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Faba bean protein flour obtained by densification: A sustainable method to develop protein concentrates with food applications

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ABSTRACT

Most protein concentrates, including plant proteins, are obtained by isoelectric precipitation, followed by spray drying, which involves a large amount of wastes, as well as the loss of protein functionality. The present work used a protein concentrate obtained by densification, an eco-friendly process which yields a protein concentrate (564 g/kg) suitable for the development of food emulsions, whose microstructure strongly depends on pH value. In this sense, the pH value of the protein dispersion influences the microstructure of the emulsions obtained, and consequently their stability. Hence, the best results in terms of smaller droplet sizes (Sauter mean diameter, $D[3,2] = 1.7$ μm) and higher viscoelastic parameters are obtained at pH 2.5 (G' at 10 Hz, $G'_{10} = 2373$ Pa), since this system exhibits the development of a suitable protein network. By contrast, at pH 8.0, the lack of protein solubility lead to emulsions with the poorest rheological response ($G'_{10} = 21.9$ Pa), whereas results from droplet size distribution (DSD) indicate that the lack of surface charges found at pH 5.0 led to a high coalescence index (CI), CI at this pH value (44.1%).

1. Introduction

Legumes show excellent functional properties that can be used for the development of food products such as emulsions (Gumus, Decker, & McClements, 2017; Ladjal-Ettoumi, Chibane, & Romero, 2016). They are considered a healthy food source, containing carbohydrates, proteins, dietary fibres, vitamins and minerals (Du, Jiang, Yu, & Jane, 2014; Tharanathan & Mahadevamma, 2003). Regarding their techno-functional properties, they have higher protein content than cereals. Thus, whereas legumes have approximately 200 g/kg protein, the protein content of cereals range from 30 to 70 g/kg. (de Almeida Costa, da Silva Queiroz-Monici, Pissini Machado Reis, & de Oliveira, 2006), despite the higher percentage, this total amount of protein is not enough to stabilize food products (Day, 2013). For this reason, proteins have been typically separated from other components following a wet process, and consequently, the protein content of the final flour increases. However, this process usually involves a great deal of waste, as well as the loss of protein functionality (Nosworthy, Tulbek, & House, 2017). To avoid these inconveniences, several alternatives have been proposed, among which ultrafiltration has been the most promising one (Castel, Andrich, Netto, Santiago, & Carrara, 2012). However, it may also involve the loss of some functional properties, including protein solubility and interfacial properties. In this sense, bibliography usually relates wet processes with the use of large amounts of water and other chemicals compounds such as hexane, HCl or NaOH (Schutyser, Pelgrom, van der Goot, & Boom, 2015). Unfortunately, there are very few studies related to dry processes, which could avoid the above-mentioned inconveniences. Dry processes require a lower amount of energy input and it produces lower amount of wastes. Moreover, some potentials of using dry processes are related as well with functional properties of the protein concentrates obtained, which remains almost unaltered after this kind of process. Moreover, these protein concentrates obtained also are related to higher nutritional properties. Unfortunately, these protein powders usually exhibit lower amount of total protein content and this procedure cannot be applied to all seeds, for instance, this procedure is not recommended to soy protein (Sosulski & Youngs, 1979) since this procedure is based on the difference of size between starch and protein granules, which can be mechanically detach, and where starch granules are the larger ones (Cloutt, Walker, & Pike, 1986; Schutyser et al., 2015). Based on these principles, a centrifugal counter-flow is applied. Drag forces act as a consequence of the joint action of air flow and centrifugal forces, which appears as a consequence of a classifier wheel. Thus, particles where the drag force is higher than the centrifugal one, pass thorough the

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classifier wheel, and it is classified as the heavy effluent (Schutyser et al., 2015).

For this research, the protein concentrate is obtained from direct milling of faba bean (*Vicia faba*) seeds, followed by air-densification process on dry basis. This densification process is based on the centrifugation of the milled seeds, which leads to two different product outlets: one is rich in protein (light effluent) and the other is rich in starch (heavy effluent) (Esfahan, Jahangiri, Kaviani, Hleba, & Shariati, 2015).

The use of legume proteins is fairly justified since the food industry is highly interested in the substitution of animal proteins in food products to manufacture healthier ones. Their use would decrease the human footprint. Among other legume proteins, faba bean is extensively grown in the Mediterranean area, being used nowadays mainly for animal feeding. In addition, its nutritional quality is excellent, which makes it a prime candidate for its use in human food (Vioque, Alaiz, & Girón-Calle, 2012).

The techno-functional properties of proteins to stabilize emulsions determine their capacity to decrease the interfacial tension of oil-in-water (O/W) interfaces, allowing the formation of small oil droplets. Moreover, they are also able to form an interfacial layer, preventing droplet coalescence (Karaca, Low, & Nickerson, 2015). However, the pH of the continuous phase has a remarkable effect on emulsion formation and stability, since it affects the surface charges of proteins. In this sense, protein-stabilized emulsions are more stable at pH levels far from the isoelectric point (IEP) (Damodaran, Parkin, & Fennema, 2007).

In addition, the viscosity of the continuous phase can increase with the presence of proteins, slowing down some destabilization phenomena, such as creaming or coalescence, and contributing to stabilize emulsions (Damodaran et al., 2007; Dickinson, 2001). Otherwise, long-term emulsion stability is only a kinetic concept and they are considered stable only if the microstructure and droplet size distribution (DSD) or the aggregation of droplets do not change over the time-scale of observation. Among others, rheological measurements can be used to determine the microstructure of emulsion systems (Franco, Guerrero, & Gallegos, 1995; McClements, 2004).

The present study is focused on the development of O/W emulsions stabilized by a faba bean (FB) protein concentrate obtained by a densification process on dry basis. The emulsification process was carried out at three different pH values (2.5, 5.0 and 8.0) and the stability of the emulsions was assessed over storage time.

2. Material and methods

2.1. Protein flour

The faba bean (FB) protein concentrate (HerbaPro F65) used for the development of the emulsions were supplied by Herba Ingredients (San José de la Rinconada, Seville, Spain). These FB protein concentrates were obtained by direct milling followed by a densification process on dry basis, which does not use water in any stage. High oleic sunflower oil (Capicua) was bought in a local store (Seville, Spain). The water used was distilled grade, and all other reagents were purchased from Panreac Química, S.A. (Barcelona, Spain).

2.2. Methods

2.2.1. Protein composition

Protein content from FB protein concentrates, before and after densification, was determined by multiplying g/kg Nitrogen x 6.25. Nitrogen content was determined in quadruplicate following the Dumas combustion method (Etheridge, Pesti, & Foster, 1998). The device used was a LECO CHNS-932 nitrogen micro analyser (St. Joseph, MI, USA). Other components such as lipid, moisture and ashes were determined in quintuplicate following A.O.A.C. (2000) procedures.

2.2.2. SDS-PAGE electrophoresis

Sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE) for electrophoresis were used to determine protein molecular fractions. The continuous gel contained 100 g/kg polyacrylamide, whereas the stacking gel contained 35 g/kg polyacrylamide. Both gels were used to obtain the different protein bands, with bands appearing within the continuous gel. Precision Plus Protein standards (Bio-Rad-Calibration kit, Richmond, CA, USA), containing ten protein bands, were used as molecular markers, while protein bands were stained using Coomassie Brilliant Blue.

2.2.3. Protein solubility

The solubility of the FB protein system was determined as a function of pH values (from 2 to 12). FB aqueous dispersions at 1 mg/mL were prepared in several buffers. Firstly, the FB protein concentrates were dispersed and stirred over 30 min, then centrifuged over 15 min at 15,000 × *g*. The Lowry method, with some modifications, was used to determine the soluble protein content (Markwell, Haas, Bieber, & Tolbert, 1978).

2.2.4. Z-potential measurements

The Z-potentials of protein solutions (0.1 mg/mL) were determined as a function of pH value (from pH 2 to 12) using a Zetasizer Nano ZS from Malvern Instruments (Malvern, UK). The instrument used the Smoluchowski equation to calculate the Z-potential from the electrophoretic mobility (Sze, Erickson, Ren, & Li, 2003).

2.3. Emulsions preparation

Following Ladjal-Ettoumi, Boudries, et al. (2016) and Ladjal-Ettoumi, Chibane, et al. (2016), a two-stage method was used to prepare the emulsions assessed in this study. Although this method was slightly modified, it consisted of a first step in which high oleic sunflower oil was gradually blended with aqueous FB dispersion (30 g/kg in protein content) at the selected pH value (2.5, 5.0 or 8.0), with an oil/water ratio of 50/50. These blends were subjected to high-shear mixing in a rotor-stator mixer (T25 basic ULTRA-TURRAX, IKA-Werke, Staufen, Germany) at 5000 rpm for 2 min (pre-emulsion). Subsequently, in the second step the pre-emulsions were passed (once) through the high-pressure valve homogenizer EmulsiFlex-C5 (Avestin, Ottawa, Canada) at 200 kPa.

2.4. Emulsion characterization

2.4.1. Backscattering (BS) measurements

Backscattering (BS) was obtained as a function of the tube height. These measurements were performed by means of Turbiscan Lab Expert (L'Union, France) along the storage time at 1, 7, 14, 21 and 28 days. Additionally, the relative backscattering ($\Delta BS\%$) was calculated in the middle of the tube (50 mm in height) according to equation (1):

$$\Delta BS\ \% = \frac{BS_0 - BS_t}{BS_0} \cdot 100 \quad (1)$$

where BS_0 and the BS_t are the backscattering of the sample at day 0, and at time t (1, 7, 14 and 28 days), respectively.

2.4.2. Droplet size distribution (DSD) measurements

Droplet size distribution (DSD) was determined by means of laser diffraction (Mastersizer X, Malvern, UK). To disrupt flocs, 10 g/kg of sodium dodecyl sulphate (SDS) was added to the water/emulsion dispersion, followed by a soft stirring (Puppo et al., 2005). The mean droplet diameter, named Sauter mean diameter ($D[3,2]$), was calculated as follows:

$$D[3,2] = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$