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GRAIN RESEARCH LABORATORY

Annual Program Report

2020



Canadian Grain
Commission

Commission canadienne
des grains

Canada 

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

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It is my pleasure to present the 2020 Grain Research Laboratory Annual Report. This edition highlights some of the research and development activities in our nine scientific programs.

2020 was an unusual and challenging year on many levels, including professional, societal and personal. Because of the COVID-19 pandemic, we transformed our workplace and workflow to respect new safety measures. At first, we were only able to deliver our critical services but were quickly able to expand our work in the labs to include analyses related to requests for services, cargo monitoring, the Harvest Sample Program and plant breeder line evaluation. We also adjusted by working remotely (including the remote use of lab instruments), and attending virtual training and conferences. I am proud of the dedication and commitment of our staff who found a way to keep working through the COVID-19 pandemic.

Despite the many challenges, we had a very productive year. Two of our most notable achievements were the successful delivery of the Harvest Sample Program and the plant breeder line evaluation which were only possible with the support of more than 50 staff from different divisions of the Canadian Grain Commission.

Early winter storms in 2019 led to research on pre-harvest sprouting in wheat and the quality of canola that overwintered. We also continued to advance the marketability of Canadian grains by developing new ways to increase the value of barley and pulse ingredients. A wide variety of projects were undertaken to ensure the quality and safety of Canadian grain. These included the first multi-year survey of mycotoxins in Canadian oats, examinations of the role of proteins in grain quality, evaluation of GMO detection methods, the use of genomics to identify microbes on grains, and the continued updating of reference DNA profiles for wheat.

In 2020, there was significant investment in the Grain Research Lab of the future. This allowed us to acquire new technologies for innovation, automation and remote access, securing our role as the pre-eminent provider of science on the quality and safety of Canadian grain. Of special note is the retirement of Twylla McKendry in October as manager of Analytical Services. I acknowledge her 30 years of dedicated work and welcome Kerri Pleskach as the new manager.

As we move forward in 2021, the work of our scientists and staff will continue. We will keep adapting and finding new and innovative ways to conduct and promote our research for the benefit of you and all members of Canada's grain sector. Thank you for taking the time to read this report and I invite you to share your comments and thoughts with us. We look forward to your feedback.

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



Technology research programs include:

- Grain Biotechnology Research
- Microbiology
- Trace Organics and Trace Elements Analysis
- Variety Identification Research and Monitoring



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.



Requests for service analysis

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



Plant breeder line evaluation


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



Statistics and facts

Harvest Sample Program

We publish annual harvest and crop reports. We also publish an annual Fusarium survey report from samples we collect through the Harvest Sample Program.

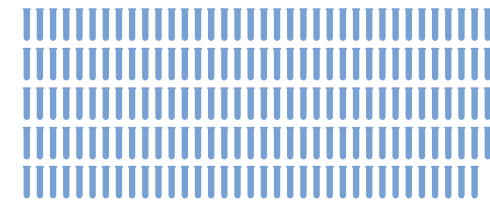
We conducted  **11,815** Falling Number and DON tests on wheat

The Harvest Sample Program received

13,783 samples for the 2019-20 crop year



 We tested **3525** cargo shipment samples



Currently, we use **179** different **test methods**



36 scientific articles were published by our scientists

We conducted **1104** tests for service requests by external clients, which included milling of **107** samples



The most popular requests were for **pathogen ID & quantity, alveographs** and **protein & free fatty acid content**

24 scientific presentations were delivered by our scientists



Out of **20** grains regulated by the Canadian Grain Commission, we analyze **14** different types of grain:

- Peas
- Oats
- Flaxseed
- Lentils
- Buckwheat
- Rye
- Wheat
- Chickpeas
- Soybeans
- Durum
- Beans
- Canola/rapeseed
- Barley
- Mustard

Grain Research Laboratory Social media

Promotion of programs and services

The Grain Research Laboratory is using social media to promote its programs and people. This gives us the ability to quickly inform and respond to producers and other members of the grain sector. We can ask producers to supply harvest samples used in research and make them more aware of changes in services, including delays or revised deadlines.



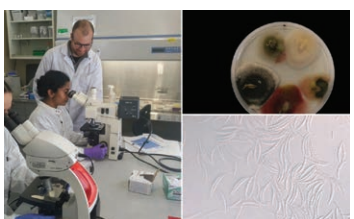
Sharing expertise

During the 2020 Science Literacy Week, Grain Research Laboratory staff were involved in two Twitter takeovers. Carly Isaak and Dr. Sean Walkowiak gave producers and other followers of the Canadian Grain Commission account a behind the scenes look at the Grain Research Laboratory.



Research reports

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



Advancements in agricultural science

Plain language summaries of scientific papers produced by Grain Research Laboratory scientists are posted on our website under [Advancements in agricultural science](#). These short articles provide the grain sector with our latest findings and show how they impact Canadian producers. In order to reach a wider audience, these summaries are also promoted on Twitter and Facebook.



“
Grain handlers and processors had concerns about free fatty acid levels in overwintered canola crops. We were able to determine that some of these samples could still be processed into good quality oil as long as they're processed as soon as possible.

*Dr. Véronique Barthelet, Program Manager,
Grain Research Laboratory*



“
It's important for us to have an effective way to determine concentrations of mycotoxins and remove them from whole oats to ensure they remain safe to eat.

*Dr. Sheryl Tittlemier, Program Manager,
Grain Research Laboratory*

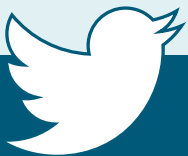
Grain Research Laboratory History

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

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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Investigating the impact of pre-harvest sprouting on the performance of bread wheat and durum

In western Canada the wet harvest of 2019 made sprout damage a major grading factor for two premium wheat classes, Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD). To investigate the impact of pre-harvest sprouting on the performance of bread wheat and durum, the Bread Wheat and Durum Research Program employed a new approach this year, examining the relationship between falling number (FN) values and the functional properties of CWRS and CWAD.

Measuring functionality in field-sprouted wheat

Most of our knowledge on how sprouting affects wheat quality comes from laboratory studies where kernels are soaked under controlled conditions. Structural and biochemical changes can differ greatly, however, between wheat kernels that have sprouted on stems standing in the field and those soaked in the laboratory. We cannot use lab-sprouted kernels as a substitute for field-sprouted wheat when assessing the impact of sprout damage on wheat functionality.

We placed harvest samples provided by producers into groups based on their FN values to form composite samples. The composite samples ranged in FN from just over 60 seconds to well over 350 seconds and were divided by 50 second increments. A comprehensive analysis of quality and physicochemical properties was conducted for each composite sample (Table 1).

Key findings

Wheat is generally considered sound when FN is above 300 seconds. Our research showed that CWRS wheat with FN values as low as 200 to 250 seconds had reasonable milling performance, dough handling and baking qualities. CWAD wheat still had reasonably good milling, semolina, and pasta qualities when FN values were as low as 100 to 150 seconds. CWRS and CWAD respond very differently to quality loss due to pre-harvest sprouting.



Test weight and milling yield

We found that the test weight and flour yield of CWRS composites decreased gradually as FN decreased to just above 200 seconds. Once FN fell below 200 seconds, the value of these properties drastically dropped. For CWAD composites, a decrease in FN from 450 seconds to 70 seconds did not significantly alter quality properties, especially the milling yields (Figure 1).

Maltose content

The endosperm of durum wheat, and the starch and gluten proteins it contains, appears to be much less damaged during field sprouting than that of bread wheat. This is demonstrated by levels of maltose, a sugar produced by the action of alpha-amylase on starch. The maltose content of the CWAD composite with the lowest FN was similar to that of sound CWRS wheat. This is most likely due to durum wheat having harder and denser kernels than bread wheat.

Dough properties and gluten proteins

The deterioration in dough properties was very evident for CWRS once the FN value dropped below 150 seconds with a significant decrease in stability during dough mixing and elasticity during stretching (Table 2). The dough was also hard to handle due to elevated stickiness. On the contrary, there was hardly any change in gluten strength and semolina dough viscoelasticity for the CWAD composites with FN values ranging from 450 to 79 seconds.

There was no significant change in the proportion of insoluble glutenin, the part of the wheat gluten protein that is responsible for the strength and elasticity of dough, across a wide range of FN values for CWRS and CWAD composites. This indicates that gluten proteins were not damaged in the kernel and that the change in dough properties was most likely due to the activation of enzymes when the flour was mixed with water.

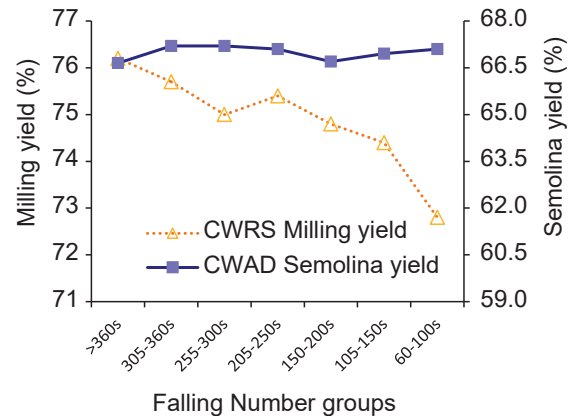


Figure 1 Milling yields obtained from Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD) composite samples grouped by falling number (seconds).

Table 1 Quality and physiochemical properties examined in composite samples of Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD).

	CWRS	CWAD
Wheat properties	test weight, thousand kernel weight, ash content, falling number	
Biochemical analysis	alpha-amylase activity, maltose content, gluten protein composition, functional protein solubility distribution	
Milling quality	flour yield, flour ash, starch damage	semolina yield and total milling yield, semolina ash, speck count
Dough properties	absorption, dough development time and stability, dough viscoelasticity and handling properties	gluten strength, and semolina dough viscoelasticity
End product quality	bread, volume and crumb texture	pasta, colour and cooking quality

Table 2 Dough properties of composite Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD) samples grouped by falling number values.

Falling Number Groups	CWRS						CWAD			
	Farinograph			Extensograph			Gluten index (%)	Alveograph		
	Absorption (%)	Dough development time (min)	Dough stability (min)	Dough elasticity (BU)	Dough extensibility (cm)	Dough handling properties		Dough resistance (P)	Dough extensibility (L)	Dough energy (W)
>360s	65.3	6.0	10.0	458	19.9	Viscoelastic	89	81	97	242
305-360s	66.0	6.5	8.5	420	19.6	Viscoelastic	88	77	100	233
255-300s	65.3	4.3	7.5	400	20.2	Viscoelastic	87	79	100	237
205-250s	65.5	4.8	6.5	410	20.7	Viscoelastic	84	75	98	216
150-200s	64.2	4.8	6.5	433	22.4	Slightly sticky	88	79	110	247
105-150s	64.3	3.5	5.0	359	24.2	Sticky	86	72	116	232
60-100s	63.9	2.5	3.0	363	24.2	Very sticky	88	75	110	240

Table 3 The effects of pre-harvest sprouting on bread qualities in Canada Western Red Spring (CWRS) samples grouped by falling number values.

Falling Number Groups	Specific volume (cm ³ /g)	Bread crumb texture		
		Adhesiveness (g*sec)	Resilience (%)	Springiness (%)
>360s	9.8	3.3	38.9	95.8
305-360s	9.6	5.0	36.7	93.8
255-300s	9.7	6.8	34.1	93.0
205-250s	9.6	5.9	34.1	93.9
150-200s	9.7	9.5	31.4	91.9
105-150s	9.5	15.4	29.2	89.1
60-100s	9.3	19.6	25.6	88.7

Table 4 The effects of pre-harvest sprouting on pasta qualities in Canada Western Amber Durum (CWAD) samples grouped by falling number values.

Falling Number groups	Cooking quality			Pasta color	
	Pasta firmness (g)	Cooking loss (%)	Brightness (L*)	Redness (a*)	Yellowness (*)
>360s	636	5.4	71.6	4.9	63.2
305-360s	647	5.5	71.0	5.2	61.6
255-300s	658	5.4	70.6	5.4	60.6
205-250s	598	5.6	70.3	5.5	60.2
150-200s	636	5.5	69.7	6.1	60.5
105-150s	620	5.6	69.1	6.4	60.2
60-100s	601	5.9	69.1	6.4	60.3

End product quality

We found that the texture of bread deteriorated with a decrease in FN values in CWRS composite samples. There was a gradual decrease in crumb resilience and crumb springiness and an increase in adhesiveness (Table 3) but loaf volume and exterior shape showed little change. (Figure 2). There were limited effects on pasta cooking quality and pasta colour for the CWAD composites (Table 4).

The loss of functionality caused by sprout damage is related to the activity of enzymes and the availability of the starch and protein that they act on. We expected bread wheat to be more affected by pre-harvest sprouting since water is required to activate enzymes and bread making requires much more water absorption than does pasta making. In addition, semolina used to make pasta consists of larger particles and less damaged starch than bread flour. These features limit the impacts of enzymes on semolina since there is less surface area for them to access and damaged starch is degraded first. The high temperature used in pasta processing also helps to maintain the functionality of durum wheat by destroying enzymes.

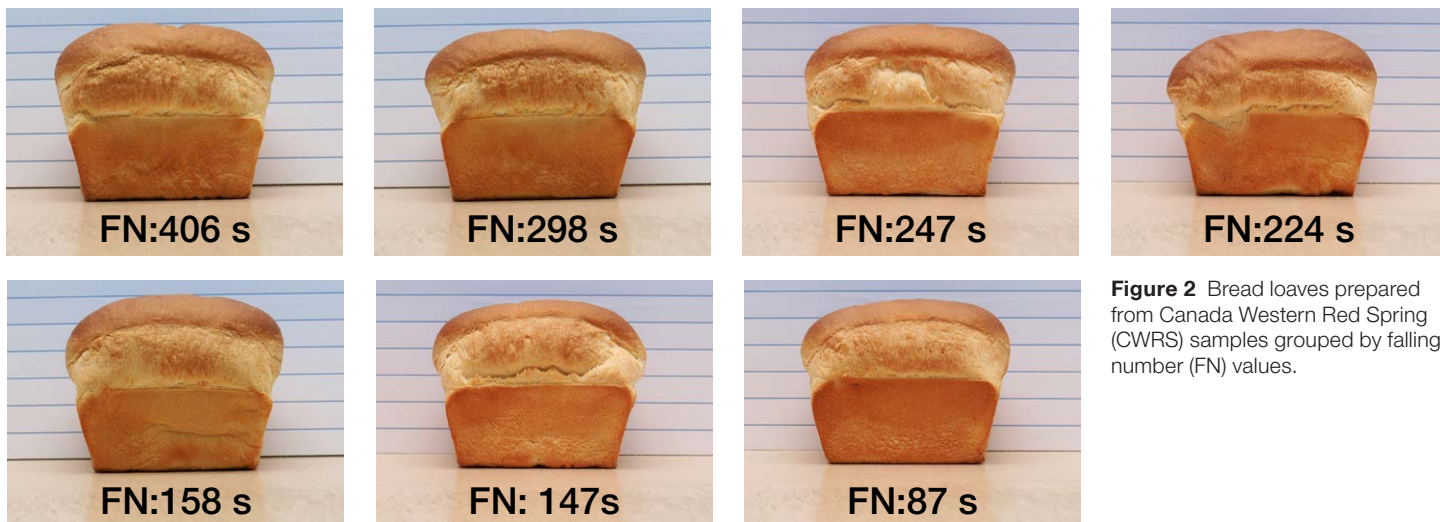


Figure 2 Bread loaves prepared from Canada Western Red Spring (CWRS) samples grouped by falling number (FN) values.

Future research

In the future we hope to conduct a more detailed investigation on the structural and biochemical changes in wheat kernels associated with progressive sprout damage.

Bibliography

Wang, K., Isaak, C. and B.X. Fu. 2020, Oct. 25 – Nov. 13. Contrasting impacts of pre-harvest field sprouting on the functionality of bread and durum wheats. Paper presented at the meeting of the Cereals & Grains 20 Online Conference.

<https://aaccnet.confex.com/aaccnet/2020/meetingapp.cgi/Paper/5334>

Isaak, C. Wang, K. and B.X. Fu. 2020, Oct. 25 – Nov. 13. Functional properties of Canada Western Red Spring Wheat in relation to the degree of pre-harvest sprouting in the field. Paper presented at the meeting of the Cereals & Grains 20 Online Conference.

<https://aaccnet.confex.com/aaccnet/2020/meetingapp.cgi/Paper/5339>

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Milling and Malting / Research on Barley and Other Grains

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Increasing the value of barley through innovation and research

This year the Milling and Malting / Research on Barley and other Grains Program looked at innovative ways to increase the value of barley. We determined which fractions of high protein barley have potential for use in food products and malting, and evaluated the quality of bulgur made from two barley cultivars. We also examined how the malting performance of barley was affected by plant growth regulators (PGRs).

Adding value to high protein malting barley through fractionation

Malting barley has the potential to be the most profitable commodity for producers but it must meet strict quality specifications. If protein concentration is greater than 13%, barley is often rejected for malting grade and sold at a lower price on the feed market. Dividing high protein malting barley into fractions may increase its value since fibre/protein-rich fractions could be used as food ingredients and starchy fractions as adjuncts to supplement the main mash in brewing.

We milled several barley varieties with grain protein concentrations above 14% dry basis (db) on a Bühler laboratory mill which resulted in six flour streams. Coarse and fine shorts were combined to make a fibre/protein-rich fraction and three flour streams made up a flour fraction. The fibre/protein-rich fraction for each variety was higher in beta-glucans, proteins, arabinoxylans and vitamin E than the whole grain (Figure 1 and Figure 2). The combined flour fractions were depleted of beta-glucans and arabinoxylans but contained acceptable levels of proteins and high levels of starch (Figure 2). Mashing experiments that replaced up to 40% of malt with flour fractions had significant improvements in malt extract without any negative effects on malting qualities.

We demonstrated that barley can be milled using equipment commonly used for wheat without needing to remove the outer hull. This makes the production of fibre/protein-rich fractions and flour fractions commercially feasible.

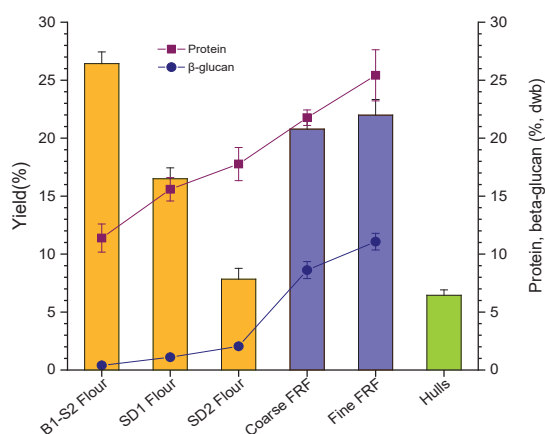


Figure 1 Average yields (%) of various milling streams from six barley cultivars and their concentrations of beta-glucans and proteins (B1S2 = flour from all break roll sets and one sizing roll set, SD1 = flour from first passage through a shorts duster, SD2 = flour from second passage through a shorts duster, FRF = fibre /protein-rich fraction).

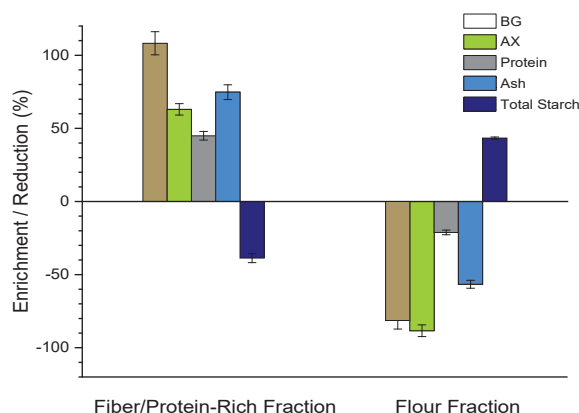


Figure 2 Average change (%) in selected constituents of fibre/protein-rich and flour fractions compared to their concentrations in the whole grains of six barley cultivars (BG = beta-glucans, AX = arabinoxylans).

Bulgur from Canadian barley and the effects of drying methods on its physicochemical and nutritional properties

Bulgur is an ancient and traditional food in the Middle East and is gaining popularity elsewhere as a nutritious and convenient cereal product with a prolonged shelf life. We set out to produce high quality bulgur from Canadian high-amylose and waxy hulless barley (*Hordeum vulgare* L.). We also investigated the effects of different drying methods on the physicochemical and nutritional properties of barley bulgur.

We prepared bulgur from a high amylose cultivar (CDC Hilose) and a waxy cultivar (CDC Marlina) by cooking, drying, and grinding kernels into smaller particles as shown in Figure 3. Hulless barley was found to be an excellent grain for preparation of bulgur, resulting in an attractive product (Figure 4).

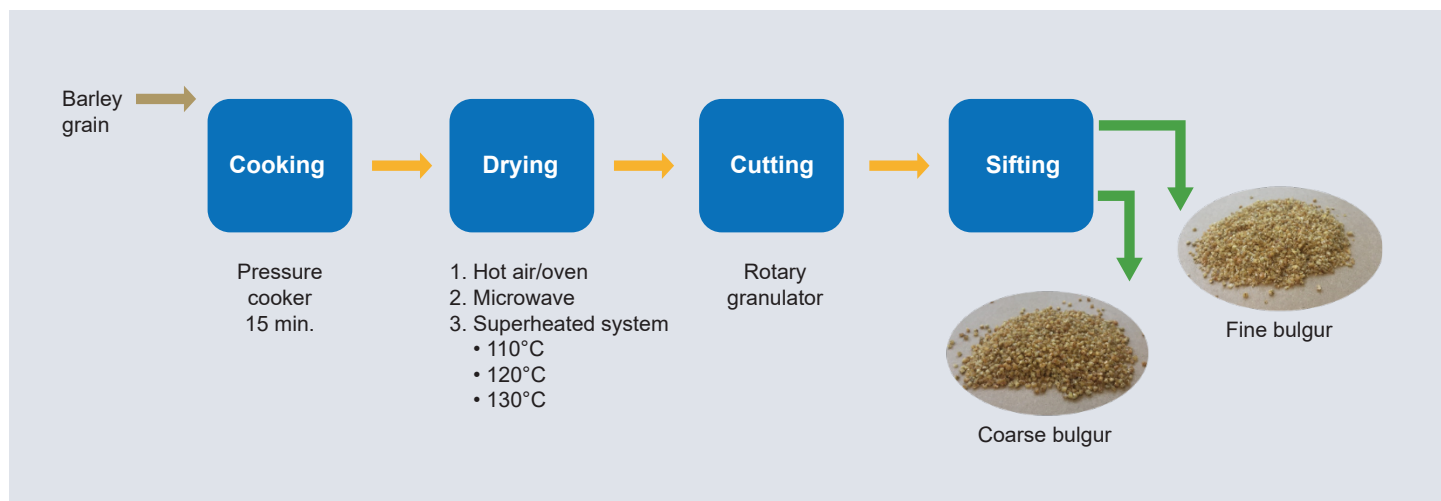


Figure 3 Processing steps in the preparation of barley bulgur.



Figure 4 Coarse (left) and fine (right) barley bulgur.

We compared the quality of bulgur dried by hot air, microwave, and superheated steam. Superheated steam at 110°C resulted in bulgur with optimal quality characteristics: high yield, short cooking time, low cooking losses, high beta-glucan solubility, and high retention of vitamin E. The next best results were obtained for bulgur prepared from grain dried in the microwave.

Some differences in composition and properties of bulgur prepared from waxy and high amylose barley were related to genetic variations and differences in starch composition. Both varieties, however, produced a bulgur product of high quality.

Effects of plant growth regulators on the malting quality of barley

Lodging, which is the bending over of stems near ground level, can negatively affect the yield and quality of barley grain. Synthetic plant growth regulators (PGRs) reduce lodging by producing shorter, thicker, and stronger stems but the effect of PGRs on the malting performance of barley is not known. We assessed the effect of three PGRs (ethephon, chlormequat chloride, and trinexapac-ethyl) in combination with different seeding rates on the malting quality of barley grown in several locations and years in western Canada.

Our study showed that the application of PGRs had a statistically significant effect on kernel weight (Figure 5) and plumpness, and no effect on the concentration of proteins and germination energy. Seeding rates significantly affected kernel weight, protein content, and germination index, but no interactions between PGRs and seeding rates were observed.

The smaller kernels of barley treated with ethephon and trinexapac had good hydration and grain modification during malting. The fine extract of malt was significantly lower only for barley treated with chlormequat and trinexapac (Figure 6).

The decision to use PGRs on malting barley needs to consider both the potential benefits of PGRs in reducing lodging and their effects on the malting performance of barley.



Barley bulgur

Cracked or cut barley berries that are partially pre-cooked for easy & fast preparation

Benefits of bulgur

1. Whole grain product
2. More available nutrients
3. High in fibre & soluble beta-glucans
4. Speed & ease of preparation

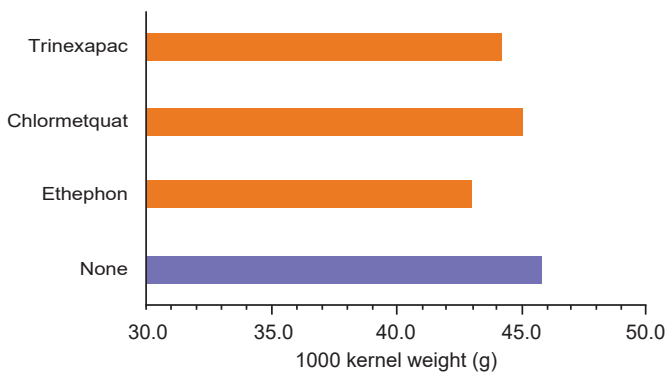


Figure 5 The effect of plant growth regulator (PGR) application on mean kernel weight when averaged across 5 environments. Orange bars indicate means that are significantly different ($p < 0.05$) from the control as determined by Proc MIXED ANOVA.

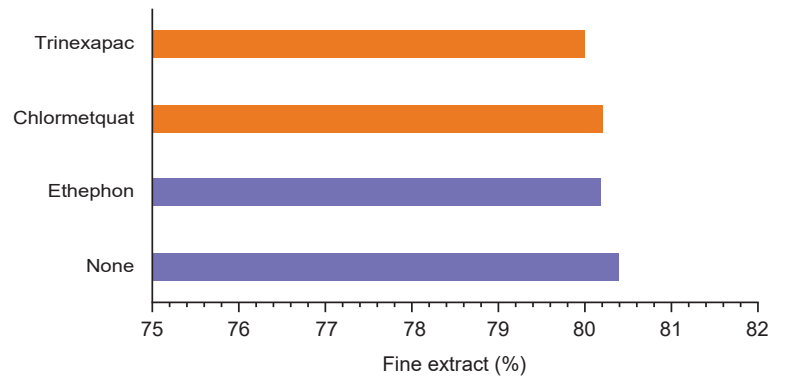


Figure 6 The effect of plant growth regulator (PGR) application on mean malt extract level produced by barley when averaged across 5 environments. Orange bars indicate means that are significantly different ($p < 0.05$) from the control as determined by Proc MIXED ANOVA.

Future research

Our future plans include studying the influence of previous crop and nitrogen management on malt barley yield and quality across Canada. We will also examine water absorption patterns in various Canadian barley genotypes during steeping. In addition, we will continue to evaluate the nutritional profile of Canadian barley as well as the malting properties of new Canadian hull-less barley varieties.

Bibliography

- McMillan, T., Tidemann, B.D., O'Donovan, J.T. and M.S. Izydorczyk. 2019. Effects of plant growth regulator application on the malting quality of barley. *J. Agri. Sci.* 100(5): 2082-2089. <https://doi.org/10.1002/jsfa.10231>
- Dueck, C., Izydorczyk, M.S. and S. Cenkowski. 2020. Effects of drying methods (hot air, microwave, and superheated steam) on physicochemical and nutritional properties of bulgur prepared from high amylose and waxy hull-less barley. *Cereal Chem.* 97 (2). <https://doi.org/10.1002/cche.10263>
- Izydorczyk, M.S., Nam, S., Sharma, A. and J. Kletke. 2021. Exploring dry grain fractionation as a means to valorize high protein malting barley. *Cereal Chem.* (in press). <https://doi.org/10.1002/cche.10426>

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Oilseeds

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Quality of 2019 overwintered canola in western Canada

In 2019, early winter storms left large amounts of snow on the ground in western Canada. This made field work impossible for producers and the harvesting of canola stretched from August 2019 to June 2020. Most of the canola that overwintered was found in northern Alberta. In the Peace-River area, 76% of canola acres overwintered as did 58.2 % of canola acres in the northwest area of Alberta (Figure 1).

In 2017, after a similar winter storm occurred, we conducted a small study to assess the quality of the overwintered canola. There were more samples than expected (34%) that graded No. 1. The free fatty acid (FFA) content in overwintered seeds stored under laboratory conditions was higher, however, than those harvested in autumn. Since FFAs have a negative effect on oil quality, grain handlers and processors had concerns about another overwintered canola crop. One of our projects this year was to assess the quality of the 2019 overwintered canola crop. We also looked for degradation changes in seeds stored in the laboratory since similar changes would take place in overwintered seeds stored on farms.

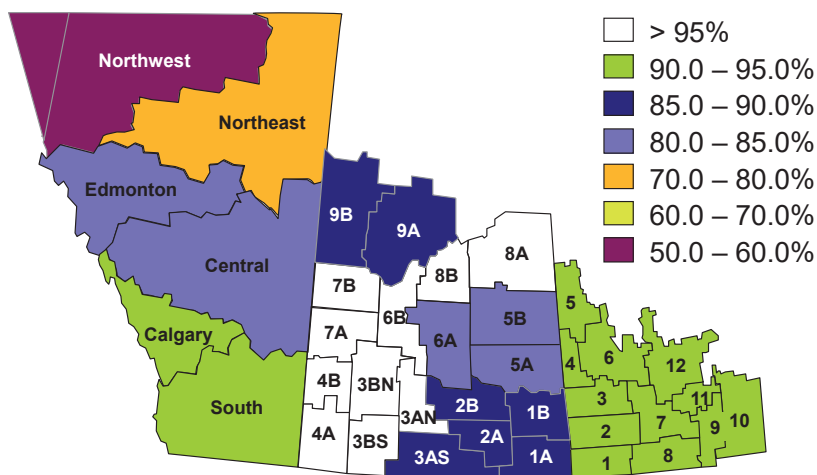


Figure 1 Percentage of canola harvested in western Canada (Prairies) as of November 2, 2019. Saskatchewan map numbers refer to census agricultural regions and Manitoba map numbers refer to agricultural districts.



How did canola that overwintered grade?

We obtained over 100 samples of 2019 overwintered canola samples from producers and processors. These were graded and analysed for all the parameters that define canola quality: oil, protein, chlorophyll, total glucosinolate, FFAs and fatty acid composition.

We found that 27.4% of 2019 overwintered canola samples were graded Canola, No.1 Canada and 34%, 20.8% and 17.9% of samples were graded Canola, No.2 Canada, No.3 Canada and Sample, respectively. The main degrading factors for samples graded Canola, No.2 and No.3 Canada were distinctively green seed (DGR) counts and the presence of other damages such as discoloration and rime. Samples downgraded to Sample grade mainly showed other damage such as discoloration due to weathering and rime, and mice excreta.

Free fatty acids and oxidative stability

The 2019 overwintered samples had a higher oil content than the autumn harvested ones. FFA content was higher in 2019 overwintered canola samples than in 2019 autumn harvested canola samples. FFA content was re-analysed after a two-month storage period. We found that it had increased even though the samples were stored under low moisture conditions. Accelerated oxidation studies were done on selected samples. We looked at fatty acid composition, oil, chlorophyll and FFA content. The results showed that oxidative stability was strongly related to the FFA content. The higher the FFA content, the faster the seed samples oxidized.

This study suggests overwintered canola can still provide good quality seeds that are top grade and have low FFA. All overwintered canola must be processed as soon as possible, however, to limit the effects of FFA content and oxidation on oil quality.

Future research

Our future plans include working with Near-Infrared Reflectance (NIR) spectrometry to predict the quality of Canadian canola meal, flaxseeds and mustard seeds. Other projects include looking at the effects of immature soybeans on end-use products and the effect of flaxseed processing on levels of cyanogenic glycoside, a plant compound that reduces the body's ability to absorb essential nutrients.

Bibliography

Barthet, V.J. 2019. Quality of western Canadian canola 2018. Final report published on the Canadian Grain Commission website.
<https://grainscanada.gc.ca/en/grain-research/export-quality/oilseeds/canola/2018/index.html>

Barthet, V.J. 2020. Quality of western Canadian canola 2019. Final report published on the Canadian Grain Commission website.
<https://grainscanada.gc.ca/en/grain-research/export-quality/oilseeds/canola/2019/index.html>

Barthet, V.J., Petryk, M.W.P. and B. Siemens. 2020. Rapid non-destructive analysis of intact canola seeds using a handheld near-infrared spectrometer. J. Am. Oil Chem. Soc. 97: 577-589.
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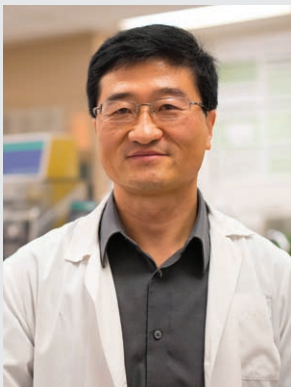
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Developing a new method for measuring the oil absorption capacity of pulse flours and protein materials

The food industry is expanding its use of pulse ingredients, such as flour, starch, fiber and protein, in a variety of products. There is a demand for these products since pulse ingredients are low in cost, high in nutritional value and have known health benefits. In order for pulse ingredients to be used successfully, we need to understand the properties that affect the way a finished product looks, tastes and feels.

The importance of oil absorption

The Pulse Research Program examines the physical, chemical and functional properties of Canadian pulses and develops new methods for measuring them. One of the functional properties we are studying is the ability of an ingredient to absorb and hold oil, referred to as oil absorption capacity (OAC). Pulse flours and proteins need to retain oil if they are to be used in foods such as baked goods and meat substitutes. OAC is usually measured by mixing a pulse sample with excess oil, centrifuging the mixture and then calculating how much oil is absorbed by the sample. When we followed the conventional procedures for measuring OAC we observed that some of the less dense ingredients drain off with the unabsorbed oil during the decanting process and that some ingredients reabsorb oil remaining in sample tubes after centrifugation. These issues resulted in OAC values that were not accurate.

Improving the accuracy of measurements

To improve accuracy, we developed a new method for measuring OAC that uses filter paper to separate unabsorbed oil from the sample during centrifugation (Figure 1). This method allows oil that is not absorbed to pass through the filter paper and collect in the bottom of the centrifuge tube while retaining all solid material within the test tube (Figure 1D). To account for any oil remaining in the filter paper a control test tube containing only oil is also centrifuged and the paper weighed after centrifugation. Our new method prevented the loss of sample material and minimized the reabsorption of oil by pulse materials.

We have used this technique to determine the OAC of pulse flours and protein concentrates/isolates (Table 1) and found that it was objective, simple, efficient, and reliable. It can also be applied to a variety of pulse and soybean ingredients. The food industry will be able to use the results generated with our new method to increase the use of pulse ingredients and create a larger market for Canadian pulses.



Other activities

The Pulse Research Program also conducts quality analysis of pulse and soybean samples submitted to the Harvest Sample Program and participates in cargo monitoring. The data collected is used to support the quality assurance system and the marketability of Canadian pulses.

Table 1 Comparison between the conventional method and our new method for measuring the oil absorption capacity (g oil/g sample, dry matter) of flours, protein concentrates and isolates from pulses and soybeans¹.

Sample	Method developed	Conventional method
Navy bean	0.19 ± 0.02b ²	0.92 ± 0.01a
Pea flour A	0.33 ± 0.02b	0.72 ± 0.02a
Kabuli chickpea	0.25 ± 0.01b	0.97 ± 0.01a
Commercial pea fibre	0.29 ± 0.01b	1.29 ± 0.02a
Extruded pea flour	0.16 ± 0.01b	1.11 ± 0.03a
Air-classified pea protein concentrate	0.75 ± 0.01b	1.04 ± 0.03a
Commercial pea protein concentrate	0.51 ± 0.03b	0.74 ± 0.03a
Commercial soy protein concentrate	0.30 ± 0.01b	1.10 ± 0.07a
Commercial soy protein isolate	0.33 ± 0.03b	0.95 ± 0.04a
Pooled standard deviation (sp)	0.018	0.034

¹Six replicates were determined for each sample (n=6).

²Means within a row with the same letter are not significantly different (P>0.05) as determined using Duncan's multiple range test.

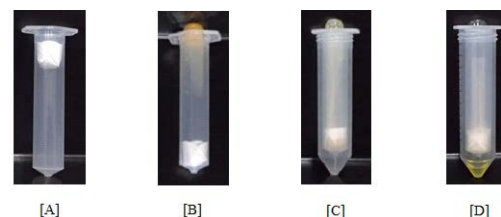


Figure 1 [A]-folded filter paper in a syringe barrel; [B]-test tube with sample and oil in the syringe barrel with filter paper at bottom; [C]-syringe assembly (syringe barrel, filter paper, inverted test tube, sample and oil) in a centrifuge tube; and [D]-syringe assembly after centrifugation with oil collected in the conical centrifuge tube.

Future research

In the future, we plan to improve methods for measuring other functional properties of pulses such as foaming and pasting. We also will investigate how different thermal processing and milling treatments affect certain chemical and functional properties of pulse flours. In addition, we will study how the yield, chemical composition and functionality of faba bean fractions produced by air-classification technology are affected by variety, growing location and year. In collaboration with the Canadian Grain Commission's Industry Services division, we will investigate how bleached and wrinkled seeds in red lentils affect dehulling. This study will provide scientific support to the grain grading system by developing tolerances for both factors in the different grades of red lentils.

Bibliography

Wang, N., and L. Maximiuk. 2019. Effect of air classification processing variables on yield, composition, and certain antinutrients of air-classified fractions from field peas by response surface methodology. *J. Food Process. Preserv.* 43 (7): e13999. <https://doi.org/10.1111/jfpp.13999>

Wang, N., Maximiuk, L., Fenn, D., Nickerson, M. and A. Hou. 2020. Development of a method for determining oil absorption capacity in pulse flours and protein materials. *Cereal Chem.* 97(6): 1111-1117. <https://doi.org/10.1002/cche.10339>

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Wheat Enzymes

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The role of proteins in determining grain quality

The Wheat Enzymes Program uses new technology to better understand quality issues in Canadian grain. We are currently involved in three projects: profiling gluten protein subunits, analysing the impact of *Fusarium* protease on gluten strength, and the proteomic analysis of barley malt and wort.

Profiling gluten protein subunits

Gluten is a large polymer made up of many protein subunits. The types of subunits, as well as their relative amounts, affect the baking performance of a flour. It is, however, the final shape and form of the gluten polymer complex that determines a flour's quality. Much of what we know about gluten comes from breaking down the polymer and studying its individual protein subunits. We use mass spectrometry to separate and simultaneously analyse all intact protein subunits in a sample using a technique known as proteoform profiling. This year we published a study describing the first application of proteoform profiling on plants and how two previously unknown modifications to gluten proteins were detected. By increasing our understanding of the protein composition of grains at a biochemical level we open doors to new discoveries. The ultimate goal is the application of this knowledge to the development of new quality tests and new markers to guide plant breeders in the future.

Investigating the impact of *Fusarium* protease on gluten strength

Fusarium head blight (FHB) is a fungal disease of cereal grains that reduces yields and produces mycotoxins that can harm humans and animals. *Fusarium* uses protease enzymes to digest proteins found in grains, allowing it to infect and feed off the host. These enzymes lie dormant in mature infected grains and are reactivated during the dough making process, weakening gluten and potentially reducing baking performance (Figure 1). Canadian grains are currently graded for *Fusarium* damage by using visual inspection to detect *Fusarium* damaged kernels (FDK) which adequately controls the potential damage from protease. Distorted FDK are thin and light and generally have more protease. They may be cleaned from grain due to their light weight and thin shape, reducing the impact of *Fusarium* protease on quality. Affected FDK are close to sound kernels in size and weight and still contain considerable protease activity. Both the number of FDK and the severity of infection contribute to the protease activity in a sample (Figure 2).

We are trying to understand the relationship between protease activity and the loss of baking quality by characterizing the biochemical properties of the proteases produced by *Fusarium* during infection. This will allow us to develop a specific test for these proteases and measure their impact on quality. Knowledge gained from this research will provide evidence for grading that is based on scientific measurements and specifically relates to functional quality in end-use products.



Proteomic analysis of barley malt and wort

We are continuing to work with the Milling and Malting / Research on Barley and Other Grains Program to detect and measure specific proteins known to cause quality issues in beer such as gushing and hazing. Wort, the liquid extracted from malt by mashing, is challenging to work with since proteins are modified and heavily degraded. Certain proteins are known to have a negative impact on malting and mashing. It may also be possible that during beer processing otherwise innocuous proteins break into fragments that affect the quality of beer. Current methods that use a combustion nitrogen analyzer or near-infrared reflectance (NIR) to measure the protein content of a sample do not give any information on which specific proteins are present, what their functional states are or how they differ between samples.

In order to obtain information on individual proteins, we are applying proteomics to wort analysis. Proteomics is the analysis of all of the proteins within a sample using high-resolution mass spectrometry and protein sequence information to identify and measure the amounts of large numbers of proteins. While genomics can indicate which proteins should be present, proteomics seeks to confirm that they were present, how they were modified, and how much of each protein was present. We have been able to simultaneously detect over 150 proteins, including all previously identified proteins, in a single analysis. We have also developed a method to measure over 90 individual proteins with high enough precision to make comparisons between samples. This will allow us to compare different malting and mashing regimes in order to see how they affect the levels of proteins known to affect beer quality. Studies like this aim to confirm the presence of known proteins and possibly identify new ones. They also help us identify processing changes that may specifically counteract the negative effects of problem proteins.



Figure 1 Bread baked with flour from sound grain (left) and 3% distorted FDK grain (right). Fusarium protease damage resulted in large, open cells and collapse of the loaf.

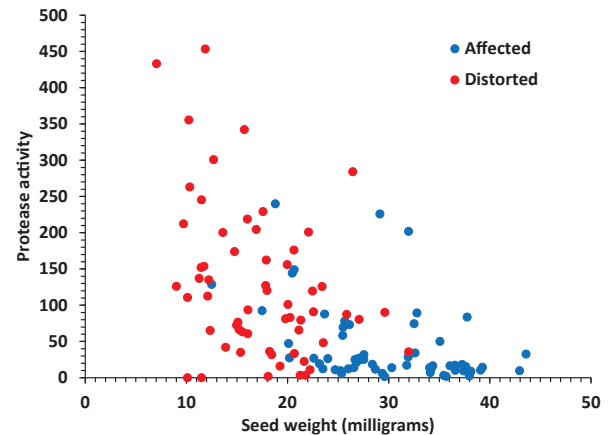


Figure 2 The relationship between protease activity and individual seed weight for *Fusarium* damaged kernels (FDK) selected from six producer samples. Both affected and distorted FDK contributed to protease activity in the samples.

Future research

Future research plans include continued examinations of how end-product quality is affected by protease damage arising from pre-harvest sprouting in wheat and durum and by differential changes in malting and mashing regimes for barley. In addition, we want to develop a method to measure granule-bound starch synthase, the enzyme responsible for the waxy trait in wheat. By understanding how much of this enzyme is present in a variety we can help guide development of partial waxy varieties to meet specific market needs. Finally, we plan to apply proteoform profiling to characterize biochemical differences between samples of wheat varieties that show unexpected differences in gluten strength between growing locations. The knowledge gained from this research supports improvements in wheat quality and a science-based quality assurance system in Canada.

Bibliography

Bacala, R., Fu, B.X., Perreault, H., and D.W. Hatcher. 2020. Quantitative LC-MS proteoform profiling of intact wheat glutenin subunits. *J. Cereal. Sci.* 94, 102963. <https://doi.org/10.1016/j.jcs.2020.102963>

Bacala, R., Fu, B.X., Hatcher, D.W., and H. Perreault. 2020. C-terminal tyrosine removal from wheat low-molecular weight glutenins (LMWGS); biologically relevant or mistaken substrate? *J. Cereal. Sci.* 95, 103060. <https://doi.org/10.1016/j.jcs.2020.103060>

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Giving producers information on grain quality through the Harvest Sample Program

The Canadian Grain Commission has been running the Harvest Sample Program since 1927. Initially, requests for samples were sent out to grain elevators but now sample envelopes are sent directly to producers who sign up for the Harvest Sample Program on a voluntary basis. In 2020, there were 39,071 envelopes sent to 6,409 producers across Canada. After submitting their samples, producers receive an unofficial grade as well as an analysis of their grain's quality. Individual producers can use this information to market their grain more effectively. The Canadian Grain Commission uses the information to help promote the quality of Canadian grain internationally.

Assessing deoxynivalenol and falling number in harvest samples

Although deoxynivalenol (DON) and falling number (FN) are not official grading factors, they can affect the end-use quality and play a role in the price that producers receive for their wheat deliveries. In 2018, the Harvest Sample Program added FN and DON tests to our analysis of wheat, durum, corn (DON only) and rye (FN only) harvest samples. These tests were included to further benefit producers, giving them more information on which to base their marketing decisions.

The Canadian maximum level for DON is 2.0 parts per million (ppm) in wheat. This is currently under review with Health Canada and some countries importing Canadian grains may have lower maximum limits with respect to DON. The Harvest Sample Program tests samples for DON using a lateral flow device (Figure 1). In 2020, we found that 99% of Canada Western Red Spring (CWRS) wheat had DON values less than 2.0 ppm and that 88% of CWRS had values lower than the detection limit of analysis which is 0.3 ppm. We also found that 98% of Canada Western Amber Durum (CWAD) wheat had DON values less than 2.0 ppm, with 85% of CWAD having levels lower than 0.3 ppm (Table 1).

The lowest possible FN value is 62 seconds which correlates to very high sprout damage. A falling number of more than 350 seconds correlates to sound grain with no sprouting. Our method at the Canadian Grain Commission (Figure 2) follows FN tests certified by the American Association of Clinical Chemistry and the International Association for Cereal Science and Technology. The 2020 Harvest Sample Program found that 74% of CWRS and 98% of CWAD were sound, with a FN of more than 350 seconds (Table 2).



Figure 1 An aliquot of extracted sample being loaded into a lateral flow test strip reader for DON analysis.

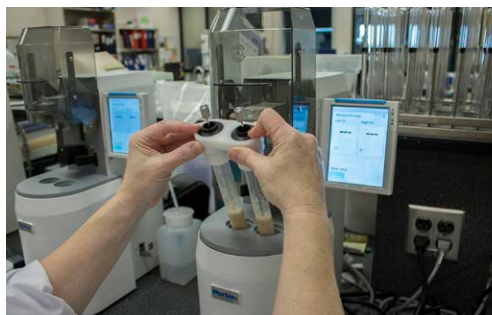


Figure 2 Test tubes of slurry being immersed in a boiling-water bath for falling number analysis.

Table 1 Results of deoxynivalenol (DON) testing on wheat samples received from the 2020 crop year. DON values are expressed in parts per million (ppm).

	Canada Western Red Spring (CWRS)	Canada Western Amber Durum (CWAD)	All wheat and durum
DON values (ppm)	Number of samples (%)	Number of samples (%)	Number of samples (%)
Below Limit (<0.3)	87.6	85.2	88.0
0.3 - 2.0	11.7	13.0	11.2
>2.0	0.7	1.8	0.9
Total number of samples	5008	1164	6936

Table 2 Results of Falling Number (FN) testing on wheat samples received from the 2020 crop year. FN values are expressed in seconds (sec).

	Canada Western Red Spring (CWRS)	Canada Western Amber Durum (CWAD)	All wheat and durum
Falling Number (sec)	Number of samples (%)	Number of samples (%)	Number of samples (%)
>350	73.9	98.1	78.5
305-350	13.6	1.5	11.4
255-300	7.0	0.6	5.7
<250	5.6	0.2	4.4
Total number of samples	5008	1164	6936

Working together during the pandemic

In 2020, restrictions implemented as a response to the COVID-19 pandemic resulted in Analytical Services relying on the help of staff from many different sections of the Canadian Grain Commission including Human Resources, Industry Services and all the Grain Research Laboratory programs. We were assisted by 42 staff members outside of the Harvest Sample Program and they spent over 8,400 hours receiving, cleaning, dividing and grinding samples as well as performing protein, moisture, DON and FN analysis. Although this led to an increased amount of training it meant that we were able to use mostly existing Canadian Grain Commission staff to complete the work of the Harvest Sample Program.

In the 2020 crop year, we received 13,783 harvest samples covering 18 different commodities. Among these samples, 59% were cereals, 31% were oilseeds and 10% were pulses. The majority of samples received were wheat, followed by canola and then durum. All samples were inspected, graded, and evaluated for protein, 7,041 samples were assessed for FN and 7,109 were analyzed for DON as well as other tests, depending on the commodity. On November 13, 2020, we received 658 samples from all commodities, setting a new record for the number of samples received in a single day.

Related links

<https://grainscanada.gc.ca/en/grain-quality/harvest-sample/>
<https://grainscanada.gc.ca/en/grain-research/statistics/grain-statistics-weekly/>

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Developing and validating detection methods for genetically modified organisms

The Grain Biotechnology Research Program develops and evaluates DNA-based methods for identifying and quantifying genetically modified organisms (GMOs). We use these methods to test for the presence of unapproved and discontinued GMOs in grains and oilseeds.

The elimination of FP967 event (CDC Triffid) in Canadian flax

In 2009, DNA from genetically modified (GM) flaxseed was detected in a Canadian shipment exported to Europe. At that time, FP967 GM flax was approved in Canada and the United States. It was not accepted by importing countries that had a zero-tolerance policy for unapproved GM products such as Japan and members of the European Union. At the peak of this FP967 flax event incident the positive detection rate was very low, approximately 0.05% to 0.01%.

Canadian flax breeders have successfully eliminated FP967 seeds from the commonly grown flax varieties. This was done by carefully selecting individual GM free plants and using strict protocols to multiply GM free seeds for each flax variety. Our role was to test flax cargo samples. We did this for several years and did not find any FP967 flax (Table 1). We also did not find any evidence of the FP967 flax when we analysed harvest samples of commercially grown flax varieties. Even though the number of samples was limited, the consistent absence of FP967 flax shows that Canadian flax varieties are now free from this GM flax.

Digital PCR tests

Our lab continues to research methods for the testing of GM events that are simple and accurate. Polymerase chain reaction (PCR) is often used for DNA testing (Figure 1). This is a technique that enables us to quickly copy, or amplify, DNA into amounts large enough to study. We see digital PCR as a promising alternative to real-time PCR, the traditional technique used in the analysis of GM events. When digital PCR is used, a sample is divided into thousands of partitions or droplets. Each partition is then tested for the presence of a given DNA sequence. The ratio of positive GM DNA partitions to that of reference DNA partitions is used to calculate the amount of GM material present in a given sample.

Detecting genetically modified DNA in samples treated by heat

DNA quality is an important consideration for the accurate detection of GM materials. Food processing can cause DNA to break down, or degrade. If a sample's DNA is degraded, it may not amplify properly and the amount of GM material it contains may not be properly measured. This year we studied the effect of heat on the accurate detection of GM events. In general, heat treatment of ground samples results in DNA degradation that is similar to that of samples processed in other ways. We heat treated canola and soybean flours containing two known GM events each. The DNA was extracted and then amplified using droplet digital PCR and real-time qualitative PCR. We also amplified DNA from untreated control samples. For three GM events, digital and real-time PCR results obtained from all samples were found to be accurate. This shows that degraded DNA has a minimal impact on PCR amplification and supports the use of PCR in detecting GM events in processed samples.

Table 1 Results of polymerase chain reaction (PCR) tests for flax cargo samples analysed from 2012 to 2019.



Figure 1 Preparation of samples for analysis by digital polymerase chain reaction (PCR) equipment.

Year	Number of flax cargo samples tested	Results of PCR tests used to detect the FP967 flax event
2019	14	Not detected
2018	16	Not detected
2017	12	Not detected
2016	22	Not detected
2015	18	Not detected
2014	9	Not detected
2013	15	Not detected
2012	12	Not detected

Future research

The Grain Biotechnology Program will continue to explore how digital PCR can be used for the detection and quantification of GMOs. We will also work on verifying detection methods for new GMOs. Specific crop varieties containing new GMOs are approved from time to time and it is important to have validated detection methods.



Bibliography

- Booker, H.M., Lamb, E.G. and S.J. Smyth. 2017. Ex-post assessment of genetically modified, low level presence in Canadian flax. *Transgenic Res.* 26: 399-409. <https://doi.org/10.1007/s11248-017-0012-7>
- 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: 1-6. <https://doi.org/10.1016/j.foodcont.2020.107291>

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Using genomics to improve grain safety

Microscopic organisms, such as bacteria and fungi, are all around us. Certain microbes are beneficial to grain crops since they contribute to the health of plants and soils. Other microbes, however, can damage crops, causing a reduction in grain yield and quality. If certain microbes that cause disease persist in food products they can result in illness in both humans and animals.

The Microbiology Program is equipped with a microbial containment laboratory where we use DNA tests to detect the presence of microbes and predict the toxins they produce. When DNA tests are not available, or we need more detailed information, we use the latest tools in DNA sequencing to capture all of their genetic information, or genome. By testing grain for microbial species and the toxins they produce, we are able to help partners in university, government, and industry stay ahead of potential threats to grain production and food safety.

High-throughput DNA testing of microbes on grain

With modern DNA testing methods, we are able to identify microbes on hundreds of grain samples in a single day. The DNA of each microbe species is unique. Using a DNA technique called polymerase chain reaction (PCR), we can test for unique sequences of DNA that tell us if a specific microbe is present. We can also use PCR to help us detect genes involved in microbe toxin production and disease potential.

For example, wheat can become infected with *Fusarium* head blight (FHB), a fungal disease caused by many different *Fusarium* species. To identify which species are present, we grind infected kernels and use PCR tests to look for DNA sequences that are unique to each *Fusarium* species. This allows us to accurately identify which species of *Fusarium* are present. Fungi that cause FHB can also make toxins that pose a risk to human and animal health. One of these toxins is deoxynivalenol (DON). There are, however, many chemical variants of the DON molecule (i.e. 3ADON, 15ADON, and NX-2) and the type of toxin that is produced depends on different sequences in their genetic material (Figure 1). Using PCR, we can monitor for these differences and predict which toxin the fungi may be producing. Our high-throughput robotic system allows us to perform more than 5000 PCR tests on a single chip (Figure 2). We use this same testing method to identify bacteria and toxin genes from bacteria found on grain.

DNA sequencing for detailed characterization of microbes and microbial communities

DNA tests that use PCR are a quick and easy screening method for detecting known DNA patterns. However, these patterns need to be preselected prior to the test, giving us an incomplete analysis of the microbes present. To complement these tests, we use whole genome DNA sequencing. This provides a more detailed analysis of the microbes on grain, including microbes that may not have been described before.

In order to capture the entire genome, we grow the microbes on grains soaked in nutrient rich liquids. We then isolate the DNA from the entire microbe community and perform targeted DNA tests for specific microbes. We also sequence all of the DNA from the entire microbial community (Figure 3). If there is a specific microbe that we are interested in, we can separate it from the community and perform genome sequencing on it. Using the genome sequencing data, we are able to perform detailed analyses of the microbial species and their genes that contribute to disease, toxin production, and resistance to antimicrobials. We can also unlock DNA differences between microbes that can allow us to develop new PCR tests that can be used for future high-throughput microbial testing.

Applying genomics to plant-pathogen interaction and crop improvement

Due to their small genome size and reduced complexity, DNA sequencing is much simpler for microbes than it is for crops. For example, the genome of *Fusarium graminearum* has been available for over a decade. The wheat genome, however, is about 400 times larger and has only become available recently. With the advent of new sequencing technologies, reduced costs and increased throughput, it is now possible to perform detailed analysis of both microbe and crop genomes. Through national and international collaborations, we are using DNA sequencing and genomics to support the research and breeding of crops with improved resistance to microbial diseases, such as FHB, rust, and wheat blast.

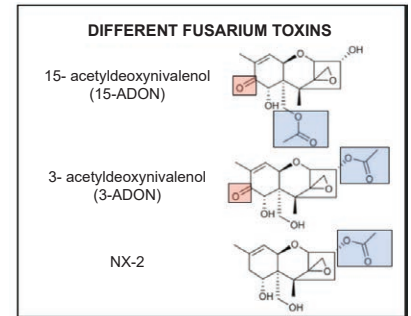


Figure 1 Chemical differences in variants of the deoxynivalenol (DON) molecule resulting from differences in the DNA of *Fusarium* genes.

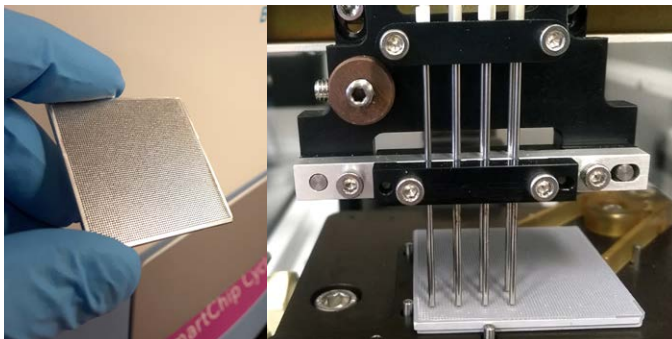


Figure 2a Single chip on which more than 5000 DNA tests for microbes and their toxins are performed.

Figure 2b High-throughput polymerase chain reaction (PCR) system and robotic liquid handling devices used to test for specific microbes.



Figure 3 Whole genome sequencing of microbes and microbial communities using technologies that read single DNA molecules.

Future research

We will continue to use genome sequencing as a foundation for developing new high-throughput DNA tests, ensuring that our testing methods are both accurate and up-to-date. These new DNA tests will allow us to address emerging needs in the grain industry.

Bibliography

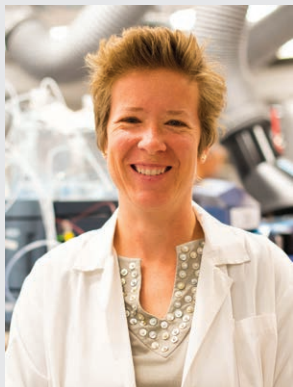
- Gao, L., et al. 2021. The *Aegilops ventricosa* 2N v S segment in bread wheat: cytology, genomics and breeding. *Theor. Appl. Genet.* 134: 529-542.
<https://doi.org/10.1007/s00122-020-03712-y>
- Sari, E. et al. 2020. Historic recombination in a durum wheat breeding panel enables high-resolution mapping of *Fusarium* head blight resistance quantitative trait loci. *Sci. Rep.* 10: 7567.
<https://doi.org/10.1038/s41598-020-64399-1>
- Walkowiak, S., Rowland, O., Rodrigue, N., and R. Subramaniam. 2016. Whole genome sequencing and comparative genomics of closely related *Fusarium* head blight fungi: *Fusarium graminearum*, *F. meridionale* and *F. asiaticum*. *BMC Genomics* 17: 1014.
<https://doi.org/10.1186/s12864-016-3371-1>
- Walkowiak, S. et al. 2020. Multiple wheat genomes reveal global variation in modern breeding. *Nature* 588:
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Reducing mycotoxin concentrations in oats

Oats, like other cereals, can become infected with fungi that produce mycotoxins and if these are eaten by humans and animals there can be negative health consequences. By finding ways to remove mycotoxins from oats and other cereals we can make them safer for consumption. The Trace Organics and Trace Elements Analysis Program is working with oats to find out where the mycotoxins are, how we can better measure them and how we can reduce their concentrations. Each link in the grain handling chain, from the farm to export, has a role to play in ensuring Canadian grain meets Canada's high standards for food safety.

The first multi-year survey of mycotoxins in Canadian oats

We conducted the first multi-year survey of mycotoxins and ergosterol, a compound that indicates the presence of fungi, in Canadian oats. Previous work on oats focused on only one year or a very limited growing area. By accessing material collected through the Harvest Sample Program and working closely with the Milling and Malting / Research on Barley and Other Grains Program at the Canadian Grain Commission, the Prairie Oat Growers Association, grain handlers and processors, we were able to obtain a much broader view of the state of mycotoxins in western Canadian oats. Harvest samples submitted by producers were analysed, as well as samples from rail shipments and truck deliveries to oat processing facilities.

We found that out of the 26 mycotoxins tested for, 7 were not detected. Among the most frequently occurring mycotoxins were those produced by *Fusarium graminearum*, such as deoxynivalenol (DON) and the newly-reported culmorin. Mycotoxins produced by *Alternaria*, such as alternariol and tentoxin, were also often found. In the harvest samples, concentrations of mycotoxins and ergosterol varied among years and growing areas. Ergosterol, as well as *Fusarium* and *Alternaria* produced mycotoxin concentrations, appeared to increase from the west toward the eastern Prairies and the province of Quebec. Ochratoxin A in deliveries and train shipments also showed annual cyclic increases in the late summer (Figure 1). All delivery and rail samples were below international limits for DON. This suggests that the bulk handling system in western Canada is preventing high concentrations from occurring in the grain handling chain. Extra scrutiny and management may be needed in years when *Fusarium* head blight is more prevalent.

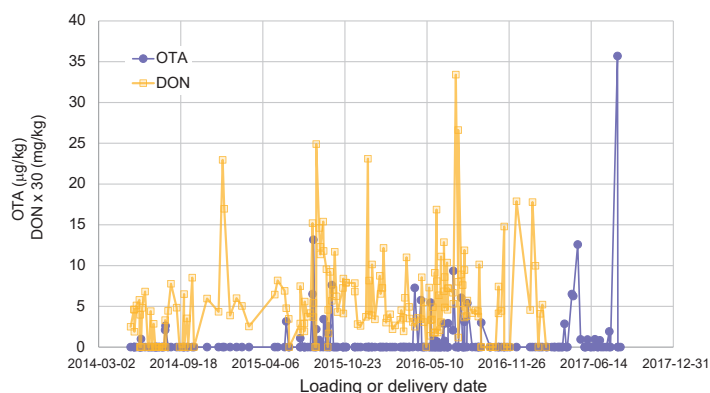


Figure 1 Annual changes in concentrations of deoxynivalenol (DON) and ochratoxin A (OTA) in oat deliveries to processors and in train shipments.



Figure 2 The rotap particle sizing unit used to separate different sized particles of ground oats.

Ensuring that laboratory equipment leads to accurate results

It's important for us to have a consistent and effective way to determine concentrations of mycotoxins in the laboratory. We assessed the ability of different grinders to produce small particles of oats (Figure 2) and looked at how sub-sampling techniques and tools affected the variability of particle sizes. We found that grinding methods do impact mycotoxin analysis. It is better to use equipment that produces smaller particle sizes, such as rotor beater mills. These grinders pulverize kernels by pushing them through a tough sieve unlike equipment such as coffee grinders and food processors (Figure 3).

Reducing mycotoxin concentrations in oats through processing

We conducted research to better understand how mycotoxin concentrations in oats change after each processing step. Laboratory scale dehulling, as well as steaming and kilning, were evaluated for how they affected the presence of fungi and mycotoxins. Our studies confirmed that mycotoxins are largely associated with oat hulls and that dehulling whole oats to produce groats removed between 60% to 100% of mycotoxins (Figure 4). Heat treatment of groats increased fungal biomass but we did not see any increase in mycotoxin concentrations. This suggests that the increase in fungi was due to species that do not produce mycotoxins. It may also mean that the conditions produced by steaming and kilning did not support the production of mycotoxins. In fact, the process of steaming and kilning further lowered concentrations of DON in groats by an average of 27%.

The mycotoxins in oats project was a collaborative venture with funding contributed by the Canadian Grain Commission, the Prairie Oat Growers Association, the Saskatchewan Ministry of Agriculture, and the Canada-Saskatchewan Growing Forward 2 bilateral agreement.



Figure 3 Four bags of whole oats ground with coffee grinders (left) and 4 bags of whole oats ground with rotor beater grinders (right).

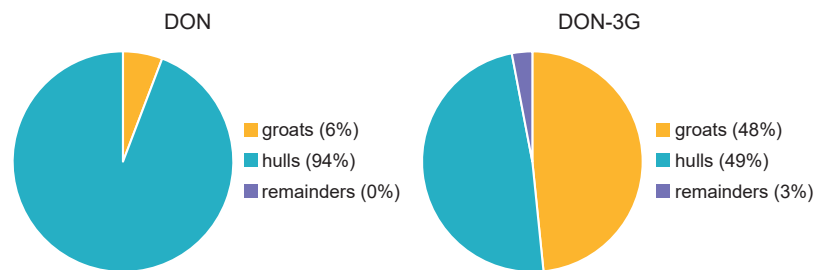


Figure 4 Distribution (based on mass) of deoxynivalenol (DON) and deoxynivalenol-3-glucoside (DON-3G) in the different fractions of dehulled oats.

Future research

We are currently looking at how processing affects the presence of pesticide residues in different grains and grain-based foods. We will also continue our monitoring of mycotoxins in exported grains to see how their occurrence changes over time.

Bibliography

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.* 36:103-114. <https://doi.org/10.1007/s12550-019-00373-9>

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.* 13:45-56. <https://doi.org/10.3920/WMJ2019.2530>

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Variety Identification Research and Monitoring

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Maintaining confidence in the quality of Canadian wheat

The Variety Identification Research and Monitoring Program plays a key role in making sure that customers have confidence in the quality of Canadian wheat. Each milling class of wheat is defined by a set of characteristics that is primarily based on performance in end-use products. 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.

Changes in wheat varieties

Monitoring shipments of wheat is challenging because the varieties being grown are constantly changing as producers gain access to newer and better performing options. Canada's strict variety registration rules ensure that new varieties also meet or exceed end-use quality requirements. New wheat varieties being considered for registration are assessed against specific points of reference to determine their appropriateness for a particular milling class.

Canada Western Red Spring (CWRS) is the most popular wheat class grown in Canada but the predominant CWRS varieties grown today are almost completely different from those grown just a few years ago (Figure 1). This change was driven in part by the Canadian Grain Commission's wheat class modernization initiative. As a result of this project, CWRS quality parameters became stricter and 25 varieties that didn't meet the new requirements were moved to the Canada Northern Hard Red (CNHR) wheat class in 2018. But even under normal circumstances, we can expect a turnover of varieties within this timeframe. This is seen in the percentage of acres planted with varieties designated as Canadian Western Amber Durum (CWAD) over the same period (Figure 2). The predominant CWAD varieties grown today are almost completely different from those grown 12 years ago despite the CWAD class being untouched by wheat class modernization.

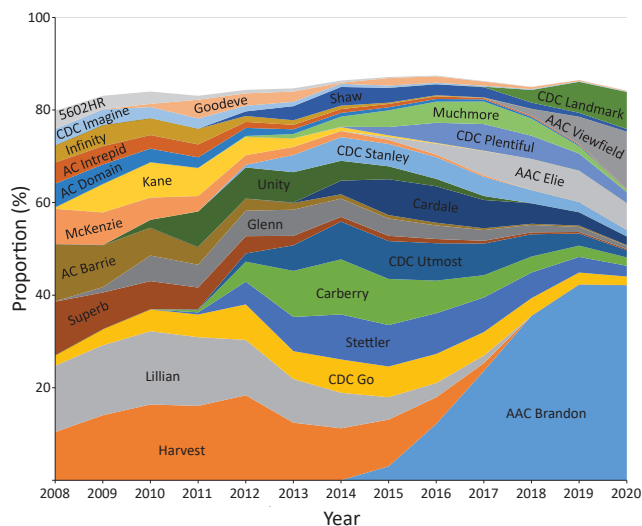


Figure 1 Acres planted with popular Canada Western Red Spring (CWRS) varieties as a proportion of total insured acreage planted with CWRS in Manitoba, Saskatchewan, and Alberta.

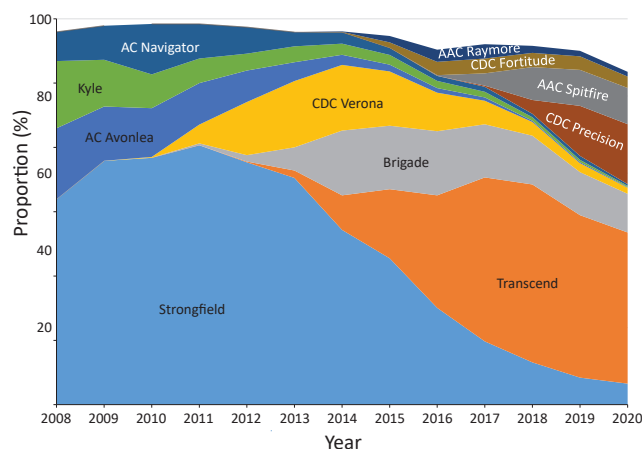


Figure 2 Acres planted with popular Canada Western Amber Durum (CWAD) varieties as a proportion of total insured acreage planted with CWAD in Manitoba, Saskatchewan, and Alberta.

There has also been a significant increase in the number of varieties eligible for delivery into these classes since 2008. Even after 25 varieties were moved out of CWRS, the number of varieties designated to that class rose from 55 to 100. Likewise, CWAD saw an increase from 15 eligible varieties to 38. The total number of wheat varieties, including durum wheat, designated for delivery into western Canadian wheat classes has more than doubled since 2008, rising from 124 to 283 (Figure 3).

Variety monitoring and DNA technology

How does the always-changing mix of varieties affect variety monitoring? It requires us to use technology that can distinguish each new variety as we encounter it. We have developed methods based on OpenArray DNA technology that allow us to look at the genetic code of individual kernels of wheat. We can examine 32 different chromosome positions, or genetic markers, and look for differences in the sequence of the DNA bases. The DNA profile of these genetic markers in a new wheat variety has a very low probability of being identical to that of an existing variety unless the two varieties are closely related. If we find that two varieties have the same DNA profile and we need to distinguish them, we must look for differences at other positions using DNA sequencing or other types of DNA testing.

We are constantly updating our database of reference DNA profiles but this requires reliable reference samples to ensure that all varieties are correctly represented. We rely on breeders and their agents to provide samples of seed as new varieties are registered.

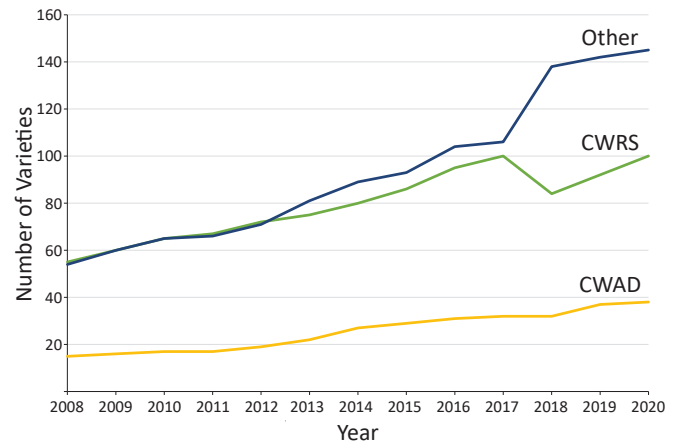


Figure 3 Numbers of varieties eligible for delivery into western Canadian wheat classes.

Future research

The varieties of wheat grown by Canadian producers will continue to change as improvements in agronomics and disease resistance are made. Our identification technology is well suited to adapt to these changes and will allow us to effectively monitor shipments for wheats of other classes and other ineligible varieties for the foreseeable future.



Bibliography

Perry, D.J. and S.J. Lee. 2015. Identification of Canadian wheat varieties using OpenArray genotyping technology. *J. Cereal Sci.* 65: 267-276. <https://doi.org/10.1016/j.jcs.2015.08.002>

Perry, D.J., and S.J. Lee. 2017. Durum wheat variety identification by OpenArray analysis. *Can. J. Plant Sci.* 97: 403-407. <https://cdnsiencepub.com/doi/abs/10.1139/cjps-2016-0300>

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