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Effect of lentil proteins isolate concentration on the formation, stability and rheological behavior of oil-in-water nanoemulsions

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ABSTRACT

The formation, stability and rheology of 5 wt% oil-in-water nanoemulsions as a function of lentil protein isolate concentration (0.5–5 wt%) at pH 3.0 was investigated for 28 days. All nanoemulsions, except 1 wt% protein, showed bimodal droplet size distribution where the larger diameter peak was ascribed to protein aggregates and entrapped oil droplets. The average droplet size for all nanoemulsions measured from the lower diameter peak ranged from 161 to 357 nm, which did not change over 28 days. Stable flowable nanoemulsions were formed at 1–2 wt% protein concentrations. Nanoemulsions with 3 and 5 wt% protein formed strong non-flowable gels which showed a two-step yielding behavior during strain-sweep rheology, indicating gel formation by interconnected clusters of proteins and oil droplets. This study demonstrated that lentil protein has a potential to be utilized as an emulsifier in nanoemulsions, as well as in the formation of emulsion gels at higher protein concentrations.

1. Introduction

An emulsion is a mixture of two or more immiscible liquids where one phase is dispersed within the continuous phase of another in the presence of an emulsifier and with the help of mechanical energy. Emulsifiers are important in the reduction of interfacial tension between two immiscible phases, which reduces the energy required to form an emulsion. They also adsorb on the freshly-formed droplet surface and provide stability against coalescence. Proteins make excellent emulsifiers as their amphiphilic nature (i.e., presence of hydrophobic and hydrophilic amino acid) enable them to become surface active, to align and aggregate at the oil-water interface to form stabilizing films. In general, during emulsion formation, proteins migrate from the bulk solution to the interface, where it absorbs and re-aligns to position its hydrophobic amino acids towards the apolar phase and the hydrophilic amino acids towards the polar phase (Damodaran, 2005; Dickinson, 1994). Protein adsorption and intermolecular interaction at the interface lead to the formation of an interfacial viscoelastic film. Improved stabilization of protein-stabilized emulsions against flocculation and coalescence occurs through electrostatic repulsive forces arising from the interfacial film at pHs away from the protein's isoelectric point, through steric stabilization and by increasing the continuous phase viscosity due to the presence of un-adsorbed proteins (McClements, 2004). In the case of globular-type proteins (e.g., lentil protein), diffusion to the interface is relatively slow, however once the film is developed, the emulsion remains quite stable due to the presence of the thick interfacial layer (Wilde, Mackie, Husband, Gunning, & Morris, 2004). In the case of a fibrous protein (e.g., gelatin), migration to the oil-water interface tends to be faster, however the interfacial film is less thick and more susceptible to rupturing (Lobo, 2002). In contrast, low molecular weight synthetic emulsifiers (e.g., phospholipids, polyoxyethylene sorbitan monolaurate (Tween 20)), diffuse rapidly to the oil-water interface and forms a thin interfacial film around the oil droplet producing smaller droplet sizes, but poorer stability under long-term storage conditions (McClements, 2005). Proteins, being natural products, are of high demand for their use in emulsion stabilization for food applications. Among the various types of proteins, plant proteins have recently become particularly popular for their ability to replace animal proteins and synthetic emulsifiers (Lam & Nickerson, 2013).

Chang, Tu, Ghosh, and Nickerson (2015) investigated the effect of pH (3, 5, and 7) on the physicochemical, interfacial and emulsifying properties of pea, lentil, canola and soy protein isolates in coarse emulsions. The authors reported the emulsifying properties were greatest for all proteins at pH 3, followed by pH 7 and then pH 5 (closer to their isoelectric point). At pH 3, protein solubility and surface hydrophobicity was higher relative to the other pHs. Further, the lentil protein isolate (LPI) at this pH was found to have higher surface hydrophobicity compared to the other proteins

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studied, and formed the strongest interfacial viscoelastic film among the pulse proteins. Karaca, Low, and Nickerson (2011) studied the emulsifying and physicochemical properties of chickpea (ChPI), faba bean, lentil and pea protein isolates (PPI), produced by isoelectric precipitation and salt extraction, in coarse emulsions. The authors reported that isolates produced by isoelectric precipitation had higher surface charge and solubility than the isolates generated by salt extraction, and that ChPI and LPI extracted by isoelectric precipitation produced the most stable coarse emulsions against creaming.

Nanoemulsions are similar to coarse emulsions, but are typically formed using high pressure homogenizers, microfluidizers or using solvent evaporation techniques and by definition should have a average droplets diameter < 200 nm (McClements & Rao, 2011). On the other hand, many researchers have used the term "nanoemulsion" for samples with average droplet diameter < 500 nm (Yerramilli & Ghosh, 2017). The majority of research in the literature involving protein-stabilized nanoemulsions has involved dairy proteins, such as casein (Dickinson, Radford, & Golding, 2003; Surh & McClements, 2008; Ye, 2008) and whey proteins (Euston, Finnigan, & Hirst, 2000; Reiffers-Magnani, Cuq, & Watzke, 2000; Ye, 2008), with very limited work involving plant proteins. Donsi, Senatore, Huang, and Ferrari (2010) developed pea protein stabilized nanoemulsions (average droplet diameter < 200 nm) using high-pressure homogenization. The authors reported that the homogenization process altered the protein's quaternary and tertiary structure by disrupting disulfide bonds to allow for a partial unraveling of its composition to expose a greater amount of hydrophobic sites. Yerramilli, Longmore, and Ghosh (2017) investigated the use of PPI to partially replace sodium caseinate in the formation and stabilization of nanoemulsions and showed an improved stability against depletion-induced destabilization. Other researchers have also developed plant protein-stabilized emulsions with droplets size in the range 200 – 500 nm (Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2016; Liang & Tang, 2013; Peng et al., 2016). However, plant protein-stabilized nanoemulsions in the droplet size range < 200 nm are rarely reported in the literature.

In the present study, oil-in-water nanoemulsions was stabilized by LPI at pH 3, building off initial work by Chang et al. (2015). Lentil protein is dominated by two types of globulin proteins: legumin and vicilin. The former is classified as an 11S (S is a Svedberg Unit) hexamer protein with a molecular mass of 350–400 kDa, with each subunit being comprised of an acidic α-chain (molecular mass ~40 kDa) and a basic β-chain (molecular mass ~20 kDa) held together by a disulfide bond (Barbana & Boye, 2011; Jarpa-Parra et al., 2015; Oomah, Patras, Rawson, Singh, & Compos-Vega, 2011). In contrast, vicilin is a 7S trimeric protein with a molecular mass of ~150 kDa. Each trimer of vicilin is ~50 kDa in mass, and contains no disulfide bridging, allowing it to unfold easier during processing or at the oil-water interface than legumin (Dagorn-Scaviner, Gueguen, & Lefebvre, 1987; Oomah et al., 2011).

The overall goal of this study is to investigate the effect of LPI concentration on the formation and stabilization of O/W nanoemulsions using high-pressure homogenization and to identify the best formulation to produce the smallest sized droplets with the greatest stability. To our knowledge, no study so far has reported development of O/W nanoemulsions with LPI as the sole emulsifier.

2. Materials and methods

2.1. Materials

Lentil protein isolate (protein content 79.5% w/w, w.b.) was kindly provided by POS Bio-sciences (Saskatoon, SK, Canada), after being produced by alkaline extraction and isoelectric precipitation pilot scale processes. Canola oil used in this study was purchased from the local supermarket (Saskatoon, SK, Canada). The citric acid was purchased from VWR International (Edmonton, AB, Canada), whereas all other chemicals were purchased Sigma-Aldrich (St. Louis, MO, USA) and were of reagent grade.

2.2. Preparation of lentil protein solutions

Lentil protein solutions were prepared by dispersing the protein powder at different concentrations (0.5, 1, 1.5, 2, 3, and 5 wt%) based on the protein content, in a 0.1 M citrate buffer (pH 3) using a magnetic stirrer at 500 rpm for 24 h at room temperature (22–23 °C). To prevent the microbial spoilage in the emulsions, an antimicrobial agent (0.02 wt% sodium azide) was added to each protein solution. Chang et al. (2015) reported the solubility of the LPI at pH 3 as 56.2 ± 0.62%.

2.3. Preparation of nanoemulsions

Nanoemulsions were prepared by adding 5 wt% canola oil to 95 wt% of the protein solutions (0.5–5 wt%), mixing with a rotor-stator blender (Polytron, Brinkman, ON, Canada) to form a coarse emulsion, followed by homogenization using a high-pressure homogenizer (Emulsiflex C3, Avestin Inc., Ottawa, ON, Canada) at 20,000 psi for 6 cycles. The temperature of the emulsions during homogenization reached ~60 °C. The stability of the nanoemulsions was recorded for 28 d (4 weeks). All nanoemulsions were prepared in triplicate.

2.4. Droplet size distribution

Droplet size and distribution was measured using a static laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Montreal, QC). The surface-weighted mean droplet diameter (d_{32}) and the size distribution were determined immediately after the preparation of the nanoemulsions and as a function of time (0, 7, 14, and 28 d). For more accurate measurement of individual un-flocculated droplet size, all samples were first diluted (1:5) with a 0.5 wt% Tween 20 in 0.1 M citrate buffer (pH 3) solution before measuring their droplet size.

2.5. Zeta potential

Surface charge or zeta potential of the oil droplets were determined using a Zetasizer Nano-ZS90 (Malvern Instruments, Westtborough, MA, USA) by measuring the electrophoretic mobility (U_E) of the LPI-coated droplets in a buffer solution (pH 3) (1 drop of emulsion added to 100 ml of citric buffer) in an electric field where the droplets move towards the oppositely charged electrode. Zeta potential (ζ, mV) was determined by measuring the electrophoretic mobility (U_E) and then applying Henry's equation:

$$U_E = \frac{2\varepsilon \times \zeta \times f(k\alpha)}{3\eta} \quad (1)$$

where ε is permittivity (F (Farad)/m), $f(k\alpha)$ is a function associated with the ratio of the particle radius (α) to the Debye length (k) and η is the viscosity (mPa·s) of the solution (water, 1 mPa·s). The Smoluchowski approximation $f(k\alpha)$ for this study was set to 1.5. Zeta potential was measured as a function of time (0, 7, 14, and 28 d) for all nanoemulsions stabilized with different LPI concentrations.