen.

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About the author

Curriculum Vitae

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Albertine Johanneke (Anja) Schröder was born on the 13th of March 1992 in Breukelen, The Netherlands. She attended Broklede, in Breukelen, where she obtained her VWO diploma in 2010, with a major in Natuur and Gezondheid (Nature and Health) and Natuur en Techniek (Nature and Technique).

In the same year, Anja started her study Food Technology at Wageningen University & Research with a minor Foods of Animal Origin, and a thesis at the laboratory of Physics and Physical Chemistry of Foods on the structural and rheological properties of gelatin gels.

After obtaining her bachelor degree in 2013, Anja continued with the master Food Technology with a specialization in Ingredient Functionality at Wageningen University & Research. For her master thesis she worked on the interfacial rheology and behavior of chemically modified food proteins and lipids at the laboratory of Food Process Engineering and the laboratory of Physics and Physical Chemistry of Foods. During her internship at Danone Nutricia Research, she investigated the interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability. She graduated with distinction for both her bachelor and master degree.

Anja continued working as PhD candidate at the laboratory of Food Process Engineering and the laboratory of Physical Chemistry and Soft Matter at Wageningen University & Research. During this research, she worked on Pickering emulsions stabilized by colloidal lipid particles for high physical and chemical stability, and the results of this research are described in this thesis.

List of publications

Berton-Carabin, C. C., **Schröder, A.**, Rovalino Cordova, A., Schroën, K., Sagis, L. M. C. Highlight Article: Protein and lipid oxidation affect the viscoelasticity of whey protein layers at the oil-water interface. **2016**, *European Journal of Lipid Science and Technology*, 118, 1630-1643.

Schröder, A., Sprakel, J., Schroën, K., Berton-Carabin, C. C. Tailored microstructure of colloidal lipid particles for Pickering emulsions with tunable properties. **2017**, *Soft Matter*, 13, 3190-3198.

Schröder, A., Berton-Carabin, C. C., Venema, P., Cornacchia, L. Interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability. **2017**, *Food Hydrocolloids*, 73, 129-140.

Schröder, A., Sprakel, J., Schroën, K., Spaen, J., Berton-Carabin, C. C. Coalescence stability of Pickering emulsions produced with lipid particles: A microfluidic study. **2018**, Journal of Food Engineering, 234, 63-72.

Schröder, A., Sprakel, J., Boerkamp, W., Schroën, K., Berton-Carabin, C. C. Can we prevent lipid oxidation in emulsions by using fat-based Pickering particles? **2019**, *Food Research International*, 120, 352-363.

Schröder, A., Sprakel, J., Schroën, K. and Berton-Carabin, C. C. Chemical stability of α-tocopherol in colloidal lipid particles with various morphologies. *Submitted.*

Schröder, A., Laguerre, M., Sprakel, J., Schroën, K. and Berton-Carabin, C. C. Pickering particles as interfacial reservoirs of antioxidants. *Submitted.*

Schröder, A., Corstens, M.N., Ho, K.K., Schroën, K., Berton-Carabin, C.C. **2019** Pickering Emulsions. *In Emulsion-based Systems for Delivery of Food Active Compounds* (eds S. Roohinejad, R. Greiner, I. Oey and J. Wen).

A.J. Schröder, M. Laguerre, J.H.B. Sprakel, S. Birtic, C.G.P.H. Schroen, C. Berton-Carabin. Emulsion comprising antioxidant particles. PCT/EP2019/067780.

Overview of completed training activities

Discipline specific activities	
Courses	
Microscopic Image Analysis: From Theory to Practice (Molecular Medicine Postgraduate School, Rotterdam, NL)	2016
Industrial wetting (TU Darmstadt, Darmstadt, DE)	2016
Multivariate analysis for food data/sciences (VLAG, Wageningen, NL)	2016
Transmission Electron Microscopy training (WUR, Wageningen, NL)	2016
Microscopy and Spectroscopy in Food and Plant Sciences (VLAG, Wageningen, NL)	2017
Han-Sur-Lesse winterschool (Wageningen / TU Delft, Han-Sur-Lesse, BE)	2017
Conferences	
16th Food Colloids Conference (Wageningen, NL)	2016
SOMATAI conference (Fodele, GR)[a]	2016
IUFoST – World Congress of Food Science and Technology (Dublin, IE)[a]	2016
21st Dutch Soft matter meeting (Wageningen, NL)[b]	2016
2017 AOCS annual meeting (Orlando, US)[b]	2017
Commercialisation of Pickering Emulsions (London, UK)[a]	2017
17th Food Colloids Conference (Leeds, UK)[a]	2018
16th Euro Fed lipids Congress (Belfast, IE)[b]	2018
Symposium: Physical and chemical stability of Pickering emulsions – 'Where food chemistry and colloid science meet' (Wageningen, NL)[b]	2018
Edible Soft Matter (Le Mans, FR)[b]	2019
General courses	
Competence assignment (WGS)	2015
PhD week (VLAG)	2016
Project and time management (WGS)	2016
Data management planning (WGS)	2016
PhD workshop carousel	2016
Teaching and supervising Thesis students (WGS)	2017
Scientific Writing (WGS)	2017
Scientific Publishing (WGS)	2018

Scientific artwork – Vector graphics and images (WGS)	2018
Start to teach (ESD)	2018
Lecturing (ESD)	2018
Career perspectives	2019
Optional courses and activities	
FPE weekly meetings & Group days[a,b]	2015-2019
PhD study tour Germany and Switserland[a,b]	2016
PhD study tour Canada[a,b]	2018

[a] Poster presentation; [b] Oral presentation.

VLAG: Advanced Studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences; WGS: Wageningen Graduate Schools; ESD: Education Staff Development.

NL: the Netherlands; BE: Belgium; DE: Germany; FR: France; GR: Greece; IE: Ireland; US: United States; UK: United Kingdom.

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Propositions

1. Making stable Pickering emulsions with biobased particles is a *contradictio in terminis*. (this thesis)

2. The location of antioxidants in emulsions is crucial for their efficiency. (this thesis)

3. Seeing may be believing, but the fact that your eyes and brain can be deceived easily is greatly overlooked in science.

4. A PhD project is like a good wine; it gets better with age, and passion determines its quality.

5. Laughter is an unrightfully ignored success factor.

6. Wisdom in cooking is the key to preventing overeating and food waste.

Propositions belonging to the thesis entitled

'Combined physical and oxidative stability of food Pickering emulsions'

Albertine Johanneke Schröder
Wageningen, 10 Feb 2020