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Emulsification properties of pea protein isolate using homogenization, microfluidization and ultrasonication

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ABSTRACT

Pea protein isolate (PPI) is used in many food formulations, due to its low cost, commercial availability and excellent amino acid profile. The objective of this study was to determine the emulsification properties of PPI. Particle size of PPI powders showed neither temperature (25–65 °C) nor time (up to 24 h) increased solubilisation of powder particles during mixing. Heating PPI dispersions (10%, w/w, protein) from 45 to 90 °C led to an increase in storage modulus (G'; Pa) at 71 °C, indicating the onset of protein aggregation. Gel formation occurred at 79 °C ($G' > 1$ Pa). Pea protein-stabilised emulsions made using homogenization (15 MPa; 1 pass) or microfluidization (50 MPa; 1 pass) resulted in the formation of cold-set gels, with gel strength increasing with increasing oil concentration and fluidic pressure. Droplet size and viscosity of pea protein-stabilised emulsions decreased and increased, respectively, with increasing ultrasonication time. Overall, ultrasonication (<50 °C) can create a uniform droplet size emulsion, while, homogenization and microfluidization can produce cold-set gels for use in a wide-range of food applications.

1. Introduction

Pea seeds accumulate multimeric storage proteins during development, namely albumin and globulin proteins. Albumins are regarded as metabolic and enzymatic proteins with cytosolic function (Harris & Croy, 1985). Unlike albumins, globulin proteins are degraded on germination of the seed to provide nutrients for the growing plant. The major seed proteins in pea are legumin and vicilin. Legumin is a hexameric molecule (molecular weight: ~380,000 Da) where each monomer (molecular weight: ~60,000 Da) contains an "acidic" and "basic" subunit linked by a disulphide bond. Vicilin is a trimeric molecule (molecular weight: ~150,000 Da) where each monomer can contain breaks in its polypeptide chain, giving rise to a variety of smaller subunits. The protein solubility of albumin and globulin fractions differ with the former considered water-soluble and the latter salt-soluble (Adebiyi & Aluko, 2011).

The extraction of protein from pea seeds for use in other food applications has become more common due to their nutritional, functional and economic benefits (Dijkink & Langelaan, 2002; Makri, Papalamprou, & Doxastakis, 2005; Singh, Kaur, Rana, & Sharma, 2010). Pea protein has a well-balanced amino acid profile, containing a high amount of lysine (Nunes, Raymundo, & Sousa, 2006). Applications for PPI include dairy-based beverages, sports and nutritional foods. Pea protein isolates (PPI) are typically produced by isoelectric precipitation (pH ~ 4.5) followed by applying membrane separation processes such as ultrafiltration and diafiltration to increase protein concentration. Adebiyi and Aluko (2011) demonstrated the extraction process of ethanol-, water-, salt- and alkaline-soluble pea protein fractions and their subsequent functional properties. Applications for pea protein include vegan style yogurts and non-dairy based sports products but is also used as partial dairy protein replacers for therapeutic beverages and powders.

Rehydration of PPI powders can be a challenging process with many available sources of pea protein being almost completely insoluble in water at neutral pH which may diminish their subsequent functional properties (Adebiyi & Aluko, 2011). The employment of ultrasound processing for the dissolution of plant- and dairy-based protein powders (i.e., soy and milk protein, respectively) has previously been assessed (Hu, Li-Chan, Wan, Tian, & Pan, 2013; McCarthy, Kelly, Maher, & Fenelon, 2014). Karaca, Low, and Nickerson (2011) showed PPI to have a low emulsification capacity due to its low surface charge and low solubility. The solubility of PPI is significantly affected by several parameters, such as temperature, pH, ionic strength and solvent type (i.e., water, ethanol, etc.). The extraction process may also play a significant role in subsequent protein solubility. The solubility of pea protein is strongly pH-dependent, with a minimum solubility between pH 4 and 6. Commercial PPI has been shown to have an extremely low solubility across a pH range of 2 to 9 (Taherian et al., 2011). The extraction and dehydration steps during protein isolation may affect the protein surface

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hydrophobicity by exposing hydrophobic residues, leading to increased hydrophobic interactions between proteins and/or peptides (Karaca et al., 2011; Tsumura et al., 2005). Previous studies (Adebiyi & Aluko, 2011; Aluko, Mofolasayo, & Watts, 2009) have examined the emulsification properties of PPI and pea protein fractions as a function of pH, whereby pea protein was observed to have less emulsification capacity at pH values close to its isoelectric point. At pH values above pH 7 emulsification capacity was much improved.

The emulsification process may be carried out by number of methods. Homogenisation involves forcing two immiscible liquids (e.g., oil and water) through a valve, which reduces the size of fat globules, due to shear forces, inertial forces and cavitation, and subsequent stabilisation by an emulsifier which reduces the interfacial tension between the oil surface and the water phase (Wilbey, 2002). The adsorption of proteins to the oil/water interface occurs in two stages: (i) transport and attachment of the molecules to the interface, and (ii) structural rearrangement of the protein molecules at the interface (Euston, 2004). A second-stage homogenisation valve operating at a lower pressure is commonly used during homogenisation to dissociate aggregates formed after the first-stage. Flow profiles of emulsions in homogenisers may be divided into three categories; laminar, turbulent or cavitational flow. Temperature plays an important role in homogenisation (Paquin, 1999). Increasing homogenisation temperature from 42 to 72 °C increases homogenisation efficiency (Michalski & Januel, 2006), with the size of fat globules depending on homogenisation pressure, protein concentration, protein structure, viscosity, pH and the time taken for protein adsorption to the interface of fat globules (Euston, 2004; Tcholakova, Denkov, Ivanov, & Campbell, 2006).

Microfluidization is a high-pressure homogenization system that can create uniform particle size reduction. Microfluidization generates high velocity micro-streams as a fluid accelerates into an interaction chamber, generating high shear and impact forces that cause the formation of fine emulsions (McCrae, 1994). Product enters the system via the inlet reservoir and is powered by a high-pressure pump into the interaction chamber at high speed. It is then cooled, if required, and collected in the output reservoir. The technology has been utilized as an alternative method for homogenizing milk (Ciron, Gee, Kelly, & Auty, 2010; Ciron, Gee, Kelly, & Auty, 2011 and McCrae, 1994,) and for processing a range of other dairy products.

Ultrasonication causes sound waves to dissipate through liquid media resulting in pressure differential cycles, with rates depending on the frequency. These pressure cycles create voids in the liquid which eventually collapse during the high-pressure cycle (Ashokkumar et al., 2009), causing high localised turbulence, shear forces, pressures and temperatures (Raviyan, Zhang, & Feng, 2005).

The aim of this study was to determine the dissolution properties of PPI powder and its subsequent functionality in terms of thermal stability, viscosity and emulsification properties.

2. Materials and methods

2.1. Materials and PPI composition

Commercially available pea protein isolate (PPI) powder was obtained from a local health food store. The protein, carbohydrate, fat, fibre and sodium content of PPI, according to the label, were 82% (w/w), 3.2% (w/w), 1.7% (w/w), 2.4% (w/w) and 1.5% (w/w), respectively. Sunflower oil was obtained from a local supermarket.

2.2. Reconstitution of PPI powders

PPI powders (10 g) were dispersed in 190 g of pre-tempered distilled water at 25, 50, 55, 60, 65 or 70 °C, using an overhead stirrer as described by McCarthy et al. (2014). After powder incorporation, samples were taken at defined time points (i.e., 30, 60, 90, 120, 150, 180, 210, 240, 270 min and 24 h) for analysis.

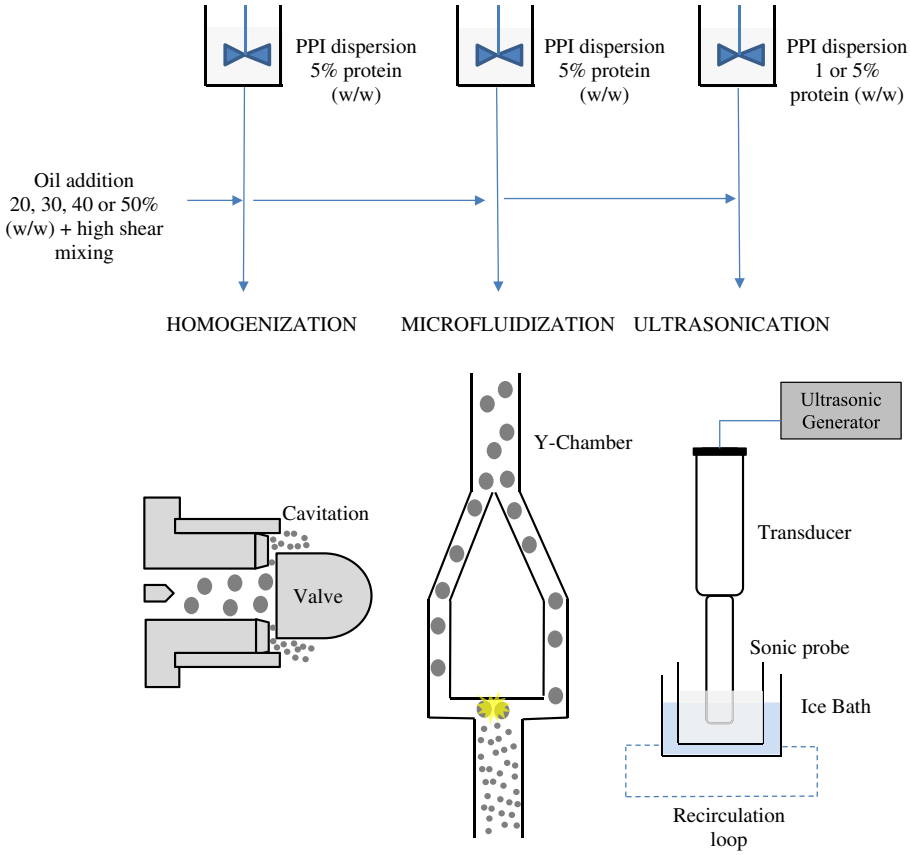

Fig. 1. Schematic diagram of emulsion preparation using homogenization, microfluidization or ultrasonication.