Upscaling of alkaline pea protein extraction from dry milled and pre-treated peas from laboratory to pilot scale: Optimization of process parameters for higher protein yields

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Abstract
The upscaling of pea protein extraction from laboratory scale with a centrifuge to pilot scale with a decanter centrifuge was investigated, and the pea protein extraction efficiency from dry milled and pre-treated peas was compared. Upscaling from laboratory to pilot scale is possible since starch was under the limit of detection (< 0.5%). The protein banding pattern of a sodium-dodecyl-sulfate polyacrylamide gel electrophoresis confirmed that albumins and globulins were extracted by alkali extraction. Protein yield increased from 59.5% to 67.1% for dry milled peas due to constant and quick discharge of dry matter in the decanter centrifuge. For pre-treated peas, the protein yield increased from 60.3% to 94.3%, which is explained by an improved cutting and improved separation in pilot scale compared to laboratory scale. The impact of acceleration, mass flow, differential speed and their respective interactions in the decanting process was determined with a design of experiments. For dry milled peas, only the mass flow exceeded the significance level. However, a mass flow of 5 kg h⁻¹, an acceleration of 1000 g × and a differential speed of 50 min⁻¹ led to the highest protein yield of 75.6%. The obtained protein yields for the pre-treated peas were in the range of 83 to 96% and therefore did not show significant differences in protein yield.

Keywords Pea protein · Protein extraction · Native proteins · Decanting · Optimization · Design of experiments

Introduction
Pulse crops are a good source for protein, carbohydrates and fiber and provide many minerals and vitamins [1, 2]. The yellow field pea (Pisum sativum L.) is a commonly grown pulse crop in central Europe [3] and provides good functionality, high acceptance, relatively low cost and a low allergenicity [4, 5].

Peas contain around 65 g·100 g⁻¹ carbohydrates, of which 45 g·100 g⁻¹ are starch. The protein content varies in a range from 20 to 30 g·100 g⁻¹. Pea proteins can be divided into its Osborne fractions: (i) salt-soluble globulin fraction; (ii) water-soluble albumin fraction; (iii) prolamins, which are soluble in a mixture of water and ethanol; (iv) insoluble glutelins [2, 6–8]. The ratio of globulins varies between 55 – 80% and 18 – 25% for albumins, respectively [9]. Globulins are classified on the basis of their sedimentation coefficient into the fractions 11S (legumin), 7S (vicilin) and convicilin. The hexameric protein legumin (11S) is composed of six subunits of 60 kDa, which are linked non-covalently. A subunit consists of an acidic (≈ 40 kDa) and a basic (≈ 20 kDa) domain, linked via a disulphide bridge. Vicilin consist of three subunits of 47 – 50 kDa. Some authors describe convicilin as a tetrameric protein with a molecular weight of 290 kDa [4, 10], other authors describe it as a trimeric protein with a molecular weight of 210 kDa [11]. The monomers are linked non-covalently and do not contain disulphide bonds [12].

Commercially available pea protein is known to be denatured and aggregated due to the processing, mainly due to heat treatment [3, 13, 14]. High pressure microfluidization shows promising results in increasing solubility of denatured pea protein isolates by weakening interactions.
between proteins. This results in a reduction of particle size and might be an interesting method to increase techno-functional properties of denatured pea protein isolate [15]. However, Fuhrmeister and Meuser [3] state that a high nativity of pea protein leads to superior techno-functional properties regarding solubility, fat-binding capacity, emulsifying capacity and foam-forming capacity. Therefore, a production of native pea protein isolate is advantageous in regard of the techno-functionality.

There are three extraction methods known to obtain pea protein isolate in its native form: (i) Micellar precipitation; (ii) salt extraction; (iii) and alkali extraction – isoelectric precipitation [16, 17]. Tanger et al. [17] reported the production of a pea protein isolate with high yield, high solubility and high nativity by alkali extraction – isoelectric precipitation. Furthermore, it was shown to be the fastest extraction procedure. Alkali extraction – isoelectric precipitation is conducted in two main steps. In the first step, pea flour is solubilized at pH 9.5 and 35 °C. The soluble globulins and albumins are separated from starch and the (at these conditions) insoluble glutelins and prolamins by centrifugation. In the second step, the precipitation step, the globulins are solubilized at pH 9.5 and 35 °C. The soluble globulins and albumins are precipitated by adjusting the pH of the supernatant to pH 4.5, which is near the isoelectric point of globulins [18]. Centrifugation leads to a pellet, mostly containing globulins, whereas albumins remain in the supernatant. The globulin pellet is then rehydrated with distilled water, adjusted to pH 7 and lyophilized. However, the setup from Tanger et al. [17] is designed for laboratory scale and only yields small amounts of pea protein isolate. To produce native pea protein fractions in bigger amounts for technical applications, a scale up of the process is indispensable. Upscaling of pea fractionation to a decanter centrifuge has been described by several authors [19, 20]. However, there are great variations between the process parameters of the different studies and an optimization has not been investigated until now.

In this study the fractionation of pea protein into globulin/albumin and glutelin/prolamin fraction via alkali extraction in laboratory scale is compared to an up scaled process in pilot scale. For reasons of comparability, laboratory scale and pilot scale extractions were carried out under the same conditions as far as possible. Furthermore, optimization of the decanting process in pilot scale was conducted with a design of experiments. Additionally, two different processing methods for grinding of peas before the alkaline extraction step were compared regarding protein fractionation efficiency: (i) dry milling of peas, yielding a pea flour; (ii) pre-treated peas (soaked in distilled water (dist. H2O) for 12 h) ground with a cutting device, yielding a pea slurry.

Material and methods

Materials

Yellow peas (P. sativum L.) were purchased from Wurzener Nahrungsmittel (Wurzen, Germany). Distilled water was used for the extraction procedure. All other chemicals and reagents were purchased from Th. Geyer (Renningen, Germany) and were of analytical grade. The dry matter of dry peas was determined to 86.81 g·100 g−1 and the protein content to 18.2 g·100 g−1 with the Dumas method using a nitrogen analyzer (Dumatherm DT N, Gerhardt, Königswinter, Germany). A protein factor of 5.36 for conversion of nitrogen content to pea protein content was used, as proposed by Mariotti, Tomé, & Mirand (2008).

Protein fractionation by alkali extraction

Alkali extraction was performed according to the method of Tanger et al. [17]. For dry milling, yellow peas were ground into pea flour with a Thermomix (Vorwerk Deutschland, Wuppertal, Germany). Peas were mixed with dist. H2O in a ratio of 1:15 (w/w). For the pre-treatment, one part of peas was soaked with three parts of dist. H2O for 12 h at 4 °C. Afterwards, the soaked peas were washed three times with dist. H2O and then crushed at 20 °C for 5 min with dist. H2O in a cutting machine (Stephan, Nazareth, Belgium) for pilot-scale and a handheld blender (ESGE, Mettle, Switzerland) for laboratory scale, respectively. The required amount of dist. H2O for crushing was calculated with equation 1, so that a total ratio pea to dist. H2O of 1:15 (w/w) was obtained.

\[
\text{m}_{\text{Wfc}} = 15\text{m}_{\text{Pea,dry}} - (\text{m}_{\text{Pea,wet}} - \text{m}_{\text{Pea,dry}})
\]  

where \( m_{\text{Wfc}} \) is weight for crushing, \( m_{\text{Pea,dry}} \) is weight of dry peas; \( m_{\text{Pea,wet}} \) is weight of wet peas.

The subsequent steps were conducted similarly for dry milled and pre-treated peas. To enable protein solubilization, the pH of the pea suspension was adjusted to pH 9.5 with 1 M NaOH and stirred for 60 min at 35 °C. In lab-scale, the pea suspension was centrifuged at 4500 g for 20 min at 4 °C (model 2–16 KHL, Sigma Laborzentrifugen, Osterode, Germany). For reasons of comparability, the extraction in pilot-scale with a decanter centrifuge (model MD 80-s, Lemitec, Berlin, Germany) had to be conducted as similarly as possible to lab scale. Therefore, the pea dispersion was decanted at 4000 g at 20 °C with a product mass flow of 5 kg h⁻¹ (Eccentric pump Type NM008BH, NETZSCH, Waldkraiburg, Germany) and a differential speed of 10 rpm (Fig. 1). The decanter bowl had a diameter of 80 mm and a length of 308 mm. The conical section was angled at 7° and the weir diameter was 56 mm. The pellet (starch, fiber
and insoluble protein) and the supernatant (globulins and albumins) were used for further analyses.

**DoE for process optimization in pilot scale**

To optimize the decanting process, a face-centered central composite design of experiments (DoE) was conducted. The target size was the protein yield in the supernatant. The three decanting parameters product mass flow, acceleration and differential speed were varied. Additionally, the boundary and the center values were coded to $-1$ (lower boundary), 0 (centered value), 1 (upper boundary). In Table 1 the values and the respective boundaries as well as the coded values are set. The design type was set to central composite design (CCD) with an axial value of 1, two centered points and 1 replicate. CCD means that there are center points and a group of axial points. As it is a face-centered central composite design of experiments, the axial points are set at the center of each face of the factorial space. Fig. 2 shows the CCD in cube form. The settings and the results of the DoE are attached in the appendix (Table 4 and 5).

$$Y = \beta_0 + \beta_1 \cdot m + \beta_2 \cdot a_c + \beta_3 \cdot \Delta n + \beta_{12} \cdot m \cdot a_c + \beta_{13} \cdot m \cdot \Delta n + \beta_{23} \cdot a_c \cdot \Delta n + \beta_{11} \cdot m^2 + \beta_{22} \cdot a_c^2 + \beta_{33} \cdot \Delta n^2 + e$$

With Eq. 2, a least square linear regression was performed. In the model, $e$ is defined as intercept and $\beta_i$ are the factors of the parameters mass flow ($m$), acceleration

**Table 1** Parameters for the DoE with the boundaries of the experimental design with real and coded values

| Parameter                  | Lower boundary | Centered value | Upper boundary |
|----------------------------|----------------|----------------|---------------|
| Mass flow in kg*h$^{-1}$   | 5              | 12.5           | 20            |
| Acceleration in g          | 1000           | 2500           | 4000          |
| Differential speed in rpm  | 10             | 30             | 50            |

| Coded Mass flow in kg*h$^{-1}$ | Coded Acceleration in g | Coded Differential speed in rpm |
|--------------------------------|-------------------------|--------------------------------|
| $-1$                           | 0                       | 0                              |

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Chemical Analysis

The dry matter of the supernatant and the pellet in lab scale and the pellet in pilot scale was determined according to the drying oven method ISO 5534:2004. The supernatant samples of the pilot scale extraction were measured via infrared drying. Hereby, water was heated and vaporized by infrared radiation and the weight difference was monitored. Preliminary experiments showed, that results of the two methods were comparable (data not shown). The protein content was determined by the Dumas method with a nitrogen analyzer (Dumatherm DT N, Gerhardt, Königswinter, Germany). A protein factor of 5.36 for conversion of nitrogen content to pea protein content was used as proposed by Mariotti et al. [22]. All analyses were performed in triplicate if not marked otherwise.

Protein yield for the extraction in lab-scale and pilot-scale (Y) was calculated with Eq. 3.

\[
Y = \frac{X_S \cdot m_S}{X_{PS} \cdot m_{PS}}
\]

With \(X_S\): protein content of the supernatant after centrifugation; \(m_S\): mass of the supernatant after centrifugation; \(X_{PS}\): protein content of the solution before centrifugation and \(m_{PS}\): mass of the dispersion before centrifugation.

Starch

The starch content of the supernatant was determined enzymatically by the CoreFacility (University of Hohenheim). In short, samples were mixed with hydrochloric acid and dimethyl sulfoxide and incubated at 60 °C. After full solubilization of the starch, an enzymatic UV test (starch determination kit, Roche-Biopharm, Darmstadt) was conducted. The limit of detection (LOD) was 0.05 g·100 g⁻¹ starch.

SDS-PAGE

Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out under non-reducing conditions (NR SDS-PAGE) to determine protein/poly-peptide masses and to identify the fractions. The protein solution (supernatant of the centrifugation/decanting step) was diluted with a Laemmlı sample buffer [23]. 20 µL of sample were loaded in each well of electrophoresis protein gels (Mini-PROTEAN TGX Stain-free gels 4–20%, 10-well comb, 50 µL, Bio-Rad Laboratories, Feldkirchen, Germany) together with a standard marker (Precision plus protein standards, Bio-Rad Laboratories, Feldkirchen, Germany) with a molecular weight ranging from 10 to 250 kDa and the SDS-PAGE was conducted at 200 V for approximately 25–30 min. Fixing (20% (v/v) acetic acid (Häberle, Lonsees-Ettlenschieß, Germany)) and staining solution (90% (v/v ethanol, Merck, Darmstadt, Germany) and 2 g·L⁻¹ of Coomassie blue (Serva Electrophoresis, Heidelberg, Germany)) were added in a 1:1 ratio. Afterwards, the gel was decolorized with decolorizing solution (200 mL·L⁻¹ ethanol, 50 mL·L⁻¹ of acetic acid and 10 g·L⁻¹ of glycerol), and qualitatively analyzed via image analysis.

Statistical analyses

For all analyses, the arithmetic mean and standard deviation (triplicate) or range (duplicate) were calculated. The experiments in lab- were performed in triplicate and in pilot-scale in duplicate. The Design of Experiments was performed with JMP 15 Pro (SAS Institute, North Carolina, USA) as a face-centered central composite design.
Results and discussion

Starch separation

One aim of the alkaline extraction is to remove starch from the supernatant, as starch would gelatinize during subsequent heating steps of the pea suspension. Determination of the starch content was done for the supernatant samples from the pilot scale process, as no difficulties regarding starch separation in lab scale were reported in literature [17]. Starch contents were below the LOD for the supernatant samples (Table 2). It was therefore concluded, that the applied process enables an almost complete separation of the starch from the aqueous phase.

Comparison of laboratory and pilot scale

Dry matter and protein content

The dry matter of the supernatant and the pellet of the alkaline extraction step in lab scale and pilot scale are shown in Table 2. As no starch was quantifiable, it was concluded that the dry matter of the supernatants was composed of proteins, minerals, and minor components such as mono-, di- and oligosaccharides, as native pea amylases could start to degrade starch during the soaking and extraction step [24, 25]. The mineral content of the supernatants is composed of native minerals from the pea and sodium from the added NaOH. The mineral content has to be taken into consideration for application of the protein fractions, as an increasing mineral content leads to an increase in denaturation temperature independent from pH, as NaCl stabilizes the quaternary structure of protein [18, 26, 27]. Due to that, gel formation is hindered and the gel stiffness is reduced. Application of ultrafiltration could be applied to decrease the mineral content.

With 1.4 ± 0.1 g·100 g⁻¹ the dry matter of the supernatant from dry milling in laboratory scale was similar to the pre-treated peas in laboratory scale with 1.5 ± 0.1 g·100 g⁻¹ dry matter. The dry matter of the pellet from dry milling and pre-treatment did not differ and had values of 23.4 ± 1.0 g·100 g⁻¹ and 20.3 ± 1.2 g·100 g⁻¹, respectively. The dry matter of the supernatant from dry milling and pre-treating in pilot scale was determined to 1.0 ± 0.3 g·100 g⁻¹ and 1.3 ± 0.2 g·100 g⁻¹, respectively, which did not differ from the laboratory scale experiments. Dry matter of the pellet from dry milling and pre-treating in pilot scale almost doubled to 48.7 ± 3.9 g·100 g⁻¹ and 43.3 ± 1.2 g·100 g⁻¹ compared to laboratory scale. The higher dry matter content of the pellet in pilot scale separation, and therefore the improvement of the separation of pellet and supernatant, was caused by the constant and immediate pellet removal via
solid discharge in the pilot scale decanting process. Further proof for the efficient separation of soluble and insoluble components of the pea in pilot scale was found by centrifuging (15,000 g, 30 min, 4 °C) the supernatant of the dry and wet milling step, as only small pellets were formed (Fig. 3).

Table 2 shows the protein content of the supernatant and pellet from lab scale and pilot scale process. The protein content of the supernatant from dry milled and pre-treated peas in lab scale showed no difference and was determined to 0.9 ± 0.1 g·100 g−1 and 1.0 ± 0.1 g·100 g−1, respectively. The protein content of the pellet in lab scale was determined to 2.1 ± 1.6 g·100 g−1 for dry milling and to 1.0 ± 0.1 g·100 g−1 for pre-treated peas. The high error of the protein content of the pellet from dry milling in lab scale was caused by inhomogeneous sampling, as complete separation of supernatant from pellet was not feasible. In pilot scale, a protein content of the supernatant of 0.8 ± 0.1 g·100 g−1 for dry milling and of 1.2 ± 0.1 g·100 g−1 for pre-treating was measured. The pellet of the pilot scale process contained 4.1 ± 1.3 g·100 g−1 protein with dry milling and 1.7 ± 0.1 g·100 g−1 protein with pre-treating. The higher protein content of the pellet in pilot scale of the dry milling process compared to the pre-treating process implies, that the protein extraction was less efficient than with the pre-treatment. To verify this, the protein yield was calculated and evaluated.

**Protein Yield**

The protein yield was calculated according to Eq. 3 and is a value for the efficiency of the protein extraction. The results for the protein yield are shown in Table 2. The protein yield for the supernatant in lab scale of 59.5 ± 4.5 g·100 g−1 for dry milling and 60.3 ± 2.5 g·100 g−1 for pre-treating did not differ. Tanger et al. [17] and Stone et al. [16] reported a slightly higher protein yield of 68.7% and 62.6–76.7%, respectively, for the supernatant of dry milled peas. A possible explanation for this could be, that the pea flour used in the study of Tanger et al. [17] was processed with a suitable mill by the pea flour producer, whereas grinding with the Thermomix yielded coarser pea flour particles. The protein yield of the pellet of dry milling in lab scale was 37.6 ± 24.7 g·100 g−1. The high errors were due to the inhomogeneous sample composition. The protein yield of the pellet of dry milling in lab scale was determined to 25.5 ± 2.9 g·100 g−1. Due to the high standard deviation of the protein yield of the pellet of dry milling in lab scale, no final conclusion regarding the comparability of the protein yield from dry milled and pre-treated peas in lab scale was possible.

The protein yield of the supernatant in pilot scale was 67.1 ± 0.8 g·100 g−1 for dry milling 94.3 ± 0.3 g·100 g−1 for pre-treating, respectively. The separation in pilot scale with the decanter centrifuge led to higher protein yields than in laboratory scale for dry milling and pre-treating. This was caused by the constant and quick discharge of the pellet in the decanter centrifuge in pilot scale (see Sect. 2.2), whereas the supernatant stayed in contact with the pellet throughout the whole centrifugation in laboratory scale. The even higher protein yield of the supernatant from pre-treating in pilot scale was probably induced by an improved wet milling compared to the cutting device in laboratory scale. Apparently, the changed rotor–stator geometry led to higher shear forces in the pea dispersions, which increased the protein–water contact area and thereby enabled higher protein solubilization in the aqueous phase due to an enhanced disentanglement of cellular components [28]. Together with the afore mentioned effect of the solid discharge, this led to an extraction of nearly 100% of the contained albumins and globulins with pre-treating in lab scale, as dry peas contain 18–25% albumins and 55–80% globulins [9]. The remaining proteins in the pellet of pre-treating in lab scale are mainly prolamins and glutelins, whereas the pellet of the dry milling in pilot scale still contains insolubilized albumins and globulins.

**Protein profile**

The protein profile of the supernatant of the different extraction methods is analyzed with a non-reducing SDS-PAGE. The bands around 70 – 75 kDa are ascribed to convicilin. By comparison with the reference protein ladder, bands at 18, 34 and 50 kDa are considered to be dissociated vicilin trimers [29]. The bands at 60 kDa are legumin monomers from the native hexameric form of legumin, which was reduced by SDS to monomers. This could be verified by conducting a SDS-PAGE under reducing conditions. By this, the disulphide bond between the alkaline (19 – 22 kDa) and acidic (38–40 kDa) subunit would be cleaved and two separate bands should be visible [18, 29].

Legumin monomers were present at all extraction and pre-treatment conditions investigated in this study. Albumins were extracted by alkali extraction too, as the bands below 15 kDa belong to the albumin fraction. In general, the same bands occurred regardless of whether the milling was done
with dry or pre-treated peas and regardless of laboratory or pilot scale. By comparing line 3 and 4 (Fig. 4), some bands on line 4, i.e. legumin at 60 kDa and some vicilin and albumin at 13 kDa, were found to be slightly more intense. This underlines the findings of Sect. 3.3, that the protein yield of the decanting step is higher when pre-treated peas instead of dry-milled are used in pilot scale. Protein extraction in laboratory- and pilot-scale was a success, as globulins and albumins (supernatant) were separated from prolams and glutelins (pellet). To verify the complete separation, the obtained supernatant was centrifuged at 15,000 g. Only minimal pellets were formed in any of the applied process conditions, which proves that prolams and glutelins as well as starch (see Sect. 3.1) were almost completely in the pellet.

**Optimization of decanting parameters**

The protein yield was chosen as target size for the DOE, as the comparison of dry matter and protein content of the supernatants showed, that almost the complete dry matter of the supernatant was protein. Optimization of the decanting parameters offers great potential to increase the protein yield of the supernatant by improving the fractionation into solid and liquid discharge. First, the influence of the decanter parameters was investigated. Therefore, the results of the DoE were evaluated using JMP Pro 15 to adjust the linear regression model. The p-values of the estimated coefficients were transformed to log-worth values for a better estimation, if the parameters and parameter combinations significantly affect the protein yield. In Table 3, the log-worth p-value of the different parameters and their interactions is shown. For dry milling, the log-worth of the significance level α = 0.05 (1.301) is only exceeded by the mass flow \( \dot{m} \) and shows a significant effect on the model.

For dry milled peas, a maximum protein yield of 75.6%, and therefore the most efficient fractionation, was obtained with parameter settings of a mass flow of 5 kg h\(^{-1}\), an acceleration of 1000 xg and a differential speed of 50 min\(^{-1}\). The process was therefore improved by approximately 13%. However, for process designs, the process time to obtain a desired amount of globulin/albumin fraction has to be considered. By increasing the mass flow to 20 kg h\(^{-1}\) the process time is reduced to a quarter of the time and protein yield is reduced to about 60% For application, it must therefore be evaluated, what the overall goal is. Low mass flows and therefore increased residence time in the decanter centrifuge leads to a higher protein yield of albumin/globulin fraction in the supernatant, whereas higher mass flows lead to larger amounts of product in less time, but at the loss of a small amount of fractionation efficiency.

A pre-treatment of peas resulted in higher protein yields at all parameter combinations than for dry milled peas. No parameter exceeded the significance level and no significant effect on the model was found. The obtained protein yields for the pre-treated peas were in the range of 83 to 96%. When conducting the process with pre-treated peas, the mass flow can be set to 20 kg h\(^{-1}\) without a significant reduction of protein yield.

**Conclusion**

The upscaling of pea protein extraction from laboratory scale with a centrifuge to pilot scale with a decanter centrifuge was proven to be feasible, as the albumin and globulin fractions of the protein could be separated of ethanol soluble glutelins, insoluble prolams and starch with the alkaline solubilization step and subsequent decanting. In general,

| Table 3 | Log-worth p-values of the parameters and parameter combinations of the dry milled- and the pre-treated peas |
|---------|---------------------------------------------------------------------------------------------------|
|         | \( m \) | \( a_c \) | \( \Delta n \) | \( m:a_c \) | \( m:\Delta n \) | \( a_c:\Delta n \) | \( m^2 \) | \( a_c^2 \) | \( \Delta n^2 \) |
| log-worth p-value | Dry milled peas | 5.02* | 0.22 | 0.15 | 1.21 | 0.5 | 0.34 | 0.76 | 0.32 | 0.07 |
|                  | Pre-treated peas | 0.35 | 0.78 | 0.11 | 0.55 | 0.27 | 0.18 | 0.86 | 0.78 | 0.69 |

*Exceeding the log-worth of the significance level \( \alpha = 0.05 \)
protein yields in pilot scale were higher than in laboratory scale due to the constant and quick solid discharge in the decanter centrifuge. In laboratory scale, no difference between dry milling and pre-treated peas was found. In pilot scale, pre-treatment proved to be superior to dry milling, with protein yields of 94.3 ± 0.3% for pre-treating and 67.1 ± 0.8% for dry milling. The increased protein extraction for pre-treating in pilot scale is ascribed to an improved solubilization of proteins due to the presoaking of the peas. By conducting the alkaline extraction in pilot scale with a decanter centrifuge, significantly larger quantities of albumin/globulin fraction (supernatant) can be produced. Starch was separated by the pilot scale process as it was not detectable in the supernatant. No significant changes in the protein profile of the supernatant were induced by upscaling the alkaline extraction. Additionally, an improvement of protein yield is possible by optimizing the decanter parameters. Thereby, a mass flow of 5 kg h⁻¹ in general leads to higher protein yield. However, the residence time and therefore the process time has to be considered for industrial applications.

In further works, the albumin/globulin fraction can be used as starting material for the fractionation into albumins and globulins by a precipitation step at the IEP and subsequent decanting or filtration.

### Appendix

See Tables 4 and 5

| Table 4 | DoE with the parameters acceleration (Acc), mass flow (Fl) and differential speed (ds) with settings as real and coded values |
|---|---|---|---|
| run | Fl in kg h⁻¹ | Acc in g | ds in min⁻¹ | Coded values |
| | Fl [-] | Acc [-] | ds [-] |
| 1 | 20 | 1000 | 10 | 1 | -1 | -1 |
| 2 | 5 | 1000 | 10 | -1 | -1 | -1 |
| 3 | 20 | 4000 | 10 | 1 | 1 | -1 |
| 4 | 12.5 | 2500 | 50 | 0 | 0 | 1 |
| 5 | 12.5 | 2500 | 30 | 0 | 0 | 0 |
| 6 | 12.5 | 1000 | 30 | 0 | -1 | 0 |
| 7 | 12.5 | 4000 | 30 | 0 | 1 | 0 |
| 8 | 20 | 4000 | 50 | 1 | 1 | 1 |
| 9 | 12.5 | 1000 | 30 | 0 | -1 | 0 |
| 10 | 5 | 1000 | 50 | -1 | -1 | 1 |
| 11 | 12.5 | 4000 | 30 | 0 | 1 | 0 |
| 12 | 20 | 1000 | 50 | 1 | -1 | 1 |
| 13 | 5 | 4000 | 50 | -1 | 1 | 1 |
| 14 | 20 | 4000 | 50 | 1 | 1 | 1 |
| 15 | 5 | 4000 | 10 | -1 | 1 | -1 |
| 16 | 12.5 | 2500 | 10 | 0 | 0 | -1 |
| 17 | 12.5 | 2500 | 30 | 0 | 0 | 0 |
| 18 | 5 | 1000 | 50 | -1 | -1 | 1 |
| 19 | 20 | 1000 | 10 | 1 | -1 | -1 |
| 20 | 5 | 4000 | 10 | -1 | 1 | -1 |
| 21 | 12.5 | 2500 | 30 | 0 | 0 | 0 |
| 22 | 20 | 1000 | 50 | 1 | -1 | 1 |
| 23 | 20 | 4000 | 10 | 1 | 1 | -1 |
| 24 | 12.5 | 2500 | 10 | 0 | 0 | -1 |
| 25 | 12.5 | 2500 | 50 | 0 | 0 | 1 |
| 26 | 20 | 2500 | 30 | 1 | 0 | 0 |
| 27 | 5 | 2500 | 30 | -1 | 0 | 0 |
| 28 | 5 | 4000 | 50 | -1 | 1 | 1 |
| 29 | 5 | 2500 | 30 | -1 | 0 | 0 |
| 30 | 20 | 2500 | 30 | 1 | 0 | 0 |
| 31 | 5 | 1000 | 10 | -1 | -1 | -1 |
| 32 | 12.5 | 2500 | 30 | 0 | 0 | 0 |
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Declarations

Conflict of interest Manuscript: Upscaling of alkaline pea protein extraction from dry milled and pre-treated peas from laboratory to pilot scale: Optimization of process parameters for higher protein yields. The authors have no relevant financial or non-financial interests to disclose.

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