Fabrication of oil-in-water nanoemulsions by dual-channel microfluidization using natural emulsifiers: saponins, phospholipids, proteins, and polysaccharides, by Bai et al

Graphical Abstract

**Fabrication of oil-in-water nanoemulsions by dual-channel
microfluidization using natural emulsifiers: saponins,
phospholipids, proteins, and polysaccharides**

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Abstract

Nanoemulsions are utilized within the food, pharmaceutical, and personal care industries because of their unique physicochemical properties and functional attributes: high optical clarity; prolonged stability; and, enhanced bioavailability. For many applications, it is desirable to utilize natural ingredients to formulate nanoemulsions so as to create “label-friendly” products. In this study, we compared the effectiveness of a number of natural emulsifiers at fabricating corn oil-in-water nanoemulsions using dual-channel microfluidization. These emulsifiers were either amphiphilic biopolymers (whey protein and gum arabic) or biosurfactants (quillaja saponin and soy lecithin). Differences in the surface activities of these emulsifiers were characterized using interfacial tension measurements. The influence of emulsifier type, concentration, and homogenization pressure on the efficiency of nanoemulsion formation was examined. The long-term stability of the fabricated nanoemulsions was also monitored during storage at ambient temperature. For all of the natural emulsifiers, nanoemulsions could be produced by dual-channel microfluidization, with the mean particle diameter decreasing with increasing emulsifier concentration and homogenization pressure. Whey protein isolate and quillaja saponin were more effective at forming nanoemulsions containing fine droplets than gum arabic and soy lecithin, with a lower amount of emulsifier required and smaller droplets being produced. This effect was attributed to faster emulsifier adsorption and a greater reduction in interfacial tension leading to more efficient droplet disruption within the homogenizer for saponins and whey proteins. This study highlights the potential of dual-channel microfluidization for efficiently producing label-friendly nanoemulsions from natural emulsifiers.

Keywords: natural emulsifier; dual-channel microfluidization; nanoemulsions; saponins; proteins; phospholipids

1. Introduction

Emulsion-based delivery systems are being increasingly investigated for their potential applications within the food, supplement and pharmaceuticals industries to encapsulate, protect, and release non-polar bioactive compounds, such as polyunsaturated (ω -3) oils, flavonoids, carotenoids, vitamins, and hydrophobic drugs (Ganta, Talekar, Singh, Coleman, & Amiji, 2014; McClements, Decker, & Weiss, 2007; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). Oil-in-water nanoemulsions are particularly promising candidates for the efficient delivery of lipophilic bioactive compounds due to their ease of fabrication, small particle size, and high surface-to-volume ratio (McClements, 2012; Sonnevile-Aubrun, Simonnet, & L'alloret, 2004). These attributes can result in high optical clarity, prolonged physical stability, and enhanced bioavailability of encapsulated compounds (McClements, 2012; Sonnevile-Aubrun, et al., 2004). Oil-in-water nanoemulsions are non-equilibrium, heterogeneous systems containing small oil droplets (diameter < 200 nm) dispersed within an aqueous phase (Wooster, Golding, & Sanguansri, 2008). Emulsifiers play a key role in the development of successful nanoemulsion formulations because they facilitate the formation of small droplets during homogenization by adsorbing to the oil-water interface and reducing the interfacial tension (Schubert & Engel, 2004b), and also protect the droplets from aggregation after their formation by generating repulsive interactions (usually steric or electrostatic) between the droplets (Tadros, Izquierdo, Esquena, & Solans, 2004; Wooster, Labbett, Sanguansri, & Andrews, 2016). A large number of emulsifiers are available for the formulation of edible emulsions and nanoemulsions, such as polysaccharides, proteins, phospholipids, natural extracts, and synthetic surfactants (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016; Mirhosseini, Tan, Hamid, & Yusof, 2008). The selection of an emulsifier for a particular application depends on its molecular and physicochemical properties, as well as its ease of utilization, legal status,

and cost (Kralova & Sjöblom, 2009). Consequently, it is necessary to compare the functional properties of emulsifiers under similar conditions so that manufacturers can select the most appropriate one for a specific product.

Recently, consumers have been demanding products that contain “label friendly” ingredients. Therefore, identifying, developing, and utilizing natural emulsifiers that can be successfully used in edible emulsion products to replace synthetic alternatives has attracted considerable attention (Ozturk & McClements, 2016). Natural biopolymer-based emulsifiers (such as proteins and polysaccharides) have been shown to be effective high molecular weight surface-active agents for forming and stabilizing oil-in-water nanoemulsions (Charoen, et al., 2011; Teo, et al., 2016). In addition, biosurfactants (such as phospholipids and saponins) have also been shown to be effective low-molecular weight emulsifiers (Ozturk, Argin, Ozilgen, & McClements, 2014; Yang, Leser, Sher, & McClements, 2013a).

In this study, we compared the ability of four natural emulsifiers to form and stabilize nanoemulsions prepared using a dual-channel microfluidizer: whey protein isolate (WPI); gum arabic; quillaja saponins; and, soy lecithin. WPI is a protein-based natural emulsifier that is widely used in the food industry (Adjonu, Doran, Torley, & Agboola, 2014). WPI contains a mixture of globular proteins that typically form a relatively thin coating around lipid droplets, and so the steric repulsion between them is relatively short range. As a consequence, the main mechanism inhibiting the aggregation of WPI-coated oil droplets is usually electrostatic repulsion (rather than steric repulsion), which means this type of emulsion is particularly sensitive to pH and ionic strength (McClements, 2004). Moreover, WPI-coated oil droplets are also highly sensitive to temperature because irreversible thermal denaturation of the adsorbed proteins increases the surface hydrophobicity of the droplets, thereby increasing the hydrophobic attraction between them (Kim, Decker, & McClements, 2002; Lam & Nickerson, 2013). Gum arabic is a glycoprotein derived from the acacia Senegal tree that contains three major

fractions (Williams & Phillips, 2009). It is one of the most widely used amphiphilic biopolymers to stabilize food emulsions, particularly soft drinks (Ozturk, Argin, Ozilgen, & McClements, 2015). The emulsifying ability of gum arabic can be attributed to a hydrophobic protein fraction that is covalently linked to hydrophilic carbohydrate fractions (Chanamai & McClements, 2001; Williams, et al., 2009). The hydrophobic part penetrates into the oil phase and therefore anchors the gum arabic molecules to the oil droplet surfaces, whereas the hydrophilic part protrudes into the surrounding aqueous phase inhibiting droplet aggregation through a combination of steric and electrostatic repulsion (Chanamai & McClements, 2002). Quillaja saponin is a natural extract isolated from the bark of the *Quillaja saponaria* Molina tree (Mitra & Dungan, 1997). The major surface-active components within this extract have been identified to be saponins, which are a kind of glycoside consisting of a sugar moiety attached to a triterpene or steroid aglycone (Mitra, et al., 1997). Quillaja saponin is an effective emulsifier because it contains hydrophobic parts (such as triterpenoid rings) that anchor it to oil droplet surfaces and hydrophilic parts (such as carbohydrate groups) that protrude into the aqueous phase and generate a steric repulsion (Pagureva, et al., 2016; Stanimirova, et al., 2011; Yang, Leser, Sher, & McClements, 2013b). Soy lecithin is a natural emulsifier derived from the cell membranes of soybeans (Klang & Valenta, 2011). Naturally, soy lecithin contains a polar head group and two non-polar tails, which means that it is good for forming bilayers and vesicles, but not particularly good at forming emulsions (Kralova, et al., 2009; Weete, Betageri, & Griffith, 1994). However, it can be chemically, physically, or enzymatically modified to improve its ability to stabilize oil-in-water nanoemulsions (Weete, et al., 1994).

Microfluidization is a widely used and highly efficient method of producing nanoemulsions containing small droplets (Lee & Norton, 2013; McClements, 2011). Conventional microfluidizers typically use a two-step procedure to produce nanoemulsions: (i) a coarse emulsion is created using a high shear mixer; (ii) this coarse

emulsion is passed through the microfluidizer to reduce the particle size (Galooyak & Dabir, 2015; Håkansson, et al., 2011; Mahdi Jafari, He, & Bhandari, 2006; McClements, 2015; Schultz, Wagner, Urban, & Ulrich, 2004). Studies have shown that the mean particle size and polydispersity index produced by a two-step microfluidizer tend to be smaller when synthetic surfactants are used rather than natural ones (Perrier-Cornet, Marie, & Gervais, 2005; Zhang, Peppard, & Reineccius, 2015). In addition, higher levels of natural emulsifiers are typically required to produce small droplets (He, et al., 2011), which often increases formulation costs. This effect can be attributed to two main physicochemical phenomena. First, the slower adsorption rate of some natural emulsifiers provides less inhibition against the re-coalescence of oil droplets after they are disrupted inside the microfluidizer (Jafari, Assadpoor, He, & Bhandari, 2008). Second, oil droplets coated by some natural emulsifiers are more difficult to break down within a homogenizer because they give a higher interfacial tension and elasticity (Mackie, Ridout, Moates, Husband, & Wilde, 2007). In particular, molecular rearrangements and cross-linking of emulsifiers may occur at the droplet surfaces between preparing the initial coarse emulsion premix, and then passing it through the microfluidizer, which may reduce the efficiency of droplet disruption during homogenization. Therefore, it is important to develop an efficient method to manufacture natural emulsifier-stabilized nanoemulsions containing small droplets.

In this study, a one-step dual-channel microfluidization method (Bai & McClements, 2016) was utilized to produce natural emulsifier-stabilized oil-in-water nanoemulsions by separately feeding oil phase and aqueous phase (containing emulsifiers) into the microfluidizer (Fig. 1a). An advantage of this method is that the premix step required to form a coarse emulsion is not required, which reduces the possibility of forming natural emulsifier-coated oil droplets that are resistant to droplet disruption inside the homogenizer. In addition, this technique only requires a single pass of material through the microfluidizer (rather than multiple passes for two-step microfluidizers), which would

make it more energy efficient (Stang, Schuchmann, & Schubert, 2001).

The objective of this study was therefore to manufacture oil-in-water nanoemulsions utilizing natural emulsifiers using a dual-channel microfluidization method. The information obtained from this study is important for the design and manufacture of “label friendly” nanoemulsions suitable for utilization within the food, supplement, pharmaceutical, and personal care industries.

2. Experimental

2.1. Materials

Corn oil (Mazola) was purchased from a local grocery store. Whey protein isolate (WPI) was provided by Davisco Foods International Inc. (Le Sueur MN). Gum Arabic was provided by TIC Gums (Belcamp, MD). Quillaja saponin (Q-Naturale[®]) was provided by Ingredion Inc. (Westchester, IL). Soy lecithin (Ultralec[®]F, defatted) was provided by Archer Daniels Midland Co. (Decatur, IL). Sodium phosphate monobasic and sodium azide (a non-food grade antimicrobial) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Double distilled water (Milli-Q) was used to prepare all solutions and nanoemulsions.

2.2. Emulsion preparation

Oil-in-water nanoemulsions were prepared by homogenizing 10 wt% oil phase (corn oil) with 90 wt% aqueous phase (0.1-10 wt% emulsifier in buffer solution). The aqueous phase consisted of emulsifier (WPI, gum Arabic, Q-Naturale, or soy lecithin) and buffer solution (10 mM sodium phosphate, pH7.0). Q-Naturale contained 14 wt% active saponins (with the remainder being mainly water), and so the concentration of this emulsifier is reported based on an active ingredient basis (rather than total mass basis). The dual-channel microfluidization method was used to manufacture nanoemulsions (Fig.

1a). The oil phase and aqueous phase were fed into two different 100 ml glass reservoirs. Fine nanoemulsions were formed by simultaneously forcing oil phase and aqueous phase through an air-driven high-pressure microfluidizer (Microfluidics PureNano, Newton, MA, USA) with a Y- and Z-type interaction chamber (Fig. 1b) for one pass at various homogenization pressures (9-19 kpsi). The Y-type chamber was placed before the Z-type chamber so that the former facilitates the initial formation of a coarse emulsion, and then the latter is mainly responsible for generating small droplets. The interaction chambers were immersed in the water-ice mixture to keep the temperature inside the interaction chamber constant. After the formation, 0.03 wt% sodium azide was added to the nanoemulsions and emulsions formed as an antimicrobial agent.

2.3. Determination of nanoemulsion composition

According to our previous publication (Bai, et al., 2016), the composition of nanoemulsions fabricated by the dual-channel microfluidization method was determined by the flow rate ratio of oil phase (f_{oil}) and aqueous phase (f_{aqu}). Briefly, the total flow rate of the microfluidizer was set as 500 ml per minute, and the flow rate of aqueous phase was adjusted by the flow rate of f_{oil} which was predefined before operation, namely if the intensifier pump was used to pressurize both aqueous phase and oil phase glass reservoirs simultaneously, the flow rate of f_{aqu} was $500 - f_{oil}$. Since the flow rates were based on volume, the density of the oil phase (ρ_{oil}) and aqueous phase (ρ_{aqu}) were needed for determination of the final nanoemulsion composition on a weight basis. The density of oil and aqueous phases were measured according to our previous method (Komaiko & McClements, 2015), and the measured densities are reported in Table 1. The composition of the nanoemulsions in mass ratio could therefore be determined using Equations (1) and (2) (Bai, et al., 2016).

$$f_{oil} + f_{aqu} = 500 \quad (1)$$

$$\frac{f_{oil} \times \rho_{oil}}{f_{aqu} \times \rho_{aqu}} = \frac{m_{oil}}{m_{aqu}} \quad (2)$$

where m_{oil} and m_{aqu} are the mass flows of oil phase and aqueous phase, respectively.

The weight percentage of oil phase can then be calculated: $\phi_m = 100/(1+m_{aqu}/m_{oil})$.

Table 1 Densities of oil phase and aqueous phase used to prepare nanoemulsions by the dual-

channel microfluidization method.^a

Type	Density (kg/m ³)
Corn oil	908 ± 3
Whey protein isolate solutions	1000 ± 0 (0.1-3%) ^b , 1001 ± 1 (5%), 1002 ± 3 (10%)
Gum Arabic solutions	1000 ± 2 (0.1-3%), 1028 ± 3 (5%), 1041 ± 2 (10%)
Q-Naturale solutions	1000 ± 0 (0.1-3%), 1022 ± 4 (5%), 1045 ± 1 (10%)
Soy lecithin solutions	1000 ± 4 (0.1-3%), 1009 ± 2 (5%), 1014 ± 4 (10%)

^a The physiochemical properties were measured at ambient temperature (20 °C).

^b % represent the concentrations of emulsifiers used in the emulsion formulations.

2.3. Particle characterization

The particle size distribution and mean particle diameter of diluted nanoemulsions were measured using a static light scattering instrument (Mastersizer 2000, Malvern Instruments, Malvern, UK). The nanoemulsions were diluted with buffer solutions of the appropriate pH prior to analysis to avoid multiple scattering effects. The refractive indices (RI) of the oil and aqueous phases used in the calculations were 1.47 and 1.33, respectively. The droplet diameter of each sample was represented as the surface-weighted mean diameter (d_{32}), which was calculated from the full particle size distribution. To determine the width of the particle size distribution, the span (also known as polydispersity) was calculated from the following equation (3).

$$\text{Span} = \frac{(d_{90} - d_{10})}{d_{50}} \quad (3)$$

where d_{90} , d_{10} , and d_{50} are the particle diameters at 10, 50, and 90% cumulative volume,

respectively. In other words, $d_{90} - d_{10}$ is the range of the data and d_{50} is the median diameter.

2.4. Interfacial tension measurements

The interfacial tension *versus* emulsifier concentration profiles of the natural emulsifiers were measured at the corn oil-water interface using a drop shape analysis instrument (DSA 100, Kruss GmbH, Hamburg Germany). Initial experiments were carried out to determine the time at which the interfacial tension reached a constant value over a limited timescale (less than 15 min) or the drop completely detached from the needle. We then used an incubation time of 15 min or complete detachment of oil drop for all of the subsequent experiments. The emulsifier concentration range used was from 0.0005% to 2.0% since over 2.0% many of the emulsifier solutions were not sufficiently transparent to make reliable measurements. The surface pressure (π) was calculated from the interfacial tension (γ) data using the following equation: $\pi = \gamma_0 - \gamma$, where γ and γ_0 are the interfacial tensions measured in the presence and absence of emulsifiers.

2.5. Nanoemulsion storage stability

Nanoemulsion samples were placed in glass test tubes and then incubated in ambient temperature (20 °C) for at least one month prior to analysis. The particle distribution of the samples was then measured by light scattering as described earlier, and the creaming stability was determined by visual observation.

2.6. Statistical analysis

All measurements were performed on three freshly prepared samples with at least two measurements per sample and are reported as means and standard deviations.

3. Results and discussion

3.1. Interfacial characteristics

The interfacial characteristics of emulsifiers play an important role in determining their ability to form and stabilize emulsions (Rodríguez Patino, Rodríguez Niño, & Sánchez, 1999). We therefore measured the interfacial tension *versus* emulsifier concentration profiles for the four natural emulsifiers used (Fig. 2). A representative image of one of the oil droplets used to measure the interfacial tension using drop shape analysis is shown in Fig. 2b.

For all of the emulsifiers, the interfacial tension decreased with increasing emulsifier concentration, which indicated that the emulsifiers adsorbed to the oil-water interface (Fig. 2a). However, there was a significant difference in the ability of the different emulsifiers to lower the interfacial tension. For the two biopolymers, a lower interfacial tension was reached for WPI (9 mN/m) than for gum arabic (16 mN/m) at high emulsifier concentrations. The quillaja saponin gave the steepest initial decrease in interfacial tension with increasing emulsifier concentration, and gave the lowest interfacial tension (5 mN/m) at high emulsifier levels. For this emulsifier, the interfacial tension reached a relatively constant value at high saponin levels indicating that the interfaces had become saturated with emulsifier (Hunter, 2001). Notably, when the concentration of soy lecithin was more than 0.03%, the solutions became so opaque that the DSA instrument could not make reliable measurements. Nonetheless, the measurements made at lower soy lecithin concentrations indicated that it was not as effective as WPI or Q-Naturale at reducing the interfacial tension.

The apparent critical micelle concentration (CMC) of emulsifiers is a useful reference to characterize and compare the emulsifying ability of different emulsifiers. CMC can be determined from the interfacial tension *versus* logarithm of emulsifier

concentration profiles using a method described previously, *i.e.*, determining the intersect between linear plots extrapolated from high and intermediate surfactant concentrations (Varona, Martín, & Cocero, 2009). We therefore estimated the CMC values of selected natural emulsifiers from the data shown in Fig. 2a: 0.3% and 0.01 wt% for WPI and Q-Naturale, respectively. These values are in reasonable agreement with reported values of 0.1% for WPI and 0.01 to 0.07 wt% for quillaja saponin from various sources (Jung, Gunes, & Mezzenga, 2010; Mitra, et al., 1997). For gum arabic and soy lecithin, the CMC could not be estimated due to the lack of interfacial tension data over a wide enough concentration range.

The surface activity (K) of an emulsifier can be conveniently characterized by the following relationship (McClements, 2015):

$$K = \frac{1}{c_{1/2}} = e^{-\frac{\Delta G_{ads}}{RT}} \quad (4)$$

where $c_{1/2}$ is the emulsifier concentration where half of the available adsorption sites on the oil-water interface are covered by emulsifier, and ΔG_{ads} is the free energy change associated with emulsifier adsorption. To a rough approximation, $c_{1/2}$ can be taken to be equal to the emulsifier concentration where the surface pressure is half its maximum value (π_{∞}) (Fig. S1). In this study, we calculated the following parameters from the interfacial pressure *versus* emulsifier concentration profiles: for WPI, $c_{1/2}$ = 0.08 wt% (43.5 μ M), K = 23,000, and ΔG_{ads} = -10.0 RT ; for gum arabic, $c_{1/2}$ = 2 wt% (110 μ M), K = 9,100, and ΔG_{ads} = -9.1 RT ; for Q-Naturale, $c_{1/2}$ = 0.003 wt% (18.2 μ M), K = 55,000, and ΔG_{ads} = -10.9 RT . The molecular weights of WPI, gum arabic, and Q-Naturale were 18,400 g/mol, 180,000 g/mol and 1650 g/mol respectively, which were obtained from published values (Duvallet, Fenyo, & Vandeveld, 1989; Mitra, et al., 1997). The value for the soy lecithin could not be calculate since there was insufficient data. It should also be noted that for the WPI and gum arabic the surface pressure had not fully reached

saturation levels (Fig. S1), and therefore the $c_{1/2}$ values should only be treated as rough estimates.

Notably, it should be emphasized that the natural emulsifiers used in this study are actually compositionally complex materials that may contain a mixture of different surface-active components with different surface activities. Consequently, the reported CMC and K values are only rough estimates rather than accurate values associated with a particular molecular species. Overall, our measurements show that all of the emulsifiers were effective at reducing the interfacial tension at an oil-water interface, but that there were appreciable differences in their interfacial properties (Fig. 2a).

3.2. Influence of emulsifier type and concentration on nanoemulsion formation

Initially, the influence of emulsifier type and concentration on the formation of oil-in-water nanoemulsions produced using the dual-channel microfluidization method was investigated (Fig. 3). Overall, there was a decrease in mean particle diameter (d_{32}) with increasing emulsifier concentration for all emulsifier types. This effect can be attributed to two main factors: (i) at higher emulsifier concentrations, there are more emulsifier molecules available to cover the oil droplet surfaces during homogenization; (ii) a higher emulsifier concentration results in faster emulsifier adsorption to the oil droplet surfaces, thereby providing more effective protection against re-coalescence (Ziani, Barish, McClements, & Goddard, 2011). In general, the dependence of the mean droplet diameter on emulsifier concentration can be divided into two regions: Region I - the particle size decreases with increasing emulsifier concentration because there is more emulsifier available to cover all the newly created surfaces formed when the oil droplets are broken up by the homogenizer; Region II - the droplet diameter remains fairly constant because there is sufficient emulsifier to cover all the oil droplets surfaces, but the homogenizer cannot produce any smaller droplets (McClements, 2015; Tcholakova, Denkov, & Danner, 2004). From Fig. 3, it can be observed that the droplet diameter *versus* emulsifier

concentration profiles for the nanoemulsions stabilized by WPI and Q-Naturale followed this two-region process. The minimum amount of emulsifier required to create droplets with relatively small constant diameters was about 1.0% for WPI and 1.0 % for Q-Naturale, and the corresponding droplet diameters were 0.132 and 0.139 μm respectively. On the other hand, the dependence of droplet diameter of nanoemulsions stabilized by gum arabic and soy lecithin on the emulsifier concentration was only fitted in Region I, suggesting that the droplet diameter was limited by emulsifier type for gum arabic and soy lecithin throughout the emulsifier concentration range. The minimum droplet diameters at 10% gum arabic and 10% soy lecithin were 0.142 and 0.197 μm , respectively.

The differences in the minimum amount of emulsifier required to reach a constant droplet diameter (C_{min}), and in the minimum droplet diameter produced (d_{min}) can be ascribed to differences in the nature of the emulsifiers. For WPI and Q-Naturale, the C_{min} and d_{min} values were relatively low because they both have relatively high surface-activities and give low interfacial tensions, thereby promoting droplet disruption by reducing the Laplace pressure. In addition, they are relatively small molecules that would be expected to rapidly adsorb onto the oil droplet surfaces during homogenization, thereby reducing the possibility of droplet re-coalescence (Schubert & Engel, 2004a). For gum arabic, the relatively high C_{min} and d_{min} can be attributed to the slow adsorption of large gum arabic molecules, lower surface-activity, and higher interfacial tension (Chanamai, et al., 2001). Interestingly, the droplet diameter variation for soy lecithin was abnormal. Soy lecithin is a low molecular weight emulsifier, however, its C_{min} and d_{min} were comparable to those of gum arabic. We speculated that this anomaly was caused by the poor water solubility of soy lecithin, and its tendency to form vesicles in solution. Indeed, insoluble substances were observed in the soy lecithin solutions when it was used at concentrations of 1% and above. Consequently, the soy lecithin used in this study was not a particularly effective emulsifier for the creation of oil-in-water nanoemulsions. In

future studies, it would be useful to measure the adsorption kinetics of the different emulsifiers under conditions that simulate those inside the homogenizer.

The surface load of the emulsifier at saturation (Γ) is an important indicator to better understand the formation process of natural emulsifier-stabilized nanoemulsions. When the droplet diameter is limited by the amount of emulsifier, the minimum emulsifier concentration required to cover all the droplets produced is given by (McClements, 2007):

$$C_s = \frac{6 \times \Gamma \times \varphi}{d_{32min}} \quad (5)$$

where C_s is the concentration of emulsifier in the emulsion (kg/m^3), Γ is the surface load of the emulsifier at saturation (kg/m^2), φ is the dispersed phase volume fraction (dimensionless), and d_{32min} is the smallest droplet diameter that can be produced by the homogenizer under specific operating conditions (m). This equation was used to calculate the surface load of the emulsifiers from knowledge of the oil droplet concentration ($\varphi = 0.1$), the emulsifier concentration (0.1 to 10%, or 1 to 100 kg/m^3), and the measured mean droplet diameters (d_{32min}). The calculated surface loads of WPI, Q-Naturelle, gum arabic, and soy lecithin were 2.2, 2.3, 24.8, and 32.8 mg/m^2 , respectively. These calculations indicate that the surface loads of WPI and Q-Naturelle are fairly similar but about ten times smaller than that of gum arabic and lecithin, which can be attributed to differences in the molecular weight and packing of the emulsifier molecules at the oil-water interface. WPI and Q-Naturelle would be expected to form thin monolayers of relatively small emulsifiers. Gum arabic would be expected to form thick monolayers of relatively large emulsifiers. Soy lecithin may have formed multiple layers of phospholipid bilayers around the lipid droplets, or it may have formed some non-adsorbed vesicles in the aqueous phase. The surface loads determined in our study are in reasonable agreement with those reported in previous publications using two-step single channel microfluidization (Berton-Carabin & Schroën, 2015; Ozturk, et al., 2014, 2015).

The nature of the particle size distribution is also important for many practical applications of nanoemulsions. We therefore plotted the particle size distribution of

nanoemulsions fabricated with different natural emulsifiers using the dual-channel microfluidization method (Fig. 4). The particle size distribution produced by the dual-channel method was monomodal at all emulsifier concentrations studied (except for when 0.1% WPI was used), indicating that it was possible to form small uniform droplets with a single pass through the dual-channel microfluidizer.

Previous studies have shown that the droplet diameters in emulsions produced by high-pressure homogenization depend on processing conditions, *e.g.*, homogenization pressure (Karbstein & Schubert, 1995; Marie, Perrier-Cornet, & Gervais, 2002; Schultz, et al., 2004). We therefore examined the influence of homogenization pressure on the size of the oil droplets produced by dual-channel microfluidization method using natural emulsifiers. Soy lecithin was not used in these studies because it was not particularly effective at forming small droplets (Section 3.2). The effect of homogenization pressure on the mean particle diameter of nanoemulsions fabricated by the dual-channel method was determined for systems containing 1 wt% WPI, 10% gum arabic or 1% Q-Naturale, respectively (Fig. 5). Overall, there was a decrease in mean droplet diameter with increasing operating pressure for all of the emulsifiers, which can be attributed to an increase in the magnitude of disruptive energy generated by the homogenizer (Schultz, et al., 2004). At the highest pressure used (19 kpsi), the mean droplet diameters were fairly similar for WPI (0.125 μm) and Q-Naturale (0.123 μm), and only slightly higher for gum arabic (0.135 μm).

For Q-Naturale there was a fairly steady decrease in mean droplet diameter with increasing homogenization pressure (Fig. 5), and a linear relationship between the logarithm of the mean droplet diameter and the logarithm of the pressure, *i.e.*, LogD *versus* LogP (Fig. S2). However, for the WPI and gum arabic, the decrease in droplet diameter with increasing pressure became less steep at higher homogenization pressures, and the LogD *versus* LogP relationship was non-linear (Fig. S2). This phenomenon may have occurred because there was insufficient emulsifier present to cover all the newly

formed droplet surfaces at high pressures for these emulsifiers, or because of some change in emulsifier properties (such as denaturation or hydrolysis) at high pressures (Walstra, 1993).

The relationship of the logarithm of the mean droplet diameter to the logarithm of the homogenization pressure for the nanoemulsions is shown in the supplementary material (Fig. S2). The slope determined from the plots was around -0.24 to -0.34 for the various emulsifiers. These values are lower than previously reported values of around -0.6 to -0.8 for high-pressure homogenizers when the fluid flow conditions are primarily turbulent-inertial (Walstra, 2002). This suggests that either droplet breakup was not primarily due to turbulent-inertial forces in the dual-channel method, or that the retardation of droplet breakup or significant re-coalescence at higher pressures for these natural emulsifiers were indeed occurred.

The particle size distributions of nanoemulsions produced using different emulsifiers at different pressures were measured (Fig. 6). For all of the emulsifiers, the particle size distributions of the nanoemulsions were monomodal at all test pressures, and the width of the distribution decreased with increasing pressure (Table S1). These results again indicate that the dual-channel method is an efficient approach of producing nanoemulsions stabilized by natural emulsifiers, even at relatively low homogenization pressures.

3.3. Influence of natural emulsifier on nanoemulsion storage stability

The long-term stability of emulsions is one of the most important factors determining their shelf life in commercial food and beverage applications (McClements, 2015; Yang, et al., 2013b). We therefore compared the stabilities of nanoemulsions formed using different natural emulsifiers during storage at ambient temperatures (20 °C) for one month. We observed no change in the visible appearance of nanoemulsions stabilized by WPI or Q-Naturelle at any emulsifier concentrations (Figs. S3 and S5). We also found only a

small increase in mean droplet diameter of both the nanoemulsions after one-month storage (Figs. S4 and S6). The relatively high storage stability of these systems is mainly attributed to the fact that the creaming rate is proportional to the square of the droplet diameter, and so a reduction in droplet size decreases the rate of gravitational separation. Additionally, Brownian motion effects that favor a homogeneous distribution of droplets throughout the system counteract the gravitational forces when the droplets are sufficiently small (McClements, 2012). On the other hand, a layer of oil droplets on top and a layer of water at the bottom of the gum arabic- and soy lecithin-stabilized nanoemulsions was observed after they were stored for one month at lower emulsifiers concentrations (Fig. 7). A distinct cream layer was observed on top of the nanoemulsions containing < 2% gum arabic and < 5% soy lecithin, which is due to the relatively large dimensions of the droplets in these systems. However, negligible or no creaming was observed at higher emulsifier concentrations in both nanoemulsions, which can be attributed to the small droplet size in these systems. Interestingly, we did not observe an appreciable change in mean droplet diameter in those systems where creaming was observed (Figs. S7 and S8). This result suggests that the long-term storage instability of nanoemulsions stabilized by gum arabic and soy lecithin was due to the relatively large size of the individual oil droplets, rather than to droplet aggregation.

4. Conclusions

This study compared the effectiveness of different natural food-grade emulsifiers in fabricating nanoemulsions using a dual-channel microfluidization method. All of the natural emulsifiers were found to be surface active, but exhibited significant differences in their interfacial characteristics. The effectiveness at lowering the interfacial tension at the oil-water interface decreased in the following manner: quillaja saponin > WPI > soy lecithin > gum arabic.

Nanoemulsions or emulsions were produced using the dual-channel microfluidizer

depending on the type of natural emulsifier used. However, WPI and Q-Naturale were much more effective at producing nanoemulsions at low emulsifier concentrations than gum arabic and soy lecithin. This effect was attributed to three major differences between the emulsifiers: (i) the faster adsorption of WPI and Q-Naturale molecules to the droplet surfaces within homogenizer; (ii) the higher surface activity and lower surface load of WPI and Q-Naturale; and (iii) the lower interfacial tension for WPI and Q-Naturale. The mean droplet diameter decreased with increasing homogenization pressure for WPI, gum arabic and Q-Naturale, but there was some evidence that the WPI and gum arabic may have lost some functionality at higher pressures. The storage experiments indicated that the nanoemulsions had good stability to droplet aggregation and gravitational separation provided the initial emulsifier concentration was sufficient to produce fine droplets.

In summary, our study shows that nanoemulsions or emulsions containing small droplets with a narrow particle size distribution can be produced from natural emulsifiers using dual-channel microfluidization. The results of this study are therefore useful to food, supplement, and pharmaceutical manufacturers who would like to use natural emulsifiers within their products.

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Table 1 Densities of oil phase and aqueous phase used to prepare nanoemulsions by the dual-channel microfluidization method. ^a

Type	Density (kg/m ³)
Corn oil	908 ± 3
Whey protein isolate solutions	1000 ± 0 (0.1-3%) ^b , 1001± 1 (5%), 1002 ± 3 (10%)
Gum Arabic solutions	1000 ± 2 (0.1-3%), 1028 ± 3 (5%), 1041 ± 2 (10%)
Q-Naturale solutions	1000 ± 0 (0.1-3%), 1022 ± 4 (5%), 1045 ±1 (10%)
Soy lecithin solutions	1000 ± 4 (0.1-3%), 1009 ± 2 (5%), 1014 ± 4 (10%)

^aThe physiochemical properties were measured at ambient temperature (20 °C).

^b% represent the concentrations of emulsifiers used in the emulsion formulations.

Figure captions

Fig.1. (a) Schematic illustration of production of corn oil-in-water nanoemulsions using one-step dual-channel microfluidization (b) Schematic drawing of Y- and Z-type homogenizer chambers.

Fig.2. (a) Influence of whey protein isolate, gum arabic, quillaja saponin, and soy lecithin concentration on the interfacial tension measured at the corn oil-water interface. (b) Photograph of the shape of corn oil droplets before measuring. *Note:* The dash line in (b) represents the starting point for calibrating the measurement.

Fig.3. Influence of emulsifier type and concentration on the mean particle diameter (d_{32}) of 10 wt% corn oil-in-water emulsions produced by dual-channel microfluidization (13 kpsi, single pass). The inset shows the mean droplet diameters of nanoemulsions prepared at low levels of WPI and quillaja saponin.

Fig.4. Particle size distributions of 10 wt% corn oil-in-water emulsions containing different concentrations of (a) whey protein isolate (b) gum arabic (c) quillaja saponin and (d) soy lecithin produced by dual-channel microfluidization (13 kpsi, single pass).

Fig.5. Influence of homogenization pressure on the mean droplet diameter (d_{32}) of 10 wt% corn oil-in-water nanoemulsions containing 1 wt% whey protein isolate, 10% gum arabic or 1% quillaja saponin produced by dual-channel microfluidization (single pass).

Fig.6. Particle size distributions of 10 wt% corn oil-in-water emulsions containing 1 wt% whey protein isolate, 10% gum arabic or 1% quillaja saponin produced by dual-channel microfluidization (single pass).

Fig.7. Photographs of storage stability of nanoemulsions stabilized by different concentrations of (a) gum arabic or (b) soy lecithin. Upper photos represent the nanoemulsions at Day-0, and lower photos represent the nanoemulsions at Day-30. The dashed ring for gum arabic after 30 days' storage shows clearing at the bottom of the tubes.

Figure

Fig.1.

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