Interim Report

Project title: Development of healthy food products by combining proteins and dietary fibers from oats and pulse

Project period: March 1, 2023, to February 29, 2024

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Project details

a. Background: A dietary pattern that provides plant protein, dietary fiber and low fat has been shown to decrease the risks of chronic diseases (obesity, cardiovascular disease). This food trend is also driven by consumers looking for sustainable plant-based options. The milling oats in Canada are good sources of both fiber and protein (10% total fiber, at least 4.5% β-glucan); some varieties contain 15-20% protein. Currently most Canadian oats is used as animal feed, and <10% for food applications. Oat fractionation into value-added ingredients has provided a strategy to promote oat human consumptions. Oat β-glucan has cholesterol-lowering and glycemic control effects. Oat protein is more nutritious than most cereal proteins with greater nutritive value. Oat starch and oil also attract interests as cosmetic ingredients. The combination of the fractionation capability and the emerging interest in plant-based ingredients has created a product development opportunity from oats. 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. 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. 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. This provides an opportunity to develop new fat-replacers of plant origin. The inclusion of dietary fiber in the formulation is positive because current fiber intake in western diets is well below the recommended 25–30g/day. Nevertheless, technology innovation is required to combine functionalities of proteins and fibers from oat and pulse for tailoring the microgel rheological properties to simulate solid fats particles.

Concerns over GMO and allergenicity have triggered industry interest in texturized vegetable protein products (TVPs) from pulses. The major constraint is the texture due to the weaker gelling capacity of the pulse proteins compared to soy. Given the excellent gelling of oat protein, its combination with pulse will potentially lead to new TVP of improved texture. Incorporating dietary fibre in meat analogue products is also highly desirable by health-conscious consumers. A similar concept has led to a commercial product “Pulled Oats®” in Finland that combine oats and beans to make the products resembling pulled pork, but the production is a trade secret. Innovations in formulation and extrusion processing are yet to be developed to combine pulse and oat to make meat substitute products balanced in protein and fiber, but also satisfactory chewy texture to simulate real meat products.

b. Objectives

The long-term objective of this research is to develop high quality protein and dietary fiber ingredients from oats for healthy food development.

The short-term objectives in the next 2 years are to:

1. Screen oat variety & optimize processing to develop high quality protein (or mixture with dietary fiber) ingredients from oat grains and oat processing by-product streams.
2. Develop new techniques to combine oat/pulse protein and dietary fiber ingredients to fabricate
    a) Microgels as fat replacers for dairy and dairy substitute products
    b) Texturized vegetable protein products (TVP) for meat analogue applications

c. Anticipated Results to Industry:

1. Recommendation of oat varieties that are advantages to produce high quality protein and dietary fiber ingredients for food development
2. New technique to combine functionalities of plant proteins and dietary fibers to develop microgel fat-replacers of plant origin
3. Optimized formulation and extrusion processing to fabricate TVPs of improved texture from oat/pulse ingredients
4. New food prototypes such as “fat free” or “low fat” yogurt and ice cream, and veggie burgers from oat and pulse ingredients to provide health food options to consumers

d. Methodology

1. Screen oat variety and by-product streams to develop quality protein/fiber ingredients

Commercial oat varieties of high protein and β-glucan content will be focused, and 4-6 varieties will be selected in consultation with POGA. The milling-air classification processing will be applied to concentrate protein and β-glucan. Two sources of food oats that allow smooth milling/air classification processing to generate fractions high in both protein and β-glucan will be studied in the first step. Then the milling/air classification processing will be fine-tuned for the selected oat varieties to product specific fractions with maximized protein and β-glucan content for food application development. This will be achieved by modulating the milling size and air classification wheel speed. In a good scenario, specific fraction with protein content of 30-40% and β-glucan contents of 12-15% will be obtained. Meanwhile, protein will be extracted from the remaining fractions by the established wet extraction method.

2. Develop microgel fat replacers for dairy/dairy substitute products

2.1 The first stage aims to combine the functional properties of protein and dietary fibers to prepare gels with viscoelastic property like solid fat particles. The proposed strategy is to modulate the gel property by modifying the protein-polysaccharide interactions. The prepared gels will be subjected to both small deformation oscillatory (rheometer) and large deformation measurements (Instron). The gel storage modulus, compressive strength and springiness will be analyzed to interpret the gel viscoelastic properties, in comparison to those of solid lipids. The gel microstructures will be observed by a confocal microscope. The second stage aims to reduce the gel size because the microgels <10um are reported to have lubrication effect. The gel formulation optimized above will be homogenized for size reduction to achieve microgels of desirable size as characterized by the Malvern Mastersizer.

2.2 The optimized microgels will be evaluated as fat replacers in low fat and fat-free yogurt and ice cream. The key food characteristics will be evaluated after replacing fat by the microgels at different inclusion levels. Sensory evaluation: The optimized yogurt and ice cream samples will be evaluated more thoroughly for their sensory quality, in comparison to the full fat counterparts. The panelists (60 consumers) will rate their overall liking of each product, liking of its sensory attributes, and complete Just-About-Right scales, which will then be analyzed to achieve a clear indication of the identity and direction the sensory attributes should be modified to increase acceptance. Additionally, participants will have a survey to identify perceived protein and fiber dietary needs and perceived benefits and barriers to low fat dairy product consumption. Penalty results will be analyzed for refinement of the fat-reduced dairy products to ensure their acceptance by consumers.

3. Develop texturized vegetable protein products (TVP) for meat analogue applications

The major constraint of the pulse protein based TVPs is the texture due to the lower gelling capacity of pulse protein compared to soy. To address this challenge, gelling proteins from oat will be combined in the formulation to fabricate TVPs of improved texture. Low moisture extrusion will be applied in this research which processes at a shorter retention time and higher temperature. When exposed to ambient pressures upon exiting the die, the moisture evaporates, and the viscoelastic mass rapidly expands to generate aerated structure to gives TVP the chewy texture resembling meat particles. Retaining dietary fibre in protein ingredient by incorporating oat fraction in TVP formulation is highly desirable.

3.1 A lab twin screw extruder in Dr. Chen's lab will be applied to prepare the TVPs. The processing will be first established to fabricate TVPs from pulse protein (pea and faba bean protein) by optimizing the protein concentration and moisture content. Then oat fraction (protein + β-glucan) will be combined with pulse protein at various mixing ratios. The extrusion conditions will be fine-tuned to modulate TVP aerated structures. The prepared TVP samples will be evaluated for their porosity, sponge-like structure, density, protein solubility index, and water holding capacity. The rehydrated TVPs will be characterized by an Instron material analyzer to evaluate their texture profiles including tensile strength, springiness and chewiness. The microstructures of the TVP samples will be observed by SEM to analyze air cell size and distribution. The optimized TVPs will be scaled up in Food Processing Development Center.

3.2 TVPs in meat analogues. The optimized TVPs will be applied to develop a set of products such as veggie burgers and crumbles as Taco fillers in the second stage. Preliminary satisfactory samples will be evaluated by the research team. Then two prototypes will be evaluated by sensory evaluation to understand the combination of protein and fibre on the chewiness, juiciness and over texture of the meat analogue products. The evaluation results will be applied to refined and optimize the products with a juicier texture and sensory characteristics similar to the real meat products.

Statistical analysis: ANOVA analysis and Tukey's multiple comparison test will be used to compare means of samples from processing of three batches. Statistical significance level will select to be 5% ($p<0.05$).

Research Progress

1. Screen oat variety and by-product streams to develop quality protein/fiber ingredients

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 protein and β-glucan from starch for the milling oats. It was expected that the spherical shape of starch particles would be selectively carried by air in contrast to the flat-shaped dietary fiber. Two sources of oats commercialized for food applications were focused in the first step 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.

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.

Enrichment of β-glucan

As shown in Figure 1, a fine fraction and a coarse fraction were obtained after the air classification separation for both Cavena Nuda and gluten-free oats. Figure 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).

Figure 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.

Enrichment of protein

Figures 5 and 6 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.

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 and next step

Our results confirm that the 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.

Therefore, in the next step, the suitable processing conditions will be fine-tuned to prepare fractions rich in both β-glucan and protein from milling oats of different varieties with the hope to recommend oat varieties to industry that are advantages to produce high quality protein and dietary fiber ingredients for food development. In addition, the remaining fine fraction will be studied for protein separation from starch by further air classification processing. The optimized processing will be scaled up to a larger pilot level to generate oat protein and dietary fiber fraction for microgel development as fat replacers and texturized vegetable protein products (TVP) development for meat analogue applications.

2. Combine oat/pulse protein and oat dietary fiber to fabricate microgels as fat replacers

The feasibility of improving pea protein gelling capacity by combining pea protein with oat β-glucan was investigated. The pea protein (89% protein) was isolated in the lab from a pea protein concentrate (60% protein) kindly provided by AGT Foods. The protein and polysaccharide concentration for appropriate gel solution preparation were identified to be 15% and 0.25-0.5%, respectively. Higher concentrations led to viscose slurries that were difficult to homogenize. The gels were prepared by heating at 95^{o}C and neutral pH in a water bath for 30 min, followed by storage in a refrigerator at 4oC overnight before analysis. As shown in Figure 2, pea protein alone could not form gels, however, self-standing gels were successfully prepared by adding 0.5% β-glucan in the formulation. This was caused by phase separation of pea protein and β-glucan by heating at neutral pH. Thus, the protein was excluded from a volume occupied by β-glucan, leading to increased protein effective concentration in its phase, which then resulted in increased protein interactions to form stronger gel networks. Addition of κ-carrageenan was also tested for the formulation with the hope to further reinforce the gel matrix due to its known gelling capacity. Figure 7 shows the gel strength measured by the Instron Materials Equipment. The gels prepared by the combination of pea protein (15%) and oat β-glucan (0.5%) demonstrated the compressive strength of 6kPa, which was further increased to 8 kPa with the addition of κ-carrageenan in the gel formulation. The formation of strong gel network was also supported by the gel microstructure observation from the Scanning electronic microscopic photos in Figure 8.

Summary and next step:

Our results demonstrated the feasibility of improving pea protein gelling capacity by combining pea protein with oat β-glucan. In the next step, the oat protein will be added in the formulation and the protein/β-glucan/κ-carrageenan ratios will be adjusted to modulate the rheological properties to better simulate solid lipids. Then microgels will be prepared by size reduction of the gels by homogenization. The performances of the microgels as a fat-replacer will be evaluated in food formulations.

3. Develop texturized vegetable protein products (TVP) for meat analogue applications

The first step aimed to establish the low moisture extrusion conditions to fabricate TVPs from pea and faba bean protein by optimizing the protein concentration and moisture content. Commercial pea (55% protein) and faba bean (60% protein) concentrates were kindly provided by Ingredion, whereas the pea (85% protein) and faba bean (90%) isolates were also kindly provided by AGT Foods. Protein isolates and concentrates were mixed at 60%, 70% and 80% final protein content concentrations. TVP were produced with a co-rotating twin screw extruder (Coperion ZSK 26, Coperion, Stuttgart, Germany) equipped with a 3mm die. All other extrusion parameters were optimized within the desired expansion range to accommodate both pea and faba proteins and held constant throughout the experiment (Table 1). Water levels for appropriate expansion of both faba and pea were identified at 50% and 55%.

Expansion ratio

As shown in Figure 9, at the moisture level of 55%, the expansion ratio (ER) significantly increased when the protein content was increased from 70% to 80% for faba bean protein, whereas such transition was observed from 60% to 70% for pea protein. This means the pea protein TVP prepared by 70% protein was not significantly different from that prepared by 80% protein in terms of the expansion ratio. For faba bean protein, higher expansion ratio was observed when TVP was extruded at the moisture level of 50% than 55% at different protein content levels. However, an adverse trend was observed for pea protein, for example at the protein concentration of 80%, significantly increased expansion ratio was obtained (from 1.67 to 1.97) when increasing the moisture level from 50 to 55%. These results suggest different performances of pea and faba bean protein in response to low moisture extrusion, which needs further investigation. When comparing these two protein sources, pea protein has significantly higher expansion ratio than faba bean protein when prepared at the extrusion same conditions. This might be related to the higher tendency of faba bean protein to form aggregates than pea protein. The strong protein interactions in the aggregates could prevent the TVP expansion by extrusion.

Bulk density

Protein source and concentration had an impact on bulk density such that pea 70% and 80% protein concentration at 55% moisture levels were significantly lower in bulk density than at those same moisture and protein levels in faba bean (Figure 10). Generally lower bulk density values of pea extrudates suggest that larger or more air pockets are formed from the pea protein structure leading to a decrease in bulk density. This indicates that the pea extrudates overall may be lighter and airier compared to the faba extrudates.

The change in faba bean expansion values correlate to the change in bulk density such that more expansion creates a less dense extrudate with more or larger air pockets. Although pea extrudates show less significant variability amongst increasing protein content and changes in moisture, the 80% pea concentration at 55% moisture showed the lowest bulk density, corresponding to the highest expansion ratio.

Water holding capacity

The water holding capacity of the TVPs was evaluated in comparison to the protein flours (F60, F70, F80, P60, P70 and P80) before extrusion. As shown in Figure 11, for faba bean protein, moisture content of extrudate did not change water holding capacity. Within faba bean extrudates the water holding content was significantly lower at the 80% protein level, possibly due to the lack of starches which tend to allow for more water absorption. This is in contradiction to the water holding capacity of the starting materials which increase as protein content increases. This indicates the conformational changes in protein structure from the extrusion process which cause a decrease in water holding capacity. The pea extrudates were more variable in water holding capacity. But the same as faba bean protein, the TVP prepared with 80% pea protein showed relatively lower water holding capacity, likely also due to the protein conformation change and less starch content.

Oil holding capacity

The oil holding capacity of the TVPs was also evaluated in comparison to the protein flours (F60, F70, F80, P60, P70 and P80) before extrusion. As shown in Figure 12, extrusion improved oil holding capacities for each protein source at every concentration and moisture level compared to the starting materials, suggesting that extrusion processing exposed hydrophobic groups of the protein, enabling stronger oil binding. Faba bean and pea extrudates had similar values of oil holding capacity as no significant difference among the samples was identified.

Summary and next step:

The low moisture extrusion processing conditions (moisture 50-55%, protein content of 70-80%) have been established to fabricate TVPs from pea and faba bean protein with reasonably good expansion ratio, bulk density and water/oil holding capacity. In the next step, the texture of the optimized TVPs will be evaluated by the Texture profile analysis using an Instron Materials Testing equipment and the porous structure of the extrudes will be observed by the Scanning Electronic Microscopy. Then oat fractions enriched with protein and β-glucan (coarse fraction) and the oat protein concentrate (extracted from the fine fraction by wet extraction) will be added in the extrudate formulations. It is expected that oat protein addition could improve the TVP texture. Meanwhile, the impact of oat β-glucan on the protein extrusion will be studied with the hope to combine protein and dietary fibers to fabricate TVP of improved nutritive value.

Research challenges and project risks

The hiring of the postdoctoral fellow (PDF) has been delayed due to the visa issue. Thus, a lab technician has been working on the project until the PDF is in place in May.

Research and action plans for upcoming reporting periods

1. Screen oat variety and by-product streams to develop quality protein/fiber ingredients

- The appropriate processing conditions identified in year 1 will be fine-tuned to prepare fractions rich in both β-glucan and protein from milling oats of different varieties.
- The remaining fine fraction will be studied for protein separation from starch by further air classification processing.
- The optimized processing will be scaled up to a larger pilot level to generate oat protein and dietary fiber fraction for microgel and texturized vegetable protein products (TVP) development

2. Combine oat/pulse protein and oat dietary fiber to fabricate microgels as fat replacers

- Oat protein will be added in the gel formulation and the protein/β-glucan/κ-carrageenan ratios will be adjusted to modulate the rheological properties to better simulate solid lipids.
- Microgels will be prepared by size reduction of the gels by homogenization.
- The performances of the microgels as a fat-replacer will be evaluated in food formulations.

3. Develop texturized vegetable protein products (TVP) for meat analogue applications

- Oat fractions enriched with protein and β-glucan and the oat protein concentrate (extracted from the fine fraction by wet extraction) will be added in the pea and faba bean protein based extrudate formulations for improved TVP texture
- The impact of oat β-glucan on the protein extrusion will be studied with the hope to combine protein and dietary fibers to fabricate TVP of improved nutritive value.

- Food prototypes will be developed from the optimized TVP and their sensory quality will be evaluated

Technology transfer activities

Completed:

Oats and Pulses Hook Up: “Let’s Get Together and Compliment Each Other’s Essential Amino Acids!”, Oat Scoop, June 2022 - Prairie Oat Growers Association (POGA)

In the future:

1. One abstract has been prepared to be submitted to the Institute of Food Technologists (IFT) Annual Meeting & Food Expo 2023, Chicago, July 16-19.

2. The research findings including the advantageous oat varieties, new ingredients (fat replacers and TVPs) and new products by combining oats and pulses (e.g., low fat yogurt and ice cream, veggie burgers) from oats will be communicated through Prairie Oat Growers Association (POGA) and Alberta Pulse Growers Commission (APGC) periodic newsletters.

3. The feasibility and advantages of using oat ingredients for health food development will be communicated to Protein Industries Canada (e.g., oral presentation, newsletter) to target its 200 members

4. Dr. Chen is one of the three key investigators of the NSERC-CREATE program: Canadian Agri-food Protein Training, Utilization & Research Enhancement (Dr. Michael Nickerson as PI). Thus, the new knowledge and techniques generated from this research will be communicated to 70 undergraduate and graduate students in the program (e.g. lectures, presentations)

5. Publications (2-3 papers) in peer reviewed journals and 3 presentations in international conferences (IFT, AOCS) are anticipated to target broad audiences from scientific community and industries