

# **Predicting Barley Neutral Detergent Fibre (NDF) by Near Infrared Spectroscopy and its Application in Malting Barley Quality Selection.**

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## **Introduction**

The dietary fibre fraction of plant material describes those component polysaccharides and lignin that cannot be digested by monogastric organisms. Fibre content is an important parameter in monogastric and ruminant feed formulations for reasons of potential digestible energy, and as a beneficial factor in human diets. It may also be important as a selection tool when breeding for improvements in barley malting quality, with the hull contributing to a significant proportion of the non-fermentable components of malt. Neutral detergent fibre (NDF) has been widely accepted as a determinant for dietary fibre in cereal grains as it estimates the content of cellulose, hemicellulose and lignin present mostly in outer grain layers. The traditional procedure for estimating NDF is the Van Soest method that requires intensive operator intervention and can limit the efficiency of the assay when assessing large numbers of samples. A method based on the Ankom filter bag technique (FBT) in a batch reflux chamber, was used as the reference for determining NDF for this investigation. The Ankom FBT procedure has previously been found not to be statistically different from the Van Soest technique for NDF in a variety of forages and cereals (Komarek et al. 1994). The utility of Near Infra Red (NIR) technology for estimating various chemical fractions and energy content in grains and forages was highlighted in recent reviews by Wrigley (1999), and Kitessa et al. (1999). Previous work in evaluating wet chemistry techniques for suitability in indicating feed and malting quality (Crosbie and Portmann, 1977), reported a significant correlation ( $r=0.65$ ,  $p<0.01$ ) between malt extract and NDF, independent of barley nitrogen. The aim of the current study was to investigate whether NDF could be predicted sufficiently well in barley by NIR spectroscopy and to determine its suitability as a selection tool in a breeding program for malting quality.

## **Materials and Methods**

### *Samples*

197 barley samples comprising 76 varieties and cultivars were selected from 13 Western Australian trial sites from the 1996 and 1997 seasons. The samples were cleaned and sieved over a 2.2mm screen prior to spectral scanning and analysis.

### *NIR scanning*

A NIRSystems 6500 scanning monochromator instrument (Foss-NIRSystems, Silver Spring, Maryland, USA) fitted with a sample transport mechanism was used to collect spectral data. Spectral analysis and calibration development was carried out using Foss-NIRSystems WinISI II v1.02a software package. Wholegrain samples were scanned in a coarse sample cell with a single up/down transport system pass, 32 scans per sample and duplicate repacks. The same samples were subsequently milled on a Cyclotec 1093 Mill (Foss-Tecator, Hoganas, Sweden) fitted with a 1.0mm screen and scanned in duplicate using the small ring cups. NIR reflectance spectral data was collected as  $\log(1/R)$  over the range 400nm-2498nm at 2nm

intervals for both sample presentations. Duplicate scans were averaged prior to spectral analysis and calibrations.

#### *Reference analysis*

NDF was determined on ground samples in duplicate using an Ankom 220 Fiber Analyzer (Ankom Technology, Fairport, NY) using a 75 minute digestion protocol with heat stable  $\alpha$ -amylase treatment. The filter bag technique (FBT) allows each sample to be digested and filtered simultaneously during reflux in a batch process with significant time savings over conventional techniques. Crude protein (N x 6.25) was determined on the ground barley samples in duplicate using the Dumas combustion method on a LECO FP2000 (LECO Corporation, Michigan, USA). Hot water extract (HWE) was determined in duplicate only on 1997 season samples (n=163). Micromalting was carried out in a Joe White Maltings micromalter. Both procedures were carried out to standard methods (Harasymow and Tarr, 1998). Moisture content was determined by EBC method 3.1 and all results were reported on a dry matter basis.

#### *Spectral analysis and calibration*

Averaged spectra from both sample presentations were ordered using the *Create Score File From Spectra File* program to establish population boundaries within a maximum standardised Mahalanobis ( $H$ ) distance of 3.0 from the average spectrum using PCA. The spectra were sorted by increasing  $H$  distance and apportioned into a test set and validation set by storing every fifth sample (n=39) for validation with the remainder (n=158) used as the full calibration set. This method was reported as ensuring equal representation in both sets and equitable cross validation splits (Shenk and Westerhaus, 1991). As an aid to determining similar or redundant samples in the full calibration set, the *Select Samples From a Spectral File* program was used with a cut-off standardised  $H$  distance of 0.6 between nearest neighbours to select a subset of samples from the full calibration set. Conventional stepwise multiple linear regression (SWMLR) and modified partial least squares (MPLS) techniques were evaluated for NDF calibration performance with full and reduced calibration sets. For MPLS, calibrations were produced with a maximum of two outlier passes and five cross validation segments and validated with the reserved validation set. The SWMLR technique was evaluated with a maximum of two outlier passes and validated with the reserved validation set. Standard normal variate (SNV) and detrend were applied to remove scatter effects and mathematical treatments (derivative, segment, smooth) of (1,4,4), (1,10,10), (2,4,4) and (2,10,10) were evaluated on both SWMLR and MPLS techniques. Wholegrain calibrations were developed utilising the spectral range 800nm-2498nm,4 and 1100nm-2498nm,4 for ground grain sets, where 4 indicates every fourth wavelength was used in the range.

#### *Statistical evaluation*

Statistical results were evaluated with JMP ver 3.1 (SAS Institute Inc., CT, USA).

## **Results and Discussion**

The correlation and statistical data for the study population is presented in tables 1 and 2. Significant ( $p<0.001$ ) correlations were found between NDF/HWE supporting the previous findings of Crosbie and Portmann (1977). A non-significant correlation between NDF/protein in this set of material indicates that NDF may have potential as a selective criterion of barley malting quality.

**Table 1. Correlations of population variables.**

<i>r</i>	<i>Protein</i>	<i>NDF</i>	<i>HWE</i>
Protein	1.00		
NDF	0.12 NS	1.00	
HWE	-0.44***	-0.46***	1.00

NDF - neutral detergent fibre.

HWE - hot water extract.

NS - not significant.

\*\*\* -  $p < 0.001$ .

**Table 2. Statistical data for reference values.**

	Protein	NDF	HWE
min	7.7	11.5	77.0
max	16.0	24.5	85.9
mean	10.5	15.6	81.8
sd	1.51	1.71	1.90
n	197	197	163
sdd lab	0.10	0.76	0.38

NDF - neutral detergent fibre.

HWE - hot water extract.

sdd lab - reference laboratory error.

The results of MPLS and SWMLR calibrations on wholegrain and ground grain sample sets are presented in tables 3 and 4. For MPLS the number of factors producing the lowest average SECV was used to develop the final equation. For SWMLR the optimum number of wavelengths was determined by the F statistic. Calibrations using different math treatments were attempted on both full calibration sets and reduced (0.6 *H* cut-off) sets to determine if sufficient variability could be modelled by a smaller calibration set (Shenk and Westerhaus, 1991). The 0.6*H* cut-off sets produced similar errors to full set calibrations. In general no single math treatment produced the best calibration and there was little difference in standard error between MPLS and SWMLR techniques for this data set. However, the ground grain calibration SEV is 35% lower than that found with the wholegrain calibration. The range in NDF for the calibrations may be a contributing factor to the calibration correlation coefficients.

**Table 3. MPLS statistics for NDF**

<b>Whole grain</b>	<b>Math</b>	<b>PC</b>	<b>SEC</b>	<b>r<sup>2</sup></b>	<b>SECV</b>	<b>SEV</b>
full set (n=158)	1,4,4	7	0.928	0.529	1.083	1.359
	1,10,10	1	1.286	0.265	1.336	1.337
	2,4,4	2	1.162	0.308	1.189	1.330
	2,10,10	4	1.079	0.404	1.176	1.378
0.6 <i>H</i> (n=75)	1,4,4	2	1.076	0.432	1.082	1.358
	1,10,10	2	1.082	0.426	1.089	1.358
	2,4,4	2	1.076	0.432	1.095	1.334
	2,10,10	3	0.985	0.524	1.087	1.369
<b>Ground grain</b>	<b>Math</b>	<b>PC</b>	<b>SEC</b>	<b>r<sup>2</sup></b>	<b>SECV</b>	<b>SEV</b>
full set (n=158)	1,4,4	4	0.947	0.567	0.994	0.900
	1,10,10	4	0.959	0.556	1.002	0.909
	2,4,4	3	0.957	0.573	1.043	0.807
	2,10,10	4	0.959	0.571	1.016	0.832
0.6 <i>H</i> (n=91)	1,4,4	3	1.049	0.541	1.149	0.898
	1,10,10	3	1.060	0.531	1.163	0.907
	2,4,4	3	0.993	0.588	1.137	0.819
	2,10,10	3	1.028	0.559	1.135	0.845

SEC - standard error of calibration.

r<sup>2</sup> - correlation coefficient.

SECV - standard error of cross validation.

SEV - standard error of validation (reserved set).

Math - derivative, segment, smooth.

PC - number of principal components.

**Table 4.** SWMLR statistics for NDF

<b>Whole grain</b>	<b>Math</b>	<b><math>\lambda</math></b>	<b>SEC</b>	<b><math>r^2</math></b>	<b>SEV</b>
full set (n=158)	1,4,4	3	1.083	0.381	1.332
	1,10,10	2	1.121	0.336	1.419
	2,4,4	3	1.069	0.397	1.412
	2,10,10	4	1.025	0.445	1.230
0.6 H (n=75)	1,4,4	2	0.916	0.576	1.514
	1,10,10	1	1.079	0.411	1.339
	2,4,4	4	0.869	0.647	1.433
	2,10,10	3	0.904	0.618	1.261
<b>Ground grain</b>	<b>Math</b>	<b><math>\lambda</math></b>	<b>SEC</b>	<b><math>r^2</math></b>	<b>SEV</b>
full set (n=158)	1,4,4	3	0.926	0.579	0.917
	1,10,10	2	0.979	0.536	0.907
	2,4,4	3	0.922	0.594	0.835
	2,10,10	3	0.991	0.542	0.880
0.6 H (n=91)	1,4,4	2	1.077	0.521	0.865
	1,10,10	2	1.059	0.537	0.880
	2,4,4	7	0.797	0.735	0.919
	2,10,10	3	1.050	0.566	0.935

SEC - standard error of calibration.

$r^2$  - correlation coefficient.

SEV - standard error of validation (reserved set).

Math - derivative, segment, smooth.

$\lambda$  - number of wavelength terms.

The calibration equation using the 2,4,4 math treatment was selected to test against the reserved validation sample set. Table 5 shows the multiple regression model results for NDF prediction. There is a significant linear relationship between ground grain predicted NDF and actual NDF, and is not significantly influenced by protein. The relationship has a coefficient of determination  $r^2 = 0.704$  for ground grain and  $r^2 = 0.202$  for wholegrain.

**Table 5.** Statistical evaluation of significance of NDF prediction.

Ground Grain #				Whole Grain #			
Term	Estimate	Std Error	Prob> t	Term	Estimate	Std Error	Prob> t
Intercept	7.029	1.335	0.000	Intercept	10.843	1.555	0.000
Protein	-0.057	0.069	0.416 NS	Protein	0.112	0.083	0.185 NS
NDF	0.593	0.066	0.000 ***	NDF	0.219	0.077	0.007 **

# - full calibration set, math treatment 2,4,4 validated against reserved set.

NDF – neutral detergent fibre.

NS – not significant.

\*\*\* -  $p < 0.001$ .

\*\* -  $p < 0.01$ .

## Conclusion

In this investigation, NDF has been shown to have a significant correlation with HWE, and low correlation with protein. NDF can be predicted by NIR in barley significantly better on ground grain than on wholegrain. Calibration technique and mathematical treatment produced little difference in standard error, although the correlation coefficients could be improved by extending the range of NDF. Statistical evaluation of performance on an

independent test set showed that NDF could be predicted independent of protein. Its utility to be used as a screening indicator in malting barley quality selection would seem to be feasible. Further work is required to fully exploit its potential.

### **Acknowledgments**

Financial support from the Grains Research and Development Corporation and staff assistance from Agriculture Western Australia Malting Barley Quality Laboratory, Biometrics and Barley Breeding is gratefully acknowledged.

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