Due Date

05/31/2024

Project Overview

Project number:	2022N006R
Project title:	Development of healthy food products by combining proteins and dietary fibers from oats and pulse
Project start date:	
Project completion date:	
This is an interim report for the reporting period to	

Research Team

Principal Investigator:

Name:	**Institution:**
Lingyun Chen	University of Alberta

Research team members:

Non-technical summary

> Provide a summary (200-300 words) of the project results which could be used by the funder(s) for communication to producers, industry stakeholders (e.g., processors, retailers, extension personnel. etc.) and/or the general public. This summary should focus on project deliverables that will advance the agriculture industry and outline the economic benefits and impact for Alberta's producers.

This is a collaborative research between the University of Alberta and the Food Processing Development Center at Leduc supported by RDAR and two industry partners including the Alberta Pulse Growers and the Prairie Oat Growers Association. The project has developed an innovative method to create microgels from pulse proteins combined with oat fractions. These microgels have the potential to partially replace fat in low-fat emulsion-based food products while maintaining desirable texture and consistency. Additionally, the research demonstrates the potential of faba bean protein as an alternative plant protein source for textured vegetable protein (TVP) production, alongside pea protein. Combining pulse proteins with oat β-glucan has resulted in TVPs with improved expansion, desirable sponge-like qualities, and enhanced water or oil absorption, which are crucial for food applications.

These successful results present significant opportunities for the protein ingredient and food manufacturing industries, enabling them to capitalize on the rapidly growing markets for fat replacers and TVPs. Furthermore, this project will provide consumers with healthier food choices and contribute to more sustainable food systems by complementing animal proteins and addressing dietary fiber deficiencies in Western diets. In the long run, the widespread application of pulse and oat based TVPs and fat replacers will help stabilize or expand the acreage of pulses and oats in Western Canada and contracts at premium prices for specific food applications will boost revenue for pulse and oat growers.

Project details

Project team

> a. Describe the contribution of each member of the R&D team to the functioning of the project.
> b. Describe any changes to the team which occurred over the course of the project.

Dr. Lingyun Chen , University of Alberta, served as the project leader. She supervised the graduate students for pulse/oat microgels and TVPs development, characterizations and food applications.

Dr. Jay Han, Food Processing Development Center at Leduc, co-supervised one graduate student for the TVPs processing and formulation development and optimizations.

Abbreviations

> Define ALL abbreviations used.

TVP: texturized vegetable protein product
LME: low moisture extrusion
HME: high moisture extrusion
ER: expansion rate
SME: specific mechanical energy
WHC: water holding capacity
OHC: oil holding capacity
SDS-Page: Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
FTIR: Fourier-transform infrared spectroscopy

Background

> a. Review the project background and update as needed.
> b. State the related scientific and development work that has been completed to date by your team and/or others.

A dietary pattern that provides plant protein, dietary fiber and low fat has been shown to decrease the risks of chronic diseases such as obesity and cardiovascular disease. This food trend is also driven by consumers looking for sustainable plant-based options. The pulses and oats grown in Canada are good sources of proteins. The Canadian milling oats are also good sources of fiber (≥4.5% β-glucan). In addition, the mixture of oat and pulse proteins provides a strategy to address the nutritive issues of plant protein-based food products. Even though pulses and oats lack some essential amino acids, produced with their mixture contain all essential amino acids.

Using protein-based fat replacers has emerged as a promising tool to reduce fat in the food products. Proteins contribute to only 4 kcal per gram (vs. 9 kcal for fat) with more satiation per calorie (Kew 2020). Whey protein currently dominates the market owing to its unique capacity to form viscoelastic microgels that enhance the smooth creamy mouthfeel and other fat-related sensory attributes (Dickinson 2018). With the increasing sustainability consideration, there has been a shift towards plant proteins. Dr. Chen’s lab has demonstrated gelling capacity of both oat and pulse proteins and the oat gel is especially strong (Nieto-Nieto 2015, Yang 2021). This provides an opportunity to develop new fat-replacers of plant origin. Nevertheless, technology innovation is required to make microgels from plant proteins and tailoring their properties to simulate solid fats particles.

Concerns over GMO and allergenicity have triggered industry interest in texturized vegetable protein products (TVPs) from pulses. Incorporating oat protein and dietary fibre in pulse protein based TVP is desirable to improve the product nutritive value. Innovations in formulation and extrusion processing are yet to be developed to combine pulse and oat ingredients to make TVPs rich in protein and fiber, but also with desirable product characteristics.

Objectives

> a. Review the original objective(s).
> b. Indicate any modifications to the objective(s) that occurred over the course of the project.

The overall objective of this research is to use high-quality protein and dietary fiber ingredients from oats and pulses for healthy food development.

The specific objectives were to:
1. Screen oat variety & optimize dry milling processing to develop ingredients enriched in protein and dietary fiber from oats
2. Develop microgels from oat and pulse proteins as fat replacers for low fat food development
3. Develop texturized vegetable protein products (TVP) by combining protein and fiber from oats and pulses for meat analogue applications

Research design and methodology

> In summary, describe the project design, methodology, laboratory and statistical analysis used to carry out the project. Please provide sufficient detail to determine the experimental and statistical validity of the work and give reference to relevant literature where appropriate. For ease of evaluation, please structure this section according to the objectives cited above.

1. Screen oat variety & optimize dry milling processing to develop ingredients enriched in protein and dietary fiber from oats

Commercial oat varieties of high protein and β-glucan content were focused. Dry fractionation techniques are energy efficient, have lower capital investments, and can better preserve the original structure of protein and β-glucan compared to wet extraction processes. Therefore, the milling/air classification processing was applied as a dry fraction method to separate oat protein and β-glucan from starch. It was expected that the spherical shape of starch particles would be selectively carried by air in contrast to the flat-shaped dietary fiber. Specifically, the oat grains were milled into flours by a pin mill followed by fractionation using a small pilot Hosokawa air classifier. Particle size is important in air classification of cereal flours and should be sufficiently small so that cell components can be separated. The preliminary experimental trials also demonstrated that the air classification wheel speeds of 2500-5000rpm were able to enrich β-glucan. Therefore, in this work, a combination of a series of particle sizes (250-500μm, 500-750μm, 500-1000μm) and air-classification wheel speeds (2500, 3500, 4500, 5500 rpm) was systematically studied for separation of protein and dietary fiber in oat flours.

The β-glucan contents were determined using enzyme kits according to Approved Methods 32-23.01(AACC International 2010). Protein content (%N × 5.7) were determined by combustion nitrogen analysis using the Leco analyzer calibrated with EDTA according to Approved Method 46-30.01 (AACC International 2010). Statistical analysis: All experiments were performed in triplicates and results were presented as mean values ± standard deviations.

2. Develop microgels from oat and pulse proteins as fat replacers

The lentil protein isolate (LPI, 85%, w/w) was prepared at the Food Processing and Development Centre. The faba bean protein isolate (FPI) was obtained from Top Health Ingredients (Edmonton, Alberta), which containing 90% protein. The oat flour with 20% protein and 11% β-glucan were prepared by using the small pilot Hosokawa air classifier from the gluten-free oats provided by Avena (Regina Saskatchewan, Canada).

The lentil and faba bean proteins were dissolved in Milli-Q water to prepare protein solutions with protein content of 15%. The oat formulation was prepared by mixing lentil protein and oat flour at the ratio of 2:1 (w/w) in solution with the total flour concentration of 10% (further increasing flour concentration led to a too viscose solution to handle). The microgels were prepared based on protein-polysaccharide phase separation phenomenon. Specifically, the protein solution was mixed with alginate solution (2%) at different volume ratios (1:4, 1:1, 2:1). The mixture solutions were heated at 90oC in a water bath. Then the cooled suspensions were centrifuged to collect the formed microgels, which were then stored at 4oC for following analysis.

The microgels were characterized for their size distribution and surface charge by established methods (Liu 2018) as well as microstructures by photographic camera and scanning electron microscopy (SEM) imaging (Jo 2023). Moreover, Fourier-transform infrared spectrum (FTIR) and the protein surface hydrophobicity were studied to understand the protein conformation changes and interactions for the microgel formation (Zhang 2021).

The microgels were then applied in a model O/W emulsion system to understand their potential to be used a fat-replacer in emulsion food systems (e.g., yogurt, creamer). The emulsions were prepared by mixing canola oil with protein microgels suspensions to have the oil concentration of 25% and 50% (w/w) in the final emulsions. Then the mixtures were homogenized by a high-speed homogenizer at the two microgel concentrations of 1.5% and 3%. The formed emulsions were then characterized for their particle size, morphology, creaming index and rheological properties (Jo 2023).

Statistical analysis: All experiments were performed at least in triplicates and results were presented as mean values standard deviations. All statistical analysis were performed by the SPSS software (SPSS, INC., Chicago, IL, USA). Student’s t-test or one–way analysis of variance (ANOVA) with post hoc Tukey’ s test were used to determine the statistically significant differences between results. The p-value <0.05 was considered significant.

3. Develop TVPs by combining protein and fiber from oats and pulses

Commercial pea (55% protein) and faba protein (60% protein) fractions by air classification were used for research in this objective, which were kindly provided by Ingredion. Whereas pea (85% protein) and faba bean (90% protein) isolates were kindly provided by AGT Foods and ingredients (Regina, Saskatchewan, Canada). Oat protein and oat β-glucan (composed of 34% soluble fiber) were purchased from Lantamman (Stockholm, Sweden).

TVP were extruded using the pilot co-rotating twin screw extruder (Coperion ZSK 26, Coperion, Stuttgart, Germany) in Alberta’s Food Processing Development Center at Leduc. Protein concentrates and isolates were mixed to achieve 60%, 70% and 80% final protein content for both pea and faba bean raw materials. Water injection rate was adjusted to reach total moisture contents of 50% and 55% in the melt. All other extrusion parameters were optimized within the desired expansion range based on preliminary work to accommodate both faba bean and pea proteins and held constant throughout the experiment. After extrusion, the TVP products were pelletized and collected. Pelletized TVP were then dried in a tray oven at 60°C for 30 minutes and stored in a sealed double plastic bag at 21°C for further analysis.

Expansion of TVP creates a porous structure, providing a sponge-like quality and enables the uptake of water or oil needed to improve meat analogue texture. Thus, the prepared TVP samples were evaluated for their expansion, bulk density, water holding and oil holding capacities by the established methods (Buchko 2024). In addition, the photo images were obtained to observe the TVP pore size and distribution. Their microstructures were observed by confocal microscopy to study the protein, starch and fiber distribution in the TVP networks. Moreover, sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-Page) and Fourier-transform infrared spectrum (FTIR) analysis were conducted to understand the protein conformation changes and interactions by extrusion for TVP network formation (Zhang 2021).

Statistical analysis: All quantitative analysis were performed at least in triplicate on two independent extrusion repetitions. Origin version 2022b (OriginLab Corporation, Northampton, MA, USA) software was used for statistical analysis and results presented as the mean ± standard deviation. Statistical analysis was conducted by one-way analysis of variance (ANOVA) at a confidence interval of 95% utilizing Tukey’s test at $p>0.05$. Two-way ANOVA was conducted to determine the interactions and impact of moisture and protein content treatments on TVP functional characteristics.

Results, discussion and conclusions

> Present the project results and discuss their implications. Discuss any variance between expected targets and those achieved. Highlight the innovative, unique nature of the new knowledge generated. Describe implications of this knowledge for the advancement of agricultural science. For ease of evaluation, please structure this section according to the objectives cited above.

1. Screen oat variety & optimize dry milling processing to develop ingredients enriched in protein and dietary fiber from oats

1.1 Enrichment of β-glucan

Two sources of oats commercialized for food applications were focused including Cavena Nuda oats (naked oats) and gluten-free oats. Both are high in protein (~16% protein) and contain similar amount of β-glucan of around 4.5%. Gluten-free oats allows food products with cleaner label. As shown in Fig.1-1, a fine fraction and a coarse fraction were obtained after the air classification separation for both Cavena Nuda and gluten-free oats. Fig. 1-2 shows the β-glucan content in the fine and coarse fractions obtained from Cavena Nude at different air classification wheel speeds when the milling particle size of a) 250-500μm, b) 500-750μm and c) 500-1000μm, respectively. The fine fractions are rich in β-glucan with the content of 7-13%. The higher β-glucan of 13% were obtained at the milling size of 500-750μm and the air classification wheel speed of 3500-4500rpm, achieving a β-glucan enrichment of around 3 folds. Beta-glucan was also enriched by air classification at the milling size of 250-500μm and 500-1000μm, but with lower enrichment degree (around 2 folds).

Fig. 1-3 shows the β-glucan content in the fine and coarse fractions obtained from the gluten-free oats at different air classification wheel speeds. The fine fractions are rich in β-glucan with the highest value reached at 11% (~2.5 folds of enrichment) at the air classification wheel speed of 2500rpm and the particle size of 250-500μm. Higher β-glucan content (~10%) was also observed at the at the milling size of 500-750μm and the air classification wheel speed of 3500-4500 rpm.

1.2 Enrichment of protein

Fig. 1-4 and 1-5 show the protein content in the fine and coarse fractions obtained from the Cavena Nude and gluten-free oats, respectively, at different milling particle sizes and air classification wheel speeds. The fine fractions also possess higher protein content (20-23.5%) than the coarse fraction (10-15%), indicating the oat protein tended to be enriched with β-glucan in the coarse fractions, possibly some protein granules have strong interactions with dietary fiber in the oat grain tissue, or entrapped in the fiber matrix during air classification process. The protein enrichment degree was relatively low with the maximum value of 1.5 folds. This is possibly due to the similarity in shape for protein and starch, which makes it difficult for their separation. Therefore, air classification is less efficient to concentrate protein than β-glucan from oat flours. Slightly higher protein enrichment was also observed for Cavena

Nude than the gluten-free oats.

On the positive side, for Cavena Nuded, the fraction with highest β-glucan content (13%) also contain the highest protein (23.5%) in it. Therefore, the identified suitable milling/air classification condition above (Milling size: 500-750μm; air classification wheel speed: 3500-4500 rpm) can be used to generate oat fractions with protein content of 23.5% and β-glucan content of 13%. Under the same processing conditions, fractions with 11% β-glucan and 20% protein were obtained from the gluten-free oats. These specific fractions also show good recovery of β-glucan (70-80%) and protein (50-60%) component. It should be mentioned that these fractions have protein content comparable to dry pea (~22% protein). Therefore, the obtained specific oat fractions can be regarded as a good source of both plant protein and β-glucan for many food applications. The consumers are look for a dietary high in plant protein, dietary fiber and low in fat due to health considerations. This food trend is also driven by consumers looking for sustainable plant-based options.

1.3 Wet extraction of protein from the remaining fine fractionation

The identified suitable milling/air classification condition above (Milling size: 500-750μm; air classification wheel speed: 3500-4500 rpm) also generate fine fractions that contain 69%-74% starch and 10-12% protein. Alkaline method was then applied to extract protein from the fine fractions under the conditions of pH 9 and the flour-to-solvent ratio of 1:10, followed by acidic precipitation at pH 5. Protein concentrates were obtained (75-82% protein) with the recovery of 58-63%. These results demonstrate the feasibility of extraction protein concentrates by wet method from the remaining fine fractions obtained by air classification processing.

Summary

- Milling/air classification processing is efficient to enrich β-glucan up to 3 folds in specific fractions for Canadian oats. In comparison, the fractionation of oat protein is less efficient (up to 1.5 folds).

- The milling size of 500-750μm and the air classification wheel speed of 3500-4500 rpm could be suitable processing conditions to generate oat fractions with protein content up to 23.5% and β-glucan content up to 13%, which can be regarded as a good source of both plant protein and β-glucan for many food applications.

- The higher β-glucan and protein enrichment efficiency observed for Cavena Nude than the gluten-free oats suggests that oats of different varieties could have significantly different performances during the milling/air classification processing.

Study innovations and implications for the advancement of agricultural science

An innovation of this objective is to obtain an oat fraction enriched in both protein and β-glucan that may be as used as an oat ingredient for food fortification, thereby increasing oat consumption in human diet. In addition, such oat fraction can be combined with pulse proteins for developing food products not only rich in protein with balanced essential amino acids, but also contain soluble dietary fiber for additional health benefits (e.g., regulate blood sugar and reduce blood cholesterol).

2. Develop microgels from oat/pulse proteins as a fat replacer for low fat foods

2.1 Microgel preparation and characterizations

From Fig 2-1, SEM images reveal preparation of spherical microgels from the lentil protein, faba protein and lentil/oat flour. Particle size (D3,2) measurements in Fig. 2-2 demonstrated that the microgels had a narrow size distribution, indicating that uniform microgels were achieved. Additionally, particle size could be precisely controlled by adjusting the biopolymer volume ratios. For the lentil protein microgels, a volume ratio of 1:4 resulted in a microgel size of 3.3 μm. Increasing the ratio to 1:1 and 2:1 led to a gradual increase in microgel size to 7.1μm and 15.2 μm, respectively. Similar trends were observed for faba and lentil/oat microgels, but the sample became irregular in shape with increase of the particle size. In general, the microgel sizes are within the oil droplet size range in food emulsion systems, thus showing potential to replace fat droplets in food formulations.

The microgels have the surface charge of -26.1 mV to -33.9 mV at neutral pH due to the deprotonation of the deprotonation of the -COO- group on protein chain. Possibly the surface adhesion of alginate contributed to the relatively high surface charge observed for the microgels. It should be mentioned that the surface charges of the microgels are favorable for their dispersion in liquid food systems as the repulsion among them will prevent microgel aggregation.

2.2 Microgel formation mechanisms discussion

The deconvoluted FTIR spectra of the amide I band region (1700 to 1600 cm-1) was used to analyzed the protein secondary structural changes during heating process and microgel formation (Fig. 2-3). Bands are assigned to different protein secondary structures as follows (Zhang 2021): 1607 cm-1 related to vibration of amino acid residues, 1615 cm-1,1616 cm-1,1630 cm-1, 1678 cm-1 and 1682 cm-1 to β-sheet, 1643 cm-1 to α-helix and random coil, 1656 cm-1 and 1658 cm-1 to α-helix, 1689 cm-1 to β- turn. All microgels exhibited two prominent peaks at 1615 cm-1/1616 cm-1 and 1682 cm-1. The band at 1615 cm-1 was assigned to the intermolecular β-sheet, which indicated the formation of strong hydrogen bonds. The band at 1682 cm-1 was associated with antiparallel β-sheet. In addition, the bands associated with protein secondary structures disappeared or were significantly reduced, indicating unfolding of protein structure. Meanwhile, surface hydrophobicity measurement of the protein before and after heating revealed markedly increase in their surface hydrophobicity after heating treatment, indicating exposure of the hydrophobic residues previously buried within the globular core of the proteins. These results revealed the microgel formation mechanisms that involving unfolding of protein to expose the hidden hydrophobic side chains and other functional groups, followed by the protein aggregation via hydrogen bonding and hydrophobic interactions to form the microgel networks.

2.3 Lentil protein (LP) microgel performances in food emulsion systems

Lentil protein microgels were focuses for the performance evaluation in emulsion systems in the first step as they show better controlled spherical shape. The emulsions were prepared with two oil volume fractions (25%, 50% oil) to simulate different food emulsions such as light coffee cream (18-30% fat), cream salad dressing (50% fat) and cream spread (~50% fat or higher). It is interesting that the microgel stabilized emulsions are much more stable than those stabilized by lentil protein itself. Rapid creaming occurred for emulsions stabilized by 3% lentil protein after 1 day of storage (Fig. 2-4). In comparison, much lower creaming index (low level of creaming) was observed for emulsions stabilized by the microgels (both 1.5% and 3%). Especially the emulsions stabilized by 3% microgels at the size of 3.3μm show almost no creaming even after 28 days of storage. The size measurement indicates that the emulsions are in the size range of 5-10μm (Fig. 2-5). At both 25% and 50% oil levels, the emulsions stabilized by 3% microgels were significantly smaller than those stabilized by 1.5% microgels. Moreover, at the oil level of 25%, the emulsions stabilized by smaller microgels shower smaller particle size. These results indicate strong capacity of the lentil protein microgels to stabilize food emulsions and those with smaller size are more effective.

2.4 Mechanism of LP protein microgel to stabilize emulsions

According to the confocal microscopic image (Fig 2-6 green: protein microgel; red: oil droplets), the small microgels (3.3μm) could be adsorbed at the oil droplet surface to form Pickering emulsions. The irreversible adsorbed microgels can form a dense surface layer to effectively prevent oil droplet coalescence, which explained the long-term stability of the emulsions stabilized by 3.3μm microgels at the concentration of 3%. On the other hand, the microgels with larger sizes (7.1 μm and 15.2μm) were rarely observed at the interface of emulsion droplets. Instead, they were dispersed in the continuous phase, which can create a jamming effect to provide steric hindrance among the emulsion droplets to prevent coagulation. Therefore, both small and larger lentil protein microgels show superior capacity to stabilize emulsions than protein itself, but through two different mechanisms.

2.5 Emulsion rheological properties

The apparent viscosity of emulsions prepared by lentil microgels with three different sizes (two different microgel concentrations and oil concentrations) was determined at the shear rates from 0.001 to 1000 s-1. The apparent viscosities of two control emulsions prepared from untreated lentil proteins were also analyzed under the same conditions. The apparent viscosity decreased with the increase in the shear rate for all flow curves, indicating that all emulsions exhibited the shear-thinning behavior (Fig. 2-7). The increased shear rate caused the droplet deformation and/or disruption, leading to decreased viscosity. The shear-thinning behavior carries practical advantages. These emulsions exhibited high viscosities at low shear rates to prevent from creaming and maintain stability, and the viscosity decreased at higher shear rate making them easier to pour out of a container.

When fixing the oil content at 25%, compared to emulsions prepared from untreated 3% lentil proteins, emulsions stabilized by microgels had significantly higher viscosity, indicating the incorporation of microgels could form thicker emulsions with stronger structures. The similar phenomenon was observed when the oil content was 50%. This is because the addition of microgels results in “jamming effect” or “filler effect”, which can limit the movement of oil droplets and form a more densely packed and stronger network. This leads to enhanced emulsion viscosity and texture.

2.6 Microgel as fat-replacer

It is worth noting that the microgels-stabilized emulsions with 25% oil achieved significantly higher structure than emulsions with 50% oil stabilized by untreated lentil protein (Fig. 2-7). As food proteins contribute to only 4 kcal per gram with high satiation (vs. 9 kcal for fat), the microgels have high potential as a fat replacer for low fat emulsion food development. For example, when the microgels are added in the formulation, the emulsion with reduced fat (25% oil) will have similar texture and consistency to their full fat counterparts (50% oil) while with reduced calories.

2.7 Microgels prepared from faba bean protein and lentil/oat formulations

The emulsions were also formed by microgels (3%) prepared from the faba bean protein and lentil/oat formulations at the microgel size of 3.4-3.6μm with two oil volume (25% and 50%). The photos in Fig. 2-8 also demonstrated their strong capacity to stabilize emulsions with minimum creaming although not as effective as the lentil protein microgels at the oil volume of 25%. Similarly, the emulsions stabilized by faba bean protein and lentil/oat formulations show shear-thinning behavior, and significantly increased viscosity and texture when compared to the emulsions stabilized by the corresponding protein without treatment. Notably, the emulsions stabilized by the microgels with 25% oil show the similar texture to those with 50% oil stabilized by the proteins without treatment Fig. 2-8. Such results demonstrate that faba bean protein and lentil/oat microgels also have good potential to be used as fat-replacers in emulsion-based food products.

Summary

- Microgels (3-23μm) are successfully prepared from lentil, faba bean protein and lentil/oat formulation

- Compared to the protein without treatment, the microgels can produce emulsions that are much more stable with minimum or no flocculation and creaming during a 28-day storage test.

- The smaller microgels were more effective to stabilize food emulsions at a higher microgel concentration of 3% owing to their capacity to adsorb at the oil droplet surface to form Pickering emulsions

- When the microgels are added in the formulation, the emulsion with reduced fat (25% oil) will have similar texture and consistency to their full fat counterparts (50% oil) while with reduced lower calories. Therefore, this work demonstrates the good potential of using microgels made from lentil and oat as fat-replacers for low fat food development.

Study innovations and implications for the advancement of agricultural science
This research has developed a facile method to prepare microgels (3-23μm) from lentil, faba bean protein and lentil/oat formulation by protein-polysaccharide phase separation phenomenon and demonstrated their capacity to replace fat for low fat emulsion food development, while maintaining the desirable texture and consistency. This will bring new ingredients (fat-replacer) and new market opportunities (fat replacers) for pulses and oats for human consumption. Furthermore, the inclusion of protein microgel (or with β-glucan) will enhance the nutritive value of emulsion-based food products. Understanding the mechanisms behind protein microgel formation and the ability to tune particle size will aid researchers in developing a wider range of applications for plant protein microgels, such as nutraceutical delivery systems, benefiting both industry and consumers.

3. Develop texturized vegetable protein products (TVP) by combining protein and fiber from oats and pulses for meat analogue applications

3.1 TVP development from pea and faba bean protein fraction from air classification

High moisture extrusion (HME) is able to create ready-to-eat products that resemble whole muscle meats with a fibrous texture, whereas low moisture extrusion (LME) products are usually incorporated into a food system before they are sold in the market. An advantage of LME over HME is that the additional cooling die is not required, which would reduce processing steps and production costs. In addition, low moisture TVP can be dried and stored at room temperature, can be developed in different sizes and textures, and adapted into a wider variety of food applications compared to HME. LME can also produce TVP using high protein flours or concentrates, reducing the need for expensive and highly purified protein isolates. In terms of LME, most works focus on soy and wheat, with much less attention given to peas and very few studies reported on faba beans. Therefore, low moisture extrusion (LME) was applied for pea and faba bean TVP development in this research. High protein fraction from air classification were used as the basic formulation and were combined with protein isolates to adjust protein content of starting materials.

3.1.1. Impact of protein type/content and moisture on TVP expansion and bulk density

Expansion of TVP creates a porous structure providing a sponge-like quality and enables the uptake of water or oil needed to improve meat analogue texture (Fig. 3-1). Our preliminary work (40%-60% dry basis) indicated that appropriate TVP expansion of both faba and pea could be obtained at the moisture level of 50% and 55% (dry basis). Inappropriate expansion took place at lower moisture (<50%) resulting in dry, crumbly, unexpanded TVP. Whereas higher moisture (>55%) led to wet and gel-like under expanded products. Protein fractions by air classification were mixed with pea protein isolates to achieve 60%, 70% and 80% final protein content (dry basis) for both pea and faba bean sources.

Moisture content impacted faba bean TVP expansion ratio (ER) at 50% moisture (1.64-1.79 ER) and 55% moisture (1.58-1.7 ER) (Fig. 3-2). Low moisture content will allow formation of a protein-based melt with higher viscosity, thus creating more shear force and higher specific mechanical energy (SME). An increase in SME then leads to enhanced protein texturization during extrusion (Lee et al., 2022). Decreased melt viscosity can also result in a stronger melt flow at the extruder die, promoting expansion of TVP (Lyu et al., 2023; Lee et al., 2022). The directional change in faba bean TVP expansion correlates to the change in bulk density where more expansion creates a less dense extrudate with more or larger air pocket pores (Fig. 3-1). For example, as moisture content decreases from 55% to 50% there is a corresponding increase in expansion (Fig. 3-2a) and a decrease in bulk density (Fig. 3-2c). When fixing moisture at 50% or 55%, an increase in faba bean protein content led to slightly better TVP expansion but a stronger increase in bulk density.

For pea TVP the expansion was not significantly impacted by moisture content, but 80% protein had better expansion (1.95-2.14 ER: expansion rate) than pea at 60% (1.78-1.82 ER) and 70% (1.82-1.87 ER) protein content (Fig.3-2). An increase in moisture from 50% to 55% caused an increase in bulk density with a corresponding decrease in expansion ratio. Higher moisture led to a less viscoelastic protein melt for expansion.

Most research on protein content and expansion or bulk density focus on protein content of expanded snack products (10-50% protein) rather than high protein content (50-80% protein) of expanded TVP products. In the extrusion of snack product, protein will compete with starch for water, therefore limiting the amount of water available for starch during extrusion (Buhler et al., 2022). Thus, in a high starch LME product, the addition of protein normally causes decreased extrudate expansion. However, TVP is a high protein LME product, the conventional understanding of higher protein content causing less expansion and higher bulk density can not be extrapolated to TVP products. It is interesting in this work that high protein content can improve the TVP expansion to certain extend. However, research on the impact of protein content on TVP products is limited and should be further explored.

Pea protein vs. faba bean protein

Faba bean TVP bulk density ranged from 0.43-0.60g/cm3, and pea TVP from 0.26-0.45g/cm3. The pea TVP showed a lower bulk density than those based on faba bean when prepared at each moisture and protein level. Bulk density has been attributed to changes in TVP cell wall structure and pore size distribution. Generally lower bulk density values of pea extrudates suggest that either larger air pockets or more air pockets are formed from the pea protein structure leading to a decrease in bulk density. This indicates that the pea TVP overall may be lighter and airier (higher expansion, lower density) compared to the faba bean extrudates.

Although pea and faba bean have similar 7S and 11S globular structures, differences in emulsion and foaming capabilities, protein solubility (Kimura et al., 2008) and their tendency to aggregate (Yang et al., 2018) may contribute to their differences in expansion and bulk density among pea and faba bean extrudates. For example, pea foams formed under pressure and heat from extrusion can lead to greater expansion. Higher solubility of pea protein could allow it to better diffuse and unfold to adsorb at the air-water interface, forming viscoelastic films around air bubble surfaces during extrusion, which could partially explain an increase in expansion and lower TVP density. Whereas faba bean protein has lower solubility and higher tendency to aggregate (Yang et al., 2018). Therefore, increasing protein molecular size could interfere with their ability to move (slower) and unfold (bound as aggregates) to adsorb on air-water interface for bubble stabilization, resulting in a less expanded, denser extrudate.

3.1.2 Water holding capacity (WHC) and oil holding capacity (OHC) of TVP

Extrudate microstructure and the presence of exposed hydrophilic and hydrophobic amino acid groups can have an impact on a protein’s ability to hold water or oil after extrusion (Webb et al., 2023). Water and oil binding content of TVP are important functionalities if being used in meat analogues to mimic the mouthfeel and texture of animal meat products, which impact not only tenderness and juiciness but also cook loss. Therefore, water and oil holding capacity of faba bean and pea TVP along with their starting materials were evaluated. Compared to the corresponding starting materials, extrusion increased WHC of faba bean TVP at 60% protein (2.57-2.65g/g) and 70% protein (2.30-2.80g/g) at 50% and 55% moisture respectively, which is most likely due to the uptake of free water filling the pores of TVP and binding to exposed hydrophilic protein groups (Fig. 3-2b (Beck et al., 2017; Webb et al., 2020). Conversely, an increase in protein content to 80% decreased WHC of faba bean TVP (1.39-1.51g/g) for both 50% and

55% moisture respectively.

WHC of pea TVP was higher than the starting material (1.57g/g) at 60% protein (2.85-3.13g/g) but the WHC was lower than the corresponding starting materials (2.21-3.12g/g) at 70% protein (1.41-1.42g/g) and 80% protein (1.39-1.29g/g) at 50% and 55% moisture respectively. The reduced water holding for TVPs made from 80% faba bean protein and from 70-80% pea protein may be due to excess protein denaturation and aggregation from exposure to heat and shear force, and therefore fewer exposed hydrophilic amino acid side chains to bind water. In addition, there were decrease in starch levels at higher protein content, and less starch could prevent additional water holding capabilities (Webb et al., 2020). Moisture at 50% or 55% had no effect on WHC for either faba bean or pea TVP.

Oil holding capacity is an important characteristic in meat analogues to improve texture and mouthfeel but to also enhance or amplify meat-like flavours. Faba bean and pea TVP had similar values of oil holding capacity (OHC) (2.25-2.44g/g and 2.19-2.57g/g for faba bean and pea TVP respectively) (Fig. 3-2). Therefore, all faba bean and pea TVP were comparable and can offer additional oil holding capabilities as an ingredient in meat analogue food products. Extrusion improved oil holding capacities in all TVP compared to the starting materials. This increase in oil holding could be attributed to denaturation of the proteins while under extrusion heat and shear conditions, causing unfolding and opening up of protein structures, thus exposing more hydrophobic groups (Osen et al., 2014). The increase in OHC in the TVP are also attributed to the formation of pore structures during the expansion of TVP at the extruder die.

3.1.3 Comparison of pea/faba protein TVPs to those based on soy/wheat proteins

The expansion of faba bean (1.56-1.75 ER) and pea (1.78-2.14 ER) tended to be slightly lower than other soy TVP ER (1.25-3) and similar in density (0.06-0.7g/cm3) (Lyu et al., 2023), but denser than wheat TVP (0.16-0.29g/cm3) (Maningat et al., 1999). Faba bean and pea proteins are similar to soy proteins (contain albumins and globulins) and are all quite different from wheat protein which consists of gliadins and glutenins. The WHC of extrudates vary drastically among literature due to extrudate size, porous structure, and WHC method. Pea, soy and wheat TVP WHC at 60% protein content range between 1.5-4g/g (Esbroeck et al., 2023; Lyu et al., 2023) with wheat having the larger WHC between 2.5-4g/g (Maningat et al., 1999). The WHC of TVP at 60% protein content and 70% faba bean content were comparable to those reported in literature. Though the 80% TVP and pea at 70% were lower in WHC but higher in protein content than reported . TVP OHC of faba bean (2.22-2.44g/g) and pea (2.19-2.57g/g) were higher compared to other TVP sourced from pea (0.93g/g), soy (0.74g/g), and wheat (0.79g/g) (Hong et al., 2022).

From this study, faba bean protein can compete with pea TVP based on WHC and OHC functionalities even though they show less expansion or higher bulk density than soy protein or wheat gluten based TVPs. Nevertheless, a dense structure may be desired in certain products that faba bean and pea TVP could provide, for instance, more resilient and springier than soy TVP patties.

3.1.3 Study of pea and faba protein structure and interactions in TVP

The SDS-PAGE gels run under reducing and non-reducing conditions for both starting materials and faba bean & pea TVP and the results are shown in Fig. 3-3. Bands characteristic of faba bean protein subunits under non-reducing conditions in the starting material (SM) were visible as legumin minor subunits (~75-80kDa), convicilin (CV) (~70kDa), legumin A (L-A) (~60kDa), legumin B (L-B) (~60kDa), vicilin (V) (~50kDa), α-legumin (L-α) (~40kDa), β-legumin (L-β) (~20kDa), and small protein aggregates below 20kDa. All major bands present in faba bean (SM) became faint in TVP, indicating extrusion caused protein denaturation and aggregation. The bands at 20kDa (β-legumin), 40kDa (α-legumin), 50kDa (vicilin) and 60kDa (legumin A and B) appeared in TVP under reducing conditions once the disulfide bonds were cleaved by sodium dodecyl and 2-mercaptoethanol. This result indicates that disulfide bonds played an important role to contribute faba bean protein aggregation after unfolding by extrusion. Faba beans have a higher content of sulfur containing amino acids (0.19-0.34% dry basis) than pea (0.23-0.15 % dry basis) of methionine and cysteine respectively, which may be contributing to increased aggregation of faba bean proteins (Martineau-Côté et al., 2022).

Bands characteristic of pea protein subunits under non-reducing conditions were visible as convicilin (CV) (~70kDa), legumin (~60kDa), vicilin (V) (~44kDa), α-legumin (L-α) (~40kDa), vicilin fragments (~30-35kDa), β-legumin (L-β) (~20kDa), and small protein aggregates below 20kDa (Fig. 3-3). Pea protein also underwent unfolding and aggregation by extrusion as the major bands became faint in TVP. On the other hand, the bands are stronger in pea protein TVPs that those of faba bean protein, supporting more protein aggregation in faba bean protein TVPs when compared to pea protein.

Fourier-transform infrared spectra of TVP are displayed in Fig. 3-4. The protein mix contained 7 dominant bands at 1628 (β-sheets), 1643 cm-1 (random coil), 1660 cm-1 (α-helix), 1670 cm-1 (β-turn), 1680-1693 cm-1 (β-sheets/turns) and 1608 cm-1 (amino acid residues). The band at 1618 cm-1 corresponds to protein aggregates of intermolecular β-sheets formed by hydrogen bonds (Yang et al., 2021). FT-IR spectra showed denaturation of proteins from extrusion processing at all protein levels and moisture contents for both pea and faba bean TVP as the intensity of the bands associated with major secondary structures were decreased significantly as expected.

3.1.4 Microstructure of TVP

Protein (red) and starch (blue) distribution within faba bean and pea TVP were analyzed by confocal imaging as shown in Fig. 3-5 and 3-6 displaying a starch-in-protein network with both a starch phase and protein phase. This suggests protein and starch phase separation, while both holding the physical structure of the TVP together. The protein content had obvious impact on the starch-in protein matrix for all faba bean and pea TVP samples. Dispersion of starch (Fig. 3-5 a, d) at the 60% protein level in pea TVP were homogenously spread within the pockets of the protein matrix, starch and oblong sized pores elongated in the same orientated direction. This elongation of the protein matrix is orientated with the length of the extruder barrel within the melting zone after expansion at the extruder die. A further degree of phase separation may have occurred in the 70% pea protein TVP (Fig. 3-5 b, e) between the starch and the protein matrix which is displayed as elongated protein structures consisting of gelatinized starch particles gathering in the larger pores of the protein matrix. The 80% pea protein TVP (Fig. 3-5 c, f) had a distinctively dense protein matrix with less homogenous distribution of starch located sporadically deep in the protein matrix.

Generally, the faba bean TVP had smaller air pockets and the protein matrix was more dense due to high level of protein aggregation, and had noticeable differences in the 80% faba bean protein TVP (Fig. 3-6 c, f) compared to the pea TVP. This dense structure was confirmed by lower expansion ratio and higher bulk density values in faba bean TVP compared to pea. This result suggests that certain amount of starch (higher than 5%) is required for even distribution and expansion and structuring of faba bean protein based TVP.

Confocal imaging suggests that 70% protein in both faba bean and pea has a higher degree of phase separation between protein and starch which can be a favourable structure to form continuous protein matrix with starch distribution in the pockets and the air pockets (shown as black) formation indicates the protein matrix in TVP structure was strong to hold together tightly during air expansion. In addition, the elongated protein matrix allowed some fiber structure formation in TVP to better simulate meat fibers.

3.1.5 Summary

- This study was able to expand beyond previous work that that is limited to investigating input and output extrusion parameters of a black box technology on TVP functionalities. By correlating microstructures modulated by protein content and moisture to expansion and subsequently TVP quality, industry ingredient and product developers can better understand the inter-molecular structures of TVP to adopt new protein sources.

- Both faba bean and pea protein can become viable high protein sources to expand the TVP market options and value. Faba bean protein TVP had greater protein aggregation lessening expansion, though microstructure and functionality was similar to that of pea protein TVP.

- TVP microstructure shifted at 60% protein from a homogenously dispersed starch-in-protein phase with evenly dispersed oblong pockets to larger sporadic air pockets within a denser protein matrix at 80% protein, implying a degree of starch phase separation is required at 70% protein to improve TVP expansion and quality.

- This study has also shown that high protein fractions from air classification can be used as a more sustainable base formulation supplemented with protein isolates to prepare TVP with high protein content, but also with desirable TVP microstructure and quality.

3.2 Pea/faba bean protein TVPs with oat flour (protein + fiber) inclusion

3.2.1 Formulation development

The fiber inclusion in extruded snack products has been shown to provide beneficial health responses by extending postprandial glucose release, possibly affecting satiety responses (Brennan et al., 2012). A Government of Canada's source of fiber health claim could be applicable in the final meat analogue product if it contained was a minimum of 2g β-glucan or pea hull fiber per 75g serving size (Food and Drug Regulations, 2024). In addition, the mixture of oat and pulse proteins provides a strategy to address the nutritive issues of plant protein-based food products as they are complementary in essential amino acids. Pulse proteins are high in lysine but lacking in sulfur containing amino acids. Whereas oat protein is high in sulfur containing amino acids but lacking in lysine. Therefore, the TVPs produced with their mixture contain all essential amino acids with improved nutritive value.

Contradicting reports on insoluble fiber either increasing or decreasing expansion based on particle size mostly involve starch based extrudates and not protein based TVP. Small insoluble particle sizes have been shown to increase expansion by increasing the number of nucleation sites so that air pockets can form while at the same time acting as a filler distributed uniformly in cell walls without causing cell rupture (Wang et al., 2017). Studies have also shown a decrease in expansion from insoluble fibers rupturing cell structure, specifically at higher inclusion rates of larger particle sizes (Wang et al., 2017; Robin et al., 2012). There is still limited knowledge on how insoluble and soluble fibers interact with the structural matrix of low moisture extruded products, especially in TVP (Xiao et al., 2023), therefore further investigation in this area is warranted for improved extrudate nutrition.

In this work, oat protein and oat β-glucan (composed of 34% soluble fiber) were purchased from Lantamman (Stockholm, Sweden). Pea hull fiber 125 and 200 was purchased from Avena (Regina Saskatchewan, Canada) composed of 79.6% insoluble fiber. The combination of 50-60% protein with 20% starch allowed TVPs with good expansion (Fig.3-7), thus for pea protein and faba bean protein raw materials with pea insoluble fiber inclusion, the formulations contain ~60% protein and ~16% starch, as well as 5% and 10% pea insoluble fiber with two different fiber sizes (150μm and 100 μm). For raw materials with soluble fiber inclusion, the formulations contain 50-60% protein and ~20% starch, as well as 5%, 10%, 20% oat β-glucan. The oat protein TVP formulation combined oat protein concentrate, pea isolate, and pea starch to reach the protein content of around 60% protein, 20% starch and 5% β-glucan (naturally containing in oat protein concentrate).

3.2.2 Impact of insoluble fiber on TVP expansion

Inclusion of insoluble fibers may cause the bubble rupture, leading to TVP with lower expansion compared to the TVPs without fiber inclusion. In addition, increase in insoluble fiber content cause further decrease in expansion from 5% fiber (1.81, 1.70 ER) to 10% fiber (1.57,1.54 ER) with inclusion of 150μm and 100 μm pea fiber respectively (Fig. 3-8). Wang et al. (2017) found that an inclusion of insoluble fiber from cherry pomace to expanded corn starch extrudates at 5% increased expansion whereas inclusion at 15% decreased expansion. Although the addition of pea hull fiber at 5% inclusion did not increase expansion, the results align with previous reports such that as insoluble fiber content increases to 10% there is a decrease in expansion. Since the insoluble fiber acts as a filler, due to its incompatibility, the increase in fiber content to 10% may be too high for the protein phase to hold the protein matrix in a continuous phase, and the additional starch is not enough to support the expanded matrix when cooled (Deng et al., 2023). Collapse or bubble rupture could occur from lack of structural integrity (interruption of protein-protein bond formations) or from fibers piercing the protein matrix, resulting in decreased expansion. A decrease in particle size from 150μm (1.81, 1.57 ER) to 100μm (1.70-1.54 ER) at 5% and 10% fiber content respectively resulted in a decrease in expansion. ER was lower than that of soy protein based TVP from 50-70% protein levels with inclusion of rice bran insoluble fiber from 2.2-2.27 ER, but within range of rice protein based TVP ranging from 1.34-1.97 ER (Pengjun et al., 2023).

3.2.3 Impact of soluble fiber (oat β-glucan) on TVP expansion

TVPs with oat β-glucan (BG) inclusion have larger expansion values (Fig. 3-8) overall at 5%, 10% and 20% inclusion (1.95-2.07 ER) than the TVPs containing insoluble fiber (1.54-1.81 ER). This can be due to its better compatibility of β-glucan within the protein-starch melt, stemming from its high-water solubility (Kristiawan et al, 2020). 10% β-glucan was highest in ER compared to 0% and even 5% β-glucan with little difference in expansion when β-glucan was increased to 20%, indicating that an addition of 10% or 20% β-glucan to TVP formulations can improve TVP expansion.

It was initially expected that the strong gelling properties of oat protein may lead to increased TVP structure and quality. However, in this work, oat protein TVPs with 5% fiber show significantly lower ER (1.43) than other TVPs samples. In addition, the oat protein TVPs show darker color and denser structure, although it still contained larger cell pockets. The oat protein raw material was then tested to have minimum gelling capacity, which is different from the oat protein concentrates extracted in the lab. Therefore, it is possible that the industry oat protein ingredient underwent some structural change (e.g., denaturation, binding of phenolics) that may negatively impact its color and functional properties. Unfortunately, the oat protein fractions generated by the pilot air classification in Dr. Chen's lab does not have CFIA certification for food and is therefore not suitable as a raw material for extrusion processing in the Food Processing Development Center at Leduc.

It should be mentioned that the insoluble and soluble fibers had a large impact visually (Fig. 3-7) on TVP expansion and the pore structure and size, however the impact on the bulk density (BD) is not significant. TVPs containing insoluble fiber and soluble fiber show BD of 0.35-0.43g/cm3 and (0.30-0.44g/cm3, respectively.

All the TVPs containing oat β-glucan have significantly lower BD than the TVPs containing insoluble fibers. According to the TVP photoimaging (Fig. 3-7), the driving force of lower BD is larger sized pores, which in turn may lead to thicker cell walls. BD of TVPs with insoluble fiber are within range of TVP made with soy or rice protein with rice bran inclusion (BD from 0.26-0.34 g/cm3 and 0.63-0.88 g/cm3 respectively) (Pengjun et al., 2023). BD of the TVPs containing β-glucans of 5%, 10% and 20% are all lower than other TVP made from faba bean protein at 5.6% β-glucan (BD of 0.48-0.52 g/cm3) (Saldanha et al., 2023), whereas the oat protein based TVPs was within that reported range.

3.2.4 Water holding capacity (WHC) and oil holding capacity (OHC) of TVP

WHC (Fig. 3-9) was significantly improved for TVPs with the addition of both insoluble fiber (2.73-3.16g/g) and soluble fiber (2.60-2.98g/g), and for the TVP made from oat protein (1.99g/g), when compared to the TVP with no added fiber (1.41g/g). The insoluble fiber TVP tended to have small pores which are more likely to hold water from capillary action (Esbroeck et al., 2024). Whereas the β-glucan can bind water effectively prior to extrusion when compared to the insoluble fiber (Sayanjali et al., 2017). The WHC of TVP with fiber inclusion was generally higher than the averages for soy (2.1g/g), pea (2.1g/g) and wheat (1.9g/g) TVP (Hong et al., 2022).

OHC was also improved by the addition of both insoluble pea hull fiber (2.42-2.54g/g) and soluble β-glucan (2.52-2.57g/g) compared to TVP with no fiber addition (2.29g/g). All TVP were higher than other reported OHC of TVP from 0.69-1.04g/g of assorted protein sources like soy, pea, gluten, chickpea, and navybean (Hong et al., 2022).

3.2.5 TVP microstructure with fiber inclusion

Confocal imaging of TVP highlights the starch and fiber (dyed blue) size, orientation, and effects on the protein matrix which is dyed red (Fig 3-10). The protein matrix is highly disrupted by the insoluble fiber compared to the TVP with no fiber addition. The rigid structure from additional hydrogen bonds contributes to higher glass transition and melt temperatures (220°C-250°C) of insoluble fiber, therefore providing resistance to deformation during extrusion, leading to thermodynamic immiscibility with the dominant protein phase (Deng et al., 2023; Ek et al., 2020). A significant decrease in ER of insoluble fiber TVP with little change in density suggests that there is an increase in small air pockets from many nucleation sites that do not grow in bubble size to cause an increase in expansion. This is supported by the observation of the micronucleation caused by insoluble pea hull fiber seen in confocal imaging.

Confocal imaging of TVP containing soluble β-glucan (Fig. 3-11) shows very different protein matrix structure and fiber distribution compared to the TVP including insoluble fiber. At 5%, β-glucan is accumulated as a separate phase from the protein and are dispersed homogenously within the formed air cells of the protein matrix. β-glucan is still homogeneously distributed throughout protein matrix at the 10% and 20% inclusion levels. In addition, the red protein becomes dominantly pinker in colour, indicating the dilution of protein and incorporation of blue starch or fiber into the protein matrix. It is possible that the blending of the soluble fiber into the protein phase helped TVP expansion, leading to a decrease in bulk density. However, the mechanisms will need to be investigated in future studies.

3.2 Summary

- Oat β-glucan inclusion at the level of 10-20% improved expansion, water holding and oil holding capacities of all pea TVPs.

- Inclusion of insoluble pea hull fiber decreased TVP expansion due to the disruption of protein matrix, although the TVPs show increased WHC and OHC.

- Based on results from this study, there were three TVP which displayed the most promising functionality (ER, BD, WHC and OHC) overall without compromising protein content; i) the pea with 70% protein content and 50% moisture content, ii) the pea TVP with 10-20% β-glucan as soluble fiber. Moreover, the addition of small amounts of pea starch (high in amylose) can improve the structural and functional quality of new or underutilized protein sources as was true for faba bean TVP.

- Inclusion of β-glucan improved TVP functionality, but the effect of this β-glucan on human health was outside of the scope of this study and yet to be investigated. Incorporation of pulse protein TVPs enriched with β-glucan into food systems such as a burger patty could provide additional health benefits on human health such as improved laxation, healthier gut microbiota, and lower cholesterol, but will need to be demonstrated by in-vivo and clinical studies in the future.

Study innovations and implications for the advancement of agricultural science
This is the first study to show evidence of faba bean protein's capability and potential as another alternative source of plant protein for TVP production in addition to pea protein. Because faba bean and pea are not regarded as major allergens, they could be a replacement for soy and wheat protein in TVP production. This results also demonstrate that it is promising to combine pulse proteins and oat β-glucan for TVP production with desirable sponge-like quality and uptake of water or oil needed to improve meat analogue texture. Therefore, this research has opened new commercialization opportunities for pea, faba and oat in human food production, and the development of texturized vegetable protein products (TVP) could be a stepping stone to increasing pulse and oat protein consumption in the human diet.

This research is also the first to prove the capability of TVP production from protein fractions by air classification supplemented with protein isolate. Air classification processing of proteins, followed by extrusion for TVP fabrication can reduce the energy and costs of the whole processing, compared to the TVP preparation from purified protein (protein isolates) by wet extraction.

The new knowledge generated by investigating deeper into the effect of protein, starch, and dietary fiber on microstructure and functionality in TVP has expanded our understanding beyond current work focusing just on input and output parameters of extrusion, thus will provide scientific insight to guide TVPs product development not only from pulse and oat, but also from other plant sources.

Tables, graphs, manuscripts, etc., may be included as appendices to this report.

Literature cited

> Provide complete reference information for all literature cited throughout the report.

Beck, S. M., Knoerzer, K., & Arcot, J. (2017b). Effect of low moisture extrusion on a pea protein isolate's expansion, solubility, molecular weight distribution and secondary structure as determined by Fourier Transform Infrared Spectroscopy (FTIR). Journal of Food Engineering, 214, 166–174.
Brennan, M. A., Derbyshire, E. J., Brennan, C. S., & Tiwari, B. K. (2012). Impact of dietary fibre-enriched ready-to-eat extruded snacks on the postprandial glycaemic response of non-diabetic patients. Molecular Nutrition and Food Research, 56(5), 834–837.
Buchko, A. (2024) MSc. thesis supervised by Dr. Lingyun Chen, University of Alberta
Bühler, J. M., van der Goot, A. J., & Bruins, M. E. (2022). Quantifying water distribution between starch and protein in doughs and gels from mildly refined faba bean fractions. Current Research in Food

Science, 5, 735–742.
Deng, Q., Wang, Z., Fu, L., He, Z., Zeng, M., Qin, F., & Chen, J. (2023). High-moisture extrusion of soy protein: Effects of insoluble dietary fiber on anisotropic extrudates. Food Hydrocolloids, 141, Atricle 108688.
Dickinson, E. (2018) On the road to understanding and control of creaminess perception in food colloids, Food hydrocolloids, 77, 372-385.
Esbroeck, T., Sala, G., Stieger, M., & Scholten, E. (2023). Effect of structural characteristics on functional properties of textured vegetable proteins. Food Hydrocolloids, 149, Article 109529.
Hong, S., Shen, Y., & Li, Y. (2022). Physicochemical and Functional Properties of Texturized Vegetable Proteins and Cooked Patty Textures: Comprehensive Characterization and Correlation Analysis. Foods, 11(17), 2619.
Jo, Y.J., Chu, Y., Chen, L. (2023) Enhanced stabilization of oil-in-water (O/W) emulsions by fibrillar gel particles from lentil proteins, Food Research International, 172, 113203.
Kew, B., Holmes, M., Stieger, M., Sarkar, A. (2020) Review on fat replacement using protein-based microgels, Trends in Food Science and Technology, 106, 457-468.
Kimura, A., Takako, F., Meili, Z., Shiori, M., Maruyama, N., & Utsumi, S. (2008). Comparison of physicochemical properties of 7S and 11S globulins from pea, fava bean, cowpea, and French bean with those of soybean-french bean 7S globulin exhibits excellent properties. Journal of Agricultural and Food Chemistry, 56(21), 10273–10279.
Kristiawan, M., & della Valle, G. (2020). Transport phenomena and material changes during extrusion. Extrusion Cooking (pg 179–204).
Lee, J. S., Oh, H., Choi, I., Yoon, C. S., & Han, J. (2022). Physico-chemical characteristics of rice protein-based novel textured vegetable proteins as meat analogues produced by low-moisture extrusion cooking technology. LWT, 157, 113056.
Liu, G., Huang, W., Babii, O., Gong, X., Tian, Z., Yang, J., Wang, Y., Jacobs, R.L., Donna, V., Lavasanifar, A., Chen, L. (2018). Novel protein-lipid composite nanoparticles with an inner aqueous compartment as delivery systems of hydrophilic nutraceutical compounds. Nanoscale,10, 10629-10640.
Lyu, J. S., Lee, J. S., Chae, T. Y., Yoon, C. S., & Han, J. (2023). Effect of screw speed and die temperature on physicochemical, textural, and morphological properties of soy protein isolate-based textured vegetable protein produced via a low-moisture extrusion. Food Science and Biotechnology, 32(5), 659–669.
Maningat, C., Demeritt, K., Chinnaswamy, R., Bassi, D. (1999). Properties and applications of texturized wheat gluten. Cereal Foods World, 44(9), 650-655.
Nieto-Nieto, V., Wang, Y., Ozimek, L., Chen, L. (2015), Inulin at low concentrations significantly improves the gelling properties of oat protein, Food Hydrocoll., 50, 116–127.
Osen, R., Toelstede, S., Wild, F., Eisner, P., & Schweiggert-Weisz, U. (2014). High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. Journal of Food Engineering, 127, 67–74.

Pengjun, T., Sringarm, C., Kunanopparat, T., Rungchang, S., Ditudompo, S., & Siriwattanayotin, S. (2023). Effect of defatted rice bran addition on properties of texturized soy and rice protein products. International Journal of Food Engineering, 19(10), 435–444.
Robin, F., Schuchmann, H. P., & Palzer, S. (2012). Dietary fiber in extruded cereals: Limitations and opportunities. Trends in Food Science and Technology, 28(1), 23–32.
Saldanha do Carmo, C., Rieder, A., Varela, P., Zobel, H., Dessev, T., Nersten, S., Gaber, S. M., Sahlstrøm, S., & Knutsen, S. H. (2023). Texturized vegetable protein from a faba bean protein concentrate and an oat fraction: Impact on physicochemical, nutritional, textural and sensory properties. Future Foods, 7, Article 100228.
Sayanjali, S., Ying, D., Sanguansri, L., Buckow, R., Augustin, M. A., & Gras, S. L. (2017). The effect of extrusion on the functional properties of oat fibre. LWT, 84, 106–113.
Wang, S., Kowalski, R. J., Kang, Y., Kiszonas, A. M., Zhu, M. J., & Ganjyal, G. M. (2017) Impacts of the Particle Sizes and Levels of Inclusions of Cherry Pomace on the Physical and Structural Properties of Direct Expanded Corn Starch. Food and Bioprocess Technology, 10(2), 394–406.
Webb, D., Plattner, B. J., Donald, E., Funk, D., Plattner, B. S., & Alavi, S. (2020). Role of chickpea flour in texturization of extruded pea protein. Journal of Food Science, 85(12), 4180–4187.
Xiao, T., Su, X., Jiang, R., zhou, H., & Xie, T. (2023). Low moisture extrusion of soybean protein isolate: Effect of β-glucan on the physicochemical properties of the product. LWT, 179, Article 114660.
Yang, J., Liu, G., Zeng, H., & Chen, L. (2018). Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. Food Hydrocolloids, 83, 275–286.
Yang, J., Zamani, S., Liang, L., Chen, L. (2021). Extraction methods significantly impact pea protein gelling properties, Food Hydrocolloids, 117, https://doi.org/10.1016/j.foodhyd.2021.106678.
Zhang, S., Huang, W., Feizollahi, E., Roopesh, MS., Chen, L. (2021) Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study, Innovative Food Science & Emerging Technologies, 67, 102567.

Benefits to the industry

> a. Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).
> b. Quantify the potential economic impact of the project results (e.g., cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

This research has developed an innovative method to prepare microgels from pulse proteins combined with oat fractions, demonstrating that these microgels can partially replace fat in low-fat emulsion-based food products while maintaining desirable texture and consistency. A patent application is currently being prepared with the assistance of the Technology Transfer Services (TTS) Team at the University of Alberta.

In the short term, we plan to engage with protein ingredient and food manufacturing industries, leveraging the support of the TTS team to attract industry investment. This collaboration aims to adapt the microgel technology for various food applications and assess the feasibility of scaling up microgel production at the pilot level. Success at this stage is expected to convince industry partners to license the technology and advance it towards large-scale commercialization in the medium term.

Additionally, this research highlights the potential of faba bean protein as an alternative plant protein source for textured vegetable protein (TVP) production, alongside pea protein. Since faba bean and pea are not considered major allergens, they present a viable replacement for soy and wheat proteins in industrial TVP production. Moreover, combining pulse proteins with oat β-glucan has resulted in TVPs with improved expansion, desirable sponge-like qualities, and enhanced water or oil absorption, which are crucial for improving the texture of meat analogues. Given that pilot-level production of TVPs has been successfully achieved at the Food Processing Development Center in Leduc, the technology is poised for rapid adoption by industry in the short term.

In the medium term, the adoption of TVP and microgel technologies by industry will lead to the development of new products, businesses, and job opportunities. The fat replacers market, valued at USD 2.6 billion in 2023, is projected to grow at a CAGR of 6.2% over the next five years, driven by evolving dietary preferences and increased health awareness. As obesity rates and lifestyle diseases rise, there is a growing demand for low-fat alternatives. Furthermore, TVP products represent a significant step towards increasing plant protein consumption in human diets.

In the long run, the wide range of applications for pulse and oat-based TVPs and fat replacers will significantly contribute to their consumption in human diets. This increased demand will help stabilize or expand the acreage of pulses and oats in Western Canada. Contracts at premium prices for specific food applications will boost revenue for pulse and oat growers.

The success of this project will offer consumers more healthy food choices that complement animal proteins. The American Heart Association (2017) recommends limiting dietary intake of saturated fat to less than 10% and gradually replacing it with other macronutrients. Incorporating oat β-glucan in TVPs is also highly desirable, as current fiber intake in Western diets falls below the recommended 25-30g per day. Additionally, producing health foods from both animal and plant ingredients will contribute to more sustainable food systems.

Attachments

Attachments

Please attach any supplemental documents

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