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Project Final Report

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Project Title: Understanding the Impact of Particle Size on Physicochemical Properties and Nutritional Benefits of Pulse and Cereal Flours

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Abstract *(maximum 500 words)*

Detail an outline on overall project objectives, methods, key findings and conclusions for use in publications and in the ministry's database. The abstract should address the following (usually 1–2 sentences per topic):

- Key aspects of the literature review
- Problem under investigation or research question(s)
- Clearly stated hypothesis or hypotheses
- Methods used (including brief descriptions of the study design, sample, and sample size)
- Study results
- Conclusions

Pulses and cereals are two important grains used to produce flour ingredients, which retain most nutrients in the whole seeds. However, the effects that processing may have on the functionality of different milled flours remain unclear. In the present project, pea, lentil, barley, and oats were selected as representative pulses and cereals to prepare flours with different particle sizes by simple milling and subsequent sieving. The sieving step was expected to efficiently separate the milled whole flours into two streams: coarse flour consisting of larger particles better preserving the original structure of cotyledon; and fine flour consisting of smaller particles having more disrupted structure (*e.g.*, more ruptured protein and fiber matrix and more obvious starch damage) (Ahmed, Taher, et al., 2016; Protonotariou et al., 2014). The physicochemical properties of the obtained whole, coarse, and fine pulse and cereal flours were comprehensively characterized using up-to-date methods and then related to their proximate compositions as well as particle size distributions and morphologies. The functional attributes and nutritional quality (*i.e.*, *in vitro* digestion of both starch and protein) of the obtained flour streams were determined and compared.

The project revealed that the flour particle size followed the order of coarse > whole > fine. For all four crops, the

three flour streams displayed the same rank order of fine > whole > coarse in their starch and damaged-starch contents but the reverse order in their ash and total dietary fiber contents. Consequently, the functional attributes closely associated with starch present in flour, such as starch gelatinization enthalpy changes, and gelling ability, also fit into the same order of fine > whole > coarse. By contrast, protein content of the three flour streams did not significantly differ in pea and lentil but showed a trend of coarse > whole > fine in barley and oats. As well, comparable foaming and emulsifying properties were observed for the three streams of pulse flours, and OBC of the barley and oat flours exhibited a consistent trend of coarse > whole > fine. The noted different particle sizes and chemical compositions among the three flour streams only caused a descending order of fine > whole > coarse in the pasting viscosities of the pulse flours but did not lead to such a clear trend in the cereal flours. In terms of the *in vitro* starch digestion, the cooked coarse and whole pea and lentil flours had lower starch digestibility than their fine counterparts. Regarding the *in vitro* protein digestibility and quality, IVPD of the three flour streams did not vary considerably for the different crops. Overall, variations in particle size had more pronounced effects on the physicochemical properties of pulse flours than cereal flours.

In the second phase of our collaborative study, we examined the nutritional and health impacts of flour particle size. The consumption of cereal grains and pulses is associated with reduced risks of type 2 diabetes and obesity and is recommended by Canada's Food Guide. However, many products (e.g. breakfast cereals, and snacks) are made using finely milled flours and are high glycemic. Our objective was to examine the impact of particle sizes in oat, pea, and lentil flours on PPG, subjective appetite, and food intake in healthy adults. Three randomized-controlled crossover experiments were conducted (n=20 per trial; age 18-43yrs; BMI 18.8-28.5kg/m²). Experiment 1 was a test of consuming a serving of oatmeal porridge prepared using 40g of coarse (675.7±19.6µm), whole (443.3±36.2µm), fine (96.0±2.1µm), or a commercial (375.9±14.8µm) oat flour. Experiment 2 investigated the consumption of 45g of wheat crackers with and without 25% pea flours of coarse (710.7±26.3µm), whole (404.4±39.7µm), or fine (83.3±2.2µm) particle size. Experiment 3 investigated the consumption of 45g of wheat crackers with and without 25% lentil flours of coarse (578.5±14.5µm), whole (473.0±42.5µm), or fine (87.8±1.6µm) particle size. After a 12 hour overnight fast, blood glucose (BG), insulin, and appetite were measured at 15-30 min intervals to 140 minutes. Food intake was measured at an *ad libitum* pizza meal at 120 minutes. Coarse flours resulted in lowest BG in all experiments (p<0.05), and lower insulin than fine flours in experiments 1 and 2 (p<0.007). Appetite AUC was lower after commercial oat flour than coarse oat flour (p<0.007). No treatment effects on food intake were observed. We conclude that controlling milling to produce coarse flour from grains and pulses to add to breakfast cereals and snack foods may have health benefits. The addition of pulse flours to wheat crackers can improve nutritional value regardless of particle size to provide potential benefits for PPG.

Extension Messages (3 to 5 bullet point in plain language)

Provide key outcomes and their importance for producers/processors and the relevant industry sector.

- (1) Variations in particle size from milling change the pasting properties, gelling ability, and *in vitro* starch digestibility of pea and lentil flours, but the influence is less pronounced for barley and oat flours.
- (2) Overall, the coarse pulse and cereal flours exhibited more desirable nutritional properties in comparison with their respective whole and fine counterparts, including less starch and more protein and dietary fiber of the coarse samples.
- (3) Controlling milling to produce coarse cereal and pulse flours to add to breakfast cereal and snack foods can be a strategy used to develop affordable and palatable products with improved health benefits.
- (4) The addition of pulse flour to wheat crackers can enhance their nutrient quality regardless of particle size, which may provide benefits for not only lowering postprandial glycemia (PPG) but also suppressing and controlling appetite.
- (5) The insightful information about the functional properties and nutritional quality of the different flour samples as influenced by both crop type and particle size will be useful for the agri-food sector to tailor the nutritional profiles of flour ingredients through simple milling and sieving, which will promote the industrial application of pulses and cereals.

Introduction (maximum 1,500 words)

Provide a brief project background and rationale.

The primary objective of this project is to investigate the effects of milling/processing of pulse and cereal flours on technological and physiological functionality in commonly consumed foods. The secondary objective is to provide evidence towards the substantiation of PPG health claims for pulse and cereal foods.

Pulses and cereal grains are two major crops grown in Saskatchewan and are of great importance to the agriculture and agri-food sector. Among the pulses, lentil and pea are the leading pulse crops in the province, and in 2016 their production reached 2.74 and 2.35 million tons, respectively. Among the cereal crops, the production of oats in 2016 was 1.65 million tons, making it a major cereal grain cultivated in Saskatchewan (statistics cited from Agricultural Statistics Pocket Reference released by the Saskatchewan Ministry of Agriculture in May 2017). However, most of the pulses and cereal grains produced in Saskatchewan are exported as raw agricultural commodities at low prices. Despite being a major producer and exporter of pulses, consumption of pulses in Canada remains very low. As a result, the agri-food sector has developed novel processing techniques to produce value-added flours for consumer use. This includes dehulling, splitting and milling of pulses to create flours for various food products, such as pasta products, meat products, snacks, batter, and breading (Pulse Canada, 2017). Furthermore, a secondary processing step (fractionation) separates pulses as well as cereals into protein concentrates and isolates, starch-rich flours, and fibers (e.g., oat fiber) for ingredient use in functional food formulations. For example, β -glucan rich fractions can be obtained by wet milling followed by sieving and solvent extractions. These approaches result in concentrates (8-30% β -glucan) or isolates (95% β -glucan) with greater purity. However, extraction of pure β -glucan isolates is complex and relatively expensive (Brennan and Cleary, 2005). Thus, oat bran or oat flour fractions are typically used by the food industry (El Khoury, Luhovyy and Anderson, 2012). Similarly, pea and lentil protein isolates can be produced but at higher costs and are more time-consuming and labour intensive than milling into flours (with no fractionation step). As a result, the food processing sectors in Saskatchewan and in Canada are exploring new strategies for ingredient development, which will add value to these crops.

The milling industry is a critical component of the food supply chain (Agriculture and Agri-Food Canada, 2016), and milling is one of the most efficient methods for the value-added processing of pulses and cereal grains. Milling can produce a diverse group of ingredients, including pulse and cereal flours, pea hull fiber, pulse protein concentrates (protein content >60% dry weight), oat bran and oat hull fiber. This increases the utility of pulses and cereal grains for use by national and international food manufacturing companies to create a broad range of food products, including snacks, breakfast cereals, porridges and pasta products, expanding their commercial uses. Their functional potential in food systems is two-fold: (1) they can impart technological functionality due to their physicochemical properties and potential to act as a gelling agent, a thickener, a binder, and a stabilizer for emulsions and foams in various food systems; (2) they can be used as ingredients in functional foods due to their favorable macro- and micronutrient profiles and deliver nutrients and physiological functionality, such as postprandial BG reductions.

However, to our knowledge no studies to date have reported the effect(s) of milling on the physicochemical properties of different pulse (e.g., pea and lentil) and/or cereal grain (e.g., oats) flours of different particle sizes while simultaneously determining in vivo health outcomes. The latter is a particularly important component of our study as dietary modifications are a cornerstone for prevention as well as management of cardiovascular disease and type 2 diabetes (T2D).

PPG is recognized as a factor to control in preventing and managing T2D. It is well documented that elevated blood glucose levels increase risk of complications in diabetic patients (Sudhir and Mohan, 2002). The Canadian Diabetes Association recommends eating nutritious meals and snacks. Canada's Food Guide emphasizes consuming pulses and cereals as part of a healthy well-balanced diet. As well, the recent Health Canada's Guiding Principles document highlights an intent to encourage consumption of plant-based foods and diets to counter current disease trends, consistent with WHO recommendations (Government of Canada, 2017). As a result, it is timely and important to identify novel ingredients

for foods that can be recommended to reduce the risk of developing markers associated with T2D.

Objectives and Progress *(add additional lines as needed)*

Please list the original objectives and/or revised objectives if ministry-approved revisions have been made to original objectives. A justification is needed for any deviation from original objectives.

Objective	Progress <i>(i.e., completed/in progress)</i>
1. To prepare pea, lentil and oat flours of coarse, granular and fine particle sizes	Completed
2. To determine the performance, starch digestibility and dietary fiber contents of the obtained flours and resultant foods	Completed
3. To examine the chemical compositions and functional properties of the flours with variations in particle size	Completed
4. To examine the relationships between flour particle size and PPG in humans	Completed

Methodology *(maximum of five pages)*

Specify project activities undertaken during this reporting period. Include approaches, experimental design, tests, materials, sites, etc. Please note that any significant changes from the original work plan will require written approval from the ministry.

Study 1. Milling and differential sieving to diversify flour functionality: A comparison between pulses and cereals

1.1 Materials

Certified seeds of pea (CDC Meadow variety), lentil (CDC Richlea variety), barley (CDC Clear variety; hulless type), and oats (Summit variety) were purchased from Penwest Seeds Company (Three Hills, AB, Canada), Simpson Seeds Inc. (Moose Jaw, SK, Canada), Lakeside Seeds (Wynyard, SK, Canada), and Ardell Seeds Ltd. (Vanscoy, SK, Canada), respectively. In the agri-food industry, they are popular cultivars used for producing flours from the respective crops. Total Starch Assay Kit, Starch Damage Assay Kit, and potato amylose standard for amylose content measurement were acquired from Megazyme International Ltd. (Co. Wicklow, Ireland). Maize amylopectin standard for amylose content measurement was procured from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Canola oil was purchased from a local grocery store. Other chemicals were reagent grade and acquired from Sigma-Aldrich Canada Co. (Oakville, ON, Canada) or Fisher Scientific Company (Ottawa, ON, Canada).

1.2 Pre-treatments of seeds and preparation of flour streams

1.2.1 Dehulling of oat seeds

The obtained oats were firstly dehulled using an impact dehuller (Model 14S, Entoleter, Hamden, CT, U.S.A.) at a rotation speed of 2,113 rpm with one pass. The dehulled seeds and seed hull were then separated using a Clipper seed cleaner (Model M-2B, A.T. Ferrell Company Inc., Bluffton, IN, U.S.A.). The dehulled oat seeds were collected for the following kilning step.

1.2.2 Kilning of hulless barley and dehulled oat seeds

The hulless barley and dehulled oat seeds were kilned to enhance the storage stability by steaming them in a rotating steam kettle (Prairie Agricultural Machinery Institute, Humboldt, SK, Canada) at 100 °C and a rotor speed of 12 rpm for 8.0 min under ambient pressure. After the heat stabilization, the seeds were cooled to room temperature and then dried in a forced-air oven at 50 °C for 16-20 h to reach a moisture level < 12%. The kilned barley and oat seeds were stored

at -30 °C for future use. The kilning process was carried out in two independent batches for each crop.

1.2.3 Milling of seeds into whole flours

The pea, lentil, and heat-stabilized barley and oat seeds were milled using a Micron Powder Systems hammer mill (Hosokawa Micron Powder Systems, Summit, NJ, U.S.A.) through a two-step method at a rotor frequency of 20 Hz. The grains were firstly milled to pass through a 5.0-mm screen, followed by a second milling step to pass through a 2.0-mm screen. The collected non-fractionated flours were designated as “whole flours” in the subsequent experiments. The weights of the used seeds and the derived whole flours were recorded, and the yields of the whole flours were calculated as:

$$\% \text{Yield of whole flour from milling} = (\text{Weight of whole flour collected from milling}) / (\text{Initial weight of seeds used for milling}) \times 100\%$$

The milling process was carried out in two independent batches for each crop (i.e., $n = 2$ for data reporting).

1.2.4 Differential sieving of whole flours into coarse and fine streams

An automatic sieve shaker (Model AS 200, Retsch GmbH, Haan, Germany) equipped with a 0.15-mm sieve was used to separate the whole flours into two different streams: the portion passing through the sieve was collected and designated as “fine flour”, and the portion remaining on the top of the sieve was collected and designated as “coarse flour”. Consequently, three streams of flours, namely “whole”, “coarse”, and “fine”, were generated from each crop type. According to our preliminary tests, the 0.15-mm sieve was chosen for two reasons: (1) the sieving efficiently yielded reasonable percentages of coarse and fine flours from the whole flours of the four crops (shown in Table 1); and (2) the generated whole, coarse, and fine flours from the same crop exhibited apparently diverse functional attributes. These two points are critical for future commercialization of the developed pulse and cereal flours. This sieving step was performed separately on the collected whole flours from the two independent batches of milling as described above (i.e., $n = 2$ for coarse and fine fractions from each crop type). The yields of coarse and fine flours from sieving were calculated as:

$$\% \text{Yield of coarse/fine flour from sieving} = (\text{Weight of collected coarse/fine flour from sieving}) / (\text{Initial weight of whole flour used for sieving}) \times 100\%$$

The whole, coarse, and fine flour streams from the four crops were stored at -30 °C before subsequent analyses.

1.3 Particle-size distributions of flours

Particle-size distributions of the whole, coarse, and fine flours were determined using Malvern Scirocco 2000 Mastersizer (Malvern Panalytical, Saint-Laurent, QC, Canada). Briefly, the flour (~2 g) was suspended in 20 mL distilled water under magnetic stirring at 250 rpm for 5 min. The flour suspension was then loaded to the dispersion cell dropwise using a disposable pipette. The particle-size distribution and volume-weighted mean particle size ($D[4,3]$) were recorded by Mastersizer 2000 Version 5.54 Software (Malvern Panalytical) after the laser obscuration reading fit into a range of 10-20%. The refractive indices of flour and dispersant were set at 1.50 and 1.33, respectively.

1.4 Morphologies of flours

The flour sample was sprinkled on a carbon tape that was attached to an aluminum stub, and the sample was then coated with gold using a Q150T ES coater (Quorum Technologies Inc., Puslinch, ON, Canada). The microscopic structure of flour was examined under a field-emission scanning electron microscope (SEM, SU8010, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada). The scanning conditions were set to 3.0 kV of acceleration voltage and 10 μA of probe current. Representative images of each sample were captured at three different magnifications: 150 \times , 500 \times and 1500 \times .

1.5 Proximate analysis of flours

Moisture contents of the whole, coarse, and fine flours were determined using AACC Method 44-15.02 (AACC, 2000). Starch contents of the flours were measured using AACC Method 76-13.01 with Megazyme Total Starch Assay Kit (AACC, 2000). Damaged-starch contents of the flours were quantitated using AACC method 76-31.01 with Megazyme Starch Damage Assay Kit (AACC, 2000). Dumas combustion method using a Nitrogen/Protein Analyzer (CN628, LECO Corporation, St. Joseph, MI, U.S.A) was employed to measure nitrogen contents of the flours. Protein contents were calculated by multiplying the nitrogen contents with a conversion factor of 6.25 according to AACC Method 46-30.01 (AACC, 2000). Lipid contents of the flours were quantitated using a Goldfish Fat Extractor (Labconco Corp., Kansas City, MO, U.S.A.) according to AOAC Method 945.16 (AOAC, 2016). Ash contents of the flours were measured following AACC Method 08-01.01 (AACC, 2000). Total dietary contents of the flours were determined using AOAC Method 2011.25 (AOAC, 2016). This experiment was completed with one replicate on each batch of flour (i.e., $n = 2$ for data reporting) by the Medallion Labs (Minneapolis, MN, U.S.A.). Amylose contents of the flours were determined using an iodine colorimetric method (Chrastil, 1987). Amylose

contents were determined on a “dry flour basis” and converted to a “dry starch basis” using the following equation:

$\% \text{Amylose content, dry starch basis} = (\% \text{Amylose content, dry flour basis}) / (\% \text{Starch content, dry flour basis}) \times 100\%$

To achieve accurate measurements of starch and amylose contents using the indicated methods, flour samples are required to pass through a sieve with openings of 0.5 mm (Ai et al., 2017). Consequently, the whole and coarse flours in this study were re-milled using a Laboratory Mill 3100 (PerkinElmer Inc., Waltham, MA, U.S.A.) installed with a 0.5-mm sieve prior to those two tests.

1.6 Color of flours

Color parameters of the whole, coarse, and fine flours were measured using Hunterlab MiniScan XE Colorimeter (Hunter Association Laboratory Inc., Reston, VA, U.S.A.) equipped with an illuminant A and 10° observer as described by Liu, Yin, Pickard, and Ai (2020). The device was standardized with black and white tiles. The flour was transferred into a transparent plastic petri dish covered with a lid before the measurement. The color of the flour was described using three parameters: L* for brightness from black (0) to white (100), a* for green (-) to red (+), and b* for blue (-) to yellow (+).

1.7 Thermal properties of flours

Thermal properties of the whole, coarse, and fine flours were measured using a differential scanning calorimeter (DSC 8000, PerkinElmer Inc.). The flour (~10 mg) was precisely weighed into a stainless-steel pan (PerkinElmer Inc.), and three volumes of distilled water (v/w) was added to fully hydrate the sample. The pan was hermetically sealed and kept at room temperature for at least 2 h before the measurement. The sample was heated from 10 to 140 °C at a ramping rate of 10 °C/min. After the first scan, the sample was immediately cooled to 10 °C at 40 °C/min and rescanned to detect the dissociation of amylose-lipid complexes (ALC) (Ai, Nelson, Birt, & Jane, 2013). The thermograms of the flour were analyzed using Pyris Software (Version 13.3.1.0014, PerkinElmer Inc.). Onset (To), peak (Tp), and conclusion (Tc) temperatures and enthalpy change (ΔH) of the endothermic peaks were calculated.

1.8 Pasting properties and gelling ability of flours

Pasting properties of the whole, coarse, and fine flours were determined using a Rapid Visco Analyser (RVA 4800, PerkinElmer Inc.). A flour slurry (28.5 g total weight containing 10.6% dry solids) was prepared and loaded to the instrument. The sample was analyzed using the following temperature profile: (1) equilibrating at 50 °C for 1 min; (2) heating to 95 °C at a rate of 6 °C/min; (3) holding at 95 °C for 5 min; (4) cooling to 50 °C at a rate of 6 °C/min; and (5) keeping at 50 °C for 2 min (Liu et al., 2019).

Immediately after the RVA run, the cooked flour paste was transferred into a plastic container (inner diameter = 33.0 mm, height = 38.0 mm) and kept at room temperature for 2.0 h for gelling to take place. TA.XT.Plus Texture Analyzer (Texture Technologies Corp., South Hamilton, MA, U.S.A.) installed with TA-10 Probe (diameter = 12.7 mm) was used to determine the flour gel hardness with the following settings (Liu et al., 2019): trigger force = 0.5 g, penetration speed = 0.5 mm/s, and penetration depth = 10.0 mm.

1.9 Water-holding and oil-binding capacity of flours

Water-holding capacity (WHC) of the whole, coarse, and fine flours was determined according to AACC Method 56-20.01 (AACC, 2000). Oil-binding capacity (OBC) of the flours was measured following the method of Setia et al. (2019). WHC and OBC were calculated on a dry basis (db) of the flours.

1.10 Foaming properties of flours

Foaming capacity (FC) and foaming stability (FS) of the whole, coarse, and fine flours were determined using the method reported by Bai, Stone, and Nickerson (2018). In brief, the flour (0.5 g) was suspended in 49.5 g distilled water, followed by adjusting the pH to 7.0 using 0.1 M HCl or 0.1 M NaOH. The suspension was magnetically stirred at 250 rpm overnight prior to the test. On the next day, the pH of the suspension was readjusted to 7.0, and 15.0 mL of the suspension was transferred to a 400-mL beaker. The suspension was homogenized using an IKA homogenizer (T10, IKA, Wilmington, NC, U.S.A.) at a speed of level 3 for 1.0 min and subsequently level 4 for 4.0 min. The generated foam was transferred into a 100-mL graduated cylinder immediately, and the initial volume of the foam was recorded as V1. After 30.0 min of sitting at room temperature, the volume of the remaining foam was recorded as V2. FC and FS were calculated using the following equations:

$$\text{FC (\%)} = \text{V1} / (15 \text{ mL initial volume}) \times 100\%$$

$$\text{FS (\%)} = (\text{V1} - \text{V2}) / \text{V1} \times 100\%$$

1.11 Emulsifying properties of flours

Emulsion activity (EA) and emulsion stability (ES) of the whole, coarse, and fine flours were measured according to

the method reported by Setia et al. (2019) with slight modifications. Briefly, the flour (4.25 g) was suspended in 75.0 g distilled water, and the pH of the suspension was adjusted to 7.0 using 0.1 M HCl or 0.1 M NaOH. The suspension was magnetically stirred at 250 rpm overnight before the test. On the next day, the pH of the suspension was readjusted to 7.0, and 75.0 mL canola oil was added to the suspension. The same IKA homogenizer was used to homogenize the sample at a speed of level 4 for 1.0 min. An aliquot (~30 mL) of the resultant emulsion was transferred into a 50-mL centrifuge tube, followed by centrifugation at 1,300 g for 5.0 min. The heights of the emulsified layer and the entire emulsion in the tube were recorded, and the EA was calculated using the following equation:

$$EA (\%) = (\text{Height of emulsified layer}) / (\text{Height of entire emulsion}) \times 100\%$$

The remaining emulsion in the beaker was heated in a water bath at 80 °C for 30.0 min and then cooled to room temperature. An aliquot (~30 mL) of the resulting emulsion was transferred into a 50-mL centrifuge tube, followed by centrifugation at 1,300 g for 5.0 min. The heights of the emulsified layer and the entire emulsion in the tube were recorded, and the ES was calculated using the following equation:

$$ES (\%) = (\text{Height of emulsified layer}) / (\text{Height of entire emulsion}) \times 100\%$$

1.12 Statistical analysis

The kilning, milling, and sieving of the pulse and cereal grains to produce whole, coarse, and fine flours were performed in two independent batches (i.e., $n = 2$ for data reporting). For each batch of sample, all the analyses were conducted in duplicate (i.e., $n = 4$ for data reporting) unless specifically indicated. The data were reported as average \pm standard deviation. Statistical differences among the data were performed using one-way ANOVA with Tukey's HSD test at a significance level of 0.05 using IBM SPSS Software Version 25 (IBM Corporation, Armonk, NY, U.S.A.).

Study 2. Milling and differential sieving as an effective approach to diversifying nutritional profiles of pulse and cereal flours

2.1 Materials

Certified seeds of pea (CDC Meadow variety), lentil (CDC Richlea variety), barley (CDC Clear variety; hulless type), and oats (Summit variety) were purchased from Penwest Seeds Company (Three Hills, AB), Simpson Seeds Inc. (Moose Jaw, SK), Lakeside Seeds (Wynyard, SK), and Ardell Seeds Ltd. (Vanscoy, SK) in Canada, respectively. In the agri-food sector, they are varieties commonly utilized for manufacturing flours from the respective crops. Total Starch Assay Kit and β -Glucan Assay Kit were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). Protease, α -amylase, amyloglucosidase, trypsin, chymotrypsin, porcine pancreatin, and invertase were purchased from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). All the other used chemicals were reagent grade and purchased from Sigma-Aldrich Canada Co. or Fisher Scientific Company (Ottawa, ON, Canada).

2.2 Pre-treatments of seeds and preparation of flour streams

2.2.1 Dehulling of oat seeds and kilning of hulless barley and dehulled oat seeds

The oat grains were dehulled using an impact dehuller (Model 14S, Entoleter, Hamden, CT, U.S.A.) at a rotation speed of 2,113 rpm with one pass. The dehulled oat seeds and seed hull were separated using a Clipper seed cleaner (Model M-2B, A.T. Ferrell Company Inc., Bluffton, IN, U.S.A.).

Kilning was performed on the hulless barley and dehulled oat grains to improve the storage stability. Briefly, the seeds were steamed in a rotating steam kettle (Prairie Agricultural Machinery Institute, Humboldt, SK, Canada) at 100 °C and a rotor speed of 12 rpm for 8.0 min under normal pressure. After this heat stabilization step, the seeds were cooled to ambient temperature and then dried in a convection oven at 50 °C for 16-20 h to reach a moisture level < 12%. The kilned barley and oat seeds were stored at -30 °C prior to the following processing. The kilning process was conducted in two independent batches for each crop.

2.2.2 Milling of seeds into “whole” flours and subsequent sieving to generate “coarse” and “fine” flours

The seeds of untreated pea and lentil and heat-stabilized barley and oats were milled into flours using a Micron Powder Systems hammer mill (Hosokawa Micron Powder Systems, Summit, NJ, U.S.A.) with a two-step method at a rotor frequency of 20 Hz: the first milling step to pass through a 5.0-mm sieve and the second to pass through a 2.0-mm sieve. The obtained non-fractionated flours were defined as “whole” flours in the subsequent tests. The milling step was carried out in two independent batches for each crop (i.e., $n = 2$ for data presentation).

The whole flour milled from each crop was sieved into two different streams using an automatic sieve shaker (Model AS 200, Retsch GmbH, Haan, Germany) equipped with a 0.15-mm sieve. After the differential sieving, the portion

remaining on the top of the sieve was defined as “coarse” flour, while the portion passing through the sieve was defined as “fine” flour. Consequently, three streams of flours, namely “whole”, “coarse”, and “fine”, were produced from each crop type. The sieving step was conducted separately on the collected whole flour from the two independent batches of milling as described above (*i.e.*, $n = 2$ for coarse and fine fractions from each crop type). The yields and particle-size distributions of the whole, coarse, and fine flour streams from the four crops were reported in our previous publication (Cheng et al., 2023). All the flour samples were stored at $-30\text{ }^{\circ}\text{C}$ before subsequent analyses.

2.3 Microscopic structures of flours

Microscopic structures of the flours were examined under a field-emission scanning electron microscope (SEM, Model SU8010, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada). The flour sample was sprinkled on a carbon tape that was attached to an aluminum stub, followed by coating with gold using a Q150T ES coater (Quorum Technologies Inc., Puslinch, ON, Canada). The SEM scanning conditions were set to 3.0 kV acceleration voltage and 10 μA probe current. Representative images of each sample were captured at 150 \times , 500 \times , and 1500 \times , respectively.

2.4 Chemical compositions of flours

Moisture contents of the flours were determined using AACC Method 44-15.02. Starch contents of the flours were determined with Megazyme Total Starch Assay Kit following AACC Method 76-13.01. Nitrogen contents of the flours were analyzed with the Dumas combustion method using a Nitrogen/Protein Analyzer (CN628, LECO Corporation, St. Joseph, MI, U.S.A.). The nitrogen contents were converted to protein contents by multiplying with a conversion factor of 6.25 according to AACC Method 46-30.01. Lipid contents of the flours were measured using a Goldfish Apparatus (Labconco, MO, U.S.A.) according to AOAC Method 945.16. β -Glucan contents of the flours were determined in accordance with AACC Method 32-23.01 with Megazyme β -Glucan Assay Kit. Total dietary fiber contents of the flours were measured by the Medallion Labs (Minneapolis, MN, U.S.A.) using AOAC Method 2011.25 with one replicate on each batch of flour (*i.e.*, $n = 2$ for data presentation). The reported dietary fiber profiles included insoluble (the fraction insoluble in water), high-molecular-weight soluble [the fraction soluble in water but precipitable by 78% (v/v) ethanol], and low-molecular-weight soluble [the fraction soluble in 78% (v/v) ethanol] dietary fiber.

To accurately measure the starch and β -glucan contents, flour samples are required to pass through a 0.5-mm sieve according to the applied methods. Therefore, the whole and coarse flours in this study were re-milled using a Laboratory Mill 3100 (PerkinElmer Inc., Waltham, MA, U.S.A.) equipped with a 0.5-mm sieve prior to these two tests.

2.5 *In vitro* starch digestibility of cooked flours

In vitro starch digestibility of the flours after cooking was determined using the Englyst method (Englyst, Kingman, & Cummings, 1992) with minor modifications (Li, Li, Zhu, & Ai, 2021). The flour sample consisting of 600 mg starch (db) was suspended in 15.0 mL of distilled water, followed by boiling in water for 10 min with vigorous magnetic stirring. Sodium acetate buffer (5.0 mL, pH 5.2, 0.4 M, containing 0.08 % sodium azide) and 50 mg guar gum were added to the cooked sample after cooling to room temperature. The sample test tube was incubated in a water bath at $37\text{ }^{\circ}\text{C}$ with shaking (160 rpm) for approximately 15 min for equilibration. Freshly prepared enzyme cocktail (5.0 mL) that contained porcine pancreatin extract and amyloglucosidase was added to the cooked flour to hydrolyze the starch in the same water bath. An aliquot (250 μL) of the hydrolyzate was sampled from the test tube at time intervals of 20 and 120 min to quantify the amounts of released glucose using Megazyme D-Glucose Assay Kit. Rapidly digestible starch (RDS; digested within 20 min), slowly digestible starch (SDS; digested between 20 and 120 min), and resistant starch (RS; undigested after 120 min) contents of the cooked flour were calculated as described before (Englyst et al., 1992; Li et al., 2021). The RDS, SDS, and RS contents of the flour were also converted to a “dry starch basis” using the equation:

$\% \text{RDS, SDS or RS content of flour, dry starch basis} = (\% \text{RDS, SDS or RS content of flour, dry basis}) / (\% \text{Starch content of flour, dry basis}) \times 100\%$

2.6 *In vitro* protein digestibility and quality of cooked flours

2.6.1 *In vitro* protein digestibility

In vitro protein digestibility (IVPD) of the flours after cooking was measured using a pH drop method as described by Setia et al. (2019) with minor modifications. Briefly, 31.0 mg chymotrypsin, 16.0 mg trypsin, and 13.0 mg protease were homogeneously mixed in 10.0 mL of deionized water and then incubated in a $37\text{ }^{\circ}\text{C}$ water bath. Sodium hydroxide solution (NaOH; 0.1 M) or hydrochloric acid solution (HCl; 0.1 M) was used to adjust the pH of the enzyme solution to 8.0 ± 0.05 . The flour sample that contained 62.5 ± 0.5 mg protein was suspended in 10.0 mL of deionized water and then cooked in a boiling water bath for 10 min with vigorous magnetic stirring. After cooking, the flour sample was equilibrated in a water

bath at 37 °C for 1 h with gentle stirring. The pH of the flour suspension was adjusted to 8.0 ± 0.05 using 0.1 M NaOH or HCl. Subsequently, 1.0 mL of the prepared multi-enzyme solution was added to the suspension to hydrolyze the protein. The pH of the suspension was recorded every 30 s for a total period of 10 min, and IVPD of the flour sample was calculated using the following equation:

$$\text{IVPD (\%)} = 65.66 + 18.10 \times \Delta\text{pH}_{10 \text{ min}}$$

where $\Delta\text{pH}_{10 \text{ min}}$ was the change in pH from the initial 8.0 to the value at the end of 10-min hydrolysis.

2.6.2 Amino acid composition and score

Amino acid compositions (including 18 amino acids) of the flours were determined at Central Testing Laboratory Ltd. (Winnipeg, MB, Canada) using ultra-performance liquid chromatography with AccQ•Tag Ultra Method (Waters Corporation, Milford, MA, U.S.A.). The essential amino acid score (AAS) was quantitated as the ratio of essential amino acid content of the target protein to that of the reference protein. The essential amino acid composition of the reference protein was set by the FAO/WHO according to the amino acid requirement for children 2 to 5 years of age: histidine, 19 (mg/g protein); isoleucine, 28; leucine, 66; lysine, 58; methionine + cysteine, 25; phenylalanine + tyrosine, 63; threonine, 34; tryptophan, 11; and valine, 35 (FAO, 1991). The lowest AAS denoted the limiting essential amino acid. The measured IVPD of the flour samples was converted to *in vitro* protein digestibility corrected amino acid score (IV-PDCAAS) using the following equation:

$$\text{IV-PDCAAS (\%)} = \text{IVPD (\%)} \times \text{limiting amino acid score}$$

2.7 Statistical analysis

The kilning, milling, and sieving of the pulse and cereal seeds to generate whole, coarse, and fine flours were carried out in two independent batches (*i.e.*, $n = 2$ for data presentation). For each batch of sample, all the analyses were performed in duplicate (*i.e.*, $n = 4$ for data presentation) unless specifically indicated. The data were reported as average \pm standard deviation. Statistical differences among the data were analyzed using one-way ANOVA with Tukey's HSD test at a significance level of 0.05 using IBM SPSS Software Version 25 (IBM Corporation, Armonk, NY, U.S.A.).

Study 3. The impact of particle size in cereal and pulse flour on PPG and appetite in healthy adults

3.1 Study Design

Three acute randomized-controlled, single-blinded crossover experiments were conducted in the Department of Nutritional Science's Nutrition Intervention Center at the University of Toronto, Temerty Faculty of Medicine (Toronto, ON, CA). All three experiments explored the use of either pulse or cereal flours in commonly consumed foods such as porridge (breakfast meal) and crackers (snack meal). Experiment 1 examined the effects of consuming oat flour in a porridge, experiment 2 investigated the effects of adding pea flour to wheat crackers, and experiment 3 involved the addition of lentil flour to wheat crackers. Throughout all 3 experiments, participants received 4 treatments in random order with a 5-to-7-day washout period in between. Study sessions took place after 12hrs of overnight fasting and started at a time between 8:00am-11:00am. Treatment effects on PPG and appetite were measured over 120min in experiment 1 and over 140min in experiments 2 and 3. At 120min, an *ad libitum* pizza meal was served to assess food intake at a second meal. Palatability of the treatments was determined. This study was approved by the University of Toronto Ethics Review Committee and was registered on ClinicalTrials.gov (NCT05291351).

3.2 Participant

The study population consisted of healthy, normoglycemic (fasting BG $<5.5\text{mmol/L}$) males and females between the ages of 18-45yrs with a BMI between $18.5\text{-}29.9\text{kg/m}^2$. Participants were recruited through social media (e.g. Facebook, Reddit, Instagram), posters around the University of Toronto campus, subway ads, and previous participant lists. Individuals were excluded if they were smokers/alcoholics, pregnant/lactating, elite athletes, regular breakfast skippers, following a restrictive eating pattern, attempting to lose/gain weight, taking medications that can influence study outcomes, allergic to treatment ingredients, or have experienced major lifestyle/physiological changes within the last 3 months. Eligible participants were invited to an in-person screening to verify their baseline weight, height, BMI, waist circumference, blood pressure, and fasting BG (BG). A sample size of $n=20$ (10 males, 10 females) was recruited for each experiment. The sample size was determined based on previous studies with a similar within-subject design which showed that 10 participants were needed to detect a 1mmol/L difference in peak BG concentration and 12 is required for examining physiological mechanisms such as a 10% change in insulin at a significance level of 0.05 and power of 80% (Mollard et al., 2014; Akhavan et al., 2010).

3.3 Treatment

All flours were processed and characterized for particle size distribution (Figure 3.1) and proximate composition (Table 3.1) according to methods outlined in Study 2. In experiment 1, the treatments included an isocaloric serving of oatmeal porridge made with coarse oat flour (COF), whole oat flour (WOF), fine oat flour (FOF), or a commercial oat flour (COMF). All porridges were prepared using 40g flour, 250g water, 1/8 tsp salt, and 1/8 tsp vanilla extract. Water was mixed with the flour until fully dissolved, then salt was added. The mixture was microwaved for 2min and stirred at 30sec intervals to avoid clumping. Vanilla extract was added after heating and the porridge was served immediately to avoid changes in viscosity. Pasting property of the oat porridges was determined by adding 3.8g of oat flour to 3.75g of water to create a flour suspension that was heated at 95°C using a Rapid Visco Analyser (PerkinElmer, Waltham, MA, US; Figure 3.2). In experiment 2, the treatments included 45g of wheat crackers with an incorporation rate of 25% coarse pea flour (CPF), 25% whole pea flour (WPF), 25% fine pea flour (FPF), or 100% all-purpose wheat flour (WF). In experiment 3, the treatments included 45g of wheat crackers with an incorporation rate of 25% coarse lentil flour (CLF), 25% whole lentil flour (WLF), 25% fine lentil flour (FLF), or WF. The crackers were formulated and produced by Saskatchewan Food Industry Development Center (Saskatoon, SK, CA). Treatments in all experiments were served with 375ml of water. The flours and crackers were stored at -20°C to prevent rancidity, defrosted at 4°C in advance to each session, and used at room temperature (Table 3.2).

Table 3.1 Nutrition composition of oat, pea, and lentil flours per 100g

	Flour Type ²	Available Carbohydrate (g)	Protein (g)	Lipid (g)	Total Dietary Fiber (g)	Energy (kcal)	D[4,3] (µm) ⁴
Exp. 1 ¹ (porridge)	COF	40.3	19.8	8.5	21.3	401.5	675.7 ± 19.6
	WOF	53.0	15.5	7.8	15.0	403.8	443.3 ± 36.2
	FOF	60.0	13.8	8.0	8.8	402.0	96.0 ± 2.1
	COMF	57.5	13.3	6.5	10.0	379.0	375.9 ± 14.8
Exp. 2 (crackers)	CPF	30.2	22.2	1.2	36.4	221.3	710.7 ± 26.3
	WPF	39.1	21.3	1.3	27.6	254.2	404.4 ± 39.7
	FPF	49.8	23.1	1.3	16.9	304.0	83.3 ± 2.2
	WF ³	69.0	12.1	2.5	2.7	346.9	212.0 ⁵
Exp. 3 (crackers)	CLF	29.2	24.7	0.6	27.8	221.0	578.5 ± 14.5
	WLF	38.4	24.5	0.7	23.0	257.9	473.0 ± 42.5
	FLF	45.9	25.1	0.8	19.1	291.2	87.8 ± 1.6
	WF ³	69.0	12.1	2.5	2.7	346.9	212.0 ⁵

¹ Exp.1, experiment 1; Exp.2, experiment 2; Exp.3, experiment 3

² COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour; CLF, coarse lentil flour; WLF, whole lentil flour; FLF, fine lentil flour

³ Nutritional information obtained from the Canadian Nutrient File

⁴ D[4,3] is particle size presented as volume weighted mean diameter ± standard deviation

⁵ The US Food and Drug Administration requires that >98% of wheat flour passes through a 212µm sieve

Table 3.2 Nutrition composition of treatments¹

	Treatment ³	Weight (g)	Available Carbohydrate (g)	Protein (g)	Lipid (g)	Total Dietary Fiber (g)	Energy (kcal)
Exp. 1 ^{2,5}	COF porridge	290 ⁴	16.1	7.9	3.4	8.5	160.6
	WOF porridge		21.2	6.2	3.1	6.0	161.5
	FOF porridge		24.0	5.5	3.2	3.5	160.8
	COMF porridge		23.0	5.3	2.6	4.0	151.6
Exp. 2	25% CPF + 75% WF crackers	45	26.7	6.6	1.0	5.0	142.2
	25% WPF + 75% WF crackers		27.7	6.5	1.0	4.0	145.8
	25% FPF + 75% WF crackers		28.9	6.7	1.0	2.8	151.4
	100% WF crackers		31.1	5.4	1.1	1.2	155.9
Exp. 3	25% CLF + 75% WF crackers	45	26.6	6.9	0.9	4.0	142.1
	25% WLF + 75% WF crackers		27.6	6.8	0.9	3.5	145.7
	25% FLF + 75% WF crackers		28.5	6.9	0.9	3.1	149.7
	100% WF crackers		31.1	5.4	1.1	1.2	155.9

¹ Values are calculated based on 40g of oat flour for Exp.1 and 11.25g of pea/lentil flour plus 33.75g of wheat flour for Exp.2 and Exp. 3

² Exp.1, experiment 1; Exp.2, experiment 2; Exp.3, experiment 3

³ COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour; CLF, coarse lentil flour; WLF, whole lentil flour; FLF, fine lentil flour

⁴ Total weight is calculated based on 40g of oat flour + 250g of water

⁵ Porridges were prepared using 40g oat flour, 250g water, 1/8 tsp salt, 1.8 tsp vanilla extract. The mixture was microwaved for 2min, stirred at 30sec intervals.

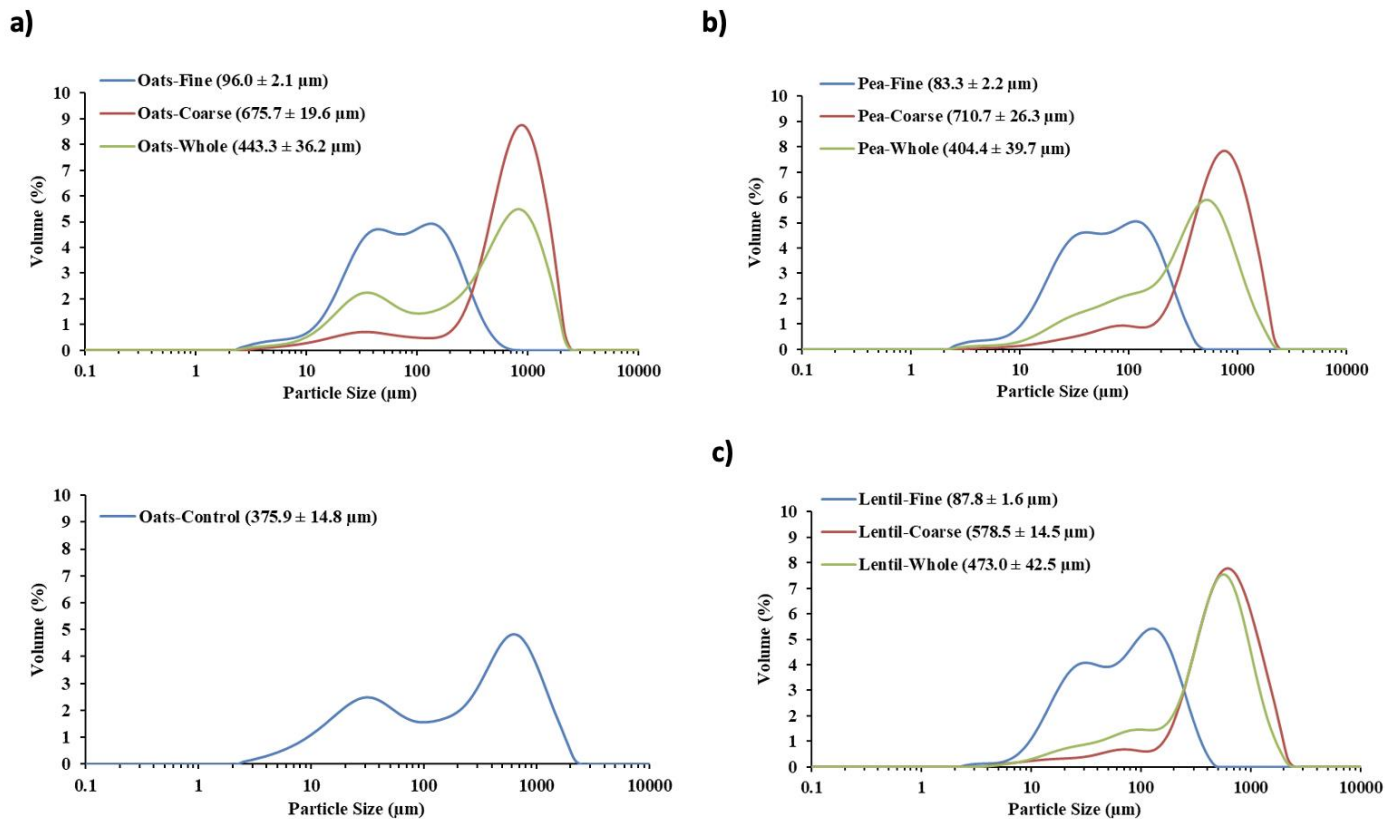


Figure 3.1 Particle-size distributions of a) oat flours, b) pea flours, and c) lentil flours. The volume-weighted mean particle size, $D[4,3]$, is presented in parentheses ($n = 3$).

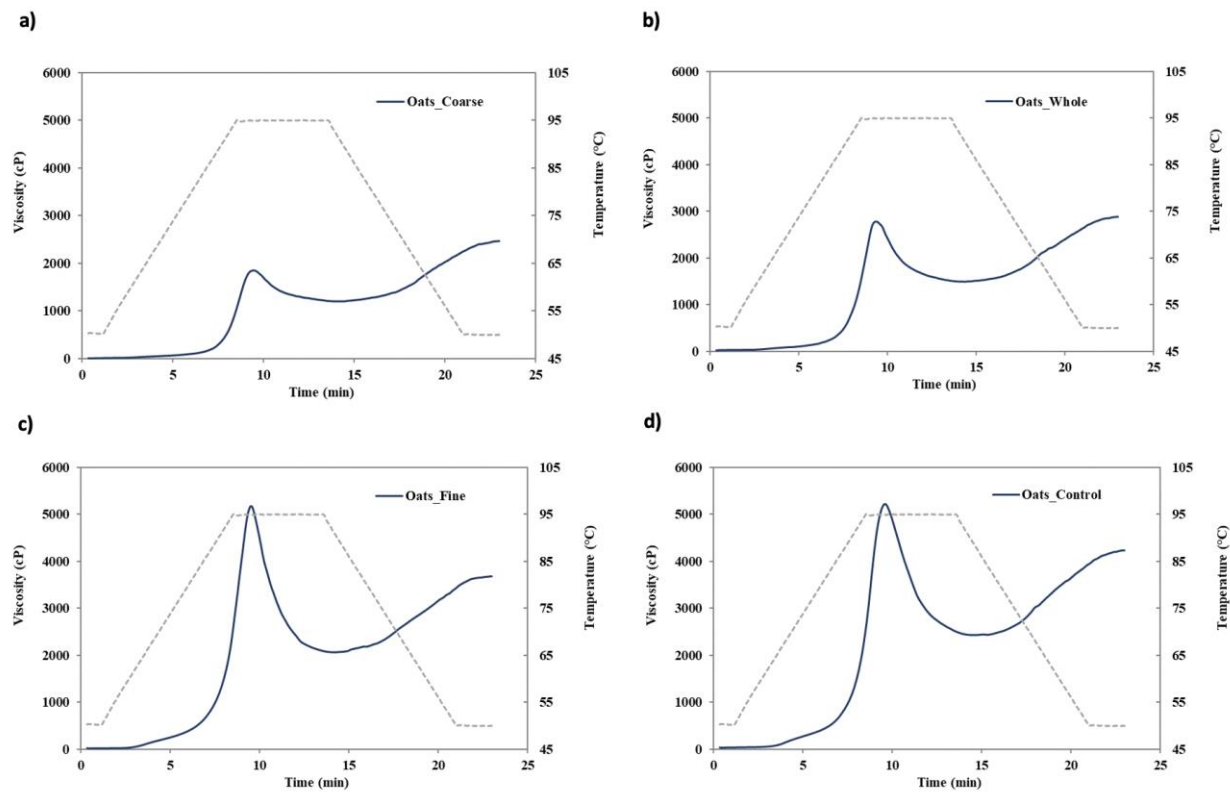


Figure 3.2 Pasting properties of oat flours: a) coarse (COF), b) whole (WOF), c) fine (FOF), d) commercial (COMF) analyzed using Rapid Visco Analyser. Flour sample (3.8 g, as-is weight) was added to 23.75 g water for flour suspension preparation (27.55 g total weight) and heated at 95°C (n=3).

3.4 Protocol

Participants were required to fast for 12hrs overnight but allowed to drink water up until 1hr before the start of their session. Participants were also informed to refrain from vigorous exercise and drinking alcohol, and to eat consistent quantities and types of food 24hrs prior to their session. Upon arrival at the study center, participants were asked to fill out baseline questionnaires assessing their recent food intake, activity, sleep, and stress levels to identify deviations that may affect their fitness for the session. They were also asked to complete a series of adaptive visual analogue scales (AVAS) to collect information about their 1) motivation to eat, 2) energy, fatigue, and stress, and 3) physical comfort. Each AVAS question was presented as a 100mm line with opposing statements flanked at each end of the line (i.e. very hungry, not hungry at all). Participants were instructed to place a 'X' at a position along the line that best denotes how they are feeling at the moment. This is transformed to provide a numerical rating between 0 to 100. Fasting glucose and insulin were next measured, and individuals were rescheduled if their BG was above 5.5mmol/L. Participants were provided the treatment to be consumed entirely within 10min. After taking the first bite, they were instructed to assess its palatability using AVAS. Subsequently, BG, insulin, and subjective appetite were measured over 140min. BG and appetite were measured at 15-30min intervals over 2 hours, and insulin was measured every 30min. At 120min, food intake was assessed through an *ad libitum* pizza meal over 20min. After the meal, BG and insulin were measured in experiments 2 and 3, and appetite was measured in all experiments.

3.4.1. BG

BG measurements were obtained from capillary blood through finger pricks using Single-Let Sterile Single-Use Safety Lancets and read instantaneously using the Contour Next Gen glucometer and test strips (Ascensia Diabetes Care, Mississauga, ON, CA). These devices have been proven to be accurate with $\geq 95\%$ of results falling within $\pm 0.83\text{mmol/L}$ when BG is $< 5.55\text{mmol/L}$, or within $\pm 15\%$ when BG is $\geq 5.55\text{mmol/L}$, as required by Health Canada. After sanitizing, the fingertip was pricked, and the first drop of blood was wiped away in case of contamination with alcohol. The second drop was used for BG reading. All glucometers were calibrated using the Contour Next normal and high control solutions (Ascensia Diabetes Care, Mississauga, ON, CA) for each lot number of test strips prior to the study. Low and high calibrated ranges for each glucometer were determined as $\pm 3\%$ of the average control solution readings. The morning of each session, quality control tests were done to ensure that the readings were within the calibrated ranges. The glucometers were re-calibrated every 3 months using new control solutions. The same glucometer and lot number of test strips were used for each participant to reduce inter-session variations.

3.4.2. Insulin

Insulin was measured in experiments 1 and 2 via finger pricks using the 16.5-gauge BD Microtainer Contact-Activated Lancet (Becton Dickinson, Franklin Lakes, NJ, US). Approximately $300\mu\text{l}$ of capillary blood was collected into a Microvette (Sarstedt AG & CO. KG, Nümbrecht, DE) and placed on ice. The blood sample was spun in a microcentrifuge (Diamed, Mississauga, ON, CA) at 10 000 RCF for 5min at 20°C and the serum was aliquoted into an Eppendorf Safe-Lock Tube (Eppendorf, Hamburg, DE). The serum samples were flash-frozen on dry ice and stored at -80°C until later analysis using a sandwich type ELISA immunoassay kit (ALPCO, Salem, NH, US).

3.4.3. Subjective appetite

Subjective appetite was assessed via AVAS questionnaires and calculated using the following equation: subjective appetite = [desire to eat + hunger + (100 – fullness) + prospective consumption] / 4.

3.4.4. Food intake

Food intake was determined as the total energy consumed at the pizza meal, calculated by multiplying the total weight of pizza eaten by the caloric value provided on the manufacturer label. Commercial frozen pizzas (Dr. Oetker

Giuseppe Pizzeria Easy Pizzi Mini Pizza, Bielefeld, DE) were defrosted for 30min and baked at 430°F for 8min. Each pizza was cut into 6 equal pieces containing approximately the same amount of toppings and was arranged nonuniformly on a serving tray. Each tray of pizza was served with 500ml of water. Participants were instructed to eat and drink until they were comfortably full over 20min. A total of 3 trays of pizza were provided in succession such that a new freshly baked tray of pizza and cup of water was provided every 7min to replace the previous one. Each tray contained 2 mini pizzas (12 pieces) so that a total of 6 pizzas (36 pieces) were provided at the meal. The weights of the pizza and water were measured before and after consumption. Participants had a choice of pepperoni, cheese, or bacon and pepperoni pizza, and the flavor was kept consistent across all their sessions (Table 3.3).

Table 3.3 Nutrition information for Dr. Oetker Giuseppe Pizzeria Easy Pizzi Mini Pizza

Flavour	Nutrients per 100g												
	Calories (kcal)	Fat (g)	Saturated Fat (g)	Trans fat (g)	Carbohydrate (g)	Fibre (g)	Sugars (g)	Protein (g)	Cholesterol (mg)	Sodium (mg)	Potassium (mg)	Calcium (mg)	Iron (mg)
Pepperoni	241	8	3.7	0.16	32	1.6	5	10	20.9	508	157	131	2
Three Cheese	235	7	3.2	0.21	33	1.6	5	10	18.7	460	134	187	2
Bacon & Pepperoni	245	9	3.5	0.15	31	1.5	5	10	22.5	565	175	125	2

3.4.5. Treatment Palatability

Palatability of the treatments was measured using AVAS after the first bite to reflect first impressions. For experiment 1, palatability was assessed based on the criteria of pleasantness, taste, texture, thickness, uniformity, coarseness, slipperiness, flavor, and smell. The overall palatability was calculated using the equation: palatability = (pleasantness + taste + texture + thickness + uniformity + coarseness + slipperiness + flavor + smell) / 9. For experiments 2 and 3, palatability was determined based on ratings for pleasantness, taste, and texture using the equation: palatability = (pleasantness + taste + texture) / 3.

3.5 Statistical Analysis

Statistical analysis was conducted using SAS version 9.4. Two-way repeated measures ANCOVA with baseline as a covariate was used to determine the effects of treatment, time, and treatment-by-time interactions on BG, insulin, and subjective appetite over 140min. When significant treatment-by-time interactions were found, a one-way ANOVA was conducted for each timepoint to determine which ones were different. BG and insulin incremental area under the curve (iAUC) between 0-120min, and subjective appetite total AUC (tAUC) between 15-120min were calculated using the trapezoid method and analyzed using one-way ANOVA. Treatment effects on food and water intake as well as palatability were also determined using one-way ANOVA. Tukey-Kramer post hoc test was used to determine significance between the means for all statistical procedures. The impacts of sex and BMI were considered for all analyses and if no effects were found, the data was pooled. All results were reported as means ± standard error of the mean (SEM), and the data was considered statistically significant if p<0.05.

Results and Discussions (maximum of 30 pages (not including figures or tables))

Describe research accomplishments during the reporting period under relevant objectives listed under “Objectives and Progress” section. Please accompany a written description of results with tables, graphs and/or other illustrations. Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.

Study 1. Milling and differential sieving to diversify flour functionality: A comparison between pulses and cereals

1.1 Yields of whole flours from milling and coarse and fine flours from differential sieving

After milling, the yields of whole flours ranged from 90.4% to 92.8% for the four different crops (Table 1.1), indicating good recovery rates of flours from this step. Upon sieving using a 0.15-mm sieve, the yields of coarse and fine flours showed broad ranges of 36.5%-63.1% and 35.6%-61.0%, respectively. Among the four crops, barley exhibited the highest yield of coarse flour but the lowest yield of fine flour, which could be linked to the largest particle size of the whole barley flour as displayed in Figure 1. Additionally, the total yields of both coarse and fine flours from the four crops were remarkably high, 97.5%-99.4%, suggesting negligible loss of flour during sieving.

Table 1.1. Yields of whole pea, lentil, barley, and oat flours from milling and corresponding coarse and fine flours from differential sieving.^a

Flour	Yield of whole flour from milling (%) ^b	Differential sieving	
		Yield of coarse flour (%) ^c	Yield of fine flour (%) ^c
Pea	92.8 ± 0.9 a	39.3 ± 0.2 a	60.0 ± 0.1 c
Lentil	90.4 ± 3.1 a	46.4 ± 2.2 b	53.0 ± 1.9 b
Barley	90.5 ± 0.7 a	63.1 ± 0.5 c	35.6 ± 0.5 a
Oats	91.8 ± 2.3 a	36.5 ± 2.6 a	61.0 ± 1.2 c

^aData are presented as average ± standard deviation (n = 2); in the same column, data with the same letter are not significantly different at $p < 0.05$.

^b%Yield of whole flour from milling = (Weight of whole flour collected from milling) / (Initial weight of seeds used for milling) × 100%.

^c%Yield of coarse/fine flour from sieving = (Weight of collected coarse/fine flour from sieving) / (Initial weight of whole flour used for sieving) × 100%.

1.2 Particle-size distributions and morphologies of flours

The particles in the whole flour samples of the four crops exhibited a bimodal distribution (Figure 1.1): the first peak (30.2, 26.3, 30.2, and 34.7 µm in the four flour samples, respectively) mainly corresponded to individual starch granules; and the second one (478.6, 363.1, 549.5, and 724.4 µm, respectively) mainly corresponded to aggregated particles consisting of starch, protein, and fiber (marked by rectangles in Figure 1.2) (Liu et al., 2020). D[4,3] of the whole flour samples was in a descending order of barley > oats > lentil > pea.

The 0.15-mm sieving was effective in separating the abovementioned two main types of particles in the whole flours: the fine stream of pea, lentil, and barley primarily consisted of individual starch granules, along with some protein and fiber debris; by contrast, the coarse counterparts predominantly comprised aggregated particles (Figures 1.1 and 1.2). Consequently, the particle-size distribution curves of the fine and coarse flours obtained from sieving were obviously different from those of the corresponding whole flours, and the D[4,3] of the three flour streams from the same crop followed an ascending order of fine < whole < coarse. For the flours within the whole, coarse, and fine groups, D[4,3] of pea and lentil samples were consistently smaller than those of the barley and oat samples. Moreover, distinct differences were observed in the morphologies of the large, aggregated particles among the coarse pulse and cereal flours: (1) Those of pea and lentil aggregated particles had fewer starch granules, as compared to the coarse barley and oat flours; and (2) Pea and lentil starch granules were more compactly entrapped in protein and fiber matrices (marked by up arrows in Figure 2), while such compact entrapment of starch granules by protein and fiber was largely absent in the coarse barley and oat flour particles. The described differences in particle morphology between coarse pulse and cereal flours generally reflected the differences in the microscopic structures of their seeds (Setia et al., 2019; Shapter et al., 2008).

Of all the four crops, it is noteworthy that oat starch granules exist in two forms in the grains: (1) single granules having approximately 2-15 µm in diameter (marked by stars in Figure 1.2); and (2) clusters composed of

compound granules having approximately 20-150 μm in diameter (marked by ovals) (Bechtel, 1981; Falsafi et al., 2019). Some of such clusters were preserved in the fine flour of oats, which explained its largest D[4,3] among all the four fine fractions.

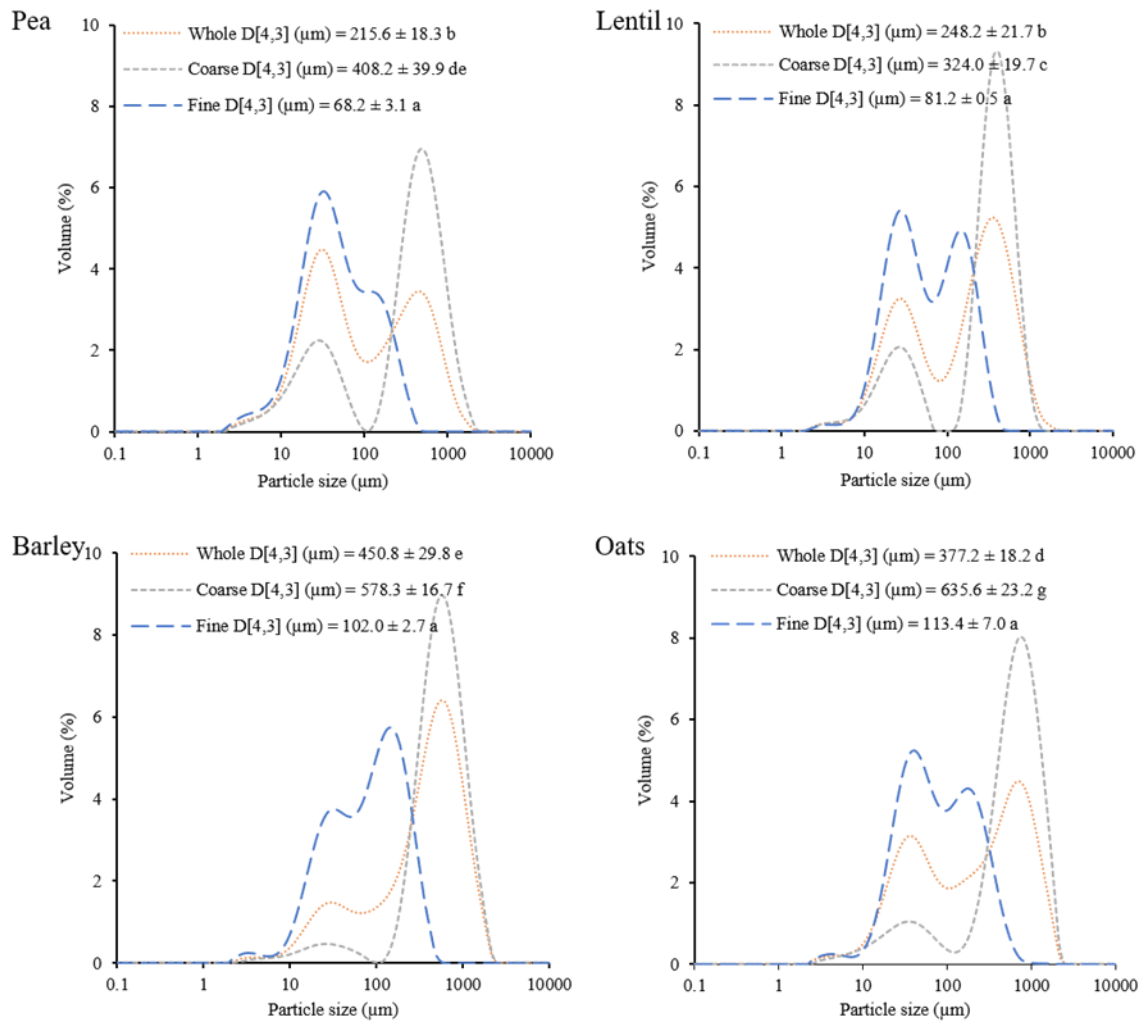
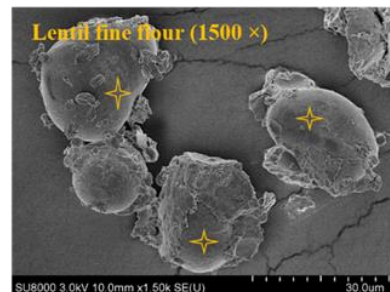
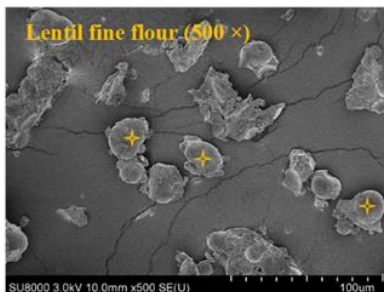
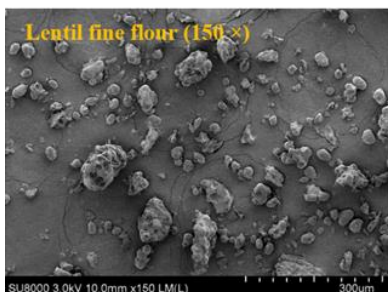
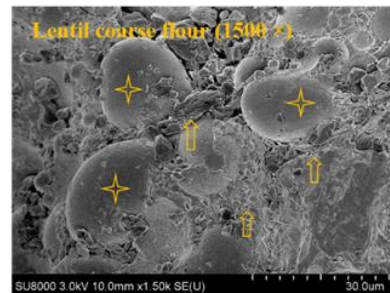
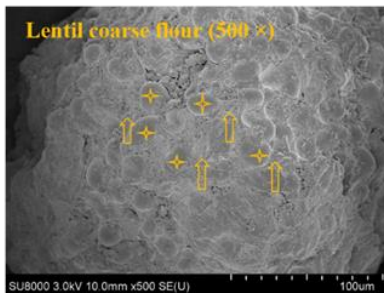
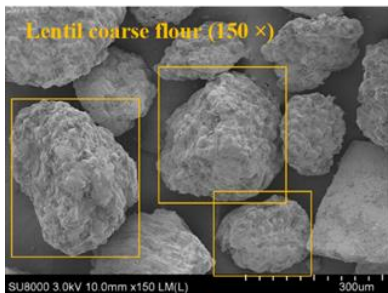
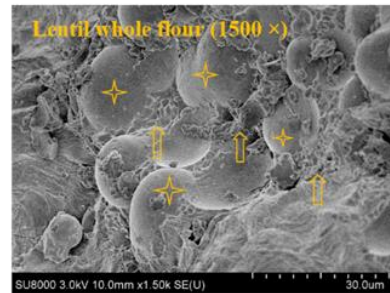
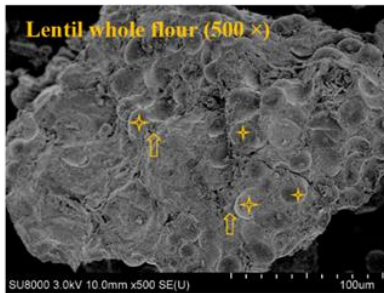
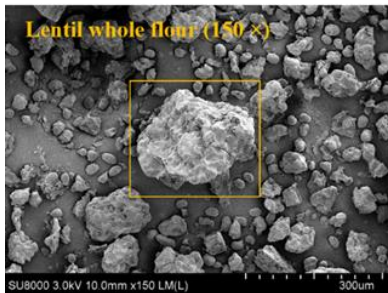
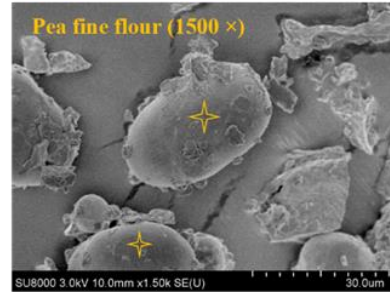
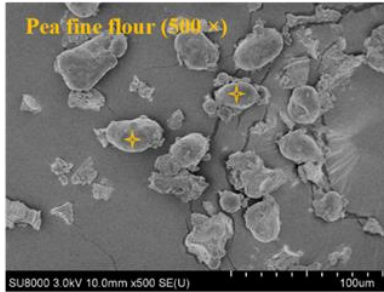
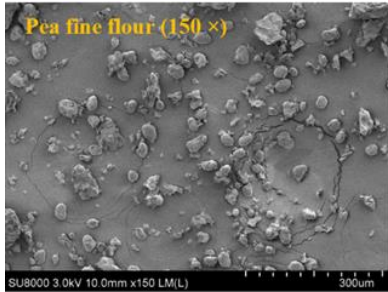
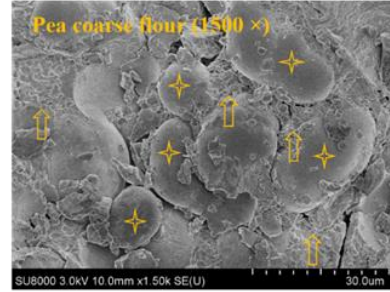
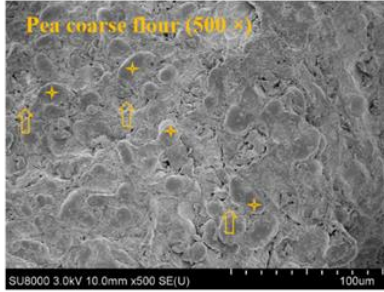
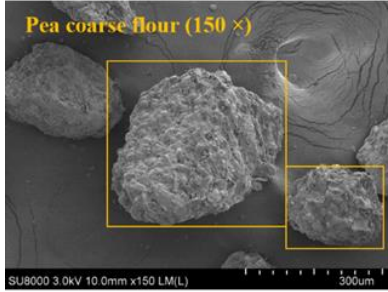
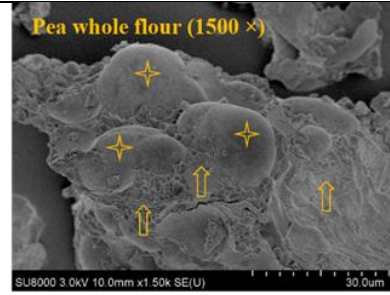
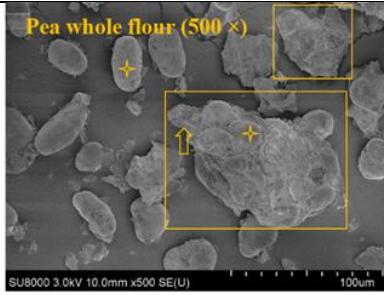
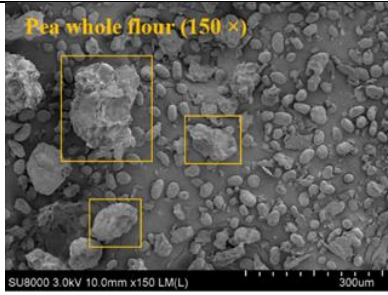


Figure 1.1. Particle-size distributions of pea, lentil, barley, and oat flours from three different streams. D[4,3] are presented as average \pm standard deviation ($n = 4$). Data with the same letter are not significantly different at $p < 0.05$ among all the samples.



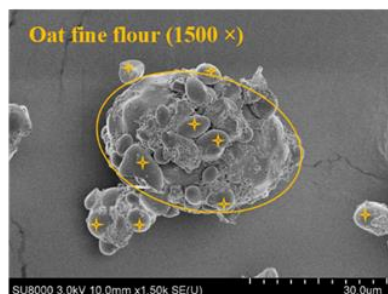
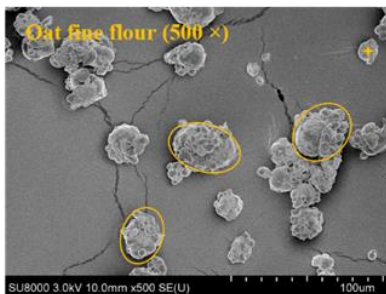
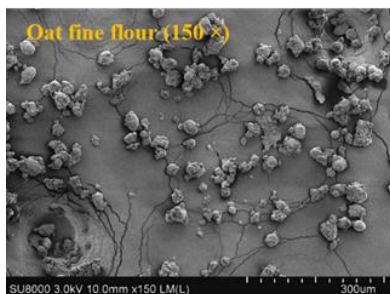
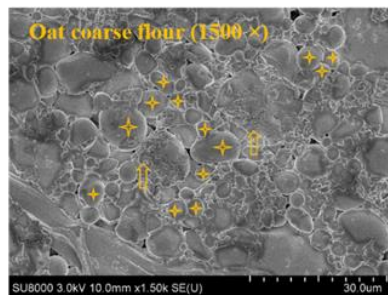
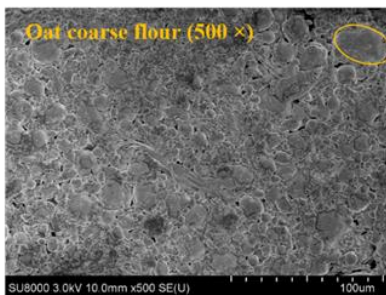
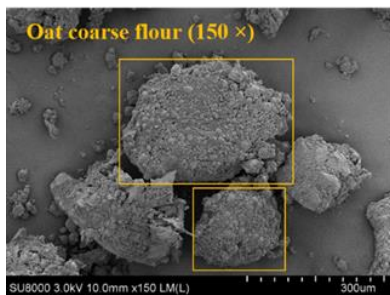
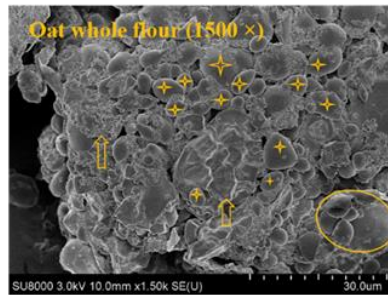
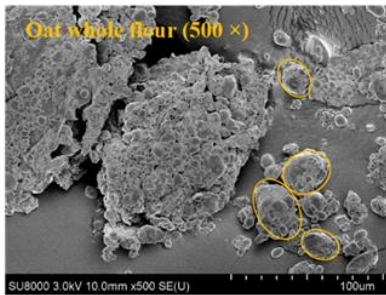
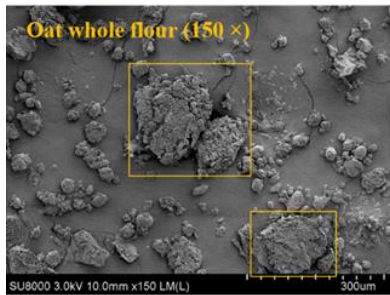
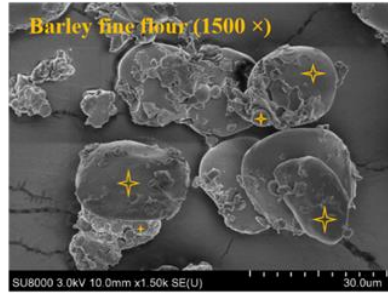
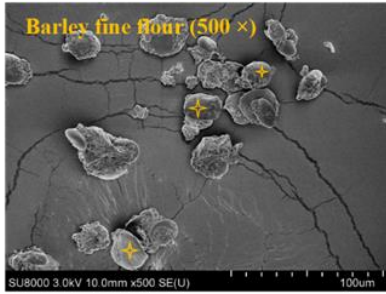
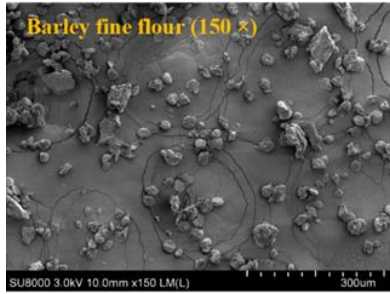
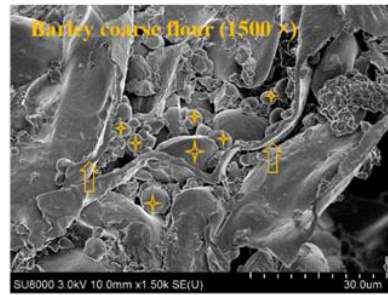
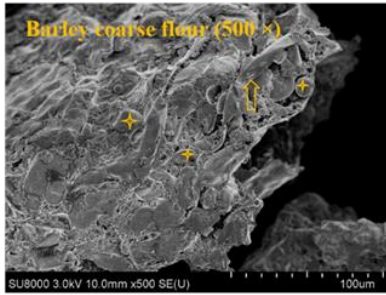
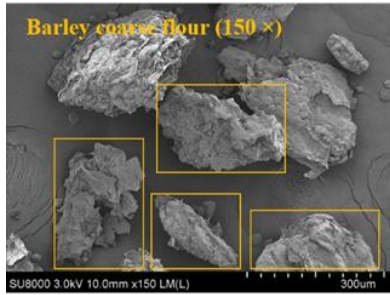
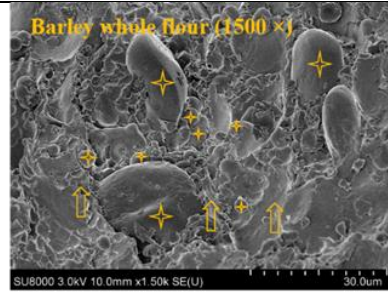
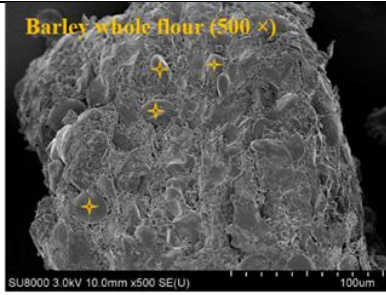
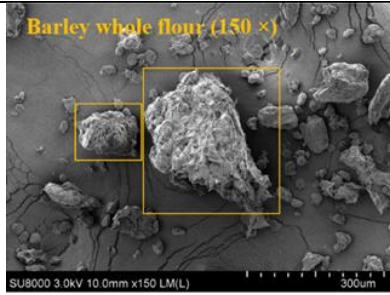


Figure 1.2. Scanning electron microscopy (SEM) images of pea, lentil, barley, and oat flours from three different streams. Magnification at which the image was captured is shown in parentheses. Rectangles indicate aggregated particles formed with starch, protein, and fiber; up arrows indicate protein and fiber matrices; stars indicate starch granules; ovals indicate clusters of compound granules

1.3 Chemical compositions of flours

Starch contents of the three flour streams followed a descending order of fine > whole > coarse for all the four crops (Table 1.2), which are in agreement with the results reported by Ahmed, Taher, et al. (2016). The trend is also consistent with the presence of starch granules in the different streams as illustrated in SEM images (Figure 1.2). Damaged-starch contents of the fine, whole, and coarse streams also fit into the same trend for all the studied crops, which could be explained by that more mechanical force was required to break cotyledon structure to achieve the fine particles and that the large particles better retained the original cotyledon structure. The milling and sieving, however, did not result in any significant difference in the amylose contents of starch (dsb) in the three flour streams of the same crop.

Protein contents of the three flour streams from pea and lentil were largely comparable, but they exhibited a trend of coarse > whole > fine for cereal flours, particularly for oats (Table 1.2). For the same crop, the difference in the lipid contents of the three resultant streams was largely insignificant. Among the different crops, the lipid concentrations of the different flours were in an ascending order of lentil < pea < barley < oats, and the remarkably higher lipid levels of oat samples correspond well with the results reported by other researchers (Liu, Bailey, & White, 2010; Sharma & Gujral, 2010; Stone et al., 2019). Ash contents of the three flour streams from the same botanical source displayed an increasing order of fine < whole < coarse, suggesting that minerals were more concentrated in the coarse fraction, which is in good accordance with the findings of Ahmed, Taher, et al. (2016). An obvious impact of particle size on the total dietary fiber contents of the flour streams was observed as the values followed an ascending order of fine < whole < coarse for all the four crops, suggesting that the coarse stream could be a more promising source of dietary fiber from the respective crops.

As clearly presented in Table 1.2, the pulse flours of the three different streams in general consisted of less starch (except for the coarse oat flour) and lipid but more protein, ash, and dietary fiber than the corresponding cereal flour streams. In addition, the starches in the former group contained more amylose (dsb) than those in the latter group. The noted differences in the proximate compositions of the pulse and cereal flours agree well with the observation in previous studies (Li et al., 2019; Stone et al., 2019), and the impacts on their physicochemical properties were comprehensively discussed in the following sections.

Table 1.2. Chemical compositions of pea, lentil, barley, and oat flours from three different streams on a dry flour basis.^a

Flour	Starch (%)	Damaged starch (%)	Amylose (%)	Amylose (% dsb) ^b	Protein (%)	Lipid (%)	Ash (%)	Total dietary fiber (%) ^c
Pea								
Whole	49.8 ± 1.3 c	0.77 ± 0.01 b	17.9 ± 0.7 cd	36.0 ± 1.4 cd	21.4 ± 1.4 f	1.36 ± 0.10 b	2.33 ± 0.13 e	27.7 ± 2.3 e
Coarse	40.1 ± 0.6 a	0.29 ± 0.03 a	14.4 ± 0.5 b	35.8 ± 1.2 cd	21.8 ± 0.3 fg	1.25 ± 0.05 b	2.51 ± 0.02 g	36.5 ± 2.3 f
Fine	53.1 ± 0.5 d	1.29 ± 0.04 c	20.6 ± 0.3 ef	38.8 ± 0.7 d	22.7 ± 0.4 g	1.36 ± 0.09 b	2.15 ± 0.03 d	16.7 ± 0.4 bc
Lentil								
Whole	49.5 ± 0.8 c	0.81 ± 0.01 b	18.1 ± 0.8 cd	36.6 ± 2.0 cd	24.5 ± 0.2 h	0.66 ± 0.03 a	2.37 ± 0.01 ef	23.0 ± 0.2 d
Coarse	43.5 ± 1.2 b	0.25 ± 0.02 a	15.3 ± 0.2 b	35.1 ± 1.2 c	24.7 ± 0.1 h	0.60 ± 0.07 a	2.46 ± 0.01 fg	27.8 ± 0.0 e
Fine	51.5 ± 1.0 cd	1.48 ± 0.04 d	17.8 ± 0.1 cd	34.6 ± 0.3 c	25.1 ± 0.4 h	0.76 ± 0.02 a	2.33 ± 0.01 e	19.1 ± 0.9 bcd
Barley								
Whole	62.2 ± 0.7 f	3.00 ± 0.04 g	19.1 ± 0.9 de	30.8 ± 1.4 b	11.4 ± 0.2 ab	2.36 ± 0.07 d	1.56 ± 0.02 b	19.1 ± 0.6 bcd

Coarse	57.7 ± 0.3 e	2.02 ± 0.06 e	16.1 ± 1.9 bc	27.9 ± 3.3 ab	12.1 ± 0.2 b	2.32 ± 0.06 cd	1.83 ± 0.00 c	21.0 ± 0.6 cd
Fine	73.3 ± 1.4 h	4.66 ± 0.10 h	21.9 ± 1.1 f	29.9 ± 1.0 ab	11.0 ± 0.2 a	1.91 ± 0.10 c	1.15 ± 0.02 a	10.4 ± 0.2 a
Oats								
Whole	59.9 ± 1.4 ef	1.91 ± 0.04 e	16.1 ± 0.5 bc	26.9 ± 0.4 a	15.5 ± 0.2 d	7.66 ± 0.17 e	1.44 ± 0.08 b	15.1 ± 0.3 b
Coarse	42.8 ± 1.0 b	1.56 ± 0.09 d	11.3 ± 0.5 a	26.4 ± 1.0 a	19.8 ± 0.1 e	8.37 ± 0.41 f	2.14 ± 0.04 d	21.2 ± 0.1 d
Fine	67.0 ± 0.9 g	2.25 ± 0.14 f	17.8 ± 1.1 cd	26.6 ± 1.4 a	13.7 ± 0.3 c	7.94 ± 0.33 e	1.18 ± 0.04 a	8.8 ± 1.3 a

^aData are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$.

^b%Amylose content, dry starch basis = (%Amylose content, dry flour basis) / (%Starch content, dry flour basis) × 100%.

^cData are presented as average ± standard deviation (n = 2); in the same column, data with the same letter are not significantly different at $p < 0.05$.

1.4 Color of flours

Overall, the color parameters of the three streams from the same crop followed the same trend: with the reduction in particle size, the L^* value increased while the a^* and b^* values decreased (Table 1.3). The enhanced L^* values in the fine flours were attributable to: (1) This stream contained more starch but less ash and dietary fiber than the whole and coarse counterparts (Table 1.2); and (2) The smaller particle size of fine flour contributed to a larger total surface area, thus allowing more reflection of light (Ahmed, Al-Jassar, & Thomas, 2015). The reported results correspond well with the work of Kaiser, Barber, Manthey, and Hall (2019) and Drakos et al. (2017). Compared with the cereal flours, the pulse flours exhibited considerably greater b^* values (*i.e.*, more yellowness), which could be associated with the nature of the seeds.

Table 1.3. Hunter color parameters of pea, lentil, barley, and oat flours from three different streams.^a

Flour	L^*	a^*	b^*
Pea			
Whole	86.4 ± 0.7 fg	2.1 ± 0.3 ef	21.0 ± 1.3 cd
Coarse	79.5 ± 0.5 cd	4.5 ± 0.3 h	31.4 ± 0.2 e
Fine	88.5 ± 0.1 h	1.6 ± 0.0 cd	20.9 ± 0.3 cd
Lentil			
Whole	80.8 ± 0.6 d	1.1 ± 0.1 b	18.1 ± 0.8 c
Coarse	70.3 ± 1.6 a	1.6 ± 0.4 cd	21.8 ± 4.5 d
Fine	84.9 ± 0.2 ef	0.5 ± 0.1 a	18.0 ± 1.3 c
Barley			
Whole	84.9 ± 0.2 ef	1.3 ± 0.0 bc	10.0 ± 0.1 a
Coarse	79.1 ± 0.6 bc	2.3 ± 0.1 f	13.1 ± 0.3 ab
Fine	87.9 ± 0.3 gh	0.9 ± 0.0 ab	9.7 ± 0.2 a
Oats			
Whole	83.9 ± 0.2 e	1.8 ± 0.0 de	13.5 ± 0.1 b
Coarse	78.0 ± 0.5 b	2.9 ± 0.1 g	17.8 ± 0.5 c
Fine	84.7 ± 0.1 e	1.6 ± 0.0 cd	13.1 ± 0.2 ab

^aData are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$.

1.5 Thermal properties of flours

The DSC thermograms of the pulse flours showed a major peak followed by an overlapping minor shoulder in the first scan (Figure S1.1 and Table 1.4): the first major peak exhibiting T_p at 70.7-73.6 °C mainly resulted from starch gelatinization, while the minor shoulder displaying T_p at 90.4-92.0 °C mainly resulted from protein denaturation (Ren et al., 2021). The second scan of pulse flours revealed the absence ALC, which is consistent with previous work (Liu et al., 2019). The DSC thermograms of the cereal flours showed a major peak followed by a separate minor peak in the first scan: (Figure S1.1 and Table 1.4): the first major peak exhibiting T_p at 65.4-70.2 °C and ΔH of 4.2-7.5 J/g primarily represented starch gelatinization, while the second minor peak displaying T_p at 95.0-101.2 °C primarily represented the dissociation of ALC, which was confirmed by the occurrence of a similar peak having T_p at 100.2-103.1 °C in the second scan (Liu et al., 2019). The ALC dissociation peak of the oat flours exhibited a wider temperature range (~88-105 °C) and a greater ΔH (0.5-0.7 J/g) in comparison with those of the barley flours (~98-106 °C and 0.2-0.3 J/g, respectively) in the second scan, suggesting more ALC formation in the former. Moreover, the oat flours distinctly showed a third peak having T_p at 115.6-116.0 °C in first scan, which represented the denaturation of protein according to Moio, Forssell, Partanen, Damerou, and Hill (2015).

Generally, for the same crop type, the starch gelatinization temperatures in the first scan did not vary significantly among the three flour streams (Table 1.4); however, the cereal flour with finer particles consistently exhibited a higher ΔH value (*i.e.*, fine > whole > coarse). Ai et al. (2017) suggested that flour with a smaller particle size could achieve more complete starch gelatinization, which thus required more ΔH for this thermal transition. In addition, the larger ΔH of finer flour could be associated with the higher starch content as compared to the other two streams (Table 1.2). Compared with the pulse flours, the barley and oat flours exhibited lower starch gelatinization temperatures, consistent with the differences in the gelatinization temperatures of isolated starches from these crops (Falsafi et al., 2019; Gao, Vasanthan, & Hoover, 2009; Li et al., 2019).

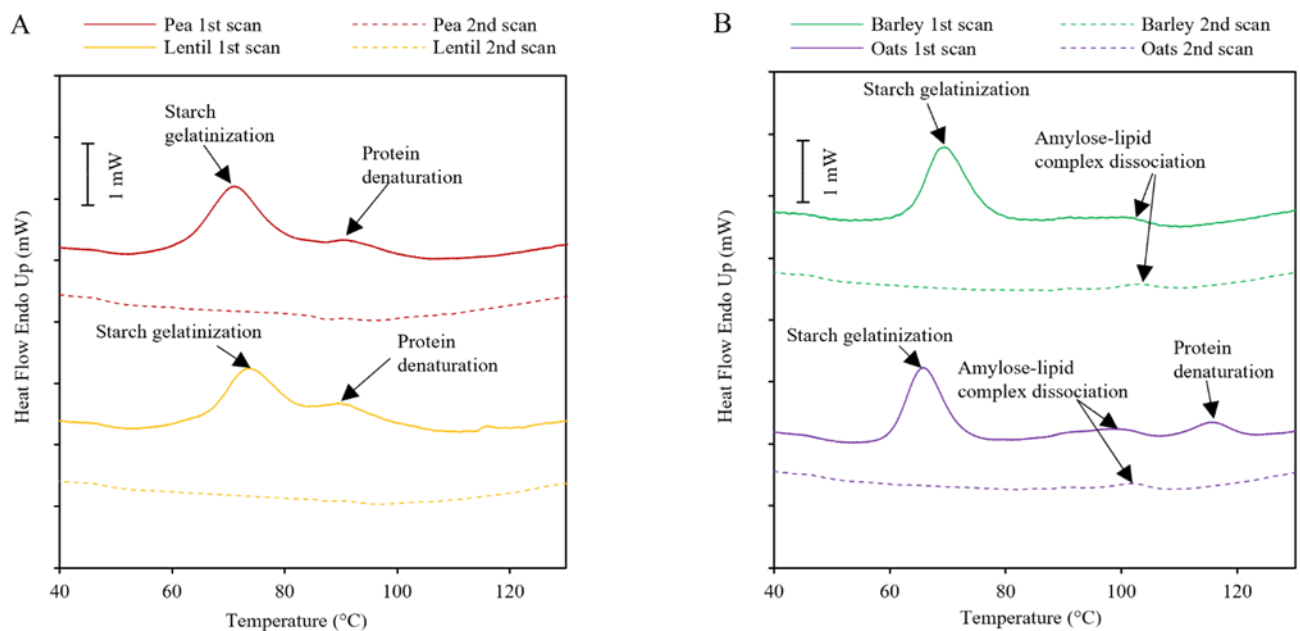


Figure S1.1. Representative differential scanning calorimetry (DSC) thermograms of fine pea and lentil flours (A) and fine barley and oat flours (B).

Table 1.4. Thermal properties of pea, lentil, barley, and oat flours from three different streams.^{a,b}

Flour	First scan						Second scan			
	Gelatinization of starch				Dissociation of amylose-lipid complexes	Denaturation of protein	Dissociation of amylose-lipid complexes in rescan			
	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)			T _p (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
Pea										
Whole	64.5 ± 0.8 cd	71.4 ± 0.3 f	78.0 ± 0.6 de	N.A. ^c	N.D. ^d	91.5 ± 0.3 b	N.D.	N.D.	N.D.	N.D.
Coarse	63.7 ± 0.4 bc	70.7 ± 0.3 ef	79.6 ± 0.5 fg	N.A.	N.D.	92.0 ± 0.3 b	N.D.	N.D.	N.D.	N.D.
Fine	63.6 ± 0.3 b	71.1 ± 0.1 f	78.8 ± 0.4 ef	N.A.	N.D.	91.3 ± 0.1 b	N.D.	N.D.	N.D.	N.D.
Lentil										
Whole	66.7 ± 0.4 e	73.5 ± 0.5 g	81.6 ± 0.5 h	N.A.	N.D.	90.4 ± 0.5 a	N.D.	N.D.	N.D.	N.D.
Coarse	67.4 ± 0.2 e	73.4 ± 0.1 g	80.4 ± 0.4 g	N.A.	N.D.	90.5 ± 0.3 a	N.D.	N.D.	N.D.	N.D.
Fine	67.3 ± 0.4 e	73.6 ± 0.3 g	81.9 ± 0.4 h	N.A.	N.D.	90.5 ± 0.3 a	N.D.	N.D.	N.D.	N.D.
Barley										
Whole	64.3 ± 0.1 bcd	69.6 ± 0.1 cd	76.5 ± 0.2 c	6.4 ± 0.3 cd	100.7 ± 0.4 bc	N.D.	98.4 ± 0.6 b	102.8 ± 0.1 c	106.5 ± 0.4 c	0.3 ± 0.0 a
Coarse	64.6 ± 0.4 d	70.2 ± 0.3 de	77.7 ± 0.7 d	5.4 ± 0.3 b	101.2 ± 0.5 b	N.D.	98.4 ± 0.5 b	102.5 ± 0.4 c	105.7 ± 0.8 bc	0.2 ± 0.0 a
Fine	63.8 ± 0.1 bcd	69.3 ± 0.3 c	76.3 ± 0.3 c	7.5 ± 0.4 e	101.7 ± 0.7 b	N.D.	98.8 ± 0.4 b	103.1 ± 0.3 c	106.3 ± 0.2 bc	0.3 ± 0.1 a
Oats										
Whole	60.3 ± 0.1 a	65.4 ± 0.2 a	71.7 ± 0.2 a	5.8 ± 0.4 bc	99.0 ± 0.4 ab	115.8 ± 0.2 c	88.5 ± 0.4 a	101.2 ± 0.3 b	105.4 ± 0.4 bc	0.7 ± 0.0 c
Coarse	60.8 ± 0.2 a	66.2 ± 0.1 b	72.8 ± 0.2 b	4.2 ± 0.1 a	98.0 ± 1.9 a	116.0 ± 0.1 c	89.1 ± 0.3 a	100.2 ± 0.5 a	104.0 ± 0.7 a	0.5 ± 0.0 b
Fine	60.3 ± 0.5 a	65.6 ± 0.3 a	71.8 ± 0.2 a	6.8 ± 0.1 d	98.7 ± 0.4 ab	115.6 ± 0.4 c	88.8 ± 0.2 a	101.7 ± 0.0 b	105.3 ± 0.4 b	0.6 ± 0.1 c

^aData are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$.

^bT_o: onset temperature; T_p: peak temperature; T_c: conclusion temperature; ΔH: enthalpy change.

^cN.A.: Not available because of the overlapping between starch gelatinization peak and protein denaturation peak.

^dN.D.: Not detectable.

1.6 Pasting properties of flours

For both pulse crops, the three flour streams explicitly showed pasting viscosities of fine > whole > coarse (Figure 1.3 and Table S1.1), corresponding well with the trends reported by Gu et al. (2021). The lowest viscosity development in the coarse pea and lentil flours could be explained by the following two important factors: (1) The coarse flours comprised significantly less starch than the corresponding whole and fine flours (Table 2), and starch is known to be the leading component responsible for viscosity development of flour during pasting (Yuan et al., 2021); and (2) The starch granules were densely packed in protein and fiber matrices in coarse flours (Figure 1.2), which was demonstrated to restrict the swelling of starch granules to provide less viscosity (Dhital et al., 2016; Setia et al., 2019).

In contrast, the different particle sizes did not have the same influence on the pasting properties of the barley and oat flours as noted above for the pea and lentil flours (Figure 1.3 and Table S1.1). The fine barley and oat flours showed higher peak viscosities than the whole and coarse counterparts, which could be mainly ascribed to the markedly greater starch contents of the fine flours (Table 1.2). Overall, the fine cereal flours displayed trough and final viscosities similar to those of the whole and coarse counterparts. Interestingly, despite the observed significant differences in their D[4,3] (Figure 1.1) and starch contents (Table 1.2), the pasting profiles of the coarse barley and oat flours were generally comparable to those of their respective whole flours. This observation could be associated with the following factors: (1) The starch granules in coarse barley and oats streams were not embedded in dense

protein and fiber matrices as in pea and lentil samples (Figure 1.2), and thus the presence of more protein and dietary fiber in the coarse cereal flours did not restrict the swelling of the granules during pasting; and (2) β -glucan tended to be concentrated in the coarse stream during differential sieving (Ahmed, 2014), and this polysaccharide, occurring at a high level in barley and oat grains, could contribute to viscosity development of their coarse flours, thereby offsetting the differences with the whole counterparts during RVA analysis.

Despite the fact that the pulse flours had higher starch gelatinization temperatures than the cereal flours (Figure S1.1 and Table 1.4), the former showed noticeably lower pasting temperatures than the latter (Figure 1.3 and Table S1.1), which could primarily result from the existence of ALC in the cereal flours because such single-helical complexes are known to restrict granular swelling of starch during heating to elevate the pasting temperatures of starch and flour (Liu et al., 2019; Yuan et al., 2021). Overall, the studied pulse flours exhibited significant lower pasting viscosities in comparison with the cereal flours, which could be attributable to less starch and the entrapment of starch in protein and fiber matrices of the former as discussed above as well as more amylose in the pulse starches (dsb; Table 1.2) (Li et al., 2019).

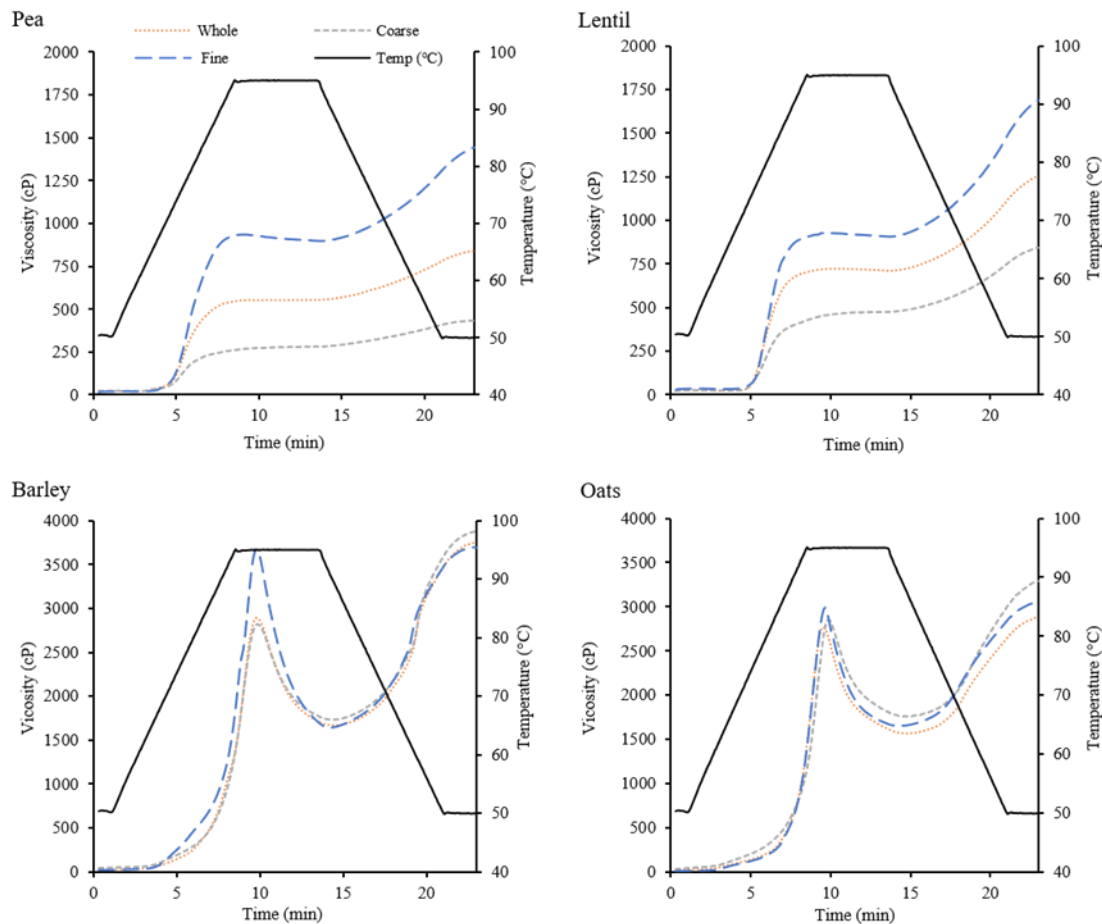


Figure 1.3. Pasting properties of pea, lentil, barley, and oat flours from three different streams. Flour suspensions (28.5 g total weight) with 10.6% concentration (w/w, dry flour basis) were used for the measurement using Rapid Visco Analyser.

Table S1.1. Pasting properties of pea, lentil, barley, and oat flours from three different streams.^a

Sample	Pasting temperature (°C)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Setback viscosity (cP)	Final viscosity (cP)
Pea						
Whole	75.1 ± 0.6 ab	556.0 ± 26.1 b	553.0 ± 26.7 b	3.0 ± 1.4 a	285.0 ± 10.0 b	838.0 ± 29.7 b
Coarse	73.8 ± 1.9 a	276.0 ± 13.7 a	275.5 ± 14.6 a	0.5 ± 1.3 a	155.5 ± 6.4 a	431.0 ± 17.3 a

Fine	74.7 ± 0.1 ab	935.5 ± 13.6 d	897.3 ± 5.1 d	38.3 ± 15.2 a	544.5 ± 12.6 c	1441.8 ± 16.0 d
Lentil						
Whole	76.0 ± 0.2 ab	724.0 ± 40.5 c	711.5 ± 36.0 c	12.5 ± 4.7 a	544.8 ± 18.8 c	1256.3 ± 54.7 c
Coarse	75.4 ± 0.5 ab	476.0 ± 21.4 b	474.8 ± 21.0 b	1.3 ± 1.0 a	368.0 ± 7.5 b	842.8 ± 20.0 b
Fine	76.9 ± 0.5 bc	944.8 ± 40.4 d	922.5 ± 43.3 d	22.3 ± 4.0 a	781.5 ± 13.4 d	1704.0 ± 54.5 e
Barley						
Whole	83.6 ± 0.7 d	2890.8 ± 38.5 fg	1664.8 ± 17.3 ef	1226.0 ± 21.4 bc	2077.8 ± 31.1 g	3742.5 ± 36.1 ij
Coarse	85.4 ± 1.1 de	2818.5 ± 60.0 ef	1730.0 ± 59.0 f	1088.5 ± 11.1 b	2139.3 ± 45.0 g	3869.3 ± 101.6 j
Fine	79.4 ± 3.4 c	3637.8 ± 93.1 h	1694.5 ± 105.9 f	1943.3 ± 193.5 d	2032.3 ± 130.0 g	3726.8 ± 92.2 i
Oats						
Whole	84.5 ± 0.2 de	2775.0 ± 30.7 e	1563.5 ± 40.7 e	1211.5 ± 71.3 bc	1322.3 ± 45.0 e	2885.8 ± 85.4 f
Coarse	86.7 ± 0.1 e	2860.5 ± 47.4 ef	1758.8 ± 31.6 f	1101.8 ± 24.0 b	1536.0 ± 16.2 f	3294.8 ± 47.3 h
Fine	85.7 ± 0.2 de	2980.3 ± 43.3 g	1657.0 ± 13.1 ef	1323.3 ± 30.3 c	1400.5 ± 13.0 e	3057.5 ± 9.3 g

^aData are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$.

1.7 Gel hardness of flours

In general, strength of the gels developed from the three flour streams of the same crop exhibited a descending order of fine > whole > coarse (except for pea; Figure 1.4), indicating that the fine streams had a tendency to form a stronger gel after cooking and storage. The observed trend is in good accordance with that reported by Nura, Kharidah, Jamilah, and Roselina (2011), in which the gel hardness of rice flour was negatively correlated with its particle size. According to previous studies, starch is the main contributor to the gelling ability of flour but the presence of protein, dietary fiber, and other components is detrimental for gel formation (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014; Yuan et al., 2021), which explained the greatest gel strength of fine flour among all the three streams (Table 1.2). Further research is needed to understand why the gelling ability of the fine pea flour did not fit into this trend.

For the same flour stream of the four studied crops, the pulse flour gels generally exhibited higher hardness than those of cereal flours (65.9-163.9 g *versus* 30.3-61.5 g; Figure 1.4), except for the coarse pea flour gel (27.9 g), although the pulse flours were composed of less starch but more protein, dietary fiber, and ash than the corresponding cereal streams (Table 1.2). The findings could be attributed to the remarkably stronger gelling ability of the pulse starches than the cereal starches (Li et al., 2019; Liu et al., 2019). The poorest gelling capability of the coarse pea flour could be related to its smallest starch content but largest dietary fiber and ash contents of all the flour samples (Table 1.2).

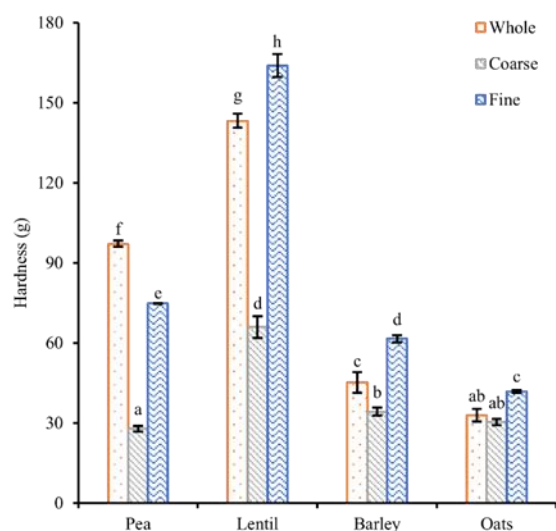


Figure 1.4. Hardness of gels prepared with pea, lentil, barley, and oat flours from three different streams. Flour suspensions (28.5 g total weight) with 10.6% concentration (w/w, dry flour basis) were cooked using RVA 4800 following the same conditions used for pasting property determination. After cooking, the flour pastes were poured into a plastic container with lid and stored at room temperature for 2.0 h before the determination of hardness. Data with the same letter are not significantly different at $p < 0.05$ among all the samples.

1.8 WHC and OBC of flours

WHC and OBC are important functional properties of food ingredients as they determine the textural properties, mouthfeel, and yield of final products (Ai et al., 2017; Lin & Zayas, 1987). Within the same crop, the coarse flour exhibited the largest WHC value followed by the whole and fine streams, indicating that WHC of the flours were reduced as the particle sizes decreased (Table 1.5), which could be partly linked to the reduced dietary fiber contents (Table 1.2) (Ahmed, Al-Attar, & Arfat, 2016). Our observation is in good accordance with the finding of Ahmed, Taher, et al. (2016). However, Rao et al. (2016) reported the opposite trend, in which WHC of sorghum flours increased as the particle sizes decreased. Within the same stream, the flours of pulses and cereals showed comparable WHC, except for the coarse group, where the coarse oat flour exhibited a noticeably greater WHC value (2.30 g/g) than the other three coarse flours (1.51-1.74 g/g). The highest WHC of the coarse oat flour could be partly explained by its largest D[4,3] as illustrated in Figure 1.1.

OBC of the three flour streams from the same pulse crop were comparable (Table 1.5), indicating that their OBC were not significantly affected by the different particle sizes, which was primarily attributed to the comparable protein contents of the different pulse flour streams (Table 1.2) (Ren et al., 2021; Stone et al., 2019). In contrast, OBC of the cereal flours were remarkably influenced by the particle sizes. For the same cereal crop, OBC of the three flour streams followed a descending order of coarse > whole > fine, suggesting that OBC of the cereal flours decreased as the particle sizes decreased, agreeing well with the data reported by Protonotariou et al. (2014), Rao et al. (2016) and Drakos et al. (2017). The lower OBC value of the fine cereal streams could be partially ascribed to their lower protein content when compared with the whole and coarse counterparts as presented in Table 1.2.

Table 1.5. Functional properties of pea, lentil, barley, and oat flours from three different streams.^{a, b}

Flour	WHC (g/g, db)	OBC (g/g, db)	FC (%)	FS (%)	EA (%)	ES (%)
Pea						
Whole	1.36 ± 0.03 b	1.49 ± 0.06 d	378 ± 17 cd	6.1 ± 1.9 a	42.4 ± 2.3 a	62.3 ± 4.3 ab
Coarse	1.73 ± 0.10 d	1.45 ± 0.03 d	390 ± 15 d	6.9 ± 2.7 ab	41.0 ± 1.8 a	59.4 ± 1.0 ab
Fine	1.23 ± 0.01 a	1.49 ± 0.04 d	404 ± 21 d	11.6 ± 1.1 b	40.9 ± 0.7 a	62.4 ± 3.8 ab

Lentil						
Whole	1.45 ± 0.03 bc	1.02 ± 0.03 ab	350 ± 22 bc	9.5 ± 3.3 ab	41.1 ± 1.8 a	65.4 ± 3.1 b
Coarse	1.74 ± 0.03 d	0.94 ± 0.01 a	303 ± 16 a	7.6 ± 1.7 ab	41.8 ± 1.8 a	56.1 ± 5.0 a
Fine	1.17 ± 0.02 a	0.99 ± 0.00 ab	333 ± 6 ab	8.8 ± 1.5 ab	39.9 ± 1.0 a	55.0 ± 2.5 a
Barley						
Whole	1.17 ± 0.02 a	1.68 ± 0.06 e	N.D.	N.D.	N.D.	N.D.
Coarse	1.51 ± 0.01 c	2.10 ± 0.07 f	N.D.	N.D.	N.D.	N.D.
Fine	1.12 ± 0.03 a	1.44 ± 0.04 d	N.D.	N.D.	N.D.	N.D.
Oats						
Whole	1.47 ± 0.02 c	1.15 ± 0.02 c	N.D.	N.D.	N.D.	N.D.
Coarse	2.30 ± 0.03 e	1.69 ± 0.06 e	N.D.	N.D.	N.D.	N.D.
Fine	1.19 ± 0.11 a	1.08 ± 0.05 bc	N.D.	N.D.	N.D.	N.D.

^aData are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$; N.D.: not determinable.

^bWHC: water-holding capacity; OBC: oil-binding capacity; FC: foaming capacity; FS: foam stability; EA: emulsion activity; ES: emulsion stability.

1.9 Foaming and emulsifying properties of flours

Foaming and emulsifying properties of flour ingredients are mainly related to the protein component (Ma et al., 2011; Stone et al., 2019). The FC values of the pea and lentil flours were 378-404% and 303-350%, respectively (Table 1.5). Generally, the three flour streams of the same pulse showed comparable FC values, which suggested an insignificant impact of particle size on the foaming properties of pulse flours, probably due to the similar levels of protein of the three streams (Table 1.2) (Stone et al., 2019). With respect to FS, a lower value indicated greater foam stability. Pea and lentil flours had FS values of 6.1-11.6% and 7.6-9.5%, respectively (Table 1.5). The fine pea flour exhibited slightly greater FS than the whole and coarse counterparts, indicating lower stability of the foam developed from the former. In contrast, the particle sizes did not show significant influence on the stability of foams generated from the lentil flours.

Within the same pulse group, both EA and ES of the three flour streams did not show a noticeable difference, except for the whole lentil flour, which exhibited a higher ES value than its coarse and fine counterparts. The results suggested that the emulsifying properties of the pulse flours were not distinctly influenced by the particle sizes, which was possibly attributed to the comparable protein contents of the three streams from the same pulse group (Table 1.2) (Stone et al., 2019).

Foaming and emulsifying properties could not be determined for the cereal flours due to the lack of foam and emulsion formation (Table 1.5). The phenomenon could be ascribed to: (1) relatively low protein contents of the cereal flours (11.0-19.8%; Table 1.2); (2) the existence of prolamin as the leading protein component in the cereal flours, which had noticeably poorer solubility than that of albumin and globulin proteins predominantly present in the pea and lentil flours (Stone et al., 2019); and (3) the greater lipid contents of the cereal flours, especially oats, thereby reducing the migration of proteins to the interface (Table 1.2) (Lam, Warkentin, Tyler, & Nickerson, 2017).

Study 2. Milling and differential sieving as an effective approach to diversifying nutritional profiles of pulse and cereal flours

2.1 Microscopic structures of flours

The whole pulse and cereal flours from milling comprised large undivided particles consisting of starch, protein, and dietary fiber (indicated by rectangles), individual starch granules, and fine protein and fiber particles (Figure 2.1). The applied differential sieving effectively separated the particles into two streams based on their sizes: the fine stream of pea, lentil, and barley mainly consisted of individual starch granules and fine protein and fiber

particles, while the coarse counterparts mainly consisted of undivided starch-protein-fiber particles. The SEM observation is in good agreement with the particle-size distributions of the three flour streams as we reported previously (Cheng et al., 2023): the volume-weighted mean particle sizes (D[4,3]) consistently displayed a rank order of coarse > whole > fine for the different crops (Table 2.1). Additionally, distinct differences were observed in the morphologies of the undivided starch-protein-fiber particles among the coarse pulse and cereal flours: (1) Undivided particles of pea and lentil contained fewer starch granules than those of barley and oats; (2) Pea and lentil starch granules were surrounded by dense and continuous protein and fiber matrices (indicated by up arrows in Figure 2.1) in the large particles, while the coarse barley and oat flour particles were more packed with starch granules that were generally not compactly surrounded by protein and fiber. The noted differences in particle morphology among the coarse pulse and cereal flours primarily resulted from the different microscopic structures of their cotyledons (Setia et al., 2019; Shapter et al., 2008). It is also important to note that oat starch granules exist in two forms in the grains: (1) single granules showing approximately 2-15 µm in diameter (indicated by stars in Figure 2.1); (2) clusters consisting of compound granules and having approximately 20-150 µm in diameter (indicated by ovals) (Bechtel, 1981; Falsafi, Maghsoudlou, Rostamabadi, Rostamabadi, Hamed, & Hosseini, 2019). The presence of such clusters in the fine oat flour contributed to its largest D[4,3] among all the four fine samples (Table 2.1).

2.2 Macronutrient compositions of flours

2.2.1 Starch, protein, lipid, and β-glucan contents of flours

Starch contents of the pulse and cereal flours consistently displayed a descending order of fine > whole > coarse within each crop (Table 2.1), corresponding well with the trend as observed by Ahmed, Taher, Mulla, Al-Hazza, and Luciano (2016). The trend is also in good accordance with the existence of different amounts of starch in the three streams as demonstrated in SEM images (Figure 2.1). For the same flour stream, the starch contents of the two pulse flours were significantly lower than those of the two cereal flours (except for the coarse oat flour).

The pulse flours of the different streams exhibited protein contents ranging from 21.4% to 25.1% (dry basis, db), higher than those of the cereal flours ranging from 11.0% to 19.8% (Table 2.1). The protein contents of the three flour streams from pea and lentil were generally comparable, but those from cereals exhibited a trend of coarse > whole > fine, especially for oats.

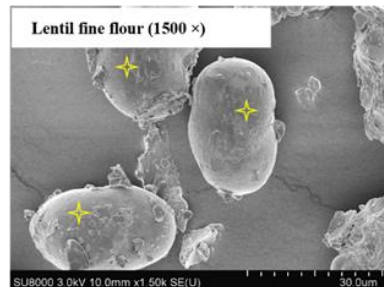
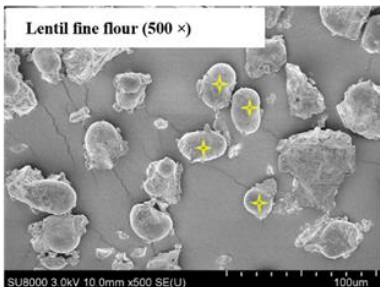
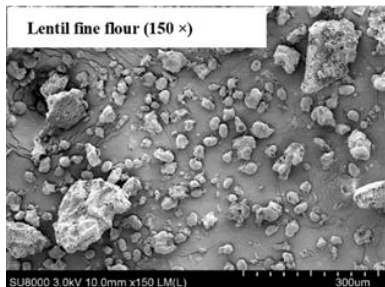
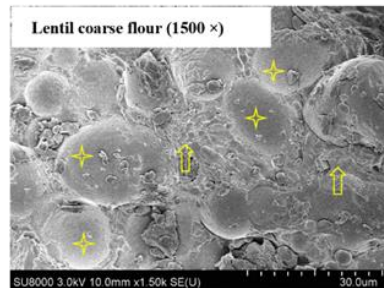
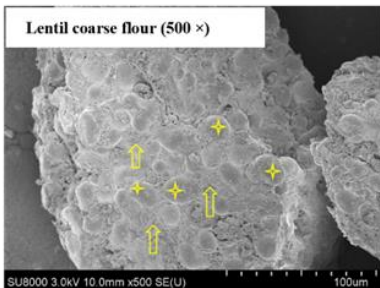
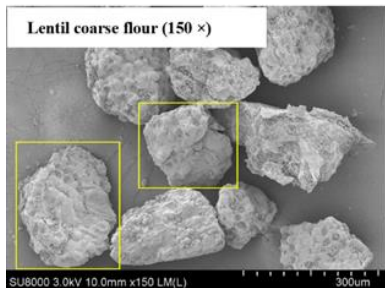
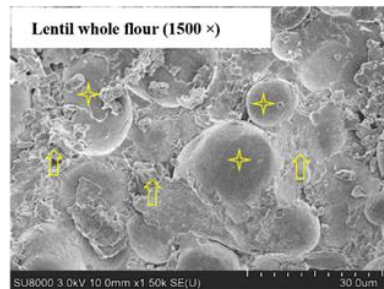
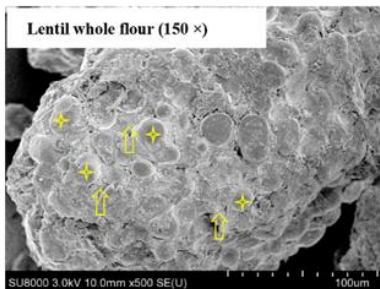
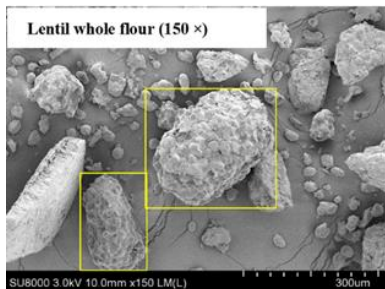
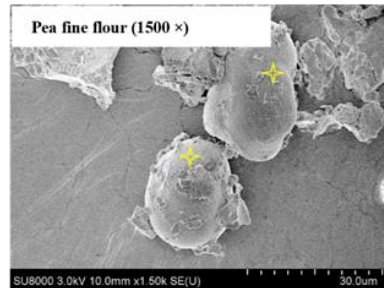
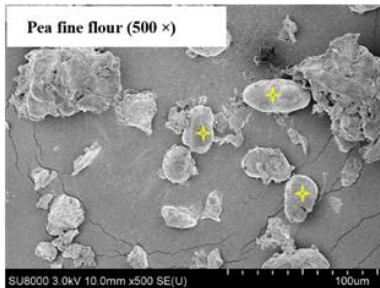
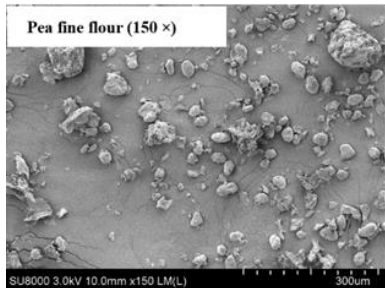
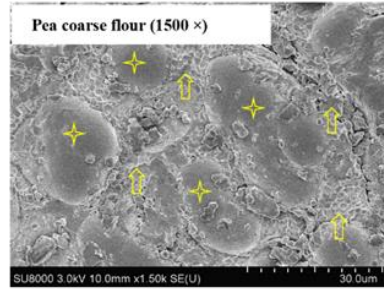
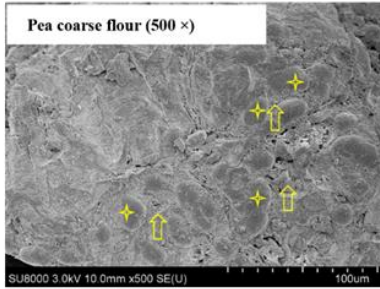
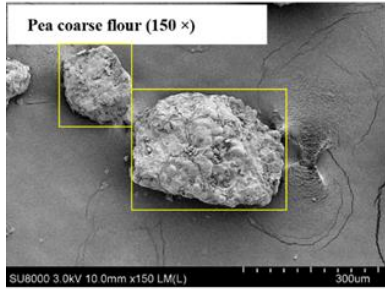
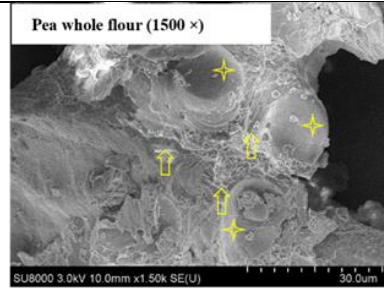
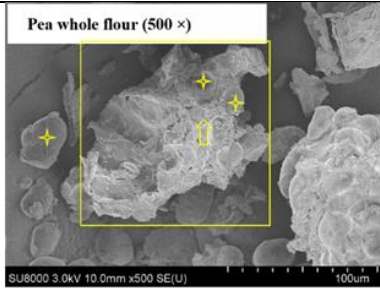
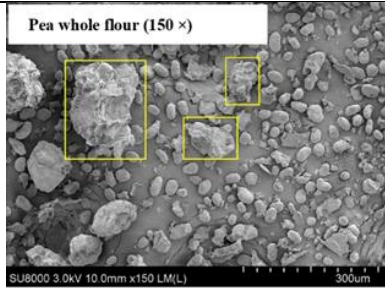
For the same grain, the differences in the lipid contents of the three streams were negligible (Table 2.1). Of the different crops, the lipid levels of the flours were in a descending order of oats > barley > pea > lentil. The noticeably greater lipid contents of oat samples correspond well with the data reported in previous literature (Liu, Bailey, & White, 2010; Sharma & Gujral, 2010; Stone et al., 2019).

The pea and lentil flours contained < 0.1% of β-glucan, indicating the absence of this type of dietary fiber in pulse grains (Table 2.1). The whole barley and oat flours consisted of 5.08% and 5.56% of β-glucan, which is in good accordance with previous work reporting that both crops are a good source of this dietary fiber (Lin et al., 2018; Tang, Wang, Cheng, Wu, & Ouyang, 2019). Sieving effectively concentrated β-glucan in the coarse flour streams of barley and oats. β-Glucan is mainly distributed in the bran of barley and oat seeds (Wood, 1994; Zheng, Li, & Wang, 2011). After milling and differential sieving, the bran tissues tended to stay in the coarse fraction as illustrated in Figure 2.1. The β-glucan level of the coarse oat stream (10.99%, db) was almost twofold that of the whole counterpart (5.56%), suggesting that the former could be a promising source of this nutritionally important dietary fiber.

2.2.2 Dietary fiber profiles of flours

Among the different grains, total dietary fiber contents of the three flour streams exhibited a consistent order of coarse > whole > fine (Table 2.2), in good agreement with the data reported by Ahmed et al. (2016); for the same stream, the pea and lentil flours contained more total dietary fiber than the barley and oat counterparts, in good accordance with the findings reported by Chen, McGee, Vandemark, Brick, and Thompson (2016) and Rainakari, Rita, Putkonen, and Pastell (2016). Accounting for the majority (47.1-73.2%) of the total dietary fiber of all the flour samples, the levels of insoluble dietary fiber also fit into the trend of coarse > whole > fine for each crop. In general, the coarse flours contained more high-molecular-weight soluble dietary fiber than the respective whole and fine counterparts, except for barley samples. The highest content of this category of dietary fiber in the coarse oat flour (8.9%, db) could be partly associated with its greatest level of β-glucan (10.99%) as presented in Table 1. The contents of low-molecular-weight soluble dietary fiber did not vary significantly among the three flour streams within the same grain. The pulse flours consisted of more low-molecular-weight soluble dietary fiber than the cereal flours, which

could be linked to the existence of more raffinose family oligosaccharides (*i.e.*, raffinose, stachyose, and verbascose) in the former group of samples (Kleintop, Echeverria, Brick, Thompson, & Brick, 2013). Because of the complex nature of dietary fibers in different plants, it will be meaningful to employ a multi-glycomic approach to characterize the structural aspects of the different categories of dietary fibers in the different flour streams as demonstrated in a recent publication (Couture et al., 2022).



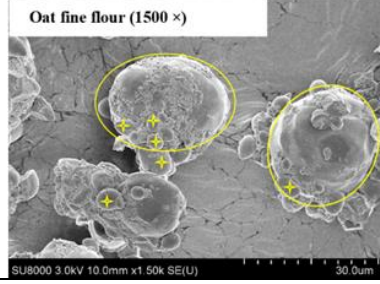
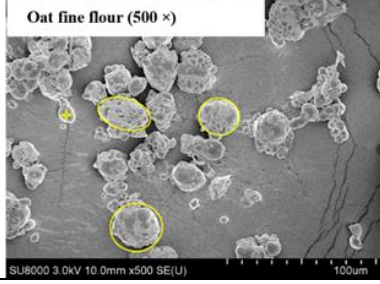
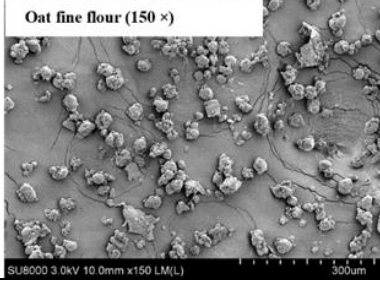
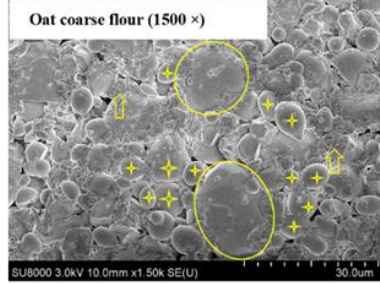
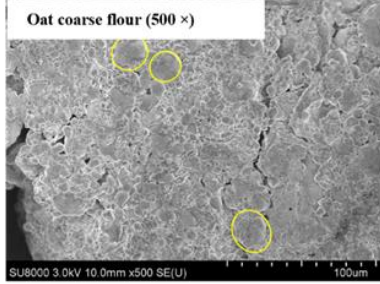
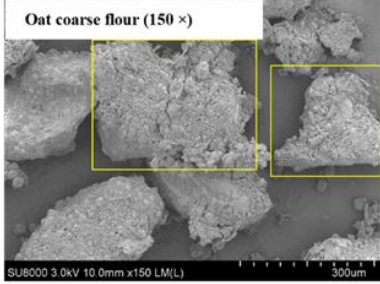
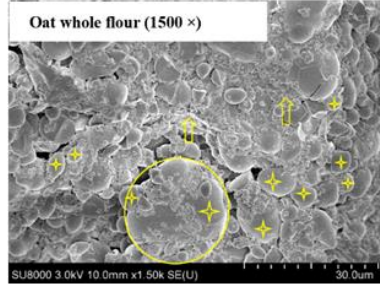
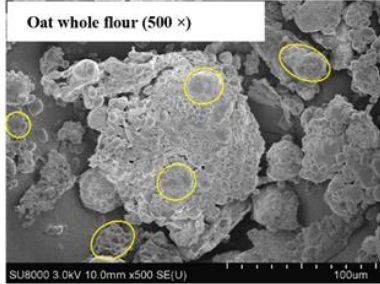
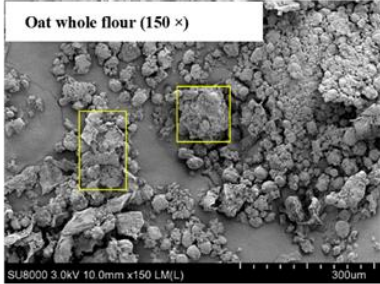
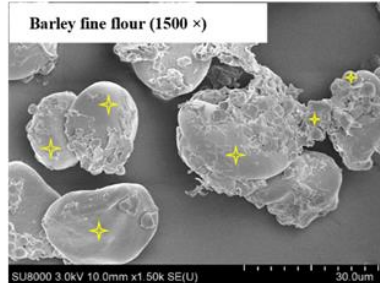
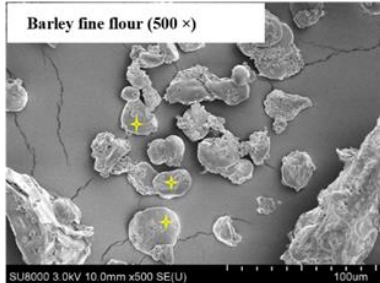
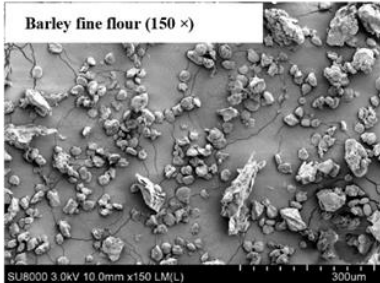
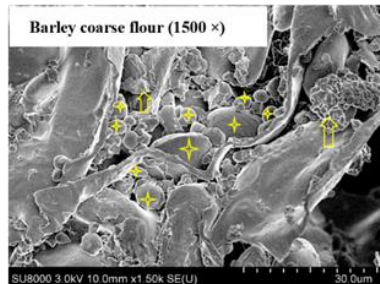
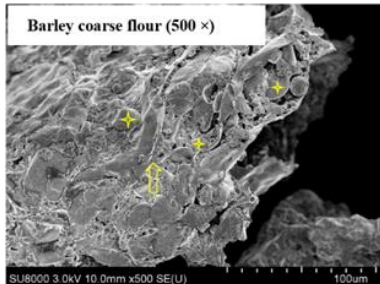
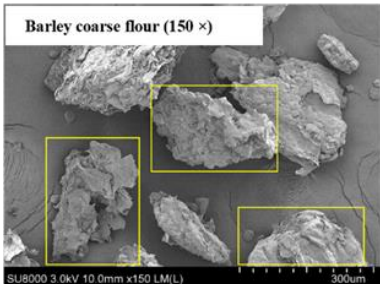
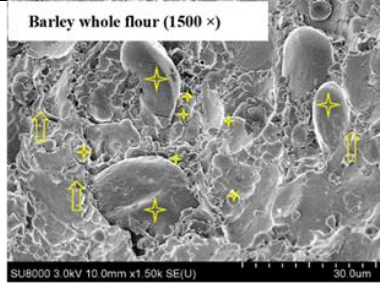
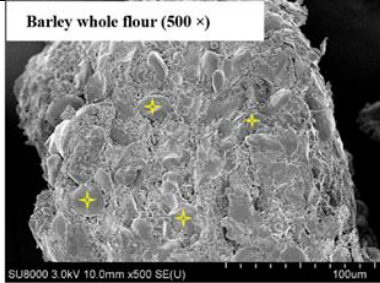
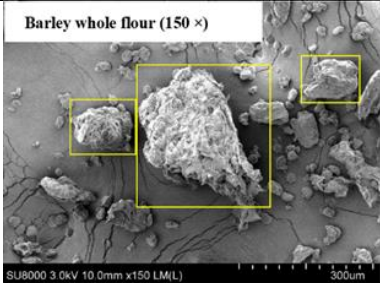


Figure 2.1. Scanning electron microscopy (SEM) images of pea, lentil, barley, and oat flours from three different streams. Magnification at which the image was captured is shown in parentheses. Rectangles mark undivided particles consisting of starch, protein, and fiber; up arrows mark protein and fiber matrices; stars mark starch granules; and ovals mark clusters of compound granules in oat flours.

Table 2.1. Volume-weighted mean particle sizes (D[4,3]) and macronutrient contents of pea, lentil, barley, and oat flours from three different streams on a dry flour basis. ^a

Sample	D[4,3] (µm) ^b	Starch (%)	Protein (%)	Lipid (%)	β-glucan (%)
Pea					
Whole	215.6 ± 18.3 b	49.8 ± 1.3 c	21.4 ± 1.4 f	1.36 ± 0.10 b	0.06 ± 0.01 a
Coarse	408.2 ± 39.9 de	40.1 ± 0.6 a	21.8 ± 0.3 fg	1.25 ± 0.05 b	0.07 ± 0.01 a
Fine	68.2 ± 3.1 a	53.1 ± 0.5 d	22.7 ± 0.4 g	1.36 ± 0.09 b	0.06 ± 0.01 a
Lentil					
Whole	248.2 ± 21.7 b	49.5 ± 0.8 c	24.5 ± 0.2 h	0.66 ± 0.03 a	0.04 ± 0.01 a
Coarse	324.0 ± 19.7 c	43.5 ± 1.2 b	24.7 ± 0.1 h	0.60 ± 0.07 a	0.04 ± 0.01 a
Fine	81.2 ± 0.5 a	51.5 ± 1.0 cd	25.1 ± 0.4 h	0.76 ± 0.02 a	0.04 ± 0.01 a
Barley					
Whole	450.8 ± 29.8 e	62.2 ± 0.7 f	11.4 ± 0.2 ab	2.36 ± 0.07 d	5.08 ± 0.08 c
Coarse	578.3 ± 16.7 f	57.7 ± 0.3 e	12.1 ± 0.2 b	2.32 ± 0.06 cd	6.25 ± 0.10 e
Fine	102.0 ± 2.7 a	73.3 ± 1.4 h	11.0 ± 0.2 a	1.91 ± 0.10 c	2.31 ± 0.11 b
Oats					
Whole	377.2 ± 18.2 d	59.9 ± 1.4 ef	15.5 ± 0.2 d	7.66 ± 0.17 e	5.56 ± 0.22 d
Coarse	635.6 ± 23.2 g	42.8 ± 1.0 b	19.8 ± 0.1 e	8.37 ± 0.41 f	10.99 ± 0.51 f
Fine	113.4 ± 7.0 a	67.0 ± 0.9 g	13.7 ± 0.3 c	7.94 ± 0.33 e	2.29 ± 0.34 b

^a Data are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at *p* < 0.05.

^b Adapted from Cheng et al. (2023).

Table 2.2. Dietary fiber compositions of pea, lentil, barley, and oat flours from three different streams. ^a

Sample	Insoluble dietary fiber (%)	High-molecular-weight soluble dietary fiber (%)	Low-molecular-weight soluble dietary fiber (%)	Total dietary fiber (%)
Pea				
Whole	19.4 ± 2.1 d	4.3 ± 0.0 abcd	4.1 ± 0.3 cde	27.7 ± 2.3 e
Coarse	26.7 ± 1.5 e	6.0 ± 0.5 de	3.7 ± 0.3 cd	36.5 ± 2.3 f
Fine	8.7 ± 0.0 b	3.9 ± 0.3 abc	4.1 ± 0.1 cde	16.7 ± 0.4 bc
Lentil				
Whole	14.7 ± 0.2 c	3.3 ± 0.4 ab	4.9 ± 0.0 de	23.0 ± 0.2 d
Coarse	18.1 ± 0.4 d	5.0 ± 0.5 bcde	4.7 ± 0.1 de	27.8 ± 0.0 e
Fine	10.1 ± 0.5 b	3.5 ± 0.2 ab	5.4 ± 0.2 e	19.1 ± 0.9 bcd
Barley				

Whole	9.6 ± 0.4 b	6.7 ± 1.1 e	2.8 ± 0.9 bc	19.1 ± 0.6 bcd
Coarse	11.4 ± 1.0 bc	6.8 ± 0.3 e	2.8 ± 0.6 bc	21.0 ± 0.6 cd
Fine	4.9 ± 0.1 a	3.8 ± 0.5 abc	1.7 ± 0.2 ab	10.4 ± 0.2 a
Oats				
Whole	8.2 ± 0.1 ab	5.6 ± 0.2 cde	1.3 ± 0.1 a	15.1 ± 0.3 b
Coarse	11.2 ± 0.1 b	8.9 ± 0.2 f	1.0 ± 0.2 a	21.2 ± 0.1 d
Fine	4.9 ± 0.8 a	3.0 ± 0.4 a	0.9 ± 0.2 a	8.8 ± 1.3 a

^a Data are presented as average ± standard deviation (n = 2); in the same column, data with the same letter are not significantly different at $p < 0.05$.

2.3 *In vitro* starch digestibility of cooked flours

As shown in Table 2.3, the cooked pulse flours contained 33.1-49.6% RDS, 1.6-3.0% SDS, and 1.9-6.2% RS (db), respectively. To better compare the enzymatic resistance of the starches in the samples, the data were converted to values on a dry starch basis (dsb). After the conversion, the RDS, SDS, and RS contents of the cooked pulse flours were 80.1-93.4%, 3.0-7.5%, and 3.6-14.3% (dsb), respectively. For the three flours of the same pulse, the RDS contents of the flours decreased as the particle size increased (fine > whole > coarse; dsb). In addition, the fine flour showed noticeably less RS than its whole and coarse counterparts. The results indicated that the starches in the fine pulse flours possessed lower resistance against enzymatic hydrolysis than those in the whole and coarse streams, corresponding well the findings of previous studies (Farooq et al., 2018; Kathirvel et al., 2019; Ren, Setia, Warkentin, & Ai, 2021). The considerably higher enzymatic resistance of starches in the whole and coarse pulse flours was attributed to: (1) higher dietary fiber contents of the whole and coarse flours (Table 2.2), which formed a compact and continuous matrix structure along with protein to provide a physical barrier surrounding starch granules/molecules (indicated by up arrows in Figure 2.1) to reduce the susceptibility to amylolysis (Dhital et al., 2016; Ren et al., 2021); however, such a matrix structure was generally absent in the fine flours; and (2) smaller relative surface area of the whole and coarse flour particles, resulting in reduced extent of water diffusion and enzymatic susceptibility of the contained starches (Farooq et al., 2018).

The RDS, SDS, and RS contents of the cooked cereal flours were 40.2-68.0%, 0.7-1.6%, and 1.1-4.6% (db), respectively (Table 2.3). After the same conversion as described above, the cereal flours consisted of 91.0-93.9% RDS, 1.2-3.6% SDS, and 2.5-7.0% RS (dsb), respectively. The RDS, SDS, and RS contents (dsb) of the barley flours and the RDS contents (dsb) of the oat flours did not differ significantly among the three streams, and the three different particle sizes did not show a clear effect on the SDS and RS contents of the oat flours. Overall, the results in Table 2.3 suggested that the differential sieving did not lead to the same rank order of fine < whole < coarse with respect to the enzymatic resistance of starch in the cooked cereal flours as emphasized for the cooked pulse flours. The discrepancy could be linked to the different microscopic structures of the flour particles from the two types of crops: Figure 2.1 revealed that starch granules in the coarse and whole barley and oat flours were not entrapped in compact and continuous protein and fiber matrix as those in the pea and lentil counterparts. Despite the significantly higher levels of dietary fiber of the coarse and whole barley and oat flours (Table 2.2), the starch granules in these two streams tended to swell to a similar degree as those in the fine barley and oat flours during cooking (Cheng et al., 2023). Therefore, the starch granules/molecules in the three streams of cereal flours in general exhibited comparable *in vitro* digestibility in this study.

A comparison of the pulse and cereal flours revealed that the whole and coarse pea and lentil flours possessed markedly less RDS but more RS than the cereal counterparts (db and dsb; Table 2.3), which could be attributable to the lower levels of starch (Table 2.1) and the entrapment of starch granules in protein and fiber matrices (Figure 2.1) in the whole and coarse pulse flours as discussed above. In respect to carbohydrate nutritional value, whole and coarse pea and lentil flours are more promising ingredients because of their higher levels of dietary fiber (Table 2.2) and lower starch digestibility than the other flour streams (Table 2.3).

Table 2.3. *In vitro* starch digestibility of cooked pea, lentil, barley, and oat flours from three different streams. ^{a, b, c, d}

Sample	RDS (%)		SDS (%)		RS (%)	
	Dry basis	Dry starch basis	Dry basis	Dry starch basis	Dry basis	Dry starch basis
Pea						
Whole	42.7 ± 1.6 c	85.6 ± 1.2 bc	1.9 ± 0.4 ab	3.8 ± 1.0 bcd	5.3 ± 0.3 de	10.6 ± 0.8 e
Coarse	33.1 ± 0.2 a	82.5 ± 0.4 ab	3.0 ± 0.2 c	7.5 ± 0.4 e	4.0 ± 0.3 cd	10.0 ± 0.8 de
Fine	49.6 ± 0.6 e	93.4 ± 1.4 e	1.6 ± 0.3 ab	3.0 ± 0.5 abc	1.9 ± 0.5 ab	3.6 ± 0.9 ab
Lentil						
Whole	42.5 ± 0.8 c	85.7 ± 1.0 c	1.6 ± 0.4 ab	3.2 ± 0.9 abc	5.5 ± 0.1 de	11.1 ± 0.1 e
Coarse	34.8 ± 0.3 a	80.1 ± 2.2 a	2.4 ± 0.6 bc	5.6 ± 1.3 de	6.2 ± 1.3 e	14.3 ± 2.6 f
Fine	46.4 ± 0.4 d	90.1 ± 0.7 d	2.2 ± 0.3 bc	4.3 ± 0.6 cd	2.9 ± 0.3 bc	5.6 ± 0.6 bc
Barley						
Whole	58.3 ± 0.8 g	93.8 ± 1.1 e	1.0 ± 0.5 a	1.7 ± 0.8 ab	2.8 ± 0.5 abc	4.5 ± 0.8 abc
Coarse	54.1 ± 0.3 f	93.8 ± 0.5 e	0.7 ± 0.2 a	1.3 ± 0.4 a	2.8 ± 0.2 abc	4.9 ± 0.3 abc
Fine	68.0 ± 1.1 i	92.8 ± 0.9 de	0.9 ± 0.6 a	1.2 ± 0.8 a	4.4 ± 1.0 cde	6.0 ± 1.2 bc
Oats						
Whole	55.0 ± 0.2 f	91.8 ± 1.8 de	0.7 ± 0.6 a	1.2 ± 1.0 a	4.2 ± 1.4 cd	7.0 ± 2.2 cd
Coarse	40.2 ± 0.3 b	93.9 ± 1.6 e	1.6 ± 0.6 ab	3.6 ± 1.3 bcd	1.1 ± 0.2 a	2.5 ± 0.4 a
Fine	61.0 ± 0.8 h	91.0 ± 1.2 de	1.4 ± 0.5 ab	2.1 ± 0.8 ab	4.6 ± 1.0 cde	6.9 ± 1.5 c

^a Data are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$.

^b Flours were cooked in a boiling water bath for 10 min.

^c RDS: rapidly digestible starch, SDS: slowly digestible starch, and RS: resistant starch; values were calculated on a dry basis.

^d %RDS, SDS or RS content of flour, dry starch basis = (%RDS, SDS or RS content of flour, dry basis) / (%Starch content of flour, dry basis) × 100%.

2.4 *In vitro* protein digestibility and quality of cooked flours

Differential sieving did not lead to significant variations in the *in vitro* protein digestibility (IVPD) of the three flour streams of the crops, except for the barley samples (Table 2.4), in which the fine barley flour exhibited a significantly higher IVPD value than the coarse counterpart (77.1% versus 73.0%). Mandalari et al. (2018) also observed that protein bioaccessibility of roller-milled wheat flours increased as the particle sizes decreased. The fine barley flour with a smaller particle size (Table 2.1) had greater relative surface area for enhanced efficiency of proteolysis, resulting in a higher extent of protein digestion (Mandalari et al., 2018). By contrast, the similar IVPD of the three flour streams of pea, lentil, and oats agrees well with the data reported by Nguyen et al. (2015) and Tinus, Damour, Van Riel, and Sopade (2012). The authors found that varying particle sizes did not noticeably influence the protein digestibility of hammer-milled flours. Further research is required to explain the discrepancy in the impact of particle size on the protein digestion of flours from different botanical sources.

In terms of amino acid composition (as-is basis), the three flour streams from pea, lentil, and barley showed largely consistent amino acid profiles; however, the amino acid contents of oat flours basically followed the order of coarse > whole > fine (Table S2.1). The results of oat flours correspond well with their protein contents (Table 2.1).

Overall, the results suggested that the effect of milling and sieving on the amino acid compositions of the flours was mainly negligible (except for oat flours), which could be attributed to the similar protein contents of the three flour streams from the same crop (Table 2.1). The essential amino acid compositions of the pulse and cereal flours are shown in Table 5, along with that of the FAO reference for children from 2 to 5 years of age. Within the same grain, the essential amino acid contents were largely comparable among the three flour streams. In general, the contents of threonine and methionine + cysteine (sulphur-containing amino acids) of the pulse flours were lower than the FAO reference levels, consistent with previous findings (Laing et al., 2023; Liu, Ren, Yin, Nickerson, Pickard, & Ai, 2022). With respect to the cereal flours, the contents of threonine and lysine were lower than those of the reference. Proteins in most cereal crops are known to be deficient in lysine for human nutrition (Bai, Nosworthy, House, & Nickerson, 2018). When compared the same stream of the pulse and cereal flours, the main differences were observed in the contents of methionine + cysteine (pulses < cereals), lysine (pulses > cereals), and tryptophan (pulses < cereals), which is in good agreement with the data reported by Stone et al. (2019).

The limiting amino acids and calculated amino acid scores of the pulse and cereal flours are summarized in Table 2.4. The pulse flours were mostly limited in threonine or methionine + cysteine (with the exception of leucine for coarse pea stream), and thus they exhibited limiting amino acid scores of 0.65-0.96 (Table 2.4). The comparatively higher limiting amino acid scores of the coarse and fine pea streams (0.96 and 0.93, respectively) indicated their more balanced essential amino acid profiles. All the cereal flours had lysine as the limiting amino acid and exhibited limiting amino acid scores of 0.54-0.65, generally lower than those of the pulse flours. After factoring in IVPD, IV-PDCAAS of the flours were obtained (Table 2.4), which is a good indicator of protein nutritional quality of flour ingredients (Nosworthy et al., 2017). In pea and lentil samples, the coarse streams possessed significantly higher IV-PDCAAS than the corresponding whole and fine counterparts; in barley and oat samples, the fine streams possessed significantly lower IV-PDCAAS than the corresponding whole and coarse counterparts. The noted variations mainly resulted from the differences in their limiting amino acid scores rather than IVPD.

IVPD, limiting amino acid scores, and IV-PDCAAS of the pulse flours were in general higher than those of the cereal flours, regardless of the streams. Previous research has illustrated that the existence of multiple anti-nutritional factors (*e.g.*, proteolytic inhibitors, tannins, and phytic acid) inhibited protein digestion in raw pulse flours (Nosworthy et al., 2017; Oomah, Caspar, Malcolmson, & Bellido, 2011). However, the cooking step applied before the IVPD assay as described in Methodology Section 2.6.1 could inactivate these anti-nutritional factors, which could partly explain the higher IVPD of pulse flours than cereal flours in this research (Nosworthy et al., 2017). Our data suggested that pea and lentil flours could be a better source of plant proteins than barley and oat flours with respect to both the quantity (Table 2.1) and quality (Tables 2.4 and 2.5).

Table 2.4. *In vitro* protein digestibility, limiting amino acid scores, and *in vitro* protein digestibility corrected amino acid scores of pea, lentil, barley, and oat flours from three different streams. ^{a, b, c, d}

Sample	IVPD (% protein basis) ^{b, c}	Limiting amino acid	Limiting amino acid score	IV-PDCAAS (%) ^{b, d}
Pea				
Whole	82.6 ± 0.8 e	Threonine	0.89	73.7 ± 0.7 f
Coarse	81.8 ± 0.5 e	Leucine	0.96	78.9 ± 0.5 h
Fine	83.1 ± 0.6 e	Threonine	0.93	77.0 ± 0.6 g
Lentil				
Whole	81.0 ± 0.2 de	Methionine + cysteine	0.67	54.1 ± 0.2 d
Coarse	80.6 ± 0.7 cde	Threonine	0.78	62.7 ± 0.5 e
Fine	82.3 ± 1.2 e	Methionine + cysteine	0.65	53.3 ± 0.8 d
Barley				
Whole	75.8 ± 0.7 ab	Lysine	0.60	45.7 ± 0.4 b
Coarse	73.0 ± 2.6 a	Lysine	0.60	43.9 ± 1.5 b

Fine	77.1 ± 1.2 b	Lysine	0.54	41.8 ± 0.6 a
Oats				
Whole	78.0 ± 1.1 bc	Lysine	0.65	51.1 ± 0.7 c
Coarse	78.7 ± 1.5 bcd	Lysine	0.63	49.7 ± 1.0 c
Fine	77.2 ± 1.0 b	Lysine	0.59	45.3 ± 0.6 b

^a Flours were cooked in a boiling water bath for 10 min.

^b Data are presented as average ± standard deviation (n = 4); in the same column, data with the same letter are not significantly different at $p < 0.05$.

^c IVPD: *in vitro* protein digestibility; IV-PDCAAS: *in vitro* protein digestibility corrected amino acid score.

^d IV-PDCAAS (%) = IVPD (%) × limiting amino acid score.

Table S2.1. Amino acid compositions of pea, lentil, barley, and oat flours from three different streams.^a

Sample	Amino acid (g/100 g of flour, as-is basis)																	
	ASP	GLU	SER	GLY	HIS	ARG	THR	ALA	PRO	TYR	VAL	MET	CYS	ILE	LEU	PHE	LYS	TRP
Pea																		
Whole flour	1.94	3.01	0.72	0.96	0.50	1.65	0.60	0.83	0.82	0.63	0.98	0.19	0.28	0.90	1.43	1.04	1.29	0.31
Coarse flour	2.04	3.27	0.78	0.93	0.57	1.57	0.67	0.83	0.87	0.65	1.00	0.20	0.30	0.98	1.27	1.16	1.29	0.31
Fine flour	2.12	3.27	0.79	1.04	0.56	1.75	0.65	0.88	0.86	0.62	1.04	0.26	0.31	0.95	1.52	1.12	1.38	0.31
Lentil																		
Whole flour	2.29	3.42	0.90	1.03	0.60	1.79	0.68	0.93	1.00	0.67	1.14	0.22	0.15	1.05	1.69	1.14	1.44	0.24
Coarse flour	1.99	3.36	0.74	0.94	0.52	1.81	0.60	0.94	0.89	0.69	1.07	0.22	0.24	0.92	1.39	1.29	1.12	0.23
Fine flour	2.30	3.43	0.90	1.01	0.60	1.83	0.69	0.92	0.99	0.70	1.13	0.20	0.17	1.04	1.67	1.31	1.38	0.28
Barley																		
Whole flour	0.54	2.23	0.36	0.44	0.28	0.51	0.30	0.24	1.18	0.30	0.51	0.20	0.10	0.42	0.71	0.59	0.35	0.17
Coarse flour	0.63	2.33	0.38	0.53	0.28	0.60	0.32	0.44	1.18	0.35	0.57	0.18	0.09	0.43	0.78	0.64	0.37	0.21
Fine flour	0.51	2.28	0.32	0.43	0.24	0.49	0.27	0.37	1.13	0.30	0.54	0.19	0.15	0.38	0.70	0.57	0.30	0.18
Oats																		
Whole flour	1.11	3.07	0.58	0.82	0.37	1.04	0.43	0.68	0.97	0.41	0.80	0.36	0.17	0.60	1.13	0.85	0.54	0.31
Coarse flour	1.22	3.90	0.71	1.05	0.48	1.27	0.55	0.84	1.09	0.73	1.03	0.31	0.18	0.72	1.41	1.05	0.68	0.33
Fine flour	0.76	1.12	0.41	0.69	0.32	0.64	0.36	0.51	1.03	0.41	0.52	0.21	0.17	0.46	0.69	0.86	0.43	0.20

^aASP: aspartic acid; THR: threonine; SER: serine; GLU: glutamic acid; PRO: proline; GLY: glycine; ALA: alanine; CYS: cysteine; VAL: valine; MET: methionine; ILE: isoleucine; LEU: leucine; TYR: tyrosine; PHE: phenylalanine; HIS: histidine; LYS: lysine; ARG: arginine; and TRP: tryptophan.

Table 2.5. Essential amino acid compositions and scores of pea, lentil, barley, and oat flours from three different streams.^a

Sample	Essential amino acid ^b								
	THR	VAL	MET + CYS	ILE	LEU	PHE + TYR	HIS	LYS	TRP
Content (mg/g protein)									
Pea									
Whole	30 ± 1 a	49 ± 1 ab	24 ± 4 ab	45 ± 1 ab	72 ± 2 ab	84 ± 3 a	25 ± 1 a	65 ± 0 d	16 ± 1 abcd
Coarse	33 ± 2 a	50 ± 0 ab	25 ± 8 ab	49 ± 5 a	64 ± 12 ab	91 ± 9 a	29 ± 4 a	64 ± 4 d	15 ± 1 abcd
Fine	32 ± 5 a	51 ± 3 ab	28 ± 5 ab	46 ± 3 ab	74 ± 6 ab	85 ± 13 a	27 ± 2 a	67 ± 6 d	15 ± 1 abcd

Lentil									
Whole	30 ± 3 a	51 ± 2 ab	17 ± 3 a	47 ± 2 ab	75 ± 4 ab	81 ± 1 a	27 ± 2 a	64 ± 3 d	11 ± 2 ab
Coarse	26 ± 0 a	47 ± 0 ab	20 ± 3 ab	40 ± 4 ab	61 ± 10 ab	87 ± 1 a	23 ± 4 a	49 ± 7 bc	10 ± 0 a
Fine	30 ± 0 a	49 ± 2 ab	16 ± 1 a	45 ± 1 ab	72 ± 2 ab	87 ± 3 a	26 ± 0 a	60 ± 1 cd	12 ± 0 abc
Barley									
Whole	29 ± 1 a	51 ± 2 ab	29 ± 7 ab	42 ± 4 ab	70 ± 2 ab	88 ± 5 a	27 ± 3 a	35 ± 0 a	17 ± 4 abcd
Coarse	30 ± 0 a	53 ± 1 b	26 ± 3 ab	40 ± 1 ab	73 ± 1 ab	93 ± 1 a	26 ± 0 a	35 ± 1 a	19 ± 1 cd
Fine	28 ± 2 a	56 ± 7 b	36 ± 4 b	40 ± 2 ab	73 ± 3 ab	91 ± 9 a	25 ± 2 a	31 ± 1 a	19 ± 2 cd
Oats									
Whole	30 ± 1 a	57 ± 1 b	37 ± 1 b	42 ± 1 ab	80 ± 1 b	89 ± 16 a	26 ± 1 a	38 ± 0 ab	22 ± 2 d
Coarse	30 ± 0 a	56 ± 0 b	27 ± 2 ab	39 ± 3 ab	76 ± 4 ab	96 ± 2 a	26 ± 0 a	37 ± 1 a	18 ± 0 bcd
Fine	29 ± 4 a	42 ± 3 a	30 ± 6 ab	36 ± 1 a	55 ± 1 a	101 ± 18 a	25 ± 2 a	34 ± 1 a	16 ± 3 abcd
FAO reference	34	35	25	28	66	63	19	58	11
Essential amino acid score^c									
Pea									
Whole	0.89*	1.41	0.97	1.62	1.09	1.33	1.34	1.12	1.43
Coarse	0.98	1.43	1.01	1.75	0.96*	1.44	1.51	1.11	1.35
Fine	0.93*	1.44	1.10	1.65	1.12	1.34	1.44	1.16	1.35
Lentil									
Whole	0.89	1.46	0.67*	1.67	1.14	1.28	1.42	1.11	0.98
Coarse	0.78*	1.34	0.79	1.43	0.93	1.38	1.21	0.85	0.92
Fine	0.88	1.41	0.65*	1.61	1.10	1.38	1.37	1.04	1.10
Barley									
Whole	0.86	1.45	1.15	1.49	1.06	1.40	1.44	0.60*	1.55
Coarse	0.89	1.53	1.02	1.42	1.11	1.48	1.38	0.60*	1.76
Fine	0.84	1.60	1.45	1.41	1.10	1.44	1.34	0.54*	1.73
Oats									
Whole	0.89	1.62	1.50	1.52	1.21	1.41	1.37	0.65*	1.97
Coarse	0.88	1.59	1.06	1.40	1.15	1.53	1.37	0.63*	1.64
Fine	0.85	1.19	1.21	1.30	0.84	1.60	1.34	0.59*	1.42

^a THR: threonine; VAL: valine; MET: methionine; CYS: cysteine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TYR: tyrosine; HIS: histidine; LYS: lysine; and TRP: tryptophan.

^b Essential amino acid data are presented as average ± standard deviation (n = 2); in the same column, data with the same letter are not significantly different at $p < 0.05$.

^c Essential amino acid score = (Essential amino acid content of the target protein) / (FAO reference value).

*Limiting amino acid.

Study 3. The impact of particle size in cereal and pulse flour on PPG and appetite in healthy adults

3.1 Participant characteristics

A total of 20 participants (10 males, 10 females) completed the study and were included in the data analysis for each experiment. Participants had an average age of 25.7yrs and BMI of 23.2kg/m². All participants had fasting BG less than 5.5mmol/L. Sex and BMI did not have an impact on any of the study variable except for a sex difference in appetite in experiment 1 (Table 3.4).

Table 3.4 Baseline participant characteristics¹

	Sex	N	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m ²) ³	WC (cm) ⁴	BG (mmol/L) ⁵
Exp. 1²	Male	10	25.0 ± 1.8	171.5 ± 2.6	69.2 ± 2.2	23.3 ± 0.6	84.9 ± 2.1	5.3 ± 0.04
	Female	10	25.5 ± 1.1	163.7 ± 2.1	61.3 ± 3.1	23.1 ± 1.0	78.4 ± 2.9	5.2 ± 0.08
	Combined	20	25.3 ± 1.0	167.6 ± 1.8	65.2 ± 2.1	23.2 ± 0.6	81.6 ± 1.9	5.3 ± 0.04
Exp. 2	Male	10	27.5 ± 2.6	176.3 ± 2.0	74.2 ± 2.9	23.8 ± 0.8	86.5 ± 2.8	5.3 ± 0.04
	Female	10	24.7 ± 0.8	162.6 ± 2.6	60.9 ± 3.5	22.9 ± 1.0	76.3 ± 2.3	5.1 ± 0.10
	Combined	20	26.1 ± 1.4	169.5 ± 2.2	67.5 ± 2.7	23.4 ± 0.6	81.4 ± 2.1	5.2 ± 0.06
Exp. 3	Male	10	26.7 ± 2.4	176.5 ± 1.2	74.7 ± 1.5	23.8 ± 0.6	88.5 ± 1.2	5.2 ± 0.09
	Female	10	24.4 ± 1.0	161.2 ± 2.0	58.9 ± 3.7	22.6 ± 1.0	72.3 ± 2.6	5.0 ± 0.1
	Combined	20	25.6 ± 1.3	168.8 ± 2.1	66.8 ± 2.6	23.2 ± 0.6	80.4 ± 2.3	5.1 ± 0.07

¹ Values are means ± SEM.

² Exp.1, experiment 1; Exp.2, experiment 2; Exp.3, experiment 3

³ BMI, body mass index

⁴ WC, waist circumference

⁵ BG, BG

3.2 PPG

In experiment 1, there were significant treatment ($p < 0.0001$), time ($p < 0.0001$), and treatment-by-time interaction ($p < 0.02$) effects on BG concentration over 120min. BG peaked at 30min for all treatments, with COF (7.50 ± 0.18 mmol/L; $p < 0.001$) resulting in a significantly lower peak than FOF (8.24 ± 0.21 mmol/L). At 45min, BG was lower after WOF and COF than FOF ($p < 0.02$), and after COF than COMF ($p < 0.04$). WOF and COF continued to result in lower BG than FOF and COMF at 60min ($p < 0.007$). No significant differences were seen at 0, 15, 90, and 120min when BG was near baseline levels (Figure 3.3). Mean BG was lower after COF (6.05 ± 0.09 mmol/L) than FOF (6.40 ± 0.12 mmol/L; $p < 0.0001$) and COMF (6.28 ± 0.11 mmol/L; $p < 0.02$), and after WOF (6.11 ± 0.11 mmol/L) than FOF ($p = 0.001$; Table 3.4). BG iAUC was lower after COF than FOF and COMF ($p < 0.03$), and after WOF than FOF ($p < 0.002$; Figure 3.4). Although no treatment-by-time interaction effects were found, mean insulin was lower after COF (19.63 ± 2.6 µU/mL) than FOF (30.43 ± 4.7 µU/mL; $p < 0.005$; Table 3.5, Figure 3.5). Insulin iAUC was lower after COF and WOF in comparison to FOF and COMF (Figure 3.6).

In experiment 2, significant treatment and time effects ($p < 0.0001$) were found for BG over 140min but there were no treatment-by-time interactions (Figure 3.3). FPF (6.23 ± 0.13 mmol/L) resulted in higher mean BG ($p < 0.04$) and higher BG iAUC ($p < 0.002$) than all other crackers which were not statistically different from one another. No differences in post-meal BG were seen (Table 3.4, Figure 3.4). No treatment-by-time effects were found for insulin but premeal insulin was significantly higher after FPF (27.18 ± 2.8 µU/mL) than CPF (22.87 ± 2.7 µU/mL) and WF (22.78 ± 2.8 µU/mL; $p < 0.007$; Table 3.5, Figure 3.5). FPF also led to higher insulin iAUC than WF ($p < 0.01$; Figure 3.6). Only a difference in post-meal BG was found in experiment 3 where concentration was lower after CLF than WLF ($p < 0.05$; Table 3.4).

Our findings show that when consumed in porridge and crackers, coarse and whole cereal and pulse flours resulted in lower PPG coupled with lower insulin responses, suggesting that the larger particle sized flours were able to attenuate BG without a disproportionate increase in insulin levels, thus aligning with Health Canada's requirement

for PPG health claims (Health Canada, 2013). The current findings are supported by the earlier work of Mackie et al. (2017) who found that reducing particle size of oats from flakes to flour resulted in markedly higher BG iAUC over 170min and peak insulin when consumed in a porridge. However, we demonstrate that the effects of particle size can extend to different grinds of oat flours as well. Previous studies conducted by Kathirvel et al. (2019) and Byars et al. (2021) have also demonstrated that finer lentil and navy bean flours led to greater *in vitro* glucose release rate and starch digestibility. The current findings help to demonstrate these effects *in vivo*.

The differences in PPG observed may be explained by structural and compositional differences between the flours resulting from the processing and sieving methods as described in our analyses above. Available carbohydrate content decreased with increasing particle size, which may be due to lower starch damage in the coarser flours, thus leading to lower glucose release after consumption (Gu et al., 2022) as well as lower surface area for the granules to be digested. In addition, dietary fiber and protein contents were more abundant in the larger particle sized flours, likely due to higher retainment of bran and seedcoat components. Higher oat β -glucan concentrations can increase digesta viscosity resulting in slower glucose absorption through delaying gastric emptying as well as reducing the diffusion rate of amylolytic enzymes in the small intestine. As well, it can impede starch gelatinization during cooking (Rebello et al., 2016; Tosh, 2014). Likewise, the higher levels of insoluble dietary fiber in pulse flour can not only speed up transit time, but also increase the production of short chain fatty acids (SCFAs) through fermentation in the colon, which can reduce BG by upregulating glucose transporters to promote glucose absorption into peripheral tissues (McCrory et al., 2010; Samra & Anderson, 2007). Higher protein content may also enhance insulin sensitivity and secretion to promote glucose uptake (Overduin et al., 2015).

In contrast to experiments 1 and 2, we did not observe lentil flour particle size to impact the BG response over the premeal period. This may be due to smaller particle size differences between the flours. While the fine and whole flours were similar in size for peas and lentils, the coarse lentil flour was much finer ($578.5 \pm 14.5\mu\text{m}$) than the coarse pea flour ($710.7 \pm 26.3\mu\text{m}$). As a result, the difference in the available carbohydrate and dietary fiber contents between the flours were also smaller. A possible explanation is that lentils have softer seeds that are more prone to structural damage during milling, thus resulting in more uniform flours and even distribution of particles during the sieving process (Bourré et al., 2019). As such, the impact of milling and particle size on PPG may vary between different crop varieties based on their structure and composition. However, a post meal BG difference between coarse and whole lentil flours was found, suggesting that coarse flour may exert delayed effects beyond 120min. The higher insoluble fiber content in coarse lentil flour can increase SCFA production to stimulate the secretion of hormones that promote glycemic regulation. It has been previously shown that consuming cereals high in insoluble fiber reduced glycemic response after a second meal served at 75min over a 150min period in healthy adults (Samra & Anderson, 2007).

Although the glycemic lowering effects of pulse flour have been established, the addition of pea and lentil flours to wheat crackers did not result in lowered PPG (Anderson et al., 2014). Previously, pasta incorporated with 25% faba bean flour also resulted in similar post-treatment BG as pasta made with 100% durum wheat semolina. However, the response was lower after pasta added with 25% faba bean protein flour, which contained approximately 2-fold more protein and 25% less carbohydrate than the wheat pasta (Chan et al., 2019). As such, increasing the incorporation rate of pulse flour in the crackers to create larger differences in their nutritional content may allow for the detection of more significant effects.

Table 3.4 Treatment effects on BG mean, pre-meal, and post-meal concentrations, and iAUC¹

	Treatment ³	BG (mmol/L) ⁴	Pre-meal (mmol/L) ⁵	Post-meal (mmol/L) ⁶	iAUC (mmol*min/L) ⁷
Exp.1 ²	COF	6.05 ± 0.09 ^a	-	-	103.58 ± 10.7 ^a
	WOF	6.11 ± 0.11 ^{ab}	-	-	109.76 ± 11.8 ^{ab}

	FOF	6.40 ± 0.12 ^c	-	-	152.10 ± 15.5 ^c
	COMF	6.28 ± 0.11 ^{bc}	-	-	135.71 ± 10.3 ^{bc}
Exp.2	CPF	5.93 ± 0.08 ^a	5.80 ± 0.06 ^a	6.84 ± 0.15	82.65 ± 10.0 ^a
	WPF	5.98 ± 0.10 ^a	5.83 ± 0.07 ^a	7.06 ± 0.22	93.56 ± 9.4 ^a
	FPF	6.23 ± 0.13 ^b	6.11 ± 0.09 ^b	7.09 ± 0.17	125.05 ± 16.3 ^b
	WF	6.08 ± 0.08 ^a	5.94 ± 0.06 ^a	7.09 ± 0.18	96.19 ± 9.7 ^a
Exp. 3	CLF	6.04 ± 0.07	5.89 ± 0.09	7.12 ± 0.15 ^a	99.47 ± 10.7
	WLF	6.14 ± 0.08	5.96 ± 0.10	7.55 ± 0.18 ^b	119.12 ± 18.8
	FLF	6.10 ± 0.07	5.94 ± 0.09	7.22 ± 0.17 ^{ab}	102.83 ± 15.1
	WF	6.15 ± 0.08	5.99 ± 0.11	7.27 ± 0.22 ^{ab}	121.57 ± 16.3

¹ Different superscript within each column for each experiment denotes statistically significant differences; Values are presented as means ± SEM; p<0.05 is statistically significant

² Exp.1, experiment 1 (n=20); Exp.2, experiment 2 (n=20); Exp. 3, experiment 3 (n=20)

³ COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour; CLF, coarse lentil flour; WLF, whole lentil flour; FLF, fine lentil flour

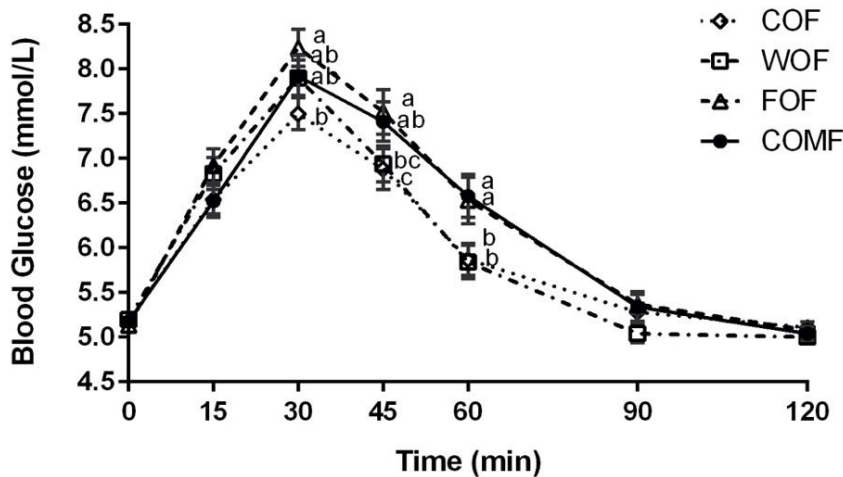
⁴ BG, BG, is calculated as the mean from 0-120min in Exp.1 and 0-140min in Exp.2&3

⁵ Pre-meal is the mean from 0-120min

⁶ Post-meal is the mean at 140min

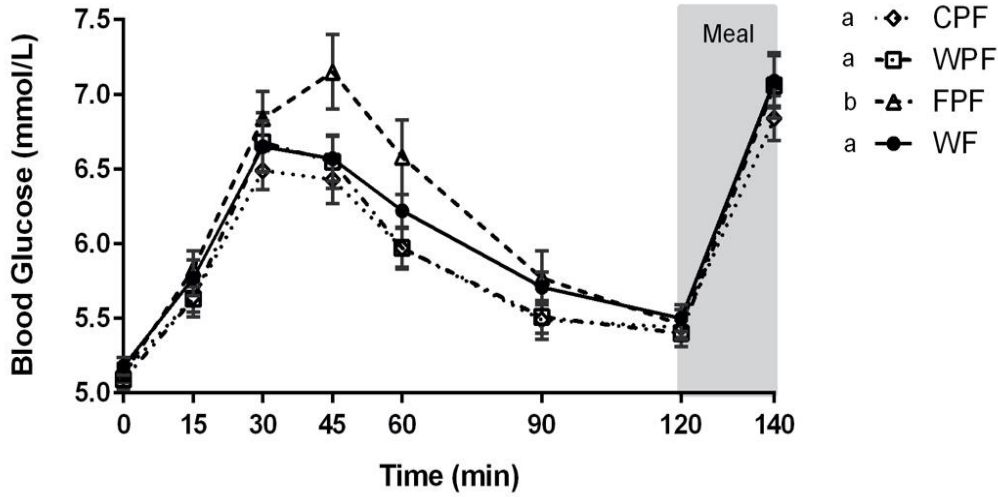
⁷ iAUC, incremental area under the curve, is calculated between 0-120min

a)



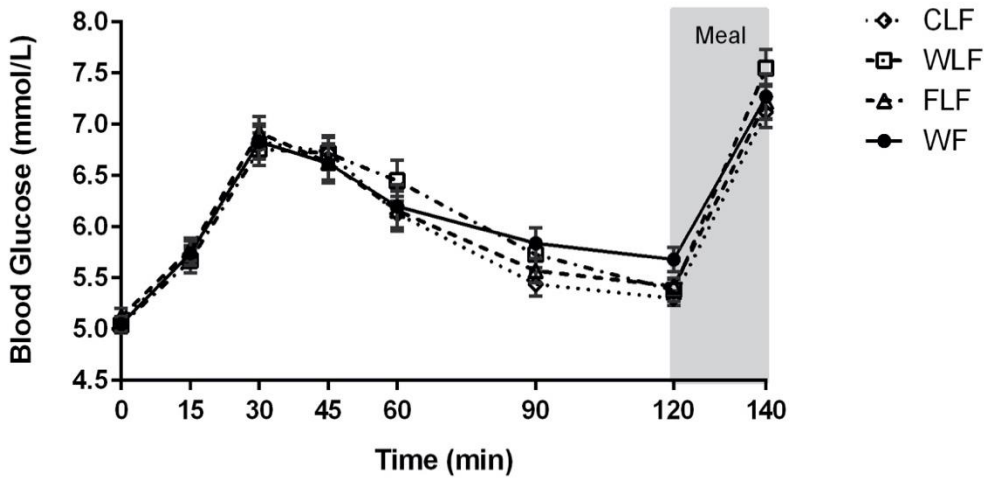
Values are mean ± SEM; n=20. Means with different letters are significantly different at each timepoint. Two-way ANCOVA (treatment, p<0.001; time, p<0.001; treatment*time, p<0.02).

b)



Values are mean \pm SEM; n=20. Means with different letters are significantly different. Two-way ANCOVA (treatment, $p < 0.002$; time, $p < 0.0001$; treatment*time, NS).

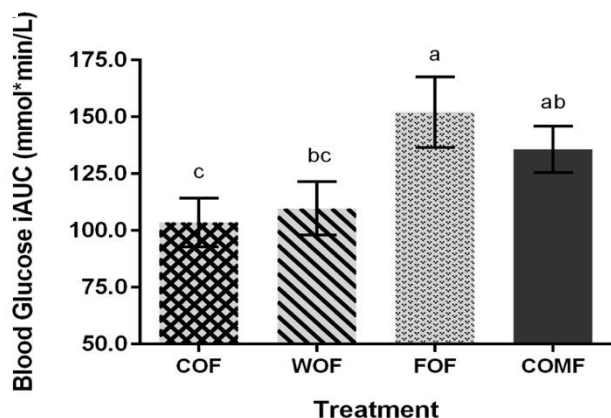
c)



Values are mean \pm SEM; n=20. Two-way ANCOVA (treatment, NS; time, $p < 0.0001$; treatment*time, NS).

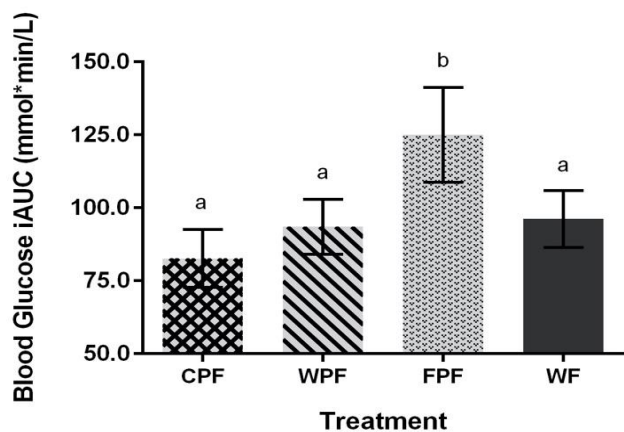
Figure 3.3 Treatment effects on BG over time for a) experiment 1 (oat), b) experiment 2 (pea), and c) experiment 3 (lentil).

a)



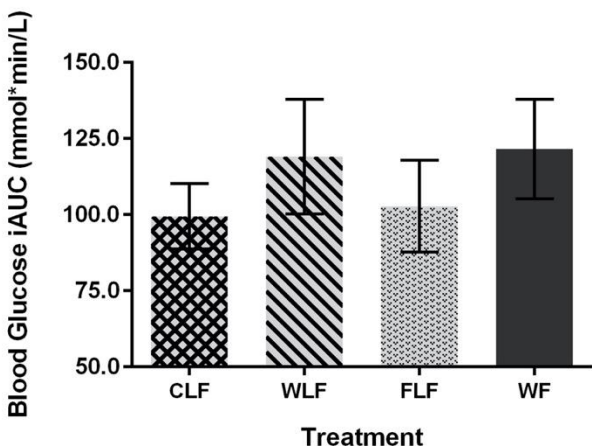
Values are mean \pm SEM; n=20. Means with different letters are significantly different. One-way ANOVA (treatment, $p < 0.0001$).

b)



Values are mean \pm SEM; n=20. Means with different letters are significantly different. One-way ANOVA (treatment, $p < 0.002$).

c)



Values are mean \pm SEM; n=20.. One-way ANOVA (treatment, NS).

Figure 3.4 Treatment effects on BG iAUC for a) experiment 1 (oat trial), b) experiment 2 (pea trial), and c) experiment 3 (lentil trial).

Table 3.5 Treatment effects on insulin mean and iAUC¹

	Treatment ³	Insulin (µIU/mL) ⁴	Pre-meal (µIU/mL) ⁵	Post-meal (µIU/mL) ⁶	iAUC (µIU*min/mL) ⁷
Exp.1 ²	COF	19.63 ± 2.6 ^a	-	-	1870.83 ± 269.1 ^a
	WOF	19.43 ± 2.6 ^{ab}	-	-	2016.60 ± 312.4 ^a
	FOF	30.43 ± 4.7 ^b	-	-	3457.85 ± 620.2 ^b
	COMF	25.17 ± 3.6 ^{ab}	-	-	2808.69 ± 463.4 ^b
Exp.2	CPF	36.41 ± 3.9	22.87 ± 2.7 ^{ac}	101.49 ± 13.6	2113.26 ± 354.2
	WPF	36.98 ± 4.2	24.20 ± 3.0 ^{abc}	100.88 ± 16.2	2081.33 ± 309.3
	FPF	40.14 ± 3.8	27.18 ± 3.3 ^b	101.95 ± 9.5	2730.91 ± 438.1
	WF	36.40 ± 4.1	22.78 ± 2.8 ^{ac}	103.52 ± 15.1	1873.76 ± 234.9

¹ Different superscript within each column for each experiment denotes statistically significant differences; Values are presented as means ± SEM; p<0.05 is statistically significant

² Exp.1, experiment 1 (n=20); Exp.2, experiment 2 (n=20)

³ COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour

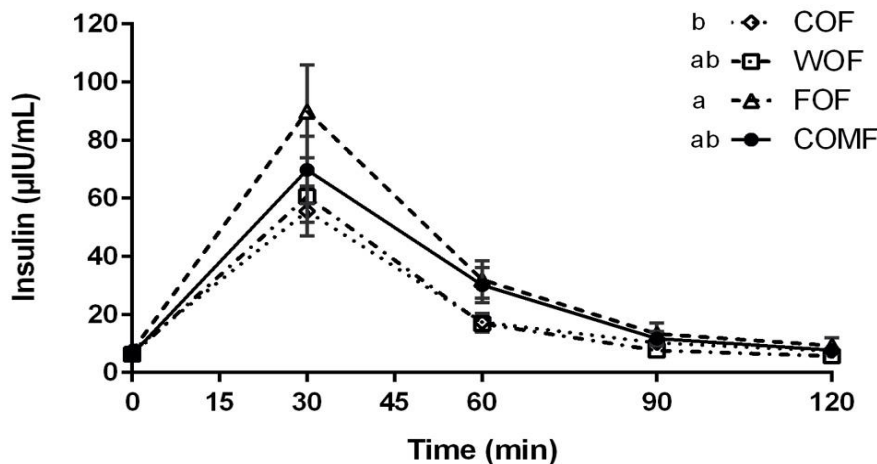
⁴ Insulin is calculated as the mean from 0-120min

⁵ Pre-meal is the mean from 0-120min

⁶ Post-meal is the mean at 140min

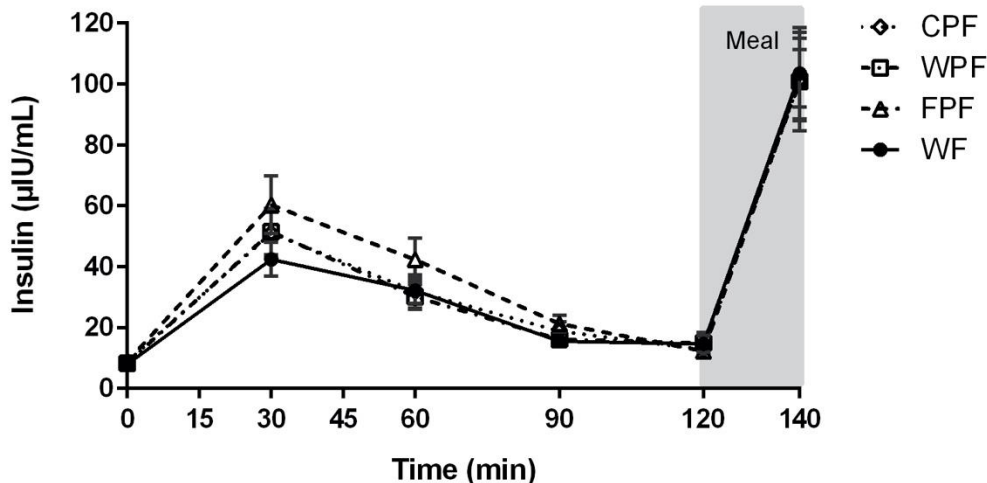
⁷ iAUC, incremental area under the curve, is calculated between 0-120min

a)



Values are mean ± SEM; n=20. Means with different letters are significantly different. Two-way ANCOVA (treatment, p<0.005; time, p<0.001, treatment*time, NS).

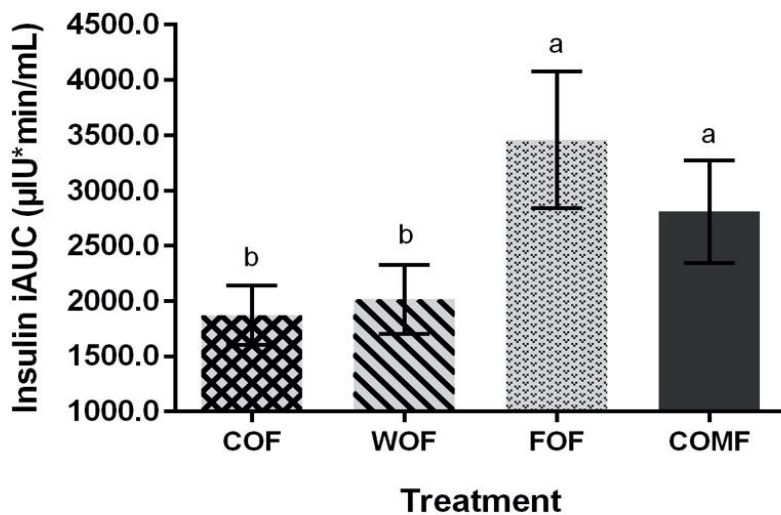
b)



Values are mean \pm SEM; n=20. Two-way ANCOVA (treatment, ns; time, $p < 0.0001$, treatment*time, NS).

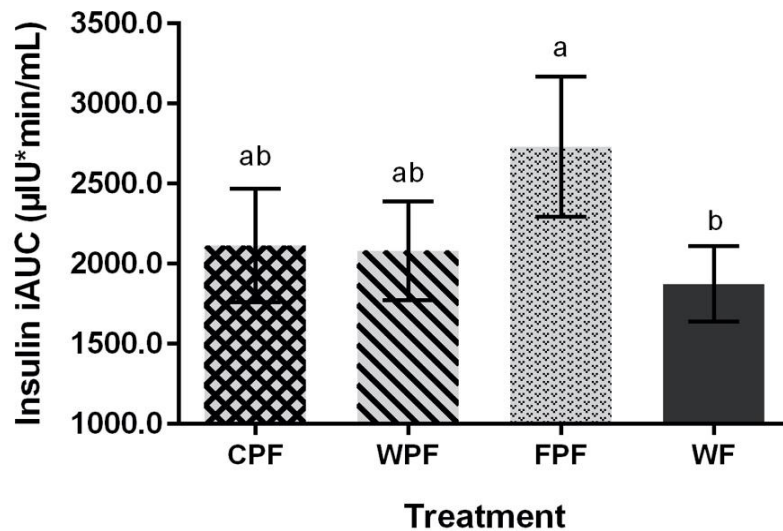
Figure 3.5 Treatment effects on insulin over time for a) experiment 1 (oat), and b) experiment 2 (pea).

a)



Values are mean \pm SEM; n=20. Means with different letters are significantly different. One-way ANOVA (treatment, $p < 0.0001$).

b)



Values are mean \pm SEM; n=20. Means with different letters are significantly different. One-way ANOVA (treatment, $p < 0.01$).

Figure 3.6 Treatment effects on insulin iAUC for a) experiment 1 (oat trial), and b) experiment 2 (pea trial).

3.3 Subjective appetite

Although no treatment-by-time effects were found, FOF and COMF porridges resulted in lower mean appetite than COF porridge in experiment 1 ($p < 0.02$). COMF was also more satiating compared to WOF ($p < 0.04$). No differences in post-meal appetite at 140min were discovered (Table 3.6, Figure 3.7). Males had greater mean appetite than females ($p < 0.04$), but no sex-by-treatment differences were observed. Appetite tAUC was found to be greater after COF than COMF ($p < 0.007$; Figure 3.8). No differences in appetite were found in experiments 2 and 3 (Table 3.6, Figure 3.7, 3.8).

Oat flours with smaller particle size were found to have greater appetite suppressing effects which may be attributable to four reasons. 1) Faster glucose release rate resulting from the greater starch availability in finer flours can quickly signal the brain to release hormones that decrease appetite, such as CCK, GLP-1, and PYY (Chaput & Tremblay, 2009; Druce & Bloom, 2006); 2) The fine and commercial oat flour porridges had higher pasting viscosities which can increase stomach distention and delay gastric emptying (Rebello et al., 2016); 3) A reduction in β -glucan particle size may lead to better extractability and water absorbing capacity further increasing viscosity (Johansson et al., 2018; Kurek et al., 2016); 4) Although protein content decreases with decreasing particle size, its digestibility and subsequent bioavailability may be increased resulting in more satiating effects (Byars et al., 2021; Gu et al., 2022). In contrast, the addition of pea flour to wheat crackers and its particle size did not impact appetite. Pletsch et al. (2022) demonstrated that when composition was controlled, particle size did not cause differences in gastric emptying rate and satiety for whole grain and refined wheat. However, Fahmi et al. (2022) found that pan bread enriched with 20% split-pea flour resulted in 18% higher feelings of fullness compared to 100% wheat pan bread. Nonetheless, this study provided 100g of treatments containing at least double the amount of energy and carbohydrates than the crackers provided and used a larger sample size of $n=24$, suggesting that the portion and sample sizes may need to be increased to observe differences in appetite.

Table 3.6 Treatment effects on subjective appetite mean, pre-meal and post-meal ratings, and tAUC¹

	Treatment ³	Appetite (score out of 100) ⁴	Pre-meal (score out of 100) ⁵	Post-meal (score out of 100) ⁶	tAUC (score out of 100*min) ⁷
Exp.1 ²	COF	46.83 ± 1.8 ^a	51.30 ± 1.7 ^a	15.75 ± 2.8	5282.61 ± 392.8 ^a
	WOF	43.21 ± 1.7 ^{ab}	47.56 ± 1.7 ^{ab}	12.71 ± 1.9	4951.81 ± 359.9 ^{ab}
	FOF	41.37 ± 1.9 ^{bc}	45.09 ± 1.9 ^{bc}	15.39 ± 2.7	4609.01 ± 409.7 ^{ab}
	COMF	39.58 ± 1.7 ^c	43.35 ± 1.6 ^c	13.19 ± 1.7	4316.62 ± 331.7 ^b
Exp.2	CPF	47.12 ± 2.0	52.09 ± 3.8	12.37 ± 2.2	5420.27 ± 456.7
	WPF	46.95 ± 1.8	51.43 ± 3.0	15.56 ± 3.0	5376.26 ± 371.2
	FPF	47.27 ± 1.9	52.13 ± 3.5	13.24 ± 1.9	5435.02 ± 419.2
	WF	48.60 ± 1.8	53.67 ± 3.2	13.08 ± 2.4	5609.25 ± 350.3
Exp.3	CLF	49.46 ± 1.9	54.50 ± 3.5	14.20 ± 2.7	5697.38 ± 398.4
	WLF	47.90 ± 1.9	53.03 ± 3.5	12.00 ± 2.5	5396.42 ± 383.6
	FLF	50.39 ± 1.8	55.46 ± 3.5	14.85 ± 2.5	5691.33 ± 389.6
	WF	50.26 ± 1.8	55.71 ± 3.2	12.11 ± 2.2	5737.97 ± 371.9

¹ Different superscript within each column for each experiment denotes statistically significant differences; Values are presented as means ± SEM; p<0.05 is statistically significant

² Exp.1, experiment 1 (n=20); Exp.2, experiment 2 (n=20); Exp. 3, experiment 3 (n=20)

³ COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour; CLF, coarse lentil flour; WLF, whole lentil flour; FLF, Fine lentil flour

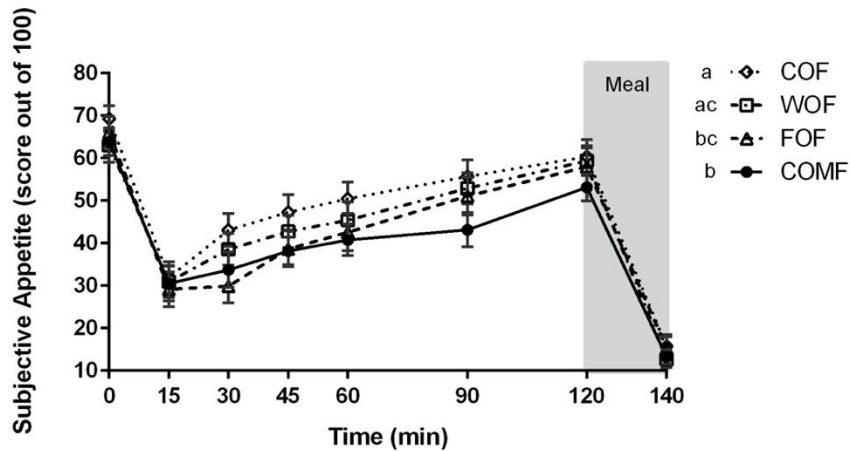
⁴ Appetite is calculated as the mean from 0-140min

⁵ Pre-meal is the mean from 0-120min

⁶ Post-meal is the mean at 140min

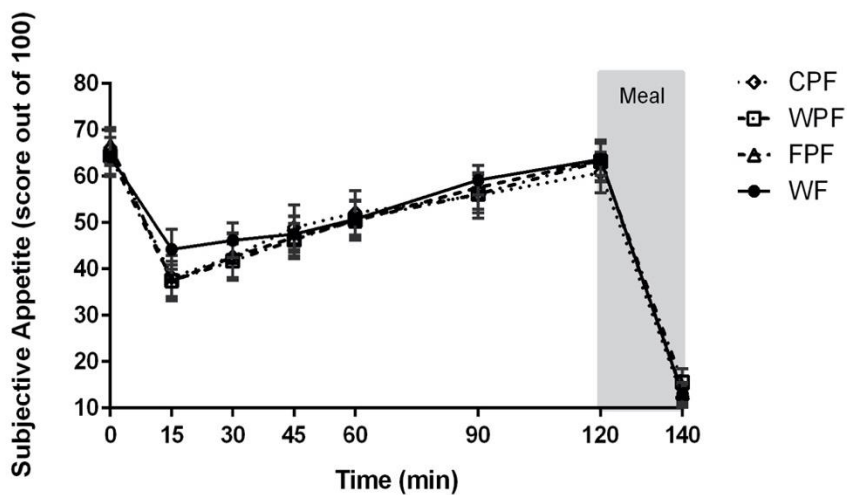
⁷ tAUC, total area under the curve, is calculated between 15-120min

a)



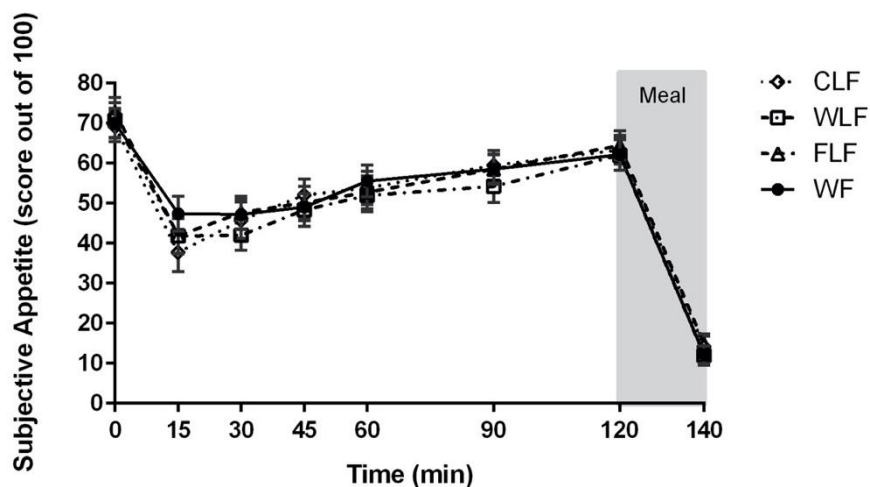
Values are mean \pm SEM; n=20. Means with different letters are significantly different. Two-way ANCOVA (treatment, $p < 0.05$; time $p < 0.0001$; treatment*time p NS).

b)



Values are mean \pm SEM; n=20. Two-way ANCOVA (treatment, NS; time, $p < 0.0001$; treatment*time, NS).

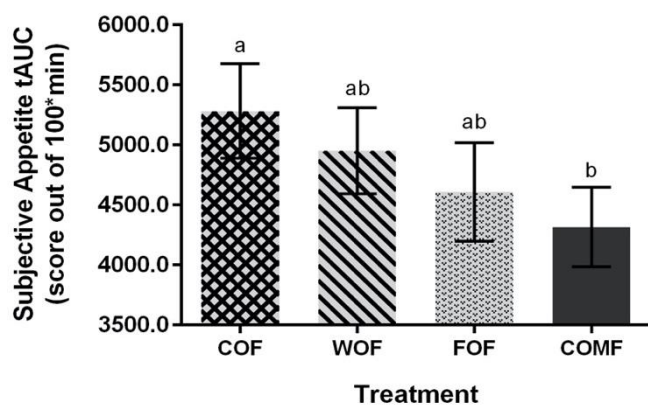
c)



Values are mean \pm SEM; n=20. Two-way ANCOVA (treatment, NS; time, $p < 0.0001$; treatment*time, NS).

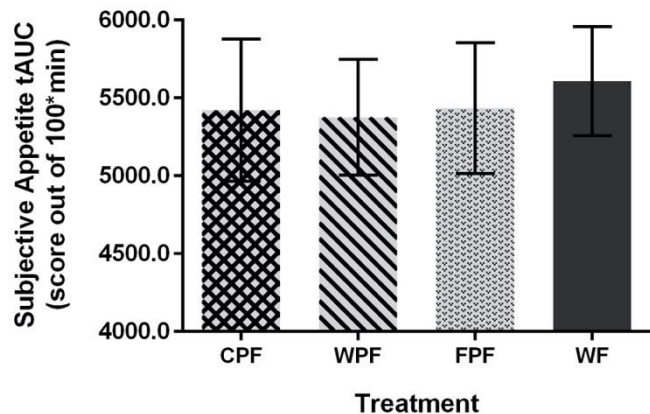
Figure 3.7 Treatment effects on subjective appetite over time for a) experiment 1 (oat trial), b) experiment 2 (pea trial), and c) experiment 3 (lentil trial).

a)



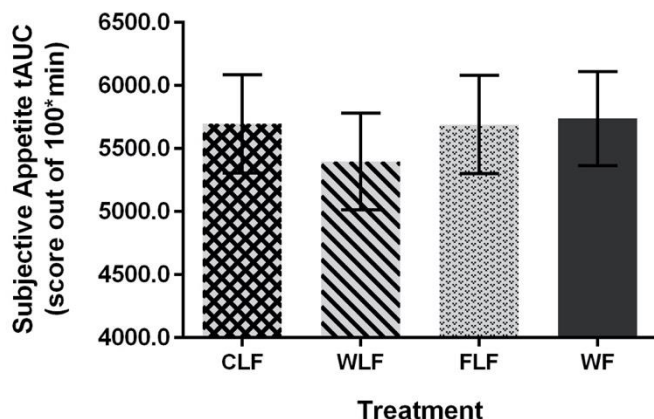
Values are mean \pm SEM; n=20. Means with different letters are significantly different. One-way ANOVA (treatment, $p < 0.008$).

b)



Values are mean \pm SEM; n=20. One-way ANOVA (treatment, NS).

c)



Values are mean \pm SEM; n=20. One-way ANOVA (treatment, NS).

Figure 3.8 Treatment effects on subjective appetite tAUC for a) experiment 1 (oat trial), b) experiment 2 (pea trial), and c) experiment 3 (lentil trial).

3.4 Food intake

No significant differences in energy consumption among the treatments were observed at the pizza meal in all experiments. The average food intake was 1097.5 ± 86.7 kcal for experiment 1, 1035.8 ± 87.5 kcal for experiment 2, and 1144.4 ± 131.3 kcal for experiment 3 (Table 3.7). WLF crackers resulted in higher water intake (425.62g) during the meal than WF crackers 327.18 g; $p < 0.004$) in experiment 3.

The results show that food intake at a second meal was not affected, suggesting that the differences in appetite were too minor to drive changes in energy intake. Various authors have made similar findings, including studies involving complete or partial replacement of pasta, muffins, pizza, and snack bars with pulse flour, which found no significant changes in appetite and food intake at a later meal. These studies suggest that short-term food intake at a meal may rely primarily on the energy contents of the treatment consumed previously, not so much on its composition and viscosity differences (Chan et al., 2019; Clark, et al., 2019; Johnston et al., 2021; King, 2022; Thamocharampillai et al., 2022; Wolever et al., 2020). Nonetheless, the sample size of the current study may be too small to detect differences in food intake, as previous studies have indicated that 26-30 subjects may be necessary (Chan et al., 2019; Johnston et al., 2021). It is uncertain why crackers containing whole lentil flour led to higher water

intake than wheat crackers but perhaps the combination of its starch and dietary fibre contents enhanced thirst. Nonetheless, this difference was unlikely to have impacted the amount of food consumed at the meals.

Table 3.7 Treatment effects on food and water intake at *ad libitum* pizza meal¹

	Treatment ³	Energy Intake (kcal) ⁴	Water Intake (g)
Exp.1 ²	COF	1116.06 ± 81.6	436.84 ± 35.6
	WOF	1091.40 ± 86.8	424.60 ± 41.2
	FOF	1102.35 ± 91.2	436.47 ± 55.4
	COMF	1080.14 ± 87.3	395.97 ± 39.5
Exp.2	CPF	984.47 ± 72.4	415.73 ± 47.6
	WPF	1038.98 ± 85.8	375.58 ± 46.4
	FPF	1081.97 ± 100.7	415.73 ± 54.5
	WF	1037.93 ± 91.1	429.51 ± 50.4
Exp.3	CLF	1120.61 ± 133.9	367.30 ± 34.1 ^{ab}
	WLF	1142.40 ± 140.8	425.62 ± 39.9 ^a
	FLF	1161.49 ± 129.1	371.99 ± 35.6 ^{ab}
	WF	1152.96 ± 121.5	327.18 ± 36.2 ^b

¹ Values are presented as means ± SEM; p<0.05 is statistically significant

² Exp.1, experiment 1 (n=20); Exp.2, experiment 2 (n=20); Exp.3, experiment 3 (n=20)

³ COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour; CLF, coarse lentil flour; WLF, whole lentil flour; FLF, fine lentil flour

⁴ Energy intake is calculated from the weight of pizza consumed converted into calories based on the manufacturer label

3.5 Treatment palatability

In experiment 1, COMF porridge was rated to be more palatable than FOF porridge (p<0.04), but indifferent from COF and WOF porridges. Significant differences were seen for ratings of thickness (p<0.0001), coarseness (p<0.0001), and flavor (p<0.03). FOF and COMF porridges were reported to have higher thickness and lower coarseness than COF and WOF porridges. COMF porridge was rated to have better flavor than FOF porridge. The crackers did not differ in pleasantness, taste, texture, and overall palatability in experiments 2 and 3 (Table 3.8).

Despite the popularity of using refined flours in the formulation of many products, porridge made using the fine oat flour was found to be less palatable than that made using the commercial oat flour. This may be due to changes in the amount of volatile compounds, phenolic acids, peptides, sugars, and fatty acids that contribute to flavor through processing (Salmenkallio-Martila et al., 2011). In contrast, the coarse and whole oat flour porridges were found to have comparable palatability as the commercial flour porridge, conferring them good potential as functional ingredients. Palatability ratings were similar for all crackers, in line with previous studies showing that the addition of yellow pea flour to crackers led to products with good appearance, texture, mouthfeel, and color compared to regular wheat crackers (Han et al., 2010; Millar et al., 2017). Therefore, pea flour, particularly coarser grinds, can be used as a substitute for wheat flour in cereal-grain based foods to create palatable products with higher nutrient density.

Table 3.8 Palatability scores for treatment porridges and crackers¹

Treatment ³	Pleasantness	Taste	Texture	Thickness ⁴	Uniformity	Coarseness ⁵	Slipperiness	Flavour ⁶	Smell	Overall Palatability	
Exp.1 ²	COF	50.63 ± 5.3	45.11 ± 5.7	42.68 ± 5.8	49.94 ± 4.3 ^a	59.28 ± 5.7	61.00 ± 5.2 ^a	41.80 ± 4.0	30.68 ± 4.8 ^{ab}	22.05 ± 4.2	44.80 ± 2.3 ^{ab}
	WOF	53.52 ± 5.5	44.04 ± 5.1	48.78 ± 5.6	62.46 ± 4.6 ^{ab}	55.30 ± 4.3	56.19 ± 4.7 ^a	45.84 ± 4.5	24.81 ± 4.3 ^{ab}	22.29 ± 3.1	44.38 ± 2.5 ^{ab}
	FOF	51.38 ± 4.5	36.38 ± 5.2	43.41 ± 6.1	75.91 ± 3.1 ^c	71.38 ± 5.1	25.65 ± 5.8 ^b	47.36 ± 7.2	23.16 ± 4.4 ^a	18.96 ± 4.3	43.73 ± 1.5 ^a
	COMF	61.04 ± 4.3	61.04 ± 5.2	44.35 ± 5.6	71.01 ± 3.5 ^{bc}	70.06 ± 5.1	36.61 ± 5.7 ^b	48.25 ± 4.5	35.31 ± 5.4 ^b	25.18 ± 4.8	50.45 ± 2.5 ^b
Exp.2	CPF	71.03 ± 4.9	67.10 ± 5.9	76.93 ± 4.2	-	-	-	-	-	-	71.69 ± 4.6
	WPF	62.65 ± 4.1	61.66 ± 4.4	74.86 ± 4.3	-	-	-	-	-	-	66.40 ± 3.8
	FPF	72.13 ± 4.5	66.43 ± 5.2	80.39 ± 4.5	-	-	-	-	-	-	72.99 ± 4.4
	WF	65.85 ± 3.7	65.84 ± 4.3	75.73 ± 4.9	-	-	-	-	-	-	69.15 ± 3.7
Exp.3	CLF	73.50 ± 4.6	65.68 ± 4.6	72.89 ± 3.6	-	-	-	-	-	-	70.70 ± 3.9
	WLF	66.56 ± 5.1	66.04 ± 4.5	74.50 ± 4.7	-	-	-	-	-	-	69.04 ± 4.4
	FLF	66.75 ± 5.5	61.49 ± 5.3	73.71 ± 4.7	-	-	-	-	-	-	67.32 ± 4.9
	WF	67.16 ± 4.2	64.36 ± 5.3	72.88 ± 4.1	-	-	-	-	-	-	68.14 ± 4.2

¹ Different superscript within each column for each experiment denotes statistically significant differences; Values are presented as means ± standard error of the mean

² Exp.1, experiment 1 (n=20); Exp.2, experiment 2 (n=20); Exp.3, experiment 3 (n=20)

³ COF, coarse oat flour; WOF, whole oat flour; FOF, fine oat flour; COMF, commercial oat flour; CPF, coarse pea flour; WPF, whole pea flour; FPF, fine pea flour; WF, wheat flour; CLF, coarse lentil flour; WLF, whole lentil flour; FLF, fine lentil flour

⁴ Thickness, p<0.001

⁵ Coarseness, p<0.001

⁶ Flavor, p<0.03

⁷ p<0.05 is statistically significant; ns, not significant

Conclusions and Recommendations (maximum 500 words)

Highlight significant conclusions based on the findings of this project, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project findings.

Both particle-size analysis and SEM observation confirmed the order of coarse > whole > fine in the flour particle sizes. For all the four crops, the three flour streams displayed the same rank order of fine > whole > coarse in their starch and damaged-starch contents but the reverse order in their ash, β-glucan (barley and oats only), insoluble dietary fiber, and total dietary fiber contents. Consequently, the functional attributes closely associated with starch present in flour, such as *L** value, starch gelatinization ΔH, and gelling ability, also fit into the same order of fine > whole > coarse. In contrast, protein contents of the three flour streams did not significantly differ in pea and lentil but showed a trend of coarse > whole > fine in barley and oats. The noted different particle sizes and chemical compositions among the three flour streams only caused a descending order of fine > whole > coarse in the pasting viscosities of the pulse flours but did not lead to such a clear trend in the cereal flours. With respect to the *in vitro* starch digestion, the cooked coarse and whole pea and lentil flours had lower starch digestibility than their fine counterparts, which was attributable to that the entrapment of starch granules by the dense and continuous protein-fiber matrix in the coarse and whole samples reduced the accessibility of starch to amylolysis. Without such a matrix structure in the cotyledons, the three flour streams of barley and oats showed largely similar starch digestibility after cooking. Overall, the coarse pulse and cereal flours exhibited more desirable nutritional quality in comparison with their respective whole and fine counterparts. For the pulse samples, the coarse streams had less starch and RDS, more dietary fiber and RS, and higher IV-PDCAAS than the whole and fine streams; for the cereal samples, the coarse streams possessed less starch but more protein, β-glucan, and total dietary fiber.

The processing of oat, pea, and lentil flours to different particle sizes can impact their health effects when consumed in the contexts of porridge or crackers. Larger particle sized flours (400-710µm), particularly coarse flours, led to lower PPG response and did not result in a disproportionate increase in insulin levels. Flour particle size effects on appetite were found but were insufficient in driving changes in food intake at a second meal. The addition of larger particle sized oat and pulse flours to porridge and crackers did not impact their palatability. Our findings demonstrate that controlling milling to produce coarse oat, pea, and lentil flours to add to cereal-grain based foods can be done to develop palatable products with improved functionality. Furthermore, the addition of pulse flours to wheat crackers can increase their nutritional density to provide potential benefits for PPG, regardless of particle size. Overall, this study has identified opportunities for the food industry to produce processed foods containing cereal and pulse flours with improved health benefits.

Follow-up Research

Please identify if there is a need to conduct further research. Detail any further research, development and/or communication needs arising from this project.

Future research that is worth exploring based on the significant findings from the current project can include:

- To evaluate the performance of pea, lentil, barley, and oat flours with variations in particle sizes in different food products, such as extruded snacks, meat products, bakery goods.
- To investigate the effects of particles on the flavor profiles of pea and lentil flours during storage.
- To investigate the effect of particle size of pea, lentil, barley, and oat flours on gut hormone responses responsible for regulating PPG and appetite
- To examine the protein quality (amino acid release measured in humans) of pulses and oats as components of mixed meals

Patents/ IP generated/ Commercialized Products

List any products developed from this research.

Although our collaborative research has not directly led to patents/IP/commercialized products at the current stage, the generated new knowledge and technologies can be utilized by the agriculture and agri-food sector in Canada to produce functional pulse and cereal flours with low-glycemic benefits.

Sustainable Canadian Agricultural Partnership (Sustainable CAP) Performance Indicators

a) List of performance indicators

Sustainable CAP Indicator	Total Number
Scientific publications from this project (List the publications under section b)	
<ul style="list-style-type: none"> • Published 	1

<ul style="list-style-type: none"> Accepted for publication 	0
HQPs trained during this project	
<ul style="list-style-type: none"> Master's students 	1
<ul style="list-style-type: none"> PhD students 	1
<ul style="list-style-type: none"> Post docs 	
Knowledge transfer products developed based on this project (presentations, brochures, factsheets, flyers, guides, extension articles, podcasts, videos)¹	8 presentations

¹ Please only include the number of unique knowledge transfer products.

b) List of scientific journal articles published/accepted for publication from this project.

Title	Author(s)	Journal	Date Published or Accepted for Publication	Link (if available)
Milling and differential sieving to diversify flour functionality: A comparison between pulses and cereals	Fan Cheng, Candy Ding, Hanyue Yin, Mehmet Tulbek, Claire Maria Chigwedere, Yongfeng Ai	Food Research International	Nov. 20, 2022	https://www.sciencedirect.com/science/article/pii/S0963996922012819

Technology Transfer Activities

List any technology transfer activities. Include presentations to conferences, producer groups or articles published in magazines except scientific journals.

<p>Presentations to conferences:</p> <ul style="list-style-type: none"> Zhou, C. Z. C., Fabek H., Fan W., Vien S., Tulbek, M., Ai, Y., and Anderson, G. H. The impact of particle size in cereal and pulse flour on postprandial glycemia and appetite in healthy adults. Upcoming annual meeting at Canadian Nutrition Society, Edmonton, AB (May 2 – 4, 2024) Zhou CZC., Fabek H., Fan W., Vien S., Ai Y., Tulbek, M., Anderson GH. (2023). The relationship between oat flour particle size and glycemic response in healthy adults [poster presentation]. Canadian Nutrition Society Annual Conference, May 2023. Ai, Y., Cheng, F., Lee, D.-J., Setia, R., Tulbek, M. C., Fabek, H., & Anderson, G. H. (2023). Critical roles of cotyledon microstructure in the processing, functionality, and nutritional quality of pulse flours. 12th Canadian Pulse Research Workshop, Windsor, Canada, February 20, 2023. Shadow, W., Kauffman, C., & Ai, Y. (2022). Performance of Plant-Based Ingredients. Cereals & Grains Webinar, December 14, 2022. Ai, Y. (2022). Impact of particle size on functional properties and nutritional benefits of oat and pulse flours. 25th Prairie Oat Growers Association Annual General Meeting, Saskatoon, Canada, December 1, 2022. Ai, Y. (2022). Interplay of variety and processing on the performance of pulse ingredients in food systems. Institute of Food Technologists Webcast, November 01, 2022. Ai, Y. (2022). Differential sieving to diversify techno-functional attributes of pulse and cereal flours: A close look at seed microstructure. Canadian Food Summit, Guelph, Canada, June 02, 2022. Cheng, F., Ding, C., Yin H., Ai, Y. (2021). Nutritional attributes of pulse and cereal flours varying in particle size. Cereals & Grains Association Annual Meeting, Virtual, November 2021.
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Contributions and Support

List any industry contributions or support received.

- All treatment crackers used in study 3 were formulated and produced by the Saskatchewan Food Industry Development Center in consultation with Dr. Mehmet Tulbek.

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- 1 refereed journal publication
- 8 presentations at academic conferences and to the private sector
- 3 manuscripts are in preparation for submission to peer-reviewed journals
- Ms. Corrina Zhou will be presenting the findings of this work to industry and government scientists at an upcoming meeting of the NSERC Program in Food Safety, Nutrition & Regulatory Affairs

Appendices

Identify any changes expected to industry contributions, in-kind support, collaborations or other resources.

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