



GRAIN RESEARCH LABORATORY

Annual Program Report

2021







Canadian Grain Commission **MANDATE**

The Canadian Grain Commission works in the interests of grain producers. Guided by the Canada Grain Act, the Canadian Grain Commission establishes and maintains standards of quality for Canadian grain. It regulates grain handling in Canada and ensures that grain is a dependable commodity for domestic and export markets.

Grain Research Laboratory **VISION**

Be the pre-eminent provider of science to ensure grain quality and safety for Canada's grain sector and stakeholders.

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.

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Director's message

Dr. Esther Salvano
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It is my pleasure to present the 2021 Grain Research Laboratory Annual Report. While respecting all COVID-19 restrictions, we continued our research, monitoring, testing and development activities. Off-site work was enhanced this year by the implementation of systems that gave virtual access to some lab equipment. I am proud of the commitment and dedication shown by staff as they successfully delivered our programs. Our Oilseeds Program was awarded the top prize in gas chromatography analysis this year by the American Oil Chemists' Society and our Analytical Services Unit achieved accreditation for 3 testing methods from the Standards Council of Canada, a member of the International Organization for Standardization (ISO).

Several important changes occurred within the Grain Research Laboratory in 2021. Dr. Daniel Perry retired after providing 20 years of service and I thank him for all his contributions to the Variety Identification Research and Monitoring Program. Research related to variety identification is now the responsibility of the Microbiology and Grain Genomics Program while the Grain Biotechnology Program has taken over variety monitoring. In addition, the analytical services offered by Industry Services have been incorporated into the Analytical Services Unit in the Grain Research Laboratory. This move will strengthen the scientific guidance of these analytic activities, improve service delivery and maintain strong ties with sector stakeholders.

With a look to the future, we developed the 2022-2027 Grain Research Laboratory Operational Framework. This strategic plan will guide our operations for the next 5 years and is built on 4 interconnected pillars:

- Science and Innovation
- People
- Communication and Knowledge Transfer
- State-of-the Art Infrastructure and Technology

To help ensure the successful implementation of the five-year plan, we began working on a Science Strategy consultation aimed at allowing interested parties to provide their input on the research and science-based activities of the Canadian Grain Commission.

This year's annual report highlights some of the many ways in which we support producers and other members of the grain sector. The crop research labs looked at how mildew affects the functionality of wheat, studied the impact of pre-treatments on pulse flour quality, evaluated the malting potential of hulless barley and continued developing mathematical models to assess oilseed quality. Articles from our technology labs show how they verify instrument accuracy through check test services, use chemical fingerprints to identify microbes, analyze seeds suspected of being treated with pesticides and use DNA-based tests to monitor grain exports.

We are excited to continue our work in 2022. 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 reading this report and we look forward to your feedback!

Canadian Grain Commission

Grain Research Laboratory

The research conducted by the Canadian Grain Commission's Grain Research Laboratory falls under two categories: crop research and technology research.

Research related to crops allows us to assess Canadian grain harvest quality and studies how grading factors affect end-use properties. Crop research also develops new uses for Canadian grain and evaluates new varieties as part of the variety registration process.

Research related to technology evaluates and develops methods used to assess the quality and safety of Canadian grain.

Crop research programs include:

- Bread Wheat and Durum Research
- Milling and Malting / Research on Barley and Other Grains
- Oilseeds
- Pulse Research



Technology research programs include:

- Analytical Services
- Grain Biotechnology
- Microbiology and Grain Genomics
- Trace Organics and Trace Elements Analysis



Beyond each program's own testing and research, all of the programs support four key activities:

Cargo quality monitoring

Provides analytical testing of export grain shipments (e.g. mycotoxins, pesticides, variety composition) to ensure they meet Canada's grading and quality parameters.

Harvest Sample Program

Producers send in a voluntary sample of their harvest, and in return receive a personalized report on the quality of their crop.

Harvest Sample Program



Requests for service analysis

Provides analytical services of samples submitted by the industry for testing, at times for a fee.

Services



Plant breeder line evaluation

Provides testing and recommendations for the advancement of breeder line seed.



Grain Research Laboratory

Statistics and facts

Quality reports

We publish annual harvest and crop reports. We also publish annual <u>Fusarium survey reports</u> from samples we collect through the Harvest Sample Program.

We conducted

Falling Number and DON tests on wheat

The Harvest Sample Program received

10,958 samples for the 2020-21 crop year





cargo shipment samples



Currently, we use 107 different test methods



24 articles

were published in scientific journals

We conducted
1606
tests for
service requests
by external clients,
which included
170
breeding trials

The most popular requests
were for NIR whole seed analyses
and fatty acid composition of
canola, DNA testing of bacteria
and fungi, and rapid moisture
tests

23 presentations

were delivered at scientific meetings



All 21 grains regulated by the Canadian Grain Commission fall within the scope of the GRL's activities:

- Barley
- Beans
- Buckwheat
- Canary seed
- Canola
- Chick peas
- Corn

- Fababeans
- Flaxseed
- Lentils
- Mixed grain
- Mustard seed
- Oats
- Peas

- Rapeseed
- Rye
- Safflower seed
- Sovbeans
- Sunflower seed
- Triticale
- Wheat

Grain Research Laboratory

Social media

Promotion of programs and services

The Grain Research Laboratory uses social media to connect with producers and other members of the grain sector. We promote our programs and people, share up-to-date information about our services and research, and respond to questions and concerns.











Sharing expertise

In 2021, Grain Research Laboratory staff were involved in three Twitter takeovers that demonstrated our methods and equipment to producers and other followers of the Canadian Grain Commission account. Kerri Pleskach gave a tour of the Harvest Sample Program labs by following a sample through its journey at the Canadian Grain Commission. During Science Literacy Week, Ann Puvirajah gave a behind-the-scenes look at the oilseed lab and Dr. Kun Wang explained the durum lab.

Research reports

In addition to our annual report, the Grain Research Laboratory produces reports on the end-use quality of Canadian grain. The promotion of these reports to the grain sector includes our posts on social media.

Grain harvest and export quality













Advancements in agricultural science

Plain language summaries of research published by Grain Research Laboratory scientists are posted on our website under Advancements in agricultural science. These short articles provide the grain sector and the general public with our latest findings and show how they impact Canadian producers. To reach a wider audience, these summaries are also promoted on Twitter and Facebook.



Our study showed that barley producers with a crop that's too high in protein for malting grade could have another option for their crop.

Dr. Marta S. Izydorczyk Scientist and Program Manager, Milling and Malting / Research on Barley and Other Grains Grain Research Laboratory



The agriculture and food sectors need fast and reliable ways to predict the functional and end-use quality of wheat and flours. Using wholemeal samples for the GlutoPeak test helps fill these gaps.

Dr. Bin Xiao Fu Scientist and Program Manager, Bread and Durum Wheat Research Grain Research Laboratory

Grain Research Laboratory History

To illustrate how the Grain Research Laboratory has changed over the years in terms of tools, techniques and personnel, we post historical photos using #ThrowbackThursday.











Up-to-date quality data

Updates to data on the quality of grains in harvests and exports are regularly released on our website. By following our social media feeds, grain sector stakeholders have access to the most up-to-date information available from the Grain Research Laboratory.









To stay up-to-date on our activities, to ask questions or receive updates follow us on Twitter using @Grain_Canada or on Facebook using @CanadianGrainCommission



Bread Wheat and Durum Research

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The science behind wheat grading

The Bread Wheat and Durum Research Program supports all the participants in the grain value chain: wheat breeders, producers, exporters and customers. Our research focuses on understanding how the physicochemical and biochemical properties of wheat influence its quality and the results of our research are shared with both the scientific community and grain sector stakeholders. We also work on developing new methods for evaluating wheat quality. One of our major activities is providing a scientific basis for the establishment of grade tolerances for wheat. We do this by determining the relationships between visually assessed damage and the functional qualities of bread wheat and durum. Other activities include conducting the quality evaluations needed for the registration of new wheat lines developed by breeders and monitoring the quality of wheat cargoes at export.

The effect of mildew damage on the functionality of wheat

Mildew damage on wheat kernels appears as a grayish to black discolouration and is caused by fungi that develop due to excessive moisture prior to harvest. Mildew is generally not considered a toxicological hazard, but it can affect the end-use quality of wheat. Kernels affected by mildew are graded using standard samples that visually correspond to different degrees of soundness.

Damage from mildew can have a significant economic impact on Canadian producers. To address their concerns that current visual standards for mildew are too stringent, we undertook a study to better understand the relationship between mildew damage and the end-use qualities of Canada Western Red Spring (CWRS) wheat. The goal of our study was to provide a scientific basis for any adjustments to the mildew standards used to grade CWRS wheat.

Samples with different degrees of mildew infection were tested for their milling performance, dough sheet colour and specks, alphaamylase activity (Amylograph peak viscosity), dough properties, gluten strength, and baking quality. To ensure that mildew was the only factor being evaluated, samples that were concurrently damaged by other grading factors were excluded from this study.

The results of our study demonstrated that mildew, as it is currently assessed, had a relatively small impact on end-use quality. Mildew might, however, act as an indicator of potential elevated α -amylase activity when sprouting is not visible. Our results were presented to the Western Standards Committee and Eastern Standards Committee meetings in 2021 and provided the scientific basis for a further increase in allowable mildew levels in the grading of CWRS wheat. The proposed higher presence of mildew in visual standards, once approved and implemented, will have a significant positive economic impact on Canadian wheat producers.

Key findings

- The impact of mildew on wheat milling yield and flour refinement (ash) was minimal. Flour yield was marginally lower only for samples graded CW Feed (Table 1) and flour refinement was comparable between different grades.
- No relationship was found between the degree of mildew damage and gluten strength, dough properties and baking quality (Figures 1 and 2). Farinograph water absorption and stability time were, however, generally lower for samples graded CW Feed.
- The proportion of hard vitreous kernels was low for samples graded No. 3 and CW Feed. Test weight was slightly lower for samples graded CW Feed.
- A higher degree of mildew is generally associated with higher α-amylase activity as indicated by lower Amylograph peak viscosity (Table 1). However, samples graded CW Feed due to mildew can have Falling Number above 300 seconds.

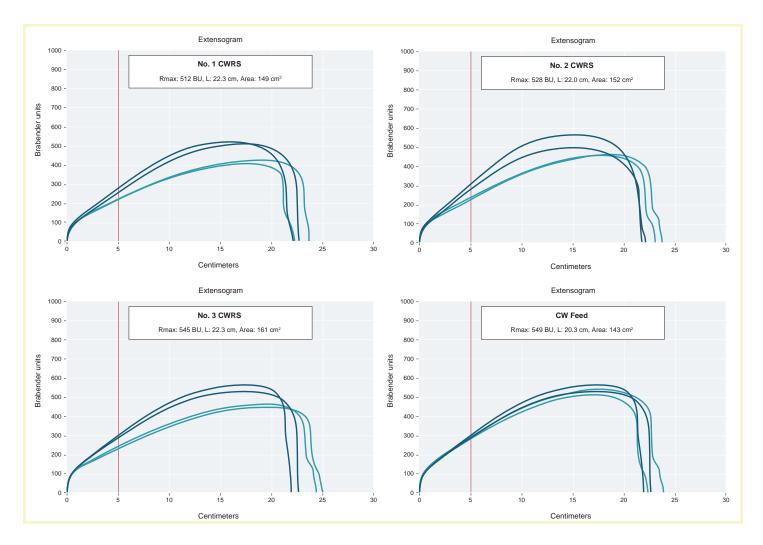


Figure 1 Extensograms of dough prepared from Canada Western Red Spring (CWRS) wheat graded for different levels of mildew damage. The maximum height of a curve (Rmax) in Brabender units (BU) indicates resistence to extension and the length of a curve (L) in centimetres (cm) indicates extensibility. The area under a curve is a measure of resistance and extensibility combined.

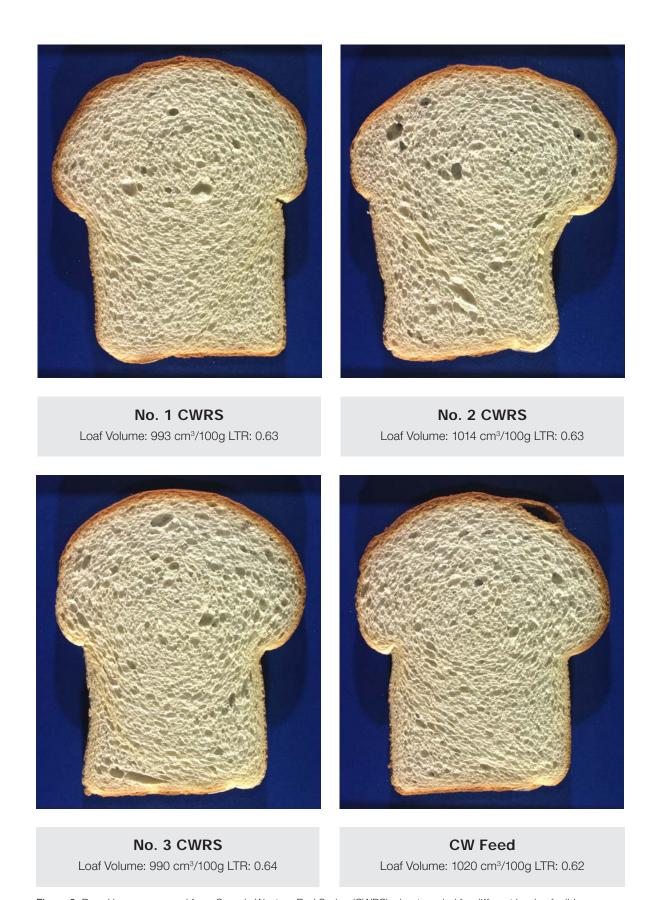


Figure 2 Bread loaves prepared from Canada Western Red Spring (CWRS) wheat graded for different levels of mildew damage. Loaf volume is measured in cubic centimetres (cm³) per 100 grams (g) of flour. Loaf top ratio (LTR) = (loaf height - pan height) / loaf width and is a measure of the shape of the loaf top.

Table 1 Milling performance and α-amylase activity of Canada Western Red Spring (CWRS) wheat graded for different levels of mildew damage.

Quality parameter	No. 1 CWRS	No. 2 CWRS	No. 3 CWRS	CW Feed
Milling yield (clean wheat basis), %	75.4	75.8	75.7	75.1
Flour ash (74% extraction), %	0.48	0.44	0.43	0.44
Falling Number (ground wholemeal), seconds	420	335	315	300
Flour Amylograph Peak Viscosity (74% extraction), BU	695	410	365	290

Engagement with the grain sector

In 2021, the Bread Wheat and Durum Research Program shared their results from mildew and test weight research with members of the Western Standards Committee and the Eastern Standards Committee to aid their science-based decision-making process. We also conducted quality evaluations for nine wheat registration trials in 2021 and generated quality data for over 150 new wheat lines to support the Canadian wheat registration process. We participated in the 2021 Prairie Grain Development Committee meetings as voting members of the wheat quality evaluation team. Drs. Fu and Wang, and Ms. Isaak participated in over 30 one-on-one meetings with key Canadian wheat customers around the world to share information on the quality characteristics of the 2021 wheat crop based on samples collected through the Harvest Sample Program.

Recent publications

Wang, K., Sangha, J., Cuthbert, R. and B.X. Fu. 2021. Effectiveness and biochemical basis of wholemeal GlutoPeak test in predicting water absorption and gluten strength of Canadian hard red spring wheat. Cereal Chem. 98 (4): 878–890. https://doi.org/10.1002/cche.10430

Bacala, R., Fu, B.X., Cordova, K. and D. Hatcher. 2021. Wheat *Fusarium* protease specificity and effect on dough properties. Foods 10 (7): 1585. https://doi.org/10.3390/foods10071585

Ruan, Y., Yu, B., Knox, R.E., Zhang, W., Singh, A.K., Cuthbert, R., Fobert, P., DePauw, R., Berraies, S., Sharpe, A., Fu, B.X. and J. Sangha. 2021. Conditional mapping identified quantitative trait loci for grain protein concentration expressing independently of grain yield in Canadian durum wheat. Front. Plant Sci. 12:642955. https://doi.org/10.3389/fpls.2021.642955

Iwaki, S.; Hayakawa, K., Fu, B.X. and C. Otobe. 2021. Changes in hydrophobic interactions among gluten proteins during dough formation. Processes 9 (7): 1244. https://doi.org/10.3390/pr9071244

Wang, K., Taylor, D., Chen, Y., Suchy, J. and B.X. Fu. 2021. Effect of kernel size and its potential interaction with genotype on key quality traits of durum wheat. Foods 10 (12): 2992.

https://doi.org/10.3390/foods10122992

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Milling and Malting / Research on Barley and Other Grains

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Evaluating new lines of hulless barley

The Milling and Malting / Research on Barley and other Grains Program conducts monitoring and research on the quality and processing properties of Canadian grains. We want to determine the relationships between the physiochemical characteristics of grains and their end-use functionality. We also want to understand how variety, environment and agronomic factors influence quality. Innovation is an important aspect of our program, as is demonstrated both by our exploration of new uses for Canadian barley and oats and our development and evaluation of new technologies for measuring quality. Our work provides breeders, producers, the malting and brewing industry, and the Canadian public with meaningful information on the reliability and quality of Canadian barley and oats.

The malting potential of hulless barley

Hulless barley (HB) has the potential to produce very high levels of malt extract and this has attracted the interest of the brewing industry. The lack of hulls and the organic substances they contain, such as tannins, other polyphenols and polysaccharides, also makes HB an appealing choice as this improves beer quality and taste. The use of HB has not been widespread, however, since many initial malting trials resulted in inadequate grain modification and other processing problems.

We undertook a study in 2021, in collaboration with Canadian barley breeders, to highlight improvements in the quality of recently developed lines of HB since no new HB cultivars have been registered in Canada since CDC Clear in 2012. Our goal was to identify and understand the similarities and differences in malting performance between HB and covered barley (Figure 1). To do this, we compared AAC Synergy (covered barley), CDC clear and 5 new HB lines.





Figure 1 Germinated covered barley (left photo) and hulless barley (right photo).

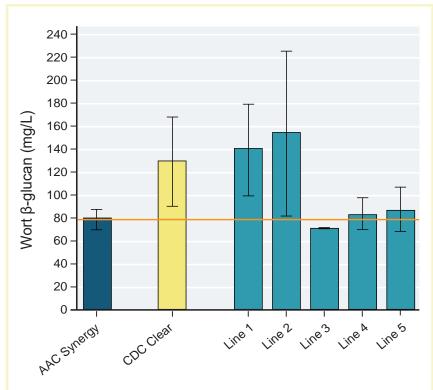


Figure 2 Concentration of β -glucan in wort produced from covered barley (AAC Synergy) and hulless barley (CDC Clear and Lines 1-5).

Methodologies and equipment

Malt analyses were conducted using the standard methods of the American Society of Brewing Chemists. We tested many parameters, including friability, alpha-amylase, malt diastatic power, wort soluble proteins and free amino nitrogen (FAN), wort β -glucans and wort viscosity. We also used state-of-the-art research techniques, including differential scanning calorimetry, high-performance anion exchange chromatography with pulsed amperometric detector and a laser light particle analyser, to isolate starch molecules and characterize their structure and physicochemical properties.





Germination drums

Table 1 Select quality parameters for covered barley (AAC Synergy) and hulless barley (CDC Clear and Lines 1-4) malts. Results with the same lowercase letters are not significantly different from each other. DU = dextrinizing units and FAN = free amino nitrogen.

	Diastatic Power (°, dry basis)	Alpha Amylase (DU, dry basis)	Fine Extract (%, dry basis)	FAN (mg/L)
AAC Synergy	123 ± 13°	73.0 ± 5.1 ^{bc}	80.4 ± 1.2°	148 ± 25°
CDC Clear	140 ± 12 ^{abc}	94.4 ± 7.0 ^a	87.5 ± 1.4 ^{ab}	165 ± 10 ^{bc}
Line 1	155 ± 9ª	83.7 ± 1.7 ^{ab}	86.9 ± 1.1 ^{ab}	143 ± 14°
Line 2	132 ± 10 ^{bc}	84.0 ± 2.8^{ab}	86.4 ± 1.1 ^{ab}	148 ± 10°
Line 3	129 ± 8 ^{bc}	69.7 ± 4.9°	88.6 ± 0.5ª	195 ± 15ª
Line 4	135 ± 3 ^{bc}	83.8 ± 5.6^{ab}	88.5 ± 0.6 ^a	184 ± 5 ^{ab}











Phoenix Micromalting Units



Steeping tanks



Kilns

Physiochemical properties and malting performance of hulless barley

Our study found that the structure of starch in HB was only slightly different from that in covered barley and that it did not negatively affect starch hydrolysis and production of fermentable sugars during mashing. HB malts exhibited low friability values but still had a crumbly texture with good solubility and enzyme accessibility. The concentration of β -glucans in wort, which indicates the extent of cell wall breakdown, was comparable in some HB lines to covered barley but the viscosity of all HB worts was higher. To understand the reason for this, we measured the concentration and average chain length of arabinoxylans which is something not usually included in quality assessments. Arabinoxylans, a type of cellulose found in cell walls, were found to have much higher concentrations and average chain lengths in all HB lines and that this partly explains the higher viscosity of HB wort. Overall, we found that the recently developed Canadian HB lines exhibited excellent malting potential with notable improvements in the levels of malt enzymes, extract, concentration of FAN compounds (Table 1) and β -glucans in wort (Figure 2).

This study provides new information on the quality traits of HB that will be useful to the malting and brewing industry. It also will help Canadian breeders promote their HB lines and develop improved genotypes.

Engagement with the grain sector

Dr. Izydorczyk, is active in many institutions, associations and committees, including:

- Cereal & Grains Association (2020-2021 President, 2021-2022 Past President)
- Brewing and Malting Barley Research Institute Technical Committee (scientific advisor)
- > Barley Council of Canada (scientific advisor and sector representative of the Research and Innovation Committee)
- Canadian Malting Barley Technical Centre (Secretary, Board of Directors)
- Prairie Recommending Committee for Oat and Barley (member of the Barley Quality Evaluation Team)
- Barley and Other Cereal Grains Sub-Committee to the Western Standards Committee (member)
- Ontario Cereal Crops Committee (member)
- University of Manitoba, Department of Food Science (adjunct professor)

Dr. Izydorczyk has also worked on several barley cluster collaborative projects under the Canadian Agricultural Partnership AgriScience Program. Tricia McMillan is a member of the malt subcommittee of the American Society of Brewing Chemists.

Recent publications

Mills, A.A.S., Izydorczyk, M.S., Choo, T.M., Sorrells, M.E., Durand, J., Mountain, N., and S. Fillmore. 2020. Cultural practices to improve malt barley quality in the northeast with focus on the craft sector. Can. J. Plant Sci. 101 (1): 39–52.

https://doi.org/10.1139/cjps-2020-0011

Tittlemier, S.A., Bestvater, L., Carlson, J., Kletke, J., Izydorczyk, M.S. 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

Izydorczyk, M.S., Nam, S., Sharma, A., and Kletke, J. 2021. Exploring dry grain fractionation as a means to valorize high protein malting barley. Cereal Chem. 98 (4): 840-850. https://doi.org/10.1002/cche.10426

Izydorczyk, M.S. and T. McMillan. Barley Harvest Annual Report 2021. Barley Production and Quality of Western Canadian Malting Barley.

https://www.grainscanada.gc.ca/en/grain-research/export-quality/cereals/malting-barley/

Izydorczyk, M.S., Badea, A., and A.D. Beattie. 2022. Physicochemical properties and malting potential of new Canadian hulless barley genotypes. J. Am. Soc. Brew. Chem. (in press)

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Oilseeds Dr. Véronique Barthet

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Using mathematical models to determine oilseed quality

The Oilseeds Program conducts research on Canadian canola, rapeseed, flaxseed, soybeans and mustard seed. We analyse how grading factors and biochemical compounds, such as free fatty acids, affect seed quality and the products made from them. Another important part of our work is assessing the methods used to measure oilseed quality. This is done by participating in the international validation of reference methods and developing and updating mathematical models to predict oilseed quality. We also pioneer and validate our own methods to analyse minor seed compounds such as the cyanogenic glycosides found in flaxseeds and flaxseed products. In addition, we conduct the quality assessments of most Canadian oilseeds submitted to the Harvest Sample Program and export shipment samples (Figure 1). The results of our research and monitoring are presented in a variety of ways, including quality reports, spreadsheets and presentations, to ensure information reaches producers, export customers and other stakeholders in a timely manner.

Obtaining canola quality data

New canola varieties are fully registered based on the merit of their qualities after 2 years of testing. In the first year, data is obtained privately while in the second year, testing is done under a co-operative system. The Oilseeds Program is responsible for conducting the quality analyses of the co-op samples and providing the data that will be part of the variety registration documentation. From September to December, we analyse approximately 2,500 canola samples, generating over 12,500 data points. Near infra-red (NIR) spectroscopy is used to test for oil, protein and total glucosinolate content while total saturated fatty acid and erucic acid content are analysed using gas chromatography (Figure 2).

Verifying the accuracy of models

Much work is done to ensure that the results reported by the Oilseeds Program are correct and trustworthy. NIR analysis gives us prediction data that we use in mathematical models that define the relationship between NIR scans and chemical data from many hundreds of reference samples. To obtain a specific quality result, data from an NIR scan is put into the mathematical model that was developed for that quality factor. A single scan can be used to predict multiple quality factors since a different mathematical model can be developed for each quality factor. This allows many samples to be analysed in a small amount of time. Although our models are based on more than 20 years of canola data, it is still essential for accuracy that they be continually verified. Sample location, variety, growing conditions, defects and contamination by other seeds can affect the models. Every year, we assess the quality of the mathematical models for all crops and all quality factors by analyzing random samples with both NIR spectroscopy and reference chemical methods. We then perform statistical analyses to compare the results obtained by both methods. To ensure quality control, it is important to have also an external check of the data. To do this the Oilseeds Program participates in international proficiency testing panels, reporting the data we obtain by NIR spectroscopy and chemical reference methods.









Oilseed Program services

The Oilseeds Program provides several analyses on a <u>fee for service</u> basis. In 2021, we conducted the following number of tests:

- 146 for oil content using NIR spectroscopy
- 3 for oil content using chemical extraction
- > 12 for protein content
- 25 for total glucosinolate content
- > 54 for free fatty acid content
- 59 for fatty acid composition of oil
- 6 for chlorophyll content



In 2021, Dr. Barthet presented data to the Canada-Japan Canola Consultations, Canola Week conference, the Western Canadian Canola/Rapeseed Recommending Committee (WCC/RRC) and the oilseeds sub-committee of the Western Standards Committee. Dr. Barthet is also a member of the WCC/RRC, the Prairie Recommending Committee for Oilseeds of the Prairie Grain Development Committee and the Global Council for Innovation in Rapeseed and Canola (GCIRC). In September 2021, Dr. Barthet organized and chaired the second day of the GCIRC technical meeting which discussed research issues on rapeseed protein production and added value.

Dr. Barthet participated as an expert in the International Organization for Standardization (ISO) meeting on oils and fats (TC34SC11) and was head of the Canadian delegation at the ISO meeting on oilseeds and oilseed meals (TC4SC2). In 2021, the Oilseeds team was awarded the top prize in gas chromatography for the analysis of fatty acids by the American Oil Chemists' Society



Figure 1 Pulse nuclear magnetic resonance (NMR) instrument used to analyse crude oil content.

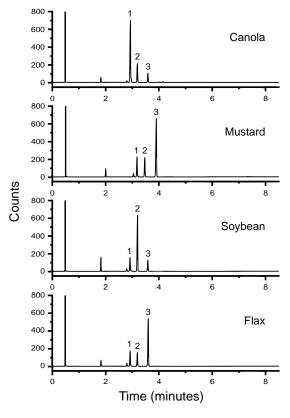


Figure 2 Chromatograms showing the results of fatty acid analysis.

Recent publications

Barthet, V.J., Petryk, M.W.P. and B. Siemens. Prediction of intact canola seed quality using handheld near-infrared spectrometer. INFORMS annual meeting, October 2021.

Barthet, V.J., Petryk, M.W.P. and B. Siemens. 2020. Rapid nondestructive analysis of intact canola seeds using a handheld near-infrared spectrometer. J. Am. Oil Chem. Soc. 97(6): 577-589.

https://doi.org/10.1002/aocs.12335

Team members

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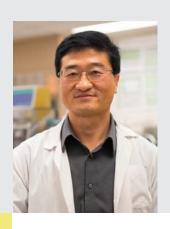
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Pulse Research

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Helping the food industry increase their use of Canadian pulses

The Pulse Research Program supports Canada's grain quality assurance system and the marketability of Canadian pulses in several ways. We investigate how variety, growing conditions, processing, and grading factors affect the quality of pulses and their end products. We research new ways to use and process pulses, and we develop methods that can be used by the pulse industry to assess pulse end-use quality and functionality in a consistent and objective way. We also conduct the annual pulse and food-type soybean quality analysis for the Harvest Sample Program and take part in cargo monitoring.

The impact of pre-treatments on the quality of pulse flours

Canada has long been a global player in the export of whole and split pulses. There is tremendous potential, however, for the secondary processing sector to expand and add value to Canadian pulses, both domestically and prior to export, in the form of products such as flours and protein concentrates. Food manufacturers are increasing their use of these types of pulse ingredients since they are low in cost, high in nutritional value and help to meet the growing demand for gluten-free items. The successful development of new food products requires, however, a thorough understanding of how processing affects the physiochemical, functional and nutritional properties of pulse ingredients. Our research team is working on a collaborative project that is studying the effects of several pre-treatments on pulse flours, including infrared heating, germination and roasting. The collaborators include researchers from Agriculture and Agri-Food Canada's Morden Research and Development Centre, Cereals Canada, the University of Saskatchewan and the University of Manitoba.



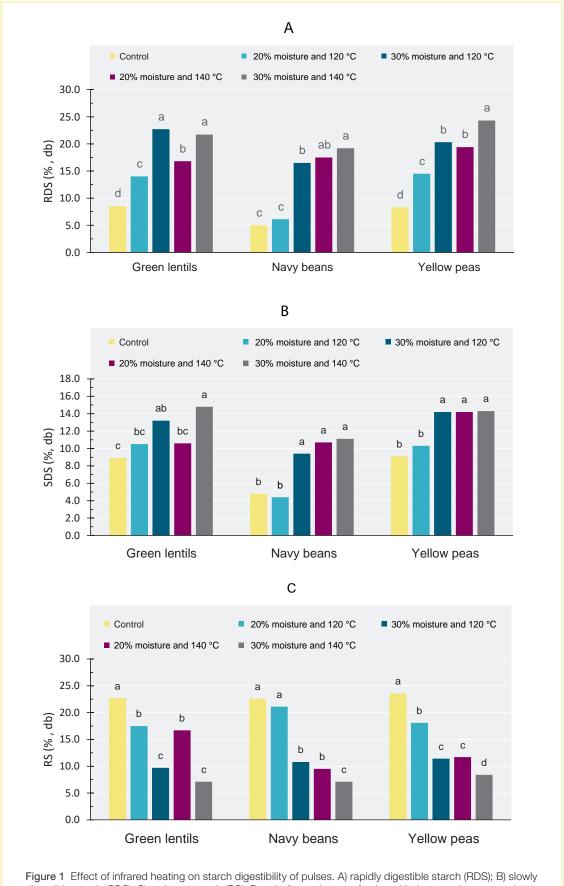
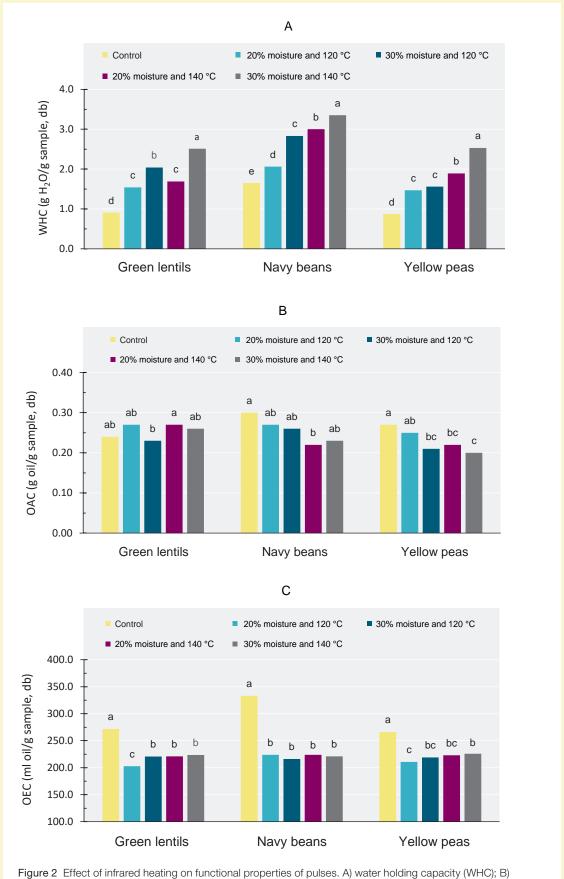


Figure 1 Effect of infrared heating on starch digestibility of pulses. A) rapidly digestible starch (RDS); B) slowly digestible starch (SDS); C) resistant starch (RS). Results for each type of pulse with the same lower-case letters are not significantly different.



oil absorption capacity (OAC); C) oil emulsion capacity (OEC). Results for each type of pulse with the same lower-case letters are not significantly different.

Infra-red heating of pulses

One of the pre-treatments, infrared-heating, uses electromagnetic radiation to cause water molecules within a seed to vibrate and generate heat. This causes rapid internal heating and a rise in water vapor pressure inside the seed. Our project evaluated the effect of infrared heating on the starch digestibility and functionality of yellow pea, green lentil and navy bean flours.

Samples of green lentils, yellow peas and navy beans were tempered to 20% or 30% moisture and then exposed to infrared heating at a temperature of 120°C or 140°C. We analysed rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) using standard methods. Our results indicate that flours made from seeds exposed to infrared heating had higher levels of digestible starch than flours made from untreated seeds. The greatest effect was seen in the amount of RDS in yellow pea flour made from seeds with 30% moisture and heated at 140°C (Figure 1). Higher moisture and temperature have been found to be associated with a higher degree of gelatinized starch. Gelatinization causes starch granules to disperse, increasing the void areas around them and making them more vulnerable to digestion by enzymes.

Infrared heating treatments also affected the functionality of the pulse flours. We found that higher moisture levels and temperatures increased water holding capacity (WHC) but decreased the oil absorption capacity (OAC) and oil emulsion capacity (OEC) of pulse flours. Infrared heating had the greatest effect on the OEC of navy bean flour (Figure 2).

The knowledge generated from this study will help Canadian food manufacturers identify pulse processing strategies that produce pulse flours with the functional and nutritional specifications needed for a wide range of food applications. This has the potential to increase the use of pulses and create diversity in the ingredients which will benefit the Canadian pulse sector.

Engagement with the grain sector

Dr. Wang, program manager of Pulse Research, is a member of the following national and international committees:

- Prairie Grain Development Committee Prairie Recommending Committee for Pulse and Special Crops (PRCPSC)
- Quality Evaluation Team for PRCPSC
- Pulse Sub-committee reporting to the Western Standards Committee
- Cereals & Grains Association, including Approved Methods Technical Leadership Committee

Dr. Wang is also the co-chair of the Pulse and Legume Technical Committee of the Cereals & Grains Association, has participated in the Manitoba Sustainable Protein Innovation Forum and provided input to the Manitoba Sustainable Protein Research Strategy through surveys and workshops. He was also an invited speaker at the annual meeting of the Cereals & Grains Association, Cereal & Grains 21.

Recent publications

Fenn, D., Wang, N. and L. Maximiuk. 2022. Physicochemical, anti-nutritional and functional properties of air-classified protein concentrates from commercially grown Canadian yellow pea (*Pisum sativum*) varieties with variable protein levels. Cereal Chem. 99: 157-168. https://doi.org/10.1002/cche.10506

Guldiken, B., Franczyk, A., Boyd, L., Wang, N., Choo, K., Sopiwnyk, E., House, J.D., Paliwal, J. and M.T. Nickerson. 2022. Impact of milling on the functional and physicochemical properties of green lentil and yellow pea flours. Cereal Chem. 99: 218-229. https://doi.org/10.1002/cche.10504

Guldiken, B., Konieczny, D., Wang, N., Hou, A., House, J.D., Tu, K., Rosendahl, S., Lavier, M. and M.T. Nickerson. 2021. Effect of variety and environment on the physicochemical, functional, and nutritional properties of navy bean flours. Eur. Food Res. Technol. 247: 1745-1756. https://doi.org/10.1007/s00217-021-03745-7

Stone, A.K., Parolia, S., House, J.D., Wang, N. and M.T. Nickerson. 2021. Effect of roasting pulse seeds at different tempering moisture on the flour functional properties and nutritional quality. Food Res. Intl., 147: 1-10. https://doi.org/10.1016/j.foodres.2021.110489

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Analytical Services

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Verifying the accuracy of grain quality measurements

Analytical Services conducts many different types of tests for thousands of clients each year. We analyse samples for a wide range of quality and grading factors, including protein content, dough strength, deoxynivalenol levels, moisture content, ash content, particle size index, Falling Number, gluten index and wet gluten content. We also oversee and maintain the Harvest Sample Program. Since 1927, this program has supported producers by giving them a grade and quality assessment report at no cost on samples they submit. Samples collected through this program are also used for various research projects throughout the Grain Research Laboratory.

Another important responsibility of Analytical Services is providing the reference data for protein testing using combustion nitrogen analysis (CNA). Labs within the Canadian Grain Commission use our CNA results to accurately calibrate near infra-red (NIR) instruments used in monitoring activities. We also monitor and maintain the Canadian calibrations for two common types of grain moisture meters. In 2021, we received ISO /IEC 17025 accreditation for measuring moisture content by forced air oven, ash content and crude protein using CNA. International accreditation confirms that our assessments are accurate and impartial, giving international customers confidence in the quality of Canadian grain.

Check test services

Analytical Services offers three types of services to stakeholders in the grain industry that allow them to regularly check the performance of their instruments:

- monthly Falling Number check tests for Canada Western Red Spring wheat and/or Canada Western Amber Durum
- monthly moisture check tests for Canada Western Red Spring wheat
- protein check test once every two months for Canada Western Red Spring wheat and/or Canada Western Amber Durum

The check tests are fee-based and by subscription only, with stakeholders being able to sign up at any time during the year.

Throughout the year, we source material to prepare the check test samples we send to clients. We obtain more than 680 kilograms of grain from cargo samples that are no longer needed for testing in the Grain Research Laboratory and from producers through bulk orders. Each month we make up a different sample to ensure that different values are measured. All check test samples are thoroughly mixed and a representative sub-sample is taken for each client. Samples are packaged (Figure 1) and for moisture tests, double bagged to protect the integrity of the moisture level.

For the Falling Number check test subscription (Figures 2 and 3), each client is assigned a lab number. Clients send in their results to our enzyme lab where all the results are compiled. A report is prepared and distributed to clients. Once the moisture samples are sent to clients, our lab randomly selects 4 representative sub-samples. These are tested on 3 different types of moisture meters: 10 Unified Grain Moisture Algorithm (UGMA) instruments (Figure 4), 6 model 919/3.5" instruments and 1 near infra-red transmittance (NIT) instrument. The samples are also analysed with the air oven reference method. The data are analysed and the official moisture result is emailed to all clients within 7 days of the sample being sent to them. Each protein check test sample is analysed over a 2-month period using 6 different NIT instruments, with 10 replicates for each. Each sample is also analysed on 2 CNA instruments using our ISO accredited method, also with 10 replicates. The results are averaged and printed on the label of each sub-sample as the official result. Clients submit their results to our protein lab and a report is created for them.

The check test subscriptions give the grain industry an opportunity to compare the measurements obtained from their instruments with those from other laboratories and verify their accuracy. If a client's result doesn't align with ours, it is their responsibility to determine the cause. Inaccurate measurements may be due to such things as errors in sample preparation, malfunctioning instruments or improper methods. Currently, the Falling Number check test has 16 subscribers, the moisture check test has 102 subscribers and the protein check test has 58 subscribers. Inquiries about the Falling Number and the moisture check subscriptions can be sent to moistureandenzymes-eauetenzymes@grainscanada.gc.ca and inquiries about the protein check subscription can be sent to protein-proteines@grainscanada.gc.ca.



Figure 1 Canada Western Red Spring wheat samples being prepared for distribution for the bi-monthly protein check test.



Figure 2 Front view of rotary divider; representatively dividing samples for the falling number check test.



Figure 3 Running monthly Falling Number check test

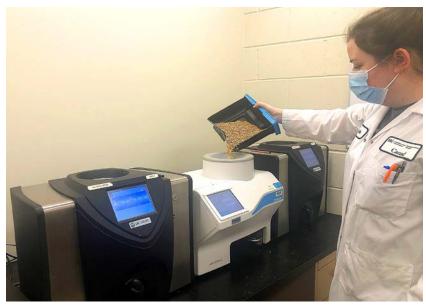


Figure 4 Moisture meters used for analysis of the moisture check test sample: AM522 Perkin Elmer and GAC Dickie John.

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Grain Biotechnology Research

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DNA-based testing of Canada's grain exports

A major focus of the Grain Biotechnology Program is the use of DNA-based methods for detecting genetically modified (GM) events in grains. Our priorities in this area are developing tests for new GM events and the overall improvement of our detection capability. Real-time qualitative and quantitative polymerase chain reaction (PCR) assays are routinely used for the detection of GM events. We also conduct research on how to optimize droplet digital PCR tests for detecting and quantifying GM events. For Canadian grain exports to have continuous access to markets, it is critical that our testing methods are accurate. Our technical experience is recognized internationally, and we have been ISO/IEC 17025 accredited since 2013 to test for GM events.

In August 2021, the Variety Identification Monitoring Program merged with the Gain Biotechnology Program. The variety identification aspect of our program is responsible for analysing the composition of all bulk export shipments of durum and bread wheat. All varieties assigned to a specific class share the same end-use properties. By confirming that the correct varieties are present in shipments, we provide assurance that each class of wheat will perform consistently and that customer expectations will be met. Additionally, we offer varietal purity certification of submitted cargo samples of barley on a fee for service basis. We also provide variety identification and composition analyses to support other research in the Grain Research Laboratory, including the Harvest Sample Program.











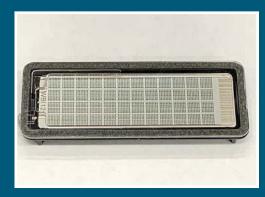
QuantStudio Accufill

Automated sample loading



QuantStudio 12K-Flex

PCR instrument



Open array plate

- Durum: 16 assays, 144 kernels / plate
- Bread wheat: 32 assays, 96 kernels / plate

Figure 1 OpenArray Technology used for monitoring of bread and durum wheat varieties

Monitoring for an unapproved GM event

In June 2018, the Canadian Food Inspection Agency announced that a few wheat plants on an access road in Southern Alberta had survived glyphosate herbicide spraying. These plants were found to be an unregistered variety of wheat that contained a GM event (MON71200). No GM wheat is currently authorized for commercial production in Canada or any other country. Since the discovery of MON71200, we have been testing bulk wheat export shipments and submitted samples for its presence. We have also been providing letters of analysis that contain our test results to exporters upon request. In the more than 3,200 samples we have analysed, no evidence of this GM event in the Canadian grain value chain has been found.

Identification of durum and bread wheat varieties

We use OpenArray-DNA technology for the identification of both durum and bread wheat varieties (Figure 1). This technology uses a plate the size of a microscope slide. We place DNA of 96 kernels of bread wheat on one OpenArray plate and use 32 DNA markers called single nucleotide polymorphisms (SNPs) to genotype the kernels. For durum wheat, we place DNA of 144 kernels on one plate and use 16 SNPs for genotyping. We use VID Inspector software to analyse the data generated by the OpenArray technology. To analyse the varietal purity of submitted barley samples, we use a Li-Cor system that is based on DNA markers called microsatellites.

Recent monitoring activities

Between August 1, 2020, and July 31, 2021, we analysed many cargo samples and submitted samples, including:

- > 38 malting barley cargo samples for varietal purity
- 707 bread wheat cargo samples for variety composition
- 263 durum wheat cargo samples for variety composition
- 67 wheat cargo composites
- > 55 co-op wheat samples
- 1,083 wheat samples for the presence of MON71200 GM event

Engagement with the grain sector

Dr. Demeke is a member of the Canadian Advisory Committee for ISO/TC 34 SC 16 (Molecular Biomarker Analysis) and the Cereals and Grains Association. He is also a special issue editor for "Digital PCR and Plant Studies" in the journal Biology (https://www.mdpi.com/journal/biology/special_issues/Digital_PCR)

Recent publications

Demeke, T., Eng, M., Holigroski, M. and S-J. Lee. 2021. Effect of amount of DNA on digital PCR assessment of genetically engineered canola and soybean events. Food Anal. Methods. 14: 372-379.

https://doi.org/10.1007/s12161-020-01889-y

Demeke, T., Beecher, B. and M. Eng. 2020. Assessment of genetically engineered events in heat-treated and non-treated samples using droplet digital PCR and real-time quantitative PCR. Food Control 115:107291.

https://doi.org/10.1016/j.foodcont.2020.107291

Demeke, T. and D. Dobnik. 2018. Critical assessment of digital PCR for the detection and quantification of genetically modified organisms. Anal. Bioanal. Chem. 410 (17): 4039-4050. https://doi.org/10.1007/s00216-018-1010-1

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Microbiology and Grain Genomics

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Improving microbe identification and surveillance

The Microbiology and Grain Genomics Program uses the latest tools in molecular biology and genomics to develop high-throughput DNA tests for the identification and characterization of grain varieties. We also perform DNA testing on thousands of samples each year for bacteria and fungi. Results from our tests provide assurance of quality to customers in the grain handling system and aid in disease management. The surveillance and applied research we conduct is done in collaboration with partners in government, academia, and industry, and supports the Canadian Grain Commission's Harvest Sample Program.

Identifying pathogens on wheat

Plant pathogenic fungi are one of the major challenges in wheat production because they reduce both yield and quality. Some of the most damaging fungi of wheat are Puccinia species that cause stem, stripe, and leaf rust. Fusarium species that cause Fusarium head blight also affect wheat yield and quality and can contaminate grain with toxins that affect food safety (Figure 1). Both rust and Fusarium fungi are very diverse, with different species and races that cause varying levels of infection on different wheat cultivars. Identification of species and races is important for disease surveillance and ensures that the grain industry stays ahead of emerging fungal strains.

Current methods for Fusarium and rust identification involve DNA testing, plant infection assays, and microscopy. In an infection assay, different crop lines are inoculated with fundi and a visual assessment of disease is performed to identify their race. Microscopy can also be used to identify microbes by their shape and size. Infection assays and microscopy are laborious and costly methods for microbial identification, and it is usually better to perform DNA tests when they are available. Even though DNA testing provides a high-throughput and low-cost solution to microbe identification, we continue to develop new methods that can better support the grain industry and its stakeholders.



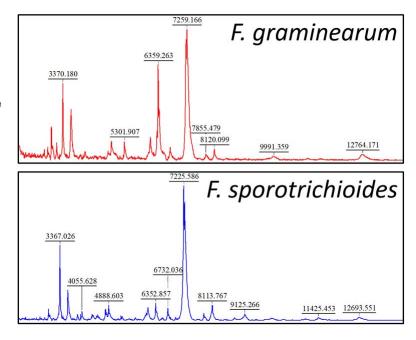
Figure 1 Fusarium graminearum growing on a Petri dish.



Figure 2 Staff using the MALDIT-TOF MS to identify fungi from grain samples.

Creating chemical fingerprints using mass spectrometry

One of our projects focuses on identifying plant pathogenic fungi using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). This technique ionizes the contents of microbe samples and analyses them using mass spectrometry, allowing for each chemical within the sample to be separated based on their mass and their charge after being ionized (Figure 2). The chemical profile of each microbe species is unique and acts like a fingerprint for identification. MALDI-TOF MS is inexpensive to run and can process hundreds of samples a day. Using this method, we have been able to create unique chemical fingerprints for different Fusarium (Figure 3) and rust species that can be used for microbe identification and surveillance, giving improved analytical support for partners in the grain sector. This project is a collaboration between the Canadian Grain Commission, Agriculture and Agri-Food Canada, the University of Manitoba, the University of Saskatchewan, and the University of British Columbia, and is supported by the Manitoba Crop Alliance, the Saskatchewan Wheat Development Commission, and the Western Grains Research Foundation.



Mass-to-charge ratio

Figure 3 Example profiles of *Fusarium graminearum* (top, red) and *Fusarium sporotrichioides* (bottom, blue) generated using MALDI-TOF MS.

Engagement with the grain sector

Dr. Walkowiak is a member of the disease evaluation team for the Prairie Grain Development Committee, which makes recommendations for crop variety registration. He is also a member of the International Wheat Genome Sequencing Consortium, 10+ Wheat Genomes Project, Canadian Phytopathological Society, Association for Biosafety and Biosecurity, and International Seed Testing Association. He also regularly participates in outreach sessions with government, industry, crop commissions, and national and international societies. In July 2021, Dr. Walkowiak was a keynote speaker for the TriSociety meeting of The Canadian Phytopathological Society, the Canadian Society of Agronomy and the Canadian Society for Horticultural Science. Dr. Walkowiak is also an Adjunct Professor in the Department of Plant Science at the University of Manitoba and trains students in the latest tools in microbiology and grain genomics.

Recent publications

Walkowiak, S., Pozniak, C.J. and K.T. Nilsen. 2022. Recent advances in sequencing of cereal genomes. In: Bilichak A., Laurie J.D., editors. Accelerated breeding of cereal crops. Springer Protocols Handbooks. Humana, New York, NY.

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Gamage, N.W., Bamforth, J., Ashfaq, T., Bernard, K., Gräfenhan, T. and S. Walkowiak. 2021. Profiling of *Bacillus cereus* on Canadian grain. PLos One 16 (11): e0259209. https://doi.org/10.1371/journal.pone.0259209

Walkowiak, S., Gao, L., Monat, C., Haberer, G., Kassa, M.T., Brinton, J., Ramirez-Gonzalez, R.H., Kolodziej, M.C., Delorean, E., Thambugala, D., et al. 2020. Multiple wheat genomes reveal global variation in modern breeding. Nature 588: 277-283. https://doi.org/10.1038/s41586-020-2961-x

Nilsen, K.T., Walkowiak, S., Kumar, S., Molina, O.I., Randhawa, H.S., Dhariwal, R., Byrns, B., Pozniak, C.J. and M.A. Henriquez. 2021. Histology and RNA sequencing provide insights into Fusarium head blight resistance in AAC Tenacious. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.570418

Rabanus-Wallace, M.T., Hackauf, B., Mascher, M., Lux, T. Wicker, T., Gundlach, H., Baez, M., Houben, A., Mayer, K.F.X., Guo, L. et al. 2021. Chromosome-scale genome assembly provides insights into rye biology, evolution and agronomic potential. Nat. Genet. 53 (4): 564-573. https://doi.org/10.1038/s41588-021-00807-0

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Trace Organics and Trace Elements Analysis

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Accurate analysis in support of grain safety

The Trace Organics and Trace Elements Analysis Program focuses on the monitoring, surveillance and research of pesticide residues, mycotoxins, and trace elements in grain. We examine the factors that cause these substances to occur in grain and study how they are affected by processing during the production of grain-based foods. Data from our monitoring, surveillance, and research activities give commodity associations, exporters, government partners, and end users confidence in the safety and reliability of Canadian grain. We also research ways to make methods for sampling and analyzing more accurate and precise. Producers and exporters rely on our work to accurately assess pesticide residues, mycotoxins and heavy metals in their grain.

Efficient identification of treated seeds

One example of how our work supports stakeholders along the grain value chain is our analysis of seeds that are suspected of being treated with pesticides. To protect crops during seeding and initial plant development, seed from a variety of grains is sometimes treated with registered pesticide products. Improper cleaning of equipment, storage spaces, trucks, railcars, and other forms of transportation, can, however, lead to treated seed contaminating grain that has been harvested.

Grain that is suspected of being treated is visually identified by grain inspectors or handlers (Figures 1 and 2) and samples are sent to the Trace Organics and Trace Elements group for analysis (Figures 3). We use a new liquid chromatography high resolution mass spectrometric method to quickly determine if pesticides are present on the grain (Figure 4). This is an important step because other substances used in agricultural practices, such as spray indicators, can give seeds the appearance of being treated with pesticide.



Figure 1 Seed suspected of being treated with pesticide.

Once the presence of pesticides is confirmed, we identify which pesticides are present and measure their concentrations. Our research on the distribution of seed treatments in grains enabled us to develop more accurate analysis methods that ensure high quality data is produced. The results of our analyses are used by the Chief Grain Inspector for Canada and grain handlers to prevent contaminated grain from moving through the Canadian grain handling system. Each year, the Trace Organics and Trace Elements group averages around 55 analyses of seed suspected of being treated with pesticides. To date, we have found that almost all suspect seed has had pesticide concentrations low enough to meet Canadian regulations on residue limits.

Additional testing services

In addition to suspect treated seed analysis, the Trace Organics and Trace Elements group conducts testing of quality factors and uses specific methodology to confirm the presence of:

- <u>mycotoxins</u>
- glyphosate

We also offer testing of grain for cadmium, lead, arsenic, and other <u>trace elements</u> upon request.



Figure 2 Grain inspector examining grain.

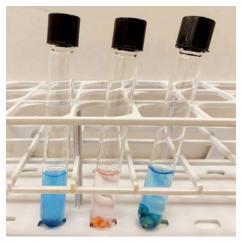


Figure 3 Sample preparation of suspect treated seed.

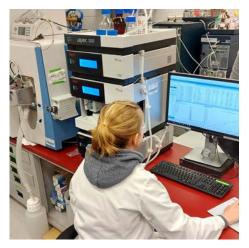


Figure 4 LC-Orbitrap used in liquid chromatography high resolution mass spectrometry.

Engagement with the grain sector

Over the past year, the Trace Organics and Trace Elements Analysis Program collaborated with Japan's Ministry of Agriculture, Fisheries and Forestry, the Canadian Food Inspection Agency, Agriculture and Agri-Food Canada and the University of Manitoba on research projects related to the occurrence and analysis of grain contaminants. We also worked with the following commodity and scientific associations on surveillance activities, the development of new research projects, and the assessment of new analytical technology:

Pulse Canada

- > Flax Council of Canada
- Canadian Special Crops Association
- Cereals & Grains Association

In response to new contaminant regulations adopted in the past year by the European Union and other countries, data from our monitoring and surveillance activities was used in meetings with the World Trade Organization and trading partners. These data also provided the framework for over 4,400 individual documents issued by the Canadian Grain Commission in support of the safety of Canadian grain exports.

Recent publications

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

Tittlemier, S.A., Blagden, R., Chan, J., Roscoe, M. and K. Pleskach. 2019. A multi-year survey of mycotoxins and ergosterol in Canadian oats. Mycotoxin Res. 36 (1): 103-114. https://doi.org/10.1007/s12550-019-00373-9

Tittlemier, S.A., Chan, J., Gaba, D., Pleskach, K., Osborne, J., Slate, A.B. and T.B. Whitaker. 2019. Revisiting the sampling, sample preparation, and analytical variability associated with testing wheat for deoxynivalenol. World Mycotoxin J. 12 (4): 319-332. https://doi.org/10.3920/WMJ2019.2450

Tittlemier, S.A., Arsiuta, J., Mohammad, U., Hainrich, C., Bowler, K., Croom, R., Olubodun, A., Blagden, R., McKendry, T., Gräfenhan, T. and K. Pleskach. 2019. Variable relationships between *Fusarium* damage and deoxynivalenol concentrations in wheat in western Canada in 2016. Can. J. Plant Pathol. 42 (1): 41-51. https://doi.org/10.1080/07060661.2019.1620861

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