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Pea variety and dehulling effects on biorefinery efficiency and fraction quality

Busra Gultekin Subasi [a,b,**], Santanu Basu [c], Roger Andersson [c], Mehdi Abdollahi [a,*]

[a] Department of Life Sciences, Food and Nutrition Science, Chalmers University of Technology, SE-41296 Gothenburg, Sweden
[b] Centre for Innovative Food (CiFOOD), Department of Food Science, Aarhus University, Agro Food Park 48, Aarhus N 8200, Denmark
[c] Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-750 07 Uppsala, Sweden

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ABSTRACT

This study investigated the potential of yellow peas, with and without dehulling, as a biorefinery feedstock for sequentially producing multiple products such as fiber, starch, and proteins. It also investigated the effect of two different pea varieties, Ingrid and Clara, on the biorefinery efficiency and the quality of the recovered fractions by analyzing the composition and mass balance of four main fractions (hull, starch-rich, soluble dietary fiber/protein-rich, and main protein-rich fractions) generated during wet fractionation via the pH-shift method. Among dehulled samples (pea flour and crude protein isolate) Ingrid resulted in higher purity in specific fractions such as protein, starch, and dietary fiber than Clara while no difference was observed between the whole (with hull) and dehulled pea seed samples. Protein extraction efficiency, amino acid, and fatty acid profile did not show significant differences between whole and dehulled samples. Overall, these findings underscore the versatility of peas as a multiple-product biorefinery feedstock using the pH-shift method with the impact of variety being more pronounced on protein fraction quality, while preprocessing steps like dehulling play a decisive role in optimizing the composition and purity of fiber- and starch-rich fractions for targeted applications.

1. Introduction

Plant-based product development from pulses is a major global goal in the food sector as demand for environmentally sustainable food consumption is increasing. Yellow Pea (*Pisum sativum* L.) is an important legume with significant potential as a plant-based food source. This is due to peas' diverse compositional profile, broad geographical cultivation, and global production volume of approximately 14–15 million metric tons annually (Boukid et al., 2021). It is widely grown in regions spanning Europe, North America, Asia, and Oceania, making it a versatile crop adaptable to diverse climatic conditions and agricultural systems (Tulbek, Wang, & Hounjet, 2024). As a legume, it contributes to sustainable agriculture by fixing atmospheric nitrogen through symbiosis with Rhizobia, reducing the need for synthetic nitrogen fertilizers, and improving soil health (Mng'ong'o et al., 2023).

Pea contains approximately 20–25 % protein, 40–50 % starch, and 10–15 % dietary fiber, along with essential micronutrients, making it a valuable raw material for various applications (Daba & Morris, 2022). The protein fraction of pea, known for its favorable amino acid profile and functional properties, has gained extensive interest in food applications, particularly in the growing plant-based and hybrid product markets (Shanthakumar et al., 2022). Due to the higher value of the protein fraction and growing global demand for proteins, it has gained the highest attention while it represents a minor component of peas. However, to establish peas as a sustainable food source, it is essential to fully utilize other valuable fractions generated during pea fractionization for protein production (Tassoni et al., 2020). Here, embracing the biorefinery concept presents a promising approach to maximize the utilization of pea's diverse components, unlocking its full potential in food, feed, and bio-based applications.

A biorefinery is a processing system that converts biomass into multiple high-value products, including food, feed, bio-based materials, and even energy, with the goal of maximizing resource efficiency and minimizing waste. Successful examples include corn wet milling, which produces starch, sweeteners, ethanol, and feed, and sugarcane biorefineries, which generate sugar, ethanol, and bioelectricity (Sorita et al., 2020). These systems exemplify how the integrated valorization of all components can enhance economic viability and sustainability.

* Corresponding author.
** Corresponding author at: Centre for Innovative Food (CiFOOD), Department of Food Science, Aarhus University, Agro Food Park 48, Aarhus N 8200, Denmark.
E-mail addresses: bgs@food.au.dk (B.G. Subasi), khozaghi@chalmers.se (M. Abdollahi).

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While biorefineries for crops like corn and sugarcane are well established, the concept has not been fully expanded to peas, a promising source of protein, starch, and fiber. For example, the starch fraction, the largest component, can be processed into bio-based products such as bioplastics, bioethanol, or functional food ingredients. The fiber fraction, rich in prebiotic components, finds applications in food, feed, and bioenergy production. Leveraging a biorefinery approach for peas could offer similar benefits, enabling the production of multiple high-value fractions while addressing the growing demand for sustainable and versatile food systems.

The conventional method for pea fractionation which can be transformed to a biorefinery platform uses wet extraction by pH-shift method (Fig. 1) and generates different fractions rich in fibers (FR1 and FR3), starch (FR2), and protein (FR4). However, the full detailed profile and potential of each fraction generated during the pH-shift processing of pea, as a biorefinery feedstock, has not yet been systematically reported to the best of our knowledge.

In addition, finding the right cultivar is extremely important from a commercial point of view when establishing peas as a biorefinery feedstock. The composition of peas, in terms of protein content, amino acid profile, type of starch, their protein-fiber ratio, and distribution of soluble and insoluble fiber differs substantially among pea varieties (Daveby et al., 1993). There are also wide compositional profile differences between the pulse hull and cotyledon (Wood et al., 2017; Wood & Malcolmson, 2021). These compositional variations can substantially impact the pea fractionation efficiency as well as the quality of the pea constituents (protein, fiber, and starch) which needs careful investigation.

The present study aimed to evaluate (i) the potential of yellow pea (± dehulling) as a biorefinery feedstock for parallel or sequential production of multiple products, including fiber, starch, and proteins, by investigating the composition of four fractions emerging during its processing using the pH-shift method and their mass balance for further cascading (ii) the effect of two different pea varieties, Ingrid and Clara on the biorefinery process efficiency and quality of the recovered fractions.

2. Materials and methods

2.1. Materials

The yellow pea (*Pisum sativum* L.) varieties Ingrid and Clara were harvested in season 2019, in Svalöv, Sweden. Pea seeds were dried to 14 % moisture content by air blowing at 30 °C and stored in farmhouse facilities within plastic bags right after their harvesting by Lantmännen Lantbruk Sweden. Pea samples were transferred as 1 kg vacuumed packages to our laboratories and stored at 4 °C in the dark, until further use. All used chemicals were analytical grade and purchased from Sigma-Aldrich unless otherwise specified.

2.2. Pre-processing/fractionation of pea samples and protein isolation

The dehulling process was conducted by a Satake TM05 abrasive mill (Satake, Japan) (Möller et al., 2021), followed by aspiration. The removed hull fraction was named FR1 (Fig. 1). Dehulled pea splits and cotyledons were ground at 12,000 rpm in a Retsch ZM 200 ultra-centrifugal mill (Retsch, Germany) with a 500 µm screen (Gu et al., 2021). Pea flour was stored in the dark at 4 °C in 150 mL sealed plastic jars until further usage.

The wet fractionation was applied to pea flour for isolating crude pea protein. Pea flour and distilled water (1:15) were homogenized for 1 h at pH 9.0 using 2 M NaOH (Karaca et al., 2011). Starch-rich fraction of the pea flour was removed from the mixture by the first decantation (4000 x g, 20 min, 20 °C) and named FR2 (Fig. 1). The supernatant was collected, and dissolved proteins were precipitated at pH 4.5 using 2 M HCL at the end of 10 min incubation. Following the second decantation with the same parameters, residual supernatant FR3 (Fig. 1), which is the soluble fiber and protein-rich fraction, was freeze-dried and stored as a powder. The precipitated crude protein named FR4, the protein-rich fraction, was collected, neutralized, freeze-dried, and stored in zipped plastic bags in the dark at 4 °C for further analysis.

2.3. Protein extraction efficiency analysis

Various yield and recovery scores could be calculated during the protein isolation process applying specified equations that are described below, where H, S1, and S2 denote the soluble protein content of the

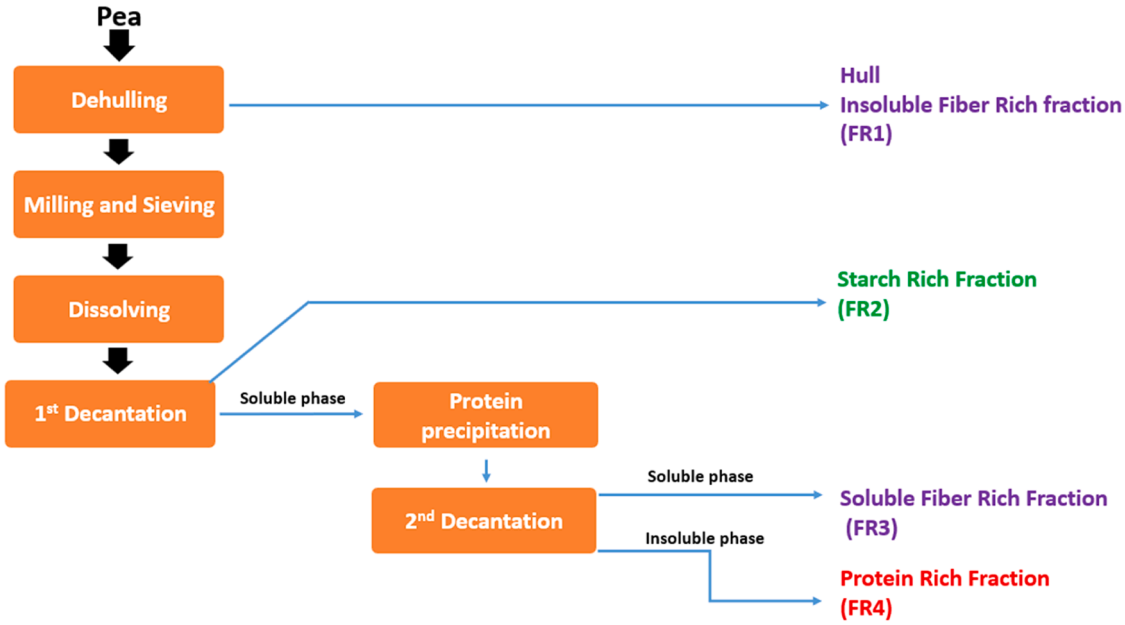

Fig. 1. Simplified pea biorefinery process flow diagram for simultaneous fiber, protein, and starch fractions recovery.

homogenate at selected pH, at supernatant after the first centrifugation, and second centrifugation, respectively (Sajib et al., 2023). Soluble protein content in the biomass was measured using an adapted version of the Lowry method (Lowry et al., 1951).

$$\text{Solubilization yield } (\%) = \frac{\text{Total Soluble protein content in S1}}{\text{Total Soluble protein content in homogenate}} \times 100 \quad (1)$$

$$\text{Precipitation yield } (\%) = \frac{\text{Total soluble protein content in the } (S1 - S2)}{\text{Total soluble protein content in S1}} \times 100 \quad (2)$$

$$\text{Total yield } (\%) = \frac{\text{Toral protein content in the } (\text{Homogenate} - S2)}{\text{Total protein content in the homgenate}} \times 100 \quad (3)$$

$$\text{Mass yield } (\%) = \frac{\text{Amount of dry protein isolate}}{\text{Amount of dry starting material}} \times 100 \quad (4)$$

$$\text{Protein recovery } (\%) = \frac{\text{Amount of final product x protein content}}{\text{Amount of starting material x protein content}} \times 100 \quad (5)$$

2.4. Chemical and nutritional properties

2.4.1. Proximate composition analysis

Moisture content was determined by gravimetric analysis based on the method AOAC 930.15, ash content was measured according to AOAC 942.05, and total protein content was determined following the Kjeldahl method based on AOAC 920.53 using 6.25 as the nitrogen conversion factor (AOAC, 2012). The total fat content was determined based on a modified method of Lee et al. (1996) to ensure the extraction is more suitable for lower amounts of fat-containing biomasses. Around 0.5 g sample was mixed with 20 ml ice-cold chloroform: methanol (2:1) solution with 0.1 % (w/v) BHT. At this stage, 50 µl C17 (1000 ppm) was added as an internal standard, and samples were exposed to mixing for 30 min. To clarify the slurry, 8 ml 0.5 % NaCl was added to each sample and then vortexed for 30 s. The final solution was centrifuged for 6 min at 3000x*g* (4 °C), and the bottom part was transferred for evaporation. Dried samples were then weighed to calculate the lipid content gravimetrically. The starch content in flour was determined by selective hydrolysis with thermostable α-amylase and amyloglucosidase as suggested by Åman et al. (1994).

2.4.2. Dietary fiber measurement

Dietary fiber and its components were quantified by the Uppsala method (Theander et al., 1995), subsequently modified by Andersson et al. (1999) for separate measurements of soluble and insoluble dietary fiber. Uronic acid residues were determined colorimetrically, and Klason lignin was determined gravimetrically as the acid-insoluble material. Total dietary fiber was calculated as the sum of sugar residues, uronic acid residues, and Klason lignin for the Ingrid and Clara whole seed samples. For the cotyledon and hull portion of the pea samples, we analyzed soluble and insoluble dietary fiber separately to find the differences.

2.4.5. Amino acid profiling

Acidic hydrolysis was conducted by adding 4 mL of 6 N HCl into freeze-dried and powdered samples then the acidic mixture was incubated at 110 °C for 24 h. The solutions were filtered through a syringe filter (0.45 µm) and clear samples were diluted before injection to LC/APCI-MS. Agilent 6120 quadrupole operating in SIM positive mode with Phenomenex 250 × 4.6 mm × 3 µm C18 column was used to detect the amino acid profile of precisely injected 2 µL sample via the LC-MS system (Agilent Technologies, Waldbron, Germany). Agilent Mass Hunter software was used to quantify the detected peak area against the AA standard mixture (ref no NCI0180. 20,088, Thermo Scientific Pierce, Rockford, IL, USA).

2.4.6. Fatty acid methyl esters (FAME) profiling

The previously extracted fats used to determine total fat content were then methylated by the addition of 1 mL toluene and 1 mL methanol:acetyl chloride (10 %, v/v) solution and exposed the incubation at 60 °C for 120 min Fredrikson et al. (2002). At the end of the incubation period, 1 mL Milli-Q water and 1.5 mL petroleum ether were added to the mixture and centrifuged (2500x*g* for 5 min). The methylated fatty acids containing upper parts were transferred for evaporation then redissolved in 500 µL isooctane for injection. Identification and quantification were conducted by GC–MS, Agilent 7890A GC system, and Agilent 5975C triple-axis MS detector (Agilent Technologies, Santa Clara, CA, USA). Following the injection of 1 µL into the system (15:1 split ratio), the separation of fatty acids was conducted by VF-WAX column (30 m × 250 µm × 0.25 µm) (Phenomenex, Torrance, CA, USA). The temperature ramp was 4 °C for between 100 °C and 205 °C while the ramp was 1 °C for between 205 °C and 230 °C with a holding time for 5 min and the inlet temperature was set at 275 °C. Helium was used as the carrier gas with a constant flow rate (1 mL/min). GLC 463 (Nu-Check Prep, Inc., Elysian, USA) was used as the standard mixture to identify the fatty acid methyl esters profiles.

2.5. Statistical analysis

The differences between the measurements with standard deviation were analyzed using a one-way analysis of variance test (ANOVA) ($p < 0.05$) followed by Tukey's test as a post-hoc analysis using the Statistical Package for the Social Science software (SPSS 22.0. SPSS Inc., Chicago. IL. USA). The number of observations was three to conduct for statistical analysis unless otherwise specified.

3. Results and discussion

3.1. Mass balance

In Fig. 2, the mass balance belonging to the biorefinery process is presented over one of the chosen varieties (Ingrid) as a proof of concept. Displaying the protein amount in each fraction in the different biorefinery steps is important for finding the selected pea variety`s upscaling potential as a suitable raw material for industrial protein isolation. Furthermore, protein quantities that each fraction had are also pointed out to highlight the protein loss and recovery potential of the applied wet fractionation technique.

The mass balance is of importance in terms of both industrial and technological perspectives since it was demonstrated that "at least" 42.6 % of the initial protein (initial protein content was 19.5 g/100 g in whole pea while the collected protein content was 11.2 g (FR4)) within the whole pea is being lost during the wet fractionation process. The content was emphasized with the term "at least" since a higher amount of loss should be expected at industrial-scale wet fractionation. During the preparation and wet fractionation processes, the utmost level of attention was paid at every level to prevent any loss thus it was aimed to obtain one of the highest possible protein recovery rates. This highly considerable amount of loss is quite concerning in terms of sustainability hence, novel alternative approaches are urgently required to prevent the protein loss during wet fractionation. The improvement strategies should focus on the FR1 (dehulling) and FR3 (second decantation) stages to avoid protein losses at any level since these fractions contain 85.8 %

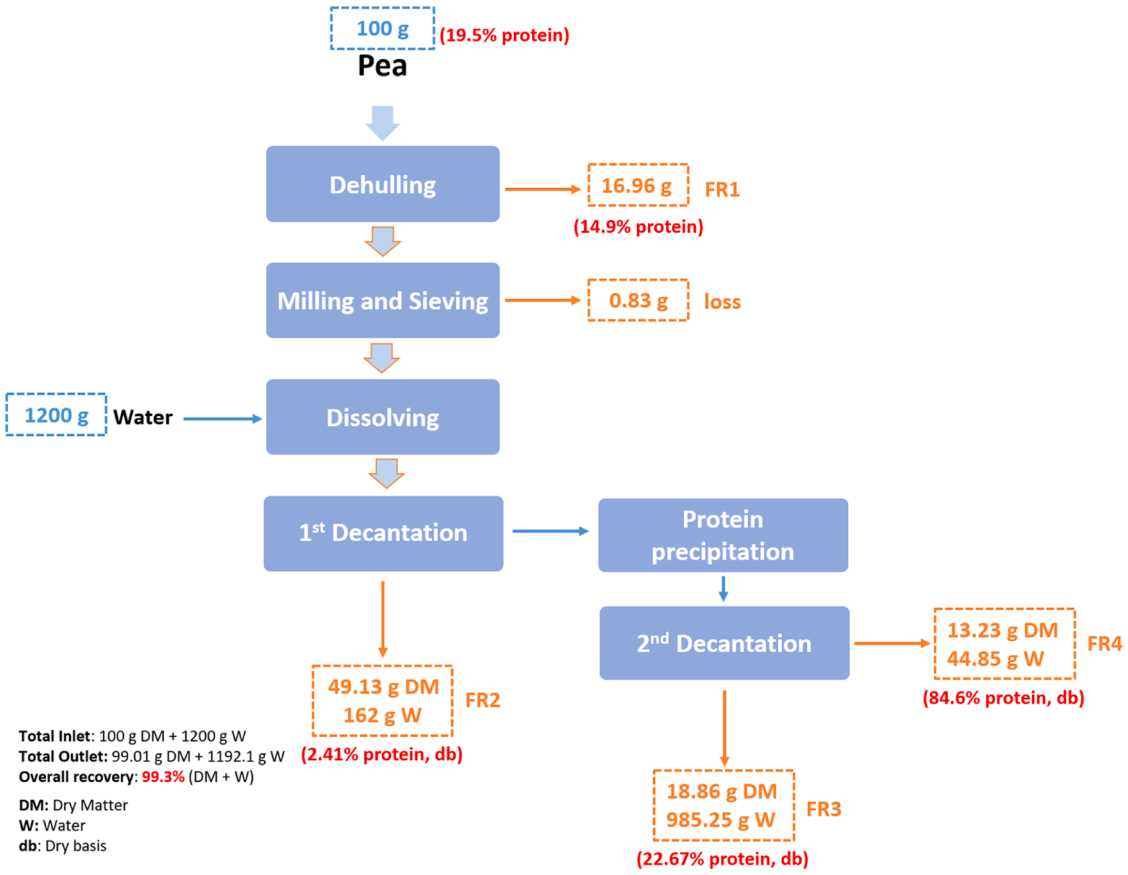

Fig. 2. Mass balance for the pea variety Ingrid during wet fractionation, focusing on protein quantity.

of the total lost protein (7.13 g out of 8.31 g). In addition, the FR2 possesses 14.2 % of the total lost protein (1.18 g out of 8.31 g). From another point of view, 30 % of the total lost protein can be recovered with an improved dehulling process while 70 % of the total lost protein can be recovered from overall decanted water fractions.

3.2. *Dietary fibers in the whole seed, cotyledon, and fraction 1 (FR1)*

The variation in dietary fiber in different portions (whole pea, cotyledon, and hull) of Ingrid and Clara pea varieties are presented in Table 1. The content of total dietary fiber in the whole seed was slightly higher for Clara (12.35 %) compared to Ingrid (11.65 %). Hulls (FR1) were obtained after dehulling of the whole pea seeds which constitute

Table 1
Dietary fibers in different parts (whole seed, cotyledon and hulls) of Ingrid and Clara pea samples (g/100 g dry matter).

Sample	Total dietary fibre	Klason lignin	Sugar residues							
			Uronic acid	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Ingrid whole	11.65 ± 1.52^{a}	0.04^{a}	0.92^{c}	0.17^{b}	NF	3.01^{a}	1.15^{b}	0.23^{b}	0.54^{b}	5.77^{b}
Clara whole	12.35 ± 0.46^{a}	0.12^{a}	0.99^{c}	0.21^{b}	NF	2.93^{a}	1.18^{b}	0.26^{b}	0.59^{b}	6.05^{b}
	Soluble dietary fibre	**Klason lignin**	**Soluble uronic acid**	**Soluble Rha**	**Soluble Fuc**	**Soluble Ara**	**Soluble Xyl**	**Soluble Man**	**Soluble Gal**	**Soluble Glc**
Ingrid cotyledon	0.48 ± 0.01^{b}	NF	0.18^{d}	NF	NF	0.13^{b}	0.0 ^{d}	0.05^{c}	0.04 ^{c}	0.06^{d}
Ingrid hull	3.71 ± 0.22^{c}	NF	2.38^{b}	0.14^{b}	0.01^{c}	0.48^{b}	0.32^{cd}	0.13^{c}	0.18^{d}	0.07^{d}
Clara cotyledon	0.48 ± 0.04^{b}	NF	0.20^{d}	NF	NF	0.11^{b}	0.02^{d}	0.05^{c}	0.04 ^{c}	0.06^{d}
Clara hull	3.70 ± 0.12^{c}	NF	2.49^{b}	0.16^{b}	0.01^{c}	0.31^{b}	0.27^{cd}	0.15^{c}	0.25^{d}	0.06^{d}
	Insoluble dietary fibre	**Klason lignin**	**Insoluble uronic acid**	**Insoluble Rha**	**Insoluble Fuc**	**Insoluble Ara**	**Insoluble Xyl**	**Insoluble Man**	**Insoluble Gal**	**Insoluble Glc**
Ingrid cotyledon	7.88 ± 0.32^{d}	0.23^{a}	1.18^{c}	0.17^{b}	0.04^{b}	3.25^{a}	0.35^{c}	0.28^{b}	0.52^{b}	1.86^{c}
Ingrid hull	75.87 ± 0.46^{e}	0.60^{b}	10.71^{a}	1.12^{a}	0.17^{a}	3.34^{a}	9.84^{a}	0.39^{a}	1.04^{a}	48.65^{a}
Clara cotyledon	7.07 ± 0.39^{d}	0.11^{a}	1.14^{c}	0.12^{b}	0.04^{b}	2.83^{a}	0.31 ^{c}	0.24^{b}	0.52^{b}	1.77^{c}
Clara hull	77.43 ± 0.47^{e}	0.40^{b}	10.88^{a}	1.13^{a}	0.19^{a}	1.67^{a}	10.35^{a}	0.33^{a}	1.09 ^{a}	51.38^{a}

Values are means of the analysis of two samples. Superscript letters represent significant difference for different samples ($p < 0.05$). Standard deviation values are only shown for total dietary fibers to increase readability of the table. Analysis of fructans was not performed for these samples. Sol: Soluble and Insol: Insoluble, NF: not found. The abbreviation of sugar residues, Rha: Rhamnose, Fuc: Fucose, Ara: Arabinose, Xyl: xylose, Man: Manose, Gal: galactose, Glc: Glucose.

around 17 % of the initial mass (Fig. 2). Total soluble and insoluble dietary fiber content were significantly higher in the pea hulls (78–80 %) compared to the cotyledons (7.5–8.5 %). Scientific literature also reports that pea hulls are rich in cellulose, xyloglucan, pectin (uronic acid derivatives), and lignin. (Daveby et al., 1993; Ralet, Della Valle et al., 1993, 1993). Furthermore, the pea hulls contain around 15 % crude protein (Fig. 2). The protein content in the Ingrid pea hull is slightly higher than some reported data in the literature such as around 13 % hull protein content of a Canadian pea variety (Ramirez et al., 2021). The difference in the hull protein ratios might be due to the efficiency of the dehulling process since tiny cotyledon pieces might contaminate the hull fractions during the abrasive milling.

The dietary fiber compositional profiles of whole seed Clara and Ingrid were very similar. The compositional profile of Clara whole seed showed a slightly higher amount of Klason lignin, uronic acid, and total sugar residues compared to Ingrid whole seed (Table 1). There were no significant differences between Clara and Ingrid fractions. Insoluble fiber dominated the total dietary fiber content for both the cotyledon and hull portions in both the pea varieties. Uronic acid, xylose, and glucose residues were found to be significantly higher in the hulls compared to cotyledons. Glucose residues come from cellulose and xyloglucan, while uronic acid residues come from pectic substances (Daveby et al., 1993; Ralet, Della Valle et al., 1993). Other important sugar residues found in the insoluble dietary fiber fractions were arabinose and xylose. Insoluble arabinose residues were not significantly different in the cotyledon and hulls or between the pea varieties (Ingrid and Clara). For the soluble dietary fiber profile, uronic acid content was dominating and was found significantly higher in the hulls compared to cotyledons. Both Ingrid and Clara had similar soluble uronic acid content, and it was expected to be coming from the pectic components present in the pea seeds (Daveby et al., 1993; Ralet, Della Valle et al., 1993). Soluble rhamnose, xylose, and fucose residues were either not found or almost negligible in the cotyledons for the two pea varieties. Based on the sugar residue analysis, we can infer that Ingrid and Clara cotyledon, and hulls comprise cellulose (glucose), xyloglucan (xylose, glucose, galactose), arabinan (arabinose) and pectin (arabinose, rhamnose, uronic acid, galactose) in distinctively different amount in the whole seed, hull, and cotyledons. The maximum cellulose and pectin content of the pea seeds comes from the pea hull fractions for both the pea cultivars. Soluble (extractable) and insoluble (unextractable) dietary fibers and their relative distributions play many important roles in our bodies. Broadly these roles fall into two categories: physiological and technological. Physiological roles are nutrient digestion and uptake, colonic fermentation, etc. Technological roles are bulk structuring, water holding, viscosity, gel-forming, and residence time in the digestive tract. The soluble dietary fibers include compounds such as hemicellulose (e.g. xyloglucans, galactomannans mixed-linkage glucans), pectin, gums, and mucilage. On the other hand, cellulose, lignin, and resistant starch are considered insoluble dietary fibers. However, depending on the plant source, and processing operation conditions, many of these polymer types can be either soluble or insoluble. All of these fibers differ in their monosaccharide components and the glycosidic linkages. Therefore, information and knowledge about soluble and insoluble dietary fraction proportions in pulse seed flour like Swedish peas can help in food product development strategies.

Clara (51.4 % and 51.7 %, dry basis-db) also showed higher starch content in the whole seed and cotyledon, respectively, compared to Ingrid which were 47.6 % and 49.8 % (db), respectively. Both, Ingrid and Clara pea varieties had only 1.1 % and 0.5 % starch content (db) in the hulls respectively.

The total starch and dietary fiber content of the pea samples and their relative distribution in the hull and cotyledons are dependent on various factors like genotype and environmental conditions (Wood et al., 2018). The protein extraction efficiency during the biorefinery process was influenced by the amounts of starch and fibers. One can also speculate that their interactions with protein and phenolics may have played a role in the yield of different components in the four fractions obtained from the biorefinery process (Rashwan et al., 2023).

Altogether, the dietary profile of the seed and their distribution in two fractions of hulls and cotyledon showed that there is high potential for further processing and application of pea hulls as a dietary fiber source, especially non-soluble fibers which are dominant in the hull and their quantity and composition was negligibly affected by pea variety. It also revealed the importance of dehulling pea seeds as a simple step for the more efficient valorization of this fraction.

3.3. Starch-rich fraction (FR2)

The bulk of the pea components during the wet fractionation using the pH-shift method ends up in fraction 2 (FR2), which is the starch-rich fraction and has low solubility in the alkaline conditions used for the extraction of proteins. The total amount of starch content obtained from the fractionation process in FR2 with whole seed flour (Ingrid (61.55 %), Clara (58.92 %)) was significantly ($p < 0.05$) lower than the cotyledon flour (Ingrid (68.98 %), Clara (70.45 %)). This higher starch yield was due to the removal of the hull before the extraction and starting of the biorefinery process with a biomass having less insoluble dietary fiber. The total dietary fiber amount found in the FR2 was significantly higher for both Ingrid and Clara whole seed flour based biorefinery process compared to the cotyledon flour-based process. Uronic acid, arabinose, and glucose residues were the dominant types of dietary fibers found in the FR2 or whole seed FR2 (WFR2) fractions (Table 2). This also highlights the importance of dehulling for achieving a FR2 fraction if targeted as a feedstock for production of pea starch. Uronic acid, xylose and glucose residues were present in much higher amounts in WFR2 for both Ingrid and Clara compared to FR2. No marked differences were observed for arabinose residues in both FR2 and WFR2 for Ingrid and Clara. Therefore, starch-rich fractions (both FR2 and WFR2) had high dietary fiber amounts (Ingrid: 11.27 and 20.89 %, Clara: 10.49 and 19.76 %), respectively. The crude protein content of the FR2 for the variety Ingrid is determined as 2.4 %, db (Fig. 2).

According to the results obtained, while FR2 shows significant potential as a starch source in pea biorefinery, the initial preprocessing, such as dehulling, plays a more critical role than the choice of variety in determining its composition and suitability for specific applications in food and industrial sectors.

3.4. Soluble fiber-rich fraction (FR3)

Fraction 3, is the processing water at the isoelectric point of proteins, containing proteins remaining soluble at this condition and soluble dietary fiber which was a diluted fraction being collected from the pea biorefinery process. FR3 is generally obtained like a very dilute slurry where the total solid content varied between 1–1.5 % for the whole and dehulled samples of varieties Ingrid and Clara. From this dilute slurry, it is not feasible for complete drying to recover the soluble fiber and protein content due to the very high water content and therefore is generally discarded during plant-protein isolation. However, this fraction possesses around 23 % protein content (db), which is mainly albumin (Fig. 2). Despite the lack of industrial recovery feasibility of FR3, it has an economic potential due to the significant functionality behaviors (such as foam-producing and stabilizing abilities) of the albumins (Yang & Sagis, 2021). Further research is required to find an optimum approach for the recovery and utilization of this soluble fiber and albumin-rich fraction.

3.5. Protein-rich fraction (FR4)

Fraction 4 is the protein-rich fraction obtained from the pea biorefinery process. The success of any biorefinery process with the primary target of protein extraction particularly from plant-based sources, should achieve maximum protein recovery as well as minimal

Table 2
Dietary fiber content (g/100 g dry matter) in the different fractions obtained during the biorefinery process for Ingrid and Clara pea samples.

Cultivar	Code	Fraction name	Total dietary fibre	Klason lignin	Uronic acid	Sugar residues						
						Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Ingrid	FR2	IngridFR2	11.27 ± 0.12 ^{b}	0.08^{ab}	1.74 ^{b}	0.23 ^{b}	0.05 ^{b}	5.13 ^{a}	0.50 ^{b}	0.17 ^{ab}	0.66 ^{b}	2.72 ^{b}
Ingrid	WFR2	IngridWholeFR2	20.89 ± 0.28 ^{a}	0.18 ^{a}	3.09 ^{a}	0.36 ^{a}	0.07 ^{a}	5.16 ^{a}	1.75 ^{a}	0.23 ^{a}	0.75 ^{b}	9.31 ^{a}
Ingrid	FR4	IngridFR4	2.79 ± 0.21 ^{c}	0.11 ^{ab}	0.22 ^{c}	NF	NF	0.35 ^{b}	0.07 ^{d}	0.25 ^{ab}	1.27 ^{a}	0.53 ^{c}
Ingrid	WFR4	IngridWholeFR4	4.29 ± 0.41 ^{c}	0.01 ^{c}	0.26 ^{c}	NF	NF	0.37 ^{b}	0.10 ^{c}	0.16 ^{b}	2.44 ^{a}	0.96 ^{c}
Clara	FR2	ClaraFR2	10.49 ± 0.09 ^{b}	0.05 ^{ab}	1.56 ^{b}	0.21 ^{b}	0.05 ^{b}	4.53 ^{a}	0.46 ^{b}	0.17 ^{ab}	0.67 ^{b}	2.79 ^{b}
Clara	WFR2	ClaraWholeFR2	19.76 ± 0.14 ^{a}	0.01 ^{a}	2.97 ^{a}	0.35 ^{a}	0.07 ^{a}	4.14 ^{a}	1.74 ^{a}	0.21 ^{a}	0.73 ^{b}	9.44 ^{a}
Clara	FR4	ClaraFR4	3.49 ± 0.32 ^{c}	NF	0.17 ^{c}	NF	NF	0.29 ^{b}	0.03 ^{d}	0.15 ^{ab}	2.01 ^{a}	0.84 ^{c}
Clara	WFR4	ClaraWholeFR4	2.81 ± 0.01 ^{c}	NF	0.23 ^{c}	NF	NF	0.32 ^{b}	0.11 ^{c}	0.15 ^{b}	1.47 ^{a}	0.53 ^{c}

Values are means of the analysis of two samples. Superscript letters represent significant differences for different samples ($p < 0.05$). NF=not found.

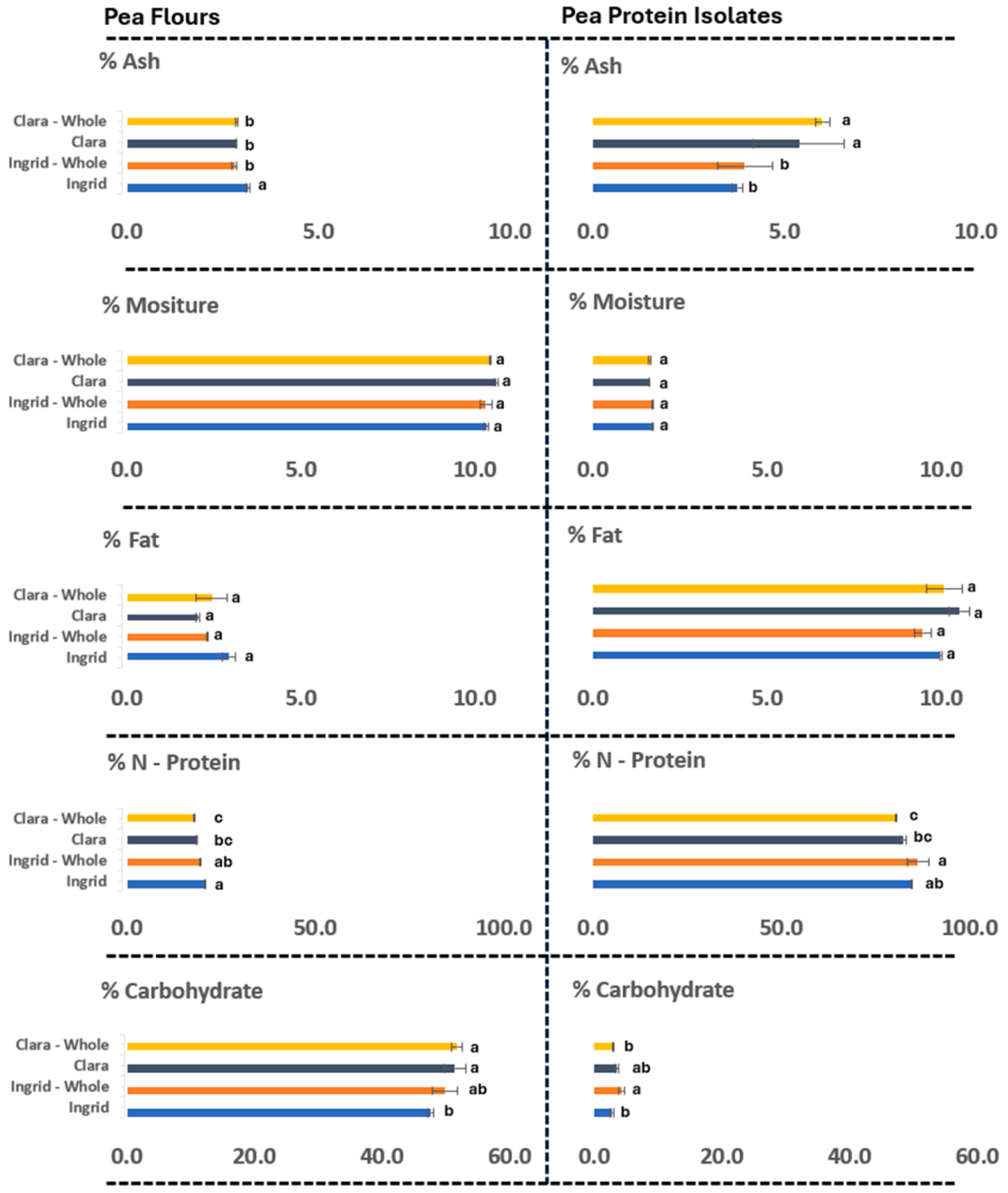

Fig. 3. Proximate compositions of pea flours and crude protein isolates of whole and dehulled Ingrid and Clara pea varieties. Different letters represent significant differences ($p < 0.05$) within groups. $n = 3$.

carbohydrate/fiber contents in this fraction for both dehulled and whole seed samples. On the other hand, the characteristic properties of the isolated proteins from different pea varieties in the form of dehulled and whole seed samples are of crucial importance.

3.5.1. Proximate composition of pea flours and protein isolates

Pea flours of dehulled and whole seeds of varieties Ingrid and Clara, together with their crude protein isolates were analyzed for the proximate compositions which can be seen in Fig. 3. Focusing on ash content, dehulled Ingrid flour had a higher value than all other flours while for the crude protein isolates, Clara (both dehulled and whole seed isolates) had the significantly higher ash content than the Ingrid isolates. There was not any significant difference in moisture and crude fat content between the whole/dehulled seed flour samples as well as in the pea varieties. However, a 4-fold reduction in moisture content and around 2.5-fold increase in fat content were observed following the protein isolation yet no significant difference was detected between the varieties.

Ingrid pea flour had a higher protein content than variety Clara and the presence of hulls did not yield any significant difference compared to dehulled flours for both varieties. The same situation was observed for the protein isolates as well. Overall, protein content is up-concentrated at least 4-fold once isolated from the whole pea seed. In the literature there is not a recent study comparing the protein (and proximate composition) content difference between dehulled and whole pea flours/isolates however, the proximate composition of 12 different European-based pea varieties was comparable for some components with Ingrid and Clara flour and protein isolates. In addition, the protein content of the Swedish pea varieties' flours (average of 19 %) was lower than the European pea flours, with an average ratio of 24 % (Arteaga et al., 2021). In another study, different types of whole peas were used to isolate pea protein, and similarly, protein content in the crude isolate was consistent with the varieties Ingrid and Clara. Nonetheless, the protein content in those six different Serbian pea flours was higher than in the Swedish pea varieties (Barac et al., 2010). A similar trend could be observed against Canadian pea varieties but this time, the protein content in the isolates was also higher (above 90 %) than that of Swedish pea varieties together with way lower crude fat content in the flours (0.3 %) and isolates (around 2.5 %) whereas Ingrid and Clara have around 3.5 % crude fat in flours and around 10 % fat in the protein isolates (Lam et al., 2017). It should be noted that the Canadian study was conducted with de-fatted pea flour and isolate samples.

Total carbohydrate for the flour samples represents the total starch and dietary fiber content, and for the protein isolates samples, it comes from mainly residual dietary fibers (Fig. 3). Ingrid (dehulled and whole) variety had the lower starch content in flour form but for the protein isolates, not a significant difference was detected between the varieties. Similarly, total starch and dietary fiber content results were found compatible with the literature data as explained earlier (Arteaga et al., 2021; Barac et al., 2010; Lam et al., 2017).

3.5.2. Protein extraction efficiency analysis

Protein extraction efficiency calculations are based on protein solubilization yield, precipitation yield, total yield, mass yield and total protein recovery (Sajib et al., 2023) (Fig. 4). Mass yield and total protein recovery ratios were determined as the same for all samples without any significant differences between them. Regarding the solubilization yield, there was not any significant difference between pea varieties, however the values were lower when whole seed flours were used. Ingrid variety presented a higher protein precipitation yield and total yield than Clara variety however their whole flour versions had the lowest values for these scores. Consequently, protein extraction efficiencies of different varieties and the presence of the hull fractions in the biomasses are not differentiated by the conducted wet fractionation process despite their slight differences in protein solubility and precipitation ratios. This might indicate the robustness of the applied wet fractionation process ending up with similar mass yield and protein recovery scores. Protein recovery (or extractability) of Swedish pea varieties was found significantly higher than the Serbian pea varieties where it was around 66 % for Ingrid and Clara but was around 40 % (at pH 8.0) for Serbian varieties, on average (Barac et al., 2010). On the other hand, the protein recovery of a Canadian pea variety was declared as 56–70 % in a pH interval-extraction study, which is more consistent with the Swedish varieties (Hansen et al., 2022).

3.5.3. Amino acid and fatty acid profiles

Amino acid and fatty acid profiles of any protein isolate are as critical as the quantity of the isolated protein content in terms of their

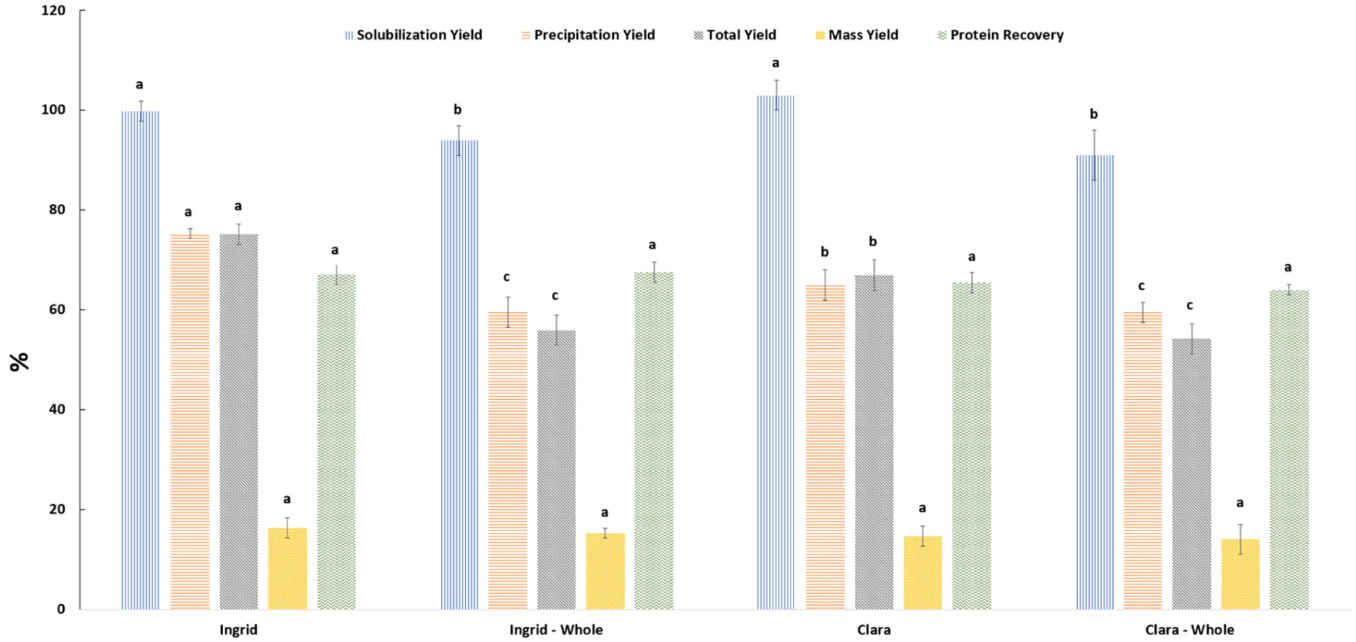

Fig. 4. Protein extraction efficiency analysis of whole and dehulled Ingrid and Clara pea varieties. Different letters represent significant differences ($p < 0.05$) within groups, $n = 3$.

nutritional and technological characteristics (Lu et al., 2020). Assessment of pea flours and protein isolates for their nutritional scores, together with digestion and amino acid bioavailability, are not in the scope of this work however, profile differences between two different varieties, absence/presence of hull fraction in the biomass towards the protein isolation was evaluated.

Amino acid profiles of the flour and protein isolate samples of varieties Ingrid and Clara are presented in Fig. 5. At first glimpse, the general profile pattern seems unchanged for all samples (both varieties of whole/dehulled). Only histidine, cysteine, and asparagine concentrations were increased due to the up-concentration and induced a slight change in the spider-web pattern. Dehulled Ingrid flour and protein isolates have the highest total amino acid content rather than the other samples. Considering the pea flours, the presence/absence of hull fraction did not affect the amino acid quantities and Ingrid samples had around 16 % higher total amino acid content than Clara samples. Among the protein isolates, Ingrid (whole) and Clara (whole and dehulled) samples had statistically the same total amino acid content while Ingrid dehulled protein isolates were 15 % higher than those. It is not feasible to make a one-by-one comparison, but it should be declared that the contents of individual amino acids of Swedish pea varieties are in line with the literature data such as the profiles of Canadian pea varieties, with some inherent fluctuations (Wang et al., 2020). However, the contents of the individual amino acids of Swedish pea variety flours were 1 order of magnitude lower than some United Kingdom pea varieties (Millar et al., 2019). This difference could be due to the variance between the used analytical tools/methods. The amino acid content is increased 1 order of magnitude in protein isolates compared to the flour samples (Fig. 5) which is in line with some Canadian pea varieties as the same amount of up-concentration was observed when they were

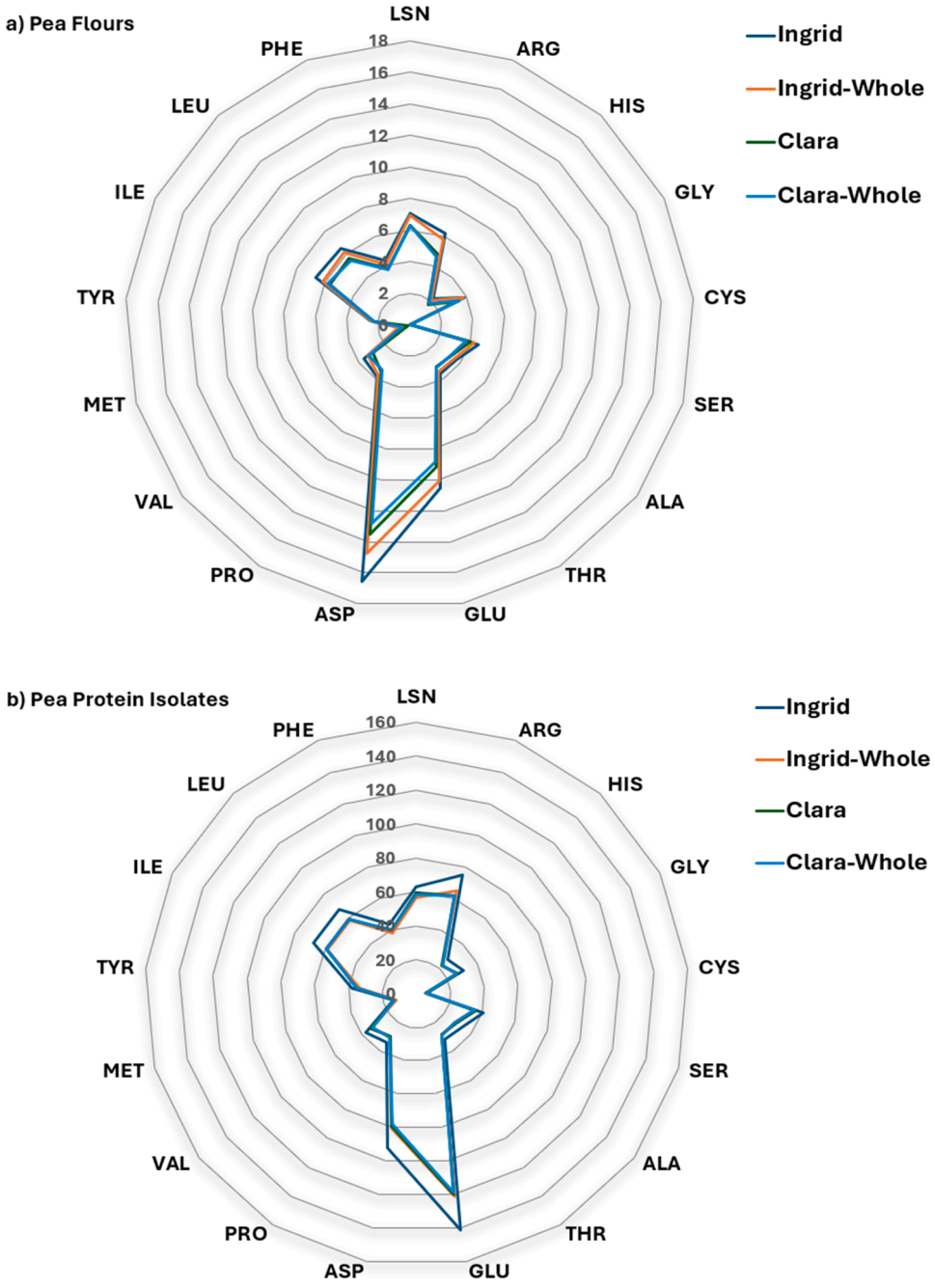

Fig. 5. Amino acid profile of whole grain and dehulled pea flours (a) and protein isolates (b) of varieties Ingrid and Clara (u/1000 u).

exposed to the protein isolation process (Chigwedere et al., 2023).

Fatty acid methyl esters distributions of all samples are presented in Table 3. The presence and/or absence of hull fraction within the flour and protein isolate did not yield any significant difference for the individual fatty acids. Furthermore, a significant difference between the varieties was not detected with some exceptions such as oleic, linoleic, and linolenic acids. Despite the ratios of fatty acids in the bulk like total saturated, and mono/polyunsaturated fatty acids remained statistically the same, their total content increased from around 60 ug/mL to 420 ug/mL once the protein fraction was isolated from the pea flour. It should be noted that the total fat content for all samples increased around 4–5 times in the final protein isolates while the total fatty acid concentration increased around 7–8 times compared to that of the flour samples. The obtained fatty acid profile (in terms of g/100 g) for the Swedish pea varieties was comparable with the literate data. The content of fatty acids for Canadian pea varieties corresponds to the result for Swedish pea varieties, particularly with oleic, linoleic, and linolenic acids (Padhi et al., 2017). Consequently, the presence/absence of hull for the pea seed had not have a significant effect on fatty acid distributions in both pea flour and crude pea protein isolates. Furthermore, varieties Ingrid and Clara did not differ from each other in terms of fatty acid distributions.

3.5.4. *Carbohydrate profiling*

Total dietary fibers present in the protein fraction (WFR4) obtained from Ingrid and Clara whole seeds were 4.29 and 2.81 % respectively (Table 2). However, dietary fiber residues in the FR4 were 2.79 and 3.49 % respectively for Ingrid and Clara, when the extraction process was

Table 3
Fatty acids composition of flour and protein isolate Ingrid and Clara pea samples (% of total fatty acid methyl esters).

	Pea Flours				Pea Protein Isolates			
Fatty acid methyl esters	**I**	**I - W**	**C**	**C - W**	**I**	**I - W**	**C**	**C - W**
Nonanoic acid (C9)		0.03				0.02		
Capric acid (C10)		0.07				0.03		
Undecanoic acid (C11)		0.04				0.02		
10-Undecenoic acid (C11:1)		0.04				0.02		
Lauric acid (C12)		0.17				0.90		
cis-5-Dodecenoic acid (C12:1)		0.10				0.05		
Tridecanoic acid (C13)		0.07				0.04		
C13:1		0.07				0.04		
Myristic acid (C14)	0.39	0.40	0.48	0.49	0.29	0.30	0.39	0.40
Methyl myristoleate (C14:1n5)		0.13				0.07		
Pentadecanoic acid (C15)		0.20				0.18		
C15:1 N5 cis		0.07				0.04		
Palmitic acid (C16)	13.49	13.51	14.14	14.06	13.53	13.61	14.71	14.69
Palmitoleic acid (C16:1n7)		0.24				0.15		
Margaric acid (C17)	0.32	0.34	0.30	0.32	0.27	0.28	0.24	0.24
cis-10-Heptadecenoic acid (C17:1n7)		0.16				0.08		
Stearic acid (C18)	3.84	3.78	3.91	3.90	3.54	3.57	3.85	3.77
Oleic acid (C18:1n9)	21.43	21.66	18.59	18.86	22.46	22.87	19.95	19.98
Vaccenic acid (C18:1n11)	0.28	0.34	0.33	0.31	0.39	0.39	0.35	0.36
Linoleic acid (C18:2n6)-cis	46.94	46.26	45.73	45.47	48.18	47.53	46.08	46.32
Gamma Linolenic acid (C18:3n6)		0.09				0.05		
cis-10-Nonadecenoic acid (C19:1n9)		0.11				0.07		
Linolenic acid (C18:3n3)	8.11	8.17	11.31	11.36	8.07	8.14	11.10	11.42
Arachidic acid (C20)	0.57	0.59	0.65	0.65	0.46	0.48	0.55	0.55
11-Eicosanoic acid (C20:1n11)		0.18			0.11	0.11	0.09	0.09
Eicosadienoic acid (C20:2n6)		0.25				0.17		
Eicosatrienoic acid - trans (C20:3n6)		0.09				0.05		
Arachidonic acid (C20:4n6)		0.11				0.06		
Eicosatrienoic acid - cis (C20:3n3)		0.10				0.06		
Eicosapentaenoic acid (C20:5n3)		0.25				0.13		
Docosanoic acid (C22)	0.35	0.37	0.35	0.40	0.25	0.28	0.30	0.31
Docosenoic acid (C22:1n9)	0.65	0.70	0.66	0.68	0.37	0.38	0.41	0.35
Docasadienoic acid (C22:2n6)		0.16				0.08		
Docosatetraenoic acid (C22:4n6)		0.16				0.08		
Docosatrienoic acid (C22:3n3)		0.18				0.75		
Docosapentaenoate (C22:5n3)		0.37				0.20		
Docosahexaenoic acid (C22:6n3)		0.53			0.30	0.31	0.27	0.28
∑ SFA	19.54	18.98	19.83	19.82	19.52	18.51	20.03	19.96
∑ UFA	80.81	77.13	76.62	76.67	82.03	79.73	78.26	78.80
∑ MUFA	23.47	22.69	19.58	19.85	23.84	23.75	20.81	20.78
∑ PUFA	57.34	54.43	57.04	56.82	58.19	55.98	57.45	58.02
∑ (ug/mL)	61.69 [b]	59.27 [b]	62.25 [b]	61.15 [b]	445.12 [a]	424.06 [a]	477.78 [a]	479.19 [a]

SFA: saturated fatty acids.
UFA: unsaturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids.
I: Ingrid
C: Clara
W: Whole
Values are the average of the analysis of two samples. Standard deviation values are not shown since the magnitude is on the third digit after the comma.
Small letters representing the statistical difference are only shown for total fatty acid content since the ratios did not change for the individual compounds between the samples. Avoiding standard deviation and statistical insignificance aimed to avoid redundancy.
One common numerical value in one row but for multiple columns represents the mutual columns have the same numerical value. It is applied to reduce repeating statements.

carried out with only cotyledon flour after the removal of the hulls from the whole seeds. Interestingly, Clara variety showed an unexpected behavior as the dietary fiber residues were higher in FR4 when the extraction was done with cotyledon fiber only. As the hulls of pea seeds contain higher dietary fibers, this result is rather surprising and interesting. Our measurements are just an average of two, it may be better in the future to check the data consistency by carrying out the biorefinery process in optimized conditions several times. Klason lignin, a phenolic constituent, which was found in the whole seed, cotyledon, and hulls, was not found in the protein fractions (FR4 and WFR4) for Clara. The major sugar residues found in the protein fractions (WFR4 and FR4) obtained from whole seed as well as cotyledon flours were galactose, glucose, and arabinose for both Ingrid and Clara pea varieties. Moreover, protein-rich fractions (FR4 or WFR4) also showed a significantly higher galactose residue content compared to starch-rich fractions (FR2 and WFR2) for both the pea cultivars. It is pertinent to mention here that peas are a rich source of free and bound galactose, which is present in the form of glycoprotein or glycolipids or pectin, etc. (Acosta & Gross, 1995). Therefore, it may be speculated that some of the galactose residues originate from glycoproteins which end up in the FR4 or WFR4. Also, the pH-shift processing for protein extraction may be completely in sync with the solubility of the galactose-containing polymers during the biorefinery process and resulted in higher galactose residues in the protein-rich fraction.

4. Conclusion

The potential of two different Swedish pea varieties, Ingrid and Clara as whole and dehulled seeds, as feedstock for multiple product biorefinery using the classic wet fractionation process (pH-shift process) as well as the protein and fiber quality of the major emerging fractions were investigated. As the first fraction optionally emerging before the wet fractionation process, pea hulls showed a high concentration of dietary fibers (78–80 %) making it an ideal candidate for either direct application or further downstream processing as a fiber source, especially non-soluble fibers. The second fraction emerging during the first decanting step of the pH-shift process showed significant potential as a starch source in pea biorefinery where the initial dehulling showed a more critical role than the choice of variety in determining its composition and suitability for specific applications. The pea varieties had a significant effect on the recovery, composition and nutritional quality of the third protein-rich fraction as the typical product targeted in the conventional single-product approach. On the other hand, we did not find an absolute need for the dehulling process to obtain a high-quality protein product. However, it might be obligatory to achieve another fiber-rich product but also obtain a higher purity in the starch fraction before its subsequent downstream processing. Further, studies on the fourth emerging fraction (the remaining processing water) which is highly diluted are needed. Overall, this study highlights the versatility of peas as a promising biorefinery feedstock, enabling the production of diverse products such as dietary fiber, starch, and high-quality protein, with the impact of variety being more pronounced on protein fraction quality, while preprocessing steps like dehulling play a decisive role in optimizing the composition and purity of fiber- and starch-rich fractions for targeted applications.

Ethical statement

We hereby would like to declare that no studies in humans and animals were conducted for the manuscript titled “Pea biorefinery: Impact of pea variety on fractionation efficiency and fractions quality” for the consideration of publication in *Applied Food Research* as a Research Article.

CRediT authorship contribution statement

Busra Gultekin Subasi: Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Santanu Basu:** Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Roger Andersson:** Writing – review & editing. **Mehdi Abdollahi:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mehdi Abdollahi reports financial support was provided by Sweden’s Innovation Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Acosta, P. B., & Gross, K. C. (1995). Hidden sources of galactose in the environment. *European Journal of Pediatrics, 154*(2), S87–S92. https://doi.org/10.1007/BF02143811

Åman, P., Westerlund, E., & Theander, O. (1994). Determination of starch using a thermostable α-amylase. methods in carbohydrate chemistry. In J. N. BeMiller, D. J. Manners, & R. J. Sturgeon (Eds.), *Enzymic methods: X. Enzymic methods* (pp. 111–115). John Wiley & Sons, Wiley Online Library.

Andersson, A. A. M., Merker, A., Nilsson, P., Sørensen, H., & Åman, P. (1999). Chemical composition of the potential new oilseed crops Barbarea vulgaris, Barbarea verna and Lepidium campestre. *Journal of the Science of Food and Agriculture, 79*(2), 179–186. https://doi.org/10.1002/(SICI)1097-0010(199902)79:2<179::AIDJSFA163>3.0.CO;2-N

AOAC. (2012). *AOAC official methods of analysis* (18th ed.). AOAC International.

Arteaga, V. G., Kraus, S., Schott, M., Muranyi, I., Schweiggert-Weisz, U., & Eisner, P. (2021). Screening of twelve pea (Pisum sativum l.) cultivars and their isolates focusing on the protein characterization, functionality, and sensory profiles. *Foods (Basel, Switzerland), 10*(4). https://doi.org/10.3390/foods10040758

Barac, M., Cabrilo, S., Pesic, M., Stanojevic, S., Zilic, S., Macej, O., & Ristic, N. (2010). Profile and functional properties of seed proteins from six pea (Pisum sativum) genotypes. *International Journal of Molecular Sciences, 11*(12), 4973–4990. https://doi.org/10.3390/ijms11124973

Boukid, F., Rosell, C. M., & Castellari, M. (2021). Pea protein ingredients: A mainstream ingredient to (re)formulate innovative foods and beverages. *Trends in Food Science & Technology, 110*, 729–742. https://doi.org/10.1016/j.tifs.2021.02.040

Chigwedere, C. M., Stone, A., Konieczny, D., Lindsay, D., Huang, S., Glahn, R., House, J. D., Warkentin, T. D., & Nickerson, M. (2023). Examination of the functional properties, protein quality, and iron bioavailability of low-phytate pea protein ingredients. *European Food Research and Technology*. https://doi.org/10.1007/s00217-023-04232-x

Daba, S. D., & Morris, C. F. (2022). Pea proteins: Variation, composition, genetics, and functional properties. *Cereal Chemistry, 99*(1), 8–20. https://doi.org/10.1002/cche.10439

Daveby, Y. D., Abrahamsson, M., & Åman, P. (1993). Changes in chemical composition during development of three different types of peas. *Journal of the Science of Food and Agriculture, 63*(1), 21–28. https://doi.org/10.1002/jsfa.2740630105

Fredrikson, M., Carlsson, N.-G., Almgren, A., & Sandberg, A.-S. (2002). Simultaneous and sensitive analysis of Cu, Ni, Zn, Co, Mn, and Fe in food and biological samples by ion chromatography. *Journal of Agricultural and Food Chemistry, 50*(1), 59–65. https://doi.org/10.1021/jf010792w

Gu, Z., Jiang, H., Zha, F., Manthey, F., Rao, J., & Chen, B. (2021). Toward a comprehensive understanding of ultracentrifugal milling on the physicochemical properties and aromatic profile of yellow pea flour. *Food Chemistry*, 345. https://doi.org/10.1016/j.foodchem.2020.128760

Hansen, L., Bu, F., & Ismail, B. P. (2022). Structure-function guided extraction and scale-up of pea protein isolate production. *Foods (Basel, Switzerland), 11*(23). https://doi.org/10.3390/foods11233773

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2742–2750. https://doi.org/10.1016/j.foodres.2011.06.012

Lam, A. C. Y., Warkentin, T. D., Tyler, R. T., & Nickerson, M. T. (2017). Physicochemical and functional properties of protein isolates obtained from several pea cultivars. *Cereal Chemistry, 94*(1), 89–97. https://doi.org/10.1094/CCHEM-04-16-0097-FI

Lee, C. M., Trevino, B., & Chaiyawat, M. (1996). A simple and rapid solvent ExtractionMethod for determining total lipids in fish tissue. *Journal of AOAC International, 79*(2), 487–492. https://doi.org/10.1093/jaoac/79.2.487

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry, 193*(1), 265–275. https://doi.org/10.1016/s0021-9258(19)52451-6

Lu, Z. X., He, J. F., Zhang, Y. C., & Bing, D. J. (2020). Composition, physicochemical properties of pea protein and its application in functional foods. *Critical Reviews in Food Science and Nutrition, 60*(15), 2593–2605. https://doi.org/10.1080/10408398.2019.1651248

Millar, K. A., Gallagher, E., Burke, R., McCarthy, S., & Barry-Ryan, C. (2019). Proximate composition and anti-nutritional factors of fava-bean (Vicia faba), green-pea and yellow-pea (Pisum sativum) flour. *Journal of Food Composition and Analysis, 82*, Article 103233. https://doi.org/10.1016/j.jfca.2019.103233

Mng'ong'o, M. E., Ojija, F., & Aloo, B. N. (2023). The role of Rhizobia toward food production, food and soil security through microbial agro-input utilization in developing countries. *Case Studies in Chemical and Environmental Engineering, 8*, Article 100404. https://doi.org/10.1016/j.cscee.2023.100404

Möller, A. C., van der Padt, A., & van der Goot, A. J. (2021). Abrasive milling: A method to pre-fractionate testa and embryonic axis from yellow pea. *LWT, 151*. https://doi.org/10.1016/j.lwt.2021.112087

Padhi, E. M. T., Liu, R., Hernandez, M., Tsao, R., & Ramdath, D. D. (2017). Total polyphenol content, carotenoid, tocopherol and fatty acid composition of commonly consumed Canadian pulses and their contribution to antioxidant activity. *Journal of Functional Foods, 38*, 602–611. https://doi.org/10.1016/j.jff.2016.11.006

Ralet, M.-C., Della Valle, G., & Thibault, J.-F. (1993a). Raw and extruded fibre from pea hulls. Part I: Composition and physico-chemical properties. *Carbohydrate Polymers, 20*(1), 17–23. https://doi.org/10.1016/0144-8617(93)90028-3

Ralet, M.-C., Saulnier, L., & Thibault, J.-F. (1993b). Raw and extruded fibre from pea hulls. Part II: Structural study of the water-soluble polysaccharides. *Carbohydrate Polymers, 20*(1), 25–34. https://doi.org/10.1016/0144-8617(93)90029-4

Ramirez, C. S. V., Temelli, F., & Saldaña, M. D. A. (2021). Production of pea hull soluble fiber-derived oligosaccharides using subcritical water with carboxylic acids. *The Journal of Supercritical Fluids, 178*, Article 105349. https://doi.org/10.1016/j.supflu.2021.105349

Rashwan, A. K., Bai, H., Osman, A. I., Eltohamy, K. M., Chen, Z., Younis, H. A., Al-Fatesh, A., Rooney, D. W., & Yap, P.-S. (2023). Recycling food and agriculture by-products to mitigate climate change: A review. *Environmental Chemistry Letters, 21*(6), 3351–3375. https://doi.org/10.1007/s10311-023-01639-6

Sajib, M., Forghani, B., Kumar Vate, N., & Abdollahi, M. (2023). Combined effects of isolation temperature and pH on functionality and beany flavor of pea protein isolates for meat analogue applications. *Food Chemistry, 412*, Article 135585. https://doi.org/10.1016/j.foodchem.2023.135585

Shanthakumar, P., Klepacka, J., Bains, A., Chawla, P., Dhull, S. B., & Najda, A. (2022). The current situation of pea protein and its application in the food industry. *Molecules (Basel, Switzerland), 27*(16). https://doi.org/10.3390/molecules27165354

Sorita, G. D., Leimann, F. V., & Ferreira, S. R. S. (2020). Biorefinery approach: Is it an upgrade opportunity for peanut by-products? *Trends in Food Science & Technology, 105*, 56–69. https://doi.org/10.1016/j.tifs.2020.08.011

Tassoni, A., Tedeschi, T., Zurlini, C., Cigognini, I. M., Petrusan, J.-I., Rodríguez, Ó., Neri, S., Celli, A., Sisti, L., Cinelli, P., Signori, F., Tsatsos, G., Bondi, M., Verstringe, S., Bruggerman, G., & Corvini, P. F. X. (2020). State-of-the-art production chains for peas, beans and chickpeas—Valorization of agro-industrial residues and applications of derived extracts. *Molecules (Basel, Switzerland), 25*(6). https://doi.org/10.3390/molecules25061383

Theander, O., Åman, P., Westerlund, E., Andersson, R., & Pettersson, D. (1995). Total dietary Fiber determined as neutral sugar residues, uronic acid residues, and Klason Lignin (The Uppsala Method): Collaborative study. *Journal of AOAC International, 78*(4), 1030–1044. https://doi.org/10.1093/jaoac/78.4.1030

Tulbek, M. C., Wang, Y. L., & Hounjet, M. (2024). Chapter 7 - Pea—A sustainable vegetable protein crop. In S. Nadathur, J. P. D. Wanasundara, & L. B. T.-S. P. S. Scanlin (Eds.), *Sustainable Protein Sources* (Second Edition, pp. 143–162). Academic Press. https://doi.org/10.1016/B978-0-323-91652-3.00027-7.

Wang, Y., Guldiken, B., Tulbek, M., House, J. D., & Nickerson, M. (2020). Impact of alcohol washing on the flavour profiles, functionality and protein quality of air classified pea protein enriched flour. *Food Research International, 132*, Article 109085. https://doi.org/10.1016/j.foodres.2020.109085

Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2017). Near-isogenic lines of desi chickpea (Cicer arietinum L.) that differ in milling ease: Differences in chemical composition. *Journal of Food Science and Technology, 54*(4), 1002–1013. https://doi.org/10.1007/s13197-016-2483-6

Wood, J. A., & Malcolmson, L. J. (2021). Chapter 10 - Pulse milling technologies. In B. K. Tiwari, A. Gowen, & B. B. T.-P. F. McKenna (Eds.), *PulseFoods: Processing, Quality and Nutraceutical Applications* (Second Edition, pp. 213–263). Academic Press. https://doi.org/10.1016/B978-0-12-818184-3.00010-6.

Wood, J. A., Tan, H.-T., Collins, H. M., Yap, K., Khor, S. F., Lim, W. L., Xing, X., Bulone, V., Burton, R. A., Fincher, G. B., & Tucker, M. R. (2018). Genetic and environmental factors contribute to variation in cell wall composition in mature desi chickpea (Cicer arietinum L.) cotyledons. *Plant, Cell & Environment, 41*(9), 2195–2208. https://doi.org/10.1111/pce.13196

Yang, J., & Sagis, L. M. C. (2021). Interfacial behavior of plant proteins — Novel sources and extraction methods. *Current Opinion in Colloid & Interface Science, 56*, Article 101499. https://doi.org/10.1016/j.cocis.2021.101499