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GRAIN RESEARCH LABORATORY

Annual Program Report

2023

Canadian Grain Commission
Commission canadienne des grains

Canada

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La présente publication est offerte en français.

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
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I am delighted to present the 2023 Annual Program Report from the Canadian Grain Commission's Grain Research Laboratory. During the past year our dedicated team undertook a diverse range of activities to enhance the understanding of grain science. Grain samples collected through our Harvest Sample Program allowed us to evaluate grain grading standards, investigate quality and safety issues, support the development of new markets for grain and improve our analytical methods. Our teams also collaborated with the Canadian Food Inspection Agency, breeders and other sector partners to investigate samples of CDC Copeland barley that contained a relatively high percentage of an undesignated DNA profile and found that the characteristics and malting quality of these samples did not differ from samples with very low levels of the undesignated DNA.

An important achievement in 2023 was ISO17025 reaccreditation for 4 testing methods and new accreditation for 3 malt testing methods from the Standards Council of Canada. Achieving this accreditation promotes confidence in our work nationally and around the world.

We also published our Science Strategy which lays out a vision for the future of science and research at the Canadian Grain Commission and positions us to respond to the latest trends and developments in the grain sector. This strategy was developed in consultation with producer and industry organizations, end users, academia, federal government, provincial governments, and Canadian Grain Commission staff. It identifies 5 drivers that will shape the future of our science and research:

- global trends and emerging market issues
- advances in technology
- evolving end uses
- climate change and extreme weather
- food safety and nutrition

This year's report highlights research that aligns with these drivers. You will learn about our work on understanding how drought affects the quality of malting barley and how heat stress influences the dough properties of wheat. You will discover how we improved the analysis of cyanogenic glycosides in flaxseed, studied the factors that impact the quality of faba beans and evaluated the accuracy and precision of our sampling methods. The 2023 report also describes how we implemented new methods for high throughput DNA-based identification of Canadian barley varieties and developed predictive models that can help forecast the risk of microbial diseases such as Fusarium head blight, ergot and blackleg.

I extend my gratitude to our exceptional team for their commitment to excellence in research and innovation, which is demonstrated by their hard work, dedication and passion. I also thank our valued stakeholders for their collaboration and support. Together, we will continue to make significant strides in grain science and in our understanding of the factors that affect the quality and safety of Canadian grains. Thank you for your interest in the Grain Research Laboratory's work. I invite you to share your comments and thoughts with us and look forward to your feedback.

Bread Wheat and Durum Research

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Understanding and addressing concerns from Japanese customers about changes in the gluten properties of CWRS wheat exposed to heat stress

The Bread Wheat and Durum Research Program supports the quality assurance system for Canadian wheat. We investigate the impact of various grading factors on functionality to provide the scientific basis for their tolerances in wheat classes and grades. We also analyze the quality of new crops and monitor wheat cargos and evaluate new wheat varieties recommended for registration for designation into Canadian wheat classes based on their merit. Our research focuses on understanding how the physicochemical and biochemical properties of wheat influence its quality and developing new techniques for evaluating wheat quality.

Dough qualities of CWRS wheat exposed to heat stress

We recently started a collaboration with the milling and baking industry in Japan, which is the largest customer of No. 1 Canada Western Red Spring (CWRS) wheat. This was done to address concerns that Japanese bakers had about the long mixing requirements and low extensibility of dough made from CWRS wheat grown in the hot and dry years of 2021 and 2023. They described the dough as “strong but brittle and inextensible” and reported that their processes had to be adjusted. This resulted in increased production costs and an undesirable variation in end product quality.

We compared the results of our Extensograph and Farinograph tests from 2020 to 2023 and confirmed that doughs made from CWRS wheat exposed to heat stress were significantly stronger, as indicated by higher maximum resistance (R_{max}) values, and longer development and stability times (Table 1). The extensibility of dough was similar, however, for all four years. Our standard test methods, widely used internationally, did not capture the brittle and inextensible dough properties described by Japanese customers.

Does gluten protein composition change under heat stress?

To better understand the effect of heat stress on dough, we fractionated the gluten proteins in flour samples of CWRS wheat from 2020 to 2023 into insoluble glutenin, soluble glutenin and monomeric

Team members

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proteins. Insoluble glutenin contributes to dough strength and the latter two fractions contribute to the extensibility of dough. The results show a higher ratio of insoluble glutenin to soluble glutenin in wheat exposed to heat stress (Figure 1), which is consistent with a stronger dough and longer mixing time. However, the brittle and inextensible dough properties are not well explained by the distribution of gluten protein fractions.

Assessment of commercial flour samples from Japan

We then assessed six commercial flour samples, milled mostly from CWRS wheat, that were provided by a major wheat milling company in Japan. Our collaborator reported that the dough properties of the samples ranged from well balanced to brittle and inextensible. We found no significant differences in extensibility between the six samples, however, using our standard test methods (Table 2).

Table 1 Properties of dough made with CWRS wheat from 2020 to 2023[1]

Dough properties	2020	2021[2]	2022	2023[2]
Extensograph				
R_{max}, BU	552	712	631	796
Extensibility, cm	19.8	19.1	20.1	18.6
Area, cm2	137	169	160	183
Farinograph				
Absorption, %	65	65	65	65
Development time, min	6.3	10.8	7.3	8.8
Stability, min	11.5	21.0	14.5	25.8

[1] Compiled from harvest quality reports for CWRS wheat
[2] Drought years in western Canada

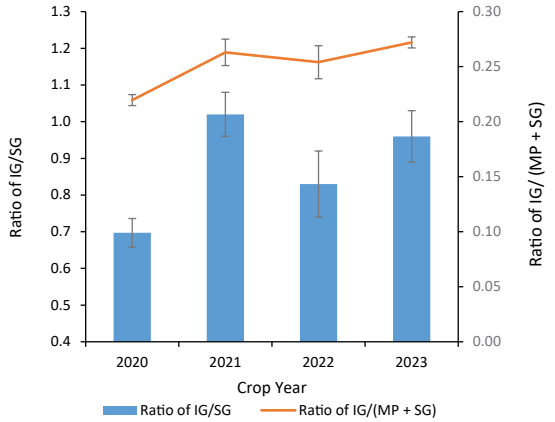

Figure 1 Changes in the ratio of insoluble glutenin to soluble glutenin (IG/SG) and the ratio of insoluble glutenin to the sum of monomeric proteins and soluble glutenin (IG/(MP+SG)) in CWRS wheat from 2020 to 2023.

Technical exchange with Japan

An in-person technical exchange was arranged with Japanese experts who have extensive experience in assessing dough handling properties. Our Sponge and Dough bake method was compared with the Shokupan bake method used in Japan and some notable differences in formulation and methodology were observed. With input from the Japanese experts, the Shokupan bake method was adapted for use with our equipment and our staff were trained in the subjective scoring system used in Japan to assess dough qualities. For example, extensibility is evaluated by how far the dough stretches before tearing and thinly stretched dough is considered brittle if it breaks easily when lightly touched. In a blind re-test of the Japanese commercial flour samples using the Shokupan bake method, our staff were able to differentiate the dough properties important to the Japanese baking industry (Figure 2).

We also evaluated the Shokupan dough properties of the 2023 CWRS wheat samples received by the Harvest Sample Program and the results were consistent with the description of dough made from 2023 wheat cargos received by Japan.

Figure 2 Test bakers in the Grain Research Laboratory assessing dough made using the Japanese Shokupan bake method.

Table 2 Properties of dough made with commercial flour samples from Japan

Dough properties	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Characteristics reported by Japanese collaborator	Well balanced	Balanced; slightly lower in extensibility	Strong but insufficient extensibility	Strong but brittle and inextensible	Fragile, low extensibility	Weak, brittle and inextensible
Extensograph						
R_{max}, BU	906	907	964	892	950	803
Extensibility, cm	15.3	16.1	14.9	16.7	15.1	17.2
Area, cm2	168	177	170	183	173	172
Farinograph						
Absorption, %	64.0	64.6	64.1	64.0	63.5	63.6
Development time, min	17.0	17.3	18.0	14.3	17.5	13.3
Stability, min	20.0	22.0	25.0	23.0	23.0	21.0

Next Steps

With the cooperation of our collaborator, we will continue receiving samples of commercial flour from Japan with good, average, and poor dough properties to be used as references to continue the training of our staff and for the evaluation of cargo and new crop No. 1 CWRS wheat aggregates. We also plan to conduct research to understand the biochemical basis of the brittle and inextensible dough properties that result from heat stress. Gluten consists of many types of proteins and their size distribution, relative proportions, cross-linking capability, hydrophobicity, and various protein-protein interactions all contribute to gluten viscoelasticity. Although these properties are mostly controlled by genetics, they can also be significantly modified by heat stress. Knowledge at the molecular level is critical for the selection or development of wheat varieties with improved consistency in gluten functionality under heat or other environmental stresses.

Recent publications

Bacala, R., Hatcher, D.W., Perreault, H., and **B.X. Fu.** 2023. Partial C-terminal truncation of Bx and Dy high molecular weight glutenin subunits after conserved aspartate. J. Cereal Sci. 114:103805. https://doi.org/10.1016/j.jcs.2023.103805

Wang, K., Taylor, D., Ruan Y., Pozniak, C.J., Izydorczyk, M. and **B.X. Fu.** 2023. Unveiling the factors affecting milling quality of durum wheat: influence of kernel physical properties, grain morphology and intrinsic milling behaviours. J. Cereal Sci. 113: 103755. https://doi.org/10.1016/j.jcs.2023.103755

Iwaki, S, **Fu, B.X.**, and K. Hayakawa. 2023. Behavior of protein aggregates via electrostatic interactions or hydrogen bonds during dough formation. J. Cereal Sci. 111: 103683. https://doi.org/10.1016/j.jcs.2023.103683

Tittlemier, S.A., Bestvater, L., Chan, J., Timofeiev, V., Richter, A., Wang, K., Ruan, Y., Izydorczyk, M., and **B.X. Fu.** 2023. Diverging fates of cadmium and glyphosate during pasta cooking. Food Addit. Contam. Part A 40(11) 1459-1469. https://doi.org/10.1080/19440049.2023.2264976

Milling and Malting / Research on Barley and Other Grains

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Effects of recent drought conditions on the Canadian Prairies on the malting quality of barley

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

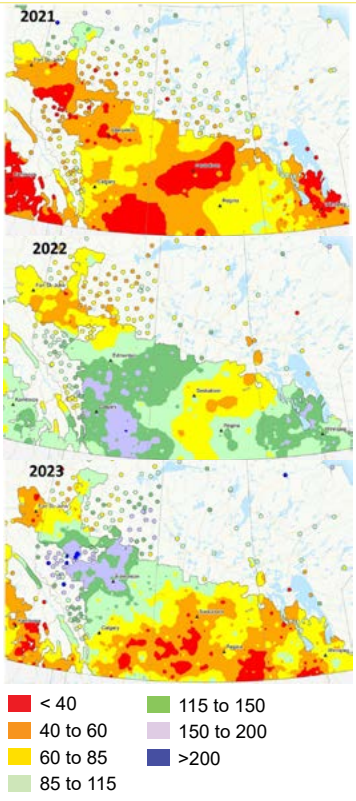

Figure 1 Percent of average precipitation in June and July for 2021, 2022 and 2023 (Agriculture and Agri-Food Canada).

Recent droughts

In 2021 and 2023, the Canadian Prairies experienced above average temperatures and varying degrees of drought. In 2022, however, temperatures and precipitation levels were close to average (Figure 1) These conditions allowed us to investigate the effects of high temperature and drought on the grain quality, processing performance, and malt properties of barley by comparing data collected for our annual reports on barley quality in 2021, 2022 and 2023.

Chemical and physical grain characteristics in drought years

High temperatures and drought in 2021 resulted in an average protein content of 13.2% for malting barley, which is significantly higher than the average protein content of 12.3% reported in 2022. Although the 2023 growing season began with high temperatures and below average precipitation, cooler temperatures in July helped relieve crop stress and malting barley from 2023

Team members

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was found to have the same average protein content as in 2022 (Figure 2). The growing conditions in 2021 and 2023 did, however, produce malting barley with significantly lower test weights and kernel densities, but greater average kernel lengths, compared to 2022 (Figure 2). The latter observation suggests heat and drought may play a role in defining kernel shape. We found that growing conditions did not affect the β-glucan content of malting barley grain, but the average arabinoxylan content was greater in 2021 and 2023 compared to 2022.

Effects of drought on malting qualities of barley

The combination of lower test weight, lower kernel density and elongated kernel shape contributed to the easy and rapid absorption of water during steeping for 2021 and 2023 barley (Figure 3). The occurrence of pre-harvest sprouting in 2021 and 2023, and a lower hardness index in 2023, also helped to decrease hydration time. This resulted in excellent cell wall modification during malting and very low viscosity and concentration of β-glucans in wort (Figure 4). High protein content in grain can reduce malt extract yield and, as expected, the malt made from 2021 barley resulted in the lowest average malt extract level (78.7%) (Figure 4). Despite the protein content of barley in 2022 and 2023 being similar, the malt extract level was lower in 2023 (80.4%) compared to 2022 (81.2%). The lower-than-expected malt extract levels in 2023 barley may be partially due to a slightly lower starch content and lower levels of proteolytic and starch degrading enzymes, especially alpha-amylase, as demonstrated by a lower concentration of soluble proteins and free amino nitrogen.

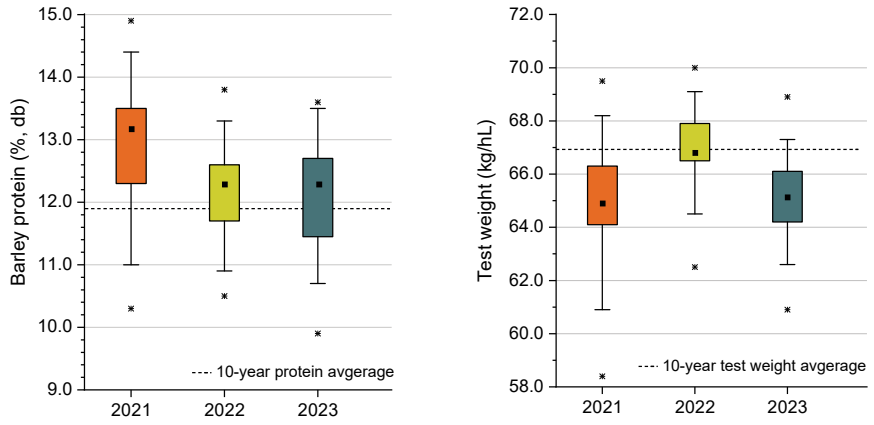
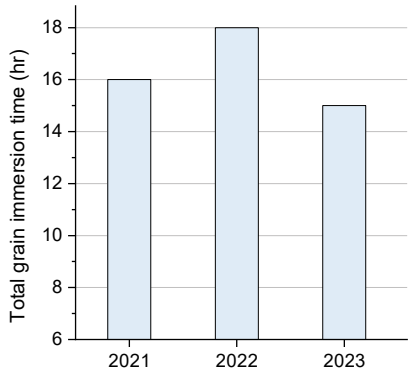


Figure 2 Protein content and test weight for western Canadian malting barley grown in 2021, 2022 and 2023.

During malting there is a specific temperature range, referred to as the gelatinization temperature (Tg), at which starch granules lose their crystalline structure and are solubilized. Barley from the 2021 drought had a Tg that was up to 2°C higher than 2022 barley. This increase in Tg can be partly attributed to a higher protein content in barley grown in 2021, stronger starch/protein interactions, and partly to starch properties. We found a strong correlation between the Tg of isolated starches and the Tg of grain, indicating that differences observed in Tg between crop years are due to the physical and molecular characteristics of starches and not just protein content. The isolated starches exhibited a bimodal distribution of large



Faster water uptake during steeping in 2021 and 2023 due to:

1. Lower test weight and kernel density of grain in 2021 and 2023
2. Elongated kernel shape in 2021 and 2023
3. Occurrence of pre-harvest sprouting in 2021 and 2023
4. Lower hardness index in grain in 2023

Figure 3 Time required for adequate hydration during steeping for western Canadian malting barley grown in 2021, 2022 and 2023.

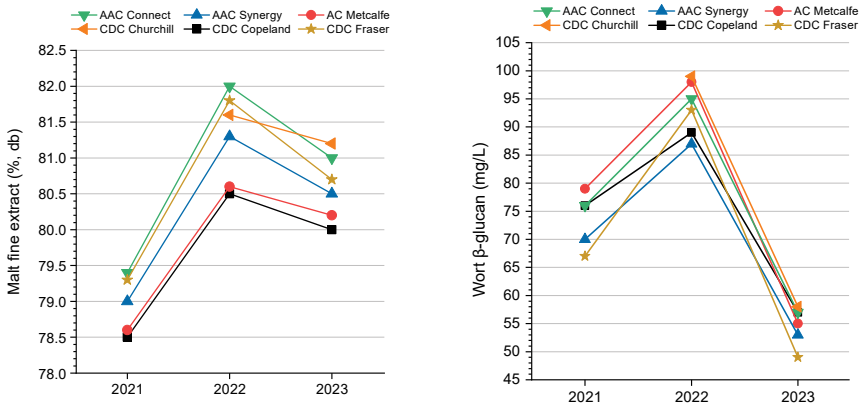


Figure 4 Average fine extract levels and wort β-glucans from the malt of six different barley varieties grown in 2021, 2022 and 2023.

and small granules. Figure 5 shows the variation in Tg and the distribution of small and large starch granules for the variety AAC Connect grown in 2021, 2022 and 2023. The volume of small granules showed a positive correlation with Tg, suggesting that drought conditions may increase the volume of small starch granules, which are known to have a higher Tg than large granules.

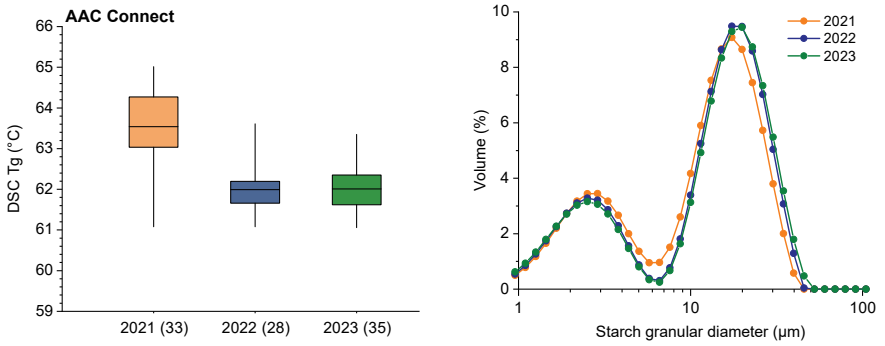


Figure 5 Gelatinization temperature (Tg) and distribution of small and large starch granules isolated from AAC Connect grown in 2021, 2022 and 2023. The number of samples in indicated in parentheses.

Conclusions

Taken together, the results of barley harvest surveys in recent years clearly indicate that weather conditions affect both the composition and physical grain properties. However, the yearly overall malting quality of barley is highly variable due to the complex interactions between specific weather patterns and their effects on different grain characteristics.

Recent publications

O'Donovan, J.T., Kubota, H., Harker, K.N., Turkington, T.K., May, W.E., Johnson, E.N., Beres, B.L., **Izydorczyk, M.,** et al. 2024. Effect of Pre-Harvest Glyphosate Rate and Timing on Yield and Pre-Malt Quality of Malting Barley. Can. J. Plant Sci. https://doi.org/10.1139/CJPS-2023-0167

Lee, S-J., Eckhardt, M., Dusabenyagasani, M., **Izydorczyk, M.** Demeke, T., Perry, D, and S. Walkowiak. 2024. Identification of Canadian Barley Varieties by High-throughput SNP Genotyping. Can. J. Plant Sci. https://doi.org/10.1139/CJPS-2023-0187

Evans, D.E., Paynter, B.H., **Izydorczyk, M.S.** and Li Chengdao. 2023. The impact of terroir on barley and malt quality—a critical review. J. Inst. Brew. 129(4): 211-258. https://doi.org/10.58430/jib.v129i4.38

Kaur, G., Toora, P.K., Tuan, P.A., McCartney, C.A., **Izydorczyk, M.S.** et al. 2023. Genome-wide association and targeted transcriptomic analyses reveal loci and novel candidate genes regulating preharvest sprouting in barley. Theor. Appl. Genet. 136: 202. https://doi.org/10.1007/s00122-023-04449-0

Acar, O. **Izydorczyk, M.S.**, McMillan, T. et al. 2023. A research on milling fractions of biofortified and non-biofortified hull-less oats in terms of minerals, arabinoxylans and other chemical properties. Cereal Chem. 100(5): 1–11. https://doi.org/10.1002/cche.10702

Oilseeds

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Improving the measurement of cyanogenic glycosides in flaxseed

The Oilseeds Program conducts research on Canadian oilseeds such as canola, rapeseed, flaxseed, soybean and mustard seed. We assess the effects of grading factors on oilseed quality and analyze how the biochemical components of oilseeds 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 models to predict oilseed quality using near infra-red spectroscopy, and pioneering and validating our own methods to analyze minor seed compounds. The Oilseeds Program also evaluates the quality of oilseeds in samples from the Harvest Sample Program and export shipments.

Cyanogenic glycosides in flaxseed

We analyze minor compounds in oilseeds because they can have physiological importance or can be perceived as negatively affecting health. One group of minor compounds that our lab studies in flaxseed is cyanogenic glycosides. There are about 75 known cyanogenic glycosides in more than 2,500 plant species and they can be found in an entire plant or just in certain structures such as roots or seeds. Although cyanogenic glycosides occur in low levels in flaxseed, they are a concern because hydrogen cyanide (HCN), a well-known toxin, is released when they break down through hydrolysis. HCN is only released if the plant or seed tissues are damaged and the enzymes responsible for hydrolysis come into contact with the cyanogenic glycosides.

The European Union and Japan have set limits on the total amount of cyanogenic glycosides allowed in flaxseed that is used in food products and feed. In Canada and the United States flaxseed is considered safe for consumption and numerous studies have documented its health benefits due to the presence of alpha-linolenic acid (an omega-3 fatty acid), fiber and lignans.

Team members

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Student
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Traditional methods for measuring cyanogenic compounds

Two cyanogenic diglycosides, linustatin and neolinustatin, are found in sound (undamaged) flaxseeds and two cyanogenic monoglycosides, linamarin and lotaustralin, are found in seedlings, developing plants, flowers, damaged seeds and immature seeds (Figure 1).

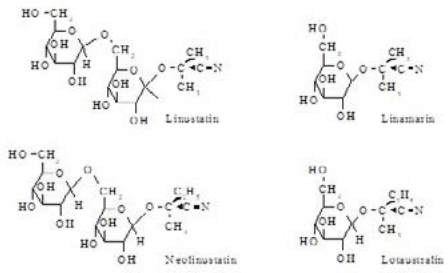


Figure 1 Molecular structure of cyanogenic glycosides found in flax.

The traditional method for analyzing these compounds measures the amount of HCN produced after the cyanogenic glycosides are hydrolyzed. To be accurate, this method requires the complete hydrolysis of the cyanogenic glycosides and the compete capture of the HCN produced. Hydrolysis may be incomplete, however, if the acids or enzymes being used to cause hydrolysis interact with other compounds or are not specific to the cyanogenic glycosides being hydrolyzed. For example, flaxseed cyanogenic glycosides need two active enzymes in sequence to be hydrolyzed and using only one will lead to almost no HCN production. This traditional method gives highly variable results with very low reproducibility.

A better approach to the analysis of cyanogenic glycosides is to quantify the intact cyanogenic compounds. An accurate high pressure liquid chromatography (HPLC) method for this has been reported in the scientific literature, but the method is very long as several steps are necessary to improve the detection limit.

Analytical improvements

Our team has been working to improve the analysis of cyanogenic glycosides in flaxseeds and to develop an accurate, repeatable and reproducible method to measure them. We first were able to lower the detection limit using gas chromatography (GC) with flame ionization detection (FID) instead of HPLC. We also improved the extraction of cyanogenic glycosides from seeds and then developed a GC-mass spectrometry (GC-MS) method to analyze baked products which further decreased the limit of detection. We also found that one of the cyanogenic monoglycosides was not commercially available in its natural form, so we developed a method to obtain pure compounds to use as reference material, which led to an improvement in the quantification of cyanogenic monoglycosides.

Comparison study with Health Canada

The Oilseeds team was also involved in a project with Health Canada that compared our GC-MS method with a liquid chromatography-mass spectrometry (LC-MS) method used to directly quantify cyanogenic glycosides. Although they can be cost prohibitive, LC-MS methods are often considered to be the best since compounds are analyzed directly without transformation into a gaseous state. They are also very specific since the mass spectra allow compounds to be identified.

The results of the study (Table 1) show that our GC-MS method has very good reproducibility with the relative standard deviation (RSD) of analysis being 0.90%. A comparison of the GC-MS and LC-MS results showed no statistical difference between the two methods. These results indicated that the analysis of intact cyanogenic glycosides should be the method of choice for their quantification.

Table 1 LC-MS and GC-MS results for total cyanogenic glycoside content in flaxseed measured as the hydrogen cyanide equivalent (mg/kg)

Statistical measure[1]	LC-MS results	GC-MS results
Average	306.82	283.44
Median	285.20	277.22
Maximum	657.20	404.46
Minimum	128.42	109.36

[1] Number of samples for each method was 22

Recent publications

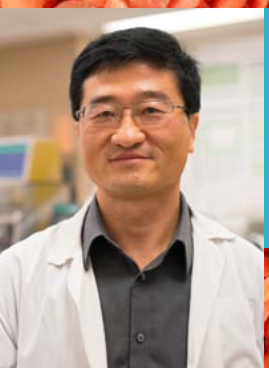
Fan, T. and **V.J. Barthet**. 2018, Nov. 29-Dec. 1. Development of GC/MS methods for the quantification of cyanogenic glycoside in breads with flaxseed. Poster session presented at: Lake Louise XXXI. 31st Workshop on Tandem Mass Spectrometry; Lake Louise, AB, CA.

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.

T. Fan and **V.J. Barthet**. 2016, June 5-9. Analysis of trimethylsilyl derivatives of cyanogenic glycosides from flaxseed (Linum usitatissimum) by GC/MS. Abstract in Proceedings of the 64th ASMS Conference on Mass Spectrometry and Allied Topics; San Antonio, TX, USA.

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

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



Pulse Research

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Understanding the factors affecting faba bean quality

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 also develop and evaluate new methods for quantifying the functional characteristics of pulses. To support the marketability of Canadian pulses, we conduct an analysis of the pulse and food-type soybean samples submitted to the Harvest Sample Program each year and take part in cargo monitoring.

Increasing use of faba beans

Faba bean production in Canada has increased in recent years due to the rising demand for plant-based proteins. Faba beans are a rich source of proteins, carbohydrates, vitamins and minerals, and can be used as whole or split beans in food products such as soups, stews and pastes. They can also be processed into flours and protein concentrates/isolates that can be used in baked goods, pasta, meat products and meat replacements. There is limited information available, however, on the quality and functionality of Canadian faba beans. Identifying varieties that can grow in different environments and have characteristics that make them valuable to the food industry is important for supporting their use in traditional and new ways.

We conducted a study to determine how variety, growing location and year affected the quality and functionality of Canadian faba beans. Location represents predictable environmental variation, such as the type of soil, and year represents unpredictable environmental variation, such as weather conditions. Data was collected for three different varieties (Fabelle, Malik and Snowbird) grown at three different locations in Saskatchewan in 2019 and 2020. The component of variance, which is the relative contribution of each experimental factor to the overall variability of the data, was calculated for several quality and functional parameters. Understanding data variability helps us to identify which factors are major contributors to the quality parameters.

Team members

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Factors affecting faba bean quality

We found that variety, growing environment (location and/or year) and their interactions had a significant effect on the protein, starch and total dietary fiber (TDF) content of faba beans. As shown in Figure 1, the largest component of variance in protein content was growing location (45.7%), followed by location x year interaction (16.6%), variety (14.4%) and year (14.2%). This indicates that growing location had the greatest effect on protein content. Variety (39.8%) and growing location (30.7%) accounted for most of the variability in starch content while variety (36.2%) and location x year interaction (19.9%) accounted for most of the variability in TDF content. Protein content ranged from 25.8% to 32.3%, starch content from 38.7% to 45.2% and TDF content from 15.7% to 18.1% on a dry matter basis. We also observed a negative correlation between protein and starch content in faba beans.

Factors affecting faba bean functionality

The ability of pulse flours and protein concentrates/isolates to absorb and retain water or oil affects the texture, mouthfeel and flavor of food, and can reduce loss for products such as meat substitutes. We found that most of the variability in the water holding capacity (WHC) of faba beans was attributed to growing year (30.8%), location x year interaction (26.1%) and location (16.7%) (Figure 2). Variability in the oil absorption capacity (OAC) was largely affected by variety x location interaction (39.8%) and variety (32.4%). WHC and OAC were both positively correlated with TDF, whereas WHC was negatively correlated with protein content.

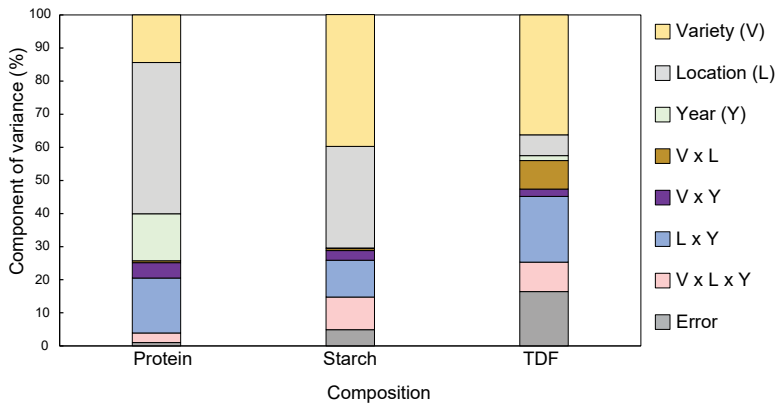

Figure 1 Relative contribution to the variance of protein, starch and total dietary fiber (TDF) content in faba beans grown in Saskatchewan in 2019 and 2020.

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. These two properties can affect the performance of pulse flours and protein concentrates/isolates in foods such as mousses, sweets and whipped desserts. We found that variability in FC was mostly accounted for by variety x location x year interaction (27.3%), variety x year interaction (25.0%) and variety x location interaction (18.0%) (Figure 2). Variety accounted for 67.8% variation in FS (Figure 2). It was observed that FS was correlated negatively with starch content. Knowledge gained from this study can help breeders and growers improve the quality of faba beans and supports the increased use and marketability of Canadian faba beans.

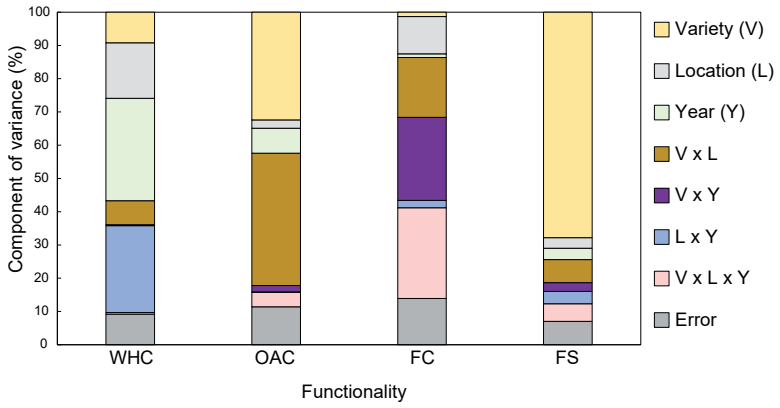

Figure 2 Relative contributions to the variance of water holding capacity (WHC), oil absorption capacity (OAC), foaming capacity (FC) and foaming stability (FS) for faba beans grown in Saskatchewan in 2019 and 2020.

Recent publications

Laing, E., Stone, A. K., Shi, D., Pickard, M., **Wang, N.** and Nickerson, M.T. 2023. Effect of infrared heating on the functional properties of yellow pea and lentil flours. Cereal Chem. 100(3): 601-613. https://doi.org/10.1002/cche.10662

Laing, E., Stone, A. K., Shi, D., Pickard, M., House, J. D., **Wang, N.** and Nickerson, M.T. 2023. Effect of infrared heating on the nutritional properties of yellow pea and lentil flours. Cereal Chem. 100(3):614-627. https://doi.org/10.1002/cche.10653

Analytical Services

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The evolution of rapid moisture testing at the Canadian Grain Commission

The Analytical Services Program conducts many different types of analyses for thousands of clients each year. This includes rapid moisture testing of grain on a fee for service basis for cargo samples and samples submitted by grain companies, producers and commodity groups. Clients can also sign up for a fee-based check test service that allows them to regularly check the accuracy of their moisture meters using samples of Canada Western Red Spring wheat prepared by our team each month.

Moisture content in grain

The moisture content of grain affects its quality, safety and storage life. High moisture levels encourage the growth of insects, fungi and bacteria and affect physiological processes, such as sprouting. The most accurate method for determining the amount of moisture in a sample of grain is to dry it in an air oven and measure the change in mass. For many years, however, the grain sector has used moisture meters to rapidly predict moisture levels in grain. These meters are based on the principle that the electrical conductivity of grain increases as moisture increases but to give accurate results, they must be calibrated using the air oven reference method. Our lab is responsible for monitoring and maintaining the calibrations for the commonly used moisture meters in Canada.

Early advances in moisture testing technology

The methods used for rapid moisture testing at the Canadian Grain Commission have changed with innovations in technology. The Board of Grain Commissioners for Canada (the precursor to the Canadian Grain Commission) first began evaluating moisture meters in 1934 and found that the motor operated Tag-Heppenstall

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meter, which measured the electrical resistance of a current passing through grain, was the most accurate for wheat and barley at that time.

Advances in electronics led to the availability of moisture meters that measured the electrical capacitance of grain samples at a frequency of 18 MHz. By 1959, the Canadian Grain Commission was using this technology in 919/3" moisture meters and in the early 1980's changed to 919/3.5" meters which could accommodate larger seeds. In the late 1990's, the Canadian Grain Commission began evaluating moisture meters that used the same technology as 919/3.5" meters but offered a higher degree of automation. In 2005, Seedburo Model 1200A became the official moisture meter at the Canadian Grain Commission.

Introduction of the Unified Grain Moisture Algorithm

In 2007 a new method for measuring moisture content, the Unified Grain Moisture Algorithm (UGMA), was developed and made available for use in moisture meters. This method uses a measurement frequency of 149 MHz, which was found to reduce the influence of grain parameters such as weight, grain type, seed size or shape and growing conditions. As a result, the accuracy and stability of the calibrations for all grain types were improved over a wider temperature range and separate grain calibrations were not needed for lightweight samples. The Canadian Grain Commission began evaluating UGMA meters in 2012 and found that their moisture predictions were equal to or better than those from 919 meters. In 2015, after using 919 meters for more than 55 years, the Canadian Grain Commission began using UGMA meters for official moisture testing (Figure 1).

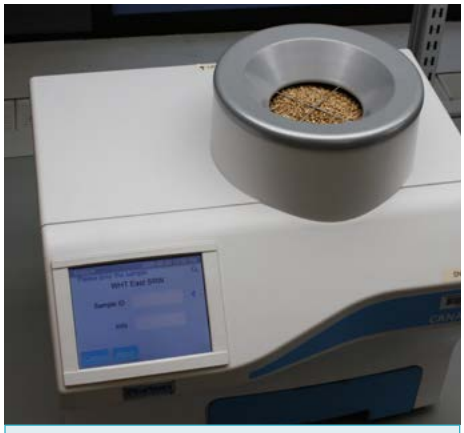

Figure 1 UGMA-type moisture meter.

Calibration of moisture meters

We monitor and maintain calibrations for UGMA moisture meters and the conversion table for 919/3.5” meters (Figure 2) on an annual basis for all regulated grains depending on sample availability. Our monitoring process evaluates the differences between results from the air oven reference method and the moisture meters. Changes to calibrations are made when we find a consistent difference, outside the range of assigned tolerance, with the air oven results for at least 3 years and sufficient samples have been tested across the full moisture range. Calibration changes to UGMA moisture meters are made in conjunction with the manufacturers and calibration updates are released annually. The 919/3.5” conversion tables are available on our website. Even though both types of moisture meters are calibrated against our reference air oven method, they can give slightly different results due to factors such as grain density, sample weight and operating temperatures.

We may use UGMA technology for the next 50 years or technology may advance again. The Canadian Grain Commission will, however, continue to evaluate improvements in moisture meter technology as they develop.

Figure 2 Model 919 moisture meter.

Related resources

Moisture content for Canadian grains

Moisture meter comparisons

Conversion tables for model 919/3.5” or equivalent

Grain Biotechnology

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DNA-based testing provides assurance of grain quality

The Grain Biotechnology Program develops and evaluates DNA-based methods for detecting genetically modified (GM) events in grain and uses these methods to monitor grain when required. Many countries have regulations that govern the approval and traceability of GM food ingredients and having the ability to detect and quantify unapproved and discontinued GM events is important for ensuring market access for Canadian grain. We also use DNA-based tests to monitor wheat shipments for varieties that do not meet the requirements for wheat classes and to certify the varietal purity of malting barley cargos.

Detecting multiple GM events in soybeans

The number of new GM events in soybeans and canola has been steadily increasing since the mid-1990's and we currently have validated methods for detecting and quantifying 18 different GM events in soybeans (Table 1). These methods are based on polymerase chain reaction (PCR) and have a limit of detection and limit of quantification of 0.05%.

The PCR detection of each GM event in a single soybean sample can be time consuming but our methods allow us to detect many GM events at the same time. We dispense the required primer and probe concentrations into 96-well plates and freeze them. After the addition of the sample DNA and other ingredients needed for testing, we analyze the sample DNA for all 18 GM events using four PCR tests for each event. Once the positive GM events in the sample are identified, we determine their quantities using a real-time quantitative PCR assay. Our lab uses the QuantStudioTM 7 Pro real-time PCR system to detect and quantify GM events in grain samples (Figure 1). This equipment has improved our capacity for PCR testing and is a replacement for the Applied BiosystemsTM 7500 System that was used in our lab from 2004 to 2023.

Varietal purity of CDC Copeland

Variety is an important consideration when barley is selected for malting purposes and most buyers require a minimum of 95% varietal purity. The purity of the variety CDC Copeland

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recently became a major concern when a relatively high percentage of an undesignated DNA profile was found in some samples from the 2022 growing season. We collaborated with the Grain Research Laboratory's Microbiology and Grain Genomics Program, the Canadian Food Inspection Agency, breeders and industry partners to test samples of CDC Copeland for this undesignated DNA profile. It was subsequently confirmed that initial breeder seed of CDC Copeland contains low levels of the undesignated DNA profile and that its presence was not a contamination event. A study by the Grain Research Laboratory's Barley and Other Grains Research/Milling and Malting Program found that the grain and malting characteristics did not differ in samples of CDC Copeland with high levels of the undesignated DNA profile. We also collaborated with the Barley and other Grains Research/Milling and Malting Program in testing selected cargo and harvest samples.

Table 1 Validated real-time quantitative PCR methods for GM events in soybeans

GMO event	Trait
A2704-12	Glufosinate (Liberty) herbicide tolerance
A5547	Glufosinate herbicide tolerance
BPS-CV127	Imidazolinone herbicide tolerance
DAS44406-6	Glufosinate, glyphosate and 2,4-D herbicide tolerance
DAS68416-4	Glufosinate and 2,4-D herbicide tolerance
DAS81419-2	Glufosinate herbicide tolerance and lepidopteran insect resistance
DP305423	High oleic/low linoleic and linolenic acid
DP356043	Glyphosate and sulfonylurea herbicide tolerance
FG72	Glyphosate and isoxaflutole herbicide tolerance
GMB151	Isoxaflutole herbicide tolerance and cyst nematode resistance
MON40-3-2	Glyphosate herbicide tolerance (Roundup Ready I)
MON88701	Lepidopteran insect resistance
MON87705	Glyphosate herbicide tolerance; modified oil/fatty acid
MON87708	Dicamba herbicide tolerance
MON87751	Lepidopteran insect resistance
MON87769	Glyphosate herbicide tolerance; modified oil/fatty acid
MON89788	Glyphosate herbicide tolerance (Roundup Ready II)
SYHTOH2	Glufosinate and Mesotrione herbicide tolerance

Figure 1 QuantStudio™ 7 Pro instrument used for real-time PCR testing

Technology used for varietal identification monitoring

The cooperation of breeders in supplying samples of the new breeder seeds of barley and wheat is essential to ensure that all varieties are represented in our database.

For many years we assessed the varietal purity of barley samples using a Li-Cor system that is based on DNA markers called microsatellites. Although this method is precise, it is not efficient since only a limited number of samples can be analyzed at one time. The Takara SmartChip system validated by the Grain Research Laboratory's Microbiology and Grain Genomics Program is now being used since it allows high throughput identification of Canadian barley varieties using 24 DNA markers.

To test wheat cargo samples, we use OpenArrayTM genotyping technology and have the DNA profiles of many wheat varieties in our database. As new varieties are released this database is updated.

Recent monitoring activities

Between August 1, 2022, and July 31, 2023, we analyzed the variety composition of the following:

- 612 samples from wheat cargo, with 178 samples re-tested (drill down testing)
- 200 samples from durum cargo
- 36 samples of cargo aggregates (previously called monthly cargo composites)
- 60 samples for wheat co-op trials
- 68 wheat samples from the Harvest Sample Program
- 23 submitted barley cargo samples

In addition, 76 wheat cargo samples were analyzed for the presence of the MON71200 GM event. This GM event has not been detected since testing for it started in 2018, but wheat exporters can request testing on a fee for service basis.

Recent publications

Lee, S-J., Eckhardt, M., Dusabenyagasani, M., Izydorczyk, **M., Demeke**, T., Perry, D. and S. Walkowiak. 2024. Identification of Canadian barley varieties by high-throughput SNP genotyping. Can. J. Plant Sci. https://doi.org/10.1139/cjps-2023-0187

Microbiology and Grain Genomics

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A multidisciplinary approach to the surveillance of microorganisms on grain

The Microbiology and Grain Genomics program uses the latest technologies to investigate the bacteria and fungi found on grain. We identify which species are present and explore how they are affected by environmental factors, such as geography and climate. This helps the grain sector understand the trends and risks associated with both the microorganisms that are beneficial and those that are harmful to plants, humans or livestock. We also identify crop varieties in grain shipments using DNA testing and currently have a database that contains the DNA information for more than 800 varieties. By monitoring microorganisms and grain varieties, we help ensure the safety, integrity and marketability of Canadian grain.

Monitoring microbial communities using DNA

Bacteria and fungi often exist in diverse communities and have complex interactions with each other and their environment. As they adapt to different conditions, changes occur in microbial communities that could have implications for the production, quality and safety of grain. One tool that we use to study microorganisms is targeted DNA testing. This type of testing allows us to determine if a microorganism is present or absent, the species it belongs to and the toxins it may produce. For example, we grow bacteria from grain samples in our laboratory and test the DNA to find out if the bacterial community contains plant pathogens, such as bacterial leaf streak (*Xanthamonas sp.*), or bacteria that cause foodborne illnesses, such as *E. coli* (Figure 1). We also perform high-throughput DNA tests on grain affected by fungi, such as *Fusarium* damaged kernels or ergot bodies (Figure 2). These types of fungal infections can reduce the production and quality of cereal grains and contaminate grain with toxins. Our tests can identify the fungal species that are present and also determine if they have the genes that are responsible for producing the toxins that may be a concern for food safety.

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Biochemical profiles of fungi

Another part of our work is developing new methods that improve the efficiency of testing and enhance the quality of the information we collect. In partnership with the Western Grains Research Foundation, Saskatchewan Wheat Development Commission and Manitoba Crop Alliance, we developed a method to identify and characterize *Fusarium* and rust fungi using Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (Figure 3). This technique ionizes the contents of a fungal sample, allowing each chemical within the sample to be separated based on its mass and charge. The pattern of biochemicals, referred to as a profile, is unique for each fungal species and acts like a fingerprint for identification. Compared to traditional methods, this technique gives results more rapidly and is more cost effective.

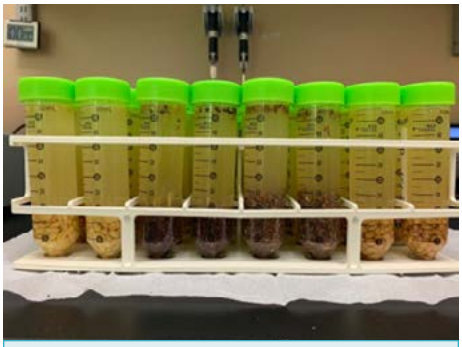

Figure 1 Bacteria growing in media exposed to grain.

Figure 2 Ergot bodies (top) and Fusarium damaged kernels (bottom).

Figure 3 Student operating equipment used for the analysis of microbial DNA and biochemical profiles.

Forecasting the risk of microbial disease

To better understand the potential for diseases of grain to change over time, we combine the DNA and biochemical data we collect with information on the pathogen, the host plant, farming practices and environmental factors. Using advanced statistics, analytics and predictive modelling, we are developing models that can forecast the risk of microbial diseases. We have applied this approach to fungal diseases of cereals, such as *Fusarium* and ergot, and blackleg of canola. This information helps the grains sector to stay informed on how the microorganisms on grain may respond to changes in climate.

Recent publications

Gamage, N.W., Ashfaq, T., Chin, T., Bamforth, J. and **S. Walkowiak**. 2023. Draft genome sequence of *Enterobacter sp.* from wheat grain. Microbiol. Resour. Announc. 13e00576-23. https://doi.org/10.1128/mra.00576-23

Chin, T., Pleskach, K., Tittlemier, S.A., Henriquez, M.A., Bamforth, J., Gamage, N.W., Ashfaq, T., Lee, S.-J., Kurera, M.S., Patel, B. and **S. Walkowiak**. 2023. A status update on fusarium head blight on Western Canadian wheat. Can. J. Plant Path. 45 (3): 277-289. https://doi.org/10.1080/07060661.2023.2177352

Kannangara, S.K., Bullock, P., **Walkowiak, S.** and W.G.D. Fernando. 2023. Species diversity of Fusarium head blight and deoxynivalenol (DON) levels in Western Canadian wheat fields. Can. J. Plant Path. 46(2): 128-141. https://doi.org/10.1080/07060661.2023.2290034

Zhao, L., Harding, M.W., Peng, G., Lange, R., **Walkowiak, S.** and W.G.D. Fernando. 2023. Artificial intelligence analysis of contributive factors in determining blackleg disease severity in canola farmlands. Can. J. Plant Path. 46(2): 114-127. https://doi.org/10.1080/07060661.2023.2290039

Rowland, B.E., Henriquez, M.A., Nilsen, K.T., Subramaniam, R. and **S. Walkowiak**. 2023. Unraveling plant-pathogen Interactions in cereals using RNA-seq. In: Foroud, N.A., Neilson, J.A.D. (eds) Plant-Pathogen Interactions. Methods Mol. Biol. 2659: 103-118. https://doi.org/10.1007/978-1-0716-3159-1_9

Trace Organics and Trace Elements Analysis

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Grain safety research to support market access for Canadian grain

The Trace Organics and Trace Elements Analysis Program focuses on researching and monitoring the pesticides, mycotoxins, and trace elements, particularly heavy metals, found in grain. We want to understand what causes these substances to accumulate in grain and how they behave during processing. We also evaluate the accuracy and precision of the methods we use for sampling and analyses, and look for ways to improve them. The information we gather and share helps instill confidence in the safety and reliability of Canadian grain for producers, commodity associations, exporters, government partners, and end users.

Sampling plans for measuring mycotoxins

Sampling methods and sample processing are the most important steps in the analytical process for many grain contaminants. The open access Mycotoxin Sampling Tool from the Food and Agriculture Organization lets users change parameters, such as sample size and sample number, to estimate the risk of misclassifying cargos as compliant with defined maximum levels of contaminants without having to perform laboratory analyses. We evaluated the potential of data to vary using different sampling plans and found that the current Codex General Standard on Contaminants and Toxins in Food and Feed sampling plans for maize and wheat could result in a total measurement error greater than or equal to 90% of the current and proposed maximum levels for ochratoxin A in wheat and aflatoxins in maize, respectively. We also found that increasing the laboratory sample size from 1 kg to 5 kg had the greatest impact on minimizing the variance due to sampling (Figure 1).

A better understanding of food safety

In another study, we looked at what happens to two chemicals, cadmium and glyphosate, when pasta made from durum wheat is cooked. Cadmium is a heavy metal that is present in soil and can accumulate in durum, while glyphosate is a herbicide used in weed management. We partnered with the durum breeding group from Agriculture and Agri-Food Canada at Swift Current, Saskatchewan, to

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produce durum wheat containing a range of cadmium and glyphosate levels. With help from other programs in the Grain Research Laboratory, the durum was milled and made into dry spaghetti. We found that the cooked spaghetti contained less glyphosate, but that the amount of cadmium stayed the same. We also found that more glyphosate than cadmium moves from the spaghetti into the water used to cook the pasta. By cooking the spaghetti for 9 minutes to al dente, approximately 65% of the glyphosate in the pasta transferred into the cooking water (Figure 2). This study helps us better understand how these chemicals behave during cooking and helps regulators set realistic and relevant regulations to manage the presence of these chemicals in grain.

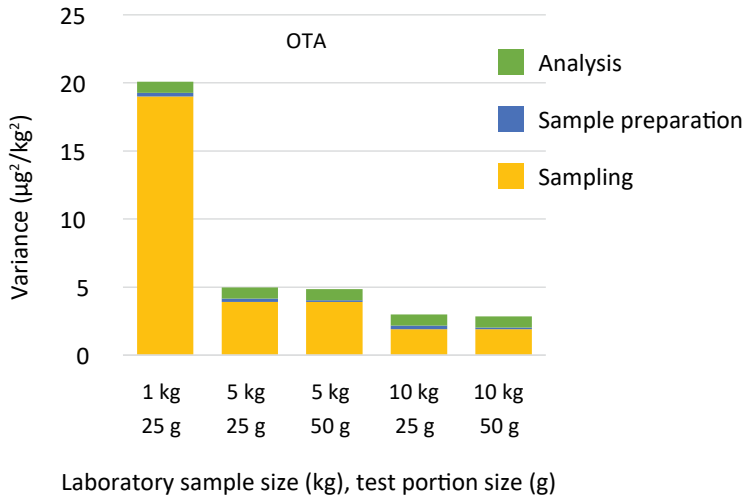

Figure 1 Estimated variance due to sampling, sample preparation and analysis in the measurement of ochratoxin A (OTA) in wheat using different sampling plans.

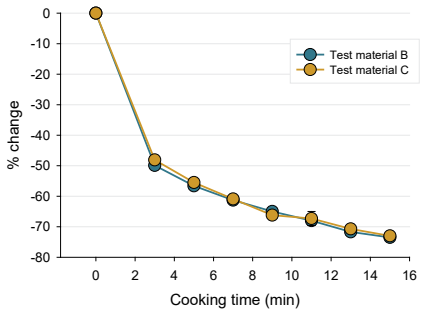

Figure 2 Change in glyphosate content of durum pasta as cooking time increases.

Improved technology reduces bias

In the past year we also improved our handling and processing of grain samples for the cargo monitoring program by modifying technology used in our laboratory. When we receive 10-kilogram samples for contaminant analysis, we make sub-samples using a rotary sample divider. We tested new rotary dividers with canola, flaxseed, peas, soybeans and wheat, and observed that variable amounts of grain ricocheted off the divider's carousel and accumulated in the corners of the housing instead of being directed into the carousel receptacles (Figure 3). The mass of lost sample was inversely correlated with the mass of individual kernels, with canola (average mass 0.004 g) affected the most and peas and soybeans (average mass 0.2 g) affected the least.

To minimize sample loss, we had a metal collar fabricated and installed on the divider carousel (Figure 4). The modification resulted in a decrease by at least 100x in the amount of sample lost, falling from 3% to 0.03% for peas and 9% to 0.06% for canola). This improvement in equipment used for sample processing will prevent lighter, lower quality kernels, from being lost and biasing the results of grain quality and safety assessments.

Figure 3 Accumulation of grain in the housing of a rotary sample divider that is lost from subsamples.

Figure 4 Rotary divider modified with a metal collar that minimizes grain loss in subsamples.

Recent publications

Tittlemier, S.A., Bestvater, L., Chan, J., Timofeiev, V., Richter, A., Wang, K., Ruan, Y., Izydorczyk, M. and B.X. Fu. 2023. Diverging fates of cadmium and glyphosate during pasta cooking. Food Addit. Contam. Part A 40(11): 1459-1469. https://doi.org/10.1080/19440049.2023.2264976

Tittlemier, S.A. and T.B. Whitaker. 2023. Current sampling plans can introduce high variance in mycotoxin testing results as demonstrated by the online FAO Mycotoxin Sampling Tool. World Mycotoxin J. 16(2): 115-126. https://doi.org/10.3920/WMJ2022.2804