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Ultra-High Pressure Homogenization improves oxidative stability and interfacial properties of soy protein isolate-stabilized emulsions

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ABSTRACT

Ultra-High Pressure Homogenization (100–300 MPa) has great potential for technological, microbiological and nutritional aspects of fluid processing. Its effect on the oxidative stability and interfacial properties of oil-in-water emulsions prepared with 4% (w/v) of soy protein isolate and soybean oil (10 and 20%, v/v) were studied and compared to emulsions treated by conventional homogenization (15 MPa). Emulsions were characterized by particle size, emulsifying activity index, surface protein concentration at the interface and by transmission electron microscopy. Primary and secondary lipid oxidation products were evaluated in emulsions upon storage. Emulsions with 20% oil treated at 100 and 200 MPa exhibited the most oxidative stability due to higher amount of oil and protein surface load at the interface. This manuscript addresses the improvement in oxidative stability in emulsions treated by UHPH when compared to conventional emulsions.

1. Introduction

A current major health issue at the heart of our understanding is to follow the most recent nutritional recommendations (EFSA Panel on Dietetic Product, 2010), one being, increasing amounts of polyunsaturated fats incorporated in foods (Walker, Decker, & McClements, 2015). At the same time, consumers look for foods containing fewer additives, especially synthetic antioxidants. However, synthetic antioxidants are added to food containing chemically unstable compounds, such as polyunsaturated fats. This is why lipid oxidation in formulated foods has become a renewed concern for the food industry and it is important to develop new technologies to replace synthetic antioxidants. Lipid oxidation consists of the reaction of molecular oxygen with unsaturated fatty acid, resulting in unsaturated hydroperoxides (Gunstone & Martini, 2010). Lipid hydroperoxides are unstable molecules that break down into other products, particularly aldehydes, responsible for undesirable odours and flavours. Thus, lipid oxidation is the main reason for the deterioration of fats and oils (Matthäus, 2010). Many food products contain oil and water. These phases are immiscible, but can coexist in a stable form in food products through emulsification, with one of the liquids dispersed as small spherical droplets in the other. In particular, oil-in-water (ow) emulsions consist of oil droplets dispersed in an aqueous phase (McClements, 2005).

Since the late 1980s, substantial work has been performed with respect to lipid oxidation in simplified model emulsions (Berton-Carabin, Ropers, & Genot, 2014; Waraho, Cardenia, Decker, & McClements, 2010). The rates and pathways of oxidation reactions are influenced by the physical environment of the molecules involved, for example, whether they are located in the oil, water or interfacial region (Berton-Carabin et al., 2014; Faraji, McClements, & Decker, 2004; Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2015). The interfacial region, which is the contact region between the oil phase and the aqueous phase, represents a particularly critical area in the system with regard to the development of lipid oxidation. For these reasons, it is important to have a better understanding of the mechanisms of lipid oxidation in food dispersions so that novel antioxidant technologies can be developed (Waraho et al., 2010).

Ultra-High Pressure Homogenization (UHPH) is an emergent technology to produce fine and stable submicron emulsions (<1 μm) (Dumay et al., 2013; Fernández-Ávila, Escriu, & Trujillo, 2015; Hebishy, Buffa, Guamis, Blasco-Moreno, & Trujillo, 2015). UHPH modifies emulsion properties due to conformational changes of proteins, which results in changes of the physical stability of emulsions. These changes in particle size and proteins structure are caused by different phenomena occurring at the exit of the UHPH valve, such as high turbulence, cavitation and shear

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stress (Desrumaux & Marcand, 2002; Floury, Desrumaux, & Legrand, 2002). Over the past decade, the changes experienced by UHPH treatment compared to conventional processes (high speed blenders, high-pressure valve homogenizers and colloid mills) remain unprecedented. The benefits obtained by applying a single stage of homogenization with UHPH technology are supported by time and cost savings in the food industry. The most significant improvements of these emulsions are their physical and microbiological stabilities, which allow maintaining emulsions in stock (Dumay et al., 2013). In fact, recent research at our laboratory has allowed to develop aseptically packaged beverages by UHPH-processing at 300 MPa (Amador-Espejo, Suàrez-Berencia, Juan, Bárcenas, & Trujillo, 2014; Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2014). However, a major problem with this kind of technology is the lower particle size associated with UHPH in products when compared to conventional treatments. It is widely known that when emulsions show higher specific surface area, it is likely for lipid oxidation to easily occur (McClements & Li, 2010). Nonetheless, the debate continues about the best strategies for management of lipid oxidation, and many other factors that affect it, such as the physical structure of emulsions, amount and type of emulsifiers or bulk oil-phase (Berton-Carabin et al., 2014). Most lipid oxidation studies in emulsions have been carried out using only antioxidants or other emulsification techniques (Cui, Kong, Chen, Zhang, & Hua, 2014; Shao & Tang, 2014; Wan, Wang, Wang, Yuan, & Yang, 2014). Hebishy et al. (2015) has been the only research which has examined the effect of UHPH (100 and 200 MPa) on oxidative stability thus far. UHPH emulsions were found to be more oxidatively stable than their CH counterparts. In particular, emulsions stabilized with 1 and 2% whey protein isolate (WPI) and treated with 100 MPa and emulsions stabilized with 4% WPI and treated with 200 MPa showed the most promising results against lipid oxidation. However, further research on this topic needs to be accomplished before the association between UHPH technology and lipid oxidation is more clearly understood. The aim of this study was to investigate the effect of UHPH (100–300 MPa) compared to conventional homogenization (CH) at 15 MPa on oxidative stability of soybean oil emulsions stabilized by SPI. Our previous research showed that o/w emulsions stabilized with SPI and treated by UHPH were more physically stable than CH emulsions (Fernández-Ávila et al., 2015). Particularly, UHPH emulsions treated at 100 and 200 MPa with 20% oil were the most stable due to lower particle size, greater viscosity and partial protein denaturation (Fernández-Ávila et al., 2015). It is important to note that the studied composition of the emulsions in this manuscript proposed the combination of soybean oil as a source of polyunsaturated fat with high propensity to be oxidized and soy protein isolate (SPI) as an emulsifier. Additionally, a thermal treatment was carried out on the protein solutions before CH treatment, to determine whether complete denaturation of proteins would similarly affect the emulsions characteristics or improve the oxidative stability. Droplet size distribution, emulsifying activity index, microstructure, surface protein concentration and lipid oxidation of prepared emulsions were evaluated for various soybean oil concentrations (10 and 20%, v/v) emulsified with SPI.

2. Material and methods

2.1. Materials

A commercial SPI (PRO-FAM 974) was purchased from Lactotecnia (Barcelona, Spain). The composition of this commercial SPI according to the manufacturer was: ⩾90% protein, <4% fat, <6% moisture, and less than 5% ash (dry basis, w/w). SPI PRO-FAM 974 has acid character and an isoelectric point of 4.6 (Kinsella, 1979) due to the high content of glutamic acid (Glu, 19.2%) and aspartic acid (Asp, 11.5%). Solubility of PRO-FAM 974 at pH = 7 is 39.5% (Bissegger, 2007). Soybean oil was purchased from Gustav Heess (Barcelona, Spain). Peroxide value of the soybean oil was below 5 mmol O_2/kg and the acidity index was below 8.9×10^{-3} mmol KOH/g. All other chemicals used were of analytical or better grade.

2.2. Preparation of oil-in-water emulsions

Oil-in-water emulsions were prepared with a fixed content of SPI (4%, w/v) and different contents of soybean oil (10 and 20%, v/v). Firstly, the stock protein dispersion (4%, w/v) was prepared by dispersing SPI in deionized water using a high-speed dispersing unit at a rate of about 250 rpm for 1 h at 25 °C. The heated SPI dispersions were prepared by heating the stock dispersion at 95 °C for 15 min to allow complete denaturation of soy protein (Keerati-u-rai & Corredig, 2009), and then cooled immediately in an ice bath to room temperature. Protein dispersions were stored overnight at 4 °C to allow complete hydration. Protein dispersions and oil were equilibrated at 20 °C (inlet temperature) before mixing. Pre-emulsions (or coarse emulsions) were prepared by mixing the native or denatured protein dispersions with the soybean oil using a rotor-stator emulsifying unit (model Diax 900, Heidolph, Kehlheim, Germany) at 15000 rpm for 4 min. The coarse emulsions were further homogenized through a high-pressure homogenizer or by conventional homogenization (Stansted Benchtop Homogenizator nG12500, Stansted Fluid Power Ltd., Essex, UK). The emulsions were treated at 100, 200 and 300 MPa (single-stage) by the high-pressure homogenizer (flow rate of 8 L/h) provided with a high-pressure ceramic needle-seat valve. The pre-homogenized native and denatured dispersions were further homogenized at 15 MPa with a ceramic ball-seat valve (conventional homogenization treatments, single-stage). Pre-emulsions were passed through both devices with an inlet temperature (T_{in}) of 20 °C. The outlet temperature of emulsions were controlled by a heat exchanger (Inmasa, Reus, Spain) located immediately after the high-pressure valve or ball-seat valve. During treatments, the T_{in}, the temperature after the high pressure valve (T_1), and outlet temperature were monitored. The pH of all the resulting emulsions was 7.14.

2.3. Determination of oil droplet size distribution

The particle size distribution in the fresh emulsion samples (UHPH and CH emulsions) was determined using a Beckman Coulter laser diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA). Emulsion samples were diluted in distilled water until an appropriate obscuration was obtained in the diffractometer cell. The optical parameters used were: a refractive index of 1.475 for the soybean oil and a refractive index of 1.332 for water. The volume-weighted mean diameter ($d_{4.3}$, μm), the median particle size by volume (d_{50}) and the specific surface area (SSA, m^2/mL) were determined. The specific surface area (SSA) was calculated according to the following Eq. (1):

$$SSA\,(m^2/mL) = \left(\frac{6\varnothing}{d_{3.2}}\right) \quad (1)$$

where $\varnothing$ is the oil volume fraction and the $d_{3.2}$ the volume-surface average diameter of the particles.