Near infrared spectroscopy for selection of malting barley in South African breeding programmes

Evette Roux[1], Cushla McGoverin[1], Glen Fox[2] and Marena Manley[1]*

[1]Department of Food Science, Stellenbosch University, Stellenbosch 7602, South Africa
[2]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Australia
*Email: mman@sun.ac.za

Introduction

After wheat, barley is the most important small grain in South Africa[1] and is used mostly for the production of malt.[2,3] Barley malt is a basic raw material in the brewing process and the quality of the malt is related to certain properties of the raw barley grain.[4-6] Breeding of malting barley cultivars involves the evaluation of large numbers of samples and requires a rapid, non-destructive analytical method that can be applied to small sample sizes. Micro-malting can be used as an indication of malting behavior but this technique is destructive, requires large sample sizes and experienced personnel.[7] The ability to predict barley quality for malting purposes in early generations would allow selection of suitable lines to deliver malt of the highest quality. Although numerous reports regarding NIR studies on malting barley exist, no information was found for barley in a South African breeding programme. The aim of this study was to develop NIR calibration models for the prediction of malt quality properties (extract, total nitrogen (TN), total soluble nitrogen (TSN), free amino nitrogen (FAN) and diastatic power (DP)), from whole grain barley over two consecutive harvest seasons.

Materials and Methods

Samples and reference data

Samples were obtained from the South African Barley Breeding Institute (SABBI) 2008 and 2009 breeding trials; these included samples grown under irrigation (5 localities) and dry land (7 localities) conditions (**Table 1**). Sample replicates within localities from the 2008 season were bulked before micro-malting and reference testing whereas locality replicates from the 2009 season were malted individually. Samples were malted on a small scale in Seeger, Joe White or Phoenix micro-malting machines. The steep cycle was carried out with 9 hrs steeping at 15˚C, 14 hrs air rest at 17˚C, 14 hrs steeping at 15˚C and 6 hrs air rest at 17˚C followed by two germinations; 24 hrs at 19˚C and 72 hrs at 17˚C. The kilning stage was 14 hrs at 65˚C followed by 4 hrs at 80˚C. Malt was cooled down to 30˚C after which reference data were collected for extract, TN, TSN, FAN and DP according to SAB Maltings in-house methods.[8]

Table 1. Samples obtained for the respective areas from the two harvest seasons.

Sample type	2008			2009		
	Total sample set	Calibration set	Validation set	Total sample set	Calibration set	Validation set
Dry land	139	95	44	193	131	62
Irrigation	99	68	31	91	64	27

Near infrared spectroscopy (Spectral data collection)

Spectra of whole grain barley (both seasons and sample types) were collected using a NIRLab N-200 spectrophotometer (Büchi, Flawil, Switzerland) from 1000-2500 nm as averages of 32 scans at a resolution of 16 cm^{-1}.

NIR analysis (Data analysis)

PLS regression models were developed with The Unscrambler (Version 9.2; CAMO, Oslo, Norway) data analysis software. Samples were split into calibration and test sets by selecting every third value from a list of ascending values for each property (Table 1). Outliers were identified and removed and various pre-processing techniques were applied and evaluated; these included no spectral pre-treatment, mean normalisation, standard normal variate (SNV), 1st derivative Savitzky-Golay (9 points), 2nd derivative Savitzky-Golay (17 points), 1st derivative (9 points) and SNV, 2nd derivative (17 points) and SNV. The accuracy of each calibration model was determined from the standard error of prediction (SEP), the coefficient of determination (r^2) and the ratio of the SEP to the standard deviation of the validation set (RPD) with the aim of obtaining the lowest SEP with the highest r^2 and RPD values.[9]

Reference paper as:
E. Roux, C. McGoverin, G. Fox and M. Manley (2012).Near infrared spectroscopy for selection of malting barley in South African breeding programmes, in: Proceedings of the15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 368-371.

Results and Discussion

Calibration and validation results for the 2008 and 2009 harvest seasons are summarised in Tables 2 and 3 respectively.

Table 2. Calibration and validation statistics for 2008 samples.

	Property	Sample range	Pre-treatment	PLS	Calibration set		Validation set		
					R^2	SEC	r^2	SEP	RPD
Dry land	Extract (%)	78.4-83.4	2nd der	5	0.58	0.79	0.56	0.73	1.46
	TN (%)	1-2.05	2nd der	6	0.85	0.10	0.77	0.11	2.10
	TSN (%)	0.45-0.95	2nd der	8	0.87	0.04	0.55	0.07	1.47
	FAN (mg/L)	107-286	None	6	0.21	33.41	0.36	26.26	1.25
	DP (W.K.)	170-635	2nd der	4	0.65	0.80	0.57	69.68	1.50
Irrigation	Extract (%)	77.6-83.6	2nd der	1	0.05	1.20	0.34	0.87	1.12
	TN (%)	1.28-2.08	2nd der	5	0.68	0.10	0.27	0.15	1.06
	TSN (%)	0.46-0.96	None	1	0.11	0.11	0.18	0.10	0.97
	FAN (mg/L)	99-252	None	2	0.10	35.05	0.36	30.87	1.07
	DP (W.K.)	170-554	None	2	0.02	84.30	0.38	71.90	1.06

TN=total nitrogen; TSN=total soluble nitrogen; FAN=free amino nitrogen; DP=diastatic power; R^2=coefficient of determination for calibration; PLS=number of partial least square factors; SEC=standard error of calibration; r^2=coefficient of determination for validation; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; 2nd der=second derivative Savitzky-Golay, 17 points; none=no spectral pre-treatment

Table 3. Calibration and validation statistics for 2009 samples.

	Property	Sample range	Pre-treatment	PLS	Calibration set		Validation set		
					R^2	SEC	r^2	SEP	RPD
Dry land	Extract (%)	75.4-84.9	None	10	0.51	1.22	0.53	1.12	1.57
	TN (%)	1.04-2.57	SNV	9	0.82	0.13	0.82	0.14	2.19
	TSN (%)	0.49-1.26	2nd der +SNV	4	0.54	0.11	0.59	0.09	1.52
	FAN (mg/L)	75-406	1st der	5	0.30	41.4	0.26	39.1	1.22
	DP (W.K.)	122-742	SNV	7	0.61	74.74	0.56	82.45	1.45
Irrigation	Extract (%)	72.4-83.5	1st der +SNV	10	0.94	0.26	0.47	0.73	1.88
	TN (%)	1.28-2.2	Mean norm	9	0.87	0.08	0.85	0.07	2.89
	TSN (%)	0.6-1.02	SNV	6	0.50	0.07	0.46	0.07	1.28
	FAN (mg/L)	138-260	None	8	0.45	21.82	0.25	20.18	1.36
	DP (W.K.)	341-714	SNV	10	0.72	49.79	0.25	75.15	2.27

TN=total nitrogen; TSN=total soluble nitrogen; FAN=free amino nitrogen; DP=diastatic power; R^2=coefficient of determination for calibration; PLS=number of partial least square factors; SEC=standard error of calibration; r^2=coefficient of determination for validation; SEP=standard error of prediction; RPD= Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; None=no spectral pre-treatment; SNV=standard normal variate; 2nd der=second derivative Savitzky-Golay, 17 points; 1st der=first derivative, 9 points; Mean norm=mean normalisation

Extract

Similar extract prediction results were obtained for dry land samples for both seasons (r^2 = 0.56 - 0.53) and were acceptable for screening purposes. Better results were obtained for the 2009 irrigation samples than the 2008 samples, probably due to the wider range in reference values obtained for the former. However, irrigation results were not acceptable for screening purposes in either harvest season. Results from this study did not compare well with that of previous researchers who developed promising calibrations for predicting the extract of whole grain barley (r^2 = 0.78 - 0.85).[10-12] This property is influenced by the malting process since enzyme activity during malting influences the malt extract; this therefore limits the accuracy of any NIR prediction based on unmalted barley.[13]

TN

For both the dry land and irrigation areas, more accurate TN prediction results were obtained for the 2009 season, although a greater improvement was observed for the irrigation areas in 2009 (r^2 = 0.85) compared to 2008 (r^2 = 0.27). Prediction results for the 2008 irrigation samples were not acceptable for screening purposes. Dry land results (r^2 = 0.77 - 0.82) from both seasons were acceptable for screening purposes. The prediction of nitrogen content from whole grain barley is well-established in the literature and the results from this study compared well with those of previous reports (r^2 = 0.71, r^2 = 0.83)[10,11] although some workers were able to develop excellent calibration models for whole grain barley (r^2 = 0.94 and r^2 = 0.95).[14-15]

TSN

For the irrigation samples, more accurate prediction results were observed for the 2009 season although a larger sample range was obtained for the 2008 samples (0.46% - 0.96%) compared to the 2009 samples (0.60% - 1.02%). Irrigation sample results were, however, not acceptable for screening purposes in either

Reference paper as:
E. Roux, C. McGoverin, G. Fox and M. Manley (2012).Near infrared spectroscopy for selection of malting barley in South African breeding programmes, in: Proceedings of the15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 368-371.

season (2008: r^2 = 0.18; 2009: r^2 = 0.46). Similar results were observed for dry land samples over both seasons (r^2 = 0.55 - 0.59) which were acceptable for screening purposes. These predictions were an improvement on those reported in the literature where a very poor model (r^2 = 0.01) was obtained when TSN was predicted from whole grain barley.[12]

FAN

Less accurate prediction results were observed for the 2009 season (dry land r^2 = 0.26; irrigation r^2 = 0.25), compared to the 2008 season (dry land r^2 = 0.36; irrigation r^2 = 0.36). These results were not acceptable for screening purposes. Worse results for FAN prediction from whole grain barley (r^2 = 0.10)[12] have been reported and were attributed to the complex nature of this constituent within unmalted barley. The smaller sample range used[12] may also have resulted in poor prediction of this property.

DP

Poor results were observed for both seasons when predicting DP from irrigation samples. Only the dry land results (r^2 = 0.56 - 0.57) were acceptable for screening purposes. The smaller DP ranges of the irrigation samples compared to those of the dry land samples may have influenced calibration accuracy. Acceptable calibrations (r^2 = 0.59) for predicting DP from whole grain barley have been reported in the literature.[11] A very poor calibration was also reported (r^2 = 0.39) for DP prediction from whole grain barley;[12] the small sample range used by these researchers resulted in poor prediction. Poor results can also be attributed to the inability of the NIR method to account for the complex interactions of barley endosperm substrates and enzymes during malting and the resulting extent of endosperm modification[11,13]

Conclusion

Predictions for the 2009 irrigation samples proved to be more acceptable than predictions for 2008 irrigation samples for extract, TN and TSN. Calibration results for the 2008 and 2009 dry land areas were similar for all properties. Sample bulking may have had an effect on calibration results for irrigation samples but not for dry land samples. The 2008 sample replicates were bulked before micro-malting but the three samples were scanned separately, resulting in three spectra with an averaged malt quality reference value for all malt properties. The reference values of the respective malt properties were therefore not representative of the specific sample spectra that were recorded and it was expected that this would influence calibration accuracy. The standard error of laboratory (SEL) for the micro-malting technique could not be obtained in this study and therefore there is no knowledge on the precision of the reference methods compared to that of the NIR method. Although NIR prediction of malt properties from whole grain barley cannot account for enzyme action during malting, the technique shows potential to be used as a screening method in earlier generations.

Acknowledgements

The authors wish to thank Francois Smit and Francois Potgieter at the South African Barley Breeding Institute for supplying barley samples and South African Breweries Maltings, Caledon, for micro-malting and reference data determinations. We also thank the Winter Cereal Trust for funding and study grant (ER).

References

1. G.J. Kotze, *Guidelines for the production of malting barley: winter dry land 2009*. SAB Maltings, Kimberley, South Africa. (2009).
2. J.M. Poehlman, "Adaptation and distribution", in *Barley*, Ed by D.C. Rasmusson, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, pp 1-17 (1985).
3. S.E. Ullrich, "Genetics and breeding of barley quality attributes", in *Barley Science: Recent advances from molecular biology to agronomy of yield and quality*, Ed by G.A. Slafer, J.L. Molina-Cano, R. Savin, J.L. Araus and I. Romagosa, The Haworth Press, Binghamton, New York, pp. 115-142 (2002).
4. W.O.S. Meredith, J. A. Anderson.and L. E. Hudson, "Evaluation of malting barley", in *Barley and Malt: Biology, Biochemistry, Technology*, Ed by A.H. Cook, Academic Press, Inc., London, pp. 207-269 (1962).
5. Burger, W.C. and LaBerge, D.E. "Malting and brewing quality" in *Barley*, Ed by D.C. Rasmusson, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin. pp. 367-401 (1985).
6. R. Savin and J.L. Molina-Cano, "Changes in malting quality and its determinants in response to abiotic stresses" in *Barley Science: Recent advances from molecular biology to agronomy of yield and quality*, Ed by G.A. Slafer, J.L. Molina-Cano, R. Savin, J.L. Araus and I. Romagosa, The Haworth Press, Binghamton, New York, pp. 523-549 (2002).

Reference paper as:
E. Roux, C. McGoverin, G. Fox and M. Manley (2012).Near infrared spectroscopy for selection of malting barley in South African breeding programmes, in: Proceedings of the15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 368-371.

7. F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009.
8. H. van Wyk, South African Breweries Maltings (SABM), Caledon, South Africa, Personal communication, 2009.
9. P.C. Williams, "Implementation of near-infrared technology", in *Near-Infrared Technology in the Agricultural and Food Industries*, Ed by P.C. Williams and K.H. Norris, The American Association of Cereal Chemists Inc., St. Paul, Minnesota, USA, pp. 145-169 (2001).
10. S.A. Halsey, *J. Inst. Brew.* **93**, 461-464 (1987).
11. Y. Li, G. Laycock and W. Fernets, "Rapid assessment of potential malting quality of barley by near infrared diffuse reflectance spectroscopy", in *Near Infrared Spectroscopy: The Future waves*, Ed. A.M.C. Davies and P.C. Williams, NIR Publications, Chichester, UK, pp. 475-478 (1995).
12. C. Black and J. F. Panozzo, *Proceedings of the 10th Australian Barley Technical Symposium, Canberra, Australia,* (2001).
13. R.J. Henry, *J. Sci. Food Agric.* **36**, 249-254 (1985).
14. M.J. Edney, J.E. Morgan, P.C. Williams, and L.D. Campbell, *J. Near Infrared Spectrosc.* **2**, 33-41 (1994).
15. M. Sohn, D.S. Himmelsbach, F.E. Barton, C.A. Griffey, W. Brooks and K.B. Hicks, *Appl. Spectrosc.* **62**, 427-432 (2008).

Reference paper as:
E. Roux, C. McGoverin, G. Fox and M. Manley (2012).Near infrared spectroscopy for selection of malting barley in South African breeding programmes, in: Proceedings of the15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 368-371.