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Design of fish oil-in-water nanoemulsion by microfluidization

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ABSTRACT

In the last years, the consumption of polyunsaturated fatty acids (PUFAs) has been promoted due to the prevention and treatment of different diseases. When these are marketed as emulsions, their therapeutic efficacy depends on their charge and size. Microfluidization is an emerging technology able to produce smaller droplet sizes. The aim of this study was to evaluate the capacity of mesquite gum to produce fish oil emulsion by microfluidization as a function of pressure and number of passes. Emulsions were produced according to a two factor, three level with two central points: pressure from 34.5 to 206.8 MPa and 1 to 5 passes. The zeta potential and droplet size distributions were evaluated using electrophoretic and dynamic light scattering equipment, respectively. Coarse emulsion produced by high-shear mixer shows a droplet size up to 1.5 μm, while microfluidization process produce smaller droplet size (≥200 nm). Also, zeta potential values increased around -30 ± 2 mV. The optimal conditions were estimated at 144 MPa and two passes of microfluidization. Nanoemulsions with an average droplet size around 200 nm could be used to improve their absorption in the digestive tract.

1. Introduction

Today we know that polyunsaturated fatty acids (PUFAs) are essential nutrients for normal growth and development, and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes mellitus, arthritis, autoimmune disorders, and some types of cancers (Arab-Tehrany et al., 2012; Garg, Wood, Singh, & Moughan, 2006). Fish oils are rich sources of PUFAs, especially docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), eicosatetraenoic acid (ETA), and α-linolenic acid (ALA). However, these PUFAs have high susceptibility to oxidation and deterioration reducing their shelf-life (Arab-Tehrany et al., 2012; Johnson & Decker, 2015). Actually, there are a number of functional foods enriched through the addition of PUFAs as bulk oils, oil-in-water emulsions (O/W), or powders (Walker, Decker, & McClements, 2015). In food emulsions, the droplet size distribution, is one of the most important properties that could determine the shelf-life, appearance, texture, and flavor. The selection of emulsifying devices depends on: (a) emulsion volume; (b) viscosity of emulsion and its phases; (c) surfactant type and concentration; (d) temperature; and (e) final droplet size and distribution (Jafari, Assadpoor, He, & Bhandari, 2008). Based on the droplet size, emulsions are divided into microemulsions (5–100 nm), nanoemulsions (20–200 nm) and macroemulsions (100 nm–100 μm). Microemulsions are thermodynamically stable, formed by low energy emulsification methods (e.g., self-assembly), while nanoemulsions are thermodynamically unstable stabilized by high energy emulsification methods (e.g., ultrasonic processors, or microfluidizers) (McClements, 2012; McClements & Rao, 2011; Rao & McClements, 2011; Walker et al., 2015). Microfluidizer is a continuous process, where a process fluid (e.g., emulsion, suspension or solution) is compressed at high pressures (50–400 MPa), and forced through a homogenization chamber, where intense shear and elongation stresses, turbulence, and cavitation are developed (Donsì & Ferrari, 2016). Several studies have shown that, the major factors that influence the droplets size distribution, include oil-water interfacial tension, oil-water viscosity ratio, emulsifier type, and emulsifier-oil or surfactant-to-oil ratio (SOR) (Bai & McClements, 2016; Donsì & Ferrari, 2016; Donsì, Senatore, Huang, & Ferrari, 2010; Jafari et al., 2008; Jafari, He, & Bhandari, 2007; Jo et al., 2015; Kentish et al., 2008; Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal, 2016).

Mesquite gum (MG) is an exudate gum produced by several species of *Prosopis* spp. Chemically, it is a neutral salt of a complex acidic-branched polysaccharide formed by a core of β-D-galactose residues, comprising a (1,3)-linked backbone with (1,6)-linked branches, bearing L-arabinose (pyranose and furanose ring forms), L-rhamnose, *b*-D-glucuronate and 4-*O*-methyl-*b*-D-glucuronate as single sugar or oligosaccharide side chains, linked to a small amount of protein (2–4%) (López-Franco, Higuera-Ciapara, Goycoolea, & Wang, 2009). Just like gum arabic, which is the most widely used and traded gum, MG has

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been used to produce O/W emulsions (Orozco-Villafuerte, Cruz-Sosa, Ponce-Alquicira, & Vernon-Carter, 2003; Román-Guerrero et al., 2009). Actually, López-Franco, Cervantes-Montaño, Martínez-Robinson, Lizardi-Mendoza, and Robles-Ozuna (2013) reported the physicochemical characteristics and emulsion properties of MG. They concluded that, MG at low SOR values (<1) have an emulsion capacity and stability of 95% and 92%, respectively. However, high SOR values (≥1) have been used to form emulsions with n-alkanes of different chain length, carotenoids, and essential oils using a high-speed mixer (Acedo-Carrillo et al., 2006; García-Márquez et al., 2015; López-Franco et al., 2009; Román-Guerrero et al., 2009). On the other hand, there are not studies of MG as emulsifier agent by microfluidization. The aim of this study was to evaluate the capacity of mesquite gum to produce fish oil nanoemulsions by microfluidization. The influence of pressure, and number of passes onto the droplet size and zeta potential of the emulsion were optimized using the response surface methodology.

2. Materials & methods

2.1. Materials

Fish oil (55.69 μg/mL of DHA, 11.31 μg/mL of EPA, 0.96 μg/mL of ETA, and 0.69 μg/mL of ALA) was purchased from "Omegamex SA de CV" (Baja California Sur, Mexico). Mesquite gum (MG, *Prosopis laevigata*) was purchased from "Natural Products of Mexico SA de CV" (Morelos, Mexico). Deionized water was used for the preparation of all solutions.

2.2. Emulsions

MG solution (20% w/w) was gently stirred for 12 h and stored overnight at 4 °C to allow their complete hydration (pH = 4.5). Fish oil was added to MG solution and stirred using a high-speed mixer (Silverson L5M, UK) at 5000 rpm for 10 min at 20 °C. Both, fish oil volume fraction (ϕ) = 0.1, and MG/oil ratio = 2, were kept constant. Coarse emulsion (CE) was homogenized using a microfluidizer M-110 PS (Microfluidics International Corporation, USA). The microfluidizer interaction chamber was a 75 μm-diameter diamond Y type (F12Y). Upon exit the interaction chamber, a tubular heat exchanger was used in-line, in order to cool the emulsion at 20 °C. Eleven emulsions were produced according to a two-factor, three-level central composite design including two replicates at the center point (Table 1): Pressure: 34.5 MPa to 206.8 MPa, and number of passes (N): 1 to 5. The droplet size distributions and zeta potentials were evaluated.

2.3. Average particle size and distribution

The hydrodynamic size (Z-average diameter, d_h) and distribution of the emulsions were estimated using a Zetasizer Nano ZS90 (Malvern Instrument, UK). The d_h was calculated by the Stokes–Einstein equation: $d_h = k_B T/3\pi\eta_s D$; where k_B is the Boltzmann constant, T is the temperature (K), η_s is the viscosity of the solvent and D is the Z-average translational diffusion coefficient. Each emulsion was diluted at a concentration of 0.01 g/mL with deionized water. The dipersant properties of water at 20 °C were used, i.e. refractive index of 1.33 and viscosity of 1.0031 cP. The emulsion refractive index and absorption values of 1.39, and 0.01 were used, respectively. All measurements were made in a glass cuvette at 20 °C. Polydispersity index (PDI) was used for comparative purposes (i.e., broader distributions, PDI ≥ 0.5; monomodal emulsions, PDI ≤ 0.1) (Zetasizer-nano, 2007).

Table 1
Experimental design with 2 central points.

Experiment code	Pressure (MPa)	N
LP1	34.5	1
LP3	34.5	3
LP5	34.5	5
MP1	120.6	1
MP3	120.6	3
MP5	120.6	5
HP1	206.8	1
HP3	206.8	3
HP5	206.8	5
CP1	120.6	3
CP2	120.6	3

2.4. Zeta potential

The zeta potential was determined using an electrophoretic light scattering equipment Zetasizer Nano ZS90. The measurements were carried out using an universal dip cell (ZEN1002, Malvern Instrument) at 20 °C using the diluted emulsions described previously. The zeta potential is related to the velocity of the emulsion droplets into an electric field. The equipment software converted the electrophoretic mobility measurements into zeta potential values using the Smoluchowsky model. The zeta potential was calculated from the average of three measurements on the diluted emulsion, and the results were reported as the mean and standard deviation (Onsaard, Vittayanont, Srigam, & McClements, 2006).

2.5. Statistics

Statgraphics Centurion XV (version 2.15.06) was used to determine differences between treatments means ($p < 0.05$) according to Tukey's test. Response surface methodology was used to determine the effect of pressure level (P) and number of passes (N) on the particle size (d_h) and zeta potential (ζ_p) of O/W emulsions, and performed according to a two-factor, three-level central composite design including two replicates at the center point (Table 1). The variables of response were evaluated by statistic software using quadratic model ($Y \equiv d_h$ or ζ_p):

$$Y = b_o + b_1 P + b_2 N + b_{11} P^2 + b_{22}\, PN + b_{22}\, N^{2.}$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22} (quadratic effects), and b_{12} (interaction effects). The quality of the fit of second-order polynomial model was expressed with the coefficient of the determination (R^2):

$$R^2 = 1 - SS_{residual}/SS_{model} + SS_{residual}.$$

The response surface and contour plots of the model predicted were used to specify the interrelationships between significant variables (Sadeghpour Galooyak & Dabir, 2015).

3. Results & discussion

Fig. 1 shows the droplet size distributions of O/W emulsions produced by high-speed shear and microfluidization at different pressures and number of passes. CE was characterized by oil droplets up to 1.5 μm and high PDI. Similar droplet size distribution has been obtained by light scattering equipment (data not show). After microfluidizer process, droplets size and PDI values of the emulsion decreased, as the pressure level and number of passes increased (Figs. 1 & 2). Inside the microfluidizer chamber, the droplets are exposed to a high shear and more complex mixing patterns, that reduce the droplet size and PDI of the emulsion (Jafari et al., 2007; Perrier-Cornet, Marie, & Gervais, 2005). In general terms, all experimental conditions produce fine O/W emulsions, but only in a few experimental conditions, "nanoemulsions" were produced. For example, after three passes at 120.6 MPa (code: MP3) the droplet size and PDI of the emulsion decreased to 155 nm and 0.15, respectively. However, at high pressure (206.8 MPa), when the number of passes was increased from three to five (i.e., HP3 to HP5), the droplet size of the emulsion increased (over-processing).