

ISSN 2371-0411



GRAIN RESEARCH LABORATORY

Annual Program Report

2022



Canadian Grain
Commission

Commission canadienne
des grains

Canada 

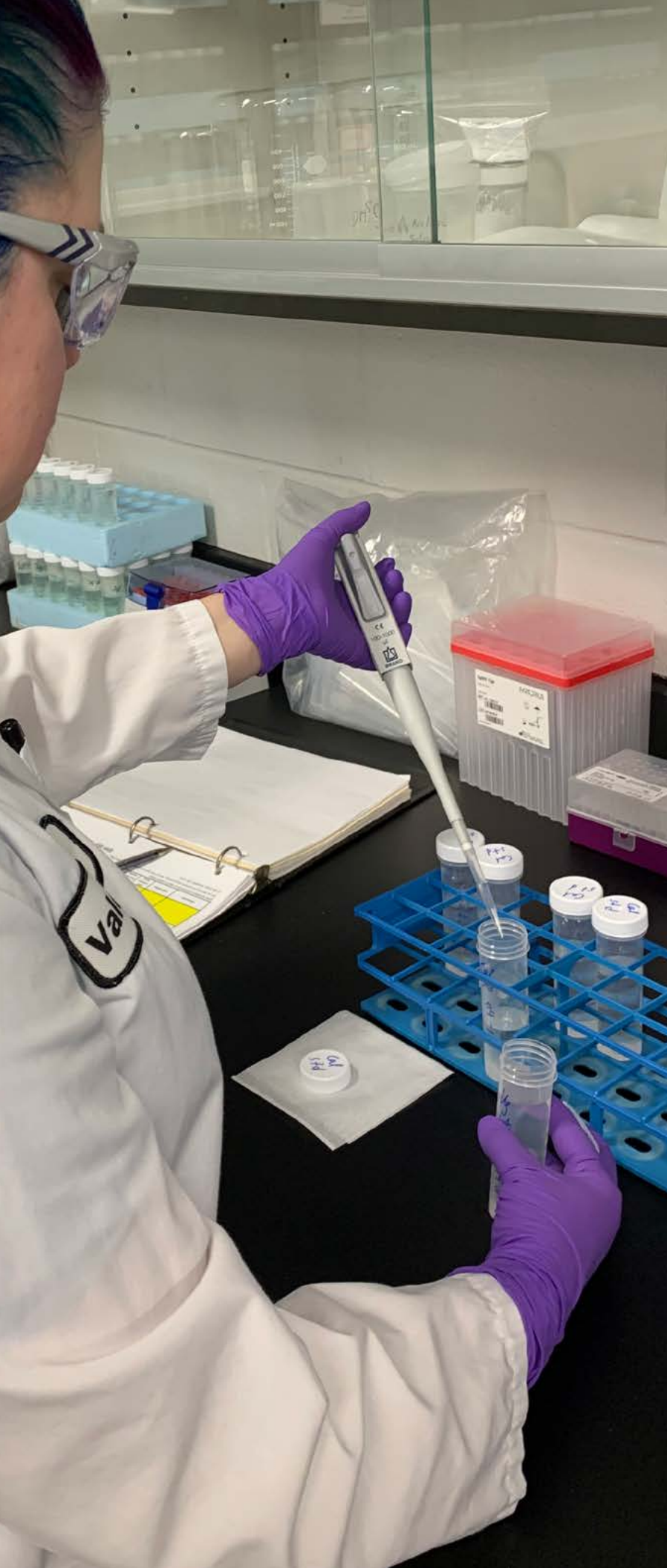


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Grain Research Laboratory

MISSION

- Undertake and promote scientific research on grains and grain products to ensure the quality and safety of Canadian grain for domestic and export markets.
- Enhance the marketability of Canadian grains through research, end-use functionality evaluation, monitoring and analytical services.
- Anticipate and respond to the needs of the grain value chain, through interaction with the grain sector and stakeholders.
- Provide the scientific basis for establishing and maintaining standards of quality and safety for Canadian grain.

In addition to their own research and testing, each of our 8 programs support four key activities:



Cargo quality monitoring

Samples from export shipments are tested to ensure they meet Canada's grain grading and quality parameters.



Harvest Sample Program

Canadian grain producers who voluntarily submit a sample of their harvested crop receive a personalized quality report at no cost.

[Harvest Sample Program](#)



Requests for service analysis

Samples submitted by the grain industry for testing are analyzed, at times for a fee.

[Services](#)



Plant breeder line evaluation

Tests and recommendations are made for the advancement of breeder line seed.



Director's message

Dr. Esther Salvano
Director General
Grain Research Laboratory
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It is my pleasure to share with you the 2022 Grain Research Laboratory Annual Program Report. We had another productive and successful year even as we continued to work and manage our activities in the context of COVID-19. Most of our employees continued working on site while taking measures to protect their health and safety. A new flexible hybrid work model that allowed for a blend of onsite and remote work was implemented in the fall to support those who had been primarily teleworking. The engagement and collaboration of employees resulted in a successful transition to a post-pandemic work environment, which was greatly appreciated.

We concluded our consultation with producers, other members of the grain sector and employees on the research and science-based activities carried out at the Canadian Grain Commission. The responses were compiled into a [report](#) which was made available on our website. The consultation helped us identify trends and challenges in the grain sector that could affect the direction of our research. We used the feedback to start the development of a science strategy that will help us meet the current and future needs of the grain sector.

We also implemented a robust science integrity policy to ensure that our research processes adhere to the strictest ethical principles, promoting transparency, accountability, and reliability in all our scientific endeavors. Guidelines were developed in support of the policy and numerous staff presentations were made.

An important highlight of 2022 was the 95th anniversary of the Harvest Sample Program. The program began in 1927 as a survey of protein levels in wheat and has evolved to include all 21 grains regulated by the Canadian Grain Commission. In 2022, over 10,000 samples of grain were received from producers allowing us to provide valuable information on the quality of crops.

This year's report highlights some of the innovative grain research projects taking place in our labs. We studied the relationship between protein content and bread wheat functionality, and looked at how grain size and shape affect the dehulling performance of oats. We developed a way to produce cost-effective reference standards for cyanogenic glycosides in flaxseed and designed new methods for measuring functional properties of pulses. Our labs also conducted a study to verify the accuracy of our protein calculations, showed how processing can reduce the amount of chemical contamination in wheat, used DNA profiling to monitor grain quality and increased the efficiency of DNA-based methods for detecting genetically modified plants.

We are excited to continue our work in 2023. We will keep adapting and finding new and innovative ways to conduct and promote our research for the benefit of Canada's grain sector. Thank you for taking the time to read this report and I invite you to share your comments and thoughts with us. We look forward to your feedback.

Highlights from 2022

Awards and recognition

The Grain Research Laboratory is committed to pursuing scientific excellence by using and adhering to internationally recognized standards, methods and processes. In 2022 our scientists and labs received a number of awards for their contributions to grain research.

Dr. Marta Izydorczyk

Presidential award, Cereals & Grains Association

Dr. Izydorczyk was elected president of the Cereals & Grains Association in 2019. She served as president elect in 2019 and 2020, as president in 2020 and 2021, and as immediate past president in 2021 and 2022. The presidential award is in appreciation and recognition of Dr. Izydorczyk contributions and services to the organization.

Dr. Sheryl Tittlemier

Edith A. Christensen Award for Outstanding Contributions in Analytical Methodology, Cereals & Grains Association

Dr. Tittlemier was recognized for developing and evaluating analytical tools to measure pesticide residues in grains and investigating the fate of glyphosate during milling and bread making.

Dr. Sean Walkowiak

Outstanding Young Scientist Award, Canadian Phytopathological Society

This award recognized Dr. Walkowiak for having made an outstanding contribution to plant pathology in Canada through superior research accomplishment and practical application of scientific or technological expertise.

Dr. Bin Xiao Fu

Fellow, International Association of Cereal Science and Technology

Dr. Fu was recognized for his contributions to wheat quality research and method development, which benefits quality improvement of wheat staple foods such as bread, pasta, and Asian noodles. He is the 5th Canadian to be awarded this honour, and the 3rd scientist from the Grain Research Laboratory.

Oilseeds Program

Top prize in gas chromatography analysis, American Oil Chemists' Society

For the second year in a row, Dr. Véronique Barthet and her team were awarded the top prize in the International Laboratory Proficiency Program. By winning first place in chromatography analysis, our Oilseeds lab ranks first in the top 10% of labs analyzing fatty acids.

Dr. Daniel Perry

Mentor Who Matters, Germination magazine

Dr. Perry, who retired from the Grain Research Laboratory after 21 years, was selected as a mentor that mattered for developing and sharing with colleagues his methods for routine DNA-based testing.



Highlights from 2022

95th Harvest Sample Program anniversary

2022 marked the 95th anniversary of the [Harvest Sample Program](#). This program has expanded over the years and now accepts all 21 grains regulated by the Canadian Grain Commission.

In 2022 we received **10,238 samples** from grain producers.

In addition to providing an unofficial grade for each sample, we analyzed:

- Dockage assessment on canola and mustard
- Protein content on barley, beans, chickpeas, lentils, oats, peas and wheat
- Oil, protein and chlorophyll content for canola
- Oil and protein content and iodine value for flaxseed
- Oil and protein for mustard seed and soybeans
- Falling number for wheat and rye
- Vomitoxin (deoxynivalenol or DON) levels for wheat, corn, barley and oats

Quality reports

Each year we [report on the end-use quality](#) of Canadian grain from the annual harvest and in exports. In 2022, we published 39 quality reports on the preliminary data and the final assessment of 10 different types of grain. These reports evaluated different grades, classes and varieties, depending on the grain. Preliminary data reports were regularly updated as results became available.

Highlights from 2022

Outreach and sharing of expertise

In 2022, we shared our expertise and research results in many different ways. Our staff published articles in peer-reviewed journals, attended scientific conferences and hosted seminars. We regularly met with stakeholder groups from across Canada and around the world and participated in agricultural tradeshow.

We also used social media to connect with producers and other members of the grain sector. We promoted our programs and people, shared up-to-date information about our services and research, and responded to questions and concerns.

In 2022, some of our most popular posts featured:



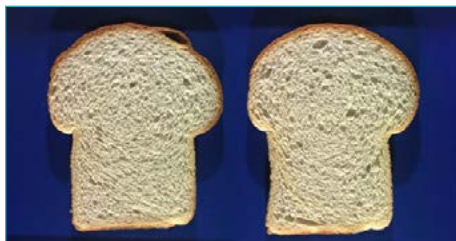
Twitter takeovers

Grain Research Laboratory staff were involved in 4 Twitter takeovers that demonstrated our methods and equipment to producers and other followers of the Canadian Grain Commission account.



Crop quality and Fusarium survey reports

Each year we report on the quality of crops as well as the incidence and severity of Fusarium damaged kernels in Canada Western Red Spring and Canada Western Amber Durum in samples submitted to the Harvest Sample Program.



Advancements in agricultural science

These short articles written in plain language provide the grain sector and the general public with some of our latest findings and show how they impact Canadian producers.



Bread Wheat and Durum Research

Dr. Bin Xiao Fu

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How does protein content affect bread wheat functionality?

The Bread Wheat and Durum Research Program supports the Canadian wheat quality assurance system in many ways. We analyze the quality of new crops, evaluate new wheat lines in registration trials, provide the scientific basis for wheat grade tolerances and monitor wheat cargoes. Our research focuses on understanding how the physicochemical and biochemical properties of wheat influence its quality and we develop new techniques for evaluating wheat quality.

Team members

Research scientist/ program manager

Dr. Bin Xiao Fu

Chemist

Dr. Kun Wang

Carly Isaak

Ray Bacala

Technicians

Altash Yirdaw

Alyssa Hilapo

Angelique Parajas

Andrea Iverson

Dale Taylor

Jerry Suchy (retired)

Joseffus Santos

Katherine Cordova

Shermy Jayasekara

Ofelia Francisco-Pabalan, (retired)

Yuming Chen

We recently undertook a study to better understand the influence of protein on the processing performance of AAC Brandon, the dominant variety of Canada Western Red Spring (CWRS) wheat. Although both protein content and composition are crucial to wheat functional quality, it has been challenging to isolate one from another in understanding their contribution to wheat processing performance. During drought, protein levels in wheat can increase and the hot and dry growing season of 2021 in Western Canada presented a unique opportunity to source samples of AAC Brandon with a larger than usual range of protein content. Using these samples, we measured variations in milling performance, dough properties and baking quality with changing protein content. We also examined protein composition to understand the

biochemical basis for the variations in functionality that were observed.

Wheat samples and flour quality

Samples of AAC Brandon (No. 2 CWRS or better) were blended into 7 aggregates according to their protein content. Aggregates were divided into groups of 11.5%, 12.6%, 13.6%, 14.5%, 15.6%, 16.6%, and 17.7% protein. We found that test weight decreased gradually with increasing protein (Figure 1a). The test weight for each aggregate was, however, above the minimum requirement for No. 1 CWRS (79 kg/hL). Flour samples were prepared using a Bühler MLU 202 laboratory mill with a constant extraction rate of 74% for comparative analysis. Milling yield increased for aggregates with protein content ranging from 11.5%

to 14.5% but decreased for aggregates with higher protein content due to a decrease in kernel size and test weight (Figure 1b).

Dough properties and end-product quality

Dough properties were measured by Farinograph and Extensigraph following [AACC International Methods](#). The results from our tests on the AAC Brandon

aggregates are given in Table 1. The mixing requirement, as indicated by dough development time, was found to increase as protein content increased. Dough strength, measured by maximum resistance (Rmax) and stability, was also found to increase with protein content. Dough extensibility showed an upward trend with increased protein content. Baking quality was determined with the Canadian Short Process (CSP) and the Sponge and Dough (S&D) bake

tests. Results from the S&D bake test showed that loaf volume increased with increasing protein content (Figure 2) but no change in volume was observed using the CSP bake test. We analyzed bread crumb texture with a TA.XT2 Texture Analyzer using a Texture Profile Analysis test. Loaves produced by both the CSP and S&D bake tests showed a decrease in crumb hardness as protein content increased (Figure 3).

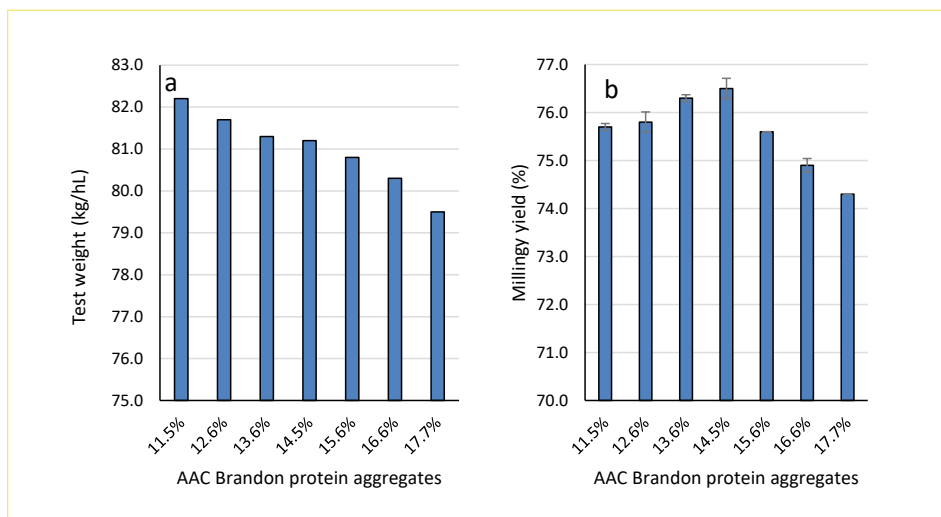


Figure 1 Test weight (a) and milling yield (b) for 7 AAC Brandon aggregates based on their percentage of protein content.

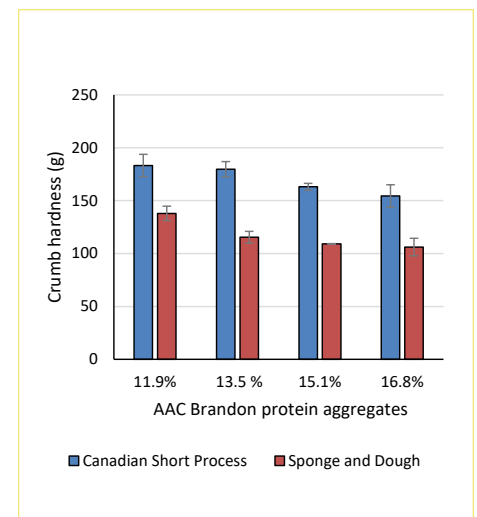


Figure 3 Crumb hardness according to protein content for AAC Brandon aggregates.



Figure 2 The Sponge and Dough bake test resulted in loaf volumes that increased as protein content increased.

Flour protein and functional protein fractions

Flour proteins were fractionated into monomeric proteins (MP), soluble glutenins (SG), and insoluble glutenins (IG). The absolute quantity of IG in flour from AAC Brandon aggregates was found to increase linearly with protein content while the percentage of IG in the

total amount of protein remained largely unchanged (Figure 4). We also found that the sum amount of SG and MP increased proportionally with IG (Figure 5).

Insoluble glutenins were analyzed using reversed-phase ultra-performance liquid chromatography (RP UPLC). The 45% 1-propanol insoluble glutenin fraction was reduced and alkylated before separation with a BEH C4 300Å column. Our results

confirmed that the total amount of IG was directly related to flour protein content and that little change was seen in the proportion of IG in total protein when the results were normalized to a protein content of 13.5%. (Table 2).

Table 1 Comparison of dough properties for AAC Brandon aggregates with increasing protein content.

Wheat Protein Content	11.5%	12.6%	13.6%	14.5%	15.6%	16.6%	17.7%
Farinogram							
Absorption, %	62.5	62.9	63.3	63.6	64.5	66.4	67.5
Dough development time, min	3.25	6.25	6.75	9.25	11.25	9.50	14.50
Stability, min	8.0	12.0	13.5	14.5	16.5	15.5	20.0
Extensigraph							
Strength (Rmax), BU	465	556	554	614	646	598	732
Extensibility (length), cm	19.5	18.4	20.4	19.3	20.5	22.0	21.2
Area, cm ²	119	130	146	153	171	168	197

Table 2 Subunit composition of 45% 1-propanol insoluble glutenins in relation to flour protein content.*

Subunit composition	11.5%	12.6%	13.6%	14.5%	15.6%	16.6%	17.7%
Insoluble glutenins (peak area x 10 ⁶)							
High molecular weight (HMW) glutenin subunits	4.6	5.4	5.5	5.7	5.5	5.9	5.8
Low molecular weight (LMW) glutenin subunits	11.7	12.0	12.0	11.8	11.5	11.6	11.6
Total area insoluble glutenins	16.3	17.5	17.5	17.5	17.1	17.4	17.4
HMW/LMW ratio	0.43	0.45	0.46	0.48	0.48	0.51	0.50

*all results were corrected to 13.5% flour protein content

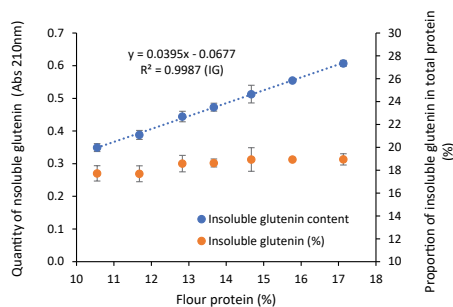


Figure 4 Effect of protein content on the quantity and proportion of insoluble glutenin in AAC Brandon aggregates.

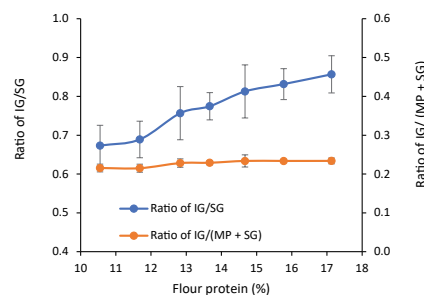


Figure 5 Effect of protein content on the ratio of insoluble glutenins to soluble glutenins (IG/SG) and the ratio of insoluble glutenins to the sum of monomeric proteins and soluble glutenins (IG/(MP + SG)).

Conclusions

- Milling yield increased as protein content increased from 11.5% to 14.5% but decreased as protein content increased further.
- AAC Brandon had well-balanced dough properties across a wide range of protein content: dough strength and dough development time increased with protein content, and extensibility had an upward trend.
- The positive correlation between dough strength and protein content in AAC Brandon was due to an increase in the total amount of insoluble glutenins, not its proportion in total protein.
- The well-balanced dough properties of all AAC Brandon aggregates can be attributed to the relatively constant ratio of insoluble glutenins to the sum of monomeric proteins and soluble glutenins.
- Loaf volume increased with protein content when measured using the S&D bake test.
- Bread crumb hardness decreased with increasing protein content.

Recent publications

- Wang, K., Pozniak, C.J., Ruan, Y. and B.X. Fu. 2022. Unveiling the impact of durum wheat protein quantity and quality on textural properties and micro-structure of cooked pasta. *Cereal Chem.* 100 (2): 484-499. <https://doi.org/10.1002/cche.10627>
- Bacala, R., Hatcher, D.W., Perreault, H. and B.X. Fu. 2022. Challenges and opportunities for proteomics and the improvement of bread wheat quality. *J. Plant Physiol.* 275: 153743. <https://doi.org/10.1016/j.jplph.2022.153743>
- Sarkar, A.; and B.X. Fu. 2022. Impact of quality improvement and milling innovations on durum wheat and end products. *Foods* 11 (12): 1796. <https://doi.org/10.3390/foods11121796>
- Walkowiak, S., Taylor, D., Fu, B.X., Drul, D., Pleskach, K. and S.A. Tittlemier. 2022. Ergot in Canadian cereals – relevance, occurrence, and current status. *Can. J. Plant Pathol.* 44(6): 793-805. <https://doi.org/10.1080/07060661.2022.2077451>
- Oduro-Obeng, H., Apea-Bah, F.B., Wang, K., Fu, B.X. and T. Beta. 2022. Effect of cooking duration on carotenoid content, digestion and potential absorption efficiencies among refined semolina and whole wheat pasta products. *Food Funct.* 13: 5953-5970. <https://doi.org/10.1039/D2FO00611A>



Milling and Malting / Research on Barley and Other Grains

Dr. Marta Izydorczyk

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Studies on physical factors and bioactive components that affect grain quality

The Milling and Malting / Research on Barley and other Grains Program conducts research to identify, characterize, and quantify the factors and molecular mechanisms responsible for the quality, functionality and performance of Canadian barley and other grains, such as oats and buckwheat. We develop new technologies for measuring quality and explore innovative ways to use barley and other grains. We monitor the quality of barley destined for export, evaluate new barley lines and assess the quality of malting barley produced in western Canada.

Team members

Research scientist/ program manager

Dr. Marta S. Izydorczyk

Chemist

Tricia McMillan
Arzoo Sharma

Technicians

Anna Chepurna
Debby Kelly
Jerry Kletke
Cherianne McClure
Shin Nam
Shawn Parsons
Dave Turnock

The effect of kernel shape on test weight and dehulling of oats

We recently conducted a study to understand how physical factors affect test weight and the dehulling performance of two oat cultivars commonly grown in western Canada: Summit and CS Camden.

A comparison of Summit and CS Camden oats from 2020 and 2021 showed that they had similar kernel widths but that Summit kernels were shorter in length, giving them a smaller and more rounded shape (Figure 1). Although the smaller size of Summit kernels coincided with lower kernel

weights, Summit tended to show a higher test weight than CS Camden when equivalent weights were compared (Figure 2). This suggests that the smaller and rounder kernels of Summit can pack more efficiently than those of CS Camden. The elongated shape of CS Camden kernels results in longer hulls that are more likely to trap air when they overlap, leading to a lower packing efficiency and lower test weight.

Kernel shape was also found to affect the dehulling performance of Summit and CS Camden. The shorter and rounder kernels of Summit oats resulted in a greater percentage of groats after dehulling than CS Camden (Figure 3). Although the longer hulls of CS Camden

kernels seemed to protect the groats from breakage during dehulling, they also resulted in less hulled kernels (Table 1). The groats of CS Camden

were, however, slightly larger than those of Summit and groat density was also higher for CS Camden. We found no significant differences in the content of

proteins and oil between the two types of groats, but CS Camden contained more β -glucans and less arabinoxylans than Summit.

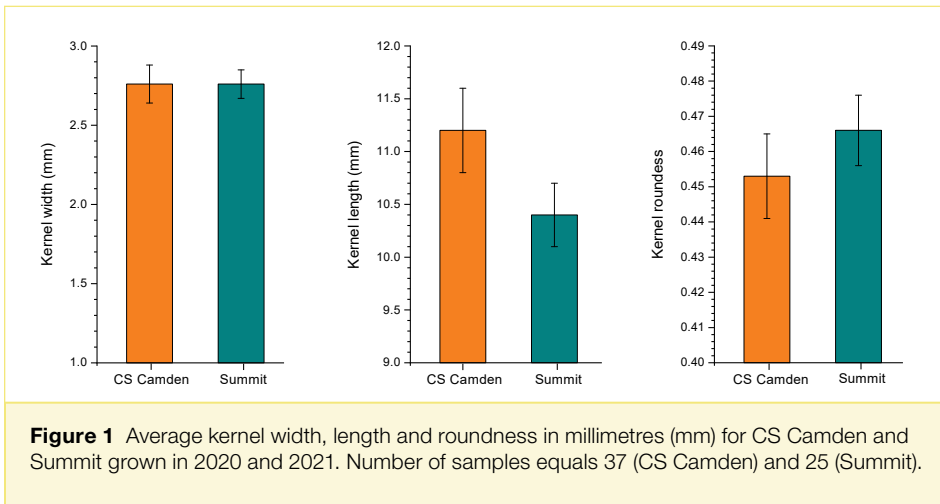


Figure 1 Average kernel width, length and roundness in millimetres (mm) for CS Camden and Summit grown in 2020 and 2021. Number of samples equals 37 (CS Camden) and 25 (Summit).

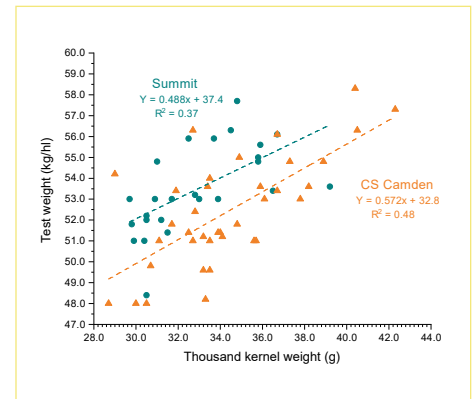


Figure 2 Scatter plot showing the relationship between thousand kernel weight in grams (g) and test weight in kilograms per hectolitre (kg/hL) for CS Camden and Summit grown in 2020 and 2021. Number of samples equals 37 (CS Camden) and 25 (Summit).

Table 1 Dehulling results for CS Camden and Summit in 2020 and 2021. The category of total groats includes whole and broken groats.

Cultivar	Year	Total groats (%)		Broken groats (%)		Hulls (%)		Hulled grain (%)	
		mean	SD ¹	mean	SD	mean	SD	mean	SD
CS Camden	2020	73.5	1.5	0.8	0.8	26.4	1.5	4.2	2.9
	2021	71.8	1.9	1.9	1.2	27.9	1.9	5.1	3.0
Summit	2020	76.3	3.1	1.8	1.6	23.7	3.1	1.2	1.3
	2021	74.8	1.7	2.5	1.2	25.2	1.7	1.1	0.6

¹ SD = standard deviation

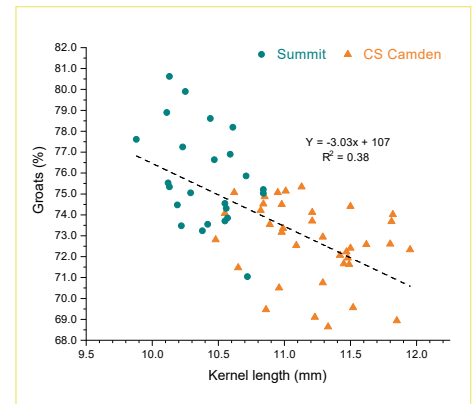


Figure 3 Scatter plot showing the relationship between kernel length in millimeters (m) and percentage of groats obtained after dehulling for CS Camden and Summit grown in 2020 and 2021.

Influence of germination time on the structure and mass of β -glucans and arabinoxylan in wort

In another recent study, we investigated the effects of germination time on the concentration, molecular mass and structure of β -glucans and arabinoxylans

in wort. These compounds are the main types of non-starch polysaccharides found in the cell walls of barley grains. During malting, the controlled germination process breaks them down but if β -glucans are not sufficiently degraded they can affect the brewing process and quality of beer. They can increase viscosity, form gels, and contribute to haze. The role and exact

mechanism of how arabinoxylans contribute to these phenomena are not fully understood. We took samples of several different varieties of Canadian covered and hullless barley and steeped them at 13°C for 48 hours and germinated them at 15°C. Five different germination times were compared: 24h (G1), 48h (G2), 72h (G3), 96h (G4), and 120h (G5).

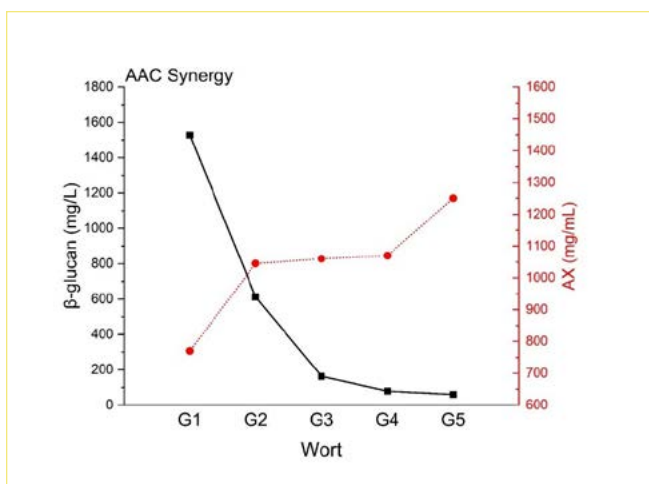


Figure 4 Effect of germination time on the concentration in milligrams per litre (mg/mL) of β -glucans and arabinoxylans (AX) in wort made from AAC Synergy. Time was measured in hours (h) and samples identified as G1 (24h), G2 (48h), G3 (72h), G4 (96h) and G5 (120h).

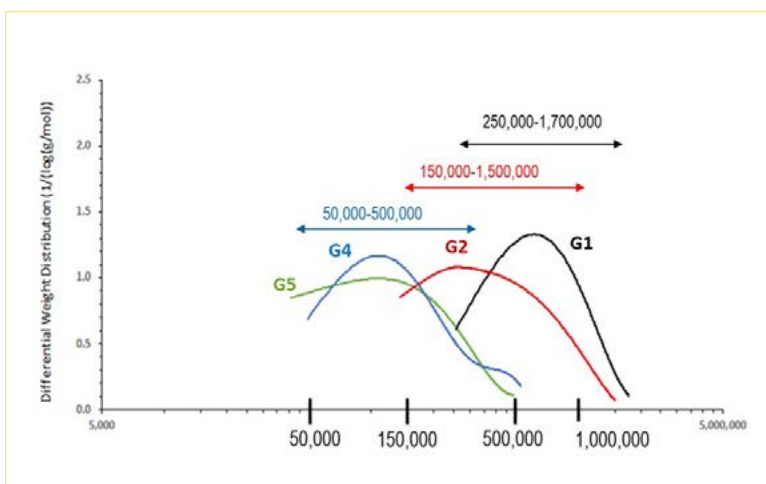


Figure 5 Effect of different germination time on the molar mass of non-starch polysaccharides (β -glucans and arabinoxylans) in wort. Time was measured in hours (h) and samples identified as G1 (24h), G2 (48h), G4 (96h) and G5 (120h).

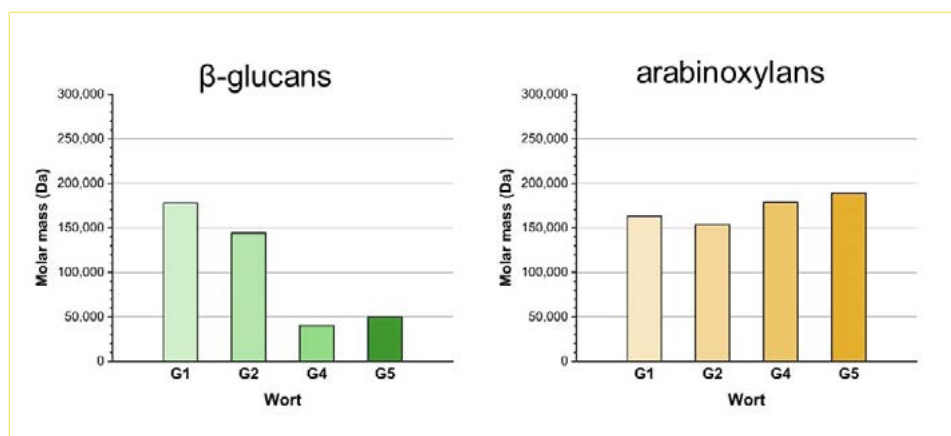


Figure 6 The average molar mass (Daltons) of β -glucans and arabinoxylans in wort when barley was germinated for different lengths of time. Time was measured in hours (h) and samples identified as G1 (24h), G2 (48h), G4 (96h) and G5 (120h).

Results of our study

- As germination time increased, the concentration of β -glucans in wort significantly decreased but the concentration of arabinoxylans increased (Figure 4).
- The molecular mass of both β -glucans and arabinoxylans decreased significantly with increasing germination time (Figure 5).
- Conditions favourable to the breakdown of β -glucans (high hydration, longer germination time) resulted in the β -glucans remaining in the wort having long polymeric chains that exhibit a relatively high ratio of cellotriosyl/cellotetraosyl units (DP3/DP4). These molecular properties likely contributed to the tendency of the chains to separate from wort if there is a change in solvent conditions such as temperature, sugar or alcohol concentrations, and shear forces.
- Arabinoxylans that remained in wort resisted enzymatic hydrolysis due to their highly branched structure and the presence of other substituents along the xylan chains (ferulic acid residues).
- Targeted hydrolysis of non-starch polysaccharides in wort revealed that the average molar mass of wort AX was consistently higher than that of BG (Figure 6).

Conclusions

Our study showed that increasing germination time can effectively reduce the content and molar mass of β -glucans in wort, but the length of germination time needs to be optimized to avoid excessive solubilization of AX during malting and mashing.

Recent publications

Izydorczyk, M.S., Badea, A. and A.D. Beattie. 2022. Physicochemical properties and malting potential of new Canadian hullless barley genotypes. *J Am Soc Brew Chem* 81 (2): 299-307. <https://doi.org/10.1080/03610470.2022.2065453>

Chen, W., Cheung, H.Y.K., McMillan, M., Turkington, T.K., Izydorczyk, M.S. and T. Gräfenhan. 2022. Dynamics of indigenous epiphytic bacterial and fungal communities of barley grains through the commercial malting process in western Canada. *CRFS* 5: 1352-1364. <https://doi.org/10.1016/j.crfs.2022.08.009>

Izydorczyk, M.S. 2022 Sept. 21-25. Effects of grain hydration and germination time during malting on the molecular structure and mass of β -glucans and arabinoxylan in wort [abstract]. 23rd North American Barley Researchers Workshop, Davis, CA, USA.

Izydorczyk, M.S. and T. McMillan. Barley Harvest Annual Report 2022. Barley Production and Quality of Western Canadian Malting Barley. <https://www.grainscanada.gc.ca/en/grain-research/export-quality/cereals/malting-barley/2022/>

Izydorczyk, M.S., Kletke, J., Sharma, A., Chepurna, A. and S. Nam. 2022 Nov. 9-11. Effects of grain steaming on the milling performance and physicochemical properties of dietary fibre in hullless food barley [abstract]. *Cereals & Grains 2022*, Bloomington, MN, USA.



Oilseeds

Dr. Véronique Barthelet

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Developing reference standards for measuring cyanogenic glycosides in flaxseed

The Oilseeds Program conducts research on Canadian oilseeds including canola, rapeseed, flaxseed, soybean and mustard. We assess the effects of grading factors on oilseed quality and analyze how various components of oilseeds, such as free fatty acids, affect their quality and the products made from them. Another important part of our work is assessing and developing methods used to analyze oilseed quality. This includes validating our reference methods to international standards, developing near infrared spectroscopy models to predict oilseed quality and pioneering new methods for analyzing minor compounds in oilseeds. The Oilseeds Program also evaluates the quality of oilseed samples from the Harvest Sample Program and export shipments.

Team members

**Research scientist/
program manager**

Dr. Véronique J. Barthelet

Chemist

Ann Puvirajah
Tao Fan

Technicians

Brad Speiss
Colleen Kobialka (Harvest Sample Program)
Hayeon Oh
Marnie McLean
Nicole Pogorzelec
Katharine Schulz

Cyanogenic glycosides in plants

Although some compounds can occur at low levels in oilseeds, they may still limit their use in food and feed applications. One such group of compounds that are of interest to us in flaxseed are cyanogenic glycosides. There are about 75 known cyanogenic glycosides in more than 2500 species of plants. These compounds are considered to be antinutrients and their concentrations are regulated in some food products, such as cassava flour. In some species they are found in every part of the plant (e.g., cassava and flax) while in others, only certain structures contain cyanogenic glycosides (e.g., apple seeds). The role of cyanogenic glycosides in plants is

not completely understood but it has been suggested that they provide plants with resistance to predators such as herbivores, nematodes or fungi.

The role of reference standards

Two cyanogenic diglycosides, linustatin and neolinustatin, are known to occur in sound (undamaged) flaxseeds. Two cyanogenic monoglycosides, linamarin and lotaustralin, have been found in seedlings, developing plants, flowers, damaged seeds and immature seeds. We use gas chromatography to identify and measure levels of cyanogenic glycosides in flaxseed by comparing the chromatographic profiles of flaxseed extracts with those of highly purified

reference standards. The high cost and unavailability of some cyanogenic glycoside standards often makes the quantification of these compounds difficult, however, so we investigated the feasibility of developing a rapid and cost-effective method for producing pure standards for use in the routine analysis of cyanogenic glycosides in flaxseed.

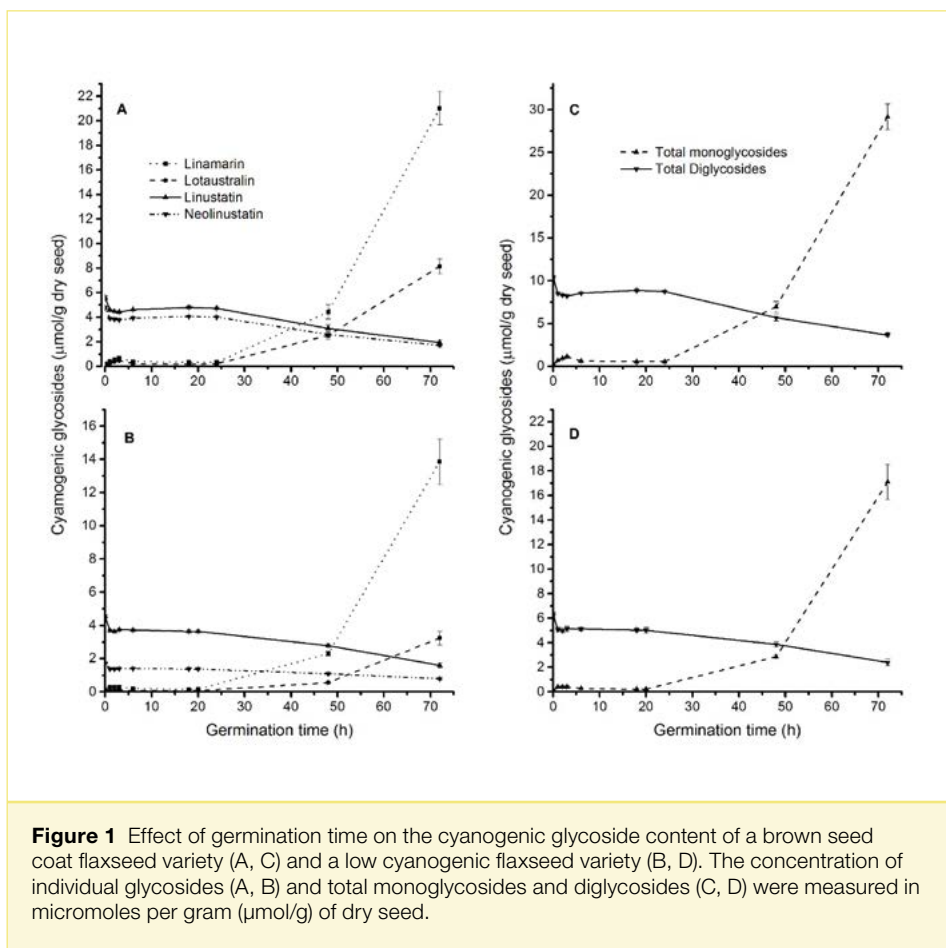
Producing purified cyanogenic glycosides for routine analysis

A review of the scientific literature revealed that germinated flaxseeds contain all of the four cyanogenic compounds we wanted to investigate.

We tested germinated flaxseeds and determined that 72 hours in a germination tray was sufficient time for the production of cyanogenic glycosides. Several samples and composites were tested to find samples that would produce cyanogenic glycosides in amounts high enough to be used as reference standards (Figure 1). We extracted the cyanogenic glycosides using the method that we previously developed, separated them using preparative high performance liquid chromatography (Prep-HPLC) (Figure 2), collected the HPLC fractions and identified the fractions

containing the cyanogenic glycosides (Figure 3). The last step was to analyze the concentrated fractions corresponding to each cyanogenic glycosides using gas chromatography-mass spectrometry to determine if they were suitable for

use in routine analyses (Figure 4). Our results show that we were able to produce purified cyanogenic glycosides in their natural form to complement the commercially available standards for all our routine analyses of flaxseed.



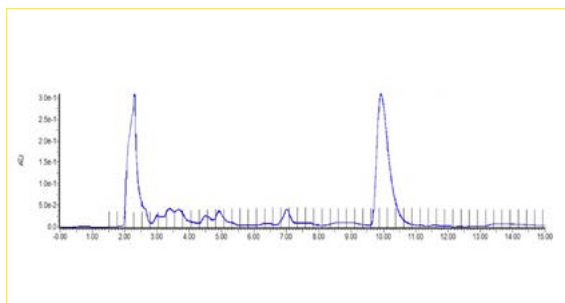


Figure 2 Typical chromatogram of a sound flaxseed extract showing ultraviolet (UV) detection at 219 nanometres, with each vertical bar representing a collected fraction.

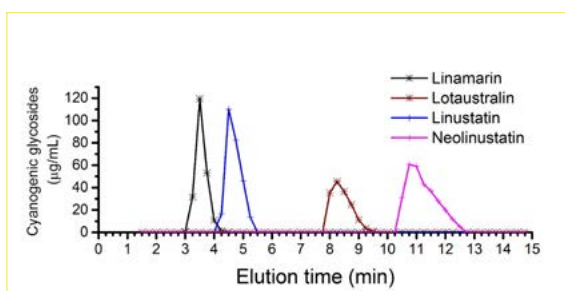


Figure 3 Concentration of cyanogenic glycosides in germinated flaxseed extract as a function of elution time (min). Concentrations are measured in micrograms per millilitres ($\mu\text{g}/\text{mL}$).

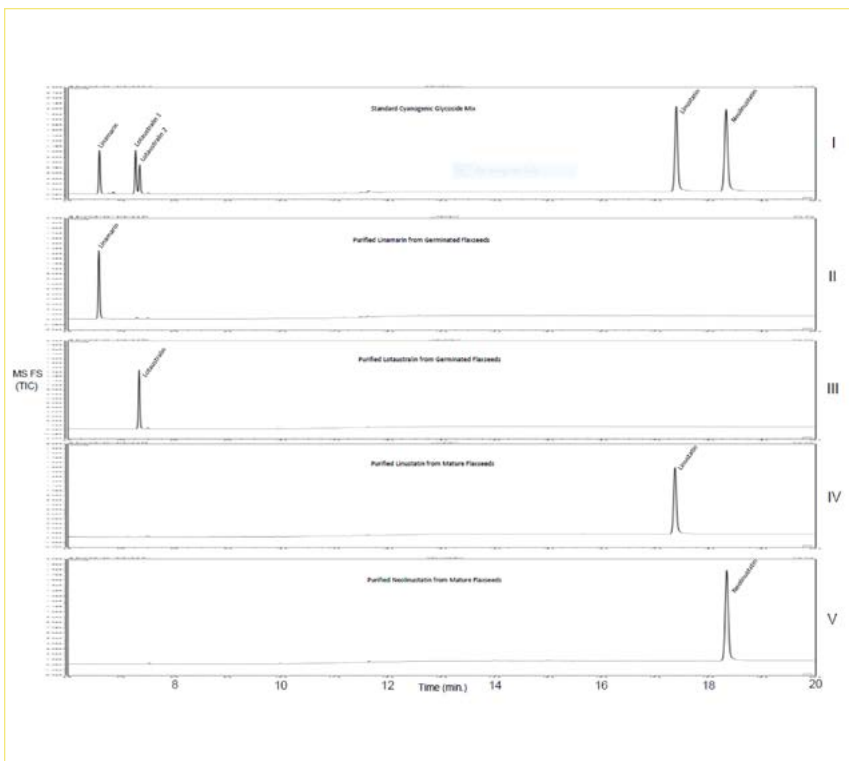


Figure 4 Typical chromatograms of partially purified extracts and commercial standards.

Recent publications

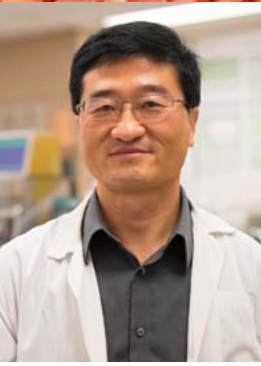
Bacala, R. and V.J. Barthet. 2007. Development of extraction and gas chromatography analytical methodology for cyanogenic glycosides in flaxseed (*Linum usitatissimum*). J. AOAC Int. 90(1): 153-161. <https://doi.org/10.1093/jaoac/90.1.153>

Barthet, V.J. and R. Bacala. 2010. Development of optimized extraction methodology for cyanogenic glycosides from flaxseed (*Linum usitatissimum*). J. AOAC Int. 93(2): 478-484. <https://doi.org/10.1093/jaoac/93.2.478>

Barthet, V.J. and T. Fan. 2018 May 6-9. Purification of cyanogenic glycosides from flaxseeds. Poster session presented at: 2018 AOCS Annual Meeting & Expo; Minneapolis, MN, USA.

Fan, T. and V.J. Barthet. 2018 Nov. 29 -Dec. 1. Development of GC/MS methods for the quantification of cyanogenic glycoside in breads with flaxseed. Poster session presented at: Lake Louise XXXI. 31st Workshop on Tandem Mass Spectrometry; Lake Louise, AB, CA.

V.J. Barthet and T. Fan. 2016 Sept. 16-19. Determination of the cyanogenic glycoside contents of flaxseed breads by GC/MS. Poster session presented at: 16th Euro Fed Lipid Congress and Expo; Belfast, UK.



Pulse Research
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New methods for evaluating functional properties of pulses

The Pulse Research Program investigates the physical and chemical properties of pulses to better understand how grading factors, processing methods, environmental conditions, and genetics affect their quality and end-use functionality. We develop and evaluate new methods for quantifying the functional characteristics of pulses. To support the marketability of Canadian pulses, we conduct the annual pulse and food-type soybean quality analysis for the Harvest Sample Program and take part in cargo monitoring.

Team members

**Research scientist/
 program manager**
 Dr. Ning Wang

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 Dora Fenn

Technicians
 Lisa Maximiuk
 Monica Cabral

Foaming properties of pulses

In food applications, foam can be created by trapping air bubbles in a liquid using mechanical energy, usually in the form of mixing. Plant-based protein ingredients that are able to foam can be used to make foods such as mousses, pastries and whipped desserts. Foaming capacity (FC) is a measure of the increase in volume achieved by mixing and foaming stability (FS) measures the change in volume of a foam over a defined period of time. Although FC and FS are two important properties that affect how well a food ingredient performs as a foam, there currently are no widely accepted procedures for measuring them.

We studied a variety of pulse ingredients to develop a standard method for

quantifying FC and FS under optimal conditions. Samples of each pulse ingredient were homogenized in distilled water and mixed at different speeds and periods of time (Figures 1 and 2). Foam volume was measured using a graduated cylinder and FC and FS were calculated as a percentage using the following:

$$FC (\%) = (\text{volume of foam at 1 minute} / \text{volume of liquid prior to mixing}) \times 100$$

$$FS (\%) = (\text{volume of foam at 60 minutes} / \text{volume of foam at 1 minute}) \times 100$$

By measuring the effect of mixing speed and mixing time on FC and FS, we were able to establish the optimum conditions for producing foam. This method is simple, has good within-laboratory reproducibility and can be applied to a variety of pulse ingredients.

Determining seed hardness in pulses

Seed hardness is an important factor affecting the physicochemical and functional properties of pulse flours produced by milling. Currently, the published methods for determining seed hardness are mainly applicable to wheat and procedures for determining the seed hardness of pulses are not readily available. We developed a method for measuring seed hardness using a texture analyzer attached with a round compression plate (Figure 3). A single pulse seed is placed on a platform with its hilum (seed scar) parallel to the compression plate and compressed at a fixed rate and strain. Seed hardness is expressed as the amount of work required to compress a seed and is determined by multiplying the force required (Newtons) by the distance compressed (meters) (Figure 4). We found that this method was objective and could be successfully applied to various pulses.

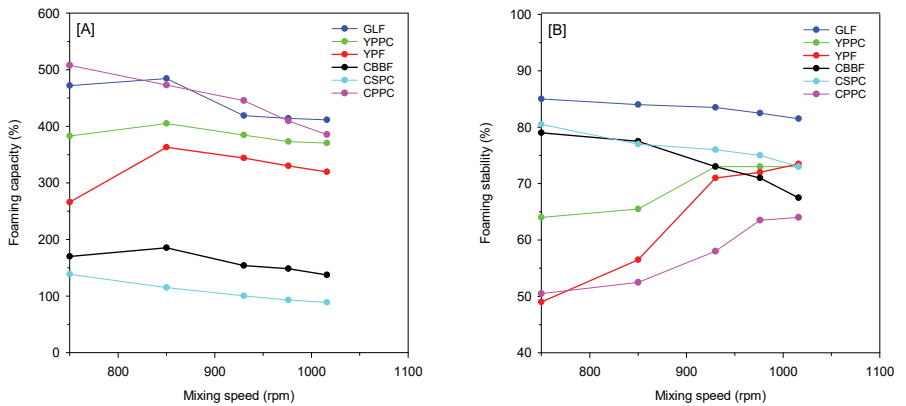


Figure 1 Effect of mixing speed on foaming capacity (A) and foaming stability (B). GLF=green lentil flour; YPF=yellow pea flour; CBBF=cranberry bean flour; YPPC=yellow pea protein isolate; CPPC=commercial pea protein isolate; CSPC=commercial soy protein isolate.

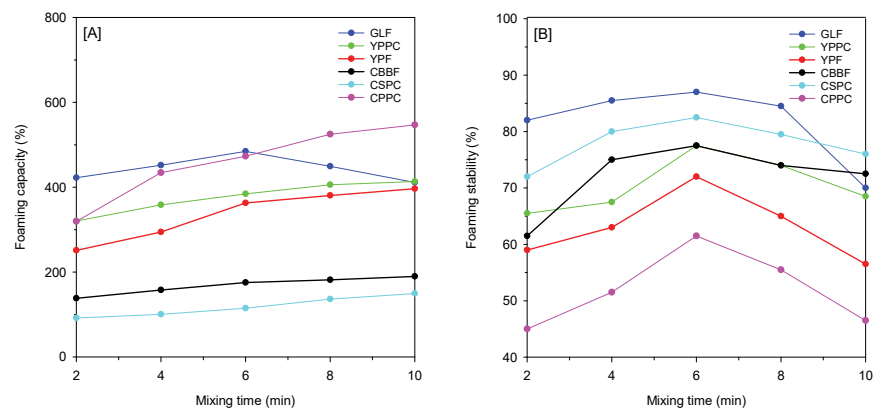


Figure 2 Effect of mixing time on foaming capacity (A) and foaming stability (B). GLF=green lentil flour; YPF=yellow pea flour; CBBF=cranberry bean flour; YPPC=yellow pea protein isolate; CPPC=commercial pea protein isolate; CSPC=commercial soy protein isolate.



Figure 3 Texture analyzer used for determining the hardness of pulse seeds.

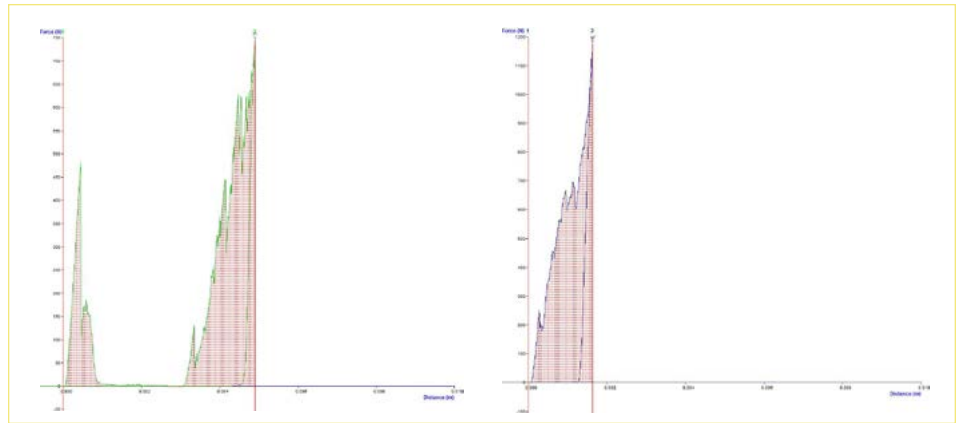


Figure 4 Typical compression curve for yellow pea (A) and green lentil (B).

Other research projects

- In collaboration with Pulse Canada, we are developing a database of pulse protein ingredients produced by wet and dry milling using published or standard testing methods. This will provide consistent results on the composition and functionality of pulse ingredients.
- We are investigating how variety, growing location and growing year affect the chemical composition and functionality of fababeans. The results of this study will support the increased use of pulse ingredients and the marketability of Canadian fababeans.
- In collaboration with the Canadian Grain Commission's Industry Services, we investigated the impact of wrinkled red lentil seeds on dehulling quality. Our results assisted Industry Services in confirming tolerances for the wrinkled seed grading factor for red lentils.

Recent publications

Guldiken, B., Konieczny, D., Franczyk, A., Sapiro, V., Pickard, M., Wang, N., House, J.D. and M.T. Nickerson. 2022. Impacts of infrared heating and tempering on the composition, morphological, functional properties of navy bean and chickpea flours. *Eur. Food Res. Technol.* 248:767-781. <https://doi.org/10.1007/s00217-021-03918-4>

Guldiken, B., Franczyk, A., Boyd, L., Wang, N., Choo, K., Spoiwnyk, E., House, J., Paliwal, J. and M.T. Nickerson. 2022. Physicochemical, nutritional and functional properties of chickpea (*Cicer arietinum*) and navy bean (*Phaseolus vulgaris*) flours from different mills. *Eur. Food Res. Technol.* 248: 1847-1858. <https://doi.org/10.1007/s00217-022-04010-1>



Analytical Services

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Comparing and validating methods for measuring protein content in wheat

Analytical Services conducts many different types of tests for thousands of clients each year. We also oversee and maintain the Harvest Sample Program, which plays a key role in helping the Canadian Grain Commission meet its responsibilities to the grain sector. By testing samples of grain submitted to the program, we get an indication of the quality of new crops and can identify potential issues of concern to producers, grain marketers and customers. The program started in 1927 as a survey of protein content in Canada Western Red Spring (CWRS) wheat and has expanded over time to where it now accepts and analyzes all 21 grains regulated by the Canadian Grain Commission. We received 10,238 samples from the 2022 crop year, which marked the 95th anniversary of the Harvest Sample Program.

Team members

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Moisture and enzyme section

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Technicians

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Technicians

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Hong Yue, Andy Peng

Sample handling section

Supervisor Janice Bamforth

Technicians

Pam Lavallee, Courtney Freeth

Harvest Sample Program technicians

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Carlie Lau, Conor Nedelec, Crystal Strelczik,
Faith Geronimo, Garrett Bourque, Genesis
Esguerra, Joshua Crellin, Julia Cann,
Krystin Polden, Susan Fatla, Tyler Traeger,
Victoria Moreau

Comparison of protein methods

Protein content continues to be an important quality factor for producers and exporters, affecting both the functionality and marketability of grain. We calculate protein content in grain by measuring the amount of nitrogen in a sample and multiplying it with a nitrogen-to-protein conversion factor. The reference method for determining protein content in wheat at the Canadian Grain Commission was Kjeldahl analysis until combustion nitrogen analysis (CNA) was implemented in the 1990s. Compared to the Kjeldahl method, CNA is more precise, takes less time, does

not use corrosive chemicals and is more commonly used in labs worldwide. Kjeldahl analysis and CNA both require samples that have been ground. In 1992, near-infrared transmittance (NIT) instruments were introduced at the Canadian Grain Commission for the protein analysis of whole grains. These instruments are calibrated against CNA results, allowing us to predict protein content from NIT data using the mathematical relationship between NIT and CNA results.

Before CNA was implemented as the new protein reference method for wheat at the Canadian Grain Commission, a study was conducted to compare the

results of Kjeldahl analysis and CNA. The study found that CNA was more efficient at recovering nitrogen than Kjeldahl analysis and that protein values obtained using CNA were 0.15% to 0.25% higher. The Canadian Grain Commission used these results to calculate wheat protein in the years following the study. In 2022, we conducted a new study to verify the accuracy of our protein calculations for each method of analysis.

Validating protein calculations

We analyzed 19 samples of CWRS wheat that had a protein content ranging from 11% to 15.5% using CNA (AACC International Method 46-30.0), Kjeldahl analysis (AOAC Method 2001.11), and NIT spectroscopy (Foss Infratec™ 2019). Whole grain samples of wheat were divided into equal portions using a Boerner divider. The samples were first analyzed using NIT and then ground using a Perten Labmill 3100. Ground samples were further divided into test

portions using a rotary divider and duplicate samples were evaluated using CNA and Kjeldahl analysis. We repeated our analysis on 3 different days.

We found that there was a Kjeldahl and CNA results gave similar results across the range of protein values (Figure 1). Based on our statistical analysis of the data, we can calculate Kjeldahl protein results from CNA results using the following equation:

$$\text{Kjeldahl protein} = (0.9891 \times \text{CNA protein result}) - 0.2162$$

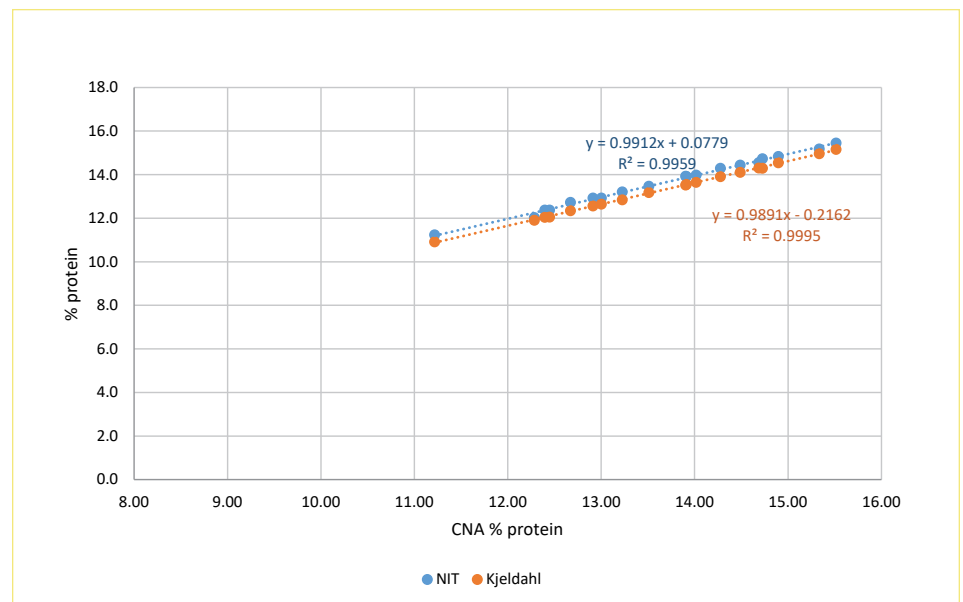


Figure 1 Scatter plot showing the relationship between protein content measured using CNA and Kjeldahl and NIT protein results.

Our statistical analysis also showed that there was good agreement between NIT and CNA results (Figure 1).

The results of our study confirm that our calculations of wheat protein

based on CNA and Kjeldahl analysis were accurate. In addition, our NIT results indicate that the monitoring and maintaining of our NIT calibrations were valid and resulted in accurate protein

calculations. Every crop year, we will again verify that the calculations used to determine protein are accurate for each method of analysis used at the Canadian Grain Commission.



Figure 2 Measuring protein content using Kjeldahl analysis.



Figure 3 Equipment used for combustion nitrogen analysis (CNA).



Figure 4 Near infrared transmittance (NIT) instruments used to predict protein content.

References

Fowler, D.B., Geddes, W.E., Johnston, A.M., and Preston, K.R. c1998. Protein testing methods. In: Williams, P.P., Sobering, D., Antoniszyn, J., editors. Wheat protein production and marketing. Proceedings of the Wheat Protein Symposium; 1998 March 9-10; Saskatoon (SK); University Extension Press, University of Saskatchewan p. 37-47.

AACC Approved Methods of Analysis, 11th Ed. Method 46-30.01. Crude Protein-Combustion Method. Approved November 3, 1999. Cereals & Grains Association, St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIntMethod-46-30.01>

Official Methods of Analysis of AOAC International (2012) 19th Ed., AOAC International, Gaithersburg, MD, USA, Official Method 20001.11-2005 Protein (crude) in animal, feed, forage (plant tissue), grain and oilseeds. <https://img.21food.cn/img/biaozhun/20100108/177/11285182.pdf>

Foss Infratec™ manual. 2019. FOSS Analytical A/S, Foxx Allé 1, DK-3400 Hillerød, Denmark

[Infratec™](http://www.fossanalytical.com) - NIR Grain Analyser (fossanalytical.com)



Grain Biotechnology

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Applying and developing DNA-based methods for detecting genetically modified plants

The Grain Biotechnology Program develops and evaluates DNA-based methods for detecting genetically modified (GM) plant materials. When required, we test for specific GM events. We also use DNA-based tests to monitor wheat shipments for varieties that do not meet the requirements for wheat classes (including non-registered varieties) and to certify the varietal purity of submitted malting barley cargo samples.

Team members

**Research scientist/
program manager**

Dr. Tigst Demeke

Biologists

Michelle Holigroski

Technicians

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Danny Saydak

Mathieu Dusabenyagasani

Testing for GM plant materials in dockage

Some importing countries have concerns about the possible presence of unapproved or discontinued GM events in dockage materials found in wheat and barley cargo shipments. In a recent study, we looked for the presence of unapproved GM events in dockage materials collected from wheat and barley grain shipments. The most frequent type of dockage material found in barley and wheat shipments was canola seed. We tested canola dockage samples for the presence of different GM events using polymerase chain reaction (PCR) and pre-spotted plates. Pre-spotted plates use aliquots

of primers and probes specific for each GM event. This method allowed us to detect 11 different GM events in canola at concentrations as low as 0.01% (Table 1). The most commonly detected GM canola events were GT73, MS8 and RF3. These GM events have received regulatory approval in the European Union and many other countries and pose no regulatory issues.

We analyzed 1,038 samples of dockage material and did not detect the unauthorized OXY235 and the discontinued HCN92 GM canola events. Only trace levels ($\leq 0.1\%$) of GM events RF1 (3 samples), MS1 (3 samples), T45 (2 samples) and RF2 (1 sample) were found. These discontinued GM events had approval in the EU but are now

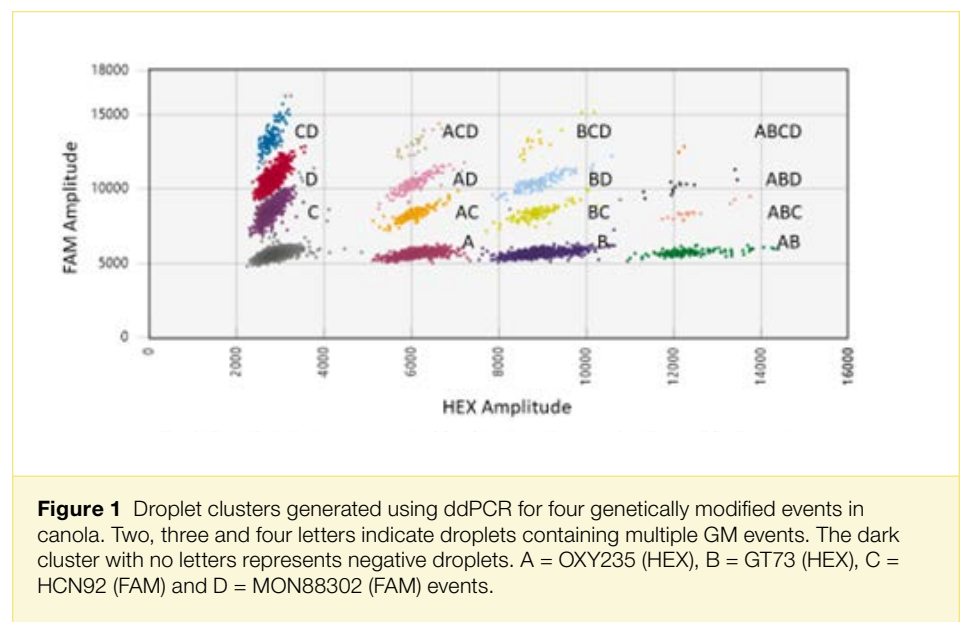
being phased out. Overall, the risk of unapproved or discontinued GM canola events being found in dockage materials collected from barely and wheat shipments was found to be very low.

Increasing the efficiency of GM event detection

We use droplet digital PCR (ddPCR) technology to measure the absolute amount of a GM event in a target sample without comparing it to a reference sample. The target sample is divided into thousands of partitions or droplets and each droplet is tested for the presence of a given GM event. Usually, only one GM event is detected with a ddPCR assay and if we need to measure more than one GM event in a sample, the assay is performed multiple times. We investigated the feasibility of absolute quantification of two, three and four GM events at the same time using the QX200 Droplet Digital PCR system. To successfully use multiplex ddPCR, an

adjustment of probe concentrations and labels was needed for some assays. For probe optimization, all four GM events were run individually using FAM and HEX probe dyes in triplicates to determine the signal intensity and establish the cluster location. Based on the results, the probes for the GM events to be labeled with either FAM or HEX were determined. Then, all four GM events were run together to assess the pattern and separation of clusters (Figure 1).

We achieved the absolute quantification of GM events in canola and soybean using duplex, triplex and tetraplex ddPCR assays at concentrations of 0.1%, 1% and 5%. The multiplex ddPCR will allow us to detect and quantify GM events more efficiently, reducing the time and resources required for monitoring.



Recent monitoring activities

Between August 1, 2021, and July 31, 2022, we analyzed the variety composition of the following:

- 380 samples from wheat cargo
- 135 samples from durum cargo
- 62 samples from monthly cargo composites
- 64 samples from wheat co-op trials
- 36 wheat samples from the Harvest Sample Program
- 14 submitted barley cargo samples

In addition, 419 wheat cargo samples were analyzed for the presence of MON71200 GM event. Bulk testing of wheat cargo samples for the presence of this GM event began in March 2018 and was discontinued on April 1, 2022. In all the samples we analyzed, MON71200 was never detected. The test is still available for submitted samples on a fee-for-service basis.

Table 1 Detection of GM canola events at concentrations of 0.1%, 0.05% and 0.01% using pre-spotted plates.

Event	Average Ct ¹ 0.1%	Average Ct 0.05%	Average Ct 0.01%
GT73	33.69 + 0.45	34.99 + 0.27	37.01 + 0.63
MS8	34.64 + 0.17	35.31 + 0.34	37.97 + 0.28
RF1	34.49 + 0.29	35.71 + 0.27	37.90 + 0.66
RF2	29.95 + 0.82	32.62 + 0.45	34.27 + 0.86
RF3	32.68 + 0.16	33.95 + 0.24	35.84 + 0.56
MS1	34.72 + 0.50	35.91 + 0.24	37.88 + 0.50
HCN92	34.65 + 0.19	35.75 + 0.22	37.68 + 0.60
T45	33.93 + 0.22	34.99 + 0.23	37.07 + 0.45
OXY235	33.28 + 0.34	34.51 + 0.27	36.66 + 0.36
MON88302	33.83 + 0.16	34.87 + 0.15	37.19 + 0.36
DP73496	34.54 + 0.13	35.85 + 0.08	38.13 + 0.45
CruA ²	23.88 + 0.07	23.66 + 0.48	23.75 + 0.05

¹Ct = threshold cycle value (cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold); average is calculated from eight independent samples

²CruA is the reference gene

Recent publications

Demeke, T., Lee, S.-J. and M. Eng. 2022. Increasing the efficiency of canola and soybean GMO detection and quantification using multiplex droplet digital PCR. *Biology* 11 (2): 201. <https://doi.org/10.3390/biology11020201>



Microbiology and Grain Genomics

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The role of DNA profiling in monitoring grain quality

The Microbiology and Grain Genomics program conducts research and develops new testing methods in our state-of-the-art DNA profiling lab. We identify the bacteria and fungi found on grains using their DNA and develop DNA-based tests to detect their presence in grain samples. We also use DNA testing to identify crop varieties in grain shipments and currently have a database containing the DNA profiles of more than 800 varieties. The monitoring of microorganisms and grain varieties helps ensure the integrity and marketability of Canadian grain and that the needs of end-users are met.

Team members

Research scientist/ program manager

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Biologists

Niradha Withana Gamage

Dr. Sung-Jong Lee

Dr. Tiffany Chin

Technicians

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Tehreem Ashfaq

Maria Eckhardt

Students

Mayantha Shimosh Kurera

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Studying microbial communities on grain

The bacteria and fungi that make up microbial communities on grains are quite diverse. Some of these microorganisms contribute to the health of plants and soils, some cause harm and others have no known effects. When we find an unfamiliar bacterium

or fungus on grain, we determine its DNA makeup through a process called sequencing. The DNA of the microorganism under investigation is compared to that of related species to determine how it differs (Figure 1). We can then target the differences in the DNA using highly specific tests that can accurately distinguish between two DNA sequences. We use the results of



Figure 1 High-throughput robotic DNA sequencing equipment used for gathering whole genome information on microorganisms and grain crops.

these tests to create a DNA profile that is unique for a certain species and can be used to identify it in a sample (Figure 2).

Our high-throughput robotic systems allow us to accurately and efficiently perform DNA tests on thousands of Canadian grain samples every year (Figure 3). The data generated from these tests help us monitor disease-causing organisms in grain more accurately. For example, we have been able to determine the geographical and temporal trends in the fungal species responsible for Fusarium head blight in Canadian wheat (Figure 4). This disease can affect the yield, quality and safety of grain.

Understanding how *Fusarium* species vary across regions and time plays an important role in its management.

The genome data we collect also gives us detailed information about the biology of microorganisms that can be used to assess risks and benefits to crop quality and safety. For example, we can determine which species contain the genes responsible for certain diseases or toxins. Understanding plant pathogens on a genomic level helps producers, breeders, pathologists, and agronomists develop mitigation strategies that target emerging species and strains.

DNA testing of grain varieties

Varieties of grain that are indistinguishable from each other based on physical characteristics or chemical analysis can still differ in their end-use qualities. The DNA-based tests that we develop enable us to accurately identify all varieties present in grain samples, including new varieties of grain that are developed and released by breeders. By sequencing the genomes of new crop varieties (Figure 1), we can detect



Figure 3 DNA arrays that allow for thousands of targeted DNA genotyping tests to be performed at once. Pictured above are a Takara SmartChip plate (left) and an OpenArray™ plate (right).

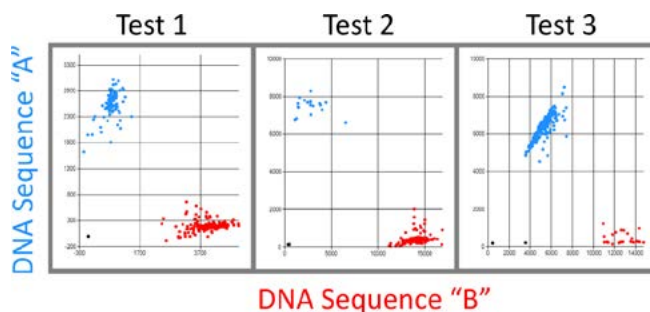


Figure 2 Example data for three different DNA tests that target a specific area in the DNA. Individuals differ in their DNA sequence and either have "Sequence A" or "Sequence B".

differences in their DNA and use this information to perform thousands of targeted DNA genotyping tests at the same time (Figure 2). The genome information we gather is also used to identify the genes responsible for traits that are in demand, which helps breeders develop new varieties that meet the changing needs of producers and customers of Canadian grain.

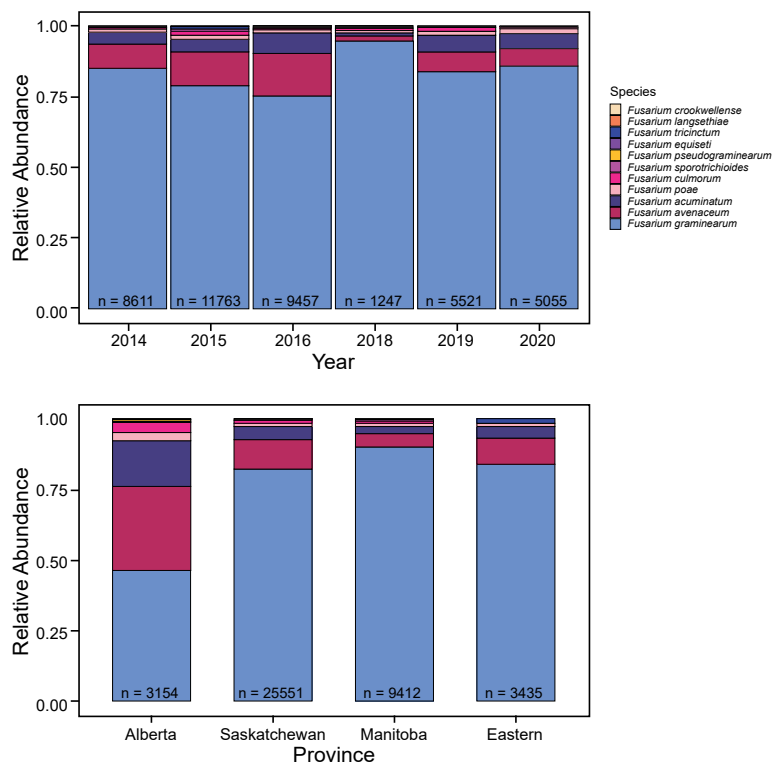


Figure 4 Relative abundance and diversity of *Fusarium* species on Canadian wheat by year (top) and province (bottom) based on DNA testing (n = the number of samples).

Recent publications

Borrill, P., Mago, R., Xu, T., Ford, B., Williams, S.J., Derkx, A., Bovill, W.D., Hyles, J., Bhatt, D., Xia, X., MacMillan, C., White, R., Buss, W., Molnár, I., Walkowiak, S., et al. 2022. An autoactive NB-LRR gene causes Rht13 dwarfism in wheat. *Proc. Natl. Acad. Sci. U.S.A.* 119(48): e2209875119. <https://doi.org/10.1073/pnas.2209875119>

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Lee, S.-J., Demeke, T., Dusabenyagasani, M., Saydak, D., Perry, D. and S. Walkowiak. 2023. Evaluation of two high-throughput genotyping systems for rapid identification of Canadian wheat varieties. *Can. J. Plant Sci.* <https://doi.org/10.1139/cjps-2022-0192>

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Trace Organics and Trace Elements Analysis

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Research demonstrates reduced chemical contaminants in wheat

The research and monitoring activities of the Trace Organics and Trace Elements Analysis Program relate to pesticides, mycotoxins, fungal biomarkers, and elemental analysis, including heavy metals. We examine the factors that cause these substances to occur in grain and study how they are affected by processing. We also research ways to make methods for sampling and analyzing more accurate and precise. The data we collect give producers, commodity associations, exporters, government partners, and end users confidence in the safety and reliability of Canadian grain.

Team members

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Anja Richter

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Richard Blagden
Daniel Bockru
Jason Chan
Dainna Drul
Valentina Timofeiev
Michael Tran
Robert Trelka (prior to November 2022)
Tanya Zirdum

Removing bran removes glyphosate residues in wheat

Glyphosate is the active ingredient in a number of herbicide products used by Canadian producers to control invasive weeds. Residues of glyphosate have been found in some grains and grain-based foods, but in almost all instances they were proven to be lower than the maximum glyphosate residue limits set by Health Canada.

We studied glyphosate in Canada Western Red Spring (CWRS) wheat to determine where it accumulates in a wheat kernel and if milling and baking changes the levels of glyphosate residues. Eight samples of CWRS wheat, containing different concentrations of

glyphosate, were selected for our study. Each sample was pearled, a process where kernels are abraded to remove their outer layers. The material removed was collected and analyzed, as was the remaining portion of the kernels. We found that 50% of the glyphosate was contained in the outer 16% of a kernel, regardless of the initial concentration in the sample.

We then milled wheat samples to remove all the bran and germ. Our analysis showed that an average of 81% of the glyphosate was contained in the bran, shorts (fine bran), and feeds (germ) milling fractions (Figure 1). The remaining flour contained 19% of the glyphosate, indicating that milling can dramatically reduce exposure to glyphosate residues.

Baking does not affect glyphosate residues

Duplicate batches of dough and bread were prepared from the flours of CWRS wheat samples that had the highest glyphosate concentrations. Dough, fermented dough, and the crust and crumb from baked bread (Figure 2) were all analyzed for glyphosate. There was no difference in glyphosate concentrations when going from flour to finished bread, demonstrating that baking does not affect glyphosate residues, confirming that milling provides the best opportunity for reducing exposure to glyphosate.

Decreased cadmium accumulation in Canadian durum wheat

Cadmium is a heavy metal that is found in soil naturally and as a result of human activities. It can enter the food chain if it is absorbed and stored in edible plants. Some cereal grains accumulate cadmium at higher levels than others, including many durum wheat varieties grown in North America. Since chronic exposure to cadmium can result in negative health effects for humans, a durum breeding program was established in the 1990s to develop varieties that accumulated less cadmium while maintaining valuable quality characteristics. In 2004, low

cadmium accumulation was established as a requirement for all new durum cultivars registered in Canada.

We have been monitoring cadmium in durum exports since the early 1990s. As part of our cargo monitoring program, shipments are systematically selected and sampled by Canadian Grain Commission inspectors using approved automated samplers and standardized procedures at terminal grain elevators (Figure 3). Since 1992, 2,239 samples of exported Canada Western Amber Durum (CWAD) wheat and 1,546 samples of exported CWRS wheat have been analyzed for cadmium.

Cadmium concentrations in bulk exports of CWAD wheat, measured in

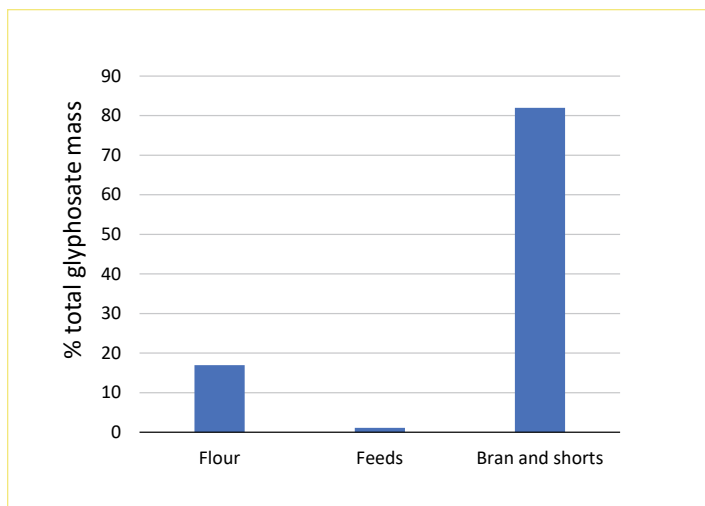


Figure 1 Proportion of glyphosate residue (%) found in milling fractions of Canada Western Red Spring wheat.



Figure 2 Bread loaf (left) and separated crust and crumb material (right) for glyphosate analysis.

milligrams per kilogram (mg/kg), began to decrease in the mid-2000s. The highest concentrations in CWAD exports (0.16 mg/kg) were from the 2003/2004 shipment year. In the 2019/2020 shipment year, cadmium concentrations were 0.07 mg/kg, representing a decrease of over 56% (Figure 4). We also measured cadmium concentrations in CWRS wheat over the same period of

time and found that there was no similar decrease. This is an important finding as it demonstrates that reduced levels of cadmium in CWAD wheat are not related to changes in the procedures and tools used in our analyses.

The decrease in cadmium in durum wheat exports mirrored the decrease in production of higher cadmium accumulating varieties. By 2019, lower

accumulating varieties accounted for 98% of the insured CWAD wheat acres. Decreasing durum wheat cadmium concentrations over the past three decades demonstrates the success of the Canadian breeding programs, variety registration requirements, and adoption of low accumulating varieties by producers.

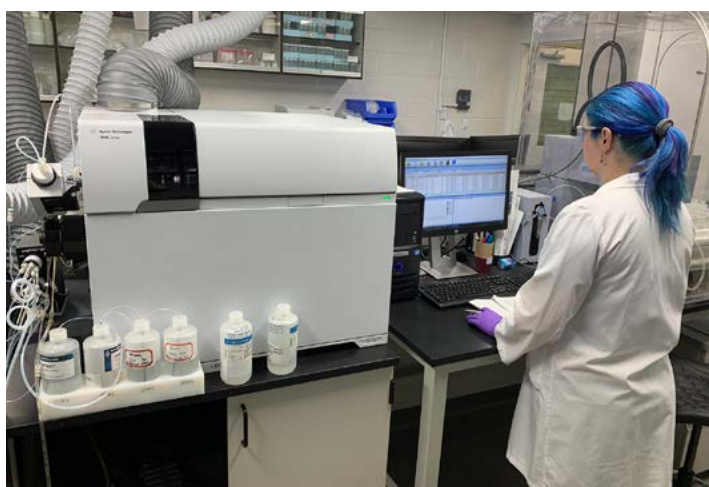


Figure 3 Samples of CWAD being analyzed for cadmium using inductively coupled plasma mass spectrometry.

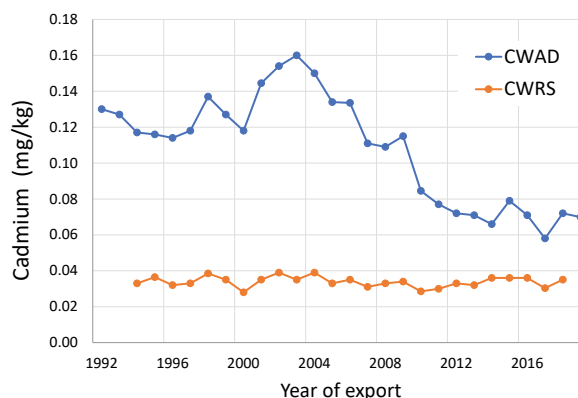


Figure 4 Cadmium, measured in milligrams per kilogram, in exports of Canada Western Amber Durum (blue) and Canada Western Red Spring (red) wheat.

Recent publications

Tittlemier, S.A. and A. Richter. 2022. Cadmium concentrations in Canadian durum exports decreased with the adoption of low accumulating cultivars. *Food Addit. Contam: Part A* 39(12): 1953-1962. <https://doi.org/10.1080/19440049.2022.2130441>

Walkowiak, S., Taylor, D., Fu, B.X., Drul, D., Pleskach, K. and S.A. Tittlemier. 2022. Ergot in Canadian cereals – relevance, occurrence, and current status. *Can. J. Plant Pathol.* 44(6): 793-805. <https://doi.org/10.1080/07060661.2022.2077451>

Tittlemier, S.A., Bestvater, L., Carlson, J., Kletke, J., Izydorczyk, M. and B.X. Fu. 2021. Fate of glyphosate in wheat during milling and bread production. *Cereal Chem.* 98 (1):100-108. <https://doi.org/10.1002/cche.10369>

Tittlemier, S.A., Blagden, R., Chan, J., Roscoe, M., McMillan, T.L., Pleskach, K. and M.S. Izydorczyk. 2020. Effects of processing whole oats on the analysis and fate of mycotoxins and ergosterol. *World Mycotoxin J.* 13 (1): 45-56. <https://doi.org/10.3920/WMJ2019.2530>