From raw material to mildly refined ingredient – Linking structure to composition to understand fractionation processes

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ABSTRACT

The relation between the structure of yellow pea and its optimal fractionation process was investigated using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). Different fractionations obtained by milling and various methods were investigated and compared to pea flour. Milling resulted in structure break-up and a distinction of particles in shape and size. Different particles were identified using the mapping of the elements (EDS), carbon, oxygen and nitrogen, indicating the presence of starch, protein and other carbohydrates, in the form of protein bodies, starch granules and cell wall fragments. It was further observed that not only protein bodies contained protein, but also particles that were presumed to be cell wall material contained protein, although in considerably lower concentrations. With both fractionation methods protein and starch could be partly separated, but wet fractionation resulted in a higher purity in both the soluble and non-soluble protein fractions.

1. Introduction

Most ingredients that are used in food products are highly purified, i.e. starch, sugar, oils or proteins. The production of these ingredients requires large amounts of water and energy, and results in large side streams (Augustin et al., 2016; Ladha-Sabur et al., 2019; Van Der Goot et al., 2016). Therefore, alternative processing and fractionation routes were investigated, where either the valorization of the produced side streams is investigated (Schieber, 2017) or routes to omit side streams were investigated, where either the valorization of the produced side streams is investigated (Schieber, 2017) or routes to omit side streams by producing less pure ingredients (Geerts, Memis, Nikiforidis, van der Padt and van der Goot, 2017a,b,c; Pelgrom, Berghout, van der Goot, Boom and Schutyser, 2014a; Pelgrom, Vissers, Boom and Schutyser, 2013b). So far, a relation seems to exist between the harshness of the process and the obtained purification. It is therefore interesting to better understand which factors determine possible limits in purification and further develop the proposed route towards less refined ingredients to increase the process efficiency.

Yellow field pea has been widely studied before and is gaining increasing popularity as an ingredient in plant-based products. The pea is a spherical seed with an outer skin, the Testa or hull. The core of the seed is di-cotyledinous, hence contains two embryonic leaves, which together form the spherical shape. The cotyledons function as storage organs in the pea and are connected by the embryonic axis. Within the cotyledons, protein bodies and starch granules are embedded in the storage tissues of pea cells. The protein bodies are spherical organelles and, in the field-pea have a diameter of around 1–3 μm. The starch granules have round elongated shapes, ranging in size from 2 to 40 μm (Ramayake et al., 2002) and are tightly embedded in a matrix of the protein bodies (Huang, 1985; Peronollet, 1976). The field pea contains around 21–30% protein, about 50% carbohydrates, 10% crude fiber and about 2–3% lipids (de Almeida Costa et al., 2006).

A range of fractionation processes have been investigated for yellow field pea, including air classification as a dry fractionation process (Pelgrom, Vissers, et al., 2013b; Silventoinen et al., 2018; Wu and Nichols, 2005) and mild wet fractionation, where chemicals are omitted (Geerts et al., 2017a,b,c; Peng, Kersten, Kyriakopoulou and van der Goot, 2020).

Milling is usually the first step when producing a concentrate or an isolate (Assatory et al., 2019; Boursier, B; Delevarre, M; Lis, J; Marquilly, 2008; Geerts et al., 2017a,b,c; Muneer et al., 2018; Pelgrom, Vissers, et al., 2013b). Ideally, milling leads to initial structure break-up that separates the components into discrete particles already. In yellow pea, starch and protein are naturally present in separate entities, which suggests potential of being broken up along those entities (Pelgrom...
et al., 2015). In this paper, we investigate how efficient peas are broken-up along the different entities. The different structural levels of the pea seed have been visualized and analyzed using microscopic imaging before. Especially the structures in the pea storage cell and the disentangled starch granules were imaged by various authors using different microscopic methods (Bertolf et al., 1993; Colonna et al., 1980; Craig et al., 1979; Kornet et al., 2019; Otto et al., 1997; Pelgrom, Schutyser and Boom, 2013a; Reichert and Youngs, 1978). The extensive research on the microstructure of the pea storage cell lead to an identification of the structures in the cell, based on their composition. During structure break-up and fractionation however, these structures are disentangled and might be modified in size and shape, which limits the particle identification in the obtained fractions. Therefore, the aim of this work was to investigate the structure break-up during milling and investigate the structural composition during dry and mild wet fractionation. By understanding the structure and composition of the flour particles we aim at understanding the potential of the fractionation methods to create possibilities for further optimization thereof.

2. Materials and methods

Pre-dried yellow peas (Pisum sativum L.), were purchased from Alimedex (Sint Kruis, The Netherlands).

2.1. Milling

The peas were pre-milled into grits using a pin mill (LV 15M, Condex-Werk, Wolfgang bei Hanau, Germany) at room temperature. The grits were subsequently milled into fine flour using a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany), with an impact mill speed of 8000 rpm, the air flow at 52 m$^3$/h, the classifier wheel speed at 5000 rpm and a feed rate of 2 rpm (method adopted from Pelgrom et al., 2015). A thermometer inside the mill was used to control the temperature between 16 and 34 °C.

2.2. Dry fractionation

Air classification was used according to the method of Pelgrom, Vissers, Boom and Schutyser (2013b) using an ATP50 classifier (Hosokawa-Alpine, Augsburg, Germany). With a fixed air flow at 52 m$^3$/h, the classifier wheel speed at 5000 rpm and the feed rate at 20 rpm a protein fraction (FF), enriched in protein and a starch fraction (DF), enriched in starch were obtained.

2.3. Mild wet fractionation

Secondly mild wet fractionation was performed according to the method of Geerts, Nikiforidis, van der Goot, & van der Padt (2017a), 11.4 g of pea flour was suspended in 100 g ultrapure water (Milli-Q) and stirred for at least an hour. The suspension was then centrifuged at 1500 g at 20 °C for 1 s, resulting in a starch enriched pellet, defined as the starch fraction (SF) and a supernatant. The supernatant was then subjected to a second centrifugation step of 10000 g at 20 °C for 30 min, resulting in a pellet further called non-soluble protein fraction (NSPF) and a supernatant, soluble protein fraction (SPF). All three fractions were freeze dried for further analysis using a Pilot freeze dryer (Christ Epsilon 2-6D, Osterode am Harz, Germany).

A batch of the pre-dried peas was dehulled in a Satake TM05 pearling machine (Japan) to obtain a hull fraction. The remaining dehulled peas were not considered for further analysis.

2.4. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

SEM was used to visualize the morphology of the pea flours and the respective fractions. The samples were mounted on SEM stubs (Aluminium Pin-Type Mounts 12.7 mm, Jeol, Nieuw-Venneh, The Netherlands) by carbon adhesive tabs (12 mm carbon tabs, SPI Supplies Division of Structure Probe, Inc., West Chester, PA, USA) and subsequently coated with 12 nm Iridium using a High Vacuum Coating system (Leica MED 020, Leica Microsystems B.V., Amsterdam, The Netherlands). Samples were analyzed at 2 kV, 13 pA, in a field emission scanning electron microscope including the energy-dispersive X-ray spectroscopy detector (Magellan 400, FEI, Eindhoven, The Netherlands). Elemental maps of the samples were produced using energy-dispersive X-ray spectroscopy. An electron beam is focused on the sample, the interaction of the beam with the specimen produces backscattered x-rays whose specific energies provide a fingerprint that is specific to each element due to their different and unique atomic numbers (Goldstein et al., 2018). In addition to the elemental maps, spectral analysis was performed on the surface of differently shaped particles to aid particle identification. Therefore, a scan area is selected. With the elemental scans the atomic percentages were calculated from the weight percentages using the following equation (Goldstein et al., 2018). Where $n$ is the number of elements detected in the scan area.

$$\text{atomic} \% = \frac{\text{weight} \% \text{ of an element}}{\sum \text{weight} \% \text{ of all elements}} \times 100\%$$

2.5. Image analysis

Image analyses was performed using the image processing and analysis open source software ImageJ (version 1.52, Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, USA). The scale bar of the image was converted into the software and the measures of the different shapes in the images were added.

3. Results and discussion

Pea structure break-up during milling was investigated by identifying the particles based on their composition using Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS). Two different fractionation methods were performed, and the protein and starch separation potentials were analyzed by combining the compositional analysis of the fractions with elemental maps and image analysis of the particles.

3.1. Structure break-up from pea storage cell to pea flour

The pea cotyledon consists of storage cells in which starch granules are embedded in a cellular matrix, rich in protein. The protein in this matrix was reported to be present in protein bodies (Pernollet, 1978). An identification of the structures in the storage cell using cryo-scanning electron microscopy and energy-dispersive X-ray spectroscopy was previously reported by (Kornet et al., 2019). In this study, elongated particles in the cell, of rounded shapes and sizes of 10–30 μm were identified as starch granules and the matrix surrounding these particles was confirmed to contain proteins. The protein in this matrix was reported to be present in protein bodies (Pernollet, 1978). The elemental composition of the cell wall was not discussed and can also not be clearly identified in the images presented. Reichert and Youngs (1978) however reported that the cell wall of the pea storage cell contains 5 wt% protein in dry matter.

Fig. 1 shows a SEM image of a pea storage cell. The elongated particles in the cell have a length of about 10–20 μm, which according to Kornet et al. (2019) are starch granules. These granules are embedded in a matrix of smaller round particles (Fig. 1, black arrow), presumably protein bodies. Further, the storage cell is surrounded by sheet-like structures (Fig. 1, white arrow), which might form the cell wall.

Fig. 1 underlines that thorough disentanglement of protein and
starch requires structure break-up of the pea cotyledon to a particle size range of 10 μm < $D_{0.5}$ < 40 μm (Pelgrom, Vissers, et al., 2013b). They found that impact milling at a classification speed of 4000 rpm resulted in optimal disentanglement of protein bodies and starch granules, with limited to no starch damage. This further resulted in a good protein and starch separation during air classification. The results are in line with previous findings. The non-uniform particle size of the starch granules makes full disentanglement without damage of the granules difficult to achieve in practice (Bertoft et al., 1993; Huang, 1985; Pernollet, 1978; Ratnayake et al., 2002; Vose, Basterrechea, Gorin, Finlayson, & G, 1976). Too coarse milling leads to aggregates of protein bodies, starch granules and other cell material, while too fine milling results in extensive starch damage, which was reported to negatively affect the component separation (Boye et al., 2010). Fig. 2 shows a fraction of the pea flour, milled according to Pelgrom et al. (2013a,b), where the cells were successfully broken-up into small particles. The elongated and round shapes of around 10–30 μm, representing starch granules, are disentangled from the cellular structure. However, upon close observation, particles of different sizes and roughness are still attached to the starch granules. Other particles of varying shapes and sizes are also visible, among others also larger particles, of which the morphology suggest insufficient break-up of the cell structures, i.e. a round, starch granule like particle, still entangled with the particulate cellular matrix (Fig. 2, white arrows). However, these particles are similar in size as some starch granules, hence their structure break-up would also induce starch damage. For a better visualization of the structure break-up of the pea an illustration was added (Fig. 3). The illustration depicts a pea storage cell, containing starch granules, embedded in a particulate matrix and surrounded by a sheet like cell wall. Further a particle >30 μm is depicted which is a representation of an insufficiently broken up cell fragment. Structure break-up to <40 μm depicts the fully disentangled particles and some insufficient structure break-up, in form of an aggregate of a starch granule and protein bodies, which have approximately the same size as the fully disentangled starch granules. Lastly, structure break-up to <30 μm is depicted to illustrate starch damage.

3.2. SEM and EDS of pea flour

Fig. 4 shows SEM images and elemental maps of particles obtained after milling. The elongated, round particle in (A) is a starch granule, given its size (27.35 μm) and shape. It is further, poor in nitrogen, see Fig. 4(B). The particles adhering to the surface of the granule, however, are rich in nitrogen, they have a diameter of about 3 μm and vary in shape and surface roughness. The size of these particles corresponds to the approximate sizes of protein bodies (~3 μm) reported in previous studies (Pernollet, 1978). However, the difference of the two particles,
The second particle (see Fig. 4(C)) looks distinctly different in shape and structure as the starch granule. The particle itself consists of smaller round particles sticking together, indicated by the black arrow. Large dents with a width of around 10 μm and a smooth surface are visible in the particle structure that might be the negative forms of starch granules according to their size and round shape. These observations suggest that the particle is a fragment of the intercellular matrix in which the starch granules were embedded (Huang, 1985; Pernollet, 1978). On its surface additional round particles (~3 μm) are adhering, which seem to have adhered to the fragment only after structure break-up, as they are positioned on the smooth surfaces where the starch granules were assumed to be in the original structure (see Fig. 1). The small particles adhering to the surface of the fragment also contain nitrogen and could according to their shape, size (3 μm) and composition be protein bodies that were disentangled from the intercellular matrix and are re-adhering to the fragment. The elemental map reveals an even distribution of nitrogen over the whole particle, indicating that the whole fragment contains protein. This underlines the assumption that the particle is indeed a fragment of the intercellular matrix, rich in protein.

The identification of the particles, which adhere to the starch granules and matrix fragment, was extended by the spectral analysis of the clear surfaces of a starch granule and from two differently shaped particles adhering to the granule (Fig. 5). Spectra 1 detected the elements on a clear starch granules’ surface. In the scan area, no nitrogen was detected (see Table 1), which correlates with the results from the overall elemental map (Fig. 4 B, D). The ratio of carbon to oxygen in starch is 6:5 according to the molecular structure of amylose and amylopectin. The large difference in the proportion of carbon to oxygen (5:1), might be induced by the carbonization of molecules by the electron beam leading...
to dehydration and thereby a loss of oxygen (Funke and Ziegler, 2010). The area of spectra 2 is from the surface of a round shaped particle, supposedly a protein body, adhering to the starch granule. The atomic nitrogen percentage of the surface is 15%, if converted to protein using the nitrogen conversion factor of 5.52 for pea (Holt and Sosulski, 1979), this corresponds to 80% protein. The organelle protein concentration in
a protein body of different legume species was reported to be on average between 60 and 90% (on dry weight basis) (Pernollet, 1978). That supports the hypothesis that the particle is a protein body. The average ratio of carbon to nitrogen based on the amino acid composition of pea proteins, was calculated to be around 4 carbon atoms per 1 nitrogen atom. This corresponds to the ratio of carbon to nitrogen detected on the surface of the protein body. It is therefore assumed that no depletion of nitrogen is occurring through, i.e. the electron beam. Spectra 3 is of the surface of a small flat shaped particle, which as well is adhering to the starch granule, but according to its flat shape might not be a protein body. It contains 2.26 atomic % nitrogen (see Table 1), corresponding to 12.45% protein (Nx5.52) which is a considerably lower amount when compared to the protein body of Spectra 2. The cell wall of legumes contains next to dietary fiber also some cell wall proteins (Reichert and Youngs, 1978), therefore the flat particle is probably a fragment of the cell wall. Although the spectra are only a representation of the surface of the particle and limited by the penetration depth of the x-rays, the atomic percentages of the surfaces allow an estimation of the total protein content of the particles. A more reliable insight, however is derived from the ratios between the elements that make a distinction and identification of the particles, based on their composition possible. This distinction and identification of the particles, might help to predict the separation potentials of fractionation methods for different crops. If the particle structure serves to determine the composition, i.e. using morphology analysis techniques, together with the cut-off size of the air classification method a rough prediction of the separation potential might be possible.

The particle adhesion of the smaller particles, i.e. protein bodies and cell wall fragments, to the bigger particles, i.e starch granules and cellular matrix fragments (Figs. 4 and 5), could be caused by different mechanisms. Firstly, the particles are not fully disentangled from each other during milling, and therefore still adhere to the granule. Another possibility is that the particles are charged during milling, resulting in attraction and adhesion of smaller to bigger particles. The charging is caused by contact electrification when two surfaces contact and separate again and is dependent on the surface properties of the material (Horn and Smith, 1992; Kweitkus, 1998). These contacting surfaces are particle-wall contact surfaces between the particles and the mill, and particle-particle contact surfaces. With decreasing particle size the electrification of the particles increases, due to the increased surface-volume ratio of smaller particles (Gajewski, 1989), which means the charging also depends on the particle size of the flour. To investigate the adhesion mechanisms, different milling materials and speeds would need to be investigated. This was however out of scope for this research question. Instead, the SEM images after fractionation might provide an indication of the cause of the adhesion.

### 3.3. SEM and EDS of dry fractionated samples

Fig. 6 depicts a protein-enriched fraction and a starch-enriched fraction, which were obtained using air classification. Compared to the SEM images of the flour, the starch-enriched fraction (see Fig. 6–A) shows mostly starch granules, and few other bigger fragments, indicated by the black arrows. The surface of the starch granules is much cleaner after air classification when compared to the starch particles present in the flour. The protein-enriched fraction contains a much broader variety of particles. Here still some starch granules are visible (white arrows), as well as irregularly shaped particles (black arrows). Further, smaller particles are visible that adhere to the present bigger particles. In the protein fraction (A), more particle adhesion is visible, compared to the starch fraction. Next to the imaging, the protein and starch content of the protein-enriched fraction and the starch-enriched fraction was determined, the results are listed in Table 2. According to the compositional analysis there is still around 13% (Nx5.52) of protein present in the starch-enriched fraction. The starch granules in the starch fraction (DF) show, however, clean surfaces, indicating no protein is adhering to the granules which could explain the remaining protein content. Hence, air classification leads to loosening of the adhering particles to the starch granules allowing their separation, based on the particle size. This supports the hypothesis that the smaller particles are only adhering to the granules after structure break-up. The remaining protein is probably due to the presence of minor impurities or might be entangled in the insufficiently broken up particles (Fig. 6 (B), black arrows) of >30 μm present in the starch-enriched fraction. The protein fraction (A) has a remaining starch content of 11% (Table 2), which can be explained by the starch granules present in the fraction. Overall, the dry fractionation method leads to good protein – starch separation, however, the method is limited by the insufficient disentanglement during milling. Pelgrom et al. (2015) investigated a broad range of process conditions to find the optimal milling conditions, for maximal protein separation efficiency during air classification. The trade-off found was between highest disentanglement of protein and starch at lowest damage of starch granules.

### 3.4. SEM and EDS of mild wet fractionated samples

Mild wet fractionation was used to produce a starch enriched and two protein enriched fractions. Freeze drying was chosen as a mild drying process. These fractions were compared to the coarse and protein fraction (DF) from air classification and the native pea flour. Fig. 7 (A) shows an image of the freeze dried and crushed starch fraction obtained after the first centrifugation step, (B) depicts the soluble protein fraction, freeze dried and crushed, and (C) the non-soluble protein fraction respectively. In addition to the SEM images, the compositional analysis of the fractions was performed, and the results are listed in Table 2 under mild wet fractionation.

#### Table 1

Results of the spectral elemental analysis using SEM EDX as depicted in Fig. 6, with the weight atomic percentages of the elements present within the area of the different spectral scars.

<table>
<thead>
<tr>
<th>chemical element</th>
<th>Weight % Spectra 1</th>
<th>Atomic % Spectra 1</th>
<th>Weight % Spectra 2</th>
<th>Atomic % Spectra 2</th>
<th>Weight % Spectra 3</th>
<th>Atomic % Spectra 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>77.42</td>
<td>82.35</td>
<td>61.62</td>
<td>67.33</td>
<td>80.23</td>
<td>84.47</td>
</tr>
<tr>
<td>N</td>
<td>21.80</td>
<td>17.39</td>
<td>21.49</td>
<td>17.61</td>
<td>16.49</td>
<td>13.02</td>
</tr>
<tr>
<td>O</td>
<td>0.78</td>
<td>0.25</td>
<td>1.17</td>
<td>0.39</td>
<td>0.78</td>
<td>0.25</td>
</tr>
<tr>
<td>Mg</td>
<td>0.16</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **SPF** and **NSPF** are equal with around 52%, see Table 2. This shows that the nitrogen detection of a particle surface does not exactly represent the protein content of the whole fraction. The compositions of the particles within the fraction are likely to be a bit different from each other which
Fig. 6. Scanning electron microscopy (SEM) images of pea starch-enriched fraction (A) and protein-enriched fraction (B) obtained by air classification of finely milled pea flour as adopted from (Pelgrom et al., 2015). In (A) the measure of a starch granule is included (34.06 μm), insufficiently broken up particles are highlighted with black arrows. In (B) starch granules are highlighted with white and irregularly shaped fragments are highlighted with black arrows.

Table 2
Compositional analysis of pea flour and the protein and starch enriched fractions obtained through dry fractionation and mild wet fractionation. The nitrogen conversion factor used was N\textsubscript{x}5.52 (N\textsubscript{x}6.25 is provided in brackets for comparison) and the values are given in g/100g dry matter.

<table>
<thead>
<tr>
<th>Component</th>
<th>Before fractionation</th>
<th>Dry Fractionation</th>
<th>MWF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flour</td>
<td>Starch fraction</td>
<td>Protein</td>
</tr>
<tr>
<td>Protein</td>
<td>18.5 ± 0.6 (23.4 ± 0.7)</td>
<td>13.2 ± 0.7 (14.2 ± 0.8)</td>
<td>37.0 ± 3.6 (41.9 ± 4.0)</td>
</tr>
<tr>
<td>Starch</td>
<td>44.7 ± 1.5</td>
<td>62.3 ± 0.0</td>
<td>11.4 ± 0.0</td>
</tr>
<tr>
<td>Other</td>
<td>36.8 ± 2.1</td>
<td>24.5 ± 0.7</td>
<td>51.6 ± 3.6</td>
</tr>
</tbody>
</table>

Fig. 7. Scanning electron microscopy (SEM) of (A) starch enriched fraction, (B) soluble protein fraction (C) non-soluble protein fraction obtained by mild wet fractionation according to the method of (Marlies E.J. Geerts et al., 2017b,c). (A) The measure of a starch granule is included (27.28 μm).
would explain over- or underestimations of the actual composition, when just looking at small sections. Further analyses showed that NSPF contains a slightly higher amount of starch than SPF, resulting from remaining starch from the first centrifugation step, which sedimented during the second step due to the longer time and higher force.

The SEM images of the starch fraction (MWF) and the starch fraction (DF) both depict mainly elongated round particles of around 30 μm. Based on their compositional analysis, both fractions contain similar amounts of other components (Table 2), but the starch fraction (MWF) is more enriched in starch and more depleted of protein. The starch granules of the starch fraction (MWF) have a similarly clean surface compared to the granules in the starch fraction (DF), however thin lines or marks can be observed spun across the surface, see Fig. 8-A, white arrows. To get a better understanding of the origin of these marks an elemental map of a starch granule is depicted in Fig. 8-B. When investigating the SEM image, no distinct particles or protein bodies are attached to the starch granules anymore, but the marks or lines which are now visual on the surface are enriched in nitrogen, hence protein is present (Fig. 8-B). In the SEM images of the SPF and NSPF also no protein bodies were observable, instead platelet structures typical for freeze drying were formed (Fig. 7 B, C). Both the observation in the starch fraction (MWF) and the SPF and NSPF suggest that the protein bodies were partly of fully dissolved upon addition of water and formed new structures during freeze drying. For the starch fraction the solubilization of the proteins most likely resulted in films around the granules upon freeze-drying, which explains the presence of nitrogen on the whole granule (Fig. 8-B). Hence, with centrifugation dissolved proteins remain in the pellet with the interstitial water and form films around the granules upon drying. The findings show that the solubilization with water lead to the washing off of the smaller particles form the starch granules, resulting in a starch-enriched fraction of ~70% and two protein enriched fractions of each ~50% (Table 2). The solubilization of the protein in water and the fact that some water remains in the pellet in form of interstitial water, point out the potential of the fractionation method, as an increase in purity could be achieved by additional pellet washing steps. Further, protein is not the only component solubilizing in water, i.e. soluble dietary fiber and small sugars probably also solubilize. This indicates that with water, soluble and insoluble components can be separated to a certain extent.

The comparison of fractions obtained through mild wet fractionation to those obtained through dry fractionation, highlights that the MWF fractions are all more enriched in either protein or starch. The protein content of the SPF and NSPF is around 10% higher and the starch content in SPF and NSPF is 10% and 6% lower, respectively, compared to those in the protein fraction (DF). Similar results are found for the starch fractions, where the starch content of the starch fraction (MWF) is 8% higher than the starch content of the starch fraction (DF). The protein content in the starch fraction (MWF) is about 8% lower than the protein content in the starch fraction (DF). This indicates that MWF is more efficient when compared to DF, based on component separation. In both dry and mild wet fractionation, the smaller particles are disentangled from the granules. Hence the adhesion between the particles could be overcome using both methods. Therefore, the remaining explanation for the further enrichment of the fractions obtained through mild wet fractionation, is the addition of water and hence the solubilization of some components in the flour. It is assumed that in MWF the insufficiently milled flour fragments could dissolve in the water, which lead to more disentangled starch granules and protein bodies that could be fractionated into the respective fractions.

The MWF process offers potential to extract more protein from the interstitial water in the starch fraction, applying washing steps. However, the complete extraction is limited by the simple techniques of using only water and centrifugal forces. Additionally, to increase the purity in the respective fractions even further, a removal of other components is necessary. Such a removal, is limited by the solubility properties of the components, i.e. the separation of soluble dietary fiber and soluble protein might require other methods or additional solvents. These other methods or solvents would, in turn, interfere with the mildness of the conditions. Therefore both, the trade-off between mild fractionation and protein enrichment, as well as, yield reduction and protein enrichment would need to be assessed together with a method on how to further enrich the respective fraction. Finally, the observation of the equally high protein purity of the NSPF compared to the SPF offers further potential to investigate the route with emphasis on the properties of NSPF, to make full use of all proteins obtained. The presented approach could be a suitable method to also investigate the structure break-up and fractionation potential of other starch rich legumes and cereals. The application of the fractions obtained through DF and MWF is next to their composition also dependent on their functional properties. MWF fractions would be more relevant in food products, where solubility characteristics and a higher purity is required, i.e. the SPF in milk-like systems, high protein drinks, etc. The functional properties of the NSPF still have to be determined in future studies, especially as it can be expected that due to their difference in solubility, the SPF and NSPF also differ in their functional properties. Both the dry and mild wet fractionated starch fraction show good gelling behavior and could therefore

![Fig. 8.](image-url)
serve as ingredients to enrich or stabilize gels. The other components present in the fractions were shown to rather improve the functional properties of the fractions (Geerts, Strijbos, van der Padt and van der Goot, 2017c; Pelgrom, Boom and Schutyser, 2014b). A tool to predict the functional properties of the fractions based on their composition could help to close this gap. A key element of such a tool would be the inclusion of potential interactions between the components.

4. Conclusion

This study revealed that both dry and wet fractionation can be used to enrich and purify yellow peas, but adhesion between small and big particles occurring after structure break-up hinders full separation of yellow peas into their pure components. Enhanced separation in case of wet fractionation was explained by the fact that water addition solubilized fragments that were not fully disentangled and would by default in dry fractionation reach the coarse fraction. Thus, water addition provided an additional driving force that resulted in further disentanglement of fragments and consequently better separation. The results presented above show that a relation between the structure and purity of the fractions can be established and used to judge on the potential of the fractionation method.

CRediT authorship contribution statement

Anna Cacilie Möller: Formal analysis, Investigation, Data curation, Writing - original draft. Albert van der Padt: Conceptualization, Writing - review & editing, Supervision. Atze van der Goot: Conceptualization, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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References


