



Utilising Near Infrared Spectroscopy for Predicting Malting Quality in Whole Grain Barley and Whole Grain Malt

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Introduction

Near infra-red reflectance (NIR) technology is a non-destructive, cost effective and rapid tool for simultaneous prediction of multiple constituents in agricultural products. Australian barley breeding programs are utilising NIR technology to streamline the selection of new cultivars with improved malting barley (Nilsen and Panozzo, 1995; Roumeliotis *et al.*, 2000). Constituents of particular interest to the malting and brewing industry include hot water extract, diastatic power, free α -amino nitrogen (FAAN), soluble protein, wort β -glucan and β -glucanase.

After harvest barley requires approximately 2 months of post-harvest maturation to break dormancy and allow the assessment of malting quality constituents. Post-harvest dormancy in barley primarily contributes to delays in providing quality data to the breeder for the selection of superior malting quality breeding lines before the next sowing season.

For the past 3 years, NIR calibrations developed on whole grain barley have enabled the Victorian Institute for Dryland Agriculture (VIDA) barley breeding program to characterise the malting quality of early generation breeding lines. More recently calibrations for whole grain malt have been developed and introduced to aid in selecting malted samples for more complex malting quality traits.

Therefore, the aim of this study was to determine where calibrations developed for predicting malting quality in whole grain barley and whole grain malt can be applied to a barley breeding program.

Materials and Methods

Barley samples

The samples used in this study were from the barley breeding trials at VIDA. The samples represented a range of breeding generations grown at various sites throughout Victoria in 1999. The breeding generations included: early generation (F₄ and F₅), the first two years of field trials; F₇, the third year of field trials; F₈, the fourth year of field trials; and F₉, the fifth year of field trials. The barley samples were passed over a 2.2mm sieve prior to NIR-testing and analysis.

Laboratory reference methods

The barley samples were malted according to Nilsen and Panozzo (1995) and analysed for hot water extract according to a small-scale version of the European Brewing Convention (EBC) fine grind method, with a grist:liquor ratio of 10g:70mL (Macleod *et al.*, 1991). Malt samples were ground on a FN3100 Mill (Falling Number, Stockholm, Sweden) to pass a 0.8mm sieve and analysed for diastatic power (Fox *et al.*, 1999) and β -glucanase according to the Megazyme method (Megazyme, Australia). The wort was analysed for: FAAN using the ninhydrin method (Institute of Brewing, 1982); soluble protein using the Dumas combustion method on a CNS-2000 (LECO Corporation, St Joseph, MI, USA); and wort β -glucan using the Megazyme method (Megazyme Australia).

NIR analysis

The reflectance spectra, Log (1/R), were collected on a Model 6500 monochromator (NIRSystems, Silver Springs, MD) equipped with transport module and a standard coarse NIR sample cell. Spectra were recorded across a range of 400-2498 nm and 2 nm wavelength increment. Diffuse reflectance readings of a ceramic tile were referenced before and after the sample scan. The barley and malt samples were equilibrated to 21°C for 24 hours prior to analysis.

The spectra were corrected for scatter with standard normal variance (SNV) and detrending. A second derivative mathematical treatment was applied with gap and smooth sizes of 5 and 5, respectively.

The spectral population was structured with three Global H units. The calibration set was optimised with Neighbourhood H units of 0.3. Calibrations were developed by using a modified partial least squares (mPLS) algorithm and cross-validation technique. ISI software V 4.01 (Infrasoft International, Silver Springs, MD) was used for all data processing.

A population of 131 barley samples from separate trials of the 1999 harvest was selected for validating the performance of developed calibrations.

Results and Discussion

The NIR calibration and validation statistics for each constituent are presented in Table 1.

Table 1. Statistical data of NIR calibration and validation samples for malt quality constituents of whole grain barley and malt

Quality Parameter	Calibration Set					Validation Set			
	N ^a	Range	No. of Terms	R ² ^b	SEC ^c	N ^a	Range	R ² ^b	SEV ^d
Whole Grain Barley Set									
Hot Water Extract (%)	277	77-87	7	0.87	0.6	131	76-81	0.78	1.1
Diastatic Power (WKE)	279	179-549	5	0.57	45	131	225-545	0.39	57
FAAN (mg/L)	263	92-228	5	0.54	16	131	116-239	0.10	31
Soluble Protein (%)	201	4-6	5	0.60	0.2	131	4-6	0.01	0.5
Wort β -Glucan (mg/L)	267	0-1089	9	0.77	104	131	0-760	0.25	240
β -Glucanase (units/kg)	276	297-868	7	0.60	66	131	322-788	0.02	135
Whole Grain Malt Set									
Hot Water Extract (%)	276	77-87	7	0.89	0.6	131	76-81	0.76	1.0
Diastatic Power (WKE)	279	179-549	8	0.75	35	131	225-545	0.54	54
FAAN (mg/L)	268	92-228	9	0.89	8	131	116-239	0.63	17
Soluble Protein (%)	203	4-6	9	0.79	0.2	131	4-6	0.53	0.3
Wort β -Glucan (mg/L)	268	0-1089	9	0.83	93	131	0-760	0.51	165
β -Glucanase (units/kg)	271	297-868	8	0.70	56	131	322-788	0.47	97

^a Number of Samples

^b Coefficient of determination

^c Standard error of calibration

^d Standard error of validation

The NIR predicted hot water extract values of the calibration samples were highly correlated for both whole grain barley ($R^2=0.87$) and whole grain malt ($R^2=0.89$) to the laboratory reference data and were associated with low standard errors of calibration, 0.6 and 0.6, respectively. The validation data for hot water extract reflected those of the calibration samples, displaying good correlations and low

standard errors (Table 1). The data in this study are in agreement with findings of other researchers and this constituent has been routinely analysed by NIR on whole grain barley to aid in the screening of early generation lines in southern Australian barley breeding programs (Nilsen and Panozzo, 1995; Roumeliotis *et al.*, 2000).

The NIR predicted values for diastatic power were moderately correlated to the laboratory reference values ($R^2=0.57$) for calibrations developed for whole grain barley (Table 1). Validation statistics for diastatic power indicated a weaker relationship between the NIR predicted values and the reference data than was observed for hot water extract (Table 1). However, the size of the standard error of validation suggested that NIR analysis could be useful for classification of early generation barley cultivars into high or low groups for diastatic power. In contrast, a higher correlation was observed between NIR predicted values and laboratory method for diastatic power in whole grain malt ($R^2=0.75$). Calibrations developed on whole grain malt are currently used for the evaluation of diastatic power in the breeder's lines that are retained after applying whole barley calibrations (Figure 1).

Calibrations for soluble protein, FAAN, wort β -glucan and β -glucanase, developed on whole grain barley, displayed a reasonably good relationship between the NIR predicted and laboratory reference values (Table 1). This relationship was not confirmed by statistics observed for the validation samples (Table 1), possibly due to the complex nature of these constituents. The unmalted barley contains proteins and starches in the storage form. The composition of barley is modified by the action of enzymes throughout the steeping and germination stages and by heating during the kilning stage of the malting process.

Calibrations developed on whole grain malt for soluble protein, FAAN, wort β -glucan and β -glucanase had high values of the coefficient of determination ($R^2>0.70$) and were associated with low standard errors of calibration (Table 1). A combination of relatively high coefficients of determination and low standard errors for the validation samples indicated that the calibrations developed for these complex constituents on whole grain malt are suitable for the evaluation of F_4 to F_8 generation barley lines retained for malting in a breeding program (Figure 1).

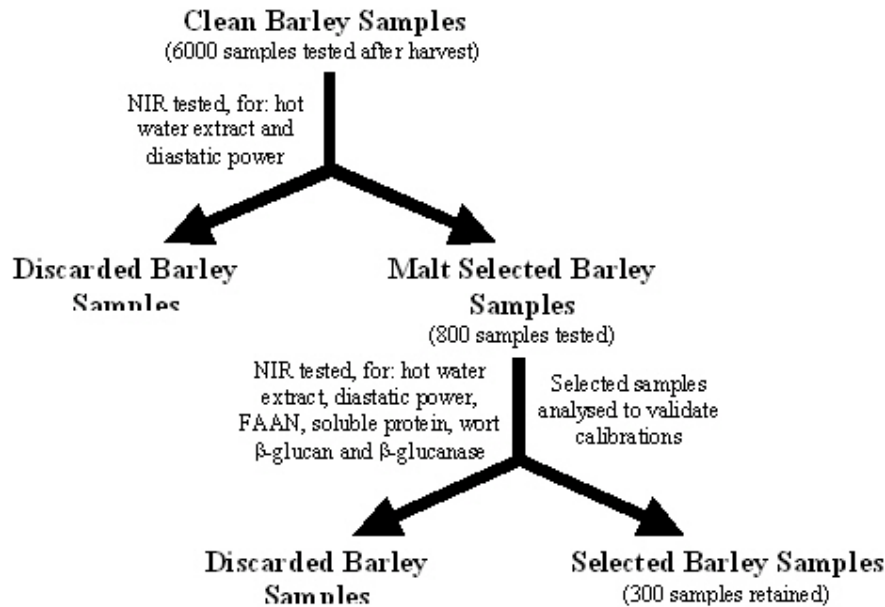


Figure 1. Protocol for the application of NIR testing to early generation samples (F₄ and F₅) of the VIDA barley breeding program

A two-step operation was employed for the analysis of barley breeding sample by NIR (Figure 1). The calibrations developed on whole grain barley for hot water extract and diastatic power were used to categorise early generation barley samples into low-high groups for the two quality constituents. In a typical year approximately 80% of the barley lines are discarded each year by this protocol. The selected samples of interest were malted and predicted for the constituents, hot water extract, diastatic power, wort β -glucan, FAAN, soluble protein and β -glucanase. Of these barley lines that contain the desired malting quality characteristics, approximately 4-5% of early generation barley breeding lines, are then advanced to the next generation of the breeding program.

Conclusion

NIR calibrations developed on whole grain barley for hot water extract display similar precision and accuracy as calibrations developed on malt. The validation data for diastatic power indicated a weaker relationship between NIR predicted values and reference values, but acceptable for classifying samples into low-high groups. The validation data of soluble protein, FAAN, wort β -glucan and β -glucanase demonstrated that the calibrations developed on whole grain barley could not be utilised in the selection of these constituents. These constituents are analysed in malted barley and it is difficult to develop NIR calibration for unmalted barley seed.

Calibrations developed for hot water extract, diastatic power, soluble protein, FAAN, wort β -glucan and β -glucanase on malted barley are suitable for the evaluation of F₄ to F₈ generation barley lines from a breeding program, although there is the added expense of micro-malting the samples.

After evaluating early generation barley lines, a large percentage of the population can be discarded using NIR testing protocols. The two-step protocol includes first applying hot water extract and diastatic power calibrations on whole grain barley, then malting the retained samples and applying malting quality calibrations on whole grain malt. This is an advantage to a barley breeding program, where the gain is in the reduced number of samples going through the lengthy process of micro-malting and laboratory analysis.

Acknowledgments

Thanks to staff of the Grains Chemistry Laboratory at VIDA - Horsham for their contribution. We acknowledge the financial support of the Malting Barley Quality Improvement Program (MBQIP), the Victorian Department of Natural Resources and Environment (NRE) and the Grains Research and Development Corporation (GRDC).

References

1. Fox, G., Logue, S., Harasymow, S., Taylor, H., Ratcliffe, M., Roumeliotis, S., Onley, K., Tansing, P., Ferguson, R., Glennie-Holmes, M., Inkerman, A., Tarr, A., Evans, B., Panozzo, J., Osman, A. and Smith, A. (1999) In: The Proc. 9th Barley Tech. Sym., Melbourne, Australia, p. 2.35.1-2.35.5.
 2. Institute of Brewing, Recommended Methods of Analysis. IOB, London (1982).
 3. Macleod, L.C., Dowling, M.A., Sparrow, D.H.B. and Lance, R.C.M. (1991) In: The Proc. 5th Barley Tech. Sym., Australia, p. 125-128.
 4. Nilsen, M. and Panozzo, J. (1995) In: *Leaping ahead with Near Infrared Spectroscopy*, Ed by Batten, G.D., Flinn, P.C., Welsh, L.A. and Blakeney, A.B. NIR Spectroscopy Group, Australia, p. 174-177.
 5. Roumeliotis, S., Logue, S.J., Jefferies, S.P. and Barr, A.R. (2000) In: *Near Infrared Spectroscopy: Proceedings of the 9th International Conference*, NIR Publications, UK, p. 673-678.
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