

Evaluation of pea (*Pisum sativum* L.) varieties for suitability in protein isolate production

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Abstract. As global interest in sustainable and plant-based protein sources grows, peas (*Pisum sativum* L.) have become an important crop due to their high protein content, favourable amino acid composition, and agronomic adaptability. Pea protein isolates are increasingly utilised in food production as functional, allergen-reduced alternatives to animal-derived proteins. However, the effectiveness of different pea varieties in isolate production varies, influenced by genotype and environmental conditions. This study evaluated 14 semi-leafless pea varieties cultivated in Latvia over two growing seasons to determine their suitability for protein isolate production. Analyses included proximate composition protein, amino acid profiling (e.g., alanine, glutamic acid, leucine, methionine, tryptophan), and agronomic traits such as yield and thousand-grain weight. The results revealed significant biochemical differences among varieties. The ‘Bruno’ variety, which was distinct from all others due to its pink flowers and brown-marbled seed coat, showed the highest levels of protein, alanine, and glutamic acid, indicating strong potential for isolate production and breeding programmes aimed at nutritional enhancement. Among the white-flowered, yellow-seeded varieties, several, including ‘Trendy’, ‘Kidam’, and ‘Eso’, also demonstrated favourable protein and amino acid profiles, making them promising candidates for further development. In contrast, varieties such as ‘Salamanca’, ‘Respect’, ‘Manager’, and ‘Alvesta’ were less suitable due to consistently lower protein and amino acid values. These findings provide insights into how regionally adapted pea varieties can support the development of a sustainable plant protein industry in Latvia and contribute to broader goals in food innovation and environmentally responsible agriculture.

Key words: Pea, protein profiling, legume breeding, plant-based, amino acid.

Introduction

The growing global demand for sustainable, plant-based protein sources has increased the attention focussed on legume crops, particularly peas (*Pisum sativum* L.), which combine high nutritional value with agronomic sustainability (Yanni *et al.*, 2023). Peas are rich in protein (typically 20 – 25%), complex carbohydrates (e.g., starch and dietary fibre), essential amino acids such as methionine, lysine, and cystine, as well as B-vitamins and important minerals including iron, magnesium, and potassium (Lam *et al.*, 2018; Thavarajah *et al.*, 2023; Tulbek *et al.*, 2024). Among these nutrients, protein is the most commercially significant, driving the increasing use of peas in protein isolate production. These isolates are high-purity protein fractions used in a range of food products including meat analogues, dairy alternatives,

bakery goods, and functional foods. The technological properties of pea protein, such as emulsifying, gelling, and foaming capacity, enhance its value in food formulations (Tulbek *et al.*, 2024). Despite their potential, pea proteins face certain limitations, such as suboptimal functional properties and a characteristic “beany” flavour. These challenges can be addressed through targeted processing and protein modification techniques (Shen *et al.*, 2022). Market interest in pea-derived ingredients continues to grow, supported by innovation in both breeding and processing. This includes improvements in extraction efficiency, protein quality, and suitability for specific applications (Hansen *et al.*, 2022). Globally, pea production has increased, with countries like Canada, Russia, China, and several EU member states among the top producers. According to FAOSTAT (2023), annual

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dry pea production now exceeds 14 million tonnes. Beyond their nutritional benefits, peas also provide agronomic advantages as nitrogen-fixing crops that improve soil fertility and reduce fertiliser input needs (Karkanis *et al.*, 2016; Yuvaraj *et al.*, 2020).

The success of protein isolate production depends heavily on the physicochemical properties of the raw material. Key factors include protein content, amino acid composition, solubility, and functional behaviour during processing (Lam *et al.*, 2017; Chudasama & Goyray, 2023). These traits are strongly influenced by genotype, environmental conditions, and cultivation practices. In particular, differences in seed morphology, cotyledon structure, and anti-nutritional components (e.g., trypsin inhibitors, phytic acid) can significantly impact protein recovery and isolate quality (Taghvaei *et al.*, 2022; Emkani *et al.*, 2023).

Given this variability, it is crucial to evaluate locally adapted cultivars to ensure high-quality, efficient isolate production under specific agroecological conditions. In Latvia, pea varieties have not been extensively studied in this context, despite the country’s unique climate and soil characteristics, which may influence seed composition and protein extractability. This study aims to evaluate 14 semi-leafless pea varieties grown in Latvia over two years, focussing on their protein content, amino acid composition, and agronomic traits. The goal is to identify the varieties most suitable for protein isolate production and to contribute to the development of a regionally adapted, sustainable plant protein supply chain.

Materials and Methods

Experimental design

This study evaluated 14 semi-leafless pea (*Pisum sativum* L.) varieties characterised by yellow cotyledons (Table 1), except for the variety Bruno, which has purple flowers and a brown marble seed coat. Field trials were conducted over two consecutive growing seasons (2023–2024) using a randomised complete block design with three replicates per variety. Each replicate consisted of a 12.5 m² plot managed under conventional agronomic practices.

Table 1

Sample code description

Sample code	Pea variety
Al	Alvesta
Ba	Bagoo
Br	Bruno
Es	Eso
In	Ingrid

Ka	Karakter
Kar	Karpate
Kid	Kidam
Ma	Manager
Or	Orchestra
Re	Respect
Sa	Salamanca
Sax	Saxon
Tr	Trendy

The trials were carried out at the Priekuļi Research Centre of the Institute of Agricultural Resources and Economics, located in Latvia (57°18'54" N, 25°21'41.292" E). This location was chosen due to its representative agroclimatic conditions for pea cultivation in the region.

The soil characteristics and crop management systems of the trials

A conventional soil tillage system, involving soil ploughing in autumn, was applied in both years. Field characteristics: soil sod podzolic (Vg) loamy sand (mS), with a weakly acidic pH in KCl solution 5.27 (2023) and acidic pH in KCl solution 5.0 (2024) soil reaction; organic matter content in the soil 1.8 and 2.3%; P₂O₅ content available to plants high 278 – 163 mg kg⁻¹, K₂O content medium 162 – 107 mg kg⁻¹ respectively, pre-crop in both years – summer barley. Sowing rate was 120 germinating seeds per m². Sowing was carried out on 26.04.2023 and 1.05.2024. In both years, basic fertiliser NPK (15-15-15) 300 kg ha⁻¹ was incorporated into the soil before sowing. To control weeds, immediately after sowing, the soil herbicide Fenix (aclonifen 600 g L⁻¹), 3.0 L ha⁻¹, was used, and later with the appearance of dicotyledonous weeds, in the elongation phase of the pea plants (AS 31 – 35), in 2023 spraying Bazagran 480 (bentazon 480 g L⁻¹) 3.0 L ha⁻¹, in 2024 Corum (imazamox 22.4 g L⁻¹ and bentazone 480 g L⁻¹) 1.0 L ha⁻¹, with the addition of Dash 1.0 L ha⁻¹. To control the black pea borer (*Cydia nigricana*) at the beginning of flowering (GS 60), the insecticide Decis Mega (deltamethrin 50 g L⁻¹) was applied at a dose of 0.150 L ha⁻¹ in 2023, and Evure (tau-fluvalinate 240 g L⁻¹) 0.2 L ha⁻¹ in 2023.

Meteorological conditions

At the end of April 2023, at the time of sowing, a pronounced moisture deficit was observed (Fig. 1), as a result of which the pea germinated and developed slowly. In 2024, sowing took place in good moisture conditions, which ensured uniform germination and further development of the plants. In both years, the amount of precipitation in May and June was below normal. The moisture deficit was particularly pronounced in 2023, when the amount of precipitation

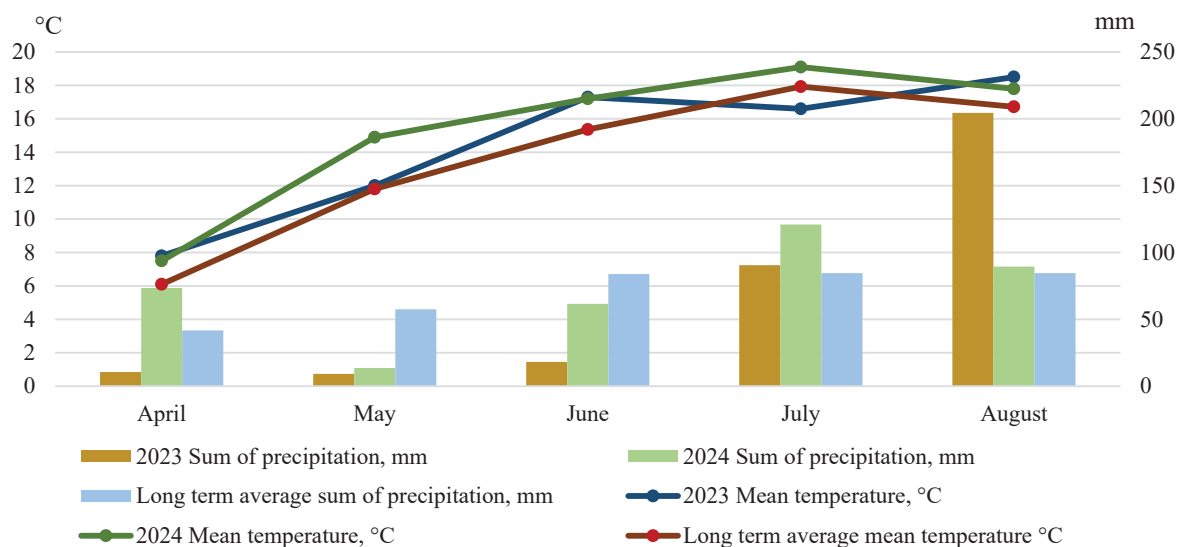


Figure 1. Amount of precipitation and average air temperature at Priekuli research centre 2023 – 2024.

in April, May, and June was significantly below normal. In 2024, at the end of July, during the pea ripening phase, heavy precipitation occurred. Some pea varieties lodged, making harvesting difficult and resulting in significant crop losses.

Parameters

All data were collected from field trials conducted over two consecutive growing seasons (2023 – 2024), with each of the 14 pea varieties sown in three replicates per year. For each replicate, representative seed samples were harvested and analysed. Initial data processing was performed separately for each year, after which mean values across the two years were calculated for further analysis. Sample analysis and data processing were carried out at the Plant Quality Laboratory of the Institute of Agricultural Resources and Economics, Department of Field Plant Breeding and Agroecology, Latvia.

Yield. Pea yield was determined from cleaned and dried seeds harvested per plot, expressed in tons per hectare ($t\ ha^{-1}$) at 14% standard moisture content. Data were recorded for each of the three replicates per variety per year.

Thousand-seed weight (TSW) was assessed by randomly selecting and weighing 1,000 seeds per replicate, using an analytical balance in accordance with International Seed Testing Association protocols. Results were expressed in grams (g), and three measurements were recorded per variety annually.

Protein and amino acid. A representative seed sample of approximately 50 g from each replicate of the trial was collected and milled into flour. Approximately 4.5 ± 0.5 g of flour from each replicate was then subjected to protein and amino

acid analysis. Protein and amino acid concentrations were analysed using a Rapid Content Analyser XDS (FOSS, Denmark), operating in the near-infrared (NIR) spectral range. The NIR system uses diffuse reflectance to acquire spectral data from ground samples placed directly into the sample holder, ensuring a homogeneous surface for accurate measurement. Each sample was scanned in triplicate across a spectral range of 400 – 2500 nm. Quantification of protein and amino acids was based on established calibration models developed from reference samples analysed using conventional wet chemistry. The amino acids measured included alanine, arginine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, proline, tryptophan, and valine. Multivariate regression methods, including Partial Least Squares, were applied to correlate spectral data with constituent concentrations. Model performance was validated through calibration and validation plots, and results were generated via integrated analytical software (WinISI 4).

Statistical analysis

Analysis of variance (ANOVA) was performed by using GENSTAT 14.0 (2011). ANOVA was used to determine the impact of variety, year, and their interaction on yield and quality parameters. A p -value of less than 0.05 ($p < 0.05$) was considered statistically significant. The proportion of the influence of factors was calculated from the variance components. Principal Component Analysis (PCA) was conducted to explore the multivariate variation among the 14 pea varieties based on their seed composition. PCA was performed using scaled and centred data in ClustVis software (Metsalu & Vilo, 2015).

Results and Discussion

The main parameters, such as pea yield, protein content, and thousand seed weight (TSW) were significantly influenced ($p < 0.01$) by both variety and year of cultivation, as well as the interaction between these two factors (Table 2).

The average yield level in 2024 was almost half (3.2 t ha^{-1}) higher compared to 2023 (Figure 3). The unusually dry and hot summer in Latvia in 2023 was the limiting factor for yields. In 2023, the lack of moisture during sowing, which continued until the beginning of July, affected the yield level. At the beginning of July, which coincided with the reproductive phase of peas, precipitation occurred, and the situation improved, but could not compensate for the effects of the drought. Other studies have also indicated that the lack of moisture is the most essential limiting factor for yields in peas, and its impact depends on its intensity, duration, and the growing stage of peas in which it occurs (Prudent *et al.*, 2015; Henriët *et al.*, 2019). Henriët *et al.* (2019)

indicate that a lack of water during the flowering phase (6 days) does not reduce biomass. However, as the author points out, the weight of grains per plant and the number of productive stages decreases, which, as a result, negatively affect the yield level.

The choice of variety is increasingly essential in sustainable crop production, ensuring high yield potential and quality. Pea varieties are characterised by certain morphological characteristics that allow growers to choose a variety in accordance with farm conditions and management practices. Semi-leafless pea varieties grown in Latvia were selected for the study. Between the two study years, the yield of most varieties differed significantly ($p < 0.01$). In 2023, under dry conditions, the highest yields were achieved by the varieties: 'Karpate', 'Saxon', and 'Orchestra' (Table 3). The yield level of most varieties in 2024 under conditions of better moisture supply was significantly higher compared to 2023. The most significant yield increase in 2024 was for the varieties: 'Eso', 'Respect', 'Manager', 'Orchestra',

Table 2

Proportion (%) of factors influencing yield and protein content

Factor	Genotype (G)	Year (Y)	Year × genotype interaction (G×Y)	Residual
Yield	0.1**	23.9**	52.3**	23.7
Protein content	10.2**	18.3**	53.3**	18.3
TSW	65.4**	6.2**	22.3**	6.0

** – significant influence of factor at $p < 0.01$.

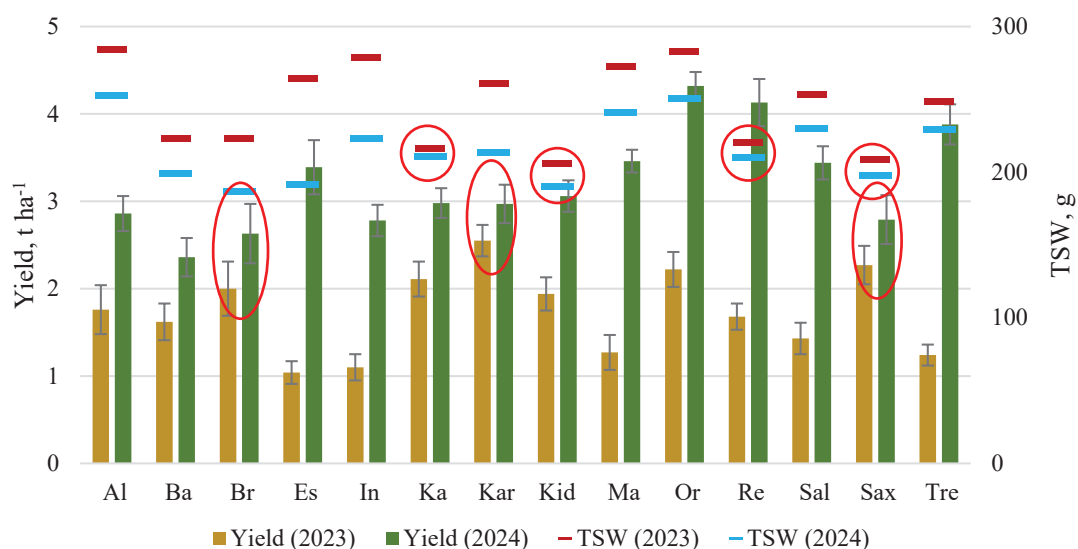


Figure 2. Yield (t ha^{-1}) and Thousand Seed Weight (TSW, g) in pea varieties in 2023 and 2024. The red oval marks the yield and Thousand Seed Weight of varieties that did not change significantly ($p > 0.01$) between the two study years.

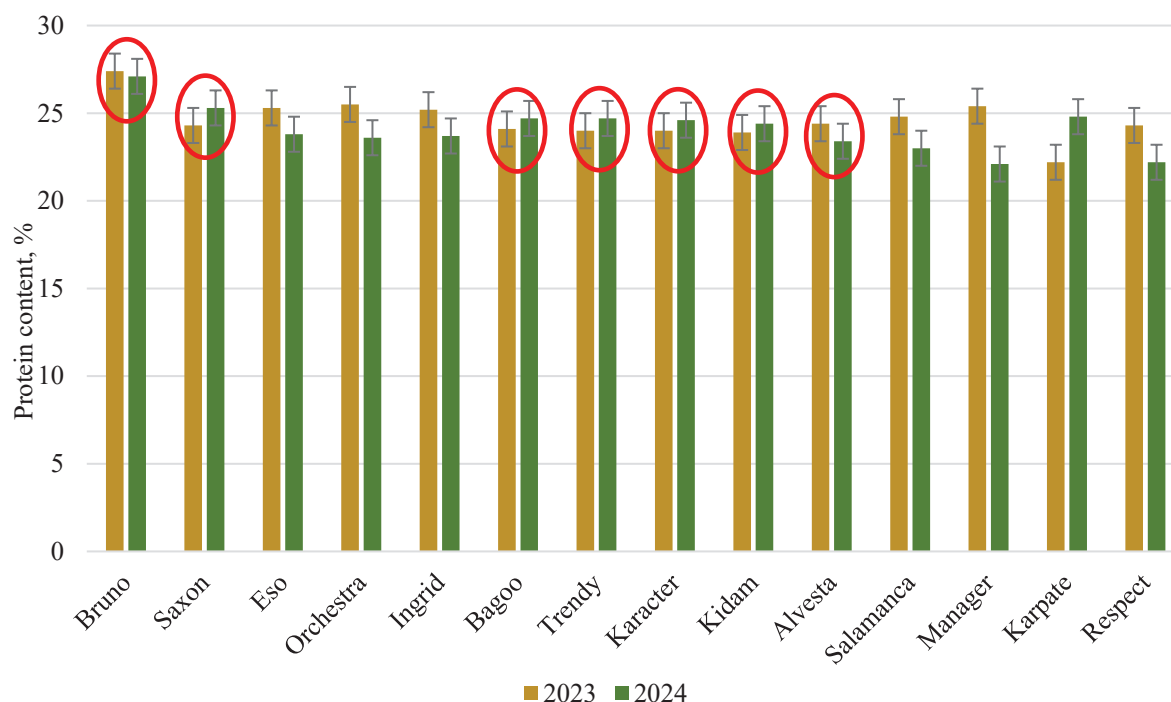


Figure 3. Protein content (%) in pea varieties in 2023 and 2024. The red oval marks the protein content of varieties that did not change significantly ($p > 0.01$) in both years of the study

and ‘Trendy’. In contrast, the yield of the varieties ‘Bruno’, ‘Karpate’, and ‘Saxon’ did not change significantly ($p < 0.01$) in both years of the study, which could indicate the ability of these varieties to adapt to changing weather conditions.

The thousand-seed weight (TSW) is a key agronomic trait reflecting seed size and mass, which can affect planting precision, mechanical harvesting efficiency, and post-harvest processing. The TSW for varieties was considerably higher in 2023 compared to 2024. In the context of protein isolate production, TSW may indirectly influence milling efficiency and protein extraction yield (Lam *et al.*, 2017). In this study, TSW values (Fig. 2.) among the evaluated varieties ranged from 198.09 g (Kidam) to 268.53 g (Alvesta), indicating substantial genotypic variation. Larger-seeded varieties such as Alvesta (268.53 g), Orchestra (266.95 g), and Manager (256.81 g) exhibited the highest TSW values, whereas smaller-seeded varieties such as Kidam (198.09 g), Saxon (203.33 g), and Bruno (205.04 g) displayed the lowest values. Although TSW does not directly correlate with protein concentration, it is an important factor in seed handling and processing (Gu, 2014). Larger seeds may offer processing advantages in dehulling and flour production, which are relevant to protein isolate extraction workflows. Conversely, smaller seeds may result in greater surface area relative to mass, potentially affecting extractability and functional quality.

The impact of climate-related parameters, such as drought and temperature, on the protein content of seeds is also significant due to expected climate change. Compared to 2023, which was characterised by a relatively large moisture deficit, thus contributing to the formation of high protein content (average 24.6%) in peas, a small but significant ($p = 0.03$) decrease in protein content was observed in 2024 (average protein content 24.1%). Other studies in Germany and the USA, which analysed pea samples collected from production fields, also indicated that high air temperatures and lack of moisture are the main climate-related parameters determining high protein content in seeds (Bestwick *et al.*, 2018; Walter *et al.*, 2022). The study’s results showed that the effect of variety on protein content in seeds was significant ($p = 0.004$).

The highest protein content in both years was in the pink-flowered variety ‘Bruno’, which has brown marbled seeds. Due to its high protein content and good taste, the variety is used more as a niche product, in traditional Latvian cuisine and in producing various food products. Tzitzikas *et al.*, (2006) and Bastianelli *et al.*, (1998) indicate that the brown seed coat colour may be associated with a higher protein content than that of yellow peas. Thus, the variety ‘Bruno’ was removed from further statistical data analysis. The other varieties included in the trial were white-flowered with yellow peas; among them, the

protein content in the seeds varied from 23.3% to 24.8%. In the group of white-flowered varieties, the protein content in the seeds above 24.0% was found in the varieties: 'Saxon', 'Orchestra', 'Eso', 'Ingrid', 'Trendy', 'Bagoo', 'Karacter' and 'Kidam'. It should be noted that the varieties responded differently to changes in agroclimatic conditions. Of the above-mentioned varieties, the most minor changes in protein content between the two study years were in the seeds of the varieties 'Saxon', 'Bagoo', 'Trendy', 'Karacter' and 'Kidam'. Regarding the other pea varieties, the protein content in the seeds changed significantly between the two study years. In a study involving peas, Mohammed *et al.* (2018) indicate that the variation in protein content in seeds between different varieties is smaller than the variation between years and different growing locations. The author attributes this to differences in moisture supply during the season, soil properties, temperature during the flowering phase, etc. The results of our study showed that the protein content in seeds varied significantly between varieties compared to the differences between years. However, this finding is not yet substantiated, since the trial was conducted for only two years and at one growing location in Latvia.

The amino acid profile was grouped into essential amino acids and non-essential amino acids (see Fig. 4)

Among the evaluated pea varieties, 'Bruno' exhibited the highest concentrations of both essential and non-essential amino acids, indicating a superior nutritional profile. These elevated levels suggest

a strong potential for high-quality protein isolate production, as essential amino acids contribute directly to the nutritional completeness of the isolate, while non-essential amino acids such as glutamic acid and alanine can enhance flavour and functionality during processing (Lam *et al.*, 2017). In contrast, varieties such as 'Salamanca', 'Respect', 'Manager', and 'Alvesta' displayed consistently lower concentrations of essential amino acids, particularly methionine and leucine, as well as reduced levels of non-essential amino acids like alanine and glutamic acid. These deficiencies may limit their suitability for isolate production where both nutritional value and sensory performance are important. Intermediate profiles were observed in varieties such as 'Trendy', 'Kidam', and 'Eso', which showed moderately high levels of both amino acid groups, supporting their potential for use in standard protein isolate formulations. Differences among other genotypes were relatively minor and not statistically significant. Pea proteins in general provide a well-balanced profile of essential amino acids but are typically limited by sulphur-containing amino acids such as methionine (Ciurescu *et al.*, 2018; Wu *et al.*, 2023). In contrast, branched-chain amino acids (leucine, isoleucine, valine), along with histidine, account for approximately 33 – 34% of total protein content in peas (Gorissen *et al.*, 2018), making them relevant for functional food applications. Reported total amino acid content in peas ranges from 19.7 to 23.5 g 100 g⁻¹ seeds (Arslan, 2017).

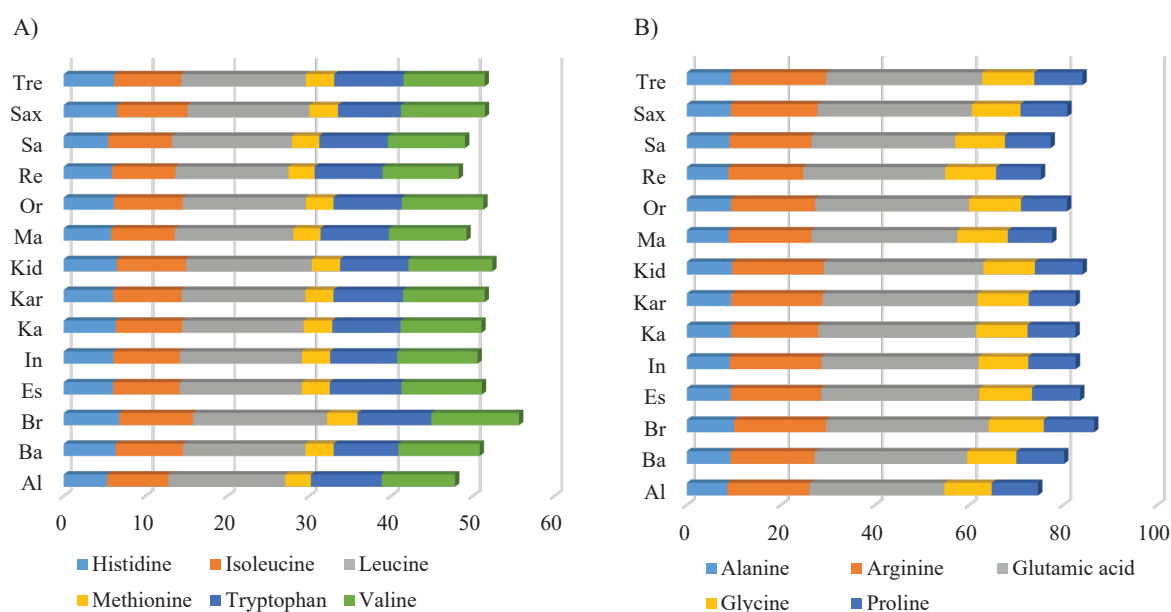


Figure 4. Amino acid (g kg⁻¹) composition of pea varieties averaged over two growing seasons: (A) Essential amino acid profile including histidine, isoleucine, leucine, methionine, tryptophan, and valine; (B) Non-essential amino acid profile including alanine, arginine, glutamic acid, glycine, and proline.

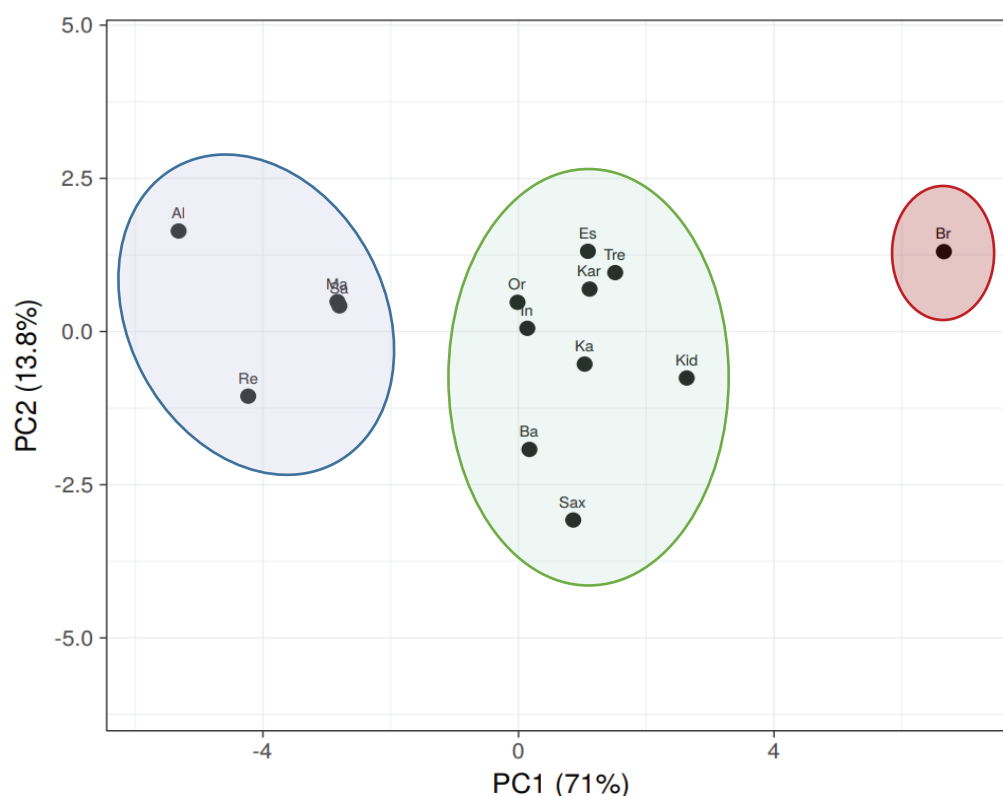


Figure 5. Principal component analysis of protein content, amino acids, and thousand seed weight in pea (*Pisum sativum* L.) varieties

Principal Component Analysis (PCA) was conducted to explore the multivariate variation among the 14 pea varieties (see Fig. 5.) based on their seed composition. The analysis included the average values of two growing seasons (2023 – 2024) for protein content, essential and non-essential amino acid concentrations, and thousand seed weight (TSW). PCA was performed using scaled and centred data in ClustVis software (Metsalu & Vilo, 2015). The first two principal components (PC1 and PC2) explained 71.0% and 13.8% of the total variance, respectively. PC1 was primarily driven by variation in protein content and essential amino acids such as valine, leucine, and isoleucine, while PC2 reflected differences in TSW and non-essential amino acids, particularly glutamic acid and glycine. The distribution of pea varieties in the PCA biplot thus provides insight into compositional and morphological characteristics relevant to protein isolate production, as TSW is closely related to seed size and processing efficiency.

To identify genotype clusters based on these biochemical and physical characteristics, hierarchical clustering and one-way ANOVA were applied. This approach grouped the pea varieties into three distinct clusters:

Group 1: 'Bruno';

Group 2: 'Bagoo', 'Eso', 'Ingrid', 'Karacter', 'Karpate', 'Kidam', 'Orchestra', 'Saxon', 'Trendy';
Group 3: 'Alvesta', 'Manager', 'Respect', 'Salamanca'.

Based on PCA separation and trait-driven classification. The use of PCA for uncovering hidden structure in multivariate nutritional data is well-established in plant breeding and food sciences (Granato *et al.*, 2018). One-way ANOVA revealed statistically significant differences ($p < 0.05$) among the clusters for the following traits: protein content ($F = 16.12$, $p = 0.0005$); alanine ($F = 36.26$, $p = 0.00001$); arginine ($F = 8.76$, $p = 0.0053$); glutamic acid ($F = 33.50$, $p = 0.00002$). These traits are known to be important contributors to the nutritional quality of pulses and have high heritability, making them suitable targets in breeding programmes (Jha & Warkentin, 2020; WHO/FAO/UNU, 2007). These biochemical traits contributed most to the separation between clusters in the PCA plot. Group 1 represents a genotype with a distinct amino acid profile, exhibiting high levels of alanine and glutamic acid, as well as above-average protein content. Notably, this group includes the pink-flowered variety 'Bruno', characterised by its brown marbled seeds. This variety stood out visually even before the analysis, and its biochemical

uniqueness was confirmed by PCA, under which it clearly separated from all other genotypes and did not cluster with any major group. The consistently high protein content observed across both years further reinforces its exceptional profile. Such profiles have been associated with improved seed functionality and dietary value (WHO/FAO/UNU, 2007). Despite its lower TSW compared to some other varieties, 'Bruno's' exceptional protein and amino acid composition suggests significant potential for high-quality protein isolate production, particularly when breeding strategies prioritise nutritional enhancement over seed size alone. Its unique composition suggests potential as a donor genotype in biofortification strategies (Jha & Warkentin, 2020). Group 2 includes the majority of genotypes and demonstrates a balanced biochemical profile, serving as a representative reference group for breeding populations. Its intermediate protein and amino acid levels, combined with varied TSW may reflect modern cultivar development strategies prioritising both agronomic and nutritional traits, including appropriate seed size (Burstin *et al.*, 2015). Group 3 shows consistently lower protein content and amino acid values overall, with notably reduced levels of alanine and glutamic acid, both of which are non-essential but contribute to flavour and seed functionality. However, varieties like 'Alvesta' and 'Manager' presented higher TSW, indicating larger seed size, which could provide processing advantages despite their nutritional limitations. These genotypes may be more agronomically adapted or disease resistant but may require nutritional enhancement via targeted crosses. Targeted breeding efforts combining these larger-seeded genotypes with nutritionally superior varieties may enhance overall seed quality and suitability for protein isolate production (Baxter *et al.*, 2014).

This multivariate analysis reveals that pea varieties exhibit significant biochemical variation, particularly in protein content, TWG and the composition of amino acids. PCA and clustering effectively distinguished a nutritionally elite genotype (Group 1), a balanced main group (Group 2), and a group with potential for improvement (Group 3). These findings support the application of PCA and biochemical profiling in marker-assisted and trait-based selection approaches for quality seed development for protein isolate production.

Conclusions

This study assessed 14 semi-leafless pea varieties cultivated under Latvian conditions, evaluating their potential for pea protein isolate production based on agronomic performance, seed biochemical traits, and thousand-seed weight (TSW). The findings

highlighted significant differences among varieties in yield, protein concentration, amino acid composition, and TSW, emphasising the strong influence of yearly climatic variations on these parameters. Results indicated notable genotype $\times$ year interactions, particularly affecting yield, protein content, and seed size, reinforcing the necessity of evaluations across multiple growing seasons to achieve reliable cultivar selections. Varieties with brown marbled seeds, which showed an outstanding biochemical profile, may serve as candidate genotypes for breeding programmes aimed at improving the nutritional and functional properties of pea protein isolates. The varieties with larger seeds may have an advantage during processing despite their nutritional limitations. This suggests that targeted breeding combining large seeds with higher nutritional genotypes could improve the suitability for isolate production. Varieties with balanced nutritional value and physical seed properties (moderate thousand-grain weight) make them suitable candidates for applications involving protein isolates meeting nutritional standards, or for inclusion in breeding programmes targeting a balance between yield and seed processing efficiency.

Nevertheless, further research is necessary to comprehensively assess varietal suitability for commercial protein isolate production. Future studies should investigate additional crucial traits, including anti-nutritional factors (trypsin inhibitors, tannins, phytic acid), seed coat structure, and seed lipid content, all of which can significantly influence isolate purity, protein extractability, processing performance, and sensory characteristics. This research highlights the potential of selecting locally adapted pea varieties as a basis for developing a sustainable and region-specific plant protein industry in Latvia. Targeted breeding and selection approaches, incorporating biochemical and morphological parameters such as protein composition and TSW, will help meet the demand for high-quality plant-based protein ingredients. This approach supports both local agricultural sustainability and broader global objectives for diversified and environmentally responsible protein production.

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