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The potential of aqueous fractionation of lupin seeds for high-protein foods

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ABSTRACT

Aqueous fractionation of protein from lupin seeds was investigated as an alternative to the conventional wet fractionation processes, which make use of organic solvents. The effect of extraction temperature was studied and the consequences for downstream processing were analysed. Omitting the extraction of oil with organic solvents resulted in a protein isolate that contained 0.02–0.07 g oil g^{-1} protein isolate, depending on the exact extraction conditions. Nevertheless, the protein functionality of the aqueous fractionated lupin protein isolate was similar to the conventional lupin protein isolate. The protein isolate suspension could be concentrated to 0.25 g mL^{-1} using ultrafiltration, which provides a relevant concentration for a range of high-protein products. Based on the results, we conclude that aqueous fractionation can be a method to lower the environmental impact of the extraction of proteins from legumes that contain water- and dilute salt-soluble proteins.

1. Introduction

Many protein-rich plants are used as animal feed to produce protein-rich products like milk, eggs and meat. However, the conversion of plant proteins into animal proteins is inefficient (Pimentel & Pimentel, 2003). Even though plant proteins are more abundant, direct consumption of protein-rich plants, beans or seeds is limited (Day, 2013). Previous studies showed that consumers are willing to switch to plant-based products provided that those products have similar taste and texture as their animal-based equivalents (de Boer, Hoek, & Elzerman, 2006). Important conditions for the development of plant-based alternatives are the availability of plant protein concentrates and isolates with high functionality and produced in a sustainable manner. Current protein extraction processes are inefficient due to the use of organic solvents, acids and bases, and large amounts of water, as a result of which the environmental gain is less than theoretically possible (Apaiah, Linnemann, & van der Kooi, 2006; Schutyser & van der Goot, 2011).

Lupin is a legume with high protein content and is therefore an interesting raw material for plant-based, high-protein products. Additionally, the seeds of lupin are known to have beneficial health effects, while the plant accepts versatile breeding conditions (Arnoldi et al., 2007; Cerletti & Duranti, 1979; Foley et al., 2011; Fontanari, Batistuti, da Cruz, Nascimento Saldiva, & Gomes Areas, 2012). The sweet variety *Lupinus angustifolius* has a protein content of about 400 g kg^{-1} flour and is further composed of carbohydrates (480 g kg^{-1} of flour), oil (70–100 g kg^{-1} of flour), minerals and water. Its proteins have an excellent amino acid composition (El-Adawy, Rahma, El-Bedawey, & Gafar, 2001; Lqari, Vioque, Pedroche, & Millán, 2002).

For use in high-protein food products, the proteins need to be extracted from the seeds. Conventional wet extraction processes remove the oil through organic solvent extraction, followed by aqueous extraction steps with varying the pH to obtain an almost pure protein fraction (Alamanou & Doxastakis, 1995; Jayasena, Chih, & Nasar-Abbas, 2011; Kiosseoglou, Doxastakis, Alevisopoulos, & Kasapis, 1999; Lqari et al., 2002; Sironi, Sessa, & Duranti, 2005; Süssmann, Pickardt, Schweiggert, & Eisner, 2011; Wäsche, Muller, & Knauf, 2001). However, the focus on purity might not be necessary as almost no food product consists of a single ingredient only. In case the plant material should resemble the composition of animal-based food products, it has to be rich in protein, may contain oil and water and it has to be low in long-chain carbohydrates. Consequently, fractionation should aim at removing the undesired insoluble carbohydrates, rather than obtaining pure protein. For this, aqueous fractionation seems to be a suitable method. Aguilera, Gerngross, and Lusas (1983), Chew, Casey, and Johnson (2003), Hojilla-Evangelista, Sessa, and Mohamed (2004), Jung (2009) and

Abbreviations: AF, aqueous fractionation; PI, protein isolate; WHC, water holding capacity; NSI, nitrogen solubility index.

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Bader, Oviedo, Pickardt, and Eisner (2011) studied aqueous processing of lupin and in the present study we looked into possibilities to make the process more efficient, for instance by using less extraction steps. For example, the low level of alkaloids in *Lupinus angustifolius* (<200 mg kg^{-1}, Alimex, the Netherlands) offers the opportunity to omit the acidic extraction step at the beginning of the process. Performing all extraction steps with fewer repetitions will reduce the use of water and chemicals. Aguilera et al. (1983) and Hojilla-Evangelista et al. (2004) obtained protein concentrate fractions with 670–790 g protein kg^{-1} (N × 6.25), 40–66 g oil kg^{-1}, and still 60–150 g carbohydrates kg^{-1}. Jung (2009) studied aqueous processing of lupin flakes where oil and protein were separated. In our case, the presence of oil in the protein-rich fraction might be an advantage for the final product composition. Bader et al. (2011) obtained protein recoveries of only 430 g kg^{-1} for conventional fractionation and 420 g kg^{-1} for aqueous fractionation, which is lower than protein recoveries of 500–600 g kg^{-1} that are usually reported (Chew et al., 2003; D'Agostina, Antonioni, Resta, Arnoldi, Bez, Knauf, et al., 2006; Fontanari et al., 2012; Jayasena et al., 2011; King, Aguirre, & De Pablo, 1985; Ruiz & Hove, 1976; Süssmann et al., 2011). Generally, the protein isolate or concentrate is dried at the end of the process. This might be a redundant step in case the final application contains or requires water, which means that the protein powder needs to be rewetted for post-processing. From an environmental point-of-view, it is interesting to study methods to concentrate the protein isolate instead of drying it to a powder.

This paper investigates aqueous fractionation of lupin seeds in greater detail to obtain protein-enriched fractions that have the potential to be used in plant-based, high-protein foods. As explained above, the presence of a certain amount of oil in the protein fraction is acceptable, which allows the introduction of a simplified fractionation process. In addition to the omission of the oil extraction step, the acidic extraction step at the start of the process is skipped and all extraction steps are performed once. The effects of extraction temperature and pH on protein recovery, chemical composition and techno-functional properties are determined. These properties include the water holding capacity and the nitrogen solubility index. Because high-protein food products contain water, it is also explored how to concentrate the protein. The results are captured in a novel process design for aqueous processing.

2. Materials and methods

2.1. Raw materials and chemicals

Dehulled, untoasted full-fat lupin seeds (*Lupinus angustifolius*) were obtained from Alimex (the Netherlands). All chemicals and reagents used in this study were of analytical grade. Tap water was used throughout unless stated otherwise.

2.2. Pre-treatment of the seeds

Lupin was pre-milled to grits with a Condux-Werk pin mill LV 15 M (Condux-Werk, Wolfgang bei Hanau, Germany). The grits were further milled into a full-fat flour with a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany) with a classifier wheel set at 1000 rpm and the air flow at 80 m^3 h^{-1}. The screw feeder was set at 2 rpm (corresponding to circa 0.75 kg/h), the impact mill speed was 8000 rpm and the batch size was 1 kg.

Part of the full-fat lupin flour was used to prepare defatted lupin flour. To extract the oil from the flour, 45 g of full-fat lupin flour was weighed into a cellulose thimble. Four thimbles (with in total 140 g of full fat flour) were mounted onto the fully automated Büchi extraction system B-811 LSV (Büchi Labortechnik AG, Flawil, Switzerland). The oil extraction was performed with petroleum ether (boiling range 40–60 °C) in Standard Soxhlet mode for 3 h with a sample-to-solvent ratio of 1:6. The extracted oil and the defatted samples were dried in an oven at 105 °C until constant weight. The petroleum ether was removed by evaporation and recovered within the Büchi extraction system.

2.3. Preparation of protein isolates

The process conditions used for protein extraction were chosen based on explorative experiments and literature data (Fontanari, Martins et al., 2012; Lqari et al., 2002; Süssmann et al., 2011). Seven different protein isolates (PI) were produced. The processing conditions and abbreviations can be found in Fig. 1. An overview of the conventional fractionation process and the newly developed aqueous fractionation (AF) processes are also depicted in Fig. 1.

The conventional lupin PI was produced by dispersing the defatted flour in water using a sample to solvent ratio of 1:15. The pH of the dispersion was adjusted and kept at 9 through addition of 1 mol L^{-1} NaOH. The dispersion was stirred at 20 °C for 2 h and subsequently centrifuged at 11,000*g* and 20 °C for 30 min. The time between centrifugation and decanting was kept as short as possible. The pellet, containing the fibre-rich fraction was freeze dried, while the protein-rich supernatant was collected. The pH of the supernatant was adjusted to 4.5 with 1 mol L^{-1} HCl. The dispersion was stirred at 20 °C for 1 h and subsequently centrifuged again at 11,000*g* at 20 °C for 30 min. The supernatant, containing the soluble solids fractions, was collected and freeze dried. The pellet, which contains the lupin PI, was washed with Millipore water twice and subsequently neutralised to pH 7 with 1 mol L^{-1} NaOH and kept at 20 °C overnight. One-third of the protein suspension was kept for post-processing at 80 °C and the other two-third of the protein suspension was freeze-dried.

The aqueous fractionated lupin protein isolates (AF PI's) were produced by dispersing the full-fat lupin flour in water using a sample to solvent ratio of 1:15. The pH of the dispersions was adjusted to 9 through addition of 1 mol L^{-1} NaOH. The dispersions were stirred at 4 °C, 20 °C, 50 °C or 90 °C for 2 h, depending on the fractionation method (Fig. 1), and subsequently centrifuged at 11,000*g* and 20 °C for 30 min. The time between centrifugation and decanting was kept as short as possible. After centrifugation, an oily layer was visible on top of the protein-rich supernatant. This oil could be collected with the supernatant or the pellet, but part of the oil remained stuck onto the tube walls. In all experiments, it was tried to combine the oil with the supernatant, which eventually becomes the protein-rich fraction. The pellets, which contain the fibres, were freeze dried. The supernatants were collected and the pH was adjusted and kept at 4.5 using 1 mol L^{-1} HCl. The resulting dispersions were stirred at 4 °C, 20°, 50 °C or 90 °C for 1 h and then centrifuged at 11,000*g* and 20 °C for 30 min. The supernatants, representing the soluble solids fractions, were collected and freeze dried. The pellets, which contain the lupin PI, were washed twice with Millipore water to remove impurities and sodium chloride.

The protein suspensions were split into four parts for further analysis. One of the parts, prepared at 20 °C and pH 4.5 and at 4 °C and pH 4.5, were freeze dried. Another part of the protein suspension, prepared at 20 °C and pH 4.5, was kept for ultrafiltration. The remaining parts of both protein suspensions and the complete protein suspension at 90 °C were neutralised to pH 7. One part of the protein suspension, neutralized to pH 7, was kept for post-processing at 80 °C. The protein suspension, produced at 4 °C, was kept at 4 °C overnight and those produced at 20 °C, 50 °C and 90 °C were kept at 20 °C overnight. All protein extractions were performed in duplicate.