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Flaxseed oil – Whey protein isolate emulsions: Effect of high pressure homogenization

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ABSTRACT

The effect of high-pressure homogenization (20–100 MPa) and the number of homogenization cycles (1–7) on the stability of flaxseed oil - whey protein isolate emulsions was evaluated. All the emulsions were stable to creaming for at least 9 d of storage. An increase in homogenization pressure from 20 to 80 MPa and number of passes through the homogenizer up to 3, decreased the mean droplet size of the O/W emulsions despite the higher polydispersity. Emulsions homogenized at lower pressures (20 MPa) showed a monomodal distribution of the particles, whereas, an increase in pressure to 80 MPa led to a bimodal distribution, indicating droplets coalescence. High-pressure homogenization (80 MPa) and an increase in the number of homogenization cycles, led to the formation of high molecular weight aggregates (>200 kDa), which favored an increase in viscosity of the emulsions. The increase in homogenization pressure also increased the formation of primary oxidation products, which could be explained by the increase in temperature and in the surface area of the droplets.

1. Introduction

An emulsion can be defined simply as "a system comprised of two immiscible liquids, one of which is dispersed as droplets (the dispersed or internal phase) in the other (the continuous or external phase)" (Jafari et al., 2008). Emulsions are thermodynamically unfavorable systems that tend to break down over time, due to a variety of physicochemical mechanisms. However, it is possible to form emulsions that are kinetically stable for a reasonable period of time, by using intense mechanical forces and/or including substances known as stabilizers, for example, emulsifiers (McClements et al., 2007).

High-pressure homogenizers are the most frequently used to produce food emulsions, providing fine emulsions with good texture properties and greater stability (Desrumaux and Marcand, 2002). In this process, the combination of intense shear, cavitation and turbulent flow conditions leads to disruption of the fat droplets (McClements, 2005). The decrease in average size of the fat droplets reduces the creaming velocity (Stokes law) and increases the stability of the emulsion (Desrumaux and Marcand, 2002). On the other hand, emulsifiers are surface-active molecules that adsorb to the surface of freshly formed droplets during homogenization. Once adsorbed onto the droplet surface, they act in two ways: lowering interfacial tension and forming a protective layer that prevents the droplets from aggregating (Guzey and McClements, 2006; McClements et al., 2007; Walstra, 2003).

Proteins are ingredients widely used in food emulsions as emulsifiers/stabilizers due to their amphiphilic character. The ability of proteins to generate repulsive interactions (e.g., steric and electrostatic) between oil droplets, and at the same time form an interfacial membrane that is resistant to rupture, plays an important role in stabilizing the droplets against flocculation and coalescence during long-term storage (McClements, 2004). Whey proteins are often used in food emulsion systems because of their ability to stabilize oil-in-water (O/W) emulsions (Dalgleish, 1996; Dickinson, 2001; Kerstens et al., 2006; Ye, 2010). Whey proteins adsorbed at an oil/water interface interact with neighboring molecules adsorbed on the same droplet, or on different droplets via a combination of non-covalent bonds or covalent disulfide bonding, increasing stability to coalescence (McClements et al., 1993). Moreover, whey protein isolate (WPI) has been reported to possess antioxidant activity (Hu et al., 2003; Sun and Gunasekaran, 2009; Tong et al., 2000), which could be extremely beneficial to systems containing labile oxidative components as the dispersed phase (Sun and Gunasekaran, 2009).

Flaxseed oil is very rich in unsaturated fatty acids, being recognized as one of the greatest sources of Omega-3 in nature, this having a positive effect on human health, being increasingly recognized for their role in reducing the risk of cardiovascular diseases (Zhao et al., 2007). However, during processing, distribution and handling, these oils can easily oxidize, due to their high degree of unsaturation (Tonon et al., 2011). Oxidation leads to the formation

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of unpleasant tastes and odors, and consequently to a reduction in product shelf life, besides promoting the generation of free radicals, which may have negative physiological effects on the organism (Tonon et al., 2011).

Thus, the objective of the present research was to study the best conditions to prepare O/W emulsions stabilized by whey proteins, aiming at greater stability and decreased oxidation of the flaxseed oil in these systems during the storage period. The effect of high-pressure homogenization (20–100 MPa) and the number of homogenization cycles (1–7) on the creaming stability, droplet size, rheology, oxidative stability and molecular weight distribution of the emulsions was evaluated, the latter being evaluated by polyacrylamide gel electrophoresis.

2. Material and methods

2.1. Material

The whey protein isolate (WPI) was obtained from New Zealand Milk Products (ALACEN 895, New Zealand) and the flaxseed oil purchased from Cisbra (Panambi, RS, Brazil), showing the following fatty acid composition: 6.2% C16:0, 5.3% C18:0, 20.1% C18:1, 13.7% C18:2 and 52.3% C18:3. The protein concentration, determined by the Kjeldahl procedure (AOAC, 1997), lipid (Bligh and Dyer, 1959), moisture (AOAC, 1997) and ash (AOAC, 1997) contents (w/w wet basis) were 87.66 ± 0.91% (N × 6.38), 0.36 ± 0.02%, 4.54 ± 0.11% and 1.36 ± 0.07%, respectively. All other reagents were of analytical grade.

2.2. Preparation of the WPI stock solution

The WPI stock solution (5% w/v) was prepared by dissolution of the powder in deionized water (pH 6.7) with magnetic stirring for 90 min at room temperature (25 °C). The pH of the solution was then adjusted to 7.0 ± 0.2 using 2.0 M NaOH. The solution was kept overnight at 10 °C to allow for complete protein dissolution.

2.3. Preparation of the emulsions

Oil-in-water (O/W) emulsions were prepared at 25 °C by homogenizing the oil in the aqueous phase using two sequential homogenization methods. The first method involved mixing the solutions in an Ultra Turrax model T18 homogenizer (IKA, Germany) for 4 min at 14000 rpm. The second method involved subjecting the previously prepared macroemulsion to a high-pressure homogenization process using a Panda 2K NS1001L double stage homogenizer (Niro Soavi, Italy). The pressure in the first stage was from 20, 40, 60, 80 or 100 MPa and in the second stage it was fixed at 5 MPa. The number of passes through the homogenizer (1, 2, 3, 4, 5, 6 or 7) at each pressure, totaling 35 emulsions produced, was also evaluated. The WPI and flaxseed oil contents were fixed at 3% (w/v) and 30% (v/v), respectively. Sodium azide (0.02% w/v) was added to the emulsions to prevent microbial growth and the pH was adjusted to 7.0 using 2.0 M NaOH.

2.4. Creaming stability

Immediately after preparation, 10 mL of each emulsion were poured into a cylindrical glass tube (internal diameter = 11 mm, height = 94 mm), sealed with a plastic cap and stored at 25 °C for a period of 9 d. The emulsion stability was measured by the change in height of the bottom serum phase (H) with storage time. The creaming index (CI) was determined according to Eq. (1).

$$CI(\%) = (H/H_0) \times 100 \quad (1)$$

where H_0 represents the initial height of the emulsion. To facilitate visualization of the phase separation, Sudam III (reddish dye) was added to the flaxseed oil. The analyzes were carried out in duplicate. Emulsions homogenized at 20 and 80 MPa were also stored in glass bottles (~60 mL of emulsion, internal diameter = 45 mm, height = 70 mm), at 25 °C, and evaluated for 3 months.

2.5. Optical microscopy

The microstructure of the emulsions was evaluated after 1 d of storage. The samples were poured onto microscopes slides, covered with glass cover slips and observed using a Carl Zeiss Model Axio Scope.A1 optical microscope (Zeiss, Germany) with the x100 objective lenses.

2.6. Particle size distribution

A Mastersizer 2000 (Malvern Instruments Ltd., UK) was used to determine the particle size distribution of the emulsions. About 0.1 mL of the emulsion was added to 150 mL distilled water (0.06% v/v) while stirring in the dispersion unit. The mean diameter of the oil droplets was expressed as the volume mean diameter (d_{43}) and the dispersion index (span) was also reported, according to Eqs. (2) and (3), respectively. The emulsions were analyzed 1 d after their preparation and each sample measured in triplicate.

$$d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3 \quad (2)$$

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

where n_i was the number of particles with diameter d_i, and d_{10}, d_{50} and d_{90} were diameters at 10%, 50% and 90% cumulative volume, respectively.

2.7. Polyacrylamide gel electrophoresis (SDS–PAGE)

Molecular weight distribution of proteins resulting from the high-pressure homogenization of the O/W emulsions were evaluated by polyacrylamide gel electrophoresis (SDS–PAGE) under reducing and non-reducing conditions, according to Laemmli (1970). Under dissociating non-reducing conditions, occurs the rupture of protein aggregates stabilized by electrostatic and hydrophobic interactions. While that, under dissociating reducing conditions, occurs the rupture of disulfide interactions formed between free sulfhydryl groups of proteins. A vertical slab Mini-Protean electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) was used and the resolving and stacking gels contained 15% and 5% of acrylamide, respectively. The whole emulsions, without phase separation, were diluted in deionized water (2 mg protein/mL) and the resulting solution was diluted in a sample buffer (1:1) to obtain dissociating non-reducing conditions (50 mM Tris–HCl (pH 6.8), plus 2% SDS (w/v), 10% glycerol (v/v) and 0.1% (w/v) bromophenol blue) and dissociating reducing conditions (50 mM Tris–HCl (pH 6.8), plus 2% SDS (w/v), 10% glycerol (v/v), 0.1% (w/v) bromophenol blue and 5% (v/v) β-mercaptoethanol). Under dissociating conditions, the samples were heat treated at 70 °C for 5 min and 10 μL aliquots then loaded into the polyacrylamide wells in the stacking gel. The gels were run at 100 V with a running buffer (pH 8.3) containing 25 mM Tris–HCl, 250 mM glycine and 0.1% (w/v) SDS. The gels were then stained with 0.25% (w/v) Coomassie Brilliant Blue in ethanol:acetic acid:water (45:10:45 v/v), and diffusion-destained by repeated washing in an ethanol:acetic acid:water solution (10:5:85 v/v). A native whey protein solution (non-denatured) was used as reference, and a commercial molecular weight marker (Pre-Stained Invitrogen™