Recent advances on food-grade particles stabilized Pickering emulsions: fabrication, characterization and research trends

Jie Xiao [a], Yunqi Li [b], Qingrong Huang [a, *]

[a] Department of Food Science, Rutgers, the State University of New Jersey, 65 Dudley Road, New Brunswick, New Jersey 08901, USA

[b] Key Laboratory of Low Carbon Chemical Power of Jilin Province, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China

*To whom correspondence should be addressed. Tel (848)-932-5514. Fax (732)-932-6776. Email qhuang@aesop.rutgers.edu

Abstract

Background

Colloidal particles assembled from food grade materials with proper fabrication and/or modification can function as Pickering emulsion stabilizers.

Scope and Approach

This paper summarized recent research practices in developing food-grade particles stabilized Pickering emulsions. Recent advances in methods for their fabrication and characterization were reviewed. Research progresses in clarifying their stabilization mechanisms based on interfacial microstructure observation as well as promising research trends in basic research and fields of applications were highlighted.

Key Findings and Conclusions

Food-grade materials can be used to engineer colloidal particles through five commonly used methods. Chemical modification, physical deposition and complex formation with surfactants were emerging strategies for improving their interfacial attachment efficiency. Current approaches and results in the study of food-grade particles stabilized Pickering emulsions, including contact angle and microstructure characterization, as well as stabilization mechanisms and rheological properties were summarized. Promising research trends in food-grade particles stabilized Pickering emulsions include: (1) to develop tunable interfacial structure, (2) to clarify their digestion profile under oral conditions, and (3) to expand their applications in fields like target delivery and double emulsions with enhanced stability.

Keywords: Pickering emulsion; edible particles; fabrication; characterization; stability

1. Challenges and added-values associated with edible particles stabilized Pickering emulsions

Stabilization of emulsion droplets can be realized by either small molecular weight surfactants through interfacial tension reduction, or amphiphilic macromolecules (e.g. proteins and polysaccharides) via the formation of steric elastic film in addition to the reduction of interfacial tension. Although well understood and widely utilized, they are not the only possible sources for emulsion stabilization. Dispersed colloidal particles were discovered to function as emulsion stabilizers in a fundamentally different way, and this concept was formally recognized since the publication of Pickering (Pickering, 1907), thus gaining the name of "Pickering emulsion". Although Pickering emulsions have been proposed for over one hundred years, the in-depth understanding towards stability mechanisms as well as explorations in applicable fields were raised mainly in the last two decades.

General principles for solid particles to function as Pickering emulsion stabilizers can be summarized as: i) particles should be partially wetted by both continuous and dispersed phase, yet should not be soluble in either phase; ii) particles should preserve proper partial wettability to gain sufficient interface absorption efficiency; iii) particle size should be substantially smaller than the targeted emulsion droplet size (at least one order of magnitude). Just as amphiphilic property (defined as Hydrophilic-Lipophilic Balance, HLB value) plays an essential role in conventional emulsifiers, the wettability of solid particle is the key property governing the formation and stabilization of Pickering emulsions. And three-phase contact angle θ (angle formed at the three-phase boundary where solid particles, continuous phase and dispersed phase intersect), expressed by classical Young's equation $\cos\theta = (\gamma_{so} - \gamma_{sw})/\gamma_{ow}$ (γ_{ij} refers to interfacial tension among solid phase, oil phase or water phase, respectively) (Chevalier &

Bolzinger, 2013; Johansson, Bergenstahl, & Lundgren, 1995), can be used to semi-quantify this property. This is the most important parameter dictating the location of an individual particle with respect to the oil-water interface and thus the emulsion type: oil-in-water or water-in-oil. For instance, if particles are preferentially wetted by water phase over oil phase, colloidal particles tend to bend over towards the oil phase thus stabilize oil-in-water (O/W) type emulsions. Based on the energy and maximum capillary pressure, Kaptay (Kaptay, 2006) claimed that for an emulsion stabilized by a single layer of particles, the contact angle for O/W emulsions must be $15^{o} < \theta < 90^{o}$ and for W/O emulsions, the contact angle must be $90^{o} < \theta < 165^{o}$; for emulsions stabilized by a double layer of particles, O/W emulsions are stable for contact angle values of $15^{o} < \theta < 129.3^{o}$ and W/O emulsions are stable for contact angle values of $50.7^{o} < \theta < 165^{o}$. For a single spherical particle of radius R resting at an oil-water interface, the depth of immersion height into water (h), the contact area (s) between particle and water (if water is the continuous phase) and the energy for detachment (ΔE) can be derived from the following empirical equations (1-3) (Rayner, et al., 2014):

$$h = R(1 + \cos\theta) \quad (1)$$

$$s = 2\pi R\hat{}2\ (1 + cos\theta) \quad (2)$$

$$\Delta E = \gamma_{ow}\pi R^2(1 - |cos\theta|)^2 \quad (3)$$

For colloidal particles, if θ falls in the range of 30^{o} to 150^{o}, desorption energy will be several orders of magnitude larger than the thermal energy of Brownian motion, which manifests their irreversible adsorption processes. This is essentially different with the dynamic equilibrium process between interface and bulk phase in the case of conventional emulsifiers stabilized emulsions.

Historically, inorganic particles received extensive research attention in Pickering emulsions and their extended application fields (Binks, 2002; Ji, Fuji, Watanabe, & Shirai, 2012). Vast majority of current understandings towards Pickering emulsions were extracted from model Pickering systems stabilized by silica nanoparticles with or without surface modification. However, their applications in food and pharmaceutical industries are largely limited due to the biodegradability and biocompatibility concerns. The ongoing challenges as well as emerging research trends are to utilize natural biopolymer particles that are both effective as Pickering stabilizers and acceptable for use in food and pharmaceutics on the commercial scale (Dickinson, 2012b; Rayner, et al., 2014).

In contrast to the well-established theories and remarkable research progresses on inorganic and synthetic polymers based Pickering emulsions, there are much less research practices on edible colloidal particles stabilized emulsions (Figure 2). Relatively small amount in case studies is due to few food ingredients can simultaneously satisfy the above-mentioned three rules. To be specific, for any food-grade colloidal particles to function as Pickering emulsifiers, the basic prerequisite is to remain insoluble and intact in both phases over the lifetime of emulsion system. This requirement is not as easy to fulfill for food-grade materials since most of proteins are initially water-soluble while polysaccharides-based materials may undergo swelling in aqueous solution. Specific procedures are thus needed to fabricate colloidal particles out of these food grade materials. Even for those intrinsically insoluble food grade materials, such as cellulose and chitin, additional energy input is sometimes necessary to regulate their particle sizes and size distributions, and surface polarity within suitable range. Furthermore, to perform as effective Pickering emulsifiers for practical commercial applications, food-grade particles should bear a proper partial wettability. For instance, excessive affinity to water phase, which

results in insufficient interfacial packing, prevents most of the unmodified polysaccharides based materials from functioning as effective Pickering emulsifier candidates.

Despite the challenges or inconveniences associated with introducing food grade materials as a source for Pickering stabilizers, the concept of food-grade particles-stabilized emulsions is increasingly attractive in both theoretical and practical point of view. The added-values of edible particles stabilized emulsions as compared to inorganic particles or conventional emulsifiers stabilized ones are expected in many ways: 1) Raw materials such as starch and cellulose are readily available and inexpensive, edible particles can be produced in a large-scale way as the mass production of inorganic particles without environment pollution; 2) The rigid elastic interface is expected to lift the resistance towards coalescence of emulsion droplet and chemical degradation of encapsulates; 3) The organic, soft feature of edible particles is readily open to physical or chemical modifications, which makes it possible to bring additional functionality at emulsion interface. Among which, the stimuli (temperature, pH, ionic strength, etc.) triggered responsive interface properties have much to offer in fulfilling development of versatile Pickering emulsion systems (Tang, Quinlan, & Tam, 2015); 4) When utilized as encapsulation vehicles, they exhibited the potential for control release and bring novel texture and sensory properties to emulsion based products; 5) When functioned as templates for preparing advanced materials, such as colloidosome, they have immediate applications in producing macrocapsule for nutrient delivery vehicles or gel power to mimic the semisolid texture of trans or saturated fats (Berton-Carabin & Schroen, 2015); 6) With regard to their acceptability in human consumption, they show low risk of toxicity due to their digestible nature. Besides, the consumers' perception of pursuing "clean label" surfactant-free products will be fulfilled. These attractive features have clearly triggered a new wave of research boom as manifested by the

exponential increase in the number of publications during the last ten years (Figure 2). Representative research practices in developing food grade particles based Pickering emulsions are summarized in Table 1.

2. Methods to fabricate colloidal particles out of food grade materials

2.1. Mechanical or chemical breakdown methods

To stabilize emulsion droplets of micron sizes, the particle size should be at least one order of magnitude smaller. Thus, particle or fiber with size larger than submicron should be reduced in size; this is especially relevant for native starches and cellulose, whose native dimensions are at micron level. Top-down strategies including mechanical cut off methods, such as ball milling, cryogenic milling, wet milling and high-pressure homogenizer provide stringent solutions. For mechanical breakdown methods, high shear, turbulence, and cavitation are the responsible mechanisms for reducing size, crystallinity or relative heterogeneity of a system. A typical example for mechanically reducing particle size for further usage of stabilizing emulsions can be found in the work done by Yusoff et al. (Yusoff & Murray, 2011). They utilized freezer milling to treat the modified starch before forming emulsions, and the sizes of particulates and their aggregates were reduced considerably from tens of microns to 0.5-15 μm.

For materials that are insoluble in water and most organic solvents due to the existence of crystalline structures, particularly cellulose and chitin, dimension of particles can be reduced dramatically via acid hydrolysis method. Strong inorganic acids such as hydrochloric acid or sulfuric acid dissolve the more susceptible amorphous regions of cellulose leaving the crystal structure intact. As an example, Tzoumaki et al. (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011) prepared chitin crystals with nano-scale dimension by acid hydrolysis (3 N HCl,

95 ℃, 90 min) of crude chitin from crab shells. Similar strategy was applied in fabrication of cellulose nanocrystals (Kalashnikova, Bizot, Cathala, & Capron, 2012; Liu, et al., 2015).

2.2. Heating or solvent-induced protein aggregates or microgels

Proteins have long been recognized as excellent natural building blocks to prepare nano/microparticles due to their versatility in tunable conformations. The bottom-up assembly of protein molecules into aggregates or microgels can be realized through heat treatment or manipulation of solvent conditions. Thermal treatment causes the formation of protein particulates through disulfide bonds and hydrophobic interactions (Ren, Tang, Zhang, & Guo, 2009). Changes in pH or salt concentration alter electrostatic interaction among charged amino acids thus promote conformational changes at tertiary and quaternary structural levels (Tang, Zhang, Wen, & Huang, 2010). Recent practices in combining protein aggregates with Pickering emulsion stabilization have involved whey protein (Wu, et al., 2015), soy protein (Liu & Tang, 2013) and pea protein (Liang & Tang, 2014). Through similar fabrication methods, sometimes combined with covalent cross-linking methods (Farjami, Madadlou, & Labbafi, 2015), some protein species can form into a special class of soft colloidal entities consisting of highly swollen cross-linked protein molecules, which were referred to as protein microgels. Special properties such as deformation and structural rearrangement after absorption and stimuli responsive properties due to its swelling property were revealed through this type of Pickering emulsifier (Dickinson, 2016; M. Rayner, 2015). Among which, the heating induced whey protein microgels and Pickering emulsion formed thereafter were most frequently cited (Donato, Schmitt, Bovetto, & Rouvet, 2009; Nicolai, 2016; Schmitt, et al., 2009; Destribats, et al., 2014).

2.3. Anti-solvent precipitation method

In practice, producing particles based on anti-solvent precipitation method suits

169 especially for those water-insoluble food materials. The synthesis process basically consisted of
170 three steps: first, materials are fully dissolved in their good solvents; and then, the organic phase
171 is dispersed in an aqueous phase under various mixing conditions (e.g. vortex stirring, high-
172 speed or high-pressure homogenization and ultrasonication) with or without the presence of
173 stabilizer. Finally, the organic phase in the resultant dispersion is removed. During this process a
174 material lost its solubility due to the attrition of solvent into bulk water phase, and consequently
175 solidified to form nana/micro particles. Major factors that had critical effects on nanoparticle size
176 and shape include the nature of material, concentration of polymer in stock solution, the volume
177 ratio of dispersed organic solvent to the bulk water phase, the sequence of adding solution (stock
178 solution drop into bulk water or bulk water drop into stock solution), and the dispersion method
179 selected (Joye & McClements, 2013). Based on this method, our lab has fabricated nanoparticles
180 out of kafirin protein (a prolamin protein from sorghum grain) (Xiao, et al., 2015; Xiao, Nian, &
181 Huang, 2015), and utilized them to stabilize oil in water type of Pickering emulsion (Xiao,
182 Wang, Gonzalez, & Huang, 2016). Similar practices included zein nanoparticles (Folter,
183 Ruijven, & Velikov, 2012; Zou, Guo, Yin, Wang, & Yang, 2015) and phytosterols particles
184 stabilized Pickering emulsion (Liu & Tang, 2014). While for those materials with acceptable
185 initial water solubility, anti-solvent precipitation method can be used in another sequence: for
186 instance, starch-based nanoparticles were prepared by dropwise addition of water-soluble starch
187 into polymer solution (Tan, et al., 2009; Tan, et al., 2014).

188 *2.4. Formation of protein-polysaccharides composite particles*

189 Another appealing conceptual approach for producing Pickering emulsifier candidates
190 involves the exploitation of protein-polysaccharide complexes based nanoparticles. One of the
191 possible routines is driven by electrostatic interactions between protein and polysaccharides. For

192 instance, the crosslinking between positively changed NH_3+ groups of chitosan and negatively
193 charged phosphorylated groups of caseinophosphopeptides resulted in the assembly of nano-
194 complex (Hu, Ting, Zeng, & Huang, 2012; Hu, Wang, Li, Zeng, & Huang, 2011). The other
195 particle formation mechanism involves the heat denatured proteins aggregate with each other to
196 form the protein nanoparticle core, which is then coated with polysaccharides through
197 electrostatic interaction by adjusting pH (Jones & McClements, 2010). The size of the
198 biopolymer nanoparticles formed can be manipulated by controlling the holding temperature,
199 holding time, composition concentration, pH, and ionic strength by affecting the nucleation and
200 growth of biopolymer particles. And they show striking pH responsiveness since the formation
201 relies on a proper pH range in which attractive electronic interactions dominate. Up to now,
202 successful Pickering emulsions stabilized by lactoferrin/polysaccharides particles (David-
203 Birman, Mackie, & Lesmes, 2013; Shimoni, Levi, Tal, & Lesmes, 2013) and zein/chitosan
204 complex particles have been investigated (Wang, et al., 2015).

205 *2.5. In situ interfacial fat crystallization*

206 Although oil in water type of emulsions take up large proportion of emulsion based
207 products in market, food products such as margarine and butter as well as some pharmaceutical,
208 cosmetic products function through water in oil type of emulsions. In the context of Pickering
209 emulsions, surface-active fat crystals are the most commonly referred Pickering type emulsifiers
210 at the oil-water interface. Fat crystals are usually formed via, with or without surfactant-
211 mediated, cooling induced interfacial fat crystallization (Rousseau, 2013). Surfactant mediated
212 fat crystallization results from the direct solidification of surfactants at the oil–water interface
213 during the post-homogenization cooling of W/O emulsions. During homogenization, surfactants
214 will arrange their hydrophobic tails and polar heads at the oil-water interface. Under a suitable

thermal gradient, surfactants (e.g. monomer glycerol monooleate (GMO) and monomer glycerol monostearate (GMS)) will undergo a liquid–solid phase transition, which then promote the heterogeneous nucleation for fat crystallization and thereby conferring a Pickering-like stabilization shell (Ghosh & Rousseau, 2011; Rousseau, 2000).

3. Methods for tuning interfacial attachment efficiency of edible particles

Compared to the difficulty of fabricating colloidal particles out of food grade materials, even challenging obstacle lies in endowing the formulated particles with sufficient interfacial attachment efficiency. Limited practical experiences have been developed up to now, since major research works focus merely on screening native food grade particles as potential Pickering stabilizers. Herein, we summarized two categories of regulation strategies emerging from current research efforts.

3.1. Chemical modification and physical treatments

Native starch granule without modification can not function as Pickering stabilizer or, at the best, stabilized emulsion with low efficiency (Li, Li, Sun, & Yang, 2013), largely due to their highly hydrophilic nature. The most commonly utilized chemical modification practice for tuning native starch granules into more effective Pickering emulsion stabilizer is to introduce hydrophobicity through esterification with octenyl succinic anhydride (OSA), acetic anhydride or phthalic anhydride (Song, Pei, Zhu, Fu, & Ren, 2014; Tan, et al., 2012). By changing the highly hydrophilic hydroxyl groups of starch to more hydrophobic ester groups, hydrophobicity of starch can be increased. Here we should be cautious at the degree of chemical reagents substitution, since starch modified with the OSA substitution higher than the order of 0.03 will be excluded from food related application (FDA, 1981). Apart from chemical modification, physical treatments such as dry heating (M. Rayner, Sjoo, Timgren, & Dejmek, 2012; Timgren,

238 Rayner, Dejmek, Marku, & Sjöö, 2013) or physically absorption of relatively
239 hydrophobic/hydrophilic components are also effective in tuning particle wettability. As an
240 example, tencel fibers were hydrophobically modified by deposition with ethyl cellulose and
241 then function as oil in water Pickering emulsion stabilizer (Murray, Durga, Yusoff, & Stoyanov,
242 2011).

243 *3.2. Forming complex system with surfactants*

244 A two-stage synergistic mechanism was proposed to explain the enhanced long-term
245 stability of the combination system of monoolein and silica nanoparticles (Pichot, Spyropoulos,
246 & Norton, 2009): By rapidly lowering the interfacial tension, the small-molecule emulsifier is
247 able to delay coalescence and facilitate droplet break-up during emulsification. The short-term
248 interfacial stabilization provided by small-molecule emulsifier allows time for the nanoparticles
249 to accumulate in a coherent layer at the oil-water interface, thereby providing the system with
250 long-term stability. Following similar mechanism, Gao et al. (Gao, et al., 2014) fabricated
251 complexes of water-insoluble zein colloidal particles and surfactant sodium stearate (SS) by
252 nonspecific hydrophobic interaction. Increase of SS concentration resulted in partial unfolding of
253 zein particles and the exposure of hydrophobic micro-domains. Not only did this effect improve
254 the diffusive mobility of zein particles but also endowed zein particles with equilibrium
255 interfacial wetting properties, which then facilitated efficient packing of zein colloidal particles
256 at the oil-water interface, producing Pickering emulsion with superior stability against both
257 coalescence and creaming. Two-way effects of Tween 80 and soybean lecithin (PL) on the
258 stability of α-cyclodextrin microcrystals stabilized Pickering emulsions were then reported by Li
259 et al. (Li, et al., 2014): Tween 80 enhanced emulsion stability by decreasing the interfacial
260 tension and increasing in viscosity. The replacement of a-cyclodextrin microcrystals by a-

cyclodextrin/PL particles at the O/W interface happened at low PL concentrations, leading to inhibitory effects, while huge reduction in interfacial tension under high concentration of PL resulted in emulsions with enhanced stability. Recently, the concentration dependent surfactants (didecyldimethylammonium bromide and cetyltrimethyl-ammonium bromide) - enhanced cellulose nanocrystal Pickering emulsions phenomena were also reported (Hu, Ballinger, Pelton, & Cranston, 2015).

4. Methods and findings in characterization of edible particles stabilized emulsions

4.1. Predicting partial wettability

Since partial wettability of colloid particles plays essential role in determining the type as well as the stability of Pickering emulsion, various techniques have been designed for the measurement of contact angle at interface. Direct measurement of contact angle while particle attached to the liquid interface by an optical microscope seems rather straightforward and simple for operation. However, this method only suit for particles with size above 30 μm, a situation not often happens in fabricated food grade particles. In 2012, de Folter et al. (de Folter, et al., 2012) applied the captive drop method in measuring the contact angle of zein colloidal particles. In their experiment, a homogeneous zein film was formed in the first place, and the film was then placed on top of the water subphase. Next, an oil droplet was attached to the film surface in the water phase with the aid of a bended needle tip. The static contact angle of oil droplet at the zein film was measured using a Data Physics OCA15 setup. Clearly, the contact angle between particle film- oil -water was not a perfect replica of three-phase contact of particle at the water-oil interface. Nevertheless, this method served well as a predicator of wetting property of zein particles, since the measured contact value (close to 90 ℃) demonstrated the potential of zein particles as Pickering emulsifier, and it is sensitive to medium condition (i.e., ionic strength and

pH) changes.

Recently a conceptually novel approach for detecting three-phase contact angle of solid particles adsorbed at oil-water interface was pioneered based on a gel trapping technique (GTT) (Cayre & Paunov, 2004). According to this method, particles are spread and adsorbed at the oil–water interface followed by gelling of the aqueous sub-phase with a non-adsorbing hydrocolloid (e.g. gellan gum). The particles are trapped at the gel surface which allows the top phase (air or oil) to be replaced with curable poly(dimethylsiloxane) (PDMS). Once polymerized, the PDMS is peeled off from the aqueous gel together with the trapped particles that are subsequently imaged on the polymer surface by scanning electron microscopy (SEM) or atomic force microscopy (AFM) (Arnaudov, Cayre, Stuart, Stoyanov, & Paunov, 2010). The particle position on the PDMS surface is complementary to the one at the original liquid interface, which allows its contact angle at the liquid interface to be determined. This method gives much higher resolution than optical microscopy, which makes it applicable even for particles of submicrometer size. The contact angles of fat crystal particles and spray-dried soy protein/calcium phosphate particles have been characterized with the GTT method (Paunov, et al., 2007), results suggested that the wettability data obtained correlated well with their preferred type of emulsions they stabilized.

It needs to emphasize that the three phase contact angle concept as well as measurement methods are based on perfect spherical particles, while in reality the particles fabricated from edible origin materials are usually in non-spherical shape with heterogeneous local surface property. Thus, the role of contact angle plays in guiding realistic application of food grade particles is far less than its theoretical value. That is why, in practices, researchers seek after the

interfacial microstructure observation and emulsion stability index as more direct criteria for evaluation.

4.2. Microstructure observation

4.2.1. Optical microscopy

Optical microscopy or light microscope uses visible light and a series of lenses to obtain magnified images. The maximum normal magnification is 1000 × (with magnification of eyepiece and objective being 10 × and 100 ×, respectively). It is thus impossible for optical microscopy to visualize the packing and organization structure of edible particles with size smaller than 1 micron, and out-of-focus blurring effect makes it impossible to record the morphology of each single emulsion droplet. However, it serves quite well in visualizing interface structure of emulsion droplets stabilized by large particles and calculating the size distribution of emulsion droplets.

4.2.2.Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) employs a laser beam to acquire in-focus images with submicron resolution from selected depths. The key feature is that the fluorescence emitted from sample is passed through an adjustable pinhole set before the detectors, thereby eliminating the out-of-focus blurring (Dinsmore, Weeks, Prasad, Levitt, & Weitz, 2001). This setup allows researchers to obtain fine two-dimensional structure images, three-dimensional images reconstruction and quantitative measurements of depth of particle coating layer. To identify the position and morphology of particles at interface, the particles should be fluorescent by itself or have to be stained with fluorescent dye, in some cases, oil phase can also be labeled with fluorescent dye to ease phase identification. In field of food grade particles stabilized emulsion droplet, CLSM collected direct evidences of adsorption of micro fibrillated cellulose

particles, starch granules around droplets interface (Shao & Tang, 2014; Tang & Liu, 2013), representative interfacial structures were presented in Figure 3 (Winuprasith & Suphantharika, 2013; Yusoff & Murray, 2011).

4.2.3. Cryogenic scanning electron microscopy (Cryo-SEM)

Both optical microscopy and CLSM fail to investigate the morphology of individual particles smaller than 500 nm. Cryogenic scanning electron microscopy can visualize the packing structure at interfaces of particles with average size below 100 nm, which enables in situ characterization of nanoscale particles at liquid-liquid interface (Isa, 2013). Typical operation steps involves: 1) to load emulsion sample onto a suitable sample mount; 2) to freeze sample rapidly by quenching it in liquid nitrogen slush, during this procedure the structure and morphology of emulsion droplets are fixed; 3) to transfer sample under vacuum to the preparation chamber where the sample is then freeze fractured by a cold knife to expose the internal microstructure. When fracturing the emulsion samples, the fracture usually follows the mechanically weakest regions of the frozen emulsions (Limage, Schmitt, Vincent-Bonnieu, Dominici, & Antoni, 2010), that is why direct observation of external surface of a drop viewed from the continuous phase is possible under proper fracture; 4) to control sublimation of the sample is carried out under designed temperature and time to remove a controlled amount of water or oil (i.e., decane, cyclohexane in the inverse water-in-oil emulsions), leaving voids between drops, after which the sample is coated with a thin gold layer; and 5) to transfer the sample to SEM chamber and observe at low pressure and temperature. One of the most exciting case studies utilizing Cryo-SEM in edible particles stabilized Pickering emulsion was conducted by Destribats et al. (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014). Direct visualization of whey protein microgel residing at interfaces by cryo-SEM gave insights to

correlate the interrelationship among formation parameter, interfacial structure and emulsion stability. The high resolution of SEM system enables the discovery of different packing structures of particles at interface and the continuous protein thin films between protein aggregates (Figure. 4).

4.3. Stability of food grade particles based Pickering emulsions

Emulsions stabilized by colloidal particles are generally considered as metastable. Their tendency and capacity in suppressing phenomenon that leading to emulsion instability differs fundamentally with those stabilized by surfactants or macromolecules.

4.3.1. Stability against creaming

Currently, several research works reported that Pickering emulsions undergo "limited coalescence" during emulsification process (Arditty, Whitby, Binks, Schmitt, & Calderon, 2003): firstly, a large excess of oil-water interface compared with the amount that can be covered by the presence of solid particles were produced during agitation; secondly, when agitation process stopped, the partially protected droplets coalescence, thus reducing the total amount of oil-water interface until the interface is sufficiently covered. Creaming or sedimentation process then take place during the post emulsification process. Stokes law $\left(U = \frac{2r^2(\rho_1-\rho_2)g}{9\eta_1}\right)$ suggested that the rate of creaming was correlated to the droplet size, density difference between continuous and dispersed phase, and viscosity of continuous phase. Without thickening agent, Pickering emulsions easily undergo creaming/precipitation since the dimension of Pickering emulsion droplets easily falls in the range of several to hundreds of microns. This is a common feature for food grade particles stabilized Pickering emulsions if the result of visual observation or creaming index were reported.

Recording the creaming index (CI) and emulsified phase volume fraction are of routine strategies in screening possible candidates for Pickering emulsion stabilization out of food grade particles. The common protocol is to storage the fresh prepared emulsions in a transparent cylindrical glass vial, and then measure the height of emulsified phase (H_e), height of serum phase (H_s), height of oil layer (H_o) if any and the total height of formulation (H_t) along specific incubation periods of storage. The emulsified phase volume fraction was reported as (H_e/H_t) × 100, and creaming index was calculated as (H_s/H_t) × 100. Internal oil phase in emulsified phase (in case of oil-in-water emulsion) was calculated as the emulsified phase volume fraction divided by oil phase fraction. This value, if extracted from the current reported case studies, can easily approach or exceed 0.74, indicating the formation of stable high internal phase emulsions (HIPE) (Xiao, et al., 2016), a phenomena cannot be sustained without high dosage of surfactants traditionally.

4.3.2. Stability against coalescence

Usually, closely approaching emulsion droplets in the creaming layer are under higher risk of further coalescence. Unlike the vulnerability to droplets merging in cases of surfactants stabilized emulsions, particles stabilized emulsions suppress the risk and demonstrate long-term stability via various evidenced mechanisms. For emulsions with densely packed particles surfaces, due to the irreversible absorption process, neighboring particle layers come into physical contact yet remain separate on the adjacent oil–water interfaces (Figure 5 a). This would effectively suppress the risk of coalescence and demonstrate long-term stability. For emulsion systems with low surface coverage, which is the more general circumstance, particle spread at oil-water interface and separated by particle free domains due to the diffusion limited cluster aggregation (Tarimala & Dai, 2004). When droplets come into contact, adsorbed particles in

adjacent layer relocated at the contact area and forming a shared particle layer ("bridging flocculation") (Ashby, Binks, & Paunov, 2004), which effectively remain the integrity of emulsion droplets (Figure5 b). Particle aggregates or fibrous particles adsorbed at the interface with several endpoints, with only a small proportion of these aggregates associated with droplet surfaces, while the rest parts protruding in between neighboring oil–water interface and forming a steric barrier to prevent further droplet contact (Figure 5c, d). In situ interfacial fat crystallization provide their functionality either by forming a rigid steric barrier at the oil-water interface, or by forming a network in the continuous phase, or by a combination of the two (Ghosh & Rousseau, 2011; Nadin, Rousseau, & Ghosh, 2014). Besides, for particles with residual surface charge, long-range repulsive forces prevent droplet flocculation induced by Van de Waals and capillary attractive forces to some extent (Levine & Bowen, 1993).

Based on interfacial structure observation, direct stabilization evidences of food grade particles stabilized emulsions were presented in Figure 6. To be specific, optical microscopy evidenced the stability of oil droplets stemming from the densely packed starch granules interfacial layer (Li, et al., 2013) (Figure 6 a, a'). A higher local protein particle concentration at the contact zone between neighboring emulsion droplets were captured under cryo-SEM (Destribats, et al., 2014) (Figure. 6 b, b'). Figure 6 c, c' manifested the effectiveness of cellulose fiber network in stabilizing emulsion droplets (Winuprasith & Suphantharika, 2013). In situ nucleation of fat crystals stabilized water droplets by the physical barrier effect of integral fat crystalline shell (Figure 6d) (Rousseau, 2013). Additional stabilization arises when crystallization extended into continuous phase and formed a larger scale of three-dimensional network (Figure 6d').

4.4 Rheological properties of food grade particles based Pickering emulsion

Due to its susceptibility to gravity induced creaming during storage, the concentrated creaming layer of Pickering emulsion usually behave as weak gel like emulsion with viscoelastic rheological behavior. Hallmarks of typical Pickering emulsions' rheological properties is $G' > G''$ in frequency sweep tests demonstrating the gel-like elastic emulsion. Underlying principle has been accumulated based on available experiences and insights: Due to the tendency of creaming and flocculation, three-dimensional network of closely packed emulsion droplets formed after emulsification (Dickinson, 2012a). The presence of particle at emulsion interface with high droplet volume fraction then yields a rigid interface that is ultimately characterized by surface elasticity. The elastic storage modulus G' is contributed both by the compression of emulsion droplets, which permits the storage of interfacial energy by deforming the droplet interfaces (Mason, Bibette, & Weitz, 1995) and interfacial elasticity resulting from the strong adhesion between solid particles adsorbed at the oil–water interface (Arditty, Schmitt, Giermanska-Kahn, & Leal-Calderon, 2004). The elasticity of compressed emulsions is thus closely related to the emulsified phase volume, the strength of the inter-particle attraction and packing geometry of droplets.

For the available case studies based on food grade particles stabilized emulsions, if rheological measurements were conducted, their rheological properties followed the above-mentioned trend. Available published results suggested that starch based Pickering emulsion exhibited typical elastic behavior with a definite yield stress and G' increase as oil phase ratio in formulation increased (Marku, Wahlgren, Rayner, Sjoo, & Timgren, 2012; Song, et al., 2015). Authors attributed this phenomenon to the formation of percolating network of colloidal particles that works as scaffolds between the oil droplets. In both kafirin nanoparticles (Xiao, et al., 2016) and phytosterol particles (Liu & Tang, 2014) stabilized Pickering emulsion systems,

443 formulations were reported to exhibit predominantly elastic gel-like behavior with shear-thinning
444 behavior. When utilized amorphous cellulose as Pickering emulsifier, as the concentration of
445 particles increased, gravitationally unstable liquid-like emulsions transformed into stable gel-like
446 emulsions with typical shear-thinning rheological characteristics, authors attributes this
447 phenomenon to a combination of Pickering and network mechanisms (Jia, et al., 2015).

448 **5. Research trends**

449 Inspired by the striking achievements in inorganic particles based Pickering emulsions
450 and emerging research attempts in edible particles stabilized emulsions, we proposed several
451 promising research prospects in both basic research areas as well as fields of application.

452 *5.1. Filling gaps in basic research areas*

453 *5.1.1. To develop tunable interfacial structure*

454 "Tunable interfacial structure" is the interface structure that tunable in terms of
455 composition of coated particles, thickness of coating layer, surface coverage and environmental
456 responsive properties. To develop tunable interfacial structure is actually to enrich the flexibility
457 in emulsion droplet size, thickness of interface, permeability and environmental responsive
458 properties. Ultimately, edible Pickering emulsions with improved stability against environmental
459 stresses, or controllable release properties may be obtained. Intriguing yet simple strategies to
460 accomplish this goal may include: Stabilize emulsion droplet with mixture of food grade
461 particles of different sources and/or hydrophobicity, which would hopefully lead to the discovery
462 of species with synergistic effect, thus expanding stabilization candidates and improving the
463 emulsification capacity. Deposit particles with opposite charges onto droplet interface by a
464 premix method may lead to enhanced surface coverage (Nallamilli, Binks, Mani, & Basavaraj,
465 2015; Mao & McClements, 2011), and applied in a layer-by-layer manner would theoretically

result in multi-layered droplet interface. Meanwhile, Pickering emulsions with environmental responsive features may be fabricated by incorporating stimuli sensitive coating materials (e.g., temperature sensitive fat crystals, pH sensitive protein-polysaccharides complex), prior to emulsification or post decoration at interface. Furthermore, duel delivery and functional interface can be achieved via utilizing edible particles as interfacial cargo of functional agents. A recent research practice performed in this direction was to develop curcumine - rich particle layer to retard the lipid peroxidation rate in Pickering emulsion (Wang, et al., 2015). To assist the above-mentioned experimental exploiting process, computer modeling and simulation, which take into consideration of both the peculiar characteristics of organic material as well as their dynamic absorption-desorption behavior, would be a worth trying strategy.

5.1.2. To clarify digestion profile under oral conditions

Emulsion behavior under oral conditions is strongly linked to emulsion sensorial perception and their effectiveness in functioning as drug delivery vehicle. Thus, before introducing Pickering emulsions as novel food formats or encapsulation/delivery vehicles, their integrity as well as digestion profiles under oral administration should be clearly addressed in the first place. However, current research evidences only limited to the digestion of lactoferrin nanoparticles stabilized emulsion through artificial saliva and simulated gastric conditions (David-Birman, et al., 2013; Shimoni, et al., 2013). And the digestion of chitin nanocrystal stabilized emulsion in simulated intestinal fluid (Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013). More research practices need to be conducted to enrich our knowledge in this field.

5.2. To Exploit fields of applications

5.2.1. Protective storage and delivery vehicles for active components

The robust interfacial particle layer of Pickering emulsions promises their advantages in serving as novel encapsulation or delivery vehicles for active compounds. For instance, one potential approach to enhance the lipid oxidation stability is to store oil in food-grade particles stabilized emulsion droplets. By forming a thick interface around the oil droplets, chances of interaction between transition metals (sited in aqueous phase) and lipid hydroperoxide (in oil phase) would be largely reduced. This concept was proved recently by the enhanced lipid oxidative stability in Pickering emulsion stabilized by microcrystalline cellulose or modified starch-stabilized emulsions (Kargar, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012), kafirin nanaoparticles (Xiao, Li, & Huang, 2016) and antioxidant encapsulated zein/chitosan particles (Wang, et al., 2015). As for delivery, O/W and W/O type of Pickering emulsions have the potential of providing improved stability as well as controlled release in gastrointestinal tract for hydrophilic or lipophilic compounds by encapsulation within the respective inner phases. However, this concept was only demonstrated based on silica particles stabilized emulsion (Tikekar, Pan, & Nitin, 2013), research effort in this direction based on food grade particles stabilized emulsions is scarce (Xiao, Li, et al., 2016). Another intriguing drug delivery format is Pickering emulsion based topical drug delivery. Skin absorption from Pickering emulsions was first tried out in silica particles stabilized emulsion (Frelichowska, et al., 2009). Diana Marku (Marku, et al., 2012) then evaluated starch based Pickering emulsions as a vehicle for topical drug delivery. The presence of starch stabilize emulsion system resulted in two times higher penetration flux of methyl salicylate over skin than that of buffer solution. Although theoretically promising, much more research efforts in above mentioned directions are necessary to manifest their advantages.

5.2.2. Promising formulation routine for long-term stable double emulsions

Double emulsions, having either a water-in-oil-in-water or an oil-in-water-in-oil structure, provide additional protection and controlled release property to the inner phase, which leads to a number of potential applications in fields of food, pharmacy and cosmetics. For instance, W/O/W type emulsions have promising characteristics in producing reduced fat-food products (by replacing the volume of oil phase with entrapped water drops) and vehicles for encapsulation and delivery of hydrophilic nutrients. Classical double emulsions stabilize inner and outer interface with two sets of monomeric and/or polymeric emulsifiers, which easily undergo molecular diffusion controlled transport across middle layer (Wronski, Vladimirov, & Adach, 2012). Absence of long-term stability thus limits their application in practice, leaving their fancy properties remain tempting on a theoretic basis.

Forming strong and rigid layer at the inner and/or the outer emulsion interface by colloidal particles is one of the most promising solutions for producing stable double emulsions. Oza and Frank (Oza & Frank, 1989) first tried out the idea of using colloidal microcrystalline cellulose (CMCC) to stabilize the inner or external interface of a w/o/w emulsion. And their results proved that addition of CMCC helped to slow down the release of electrolytes to outer phase. Garti et al. (Garti, Aserin, Tiunova, & Binyamin, 1999) applied the α-form solid microcrystalline fat particles as the stabilizer for inner W/O interface of a W/O/W double emulsion. The encapsulated marker (NaCl) did not release with time, confirming that the fat particles interface totally sealed the water from releasing its addenda. Most recently, Matos et al. utilized quinoa starch granule to stabilize external interface of W/O/W double Pickering emulsions (Matos, Timgren, Sjoo, Dejmek, & Rayner, 2013). And Marefati et al. employed OSA modified quinoa starch granules stabilized W/O/W double Pickering emulsions followed by freeze-drying to produce “food-grade oil filled powders” (Marefati, Sjoo, Timgren, Dejmek, &

Rayner, 2015). Up to now, the attempts of utilizing two sets of inorganic colloidal particles to stabilize stable double emulsion have been demonstrated recently (Williams, Armes, Verstraete, & Smets, 2014). However, the practice of using exclusively two sets of food grade particles to form stable double emulsion is not yet available, proper combinations of hydrophilic food grade particles and fat crystals are worth trying strategies.

6. Conclusions

Colloidal particles origin from food grade materials with proper fabrication or modification can function as Pickering emulsion stabilizers. The superiority of such Pickering emulsions is manifested in several aspects: irreversible interface absorption, outstanding stability against coalescence and peculiar rheology property. Further, they are expected to be advantageous over inorganic nanoparticles based ones when biocompatibility, degradability, cost issues and amenability for surface modification are considered. Novel applications in food texture modification, encapsulation and delivery of active ingredients, and the possibility of fabricating emulsion with flexible interfacial properties are promising perspectives. In view of these, food grade particles based Pickering emulsion is not only fascinating from an academic viewpoint but would have great impact on emulsion fabrication industries. However the advantages offered by this category of emulsions are not yet fully exploited or commercially available, largely due to insufficient theoretical basis and shortage in experimental attempts. Future research attentions may direct towards both directions and we expect that their applicable portion in food industry may soon increase.

Acknowledgments. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2014-67021-21630 from the USDA National Institute of Food and

556 Agriculture. The financial support from the China Scholarship Council to the first author is also
557 gratefully acknowledged.

References

Arditty, S., Schmitt, V., Giermanska-Kahn, J., & Leal-Calderon, F. (2004). Materials based on solid-stabilized emulsions. *Journal of Colloid and Interface Science, 275*, 659-664.

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*, 273-281.

Arnaudov, L. N., Cayre, O. J., Stuart, M. A. C., Stoyanov, S. D., & Paunov, V. N. (2010). Measuring the three-phase contact angle of nanoparticles at fluid interfaces. *Physical Chemistry Chemical Physics, 12*, 328-331.

Ashby, N. P., Binks, B. P., & Paunov, V. N. (2004). Bridging interaction between a water drop stabilised by solid particles and a planar oil/water interface. *Chemical Communications*, 436-437.

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

Binks, B. P. (2002). Macroporous silica from solid-stabilized emulsion templates. *Advanced Materials, 14*, 1824-1827.

Capron, I., & Cathala, B. (2013). Surfactant-free high internal phase emulsions stabilized by cellulose nanocrystals. *Biomacromolecules, 14*, 291-296.

Cayre, O. J., & Paunov, V. N. (2004). Contact angles of colloid silica and gold particles at air-water and oil-water interfaces determined with the gel trapping technique. *Langmuir, 20*, 9594-9599.

581 Chevalier, Y., & Bolzinger, M. A. (2013). Emulsions stabilized with solid nanoparticles:
582 Pickering emulsions. *Colloids and Surfaces a-Physicochemical and Engineering*
583 *Aspects, 439*, 23-34.
584 David-Birman, T., Mackie, A., & Lesmes, U. (2013). Impact of dietary fibers on the
585 properties and proteolytic digestibility of lactoferrin nano-particles. *Food*
586 *Hydrocolloids, 31*, 33-41.
587 Dickinson, E. (2012a). Emulsion gels: The structuring of soft solids with protein-
588 stabilized oil droplets. *Food Hydrocolloids, 28*, 224-241.
589 Dickinson, E. (2012b). Use of nanoparticles and microparticles in the formation and
590 stabilization of food emulsions. *Trends in Food Science & Technology, 24*, 4-12.
591 Dickinson, E. (2016). Exploring the frontiers of colloidal behaviour where polymers and
592 particles meet. *Food Hydrocolloids*, 52, 497-509.
593 Dinsmore, A. D., Weeks, E. R., Prasad, V., Levitt, A. C., & Weitz, D. A. (2001). Three-
594 dimensional confocal microscopy of colloids. *Applied Optics, 40*, 4152-4159.
595 de Folter, J. W. J., van Ruijven, M. W. M., & Velikov, K. P. (2012). Oil-in-water
596 Pickering emulsions stabilized by colloidal particles from the water-insoluble
597 protein zein. *Soft Matter, 8*, 6807-6815.
598 Destribats, M., Rouvet, M., Gehin-Delval, C., Schmitt, C., & Binks, B. P. (2014).
599 Emulsions stabilised by whey protein microgel particles: towards food-grade
600 Pickering emulsions. *Soft Matter, 10*, 6941-6954.
601 Donato, L., Schmitt, C., Bovetto, L., & Rouvet, M. (2009). Mechanism of formation of
602 stable heat-induced beta-lactoglobulin microgels. *International Dairy Journal*, 19,
603 295-306.

604 Farjami, T., Madadlou, A., & Labbafi, M. (2015). Characteristics of the bulk hydrogels
605 made of the citric acid cross-linked whey protein microgels. *Food Hydrocolloids,*
606 50, 159-165.
607 FDA. (1981). Code of federal regulations, title 21, part 172. In F. a. D. Administration
608 (Ed.). Washington, DC: US Government Printing Office.
609 Frasch-Melnik, S., Norton, I. T., & Spyropoulos, F. (2010). Fat-crystal stabilised w/o
610 emulsions for controlled salt release. *Journal of Food Engineering, 98*, 437-442.
611 Frelichowska, J., Bolzinger, M. A., Valour, J. P., Mouaziz, H., Pelletier, J., & Chevalier,
612 Y. (2009). Pickering w/o emulsions: Drug release and topical delivery.
613 *International Journal of Pharmaceutics, 368*, 7-15.
614 Gao, Z. M., Yang, X. Q., Wu, N. N., Wang, L. J., Wang, J. M., Guo, J., & Yin, S. W.
615 (2014). Protein-based Pickering emulsion and oil gel prepared by complexes of
616 zein colloidal particles and stearate. *Journal of Agricultural and Food Chemistry,*
617 *62*, 2672-2678.
618 Garti, N., Aserin, A., Tiunova, I., & Binyamin, H. (1999). Double emulsions of water-in-
619 oil-in-water stabilized by alpha-form fat microcrystals. Part 1: Selection of
620 emulsifiers and fat microcrystalline particles. *Journal of the American Oil*
621 *Chemists Society, 76*, 383-389.
622 Ghosh, S., & Rousseau, D. (2011). Fat crystals and water-in-oil emulsion stability.
623 *Current Opinion in Colloid & Interface Science, 16*, 421-431.
624 Haaj, S. B., Thielemans, W., Magnin, A., & Boufi, S. (2014). Starch nanocrystal
625 stabilized Pickering emulsion polymerization for nanocomposites with improved
626 performance. *Acs Applied Materials & Interfaces, 6*, 8263-8273.

627 Hu, B., Ting, Y. W., Zeng, X. X., & Huang, Q. R. (2012). Cellular uptake and
628 cytotoxicity of chitosan-caseinophosphopeptides nanocomplexes loaded with
629 epigallocatechin gallate. *Carbohydrate Polymers, 89*, 362-370.
630 Hu, B., Wang, S. S., Li, J., Zeng, X. X., & Huang, Q. R. (2011). Assembly of bioactive
631 peptides and chitosan nanocomplexes. *Journal of Physical Chemistry B, 115*,
632 7515-7523.
633 Hu, Z., Ballinger, S., Pelton, R., & Cranston, E. D. (2015). Surfactant-enhanced cellulose
634 nanocrystal Pickering emulsions. *Journal of Colloid and Interface Science, 439*,
635 139-148.
636 Isa, L. (2013). Freeze-fracture shadow-casting (FreSCa) Cryo-SEM as a tool to
637 investigate the wetting of micro- and nanoparticies at liquid-liquid interfaces.
638 *Chimia, 67*, 231-235.
639 Ji, J., Fuji, M., Watanabe, H., & Shirai, T. (2012). Partially functionalized Janus ZnO
640 spheres prepared by protecting mask techniques. *Colloids and Surfaces a-*
641 *Physicochemical and Engineering Aspects, 393*, 6-10.
642 Jia, X., Xu, R., Shen, W., Xie, M., Abid, M., Jabbar, S., Wang, P., Zeng, X., & TaoWu.
643 (2015). Stabilizing oil-in-water emulsion with amorphous cellulose. *Food*
644 *Hydrocolloids, 43* 275-282.
645 Johansson, D., Bergenstahl, B., & Lundgren, E. (1995). Wetting of Fat Crystals by
646 Triglyceride Oil and Water .1. The Effect of Additives. *Journal of the American*
647 *Oil Chemists Society, 72*, 921-931.

Jones, O. G., & McClements, D. J. (2010). Biopolymer nanoparticles from heat-treated electrostatic protein-polysaccharide complexes: Factors affecting particle characteristics. *Journal of Food Science, 75*, N36-N43.

Joye, I. J., & McClements, D. J. (2013). Production of nanoparticles by anti-solvent precipitation for use in food systems. *Trends in Food Science & Technology, 34*, 109-123.

Kalashnikova, I., Bizot, H., Cathala, B., & Capron, I. (2012). Modulation of cellulose nanocrystals amphiphilic properties to stabilize oil/water interface. *Biomacromolecules, 13*, 267-275.

Kaptay, G. (2006). On the equation of the maximum capillary pressure induced by solid particles to stabilize emulsions and foams and on the emulsion stability diagrams. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 282*, 387-401.

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*, 209-215.

Levine, S., & Bowen, B. D. (1993). Capillary interaction of spherical-particles adsorbed on the surface of an oil-water droplet stabilized by the particles .3. Effective interfacial-tension. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 70*, 33-45.

669 Li, C., Li, Y. X., Sun, P. D., & Yang, C. (2013). Pickering emulsions stabilized by native
670 starch granules. *Colloids and Surfaces a-Physicochemical and Engineering*
671 *Aspects, 431*, 142-149.
672 Li, X., Li, H. Y., Xiao, Q., Wang, L. Y., Wang, M. L., Lu, X. L., York, P., Shi, S. L., &
673 Zhang, J. W. (2014). Two-way effects of surfactants on Pickering emulsions
674 stabilized by the self-assembled microcrystals of alpha-cyclodextrin and oil.
675 *Physical Chemistry Chemical Physics, 16*, 14059-14069.
676 Liang, H. N., & Tang, C. H. (2014). Pea protein exhibits a novel Pickering stabilization
677 for oil-in-water emulsions at pH 3.0. *Lwt-Food Science and Technology, 58*, 463-
678 469.
679 Limage, S., Schmitt, M., Vincent-Bonnieu, S., Dominici, C., & Antoni, M. (2010).
680 Characterization of solid-stabilized water/oil emulsions by scanning electron
681 microscopy. *Colloids and Surfaces a-Physicochemical and Engineering Aspects,*
682 *365*, 154-161.
683 Liu, F., & Tang, C.-H. (2013). Soy protein nanoparticle aggregates as pickering
684 stabilizers for oil-in-water emulsions. *Journal of Agricultural and Food*
685 *Chemistry, 61*, 8888−8898.
686 Liu, F., & Tang, C. H. (2014). Phytosterol colloidal particles as Pickering stabilizers for
687 emulsions. *Journal of Agricultural and Food Chemistry, 62*, 5133-5141.
688 Liu, H. X., Geng, S. M., Hu, P. P., Qin, Q. Y., Wei, C., & Lv, J. (2015). Study of
689 Pickering emulsion stabilized by sulfonated cellulose nanowhiskers extracted
690 from sisal fiber. *Colloid and Polymer Science, 293*, 963-974.

691 Marku, D., Wahlgren, M., Rayner, M., Sjoo, M., & Timgren, A. (2012). Characterization
692 of starch Pickering emulsions for potential applications in topical formulations.
693 *International Journal of Pharmaceutics, 428*, 1-7.
694 Mao, Y. Y., & McClements, D. J. (2011). Modulation of bulk physicochemical properties
695 of emulsions by hetero-aggregation of oppositely charged protein-coated lipid
696 droplets. *Food Hydrocolloids,* 25, 1201-1209.
697 Marefati, A., Sjoo, M., Timgren, A., Dejmek, P., & Rayner, M. (2015). Fabrication of
698 encapsulated oil powders from starch granule stabilized W/O/W Pickering
699 emulsions by freeze-drying. *Food Hydrocolloids*, 51, 261-271.
700 Mason, T. G., Bibette, J., & Weitz, D. A. (1995). Elasticity of compressed emulsions.
701 *Physical Review Letters, 75*, 2051-2054.
702 Mathapa, B. G., & Paunov, V. N. (2013). Cyclodextrin stabilised emulsions and
703 cyclodextrinosomes. *Physical Chemistry Chemical Physics, 15*, 17903-17914.
704 Matos, M., Timgren, A., Sjoo, M., Dejmek, P., & Rayner, M. (2013). Preparation and
705 encapsulation properties of double Pickering emulsions stabilized by quinoa
706 starch granules. *Colloids and Surfaces a-Physicochemical and Engineering*
707 *Aspects*, 423, 147-153.
708 Murray, B. S., Durga, K., Yusoff, A., & Stoyanov, S. D. (2011). Stabilization of foams
709 and emulsions by mixtures of surface active food-grade particles and proteins.
710 *Food Hydrocolloids, 25*, 627-638.
711 Nadin, M., Rousseau, D., & Ghosh, S. (2014). Fat crystal-stabilized water-in-oil
712 emulsions as controlled release systems. *Lwt-Food Science and Technology, 56*,
713 248-255.

714 Nallamilli, T., Binks, B. P., Mani, E., & Basavaraj, M. G. (2015). Stabilization of
715 Pickering emulsions with oppositely charged latex particles: Influence of various
716 parameters and particle arrangement around droplets. *Langmuir*, 31, 11200-
717 11208.
718 Nicolai, T. (2016). Formation and functionality of self-assembled whey protein
719 microgels. *Colloids and Surfaces B-Biointerfaces*, 137, 32-38.
720 Oza, K. P., & Frank, S. G. (1989). Drug release from emulsions stabilized by colloidal
721 microcrystalline cellulose. *Journal of Dispersion Science and Technology, 10*,
722 187-210.
723 Paunov, V. N., Cayre, O. J., Noble, P. F., Stoyanov, S. D., Velikov, K. P., & Golding, M.
724 (2007). Emulsions stabilised by food colloid particles: Role of particle adsorption
725 and wettability at the liquid interface. *Journal of Colloid and Interface Science,*
726 *312*, 381-389.
727 Perrin, E., Bizot, H., Cathala, B., & Capron, I. (2014) Chitin nanocrystals for Pickering
728 high internal phase emulsions. *Biomacromolecules*. *15*, 3766–3771.
729 Pichot, R., Spyropoulos, F., & Norton, I. T. (2009). Mixed-emulsifier stabilised
730 emulsions: Investigation of the effect of monoolein and hydrophilic silica particle
731 mixtures on the stability against coalescence. *Journal of Colloid and Interface*
732 *Science, 329*, 284-291.
733 Pickering, S. U. (1907). Emulsions. *Journal of the Chemical Society, 91*, 2001-2021.
734 Rayner, M. (2015). Current status on novel ways for stabilizing food dispersions by
735 oleosins, particles and microgels. *Current Opinion in Food Science*, 3, 94-109.

736 Rayner, M., Marku, D., Eriksson, M., Sjöö, M., Dejmek, P., & Wahlgren, M. (2014).
737 Biomass-based particles for the formulation of Pickering typeemulsions in food
738 and topical applications. *Colloids and Surfaces A: Physicochem. Eng. Aspects,*
739 *458*, 48-62.
740 Rayner, M., Sjoo, M., Timgren, A., & Dejmek, P. (2012). Quinoa starch granules as
741 stabilizing particles for production of Pickering emulsions. *Faraday Discussions*,
742 158, 139-155.
743 Ren, C. G., Tang, L., Zhang, M., & Guo, S. T. (2009). Structural characterization of heat-
744 induced protein particles in soy milk. *Journal of Agricultural and Food*
745 *Chemistry, 57*, 1921-1926.
746 Rousseau, D. (2000). Fat crystals and emulsion stability - a review. *Food Research*
747 *International, 33*, 3-14.
748 Rousseau, D. (2013). Trends in structuring edible emulsions with Pickering fat crystals.
749 *Current Opinion in Colloid & Interface Science, 18*, 283-291.
750 Schmitt, C., Bovay, C., Vuilliomenet, A. M., Rouvet, M., Bovetto, L., Barbar, R., &
751 Sanchez, C. (2009). Multiscale characterization of individualized beta-
752 lactoglobulin microgels formed upon heat treatment under narrow pH range
753 conditions. *Langmuir*, 25, 7899-7909.
754 Shao, Y., & Tang, C. H. (2014). Characteristics and oxidative stability of soy protein-
755 stabilized oil-in-water emulsions: Influence of ionic strength and heat
756 pretreatment. *Food Hydrocolloids, 37*, 149-158.

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

Song, X. Y., Pei, Y. Q., Qiao, M. W., Ma, F. L., Ren, H. T., & Zhao, Q. Z. (2015). Preparation and characterizations of Pickering emulsions stabilized by hydrophobic starch particles. *Food Hydrocolloids, 45*, 256-263.

Song, X. Y., Pei, Y. Q., Zhu, W., Fu, D., & Ren, H. T. (2014). Particle-stabilizers modified from indica rice starches differing in amylose content. *Food Chemistry, 153*, 74-80.

Tan, Y., Xu, K., Li, L. L., Liu, C., Song, C. L., & Wang, P. X. (2009). Fabrication of size-controlled starch-based nanospheres by nanoprecipitation. *ACS Applied Materials & Interfaces,* 1, 956-959.

Tan, Y., Xu, K., Liu, C., Li, Y. L., Lua, C. G., & Wang, P. X. (2012). Fabrication of starch-based nanospheres to stabilize pickering emulsion. *Carbohydrate Polymers, 88*, 1358-1363.

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

Tang, C. H., & Liu, F. (2013). Cold, gel-like soy protein emulsions by microfluidization: Emulsion characteristics, rheological and microstructural properties, and gelling mechanism. *Food Hydrocolloids, 30*, 61-72.

778 Tang, C. H., Zhang, Y. H., Wen, Q. B., & Huang, Q. R. (2010). Formation of amyloid
779 fibrils from kidney bean 7S globulin (phaseolin) at pH 2.0. *Journal of*
780 *Agricultural and Food Chemistry, 58*, 8061-8068.
781 Tang, J. T., Quinlan, P. J., & Tam, K. C. (2015). Stimuli-responsive Pickering emulsions:
782 recent advances and potential applications. *Soft Matter*, 11, 3512-3529.
783 Tarimala, S., & Dai, L. L. (2004). Structure of microparticles in solid-stabilized
784 emulsions. *Langmuir, 20*, 3492-3494.
785 Tikekar, R. V., Pan, Y. J., & Nitin, N. (2013). Fate of curcumin encapsulated in silica
786 nanoparticle stabilized Pickering emulsion during storage and simulated
787 digestion. *Food Research International, 51*, 370-377.
788 Timgren, A., Rayner, M., Dejmek, P., Marku, D., & Sjöö, M. (2013). Emulsion
789 stabilizing capacity of intact starch granules modified by heat treatment or octenyl
790 succinic anhydride. *Food Scienc and Nutrition,* 1, 157–171.
791 Tzoumaki, M. V., Moschakis, T., Kiosseoglou, V., & Biliaderis, C. G. (2011). Oil-in-
792 water emulsions stabilized by chitin nanocrystal particles. *Food Hydrocolloids,*
793 *25*, 1521-1529.
794 Tzoumaki, M. V., Moschakis, T., Scholten, E., & Biliaderis, C. G. (2013). In vitro lipid
795 digestion of chitin nanocrystal stabilized o/w emulsions. *Food & Function, 4*,
796 121-129.
797 Wang, L. J., Hu, Y. Q., Yin, S. W., Yang, X. Q., Lai, F. R., & Wang, S. Q. (2015).
798 Fabrication and Characterization of Antioxidant Pickering Emulsions Stabilized
799 by Zein/Chitosan Complex Particles (ZCPs). *Journal of Agricultural and Food*
800 *Chemistry, 63*, 2514-2524.

Williams, M., Armes, S. P., Verstraete, P., & Smets, J. (2014). Double emulsions and colloidosomes-in-colloidosomes using silica-based Pickering emulsifiers. *Langmuir, 30*, 2703-2711.

Winuprasith, T., & Suphantharika, M. (2013). Microfibrillated cellulose from mangosteen (Garcinia mangostana L.) rind: Preparation, characterization, and evaluation as an emulsion stabilizer. *Food Hydrocolloids, 32*, 383-394.

Wronski, S., Vladimirov, V., & Adach, A. (2012). Modelling of mass transfer from multiple emulsions. *International Journal of Heat and Mass Transfer, 55*, 4241-4245.

Wu, J. D., Shi, M. X., Li, W., Zhao, L. H., Wang, Z., Yan, X. Z., Norde, W., & Li, Y. (2015). Pickering emulsions stabilized by whey protein nanoparticles prepared by thermal cross-linking. *Colloids and Surfaces B-Biointerfaces, 127*, 96-104.

Xiao, J., Li, C., & Huang, Q. (2016). Kafirin nanoparticles-stabilized Pickering emulsions as oral delivery vehicles: physicochemical stability and in vitro digestion profile. *J. Agric. Food Chem, 63,* 10263-10270.

Xiao, J., Li, Y., Li, J., Gonzalez, A. P., Xia, Q., & Huang, Q. (2015). Structure, morphology and assembly behavior of kafirin. *Journal of Agricultural and Food Chemistry, 63*, 216–224.

Xiao, J., Nian, S., & Huang, Q. R. (2015). Assembly of kafirin/carboxymethyl chitosan nanoparticles to enhance the cellular uptake of curcumin. *Food Hydrocolloids, 51*, 166-175.

822 Xiao, J., Wang, X. a., Gonzalez, A. J. P., & Huang, Q. (2016). Kafirin nanoparticles-
823 stabilized Pickering emulsions: Microstructure and rheological behavior. *Food*
824 *Hydrocolloids, 54*, 30-39.
825 Yusoff, A., & Murray, B. S. (2011). Modified starch granules as particle-stabilizers of
826 oil-in-water emulsions. *Food Hydrocolloids, 25*, 42-55.
827 Zoppe, J. O., Venditti, R. A., & Rojas, O. J. (2012). Pickering emulsions stabilized by
828 cellulose nanocrystals grafted with thermo-responsive polymer brushes. *Journal*
829 *of Colloid and Interface Science, 369*, 202-209.
830 Zou, Y., Guo, J., Yin, S. W., Wang, J. M., & Yang, X. Q. (2015). Pickering Emulsion
831 Gels Prepared by Hydrogen-Bonded Zein/Tannic Acid Complex Colloidal
832 Particles. *Journal of Agricultural and Food Chemistry, 63*, 7405-7414.

Table. 1. Food grade colloidal particles stabilized Pickering emulsions.

Particle origin	Formation/modification approach	Particle shape size or shape	Emulsion droplet size	Evidence for stabilization mechanism	References
Polysaccharides					
Waxy maize starch (Mw=180,000)	Esterified by acetic anhydride and phthalic anhydride; formed by nano precipitation method	Mean diameter $d_{4,3}$=360 nm	20-100μm	CLSM showed nanospheres with a dense film to prevent coalescence	(Tan, et al., 2012; Tan, et al., 2014)
Waxy maize starch	HCl and H_2SO_4 hydrolyzed	51 nm and 58 nm for HCl and H_2SO_4 hydrolyzed ones	342-825 nm (size after polymerization)		(Haaj, Thielemans, Magnin, & Boufi, 2014)
Native rice, waxy maize, wheat starch	Without modification	Rice: polygonal shape with $d_{4,3}$~5.2 μm; Waxy maize: spherical and polygonal with $d_{4,3}$~11.3 μm; Wheat starch: oval and spherical	Average $d_{4,3}$~200 μm for rice starch stabilized emulsion	Close-packed "clumps" or densely packed particle layer under optical microscopy observation	(Li, et al., 2013)
Indica rice starches	Octenyl succinic anhydride (OSA) modified	Average particle size range 4.95-5.29 μm	Average droplet size range10-70	Optical microscopy showed particles accumulated at oil–	(Song, et al., 2014)

			μm	water interface in the form of a densely packed layer	
Gelose 80 starch	Cryogenic milling to reduce granule size; Hydrophobic modified with OSA	Particle size range 0.5-15μm	Droplet size range1- 20 μm	Incomplete coverage by starch particles and particle aggregates are observed by CLSM.	(Murray, et al., 2011; Yusoff & Murray, 2011)
Microcrystals of α-, β-Cyclodextrin (CD)	Used without modification	Length of microrod: α-C D>100 μm, β-CD< 10 μm		Optical micrograph showed the presence of densely packed surface layer with "pasta-like" appearance	(Mathapa & Paunov, 2013)
Cellulose from mangosteen rind	Passed through high pressure homogenizer at 500 bar		Average droplet diameters$d_{4,3}$ 24.3-61.2 μm	Strongly entangled and disordered network structure was observed under freeze-fracture SEM	(Winuprasith & Suphantharika, 2013)
Chitin from crab shells	Acid hydrolysis (3 N HCl, 95 ℃, 90 min)	240nm × 20 nm	10-100 μm	Polarized light optical micrograph showed ChN particles and aggregates at the droplet interface	(Tzoumaki, et al., 2011)

Cellulose nanocrystal (CNC) from ramie fiber	Grafted with Poly(NIPAM) chains by radical polymerization	3-15 nm in width 50-250 nm in length	Droplet size range 10-100 μm Centered ~38 μm	TEM images showed anisotropically aligned rectangular-shaped inclusions	(Zoppe, Venditti, & Rojas, 2012)
Chitin nanocyrstals	Hydrolysis of chitin with 3N HCl for 90 min.	Average 160 ± 77 nm in length, 16 ± 5 nm in width	Droplet size range 1-20 μm	Droplets with high CNC surface coverage were observed under SEM	(Capron & Cathala, 2013; Perrin, Bizot, Cathala, & Capron, 2014)
Amorphous cellulose	Dissolution of cellulose in H_3PO_4, then regenerated in DI water		Droplet size range 10-30 μm	Optical micrographs showed the absorption of amorphous cellulose on the droplet interface	(Jia, et al., 2015)
Cotton linters and bacterial cellulose	Acid hydrolysis under H_2SO_4 or HCl	Length range in 117-855 nm Width range in 13-17 nm	Average size: 3.4-18.6 μm	Evenly distributed on the surface and curved along the droplets, building an armored layer under SEM	(Kalashnikova, et al., 2012)
Tencel fibres (initial mean length 3 mm, mean width 20 μm)	Physical breakdown by hammer mill and cryogenic freezer mill; Hydrophobic modified by ethyl cellulose	Width: 5 - 20 μm Length: 0.5 - 70 μm			(Murray, et al., 2011)
Proteins					

Kafirin	Dissolve in acetic acid and mix with water followed by dialysis	Spherical particles with size range of 90-340 nm	40 – 130 μm	Evidence of particles anchoring at interface by Cryo-SEM, CLSM images	(Xiao, Wang, et al., 2016)
Zein	Dissolve in a 80 % EtOH and mix with water to final 20% EtOH followed by EtOH evaporation	Spherical shape with average size of 82 ± 16 nm	10 - 100 μm		(de Folter, et al., 2012)
Zein/Tannic acid complex particles	Zein-Tannic acid complex first dissolve in ethonal solution then formed by anti-solvent precipitation method	100 - 300 nm	13 – 44 μm	CLSM images showed emulsion droplets stabilized by particles	(Zou, et al., 2015)
Pea protein isolate	Adjust pH to 3.0	134-165 nm	1.3-12 μm		(Liang & Tang, 2014)
Soy protein isolate (SPI)	Thermal treatment of SPI solution followed by addition of NaCl	50-200 nm	0.6-300 μm Major peak at 50-70 μm		(Liu & Tang, 2013)
Whey protein isolate	Heating whey protein within o/w emulsion at 80 ℃ for 15	200 - 500 nm	1-5 μm	CLSM images of droplets stabilized by whey protein	(Wu, et al., 2015)

	min			nanoparticles was observed	
Whey protein microgel	Pre-heated to 60 °C and then heated to 85 °C followed by a holding time of 15 min	Mean diameter ~280 nm		Cryo-SEM revealed a continuous protein membrane that covered the interface area between the aggregates.	(Destribats, et al., 2014)
Lactoferrin protein	Adjust pH to 7 and heated for 20 min at 90 °C, cool to ambient and adjust pH to 8	50-70 nm	60-120 μm		(David-Birman, et al., 2013; Shimoni, et al., 2013)
Fat crystals					
Microcrystalline fat of fully hydrogenated tristearin (TS)	Clear solution of TS was flash-cooled in liquid nitrogen	285 to 1858 nm	6–10 μm for final double emulsion	Optical microscope showed relatively stable w-o-w type of double emulsion	(Garti, et al., 1999)
Mixture of mono- and triglyceride crystals	Cooling down by a scraped-surface heat exchanger during emulsification		$d_{3,2}$ varied from 3.4 to 11.1 μm	SEM pictures showed solid shell of fat crystals give rough and uneven water droplet surface	(Frasch-Melnik, Norton, & Spyropoulos, 2010)
Hydrogenated canola oil(HCO) crystal seeded	Emulsions were cooled from >70 °C to room temp.		Average droplet sizes ~14 μm	Crystalline fat Pickering shells surrounded the water	(Nadin, et al., 2014)

by glycerol monostearate (GMS)	Continuous stirring to crystalline GMS and HCO			droplet was observed under Polarized light microscopy	
Nutraceuticals					
Phytosterol	Dissolve in 100% EtOH, mixed with water solution with whey protein isolate as stabilizer	Platelet-like sheets, had a mean volume- and surface-averaged diameter of 44.7 and 24.7 μm	230-50 μm		(Liu & Tang, 2014)

Figure captions:

Fig. 1. Schematic diagram of preferred emulsion type and corresponding three phase contact angle

Fig.2. Publication per year analyzed by Sci-finder using search item “Particle stabilized emulsions” or “Pickering emulsion”; inserted figure highlights publication per year for food grade particle stabilized Pickering emulsions

Fig. 3. (a) CLSM micrograph of emulsion droplets stabilized bymicrofibril cellulose;(b) Reverse contrast CLSM image of Gelose 80 starch granules stabilized emulsion, the enlarged image highlights the presence of starch and their aggregates at the interface. Reprinted with permission from Ref. (Winuprasith & Suphantharik, 2013) and Ref (Yusoff & Murray, 2011), respectively.

Fig. 4. Cryo-SEM images of the interface of heptane-in-water emulsion stabilized by whey protein microgel particles. At pH 4.8, the interface was covered by a continuous 2-D network of highly aggregated particles (b) particles adopted discrete configurations of either individual particles/small aggregates at pH 3 (a) or larger aggregates separated by what appears to be bare interface at pH 7 (c); Sublimation of interface at pH 4.8 reveals the continuous protein membrane between the particle aggregates (d). Reprinted with permission from Ref. (Destribats, et al., 2014).

Fig. 5. Schematic illustration of particles stabilized neighboring emulsion droplets against coalescence

Fig. 6. (a) Emulsion stabilized by rice starch; (a’) magnified from (a); (b) Optical microscopy of flat adhesive films between flocculated droplets stabilized by whey protein microgel; (b’) cryo-SEM image of the contact zone, note the lower concentration of

adsorbed particles outside of this zone; (c) SEM images of microfiber cellulose stabilized emulsion; (c') magnified from (c'); (d) Polarized light micrograph of a water droplet surrounded by a fat crystalline multilayer, size bar = 10 μm. (d') Combined interfacial crystallization and local surroundings of hydrogenated canola fat crystals. Size bar = 40 μm. Reprinted with permission from Ref. (Li, et al., 2013), Ref. (Destribats,et al., 2014), Ref. (Winuprasith & Suphantharik, 2013) and Ref. (Rousseau, 2013) successively.

Figure 1

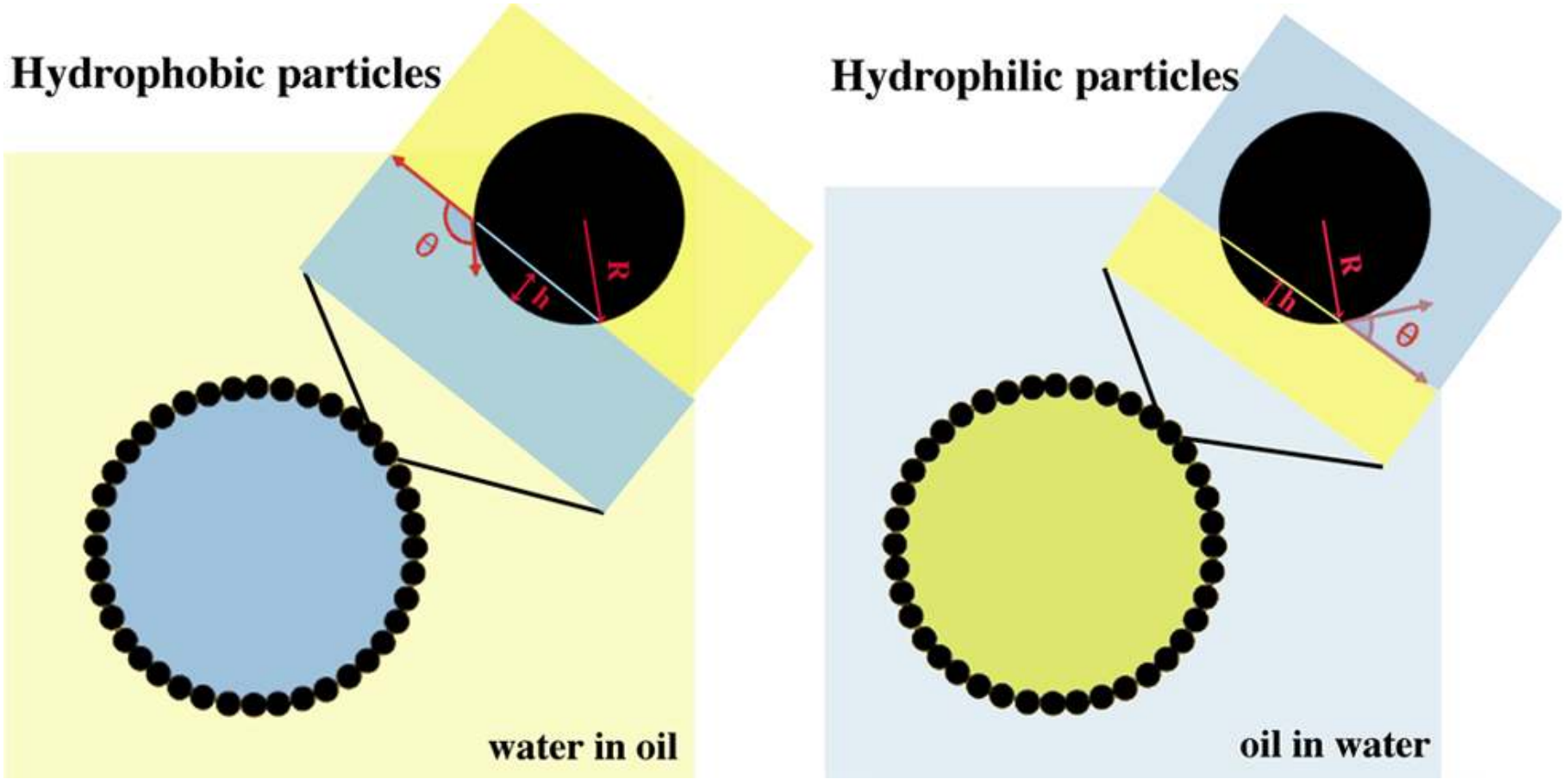
Hydrophobic particles
θ
h
R
water in oil
Hydrophilic particles
R
h
θ
oil in water

Figure 2

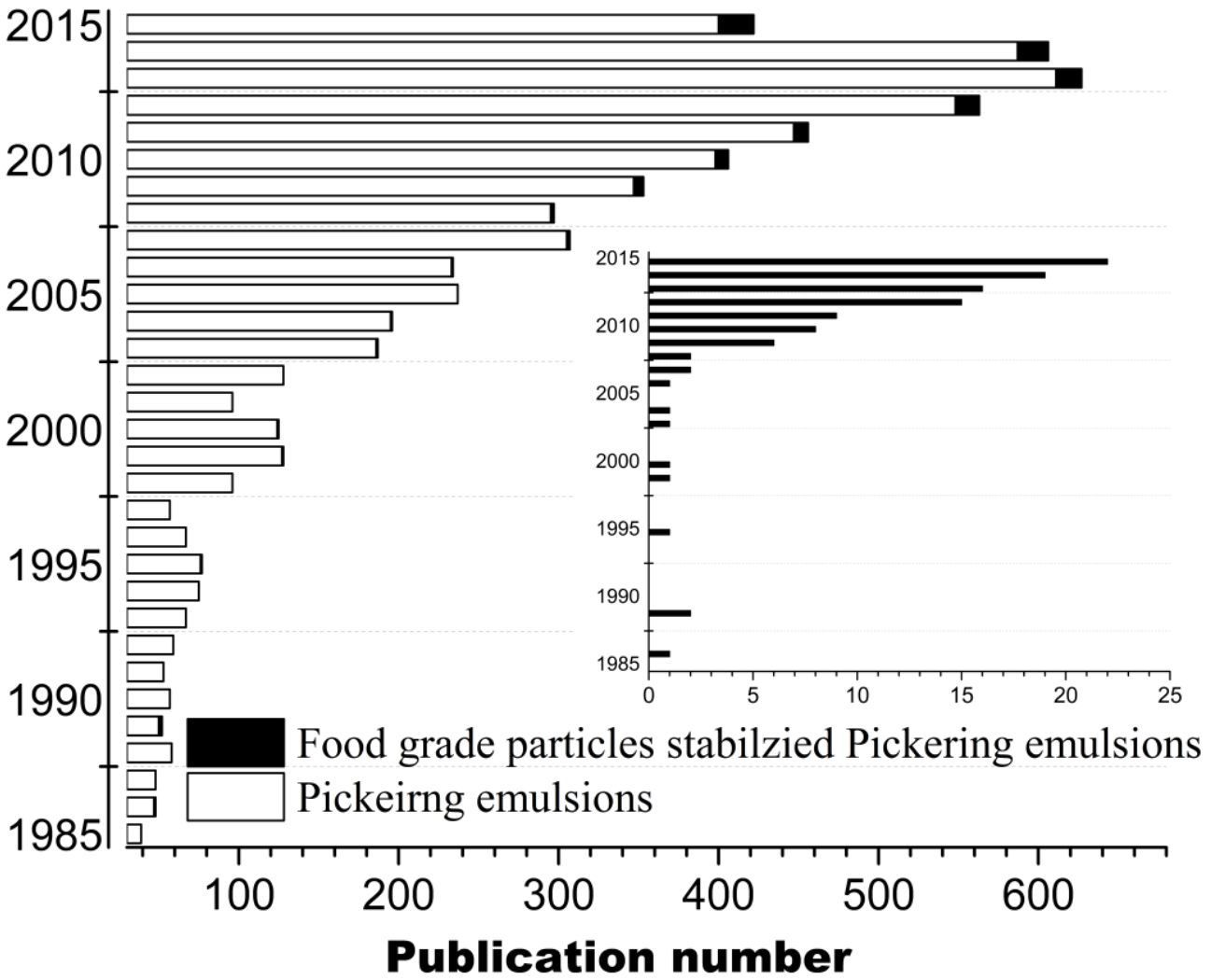
2015
2010
2005
2000
1995
1990
1985
100
200
300
400
500
600
Publication number
Food grade particles stabilzied Pickering emulsions
Pickeirng emulsions
0
5
10
15
20
25

Figure 3

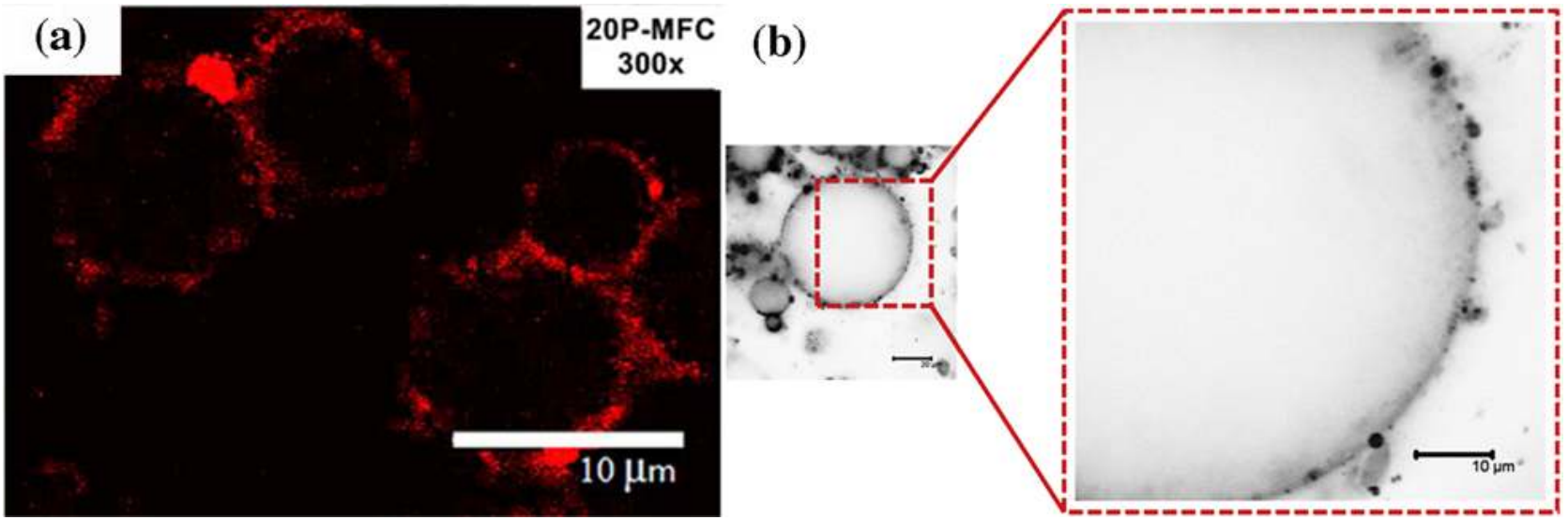

Figure 4

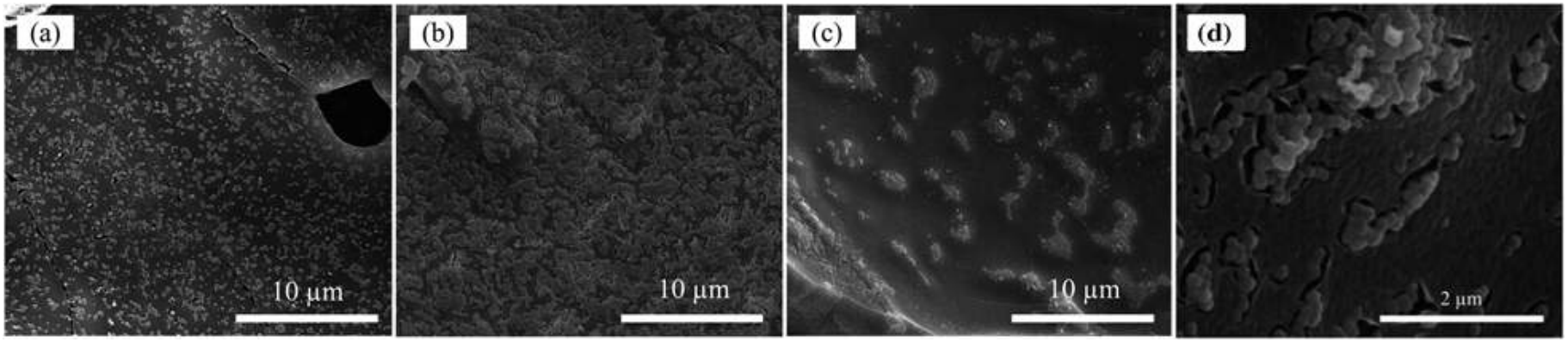

Figure 5

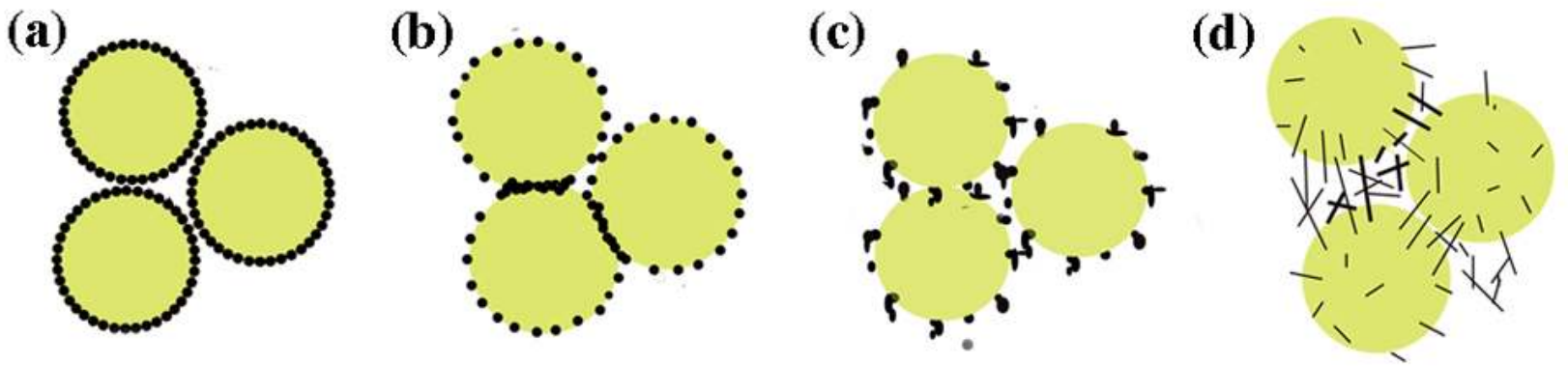

Figure 6