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Exploring Swedish pea varieties suitable for protein isolation, focusing on antinutrients and off-flavors

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ABSTRACT

Six different pea (*Pisum sativum*) varieties and their proteins isolated *via* wet fractionation were screened to find varieties with outstanding protein functionality but minimum contents of antinutrients and off-flavor volatiles. A broad difference in emulsion activity (44.7–74.2 m^2/g) and foaming capacity (163–210%) were detected between the varieties. Pea variety significantly affected LOX activity of the sample, yielding outstanding decreases (1.6–28.6 times) for all varieties following protein isolation. Variety Eso had the highest hexanal increase ratio (820 times) while variety Balder had the lowest (32 times) after the protein isolation. The total concentration of volatile off-flavors, phytate, and saponin increased during the protein isolation with distinctive degrees for each variety. The content of the antinutrients in the proteins was substantially affected by the variety. Altogether, purpose-specific selection of pea varieties based on their desired potential could enable pea proteins with fewer antinutrients and off-flavors.

1. Introduction

Due to a range of reasons such as environmental concerns, animal welfare, sustainability, and regional-based self-sufficiency demands, plant/crop-based raw materials have globally gained extra attention as a food source (Bashi et al., 2019; Utz et al., 2022). Thereby, crops such as pulses have been targeted as valuable raw materials for extracting proteins to reformulate innovative food products as an alternative to animal proteins (Boukid et al., 2021). Among pulses, peas are one of the most promising protein sources (X. Yang et al., 2022). Peas contain majority of essential amino acids with high protein content (21–24%, whole pea), low allergenicity, and non-GMO responses which turn them into a good alternative to soy as a protein source, particularly in cold climate regions in Europe and North America (Lam et al., 2018; Tulbek et al., 2016). Despite all the mentioned advantages in addition to the abundance of pea varieties with high production rates, almost half of the world's production is still being utilized as animal feed instead of food for human consumption (X. Yang et al., 2022). For instance, in 2022, 86,000 tons of peas were produced in Sweden. However only 5% of this production was employed for food production; the rest was used as feed material for livestock (Statistics Sweden, 2022).

In the last decade, the impact of protein extraction technologies and pea varieties (Stone et al., 2015, Lam et al., 2017, Cui et al., 2020) on physicochemical (Lu et al., 2020) and functional properties (Shen et al., 2022) of pea proteins have been investigated. Furthermore, their potential applications in the food industry (Shanthakumar et al., 2022), such as meat analogs (Sajib et al., 2023) or conventional foods and beverages (Boukid et al., 2021) developments and the possibilities to improve their functional properties (J. Yang et al., 2023) have been widely reported in the literature. However, undesired sensorial/flavor attributes of pea proteins are limiting the usage of pea proteins for innovative food developments (Xiang et al., 2023). A full perspective of the flavor profile of pea proteins like many other pulse proteins is still limited. However, hexanal, 1-pentanol, 1-nonanol, (*E,E*)− 2,4-nonadienal, (*E,E*)− 2,4-decadienal, and 3-methyl-1-butanol are some of the volatile off-flavor compounds which are commonly identified in pea protein isolates (Murat et al., 2013). Undesired volatile compounds are mainly from the chemical groups of alcohols, aldehydes, ketones, pyrazines, esters, and hydrocarbons while non-volatile compounds include saponins, phenolic compounds, phytic acids, and peptides. Off-flavors can be inherent and/or be developed during pre- and post-harvesting depending on varying external conditions (Roland et al., 2017). Those compounds can be removed, masked, or modified but complete prevention is not possible yet. It has been recently (Arteaga et al., 2021)

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shown that the difference among the cultivars is not only limited to their composition, with large impacts on the sensorial profile of the pea protein isolates. Therefore, screening and choosing the pea variety that has the least off-flavor-inducing potential can be a very promising scenario for isolating more acceptable proteins from pea seeds (Roland et al., 2017).

Another big challenge ahead of the widespread applications of plant proteins and peas is the presence of antinutritional compounds such as phytic acid, saponins, polyphenols, tannins, *etc.* For example, a study recently has shown that almost all plant-based meat substitutes existing in the Swedish market suffer from high content of phytic acid (Mayer Labba et al., 2021). Some studies have shown that the content of antinutritional compounds in some legumes such as fava beans can be highly affected by their cultivar (Mayer Labba et al., 2021). However, the impact of pea varieties and the wet protein extraction process on the content of these antinutrients has not been reported previously. Furthermore, screening out different pea varieties in terms of their protein yields, techno-functional properties, nutritional values along with antinutrient components, and sensorial attributes which are of crucial importance for their future application potential has not been investigated yet, within a holistic perspective in the literature. Finding pea varieties with a minimum level of off-flavor and antinutritional compounds but optimum protein yield and functionality could facilitate their wider application as a protein source but also minimize the required level of pre-and post-processing to improve their quality. This research aims to propose the mentioned holistic and broader approach in the same study for pea protein but might also be inspiring for other plant-based proteins that are being studied nowadays. According to the best of our knowledge, a range of analyses is needed to characterize pea varieties from different parts of the world to understand which aspects of pea proteins can be optimized by choosing the right cultivars.

Therefore, the present study aimed to screen out six different pea varieties cultivated in Northern Europe and their protein isolates in terms of their molecular and structural properties, and functional properties such as protein solubility, emulsion activity, and foaming capacity. Off-flavor volatile compounds were also analyzed and the potential contribution of inherent lipoxygenase (LOX) enzyme activity was also determined and discussed critically. Finally, the effect of pea variety on the content of antinutritional compounds including phenolic compounds, tannins, saponins, and phytic acid in their protein isolates have been investigated.

2. Material and methods

2.1. Materials

The chosen pea (*Pisum sativum* L.) varieties belong to harvest season 2019 (Ingrid, Clara, Rokka, Balder) and 2021 (Eso, Bagoo) from the location Svalöv, Sweden. All varieties are yellow except Rokka, which has a green cotyledon color. Pea varieties were dried to 14% relative humidity, filled into paper bags then stored under farmhouse storage following the harvest by Lantmännen Lantbruk Sweden until the present study. Immediately after receiving each sample (around 1 kg, without vacuum) in sealed plastic bags at the division laboratory, they were placed at 4 °C in dark conditions until further use.

All chemicals used were of analytical grade and purchased from Sigma-Aldrich unless otherwise specified.

2.2. Pre-processing of pea samples and protein isolation

Whole dry pea samples were dehulled using a Satake TM05 abrasive mill (Satake, Japan) (Möller et al., 2021), and hull fractions were removed. The obtained split peas were ground using a Retsch ZM 200 ultra-centrifugal mill (Retsch, Haan, Germany) with a 500 μm screen at 12,000 rpm based on the method of Gu et al. (2021). Fine ground pea flour was either stored in plastic containers with a lid (max 100 g, dark at 4 °C) for further use or directly utilized for protein extraction.

No initial de-fatting step was applied to the flours and the alkaline solubilization and isoelectric precipitation (pH shift) method was chosen for pea protein isolation. Pea flour was mixed with distilled water in a ratio of 1:15, at pH 9.0 using 2 M NaOH and held under stirring for 1 h (Karaca et al., 2011). The supernatant of the slurry was collected after centrifugation (4000 *g*, 20 min, 20 °C) then proteins coagulated following pH adjustment to 4.5 using 2 M HCL during 10 min incubation. Obtained protein pellets following the second centrifugation (4000 *g*, 20 min, 20 °C) were dissolved in distilled water (1:1) for pH neutralization. Protein slurry at pH 7.0 was frozen at –80 °C, freeze-dried, and stored in zipped plastic bags in the dark at 4 °C for further analysis. Distilled water is used for every step of protein extraction, isolation, and characterization unless otherwise specified such as for chromatographic analysis, where only Milli-Q water is used.

The protein content of the pea flours and pea isolates was determined using the Kjeldahl method by Eurofins, Lidköping, Sweden. Nitrogen-to-protein conversion factor was chosen as 6.25 (Karaca et al., 2011). Protein content ranges for different pea varieties were 18.5–21.7% and 82.9–86.2% for flour and protein isolate samples, respectively.

2.3. Structural properties

2.3.1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The polypeptide profiles of the proteins within the flour and protein isolate samples of different pea varieties were determined with SDS-PAGE, according to the method proposed by Laemmli, (1970). Electrophoresis was conducted using Mini-Protean TGX 4–20% pre-cast gels (Bio-Rad Laboratories, Hercules, CA). All samples were diluted with 0.1 M NaOH and mixed 1:1 (*v/v*) with the Laemmli sample buffer containing 2-mercaptoethanol to obtain a final protein concentration of 2 μg/μL and 10 μL of each protein solution were loaded into each well per sample. A broad range (10–250 kDa) polypeptide molecular standard (Bio-Rad Laboratories) was used to identify the bands. Protein bands were stained by Coomassie brilliant blue (0.02% *w/v*) mixture in 50% methanol and 7.5% acetic acid (*v/v*) for 45 min and destained in 50% methanol and 7.5% acetic acid (*v/v*) for 90 min. Gel was imaged with Bio-Rad's Gel Doc 2000 after overnight refrigeration. Quantification of bands was conducted using Bio-Rad Image Lab 6.1.0. software.

2.3.2. Molecular weight distribution analysis using size-exclusion chromatography

The molecular weight distributions of soluble proteins and peptides were analyzed by high-performance size exclusion chromatography (HP-SEC) (Dionex HPLC; Dionex GmbH. Idstein. Germany) according to a modified method which was adopted from Cui et al. (2020), and Gao et al. (2020). Firstly, 3 mg of samples (both flour and protein isolates) were dissolved in 10 mM (pH 7.0) phosphate buffer and stored overnight for full hydration. Samples were then sonicated at room temperature for 5 min and shaken for 1 h in a rotary shaker. The samples were then centrifuged at 10,000 *g* for 10 min, the supernatants were filtered using 0.45 μm pore size cellulose acetate membrane and the filtrates were directly injected into the HPLC system equipped with an Agilent Bio SEC-5 guard column (5 μm, 150 Å, 4.6 × 50 mm) followed by chromatographic separation using an SEC-5 300 Å column (Agilent Bio, 5 μm, 300 Å, 7.8 × 300 mm). PBS buffer (10 mM, pH 7.0) containing 100 mM NaCl was used as the mobile phase. The molecular weight of the samples was calculated based on AdvanceBio SEC 300 Å protein standard (Agilent Technologies).

2.4. Basic functional properties

The solubility behavior of pea protein isolates was investigated as a function of pH using a modification of the methods described by Tanger et al. (2020) and M. Yang et al. (2021). Briefly, protein solutions were

prepared with distilled water at 10 mg/mL and left for stirring overnight at room temperature. pH was adjusted to the desired range (3.0–11.0) using 1.0 M NaOH or HCl, and stirred (500 rpm) for 1 h. The protein solutions were then centrifuged at 4000 *g* for 30 min and soluble protein content was determined using the modified Lowry protein determination method (Markwell et al., 1978).

The foaming capacity (FC) and foam stability (FS) of the samples were determined based on the method presented by Stone et al. (2015). Protein solutions were prepared as 1% (*w/v*) at pH 7.0 using 10 mM sodium phosphate buffer (PBS) and stirred overnight at room temperature. Around 30 mL of each protein solution were transferred into suitable glass beakers and foamed at 10,000 rpm for 2 min using a Polytron homogenizer (T18 ULTRA-TURRAX; IKA, Brazil) within an ice chamber to control excessive temperature increase. The created foam was immediately transferred into a graduated cylinder (100 mL) and foam volume was measured immediately and after 30 min. Relative FC and FS were calculated and represented as percentage changes.

Emulsifying activity (EAI) and stability (ESI) indices were determined based on the defined method by Karaca et al. (2011). The protein solution was prepared as 0.5% (*w/v*) at pH 7.0 using 10 mM sodium phosphate buffer and 5 g of the solution. They were homogenized with dropwise added 5 g sunflower oil at 8000 rpm for 5 min using a Polytron homogenizer (T18 ULTRA-TURRAX; IKA, Brazil) within an ice chamber to control excessive temperature increase. Immediately after, a 50-μL emulsion sample from the bottom of the container was taken, diluted with 7.5 mL 10 mM PBS (pH 7.0) containing 0.1% sodium dodecyl sulfate, and vortexed for 10 s. Another aliquot was taken and diluted in the same way at 10 min. The absorbance of these suspensions were measured at 500 nm using plastic cuvettes with 1-cm path lengths. The EAI (1) and ESI (2) were calculated using the following equations.

$$EAI \quad \left(\frac{m^2}{g}\right) = \frac{2 \times 2.303 \times A_0 \times DF}{C \times \varphi \times \theta \times 10000} \tag{1}$$

$$ESI \quad (\text{min}) = \frac{A_{10} \times \Delta t}{\Delta A} \tag{2}$$

A_0 is the absorbance at $t = 0$ min, A_{10} is the absorbance at $t = 10$ min, DF is the dilution factor, C is the initial protein concentration (g/mL), φ is the volume fraction of oil in the emulsion, θ is the path length of cuvette (1 cm), Δt is the elapsed time (10 min), ΔA is the absorbance difference between $t = 0$ and $t = 10$ min.

2.5. Fatty acid methyl esters (FAME) analysis

Oleic, linoleic, linolenic, and total unsaturated fatty acid distributions of both pea flours and proteins were determined using consecutive methods with slight modifications such as fat extraction (Lee et al., 1996), methylation (Fredrikson et al., 2002), identification and quantification. A sample of 0.5 g from each powder was mixed with 20 mL chloroform-methanol (2:1) solution and then C17 was added as an internal standard. Following a 30-minute extraction with a rotary shaker, 8 mL NaCl (0.5%) were added to each sample for clarification and then vortexed for 30 s. The mixtures were centrifuged (3000 *g* for 6 min) and lower parts were transferred for evaporation. The fat extracts were then methylated by the addition of 1 mL toluene and 1 mL methanol:acetyl chloride (10%, *v/v*) solution followed by incubation at 60 °C for 120 min. After the incubation, 1 mL Milli-Q water and 1.5 mL petroleum ether were added and then centrifuged at 2500 *g* for 5 min, and the upper parts were transferred for evaporation. The methylated fat extracts were finally redissolved in 500 μL isooctane for injection. Identification and quantification of the fatty acids were conducted by GC-MS, Agilent 7890 A GC system, and Agilent 5975 C triple-axis MS detector (Agilent Technologies, Santa Clara, CA, USA). GLC 463 (Nu-Check Prep, Inc., Elysian, MN) was used as the standard to identify the fatty acid methyl esters.

2.6. Iron and zinc content analysis

The total iron and zinc contents of the flour and protein isolate samples were determined using a method by Fredrikson et al. (2002) with slight modifications. Around 0.5 g of each flour and protein sample were mixed with 0.75 mL concentrated HNO_3, 0.15 mL concentrated HCl, and 3 mL of Milli-Q water in a Teflon vial. The prepared mixture was exposed to microwave digestion (Milestone microwave laboratory system; Ethos Plus, Sorisole, Italy) at 180 °C for 35 min. Following the cooling down to room temperature, the digested transparent sample was transferred into a volumetric flask to complete the final volume of up to 10 mL with Milli-Q water and analyzed with atomic absorption spectroscopy (Agilent 240FS AA Systems, Australia).

2.7. Determination of antinutrients

The extraction of total phenolic compounds from the samples and their quantification were conducted according to the method described by Capanoglu et al. (2008). For this purpose, 0.5 g of each sample were mixed with 3 mL of 75% methanol, sonicated at room temperature for 15 min, centrifuged (2500 *g* for 10 min at 20 °C) and the supernatant was collected. The same application was repeated with the same pellet again using 2 mL of 75% methanol this time. The total phenolic content of the methanolic extracts was determined by the method of Singleton & Rossi (1965) using Folin-Ciocalteu reagent. A calibration curve was prepared with gallic acid and results were expressed as mg gallic acid equivalent (GAE) in 100 g of protein isolate. The same methanolic extracts that were prepared for total phenolic content analysis were used to determine the total tannin content of the samples as well. For tannin quantification, a method that was developed and proposed by FAO/IAEA, (2000) was used. For this purpose, total phenolic content was determined as mg tannic acid equivalent (TAE) in 100 g of protein isolate, using the tannic acid to prepare the standard curve. Afterward, tannin compounds were precipitated and removed from the extract using 100 mg polyvinyl polypyrrolidone (PVPP) for 1 mL extract diluted with 1 mL distilled water. The PVPP-containing mixture was vortexed, held for 15 min at 4 °C, and centrifuged at 3000 *g* for 10 min. The obtained supernatant includes only non-tannic simple phenolics. Absorbances of the extracts before and after PVPP precipitation were measured at 725 nm using a UV–visible spectrophotometer and their differences were recorded as mg TAE in 100 g of sample on a dry basis.

The saponin content of the samples was determined colorimetrically, following a microwave-assisted extraction of targeted compounds (Akbari et al., 2019; Navarro del Hierro et al., 2018). Briefly, samples were mixed with 60% ethanol (1:10 g/mL) and irradiated under 600 W at 70 °C for 3 min (Milestone microwave laboratory system; EthosPlus, Sorisole, Italy). The treated samples were then centrifuged at 4000 *g* for 5 min, the supernatants were filtered through a 0.45 μm membrane filter and the ethanol was completely evaporated. The dried extracts were dissolved in methanol at 2 mg/mL. Aliquots of 125 μL were transferred to Eppendorf tubes, followed by adding 125 μL of freshly prepared vanillin in ethanol (8%, *w/v*) and 1.25 mL of sulfuric acid in water (72%, *v/v*). The samples were vortexed and heated at 60 °C for 10 min and then the tubes were transferred to a beaker with ice crystals. Total saponin content was detected by measuring the absorbance of this mixture at 520 nm using a UV–visible spectrophotometer at room temperature. A standard curve was prepared using oleanolic acid (50–800 μg/mL) and total saponin content was expressed as g per 100 g extract.

Phytate content was analyzed by high-performance ion chromatography (HPIC) according to the method of Carlsson et al. (2001). Samples (0.5 g) were mixed with 10 mL of 0.5 M HCl for 3 h using a laboratory shaker (Heidolph Reax 2; Heidolph Instruments GmbH, Schwabach, Germany). Then, the samples were centrifuged (12,000 *g* for 5 min at 20 °C), and the supernatants were transferred to HPLC vials. The used equipment consisted of an HPLC pump (model PU-4080i; Jasco Inc.,

Easton, MD) for the eluent and an RHPLC pump (model PU-4180; Jasco) equipped with a PA-100 guard column and a CarboPac PA-100 column. Phytate was eluted with an isocratic eluent of 80% HCl (1 mol/L) and 20% H_2O at 0.8 mL/min, exposed to a post-column reaction with ferrous nitrate, and detected at 290 nm with a UV–Vis HPLC detector (UV-4075; Jasco, Tokyo, Japan). The phytate concentration was calculated based on an external standard within a concentration range of 0.1–0.6 μmol/mL.

2.8. Lipoxygenase enzyme activity (LOX)

LOX values of the samples were measured based on a principle briefly consisting of extracting the enzymatic compounds from the samples and controlled introduction of these enzymatic extracts to a model substrate system with a concomitant visualization of their absorbance change (Gao et al., 2020; Gökmen et al., 2002). Each pea flour or protein sample (0.1 g) was mixed with 10.0 mL phosphate buffer (10 mM) and left to stir for 5 h at room temperature. The mixture was then centrifuged at 9100 *g* for 10 min and the supernatant was used as the enzyme extract. To obtain the model substrate solution linoleic acid (140 μL) and Tween 20 (140 μL) were mixed and emulsified into 8 mL of phosphate buffer. Then, 1.1 mL of 0.5 M NaOH was added to clarify the solution, and the volume was brought to 50 mL with phosphate buffer. The stock substrate solution was diluted (1:40, *v/v*) with 0.2 M sodium borate buffer (pH 9.0) before commingling with the enzymatic aliquot (1.25 mL: 50 μL), and absorbance increased due to the presence of a conjugated hydroperoxide moiety of the mixture at 234 nm was recorded for 3 min using a UV–visible spectrophotometer. The unit of LOX activity was U/g, where U is defined as the numeric increase in absorbance per minute with the following equation.

$$\text{LOX } activity\left(\frac{\text{unit}}{\text{g}}\text{protein.min}\right) = \frac{\Delta ABS \times 1000 \times 1000}{Protetin\ content\ (mg) \times 3} \quad (3)$$

where the number 1000 represents the LOX unit conversion (0001 ABS indicates 1 unit) and the conversion for mg to g, while 3 represents the total assay time (min).

2.9. Determination of selected off-flavor-related volatile compounds

Selected volatile off-flavor contributors were detected by headspace solid-phase microextraction (HS-SPME)–GC–MS based on a method defined by Sajib et al. (2023) with some modifications. A specified amount of the samples (1 g for flour and 0.05 g for protein isolates) were dissolved with 8 mL Milli-Q water in 20-mL SPME vials. SPME fiber (75 μm Carboxen/ polydimethylsiloxane (CAR/PDMS); Supelco, Bellefonte, PA) collected the volatiles from the vial headspace during a 40 min extraction at 60 °C under continuous stirring (500 rpm). The adsorbed volatile compounds were then injected (desorbed) into the GC-MS for 5 min with the splitless mode. A Shimadzu TQ8030 GC–MS setup with a ZB-1701 capillary column (30 m × 0.32 mm, 1 μm; Phenomenex, Torrance, CA) was used and the data acquisition scan was in the mass range of *m/z* 30–500. Helium as the carrier gas had a flow rate of 1.5 mL/min. The GC inlet temperature was maintained at 300 °C and GC separation was performed using a GC oven with varying temperatures in the range of 35–260 °C. MS transfer line temperature was maintained at 265 °C, while the ion source temperature was 200 °C. The marker compounds for undesired beany off-flavors in pea were chosen as hexanal, 1-hexanol, 1-octen-3-ol, benzaldehyde, 2-pentylfuran, 1-pentanol, 1-nonanol, 2-methoxy-3-isopropyl pyrazine, (*E,E*)− 2, 4-nonadienal, (*E,E*)− 2,4-decadienal, and 3-methyl-1-butanol based on recent literature data (Benavides-Paz et al., 2022; Liu et al., 2023; Murat et al., 2013). Quantification of volatile compounds was conducted by relative peak areas of targeted compounds based on external standards, against the peak area of the chosen internal standard.

2.10. Statistical analysis

The mean differences of the measurements with ± standard deviation (SD) were analyzed using 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). Triplicate observations were used for statistical analysis unless otherwise specified.

3. Results and Discussion

3.1. Chemical and molecular structure of pea flours and protein isolates

3.1.1. SDS-PAGE

The polypeptide pattern of the pea varieties for both flour and protein isolates is shown in Fig. 1. The bands were observed typically in between 10–100 kDa for all samples. The bands were mostly homologous with other results in the literature; the bands just below 75 kDa represent convicilin (α-subunits of vicilin) together with the polypeptide fractions of 7 S vicilin from the bands around 47–50, 30–34 and around 15 kDa. Subunits of hexameric legumin were seen with bands around 38–40 kDa for Leg α (acid polypeptide) and around 19–22 kDa for Leg β (basic polypeptide) (Gao et al., 2020). Exceptional findings here (compared to the literature) were the bands observed around 94 kDa and around 10–15 kDa, considered as the lipoxygenase enzyme (LOX) and albumin proteins, respectively (Barac et al., 2010). The albumin bands disappeared for the protein isolates of all varieties while they were detectable as 5% for the flour samples. Another decrease was observed in the convicilin concentration for all varieties, following protein isolation. However, the pH shift process did not affect the relative distribution of legumin and vicilin subunits significantly, while LOX content increased following the protein isolation process. The highest and lowest LOX content according to the electrophoretic profile were detected for Ingrid and Eso varieties for both flour and isolate forms.

A multifunctional term, legumin/vicilin (Lg/Vn) ratio, could be considered as an informative indicator for varying properties such as the techno-functional potential of the protein isolate or even the flavor perception of the isolates due to the lower water solubility, off-flavor attributed disulfide bonds and lower allergenicity of the legumin structure (Arteaga et al., 2021). The Lg/Vn ratios of the pea varieties were calculated for flour forms as 1.5, 1.3, 1.6, 0.9, 1.6, and 1.4, while they were 1.7, 1.1, 1.5, 1.0, 1.4, and 0.9 for Ingrid, Clara, Rokka, Balder, Eso, and Bagoo protein isolates, respectively. Isoelectric precipitation of the proteins from pea flours did not affect the Lg/Vn ratio for individuals since that process did not favor one protein over the other one (Lam et al., 2017). The observed Lg/Vn ratios were in correlation with the literature data for pea protein isolates (and flours) which are mostly observed in the range of 0.4–2.0 (Guldiken et al., 2021a) even with some exceptions such as around 8.0 (Mertens et al., 2012; Asen et al., 2023). The ratio is highly dependent on the environmental/growth conditions of the crops, rainfall ratio, and maturity of the seed (Lam et al., 2018).

3.1.2. Molecular weight distributions with HP-SEC

Relative molecular weight (Mw) distribution of soluble proteins was detected with HP-SEC and proportional quantities of the major peaks for both flour and protein isolate samples are provided in Fig. 2. Mw fractions that are higher than 1000 kDa are represented by Fraction (F) 1. The first fraction indicates the first peak that was observed in the chromatogram, and so does the rest. F1 is considered the insolubilized large protein bodies as has been reported previously (Cui et al., 2020). Aggregates were observed for all pea flours in a range of 2–4% while no F1 was detected for isolate samples. On the other hand, Fraction 2 (300–400 kDa), Fraction 3 (100–200 kDa), and Fraction 4 (<50 kDa) represent the hexameric legumin forms, trimeric vicilin forms, and vicilin subunits/albumins, respectively (Gao et al., 2020). Following the protein isolation, a significant decrease in F4 was observed for all

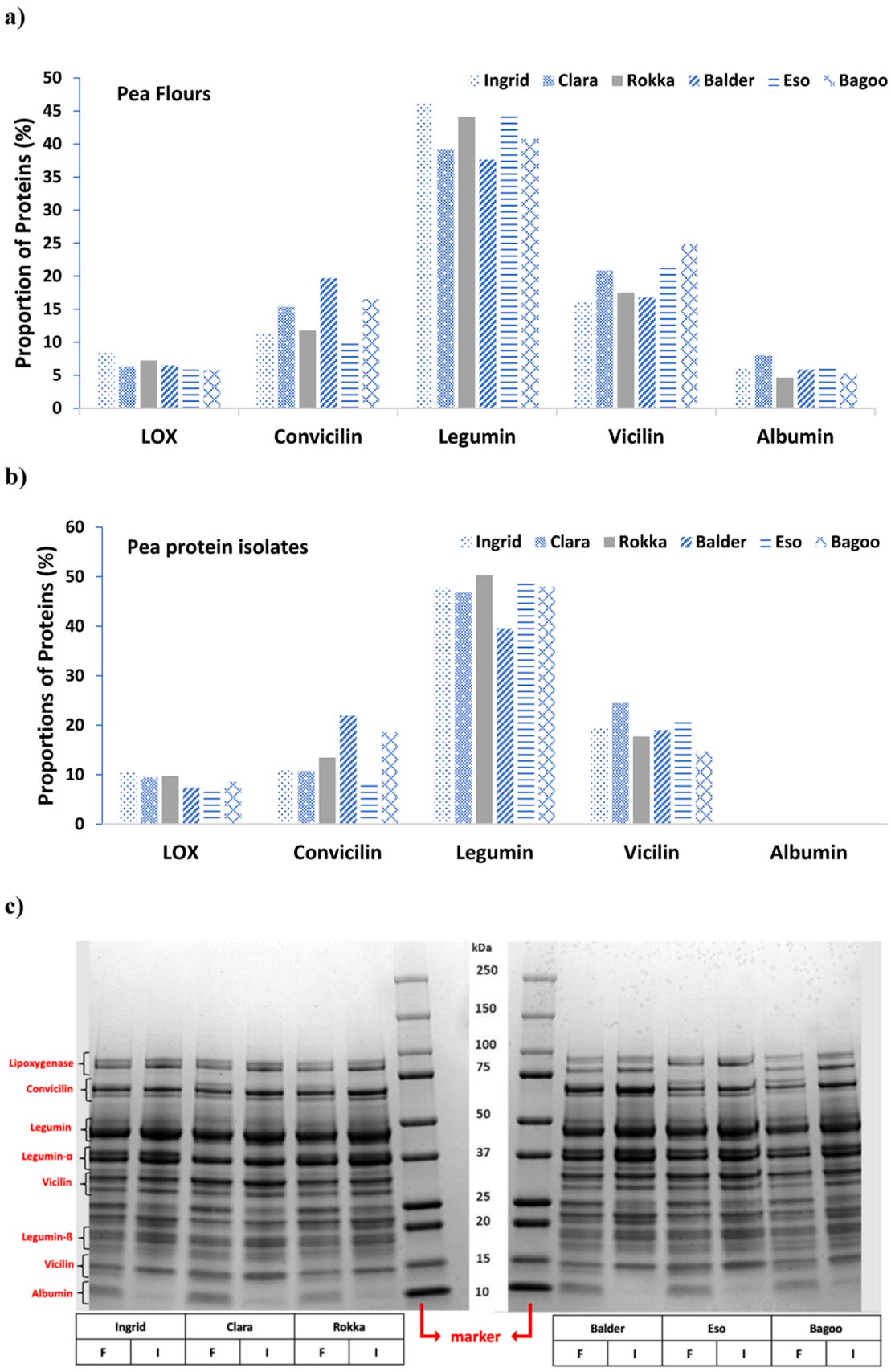

Fig. 1. Protein composition of flour (a), protein isolates (b), and electrophoretic polypeptide profiles of flour and protein isolate forms (c) of the six studies pea varieties. F and I represent "Flour" and "Isolate", respectively. LOX: lipoxygenase, Cv: convicilin, L: legumin, L-α: α subunits of legumin, V: vicilin, L-β: β subunit of legumin, A: albumin.

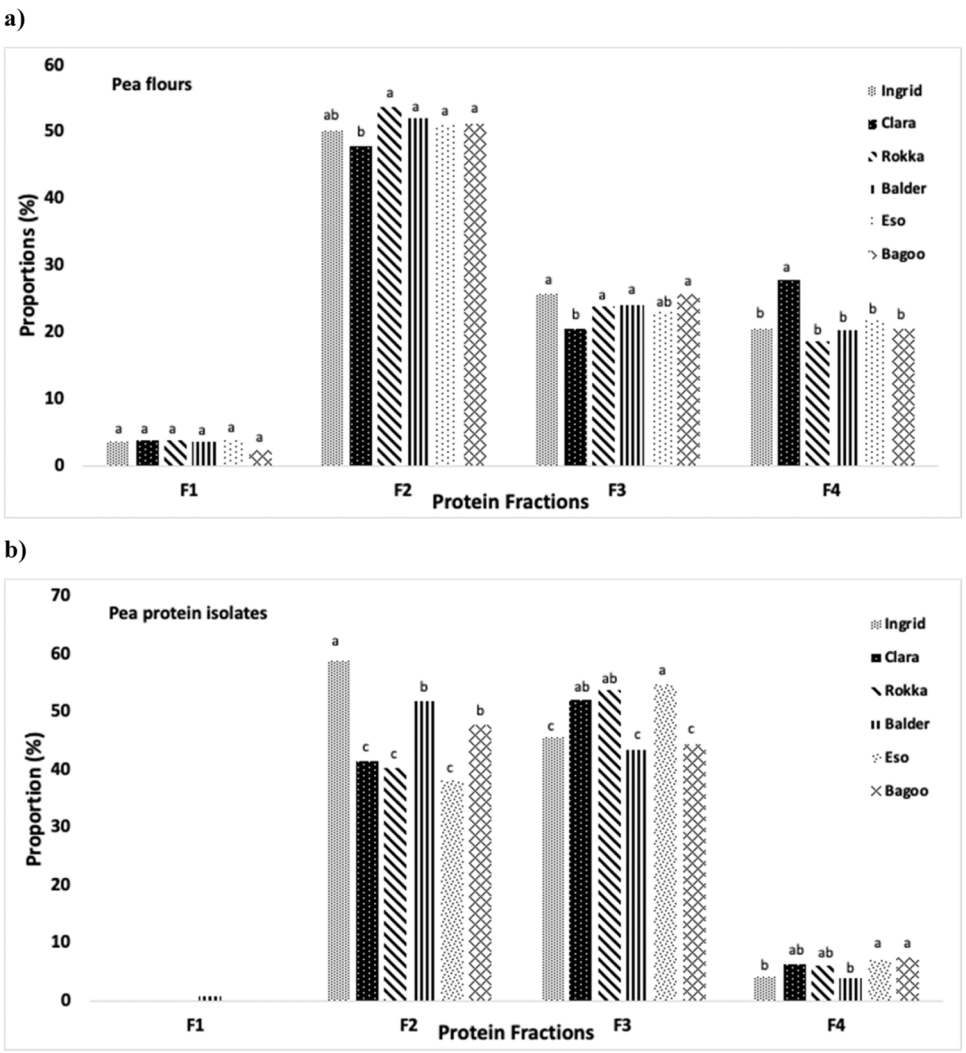

Fig. 2. Proportional molecular weight distributions of proteins in flour (a) and protein isolate (b) of the studied pea varieties identified with size exclusion chromatography. Small letters define significant differences with different letters between the varieties in each fraction/peak.

samples due to the expected albumin loss during the pH shift process. This phenomenon was also correlated and can be seen clearly on the SDS-PAGE electrophoretic profile. There was a substantially lower content of aggregates in the protein isolates while the percentage of Fraction 3 (assigned to vicilin) was doubled compared with the flours.

Protein isolate forms of the varieties yielded a distinctive Mw distribution pattern compared to the flour forms. Ingrid, Balder, and Bagoo isolates had higher hexameric legumin content while having the lowest trimeric vicilin ratios compared to the other varieties. The detected legumin contents for the varieties (except Ingrid) were lower than that of the electrophoretic profile. The reason behind this could be removing most of the particles/aggregates with centrifugation and filtration prior to injection. In addition, some other technical issues such as distinctive determinations of convicilin or trypsin inhibitors following the measurement of the same fractions with the same sensitivity both with SDS-PAGE and SEC might affect the relative distributions of the protein fractions (Klost & Drusch, 2019). Different fractions/subunits which are detected with two different approaches can provide further information about the biochemical and functional properties of the pea varieties and those differences could be inconsistent and change from variety to variety, harvest time, and storage (Arteaga et al., 2021).

3.2. Functional properties of pea protein isolates

As the key factor governing techno-functional attributes of proteins, water solubility characterization of the targeting proteins provides essential information about their application potential. Solubility distribution of protein isolates from different pea varieties as a function of pH (3.0–11.0) is provided in Fig. 3. As expected, all varieties showed their lowest solubilities in the range of pH 4.0–5.0 which is in good agreement as the indicated range for isoelectric point of pea protein isolates is pH 4.0–6.0, depending on the extraction method and the cultivar (Lam et al., 2016). In another study, the pH where four different American yellow pea varieties had the lowest solubility was defined as around pH 5.0 while pH 8.0 was determined for yellow pea varieties where they have the highest solubility ratios (around 80%) (Cui et al., 2020). The studied Swedish pea varieties yielded their highest solubilities at around pH 9.0 with 90% average solubility ratios. Among the varieties, slight differences in solubility as a function of pH were observed, except for the variety Bagoo, which had the highest solubilities close to the isoelectric point (pH 4.0–5.0) as well as at pH 9.0. Particularly, the differentiation of solubilities at pH 3.0 nominates the pea protein isolates to be potential substitutes for acidic food matrices such as fortified soft drinks or dairy analogs (Gao et al., 2020).

The functional properties of pea protein isolates are represented in Table 1. For the emulsion activity index, Balder, Eso, and Bagoo had the

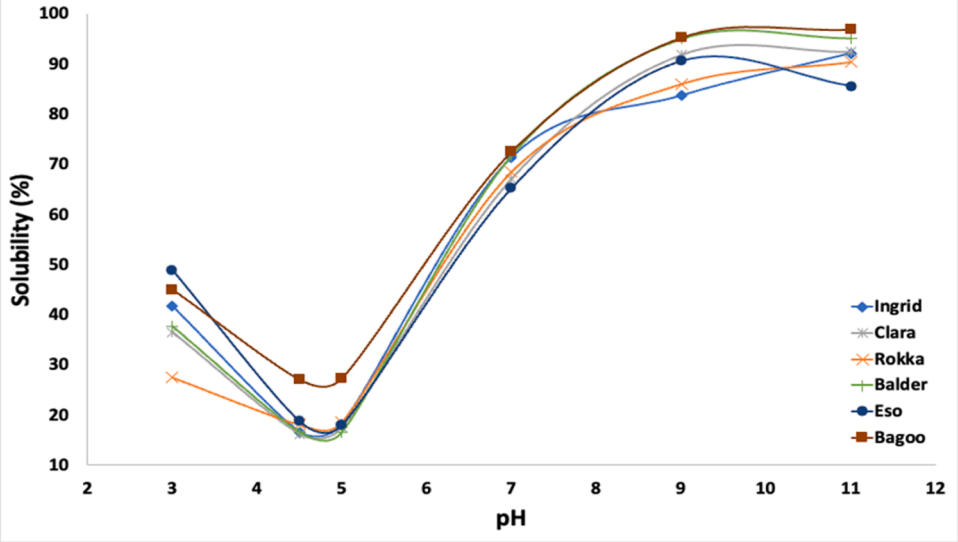

Fig. 3. Solubility of pea protein isolates from six different studied pea varieties as a function of environmental pH.

Table 1
Functional properties such as emulsion activity index (EAI), emulsion stability index (ESI), foaming capacity (FC), and foam stability (FS) of pea protein isolates from the six studied pea varieties cultivated in Sweden at pH 7.0.

Pea Variety	EAI (m^2/g)	ESI (min)	FC (%)	FS (%)
Ingrid	44.7 ± 1.7^{c}	11.3 ± 0.5^{c}	170 ± 3.3^{b}	76.3 ± 3.6^{a}
Clara	68.2 ± 4.8^{ab}	18.9 ± 2.1^{bc}	163.3 ± 3.3^{b}	73.9 ± 3.9^{a}
Rokka	52.6 ± 5.6^{b}	47.3 ± 4.1^{a}	193.3 ± 6.7^{a}	28.7 ± 2.1^{d}
Balder	72.2 ± 8.4^{a}	22.6 ± 3.4^{b}	203.3 ± 3.3^{a}	64.5 ± 2.1^{b}
Eso	72.9 ± 1.7^{a}	22.9 ± 1.7^{b}	193.3 ± 0.0^{a}	35.7 ± 0.0^{c}
Bagoo	74.2 ± 3.4^{a}	29.9 ± 2.2^{b}	210 ± 10.0^{a}	36.1 ± 2.8^{c}

n = 3 and standard deviations are defined as ± .
Superscripts define significant differences with different letters ($p < 0.05$)

highest values followed by Clara; however, the highest emulsion stability was observed for the emulsion made with Rokka protein isolate. The second most stable emulsions were observed for Balder, Eso, and Bagoo and they had no significant differences. EAI was affected by the varying pea varieties which were also indicated distinctively in the literature; Barac et al. determined significant EAI and ESI differences for six different pea protein isolates, so did Shevkani et al. for determining the differences for 5 different field pea varieties (Barac et al., 2010; Shevkani et al., 2015). According to the Lg/Vn ratio, the protein isolates that have the highest ratio of Lg/Vn are expected to yield the highest EAI and ESI (Lam et al., 2018). However, this correlation was not observed in this study. The potential reason for this could be the fact that this theory has been suggested by investigation of purified legumin and vicilin extracts while our Swedish pea protein isolates in the form of crude protein isolates and already include high-fat content, which is known to affect functional behaviors of isolates. However, the EAI of the protein isolates of the different varieties correlated with their water solubility at pH 7.0.

The foaming capacity of many different pea varieties has been previously reported in between/around 150–200% as well as for many other pulse proteins (Cui et al., 2020; Guldiken et al., 2021b). The Swedish pea varieties in this study were also confirmed to be in these ranges of 163–210%. The variety Clara showed the lowest and Bagoo the highest foaming capacity (FC%) which were to a large extent correlating with the water solubility of their proteins (see Fig. 3). On the other hand, the foam stability of varieties Ingrid and Clara was the highest among all. Variety Balder ended up with the highest volume at the end of observation time (30 min) meaning the highest FS. Overall, the results showed that screening the Swedish pea varieties is a very promising solution for optimizing pea protein functionality where variety Bagoo stands out as the most promising variety.

3.3. *Off-flavor and antinutrient attributions of pea flours and protein isolates*

3.3.1. *Unsaturated fatty acid distribution*

Oleic, linoleic, and linolenic acid compositions were selected to present here due to their high affiliation with lipid-oxidation-driven off-flavor formation out of their unsaturation status (Table 2). The Swedish pea varieties had a high content of linoleic acid both in the flours and protein isolates ranging between 41–52% for all samples. The de-fatting process was not applied to the raw material (pea flour) prior to protein isolation since it might lead to loss of functional properties of isolates as well as not being considered economically/industrially feasible, despite its potential to avoid undesired lipid oxidation (Mehle et al., 2020). Consequently, the fat content increase was around 4× for the protein isolates compared to flour samples ending up with around 9% of total fat content (data not shown). Similar fat content results were indicated for other commercial European pea protein isolates as 8–9% (Ebert et al., 2022). However, the distribution of oleic, linoleic, linolenic, and total unsaturated fatty acid distribution of the different varieties did not change significantly ($p > 0.05$) during the protein isolation process. The distribution of those selected fatty acids was in good agreement with the literature data, particularly for linoleic and linolenic acids, *i.e.*, the Canadian split yellow/green peas had similar ratios of around 41–47% and 8%, respectively (Padhi et al., 2017). On the other hand, oleic acid distribution was determined to be lower for Swedish pea varieties (15–23%) compared to Canadian peas (23–29%). The percentage of linolenic acid in the protein isolates showed some differences where Rokka showed the lowest (42%) and Bagoo showed the highest content (51%) but finally, the total content of unsaturated fats of all protein isolates from all the six varieties was very similar. The differences in fatty acid distributions might play a crucial role in the off-flavor formation and stability during the protein isolation as well as the storage periods since they are recognized as the substrates of both enzymatic/non-enzymatic lipid oxidation reactions (Roland et al., 2017). Surely, the contents and activities of the relevant enzymes such as lipoxygenase and lipase coupled with the other physical factors such as temperature and water activity of the media have significant roles equally with the fatty acid distributions for off-flavor formation (Mehle et al., 2020).

Table 2
Selected unsaturated fatty acids composition of flour and protein isolate from pea varieties (% of total fatty acid methyl esters).

	Flours						Protein Isolates					
	Ingrid	Clara	Rokka	Balder	Eso	Bagoo	Ingrid	Clara	Rokka	Balder	Eso	Bagoo
Oleic acid	21.43 ± 0.97^{a}	18.58 ± 0.84^{ab}	22.75 ± 0.93^{a}	17.23 ± 0.76^{bc}	17.10 ± 0.72^{bc}	15.41 ± 0.97^{c}	22.45 ± 1.14^{ab}	19.95 ± 0.93^{bc}	23.63 ± 0.87^{a}	19.03 ± 0.78^{bc}	18.03 ± 0.81^{c}	16.48 ± 0.68^{c}
Linoleic acid	46.94 ± 1.37^{ab}	45.73 ± 1.74^{b}	41.19 ± 1.82^{c}	50.55 ± 1.63^{a}	48.83 ± 1.26^{a}	50.34 ± 2.11^{a}	48.18 ± 1.85^{a}	46.07 ± 1.43^{ab}	42.92 ± 1.17^{b}	51.87 ± 2.34^{a}	50.46 ± 2.46^{a}	51.83 ± 2.63^{a}
Linolenic acid	8.11 ± 0.42^{b}	11.30 ± 0.67^{a}	11.91 ± 0.71^{a}	8.95 ± 0.57^{b}	10.67 ± 0.64^{ab}	10.09 ± 0.58^{ab}	8.07 ± 0.44^{b}	11.10 ± 0.56^{a}	11.94 ± 0.97^{a}	8.65 ± 0.74^{b}	10.61 ± 0.72^{ab}	9.81 ± 0.56^{ab}
∑ UFA[a]	80.68	79.81	80.31	81.52	81.27	80.74	82.01	80.36	81.91	82.98	82.58	81.75

Standard deviations are presented ± ⊡ Different letters as superscripts represent significant differences ($p < 0.05$) in the same row, enclosed for flours and protein isolate samples.
[a] UFA: unsaturated fatty acids.

3.3.2. *Antinutrients/non-volatile off-flavor compounds*

Antinutrients are inherent but undesired compounds in food biomasses due to their hindering effects for binding to nutritional compounds such as amino acids and/or minerals and lower their bioavailability and absorption in living organisms (Cirkovic Velickovic & Stanic-Vucinic, 2018). Among different types of antinutrients, phenolic compounds, tannins, saponins, and phytate are known for their contribution to the off-flavor perception of plant/legume-based proteins as well as their relative physical stability against varying food processing conditions (Tulbek et al., 2016). For instance, phytate (IP6) is a heat-stable antinutrient while polyphenols and tannins might be partially inactivated by soaking and/or heat treatment and saponins might be degraded by fermentation (Ma et al., 2017). Phenolics represent a wide range of compounds with different physicochemical properties as well as distinctive flavor characteristics such as bitter, smokey, spicy, and astringent; saponins are known for their bitter flavor in legumes; tannins are primarily responsible for astringent perception. Phytate also contributes to bitter taste formation; however it is more linked with its inhibition effect for nutrients (Leonard et al., 2022).

The total phenolic compounds, tannins, saponins, and phytate contents of flour and protein isolate from different pea varieties are presented in Table 3. The total phenolics and tannin content of the samples were observed to be slightly affected by the protein isolation process. A tentative decrease for these two antinutrients was observed in the protein isolate samples when compared to the flour samples of almost all the varieties. Among the varieties, Rokka (the only green variety) had the highest decrease for both total phenolics and tannin when it was processed into the protein isolate. The phenolic content distribution of the pea varieties was compatible with most of the literature data. However, it is a fact that a wide range of distinctions exists for peas/legumes from different regions of the world. For varying legumes, it was presented as 0.4–7 mg/100 mg GAE, for some green and yellow pea varieties from the UK and Canada, they were reported as around 0.1 mg/100 mg GAE (Millar et al., 2019; Navarro del Hierro et al., 2018; Padhi et al., 2017). Total phenolics and condensed tannin amount changes were either insignificant or slightly increased in some Canadian pea protein isolates (1.1–2.5; 0.02–0.22 mg/g) compared to their flour forms (2.9–3.6; 0.17–0.39 mg/g) (D. Shi et al., 2022). Tannin content was found to decrease by around 0.9 mg/100 g following the protein isolation from pigeon pea flour from 2.70 mg/100 g (Adenekan et al., 2018). The decrease might be more drastic for other plant seeds such as around 95% tannin content degradation observed for Sangri seed flour after the protein concentrate preparation (Garg et al., 2020). There was a big difference in the content of total phenolic compounds and tannins in protein isolates from the different pea varieties. Protein isolated from Bagoo showed the highest content and the protein from Rokka had the lowest content of the two antinutrients compared with the other varieties. The content of total phenolic compounds and tannins in proteins from Rokka was less than half of Bagoo.

Saponins are also important antinutrients which are also one of the major bitterness-contributing compounds in peas with a very low perception threshold (8 mg/L). Due to their high affinity to protein bodies, it is expected to increase their concentration following the pea protein isolation with increased unpleasant flavor attribution in the final

Table 3
Off flavor attributed non-volatile antinutrients of flour and protein isolate from six yellow pea varieties cultivated in Sweden.

		Antinutrients					
	Varieties	TPC	TTC	Saponin	Phytate	Phy/Fe	Phy/Zn
Flours	Ingrid	0.45 ± 0.01^{a}	0.44 ± 0.02^{a}	11.45 ± 0.12^{a}	578 ± 2.61^{bc}	10.58	13.71
	Clara	0.60 ± 0.01^{a}	0.59 ± 0.01^{a}	13.99 ± 0.74^{a}	601 ± 4.49^{ab}	13.24	14.72
	Balder	0.47 ± 0.01^{a}	0.38 ± 0.01^{a}	15.21 ± 0.97^{a}	445 ± 3.78^{c}	11.49	14.07
	Rokka	0.54 ± 0.01^{a}	0.59 ± 0.02^{a}	15.27 ± 0.30^{a}	640 ± 6.81^{ab}	8.63	11.79
	Eso	0.64 ± 0.01^{a}	0.59 ± 0.02^{a}	16.30 ± 0.05^{a}	470 ± 7.44^{c}	10.72	13.44
	Bagoo	0.78 ± 0.01^{a}	0.54 ± 0.01^{a}	15.81 ± 0.47^{a}	734 ± 22.7^{a}	14.06	16.93
Protein Isolates	Ingrid	0.38 ± 0.03 ^{A}	0.39 ± 0.02 ^{A}	21.78 ± 0.13^{B}	1616 ± 15.1^{B}	5.99	28.20
	Clara	0.51 ± 0.01 ^{A}	0.33 ± 0.01 ^{A}	25.32 ± 0.12^{B}	1621 ± 3.45^{B}	6.20	25.26
	Balder	0.42 ± 0.01 ^{A}	0.41 ± 0.02 ^{A}	23.46 ± 0.21^{B}	1567 ± 12.1^{B}	7.21	32.34
	Rokka	0.25 ± 0.01 ^{A}	0.24 ± 0.02 ^{A}	35.33 ± 1.66 ^{A}	1994 ± 8.54 ^{A}	5.35	46.65
	Eso	0.44 ± 0.01 ^{A}	0.39 ± 0.01 ^{A}	25.34 ± 1.49^{B}	1620 ± 6.98^{B}	6.85	32.89
	Bagoo	0.60 ± 0.01 ^{A}	0.70 ± 0.02 ^{A}	31.43 ± 0.68 ^{A}	2061 ± 6.96 ^{A}	7.23	36.84

TPC: Total phenolic content as gallic acid equivalent, mg/100 mg dry basis sample.
TTC: Total tannin content as tannic acid equivalent, mg/100 mg dry basis sample.
Saponin as g/100 g UAE extract.
Phytate as total phytic acid (IP6) (mg/100 g sample).
Molecular masses were used as 660.3 g/mol, 56 g/mol, and 65.4 g/mol for Phy, Fe, and Zn, respectively.
The molar ratio of Phy/Fe lower than 0.4 and Phy/Zn lower than 5.0 indicate high Fe and Zn bioavailability, respectively (EFSA, 2019; Hurrell & Egli, 2010).
Standard deviations are presented ± ⊡ Different letters as superscripts represent significant differences ($p < 0.05$) in the same column, enclosed for flours and protein isolate samples.

product (Gläser et al., 2021; Roland et al., 2017). This phenomenon is compatible with the detected saponin levels of Swedish pea flour and isolates; around a two-fold increase in saponin concentration was observed for protein isolate samples compared to flour forms for all varieties. Similar cases of up concentration in protein isolates rather than pea flours were provided in the studies for Belgian peas (1.6 mg/g) (Martínez Noguera et al., 2022) and German peas (0.09 g/100 g isolate) (Assad-Bustillos et al., 2023) however, saponin content of Swedish pea varieties were observed to be higher than those pea varieties. Apart from the location/variety difference of these distinctive differences of saponin content, the saponin extraction method (ethanol/ethanol: water/water) and quantification method (spectroscopic/chromatographic) yield a broad range of results for the saponins content (Navarro del Hierro et al., 2018). Pea variety also had a big impact on the content of saponins in the pea protein isolates where proteins from Ingrid had the lowest (21.78 g/100g UAE extract) and Rokka showed the highest content (35.66 g/100g UAE extract) of saponins. The rest of the varieties showed saponin content in between these two varieties.

A drastic increase (3 ×) was monitored for the phytate content of all varieties in the protein isolates compared to the flour forms, due to the up concentration of phytate with the protein bodies with which they bonded. On the other hand, phytate content differences among the pea varieties were quite clear for both flour and isolate forms. Phytate content of the Swedish pea varieties was in the range of 6.7–11.0 μmol/g (570–730 mg/100 g) which was compatible with the literature data for the various peas. Green and yellow UK peas were observed to have phytate content of around 550 mg/100 g (Millar et al., 2019), Bolivian green and dry peas had around 60 and 344 mg/100 g phytate (Castro-Alba et al., 2019), Australian field peas had phytate in the range of 3.0–13.0 g/kg (N. Wang & Daun, 2004) while Canadian yellow and green peas contents were around 12.3 mg/g (L. Shi et al., 2018. The content of phytate in the pea protein isolates was also governed by the variety of the yellow peas. The proteins from Bagoo and Balder contained significantly ($p < 0.05$) higher content of phytate compared with proteins from the four other varieties. Protein isolated from Rokka showed the lowest content of phytate among the studied varieties. The right selection of pea variety can be a promising approach for the production of pea proteins with up to 25% less phytate.

As another handicap, an increased concentration of phytate would limit the bioavailability of some nutrients and minerals such as Fe and Zn more than their lower amounts (Fredlund et al., 2006). The molar ratio of Phy/Fe lower than 0.4 and Phy/Zn lower than 5.0 indicate high Fe and Zn bioavailability, respectively (EFSA, 2019; Hurrell & Egli, 2010). None of the pea varieties either in a protein isolate of flour forms had a desired estimated relative bioavailability for Fe and Zn (Table 3). In fact, the relative bioavailability for Fe was increased with protein isolation due to the co-increasing of Fe amount with protein isolation; however, this was not the same for Zn relative bioavailability. A similar approach was also conducted in a study for Swedish fava bean varieties and different Fe and Zn relative bioavailability values were detected among the different samples (Mayer Labba et al., 2021); however, pea samples were found to have mostly better relative bioavailability for Fe and Zn (8.6–14.0 for Fe, 11.8–17.0 for Zn) compared to the fava bean study (16.0–45.0 for Fe, 20.0–80.0 for Zn). The relatively lower phytate content of peas compared to the other legumes indicates their significant potential for higher mineral and nutrient bioavailability and their future applications (Chigwedere et al., 2023).

Consequently, the antinutrient content of the chosen pea/pea flour, and pea protein isolate are highly dependent on pea variety and protein isolation process. Choosing a variety that has relative antinutrient deficiency might ensure obtaining the protein isolate that has lower total antinutrient levels.

3.3.3. Lipoxygenase enzyme activity (LOX)

Off-flavor formation in pulses as well as many other food products are mainly attributed to the oxidation of fatty acids mediated by autooxidation or enzymatic activities. Lipoxygenase (LOX) is the major enzyme that degrades unsaturated fatty acids and induces off-flavor attributed compounds (Roland et al., 2017). Inherent LOX within the pea cotyledon storage parenchymatic cells becomes activated immediately after grinding into flour but the maximum LOX activity is observed following the dispersion of flour in aqueous media, particularly at neutral and/or basic pH values (Gao et al., 2020). Due to this reason, wet fractionation of proteins is considered as the most critical stage for LOX-mediated off-flavor formation in terms of the secondary products of unsaturated fatty acid oxidation. In Fig. 4, the LOX activity changes from pea flours to isolates are provided for different pea varieties. The variety of the studies of peas had a big impact on the LOX activity both in their flour and protein isolate. Maximum LOX activity was seen in Bagoo flour which was 3 times higher than that of the Ingrid flour. The sharp difference was still visible among the protein isolates of the different varieties, but the lowest LOX activity was seen in the Balder protein which was around 15 times lower than LOX activity in the Clara protein, that had the maximum activity.

The presence and even the quantities of LOX enzyme are also observed with the gel electrophoresis images in Fig. 2. Despite the LOX quantities slightly increasing in the protein isolates for all varieties (see Fig. 2) most likely due to their concentration, sharp diminutions for their activities from flour to isolate forms are prominent. This could be due to the structural changes induced by the pH adjustment, especially during the acidification process happening in the wet fraction process used. The highest LOX activity loss was observed for variety Balder (28.6 ×) followed by Bagoo (14.7 ×) while the lowest loss was recorded for variety Ingrid (1.6 ×).

Hexanal is one of the major LOX-derived undesired volatiles contributing to beany flavor. To distinguish hexanal increase (flour to isolate) with isolation-induced formation, the ratios of hexanal increase *versus* LOX activity degradation could be targeted. Based on that, variety Eso had the highest hexanal increase ratio (820 ×) with a low LOX degradation ratio (1.9 ×) while variety Balder had the lowest hexanal increase ratio (32 ×) with the highest LOX degradation ratio (28.6 ×).

The information about the LOX activity of the pea flours and protein isolates might be promising when considering the storage potentials of pea products. For instance, the protein isolate of variety Clara could have the highest further LOX-induced off-flavor formation potential while the variety Balder might have the lowest based on their LOX activities of around 79 and 5.9 U/g, respectively. Investigation of inherent LOX content of pea varieties might be considered as an additional strategy while screening out different pea (as well as other legumes) varieties for their better stability and flavor attributes. In a previous study, it was shown that protein isolates of twelve different pea varieties that were cultivated in different locations of Europe had distinctive LOX contents based on the conducted molecular weight distribution analysis both for reducing and non-reducing conditions (Arteaga et al., 2021). The mentioned study only focused on the properties of protein isolates from different pea varieties without including the pea flours. However, the outputs were in good agreement with this present study from the pea variety screening perspective.

Considering the similar distributions of linoleic and linolenic acids in flour and protein isolate samples (those fatty acids are recognized as the primary substrates for LOX) (Table 2), varying results for the beany volatiles such as hexanal and/or 2-pentylfuran (Table 4) might be another indicator of different LOX activities of the flour and protein isolate samples.

3.3.4. Volatile off-flavor compounds

As indicated in Table 4, the volatile concentrations for all targeted compounds increased with varying constants but overall, a 10^2–10^3 (2-3 order of magnitude) rise was detected when the proteins were isolated from the pea flours. Particularly the highest quantitative increments were observed for hexanal, 1-nonanol, 1-octen-3-ol, pentanol, and 3-methyl-1-butanol in descending order. Their attributed sensorial

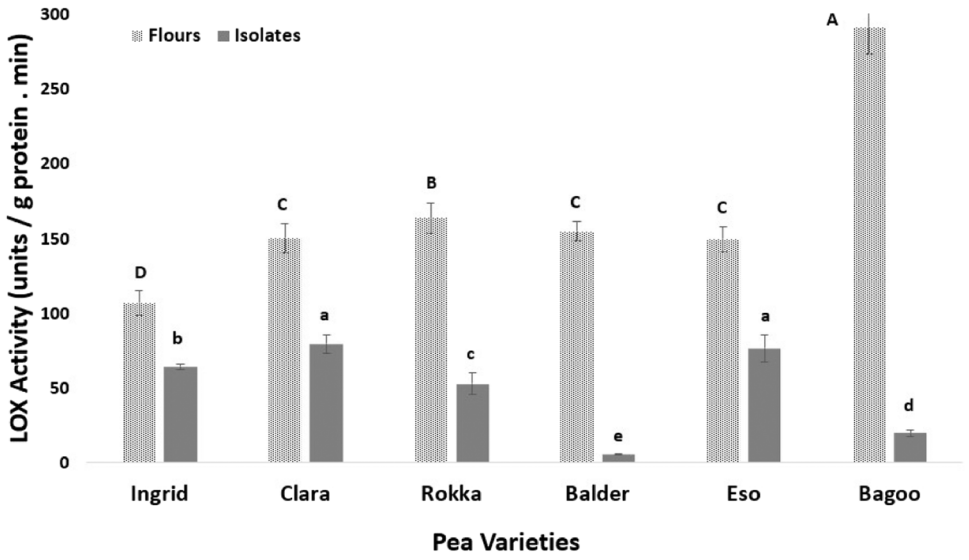

Fig. 4. Lipoxygenase activity (units × (g/min)) of flour and protein isolate of the six different studies pea varieties. Different capital and small letters indicate significant differences for pea flour and protein isolate samples, respectively ($p < 0.05$).

Table 4
Off-flavor attributed volatile compounds of flour, and protein isolate of the six studied yellow pea varieties (μM/g).

	Ingrid		Clara		Rokka		Balder		Eso		Bagoo	
uM/g	Flour	Isolate	Flour	Isolate	Flour	Isolate	Flour	Isolate	Flour	Isolate	Flour	Isolate
hexanal	4.11 ± 0.5^{b}	668.57 ± 159^{B}	2.14 ± 0.3^{b}	711.57 ± 287^{AB}	22.87 ± 6.9^{a}	1693.24 ± 365 ^{A}	22.27 ± 4.1^{a}	778.06 ± 73^{AB}	1.78 ± 0.2^{b}	1460.63 ± 114 ^{A}	1.83 ± 0.1^{b}	1154.47 ± 98^{AB}
1-hexanol	0.01 ± 0.0^{a}	0.41 ± 0.0 ^{A}	0.01 ± 0.0^{a}	0.46 ± 0.1 ^{A}	0.03 ± 0.0^{a}	1.09 ± 0.2 ^{A}	0.04 ± 0.0^{a}	0.52 ± 0.1 ^{A}	0.00 ± 0.0^{a}	0.00 ± 0.0 ^{A}	0.00 ± 0.0^{a}	0.59 ± 0.0 ^{A}
1-octen-3-ol	0.49 ± 0.0^{b}	320.57 ± 72^{B}	0.76 ± 0.0^{b}	161.03 ± 15^{B}	1.50 ± 0.4^{b}	1427.55 ± 117 ^{A}	6.23 ± 0.1^{a}	177.63 ± 31^{B}	0.91 ± 0.0^{b}	188.57 ± 8.3^{B}	0.46 ± 0.0^{b}	123.10 ± 9.5^{B}
benzaldehyde	0.06 ± 0.0^{a}	3.91 ± 2.5^{B}	0.01 ± 0.0^{a}	2.33 ± 2.3^{B}	0.04 ± 0.0^{a}	12.79 ± 2.9 ^{A}	0.09 ± 0.0^{a}	2.31 ± 0.2^{B}	0.00 ± 0.0^{a}	1.61 ± 0.3^{B}	0.00 ± 0.0^{a}	3.05 ± 0.3^{B}
2-pentylfuran	0.00 ± 0.0^{a}	0.47 ± 0.1 ^{A}	0.00 ± 0.0^{a}	0.26 ± 0.0 ^{A}	0.01 ± 0.0^{a}	1.08 ± 0.1 ^{A}	0.01 ± 0.0^{a}	0.37 ± 0.0 ^{A}	0.00 ± 0.0^{a}	0.22 ± 0.1 ^{A}	0.00 ± 0.0^{a}	0.32 ± 0.0 ^{A}
1-pentanol	0.72 ± 0.2^{a}	146.76 ± 34^{B}	0.17 ± 0.1^{a}	108.83 ± 8.9^{B}	0.81 ± 0.2^{a}	509.25 ± 97 ^{A}	0.30 ± 0.0^{a}	196.61 ± 14^{B}	0.03 ± 0.0^{a}	204.53 ± 19^{B}	0.42 ± 0.3^{a}	156.95 ± 20^{B}
1-nonanol	0.79 ± 0.1^{a}	481.58 ± 117^{B}	0.47 ± 0.0^{a}	424.86 ± 48^{B}	3.73 ± 1.2^{a}	1200.7 ± 252 ^{A}	2.99 ± 1.2^{a}	321.41 ± 47^{B}	0.17 ± 0.0^{a}	364.30 ± 36^{B}	0.21 ± 0.0^{a}	426.45 ± 38^{B}
2-methoxy-3-isopropylpyrazine	0.00 ± 0.0^{a}	1.30 ± 0.4 ^{A}	0.00 ± 0.0^{a}	0.71 ± 0.2 ^{A}	0.01 ± 0.0^{a}	0.77 ± 0.2 ^{A}	0.01 ± 0.0^{a}	0.16 ± 0.0 ^{A}	0.00 ± 0.0^{a}	0.41 ± 0.0 ^{A}	0.00 ± 0.0^{a}	0.21 ± 0.0 ^{A}
(*E,E*)− 2,4-nonadienal	0.34 ± 0.0^{a}	29.82 ± 6.6 ^{A}	0.28 ± 0.0^{a}	25.61 ± 2.8 ^{A}	2.25 ± 0.6^{a}	77.65 ± 16 ^{A}	2.33 ± 0.4^{a}	35.32 ± 3.5 ^{A}	0.25 ± 0.0^{a}	50.12 ± 5.3 ^{A}	0.18 ± 0.0^{a}	53.76 ± 5.3 ^{A}
(*E,E*)− 2,4-decadienal	0.00 ± 0.0^{a}	0.80 ± 0.3 ^{A}	0.00 ± 0.0^{a}	0.12 ± 0.0 ^{A}	0.01 ± 0.0^{a}	0.57 ± 0.0 ^{A}	0.01 ± 0.0^{a}	1.03 ± 0.8 ^{A}	0.00 ± 0.0^{a}	0.42 ± 0.1 ^{A}	0.00 ± 0.0^{a}	0.17 ± 0.0 ^{A}
3-methyl-1-butanol	0.66 ± 0.0^{a}	152.23 ± 0.0^{B}	0.21 ± 0.1^{a}	98.97 ± 16^{B}	0.43 ± 0.4^{a}	426.11 ± 87 ^{A}	0.09 ± 0.0^{a}	167.23 ± 16^{B}	0.08 ± 0.0^{a}	179.77 ± 13^{B}	0.04 ± 0.0^{a}	126.02 ± 10^{B}
∑	**7.18**	**1806.43**	**4.05**	**1534.75**	**31.68**	**5350.81**	**34.37**	**1680.65**	**3.23**	**2450.58**	**3.15**	**2045.08**

Standard deviations are presented □□□Different small letters as superscripts represent significant differences ($p < 0.05$) in the same row for flour samples. Different capital letters as superscripts represent significant differences ($p < 0.05$) in the same row for protein isolate samples.

perceptions are mostly defined as waxy/green, waxy/potato, and fatty/green while most studies pointed out hexanal (grassy) as the most dominating compound in pea protein isolates (Ebert et al., 2022; Y. Wang et al., 2020). In those studies, other compound concentrations such as 1-nonanol were also higher than the rest of the determined volatile compounds. The dominating "fatty/waxy" profile in this study could be a consequence of very high-fat content in the protein isolates (7.0–10.0%). The high-fat content of the Swedish pea varieties makes them even more susceptible to lipase/lipoxygenase-induced oxidation hence, more intense waxy/fatty off-flavors. The volatiles 1-pentanol, hexanal, 1-octen-3-ol, 2-pentylfuran, (*E,E*)− 2,4-nonadienal, (*E,E*)− 2, 4-decadienal are typically formed by the enzymatic oxidation of linoleic and linolenic acid. However, their formation is also possible under autooxidation in the absence of oxidizing enzymes (Murat et al., 2013; Trindler et al., 2022). Due to this reason, lipoxygenase and/or hydroperoxide lyase activities might be evenly contributing to off-flavor formation with autoxidation for all analyzed protein samples from different pea varieties. From another perspective, in terms of total marker volatile amounts, Clara, Balder, and Ingrid had the lowest off-flavor content compared to other varieties while the variety Rokka had 2–3 times higher total volatile concentration. Considering the fat content distribution (Table 2), it is not possible to make a clear deduction based on unsaturated fatty acid differences towards different off-flavor contents, since oleic, linoleic, and linolenic acid ratios of different pea varieties are not distinctive enough. It should be noted that Rokka and Balder already had a higher volatile concentration in their flour, particularly for hexanal. Due to this reason, the final off-flavor content of the isolate samples might also be a result of up concentration alongside

isolation-induced formation. Consequently, autoxidation-driven oleic acid degradation might be the determinant reaction for off-flavor formation.

From another perspective, the concentration of 2-pentylfuran was found to increase in another study more than that of hexanal and 1-nonanol, when the protein isolates were stored at 30 °C in the dark, for 12 months compared to storage at 20 °C (Fischer et al., 2022). It is an important indicator that storage/processing temperature has a significant importance on off-flavor formation and development.

Surely, along with the fatty acid content, inherent enzyme concentration, the potential of enzymatic activity, and presence/absence of antioxidant compounds in the biomass requires an overall elaboration for the volatile potentials of pea varieties. Also, considering extremely low perception threshold values (0.07–1.0(0.07–1.0 ng/g in water) of these volatiles, further utilization for the targeted protein isolates within the frame of consumer acceptance should be conducted with sensorial panels.

4. Conclusion

Variety selection of the crop, *i.e.*, peas, to find an inherently more compatible one for protein isolation might require less pre-/post processing, time, and energy consumption for desired quality criteria. For this purpose, six different yellow pea varieties cultivated in Sweden were screened as a protein source, and evaluated for their structural, functional, antinutritional, and off-flavor characteristics/differences in their flour and protein isolate forms. The content of antinutrients in both flours and isolates was significantly different among the different varieties where the lowest content of total phenols, tannins, and phytate were found in Rokka proteins. The content of saponins and phytate increased around 2 and 3 times during the wet extraction (flour to protein isolate) while phenolics and saponins decreased for all the varieties. As pea flours, varieties Rokka and Balder had the highest total content of the studied off-flavor volatile compounds. A significant amount of the volatiles was removed during protein isolation for Balder while it was the opposite for Rokka proteins which yielded the highest content of the beany flavor compounds. The minimum LOX activities were detected for Ingrid and Balder among the flours and proteins, respectively, which could help in selecting varieties with better stability. Altogether, screening and the right selection of yellow pea varieties can be a promising strategy to produce pea proteins with better functional, nutritional, and sensorial properties but it might be difficult to find a variety that is superior in all aspects.

Surely, many further confirmations are required for aroma characterization including perception threshold studies in model food matrices with sensory panels. On the other hand, nutritional limitations are of great importance and require in-depth investigations on both *in-vivo* and *in-vitro* scales. Exploring the pea varieties all over the world will provide important scientific and industrial know-how to progress with the urgent protein shift strategy for a more sustainable food/feed supply.

CRediT authorship contribution statement

Abdollahi Mehdi: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Gultekin Subasi Busra:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. **Forghani Bita:** Data curation, Methodology, Supervision.

Declaration of Competing Interest

The authors and the contributors from Lantmännen declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Author Agreement Statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.105988.

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