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Lupine protein enrichment by milling and electrostatic separation

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ABSTRACT

Lupine seeds are excellent source of plant protein. We here report on dry fractionation by combining milling and electrostatic separation providing an alternative to wet extraction of protein from lupine seeds. Relatively coarse milling was preferred as this provides sufficient detached protein bodies with less agglomeration of particles. After a single separation step a fraction with protein content 57.3 g/100 g dry solids was obtained. After three successive steps protein content was increased further to 65.0 g/100 g dry solids. By extra milling and recycling the fractions with comparable protein content as the flour, yield was improved without compromising protein content. A final fraction with protein content 65.1 g/100 g dry solids and yield of 6% was obtained, which means 10% of protein in the flour was recovered. Based on our findings an optimised scheme for protein enrichment from lupine seeds by combining milling and electrostatic separation is proposed.

Industrial relevance: Lupine seeds are an excellent source of plant protein. Wet extraction of protein from lupine seeds consumes large amounts of water and energy. Alternatively, dry fractionation is more sustainable and retains the native functional properties of the protein. Previously, it was shown that dry milling and electrostatic separation could be used to further enrich protein from lupine flour. In this study, the process was further investigated with a new custom-build bench scale electrostatic separator. We found that a lupine protein concentrate could be obtained with higher purity compared to conventional air classification and earlier lab-scale experiments. Subsequently, a scheme was developed to improve the yield of the lupine protein concentrate without compromising the purity, and this provides a guideline for scaling-up this technique for industrial application.

1. Introduction

The quickly growing world population requires a rapid increase in the production of protein-rich foods (Bruinsma, 2009). The production of animal protein is intensive in the use of resources, such as land, water, nutrients and especially valuable plant proteins (Aiking, 2011). Including those plant proteins directly in the human diet would be more sustainable. Lupine recently has gained attention as a 'novel' plant protein ingredient source for foods because of its high protein content in seeds (38–40 g/100 g dry seeds) (Hove, 1974; Lqari, Vioque, Pedroche, & Millán, 2002) and its ability to grow in temperate climates and different soil types (Maxted, Bennett, & Cowling, 2001). With these characteristics, lupine represents a significant alternative to soybean. Therefore, lupine protein has been investigated for its emulsifying, foaming and gelling properties etc. (Berghout, Boom, & van der Goot, 2015; Berghout, Venema, Boom, & van der Goot, 2015; Lqari et al., 2002; Pelgrom, Berghout, van der Goot, Boom, & Schutyser, 2014; Pollard, Stoddard, Popineau, Wrigley, & MacRitchie, 2002; Pozani, Doxastakis, & Kiosseoglou, 2002) and has been explored as additive to food/feed products, e.g. baking product, processed meat product and fish meal (Alamanou, Bloukas, Paneras, & Doxastakis, 1996; Dervas, Doxastakis, Hadjisavva-Zinoviadi, & Triantafillakos, 1999; Draganovic, Boom, Jonkers, & van der Goot, 2014; Drakos, Doxastakis, & Kiosseoglou, 2007; Papavergou, Bloukas, & Doxastakis, 1999).

Protein exists in lupine seeds in the form of protein bodies (Fig. 1). The protein bodies have sizes between 5 and 25 μm (Le Gal & Rey, 1986), with a protein content of 73 g/100 g protein bodies (Plant & Moore, 1983). Other main cellular components in lupine seeds are carbohydrates including fibre and soluble sugars (43–48 g/100 g dry seeds) and lipids (7–10 g/100 g dry seeds) (Lqari et al., 2002; Sujak, Kotlarz, & Strobel, 2006). Conventional extraction of protein from seeds involves large quantities of organic solvent and water to remove the lipids and soluble carbohydrates (S. Alamanou & Doxastakis, 1995; Jayasena, Chih, & Nasar-Abbas, 2011). Furthermore, the drying step afterwards is energy intensive. Aqueous fractionation that skips the defatting step was proven to be a more sustainable method (Berghout, Boom, & van der Goot, 2014; Berghout, Marmolejo-Garcia, Berton-Carabin, Nikiforidis, Boom, & van der Goot, 2015; Jung, 2009), but still cannot avoid the large consumption of water and energy because a drying step is still needed. Alternatively, dry fractionation by combining proper milling and air classification consumes no water and hardly any energy and produced functional protein enriched fractions (Pelgrom et al., 2014; Pelgrom, Vissers, Boom, & Schutyser, 2013; Schutyser, Pelgrom, van der Goot, & Boom, 2015).

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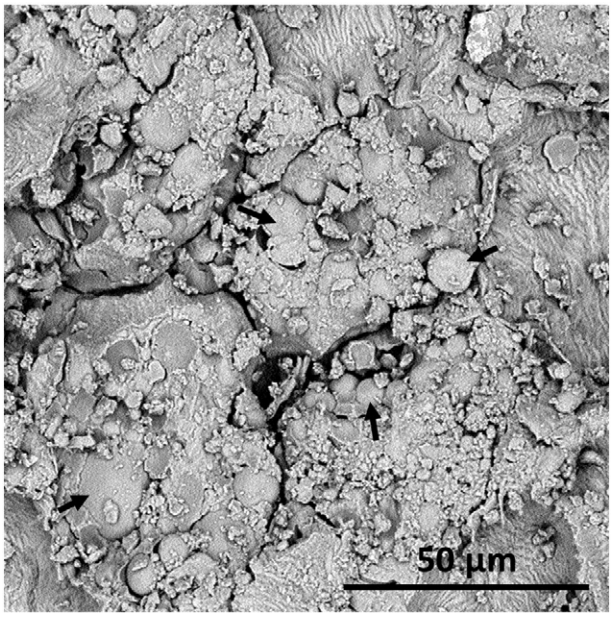

Fig. 1. Scanning electron microscope (SEM) image of a section of lupine seed. The arrows indicate the protein bodies (PB).

A disadvantage of dry fractionation is the low purity obtained, compared to wet fractionation. By applying milling and air classification to lupine seeds, the protein content in the fine fraction could reach a maximum of 59 g/100 g dry solids, with a yield of 6%–10% (Pelgrom et al., 2014). The main cause for this low purity was the presence of fine fibre fragments in the fine fraction (Pelgrom et al., 2014). Intensive milling not only opens the cellular structure and detaches the protein bodies from other cellular components, but also breaks fibre fragments to smaller sizes. Because air classification is based on particle size and density, these fine fibre fragments then accumulate in the fine fraction and reduce the overall protein content. Therefore, to improve the purity and yield of protein enriched fraction obtained by dry fractionation, a separation process based on a different driving force is needed.

Recently, electrostatic separation based on tribo-electrostatic charging and subsequent separation of particles in an electric field was explored as an alternative to dry separate food materials (Brouns, Hemery, Price, & Anson, 2012; Chen, Wang, Wang, Li, & Chen, 2014; Hemery et al., 2011; Pelgrom, Wang, Boom, & Schutyser, 2015; Sibakov, Abecassis, Barron, & Poutanen, 2014; Stone & Minifie, 1988; Wang, Smits, Boom, & Schutyser, 2015), though this technique has been applied for decades in mining industries for beneficiation of minerals and coal (Bada et al., 2010; Ban et al., 1997; Cangialosi, Notarnicola, Liberti, & Stencel, 2008; Dwari et al., 2015; Trigwell et al., 2003), and for fractionation of plastic waste materials (Bendimerad et al., 2009; Wu et al., 2013; Younes et al., 2015). Due to their different tribo-electrostatic properties, different materials such as protein and fibre, charge either positively or negatively when sliding along a surface made of a different material. Pelgrom et al. (2015) applied electrostatic separation as a post-treatment to further increase the protein content of the fractions obtained by air classification It was observed that electrostatic separation could deliver lupine protein enriched fractions (protein concentration of ~59 g/100 g dry solids) not only from the fine fraction, but also directly from the coarse fraction and whole flour.

Pelgrom et al. (2015) carried out an explorative study on a lab-scale electrostatic separator described by Wang et al. (2015b), but the influence of milling conditions on the separation performance was not considered. Proper milling is crucial to obtain good separation, because the cellular components need to be disassociated from each other and the particles need to be small enough to take sufficient charge, but at the same time the particles may not be too small to avoid risk of agglomeration of the particles (Lam & Newton, 1992; Wang, de Wit, Boom, & Schutyser, 2015). Moreover, milling also exposes the lipids, which promotes the agglomeration by liquid bridging between particles (Pelgrom et al., 2014).

Therefore, the current study aims to produce protein enriched fractions from lupine using a custom-built bench-scale electrostatic separator that allows better defined experiments (Wang, de Wit, et al., 2015a). Lupine flours with different particle size distributions obtained by impact milling were used to investigate the influence of milling conditions on electrostatic separation. After establishing the optimal milling conditions, a single-step electrostatic separation was carried out with different carrier gas flow rates. Subsequently, multiple-step electrostatic separations were explored based on the optimal conditions from the single-step process to further increase protein enrichment. Furthermore, methods that could increase both the purity and the yield were tested to fully optimise the separation. Finally, an optimised scheme is proposed for lupine by electrostatic separation.

2. Materials and methods

2.1. Preparation of lupine flour

Dry lupine seeds, *Lupinus angustifolius L.*, were purchased from L.I. Frank (Twello, The Netherlands). To obtain lupine flour, the seeds were first pre-milled with a pin mill (LV 15 M, Condux-Werk, Wolfgang bei Hanau, Germany). The grits were then further milled to flour with a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany). The air flow was 80 m^3/h and the impact mill speed was fixed at 8000 rpm. The classifier wheel speed was 2500, 4000, 6000 or 8000 rpm to obtain flours with different particle size distributions. The moisture content of the flours was in the range of 8%–10%, a range of values assumed to provide no difference in charging behaviour (Wang, de Wit, Schutyser, & Boom, 2014). Therefore, the flours were used directly for electrostatic separation without further drying. All flours and fractions were stored at −20 °C in sealed containers.

2.2. Electrostatic separation

The electrostatic separation experiments were carried out with a custom-built separator described previously in large detail (Wang et al., 2015a). For each single-step electrostatic separation experiment, ~25 g of lupine flour was dosed by the screw feeder into the system with a dosing rate of 1.25 kg/h. The flow rate of the carrier nitrogen gas was set at 10, 20 or 30 L/min. The electric field strength applied was kept at 200 kV/m by applying a voltage of 20 kV to the positive electrode and keeping the distance between the electrodes at 10 cm. From each experiment four fractions were obtained: one from the ground electrode, labelled 'GE', one from the positive electrode labelled 'PE', one from the collecting filter bag below the ground electrode labelled 'GC' and one from the collecting filter bag below the positive electrode labelled 'PC'. According to the study of Pelgrom et al. (2015), lupine protein takes positive charge and will be deflected towards the ground electrode. Therefore the fractions GE and GC are expected to be protein enriched.

For multiple-step electrostatic separation experiments (Fig. 2), ~400 g of lupine flour was used for each experiment. The other parameters were set according to the optimal settings established during the single-step experiments. After the first electrostatic separation step, the fraction GE (relabelled as 'GE1') was subject to another electrostatic separation and again resulted in four fractions: GE2, PE2, GC2 and PC2. Then a third electrostatic separation was carried out with the fraction GE2 and yielded another four fractions: GE3, PE3, GC3 and PC3. Depending on the amount of fraction GE3 and its purity, more steps of electrostatic separation could be done yielding four fractions from each step. All fractions were analysed on yield and protein content.