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Pre- and post-treatment enhance the protein enrichment from milling and air classification of legumes

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ABSTRACT

Air classification is a milder and more sustainable method to obtain protein-enriched fractions than commonly used wet fractionation. The protein content of air-classified fractions is generally lower than obtained with wet methods, therefore we applied pre- and post-treatments to increase the protein purity. A starch-rich legume, pea, and an oil-rich legume, lupine, were pre-treated by varying the moisture content, defatting, soaking or freezing cycles. Higher moisture contents and defatting of lupine increased the protein purity, but lower moisture contents increased the protein yield. Soaking and freezing cycles lowered the particle density, which impaired the separation. Electrostatic separation is based on electrostatic charging behaviour and was successfully applied to enrich air-classified fractions by separating protein and fibre into oppositely charged fractions. The results showed that pre- and post-treatments yielded protein fractions that are significantly purer than those obtained in single-step milling and air classification.

1. Introduction

Protein-enriched fractions can be obtained from starch-rich legumes such as pea and oilseeds such as lupine, by combined milling and air classification (Pelgrom et al., 2014, 2013b; Schutyser and Van der Goot, 2011; Sosulski and Youngs, 1979; Tyler et al., 1981). The milling detaches the protein from the other cellular components. Subsequently, air classification separates the smaller protein-rich fragments from the starch granules for pea, and the protein bodies from larger cell wall rich fragments for lupine. During air classification the powder particles are fluidized and contacted with a rotating classifier wheel. Small particles below the cut size are collected via the classifier wheel in the fine fraction, depending on the gas flow and the rotation speed of the classifier wheel. Larger starch granules or fibre fragments are collected via the bottom of the classifier into the coarse fraction. During previous research we found that pea fine fractions with 55.5 ± 0.5 g protein/100 g dry matter (Pelgrom et al., 2013b; Tyler et al., 1981; Wright et al., 1984) and lupine fine fractions with 58.9 ± 0.0 g protein/100 g dry matter could be obtained (Pelgrom et al., 2014). These protein levels are lower than for protein isolates which typically have 80–85 g protein/100 g dry matter.

Higher protein content of the dry-enriched fractions could widen the perspective for applications, e.g. to better meet nutritional and/or functional ingredient requirements. Theoretically, the maximum protein content that can be achieved by air classification is the actual protein content of the protein bodies (73 g protein/100 g dry matter) (Plant and Moore, 1983), which means there is still room to increase the protein content in the fractions obtained by air classification. The improvement is expected to be achieved by adding pre-treatments or post-treatments to the separation process. A wide variety of pre-treatments has been proposed in literature to enhance the protein yield and purity. Two main types were considered in this paper, namely: weakening of the cellular structure by moisture addition or freezing and removal of specific components from the seed.

Addition of moisture has been reported to enhance disentanglement of cellular components, although its effect is species dependent holder (Pelgrom et al., 2013a). A higher yield and protein content were obtained in the fine fraction for hard wheat, whereas an opposite effect was found for soft wheat, field pea and faba bean (Kent, 1965; Tyler and Panchuk, 1982). A disadvantage of moisture addition is that ductility of the seeds increase and therefore more energy is required for milling (Dijkink, 2002).

Another way to disrupt cellular structures may be freezing in the presence of water. During initial experiments fast freezing with liquid nitrogen of non-wetted pea and lupine seeds (11–13 g

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water/100 g seed) was found not effective in improving dry separation. It is hypothesized that weakening of cellular structures with ice crystals requires sufficient water and slow freezing and thawing cycles (Vertucci, 1990). Moreover, applying several freezing cycles could possibly increase the inflicted damage and thus improve the disentanglement (Fahloul et al., 1996). This pre-treatment has not been combined with dry fractionation and is a different approach compared to cryogenic milling. The latter treatment produces composite particles, which is not very desirable before air classification (Hemery, 2011a).

The second type of pre-treatment involves the removal of specific components from the seeds before milling. For example, the hulls can be removed by air classification (Wu and Nichols, 2005) or by elusieve processing, which is a combination of air classification and sieving (Srinivasan et al., 2010). Hulls are rich in fibre, 91 g/100 g dry matter (Ralet et al., 1993). Furthermore, they comprise 12–14% of the total weight of pea seeds and contain 6 g protein/100 g dry hull (Meuser et al., 1995; Vose, 1978). Removing the hull increases the protein content of pea seeds from 22 g/100 g dry matter to 24 g/100 g dry matter. Dehulling of lupine may provide a protein content of 39 g/100 g dry matter instead of 35 g/100 g dry matter. Furthermore, lipids can be removed via extraction, which not only influences the protein content, but also the powder properties, e.g. the dispersibility of the flour (Snyder et al., 1984). After defatting dry fractionation can yield a lupine protein concentrate of 61 g protein/100 g dry solids (Booth et al., 2001), although others reported no significant increase in protein content (Dijkink and Willemsen, 2006; Gueguen, 1983).

Post-treatments are another route to improve the purity and yield of dry fractionation. A post-treatment that is based on a different driving force for separation compared to particle size and density may be applied to further increase the protein content and yield (Schutyser and Van der Goot, 2011). We therefore propose to combine electrostatic separation, which relies on different tribo-electric charging properties of materials, with air classification. Electrostatic separation is not yet a common separation technique for food production, but its potential has been demonstrated for separation of wheat bran and rice flour (Hemery et al., 2011b; Noguchi et al., 1981).

The objective of this study was to evaluate selected methods for pre- and post-treatment to increase the effectiveness of dry fractionation of pea and lupine, especially in terms of protein purity. Pea and lupine were selected as raw materials to represent starch-rich and oil-rich legumes, respectively. An initial series of lab-scale experiments was carried out to evaluate the potential of different pre-treatments. Based on protein purity increase the most promising pre-treatments were selected for more elaborate investigation: varying moisture contents, defatting and freezing cycles. Pre-treated seeds were compared to untreated seeds on: cellular structure after pre-treatment, milling behaviour and protein yield with air classification. Electrostatic separation was used as a separation method for pea and lupine flour and as a post-treatment after air classification. Finally, a reflection is provided on the boundaries of protein enrichment by air classification.

2. Materials and methods

2.1. Materials

Pre-dried yellow pea seeds, *Pisum sativum* L., were purchased from Alimex (Sint Kruis, The Netherlands) and pre-dried lupine seeds, *Lupinus angustifolius* L., were purchased from L.I. Frank (Twello, The Netherlands). All experiments were done in duplicate unless stated differently.

2.2. Preparation of enriched fractions

2.2.1. Pre-treatments

Blank. Untreated pea and lupine seeds were pre-milled to grits of approximately 200 μm using a Condux-Werk LV 15 M mill (Condux-Werk, Wolfgang bei Hanau, Germany).

Moisture content. A decrease of the moisture content was obtained by oven drying at 50 °C for 6 days. An increase in moisture content was obtained by soaking pea or lupine overnight at 4 °C in a calculated amount of water.

Defatting. Four batches of pea and lupine grits (i.e. 4 ∗ 700 g) were defatted in a soxhlet using petroleum ether (boiling range 40–60 °C) with a sample-to-solvent ratio of 1:5 for 24 h.

Soaking. Two batches of pea and lupine grits (i.e. 2 ∗ 1200 g) were soaked for 1 h in 2157 g of water for peas and 2482 g of water for lupine. These amounts of water allowed complete soaking without the presence of free water at the end of the soaking time. The grits were dried in a fluidized bed (TG200 Rapid Dryer, Retsch GmbH, Haan, Germany) at an air flow of 60% (maximum: 185 m^3/h at no-load operation without material to be dried) and an air temperature of 40 °C to obtain the same moisture content as the untreated grits (13 g water/100 g pea grits and 11 g water/100 g lupine grits).

Freezing cycles. Freezing cycles were applied to whole pea and lupine seeds. The seeds were soaked overnight at 4 °C at a seed to water ratio of 1:5. After packing the seeds in plastic bags of 300 g, the seeds were frozen at −18 °C and thawed at 20 °C 3 times. A fluidized bed (TG200 Rapid Dryer, Retsch GmbH, Haan, Germany) with an air flow of 50% and an air temperature of 40 °C was used to dry the seeds to their original moisture content of 13 g water/100 g grits for pea and 11 g water/100 g grits for lupine. The seeds were pre-milled to grits after the freezing cycles.

2.2.2. Lab scale milling and sieving

Milling and separation of pea and lupine at various moisture contents was done in a lab scale mill (Fritsch Pulverisette 14, Fritsch, Idar Oberstein, Germany) because in the pilot scale mill (ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany)) the moisture content could not be controlled due to the large air flow, 52 m^3/h, and longer milling time that was required. From the pre-treated seeds, 50 g was milled at 6000 rpm for 2 min with a sieve ring size of 0.2 mm for pea and 0.5 mm for lupine. The flour was separated by air jet sieving (Alpine200 LS-N, Hosokawa-Alpine, Augsburg, Germany) for 2.5 min at 4000 Pa on a 20 μm sieve. Each experiment started with 9.9 g of flour, which was mixed with 0.1 g fumed silica (Aerosil®200, Azelis Netherlands B.V., Oosterhout, The Netherlands) to improve the flowability. The protein separation efficiency was calculated as the amount of protein (g) in the fraction smaller than 20 μm divided by the total amount of protein (g).

2.2.3. Pilot scale milling and air classification

Untreated and pre-treated grits were milled into flour using a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany). This mill contains an internal rotating classifier wheel that allows the passage of fine particles while coarse particles are returned and further milled. The impact mill speed was fixed at 8000 rpm and the feed rate at 2 rpm (circa 0.75 kg/h). For pea, the classifier wheel speed was set at 3400 rpm and the air flow at 60 m^3/h. For lupine, the classifier wheel speed was set at 1200 rpm and the air flow at 80 m^3/h. The milling yield was calculated as the weight of the milled flour divided by the weight of grits. Untreated pea grits were milled at 4000 and 8000 rpm at an air flow of 52 m^3/h and untreated lupine grits were milled at 1000 rpm with an air flow