Colloids and Surfaces A: Physicochemical and Engineering Aspects 676 (2023) 132242

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Interfacial engineering of clear emulsions: Surfactant hydrophobicity and the hidden role of chain structure

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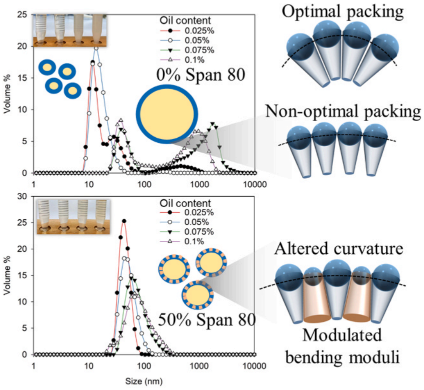
HIGHLIGHTS

- Higher loading efficiency achieved by blending surfactants.
- Blending surfactants alters optimum curvature of micelle for uniform droplets.
- Menthol acts as a co-surfactant in the oil phase improving emulsion clarity.
- Short surfactant tails made smaller droplets despite same hydrophilic/lipophilic balance.
- Surfactants with linear chains showed higher compatibility with saturated oil.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Emulsion
Surfactants
Curvature
Interface
Turbidity
Chain structure

ABSTRACT

The large surface area requires using high concentration of surfactants to produce emulsions with small droplets, which poses challenges for industries with respect to safety issues and the potential to introduce unpleasant flavors. In this work, we investigated the factors of interfacial engineering to improve oil loading capacity and reduce surfactant concentration in emulsions while still keeping high optical clarity. The interface was systematically modulated using a fixed concentration (0.05 w/w %) of sorbitan-based surfactants with different hydrophobicity, chain length, and linearity. Particle size and turbidity were evaluated using dynamic light scattering and spectrophotometry. Hydrophilic surfactants were found to produce small droplets when the oil fraction was limited but were less effective for creating a higher oil fraction load. However, blending in hydrophobic surfactants produced smaller uniform droplets, lower turbidities and higher loading capacity owing to the change in the optimum curvature. The results revealed that surfactant-to-oil ratios (SORs) played an important role in emulsion formation at different hydrophobic-lipophobic balance (HLB). In addition, with identical HLB values, surfactants with shorter chains exhibited higher loading capacity than those with longer chains. Further, surfactants with similar backbone linearity were observed to favor emulsifying oils that have similar degrees of saturation. We found that emulsification is influenced by factors beyond hydrophobicity, such

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https://doi.org/10.1016/j.colsurfa.2023.132242
Received 21 June 2023; Received in revised form 5 August 2023; Accepted 13 August 2023

as surfactant chain length and linearity, which have been usually overlooked. From the view of interfacial curvature, we report evidence that interfacial engineering can be used to achieve more efficient emulsification, which helps to reduce surfactant content from beverages. This study provides a framework for tailored emulsion systems that bridges the current gaps in knowledge regarding factors hidden behind HLB, including SORs and chain structures. This work has potential application in the food, cosmetic, and pharmaceutical industries.

1. Introduction

Emulsions have versatile applications, such as in foods, cosmetics, and pharmaceuticals, by delivering bioactive compounds or flavors while providing desirable rheological and optical properties [1–3]. In the presence of surfactants, the interfacial tension between immiscible phases is reduced; this facilitates the generation and stabilization of droplets with larger surface area [4]. Smaller and more uniform emulsion droplets are usually preferred in these applications as they provide enhanced bioavailability [5]; stronger stability against creaming [6] and Ostwald ripening [7]; and higher optical clarity [8]. A typical example is a beverage containing extracted fruit oils, where high transparency is considered a favorable attribute for a visually pleasing and refreshingly flavored drink [8]. Low turbidity, with respect to emulsions, is a result of the weaker light scattering associated with smaller oil droplets; when the droplet size decreases below 100 nm, which is far from the optimal light-scattering diameter [9], emulsions turn translucent or even optically clear [10].

However, it is challenging to prepare emulsions with ultra-fine droplets while keeping reasonable loading capacities. The specific surface area (A_s) of a population of spheres is inversely proportional to their diameter (d) and density (ρ): $A_s = 6/\rho d$ [11]. For example, reducing the size of particles with the same density from 500 nm to 50 nm would enlarge the surface area by a factor of 10. The larger surface area of emulsions with small oil droplets suggests the requirement of a larger amount of surfactants to cover them. For conventional emulsions a surfactant-to-oil ratio (SOR) of 1:10 is generally sufficient; for emulsions with droplets smaller than 100 nm the ratio could be higher than 1:1 due to the enlarged surface area [12]. The loading capacity is particularly important to the food and beverage industries, as safety regulations have been established to limit surfactant concentrations as additives posing a challenge to the food industry to produce smaller droplets with sufficient cargo [13]. Another hurdle of using surfactants in beverages is the unpleasant sensory attributes where high concentrations of surfactants can often taste bitter and soapy [14]. To improve both safety and consumer acceptance, the food industry has been continuously trying to reduce the surfactants added to drinks while keeping the emulsions clear: to do this effectively requires careful interfacial engineering using surfactants.

Hydrophilic lipophilic balance (HLB), a classic theory that describes hydrophobicity of surfactants based on chemical composition, has been used to select surfactants [15]. For example, sorbitan monooleate (Span 80) with low HLB (4.3) is hydrophobic and used for producing water-in-oil emulsions, whereas polyoxyethylene (20) sorbitan monooleate (Tween 80) with higher HLB (15.0) is used in oil-in-water emulsions [16]. An intermediate HLB can be formulated by blending surfactants and the mixture shows the proportionally weighted HLB. The theory of "required HLB" has been used to describe the optimum combination between surfactants of specific organic phases [17]. Numerous reports have been published to investigate the required HLB to produce emulsions of different oils. Most reports described using a method with a fixed SOR and identifying the required HLB which produced the smallest droplets therefore evaluating the compatibility between oil and surfactants [18–22]. However, SOR may play an important role by affecting the interfacial curvature which is largely overlooked by conventional approaches. For instance, a study used paraffin oil with an SOR of 1:4 to investigate the optimum combination of surfactants and found the required HLB was 10 [20]; but another work reported the required HLB of the same oil was 12 when the emulsion was prepared with a lower SOR (1:20) [19]. This disparity indicated that the SOR affected the optimum HLB, and the underlying reasons still need to be uncovered. Understanding the interactive roles of hydrophobicity and SOR can benefit emulsion designs that have higher loading capacity and smaller amounts of surfactants.

Another commonly overlooked characteristic hidden behind hydrophobicity is the surfactant chain structure. Surfactants can be formulated to have the same HLB, but the profile of the chain structure can be totally different. For example, the combination of 53 % Tween 20 and 47 % Span 20 shows the same HLB as the blend of 80 % Tween 60 % and 20 % Span 60, but the micelles formed by the first combination exhibited a much thinner interface due to the shorter chains (Fig. S1). Previous comparative studies using varied surfactants with different chain length usually did not control the HLB, and the different emulsifying proprieties could be attributed to the unmatched hydrophobicity instead [23–25]. Therefore, further studies where HLB is maintained while chain length is varied are required to interpret the role of chain length.

In addition to chain length, surfactant linearity, where two surfactants with the same carbon chain length may have different backbone configuration, may play a role in the emulsifying properties. For example, the backbone structure of Tween 80 and Tween 60 both have 18 carbons on their tails, but Tween 80 is less linear due an unsaturated bond (Fig. S1). The difference on backbone may affect the surfactant-surfactant interaction and also surfactant-oil interaction [25,26]. However, the compatibility between surfactants and an oil phase with a different degree of saturation was not extensively studied, and revealing the hidden effect of chain structure will also require systematic control of both hydrophobicity and chain length.

This work aims to investigate the impact of surfactant hydrophobicity with close attention paid to SOR, and then we will branch out to discuss the impacts of surfactant structures such as chain length and chain linearity when hydrophobicity is kept the same. To do this, sorbitan-based surfactants as FDA-approved food additives that have been used as emulsifiers in beverages were chosen as models. With their well-classified polar head and fatty acid tails, they can be utilized as a model system to systematically investigate the effect of interfacial engineering on emulsifying properties (Fig. S1). Understanding the roles of surfactant hydrophobicity and chain structures would help industries design emulsion systems with targeted functional properties that can be applied to foods, cosmetics, or pharmaceuticals.

2. Materials and methods

2.1. Materials

D-Limonene (purity > 90 %) was purchased from True Terpenes (OR, USA). Citric acid, trisodium citrate, sodium benzoate, polyoxyethylenesorbitan monooleate (Tween 80), polyoxyethylenesorbitan monostearate (Tween 60), polyoxyethylenesorbitan, monopalmitate (Tween 40), polyoxyethylenesorbitan monolaurate (Tween 20), sorbitan monooleate (Span 80) sorbitan monostearate (Span 60), sorbitan, monopalmitate (Span 40), and sorbitan monolaurate (Span 20) were purchased from Sigma-Aldrich (MO, USA). (-)-Menthol (purity > 99 %) was purchased from TCI America (OR, USA). Canola oil and palm oil were purchased from Crisco (NJ, USA) and Okonatur (FL, USA), respectively.

2.2. *Preparation of surfactants*

To study the effect of hydrophobicity, surfactants were prepared in different combinations using Tween 80 (HLB = 15.0) and Span 80 (HLB = 4.3), such that the mixed surfactants provided HLB ranging from 15 (with 0 % Span 80) to 9.65 (with 50 % Span 80). To study the effect of chain length, surfactants with the same fatty-acid motifs are mixed as Table S1 to control the identical HLB = 12.86. To study the effect of linear structure, the blends of Tween 80 and Tween 60 were employed to control the same chain length and HLB. The chemical structures of surfactants are shown as Fig. S1.

Citrate buffer (pH = 3) was used consistently in this study to simulate the aqueous conditions associated with commercial beverages. To obtain clear solution, surfactants were mixed in the citrate buffer and sonicated (Sonics Vibra Cell VCX-750, CT, USA) using a 13 mm diameter probe (750 W cm^{-2}, 115-μm amplitude) for 2 min at an 50 % amplitude and 20 kHz with 1 s pulse and 2 s relaxation delay between pulses. The samples were immersed in an ice bath during sonication. All aqueous phases contained 0.05 w/w % surfactants in total but different blending ratios of individual components. The concentration of surfactants used in the formulation (0.05 w/w %) falls within the permitted concentration and was kept constant throughout [13].

2.3. *Determination of interfacial tension*

Based on the pendant drop method, the interfacial tension was measured using a goniometer (ramé-hart model 500, NJ, USA) with a 22-gauge blunt-tip needle. The images of droplets were examined using DROPimage advanced (ramé-hart, NJ, USA) to analyze the pendant shape. The interfacial tension between 0.05 w/w % surfactant solution and the organic phase was calculated based on the Young–Laplace equation.

2.4. *Preparation of emulsions*

To prepare emulsions, limonene (0.025–0.1 w/w % oil to total emulsion) was mixed in a 0.05 w/w % surfactant solution, thus the surfactant-to-oil ratio (SOR) ranged from 2.0 to 0.5. The coarse emulsions (20 mL) were mixed using a higher-shear homogenizer (T25 digital ULTRA-TURRAX, IKA Works, NC, USA) with an 18-G dispersing probe for 1 min at 15,000 rpm and were further processed (20 mL a batch) by ultrasonication (Sonics Vibra Cell VCX-750, CT, USA) using a 13 mm diameter probe (750 W cm^{-2}, 115-μm amplitude) for 2 min at a 50 % amplitude and 20 kHz frequency with 1 s pulse and 2 s relaxation delay between pulses. The samples were immersed in an ice bath during sonication.

To investigate the effect of the composition of the organic phase on emulsion formation, menthol or amyl acetate was blended with limonene at a ratio of 1:1, and the mixed oil was used to replace pure limonene for emulsion preparation per the procedure stated above.

To investigate the effect of chain linearity, canola oil and palm oil were mixed in a 0.05 w/w % surfactant solution containing Tween 60 and Tween 80. The mixtures were incubated in 50 °C and immediately were mixed using a higher-shear homogenizer. Ultrasonication was carried out on the primary emulsions as stated previously.

2.5. *Measurement of turbidity*

The turbidity of the emulsion was evaluated with the transmittance mode of a UV–visible spectrophotometer (Shimadzu UC-2600, Japan) at 600 nm and room temperature (20 °C). Citric buffer was used as the blank.

2.6. *Measurement of particle size*

The particle size was measured using dynamic light scattering with a NanoZS90 zeta-sizer (Malvern Instrument Ltd., UK) with a He/Ne laser ($\lambda = 633$ nm) at 25 °C in triplicates. Signal was collected by the back detector at (173°) to minimize the effect of multiple scattering. The volume distribution in different droplet sizes was plotted to quantify the volume of oil at each peak.

2.7. *Transmission electron microscopy (TEM)*

Surfactant micelles were visualized using an FEI Tecnai 12 BioTwin TEM (Eindhoven, The Netherlands). Ultrasonicated surfactant solutions (0.05 w/w %) were negative-stained using 2 % uranyl acetate. An aliquot of the stained sample was placed on carbon-coated copper TEM grids and visualized with accelerating voltage at 120 kV and an objective aperture of 20 μm.

2.8. *Statistical analysis*

All measurements were performed in triplicates using freshly prepared samples and were reported as mean ± standard deviation (SigmaPlot 14.0, Systat Inc.). To determine the statistical significance, the means were compared using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test in JMP Pro (Version 14, SAS Institute, USA) with a significance level set at $p < 0.05$.

3. Results and discussion

3.1. *Effect of mixed surfactants on limonene emulsion*

The turbidity and transmittance of the emulsions with different oil content and surfactant combinations indicate that when the oil fraction

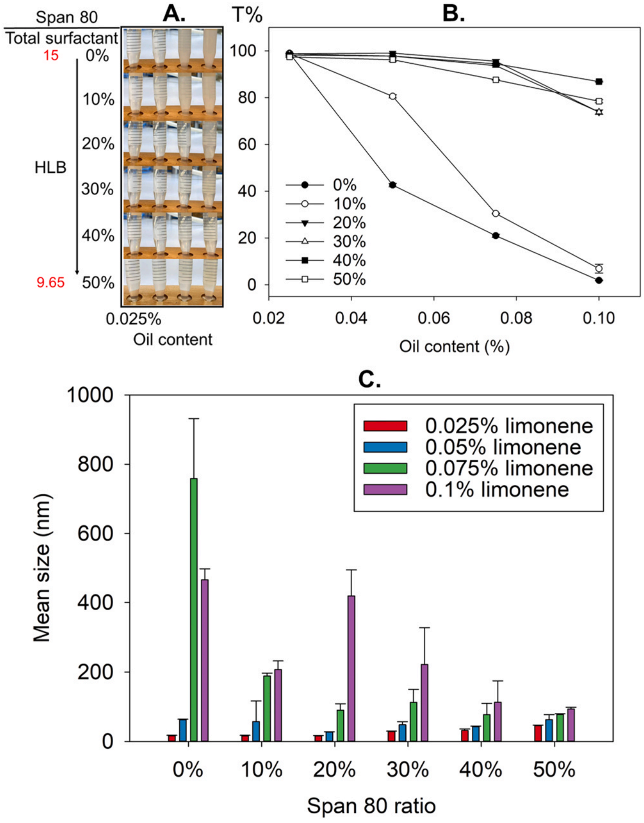

Fig. 1. Photographs (A), transmittance (B), and mean droplet size (C) of limonene emulsions prepared with different oil contents (0.025–0.1 w/w %) and different surfactant combinations (0–50 % Span 80). All emulsions have the same total surfactant concentration (0.05 w/w %).

was low (0.025 w/w %) the emulsions were clear with a transmittance higher than 97 % for all surfactant formulas (Figs. 1A and B). However, when the oil content is over 0.05 w/w %, the group with Tween 80 as the only surfactant turned translucent with the transmittance reduced to 42.6 %, and increasing the oil content further (> 0.05 w/w % oil, SOR < 1.0) turned the emulsions optically turbid with transmittances falling below 20 %. This transition of turbidity was inhibited, however, by blending a hydrophobic surfactant into the Tween 80. The data shows that when the surfactant contained 10 % Span 80, the clarity of emulsion with 0.05 w/w % oil fraction was improved with transmittance increasing to 80.6 %, and the additional increase of Span 80 further increased the clarity of the emulsions. The groups with less hydrophobic surfactants exhibited the strongest responses to adding oil while emulsions with more Span 80 showed a moderate increase of turbidity where ~80% transparency can still be maintained in the presence of 0.1 w/w % oil.

This phenomenon was also observed on droplet size (Fig. 1C). With Tween 80 as the surfactant, the droplets of 0.025 w/w % and 0.05 w/w % limonene emulsion were 16.8 nm and 62.1 nm which corresponded to their transparent and translucent appearance respectively. Increasing the oil fraction caused a sharp increase in the droplet size (> 400 nm). When 20 % Span 80 was used, the droplet size of the 0.075 w/w % limonene emulsion was reduced to less than 100 nm, but higher oil fractions still produced large droplets. However, when the ratio of Span 80 was increased to over 40%, the oil droplets remained at ~100 nm even for 0.1 w/w % limonene emulsion. This indicates that the hydrophobic surfactant, Span 80, contributed to a moderate droplet swelling (from 45.4 nm to 93.8 nm) when the oil fractions increased four times.

The group containing 50 % Span 80 has relatively large droplets (45.4 nm) at low oil content (0.025 w/w % limonene) compared with the group without Span 80 (16.8 nm). This phenomenon indicates the effect of surfactant HLB on the droplet size could be affected by oil fraction or SOR. When limited oil (high SOR) was used to prepare the emulsion, the droplets were smaller with a higher HLB (less Span 80); when more oil was added (low SOR), a lower HLB (more Span 80) produced finer droplets. This explains the disparity evident in previous studies where different optimum HLBs were reported when the oil fraction was changed [19,20]. Previous experiments have used SOR = 1:1 to prepare limonene emulsion and reported an optimal HLB at 15 [27]. This was in partial in agreement with our observed results where pure Tween 80 was able to produce small droplets at a limited oil fraction, but optimizing the HLB of the surfactant system can lower the SOR required for producing the emulsion. This suggests that future research to find and report optimum HLBs needs to take into account SOR.

The addition of Span 80 impacted both the average size and the monodispersity of the samples. Although the emulsions with low oil content (0.025 w/w % and 0.05 w/w %) had the main peak at ~13 nm, a small portion of oil was not emulsified in the small droplets and produced a long tail lag in the distribution curve (Fig. 2A). At higher oil fractions (0.075 w/w % and 0.1 w/w %) and Tween 80 as the only surfactant, a bimodal distribution of droplets was observed where the second peak is at the 1000 nm dimension. This indicates that Tween 80 alone is not an effective surfactant to emulsify limonene. However, when Span 80 was used as the co-surfactant, the emulsion exhibited monomodal distribution for all oil fractions (Fig. 2B) though the peak slightly shifted to the right with a large average size when the limonene content increased. The PDI (polydisperse index) of the emulsions stabilized by pure Tween 80 was over 0.5, but the addition of Span 80 reduced the PDI to ~0.2, which suggests the hydrophobic surfactant helped produce uniform droplets (Fig. 2C). The volume of oil not emulsified in the main peak at different oil contents was quantified (Fig. 2D). For 0.025 w/w % limonene, all groups were well emulsified as there was no extra oil outside of the main peak. However, when the oil content was increased to 0.05 w/w %, the group using Tween 80 as the only surfactant had over 30 % of the oil not emulsified. However, this failure of emulsification was ameliorated when HLB was decreased with the addition of Span 80, more oil can be incorporated into small

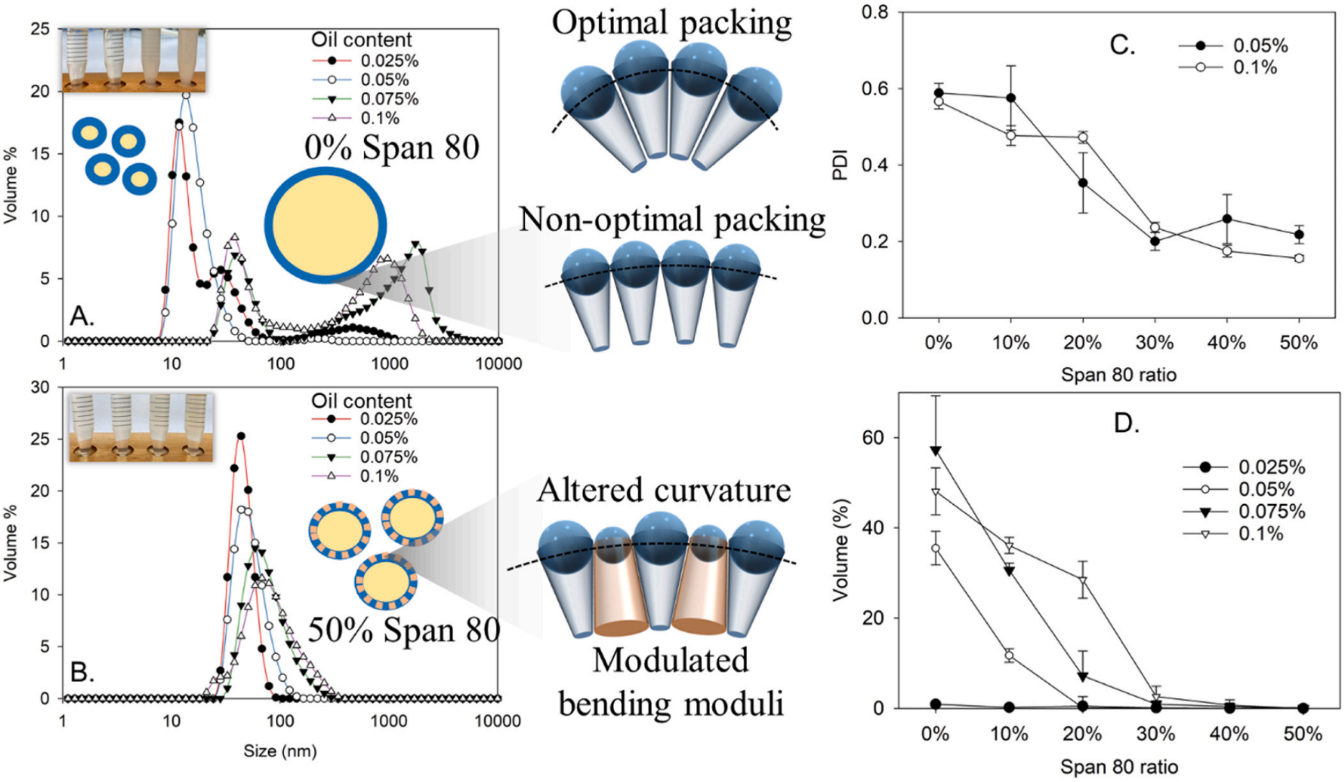

Fig. 2. Size distribution of limonene emulsions with 0 % Span 80 (A) and 50 % Span 80 (B), the polydisperse index (PDI) of 0.05 w/w % and 0.1 w/w % limonene emulsions (C), and the oil volume not emulsified in the main emulsion peaks with different surfactant combinations (D).

droplets, and for the groups with more than 40 % or 50 % Span 80, limonene was well emulsified even at low SOR, which contributed to the emulsion transparency.

The interfacial tension between limonene and the 0.05 w/w % total surfactant solutions for Tween 80 alone was 7.1 mN/m, while the tension increased with the increasing ratio of hydrophobic surfactant rising to 9.7 mN/m in the presence of 50 % Span 80 (Fig. 3 A). The stronger interfacial tension at higher Span 80 ratios suggests more energy is required to increase surface area or reduce droplet dimension. A lower interfacial tension is usually favored for emulsion preparation, which is contradictory to our results where the surfactants providing higher interfacial tension contributed significantly to the formation of small and uniform droplets.

Without Span 80, the micellar size was below 10 nm with a limited core size to load oil, with an increase in surfactant of hydrophobicity the micelles enlarged to over 100 nm (Fig. 3B). This change is due to the difference of packing parameter (*p*): Tween 80 as the hydrophilic surfactants has a cone shaped molecule with a predominant polar head thus forming a concave curvature ($p < 1/3$), whereas the hydrophobic Span 80 with the inverted cone-shaped structure forming convex curvature ($p > 1$). The micelles formed by Tween 80 exhibit a short radius of curvature due to their close packing behavior, but in the presence of Span 80, the inverted cone inserts between Tween 80 molecules and prevents close packing and increases the radius of curvature. The mixed surfactants with larger radius r exhibit lower HLB, smaller spontaneous curvature $c_0 = 1/r$, and lower bending stiffness. The TEM images (Fig. 3 C and D) demonstrated that the Tween-based micelles are ~10 nm and uniform in size, whereas when Span 80 was incorporated, the micellar size was enlarged. The mixed micelles may not be monolayered as the spheres were not hollow. The internal part of the micelles could be similar to multilayered liposomes since the HLB (9.65) of the 50 % Span 80 group was close to the HLB for phospholipids (~8) which tend to form planar interfaces. The larger micelles formed by mixed surfactants have less surface area which corroborates with the observed higher interfacial tension. This suggests that low interfacial tension may not be the only criteria by which to select a surfactant to emulsify oils.

Surfactant micelles have the optimum curvature when the molecules with specific geometric properties are packed by surfactant-surfactant interaction, and at the optimal curvature the bending energy on the interface can be minimized. We hypothesize that with pure Tween 80, the small micelles tend to form small droplets or swollen micelles (~13 nm as shown in Fig. 1C), which is close to the size of the spontaneously formed micellar (~10 nm). Large surface area requires a substantial amount of surfactants to stabilize and the SOR can reach as high as 2. When more oil is added, the amount of surfactants could be insufficient, which means that the oil droplets would have to be larger to compensate for the shortage of surfactants. The free energy of monolayer surfactants at radius *r* is calculated using the following equation [28]:

$$E_r = \gamma_0 A + 2k_c\int(c_r - c_0)^2 dA + k_c\int K dA \tag{1}$$

The c_r is the curvature at radius *r* and c_0 is the spontaneous curvature. The k_c is the bending constant. A is the surface area, and K is the Gaussian curvature. With higher oil fractions, the oil droplets would alter the curvature which would be far from the well-packed status, that is a large $c_r - c_0$, which could be thermodynamically unfavorable as the free energy E_r would increase drastically. Therefore, droplets tend to reduce their surface area to compensate for the change of free energy and the interface is prone to distortion, therefore, pure Tween 80 exhibits poor emulsifying ability at low SOR (high oil content). By

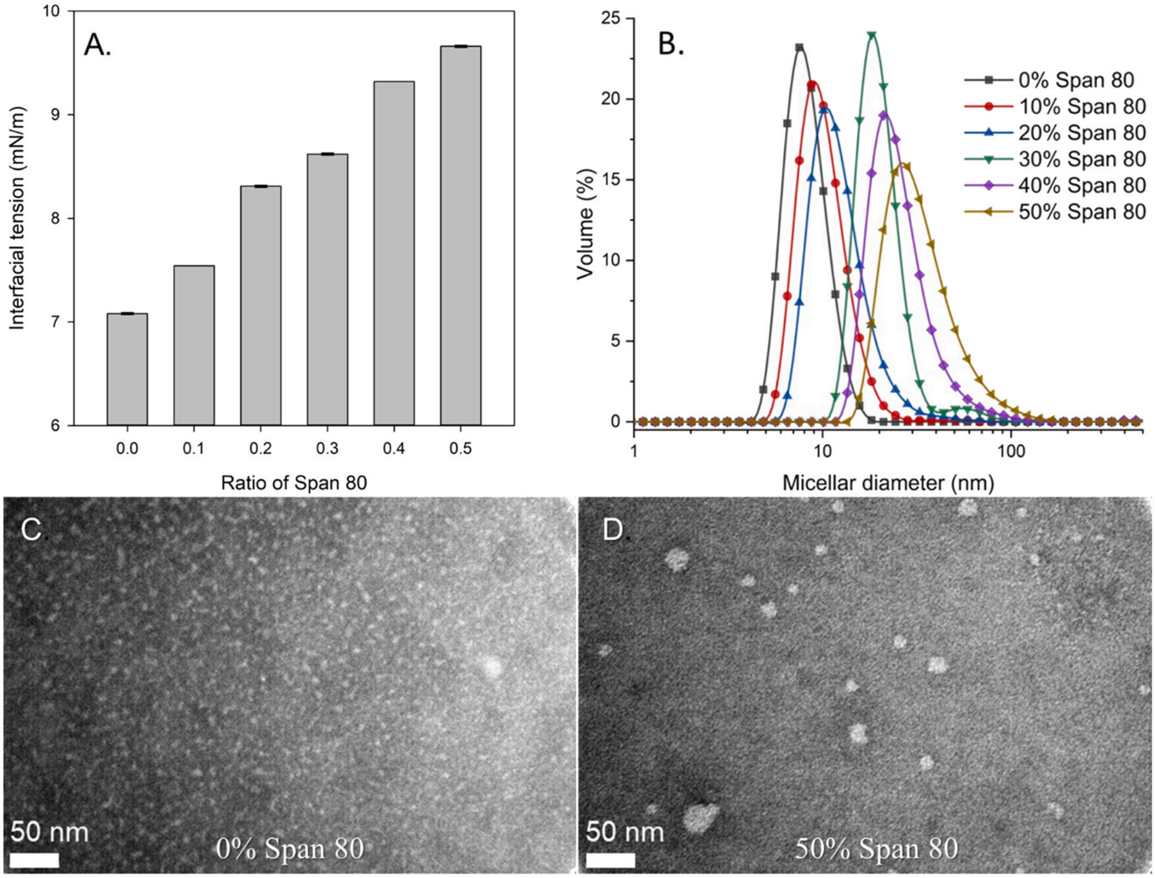

Fig. 3. (A) Interfacial tension between limonene and surfactant solutions (0.05 w/w % total surfactants); (B) micellar size of mixed surfactants; (C) TEM image of Tween 80 surfactants; (D) TEM image of surfactant surfactants with 50 % Tween 80 + 50 % Span 80 blending ratio.

contrast, for the mixed micelles (Tween 80:Span 80 = 1:1), at limited oil fraction, the droplets are larger than the micelles stabilized by Tween 80 itself due to the strong surfactant-surfactant interaction. The larger radius of the optimum curvature suggests, however, that the micelles provide a relatively large compartment to store organic molecules, which contributes to their increased resistance to droplet swelling, or a smaller c_r - c_0. Being closer to the spontaneous curvature, the emulsions with mixed surfactants were more stable against interfacial collapse, therefore they showed a lower polydispersity and higher oil loading capacity [28]. Manipulation of surfactant curvature not only showed effect on emulsion formation but were also reported as contributing to other applications, such as DNA condensation [29,30], where the electrostatic interaction between DNA and the cationic lipid membrane induces deformation and wrapping of the laminar membrane around the DNA rods. The charge density of the membrane serves as a modulator of its critical bending rigidity.

3.2. *Effect of oil composition on limonene emulsion*

Flavored oils include a wide variety of molecules with different hydrophobicities, solubility, partitioning coefficients, and diffusion kinetics, which can affect emulsion formation. Therefore, besides manipulation of the surfactant composition, we also investigated the impact of altering the composition of organic phase on emulsification. Menthol (log P = 2.66), as the typical beverage mint flavor and scent, has a similar molecular structure as limonene (log P = 3.22) which is also a 10-carbon monoterpene, but is more polar due to an alcohol group. Pure menthol is a crystalline solid and soluble in limonene due to the similar molecular structure. We used a 1:1 mixture of limonene to menthol as the oil phase in the following experiment.

When Tween 80 is used as the only surfactant, the pure limonene emulsions showed the main peak at ~10 nm with a certain volume of oil remaining larger than 100 nm; when 50 % menthol was blended with limonene the size peaks at 35.6 nm (Fig. 4A). When the oil content is doubled to 0.1 w/w % (Fig. 4B), the emulsion with limonene itself showed bimodal distribution with high turbidity, but incorporating menthol in the system the emulsion is clear with only one peak observed at a size similar to the group with low oil content (Fig. 4A). This suggests that menthol could also inhibit swelling when more oil is added. The reason for this could be due to the hydroxyl group on menthol that enables it to act as a co-surfactant. The interfacial tension of the pure limonene was 7.12 mN/m, but when 50 w/w % menthol was added, it plunged to 1.97 mN/m (Table 1). While the co-surfactants, alone, are not sufficient to emulsify the two phases, they do assist the main surfactant by anchoring it at the interface. With its smaller molecular size, menthol could insert between Tween 80 molecules and reduce the surfactant-surfactant interaction thus softening the interface, and therefore holding more oil without rupturing the interface because of improved flexibility [31].

Table 1
Interfacial tension between 0.05 % Tween 80 and different oil phase at 20 °C.

Oil phase	Tension (mN/m)
Limonene	7.12
Limoenen:menthol = 1:1	1.97
Limoenen:amyl acetate = 1:1	8.04

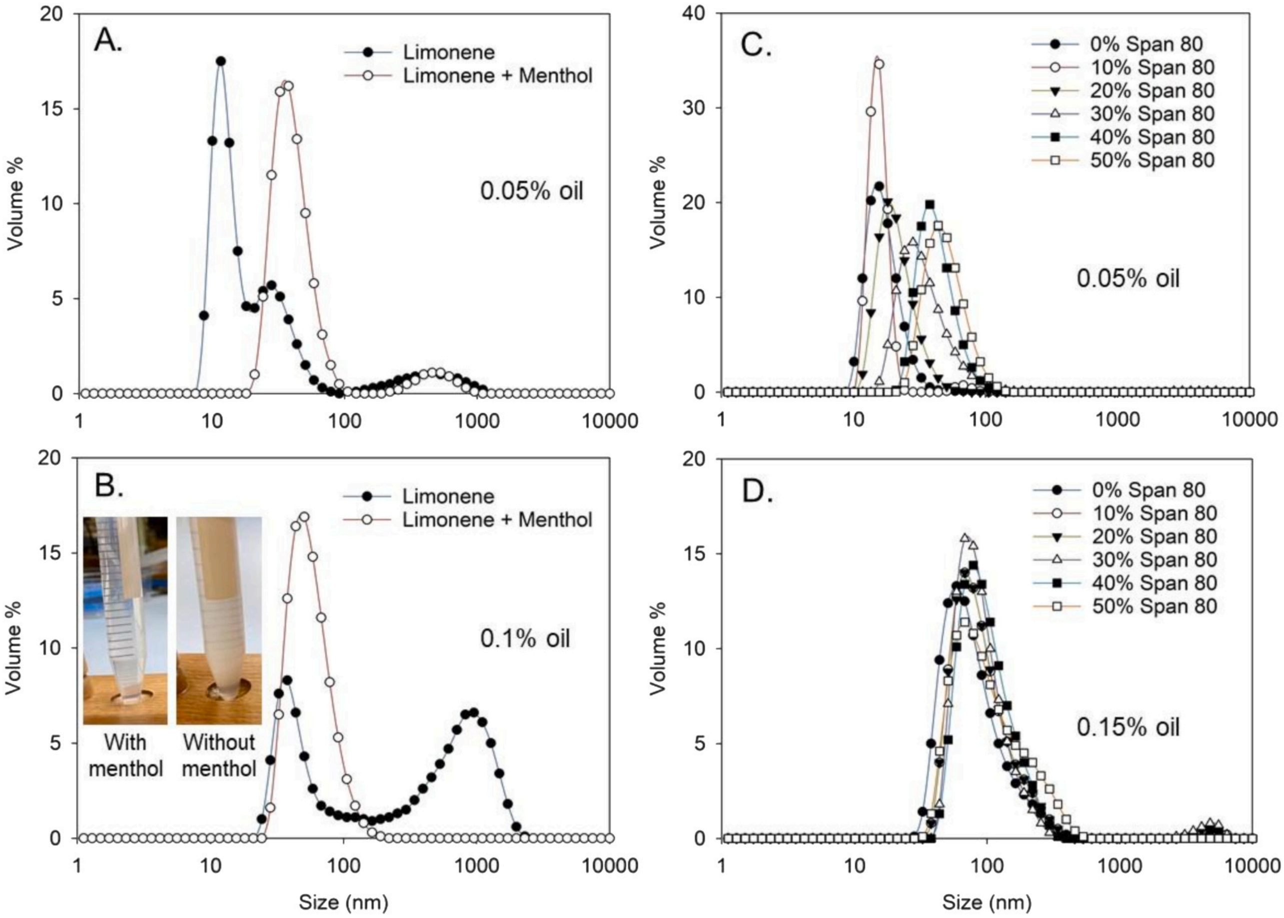

Fig. 4. Effect of menthol on droplet size distribution with 0.05 w/w % oil (A) and 0.1 w/w % oil (B), effect of Span 80 content on size distribution of menthol-limonene (1:1) emulsions with 0.05 w/w % oil (C) and 0.1 w/w % oil (D).

Taking HLB into consideration, at low SOR (oil content = 0.15 w/w %), the droplet size of the emulsion was not affected by the addition of hydrophobic surfactants as it functioned in the system with pure limonene (Fig. 4D). The reason could be that due to the presence of menthol, even without Span 80 the interface is flexible enough to encapsulate more nonpolar molecules and the system is not dominated by HLB. Several reports have demonstrated that blending hydrophobic long-chain triacylglycerols was able to improve emulsification and stability of flavored emulsions [32–34]. We explored a new approach and found that the addition of oil with higher polarity could also enhance loading capacity.

One might think the benefit of menthol is that its lower hydrophobicity (log P = 2.66) caused that menthol molecule to escape the droplets. To rule this out, amyl acetate, the main flavor compound in bananas, was blended with limonene (1:1). The molecular weight of amyl acetate was close to limonene and menthol, but the hydrophobicity was lower (log P = 1.53). The emulsions prepared by the blends of limonene and amyl acetate have higher loading capacity than those of limonene or limonene-menthol blends, which could be due to the lower hydrophobicity of amyl acetate (Fig. S2A). The ester-blended emulsion shows a similar HLB-dependent phenomenon as those with pure limonene: the emulsion droplet size increased dramatically with the increase of oil content, but droplet swelling was inhibited by blending hydrophobic surfactants. The groups emulsified by Tween 80 alone swelled to ~250 nm in average size when 0.15 w/w % of the mixed oil was added, but remained under 100 nm when 50 % Span 80 was used. The improvement was also found in PDI (Fig. S2B) where the droplets showed a wide range without the aid of hydrophobic surfactants (Fig. S2C) compared the groups with the mixed surfactants (Fig. S2D). Although both amyl acetate and menthol lower hydrophobicity, in the absence of Span 80, amyl acetate did not inhibit the enlargement of droplet size in the same way that menthol did. The reason could be that amyl acetate cannot serve as co-surfactant like menthol. Blending amyl acetate with limonene did not contribute to the lower interfacial tension (Table 1), which suggests that molecular structure does matter. The alcohol group of the menthol helped improve the interfacial flexibility similar to other alcohols as reported by others [31,35,36].

3.3. *Factors beyond hydrophobicity: structure of surfactant chains*

As discussed previously, the hydrophobicity of the surfactant controls the micellar size and this affects the resulting emulsion. In this section, we controlled the HLB keeping it the same throughout and investigated the effect of the chain structure of hydrophobic tails of surfactants. To formulate comparable surfactants, sorbitan-based surfactants with the same fatty-acid motifs were used (Fig. S1 and Table S1).

The 0.1 w/w % limonene emulsion stabilized by long-chain surfactants with 18 carbons exhibited wide droplet size distribution (Fig. 5 A) where a main peak is located at < 100 nm but also showed another peak at > 100 nm, which accounts for 30 % of total oil volume (Fig. S3). This is because that the small droplets in the first peak have a large surface area, which depletes the surfactants in the solution and causes the extra volume to stay in large droplets in order to compensate for lower the interfacial coverage. This bimodal distribution leads to a large average droplet size of emulsions stabilized by long-chain-surfactants over 400 nm (Fig. 5B) and a high PDI over 0.4 (Fig. 5 C). In contrast, the surfactants with shorter fatty-acid tails (laurate and palmitate) exhibited a monomodal distribution (Fig. 5 A), where the average droplets size is ~100 nm (Fig. 5B), and the PDI is lower than 0.2 (Fig. 5 C).

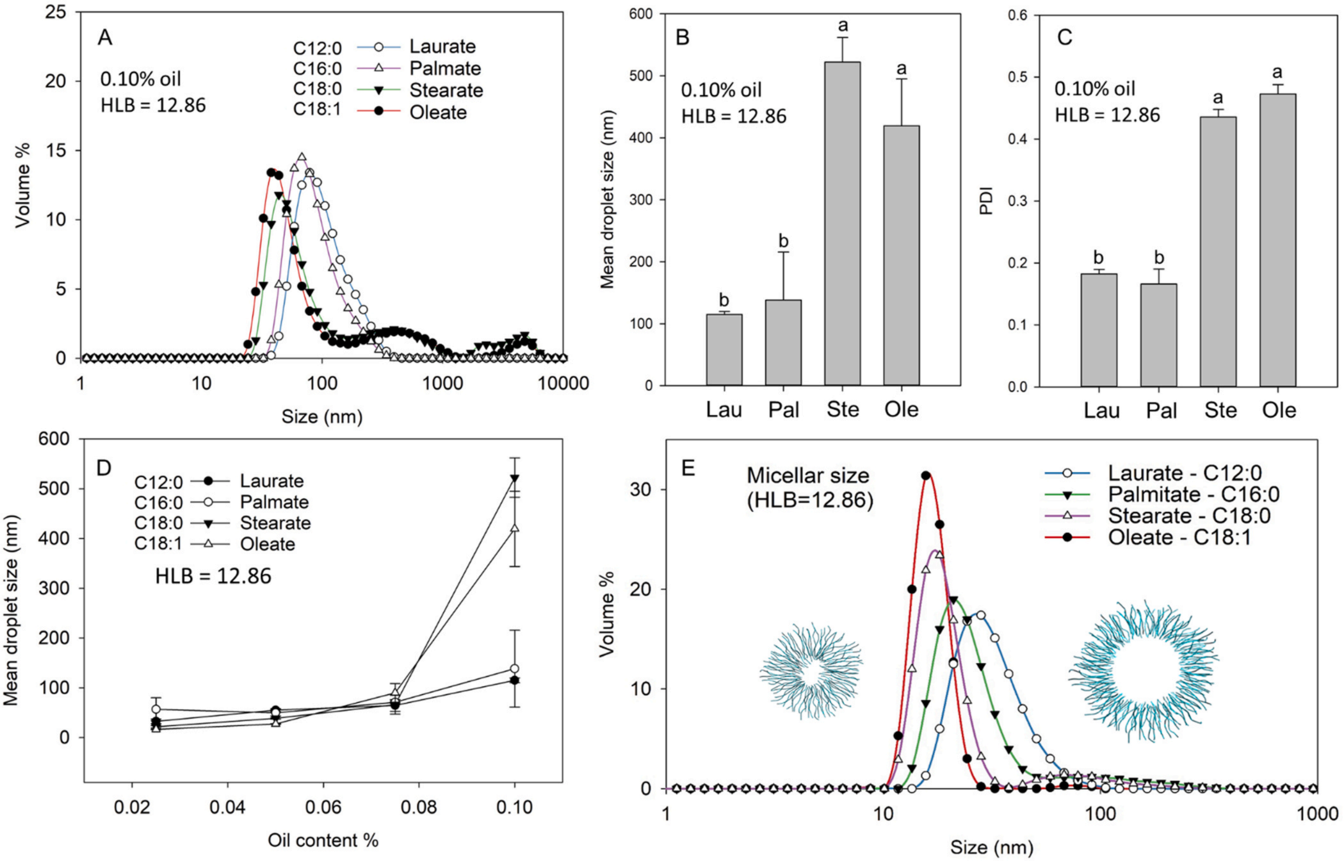

Fig. 5. (A) Droplet size distribution of 0.10 w/w % limonene emulsion stabilized by surfactants with different fatty-acid tails. (B & C) Mean droplet size and PDI of 0.10 w/w % limonene emulsion stabilized by surfactants with different fatty-acid tails. (D) Effect of surfactant with different fatty-acid tails on loading capacity. (E) Micellar size of surfactants with different fatty acids. The HLB of surfactants = 12.86.

The effect of surfactant chain length is affected by SOR (Fig. 5D): the surfactants with longer chains produced larger droplets at higher oil content, but produced smaller oil droplets at lower oil content (Fig. S4). In the absence of the oil phase, the micellar size was found to be smaller when the surfactant chain lengths are longer but still at the same HLB value (Fig. 5D). The longer chain length of the surfactant leads to a stronger interaction between surfactant molecules, causing the hydrophobic tails to aggregate and form micelles with a compact core. This limits the space within the core hindering the surfactant's ability to accommodate a large amount of oil. Consequently, adding more oil disrupts the interaction between surfactants and the curvature is altered far from the spontaneous curvature, that is, a high value of c_r - c_0, which is thermodynamically unfavorable (Eq. 1). As a result, oil emulsification is not efficiently achieved in this scenario with high oil loading. In contrast, the shorter-chain surfactants provided weaker interactions between surfactants, and their resulting larger micellar size means that the interface is more resistant to swelling which contributes to a higher oil loading capacity. Therefore, the length of surfactants is a factor beyond hydrophobicity that affects emulsifying activity.

Structural linearity is another characteristic of surfactant chain which is related to the degree of saturation of fatty-acid tails. For example, Tween 60 shows a more linear backbone with stearate than Tween 80 with oleate, though both tails are composed of 18 carbons and exhibit similar hydrophobicity. However, the emulsion was not significantly different when limonene was used as the organic phase (Fig. 5B). Therefore, to reveal the effect of surfactant linearity, canola oil and palm oil were used as the dispersed phase with different fat saturation, and the blends of Tween 80 and Tween 60 were used as surfactants (Fig. 6 A). Results showed that palm oil stabilized by Tween 80 by itself produced 61 nm droplets, but increasing the Tween 60 content in the surfactant blends reduced the size of the palm oil droplets, and a pure Tween 60 emulsion produced droplets as small as 21 nm. The turbidity of the mixtures also differed, where the palm oil emulsion stabilized by Tween 80 was cloudy, but the one emulsified with Tween 60 was clear (Fig. 6B). The opposite results were observed on emulsions of canola oil, where Tween 80 itself was able to produce a fine dispersion of droplets, but increasing the amount of Tween 60 produced larger droplets (Fig. 6 A). Therefore, a more linear chain surfactant is favorable to improve loading capacity of a saturated fat, whereas a kinked chain surfactant (unsaturated carbon chain) is favorable to improve the loading capacity of unsaturated oil (Fig. S5).

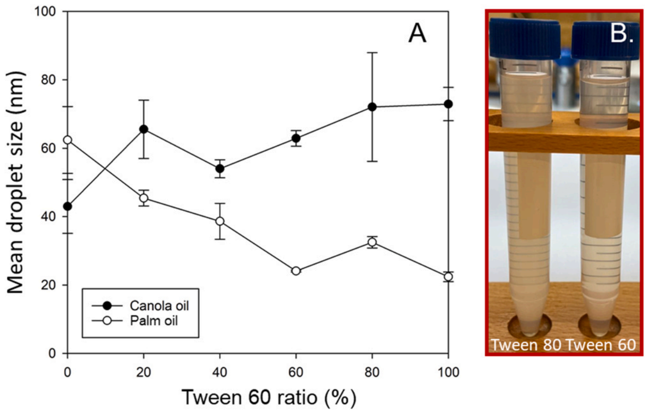

Fig. 6. (A) Droplet size of emulsions with 0.1 w/w % canola oil and palm oil stabilized the blends of Tween 60 (stearate) and Tween 80 (oleate) (total surfactant = 0.05 w/w %). (B) Clarity of 0.1 w/w % palm oil emulsions stabilized by Tween 80 and Tween 60, respectively.

4. Conclusion

This study demonstrated that more efficient emulsification can be accomplished by tailoring the oil-water interface. Hydrophilic Tween 80 by itself produced smaller droplets at low oil content, and large droplets at high oil content due to the unfavorable curvature formation. By contrast, combinations with more hydrophobic surfactants contributed to the formation of clear emulsions with uniform droplets and a higher loading capacity. This study demonstrated that surfactant hydrophobicity, which facilitates the formation of small droplets, is impacted by SOR and has been overlooked by previous studies. The surfactants with altered optimum curvature produced larger cavities which allowed for the encapsulation of higher volumes of oil without interfacial collapse.

Further, we reported that the hydrophobicity is not the only factor that determines emulsifying activity. When the HLB value is kept the same, surfactants with shorter chains showed higher loading capacity than those with longer chains. In addition to chain length, surfactants with linear backbones contributed to emulsification of saturated oil, and vice versa. This systematic study addressed the research gap by controlling variables step-by-step, thus helping to uncover the roles of SOR and chain structure on emulsifying properties. This work provides a blueprint for the design of emulsion systems with minimum surfactant dosage, while retaining desirable properties, that can be applied to various industries, including foods, cosmetics, and pharmaceuticals. Future studies are planned to explore other surfactants to show the ubiquity of this model and to further explore the impacts of factors such as processing conditions and oil composition on emulsifying properties.

CRediT authorship contribution statement

Peilong Li[1], Conceptualization, Methodology, Investigation, Writing - Original Draft, Project administration. Hao Huang[1], Investigation, Writing - Review & Editing. Yuan Fang[2], Writing - Review & Editing, Funding acquisition, Project administration. Yu Wang[2], Writing - Review & Editing, Project administration. Da Som No[2], Writing - Review & Editing. Rohil S. Bhatnagar[2], Writing - Review & Editing. Alireza Abbaspourrad[1]*, Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Data Availability

Data will be made available on request.

Acknowledgements

This work was funded by PepsiCo, Inc. Authors also thank Kelley J. Donaghy for editing this manuscript. This study made use of the Cornell Center for Materials Research Shared Facilities which are supported through the NSF MRSEC program (DMR-1719875).

Disclaimer

The views expressed in this manuscript are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2023.132242.

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