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Understanding the differences in gelling properties between lupin protein isolate and soy protein isolate

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ABSTRACT

The gelling properties of lupin protein isolate (LPI) were compared with those of soy protein isolate (SPI). It was found that LPI behaves fundamentally different than SPI, evidenced by the formation of weaker and deformable gels. Further investigation shows that both protein isolates can be considered particle gels and that LPI particles do not swell as much as SPI particles inside the network. Besides, heating hardly affects LPI particles while SPI particles show additional swelling. To explain the differences, the sulfhydryl reactivity of LPI was tested. The amount of free sulfhydryl groups on LPI was higher than the amount of free sulfhydryl groups on SPI. Upon heating the amount of free sulfhydryl groups on LPI increases. We hypothesize that the compact, heat stable structure of the protein particles suppresses the intermolecular bonding through disulphide bridge formation and favours intramolecular crosslinking. The small sulphur-rich proteins that are not incorporated within the particles but are present in the surrounding solution cannot strengthen the particle network, due to their low concentration. LPI did not form gels of similar consistency as SPI and may therefore be less useful for solid food products. The thermal stability of LPI could offer opportunities for high-protein foods that require low viscosity after heating.

1. Introduction

Legume seed proteins have gained increased attention due to their favourable nutritional and functional properties for modern food production (Batista, Portugal, Sousa, Crespo, & Raymundo, 2005; Day, 2013; Makri, Papalamprou, & Doxastakis, 2005). The traditional raw materials for many plant-based alternatives to animal-based foods are soybeans and wheat and lately also peas and lupin can be found in these alternatives. Animal-based foods are mainly composed of protein, water and oil. Soybeans and lupin seeds are rich in protein, contain oil and are low in starch, while peas and wheat are high in starch. Soybeans do not grow in temperate areas though and therefore rely on a long supply chain while lupin can be grown in moderate climates areas as Northern Europe. Therefore, more research focuses on legumes that can be grown in moderate climate countries, like pea and lupin (Batista et al., 2005; Cai, McCurdy, & Baik, 2002; Dijkstra, Linnemann, & van Boekel, 2003; Drakos, Doxastakis, & Kiosseoglou, 2007; Fontanari et al., 2012; Hojilla-Evangelista, Sessa, & Mohamed, 2004; Kiosseoglou, Doxastakis, Alevisopoulos, & Kasapis, 1999; Makri et al., 2005; Mohamed et al., 2005; Swanson, 1990). Lupin seeds are interesting as food ingredient because of their high protein content, which is at least similar to that of soybeans. Currently, soy protein isolates and concentrates are mainly used in plant-based products because of their excellent gelling and structuring behaviour (Banerjee & Bhattacharya, 2012; Day, 2013). Many other legumes and oilseeds do not possess these functional properties naturally and that is why soybeans are taken as a benchmark. For example, pea and lupin protein isolates are reported to form weaker heat-induced gels than soy protein isolates (SPI) (Batista et al., 2005). The low gelling capacity made lupin an ideal protein source for replacing fish meal in fish pellets (Draganovic, Boom, Jonkers, & van der Goot, 2013).

Food gels can be considered high-moisture, 3D polymeric networks that resist flow and retain their distinct structural shape upon deformation (Banerjee & Bhattacharya, 2012). Food gels are a continuous network of assorted macromolecules or interconnected particles dispersed in a continuous liquid phase, for which the properties are determined by the components present in the network. For example, differences in gel strength and deformability are related to differences in protein molecular

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weight and the hydrodynamic size of the polypeptides in the gel (Renkema, 2001; Totosaus, Montejano, Salazar, & Guerrero, 2002). Gel formation of plant proteins can be induced through heating, which leads to transformations such as molecular unfolding, dissociation-association and aggregation (Batista et al., 2005; Damodaran, Parkin, & Fennema, 2008). An unfolded protein exposes functional groups on the surface of the protein such as hydrophobic, hydrogen, electrostatic and sulfhydryl groups. After protein unfolding, protein aggregates are formed through hydrophobic interactions and strengthened further due to the formation of disulphide bridges (Wang & Damodaran, 1991). The role of disulphide bridges in protein gelation is related to their ability to increase the protein molecular weight and hence the chain length, rather than acting as an initial network stabilizer (Clark, 1998; Wang & Damodaran, 1990).

Soy and lupin flour both contain globular proteins, more specifically salt-soluble globulins and water-soluble albumins in a ratio of 9:1. During the production of protein isolates part of the water-soluble albumins are lost, enriching the protein isolate in globulins (Berghout, Boom, & van der Goot, 2014; Lqari, Vioque, Pedroche, & Milla, 2002). Batista et al. (2005) established a relationship between the gelling ability of soy, pea and lupin protein isolates and their resistance to thermal unfolding. SPI formed strong gels, which was associated with more protein unfolding during and after thermal treatment. LPI formed only weak gels and the authors stated that this was because the unfolding of LPI upon heating was not significant due to its high denaturation temperature. However, it remains interesting to explore the nature of those differences. The reduced thermal unfolding of LPI might be related to the ratio of polar and non-polar amino acids present in LPI and SPI. Fisher (1964) introduced the polarity ratio *p*, which is the ratio of polar to non-polar volume of amino acid residues. This ratio is 1.7 for lupin flour and 1.4 for soy flour, which means that both have very polar proteins. The small difference between lupin and soy flour probably does not explain the major differences in gelling properties of SPI and LPI. The accessibility of sulfhydryl groups on LPI upon heating might play a role, though this has not been reported yet.

In this study we further explore the differences between LPI and SPI's functional properties and investigate the effect of an altered gelling process, such as prolonged, high temperature heat treatments, on LPI's gelling properties. Small deformation rheology is used to identify the differences between LPI and SPI dispersions and gels for 12–30% (w/v) protein and at 95 °C. The swelling behaviour of LPI and SPI on macroscopic scale is studied with light microscopy and laser scattering. The differences between SPI and LPI on microscopic scale are investigated by the determination of the size of their protein subunits and by quantification of the amount of free sulfhydryl groups for disulphide bridge formation.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI), Supro 500E IP, was kindly provided by Barentz, the Netherlands. This product contained at least 90% protein (N x 6.25) and was not chemically modified after isolation according to the manufacturer's specifications. Lupin protein isolate (LPI) with a protein content higher than 90% (N x 6.25) was prepared in-house, with the aqueous fractionation method as described previously by Berghout et al. (2014), from untoasted lupin seeds (LI Frank, Twello, the Netherlands). All reagents used were of analytical grade unless otherwise stated.

2.2. Methods

2.2.1. Preparation of protein dispersions and gels

Prior to gelling, the protein isolates were dispersed into Millipore water in 15 mL Falcon tubes at room temperature. The pH of the SPI dispersions varied between 7.1 and 7.2. The pH of the LPI dispersions varied between 6.8 and 7.0. The protein dispersions were stirred with a glass rod until completely wetted. The concentrations used were 12, 15, 18 and 24% (w/v) for SPI and 12, 15, 18, 24 and 30% (w/v) for LPI. After viscosity measurements (see 2.2.4 Small deformation rheology), the dispersions were heated in a water bath at 95 °C and kept for 30 min. The dispersions were cooled with running tap water and subsequently stored at 4 °C for 24 h. Two additional LPI dispersions of 30% (w/v) were prepared; the first dispersion was heat-treated in a water bath at 80 °C and kept for 30 min, the second dispersion was heat-treated at 80 °C and kept for 8 h. Both protein dispersions were cooled with running tap water and stored at 4 °C for 24 h. The protein dispersion heated at 80 °C for 30 min was re-heated to 130 °C in an in-house developed shearing device (van der Zalm, Berghout, van der Goot, & Boom, 2012) for about 10 min and then cooled down to 10 °C. All dispersions and gels were prepared in duplicate.

2.2.2. Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a Diamond DSC (PerkinElmer, USA) using stainless steel pans. About 10 mg of sample was weighed into the pans. The DSC analyser was calibrated with indium and an empty pan was used for reference. Samples were scanned between 20°C and 130 °C with a heating rate of 10 °C/min. Measurements were analysed for peak temperature and enthalpy of denaturation.

2.2.3. Light microscopy

An upright microscope Axioscope (Carl Zeiss Microscopy, LLC, United States) with camera was used to inspect the samples. The LPI and SPI powders were dissolved in Millipore water at 1% (w/v) and mixed at 900 rpm for 1 h on a Multi Reax vibrating shaker (Heidolph, Essex, UK). One LPI dispersion and one SPI dispersion were heated at 90 °C for 30 min and cooled under running tap water. The samples were prepared on a glass slide at room temperature and covered with a cover slip. Snapshots of 100 ×, 200 × and 400 × magnification were taken.

2.2.4. Small deformation rheology

The protein dispersions were transferred to a rheometer (Anton Paar Physica MCR301, Graz, Austria) using a cone-plate geometry (CP-20-2). The samples were equilibrated for 5 min; subsequently the flow properties were determined at 25 °C using a shear rate range from 1 to 100 s^{-1}. The flow properties of the protein gels were determined with plate–plate geometry (PP-25/P2) under the same conditions as the protein dispersions. Amplitude sweeps were performed to find the linear viscoelastic region of SPI and LPI gels. A frequency sweep test was performed on the protein gels with cone-plate geometry (CP-20-2) at constant strain (0.1%) and increasing angular frequency (0.1–10 rad s^{-1}) at 25 °C. The gels were equilibrated for 10 min. Tangent delta ($\tan\delta = G''/G'$) was calculated from frequency sweep data at 1 rad s^{-1} (within LVE).

2.2.5. Static laser scattering

For particle size analysis, 1% (w/v) protein isolate was dispersed in Millipore water in a 15 mL Falcon tube. For each protein isolate, five tubes were prepared: one tube was kept at room temperature, three tubes were heated at 75 °C, 85 °C or 95 °C for 30 min, and one tube was heated at 80 °C for 8 h. Additionally, one tube of 1% (w/v) LPI was heated at 90 °C for 8 h. The particle size distribution was