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Modulation of the emulsifying properties of pea globulin soluble aggregates by dynamic high-pressure fluidization

Bonastre Oliete, François Potin, Eliane Cases, Rémi Saurel*

Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, F-21000 Dijon, France

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ABSTRACT

The effects of thermal aggregation and microfluidization on pea (*Pisum sativum L.*) globulin characteristics and emulsifying properties were investigated. The thermal treatment of native pea globulins (NPs) induced the formation of aggregates (< 150 nm) that were partially stabilized by disulphide bonds and characterized by higher surface hydrophobicity and lower surface charge vs NPs. These modified characteristics facilitated the development of O/W interfaces and smaller droplets by aggregates. Nevertheless, coalescence and flocculation were favoured in aggregate-based vs NP-based emulsions. The microfluidization process (70, 130 MPa) led to structure rearrangements within globulin aggregates, resulting in decreased protein particle size and hydrophobicity. The positive action of the new characteristics of microfluidized aggregates on emulsion stability, by reducing the flocculation and creaming phenomena, was more pronounced at the highest microfluidization pressure. Because of these enhanced inter-droplet interactions, the gel-like structure of the aggregate-stabilized emulsion was supposed to play a key role in this stability.

Industrial relevance: Microfluidization or dynamic high pressure is a novel technology that could improve techno-functional properties of pea proteins after thermal aggregation. Microfluidization process at 70 MPA or 130 MPa broke large aggregates previously induced by the protein aggregation, and lead to new protein conformations. The new characteristics of microfluidized aggregates partly hinder the negative effect of thermal aggregation on the emulsion stability of aggregated proteins, especially when high microfluidization pressures were used (130 MPa). These findings will be of crucial importance for the development of pea protein-based oily formulations.

1. Introduction

With the rapid growth of the world's population (9 billion predicted for 2050), the efficient use of plant proteins will become critical when the supply of animal proteins reaches its maximum capacity (Aiking, 2011). Furthermore, the increasing awareness of the importance of food for health and the positive effects of vegetables in avoiding several diseases has increased the awareness of the benefits of plant proteins. Among the plant proteins, pea (*Pisum Sativum L.*) proteins represent an interesting option. Peas can be grown extensively worldwide, and the hull is easily removed. However, pea proteins remain largely underused by the food industry because of insufficient information regarding the relationship between structure–function and their performance (Adebiyi & Aluko, 2011; Karaca, Low, & Nickerson, 2011). Pea proteins mainly consist of globulins (65%–80% of the total proteins), which comprise three major groups: legumin (11S), with a hexameric structure (6 × ~60 kDa); and vicilin (7S) and convicilin (7S), both of which have a trimeric structure (3 × ~50 kDa and 3 × ~70 kDa, respectively) (Casey & Domoney, 1999).

One of the main techno-functional properties of proteins is their emulsifying capacity. For a protein to be an effective emulsifier, it should be water soluble and adsorb to the oil–water interface readily, unfold at the interface and form a cohesive film around oil droplets via inter-molecular interactions (Damodaran, 2006). Studies of globular protein-stabilized emulsions have demonstrated that the stability of the emulsion depends on the composition of the solution (pH, ionic strength, surfactants and biopolymers) and environmental stresses (heating, freezing and drying) (McClements, 2004). Peng et al. (2016) observed that the administration of thermal treatment to pea globulins affected the properties of the corresponding emulsions, such as flocculation and creaming stability.

To date, many physical processes, like heat or hydrostatic pressure treatments, have been used to improve the physicochemical and techno-functional properties of pea globulins (Keerati-u-rai & Corredig, 2009; Peng et al., 2016; Shen & Tang, 2012; Speroni, Anon, & de Lamballerie, 2010). Microfluidization is a homogenization technique

* Corresponding author.
E-mail addresses: eliane.cases@agrosupdijon.fr (E. Cases), remi.saurel@grosupdijon.fr (R. Saurel).

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that applies high pressure (up to 275 MPa) by pumping a liquid at high speed into microchannels with a fixed geometry. This treatment modifies the properties of the protein sample through the combined actions of shear, collisions with the walls of microchannels and the fluid itself, turbulence inside the microlitre cell and cavitation (Liu et al., 2009). The modification of protein structure via dynamic high-pressure treatment is a growing area of interest because it may affect the techno-functional properties of proteins. Dynamic pressure treatments at relatively low pressures (approximately 15–20 MPa) are used routinely in the dairy and other food industries as a homogenization technique. Treatments involving much higher pressures are less well known: the behaviour of whey proteins during this process has been the main subject of investigation in previous works (Bouaouina, Desrumaux, Loisel, & Legrand, 2006; Dissanayake & Vasiljevic, 2009; Iordache & Jelen, 2003; Oboroceanu, Zang, Magner, & Auty, 2014). The microfluidization of legume proteins, like soy proteins, has been studied more recently. Keerati-u-rai and Corredig (2009) demonstrated that dynamic high-pressure homogenization causes changes in the supra-molecular structure of soy proteins that are mainly related to the disruption of hydrophobic domains (Shen & Tang, 2012). Microfluidization improves the emulsifying efficiency of native soy proteins and of their thermal aggregates, but the microfluidization effect depends on the temperature of aggregation applied previously (75, 85 or 95 °C) (Shen & Tang, 2012). Tang and Liu (2013) concluded that emulsions formed from native and thermally aggregated (95 °C) soy proteins at various oil/water ratios (0.2–0.6) and at appropriate NaCl concentrations exhibited gel-like properties after microfluidization at a moderate pressure (40 MPa). Aggregate-stabilized emulsions tended to form a solid gel (higher elastic moduli) more often than did native-protein-stabilized emulsions. The formation of a gel-like network was strongly associated with the bridging flocculation of oil droplets through inter-droplet hydrophobic interactions involving aggregated proteins adsorbed at the oil–water interface (Tang & Liu, 2013). This type of gel-like network formation in the soy-globulin-stabilized emulsions was favoured by the high glycinin content of the soy globulin isolate (Luo, Liu, & Tang, 2013). Data pertaining to the effect of microfluidization on the emulsifying properties of pea globulins are scarce. Liang and Tang (2014) found that, at pH 3.0, pea globulins stabilized a new type of Pickering emulsion, especially when dispersions were previously microfluidized at a moderate pressure (40 MPa). As alternative Pickering emulsions, pea globulins showed extraordinary stability against coalescence and/or creaming and formation of a flocculated droplet network at high concentrations.

The present work was undertaken to study the effect of thermal aggregation and subsequent microfluidization (70 and 130 MPa) on the emulsifying properties of pea globulins at neutral pH.

Dispersions from pea globulin aggregates obtained by thermal treatment were subjected to a microfluidization process and then characterized in terms of their thermal properties (using differential scanning calorimetry (DSC)), polypeptide composition, particle size and charge, surface hydrophobicity and sulphydryl content. Emulsions prepared using these microfluidized pea globulins were studied for their interfacial protein-adsorption capacity, charge, emulsifying and flocculation capacities and stability.

2. Material and methods

2.1. Globulin extraction

Pea globulins were extracted from pea flour provided by Cosucra (Warcoing, Belgium) using a salt extraction method on a laboratory scale, as described by Crévieu, Berot, and Gueguen (1996), with slight modifications: pea flour was defatted by vigorous stirring in 5 volumes of petroleum ether for 1 h at room temperature (twice), and in 5 volumes of ethanol for 1 h at 4 °C (twice). The slurry was vacuum filtered on a No. 2 porosity glass filter, spread out in a pan and left for 48 h for solvent evaporation. The dry and defatted flour was poured into 10 volumes of 0.1 M sodium acetate buffer, pH 4.9, and then stirred overnight at 4 °C. The suspension was centrifuged at 12,000 ×*g* for 20 min at 4 °C. Pellets containing insoluble globular proteins were washed with approximately 30 volumes of deionized water to ensure the elimination of albumin. Washed pellets were re-suspended for 2 h at 4 °C in a 0.1 M phosphate buffer containing 5% (w/v) potassium sulphate, pH 8, using a pellet-to-buffer ratio of 1:10 (w/v). The remaining insoluble material was removed by centrifugation at 12,000 ×*g* for 20 min at 4 °C. Pooled supernatants containing salt-extractable proteins were concentrated five-fold by ultra-filtration (UF), and then desalted extensively via dia-filtration (DF) with 5 mM ammonium carbonate buffer, pH 7.2. The UF/DF procedure was performed using a Millipore Pellicon 2 Mini holder (Millipore SAS, Molsheim, France) equipped with a 1115 cm^2 Kvick Lab Cassette (UFELA0010010ST, GE Healthcare, Amersham Biosciences, Uppsala, Sweden) with a molecular weight cut-off of 10 kDa. The final globular pea protein retentate was centrifuged at 12,000 ×*g* for 20 min at 4 °C, frozen and freeze dried. The resulting powder was stored at −20 °C until further use.

The protein content of the pea globulins was 91.10% ± 0.3% (w/w) on a dry basis, as determined using the Kjeldahl method as per the AOAC International method 920.87 (AOAC, 1990) with a nitrogen conversion factor of 6.25 (Mession, Sok, Assifaoui, & Saurel, 2013; Shand, Ya, Pietrasik, & Wanasundara, 2007). The dry matter (96.32% ± 1.59% (w/w)) was determined according to the AOAC International method 923.03 (AOAC, 1990).

2.2. Preparation of soluble aggregates followed by microfluidization

Soluble aggregates (SAs) were obtained using protocols described in the literature (Chihi, Mession, Sok, & Saurel, 2016). Globulin dispersions were prepared in 10 mM sodium phosphate buffer, pH 7.2, and stirred overnight at 4 °C to complete hydration. Sodium azide (0.05% w/w) was added as a preservative. The pH was adjusted to 7.2 with 0.1 M NaOH.

The dispersion was centrifuged at 12,000 ×*g* for 20 min at 20 °C to remove insoluble proteins. The supernatant (called native pea globulins (NPs)) had a protein concentration of 2.52% ± 0.06% (w/w). NPs were placed in a temperature-controlled water bath previously equilibrated at 40 °C, then heated at 1 °C/min from 40 to 90 °C, incubated at 90 °C for 60 min and rapidly cooled on ice for 10 min. SAs were obtained after centrifugation (12,000 ×*g*, 20 min, 25 °C), to eliminate possible insoluble material. Intensive thermal treatment of NPs with low ionic strength and far from the isoelectric point will produce small, curved, strand-shaped aggregates (Nicolai, Britten, & Schmitt, 2011). Before each analysis, SA samples were brought to a temperature of 20 °C. The protein concentration of the final dispersion was determined using the Kjeldahl method. The protein loss during the formation of SAs was checked and found to be negligible (data not shown).

SA solutions were subjected to microfluidization at two different dynamic pressures: 70 MPa (SA70) and 130 MPa (SA130) on a LM10 Microfluidizer (Microfluidics, Newton, MA, USA) fitted with a Z-type chamber (G10Z). Samples were passed through the system three times. SA samples without microfluidization treatment were denoted as SA0. All samples obtained were stored at 4 °C before analysis and were used within 2 weeks.

2.3. Characterization of soluble aggregates that underwent microfluidization

The thermal transition of NP and SA (SA0, SA70 and SA130) samples was examined by DSC using a Micro DSC III calorimeter (Setaram, Caluire, France). NP and SA solutions (5%, w/w) in 10 mM phosphate buffer, pH 7.2, were used. Approximately 0.5 g of sample was weighed in an aluminium pan, hermetically sealed and heated from 25 to 105 °C at 0.5 °C/min. A pan with 10 mM phosphate buffer, pH 7.2,