



Rapid dough making quality analysis of wheat flour using Fourier transform infrared spectroscopy and chemometrics

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ABSTRACT

Current methods for measuring wheat quality and dough rheology in the later stages of wheat breeding programs, including extensographs and farinographs, are costly and time-consuming. There is a significant interest in the Australian wheat industry for developing non-destructive, field-based, rapid dough-making quality assessment methods for Australian wheat varieties throughout earlier and later stages of the wheat breeding process. Fourier transform infrared (FTIR) spectroscopy is a valuable tool for analysis and quality control in the food industry as it is a simple and rapid technique requiring no sample pre-treatment before analysis.

We aimed to investigate the application of FTIR spectroscopy coupled with partial least squares (PLSR) regression data analysis to rapidly assess wheat flour's dough-making quality. Results indicated that using FTIR data, PLSR could be applied to accurately predict multiple dough-making qualities, including protein content, extensibility, water absorption, dough development time (DDT), dough stability, and maximum resistance to tension (R_{max}). FTIR spectroscopy could not only be used to accurately predict the dough making quality of wheat lines from an in-sample test dataset, but this method also outperformed genetic predictive analysis, an established quality-prediction method in wheat breeding, in predicting dough making quality using out-of-sample data.

1. Introduction

Standard methods to determine the end-product quality of newly developed wheat lines involve costly and time-consuming processes, including using expensive instruments such as farinographs and extensographs (AACC, 1999b, 2011). These instruments are designed to measure the rheological parameters of dough. They are employed during the later stages of the wheat breeding process to measure the dough quality of novel wheat lines (AACC, 2023). To measure dough quality, a large quantity of grain/flour is required so that dough can be physically made to perform multiple rheology measurements. These processes are destructive, have low repeatability and are expensive; as such, it is vital to determine early in the wheat breeding process that wheat lines selected for end-product testing are of high quality, as measuring sub-standard wheat lines is inefficient.

There remain considerable challenges to developing non-destructive and rapid methods for assessing dough-making quality in wheat varieties early in breeding. Currently, genomic prediction is the standard method for early-stage predictive analysis (Norman et al., 2017). Adopting new scientific methods and technologies can help breeding companies ensure that lines in the breeding program have suitable quality within the parameters of the tests that correlate with desirable performance for end users. Combining multiple strategies and technologies is one way breeding companies can increase the efficiency of developing novel high-quality wheat lines.

FTIR spectroscopy measures the interaction of infrared (IR) radiation with compounds of interest, altering their vibrational or rotational states (Tyner & Francis, 2017). The different types of vibrations and rotations for each compound absorb different wavelengths of infrared radiation, which results in unique spectral properties for different molecular

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species (Tyner & Francis, 2017). These unique spectral properties in biological samples, such as food products, can be regarded as a metabolic fingerprint. This has led to research into various applications throughout the food and agriculture industry (Karoui et al., 2010), including meat and meat products (Rohman, 2019), milk and dairy (Jawaid et al., 2013), fats and oils (Rohman et al., 2020) and cereal products (Amir et al., 2013; Ferrão & Davanzo, 2005). The characteristic FTIR absorbance bands that appear in the spectrum due to specific functional groups and bonds in a compound can be associated with the different macro components found in biological samples, including proteins, fats, carbohydrates and water (Alina et al., 2011; Karoui et al., 2010; Su & Sun, 2018). Both the protein and starch content in flour are important for dough quality (Salvador et al., 2006). The network of gluten proteins and their interaction with water and starch granules define the dough's viscoelastic properties (Ortolan & Steel, 2017; Schiedt et al., 2013) and provide a range of on-site testing applications for wheat breeders, growers, and buyers. Infrared spectroscopy has already shown some success in applications for variety identification and classification (Amir et al., 2013; Porker et al., 2017), ensuring provenance and quality assessments, including macromolecule content and dough quality predictions (Dowell et al., 2006; Miralbé, 2004; Sujka et al., 2017).

Wheat flour dough characteristics depend significantly on the flour's protein content, mixing conditions, the percentage of water added, and the air incorporated into the dough structure (Salvador et al., 2006). When water is added to flour, the gluten proteins gliadin and glutenin interact to create a three-dimensional gluten network (Schiedt et al., 2013). As the flour and water are mixed, the protein chains align, the starch granules become more evenly distributed, and air bubbles are incorporated into the dough structure, contributing to its rheological changes (Schiedt et al., 2013). This structure of gluten proteins, starch, water, and air bubbles comprises the gluten network and is mainly responsible for the overall dough structure and quality.

In the infrared spectrum, the peptide backbone universally present in proteins exhibits three characteristic bands: the amide I C=O stretching band (1580–1720 cm^{-1}), as well as the amide II (1480–1580 cm^{-1}) N–H bending and amide III (1200–1340 cm^{-1}) C–N stretching bands (Dhaka & Khatkar, 2016; Nawrocka et al., 2018). In the FTIR spectrum of starch, bands appear at 2900–3000 cm^{-1} (C–H single bond stretching), 1100–1150 cm^{-1} (C–O, C–C and C–O–C single bond stretching) and 1100–900 cm^{-1} (C–O–H bending) (Türker-Kaya & Huck, 2017). Despite the complexity of the molecular structure of starch and other storage carbohydrates, the bands in the region of 1100–900 cm^{-1} are characteristic of changes in starch structure, and changes in this spectral region may be used to track the structural changes of storage carbohydrates in biological samples (Türker-Kaya & Huck, 2017). When investigating the infrared spectra of wheat flour, these infrared spectrum regions can be points of focus when developing appropriate prediction models.

Despite the complexity of FTIR spectra of wheat flour, applying chemometrics-based approaches may be able to extract qualitative or quantitative information from chemical data, including spectral data. Partial least squares regression (PLSR) is a multivariate analytical technique that can extract these latent variables from the data and construct regression models to predict observations or measurements of unknown samples. PLSR is widely used to extract information from spectral data sets because it can be used to analyse data with a large amount of 'noisy' and redundant variables and model several characteristic variables simultaneously (Fu & Ying, 2016). As such, it has shown to be the multivariate regression model of choice for extracting data from spectral data sets of wheat samples (Fu & Ying, 2016; Karoui et al., 2010; Pojić & Mastilović, 2012; Qu et al., 2015; Woodcock et al., 2008).

Our objective was to investigate the application of FTIR spectroscopy coupled with multivariate analysis to create prediction models for accurate, rapid, and inexpensive wheat flour analysis. We aimed to

construct prediction models using a wide range of Australian wheat varieties to predict multiple dough-making properties with accuracy and robustness that are suitable for use in process, quality control, or breeding.

2. Materials and methods

2.1. Wheat samples

Australian Grain Technologies (AGT) supplied wheat samples and rheology data. These samples and data were used to construct dough quality prediction models using multivariate analysis. Three sample sets were provided (Table 1).

Three sample sets were sample set A consisted of 29 flour samples, including eight different Australian varieties from five sets of quality experiments collected from Western Australia field trials. All samples underwent full dough rheology and end-product testing at AGT's in-house laboratory (Roseworthy, South Australia). Varieties include Magenta, Wyalkatchem, Chief CL Plus, Scepter, Corack, Mace, Emu Rock, and Westonia. Sample set B consisted of 136 flour samples supplied by AGT, taken from six sites in Western Australia and South Australia with varying climates and soil types; Northam, Gibson, Gnowangerup, Roseworthy, Eradu and Buntine. All the samples underwent full dough rheology and end-product testing at AGT's in house laboratory (Roseworthy, South Australia). In sample set C, 131 grain samples were supplied by AGT. These samples were early generation material that had not yet undergone milling and dough rheology testing, however genomic predictions of the dough quality data was supplied. Sample set C was supplied as grain rather than flour. These samples were milled to flour for FTIR spectroscopy analysis using a NutriMill benchtop mill (NutriMill, USA) and a size 50 cell dissociation sieve to filter the bran.

2.2. Dough rheology testing

AGT supplied dough rheology analysis results. Measurements include protein content, farinograph water absorption, extensibility, and maximum resistance to tension (R_{max}). These measurements were obtained using the current standard techniques for assessing wheat quality parameters (AACC, 2023) and carried out at AGT's in-house laboratory (Roseworthy, South Australia).

The protein content of wheat flour was determined using NIR reflectance spectroscopy (AACC, 1999a). Extensibility, i.e. how far the dough can be stretched until it breaks (cm), and R_{max} , i.e. the maximum force applied to the dough (BU), were measured using a Brabender Extensograph (Brabender, Germany) following the standards

Table 1

Dough rheology data for sample sets A and B. R_{max} , maximum resistance to tension (BU). DDT, dough development time (minutes).

	Sample Set	Number of Samples	Min	Max	Average \pm SD
Protein	A	29	8.9	13.2	11.0 \pm 1.32
	B	136	5.67	13.08	9.23 \pm 1.92
Extensibility	A	29	16.14	25.49	20.7 \pm 2.65
	B	136	11.38	25.49	17.34 \pm 3.75
R_{max}	A	29	222.5	587.5	405.4 \pm 90.12
	B	136	222.5	602.5	397.37 \pm 81.23
Water Absorption	A	29	60.6	69.1	63.7 \pm 1.87
	B	136	52.9	68.8	62.39 \pm 2.51
DDT	A	29	3.61	8.59	5.69 \pm 1.23
	B	136	0.76	12.29	3.92 \pm 2.60
Stability	A	29	5.64	16.37	9.88 \pm 2.70
	B	136	0.96	31.96	8.57 \pm 4.70

procedures as described by the American Association of Cereal Chemists (AACC) (AACC, 1999b). Water absorption (14% moisture content flour), dough development time (DDT) and dough stability were measured using a Brabender Farinograph (Brabender, Germany) following the standards procedures as described by the AACC (AACC, 2011).

2.3. Genomic selection

AGT provided genomic predictions of the dough-making qualities of the sample set C wheat lines. These qualities include flour protein content, extensibility, farinograph water absorption, DDT, stability, and R_{\max} . AGT genotyped samples using a custom Axiom Affymetrix SNP array (Norman et al., 2017).

Genomic selection is a method for early-stage dough quality predictive analysis currently implemented in wheat breeding programs. Thus, to determine the viability of FTIR spectroscopy in these early stages of the wheat breeding process, comparisons were made between the accuracy of the FTIR spectroscopy predictions of dough quality and the standard methods of dough quality prediction.

2.4. FTIR spectroscopy

FTIR spectroscopy was performed using the Agilent Cary 630 ATR-FTIR analyser (Agilent Technologies, USA), a benchtop FTIR spectrophotometer with a type IIa diamond crystal ATR accessory and the Microlab software (version B.05.4, Agilent Technologies, USA). Infrared absorption of the milled grain samples was measured in five replicates, and a background calibration was performed before each measurement. Instrument parameters included: Range 4000–650 cm^{-1} , resolution 4 cm^{-1} , background scans 64, sample scans 64.

2.5. Spectral data pre-processing

Raw spectral data collected from the FTIR spectrophotometer underwent pre-processing through normalisation using Microsoft Excel and first and second derivative spectroscopy using R (version 4.3.1.) (R Development Core Team, 2019) prior to multivariate analysis to optimise the multivariate models' predictability. Multiple data pre-processing techniques were tested, including first and second derivative spectroscopy, standard normal variate (SNV) normalisation, 1-norm, 2-norm, min-max normalisation, and band normalisation.

2.6. Multivariate analysis

All the multivariate analysis was performed using the statistical computing and graphics software R (version 4.3.1.) (Hovde Liland et al., 2023; R Development Core Team, 2019). Prediction models were constructed using Partial Least Squares Regression (PLSR) with the FTIR spectral data to predict dough-making qualities, including protein content, farinograph water absorption, extensibility, extensibility without the influence of protein content (EXT-WP), DDT, stability and R_{\max} . Models were constructed to predict extensibility with and without the influence of protein content. Extensibility was regressed on protein content to remove the influence of protein content, and the residuals for protein and extensibility were calculated.

The optimal number of components (extracted latent variables) used to construct the PLSR models was determined by plotting the root mean standard error (RMSE) values of the leave-one-out (LOO) cross-validated predictions of the training data set for the first 10 components. For each PLSR model, components were chosen by minimising RMSE to construct the most accurate model without overfitting the data. Each model underwent an examination of its RPD (Ratio of Performance to Deviation), a straightforward means to swiftly evaluate the statistical strengths of multivariate spectroscopy calibrations (Williams, 2014). A prediction model with an RPD of over 1.9 is considered suitable for preliminary screening of complex materials including grain and flour, and an RPD of

over 2.9 is considered suitable for use in quality control (Williams, 2014).

For each multivariate model, the full FTIR spectrum (4000–650 cm^{-1}) was divided into spectral ranges based on the bands corresponding to specific macromolecular components. Data from the entire spectral region and specific spectral ranges were chosen to construct the regression models to maximise the predictive power of the model, as using all data points in the full range does not necessarily construct the best model for predicting output data for unknown samples. The spectral ranges chosen were 3680–2990 cm^{-1} (A1), 2990–2825 cm^{-1} (A2), 1775–1710 cm^{-1} (A3), 1710–1480 cm^{-1} (A4), 1480–1180 cm^{-1} (A5) and 1180–810 cm^{-1} (A6). These ranges were chosen based on significant bands along the spectrum and the PLSR loadings for the models constructed from the entire spectrum. Most spectral ranges involve bands corresponding to characteristic features of specific macromolecules: proteins (A4), fats (A2, A3), water (A1), and carbohydrates (A6)) (Alina et al., 2011; Karoui et al., 2010; Su & Sun, 2018). Variable importance in projection (VIP) and regression coefficient analysis was also utilised to confirm that the spectral regions selected for PLSR model calibration included the most relevant bands by scoring the importance of each variable (wavenumber) in the PLSR models and determining if there is a positive or negative correlation between each wavenumber and dough making quality respectively.

Preliminary PLSR prediction models were constructed from the FTIR spectroscopy data of sample set A and the supplied sample set A dough rheology data to predict the seven dough quality parameters. Using a sample set of control varieties, regression models were constructed to accurately predict dough-making parameters of wheat lines with a wide range of qualities. To strengthen the robustness and reduce the bias/overfitting of the preliminary sample set A models, FTIR data from sample sets A and B were used to construct the finalised PLSR models.

To validate the accuracy of the regression models, a test set consisting of randomly chosen samples from each sample set (five from sample set A and 20 from sample set A + B) were treated as flour samples with unknown dough-making quality. The data from the remaining flour samples were used to construct the prediction models for each dough-making quality parameter (i.e. the training set). The linear correlation between the predicted values of the test set samples and their measured values supplied by AGT was used to validate the accuracy of each prediction model.

The finalised PLSR models built from the sample set A + B FTIR spectroscopy data were then used to predict the dough-making qualities of the sample set C flour samples. The sample set C provided the external data needed to validate the PLSR models. It represented a set of novel wheat lines that would benefit from FTIR dough-making quality predictions in a practical setting. The results of the PLSR predictions of the sample set C flour samples were then used to select 12 of the 131 sample set C samples for further dough rheology testing. This was done to confirm if FTIR spectroscopy could accurately predict the dough-making quality of flour samples from an external data set and to compare the accuracy of the FTIR spectroscopy prediction models to the genomic predictive analysis of the sample set C flour samples.

In wheat grain, there is a strong negative genetic correlation between protein content and grain yield (Acreche & Slafer, 2009; Bogard et al., 2010). This means that wheat breeders do not select varieties based solely on protein content, as doing so would result in the selection of lines with reduced yield. This negative correlation is largely unexplained, although some researchers hypothesise that there may be competition between carbohydrates and protein production for energy (Munier-Jolain & Salon, 2005) or that protein is diluted by increased carbohydrate production (Acreche & Slafer, 2009). As such, the influence of grain yield on protein content was removed before ranking the samples for both protein content and extensibility. To remove the influence of yield, protein and extensibility were regressed on yield, and the residuals for protein and extensibility were calculated.

2.7. Comparison of FTIR spectroscopy and genomic predictive analysis

Genomic predictive analysis is the standard method for early-stage dough making quality predictive analysis. The predicted dough making qualities of the selected sample set C samples using FTIR spectroscopy were compared to the genomic predictions of dough making quality supplied by AGT. Using the statistical computing and graphics software, R (version 44.3.1.) (R Development Core Team, 2019), Kendall Tau distance and Spearman's rank correlation were used to determine if there was any correlation between the rankings for genomic and FTIR spectroscopy dough quality predictions of the 131 sample set C flour samples. The accuracy of the two prediction methods was then determined by comparing the R^2 values between the predicted dough quality values to the measured dough rheology results of the 12 selected sample set C samples.

3. Results and discussion

3.1. Training PLSR prediction models for dough making quality

Results of the PLSR predictions for the sample set A indicated that the models with the strongest predictive capabilities were the models predicting protein content ($R^2 = 0.977$), stability ($R^2 = 0.968$), water absorption ($R^2 = 0.921$), R_{\max} ($R^2 = 0.816$) and DDT ($R^2 = 0.810$) (Table 2a). The regression models for extensibility and EXT-WP were less accurate, with an R^2 value of 0.620 and 0.470, respectively (Table 2a). The RPD values of each model indicated that only the model predicting protein content was suitable for accurate process and quality control, with a value of 4.076. The PLSR models predicting R_{\max} , dough stability and water absorption all scored over 2.0, suggesting that these models were more suitable for implementation for preliminary screening. Both normalisation and differentiation methods were used to pre-process the raw spectral data. It was found that first derivative spectroscopy showed improved regression model accuracy for predicting protein content using FTIR data. However, raw spectral data was most accurate for all other PLSR models.

The dough-making quality predictions from the sample set A gave an early indication that FTIR spectroscopy combined with PLSR regression has the potential to accurately predict multiple dough-making properties for a wide variety of wheat lines with accuracy suitable for implementation in the early stages of the wheat breeding process.

Consequently, FTIR data of the sample set A flour samples was combined with that of the sample set B flour samples to construct more robust regression models and to validate the accuracy of the initial sample set A models.

As with the sample set A models, the prediction model for protein content from the combined sample set A + B data proved to be the most accurate model ($R^2 = 0.963$ Table 2b and Fig. 1), which is coherent with previous studies reporting the ability of PLSR to accurately predict protein content in wheat samples using both FTIR spectroscopy ($R^2 = 0.93$) (Sujka et al., 2017) and NIR spectroscopy ($R^2 = 0.97$) (Dowell et al., 2006). The prediction models calculated from the extensibility, farinograph water absorption and R_{\max} data also showed predictive capabilities, with R^2 values of 0.927, 0.700 and 0.620, respectively (Fig. 1). RPD values of the sample set A + B models indicated that the protein content and extensibility of PLSR models were suitable for high-quality process and quality control and that PLSR would be suitable for preliminary screening of DDT. The RPD values of the models predicting the remaining dough qualities suggested that these models still need improvement.

The strongest prediction models for protein content and extensibility were constructed from FTIR data from the A5 region of the spectrum (1480–1180 cm^{-1}). While the A4 region (1710–1480 cm^{-1}), encompassing the amide I and II bands, was initially hypothesised to be critical for predicting these qualities, it appeared that the amide III band (1330–1230 cm^{-1}), characterised by N–H bending and C–N stretching, played a more significant role in these predictions.

Investigation of the cumulative loadings of the protein and extensibility PLSR models indicated that the bands that attributed most to the predictive ability were at ca 1350 cm^{-1} and ca 1450 cm^{-1} (Fig. 2). The average spectrum of the sample set A and B samples showed that there are observable bands at 1490–1400 cm^{-1} ($\tilde{\nu}_3$) and 1400–1270 cm^{-1} ($\tilde{\nu}_4$) (Fig. 3). These bands are suggested to correlate to amide III vibrations from the polypeptide backbone or contributions from side-chain vibrations (Barth, 2000; Ji et al., 2020). Side-chain vibrations, including CH and CH_2 , may also result in less intense bands or shoulders within the 1370–1320 cm^{-1} range ($\tilde{\nu}_4$) (Barth, 2000). The exact wavenumbers and intensities of these bands depend on the abundance and types of amino acids present. Some protein-related bands also appear at ca 1450 cm^{-1} ($\tilde{\nu}_3$), associated with amide II or side-chain vibrations (Barth, 2000; Ji et al., 2020). The cumulative loadings of the DDT model also showed that the band around 1450–1420 cm^{-1} contributed most to the

Table 2
PLSR results for (a) sample set A and (b) sample set A + B using FTIR spectral data.

(a)								
Dough quality parameter	Wavenumber range	^a Components	^b Test set R^2	^c RMSE	^d RPD	^e Slope	^f Bias	Derivative
Protein	A5	3	0.977	0.429	4.076	1.064	0.215	1st
Extensibility	A5	4	0.620	1.413	1.534	1.221	−0.310	None
^g EXT-WP	A4	8	0.470	1.509	0.852	0.407	−0.001	None
^h R_{\max}	A4	6	0.816	77.06	2.597	1.100	3.200	None
ⁱ DDT	FULL	9	0.810	0.745	1.604	0.965	0.304	None
Stability	FULL	6	0.968	1.945	2.213	0.799	0.857	None
Water Absorption	A2	6	0.921	1.354	2.710	0.846	0.096	None
(b)								
Dough quality parameter	Wavenumber Range	^a Components	^b Test set R^2	^c RMSE	^d RPD	^e Slope	^f Bias	Derivative
Protein	A5	2	0.963	0.460	4.950	0.969	0.127	None
Extensibility	A5	2	0.927	1.391	3.687	0.941	−0.336	None
^g EXT-WP	A4	8	0.372	1.326	1.421	0.815	−0.411	1st
^h R_{\max}	A4	6	0.620	71.940	1.750	1.078	−1.588	1st
ⁱ DDT	A5	1	0.815	1.427	2.477	0.959	−0.270	1st
Stability	FULL	4	0.450	3.075	0.935	1.030	0.106	1st
Water Absorption	A6	4	0.700	1.302	1.887	−0.433	1.001	None

^a Latent variables. ^b Squared correlation coefficient of the predicted and measured values for the test set samples. ^c Root mean standard error for leave-one-out cross-validated predictions of the training data set. ^d Ratio of Performance to Deviation, calculated by dividing the Standard deviation of reference values by RMSE. ^e relationship between predicted values versus reference values. ^f the mean difference between the predictor variables and the response variable. ^g extensibility without the influence of protein content. ^h maximum resistance to tension (BU). ⁱ dough development time (minutes).

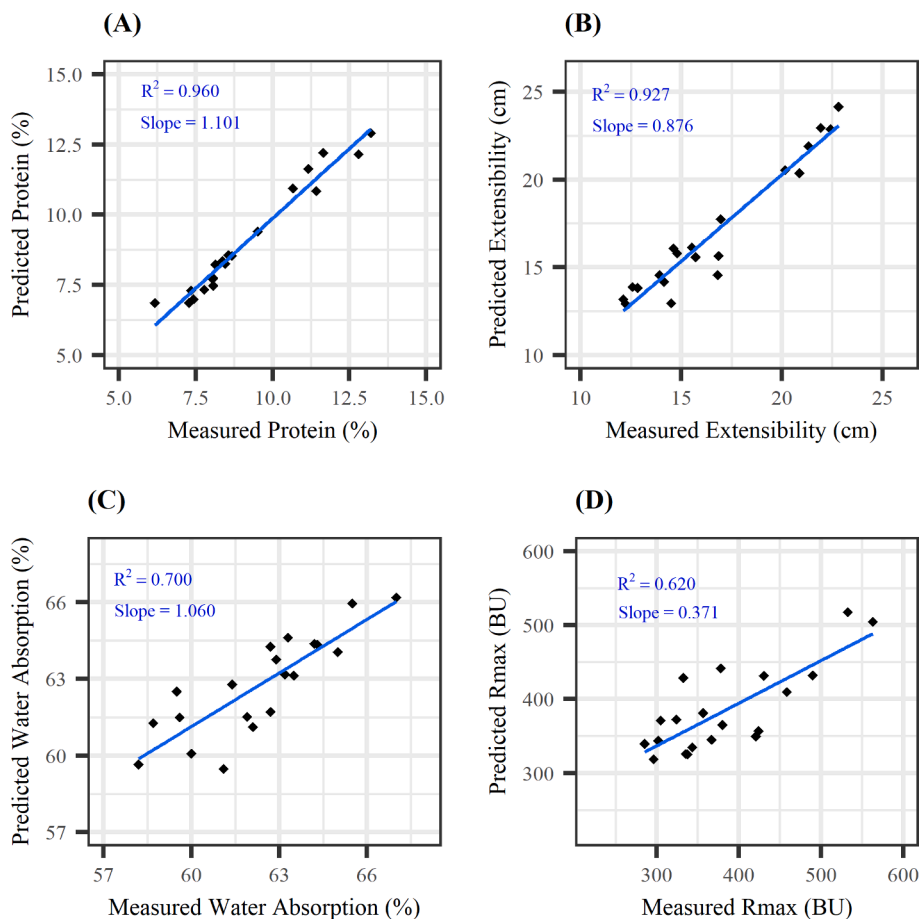


Fig. 1. Correlation plots of the predicted and measured dough qualities for the PLSR models constructed from the sample set A + B data set. (B) Protein content (%), (B) extensibility (cm), (C) water absorption (%) and (D) R_{max} (BU).

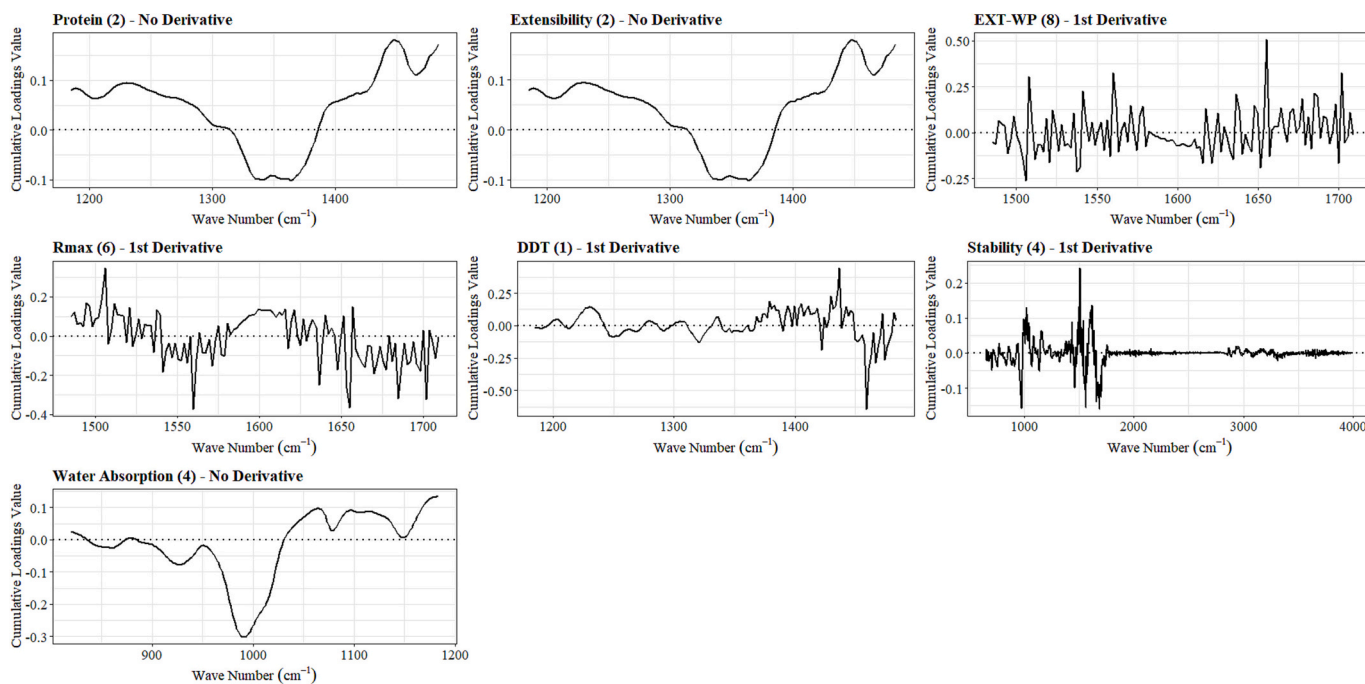


Fig. 2. Partial least squares (PLS) loadings of the optimal calibrations. Each PLSR model is constructed from the sample set A + B dataset. The optimal number of PLS loadings is in brackets.

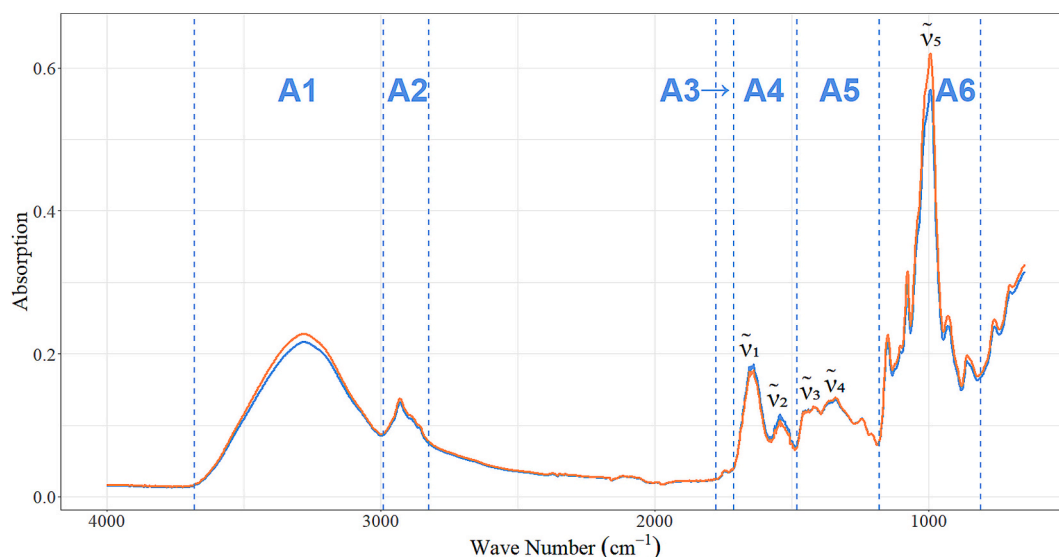


Fig. 3. Mean FTIR spectrum of the flour samples from sample sets A and B. Labelled bands ($\tilde{\nu}_1$ - $\tilde{\nu}_5$) are identified as important for the predictive analysis of dough-making quality according to the cumulative loadings of the PLSR models. Sample set A is shown as a solid line, and sample set B is shown as a dotted line. A1 to A6 are the selected spectrum regions for the PLSR models.

predictive ability of the model (Fig. 2). This suggested that amide II bands relating to protein content were also the main factor in predicting DDT, as with extensibility and flour protein content. The gluten network plays a vital role in dough extensibility and development time (Song & Zheng, 2008), which may be why protein-related FTIR bands contributed most to the PLSR models.

For the models predicting water absorption, the strongest model was constructed from region A6 (1180–810 cm^{-1}) containing bands at 1100–1150 cm^{-1} (C–O, C–C and C–O–C single bond stretching) and 1100–900 cm^{-1} (C–O–H bending), all present in starch and other carbohydrates. As starch has the most significant impact on flour's water absorption, this result was not unexpected (Lovegrove et al., 2020). Investigations of the PLSR loading values for the model predicting water absorption indicated that bands around 990 cm^{-1} contributed most to the predictive ability of the model, suggesting the importance of the presence of C–O–H bonds (Fig. 2).

The cumulative PLSR loadings of the R_{max} model indicated that multiple bands between 1500 cm^{-1} and 1700 cm^{-1} contributed to the predictive ability (Fig. 2). Two bands were observed in the sample set A + B flour samples, at 1700–1575 cm^{-1} ($\tilde{\nu}_1$) and 1575–1485 cm^{-1} ($\tilde{\nu}_2$). The band at 1700–1575 cm^{-1} is often associated with the stretching vibration of the carbonyl (C=O) group in amide bonds, whereas the band at 1575–1485 cm^{-1} is associated with amide II N–H bending and C–N stretching bands (Dhaka & Khatkar, 2016; Nawrocka et al., 2018). As with the extensibility and DDT models, the PLSR loadings of the R_{max} model indicated that the bands corresponding to protein content are the most important for predicting R_{max} .

When we compared the models built using only data from sample set A to those using data from both sample sets A and B, we observed a decrease in the accuracy of predicting R_{max} , water absorption, dough stability, and EXT-WP, but an increase in accuracy for predicting extensibility for sample set A + B. By constructing multivariate models with multiple data sets (sample set A + B), the bias of the model's predictions towards the model's training data is reduced. The higher R^2 values seen in the sample set A models for R_{max} , dough stability, EXT-WP and water absorption may be due to the increased bias seen in the models for predicting the validation sample set dough making qualities, as the validation sample set also comprised of solely sample set A data.

The results of the internal validation of the sample set A and sample set A + B PLSR models also indicated that FTIR spectroscopy combined with PLSR could accurately predict extensibility but not EXT-WP. This

suggests that PLSR is using the same latent variables that predict protein content to predict extensibility. Analysis of the loadings for the models predicting protein content and extensibility also indicated that the same range of wavenumbers had similar relative loading values for predictability (Fig. 2). Previous research has highlighted that there is often a negative correlation between wheat grain yield and protein content (Acreche & Slafer, 2009; Bogard et al., 2010; Simmonds, 1995), often leading to wheat breeders not selecting varieties based on protein content alone as this would result in selections of lines with reduced yield. As the extensibility PLSR models may be using the same variables to predict extensibility as the protein PLSR models, a similar issue may arise whereby wheat lines being selected for their high extensibility values may result in selections of lines with lower yields. This means that if the extensibility models were to be used on unknown wheat lines, the yield of these lines must be taken into consideration to affirm that varieties with high yield and high extensibility are being selected to enter the later stages of the wheat breeding process.

3.2. Validating the PLSR models using an external dataset

Dough making quality predictions of the 131 sample set C grain samples were made using the PLSR prediction models constructed from the sample set A + B data, then ranked from highest to lowest, and finally compared to the rankings of the genomic predictions for dough quality of the same samples. Both Kendall Tau distance and Spearman's rank correlation concluded that there was no association between ranks for the predictions of protein content or dough extensibility (see Supplementary data, Table S1).

Using the results from FTIR spectroscopy and genomic predictions of each dough quality, 12 wheat samples from sample set C consisting of wheat lines with low and high predicted dough making qualities were selected to be sent for further dough rheology testing. Of the 12 selected samples, only 11 were tested due to weevil damage to one sample. The predictions were compared to the results of the dough rheology tests and regression coefficients were calculated between the predicted and measured values (Table 3).

The results of the comparison between the predicted and measured values of the 11 sample set C samples indicated that FTIR spectroscopy was superior at predicting parameters including protein content, extensibility, DDT, stability, and water absorption, whereas genomic predictive analysis was more accurate at predicting R_{max} . The higher

Table 3

Correlation (R^2) between the predicted and measured dough qualities for the stage 2 tested grain samples (sample set C).

Dough Quality Parameter	FTIR spectroscopy	FTIR 1st Derivative	FTIR 2nd Derivative	Genomic prediction
Protein	0.400	0.453	0.469	0.037
Extensibility	0.355	0.343	0.379	0.056
Water Absorption	0.151	0.061	0.089	0.005
DDT	0.497	0.034	0.066	0.122
Stability	0.107	0.153	0.073	0.119
R_{\max}	0.065	0.239	0.050	0.754

accuracy for genomic analysis to predict R_{\max} may have been due to the fact that R_{\max} is dictated by the expression of specific glutenin proteins, which is determined by the allelic composition for six glutenin subunits (Kovács et al., 2013). Dough rheology measurements such as extensibility and water absorption are generally dictated by overall protein content in wheat flour, which may be why FTIR spectroscopy is more suited to predict these qualities (Abdelaleem & Al-Azab, 2021; Blakeney et al., 2009).

The PLSR prediction accuracies of each of the dough quality parameters for sample set C still showed similar relative accuracies when compared to the results of the internal validation sets for sample set A + B (Table 2), with the protein model having the strongest predictive accuracy, followed by extensibility. The drop in overall predictive accuracy when compared to the internal validation results was to be expected, as the regression models were only constructed from two sets of data. For preliminary or exploratory analysis, these 'lower' R^2 values may be acceptable, as the primary goal at this stage is to identify broad trends and make initial assessments rather than achieve precise predictions. This level of accuracy may be sufficient to measure early-stage wheat lines, allowing researchers to determine which lines fall into the bottom 30–50 % in terms of quality and subsequently eliminate them from further consideration. Nonetheless, the results still indicated that FTIR spectroscopy was more accurate than genomic analysis for this prediction. Over time, more sample sets will become available to increase the robustness of the PLSR models, which will improve the prediction models.

Although these results indicate that FTIR spectroscopy is superior to genomic analysis for predicting dough making quality, it should be noted that the dough making quality predictions came from the direct FTIR spectroscopy measurements of flour samples. On the other hand, genomic selection is a prediction based on the genotype of the wheat line using many datasets to calibrate each marker locus value, rather than a prediction formed from a direct assessment/measurement of wheat flour itself. In doing a direct comparison of predicted and measured values of the dough making quality parameters, FTIR spectroscopy was expected to outperform genomic predictive analysis. For accurate characterisation of the line's actual genetic performance, genomic selection should be more accurate for that line's long-term value.

3.3. Model limitations and future improvements

Despite the promising results of the FTIR prediction models, the accuracy of the predictions for dough stability, R_{\max} and EXT-WP suggested that the FTIR data used to build the models in this study may not have fully captured the complexity of these traits. The relatively poor performance of the EXT-WP model suggested that there is a close link between extensibility and total protein content. This makes it difficult for PLSR to identify other variables within the FTIR data that may be associated with extensibility. After removing the influence of protein content on extensibility, by regressing extensibility with protein content, the remaining variability in extensibility likely reflected measurement noise, experimental errors, or secondary factors such as starch structure or lipid composition, which may influence extensibility but to

a much smaller extent than protein content. Because extensibility is largely impacted by gluten protein composition in wheat flour, FTIR spectra may not adequately capture differences in starch or lipid structures that could have a small effect on extensibility.

To overcome these issues, future research should focus on constructing and enhancing prediction models with more diverse wheat lines that span different genetic backgrounds and environmental conditions. Combining FTIR spectroscopy with other techniques, such as NIR and Raman spectroscopy, could also enhance model performance and provide a more comprehensive analysis of dough making quality for wheat breeders by capturing a broader range of spectral features. Such models, may also capture secondary factors related to extensibility, which may help wheat breeders select lines with desired extensibility traits without selecting for total protein content.

Another limitation to consider with the prediction models in this study is that, even though the FTIR models demonstrated strong predictive power for dough making quality, their performance could vary across different wheat varieties, growing conditions and environmental stresses. Factors such as soil quality and climate could influence the chemical composition of the wheat grain/flour and, consequently, its dough-making properties. To validate the robustness of FTIR predictions, future studies should incorporate trials and samples that account for these environmental variables. Additionally, the inclusion of a broader range of wheat genotypes, including those grown under stressed conditions, would be valuable in assessing the adaptability FTIR spectroscopy for predicting dough making quality.

3.4. Future implications

Our results suggest that FTIR spectroscopy can add value to wheat breeding programs combined with genomic analysis to produce a more comprehensive analysis of the dough qualities of novel wheat lines to make more informed decisions in the early stages of the wheat breeding process. FTIR spectroscopy may be implemented in wheat breeding programs either by using dough making quality predictions to check the suitability of grain samples from a site to both cull wheat lines with poor dough-making quality and select wheat lines with acceptable dough-making quality for further dough rheology analysis, or by producing covariate values that can be used to improve the accuracy of the data collected from dough rheology testing. Both approaches would ultimately improve the accuracy of genomic selection by reducing error in the datasets used in further calibrations of the genomic models, which would in turn help wheat breeders develop wheat lines that can produce higher quality end-products for consumers.

FTIR spectroscopy has been utilised to analyse various aspects of wheat and flour quality. For instance, FTIR has been used to analyse dough rheology in wheat dough samples (Fanari et al., 2022), for flour quality control by building models to predict protein, fat, ash content, moisture, falling number, and fatty acids (Sujka et al., 2017), and for analysing flour macromolecule composition (Golea, Codina, & Oroian, 2023). However, no prior work has investigated the use of FTIR spectroscopy in a wheat breeding context to predict dough-making quality traits commonly measured in wheat breeding dough rheology laboratories. The results from this study provide a foundation for advancing the implementation of FTIR spectroscopy in the early stages of wheat breeding programs, offering an efficient method for evaluating dough-making quality parameters and improving the selection process for high-performing wheat lines.

Looking forward, the combination of FTIR spectroscopy with other high-throughput techniques, such as Raman spectroscopy, may also offer a promising avenue for enhancing predictive models for dough-making quality in the early stages of the wheat breeding process. Raman spectroscopy provides complementary insights into molecular structure and chemical bonding. Integrating these two spectroscopic techniques alongside early-stage genomic analysis could offer a more comprehensive understanding of wheat dough properties, allowing

breeders to better capture the molecular dynamics that determine dough quality.

4. Conclusion

FTIR spectroscopy combined with PLSR is capable of predicting dough making qualities including protein content, extensibility, DDT, and water absorption with an accuracy allowing for applications in screening and quality control, and predict R_{max} and dough stability in preliminary screenings. The accuracy of the PLSR models suggests that FTIR spectroscopy is superior to genomic analysis for predicting dough making quality of novel wheat lines for each studied dough quality parameter, other than R_{max} . These findings suggest that FTIR spectroscopy has the potential to be used in combination with current predictive analysis techniques, such as genomic analysis, to provide more informed decisions early in the wheat breeding process on the viability for wheat lines to be selected for further dough rheology analysis, as well as be used alongside dough rheology measurements to improve the accuracy of dough quality testing.

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CRediT authorship contribution statement

James M. Kelly: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Björn Bohman:** Writing – review & editing, Supervision. **Dion Bennett:** Writing – review & editing, Resources, Conceptualization. **Nicolas L. Taylor:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.143335>.

Data availability

Data will be made available on request.

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