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Comparing functional properties of concentrated protein isolates with freeze-dried protein isolates from lupin seeds

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ABSTRACT

Fractionation processes are used to increase the potential applications of plant materials. Unfortunately, those processes are energy-intensive and require large amounts of water and chemicals. A route to reduce the energy consumption is to replace the drying step by ultrafiltration. The latter is possible, because in many final applications, water is used to make the products. However, the dried product is still the industrial standard and altering the final steps in fractionation requires sound understanding of the differences in functional properties and the underlying reasons. This study compared the technical functionality of freeze-dried lupin protein isolates (LPIs) with concentrated 'wet' LPIs, both obtained through aqueous fractionation. It was demonstrated that freeze-drying lead to the formation of relatively large protein particles that are quite stable upon thermal treatment. The concentrated LPI dispersions were composed of smaller protein aggregates. In terms of functionality both protein isolates show similar behaviour, though some differences are observed. Additional processing of concentrated LPI however can make the properties more comparable to the freeze-dried LPI, making them suitable for most applications in which now dried protein isolates are used. In addition, the concentrated protein isolate provides additional functionality, which might offer new product opportunities.

1. Introduction

Dehulled lupin seeds from *Lupinus angustifolius* have a high protein content (about 37–40 wt%). The protein contains several essential amino acids and has a high protein digestibility due to low levels of anti-nutritional factors and protease inhibitors (Chew, Casey, & Johnson, 2003; Lqari, Vioque, Pedroche, & Millan, 2002). The applicability of lupin and other plant materials can be enhanced by fractionating these materials into their components. Plant protein isolates can be prepared using wet fractionation techniques. Prior to protein extraction from oilseeds, the flour is defatted with organic solvents like hexane or petroleum ether. The defatted flour is then solubilized in water or a buffer at alkaline pH, after which insoluble parts are separated from the protein-rich supernatant. The protein is separated from other soluble solids, like sugars, by iso-electric precipitation of the protein. The major drawback of fractionation processes is that they require large quantities of water, energy and chemicals. In a recent study, we showed that in case of oilseeds main losses occur due to material loss during fractionation, due to the oil extraction step using organic solvent, and due to the drying step (Berghout, Pelgrom, Schutyser, Boom, & van der Goot, 2015). Routes to reduce the environmental impact of fractionation processes are therefore to improve yield, and omit defatting and drying steps. Aqueous fractionation of lupin seeds without oil extraction avoids the use of organic solvents and results in lupin protein isolates (LPIs) containing a few percent of oil and with functional properties similar to those of wet-fractionated LPIs, which generally does not contain oil (Berghout, Boom, & van der Goot., 2014). Generally, protein isolates are dried after protein isolation for chemical and microbial stability and reduced transportation costs. In industry, dried products can be considered as the standard product, meaning that products produced in another manner should have similar functional properties at least.

To reduce the energy consumption of protein fractionation processes, the drying step may be replaced by ultrafiltration to concentrate the protein dispersion up to the concentration relevant for direct product application (e.g. up to 10% (w/v) for high-protein

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beverages). Concentration is possible because in many final applications, water is used to make the (food) products. After fractionation and concentration, the protein dispersion will have to be heated to ensure microbial and chemical stability or will have to be used directly after production. The properties of the resulting concentrated LPI dispersions have not yet been reported to our knowledge. Papalamprou, Doxastakis, Biliaderis, and Kiosseoglou (2009) reported that milder processing techniques, rather than the composition of the protein isolate, improved the functional properties of chickpea protein isolates in terms of increased protein solubility, reduced minimum protein concentration needed for gel formation, and improved gel elasticity. Ultrafiltration was shown to improve the solubility behaviour of soy protein concentrates and isolates (Alibhai, Mondor, Moresoli, Ippersiel, & Lamarche, 2006) and resulted in gels at lower protein concentration for LPIs (Kiosseoglou, Doxastakis, Alevisopoulos, & Kasapis, 1999). In this study, the effect of concentrating a wet LPI dispersion to 10% (w/v) on functional properties is investigated and compared with the functional properties of the dried LPI.

The effect of the drying method on protein functionality depends on the drying method and on the type of protein. Water-based plant protein dispersions are typically dried using freeze-drying, spray-drying or vacuum-drying (Hu et al., 2009; Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011; Liao, Wang, & Zhao, 2013). Freeze-drying is an expensive drying process, which is typically used for speciality ingredients and is perceived to be a relative mild drying process (Claussen, Strømmen, Egelandsdal, & Strætkvern, 2007). Spray drying is often applied in industry because of its scalability, continuous operation and standardized quality specifications (Georgetti, Casagrande, Souza, Oliveira, & Fonseca, 2008). Vacuum drying is a low cost process that is performed at low temperature, but requires long residence times (Joshi et al., 2011). Freeze-drying influences the morphology and size of the protein and the surface hydrophobicity of proteins by partial denaturation, due to stresses such as low temperatures, freezing stresses (e.g. phase separation, pH change and ice crystal formation) and drying stresses (Hu et al., 2009; Wang, 2000). Spray drying reduced the solubility of a lentil protein isolate less than vacuum drying (Joshi et al., 2011) and can lead to thermal damage in case of lupin protein isolates (D'Agostina et al., 2006). Since freeze-drying is generally perceived as the mildest form of drying, this drying method was chosen for comparison with ultrafiltration.

The objective of this paper is to better understand the differences in the properties of ultrafiltered, non-dried 'wet' LPI dispersions and freeze-dried LPI dispersions, both obtained by aqueous fractionation. The properties of the wet and freeze-dried LPIs are compared by measuring viscosity, nitrogen solubility index, volume fraction, particle size distributions and the zeta potential before and after heat treatment. These parameters reflect the influence of freeze-drying on the structural properties of LPI on a molecular and mesoscopic scale.

2. Materials and methods

2.1. Materials

Lupin seeds (*L. angustifolius)* were obtained from LI Frank (Twello, the Netherlands). Tap water (4.8°dH) was used throughout, unless stated otherwise. All reagents used were of analytical grade. Lupin flour was produced by pre-milling 1 kg of lupin seeds to grits with a Condux-Werk pin mill LV 15 M (Condux-Werk, Wolfgang bei Hanau, Germany) after which the grits were further milled into a flour with a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany) with a classifier wheel (Berghout, Boom, & van der Goot, 2014; Berghout, Boom, et.al., 2015).

2.2. Protein isolation

Freeze-dried lupin protein isolates (LPIs) were prepared by aqueous fractionation as described by Berghout et al. (2014). In short, aqueous fractionation starts with solubilizing full-fat lupin flour into tap water in a ratio of 1:15 (w/v) and adjusting the pH to 9 with 1 mol/L NaOH. The dispersions were stirred at 4 °C for 1 h and subsequently centrifuged, after which a fibre-rich pellet could be separated from a protein-rich supernatant. The protein-rich supernatant was acidified to pH 4.5 with 1 mol/L HCl and stirred at 4 °C for 1 h. Then the dispersions were centrifuged and the protein pellet was washed twice with distilled water. The protein pellet was re-dispersed in distilled water and the pH was adjusted to 7.0 with 1 mol/L NaOH. These protein dispersions at pH 7.0 were stored in the freezer at −20 °C and subsequently freeze-dried. Wet LPIs were prepared in a similar way, except for the freezing and drying step. Instead, after neutralization to pH 7.0 with 1 mol/L NaOH, the wet LPI dispersions were concentrated using membrane (ultra)filtration (Amicon cell, Millipore Co. Bedford, USA), fitted with a regenerated cellulose membrane having a molecular weight cut-off of 5 kDa (Millipore Co. Billerica, USA). Pressurized air at 4 atm was applied over the cell and the stirring speed was set at 500 rpm. To prevent bacterial growth during concentration, the water jackets surrounding the Amicon cells were cooled with a circulating water bath set at 4 °C. All protein isolations were performed in duplicate. A sample of the LPI dispersion before concentration and drying was taken for particle size distribution determination.

2.3. Sample preparation

Protein dispersions were prepared by dispersing the freeze-dried LPI in distilled water at 10% (w/v) in Falcon tubes and shaking the tubes for 30 min on a Multi Reax tube shaker (Heidolph Instruments GmbH, Schwabach, Germany) at room temperature. The tubes with the re-dispersed freeze-dried LPI were checked for sedimentation before analysis. The wet LPI dispersions were concentrated to 10% (w/v) using membrane filtration. The dispersions were at pH 7.0 and were stirred for 1 h before heat treatment. One dispersion was kept at room temperature, the second dispersion was heat-treated in a water bath at 60 °C for 30 min, the third dispersion was heat-treated in a water bath at 80 °C for 30 min, the fourth dispersion was heat-treated in an autoclave (Systec V-150, Systec GmbH, Germany) at 100 °C for 5 min and the fifth dispersion was heat-treated in an autoclave at 121 °C for 5 min. The autoclave required 30 min to reach the set temperature, cooling down required 20 min. All bottles containing the protein dispersions were cooled to room temperature with running tap water. Each heat treatment was performed in duplicate.

About 15 mg freeze-dried powder was dispersed into 1.5 mL of three different solvents. Solvent 1 was a 50 mM Bis-Tris buffer, solvent 2 a 50 mM Bis-Tris buffer with 10 mM DTT, and solvent 3 a 50 mM Bis-Tris buffer with 10 mM DTT and 6 M urea. The samples were evaluated with the upright microscope, as described in the section Light Microscopy, after incubation for 1, 2, and 24 h at room temperature.

2.4. Sample composition

The dry matter content was determined by drying 1 g of sample overnight in an oven at 105 °C. The oil content was determined by a Soxhlet extraction according to AACC method 30-25 (AACC, 1983a) on a fully-automated extractor (Büchi extractor B-811, Büchi Labortechnik, Germany). Oil extraction was performed with petroleum ether (boiling range 40–60 °C) for 3 h. The protein content was determined with the Dumas combustion method on an NA