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Compositional diversity in pea flour: Effects on functional properties

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ABSTRACT

Peas, recognized for their nutritional richness and sustainability in food systems, are increasingly important for global food security. Although flours vary widely in composition, the effects of starch and protein on their functional properties in food applications remain poorly understood. To investigate this, we strategically selected 30 pea accessions from 265 accessions based on genetic variation and explored how the composition of pea flours relates to their functional properties. Analysis of the flours revealed wide-ranging composition across the accessions, with total starch (29–51 %), protein (22–35 %), amylose (11–25 %) and amylopectin (10–36 %) content on a dry flour basis. Notably, the composition was significantly associated with pea phenotype. Peas with lower total starch and amylopectin levels, along with higher fibre and amylose content, were generally more wrinkled. Protein profiles, assessed through SDS-PAGE, revealed variations in legumin and vicilin content. Peas with lower legumin and higher vicilin and low-molecular-weight pea albumin fractions, such as PA 1 and lectins, were likely to be more wrinkled. Functional assessments highlighted diverse properties linked closely to flour composition. Higher protein content corresponded to lower protein solubility, particularly pronounced in smaller particles where protein-starch/fibre interactions may be enhanced. Thermal sensitivity, assessed by comparing volume changes in pea suspensions before and after heating, expressed as the swelling factor (SF), also differed depending on composition. Flours with lower amylopectin exhibited lower sensitivity and a reduced SF, attributed to lower total starch and legumin levels. Conversely, flours with higher amylopectin content showed higher thermal sensitivity and variable SF, associated with higher total starch and legumin levels. This study reveals the impact of pea composition on functionality, providing insights for utilizing diverse pea varieties in the development of innovative pea-based ingredients.

1. Introduction

Pea (*Pisum sativum* L.) is among the most widely cultivated pulses after soybean, with global production steadily increasing from 22.9 Mt in 2000 to 34.5 Mt in 2020 (FAOSTAT, 2022). Dried pea seeds exhibit considerable morphological diversity in size, colour, and surface wrinkling (Brhane and Hammenhag, 2024; Dueholm et al., 2024). They are broadly categorized into green and yellow peas based on hull colour, and into smooth (round) and wrinkled types based on seed shape (Dueholm et al., 2024; Ren et al., 2021).

The primary components of dried pea seeds include protein (17–38 %), starch (40–60 %), and fibre (15–30 %), with their composition varying due to differences in climate, growth conditions, and genetic diversity among accessions (Boye et al., 2010; Brhane and Hammenhag, 2024; Ratnayake et al., 2002; Shanthakumar et al., 2022). Protein and starch are primarily concentrated in the cotyledon, where proteins are organized in protein bodies, and starch in granules (Kornet et al., 2020). The cotyledon also contains soluble and insoluble fibres, while the hull primarily consists of water-insoluble fibres (Dalgetty and Baik, 2003; Tosh and Yada, 2010).

Pea-derived ingredients such as protein, starch, and fibre, have gained popularity in food formulations, particularly in plant-based alternatives (Lyu et al., 2022, 2023, 2024). These ingredients vary in refinement levels (Yang et al., 2024). For aqueous or wet fractionation, pea protein isolate contains ~85 % protein with minimal starch and fibre content, produced through intensive processes like milling, protein extraction, precipitation, and drying. Pea protein concentration contains ~50 % protein, with moderate starch and fibre, and involves milder extraction processes. However, its poor gelation properties of more-refined protein isolate and concentration pose a challenge for its

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use in pea-based ingredients. Pea flour, a less-refined product made by milling whole peas into a powder, retains all the natural components of peas, including protein (~20 %), starch, and fibre. While studies often focus on more-refined protein isolate and concentration, the functional potential of less-refined pea flour and the role of protein-starch interactions remain underexplored.

Starch content is linked to pea phenotype, primarily due to a deficiency in starch branching enzyme I (SBEI) during seed development (Bhattacharyya et al., 1993; Edwards et al., 1988). In wrinkled peas, SBEI activity is less than 15 % of that in smooth peas, resulting in reduced amylopectin biosynthesis and an accumulation of sucrose (Casey et al., 1998). This sucrose accumulation may contribute to increased amylose and fibre content, as studies have observed higher amylose fractions and fibre levels in wrinkled peas (Ren et al., 2021; Sun et al., 2023). However, limited research has clarified which components primarily arise from the excess sucrose. Differences in protein composition between smooth and wrinkled peas have also been reported. Wrinkled peas are characterized by lower legumin and higher vicilin levels compared to smooth peas (Daba et al., 2024; Schroeder, 1982). However, the variation in pea albumin fractions, such as PA2, PA1, lectins, and lipoxygenases, remains underexplored.

The functional properties of peas vary due to their compositional difference and the interactions between compositional factors. For instance, protein solubility, a critical factor for protein functionality, depends on the balance of hydrophobic protein-protein and hydrophilic protein-solvent interactions. Generally, more-refined pea isolates typically show a U-shaped solubility-pH curve, with the minimum occurring near the isoelectric point. At this pH, the proteins carry no net charge, making them least soluble and prone to aggregation. For the main storage proteins, legumin and vicilin, this point is around pH 5.0 (Barac et al., 2015). For less-refined pea flours, the protein solubility was typically lower (20–30 %) compared to protein concentrates and isolates (80–90 %) from wet fractionation (Barac et al., 2015; Kornet et al., 2020). Moreover, the interactions between compositional factors might also affect solubility. Flours obtained from wrinkled peas exhibited more fused granules composed of starch, protein, and fibre, while flours from smooth peas showed less fusion and retain oval-shaped granules (Sun et al., 2023). This suggests potential differences in their protein solubility. However, studies comparing flours from diverse pea accessions are limited.

The thermal sensitivity of pea flours is also critical, as it affects gelation properties. Unlike fibres, which underwent minimal swelling during heating (Karlsson et al., 2024), starch granules significantly changed their structure due to water absorption and swelling, governed by the amylose-to-amylopectin ratio (Ji et al., 2022). Higher amylose content reduced swelling because amylose double helices required higher temperatures for disruption compared to amylopectin's crystalline structures (Debet and Gidley, 2006). Despite its importance, research on the thermal behaviour of pea flours is scarce, and further insights could aid in optimizing gelation and textural properties of pea-based formulations.

This study aimed to investigate how starch composition (amylopectin and amylose) and protein profile relate to the functional properties of pea flours using a highly diverse set of accessions. Based on genetic diversity, flours from 30 pea accessions were examined. These accessions were strategically selected from a panel of 265 pea accessions representing high genetic variation. Differences in main components, starch composition and protein profile, protein solubility, and thermal sensitivity were evaluated across these accessions. By elucidating the interplay between pea composition and functionality, this study provides a foundation for optimizing the use of diverse pea varieties in food systems.

2. Materials and methods

2.1. Materials

Reagents for gel electrophoresis were obtained from Invitrogen (Hvidovre, Denmark). Other chemicals (ACS grade or higher) were purchased from Thermo Fisher Scientific (Roskilde, Denmark) and Sigma-Aldrich Denmark A/S (Søborg, Denmark). All samples were prepared using ultra-pure Milli-Q water.

2.2. Pea seed selection and the pea morphology

A panel of 30 pea accessions was selected from a total of 265 accessions based on marker data, which revealed significant genetic differentiation among them (Brhane and Hammenhag, 2024), with detailed information provided in Table S1. Seed morphological traits: colour, size and the degree of wrinkling were diverse to obtain the possible diverse in the composition. The seeds used for analysis were harvested from plants grown in southern Sweden (55.90°N, 13.09°E) under field conditions in 2022. Planting was made on April 26, followed coverage with fibre cloth for establishment. Tall and vining plants were supported with metal trellises. Seeds were harvested manually from mature plants between July 11 and August 26, threshed, weighed, and stored at 4 °C with a moisture content of 7 %.

The colour of the pea seeds was varied from creamy yellow, yellow-green, light green, green, dark green, and army green to orange-brown and brown according to Brhane and Hammenhag (2024).

The size of pea seeds from the 30 accessions (100–800 g) was assessed using a Marvin Proline Seed Analyser (Marvitech, Germany). High-resolution images were captured to trace seed boundaries, calculating the two-dimensional surface area (mm^2). Additionally, the thousand-kernel weight (TKW) was also recorded.

The degree of wrinkling was visually evaluated by five experienced members of the group, each assigning a specific score to represent the level of wrinkling. Higher score indicates higher degree of wrinkling. The categories are as follows: MW (more wrinkled pea) with a value of "5", W (wrinkled pea) with a value of "4", M (medium pea) with a value of "3", S (smooth pea) with a value of "2" and MS (more smooth pea) with a value of "1".

2.3. Milling of peas into flours

Pea seeds (20 g) were pre-ground using a Bosch coffee grinder to break them into small particles. The hulls were manually removed, and the cotyledons were further ground into flour. The flour was passed through a 0.25 mm sieve and stored in a fridge (4 °C) until analysis.

2.4. Protein content

The Dumas method was used to quantify nitrogen content in the pea flours using an Organic Elemental Analyzer (vario MACRO cube, Elementar, Hesse, Germany). Protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25. Each sample was analysed in triplicate.

2.5. Total starch, amylose and amylopectin content

Total starch content in the pea flour was measured using a Total Starch Assay Kit (Megazyme, Bray, Ireland) following AACC Method 76-13.01 as described by Dueholm et al. (2024). The resistant starch method (RTS-NaOH Procedure) was applied, and each sample was measured at least twice.

Starch component of amylose and amylopectin was determined enzymatically using an Amylose/Amylopectin Assay Kit (Megazyme, Bray, Ireland) following the manufacturer's protocol with some modifications. Briefly, 25 mg of flour was mixed with 0.5 mL of 80 % ethanol

and stirred gently on a vortex mixer. The suspension was heated in a boiling water bath for 15 min. It was then removed and allowed to cool to room temperature. Once cooled, 2 mL of 95 % ethanol was added, followed by an additional 4 mL of 95 % ethanol to precipitate the starch. The sample was centrifuged at 5000 g for 5 min, and the resulting pellet was processed with sodium hydroxide and buffer solutions to obtain Solution A. Further steps followed the manufacturer's protocol as described by Dueholm et al. (2024).

Amylose content in the flour was calculated using the formula:

$$\text{Amylose content}\,(\%) = \text{Total starch content} * \text{Amylose fraction in starch}\,(\%) \quad (1)$$

Amylopectin content was determined by subtracting the amylose fraction from the total starch fraction. Each measurement was conducted at least twice.

2.6. Gel electrophoresis

Gel electrophoresis was performed following the method of Joehnke et al. (2019) with modifications. Pea flours (14–20 mg, containing 4 mg of total protein) were solubilized in 0.6 mL of 0.1 M Tris-HCl buffer (containing 5 % SDS, pH 8.0). Protein extraction was carried out using a Mixer Mill (Retsch MM400, Haan, Germany) at a frequency of 30 Hz for 20 min. The samples were then heated at 80 °C for 10 min on a Microplate Shaker (350 rpm), followed by centrifugation at 22,000 g for 10 min to collect the supernatant.

The supernatant was diluted tenfold, and the protein concentration was measured using a NanoDrop 1000 Microvolume Spectrophotometer (Thermo Fisher Scientific, Waltham, USA). For non-reducing conditions, 65 μL of diluted protein solution was mixed with 25 μL of lithium dodecyl sulfate (LDS) buffer (4 ×) and 10 μL of Milli-Q water to achieve a final protein concentration of 1 mg/mL. For reducing conditions, 10 μL of 1 M DTT was added as the reducing agent instead of Milli-Q water.

Both reduced and non-reduced samples (7 μL) were loaded onto 4–12 % NuPAGE Bis-Tris gels. Protein markers (3 μL, 3–260 kDa) were loaded in lanes on both sides of the gel. Electrophoresis was run at 200 V for 30 min. The gels were stained with Coomassie brilliant blue for 40 h and rinsed with Milli-Q water until the background was clear. Scanning was performed using an Epson Perfection V850 Pro scanner (Suwa, Japan), and band intensities were analysed using TotalLab 120 software (v2008, Nonlinear Dynamics Ltd., Newcastle upon Tyne, UK) with parameters: minimum slope 100, noise reduction 5, and maximum peak 5 %. Each sample was analysed at least twice.

2.7. Protein solubility

Protein solubility was measured based on the method of Zhang et al. (2024) with modifications. Pea flour (0.1 g) was dispersed in 10 mL of 0.1 M Tris-HCl buffer (pH 7.0) and shaken continuously for 18 h to ensure complete hydration. The mixture was centrifuged at 20,000 g for 30 min, and the supernatant volume was recorded. Protein concentration in the supernatant was determined using a BCA protein assay kit. Protein solubility was calculated as the percentage of protein content in the supernatant relative to the total protein content in the flour. Each sample was analysed in duplicate.

2.8. Particle size determination and swelling factor

Flour dispersions were prepared by mixing 0.5 g of flour with 99.5 g of water (0.5 % w/w) and stirring for 30 min. This non-centrifuged dispersion served as the raw bulk solution. A portion of the solution was centrifuged at 500 g for 3 min at 20 °C served as the native bulk solution, while the remainder was heated at 95 °C for 20 min, then cooled in a 0 °C water bath for 5 min, obtaining the heated bulk solution.

Particle size distributions of both solutions were measured using a Mastersizer 3000 (Malvern Instruments Ltd., Malvern, UK) with a Hydro-SM accessory. The refractive index of water was set at 1.33. The volume-weighted mean particle diameter ($d_{4,3}$) was recorded for each sample in triplicate.

Swelling factor (SF) was calculated as the ratio of the volume of swollen particles ($V_{swollen}$) after heating to the volume of native particles (V_0) before heating, assuming spherical particles:

$$SF = \frac{V_{swollen}}{V_0} \sim \frac{(d_{4,3\text{-H}})^3}{(d_{4,3\text{-NH}})^3} \quad (2)$$

where the $d_{4,3\text{-NH}}$ and $d_{4,3\text{-H}}$ are the particle sizes of non-heated (native pea suspension) and heated pea suspension, respectively.

SF reflects the thermal sensitivity of the pea suspension. Values above or below “1” indicate swelling or fragmentation upon heating, representing higher thermal sensitivity, whereas values near “1” indicate minimal volume change and lower thermal sensitivity.

2.9. Statistical analysis

Results are presented as mean values ± standard deviation. Differences among the 30 pea samples were analysed using ANOVA with a significance threshold of $p < 0.05$. Statistical analyses were conducted using IBM SPSS 25 (IBM SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was performed with LatentiX 2.12 software (LatentiX Aps, Gilleleje, Denmark).

3. Results and discussion

3.1. Morphology and main component

As illustrated in Fig. 1, the selected 30 pea accessions displayed considerable diversity in seed morphology, including size (Area: from 16 to 77 mm^2), thousand-kernel weight (TKW: from 57 to 414 g), and colour, which varied from creamy yellow, yellow-green, light green, green, dark green, and army green to orange-brown and brown. The degree of wrinkling was used to classify the accessions into five groups: more wrinkled (MW1-5, 5 accessions), wrinkled (W1-3, 3 accessions), medium (M1-5, 5 accessions), smooth (S1-5, 5 accessions), and more smooth (MS1-12, 12 accessions).

After grinding and sieving, the pea seeds were milled into flour, with the hulls removed. The yield of the flour (seed cotyledon) was 90.3 ± 1.2 %, the hull yield was 5.7 ± 1.1 %, and the mass loss was 4.0 ± 0.7 % (Table S2). The peas naturally exhibited a wide range of main components (summarized in Table 1, all on a dry basis of the flour). Total starch content averaged 44.5 ± 5.8 %, ranging from 29.1 % to 50.9 %, with amylose at 16.3 ± 3.3 % (11.4–24.6 %) and amylopectin at 28.2 ± 8.0 % (9.7–35.5 %). Average protein content was 27.2 ± 3.1 %, ranging from 21.7 % to 34.8 %. Consequently, the combined protein-and-starch content was 71.1 ± 5.1 % (60.7–78.8 %), while other components (primarily fibre) accounted for 21.3 ± 5.1 % (14.2–32.3 %).

To visualize the differences among the 30 pea accessions, the principal component analysis (PCA) of the main components was plotted in Fig. 2A. The analysis clearly showed that the more wrinkled group was distinctly separated from the other groups (wrinkled, medium, smooth, and more smooth). Based on substantial differences in main components, the 30 pea accessions were also divided into two groups: high-amylopectin group, which includes more wrinkled group by visual assessment, and low-amylopectin group, which includes from more smooth to wrinkled groups by visual assessment. As shown in Table 1, the low-amylopectin group was characterized by lower total starch, comparable protein levels, but higher amylose and other components (primarily fibre). Consequently, the low-amylopectin group also had lower combined protein-and-starch content. This trend was reflected in Fig. 2A, where amylose and other components clustered near the low-amylopectin group, while total starch was closer to the high-

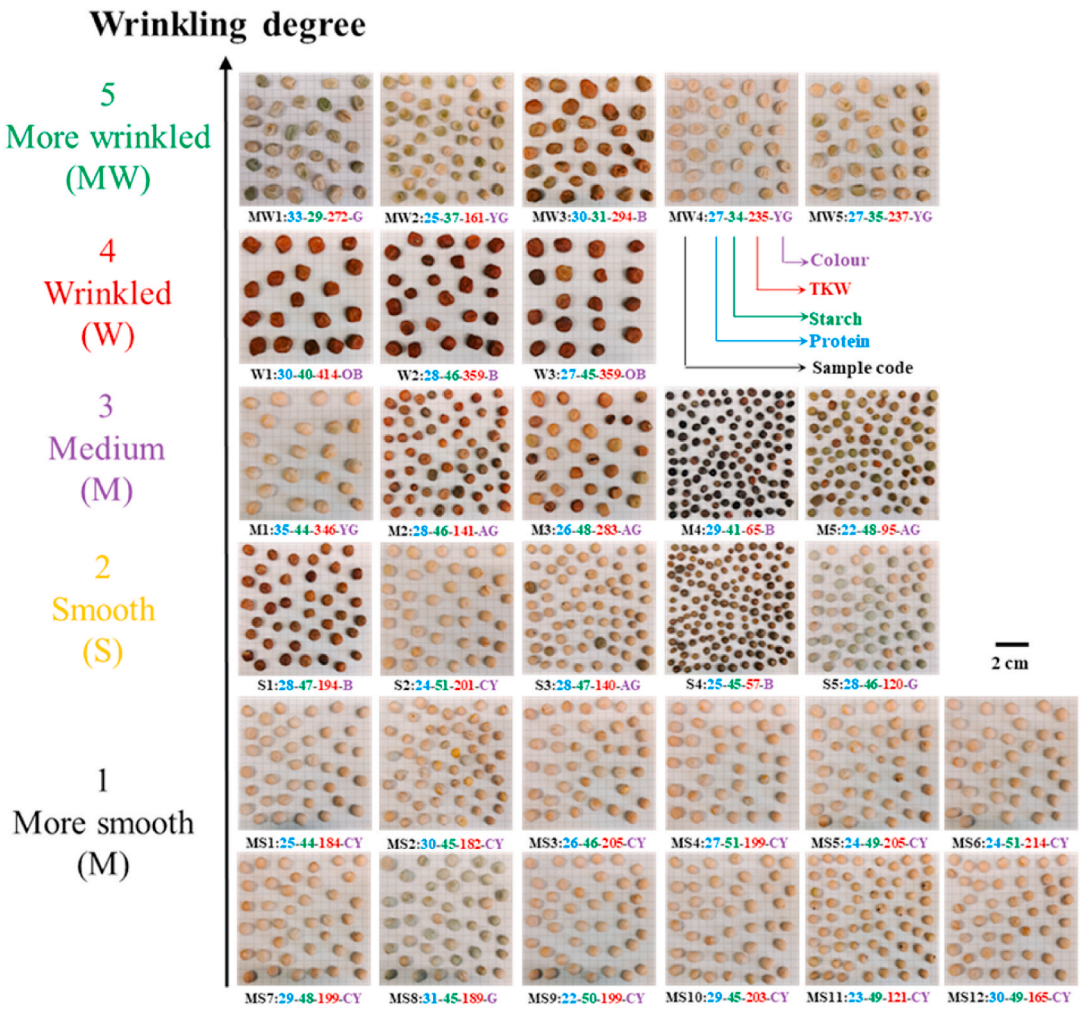


Fig. 1. Morphological diversity of 30 pea accessions categorized into five wrinkling groups [more wrinkled (MW, 5), wrinkled (W, 4), medium (M, 3), smooth (S, 2), and more smooth (MS, 1)] based on visual assessment. Background information below each accession includes sample code (black), protein content (blue), total starch content (green), TKW (red), and colour (purple). Colour codes: CY (creamy yellow), YG (yellow green), LG (light green), G (green), DG (dark green), AG (army green), OB (orange-brown), and B (brown). Scale bar: 2 cm.

amylopectin group. However, protein was far away from the two groups. Based on these findings, the wrinkling of peas was more related to the total starch content rather than the protein content, even though previous studies (Daba et al., 2024; Sun et al., 2023) have reported that more wrinkled peas tend to have higher protein content.

To explore the relationship in main components among the five groups with varying degrees of wrinkling, protein and total starch contents were plotted in Fig. 2B. These groups showed a negative linear relationship between protein and total starch content, with R^2 values of 0.95, 0.81, 0.39, 0.32, 0.29 from the more wrinkled to more smooth peas, respectively. Notably, more wrinkled peas had significantly lower total starch content (<40 %) compared to other groups, consistent with the results in Fig. 2A. This observation aligns with previous studies, which also reported that more wrinkled peas contain less total starch than smoother varieties (Bhattacharyya et al., 1990, 1993; Daba et al., 2024; Dueholm et al., 2024; Sun et al., 2023).

To further illustrate which component in the total starch is more related to the pea wrinkling phenotype, amylose and amylopectin levels in the dry flour were plotted in Fig. 3A and B. Amylose was clearly divided into two groups, showing a positive linear relationship with total starch content in both low-and high-amylopectin groups ($R^2 = 0.64$ and 0.60, respectively). Similarly, amylopectin also exhibited a positive linear relationship with total starch content in both groups ($R^2 = 0.57$ and 0.63, respectively). However, the trends of these components differed between the groups. As total starch content increased, amylose content decreased slowly, while amylopectin content increased significantly. Meanwhile, the peas appeared to be more smooth. This resulted in a much lower amylose-to-amylopectin ratio (below 0.6) in the high-amylopectin group, as shown in Table 1.

These findings indicate that amylopectin biosynthesis is a major driver of total starch accumulation and significantly influences seed development and maturation. The lower total starch content in the low-amylopectin group can be attributed to reduced levels of the starch-branching enzyme I (SBEI), which is critical for amylopectin biosynthesis (Bhattacharyya et al., 1993; Edwards et al., 1988).

3.2. Protein profile

In section 3.1, protein content appeared to have less relation with the pea phenotype. To investigate further, we quantified the composition of protein that is soluble in the SDS buffer to determine its potential impact. The protein composition of 30 pea accessions was assessed using gel electrophoresis. Representative SDS-PAGE profiles of 13 pea accessions are shown under non-reducing (Fig. 4A) and reducing (Fig. 4B) conditions.

To compare differences systematically, the gel lanes of each sample were divided into 18 areas based on molecular weight (M_W) and previously identified protein subunits in peas (Grossmann, 2024; Lam et al.,

Table 1
Main component of the 30 pea accessions on a dry basis of the flour. They are categorized into low-amylopectin and high-amylopectin groups. The degree of wrinkling is assessed visually and categorized as: MW (more wrinkled pea), W (wrinkled pea), M (medium pea), S (smooth pea) and MS (more smooth pea).

Sample code	Total starch (%)	Protein (%)	Amylose (%)	Amylopectin (%)	Amylose: amylopectin (−)	Protein and starch (%)	Other components (%)
Low-amylopectin group							
MW1	29.1 ± 0.8	32.8 ± 0.3	19.4 ± 0.6	9.7 ± 0.6	2.0 ± 0.2	62.0	31.0
MW2	37.0 ± 0.8	25.4 ± 0.5	22.6 ± 0.8	14.3 ± 0.8	1.6 ± 0.1	62.4	30.6
MW3	31.4 ± 0.6	30.1 ± 0.5	21.3 ± 0.4	10.0 ± 0.4	2.1 ± 0.1	61.4	31.6
MW4	34.1 ± 0.8	26.5 ± 0.3	24.0 ± 0.4	10.1 ± 0.4	2.4 ± 0.1	60.7	32.3
MW5	35.2 ± 0.8	27.2 ± 0.4	24.6 ± 0.4	10.6 ± 0.4	2.3 ± 0.1	62.4	30.6
High-amylopectin group							
W1	40.4 ± 0.3	29.9 ± 0.5	12.5 ± 0.3	27.9 ± 0.3	0.4 ± 0.0	70.3	22.7
W2	46.5 ± 1.9	27.6 ± 0.1	15.7 ± 0.9	30.8 ± 0.9	0.5 ± 0.0	74.1	18.9
W3	45.4 ± 1.2	26.7 ± 0.5	13.2 ± 0.5	32.2 ± 0.5	0.4 ± 0.0	72.1	20.9
M1	44.0 ± 0.5	34.8 ± 0.5	13.9 ± 0.3	30.1 ± 0.3	0.5 ± 0.0	78.8	14.2
M2	45.5 ± 0.5	28.0 ± 0.4	13.4 ± 0.9	32.1 ± 0.9	0.4 ± 0.0	73.5	19.5
M3	48.1 ± 1.5	25.9 ± 0.2	15.5 ± 1.2	32.6 ± 1.2	0.5 ± 0.1	74.1	18.9
M4	40.8 ± 1.6	29.3 ± 0.2	11.4 ± 0.2	29.4 ± 0.2	0.4 ± 0.0	70.2	22.8
M5	47.7 ± 1.6	21.7 ± 0.3	16.5 ± 0.9	31.2 ± 0.9	0.5 ± 0.0	69.3	23.7
S1	46.6 ± 0.7	27.7 ± 0.8	16.0 ± 0.5	30.7 ± 0.5	0.5 ± 0.0	74.3	18.7
S2	50.9 ± 0.2	23.7 ± 0.5	15.6 ± 0.7	35.3 ± 0.7	0.4 ± 0.0	74.5	18.5
S3	47.2 ± 0.0	27.8 ± 0.3	14.2 ± 0.6	32.9 ± 0.6	0.4 ± 0.0	75.0	18.0
S4	45.3 ± 0.6	24.8 ± 0.3	15.2 ± 1.4	30.1 ± 1.4	0.5 ± 0.1	70.1	22.9
S5	45.6 ± 0.8	27.8 ± 0.3	16.1 ± 0.0	29.5 ± 0.0	0.5 ± 0.0	73.4	19.6
MS1	44.4 ± 0.6	25.1 ± 0.3	15.4 ± 0.1	28.9 ± 0.1	0.5 ± 0.0	69.4	23.6
MS2	45.4 ± 0.4	30.0 ± 0.2	13.7 ± 0.3	31.8 ± 0.3	0.4 ± 0.0	75.4	17.6
MS3	46.3 ± 1.1	25.9 ± 0.5	14.5 ± 0.4	31.8 ± 0.4	0.5 ± 0.0	72.2	20.8
MS4	50.6 ± 2.9	26.8 ± 0.5	17.1 ± 0.3	33.5 ± 0.3	0.5 ± 0.0	77.4	15.6
MS5	49.0 ± 1.3	23.9 ± 0.1	15.3 ± 0.4	33.7 ± 0.4	0.5 ± 0.0	72.9	20.1
MS6	50.9 ± 0.3	23.5 ± 0.1	18.6 ± 0.8	32.3 ± 0.8	0.6 ± 0.0	74.5	18.5
MS7	47.7 ± 1.8	29.3 ± 0.1	16.8 ± 0.4	30.9 ± 0.4	0.5 ± 0.0	77.1	15.9
MS8	45.3 ± 0.1	30.6 ± 0.1	14.3 ± 0.6	31.0 ± 0.6	0.5 ± 0.0	75.8	17.2
MS9	50.2 ± 2.2	22.4 ± 0.4	15.3 ± 0.5	34.9 ± 0.5	0.4 ± 0.0	72.6	20.4
MS10	45.0 ± 0.3	28.9 ± 0.3	13.2 ± 0.2	31.8 ± 0.2	0.4 ± 0.0	74.0	19.0
MS11	49.3 ± 1.2	22.6 ± 0.2	18.5 ± 0.9	30.7 ± 0.9	0.6 ± 0.0	71.9	21.1
MS12	48.9 ± 2.3	29.8 ± 0.7	15.2 ± 0.4	33.6 ± 0.4	0.5 ± 0.0	78.7	14.3

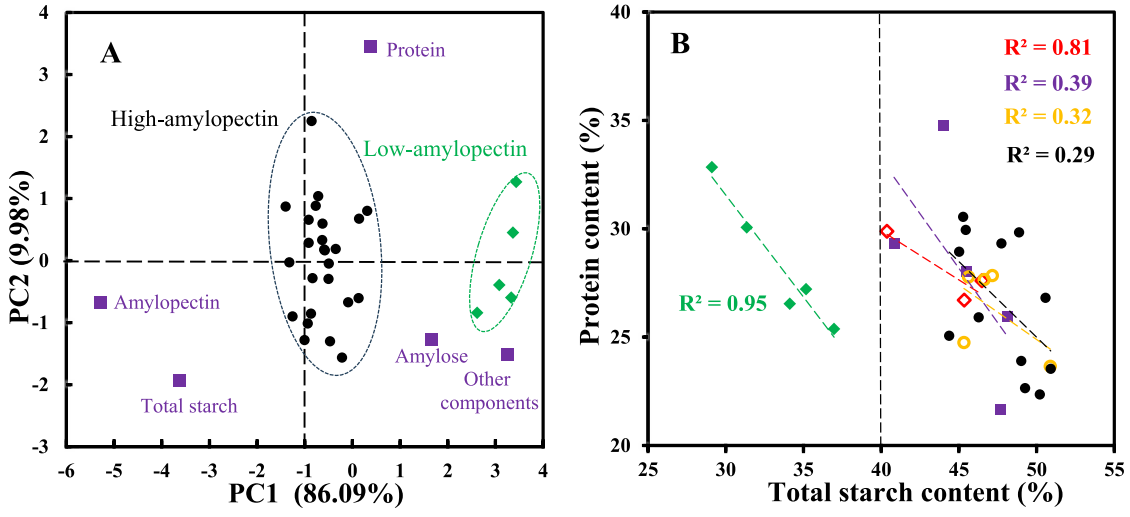


Fig. 2. (A) PCA plot of main components for the low-amylopectin (♦) and high-amylopectin (●) groups. (B) Protein vs. total starch content of 30 pea accessions grouped by wrinkling degree: more wrinkled (♦), wrinkled (◊), medium (■), smooth (○), and more smooth (●). Long-dashed lines and R^2 values indicate linear correlations.

2018; Lu et al., 2020; Mession et al., 2015; Yang et al., 2024). As shown in Fig. 4C, globulin included legumin (11S), vicilin (7S), and convicilin (7S). Legumin subunits: The legumin monomer ($L_{\alpha\beta}$) was detected in area 7 (56–60 kDa), the acidic subunit (L_α) in area 11 (36–40 kDa), and the basic subunit (L_β) in area 15 (17–22 kDa). Vicilin subunits: Vin 1, Vin 2, and Vin 3 were detected in areas 9 (48–52 kDa), 12 (30–36 kDa), and 17 (10–15 kDa), respectively. Convicilin subunits: Con 1, Con 2, and Con 3 were found in areas 4 (67–79 kDa), 5 (64–67 kDa), and 6 (60–64 kDa), respectively. Pea albumin (PA) includes PA2, PA1, lectins, and lipoxygenases. PA2 fractions (e.g., PMA-L and PMA-S) were detected in areas 8 (52–56 kDa) and 10 (40–48 kDa), while subunits PA2a and PA2b were identified in area 13 (25–30 kDa). PA1 was found in area 18 (3–10 kDa), lectins in area 14 (22–25 kDa), and lipoxygenases in areas 2 (90–100 kDa) and 3 (79–90 kDa). Areas 1 (100–160 kDa) and 16

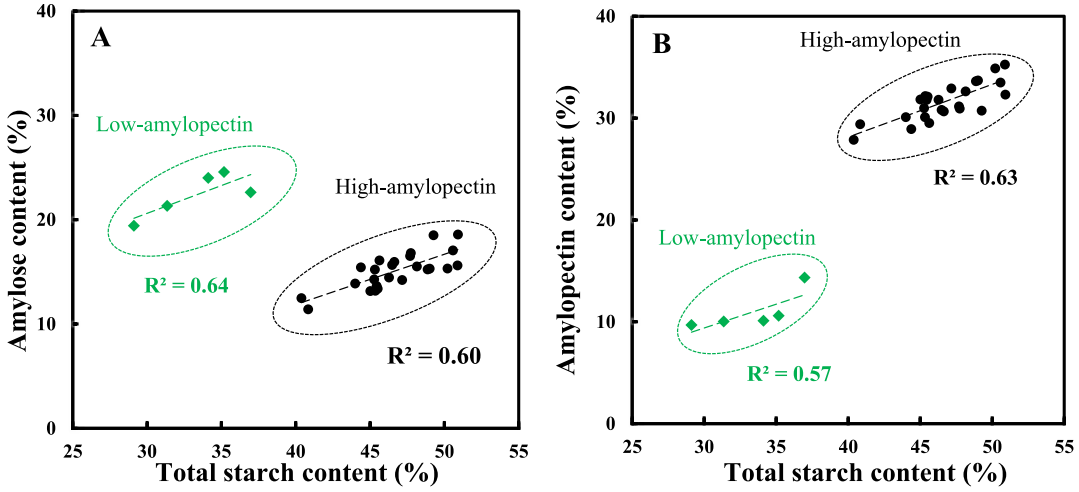


Fig. 3. (A) Amylose vs. total starch content and (B) amylopectin vs. total starch content for low-amylopectin (♦) and high-amylopectin (●) groups. Long-dashed lines and R^2 values represent linear correlations.

(10–15 kDa) were rarely reported in previous studies and they were likely protease inhibitors. Protein subunit content is summarized in Fig. 4D (non-reducing condition) and 4E (reducing condition). In the non-reducing condition, samples showed high levels of $L_{\alpha\beta}$, Vin 1, and Vin 2. In the reducing condition, $L_{\alpha\beta}$ decreased significantly, while Vin 1 and Vin 2 remained stable, and L_α and L_β increased markedly due to DTT breaking disulfide bonds in $L_{\alpha\beta}$, consistent with another pea study (Kornet et al., 2021).

Protein compositions are summarized in Table 2, revealing substantial diversity among the 30 pea accessions. Globulin: High concentrations (75.7 ± 4.4 %, ranging from 67.5 % to 82.4 %), comprising legumin (31.2 ± 4.7 %), vicilin (29.2 ± 3.8 %), and convicilin (15.3 ± 2.8 %). Pea albumin (PA): Lower concentrations (25.3 ± 4.4 %, ranging from 17.6 % to 32.5 %), including PA2 (7.5 ± 2.4 %), PA1 (2.2 ± 0.8 %), lipoxygenases (6.7 ± 1.3 %), and lectins (3.5 ± 1.4 %).

To further explore compositional differences, the PCA plot of protein composition for the 30 accessions is shown in Fig. 5. The separation between the low-amylopectin and high-amylopectin groups was clear and some trends emerged, although it was less distinct than that based on the main components (Fig. 2A). For globulin fractions, low-amylopectin group showed lower legumin, higher vicilin, and comparable convicilin content (Table 2). This resulted in lower legumin-to-vicilin ratio and lower globulin. For PA fractions, the low-amylopectin group had slightly higher levels of low-molecular-weight fractions (e.g., PA1 and lectins), though PA2 and lipoxygenases were comparable.

These results, confirmed by the PCA plot, show vicilin and low-molecular-weight PA fractions clustering near the low-amylopectin group, while legumin align with the high-amylopectin group. These protein fractions differences, particularly in legumin and vicilin, showed strong relation with the pea phenotype than other protein fractions. These align with prior studies (Daba et al., 2024; Gueguen and Barbot, 1988). They also reported lower legumin and higher vicilin content in wrinkled peas with low amylopectin content, although differences in PA fractions rarely reported.

3.3. Protein solubility

Solubility is influenced by factors such as hydrophilicity, electrostatic repulsion, and hydrophobic among proteins and peptides (Lam et al., 2018; Zhang et al., 2024). Fig. 6 presents the overall solubility of 30 pea accessions at pH 7, expressed as the percentage of protein present in the supernatant after centrifugation. Protein solubility varied across the 30 pea accessions, averaging 30.7 %. The highest solubility was observed in W2 (35.9 %), while the lowest was in S3 (26.2 %). The solubility of protein in pea flour was considerably lower than in pea protein isolates, where solubility reached 87.5 % (Barac et al., 2015). This disparity may be attribute to the presence of starch granules and other cell wall materials in the flour, which promote protein-starch and protein-fibre interactions while weakening protein-solvent interactions.

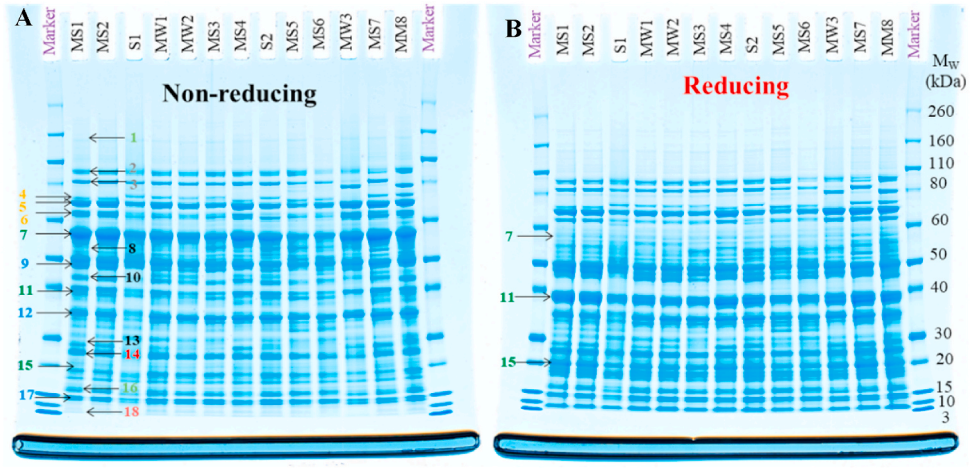
Protein solubility and content exhibited similar trends, showing minimal correlation with the pea phenotype, although some protein compositions were associated.

3.4. Swelling factor

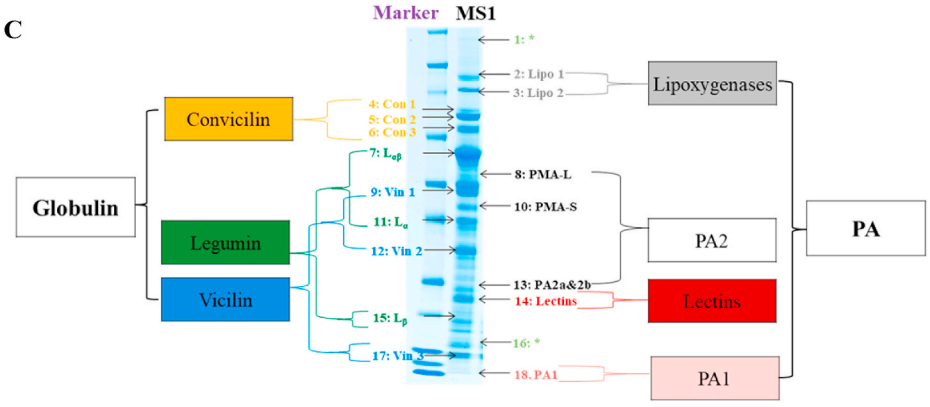
When pea flour is mixed with an aqueous medium, portions of the protein, starch, and fibre dissolve or disperse into the solvent. As shown in Fig. 7A (example: MS5), three peaks appear at particle sizes of approximately 0.8 μm, 30 μm, and 200 μm, corresponding primarily to proteins, starch, and group particles composed of starch, protein, and fibre, respectively. The volume-weighted diameter ($d_{4,3}$) of the bulk solution exceeds 80 μm, heavily influenced by the size of the group particles (third peak). A similar observation was reported by Sun et al. (2023). After centrifugation, the group particles were likely removed, revealing a more distinct size distribution for individual proteins and starch.

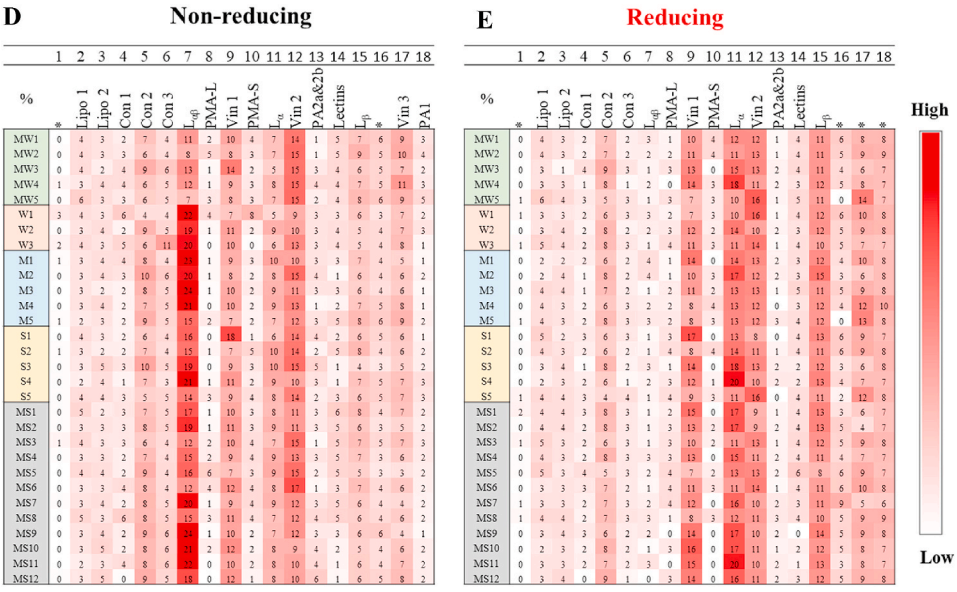
As shown in Fig. 8A, the particle size ($d_{4,3\text{-NH}}$) of the 30 pea flours after centrifugation, i.e. before heating, varied, with an average size of 6.1 μm, ranging from 3.8 to 9.7 μm. After heated at 95 °C for 20 min, the particle size ($d_{4,3\text{-H}}$) differed largely across samples, as illustrated in Fig. 8A. For most samples (25 out of 30), the particle size increased. For instance, $d_{4,3}$ in sample W1 rose from 5.4 μm to 38.8 μm (Fig. 7B). However, in a few samples, the particle size either decreased or remained unchanged. For example, in sample W3, $d_{4,3}$ decreased from 9.6 μm to 4.4 μm (Fig. 7C), while sample MW1 showed little change, maintaining a size of ~6.0 μm (Fig. 7D). Interestingly, particle size changed minimally, showing low variability ($d_{4,3\text{-H}}$ ranged from 5.4 μm to 11.1 μm) in the low-amylopectin group, while it exhibited greater variability ($d_{4,3\text{-H}}$ ranged from 3.7 μm to 38.8 μm) in the high-amylopectin group.

As shown in Fig. 8B, the low-amylopectin group had SF values close to 1, suggesting minimal volume changes and less thermal sensitivity during heating. This may be attributed to the low amylopectin content and high amylose-to-amylopectin ratio (Table 1). At low amylopectin levels, water absorption and structural changes in starch granules during heating were limited (Ji et al., 2022; Oates, 1997). An exception within the low-amylopectin group was sample MW3 (SF = 8.6), likely due to its higher globulin content and globulin-to-PA ratio (Table 2). As starch



Area	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Mw (kDa)	100~160	90~100	79~90	67~79	64~67	60~64	56~60	52~56	48~52	40~48	36~40	30~36	25~30	22~25	17~22	15~17	10~15	3~10
Protein subnuit	*	Lipo 1	Lipo 2	Con 1	Con 2	Con 3	$L_{\alpha\beta}$	PMA-L	Vin 1	PMA-S	L_{α}	Vin 2	PA2a&2b	Lectins	L_{β}	*	Vin 3	PA1





(caption on next page)

Fig. 4. SDS-PAGE profiles of 13 pea accessions under non-reducing (A) and reducing (B) conditions. Lane Marker: protein marker (3–260 kDa). Sample codes are presented on the top of each lane (C). Protein subunits (e.g., MS1). were identified by molecular weight and quantified for 30 accessions under non-reducing (D) and reducing (E) conditions. Areas in each lane is divided based on molecular weight and correspond to specific protein fractions: Lipoxygenases (Lipo 1–2), Convicilin (Con 1–3), Legumin monomers ($L_{\alpha\beta}$), acidic (L_{α}) and basic (L_{β}) subunits, Vicilin (Vin 1-3), PA2 fractions of pea major albumins (PMA-L and PMA-S), and PA 2a&2b subunits, Lectins, PA1, and unidentified fractions (*). The degree of wrinkling is assessed visually and categorized as: MW (more wrinkled pea), W (wrinkled pea), M (medium pea), S (smooth pea) and MS (more smooth pea).

Table 2
Protein composition of the 30 pea accessions. They are categorized into low-amylopectin and high-amylopectin groups. The degree of wrinkling is assessed visually and categorized as: MW (more wrinkled pea), W (wrinkled pea), M (medium pea), S (smooth pea) and MS (more smooth pea).

Sample code	Legumin (%)	Vicilin (%)	Convicilin (%)	PA2 (%)	PA1 (%)	Lipoxygenases (%)	Lectins (%)	Globulin (%)	PA (%)	Legumin: vicilin (−)	Globulin: PA (−)
Low-amylopectin group											
MW1	25 ± 2	34 ± 3	13 ± 1	7.5 ± 0.4	2.9 ± 0.6	7.1 ± 0.2	4.9 ± 1.0	72 ± 1	28 ± 1	0.7 ± 0.1	2.5 ± 0.2
MW2	24 ± 2	33 ± 2	12 ± 1	9.2 ± 1.8	3.5 ± 1.7	8.0 ± 0.2	4.6 ± 0.2	69 ± 1	31 ± 1	0.7 ± 0.1	2.2 ± 0.1
MW3	24 ± 0	36 ± 1	19 ± 2	5.4 ± 0.5	2.1 ± 0.9	4.2 ± 0.5	4.4 ± 0.2	79 ± 4	21 ± 4	0.7 ± 0.0	3.8 ± 0.9
MW4	24 ± 3	35 ± 1	13 ± 1	8.3 ± 2.2	2.6 ± 1.9	6.3 ± 1.5	4.5 ± 0.7	72 ± 4	28 ± 4	0.7 ± 0.1	2.6 ± 0.6
MW5	22 ± 0	32 ± 0	13 ± 0	8.1 ± 1.9	4.5 ± 0.5	9.0 ± 0.1	3.9 ± 1.5	67 ± 0	33 ± 0	0.7 ± 0.0	2.1 ± 0.0
High-amylopectin group											
W1	33 ± 1	22 ± 1	13 ± 1	16.1 ± 0.0	2.3 ± 1.0	3.7 ± 0.2	3.2 ± 0.0	68 ± 1	32 ± 1	1.5 ± 0.1	2.2 ± 0.1
W2	33 ± 2	27 ± 1	15 ± 0	6.6 ± 0.8	3.3 ± 1.1	6.9 ± 0.0	3.5 ± 1.7	75 ± 4	25 ± 4	1.2 ± 0.0	3.0 ± 0.6
W3	31 ± 1	30 ± 2	21 ± 1	3.7 ± 0.9	1.3 ± 0.3	3.7 ± 0.7	3.7 ± 0.2	82 ± 3	18 ± 3	1.0 ± 0.1	4.6 ± 0.9
M1	39 ± 0	23 ± 1	18 ± 0	6.6 ± 0.1	1.1 ± 0.5	6.4 ± 1.4	2.6 ± 0.3	81 ± 0	19 ± 0	1.7 ± 0.1	4.1 ± 0.1
M2	34 ± 1	28 ± 1	24 ± 1	6.4 ± 0.8	1.8 ± 1.2	6.9 ± 0.7	1.1 ± 0.9	82 ± 1	18 ± 1	1.2 ± 0.0	4.7 ± 0.8
M3	38 ± 2	26 ± 2	15 ± 0	6.1 ± 2.0	1.0 ± 0.1	7.3 ± 0.3	3.2 ± 0.3	79 ± 4	21 ± 4	1.4 ± 0.1	3.9 ± 0.9
M4	36 ± 2	31 ± 1	14 ± 0	3.3 ± 0.9	1.4 ± 0.5	7.4 ± 0.1	1.6 ± 0.5	82 ± 2	18 ± 2	1.2 ± 0.1	4.5 ± 0.7
M5	30 ± 2	27 ± 3	15 ± 1	7.3 ± 1.9	1.9 ± 0.9	6.9 ± 1.5	4.8 ± 1.1	73 ± 4	27 ± 4	1.1 ± 0.1	2.7 ± 0.6
S1	29 ± 1	38 ± 2	12 ± 1	6.2 ± 0.1	1.5 ± 0.1	6.7 ± 0.1	2.4 ± 0.1	78 ± 0	22 ± 0	0.8 ± 0.1	3.6 ± 0.1
S2	33 ± 1	27 ± 1	12 ± 0	7.8 ± 0.0	2.0 ± 0.7	8.3 ± 0.2	4.6 ± 0.1	73 ± 0	27 ± 0	1.2 ± 0.1	2.6 ± 0.0
S3	33 ± 2	27 ± 0	20 ± 1	8.3 ± 0.5	2.0 ± 0.7	5.7 ± 0.2	1.4 ± 0.6	80 ± 3	20 ± 3	1.2 ± 0.1	4.0 ± 1.6
S4	37 ± 1	28 ± 1	11 ± 0	6.5 ± 0.7	3.5 ± 1.0	5.6 ± 0.1	1.4 ± 0.7	77 ± 0	23 ± 0	1.3 ± 0.1	3.3 ± 0.0
S5	28 ± 4	30 ± 3	13 ± 1	8.8 ± 2.3	3.2 ± 0.9	8.4 ± 0.0	3.5 ± 1.6	70 ± 1	30 ± 1	0.9 ± 0.2	2.4 ± 0.1
MS1	32 ± 0	28 ± 0	15 ± 0	5.8 ± 0.2	2.4 ± 0.9	7.0 ± 0.4	5.6 ± 1.0	75 ± 0	25 ± 0	1.2 ± 0.0	2.9 ± 0.1
MS2	34 ± 1	27 ± 2	15 ± 1	6.6 ± 0.8	2.1 ± 0.5	6.2 ± 1.0	4.5 ± 0.2	77 ± 0	23 ± 0	1.3 ± 0.1	3.3 ± 0.1
MS3	27 ± 1	33 ± 2	13 ± 0	6.7 ± 0.0	2.8 ± 1.7	7.8 ± 1.7	5.2 ± 0.2	72 ± 1	28 ± 1	0.8 ± 0.1	2.6 ± 0.1
MS4	31 ± 1	28 ± 1	13 ± 1	9.3 ± 0.4	2.4 ± 0.9	8.3 ± 0.1	4.6 ± 0.1	72 ± 1	28 ± 1	1.1 ± 0.1	2.6 ± 0.1
MS5	31 ± 2	26 ± 2	15 ± 1	11.2 ± 0.9	2.1 ± 0.1	7.9 ± 0.2	4.9 ± 0.3	71 ± 0	29 ± 0	1.2 ± 0.2	2.5 ± 0.0
MS6	27 ± 2	35 ± 1	13 ± 1	8.9 ± 0.6	1.8 ± 0.4	5.6 ± 0.6	3.5 ± 1.6	76 ± 0	24 ± 0	0.8 ± 0.1	3.1 ± 0.0
MS7	35 ± 1	25 ± 0	15 ± 1	8.2 ± 0.6	1.7 ± 0.5	6.8 ± 0.5	4.6 ± 0.2	75 ± 2	25 ± 2	1.4 ± 0.1	3.0 ± 0.1
MS8	27 ± 1	28 ± 1	19 ± 1	10.3 ± 1.4	1.6 ± 0.3	4.9 ± 0.4	5.1 ± 0.4	74 ± 1	26 ± 1	1.0 ± 0.0	2.8 ± 0.1
MS9	37 ± 1	26 ± 1	17 ± 0	4.6 ± 0.8	1.0 ± 0.5	6.5 ± 0.4	2.5 ± 0.7	80 ± 0	20 ± 0	1.4 ± 0.1	3.9 ± 0.0
MS10	35 ± 2	28 ± 0	18 ± 0	7.4 ± 1.0	2.1 ± 0.8	6.4 ± 0.9	1.6 ± 0.4	80 ± 2	20 ± 2	1.3 ± 0.1	4.0 ± 0.7
MS11	35 ± 2	26 ± 1	20 ± 0	6.4 ± 0.5	1.9 ± 1.1	6.7 ± 0.9	1.3 ± 0.4	81 ± 1	19 ± 1	1.4 ± 0.1	4.3 ± 0.2
MS12	31 ± 3	31 ± 2	14 ± 0	7.1 ± 0.2	2.2 ± 0.2	7.8 ± 0.6	1.1 ± 1.0	77 ± 1	23 ± 1	1.0 ± 0.2	3.3 ± 0.2

granules may remain largely unchanged in the low-amylopectin group, the increase in protein size may contribute more significantly to the overall volume increase after heating. Globulin, which includes legumin, vicilin, and convicilin, has a higher molecular weight and more complex structure than PA. During heating, globulin likely contributed more to volume increases than PA, forming protein aggregates through hydrophobic interactions and disulfide bonding.

In contrast, the high-amylopectin group exhibited higher and more variable SF values (average: 79.2, range: 0.1–377.9), indicating more thermal sensitivity. In several samples (e.g., W1, S3, S4, S5, MS5, MS7, MS8, MS11), SF values exceeded 100, driven mainly by starch granule swelling. These samples contained higher amylopectin levels, facilitating water absorption and granule expansion. Additionally, the higher legumin-to-vicilin ratios (~1.2) in these samples may also contribute to swelling. Legumin, a hexameric protein with a molecular weight of 320–380 kDa and a quaternary structure stabilized by disulfide bonds (Barac et al., 2015; Grossmann, 2024), is likely to undergo larger size expansion during heating compared to vicilin, a trimeric protein with a lower molecular weight of 150–200 kDa.

Some samples in the high-amylopectin group (e.g., W3, M2, MS3, MS6) showed SF values below 1, indicating fragmentation and more thermal sensitivity upon heating. This may be attributed to thermal degradation of starch molecules, resulting in smaller granule fragments. For other samples, SF values ranged between 1 and 100, indicating moderate swelling. Early heating stages likely involved partial starch gelatinization and amylopectin crystallite melting, leading to granule swelling. Prolonged heating caused partial granule breakdown into smaller fragments, reducing granule size. These structural changes in starch granules were influenced by various factors, which led to a various value of swelling factor. In addition, the proteins may also play a role as we noted earlier.

The thermal sensitivity of the pea suspension may be primarily determined by starch granule structure, with protein composition playing a secondary role. Starch granules in the low-amylopectin group undergo minimal changes due to their low total starch, amylopectin, and legumin content.

3.5. Correlation between pea composition, morphology and function

To explore the relationships between composition, morphology, and functionality in the 30 pea accessions, Pearson's correlation coefficients (R) were calculated to evaluate the relationship among morphological traits, main components, protein composition, and functional properties. The results are displayed in Fig. 9, while the principal component

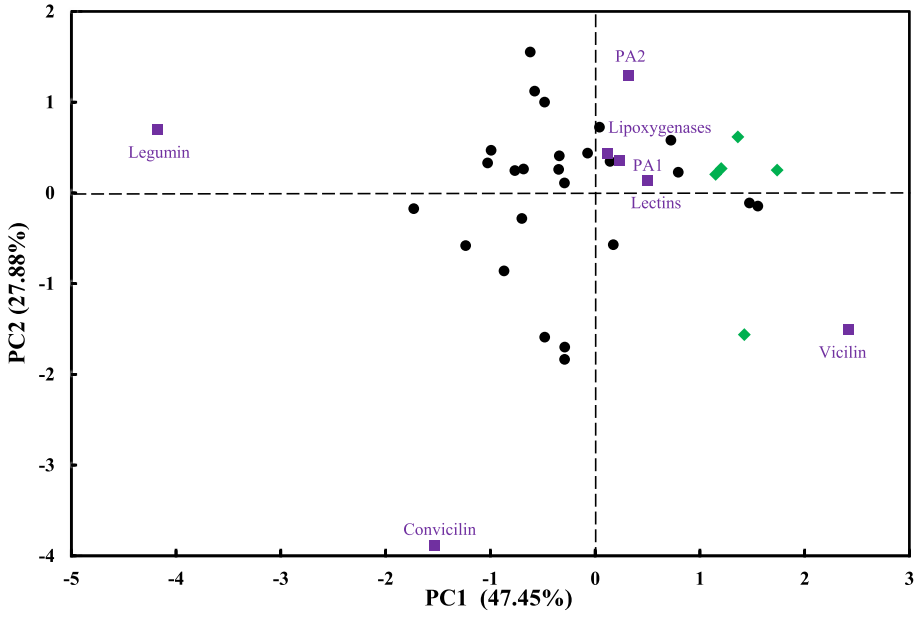


Fig. 5. PCA plot of protein composition (■) for the low-amylopectin (♦) and high-amylopectin (●) groups.

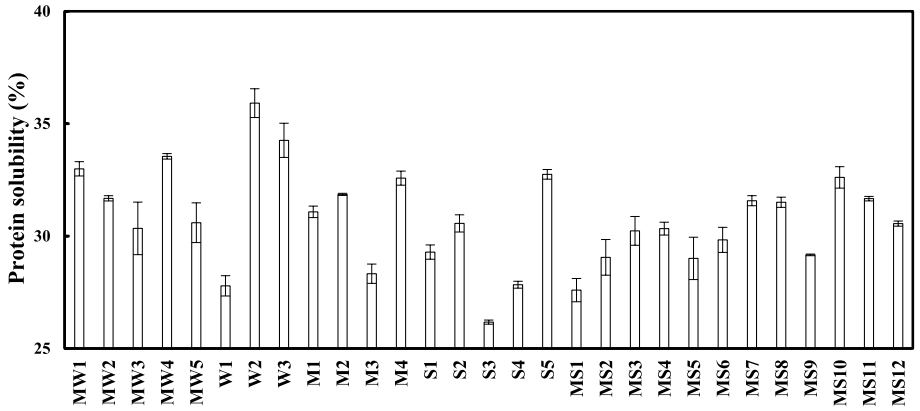


Fig. 6. Protein solubility of 0.5 % pea flour for 30 pea accessions at pH 7. The degree of wrinkling is assessed visually and categorized as: MW (more wrinkled pea), W (wrinkled pea), M (medium pea), S (smooth pea) and MS (more smooth pea).

analysis (PCA) plot in Fig. 10 provides a visual representation of these relationships.

Among the main components, total starch, amylopectin, and protein-and-total starch show strong positive correlations ($0.85 \leq R \leq 0.94$, $p<0.05$), as depicted in Fig. 9. Similarly, amylose, amylose-to-amylopectin ratio, and other components (particularly fibre), are positively correlated ($0.71 \leq R \leq 0.88$, $p<0.05$). However, the former group exhibits negative correlations with the latter ($0.54 \leq R \leq 1.00$, $p<0.05$), and these components are positioned on opposite sides in the PCA plot. For the two main components of starch: amylopectin and amylose, total starch exhibits a strong positive correlation with amylopectin ($R = 0.94$, $p<0.05$) and a weak negative correlation with amylose ($R = 0.54$, $p<0.05$). However, a study by Sun et al. (2023) on pea flours reported a strong negative correlation between amylose and starch ($R = 0.99$, $p<0.05$), likely due to the limited sample size of 10 pea accessions. These findings highlight that the correlations depend on the number of pea accessions analysed. Interestingly, within low- and high-amylopectin groups, amylose showed a positive correlation, as discussed in section 3.1. This suggests that the relationship between amylose and total starch could vary and need further investigation in other studies under larger sample size. In addition, samples with lower total starch tend to have higher fibre content, supported by a strong negative correlation between total starch and other components ($R = 0.85$, $p<0.05$). This finding is consistent with the results of Sun et al. (2023). During seed development, a deficiency in starch branching enzyme I (SBEI) hampers the biosynthesis of amylopectin from saccharides, leading to the accumulation of sucrose or unbranched oligosaccharides. These saccharides may primarily contribute to the formation of cell wall polysaccharides, a major component of dietary fibre. Protein exhibits weak correlations with these components and is distinct in the PCA plot. Only total starch shows a weak negative correlation with protein ($R = 0.46$, $p<0.05$). This may be attributed to the independent biosynthesis pathways of starch and protein.

Among protein compositions, the relationships are generally weaker, as indicated by lower correlation coefficients compared to those among the main components. Some high correlations, such as between globulin and PA, may arise from the calculation method rather than biological relevance. Certain components, including PA2, PA1, lipoxygenases and lectins, show weak correlations with specific compositions, likely due to their low concentration and minimal variability across samples. Generally, these proteins are positively correlated with PA and negatively correlated with globulin, as reflected in the PCA plot, where they

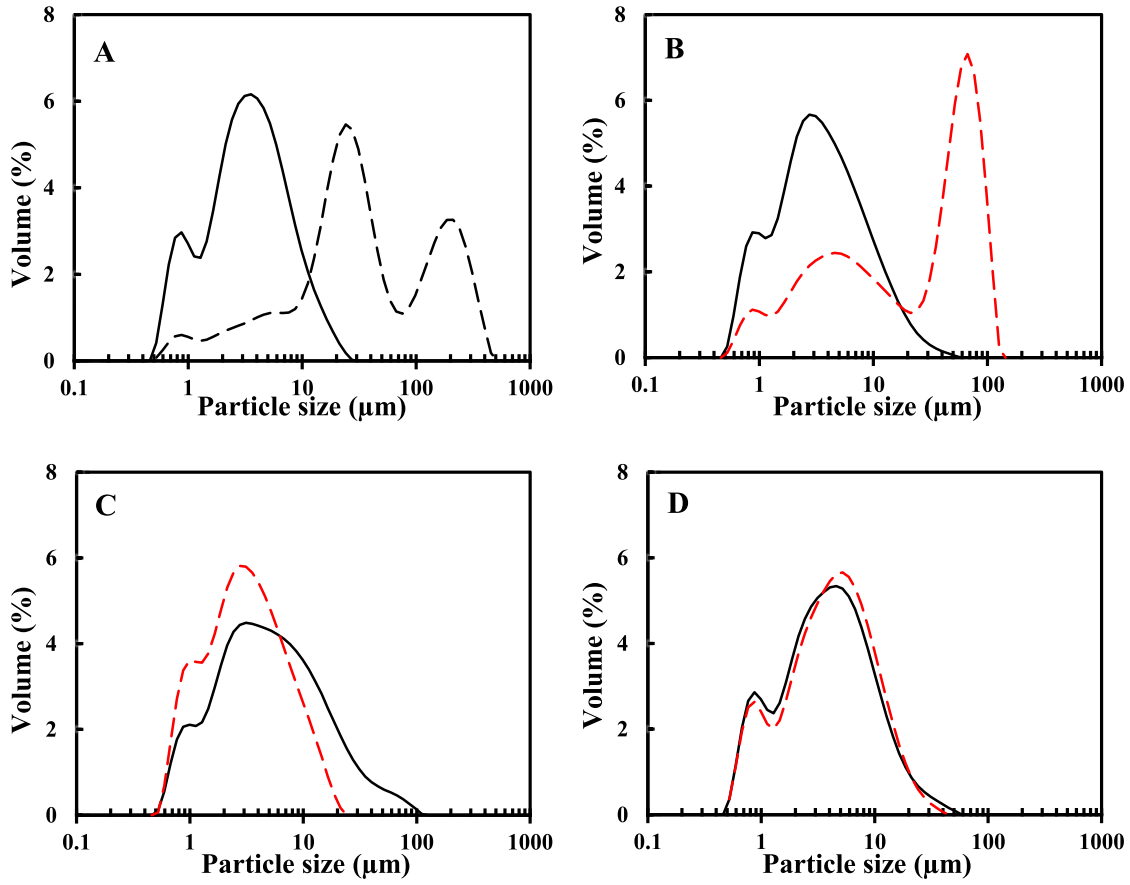


Fig. 7. (A) Particle size distribution for sample MS5 under non-centrifugation (—) and centrifugation (- -) conditions. Particle size distribution for samples W1 (B), W3 (C), and MW1 (D), corresponding to particle size increased, decreased and did not change after heating. Non-heated (- -) and heated (- -). Centrifugation: 500 g, 3 min. Heating: 90 °C, 20 min.

are positioned close to PA and opposite to globulin. Notable correlations include the strong positive relationship of the legumin-to-vicilin ratio with legumin (R = 0.95, p<0.05) and its weak negative correlation with vicilin (R = 0.73, p<0.05). This suggests that this ratio is more associated with legumin than with vicilin, as confirmed by its closer proximity to legumin in the PCA plot. Additionally, globulin shows a moderate correlation with convicilin (R = 0.69, p<0.05) and legumin (R = 0.46, p<0.05). Interestingly, vicilin, despite being a major fraction of globulin, exhibits a weaker relationship with globulin, as evidenced by their separation in the PCA plot, although it is negatively correlated with legumin (R = 0.62, p<0.05). These results indicate larger variation in legumin and vicilin compared to other protein compositions among the samples.

Some interesting correlations are observed between the main components and protein composition. Legumin, one of the main proteins in peas, shows a positive correlation with amylopectin and its associated components (such as total starch and protein-and-starch) and a negative correlation with amylose and its related components (such as the amylose-to-amylopectin ratio and other components). This is further supported by the PCA plot, where legumin is positioned close to amylopectin and its associated components, and opposite to amylose and its related components. In contrast, vicilin displays an opposing distribution in the PCA plot, despite having a low correlation coefficient. This suggests that more "active" components, such as amylopectin, total starch, and legumin, are positively correlated with each other, whereas they are negatively associated with more "default" components, including amylose, other components (primarily fibre), and vicilin. This implies that a shortage of starch branching enzyme I (SBEI) could potentially impair legumin biosynthesis, as noted by Bhattacharyya et al. (1993). Specifically, the absence of SBEI might impact the expression of storage protein genes, potentially leading to the destabilization of legumin mRNAs in high-sugar conditions (Casey et al., 1998).

As expected, the area and TKW are related, but they show weaker correlations with other characteristics compared to wrinkling. This suggests that the degree of wrinkling, described by discontinuous wrinkling values, is a more important index in respect to pea morphology. The degree of wrinkling is strongly related to the main components. It shows a negative correlation with total starch, amylopectin, and the protein-and-starch (R = 0.80, 0.78, and 0.75, respectively, p<0.05), and a positive correlation with other components, amylose-to-amylopectin ratio and amylose (R = 0.75, 0.73, and 0.51, respectively, p<0.05). This relationship is further supported by the PCA plot, where the former components were located oppositely, while the latter components were positioned closely to wrinkling. Based on these findings, we can conclude that more wrinkled peas contain lower total starch content, particularly amylopectin, and higher fibre content. Protein, a major component of peas, exhibits a weaker correlation with wrinkling. This finding contrasts with previous studies, which have linked higher protein content to more wrinkled peas. The discrepancy may be due to the limited number of pea accessions (≤10) used in those studies (Bhattacharyya et al., 1990; Daba et al., 2024; Sun et al., 2023). Nevertheless, protein composition appears to be associated with the wrinkling degree in peas. Legumin and legumin-to-vicilin ratio show a negative correlation with wrinkling (R = 0.40 and 0.49, respectively, p<0.05), whereas some low-molecular-weight pea albumin fractions, such as PA1 and lectins, exhibit a positive correlation with wrinkling (R = 0.41 and 0.42, respectively, p<0.05). These relationships are also supported by their positions in the PCA plot. Vicilin, on the other hand,

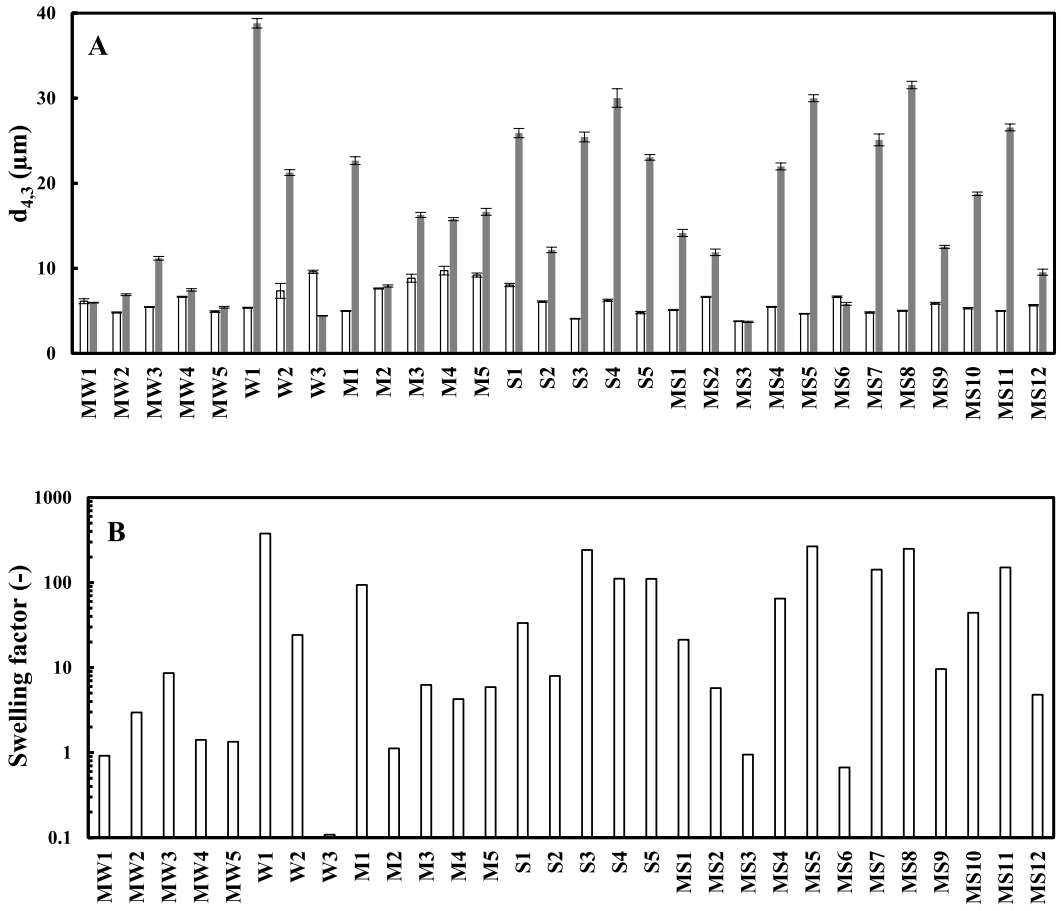


Fig. 8. (A) Volume-weighted size ($d_{4,3}$) of pea flour for 30 accessions before (□) and after heating (■). (B) Swelling factor of pea flours. Centrifugation: 500 g, 3 min. Heating: 90 °C, 20 min. The degree of wrinkling is assessed visually and categorized as: MW (more wrinkled pea), W (wrinkled pea), M (medium pea), S (smooth pea) and MS (more smooth pea).

shows a weak correlation with wrinkling (R = 0.07, p<0.05) but was positioned close to wrinkling in the PCA plot. In section 3.2, we did observe that more wrinkled peas (low-amylopectin group) tended to have higher vicilin content. This is because mutations at the r locus not only reduced the activity of the SBEI, leading to altered starch composition and seed wrinkling, but also produced several other effects, such as elevated sugar concentrations. Additionally, they affected storage-protein gene expression by specifically destabilizing legumin mRNAs in the high-sugar environment of mutant seeds, while vicilin mRNAs remained stable (Casey et al., 1998). As a result of these altered carbohydrate conditions and mRNA dynamics, the seed tended to contain high vicilin. However, within the high-amylopectin group, vicilin content varied (Table 2). Specifically, high vicilin levels were mainly observed in the more wrinkled, low-amylopectin group, while the high-amylopectin group contained a wider range of vicilin content, likely reducing its influence on seed wrinkling.

In addition to compositional factors, the size of the pea seed is also associated with the degree of wrinkling. Larger peas tend to be more wrinkled, as both seed area and TKW show positive correlations with wrinkling (R = 0.60 and 0.43, respectively, p<0.05). This may be attributed to the accumulation of sucrose in more wrinkled peas, resulting from insufficient conversion of sucrose into amylopectin. The elevated sucrose levels increase osmotic pressure, leading to greater water uptake, larger seed size, and higher fresh and dry weights of the seeds. These findings are consistent with observations reported in previous studies (Bhattacharyya et al., 1993; Casey et al., 1998; Daba et al., 2024).

Protein solubility shows limited correlation with other characteristics, but it is negatively associated with protein content (R = 0.63, p<0.05) and positively associated with $d_{4,3\text{-NH}}$ (particle size before heating, R = 0.62, p<0.05). Two factors may be associated with protein solubility in flours. 1). Protein content. As the protein content in flours increases, the volume of water or solvent available per protein particle decreases, weakening water/solvent-protein interactions and reducing protein solubility. 2). Particle size. Larger particle sizes, primarily representing starch granules in this study, typically have a lower specific surface area (SSA). This reduced SSA may weaken protein-starch interactions, allowing proteins to detach more easily from the starch surface into the water or solvent, thereby increasing solubility.

The swelling factor exhibits a stronger correlation with various characteristics compared to protein solubility. As expected, it is positively associated with $d_{4,3\text{-H}}$ (particle size after heating) and negatively associated with $d_{4,3\text{-NH}}$ (particle size before heating). Additionally, it also shows a positive correlation with the legumin-to-vicilin ratio and legumin but a negative correlation with vicilin. This is in line with the discussion in section 3.4. Total starch, amylose, and related components demonstrate weaker correlations with the swelling factor, as the swelling factor is more influenced by structural changes in starch granules during heating rather than their compositional factors. Interestingly, PA2 exhibits a strong positive correlation with the swelling factor (R = 0.72, p<0.05), and both are closely located in the PCA plot. PA2 comprises PMA-L and PMA-S, and their subunits PA2b and PA2a, with molecular weights of 53, 48, 25, and 24 kDa, respectively (Croy et al., 1984; Grossmann, 2024). Despite its small size, PA2 is rich in sulphur-containing amino acids (Grossmann, 2024; Schroeder, 1982), enabling the formation of protein aggregates through disulfide bridges.

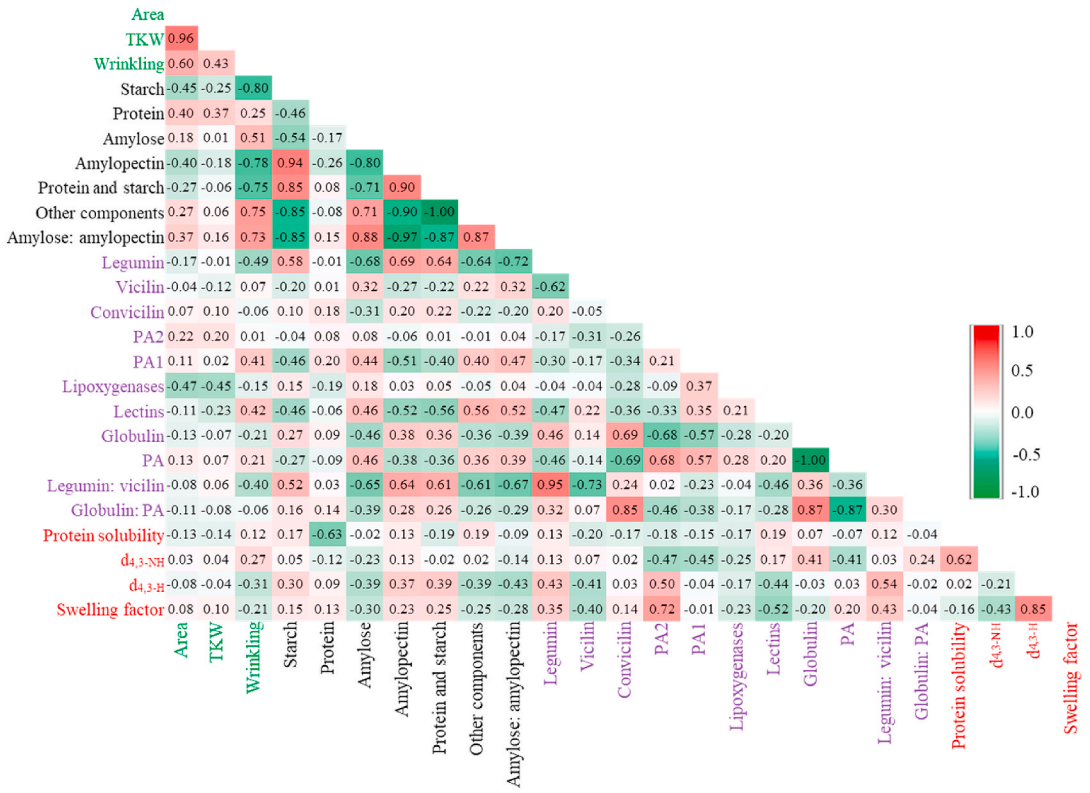


Fig. 9. Pearson's correlation coefficients (R) among morphological traits, main components, protein composition, and functional properties for 30 pea accessions at 95 % confidence. TKW: thousand-kernel weight. PA: Pea albumin.

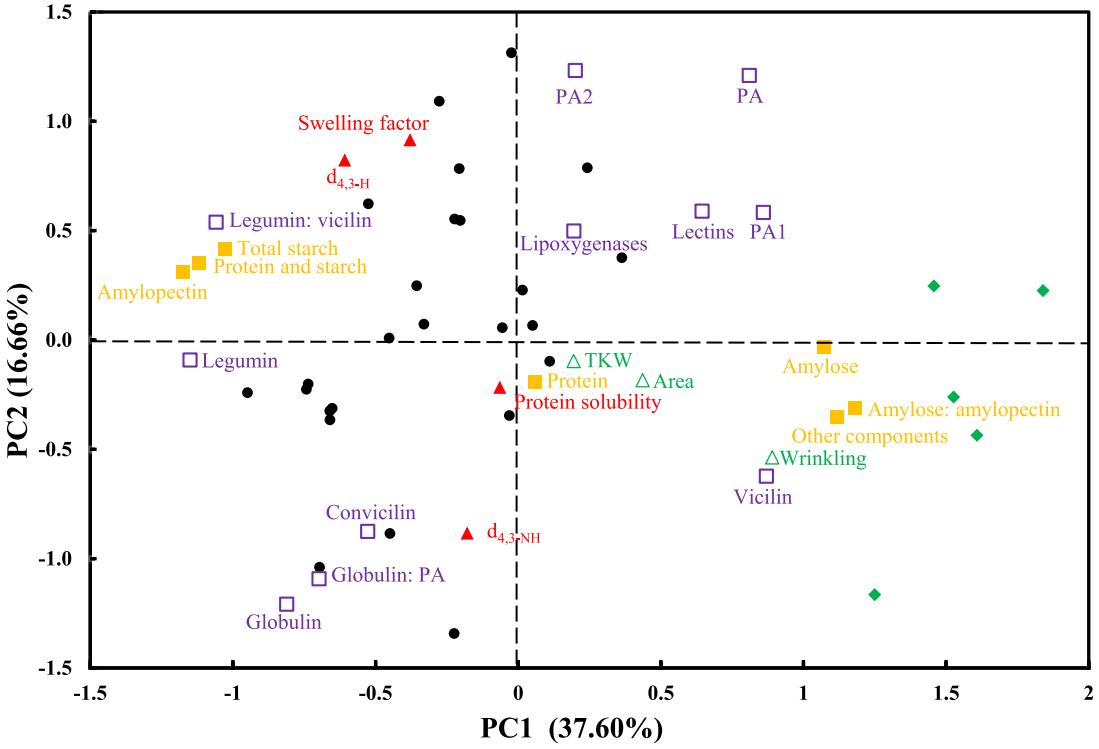


Fig. 10. PCA plot of morphological traits (△), main compositions (■), protein composition (□), and functional properties (▲) for the low-amylopectin (♦) and high-amylopectin groups. TKW: thousand-kernel weight. PA: Pea albumin.

For example, sample W1, which had the highest legumin-to-vicilin ratio, also contained the highest PA2 content (16 %). This likely enhanced disulfide cross-linking among denatured proteins, leading to larger aggregates and contributing to size increases during heating. Conversely, lectins, which are also small in size, exhibited a negative correlation with the swelling factor. This may be attributed to their low sulphur-containing amino acids, which limits their ability to form disulfide-linked protein aggregates.

4. Conclusions

This study is the first to comprehensively analyse the chemical composition and functional properties of pea flours across numerous natural accessions. Total starch, particularly amylopectin, fibre, and legumin were associated with wrinkling, while total protein and vicilin showed less correlation due to their variability across wrinkling degrees. Flours with lower total starch were associated with reduced amylopectin but higher amylose and fibre levels. In addition, their protein profiles also exhibited lower legumin, higher vicilin, and higher low-molecular-weight albumins (e.g., PA1, lectins), while large-molecular-weight fractions (e.g., PA2, lipoxygenases) remained largely unchanged. Protein solubility was lower in flours with higher total protein content or smaller particle size, likely due to decreased protein–solvent interactions or increased protein-starch/fibre interactions. Flours with lower amylopectin were less heat-sensitive, retaining intact starch granules and forming fewer protein aggregates due to reduced total starch and legumin levels. In contrast, flours with higher amylopectin exhibited more heat sensitivity. Their starch granules either swelled intactly or fragmented into smaller fractions during thermal degradation, with protein aggregation playing a secondary role. These findings offer a foundation for developing pea-based ingredients with tailored functional properties for specific applications in the agri-food industry.

CRediT authorship contribution statement

Bo Yuan: Writing - original draft, visualization, methodology, investigation, formal analysis, and conceptualization. Cecilia Hammenhag: Writing - review & editing, resources, methodology, conceptualization, and supervision. Qinhui Xing: Writing - original draft, methodology, and formal analysis. Michael F. Lyngkjær: writing - review & editing and formal analysis. René Lametsch: Writing - review & editing, visualization, methodology, investigation, formal analysis, conceptualization, supervision, and project administration.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101285.

Data availability

Data will be made available on request.

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