APPLICATION NOTE

FT-NIR Spectroscopy

The Determination of Protein and Moisture in Samples of Wheat

Summary

NIR spectroscopy has many valuable uses throughout the various stages of the manufacturing process particularly for raw material qualification and quantitation. The technique offers a fast and reliable alternative to traditional quantitative methods which often take many hours to complete. This note describes the use of FT-NIR spectroscopy to determine the protein and moisture content in ground wheat raw materials used in the agricultural industry. We have established the feasibility of determining such properties with an estimated prediction error of less than 0.5%.

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Experimental

All spectra were recorded on a PerkinElmer® FT-NIR Spectrometer fitted with an in-board solid sampling accessory. Seventy different ground wheat samples were supplied and measured with no additional milling or grinding. Spectra were recorded by filling a standard sample cup with the sample and scanning in interleaved mode. This mode of operation alternately takes a background spectrum as well as the ratioed spectrum which minimizes changes in atmospheric effects.

Three replicate measurements of each of the calibration samples were collected, and the mean spectrum used for the generation of the calibration equations. The sample cup was emptied and refilled for the collection of the three replicate spectra to obtain a more representative spectrum of the sample. A rotating sample cup is also available, which removes the need to scan multiple replicates for these types of samples.

To support the validation tests, a random set of sample spectra was collected approximately one week later. Data was collected over the range 10000 to 3800 cm^{-1} at 16 cm^{-1} resolution with approximately one minute scanning time. It may be possible to scan the samples using considerably less scanning time and still achieve the desired accuracy. Data was collected over the whole range of the NIR spectrum since this data set may be used to determine a number of other properties in wheat from these spectra. A typical spectrum representative of the wheat samples is shown in Figure 1.

A partial least squares analysis (PLS) was performed on the data (70 spectra). It is possible to predict values for protein and moisture content in wheat in the independent validation set.

Various mathematical pretreatments were tested and a second derivative function chosen to provide SEP value of 0.28 for protein and 0.49 for moisture using 6 PLS factors and full cross validation. Full cross validation excludes each standard in turn from the calibration set, performs the calibration and then predicts the excluded standard using that calibration. Smaller prediction errors may be obtained using a larger number of PLS factors.

However, it was decided to optimize the calibration for robustness which is better achieved by performing independent validation over time. Figures 2 and 2a are the illustrated plots of Estimated versus Specified values, first for protein and second for moisture. This provides an adequate starting point for the calibration model.

These graphs show that protein has a slightly tighter model than moisture. This may be due to the samples' changing moisture content in storage. It is recommended to store calibration samples in dry conditions, especially if there is a significant time lapse between reference and NIR measurements. The regression model summaries for the full cross validation model are shown in Table 1.

To support validation, a series of samples were run a week later and both the protein and moisture content predicted using the calibrated model. Table 2 shows the results along with the reference values supplied. Additional statistics in terms of the total M-distance and residual ratio give an indication of how well the model covers these samples.

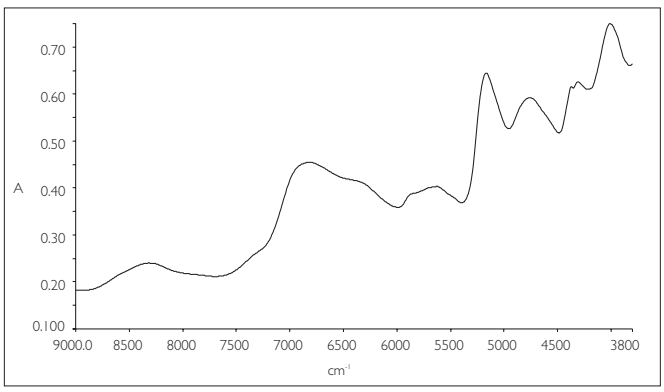

Figure 1. Typical spectrum of ground wheat.

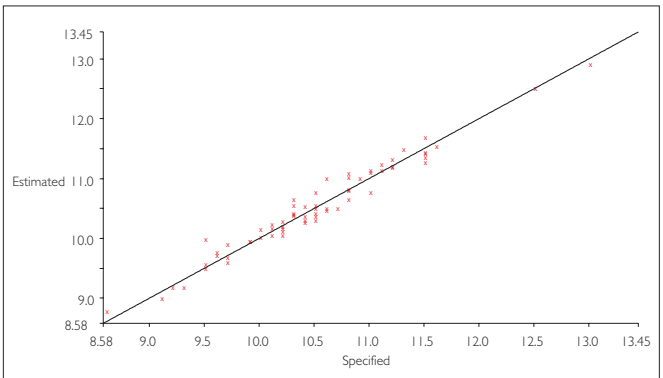

Figure 2a. Estimated vs Specified plot for Protein/Full Cross Validation.

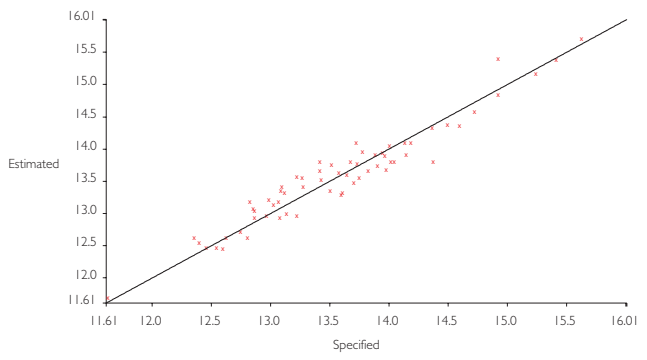

Figure 2b. Estimated vs Specified plot for Moisture/Full Cross Validation.

Table 1. **Summary of Calibration Reports for i) Protein and ii) Moisture in Wheat.**

i) Protein		**Number of LVs used : 6 + intercept**			
LV Number	**Correl. of LV with property**	**Regression Coefficient**	**Std. error of R.C.**	**t-value**	**Sig. Lev.%**
1	0.8298	5.82	0.1961	29.67	0.00
2	0.2590	1.669	0.1646	10.14	0.00
3	0.4893	2.66	0.1637	16.24	0.00
4	0.1555	0.9108	0.1656	5.50	0.00
5	0.2314	1.328	0.1635	8.12	0.00
6	0.1613	0.9859	0.1611	6.12	0.00
Intercept	0.1966	-0.06268	0.0196	-3.19	0.22
Std Error of Prediction: Estimate		**= 0.1659**	**Actual = 0.2824**		
Multiple Correlation		**= 0.9819**			
Mean Property Value		**= 10.46**			
% Variance (R squared)		**= 96.4107**			
Std Error of Estimate (SEE)		**= 0.159**			
F-value		**= 268.6**			
ii) Moisture		**Number of LVs used : 6 + intercept**			
LV Number	**Correl. of LV with property**	**Regression Coefficient**	**Std. error of R.C.**	**t-value**	**Sig. Lev.%**
1	0.5654	3.965	0.2389	16.59	0.00
2	0.5432	3.935	0.2351	16.74	0.00
3	0.2324	2.214	0.2546	8.70	0.00
4	0.2632	1.72	0.2454	7.01	0.00
5	0.3195	2.228	0.2220	10.03	0.00
6	0.0845	0.9262	0.2334	3.97	0.02
Intercept	0.2766	0.08827	0.0279	3.16	0.25
Std Error of Prediction: Estimate		**= 0.2314**	**Actual = 0.4938**		
Multiple Correlation		**= 0.9642**			
Mean Property Value		**= 13.55**			
% Variance (R squared)		**= 92.9637**			
Std Error of Estimate (SEE)		**= 0.2189**			
F-value		**= 123.3**			

Table 2. **Samples 1 and 2.**

QUANT+ V4.00 PREDICTION RESULTS PLS1								
		Sample 1			**Sample 2**			
Sample		V20030 (1 of 2)			V20033 (1 of 2)			
Calc.Name		R01V2030.SP			R01V2033.SP			
Normalization		None			None			
Method		WHEAT.MD Ver: 2 ID: 3294			WHEAT.MD Ver: 2 ID: 3294			
Total M-Distance		0.379			0.611			
Residual Ratio		1.33			1.15			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Protein	**10.13%**	**10.00**	**0.275**	**0.397**	**12.15%**	**12.50**	**0.28**	**0.595**
Total M-Distance		0.368			0.555			
Residual Ratio		1.18			1.33			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Moisture	**12.96%**	**12.34**	**0.378**	**0.387**	**12.57%**	**12.44**	**0.383**	**0.547**
Prediction complete					Prediction complete			

Table 2. **Samples 3 and 4.**

QUANT+ V4.00 PREDICTION RESULTS PLS1								
		Sample 3			**Sample 4**			
Sample		V20073 (1 of 2)			V20077 (1 of 2)			
Calc.Name		R01V2073.SP			R01V2077.SP			
Normalization		None			None			
Method		WHEAT.MD Ver: 2 ID: 3294			WHEAT.MD Ver: 2 ID: 3294			
Date		10-Apr-1997 15:55:02			10-Apr-1997 15:55:05			
RMS Error		1.807e-006			1.612e-006			
Peak to Peak Error		2.126e-005			2.116e-005			
Total M-Distance		0.652			0.573			
Residual Ratio		1.84			1.47			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Protein	**9.463%**	**9.50**	**0.281**	**0.63**	**9%**	**9.10**	**0.279**	**0.563**
RMS Error		1.691e-006			1.654e-006			
Peak to Peak Error		1.932e-005			2.022e-005			
Total M-Distance		1.06			0.508			
Residual Ratio		1.51			1.44			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Moisture	**15.94%**	**15.61**	**0.398**	**0.977**	**13.76%**	**14.03**	**0.382**	**0.507**
Prediction complete					Prediction complete			

Table 2. **Samples 5 and 6.**

QUANT+ V4.00 PREDICTION RESULTS PLS1								
		Sample 5			**Sample 6**			
Sample		V20181 (1 of 2)			V20380 (1 of 2)			
Calc.Name		R01V2181.SP			R01V2380.SP			
Normalization		None			None			
Method		WHEAT.MD Ver: 2 ID: 3294			WHEAT.MD Ver: 2 ID: 3294			
RMS Error		1.318e-006			1.441e-006			
Peak to Peak Error		1.229e-005			1.754e-005			
Total M-Distance		0.111			0.427			
Residual Ratio		0.982			1.17			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Protein	**10.89%**	**11.00**	**0.269**	**0.167**	**10.78%**	**10.50**	**0.276**	**0.438**
RMS Error		1.353e-006			1.579e-006			
Peak to Peak Error		1.423e-005			1.735e-005			
Total M-Distance		0.17			0.358			
Residual Ratio		0.963			1.31			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Moisture	**14.11%**	**13.40**	**0.372**	**0.217**	**12.52%**	**12.58**	**0.378**	**0.379**
Prediction complete					Prediction complete			

Conclusion

The example detailed here illustrates that it is possible to determine a number of properties present in ground wheat samples with accuracy which is of a similar order to that of the reference method using FT-NIR spectroscopy. Based on the samples supplied, it has been shown that FT-NIR and partial least squares can be used to determine protein and moisture in ground wheat to within 0.5% SEP.

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