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A two-step air classification and electrostatic separation process for protein enrichment of starch-containing legumes

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ABSTRACT

A two-step dry fractionation process was investigated that further enriches protein from starch-containing legumes. Legumes (pea, lentil, and chickpea) were subjected to milling, air classification, and subsequent triboelectrostatic separation. The air classification first removes starch, whereas the subsequent electrostatic separation removes fiber from the resulting protein concentrate. Successful enrichment was achieved with pea and lentil, but this was not the case for chickpea due to the smaller starch granules and higher fat content. The best conditions for pea were air classification at an air-classifier wheel speed of 8000 rpm. Subsequently, electrostatic separation was optimized with two passes. With this, a protein purity was obtained of 63.4 g/100 g dry basis and a yield of 15.8 g/100 g dry solids. For the overall two-step dry fractionation process, a protein-enriched fraction with a yield of 4.0 g/100 g pea could be obtained, leading to 7.8% of total protein recovered from yellow pea. *Industrial relevance:* To enrich protein from starch-containing legumes a novel dry method combining air classification and electrostatic separation was developed. Compared to conventional wet extraction, this dry route is much less energy-consuming and preserves the native functionality of the proteins. By adding electrostatic separation to air classification, a higher pea protein purity (up to 63.4–67.6 g/100 g) could be obtained, which is higher than that obtained by air classification only (57.1 g/100 g). It is estimated that for an improved dry fractionation process with increased recovery of material, the yield and protein recovery may be further increased with factor ~ 2.8 compared to the current results.

1. Introduction

Starch-containing grain legumes such as pea, chickpea, and lentils are a major source of dietary protein for over one billion consumers worldwide (Khazaei et al., 2019). These legumes live in symbiosis with nitrogen binding bacteria in their root nodules, which reduces the need for artificial fertilizers compared to other plant protein sources. Besides, they can grow in temperate climate zones, and therefore in proximity to many of the world's population centers. Therefore, legumes have an advantage in meeting the growing demand for sustainable dietary plant protein (M. Schutyser & Van der Goot, 2011). Proteins from legumes have been extracted as an ingredient (e.g. concentrate or isolate) and are applied in numerous food applications, where functional behavior such as foaming, gelling and emulsifying properties is critical (Stone et al., 2019). Traditionally, legume proteins are often extracted via wet extraction methods that involve energy consuming steps such as drying and lead to the loss of native functional properties due to the use of solvents or alkaline conditions during the extraction and the thermal load due to drying (Assatory et al., 2019).

Dry fractionation by dry milling and dry separation is a more resource-efficient alternative to wet extraction, while the native functional properties of the proteins are better retained (Mayer-Laigle et al., 2018). During milling, starch granules are disclosed as larger particles; the proteins and fibers are primarily present as smaller fragments. Subsequently, dry separation can be carried out via air classification, using the size or density difference as separation principle, or via electrostatic separation, which uses the different triboelectric charging properties of the materials (M. Schutyser et al., 2015). Air classification was successfully applied to separate larger starch granules from smaller protein particles to produce starch and protein-enriched fractions from pea, navy bean, faba bean and lentil (Boye et al., 2010; J. Wang et al., 2015; J. Wang et al., 2016; Xing et al., 2018). Direct electrostatic separation of starch-containing legume flours was shown to be infeasible (P.J. Pelgrom et al., 2015), despite the observation that artificial mixtures of wheat gluten and starch could be separated with this method (J. Wang, de Wit, et al., 2015). However, further protein enrichment

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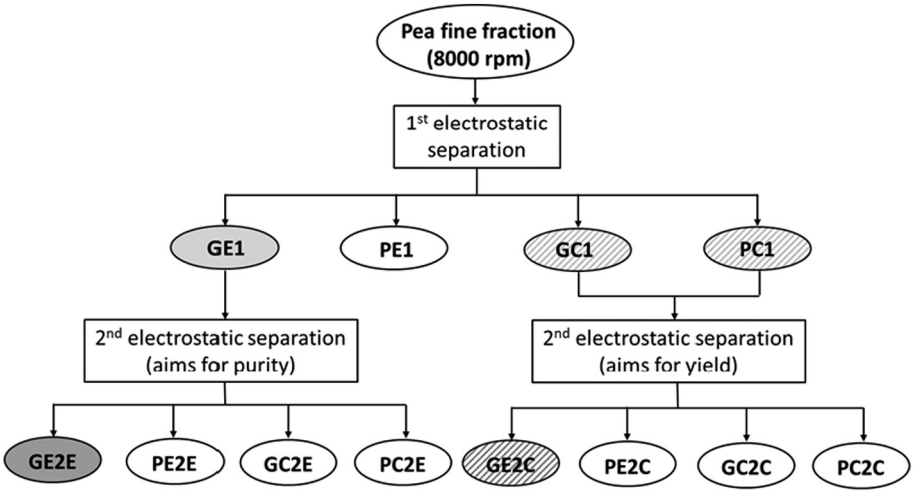

Fig. 1. The diagram of the two-step electrostatic separation in this study. GE1, PE1, GC1, and PC1 represent fractions collected from the grounded electrode, positive electrode, grounded collector, and positive collector, respectively, after the 1st separation. GE2E and GE2C represent protein-enriched fractions obtained from GE1 and the mixture of GC1 and PC1, respectively.

could be obtained by subjecting the protein-rich fine fraction obtained by air classification, to subsequent electrostatic separation (P.J. Pelgrom, Wang, et al., 2015). During triboelectric charging, the protein and fiber fragments obtain an opposite charge and thus can be separated in an electrostatic field. However, starch obtains a similar polarity as the protein and thus is attracted to the same electrode as the protein, which impairs their separation. This suggests a two-step approach by combining air classification and electrostatic separation to obtain pea protein concentrates with higher purity. This approach was only demonstrated using a lab-scale electrostatic separator in which yields could not be reported (P.J. Pelgrom, Wang, et al., 2015).

The aim of the current study is to further develop the two-step dry separation approach using yellow pea, lentil, and chickpea for protein enrichment. The protein content of these legumes have been reported 21.9 ± 1.5, 20.6 ± 0.4, and 18.5 ± 1.7 g/100 g and the starch content 48.0 ± 1.4, 46.5 ± 0.5, and 44.6 ± 1.7 g/100 g, respectively (H.J. Chung et al., 2008; de Almeida Costa et al., 2006). Fine fractions (protein-rich) produced by air classification are further purified with a custom-built bench-scale electrostatic separator and evaluated on purity and yield. Pea was selected to optimize the process parameters for obtaining fractions with the highest purities and yields.

2. Materials and methods

2.1. Materials

Dry yellow pea (*Pisum sativum*), lentil (*Lens culinaris*) and *Kabuli* chickpea (*Cicer arietinum*) seeds were purchased from a local market (Alimex, Sint Kruis, The Netherlands). All seeds were stored until use at 4 °C in tightly sealed polyethylene containers.

2.2. Milling

Legume seeds were pre-milled into grits with a pin mill (LV 15 M Condux-Werk, Wolfgang bei Hanau, Germany). Subsequently, the coarse grits were further milled into flour with a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany) at ambient temperature. The unrecovered material after this milling step is ~12 g/100 g flour. The classifier wheel speeds during milling for pea, lentil, and chickpea are 4000, 2200, and 2900 rpm respectively, based on our previous study (P.J. Pelgrom et al., 2015). An airflow rate 40 m^3/h, an impact milling speed of 8000 rpm, and a feed rate of 0.5 kg/h was used for all pulses grits during impact milling.

2.3. Air classification

Protein-rich fine fractions of the three pulses were produced using an ATP50 air-classifier (Hosokawa-Alpine, Augsburg, Germany) at ambient temperature. Based on previous experience (P.J. Pelgrom, Boom, et al., 2015), the classifier wheel speed was set at 10,000 rpm. The airflow was kept constant at 52 m^3/h and the feed rate was ~0.5 kg/h.

For a next series of experiments, pea fine fractions having different starch content were prepared. The classifier wheel speeds for these experiments were 6000, 8000 and 10,000 rpm. The airflow was set at 52 m^3/h and the feed rate was ~0.5 kg/h.

2.4. Electrostatic separation

A custom-built bench electrostatic separator was used for protein enrichment. This equipment was extensively described in a previous report (J. Wang, de Wit, et al., 2015). In the current study, a charging slit made of aluminum and a straight tube made of stainless steel with an internal diameter of 8 mm were used. The height of the charging slit was 21.8 cm, and the cross-section length and width were 4.1 cm and 0.24 cm, respectively (Xing et al., 2018). The height of the straight tube was 29.6 cm and the inner diameter was 0.8 cm. For each single step electrostatic separation experiment, 25 g raw material was used. The N_2 flow rate was fixed at 50 L/min, the distance between electrodes was 10 cm, the voltage set on the positive electrode was 20 kV and the dosing rates were 0.5 and 1.25 kg/h. After each separation, four fractions labelled as “GE”, “PE”, “GC” and “PC” were collected from the grounded electrode (protein-enriched), positive electrode (fiber-enriched), ground collector bag and positive collector bag, respectively.

During the two-step electrostatic separation experiments, 300 g raw material was used in the first step. An overview of the two-step electrostatic separation process is shown in Fig. 1. The protein-enriched fraction (GE1) and the mixture of the fractions obtained from the two collector bags (GC1 + PC1) were used as feed for the second separation step. The former strategy aims to further increase the protein content in fraction GE2E and the latter strategy was used to recover the additional protein from the fractions in the collecting bags (GC1 + PC1) into fraction GE2C.

2.5. Analyses

2.5.1. Compositional analysis

The protein content of pea, lentil, and chickpea flours and fractions was determined by the Dumas method with a Nitrogen Analyser FlashEA 1112 series (Thermo Scientific, Breda, The Netherlands). To

calculate the protein content, a nitrogen conversion factor of N × 6.25 was used. The moisture, oil, and ash contents were determined by methods AACC 44–15.02 (1999), AACC 30–25.01 (1999), and AACC 08–01 (1983), respectively. The starch content was analyzed with a Total Starch Assay Kit (Megazyme, Ireland). The content of fiber was approximated by the difference.

2.5.2. Protein enrichment

The protein enrichment is defined as the increase in protein purity of the target fraction to the protein purity relative to that of the starting material.

$$\text{Protein enrichment} = \frac{\text{Protein purity}_{\text{target fraction}} - \text{Protein purity}_{\text{starting material}}}{\text{Protein purity}_{\text{starting material}}} \times 100\%$$

The yield is defined as the mass of the target fraction divided by 100 g of the starting material.

$$\text{Yield} = \frac{\text{Mass}_{\text{target fraction}}(g)}{100\,(g)} \times 100\,(\%)$$

The protein recovery is defined as the ratio of the protein mass present in the target fraction to the protein mass present in the starting material.

$$\textit{Protein recovery} = \frac{\textit{Protein mass}_{\textit{target fraction}}}{\textit{Protein mass}_{\textit{starting material}}} \times 100\%$$

2.5.3. Scanning electron microscopy

The particles of pea, lentil, and chickpea flours and fractions were visualized using scanning electron microscopy (Phenom G2 Pure, Phenom World BV, the Netherlands). Powder samples without any pretreatment were sprinkled on 12.7 mm aluminum pin mounts (JEOL Europe BV, the Netherlands) with carbon tabs (SPI Supplies/Structure Probe Inc., West Chester, USA) and placed into the microscope chamber for observation. The acceleration voltage was set at 5 kV.

2.5.4. Particle size distribution

The particle size distributions (PSDs) of pea, lentil and chickpea flours and fractions were analyzed with a Mastersizer-3000 (Malvern Instrument Ltd., Worcestershire, UK) equipped with a module for dry powder dispersion (Aero S, UK). A dispersion pressure of 2 bar was applied and the volume-weighted particle size distribution was estimated using the Fraunhofer theory.

2.5.5. Statistical analysis

All measurements were carried out in duplicate unless indicated differently. Data were analyzed by one-way ANOVA using SPSS statistics Version 25.0 (IBM, Armonk, NY). A p-value < 0.05 meant the difference between data was statistically significant. The results are expressed as average values ± standard deviations.

3. Results and discussion

3.1. Air classification of starch-containing legumes

After air classification of the three legume flours at 10,000 rpm, the flour and their corresponding fine and coarse fractions were compared on their composition (Fig. 2). The protein content of pea, lentil, and chickpea fine fractions increased 107%, 129%, and 58% compared to the original flours, respectively. These results are consistent with those from previous research (P.J. Pelgrom, Boom, et al., 2015). It is noted however that the initial protein content and thus also the protein content after enrichment for these legumes will vary with season and in general with environmental cultivation conditions (Lascano et al., 2001). Starch was depleted in the protein-enriched fraction. Especially

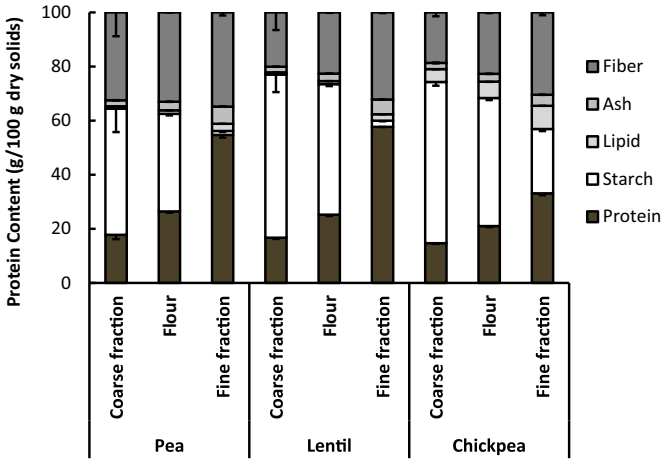

Fig. 2. Compositions of flour, coarse and fine fraction of pea, lentil, and chickpea. The amount of fiber was calculated by difference. The error bars indicate standard deviation, only minus direction is shown.

in pea and lentil fine fractions, residual starch was only 1.5 and 2.3 g/100 g dry solids, respectively, while the chickpea fine fraction had a starch content of 23.8 g/100 g dry solids (Fig. 2). The reason for the inefficient separation of starch from chickpea is probably the smaller starch granule size compared to those of pea and lentil. This leads to incomplete separation as the starch granule size is close to the cut point for separating the protein-rich particles (P.J. Pelgrom, Boom, et al., 2015). Additionally, the higher oil content of chickpea (6 g/100 g flour compared to 1 g/100 g flour in pea or lentil on dry basis) contributes probably to a higher tendency to agglomeration which negatively affects the separation (Sosulski & Youngs, 1979).

The SEM pictures show pea, lentil, and chickpea flours and fractions obtained after milling and air classification. As the cotyledons are ground into powders, starch granules are released from the cellular matrix, which also contains protein-rich particles and fibers. Pea, lentil, and chickpea starch granules can be recognized as smooth spherical or oval particles. The fragments of different sizes and irregular shapes are most probably protein and fiber particles. In pea and lentil fine fractions (Fig. 3 B and E), starch granules are hardly seen, while for chickpea, starch granules can be observed in the fine fraction (Fig. 3 H), indicating poorer separation. The size of the starch granules decreases in the order from pea (25 ± 6 μm) > lentil (23 ± 5 μm) > chickpea (22 ± 4 μm), which is in line with another study, which reported sizes of 32 ± 14 μm, 25 ± 13 μm and 22 ± 12 μm, respectively (H.J. Chung et al., 2008).

The particle size distributions of the chickpea fine and coarse fractions overlap to a larger extent than those of pea and lentil (Fig. 4). This confirms the more diffuse separation for the finely milled chickpea flour during air classification (P.J. Pelgrom, Boom, et al., 2015). To further increase the protein purity of the fine fractions, the fine fractions of the three legumes were subjected to subsequent electrostatic separation. Coarse fractions were not considered further, as the presence of larger amounts of starch content impairs effective separation (P.J. Pelgrom, Boom, et al., 2015).

3.2. Electrostatic separation of starch-containing legumes

During the first electrostatic separation experiments, a charging slit was used for separation (Xing et al., 2018) and the dosing rate was set at 1.25 kg/h. The protein content after separation is shown in Fig. 5 A. For pea and lentil fine fractions, a slight protein enrichment (6% on dry basis) was observed for the GE fractions. This is consistent with our previous research using a laboratory-scale electrostatic separation which showed ~8% protein enrichment (P.J. Pelgrom, Wang, et al., 2015). The fractions of pea and lentil collected on the positive electrode

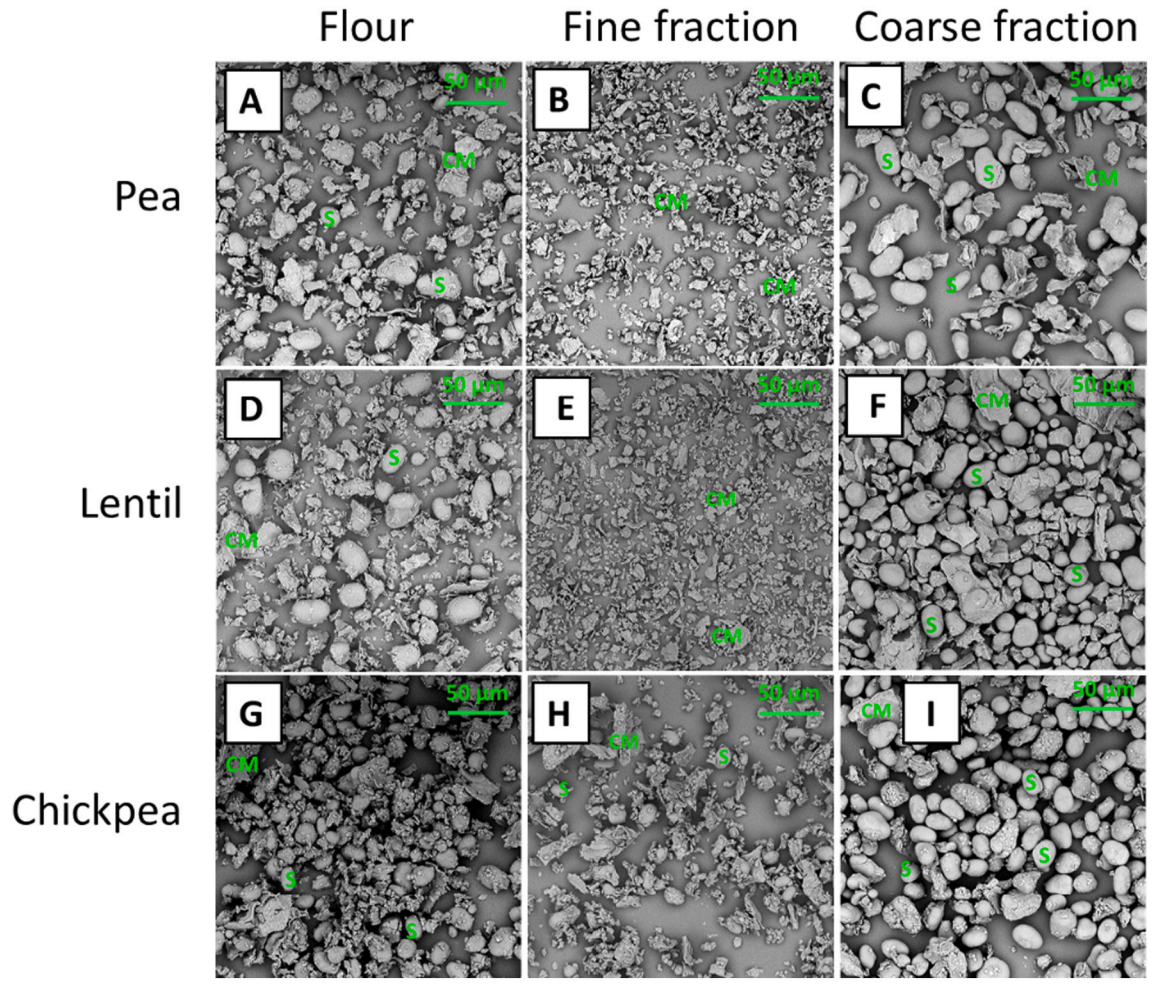

Fig. 3. Scanning electron microscopic pictures of pea, lentil, chickpea flour, and their air classified fine and coarse fractions, respectively. From A to C: pea flour, pea fine fraction, and pea coarse fraction. From D to F: lentil flour, lentil fine fraction, and lentil coarse fraction. From G to I: chickpea flour, chickpea fine fraction, and chickpea coarse fraction. S: starch granules. CM: cellular material.

(PE) were depleted in protein and thus enriched in fiber as the starch had already been removed during air classification. No protein enrichment was achieved for the chickpea fine fraction, which was expected given the presence of larger amounts of starch granules. During previous research, it was already suggested that starch granules obtain similar charges as the protein-rich particles, which impairs their separation (P.J. Pelgrom, Wang, et al., 2015). This explains the better separation for pea and lentil, which is thanks to the effective removal of the starch granules during the air classification step. It was found that the protein content of the ground collector (GC) and the positive collector (PC) are close to that of the starting material. These fractions may be recombined and subjected to a second separation pass for enlarging the overall protein yield.

The yields of the protein-enriched fractions (GE) for the three legumes were similar ($P > 0.05$) (Fig. 5 B). We expected that the yield of chickpea might be lower due to the lower protein content of the fine fraction, but this was not found. The similar yield for chickpea can be also explained by the presence of higher amounts of starch in the chickpea GE fraction. Starch attracted on the grounded electrode resulting in lower protein purity but similar mass yield. A significant amount of powder was not recovered. This is due to the experimental system (fouling inside the equipment) and will need to be reduced by improving the design of the equipment.

The yield of the fractions from the collector bags exceeded that of

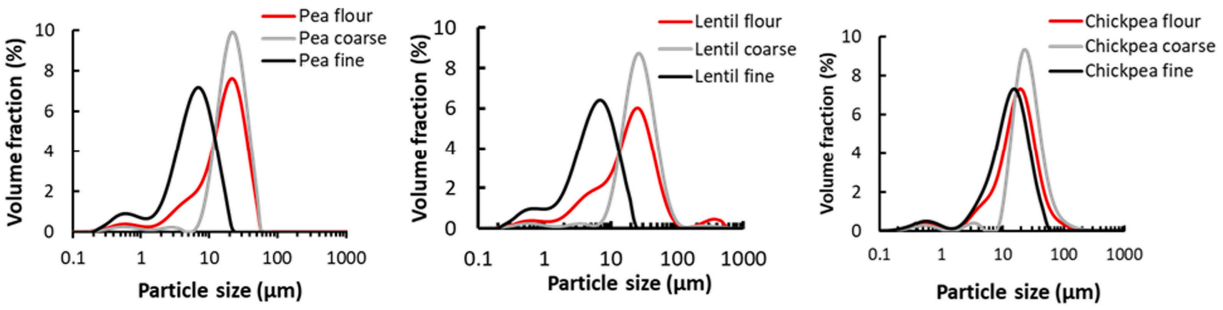

Fig. 4. Particle size distribution curves of pea, lentil, and chickpea flour compared with those of the fine and coarse fractions.

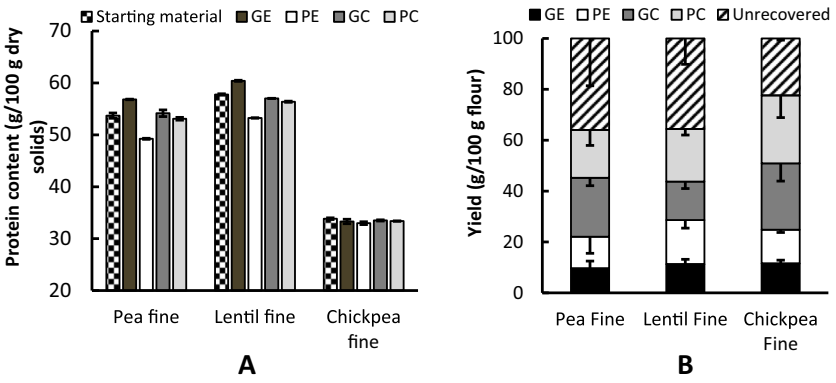

Fig. 5. One-step electrostatic separations of pea, lentil, and chickpea fine fractions (obtained at an air-classifier wheel speed of 10,000 rpm) with a charging slit. A: protein content of the starting material and four fractions collected from the grounded electrode (GE), ground collector (GC), positive electrode (PE), and positive collector (PC), respectively. B: the yield of four fractions and the weight of unrecovered material which was calculated by difference. The error bars indicate standard deviation, only minus direction is shown.

the electrodes. In the next section, we present results on increasing the recovery of pea protein by recycling the fractions in the collector bags. Pea was selected to further optimize the dry fractionation process because pea protein is increasingly being used in for example meat substitutes (Rempel et al., 2019). Moreover, we have ample prior experience with milling and air classification of yellow pea (P.J. Pelgrom et al., 2013; P.J. Pelgrom, Boom, et al., 2015). Thus, it is a good start to investigate the effect of air classification on subsequent electrostatic separation.

3.3. Dry fractionation of pea protein

Pea fine fractions were prepared by air classification using three different air-classifier wheel speeds (6000, 8000, and 10,000 rpm) providing fractions differing in composition and yield (Fig. 6). Data of the coarse fractions are not shown. As the classifier wheel speed increased, the particle size shifted to smaller sizes for the fine fraction (Fig. 6 A).

The compositions of the pea fine fractions are shown in Fig. 6 B. With increasing classifier wheel speed, the protein content of the fine fractions increased from 51.8 to 58.8 g/100 g dry solids (with 32.4 g/100 g dry solids in pea flour). The fine fractions were depleted of starch, with a negative correlation to the protein content. The fine fractions were also richer in fiber, ash, and fat. This is related to the high purity of the starch, while the protein is more integrated with the other components in the cotyledon tissue structure (Sridharan et al., 2020). Although using 10,000 rpm gave the highest protein content (Fig. 6 B), this also resulted in a decreased yield (Fig. 6 C) of the fine fraction (from 32.2 g/100 g flour at 6000 rpm to 20.0 g/100 g flour at 10000 rpm) due to the lower cut point, which is in line with a previous study on air classification of pea (Saldanha do Carmo et al., 2020). The yield went further down as the classifier wheel speed increased. This is because small particles are more prone to remain unrecovered by adhering to the inner walls of the equipment. Classifier wheel speeds below 6000 rpm (data not shown) did not lead to protein and starch separation (only one fraction was obtained). With the air-classifier wheel speed at 8000 rpm, a protein recovery of 49.0% from pea flour could be obtained.

Pea fine fractions obtained from air classification at 6000, 8000, and 10,000 rpm were subjected to electrostatic separation with a straight charging tube (Fig. 7 A). The dosing rate was kept constant at 0.5 kg/h. The separation showed protein enrichment for all the pea fine fractions in the GE fraction (Table 1). Pea protein enrichment (14.6%) at 10,000 rpm was larger compared to the electrostatic separation using

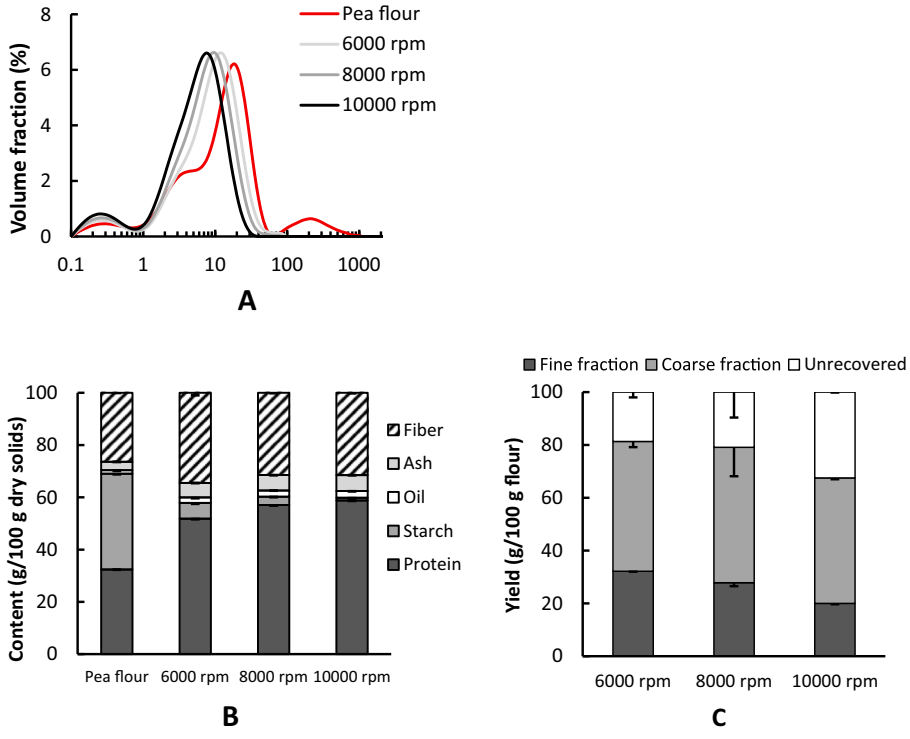

Fig. 6. A: The particle size distribution curves of pea flour and pea fine fractions obtained at different air-classifier wheel speeds. B: The compositions of pea flour and fine fractions as a function of the air-classifier wheel speed. Fiber content was calculated by difference. C: The yield of pea fine, coarse fractions, and the mass of unrecovered material as a function of the air-classifier wheel speed. The error bars indicate standard deviation, only minus direction is shown.

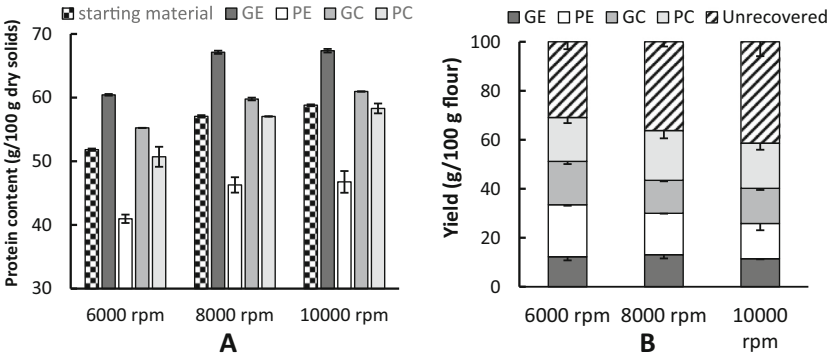

Fig. 7. One-step electrostatic separation of pea fine fractions obtained at three different air-classifier wheel speeds with the charging tube. A: protein content of starting materials and four fractions collected from the grounded electrode (GE), ground collector (GC), positive electrode (PE), and positive collector (PC), respectively. B: the yield of four fractions and the mass of unrecovered material which was calculated by difference. The error bars indicate standard deviations. Only minus direction is shown in B.

Table 1
Summary on protein enrichment and protein recovery achieved by air classification and electrostatic separation (compared to pea flour) as function of classifier wheel speed. Data marked with a different lowercase superscript in the same column indicate significant differences ($P < 0.05$).

Classifier wheel speed (rpm)	Air classification		Electrostatic separation	
	Protein enrichment (%)	Protein recovery (%)	Protein enrichment (%)	Protein recovery (%)
6000	$59.9^{a} \pm 0.9$	$51.4^{b} \pm 0.0$	$86.5^{a} \pm 0.1$	$7.4^{b} \pm 0.8$
8000	$76.1^{b} \pm 1.0$	$49.0^{b} \pm 2.6$	$107.2^{b} \pm 0.2$	$7.5^{b} \pm 0.9$
10,000	$81.5^{c} \pm 0.8$	$36.3^{a} \pm 0.8$	$107.9^{b} \pm 0.3$	$4.7^{a} \pm 0.0$

the charging slit (6%) (Fig. 5 A). The halved cross-section area of the straight tube (0.5 cm^2 compared to 1.0 cm^2 of the slit), which leads to larger gas velocity and thus increased charging may explain the improved separation performance.

The protein purity of the GE fraction increased for the fine fractions prepared with higher classifier wheel speeds between 6000 and 8000 rpm (Fig. 7 A) but did not increase further when using a classifier wheel speed of 10,000 rpm. The initial increase in the separation efficiency may be due to the better removal of pea starch granules at higher air-classifier wheel speeds enabling better electrostatic separation. A further increase in air-classifier wheel speed (10,000 rpm) did not remove additional starch granules (Fig. 6 B) and therefore also subsequent electrostatic separation did not improve further.

Pea fine fractions prepared with air classification at 8000 and 10,000 rpm and subsequently subjected to electrostatic separation yielded the highest protein content. The yield of protein-enriched fractions separated from different pea fine fractions was highest for 8000 rpm (13.1 g/100 g fine fraction), though differences are not very large (Fig. 7 B). The presence of more residual starch granules affected the yield of the 6000 rpm fine fractions, while the small particle size reduced the yield of the 10,000 rpm fraction (J. Wang et al., 2015). In summary, air classification at 8000 rpm is preferable to prepare the feed for subsequent electrostatic separation (Table 1).

3.4. Optimization of protein enrichment by repeated electrostatic separation

3.4.1. Purity improvement

To increase the protein purity and yield, fractions collected during a first electrostatic separation were collected and subjected to a second electrostatic separation step. After a 1st electrostatic separation, a protein enrichment of 11.7% was achieved (Fig. 8). Theoretically, a protein purity of maximally 76 g/100 g dry solids might be achieved, as this has been reported the protein concentration in proteosomes (also known as protein bodies), suggesting room for possible further protein enrichment (P.J. Pelgrom, Wang, et al., 2015). Therefore, as described in Fig. 1, a second electrostatic separation was carried out. Fig. 8

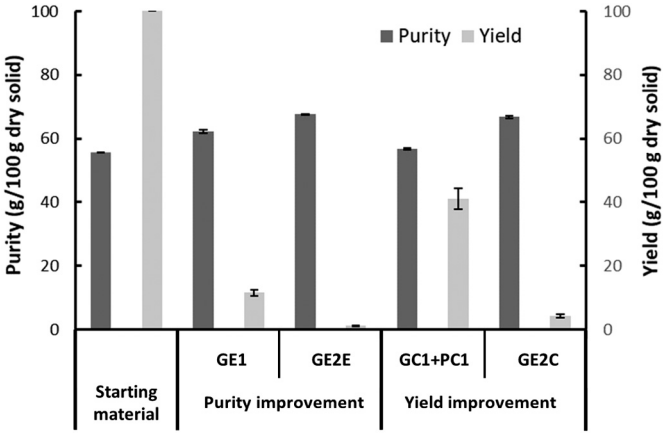

Fig. 8. Protein content and yield of protein-enriched fractions after the 1st and 2nd electrostatic separation following the purity improvement and the yield improvement strategies, respectively. The starting material was pea fine fraction obtained from air classification at 8000 rpm and the charging tube was used. The error bars represent the standard deviations.

showed that after a 2nd separation, the protein content of the protein-enriched fraction can be further increased from 62.2 to 67.6 g/100 g dry solids, i.e. a protein enrichment of 8.7%, achieving an overall enrichment of 21.4% starting from the fine fraction. However, this higher purity is at the expense of low yield (Fig. 8). In the first separation, only 12.8% of the total amount of protein in the feed (fine fraction) was recovered in the GE fraction. This was reduced further in the second step.

3.4.2. Yield improvement

The compositions of the GC and the PC fractions are approximately equal to the starting material (Fig. 7 A). The fractions collected from the two collecting bags (GC1 + PC1) were therefore mixed and subjected to a 2nd electrostatic separation (Fig. 1). As shown in Fig. 8, the protein content of GC1 + PC1 (56.7 g/100 g dry solids) was similar to that of the starting material (55.6 g/100 g dry solids). The protein content of the protein-enriched fraction (GE2C) increased in the second separation up to 66.7 g/100 g dry solids. Interestingly, this protein content is similar to the GE2E fraction obtain in the previous experiment. Apparently, there are still easily separable protein-rich particles present in the collector fractions that were not caught by the grounded electrode during a single pass. This indicates that the electrostatic separator itself can still be improved. The fraction GE2C might be added to the GE1 fraction to obtain a high purity protein concentrate with a higher yield. This is visualized in Fig. 9.

After two separation steps, a protein-enriched fraction (GE2E + GE2C) with a purity of 66.9 g/100 g dry solids and a yield of 5.6 g/100 g dry solids was obtained. This is 6.7% of the protein in the starting raw material. However, combining GE1 and GE2C fractions

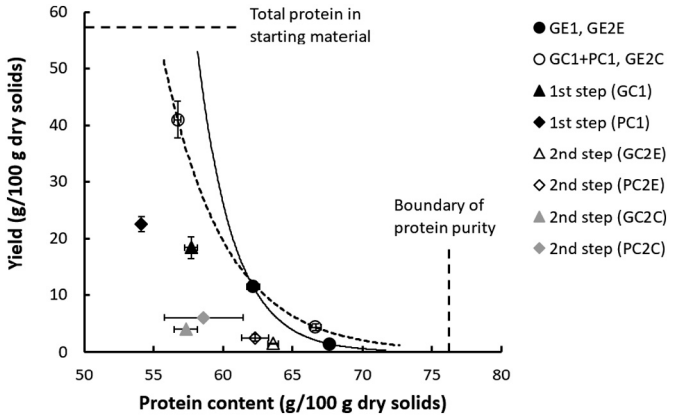

Fig. 9. The relation between yield and protein purity of fractions by 1st and 2nd electrostatic separation. The protein-depleted fractions were not plotted. The error bars represent the standard deviations. The solid line is drawn to indicate the protein-enriched fractions from 1st (GE1) and 2nd (GE2E) electrostatic separation. The dotted line is drawn to indicate the protein-enriched fraction (GE2C) by recycling of collecting bags (GC1 + PC1) from the 1st electrostatic separation. The dotted straight lines represent the upper limit of yield and protein purity for the protein-enriched fraction, respectively.

shows a better balance between protein purity and yield. A product with a protein content of 63.4 g/100 g dry solids and a yield of 15.8 g/100 g dry solids was obtained, recovering 18.0% of the original protein in the starting material (pea fine fraction). Comparison of the two lines drawn in Fig. 9 shows that it is useful to further fractionate the collector fractions by subsequent steps to achieve higher yield while maintaining the protein purity.

In summary, the obtained protein purity by combining air classification and electrostatic separation is higher (at reasonable yields) compared to that from only air classification, which indicates that electrostatic separation is a valuable additional processing step. With the optimized dry fractionation process, a protein-enriched fraction with a yield of 4.0 g/100 g pea was obtained, leading to 7.8% of total protein recovered from yellow pea (Table 2). The mass balance for the entire dry fractionation process is visualized in a Sankey diagram (Fig. 10). The yield in protein reported in this study may be further optimized by improved equipment electrostatic separator design. It was estimated that the yield for the optimized dry fractionation process may then be more than doubled to 10.9 g/100 g pea with 22.7% protein recovery (Table 2). However, of course, improving design and scale-up is still a major challenge, where ideally electrostatic separation should become a more continuous multi-stage process that enables separation to high purity and optimum yields.

4. Conclusions

Dry fractionation of three starch-containing legumes was achieved by combining air classification and electrostatic separation. By fine milling flours consisting of starch granules and smaller protein-rich fragments were prepared that could be used for subsequent air classification and electrostatic separation. Specifically, the fine fractions were subjected to electrostatic separation as it was known from a previous study that the presence of large amounts of starch impaired the electrostatic separation performance. Modest protein enrichment (4.6–5.8%) was achieved for the pea and lentil fine fractions, whereas no protein enrichment was observed for chickpea fine fraction.

Further optimization of the electrostatic separation was carried out using pea fine fraction. An optimum balance between protein purity and yield was achieved by adjusting the classifier wheel speed to 8000 rpm, where a pea fine fraction with a protein purity of 57.1 g/100 g dry solids was obtained and 49.0% of the protein was recovered from pea flour. After a single-step electrostatic separation, a protein-enriched fraction with a protein purity of 67.1 g/100 g dry solids and a yield of 13.1 g/100 g fine fraction was obtained, recovering 15.4% of the total protein in the pea fine fraction.

The protein purity and yield of the protein-enriched fraction was further improved by applying a second electrostatic separation. In the first strategy, protein-enriched fraction obtained from 1st separation was subjected to a 2nd separation. By doing so, a protein-enriched fraction with a protein purity of 67.6 g/100 g dry solids was obtained while only 1.6% protein was recovered from the starting material (fine fraction). In the second strategy, fractions obtained from the two collecting bags in the 1st separation were mixed and used for a 2nd separation. The optimum combination of protein-enriched fractions from two separation steps yielded a protein purity of 63.4 g/100 g dry solids with a yield of 15.8 g/100 g fine fraction. It means 18.0% of the protein was recovered from the pea fine fraction.

CRediT authorship contribution statement

Qinhui Xing: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft. **Dea Putri Utami:** Methodology, Investigation, Data curation. **Marta Boronat Demattey:** Investigation, Data curation. **Konstantina Kyriakopoulou:** Supervision, Validation. **Martin de Wit:** Validation, Resources. **Remko M. Boom:** Supervision. **Maarten A.I. Schutyser:** Conceptualization, Supervision, Writing - review & editing.

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Table 2
Comparison of purity, yield and protein recovery for protein-enriched fractions (compared to yellow pea with a protein purity of 32 g/100 g dry basis) obtained from three different dry fractionation processes on basis of measurements (with unrecovered material) and calculated potential assuming full recovery (e.g. for improved design). Different scenarios of electrostatic separation are included as well as air classification only. Data marked with a different lowercase superscript in the same column indicate significant differences ($P < 0.05$).

	Protein purity (g/100 g dry basis)	From current study		Calculated with fully recovered material	
		Yield (g/100 g pea)	Protein recovery (%)	Yield (g/100 g pea)	Protein recovery (%)
Air classification only	$57.1^{a} \pm 0.2$	$24.5^{c} \pm 1.1$	$49.0^{c} \pm 2.6$	$36.3^{d} \pm 2.0$	$70.5^{d} \pm 2.3$
Air classification + electrostatic separation	$67.1^{c} \pm 0.3$	$3.2^{b} \pm 0.2$	$6.6^{b} \pm 0.4$	$7.1^{b} \pm 1.2$	$13.3^{b} \pm 1.7$
Air classification +2-step electrostatic separation (GE2E)	$67.6^{c} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.7^{a} \pm 0.0$	$1.4^{a} \pm 0.3$	$2.9^{a} \pm 0.5$
Air classification +2-step electrostatic separation (GE1 + GE2C)	$63.4^{b} \pm 0.5$	$4.0^{b} \pm 0.3$	$7.8^{b} \pm 0.6$	$10.9^{c} \pm 0.5$	$22.7^{c} \pm 1.0$

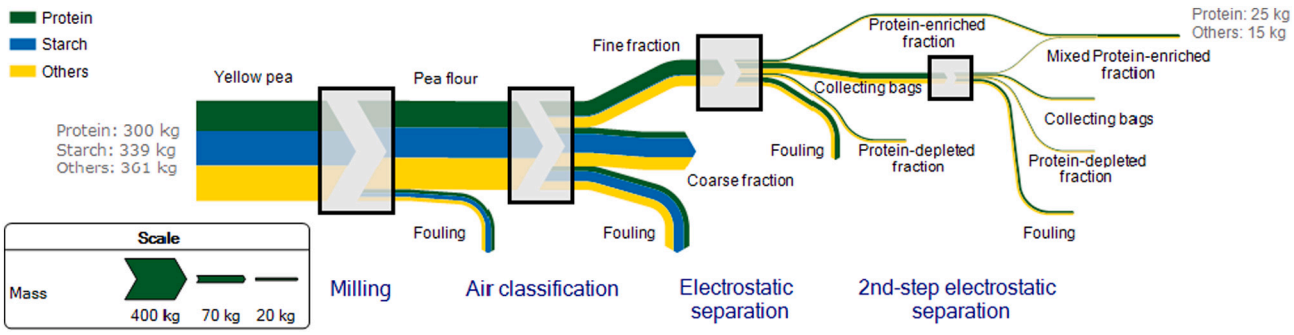

Fig. 10. Sankey diagram of the mass flow of the dry fractionation process on the yellow pea (starting with 1000 kg pea). The distribution of color indicates the dry mass of each component in each stream. For easy visualization, the "Starch" is merged into the category of "Others" after electrostatic separation.

Declaration of competing interest

The authors have no declarations of interest.

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