Effectiveness and biochemical basis of wholemeal GlutoPeak test in predicting water absorption and gluten strength of Canadian hard red spring wheat

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Abstract

Background and objectives: To further improve the efficiency of GlutoPeak test for selecting water absorption and gluten strength in wheat breeding programs, this study was conducted to investigate the suitability and effectiveness of wholemeal as testing material by eliminating flour milling process which is often the bottleneck of quality evaluation throughput.

Findings: By analyzing forty-four advanced wheat lines, strong correlations were found for wholemeal GlutoPeak parameters with conventional flour-based farinograph absorption ($r = .82, p < .001$) and stability ($r > .76, p < .001$), and extensigraph $R_{\text{max}}$ ($r > .90, p < .001$). The secondary structures of gluten proteins, collected at different mixing stages of GlutoPeak test, were examined using Fourier transform infrared (FTIR) spectroscopy. The β-turn structure increased significantly from partially developed to fully developed gluten, indicating its critical role in contributing to gluten viscoelasticity.

Conclusions: Without the preparation of refined flour, wholemeal GlutoPeak test can be a powerful tool for rapid and effective selection of key wheat quality traits.

Significance and novelty: This study demonstrated the effectiveness of wholemeal GlutoPeak test in screening key wheat quality parameters. The changes in gluten protein secondary structure during GlutoPeak test (refined flour or wholemeal) were demonstrated for the first time.

**Key Words**

gluten strength, GlutoPeak test, protein secondary structure, water absorption, wheat quality, wholemeal

**Abbreviations:** ATR-FTIR, Attenuated total reflection–Fourier transform infrared spectroscopy; CNHR, Canada Northern Hard Red; CPSR, Canada Prairie Spring Red; CWRS, Canada Western Red Spring; FAB, Farinograph absorption; GSI, GlutoPeak strength index; PA, Peak area; PT, Peak time; QJ, Quadrumat Junior; Tmax, Maximum torque.

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INTRODUCTION

Understanding quality at early stages of wheat breeding programs is crucial for selecting new varieties with improved end-use quality traits and for mapping genes related to these traits. However, the large number of grain samples with small sample size from a breeding population usually limits the use of traditional testing methods. Recent development of Quadrumat Junior (QJ) milling protocol, flour GlutoPeak test, and rapid extensigraph method in our laboratory (Fu et al., 2017a, 2017b; Wang et al., 2017) allows the prediction of flour yield, farinograph absorption (FAB), dough mixing, and gluten viscoelastic properties with much reduced amount of wheat (i.e., 200–300 g), comparing with standard methods used in the advanced Canadian wheat breeding trials (i.e., 2–3 kg of wheat). However, QJ milling still requires careful control of milling conditions and the preparation of refined flour with laboratory milling is a time-consuming process. At the early stages of breeding, a wholemeal sample can be readily available by simply grinding a small amount of wheat. However, due to the physical presence of bran materials and its interference with gluten development during mixing, the wholemeal flour is generally not suitable for empirical dough rheological measurements (Fu et al., 2017a). The results obtained from wholemeal are not always warranted as compared to the corresponding refined flour counterpart.

The recent development of high-speed shearing GlutoPeak test allows flour properties be measured within minutes with less than 10 g of flour (Chandi & Seetharaman, 2012; Melnyk et al., 2011, 2012). During the GlutoPeak test, the flour and water slurry are subjected to intense mechanical shearing action created by a rotating paddle. The counter torque resulting from the gluten network development upon mixing and the time required to reach peak resistance are registered in a torque curve. Great amount of research has demonstrated the success of this instrument in predicting flour water hydration capacity (Fu et al., 2017b; Wang et al., 2017), gluten quality (Marti et al., 2014, 2015; Sissons, 2016; Wang et al., 2017), and baking potential (Geisslitz et al., 2018; Rakita et al., 2017). Fu et al. (2017a) found that flour GlutoPeak maximum torque (T_max) was highly related to FAB by examining 83 candidate lines selected from six Canadian wheat registration trials in 2015. Wang et al. (2017) optimized flour GlutoPeak test conditions and reported that GlutoPeak peak time (PT) and peak area (PA) were positively associated with gluten strength but negatively related to FAB. For samples with similar gluten strength but with diverse FAB, higher PT and PA values can simply attributed to lower FAB, resulting an overestimation of gluten strength. To account for the negative impact of FAB on PA, Wang and co-workers introduced a new parameter, GlutoPeak strength index (GSI), to predict gluten strength by multiplying T_max values with PA. The arithmetic product GSI was found to provide higher correlations (r = .91) with dough strength (extensigraph R_max) than those of PA (r = .84) or PT (r = .57) based on the analysis of 56 advanced breeding lines with a wide range of FAB and gluten strength.

Some preliminary analysis of wholemeal gluten aggregation behavior during GlutoPeak test has shown its potential as a rapid tool for quality screening in breeding programs. Malegori et al. (2018) found strong predicting power of GlutoPeak for farinograph stability (81.8% of variation) using both wholemeal and refined flour as test materials. Sissons and Smit (2018) reported GlutoPeak PA and PT were good indicators of mixograph parameters and gluten index, which are traditionally used to measure gluten strength of durum wheat. Karaduman et al. (2019) noted T_max and torque at 15 s after T_max provided a stronger relationship with gluten quality parameters than other GlutoPeak indices. A wide range of testing conditions (i.e., solid-to-water ratio and mixing speed) has been used to evaluate wholemeal gluten aggregation properties, which makes it hard to compare results. Conflicting results have also been reported on the relationship between wholemeal GlutoPeak indices and flour quality parameters. It appears that a thorough investigation is required to justify the use of wholemeal GlutoPeak test in predicting conventional flour quality parameters and its use in wheat breeding programs.

In addition, determining flour water absorption and gluten properties is critical in assessing new lines in Canadian wheat registration trials based on market class. In order to improve wheat grain hardness, protein content, and gluten strength in elite lines for superior bread-making quality, wheat breeders rigorously aim for traits such as increased FAB capacity, strong gluten, and high wheat protein during crossing plans. Breeders are interested in removing genotypes with undesirable quality traits from their wheat populations. Early testing of the grain properties for quality such as water absorption and gluten strength is therefore important in breeding programs.

The objectives of this study were therefore to optimize wholemeal GlutoPeak testing condition, evaluate the impact of bran on GlutoPeak mixing behavior, and elucidate the relationship between wholemeal GlutoPeak indices and conventional flour quality parameters. To further understand the biochemical basis of GlutoPeak test in predicting gluten strength, secondary structure of gluten proteins at different mixing stages of GlutoPeak test was evaluated for both refined flour and wholemeal.

MATERIALS AND METHODS

2.1 Wheat samples

In the early stage of method development, two sets of wheat samples were selected from 2018 Canadian wheat variety registration trials to optimize the testing condition of wholemeal...
GlutoPeak analysis. Set I includes two wheat samples of similar FAB but exhibited very different gluten properties. Set II is composed of two candidate lines with similar gluten properties but greatly different in flour FAB. The detailed flour quality parameters for sample Sets I and II were presented in Table 1. To evaluate the relationship between flour and wholemeal GlutoPeak indices with conventional flour quality parameters, 44 advanced wheat breeding lines with a wide range of FAB (62.0%–73.8%) and dough strength (maximum resistance to extension: 179–898 BU) as measured by modified extensigraph method (Suchy et al., 2017) were chosen from 2018 Canadian wheat variety registration trials. A composite of each line was made from wheat grown at multiple locations across Western Canada. All composites were graded as No.2 Canada Western Red Spring (CWRS) or better.

2.2 Preparation of flour and wholemeal

Straight grade flour for all wheat composites was generated on an experimental Bühler MLU 202 test mill (AACC International Approved Method 26-21.02) at the Grain Research Laboratory of the Canadian Grain Commission. For sample sets I and II, refined flour was prepared based on the method previously described by Fu et al. (2017a) using a Quadrumat Junior mill (C. W. Brabender Instruments, South Hackensack, NJ, USA) with minor modification. Wheat was first tempered to a moisture content of 14% overnight (16 hr). To improve milling efficiency, optimize flour extraction rate, and avoid cross-contamination, the reel sifter originally supplied with the QJ laboratory mill was removed; thereafter, the obtained wholemeal was sifted through a Bühler MULA GM sieve (Bühler AG, Uzwil) at an opening of 250 µm to prepare refined flour by removing bran from the grounded wholemeal. The sieve was operated at 260 rpm for 1 min for each sieving. The milling yield of refined flour was within one to two percentage lower or higher than 75% (clean wheat basis). The refined flour has typical particle size distribution of the following: <63 µm: 18.4%; 63–125 µm: 40.8%; 125–180 µm: 25%; and 180–250 µm: 15.8%.

To prepare wholemeal, all wheat samples were tempered to a constant moisture content of 10% overnight (16 hr). Subsequently, the samples were ground with an Udy Cyclone Mill (UDY Corp., Fort Collins, Co) equipped with a 1.00 mm mesh screen.

2.3 Evaluation of flour quality parameters (Bühler milled flour)

Farinograph properties of Bühler milled flour were assessed based on AACCI International Approved Method 54-21.01. Dough extensional and mixing properties were measured following a modified extensigraph protocol previously reported by Suchy et al. (2017).

2.4 Flour and wholemeal GlutoPeak test

Gluten aggregation properties of water and flour slurry were measured by the GlutoPeak instrument with a high shear-based method (Wang et al., 2017). First, a 3 × 3 full factorial experiment was adapted to evaluate the impact of testing conditions, including ratio of solid-to-water (8/10, 8.25/9.75, 8.5/9.5, g/g) and mixing speed (2,400, 2,700, and 3,000 rpm), on gluten aggregation properties of wholemeal and refined flour for Sample sets I and II. The desired testing condition was selected based on the power to differentiate FAB and gluten strength for sample Sets I and II.

For sample analysis, 8 g of refined flour (14% moisture basis) was dispersed in 10 g of distilled water and mixed in a

| Sample set I | Sample set II |
|--------------|---------------|
| **Flour properties** | | |
| BW 362 | BW 5070 | CV (%) |
| BW 414 | BW 1086 | CV (%) |
| **Farinograph mixing parameters** | | |
| FAB (%) | 65.1 | 65.0 | 0.1 |
| 58.5 | 69.3 | 12.0 |
| DDT (min) | 5.25 | 8.0 | 29.4 |
| 5.25 | 6.75 | 17.7 |
| Stability (min) | 7.0 | 11.5 | 34.4 |
| 8.0 | 7.5 | 4.6 |
| **Extensigraph parameters** | | |
| Rmax (BU) | 366 | 695 | 43.9 |
| 402 | 401 | 0.2 |
| Extensibility (cm) | 18.8 | 18.4 | 1.5 |
| 17.2 | 19.2 | 7.8 |
| EA (cm²) | 87 | 155 | 39.7 |
| 87 | 96 | 7.0 |

Abbreviations: CV, Coefficient of variation based on the difference between two selected cultivar; DDT, Dough development time; EA, extensigraph area; FAB, farinograph absorption; Rmax, dough maximum resistance.

**TABLE 1** Major flour quality parameters for sample sets I and II
stainless-steel sample cup at 2,700 rpm. Sample temperature was controlled at 34°C by circulating water from an Isotemp 4100 water bath (Fisher Scientific, Pittsburgh, PA, USA) through a water jacket that surrounds the sample cup.

The GlutoPeak parameters analyzed by the GlutoPeak software (Version 2.1.1) include the following: (a) T\textsubscript{max}, corresponding to the maximum resistance occurring during mixing, expressed in Brabender units (BU); (b) PT, the time before maximum torque falls off, expressed in seconds; and (c) PA, area under the curve until PT is reached, expressed in GlutoPeak units (GPU). In addition, a new GlutoPeak parameter GlutoPeak strength index (GSI) was calculated based on GSI = T\textsubscript{max} × PA/1,000 as proposed by Wang et al. (2017). All GlutoPeak tests were conducted at least in duplicate.

To evaluate the potential impact of bran on gluten aggregation properties during GlutoPeak test, bran fraction from a bulk No. 1 CWRS was separated using QJ flour mill and subsequently reduced with Udy cyclone mill. Bran particle size distribution: <125 µm: 35.3%; 125–250 µm: 25.2%; 250–425 µm: 22.6%; 425–630 µm: 14.7%; and >630 µm: 2.3%. The reduced bran material was added to the refined flour of samples selected in Sets I and II at zero, ten and twenty percentage to research normal level of bran fraction in wholemeal samples. The resulting GlutoPeak mixing behaviors were compared to those of refined flour.

### 2.5 ATR-FTIR spectroscopy

Wholemeal and refined flour samples selected from sample Set I were mixed to 20 (under-mixed), 90 (under-mixed, but gluten partially developed), 100 (optimally mixed and gluten fully developed), and 120% (over-mixed) of their GlutoPeak peak times. A constant solid-to-water ratio of 8/10 (g/g) and mixing speed of 2,700 rpm were adopted. Fresh batter samples were collected for FTIR analysis to further understand GlutoPeak test and reveal protein conformational changes in the process of gluten aggregation.

ATR-FTIR spectra of refined flour, wholemeal, and GlutoPeak batter were collected in triplicate using a Bruker Invenio R Spectrometer (Bruker Optics Inc., Billerica, MA, USA) equipped with an extended range (10,000–380 cm\(^{-1}\)) KBr beam splitter and a Mercury Cadmium Telluride (HgCdTe) detector. The optical compartment and sampling accessory interior were purged with dry CO\(_2\)-free air generated by a purge gas generator (Parker Hannifin, Milton, ON, Canada) for at least 24 hr before measurements. Spectra were recorded in the 4000–400 cm\(^{-1}\) infrared spectral range at room temperature. For batter samples, a variable angle horizontal multi-reflectance ATR sampling accessory (Pike Technologies, Madison, WI, USA) set at 45 degrees coupled with a ZnSe crystal trough plate was used. A single reflection diamond ATR accessory (Pike Technologies, Madison, WI, USA) with a high-pressure clamp was used for dry samples. Each spectrum was an average of 32 scans at 4 cm\(^{-1}\) resolution. Three spectra were used for spectral analysis.

Spectral analysis of GlutoPeak batter was conducted using OPUS software v8.0 (Bruker Optics, Inc., Billerica, MA, USA). Atmospheric contribution of H\(_2\)O and CO\(_2\) was subtracted, and concave rubberband correction with 10 iterations and 64 baseline points was applied. The spectra were then vector normalized, and then offset corrected so that the minimum value is zero. Reference FTIR spectra of H\(_2\)O, corresponding to moisture content of GlutoPeak batter, were collected as described by Bock and Damodaran (2013). Reference H\(_2\)O spectra were subtracted from the Amide I (1,700–1,600 cm\(^{-1}\)) region of sample spectra to remove H\(_2\)O contribution. Second-derivative spectra were then calculated using Savitzky–Golay function with nine smoothing points to compare protein secondary structure changes.

Quantification of protein secondary structures was calculated based on the method previously described by Bock and Damodaran (2013). Briefly, mean frequencies of protein secondary structure were employed as reported by Kong and Yu (2017) and Dong et al. (1990). The protein secondary structures were estimated from relative band areas in the following spectral regions: β-sheet (1,620–1,644 cm\(^{-1}\)), random (1,644–1,652 cm\(^{-1}\)), α-helix (1,652–1,660 cm\(^{-1}\)), and β-turn (1,660–1,685 cm\(^{-1}\)).

### 2.6 Statistical analysis

All data analyses were conducted with Microsoft Excel and SAS 9.4 software (SAS Institute, Cary, NC, USA). Tukey’s test, which followed the analysis of variance, indicated significant differences with a level of \(p < .05\).

### 3 RESULTS AND DISCUSSION

#### 3.1 Effect of GlutoPeak testing conditions on gluten aggregation properties of wholemeal and refined flour

Tables 2 and 3 illustrates the impact of mixing speed and solid-to-water ratio on wholemeal and refined flour gluten aggregation properties for selected sample sets with diverse gluten strength (Sample Set I: BW362 & BW5070, Table 2) and FAB (Sample Set II: RW414 & BW1086, Table 3). Results demonstrate that mixing speed and ratio of solid-to-water significantly \((p < .05)\) affect gluten aggregation behaviors of all wholemeal and refined flour samples as shown by PT, T\textsubscript{max}, PA, and GSI. In general, higher mixing speed and flour-to-water ratio resulted in shorter PT, smaller PA, and higher T\textsubscript{max}. The wholemeal GlutoPeak exhibited similar
| Primary testing condition | Mixing speed (rpm) | Solid-to-water ratio (g/g) | Peak time (s) | Maximum torque (BU) | Peak area (GPU) | GSI (AU) |
|---------------------------|--------------------|----------------------------|--------------|---------------------|----------------|----------|
|                           |                    |                            | BW362 | BW5070 | CV (%) | BW362 | BW5070 | CV (%) | BW362 | BW5070 | CV (%) |
| Wholemeal                 |                    |                             |       |        |        |       |        |        |       |        |        |
|                           | 2,400              | 8/10                       | 83 ± 1 | 107 ± 2 | 18     | 48 ± 1 | 51 ± 1 | 4      | 1,157 ± 3 | 1,568 ± 16 | 21    | 55 ± 1 | 79 ± 0 | 26    |
|                           | 8.25/9.75          | 67 ± 1                     | 83 ± 0 | 15     |        | 54 ± 1 | 57 ± 1 | 4      | 1,201 ± 16 | 1,566 ± 64 | 19    | 64 ± 2 | 88 ± 2 | 22    |
|                           | 8.5/9.5            | 50 ± 2                     | 65 ± 4 | 19     |        | 61 ± 1 | 62 ± 0 | 2      | 1,092 ± 42 | 1,405 ± 72 | 18    | 66 ± 2 | 88 ± 4 | 20    |
|                           | 2,700              | 8/10                       | 75 ± 1 | 97 ± 2 | 18     | 49 ± 1 | 53 ± 0 | 6      | 1,101 ± 15 | 1,401 ± 11 | 17    | 53 ± 1 | 74 ± 1 | 23    |
|                           | 8.25/9.75          | 59 ± 1                     | 73 ± 1 | 16     |        | 56 ± 1 | 60 ± 0 | 5      | 1,091 ± 27 | 1,403 ± 8  | 18    | 61 ± 3 | 84 ± 0 | 22    |
|                           | 8.5/9.5            | 50 ± 2                     | 57 ± 2 | 9      |        | 64 ± 1 | 68 ± 1 | 5      | 1,103 ± 16 | 1,272 ± 16 | 10    | 70 ± 0 | 86 ± 3 | 15    |
|                           | 3,000              | 8/10                       | 70 ± 1 | 90 ± 0 | 18     | 53 ± 0 | 59 ± 1 | 7      | 1,049 ± 47 | 1,370 ± 30 | 19    | 56 ± 2 | 80 ± 3 | 26    |
|                           | 8.25/9.75          | 57 ± 0                     | 70 ± 1 | 14     |        | 61 ± 1 | 67 ± 1 | 7      | 973 ± 23  | 1,248 ± 18 | 18    | 59 ± 2 | 83 ± 2 | 24    |
|                           | 8.5/9.5            | 46 ± 1                     | 56 ± 0 | 14     |        | 69 ± 1 | 74 ± 1 | 5      | 985 ± 28  | 1,237 ± 61 | 16    | 67 ± 3 | 91 ± 5 | 21    |
| Refined flour             |                    |                            |       |        |        |       |        |        |       |        |        |
|                           | 2,400              | 8/10                       | 165 ± 6| 217 ± 3| 19     | 42 ± 1 | 44 ± 1 | 2      | 2,154 ± 115 | 2,885 ± 69 | 21    | 88 ± 6 | 125 ± 1 | 25   |
|                           | 8.25/9.75          | 129 ± 6                    | 171 ± 5| 20     |        | 47 ± 1 | 50 ± 2 | 4      | 2,092 ± 117 | 2,773 ± 76 | 20    | 103 ± 6 | 137 ± 2 | 20   |
|                           | 8.5/9.5            | 106 ± 6                    | 128 ± 1| 13     |        | 54 ± 2 | 55 ± 1 | 2      | 2,088 ± 51 | 2,690 ± 47 | 18    | 112 ± 2 | 148 ± 6 | 20   |
|                           | 2,700              | 8/10                       | 131 ± 1| 178 ± 2| 21     | 46 ± 1 | 48 ± 0 | 4      | 1,642 ± 85 | 2,304 ± 56 | 24    | 75 ± 5 | 111 ± 3 | 27   |
|                           | 8.25/9.75          | 103 ± 0                    | 132 ± 6| 17     |        | 54 ± 1 | 56 ± 2 | 3      | 1,715 ± 6  | 2,258 ± 44 | 19    | 92 ± 2 | 125 ± 7 | 22   |
|                           | 8.5/9.5            | 80 ± 1                     | 102 ± 1| 18     |        | 60 ± 1 | 62 ± 1 | 2      | 1,750 ± 26 | 2,272 ± 18 | 18    | 105 ± 1 | 140 ± 1 | 20   |
|                           | 3,000              | 8/10                       | 112 ± 4| 140 ± 1| 16     | 50 ± 1 | 54 ± 0 | 5      | 1,473 ± 29 | 1,897 ± 32 | 18    | 74 ± 1 | 102 ± 2 | 23   |
|                           | 8.25/9.75          | 86 ± 1                     | 104 ± 11| 13    |       | 59 ± 1 | 62 ± 1 | 3      | 1,473 ± 26 | 1,898 ± 37 | 18    | 87 ± 2 | 117 ± 1 | 21   |
|                           | 8.5/9.5            | 69 ± 1                     | 88 ± 3 | 17     |       | 68 ± 1 | 69 ± 0 | 1      | 1,565 ± 45 | 1,959 ± 28 | 16    | 106 ± 1 | 135 ± 2 | 17   |

Note: CV, Coefficient of variation based on the difference between two selected cultivars.
### TABLE 3  Effect of GlutoPeak testing conditions on wholemeal and flour Gluten aggregation behaviors: Sample Set II

| Primary testing condition | Mixing speed (rpm) | Solid-to-water ratio (g/g) | Peak time (s) | Maximum torque (BU) | Peak area (GPU) | GSI (AU) |
|---------------------------|--------------------|---------------------------|---------------|---------------------|----------------|----------|
|                           |                    |                           | RW414         | BW1086              | CV (%)         | RW414    | BW1086  | CV (%) |
| Wholemeal                 |                    |                           |               |                     |                |          |         |        |
|                           | 2,400              | 8/10                      | 114 ± 4       | 88 ± 0              | 18             | 43 ± 0   | 54 ± 1  | 15     |
|                           | 8.25/9.75          |                           | 84 ± 3        | 72 ± 2              | 11             | 49 ± 1   | 60 ± 1  | 15     |
|                           | 8.5/9.5            |                           | 69 ± 0        | 57 ± 0              | 13             | 57 ± 1   | 67 ± 0  | 12     |
|                           | 2,700              | 8/10                      | 101 ± 0       | 82 ± 1              | 15             | 46 ± 0   | 58 ± 0  | 16     |
|                           | 8.25/9.75          |                           | 79 ± 1        | 64 ± 1              | 15             | 52 ± 0   | 65 ± 0  | 16     |
|                           | 8.5/9.5            |                           | 59 ± 1        | 51 ± 1              | 10             | 59 ± 0   | 73 ± 1  | 15     |
|                           | 3,000              | 8/10                      | 101 ± 0       | 76 ± 0              | 20             | 48 ± 1   | 61 ± 0  | 18     |
|                           | 8.25/9.75          |                           | 78 ± 1        | 60 ± 4              | 19             | 56 ± 1   | 71 ± 1  | 17     |
|                           | 8.5/9.5            |                           | 59 ± 1        | 48 ± 2              | 15             | 63 ± 0   | 78 ± 1  | 15     |
| Refined flour             |                    |                           |               |                     |                |          |         |        |
|                           | 2,400              | 8/10                      | 234 ± 2       | 134 ± 1             | 39             | 37 ± 0   | 50 ± 0  | 21     |
|                           | 8.25/9.75          |                           | 169 ± 4       | 106 ± 2             | 33             | 42 ± 1   | 57 ± 1  | 22     |
|                           | 8.5/9.5            |                           | 127 ± 0       | 77 ± 1              | 35             | 49 ± 1   | 62 ± 1  | 17     |
|                           | 2,700              | 8/10                      | 185 ± 0       | 113 ± 1             | 34             | 39 ± 1   | 55 ± 0  | 25     |
|                           | 8.25/9.75          |                           | 137 ± 2       | 87 ± 1              | 32             | 47 ± 1   | 61 ± 0  | 19     |
|                           | 8.5/9.5            |                           | 104 ± 1       | 64 ± 1              | 34             | 54 ± 1   | 67 ± 0  | 16     |
|                           | 3,000              | 8/10                      | 164 ± 2       | 93 ± 1              | 39             | 38 ± 0   | 61 ± 0  | 33     |
|                           | 8.25/9.75          |                           | 119 ± 0       | 73 ± 3              | 34             | 50 ± 0   | 69 ± 1  | 22     |
|                           | 8.5/9.5            |                           | 92 ± 0        | 56 ± 1              | 35             | 60 ± 1   | 76 ± 1  | 17     |

Note: CV, Coefficient of variation based on the difference between two selected cultivars.
trends with much shorter PT, smaller PA, lower GSI, but higher \( T_{\text{max}} \) values than those of refined flour at same testing conditions.

The GlutoPeak profiles of sample Sets I and II at 8/10 (g/g) solid-to-water ratio and 2,400 rpm are illustrated in the supplementary documents (Figure 1). Due to similar FAB but contrasting in gluten strength, GlutoPeak profiles (refined flour and wholemeal) of BW362 and BW5070 were similar in \( T_{\text{max}} \) but very different in PT, PA, and GSI. BW5070 exhibited much higher PT, PA, and GSI attributed to its stronger gluten (Table 1). On the other hand, much lower \( T_{\text{max}} \) was shown for RW414 than BW1086 due to the lower FAB of RW414. The reduced FAB for RW414 resulted in significant increase in PT as previously noted by Wang et al. (2017). Interestingly, the PA values of RW414 and BW1086 were similar for both refined flour and wholemeal samples, suggesting PA provided a better prediction of gluten strength than GSI for this selected set of samples.

Comparing the wholemeal GlutoPeak parameters of BW362 and BW5070 in sample Set I (Table 2), a solid-to-water ratio of 8/10 (g/g) appeared to be most effective in discriminating strength based on coefficients of variation (CV%) for the PA and GSI. At fixed solid-to-water ratio of 8/10 (g/g), increasing the mixing speed from 2,400 to 3,000 rpm did not greatly affect GlutoPeak parameters (i.e., PA and GSI) in differentiating gluten strength. Since BW362 and BW5070 had similar flour FAB (Table 1), the greater difference in \( T_{\text{max}} \) values (53 versus 59) at higher mixing speed (i.e., 3,000 rpm) could affect the prediction of water absorption (Fu et al., 2017a). Overall, wholemeal GlutoPeak test was comparable to that based on refined flour in discriminating gluten strength.

This study also evaluated the impact of GlutoPeak testing condition on the discrimination for flour FAB with lines similar in gluten strength but diverse in FAB (Table 3). When compared at the same condition, GlutoPeak test based on refined flour appeared to be much more sensitive
to the variation in FAB than that of wholemeal, as indicated by greater difference of PT and T_max between the two selected samples. Increasing solid-to-water ratio from 8/10 to 8.5/9.5 (g/g) reduced the difference in T_max between the two samples selected, with a greater influence shown for refined flour than that of wholemeal. At solid-to-water ratio of 8/10 (g/g), increasing mixing speed from 2,400 to 3,000 rpm greatly improved the differentiation power of T_max for refined flour, but much less so for wholemeal samples. Overall, better differentiation in T_max was shown for refined flour than that of wholemeal regardless of test conditions.

To maximize the differentiation for both gluten strength parameters and T_max values for predicting FAB, a solid-to-water ratio of 8/10 (g/g) at mixing speed of 2,400 rpm was chosen for subsequent wholemeal analysis. Mixing speed of 3,000 rpm was not selected due to high shear rate which might disrupt wholemeal gluten aggregation process with no peak can be registered in the GlutoPeak analysis. Wang et al. (2018) improved the GlutoPeak test conditions for whole wheat flour samples of different particle size to predict whole wheat dough properties. They found solid-to-solvent (0.5 M CaCl_2) ratio of 8/10 (g/g), mixing speed of 3,000 rpm at 20°C provided the best testing reproducibility as measured by coefficients of variation (2%–3%). Reproducibility of GlutoPeak parameters in this study is <3% CV compared with those reported by Wang et al. (2018). This is likely due to the narrower range of testing conditions evaluated in this study.

### 3.2 Effect of bran on gluten aggregation properties in GlutoPeak test

To evaluate the impact of bran on flour GlutoPeak aggregation properties, grounded bran was added to the refined flour generated with a Bühler test mill from selected samples in Sets I and II (Table 4). GlutoPeak mixing profiles of reconstituted samples are included in the supplementary documents (Figure 2). Addition of bran materials increased the total viscosity as measured by T_max, while the PT and PA significantly reduced, especially for samples with longer PT and PA. A similar trend was also shown with sample Sets I & II when wholemeal and refined flour samples were tested at various mixing conditions (Tables 2 and 3). With the presence of bran materials, its high water-binding capacity is responsible for a more viscous slurry with less water available for flour or gluten hydration. As a result, the concentrated gluten fraction aggregates faster and the resulting gluten network behaved highly viscous as reflected by the elevated T_max values for samples with addition of bran. Despite the change in gluten aggregation behavior, addition of bran did not greatly affect the discrimination of selected samples with.

### Table 4 Effect of bran on flour GlutoPeak gluten aggregation behaviors

| GlutoPeak parameters | Percentage of bran | Peak time (s) | Maximum torque (BU) | Peak area (GPU) | GSI (AU) | Percentage of bran |
|----------------------|--------------------|---------------|---------------------|----------------|---------|--------------------|
| Sample set I         | 0%                 | 148 ± 2       | 206 ± 0             | 46.0 ± 0       | 0       | 2347 ± 60          |
|                      | 10%                | 120 ± 4       | 165 ± 2             | 47.5 ± 0.7     | 1.5     | 1853 ± 66          |
|                      | 20%                | 100 ± 1       | 128 ± 2             | 49.5 ± 0.7     | 1.4     | 1700 ± 14          |
|                      | 0%                 | 265 ± 1       | 141 ± 2             | 37.5 ± 0.7     | 3.4     | 2529 ± 77          |
|                      | 10%                | 191 ± 6       | 113 ± 1             | 53.5 ± 0.7     | 1.3     | 2120 ± 13          |
|                      | 20%                | 149 ± 2       | 100 ± 4             | 42.0 ± 0       | 3.4     | 1788 ± 37          |

Note: CV, Coefficient of variation based on the difference between two selected cultivars.
diverse gluten strength (sample Set I) or water absorption capacity (sample Set II) (Table 4).

3.3 | Relationship between Glutopeak parameters with conventional flour quality attributes

To evaluate the effectiveness of wholemeal GlutoPeak test in predicting key flour quality attributes, 44 advanced breeding lines were chosen from the 2018 Canadian wheat variety registration trials. More specifically, the 44 hard red spring wheat lines include 22 candidates from three CWRS wheat trials, 11 from the Canada Prairie Spring Red (CPSR) trial, and another 11 from the Canada Northern Hard Red (CNHR) trial. The simple correlations between GlutoPeak indices and conventional flour quality parameters as measured by farinograph and extensigraph were summarized in Table 5. Relationships of flour farinograph absorption and extensigraph R_max values with GlutoPeak T_max and PA of wholemeal (test condition: 8/10, g/g at 2,400 rpm) and refined flour (test condition: 8/10, g/g at 2,700 rpm) were also presented in Figure 1.

As shown in Table 5, highly significantly correlations \( r > .90, p < .001 \) existed between all major GlutoPeak parameters (i.e., \( T_{\text{max}}, \) PT, PA, and GSI) of refined flour and those based on wholemeal as testing material. Although a significant relationship was observed between \( T_{\text{max}} \) of wholemeal and that of refined flour \( (r = .91, p < .001) \), the overall relationship between \( T_{\text{max}} \) of wholemeal and flour FAB \( (r = .82, p < .001) \) was weaker than that predicted using refined flour \( (r = .94, p < .001) \). This is likely due to the presence of bran materials interfering with the measurement of intrinsic viscosity of flour aggregates. As expected, a highly significant relationship \( (r = .94, p < .0001) \) was observed for flour FAB and \( T_{\text{max}} \) of refined flour from the GlutoPeak test (Table 5 and Figure 1e). The correlation coefficient is comparable to the value \( r = .98, p < .001 \) reported in a previous work by Fu et al. (2017b) who tested 63 wheat lines selected from 2015 Canadian wheat variety registration trials using QJ milled refined flour. In addition, regression coefficients (Figure 1e) for predicting
FAB were highly consistent with the values reported by Fu et al. (2017a) (Predicted FAB = 0.4932 × T max + 39.271), indicating the consistent effectiveness of T max of refined flour in FAB prediction across different sets of materials grown in different environments.

In terms of gluten strength parameters, significant relationships were found between PA, GSI, and PT of wholemeal samples and flour farinograph stability, extensigraph R max and extensigraph area (Table 5 and Figure 1b, d, and f). A negative relationship was shown between FAB and PT, inferring flour FAB could affect GlutoPeak mixing behaviors as noted previously. By analyzing 22 refined flour and wholemeal samples, Malegori et al. (2018) found that the calculated indices based on data exploration with K-nearest neighbor model can be successfully used to classify hard red spring wheat with average prediction rate of 81.8% for farinograph stability. Our study showed significant correlation coefficients of 0.74–0.79 between GlutoPeak PA and GSI with farinograph stability and a stronger correlation to extensograph R max (0.88–0.93). Geisslitz et al. (2018) analyzed wholemeal GlutoPeak mixing profiles of five different wheat species, including common wheat, durum, spelt, emmer, and einkorn. Significant correlation was found between GlutoPeak parameters with FAB, dough stability, and bread volume. GlutoPeak parameters provided comparable efficiency to predict dough properties and bread volume as compared to micro-protein fractionation methods by extracting Osborne fractions and glutenin macropolymer (GMP) using SDS solution. By testing 145 durum genotypes, Sissons and Smit (2018) found GlutoPeak PA gave the best prediction of gluten index with correlation coefficient of 0.783 and 0.753 for wholemeal and semolina, respectively. GlutoPeak PT and PA successfully categorized durum wheat to weak, moderately strong and strong gluten genotypes. Similarly, according to the quality objectives of different Canadian hard red spring wheat classes, wholemeal and flour GlutoPeak parameters could be used to classify lines in a large breeding population to appropriate wheat classes based on their T max and PA values (Figure 1c,d).

### 3.4 Biochemical basis of wholemeal and flour GlutoPeak analysis

To understand the conformational changes of gluten proteins during GlutoPeak analysis, selected wholemeal and refined flour samples from sample Set I with diverse intrinsic gluten property were mixed to four different PT (20%, 90%, 100%, and 120% of PT) to represent a wide range of gluten development. Due to the rapid formation of gluten aggregates in GlutoPeak analysis, 90% of PT was chosen to represent partially developed gluten. For selected samples, the torque at 90% of PT was approximately half of the T max.

**TABLE 5** Simple correlation coefficient (r) between wholemeal and flour GlutoPeak parameters and major flour quality attributes

| GlutoPeak parameters—Refined flour | GlutoPeak parameters—Wholemeal |
|------------------------------------|--------------------------------|
| Tmax                               | Tmax |
| PT                                 | PT |
| PA                                 | PA |
| GSI                                | GSI |

**Abbreviations:** DDT, Dough development time; EA, extensigraph area; FAB, farinograph absorption; GSI, GlutoPeak strength index; PA, peak area; PT, peak time; R max, dough maximum resistance; Tmax, GlutoPeak maximum torque.

* *, **, *** = significance at 5, 1, and 0.1% levels, respectively; ns = not significant (p > .05).
at PT. Figure 2 presents the second-derivative of FTIR spectrum of gluten proteins in Amide I region (1,600–1,700 cm$^{-1}$) for refined flour (Figure 2a,b) and wholemeal (Figure 2c,d) and their GlutoPeak batter collected at different stages of mixing. Gluten proteins exhibited second-derivative FTIR spectrum bands at 1,621, 1,627, 1,648, 1,657, 1,675, and 1,679 cm$^{-1}$, which corroborates with band frequencies of secondary structural elements specified in previous report (Bock & Damodaran, 2013). The estimates for secondary structure of β-sheet, random/unordered, α-helix, and β-turn were calculated and summarized in Table 6.

In the unhydrated state (i.e., 0% PT), the secondary structure of gluten protein for wholemeal and refined flour is mainly composed of β-sheet (38%–42%), β-turn (23%–25%), random (16%–20%), and α-helix (17%–18%). No significant difference ($p > .05$) in secondary structure was found between the two genotypes with diverse gluten strength in the unhydrated state of both wholemeal and refined flour. The distribution of secondary structure of gluten proteins at unhydrated state is generally in agreement with Bock and Damodaran (2013) who reported that dry gluten power contained 39% of β-sheets, 14% of β-turn, 17% of α-helix, and 30% of random structure. With the progress of hydration and mixing during the GlutoPeak test, significant proportions of α-helix, random, and β-turn structures were transformed to β-sheet. As gluten is fully hydrated, β-sheet becomes the major secondary structure element in gluten. Issarny et al. (2017) and Cao et al. (2017) also reported that β-sheet is the dominant secondary structure of gluten proteins in the peak batter (58%–75%) and dough (32%–71%) for hard and soft winter wheat.

With the progress of mixing, the secondary structure of gluten in the GlutoPeak batter changed significantly for both refined flour and wholemeal as evidenced by the second-derivative spectrum in the amide I region (Figure 2). Using refined flour of BW362 as an example (Table 6), the β-sheet structure decreased only slightly from 68% to 64% with the increase of mixing time from 20% to 90% of PT. From partially developed gluten at 90% of PT to fully developed gluten at 100% of PT, however, the β-sheet structure drastically reduced by 17.5% from 64% to 46.5%. This was accompanied by increases in β-turn and α-helix structures from 18% to 30.5% and 9.1% to 12.7%, respectively. Over mixing (120% PT) resulted a slight decrease of β-turn structure and increase in β-sheet structure despite the changes were not statistically significant. Similar trends were shown for both refined flour and wholemeal of strong gluten genotype BW5070. These results suggest the formation of additional β-turn and α-helix structures at the expense of β-sheets during gluten aggregation. The protein conformational changes are very evident at PT when full development of gluten is achieved. The change in protein secondary structure during gluten aggregation demonstrates the critical role of β-turn in contributing to gluten viscoelasticity and provide the biochemical basis of GlutoPeak test (refined flour or wholemeal) in predicting gluten strength.

Much research has been conducted to reveal the conformation of wheat gluten proteins and its relationship to viscoelastic properties of gluten (Wellner et al., 2005). It is generally accepted that β-turn structure in gluten proteins is different from that related to the parallel and anti-parallel pleated β-sheets commonly found in globular proteins (Bock & Damodaran, 2013). Previous studies on gluten secondary

**TABLE 6** Effect of mixing on the secondary structure of gluten proteins in the Amide I region for selected samples with wide difference of gluten properties

| Peak time percentage | BW 362 | | | | BW5070 | | | |
|----------------------|--------|-------|-------|-------|--------|-------|-------|-------|
|                      | β-sheets | α-helix | random | β-turn | β-sheets | α-helix | random | β-turn |
| Refined flour         |         |        |        |       |         |        |        |       |
| 0%                   | 41.9$^{a-d}$ | 17.1$^{a-d}$ | 16.6$^{a-d}$ | 24.3$^{a-d}$ | 41.5$^{a-d}$ | 17.7$^{a-d}$ | 17.2$^{a-d}$ | 23.7$^{a-d}$ |
| 20%                  | 68.0$^{a-d}$ | 8.4$^{a-d}$ | 8.4$^{a-d}$ | 15.3$^{a-d}$ | 65.5$^{a-d}$ | 9.0$^{a-d}$ | 8.9$^{a-d}$ | 16.6$^{a-d}$ |
| 90%                  | 64.0$^{a-d}$ | 9.1$^{a-d}$ | 8.9$^{a-d}$ | 18.0$^{a-d}$ | 61.4$^{a-d}$ | 9.6$^{a-d}$ | 9.2$^{a-d}$ | 19.8$^{a-d}$ |
| 100%                 | 46.5$^{a-d}$ | 12.7$^{a-d}$ | 10.4$^{a-d}$ | 30.5$^{a-d}$ | 47.0$^{a-d}$ | 12.4$^{a-d}$ | 10.4$^{a-d}$ | 30.4$^{a-d}$ |
| 120%                 | 49.2$^{a-d}$ | 12.1$^{a-d}$ | 10.0$^{a-d}$ | 28.6$^{a-d}$ | 48.2$^{a-d}$ | 12.3$^{a-d}$ | 10.2$^{a-d}$ | 29.4$^{a-d}$ |
| Wholemeal            |         |        |        |       |         |        |        |       |
| 0%                   | 38.5$^{a-d}$ | 17.4$^{a-d}$ | 19.4$^{a-d}$ | 24.8$^{a-d}$ | 37.6$^{a-d}$ | 17.8$^{a-d}$ | 20.2$^{a-d}$ | 24.4$^{a-d}$ |
| 20%                  | 65.2$^{a-d}$ | 8.5$^{a-d}$ | 8.2$^{a-d}$ | 18.0$^{a-d}$ | 65.6$^{a-d}$ | 8.7$^{a-d}$ | 8.4$^{a-d}$ | 17.3$^{a-d}$ |
| 90%                  | 64.4$^{a-d}$ | 9.1$^{a-d}$ | 8.5$^{a-d}$ | 15.3$^{a-d}$ | 68.8$^{a-d}$ | 8.5$^{a-d}$ | 8.1$^{a-d}$ | 14.6$^{a-d}$ |
| 100%                 | 49.0$^{a-d}$ | 11.9$^{a-d}$ | 9.8$^{a-d}$ | 29.3$^{a-d}$ | 45.5$^{a-d}$ | 12.8$^{a-d}$ | 10.2$^{a-d}$ | 31.5$^{a-d}$ |
| 120%                 | 53.3$^{a-d}$ | 11.0$^{a-d}$ | 9.4$^{a-d}$ | 26.3$^{a-d}$ | 54.0$^{a-d}$ | 10.9$^{a-d}$ | 9.4$^{a-d}$ | 25.7$^{a-d}$ |

$^{a-d}$For each selected genotype and gluten secondary structure, values followed by the same superscript were not significantly different ($p > .05$).
structure have revealed that β-turn structure was associated with β-spiral domain, which present in the repetitive central region of the high molecular weight (HMW) subunits of glutenin (Wellner et al., 2005). The N- and C-terminal domains of HMW subunits are composed of α-helical structure. It is hypothesized that the repetitive β-turns in the central domain contribute the formation of β-spiral structure, which is considered as a major structural component responsible for the elastic properties of gluten. The results of this study appeared to be in agreement with the hypothesis when secondary structure of gluten proteins was measured at different stages of mixing during GlutoPeak test (Table 6). Issarny et al. (2017) studied the secondary structure of hard and soft red wheat flour blends by using dough, GlutoPeak batter and bread. Interestingly, GlutoPeak batter is the only material which showed changes in secondary structure with various blends of hard and soft wheat flour. With the increase of the proportion of hard wheat flour which has much stronger gluten than soft wheat flour, the β-turn structure increased from 1.6% to 13.9%, accompanied by a decrease in β-sheet structure from 92.7% to 73.4%. Their results emphasized the importance of β-turn structure in contributing to dough strength, further supporting the finding in Table 6. Cao et al. (2017) discovered that the secondary structure of gluten varied greatly in the formats of flour, dough, GlutoPeak batter, and bread. By evaluating 32 Ontario winter wheat samples, a significantly positive relationship was found between bread volume and β-turn content in dough, batter, and bread, suggesting protein secondary structure and conformation played an important role in contributing to bread-making quality. However, there was no significant difference in the relative proportions of secondary structure between the weak and strong gluten genotypes selected for this study. As a higher solid-to-liquid ratio of 8.5/9.5 (g/g) and a lower mixing speed (1,900 rpm) was used by Issarny et al. (2017) and Cao et al. (2017), the high hydration level (8/10, g/g) and high shear rate (2,700 rpm) used in this study could minimize the difference in protein secondary structure (particularly β-turns) between genotypes. Further analysis of secondary structure of gluten prepared with different types of mixer at different degree of development will be useful to further understand the protein secondary structure in relation to gluten functionality.

4 | CONCLUSIONS

A wholemeal GlutoPeak test protocol was developed in this study to predict flour farinograph water absorption capacity and dough physical strength of Canadian hard red spring wheat at solid-to-water ratio of 8–10 (g/g) and mixing speed of 2,400 rpm. Compared to the refined flour, wholemeal GlutoPeak test possessed significantly shorter mixing time, smaller peak area and higher \( T_{\text{max}} \) values at the same test condition. Varying mixing speed and solid-to-water ratio greatly affected gluten aggregation behaviors of wholemeal and Qf flour in GlutoPeak analysis. Addition of bran to flour greatly altered gluten aggregation behavior; however, the relative difference in \( T_{\text{max}} \), PA, and GSI between genotypes remained similar. By analyzing 44 selected Canadian hard red spring wheat cultivars with diverse FAB and gluten properties, PA and GSI from wholemeal GlutoPeak test appeared to be good indicators of conventional gluten strength parameters such as extensigraph maximum resistance and farinograph stability. The effectiveness of GlutoPeak \( T_{\text{max}} \) in FAB prediction is dependent upon the degree of flour refinement with refined flour showing greater correlation \( (r = .94, n = 44) \) to flour FAB than wholemeal \( (r = .82, n = 44) \). Analysis of secondary structure of gluten proteins using FTIR spectroscopy revealed the critical role of β-turn structure in contributing to gluten viscoelasticity and providing the biochemical basis of GlutoPeak test. By eliminating flour milling process which is often the bottle-neck of quality evaluation throughput, GlutoPeak test based on wholemeal can be a rapid and effective tool for screening key quality attributes in early stages of wheat breeding programs.

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