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Functional properties of pea protein hydrolysates in emulsions and spray-dried microcapsules

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ABSTRACT

In the present study the impact of partial enzymatic hydrolysis on the functional properties of pea protein isolate (PPI) was investigated. PPI and pea protein hydrolysates (PPH) with various degrees of hydrolysis (DH) were characterised by molecular weight distribution, interfacial activity and dilatational rheology (pendant drop tensiometry) and emulsion properties (oil droplet size and ζ-potential). Their suitability for the microencapsulation of nutritional oils by spray-drying and prevention of hydroperoxide formation during storage was evaluated. Only at very low DH (1%), could stable emulsions be produced using alcalase PPH as emulsifier, most likely due to enhanced bulk aggregation, however the oil droplet size was increased compared to PPI-stabilised emulsions. In contrast, trypsin PPH-stabilised emulsions exhibited smaller oil droplets and an increased surface charge (ζ-potential) with increasing DH in comparison to PPI-stabilised emulsions. Differences observed were reflected in dilatational rheological experiments. Depending on the enzyme used to produce PPH, the dilatational moduli increased and the phase angle decreased (trypsin), i.e. stronger and more elastic interfacial layers were formed, or vice versa (alcalase). The PPI- and trypsin-derived PPH-containing emulsions were stable during atomisation and drying, resulting in high microencapsulation efficiencies (94.5–95.6%). Trypsin PPH exhibited a higher potential than PPI to reduce lipid oxidation of rapeseed oil in spray-dried emulsions during storage as demonstrated by hydroperoxide formation. This effect may be attributed to the altered physical properties of the interfacial film as well as the antioxidative effects of the hydrolysed proteins.

1. Introduction

The creation and stabilisation of an emulsion essentially requires surfactants like proteins to reduce the interfacial tension between oil and water and to create a gradient in interfacial tension, which facilitates droplet breakup (Walstra, 1993). The rate of lowering the interfacial tension is crucial for the stabilisation of the interface (Damodaran, 2005). A protein should meet the following properties: fast diffusion and adsorption to the newly created interface during homogenisation, unfolding of its molecular structure and the formation of highly viscoelastic films by intermolecular interactions at the interface to stabilise the increased interfacial area against (re)coalescence (Damodaran, 2005). Thus, the functional properties of proteins with respect to their application in dispersed food systems depends on the physical, chemical and conformational properties, i.e. size, shape, amino acid composition and sequence, charge and charge distribution (Rodriguez Nino, Carrera Sanchez, Ruiz-Henestrosa, & Rodriguez Patino, 2005). As a result of their structural characteristics globular proteins may possess limited emulsifying properties due to their high molecular weight, compact tertiary structure, inclusion of their hydrophobic groups in the molecules interior and low molecular flexibility (Barac et al., 2011; Davis, Doucet, & Foegeding, 2005; Martinez, Carrera Sanchez, Ruiz-Henestrosa, Rodriguez Patino, & Pilosof, 2007; Ruiz-Henestrosa, Carrera Sanchez, Pedroche, Millan, & Rodriguez Patino, 2009).

An overwhelming amount of literature is available for whey protein stabilised emulsions. Legume proteins present a serious option to replace widely used proteins from animal origin, in food and pharmaceutical applications (Aberkane, Roudaut, & Saurel,

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2014; Karaca, Low, & Nickerson, 2011). Soy protein is the legume protein studied far most in the last decades (O'Kane, Happe, Vereijken, Gruppen, & van Boekel, 2004). However in comparison pea proteins possess the advantages of being non-allergenic, of high nutritive value (Gharsallaoui, Saurel, Chambin, & Voilley, 2012), and exhibit similar emulsifying properties (Aluko, Mofolasayo, & Watts, 2009). Pea protein has been used as emulsifier in liquid emulsions (Aluko et al., 2009; Amine, Dreher, Helgason, & Tadros, 2014; Barac et al., 2010, 2011, 2012; Dagorn-Scaviner, Gueguen, & Lefebvre, 1987; Gharsallaoui, Cases, Chambin, & Saurel, 2009; Gharsallaoui et al., 2012; Gharsallaoui, Yamauchi, Chambin, Cases, & Saurel, 2010; Humiski & Aluko, 2007; Karaca et al., 2011), and as emulsifier in spray-dried emulsions for the microencapsulation of oil (Aberkane et al., 2014; Gharsallaoui et al., 2012).

Due to the complex molecular structure it must be hypothesised that the emulsifying properties of pea proteins are not fully exploited in the native state. A limited enzymatic hydrolysis is a suitable technique to improve the functional properties of globular proteins (Davis et al., 2005; Ipsen et al., 2001; Martinez, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2009; Minones Conde & Rodriguez Patino, 2005; Perez, Sanchez Carrera, Rodriguez Patino, Rubiolo, & Santiago, 2012; Ruiz-Henestrosa et al., 2007, 2009; van der Ven, Gruppen, de Bont, & Voragen, 2001), however they were also found to be impaired in some studies (Martinez et al., 2009, 2007; Minones Conde & Rodriguez Patino, 2005; van der Ven et al., 2001). Generally, an enzymatic hydrolysis leads to a reduction in molecular weight, facilitating the coverage of a larger interfacial area in comparison to the unmodified protein (O'Regan & Mulvihill, 2010). Hydrolysis may result in the exposure of hydrophobic patches from the interior of the globular molecule (Damodaran & Paraf, 1997) and thus increases the interfacial activity (Minones Conde & Rodriguez Patino, 2007) initiated by a higher rate of diffusion (Ruiz-Henestrosa et al., 2007). Additionally, the molecular flexibility may be increased, enabling a more rapid unfolding and the formation of intermolecular interactions between adsorbed molecules (Perez et al., 2012). The functional properties of peptides created by enzymatic hydrolysis depend on a multitude of factors, e.g. the specificity of the enzyme used or the degree of hydrolysis (Ipsen et al., 2001). Generally, with respect to the functional properties in emulsions, a low degree of hydrolysis (1–10%) is favourable (O'Regan & Mulvihill, 2010). Apart from the evaluation of the oil droplet size distribution and ζ-potential to characterise protein-stabilised emulsions, the interfacial activity of proteins and peptides is often studied, i.e. the decrease in interfacial tension due to protein adsorption (Minones Conde, Escobar, Pedroche Jimenez, Rodriguez, & Rodriguez Patino, 2005). A decreased interfacial tension lowers the energy input required to create the emulsion and thus facilitates the creation of smaller droplets (Bos & van Vliet, 2001). Furthermore the interfacial dilatational rheology of proteins has been the objective of many investigations since compression/expansion experiments are linked to emulsion stability during emulsification (Benjamins, Lyklema, & Lucassen-Reynders, 2006).

In addition to their functional properties proteins also exhibit antioxidant properties in oil/water-emulsions (Adjonu, Doran, Torley, & Agboola, 2014; Berton-Carabin, Ropers, & Genot, 2014). These properties include the chelation of metals, free radical scavenging, binding of secondary lipid oxidation products and/or the formation of a physical barrier protecting the lipid phase (Berton-Carabin et al., 2014). Apart from extensively studied proteins, e.g. milk proteins (Serfert et al., 2014; Tamm et al., 2015), pea proteins also exhibit antioxidant properties which were improved due to enzymatic hydrolysis (Pownall, Udenigwe, & Aluko, 2010; Zhang, Xiong, Chen, & Zhou, 2013). Recently published results on hydrolysed β-lactoglobulin indicate that the antioxidant properties of peptides does also improve the oxidative stability in oils microencapsulated by spray-drying (Tamm et al., 2015), but no such approach has been reported for pea protein hydrolysates.

Aim of the present study was to investigate the impact of a limited enzymatic hydrolysis on the physicochemical properties at the oil/water-interface, encapsulation properties and antioxidant effects of pea proteins in spray-dried emulsions. The application of two proteases with varying cleaving specificity allows the generation of peptide distributions strongly differing in their functional properties. The proteins and hydrolysates were characterised by their molecular weight distribution (SEC and SDS-PAGE) and their interfacial dilatational rheology (pendant drop tensiometry). Emulsifying properties and subsequent stability of emulsions stabilised by pea protein isolate (PPI) and pea protein hydrolysates (PPH) were analysed regarding oil droplet size and ζ-potential. Rapeseed oil (as a reference for an oil with a high proportion of unsaturated fatty acids) was microencapsulated by spray-drying of emulsions stabilised by PPI or PPH and the microencapsulation efficiency and lipid oxidation in terms of hydroperoxide formation were evaluated from microcapsules.

2. Materials and methods

Pea protein isolate (PPI, Pisane F9, Cosucra, Warcoing, Belgium, 85% protein as is) was kindly provided by Georg Breuer GmbH (Königstein, Germany) and stored at 4 °C. For enzymatic hydrolysis trypsin (from bovine pancreas, cat#T8003, EC: 3.4.21.4, 12238 BAEE units/mg protein) and a serine protease (alcalase 2.4L, cat# P4860, EC: 3.4.21.62, 2.59 AU/g protein) both purchased from Sigma–Aldrich (Taufkirchen, Germany) were used. Glucose syrup (DE38, C*Dry 01934) was purchased from Cargill (Krefeld, Germany). Commercial rapeseed oil was purchased at local supermarket. All chemicals were of reagent grade.

2.1. Enzymatic hydrolysis of PPI and molecular weight distribution of PPI and PPH

Enzymatic hydrolysis of pea protein isolate was conducted as described elsewhere (Frederic Tamm et al., 2015). In the following the pea protein hydrolysates (PPH) will be abbreviated by DHX(Y), with X being the degree of hydrolysis and Y indicating the enzyme used for modification, i.e. trypsin (T) or alcalase (A). Solutions containing 5 wt% PPI were hydrolysed to a degree of hydrolysis (DH) of 1, 2, 4, 6 or 8% using the pH-Stat method of Adler-Nissen (1986) at pH 8.0. The DH was calculated using a h_{tot}-value of 8.41 meqv/g of protein (Owusu-Apenten, 2002) for the total number of peptide bonds in the protein substrate. The enzyme/substrate-ratio (w/w) was varied for each enzyme and DH, for hydrolysis using trypsin the following ratios have been used: DH1 (1:6500), DH2 (1:2000), DH4 (1:800), DH6 (1:600) and DH8 (1:200). Accordingly for hydrolysis via alcalase these ratios were applied: DH1 (1:700), DH2 (1:400), DH4 (1:300), DH6 (1:200) and DH8 (1:100). The inactivation of the enzyme was conducted by heating the reaction mixture to 75 °C for 30 min when the required DH was obtained. Samples for the determination of the molecular weight distribution were taken after hydrolysis and frozen until further analysis. Emulsions were prepared using freshly prepared hydrolysed samples.

2.1.1. Tris-tricine SDS-PAGE

The characterisation of the peptide composition of PPI and the PPH resulting from enzymatic hydrolysis was performed by SDS-PAGE under reducing and non-reducing conditions (Laemmli, 1970). The method used described in details elsewhere (Tamm