Physicochemical and nutritional properties of starches from nine Canadian-grown peas compared with six commercial starches

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Abstract
The physicochemical properties and nutritional fractions of the starches isolated from nine Canadian-grown peas, including marrowfat, green, and yellow pea types, were studied and compared with six commercial starches, to explore the unique properties of these pea starches. These nine pea starches were found to have high apparent amylose content (marrowfat peas, 51.3%–51.6%; yellow peas, 50.6%–53.8%; and green peas, 49.9%–54.2%) and a higher tendency than most commercial starches to retrograde. Although their physicochemical properties were not drastically different, a green pea variety, Limerick, stood out for its significantly high apparent amylose content (54.2%) and also the highest resistant starch (RS) content after cooking (29.5%), the latter even comparable with a commercial high amylose corn starch (29.8%). Principal component analysis indicated that amylose content, amylose leaching, and Rapid Visco Analyzer (RVA) parameters at the cooling stage are significantly positively correlated to the starch nutritional fractions of cooked samples. Cluster analysis showed a clear pattern that the RS content in cooked starches increased with the increasing amylose content in these starch samples. In general, these pea starches were rich in slowly digestible starch and high in RS after cooking (>16%). This study highlighted the unique properties of these pea starches, including their high amylose content, and ease of gelatinization yet strong tendency toward retrogradation, which resulted in superior final pasting viscosity and high RS content; thus, these pea starches could be the best alternative to commercial high amylose starches, to address the latter’s deficiencies in pasting properties when applying in gel-based low glycemic index (GI) foods.

KEYWORDS
amylose, crystallinity, digestibility, pulse, resistant starch

1 INTRODUCTION

Pulses are rich in complex carbohydrates, protein, vitamins, minerals, and phytochemicals (Singh, 2017) and are the second most important source of food for humans after cereals (Shevkani et al., 2019). Pulse foods were identified as low glycemic index (GI < 55) (Jenkins et al., 1980; Rizkalla et al., 2002), and pulse consumption was inversely associated with the risk of type-2 diabetes (Brand-Miller...
et al., 2003; Villegas et al., 2008). The rate and degree of starch digestion in pulses are lower than those in the cereals both in vitro and in vivo (Aguilera et al., 2009). Generally, starch is classified into three main fractions of nutritional importance: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst & Cummings, 1987). RDS is digested in the small intestine and leads to a rapid rise in blood glucose levels following ingestion. SDS, an intermediate starch fraction between RDS and RS, is digested slowly throughout the entire small intestine to provide sustained and prolonged glucose release with a low initial glycemia. SDS is likely associated with positive health effects including glycemic control, reduced postprandial circulated free fatty acids, and reduced oxidative stress (Zhang & Hamaker, 2009). RS has the ability to modulate postprandial blood glucose levels; it evades enzymatic hydrolysis in the small intestine, passing into the colon where it is fermented to produce butyrate-rich short chain fatty acids for energy (Topping & Clifton, 2001). Lower starch digestion allows for an increased stability in insulin response due to a decrease in blood glucose fluctuation corresponding to the slower glucose release (Rizkalla et al., 2002). These properties can help in the prevention of obesity and high blood cholesterol, as well as diabetes (Kushi et al., 1999). Therefore, consumption of pulses is recommended for all overweight individuals who have diabetes or are at risk of developing diabetes (American Diabetes Association, 2008). Except for its health benefits of low GI, little is known about pulse starch end-use functionalities compared with other commercial starches (Ren et al., 2021).

Canada is a major producer of pulses in the world and a global leader in lentil production (FAOSTAT, 2019). Pulses are favored crops in Canada due to their high protein content (Roy et al., 2010), but much less attention has been paid to another major component—starch. Starches from different plant sources exhibit different properties determining their end-use functionality and quality (Motte et al., 2021). The relationships between starch composition, structure, functional properties, and practical applications can be deduced by comparison with well-known commercial starches, such as starches from normal corn, waxy corn, high amylose corn, wheat, potato, and yellow pea, which include various types of starch crystallites: A-type (normal corn, waxy corn, and wheat starches) (Jane et al., 1997), B-type (potato starches) (Jane et al., 1997), mixture of B- and V-types (high amylose corn) (Chung et al., 2003), and C-type (mixture of A- and B-types) (yellow pea starch) (Huang et al., 2007).

The objective of this study was to characterize the physicochemical properties and nutritional fractions of the starches isolated from nine Canadian-grown peas in comparison with six commercial starches and to explore their unique properties for the greatest utilization of the pulse resources. Principal component analysis (PCA) and cluster analysis (CA) were conducted to elucidate the relationship between physicochemical properties and nutritional fractions of these pea starches and to identify the key factors influencing these properties.

2 | MATERIALS AND METHODS

2.1 | Materials

Three marrowfat peas (Pisum sativum L.) (AAC Olive, AAC Greenrich, and Bibao), four yellow peas (P. sativum L.) (AAC Profit, AAC Chrome, P1126–1857, and P1116–1114), and two green peas (P. sativum) (AAC Comfort and CDC Limerick) from the 2019 growing season were provided by the Seed Increase Unit, Agriculture and Agri-Food Canada (Indian Head, Saskatchewan, Canada). P1126–1857 and P1116–1114 are two breeding lines developed at Lacombe Research and Development Centre, Agriculture and Agri-Food Canada. The other seven peas are registered varieties in Canada. In the following sections, these variety names are simplified to Olive, Greenrich, Bibao, Profit, Chrome, P1126, P1116, Comfort, and Limerick.

Six commercial starch samples included the following: waxy corn (S9679), normal corn (S4126), wheat (S5127), and potato starch (S4251), which were purchased from Sigma-Aldrich (St. Louis, MO, USA); high amylose corn starch (Hylon VII, Ingredion, Westchester, IL, USA); and yellow pea starch (Meelunie America Inc., MI, USA). Other chemicals used were of analytical grade.

2.2 | Preparation of pea flour and isolation of pea starch

Pea seeds were first cracked with an IKA mill (M10), then milled to flour using an Udy cyclone mill (Model 3010-030; Udy Corp., Fort Collins, CO 80524, USA), and passed through a 500-μm sieve.

Pea starch was isolated from pea flour according to a procedure of Otto et al. (1997) that was modified by Chung et al. (2008). Briefly, the pea flour (100 g) was blended with 200 ml of water for 3 min using a blender at 3500 rpm (Model 34BL97, Waring Commercial, Torrington, CT, USA), and the slurry was then filtered through a 75-μm polypropylene mesh screen using a suction filtration setup with a Büchner funnel and filtration flask under vacuum. The residue was repeatedly blended with 200 ml of water followed by filtration twice. The filtrates were combined and centrifuged at 1500×g for 15 min. The supernatant was discarded, and the tailing starch was carefully separated from the bottom prime starch using a spatula. The prime starch was repeatedly purified by blending with 200 ml of water and centrifuging three more times and then air dried at 25°C, ground, and passed through a screen with 125-μm openings.

2.3 | Color, starch granular morphology, particle size distribution, and chemical composition

Color of pea seeds, flour, and isolated starch (10 g) was measured on the CIeLAB scale with a Lab Scan XE Spectrocolorimeter (Hunter Associates Laboratory, Reston, VA, USA). The colorimeter was standardized with the white standard tile (Hunter Associates Laboratory,
Reston, VA, USA). Three CIE parameters, lightness L* (0 = black, 100 = white), a* (−value = greenness and +value = redness), and b* (−value = blueness and +value = yellowness), were measured with an illuminant of D65 and a standard observer of 10°.

For the morphology of granular starch, starch samples (1% suspension) were placed on a microscope slide and viewed under brightfield and polarized light using a light microscope (AxioImager A2, Carl Zeiss Canada, Toronto, ON, Canada). Images were taken with an AxioCam MRc5 camera (Zeiss) and Zen lite 2012 software using the auto-exposure function at 200× magnification.

The particle size distribution of starch granules was measured using a Fritsch NanoTec laser particle sizer (Analysette 22, Fritsch GmbH, Idar-Oberstein, Germany). For particle size measurement, starch was evenly dispersed with ultrasound in a sample cell filled with distilled water to attain a beam obscuration of ~7%.

Crude protein content was measured by nitrogen amount times 6.25 using a protein analyzer (Flash 2000 analyzer, ThermoFisher Scientific, Waltham, MA, USA). Total starch and total free glucose content were determined by the AACC Approved Method 76-13.01 using DMSO to solubilize the starch at the beginning of the method (AACC, 1999). Amylose content of pea flour samples was determined by a concanavalin A (Con A) method using a Megazyme Amylose/Amylopectin Assay kit (K-AMYL 06/18). Apparent amylose content of the isolated pea starches and commercial starches was measured by iodine colorimetry (Williams et al., 1970).

2.4 Amylose leaching

Starch samples (20 mg, dry basis) in water (10 ml) were heated at 60°C, 70°C, 80°C, and 90°C, in sealed tubes for 30 min. The tubes were then cooled to room temperature and centrifuged at 2000× g for 10 min. Supernatant was withdrawn and its amylose content was determined according to the method of Williams et al. (1970).

2.5 Differential scanning calorimetry

Thermal analyses were performed using a differential scanning calorimeter (DSC Q20; TA Instruments, New Castle, DE, USA) equipped with an RCS90 refrigeration system. Samples (12 mg each) were weighed into high-volume pans and distilled water was added to make suspensions with 70% moisture content. The pans were sealed with an RCS90 refrigeration system. Samples (12 mg each) were heated at 2.4°C/min to 100°C, and held at 100°C for 30 min. The instrument was calibrated using indium and an empty pan as a reference. The onset temperature (T_onset), peak temperature (T_peak), conclusion temperature (T_conclusion), and transition enthalpy (∆H) of each curve were determined using TA Universal Analysis software. Enthalpies of samples were calculated as J/g of dry starch.

2.6 Fourier transform infrared spectroscopy

Infrared spectra of native and processed pea starches were recorded on a Frontier Fourier transform infrared (FTIR) spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a deuterated tri-glycine sulfate (DTGS) detector using an attenuated total reflectance (ATR) accessory at a resolution of 2 cm−1 by four scans. The processed samples were the recovered pastes from Rapid Visco Analyzer (RVA) testing, cooled down to room temperature, and stored at −20°C for 7 days before freeze-drying. The freeze-dried gel was milled to powder by an IKA mill (M10) to pass through a 250-μm sieve. The spectra were baseline corrected in the starch fingerprint region (800–1200 cm−1), and the amplitudes of absorbance were measured at 1047, 1022, and 995 cm−1 (Bernazzani et al., 2000; van Soest et al., 1995).

2.7 Rapid viscosity analysis

Pasting properties of the isolated starch were determined using an RVA (RVA-4, Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) with the STD 2 profile (AACC Method 76-21.01) (AACC, 1999). The starch slurries (2.5 g of starch on a dry basis, 25 ml of water) were equilibrated at 50°C for 1 min, heated at 6°C/min to 95°C, held at 95°C for 5 min, cooled at 6°C/min to 50°C, and held at 50°C for 2 min. Slurries were stirred with a paddle at 160 rpm, except for the first 10 s of the experiment when they were stirred at 960 rpm. Pasting temperature, peak viscosity (PV), breakdown (BD), setback (SB), and final viscosity (FV) were obtained from pasting curves. The reported values were the means of duplicate measurements.

2.8 Starch nutritional fractions

Nutritional fractions of the native starch and cooked starch (freeze-dried RVA gel samples) were determined according to a method of Englyst et al. (1992) with minor modifications (Chung et al., 2009). Briefly, porcine pancreatic α-amylase (P7545, Sigma Aldrich, St. Louis, MO) (0.45 g) was dispersed in water (4 ml) and centrifuged at 1500× g for 12 min. The supernatant (2.7 ml) was transferred to a beaker, and amyloglucosidase (Megazyme International Ireland Ltd., Co., Wicklow, Ireland) (0.32 ml) was diluted to 0.4 ml and 0.3 ml of diluted amyloglucosidase and invertase (2 mg in 0.2 ml distilled water) were added to the solution. This enzyme solution was freshly prepared. For each digestion, 100 mg of sample was added to a test tube, followed by 4 ml of 0.5-M sodium acetate buffer (pH 5.2) and 15 glass beads (4 mm diameter). The enzyme solution (1 ml) was then added and incubated in a shaking water bath (37°C, 200 strokes/min). Aliquots (0.1 ml) were taken at 20 and 120 min after enzyme addition and mixed with 1 ml of 50% ethanol to stop the enzymatic reaction, after which glucose content was measured by the glucose oxidase–peroxidase reagent. Nutritional fractions of starch, that is, percentages of RDS (% digestible starch at 20 min), SD5 (% digestible starch at 120 min – % digestible starch at 20 min),...
and RS (100% – % digestible starch at 120 min), were normalized to the total starch content.

2.9 | Statistical analyses

Samples were tested in duplicate, except starch nutritional fractions were in quadruplicate. Pearson's correlation coefficients and PCA were performed on centered and standardized data to elucidate the relationships among variables of the chemical and nutritional properties of samples. CA was conducted using furthest neighbor linkage method with Euclidean distance being the measure of similarity. Dendrogram and K-means plot were drawn to visualize the clusters of samples. PCA, CA, and statistical analyses (one-way analysis of variance [ANOVA]) followed by Tukey's post hoc test were conducted by OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA). Statistical significance was set at the 5% level of probability.

3 | RESULTS AND DISCUSSION

3.1 | Appearance and color

The appearance of the nine pea seeds is shown in Figure 1. As shown in Figure 1, the three marrowfat peas (Olive, Greenrich, and Bibao) and the two green peas (Comfort and Limerick) showed a similar green seed color except for Greenrich, which was more yellow. The four yellow peas had a similar golden yellow color. This visual appearance of pea seed color was well confirmed by the CIE color parameters (Table S1), as marrowfat peas and green peas showed negative values of $a^*$ (indicating greenness) whereas the four yellow peas had higher or the highest positive values of $b^*$ (indicating yellowness). The marrowfat pea Greenrich was an exception with its higher yellowness $b^*$ value (19.2) of seed color. Marrowfat pea seeds looked larger than the other peas and had a wrinkled, blocky, and irregularly angular shape in contrast to the round, plump seed shape and smooth surface for the green peas and yellow peas (Figure 1). These characteristics are typical of marrowfat peas (Bing et al., 2019).

3.2 | Morphology and size distribution of isolated starch granules

Microscopic images of starch granules are shown in Figure 2. As shown in the figure, no drastic visual differences were observed in the isolated starches from these nine pea seeds. Pea starch granules were relatively homogeneous in granular shape and size distribution compared with those starches from cereals and tubers (Zan et al., 2021). Most granules showed surface cracks that appeared as dark bands under the light microscope, which were actually caused by internal features when viewed under a scanning electron microscope (Ren et al., 2021), and could be cavities produced when the granules
undergo drying (Hall & Sayre, 1971). Also, most starch granules showed multiple Maltese crosses overlapped at the hilum on birefringence as seen under polarized light, which was also observed by Chung et al. (2008). Compared with the four yellow pea starches, marrowfat pea and green pea starches showed larger, irregularly shaped starch granules with multiple Maltese crosses (arrows in Figure 2). However, average starch granular sizes, as reflected by mean particle diameters ($D_{4,3}$) (Zan et al., 2021), were not significantly different among these three pea types (29.3–30.4 μm) and were comparable with the commercial yellow pea starch (29.3 μm) ($p > 0.05$), even though variances were significant among some pea varieties. Compared with the other five commercial starches, only potato starch had a significantly larger granule size (53.9 μm) than these isolated pea starches ($p < 0.05$). The order of mean particle diameter was ranked as potato starch (53.9 μm) > peas (~30 μm) > wheat (20.1 μm) > waxy corn (17.0 μm) > normal corn (16.8 μm) > high amylose corn (11.5 μm) (Table 2).

### 3.3 Chemical composition

The contents of crude protein, total starch, free glucose, and the amylose content measured by the Con A method of pea flours are shown in Table 1. The protein content of these nine peas varied from 22.7% (P1116) to 26% (Greenrich, Bibao, and Limerick), which is consistent to protein content previously reported (21.3%–26.8%) (Motte et al., 2021; Ren et al., 2021). On average, the marrowfat peas had a similar protein content (25.5%) to the green peas (25.5%), and both were slightly higher than that of yellow peas (23.8%) ($p < 0.05$). Proportionally, the total starch content of yellow peas (46.2%) was significantly higher than that of marrowfat peas (43.5%) ($p < 0.05$), but not significantly different from that of green peas (44.7%) (Table 1). Furthermore, no significant differences were observed in the amylose content measured by the Con A method among the three pea types ($p > 0.05$), that is, marrowfat peas (29.3%–29.6%), yellow peas (27.4%–30.2%), and green peas (28.3%–28.9%), except for a few random variances among varieties (Table 1). We also measured the apparent amylose content of the isolated pea starches by an iodine colorimetric method, and the values were 51.3%–51.6% (marrowfat peas), 50.6%–53.8% (yellow peas), and 49.9%–54.2% (green peas) and found no significant differences among pea types ($p > 0.05$) (Table 2). The total free glucose content of the pea flours was low (0.1%–0.14%) (Table 1). The values of total starch of these nine peas (42.6%–47.0%) are slightly lower than those in previous reports (45.7%–51.2%) (Ren et al., 2021), but the apparent amylose contents (49.9%–54.2%) of these nine

![Figure 2](image-url)
## TABLE 1  Chemical components of pea flour (% db)

| Variety       | Protein  | Total starch | Total free glucose | Amylose by Con A method |
|---------------|----------|--------------|--------------------|-------------------------|
| Marrowfat pea |          |              |                    |                         |
| Olive         | 24.6 ± 0.0c | 43.6 ± 1.3cb  | 0.13 ± 0.01a       | 29.5 ± 0.0ba            |
| Greenrich     | 26.0 ± 0.0a | 42.6 ± 0.2c   | 0.12 ± 0.01a       | 29.6 ± 0.0a             |
| Bibao         | 26.0 ± 0.1a | 44.1 ± 0.1cb  | 0.11 ± 0.03a       | 29.3 ± 0.2cba           |
| Average       | 25.5 ± 0.7A | 43.5 ± 0.9B   | 0.12 ± 0.02A       | 29.5 ± 1.8A             |
| Yellow pea    |          |              |                    |                         |
| Profit        | 25.0 ± 0.0b | 45.9 ± 0.3ba  | 0.11 ± 0.02a       | 30.2 ± 1.0a             |
| Chrome        | 23.9 ± 0.2d | 46.1 ± 0.8ba  | 0.13 ± 0.00a       | 27.4 ± 0.1d             |
| P1126         | 23.7 ± 0.1d | 47.0 ± 0.6a   | 0.11 ± 0.00a       | 27.9 ± 0.3dc            |
| P1116         | 22.7 ± 0.0e | 45.9 ± 1.3ba  | 0.14 ± 0.02a       | 29.1 ± 0.5cba           |
| Average       | 23.8 ± 0.9B | 46.2 ± 0.8A   | 0.12 ± 0.02a       | 28.7 ± 1.4A             |
| Green pea     |          |              |                    |                         |
| Comfort       | 24.9 ± 0.1b | 45.8 ± 0.3cba | 0.11 ± 0.01a       | 28.9 ± 0.1dcb           |
| Limerick      | 26.0 ± 0.0a | 43.6 ± 1.1cb  | 0.10 ± 0.02a       | 28.3 ± 0.2dcb           |
| Average       | 25.5 ± 0.6A | 44.7 ± 1.5AB  | 0.11 ± 0.01A       | 28.6 ± 0.3A             |

Note: Values are presented as the mean ± SD, n = 2. Values followed by a different lowercase letter in the same column are significantly different among pea varieties; values followed by a different uppercase letter in the same column are significantly different among pea types (p < 0.05).

## TABLE 2  Starch granule size (μm), apparent amylose content, and amylose leaching of isolated starches (%)

| Variety       | D(4, 3), μm | Apparent amylose  | Amylose leaching |
|---------------|-------------|-------------------|-----------------|
|               | 60°C        | 70°C              | 80°C            | 90°C            |
| Marrowfat pea |             |                   |                 |                 |
| Olive         | 28.9 ± 0.1j | 51.4 ± 0.94dcb    | 9.2 ± 1.3cb     | 33.0 ± 0.1ba    | 38.2 ± 0.7ba    | 46.6 ± 0.2a    |
| Greenrich     | 29.0 ± 0.3i | 51.3 ± 0.1dcb     | 11.4 ± 4.0b     | 30.8 ± 0.3c     | 37.6 ± 1.0ba    | 44.8 ± 0.7a    |
| Bibao         | 30.1 ± 0.0e | 51.6 ± 0.0dcb     | 18.4 ± 2.2a     | 34.1 ± 0.2a     | 40.1 ± 0.7a     | 47.4 ± 0.1a    |
| Average       | 29.3 ± 0.6B | 51.4 ± 0.4B       | 13.0 ± 4.8BA    | 32.6 ± 1.5A     | 38.6 ± 1.3A     | 46.3 ± 1.2A    |
| Yellow pea    |             |                   |                 |                 |
| Profit        | 29.2 ± 0.7h | 50.8 ± 0.3dc      | 15.8 ± 3.0a     | 33.2 ± 0.3ba    | 39.0 ± 0.3ba    | 47.0 ± 0.4a    |
| Chrome        | 29.5 ± 0.6f | 50.6 ± 0.7d       | 16.4 ± 1.1a     | 31.7 ± 0.4cb    | 36.4 ± 0.2ba    | 44.6 ± 0.1a    |
| P1126         | 31.3 ± 0.1d | 51.6 ± 0.4dcb     | 6.9 ± 2.2dc     | 31.2 ± 0.6cb    | 36.8 ± 0.6ba    | 44.6 ± 0.4a    |
| P1116         | 31.7 ± 0.2b | 53.8 ± 0.4cb      | 6.6 ± 2.2dc     | 32.2 ± 0.1cb    | 38.0 ± 0.0ba    | 46.1 ± 0.3a    |
| Average       | 30.4 ± 1.2B | 51.7 ± 1.4B       | 11.4 ± 5.3BA    | 32.1 ± 0.9A     | 37.6 ± 1.18A    | 45.6 ± 1.1A    |
| Green pea     |             |                   |                 |                 |
| Comfort       | 27.6 ± 0.0k | 49.9 ± 0.6d       | 10.1 ± 0.0cb    | 31.6 ± 0.5cb    | 36.6 ± 0.2ba    | 46.0 ± 0.7a    |
| Limerick      | 31.6 ± 0.2c | 54.2 ± 0.4b       | 7.2 ± 0.6dc     | 34.3 ± 0.1a     | 37.5 ± 0.1ba    | 45.4 ± 0.6a    |
| Average       | 29.6 ± 2.3B | 52.0 ± 2.5B       | 8.6 ± 1.7CB     | 32.9 ± 1.5A     | 37.0 ± 0.5BA    | 45.7 ± 0.6A    |
| Commercial starchy | |                   |                 |                 |
| Yellow pea    | 29.3 ± 0.1gB | 44.9 ± 1.2eC     | 1.4 ± 0.1eD     | 10.0 ± 0.1eC    | 25.7 ± 0.4eC    | 32.4 ± 0.6eCB  |
| Waxy corn     | 17.0 ± 0.0mC | 5.7 ± 0.1HF      | 0.1 ± 0.00eF    | 0.4 ± 0.00f    | 3.8 ± 0.1eF     | 4.7 ± 0.1eE    |
| Normal corn   | 16.8 ± 0.0nC | 32.4 ± 0.0gE     | 1.1 ± 0.1eD     | 7.5 ± 0.3fD     | 13.8 ± 0.2dD    | 31.4 ± 1.8bC   |
| High amylose corn | 11.5 ± 0.4oD | 60.8 ± 2.1aA    | 0.5 ± 0.1eD     | 0.5 ± 0.0hf    | 2.3 ± 0.0eF     | 9.0 ± 0.5cD    |
| Wheat         | 20.1 ± 0.5iC | 33.8 ± 0.1gED    | 3.2 ± 0.1edDC   | 5.0 ± 0.0gE    | 10.4 ± 0.4dE    | 34.2 ± 0.8bB   |
| Potato        | 53.9 ± 0.3sA | 37.0 ± 1.1fD    | 16.4 ± 0.4aA    | 24.0 ± 1.8dB    | 35.1 ± 3.8bB    | 46.7 ± 1.9A    |

Note: Values are presented as the mean ± SD, n = 2. Values followed by a different lowercase letter in the same column are significantly different among pea varieties and commercial starchy; values followed by a different uppercase letter in the same column are significantly different among pea varieties and commercial starchy (p < 0.05).

*Apparent amylose content is expressed on a dry weight basis as a percentage of total starch.
peas are obviously higher than those reported by others (31%-49%) (Ren et al., 2021). The higher amylose content imparts distinct functional properties. Unique physicochemical properties and functionalities of these pea starches would be expected in these newly bred pea varieties.

### 3.4 | Amylose leaching

The amylose leaching results from nine isolated pea starches and six commercial starches are shown in Table 2. Statistically, the values of amylose leaching varied significantly among these nine pea varieties (p < 0.05) but not significantly among the three pea types (p > 0.05), especially at lower temperatures (60–70°C), and the differences diminished at higher temperatures (80–90°C). This result is foreseeable because of their similar apparent amylose contents (p > 0.05) (Table 2), which is also supported by the similar amylose contents measured by the Con A method (Table 1).

Compared with the six commercial starches, the pea starches in this study showed an apparent amylose content (~51.7%) lower than only the high amylose corn starch (60.8%), yet considerably higher than those of other commercial starches, including the commercial yellow pea starch (44.9%) (p < 0.05) (Table 2). Except for commercial potato starch, which showed comparable amylose leaching, the nine pea starches showed significantly higher amylose leaching than the other commercial starches at any given temperature (p < 0.05). The commercial waxy corn starch was theoretically absent of amylose (5.7%) and thus gave an expected low amylose leaching value (4.7% at 90°C), whereas the commercial high amylose corn starch had 60.8% apparent amylose content but showed only 9.0% amylose leaching at 90°C (Table 2), which was attributed to its exceptionally high gelatinization temperature (Tg, 71.5°C; Tp, 92.3°C; and Tc, 115.4°C) (Table 3).

In fact, the high amylose corn starch showed no granular swelling or pasting peak when heated to 95°C by the RVA (Table 5). It is reported that, because of the high content, amylose molecules in common pulse starches tend to restrict granular swelling and show less leaching than most normal cereal, tuber, and root starches during leaching than most normal cereal, tuber, and root starches during cooing (Ren et al., 2021). Obviously, these nine pea starches had high amylose content but still showed higher amylose leaching than the commercial starches, with potato starch as the only exception (Table 2). These differences imply different profiles of starch nutritional fractions.

### 3.5 | Thermal properties of isolated starches (DSC)

The thermal properties (gelatinization and retrogradation) of isolated pea starches and six commercial starches are shown in Table 3. There were no significant differences in either gelatinization parameters or retrogradation parameters among pea types and also no significant differences among these nine pea varieties in most cases (p > 0.05) (Table 3). These results indicated that the thermal properties of the isolated pea starches were similar among these nine pea varieties. Their values of gelatinization temperatures (Tg, 56.2–57.1°C; Tp, 63.1–63.8°C; Tc, 75.5–76.8°C) are similar to previous reports (Tg, 58.0–60.0°C; Tp, 63.5–70.1°C; Tc, 72.3–81.1°C), but the gelatinization enthalpies (16.8–18.3 J/g) are much higher than those of previous reports (8.4–14.1 J/g) (Ren et al., 2021). Gelatinization parameters, especially the enthalpy, are a measure of crystalline structure of starch, which means reduced susceptibility to enzymes during the hydrolysis process (Li et al., 2019), thus potentially more starch nutritional fractions (i.e., SDS and RS).

Compared with the six commercial starches, including the commercial yellow pea starch, the nine isolated pea starches in this study showed lower or the lowest gelatinization temperatures (Tg, Tp, Tc) but higher or the highest gelatinization enthalpy (ΔH) (p < 0.05). After storage at 4°C for 14 days, these nine pea starches showed comparable retrogradation parameters with the commercial yellow pea starch, that is, intermediate retrogradation temperatures (Tg, Tp, Tc) but still higher or highest retrogradation enthalpy (ΔH) compared with the other five commercial starches (p < 0.05). All the nine isolated pea starches had comparable gelatinization enthalpy (~17.3 J/g) with the high amylose corn starch (17.0 J/g), potato starch (17.9 J/g), and waxy corn starch as well (16.0 J/g), and also comparable retrogradation enthalpy (~9.0 J/g) with that of the commercial potato starch (11.7 J/g) and commercial yellow pea as well (10.4 J/g) (p > 0.05). These results suggest that these nine pea starches gelatinize easily (indicated by their significantly lower gelatinization temperatures [Tg, Tp, Tc] than those of all the commercial starches except a lower Tc of commercial wheat starch), but retrograde extensively (indicated by their higher retrogradation enthalpies), and thus can be fully cooked at a lower temperature and a shorter time, but with a higher tendency to retrograde and a greater potential to form type-3 RS than other starches (Lu et al., 2019). Pulses used to be perceived as difficult to cook with long cooking times (Ren et al., 2021). The unique low gelatinization temperatures of these nine pea starches would be advantageous to promote their use in a broad spectrum of industrial applications.

### 3.6 | Fourier transform infrared spectroscopy

Table 4 shows the FTIR peak height ratio of 1047/1022 cm⁻¹ and 995/1022 cm⁻¹ of native and cooked starches from the nine pea varieties and six commercial starches. There were no significant differences observed in peak height ratio of 1047/1022 cm⁻¹ and 995/1022 cm⁻¹ among the nine native pea starches. Even after the starches were cooked, the peak height ratios showed only minor differences among the three pea types, except for a few variety-based variances that were mainly seen on the peak height ratios of 995/1022 cm⁻¹ of the cooked pea starches (Table 4).

FTIR reveals the short-range ordering on the starch granular surface, because the average ATR-FTIR penetrating depth is ~2 μm (Sevenou et al., 2002b). Within the wavenumber region from approximately 1200 to 800 cm⁻¹ (starch fingerprint) (van Soest et al., 1995),
| Variety                  | Gelatinization | Retrogradation |
|--------------------------|----------------|----------------|
|                          | $T_o$ (°C)     | $T_p$ (°C)     | $T_c$ (°C) | $\Delta H$ (J/g) | $T_o$ (°C)     | $T_p$ (°C)     | $T_c$ (°C) | $\Delta H$ (J/g) |
| **Marrowfat pea**        |                |                |            |                |                |                |            |                |
| Olive                    | 57.1 ± 0.0g    | 63.8 ± 0.2fe   | 76.8 ± 0.1f| 18.3 ± 1.1a    | 40.9 ± 0.2eb   | 58.3 ± 0.3c    | 79.0 ± 0.5c| 10.8 ± 0.6cba   |
| Greenrich                | 56.8 ± 0.1hg   | 63.7 ± 0.2fe   | 75.5 ± 0.2i| 16.8 ± 0.1ba   | 41.0 ± 0.1b    | 58.0 ± 0.1c    | 78.4 ± 0.2c| 10.7 ± 0.3cba   |
| Bibao                    | 56.4 ± 0.2hg   | 63.5 ± 0.2fe   | 76.0 ± 0.2hgf| 17.1 ± 0.5ba  | 40.7 ± 0.1dcb  | 58.0 ± 0.1c    | 78.7 ± 0.6c| 10.8 ± 0.1cba   |
| Average                  | 56.8 ± 0.4G    | 63.7 ± 0.2FE   | 76.1 ± 0.6GF| 17.4 ± 0.9BA  | 40.9 ± 0.2B    | 58.1 ± 0.2C    | 78.7 ± 0.5C| 10.7 ± 0.3BA    |
| **Yellow pea**           |                |                |            |                |                |                |            |                |
| Profit                   | 56.5 ± 0.4hg   | 63.3 ± 0.5fe   | 75.7 ± 0.6hgf| 16.9 ± 0.3ba  | 40.2 ± 0.1edcb| 57.7 ± 0.1c    | 78.5 ± 0.4c| 10.9 ± 0.1cba   |
| Chrome                   | 56.2 ± 0.0h    | 63.1 ± 0.3f    | 76.1 ± 0.0hgf| 17.0 ± 1.0ba  | 40.7 ± 0.4dcb  | 58.0 ± 0.3c    | 79.0 ± 1.0c| 10.5 ± 0.1dbb   |
| P1126                    | 56.5 ± 0.0hg   | 63.6 ± 0.1fe   | 75.9 ± 0.1hgf| 17.9 ± 0.4ba  | 40.4 ± 0.0edcb| 58.3 ± 0.1c    | 79.1 ± 0.1c| 11.5 ± 0.0ba    |
| P1116                    | 56.7 ± 0.1hg   | 63.7 ± 0.2fe   | 76.3 ± 0.1hgf| 17.0 ± 0.3ba  | 40.0 ± 0.0edcb| 58.3 ± 0.4c    | 79.2 ± 0.8c| 11.5 ± 0.1cba   |
| Average                  | 56.5 ± 0.2G    | 63.4 ± 0.3F    | 76.0 ± 0.3GF| 17.2 ± 0.6BA  | 40.3 ± 0.3DCB  | 58.1 ± 0.3C    | 78.9 ± 0.6C| 11.1 ± 0.4BA    |
| **Green pea**            |                |                |            |                |                |                |            |                |
| Comfort                  | 56.5 ± 0.1hg   | 63.7 ± 0.1fe   | 76.6 ± 0.1gf| 17.2 ± 0.5ba  | 40.7 ± 0.2dcb  | 58.2 ± 0.1c    | 79.1 ± 0.3c| 11.4 ± 0.1cba   |
| Limerick                 | 56.4 ± 0.5hg   | 63.3 ± 0.2fe   | 76.6 ± 0.6hgf| 17.3 ± 0.0ba  | 41.1 ± 0.7b    | 57.7 ± 0.0c    | 78.5 ± 0.3c| 10.7 ± 0.6cba   |
| Average                  | 56.5 ± 0.3G    | 63.5 ± 0.2F    | 76.3 ± 0.5F  | 17.2 ± 0.3BA  | 40.9 ± 0.5B    | 58.0 ± 0.3C    | 78.8 ± 0.4C| 11.0 ± 0.5BA    |
| **Commercial starches**  |                |                |            |                |                |                |            |                |
| Yellow pea               | 64.4 ± 0.4dD   | 70.4 ± 0.2cC   | 81.4 ± 0.0dD| 13.3 ± 0.6dC  | 41.0 ± 0.3bB   | 57.9 ± 0.4cC   | 79.2 ± 0.2cC| 10.4 ± 0.1dCB   |
| Waxy corn                | 65.8 ± 0.0cC   | 73.8 ± 0.3bB   | 84.8 ± 0.0bB| 16.0 ± 0.1cbB | 38.9 ± 0.2cF   | 56.9 ± 0.4cC   | 74.9 ± 0.3dD| 6.9 ± 0.5eD     |
| Normal corn              | 67.1 ± 0.1bB   | 73.0 ± 0.0bB   | 83.6 ± 0.0cC| 14.1 ± 0.1dcC | 39.7 ± 0.1feED| 54.4 ± 0.1dD   | 73.2 ± 0.4dE| 9.5 ± 0.1dC     |
| High amyllose corn       | 71.5 ± 0.4aA   | 92.3 ± 1.2aA   | 115.4 ± 0.4aA| 17.0 ± 0.4baBA| 74.7 ± 0.2aA   | 96.8 ± 0.6aA   | 119.5 ± 0.1aA| 6.5 ± 0.1eD     |
| Wheat                    | 59.9 ± 0.1fF   | 64.6 ± 0.2eE   | 75.0 ± 0.1g  | 12.9 ± 0.3dC  | 39.9 ± 0.0fedDC| 53.6 ± 0.2dD   | 70.6 ± 0.1eF| 6.0 ± 0.4eD     |
| Potato                   | 62.2 ± 0.0eE   | 68.3 ± 0.1dD   | 79.1 ± 0.1eE| 17.9 ± 0.5baA | 40.7 ± 0.2edcbCB| 64.2 ± 1.4bB   | 82.0 ± 0.2bB| 11.7 ± 0.1aA    |

Note: Values are presented as the mean ± SD, $n = 2$. Values followed by a different lowercase letter in the same column are significantly different among pea varieties and commercial starches; values followed by a different uppercase letter in the same column are significantly different among pea types and commercial starches ($p < 0.05$).
typical bands at 1047 and 995 cm$^{-1}$ have been assigned to crystalline starch (Capron et al., 2007; Sevenou et al., 2002a), and the band at 1022 cm$^{-1}$ has been assigned to amorphous forms in starch (van Soest et al., 1995). The ratios of bands at 1047/1022 cm$^{-1}$ and 995/1022 cm$^{-1}$ have been used to quantify the degree of short-range ordering in starches (Lopez-Rubio et al., 2008; Lu et al., 2018). Therefore, these results indicated that there were no significant differences in the short-range ordering in the native form of these starches and their new crystalline structure after retrogradation was also similar ($p > 0.05$). This result supports the finding of similar crystalline structure among these samples as determined by DSC (gelatinization and retrogradation) (Table 3).

The FTIR results of these nine pea starches are comparable with those of the commercial yellow pea starch, that is, showing a higher or the highest short-range ordering in starch compared with the other commercial starches, except for a few minor differences in FV and SB among individual pea starches (Table 5). Compared with the commercial starches, these nine pea starches showed the most similar pasting parameters to the commercial yellow pea starches (PV, trough, and BD) and were at an intermediate level compared with other commercial starches. However, the FV and SB of these nine pea starches were the highest whereas the BD, peak time, and peak temperature were lower or the lowest among all the starch samples ($p < 0.05$), which again indicated that these pea starches are easier to be gelatinized but have a high tendency to retrograde. These results support the DSC and FTIR results, which indicated that these nine pea starches are better options for gel foods or foods with low glycemic response for their easy gelatinization but high tendency of retrogradation in regard to their advantageous high amylose content (Tables 2–4). Ren et al. (2021) indicated that, due to the high amylose

### Table 4: FTIR peak height ratios of native and cooked pea starches and commercial starches

| Variety             | Native starch       | Cooked starch       |
|---------------------|---------------------|---------------------|
|                     | 1047/1022 ratio     | 995/1022 ratio      | 1047/1022 ratio     | 995/1022 ratio      |
| Marrowfat pea       |                     |                     |                     |
| Olive               | 0.7122 ± 0.0004cb    | 1.2034 ± 0.0010dcb  | 0.6782 ± 0.0024edcb| 1.0239 ± 0.0031h    |
| Greenrich           | 0.7172 ± 0.0007cb    | 1.2099 ± 0.0002cba  | 0.6885 ± 0.0014cba  | 1.0637 ± 0.0066e    |
| Bibao               | 0.7155 ± 0.0000cb    | 1.2068 ± 0.0019dcb  | 0.6815 ± 0.0005cba  | 1.0420 ± 0.0030g    |
| Average             | 0.7150 ± 0.0023B     | 1.2067 ± 0.0030BA   | 0.6827 ± 0.0049BA   | 1.0432 ± 0.0179ED   |
| Yellow pea          |                     |                     |                     |
| Profit              | 0.7148 ± 0.0004cb    | 1.1210 ± 0.0016cba  | 0.6892 ± 0.0016cba  | 1.0598 ± 0.0015e    |
| Chrome              | 0.7134 ± 0.0000cb    | 1.2097 ± 0.0009cba  | 0.6795 ± 0.0011edcb | 1.0728 ± 0.0016ed   |
| P1126               | 0.7172 ± 0.0008cb    | 1.1936 ± 0.0022dcb  | 0.6992 ± 0.0004a    | 1.0847 ± 0.0017d    |
| P1116               | 0.7160 ± 0.0001cb    | 1.1989 ± 0.0007dcb  | 0.6918 ± 0.0017cba  | 1.0733 ± 0.0015ed   |
| Average             | 0.7154 ± 0.0016B     | 1.2035 ± 0.0082BA   | 0.6899 ± 0.0076A    | 1.0726 ± 0.0095DC   |
| Green pea           |                     |                     |                     |
| Comfort             | 0.7132 ± 0.0007cb    | 1.1985 ± 0.0025dcb  | 0.6730 ± 0.0005edc  | 1.0276 ± 0.0025h    |
| Limerick            | 0.7149 ± 0.0004cb    | 1.2090 ± 0.0005cba  | 0.6947 ± 0.0010ba   | 1.0416 ± 0.0010g    |
| Average             | 0.7140 ± 0.0011B     | 1.2038 ± 0.0063BA   | 0.6839 ± 0.0125BA   | 1.0346 ± 0.0083E    |
| Commercial starches |                     |                     |                     |
| Yellow pea          | 0.7233 ± 0.0011B     | 1.2256 ± 0.0047baA  | 0.6863 ± 0.0006cbA  | 1.1415 ± 0.0007aA   |
| Waxy corn           | 0.7167 ± 0.0038cbB   | 1.1856 ± 0.0093edCB | 0.6781 ± 0.0030edcbA| 1.0582 ± 0.0083ED   |
| Normal corn         | 0.6964 ± 0.0010dC    | 1.1552 ± 0.0016dD   | 0.6599 ± 0.0074eB   | 1.1054 ± 0.0063cCB  |
| High amylose corn   | 0.6782 ± 0.0119eD    | 1.1117 ± 0.0352fE   | 0.6802 ± 0.0023dcbA | 1.1213 ± 0.0054bBA  |
| Wheat               | 0.7030 ± 0.0016dC    | 1.1705 ± 0.0017edDC | 0.6606 ± 0.0098edB  | 1.0604 ± 0.0015fED  |
| Potato              | 0.7421 ± 0.0059aA    | 1.2319 ± 0.0016aA   | 0.6793 ± 0.0135edcbBA| 1.1034 ± 0.0022cCB  |

Note: Values are presented as the mean ± SD, $n = 2$. Values followed by a different lowercase letter in the same column are significantly different among pea varieties and commercial starches; values followed by a different uppercase letter in the same column are significantly different among pea types and commercial starches ($p < 0.05$).

### 3.7 Pasting properties (RVA)

RVA pasting properties of isolated pea starches and six commercial starches are shown in Table 5. Again, there were no significant differences in most of the pasting parameters among these isolated pea starches ($p > 0.05$), except for a few minor differences in FV and SB among individual pea starches (Table 5). Compared with the commercial starches, these nine pea starches showed the most similar pasting parameters to the commercial yellow pea starches (PV, trough, and BD) and were at an intermediate level compared with other commercial starches. However, the FV and SB of these nine pea starches were the highest whereas the BD, peak time, and peak temperature were lower or the lowest among all the starch samples ($p < 0.05$), which again indicated that these pea starches are easier to be gelatinized but have a high tendency to retrograde. These results support the DSC and FTIR results, which indicated that these nine pea starches are better options for gel foods or foods with low glycemic response for their easy gelatinization but high tendency of retrogradation in regard to their advantageous high amylose content (Tables 2–4). Ren et al. (2021) indicated that, due to the high amylose...
content, pulse starches generally display lower PV and BD viscosity but higher FV and SB viscosity in comparison with normal maize and tapioca starches, which is supported by the results in the current study. Pasting properties are mainly attributes of the starch component and were also reported to be significantly affected by both chemical and physical parameters and thus a comprehensive multiparameter for evaluation of the functionality of pulse flour (Motte et al., 2021).

### 3.8 Starch nutritional fractions

Nutritional fractions (RDS, SDS, and RS) of native and cooked starches are shown in Table 6. Among the three pea types, the average values of starch nutritional fractions had no significant differences either in native starches or in cooked ones, except a slightly lower RDS was found in cooked green peas (69.9% RDS) compared with marrowfat peas (75.8%) and yellow peas (74.9%) (p < 0.05). The Olive marrowfat pea had the highest RDS (26.2%) but the lowest RS (39.9%) among the nine native starch samples. The remaining eight native pea starches showed similar starch digestibility profiles, with the other two native marrowfat pea starches (Greenrich and Bibao) having slightly higher RS values (≥45.5%). However, after the starches were cooked, the differences were mostly diminished, except the green pea Limerick showed the highest RS (29.5%), the lowest SDS (1.3%), and the lowest RDS (69.3%) among these nine pea varieties. Two marrowfat peas (Olive and Bibao) and two yellow peas (Profit and Chrome) had similar low RS (≤17.2%) after cooking, whereas other pea varieties showed higher RS values ranging from 20% to 23% (Table 6). The SDS content of cooked samples ranged from 1% to 9%.

Compared with the commercial starches, these nine native pea starches had high RS content (~44%) similar to the commercial pea starch (47.1%), and only lower than that of high amylose corn starch (65.2%) and potato starch (66.2%). However, after cooking, these nine pea starches had higher or the highest SDS (1.3%–8.5%) and higher RS content (16.1%–29.5%) than most of the commercial starches except the cooked high amylose corn starch (7.0% SDS and 29.8%)

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**Table 6: RVA pasting properties of isolated pea starches and commercial starches**

| Variety       | Peak viscosity, cP | Trough, cP | Breakdown, cP | Final viscosity, cP | Setback, cP | Peak time, min | Pasting temp, °C |
|---------------|-------------------|------------|---------------|---------------------|-------------|---------------|-----------------|
| **Marrowfat pea** |                   |            |               |                     |             |               |                 |
| Olive         | 1560 ± 4dc        | 1170 ± 10cb| 390 ± 6d      | 2919 ± 18b          | 1749 ± 8ba  | 6.9 ± 0.1fed  | 68 ± 0.6ed     |
| Greenrich     | 1467 ± 80dc       | 1070 ± 37dc| 397 ± 42d     | 2546 ± 103c         | 1477 ± 66c  | 6.5 ± 0.1fe   | 67.3 ± 0.3e    |
| Bibao         | 1447 ± 5dc        | 1188 ± 28cb| 259 ± 23ed    | 2912 ± 45b          | 1724 ± 17cba| 7.2 ± 0.2ed   | 67.9 ± 0.0ed   |
| Average       | 1491 ± 65c        | 1143 ± 61CB| 349 ± 73d     | 2792 ± 198BA        | 1650 ± 138A| 6.9 ± 0.3E    | 67.7 ± 0.4E    |
| **Yellow pea**  |                   |            |               |                     |             |               |                 |
| Profit        | 1492 ± 1dc        | 1175 ± 34cb| 317 ± 33ed    | 3061 ± 8b           | 1886 ± 25ba | 6.9 ± 0.2fed  | 68.4 ± 0.1ed   |
| Chrome        | 1423 ± 8dc        | 1285 ± 158cb| 138 ± 150ed   | 2869 ± 60b          | 1584 ± 98cb | 7.4 ± 0.5ed   | 67.8 ± 0.3e    |
| P1126         | 1396 ± 95d        | 1252 ± 81cb| 144 ± 176ed   | 2968 ± 252b         | 1716 ± 332cba| 7.5 ± 0.2edc  | 68.1 ± 0.3ed   |
| P1116         | 1542 ± 28dc       | 1339 ± 5b  | 204 ± 33ed    | 3373 ± 16a          | 2035 ± 21a  | 7.4 ± 0.0ed   | 68.0 ± 0.0ed   |
| Average       | 1463 ± 72C        | 1263 ± 93B | 201 ± 118ED   | 3068 ± 225A         | 1805 ± 225A| 7.3 ± 0.3ED   | 68.1 ± 0.3E    |
| **Green pea**  |                   |            |               |                     |             |               |                 |
| Comfort       | 1637 ± 23dc       | 1251 ± 26cb| 387 ± 49d     | 2971 ± 21b          | 1721 ± 47cba| 6.7 ± 0.1fe   | 67.4 ± 0.2e    |
| Limerick      | 1581 ± 23dc       | 1364 ± 89b | 217 ± 112ed   | 3116 ± 3b           | 1752 ± 92cb | 6.9 ± 0.0fed  | 67.8 ± 0.3e    |
| Average       | 1609 ± 37C        | 1307 ± 85B | 302 ± 121ED   | 3044 ± 85A          | 1736 ± 62A  | 6.8 ± 0.1E    | 67.6 ± 0.3E    |
| **Commercial starches** |           |            |               |                     |             |               |                 |
| Yellow pea    | 1544 ± 16dcC      | 1326 ± 5bB | 219 ± 11edED  | 2337 ± 16cB         | 1012 ± 11dB | 9.0 ± 0.0cabcB| 72.3 ± 0.5C    |
| Waxy corn     | 3003 ± 33bB       | 947 ± 6edDC| 2056 ± 27bB   | 1153 ± 8eD          | 207 ± 2eC   | 5.6 ± 0.0gF   | 69.1 ± 0.1eD   |
| Normal corn   | 1662 ± 1cC        | 925 ± 0edD | 737 ± 1cC     | 1798 ± 6dC          | 873 ± 6dB   | 8.5 ± 0.0dcbDC| 82.3 ± 0.5bB   |
| High amylose corn | 4 ± 0IE       | –22 ± 1E   | 26 ± 1eE      | –15 ± 1FE           | 7 ± 1eC     | 10.4 ± 1.4aA  | 0.0 ± 0.0gG    |
| Wheat         | 1054 ± 1eD        | 800 ± 7eD  | 254 ± 8eDE    | 1531 ± 11dDC        | 731 ± 4dB   | 9.9 ± 0.0baBA | 89.3 ± 0.3aA   |
| Potato        | 8987 ± 207aA      | 2230 ± 30aA| 6757 ± 177aA  | 2936 ± 52bA         | 707 ± 22dB  | 4.6 ± 0.0gF   | 64.4 ± 0.0fF   |

Note: Values are presented as the mean ± SD, n = 2. Values followed by a different lowercase letter in the same column are significantly different among pea varieties and commercial starches (p < 0.05); values followed by a different uppercase letter in the same column are significantly different among pea types and commercial starches (p < 0.05).
These results are supported by previous in vitro and in vivo studies, which revealed that the digestibility of raw pulse starches is generally lower than that of normal maize and other normal cereal starches (Ren et al., 2021). These results further suggested that these nine pea starches have a high retrogradation tendency (also indicated by DSC, FTIR, and RVA), which could promote the formation of more desirable starch nutritional fractions (SDS and RS) after cooking and would be valuable ingredients for low GI foods as well-documented healthy benefits conferred by the consumption of pulse and pulse-based foods in previous literature, such as improved glycemic and insulimemic control and prevention of type-2 diabetes (Ren et al., 2021).

3.9 | Principal component analysis

PCA provides an overview of the similarities and differences between the measured properties and was used in this study to visualize the variations in physicochemical and nutritional properties of the isolated pea starches and commercial starches. The results showed that the proportions of eigenvalues of the correlation matrix for the first three principal components (PCs) were 42.4%, 27.2%, and 14.3%. Two components accounted for 69.6% of the total variance and three components explained 83.9%, indicating that three components could provide an excellent summary of the data. A 3D score plot in which the points represent different starches and a 3D loading plot in which the vectors represent physicochemical properties and starch nutritional fractions are presented in Figure 3.

As shown in Figure 3b, the directions of the vectors indicate that PC1 explains amylose-related properties, such as amylose leaching at 70°C, 80°C, and 90°C, FV, SB, and RS content of cooked starches; PC2 distinguishes the structure properties (e.g., FTIR and DSC) and nutritional fractions in native starches; and PC3 appears to represent some of the pasting properties (e.g., RVA). The pair of variables describing RS of cooked starch, amylose content, amylose leaching, and RVA parameters during the cooling stage (FV and SB) were highly correlated and also highly negatively correlated to RDS of cooked starches and DSC gelatinization and retrogradation temperatures (\(T_o\), \(T_p\), and \(T_c\)), suggesting that amylose was a dominant factor.

### Table 6: Nutritional fractions of native and cooked isolated starches and commercial starches

| Variety          | Native       | Cooked       |
|------------------|--------------|--------------|
|                  | RDS          | SDS          | RS     |
|                  | RDS          | SDS          | RS     |
| **Marrowfat pea**|              |              |        |
| Olive            | 26.2 ± 0.5c  | 34.0 ± 1.2e  | 39.9 ± 1.1d |
| Greenrich        | 19.7 ± 1.1g  | 34.2 ± 1.2ed | 46.2 ± 2.2cb |
| Bibao            | 23.5 ± 1.2ed | 31.0 ± 0.6e  | 45.5 ± 1.1cb |
| Average          | 23.1 ± 2.9D  | 33.1 ± 1.8D  | 43.8 ± 3.3B |
|                  | 77.2 ± 1.0ed | 5.9 ± 0.5dcba | 16.9 ± 0.7dcb |
| **Yellow pea**   |              |              |        |
| Profit           | 23.4 ± 2.2ed | 33.4 ± 3.2e  | 43.2 ± 1.0c |
| Chrome           | 22.2 ± 0.3fe | 33.5 ± 1.1e  | 44.3 ± 1.2cb |
| P1126            | 21.1 ± 0.8gf | 33.6 ± 1.7e  | 45.3 ± 2.0cb |
| P1116            | 23.0 ± 1.3fe | 32.7 ± 0.7e  | 44.3 ± 1.4cb |
| Average          | 22.4 ± 1.5D  | 33.3 ± 1.8D  | 44.3 ± 1.5B |
|                  | 77.4 ± 0.3ed | 6.5 ± 0.3cba  | 16.1 ± 0.5dcb |
| **Green pea**    |              |              |        |
| Comfort          | 25.2 ± 0.2dc | 31.8 ± 2.1e  | 43.0 ± 2.2dc |
| Limerick         | 22.8 ± 0.6fe | 32.4 ± 1.1e  | 44.8 ± 1.6cb |
| Average          | 24.0 ± 1.4DC | 32.1 ± 1.6D  | 43.9 ± 2.0B |
|                  | 70.6 ± 1.2gf | 8.5 ± 2.5a   | 20.9 ± 2.3b |
| **Commercial starches** | | | |
| Yellow pea       | 15.6 ± 0.2hE | 37.3 ± 0.8dcC | 47.1 ± 0.7b |
| Waxy corn        | 52.5 ± 0.3aA | 39.5 ± 0.7cC | 8.0 ± 0.5fD |
| Normal corn      | 27.3 ± 0.3cC | 57.2 ± 0.7aA | 15.6 ± 0.9eC |
| High amylose corn| 10.4 ± 0.1fF | 24.4 ± 0.2fE | 65.2 ± 0.2aA |
| Wheat            | 44.6 ± 0.5bB | 49.6 ± 0.6bB | 5.8 ± 0.4fD |
| Potato           | 12.4 ± 0.1fF | 21.4 ± 0.4fF | 66.2 ± 0.3aA |
|                  | 82.1 ± 1.1dC | 4.5 ± 1.2edcbA | 13.5 ± 0.4edEDC |
|                  | 96.4 ± 2.0aA | -0.6 ± 1.1edB | 4.2 ± 3.1fF |
|                  | 87.9 ± 1.2bB | 1.7 ± 2.6edcbA | 10.4 ± 2.0edFE |
|                  | 63.2 ± 0.4hF | 7.0 ± 0.7cbA  | 29.8 ± 0.7aA |
|                  | 89.8 ± 0.9bB | -0.8 ± 0.5eB  | 11.0 ± 0.6fedFED |
|                  | 86.7 ± 0.6cbCB | 5.3 ± 0.6edcbBA | 8.1 ± 0.3fE |

Note: Values are presented as the mean ± SD, \(n=4\). Values followed by a different lowercase letter in the same column are significantly different among pea varieties and commercial starches (\(p < 0.05\)); values followed by a different uppercase letter in the same column are significantly different among pea types and commercial starches (\(p < 0.05\)).

Abbreviations: RDS, rapidly digestible starch; RS, resistant starch; SDS, slowly digestible starch.
determining starch thermal properties, granular swelling and amylose leaching, starch gelation, and thus, the resultant RS content in the cooked starches.

Starch samples that are clustered together in the score plot (Figure 3a) are those that have similar profiles of physicochemical and nutritional properties. The nine isolated starches in this study closely stay together because of their similar physicochemical properties as mentioned above. The six commercial starches scatter all over the PCA space for their distinct physicochemical and nutritional properties. Nonetheless, our pea starch samples are closest to the commercial yellow pea starch in Figure 3a, whereas they are far apart from waxy corn, high amylose corn, and potato starch. When projecting the score plot and loading plot together and overlapping them, it would clearly show that the pea starches in this study were aligned with the positive direction of PC1 and unique for their general high amylose content, amylose leaching, RVA FV and SB, and the RS content in the cooked starches (Figure 3). These results are further supported by Pearson’s correlation coefficients (Table S2), in which the amylose content, amylose leaching, and variables related to starch retrogradation are mostly highly significantly correlated ($p < 0.01$).

PCA was informative to differentiate and localize these starch samples with their overall physicochemical and nutritional properties. However, it did not show clear separation of starches corresponding to their starch nutritional fractions, which is one of the objectives of this study. Therefore, CA was conducted to group these 15 starch samples, specifically focusing on the amylose content and resistant starch (RS) values of cooked starches.
3.10 | Cluster analysis

Hierarchical CA and K-means CA were conducted on overall physicochemical and nutritional properties of the nine pea starches and six commercial starches. These 15 starch samples were divided into five clusters as shown in a dendrogram (Figure 4a) and a K-means plot (Figure 4b), and the grouping was consistent to that in Figure 3a from PCA. As shown in Figure 4b, which projected the five clusters to a coordinate system of the amylose content and RS content of cooked starches, there is a clear trend that the RS in cooked starch increases with the increasing amylose content. This trend is consistent with the data in Table S2, which shows that the amylose content and RS of cooked starches are highly positively correlated ($r = 0.791$, $p < 0.01$). The visual segmentation of these 15 starches in Figure 4b could be further divided into four groups by their RS content in cooked starches:

- Group 1, low RS-c (<5%) and low amylose (<6%), that is, commercial waxy corn starch;
- Group 2, intermediate RS-c (5%–12%) and intermediate amylose (30%–40%), includes commercial normal corn, wheat, and potato starches;
- Group 3, high RS-c (12%–29%) and high amylose (40%–54%), includes commercial pea starch and eight isolated pea starches (excludes Limerick pea starch) in this study; and
- Group 4, the highest RS-c (>29%) and the highest amylose (>54%), includes Limerick pea starch and commercial high amylose corn starch.

The above groupings clearly demonstrated that all nine pea starches would be valuable selections as food ingredients for low GI foods, with the most outstanding, the starch from Limerick peas.

4 | CONCLUSION

The physicochemical and nutritional properties of the isolated starches from nine pea seeds are not drastically different although the pea types include three marrowfat peas, two green peas, and four yellow peas. However, the isolated starch from a green pea, Limerick, stands out for its high amylose content and also the highest RS content in cooked starch, even compared with most of the commercial starches. Among the variables studied, amylose content, amylose leaching, and RVA parameters at the cooling stage are significantly positively correlated to the starch nutritional fractions of cooked samples. In general, the isolated starches from these pea seeds are rich in SDS and high in RS after cooking (>16%) and thus would be nutritional ingredients for a broad range of food applications. All these nine pea starches are superior to the commercial yellow pea starch for their higher amylose content and RS content after cooking. The starch from Limerick peas, in particular, could even replace commercial high amylose corn starch for its comparable RS content (29.5% vs. 29.8%) after cooking while providing superior pasting properties, as the commercial high amylose corn starch did not form a paste at normal processing conditions, which would be a major deficiency for gel-based food applications.

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ETHICS STATEMENT

Ethics approval was not required for this research.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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