HGCA

PROJECT REPORT No. 28

NEAR INFA-RED SPECTROSCOPY FOR IDENTIFYING MALTING QUALITY

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Near infra-red spectroscopy for identifying malting quality

by

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Final report of a three year project which commenced in May 1987. The studies were carried out in the Malt and Wort Production Section of The Brewing Research Foundation, Nutfield, Surrey and were supported by a grant of £136,664 from the Home-Grown Cereals Authority (Project No: 0029/1/87).

OVERALL SUMMARY OF PROJECT 0029/1/87

Objectives

The objective of this project was to investigate the use of Near Infra-Red (NIR) spectroscopy of whole grain barley to predict the concentrations of components which will be present after malting and thus to assess the malting quality of that barley.

Areas of investigation

The measurement of components by NIR relies on correlations between the sample spectra and the reference analysis. These correlations will hold for future samples only if all possible sources of variation are present in the calibration set. To achieve this a large number of samples, including different varieties, geographical sites and harvest years, were used to develop calibrations.

The diffuse reflectance spectrum of each sample was recorded using a Perstorp Analytical 6250 scanning spectrophotometer. All samples were then malted, using a standard malting regime, and analysed by recommended Institute of Brewing methods.

Calibrations have been developed for several varietal subsets, from a single variety to a range of malting and feed varieties. Winter and spring barleys were analysed separately.

Within each subset the samples were divided into those used for calibration and those used for testing the equations generated.

The spectra were derivatised, to aid peak resolution and reduce interferences, such as those caused by particle size effects. Multiple linear regression analysis was then carried out on the calibration samples. This technique identifies groups of wavelengths which correlate well with the reference data and incorporates them into predictive equations. The analytical performance of the equations was then determined by testing them on the prediction sets.

Key findings

1. Hot Water Extract (HWE), Total Nitrogen (TN), and Total Soluble Nitrogen (TSN) of the malt can be predicted from scans of whole grain barley. Once calibrations have been developed, approximately 10-12 samples/hour can be analysed for all 3 parameters.

2. Calibrations developed using several malting varieties had similar levels of error to those developed using a single variety. It is not, therefore, necessary to develop equations for each individual variety.

3. Calibrations developed using malting and feed barleys are accurate enough to provide a useful screening tool, for example, to help selection during barley breeding

programmes. Spring barleys from the 1987 harvest, which were judged acceptable for malting on the basis of reference HWE values, were correctly identified by NIR in 89% of cases. Similarly, 81% of those unacceptable for malting were correctly identified.

4. In most cases equations can be used, with similar levels of accuracy, for samples from new seasons. Combining data from more than one harvest sometimes resulted in more accurate equations.

5. Calibrations for combined malting and feed winter barleys are less stable to seasonal variations than are spring barley calibrations and combining data from more than one harvest was unsuccessful.

Terminal Report

1. Objective

The overall objective of this project was to investigate the use of Near Infra Red (NIR) spectroscopy of whole grain barley to predict the concentrations of components which will be present after malting and mashing, and thus to assess the malting quality of that barley.

2. Introduction

The prediction of components by NIR relies on correlations between the spectral and reference (wet chemical) data. To maximise the stability of these correlations a large number of samples, encompassing as many sources of variation as possible, must be used to develop the calibration.

NIR is simple and rapid to perform. Multi-parameter analysis of a sample takes about one minute. It is also a non-destructive technique. It can, therefore, be used in many situations where conventional analysis is not feasable due to limitations of time or available sample.

This project was aimed at producing two types of calibrations. The first would allow maltsters to check malting quality of barley at intake. For this application only malting grade varieties need to be considered. Wider calibrations, which include feed grade barleys, could be used as a screening tool by plant breeders.

3. Materials and Methods

3.1 Barley Samples

A wide range of barley samples were collected from the 1987, 1988 and 1989 harvests (Table 1). These included malting and feed grade barleys grown at sites in the UK from Scotland to Dorset. A large number of different spring and winter varieties, with a wide range of nitrogen contents, are represented. These samples were selected to include any variation due to climate, geographical location and agronomy.

3.2 Near Infra Red Spectroscopy

A Perstorp Analytical 6250 spectrophotometer, interfaced with an IBM PS2/50 computer, was used for this project. The barley was presented to the instrument as whole grain contained in a large sample cell. The cell has a capacity of approximately 80g of barley although scans can be obtained with as little as 20g. The reflectance spectrum of each sample was recorded, in duplicate, by averaging 50 scans from 1100nm to 2500nm at 2nm intervals.

The spectra were derivatised before regression. Derivatisation increased peak resolution and helped to overcome the heterogeneous nature of the sample such as differences in corn sizes.

3.3 Malting and Malt Analysis

All barley samples were cleaned, screened and, if necessary, dried. Only samples with viability (by a peroxide test) over 95% and dormancy (by a 4ml germination test according to the IOB Recommended Methods) under 10% were malted. All samples were malted with the following steeping, germination and drying regime:

Steeping	-	16°C
		7 hours wet
		17 hours air rest
		7 hours wet
		17 hours air rest
		1 hour wet
Germination	-	4 days at 16°C
Drying	-	8 hours at 45°C
	-	16 hours at 65°C

The resultant malts were analysed for TN, TSN and HWE (coarse grind) by the Recommended Methods of the Institute of Brewing. Moisture was also determined and the results are reported on a dry weight basis.

3.4 Statistical Manipulation

It has been shown that there was no advantage in producing separate calibrations for each barley variety (HGCA Annual Report, 1989) and single variety calibrations have, therefore, not been considered further.

The selection of training sets is an important step in the development of calibrations. These sets must have a uniform distribution over the entire range of all the constituents under investigation. The samples must be chosen to minimise any correlations between constituents. When such inter-correlations exist the measurement of one constituent can be affected by the concentration of others.

It proved impossible to fulfill these requirements for all constituents in a single set of data. Separate training sets were therefore selected for TN, HWE and TSN.

Calibration and prediction sets were chosen for malting varieties and for malting and feed varieties. For each of these groupings calibration and prediction sets were chosen for three seasonal subsets. Sets of data were also selected to assess the seasonal stability of the calibrations. The training sets used are listed in Appendix 1. Statistical summaries of the reference data for each of the training sets are presented in Appendix 2.

Regression was carried out against first and second order derivatives of the calibration spectra. Variation in the optical data at each wavelength are compared with variations in the reference data. Sets of wavelengths which correlate well with the reference data are incorporated into calibration equations. These equations are used to predict the constituent data of the prediction set. The predicted values are compared with the measured values to assess the performance of the calibration. The standard error of prediction (SEP) is the deviation of the differences between the two methods and it represents a typical discrepancy from the fitted line.

Seasonal stability was assessed by determining the predictive capability of the wavelengths in each equation for samples from subsequent harvests. This allows the calibration constants to be altered independantly to obtain the correct slope of the regression line for the new samples.

4. Results

NIR is a secondary technique and relies on calibration against reference data. It can, therefore, never be more accurate then the wet chemical methods used in the calibration process. In this project the SEPs have been compared with the reproducibility of the IOB Recommended Methods.

The accuracy of the wet chemical methods are quoted in the IOB Recommended Methods as R_{95} values for malt analysis. These figures do not take into account any of the variability introduced during malting. More realistic estimates of the combined errors of micro-malting and malt analysis were calculated from a limited number of trials where the same barley was malted and analysed by a number of different laboratories. Both sets of R_{95} values are given in Appendix 3. The figures in parenthesis are $R_{95}/2.8$, the value which may be compared directly with the standard errors of the NIR method.

Regression results for calibrations developed using malting barley are presented in Appendix 4, Tables 1 to 6. Tables 7 to 12 give regression results for calibrations developed using malting and feed barleys. The figures in bold type are for the equations which gave the lowest SEP for each of the seasonal subsets. Where different calibrations performed optimally on the various seasonal groups it may be possible to compromise and select a single calibration which performed well on all three harvests. For example SHWE1 performs almost as well as SHWE2 on

1988 samples. SHWE2 could therefore be discarded, reducing the number of calibrations to be monitored.

In all cases more accurate and stable calibrations were achieved using the second derivative of the spectral data. A segment size of 20 and gap size of 0 was used. The wavelengths used in the calibrations are presented in Appendix 5.

To ensure stable calibrations and minimise the effects of inter-correlations it was important that the wavelengths used could be understood in terms of known absorbances of the constituents. For example, 1690nm is a well documented area of protein absorbance and wavelengths close to this peak provided the primary correlation in many of the TN calibrations. This peak also appeared in some of the TSN and HWE equations, due to the effect of the TN content on these constituents. Carbohydrate absorbances are obviously important in HWE calibrations and these have been used. For example, absorbances at 2284nm and 1160nm were due to starch.

5. Discussion

5.1 Accuracy of NIR Predictions

5.1(a) Malting only calibrations

The prediction errors of calibrations for TSN and HWE in malts prepared from malting barleys are only slightly higher than the errors of the manual methods. Comparison of, for example, the R_{95} and equivalent SE for malting and malt analysis for HWE (Appendix 3) with the SE of prediction from NIR (bold figures in Appendix 4) support this. The SE for the reference method is 2.76 l°/kg and for NIR, based on the 1987 calibration set, prediction errors are 3.22, 3.13 and 3.52 l°/kg for 1987, 1988 and 1989 barleys, respectively (Appendix 4, Table 1). This loss of accuracy may be acceptable when the relative process time for each method is taken into account. Analysis by NIR takes about 1 minute compared with a week or more for conventional micro-malting and malt analysis.

In contrast, the prediction of malt TN by NIR was significantly less accurate than its manual measurement. The R_{95} and SE for TN values involving malting and malt analysis were 0.14% and 0.049%, respectively (Appendix 3). Data in Appendix 4 indicates that the SE of prediction by NIR was always much greater, being 0.0804%, 0.0634% and 0.0771% for 1987, 1988 and 1989, respectively (Appendix 4, Table 1, figures in bold), using the 1987 calibration equation. These differences probably reflect the relatively low errors in the wet chemical determination of this parameter. The need to predict malt TN from scans of malting grade barleys is, however, questionable. Malting barley is priced on the basis of a Kjeldahl determination of barley TN and this generally falls by less than 0.1% on malting. Calibrations set up to measure barley TN are likely to be more accurate than those predicting malt TN.

In spring barleys all constituents could be predicted, after slope adjustment, in 1988 and 1989 samples using calibrations developed from the 1987 harvest. Calibrations developed using the combined data from 1987 and 1988 did not result in lower prediction errors for the 1989 samples. This indicated that there was sufficient variability in the 1987 data and seasonal variability was not required. The equations produced using data from all three harvests were considerably less accurate than single season calibrations. This may be due to the unusually hot, dry summer in 1989 and particularly to the high nitrogen levels which resulted from this.

In winter barleys the SEPs for all constituents increased slightly when single seasons calibrations were used for samples from subsequent harvests. The errors for 1988 samples, especially that for HWE (Appendix 4, Table 4), may still be low enough for the equations to be useful. For all constituents the accuracy is further reduced for 1989 samples. Except in the case of TSN, the prediction errors for 1989 samples were reduced when the two season calibration was used. There was no improvement in the SEP when data from all three seasons was combined. The winter malting calibrations appear, therefore, to be slightly less stable than the corresponding equations for spring barleys. Combining data from more than one season increased the robustness of the calibrations to some extent but, again, the 1989 samples do not fit in well with the data from previous harvests.

5.1 (b) Malting and Feed Calibrations

The prediction errors for TSN and HWE generally increase when feed varieties are included in the calibrations, particularly for winter barleys (cf Appendix 4, Table 4 and Table 10). This was anticipated as the concentration of soluble components present after malting and mashing are dependent not only on the gross chemical constituents of the barley but also on physiological factors. These factors, such as the rate of water uptake and distribution and the synthesis of enzymes during germination, cannot be measured directly by NIR spectroscopy of the resting grain. They are, however, reflected in other variety dependent characteristics such as grain size and morphology which do affect the NIR spectra.

It is probable that calibrations containing both malting and feed varieties would only be used where there is no prior knowledge of variety, such as in plant breeding programmes, and a lower level of accuracy would be acceptable in these circumstances. The technique would then be used as a screening tool to differentiate between potential malting quality cultivars and feed grade barleys.

For this application the prediction of malt TN may be of more importance when it can be used to 'standardise' the other constituents. Malting barley is grown under relatively low nitrogen conditions to maximise the extract and HWE values are inversely proportional to the total nitrogen content of the grain. During barley breeding programmes higher nitrogen regimes may be used to maximise the yield of seed for the next generation. To allow an assessment of the likely extract of these samples when grown under commercial agronomic conditions and to compare samples grown under different nitrogen regimes, their HWE values can be corrected to a standard TN. Similarly, Soluble Nitrogen Ratio (SNR = TSN/TN x 100%) is often used as a quality parameter, for malts. The TN prediction errors were lower for malting and feed calibrations than for malting only calibrations (Appendix 4) and although they were still relatively high when compared with the reference method (Appendix 3), they may be low enough for screening purposes.

Single season calibrations for TN in spring barleys performed well on both 1988 and 1989 data (Appendix 4, Table 1). For TSN and HWE the errors were reasonable for 1988 samples but increased somewhat for 1989 data (Appendix 4, Table 1). Prediction errors for 1989 barleys were slightly lower when the two season calibration was used (Appendix 4, Table 2) but equations developed using the combined data from all three harvest had relatively high SEP values (Appendix 4, Table 3).

The increased prediction errors obtained with 1989 samples may have been due to the atypical nature of those barleys as a consequence of the extremely dry growing season.

The prediction errors for the winter malting and feed subset (Appendix 4, Tables 10-12) were consistently higher than the corresponding spring barley calibrations (Appendix 4, Tables 7-9) and this varietal group also showed poorer seasonal stability. Prediction errors were high when single season calibrations were used for samples from the 1988 and 1989 harvests (Appendix 4, Table 10) and even using the two season calibration to predict samples from the 1989 harvest (Appendix 4, Table 11) resulted in only a slight reduction in SEP. Again calibrations developed using data from all three harvests had relatively high prediction errors (Appendix 4, Table 12).

The poorer performance of calibrations for this varietal subset may have been due to the inclusion of both two and six row barleys, all other groups containing only two row varieties. The winter barley sets also contained samples with blue aleurone layers and this too may have had an effect on the spectra produced. Even at this lower level of accuracy the calibrations may still be seen as being suitable for using NIR as a rough screening tool.

5.2 Applications of NIR

5.2(a) Malting only Calibrations

NIR predictions can never be more accurate than the wet chemical methods used in developing the calibrations. Where very accurate determinations are required, for example, as a basis for commercial transactions, or in cases of dispute, maltsters will continue to use micromalting and malt analysis for the forseeable future. The use of NIR, however, could lead to a reduction in the amount of wet chemistry required. For instance, if a maltster had a choice of several barleys with similar TN values then NIR could be used to help identify particularly good samples which may be purchased and particularly bad samples which may be rejected. Only the intermediate samples need then be analysed by conventional methods. The efficiency of this application was tested as follows. Values for HWE, both laboratory results and from NIR prediction, were plotted against barley TN. The regression line and SE of wet chemical HWE against barley TN were then calculated and the values used to divide the plot into areas as shown in Figure 1. The maltster could adjust the limits set for different situations. Using these limits, areas A, B and C ,might be considered acceptable for malting with areas D and E being unacceptable. Alternatively samples in area A might be those requiring wet chemical analysis as they are neither particularly good or very poor. Obviously, NIR prediction of HWE would not be the maltster's only selection tool. For example, samples in area C would still be rejected if they had very high TN values.

Figure 1 is an example of this type of application, using data for 1987 harvest Triumph. To simplify the plot 32 samples which both wet chemistry and NIR placed in area A have been omitted. In this case 80% of samples in area A, B and C were correctly identified by NIR and all 16 samples with relatively low HWE, ie. in areas D and E were correctly placed by NIR. If the maltster micromalted only those samples in area A the wet chemistry required would be reduced by 37%.

Similar results, not shown here, were obtained for all varietal and seasonal groups. For example, in 1988 winter barleys 81% of above average and 83% of below average samples were identified. In no case were areas C and E confused.

5.2 (b) <u>Malting and Feed Calibrations</u>

Calibrations developed using both malting and feed grade barleys would allow plant breeders to use NIR as a screening tool. Using conventional micro-malting and malt analysis HWE can generally be determined at the fourth generation and TSN at the sixth.

Earlier determinations can be made as soon as 10g of barley are available for analysis but the number of samples involved make this impractical. As NIR is non-destructive and rapid it could be used to determine TN, TSN and HWE at the third generation. Barley breeders could therefore select for malting quality at a much earlier stage in the breeding programme.

The efficiency of screening by NIR was assessed as follows. All of the samples obtained during the three years of the project were divided into sets deemed to be acceptable or unacceptable on the basis of either wet chemistry HWE or SNR. Samples with HWE> 300 1°/kg or $35 \geq SNR \leq 42$ were said to be acceptable for malting in this exercise but these limits are arbitrary and could be altered to suit the application. The HWE and SNR values for each group of samples were predicted by the relevant calibration and the number of samples correctly identified as belonging to each of the groups was counted. These results, expressed as percentages are plotted in Figures 2 and 3.

The HWE calibrations performed well for spring barleys over all seasons. A total of 90% of acceptable and 80% of unacceptable samples were correctly identified. The segregation of samples on the basis of SNR is slightly poorer as the errors of both the TN and TSN determination are included. Even with only 60 to 70% of samples correclty assigned, however, the technique would still be a useful screening tool. Only 31% of 1989 samples acceptable for malting on the basis of SNR were correctly predicted. This was partly due to the slightly lower accuracy of the 1989 TSN calibration. There were very few samples from the 1989 which had acceptable SNR values and those which had were close to the lower limit. This also contributed to the low identification of acceptable samples.

In general, the calibrations for winter barleys did not perform as well as those for springs in predicting acceptability for malting. This is a reflection of their higher SEP values. The poorer seasonal stability of these calibrations is also seen in Figure 3.

During the early stages of breeding programmes large numbers of potential new cultivars must be discarded. Under these circumstances it is important that the low quality samples are identified so that they can be removed. The calibrations perform well in this respect in selecting those barleys deemed unacceptable for malting. For example, in 1988 winter barleys only 36% of acceptable barleys were identified but 97% of those unacceptable were correctly identified.

6. Conclusions

Near infra red spectroscopy of whole grain barley can provide a useful prediction of malt TN, TSN and HWE.

Calibrations developed using several malting varieties had similar levels of error to those developed using a single variety. It is not, therefore, necessary to develop equations for each individual variety. These calibrations could be used by maltsters to compare the quality of samples at intake and to reduce the amount wet chemistry required.

Calibrations developed using malting and feed barleys are accurate enough to be useful as a screening tool, for example, to help selecting potential cultivars of malting quality during barley breeding programmes.

In most cases equations can be used, with similar levels of accuracy, for samples from new seasons.

Combining data from more than one season sometimes resulted in a more accurate equation. Calibrations for combined malting and feed winter barleys are less accurate and less stable to seasonal variations than the other varietal groups. They are, however, still accurate enough to provide a useful screening tool.

Table Legend

Table 1 Barley samples from the 1987, 1988 and 1989 harvests. Samples were obtained from all barley growing areas in the UK and their constituent ranges are detailed in Appendix 2.

Figure Legends

Figure 1 Correlation between HWE of malts prepared from Triumph barleys of different TN values from the 1987 harvest.

- line of best fit through all data (32 samples have been omitted from Area A for clarity). Area A covers values ± 1 standard error of the mean (SEM); Area B values + 1-2 SEM; Area C values >+2 SEM; Area D values -1-2 SEM; Area E values > -2 SEM.

Figure 2 Prediction of acceptability or unacceptability of spring barleys for malting. Cut-off points for HWE and SNR were chosen arbitrarily and can be altered.

Figure 3 Prediction of acceptability or unacceptability of winter barleys for malting. Cut-off points for HWE and SNR were chosen arbitrarily and can be altered.

Table 1: Barley samples from the 1987, 1988 and 1989 harvest

a)1987	Location	No of Sites	No of Varieties	No of Samples
-Winters	Scotland	3	16	33
	England	1	1	100
	England	14	2	45
	England	4	21	84
Springs	Scotland	5	13	65
	England	14	3	67
	England	3	5	150
b)1988				
Winters	Scotland	9	3	21
	England	1	12	160
	England	12	23	78
Springs	Scotland	3	12	50
	Scotland	10	3	24
	England	12	17	58
	England	4	5	15
c)1989				
Winters	Scotland	3	14	42
	England	2	31	53
	England	1	4	18
Springs	England	3	12	17
	England	1	4	30
	England	3	8	25
	England	1	4	18

Figure 1: Correlation between malt HWE and barley TN 1987 Triumph

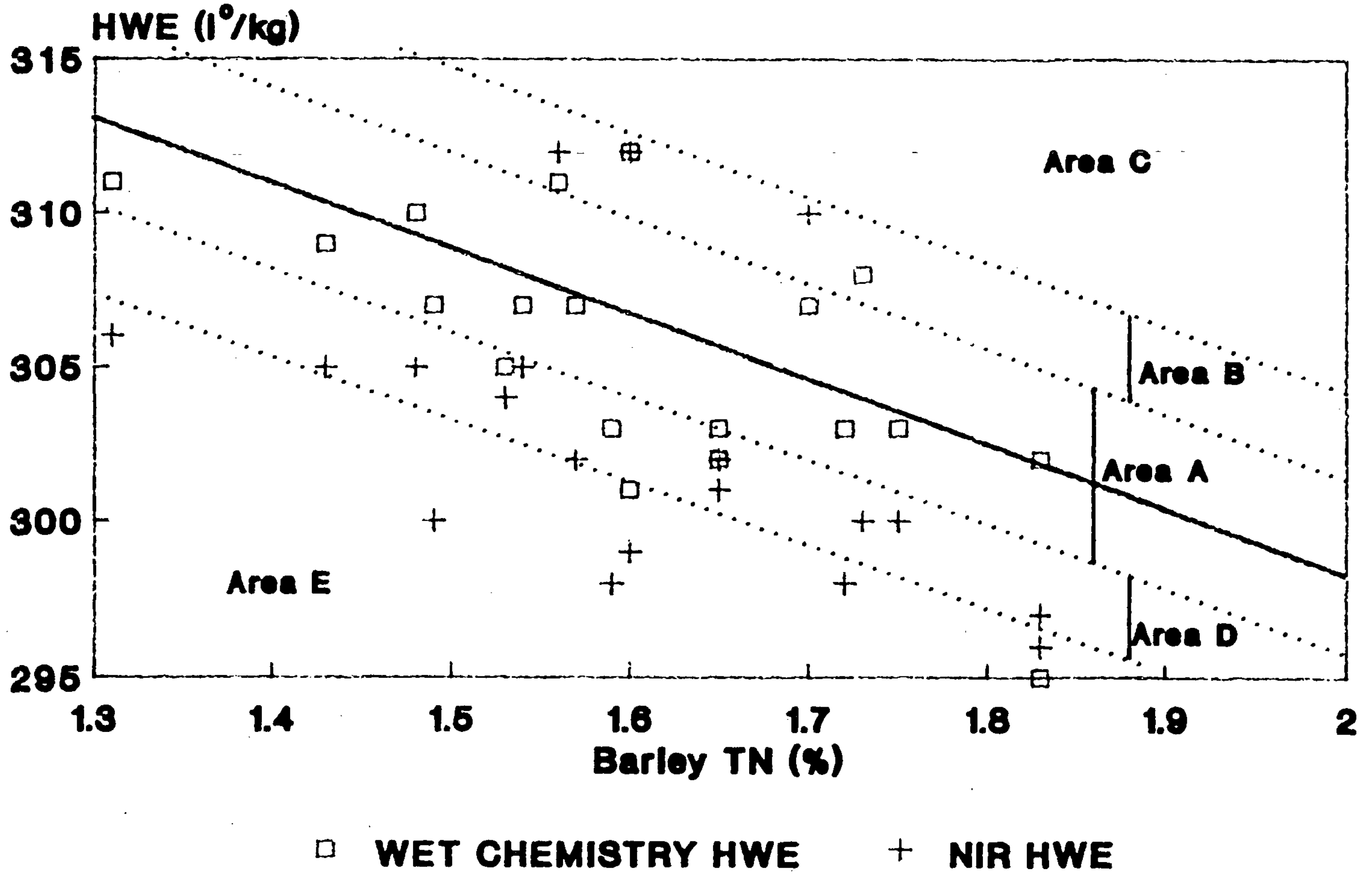


Figure 2: Prediction of malting quality

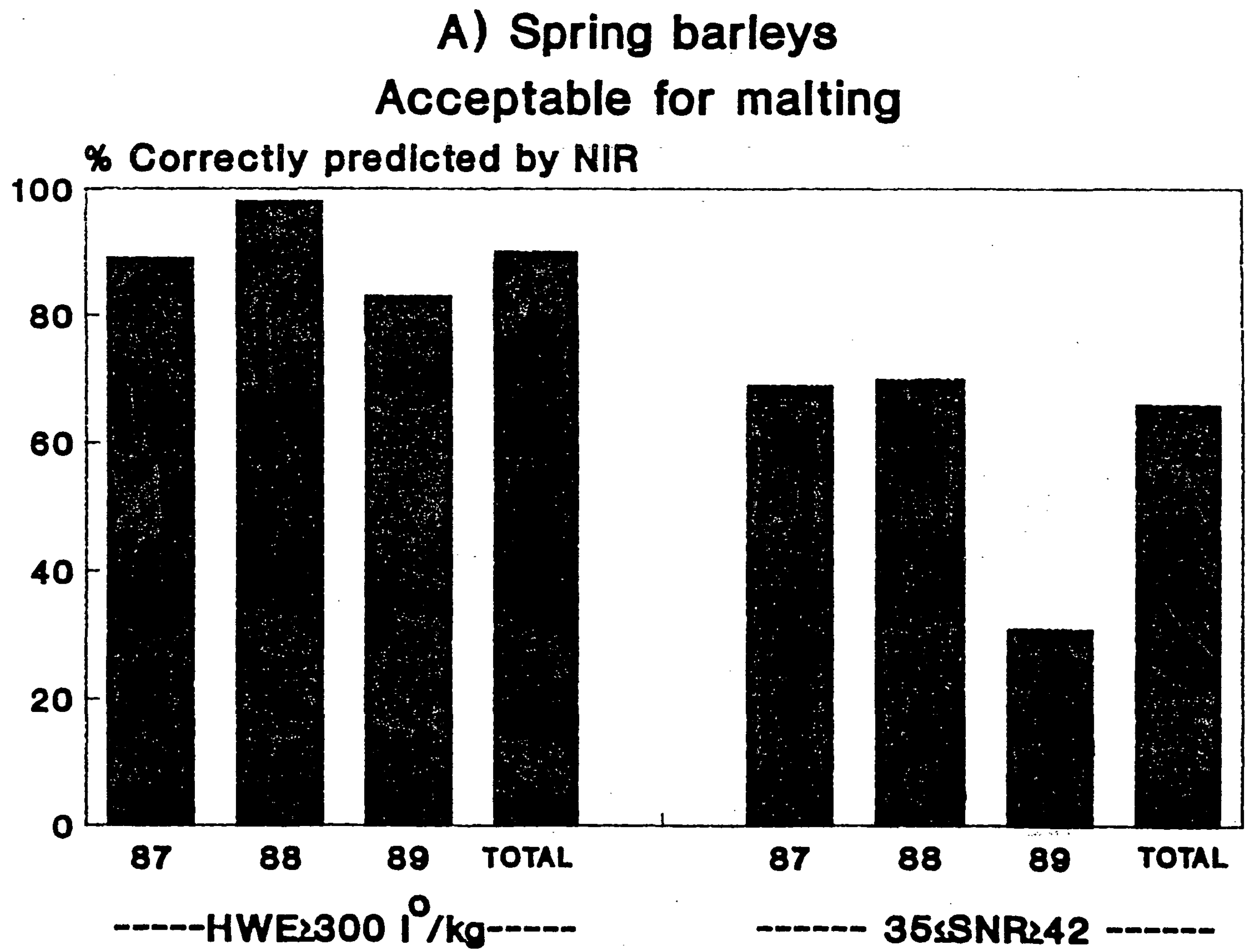
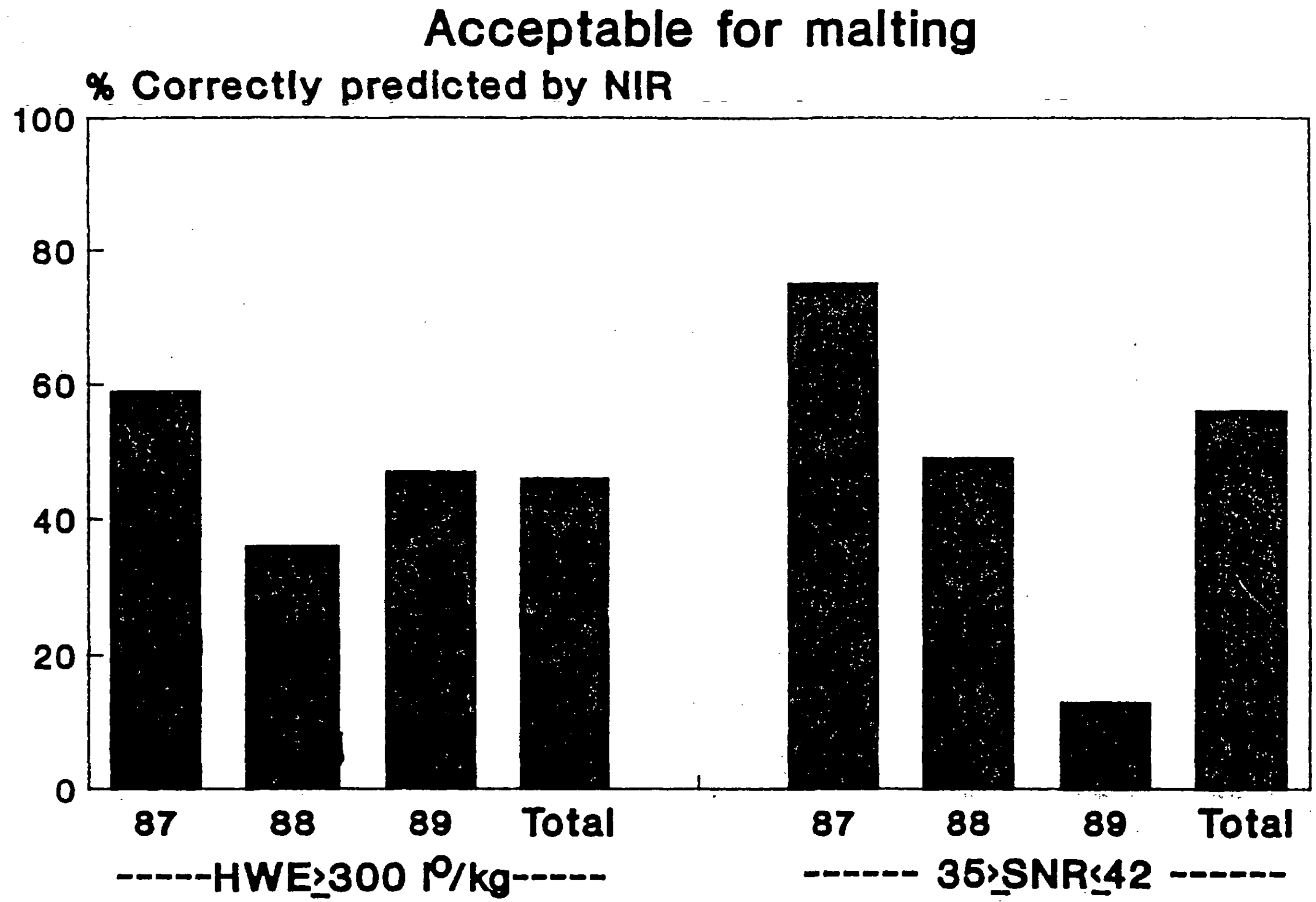


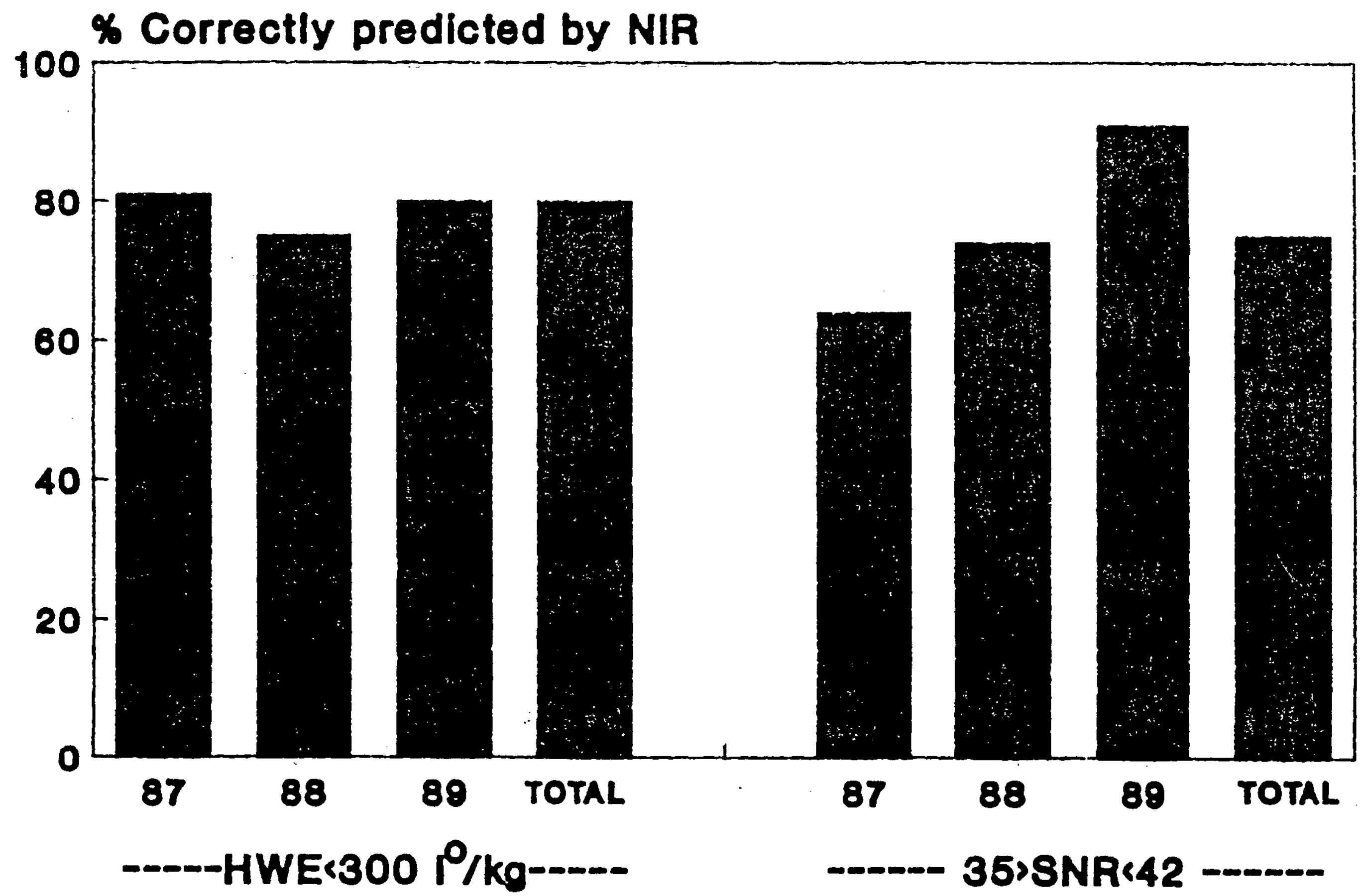
Figure 3: Prediction of malting quality

A) Winter barleys



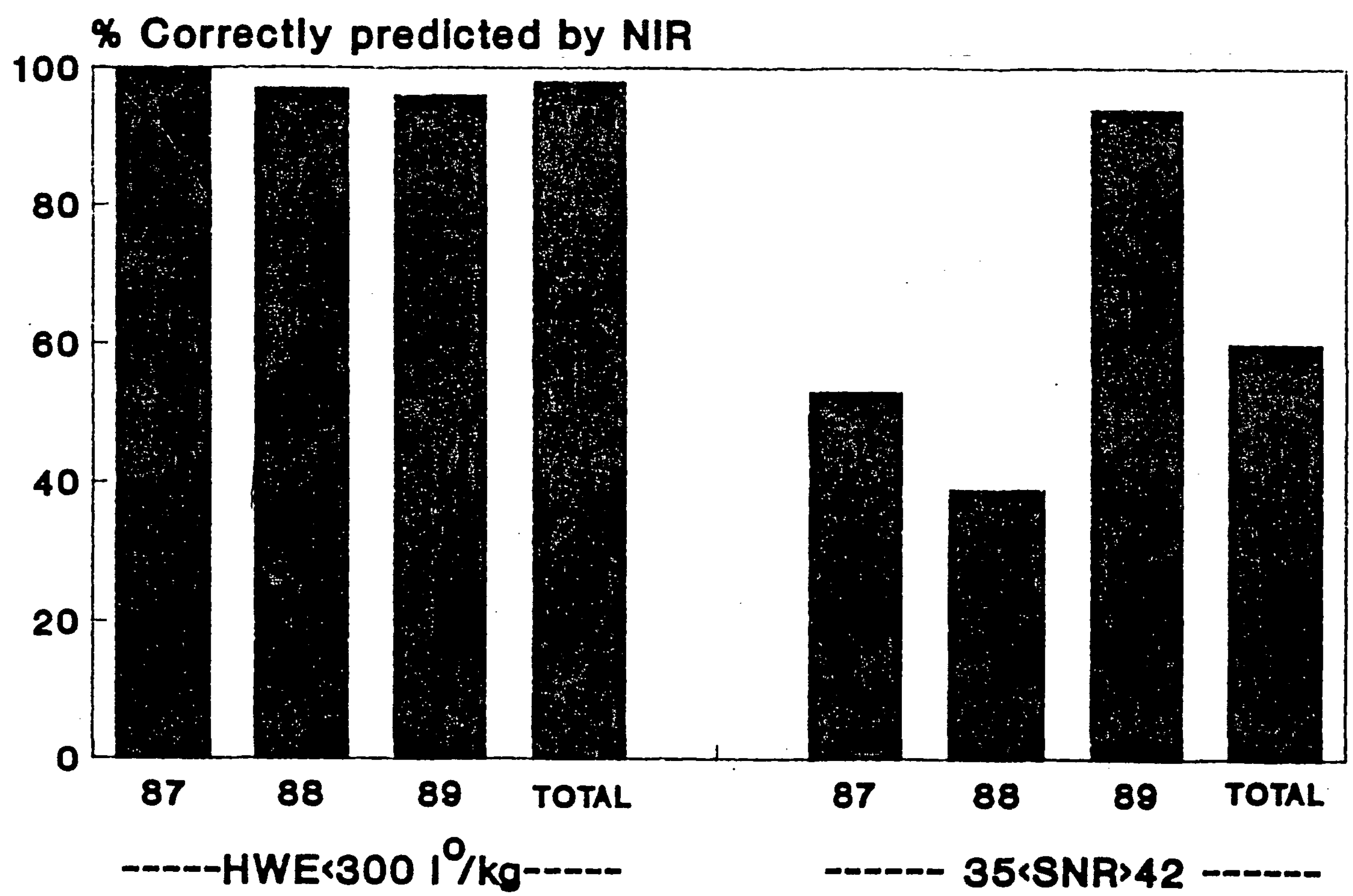
B) Spring barleys

Unacceptable for malting



B) Winter barleys

Unacceptable for mallting



Appendix 1

TRAINING SETS

VARIETAL SUBSETS

Calibration and prediction sets

(Magie)

Winter malting

Winter malting and feed

Spring malting

Spring malting and feed

SEASONAL SUBSETS

Calibration and prediction sets

Single season - samples from the 1987 harvest

Two seasons - samples from the 1987 and 1988 harvests

Three seasons - samples from the 1987, 1988 and 1989 harvests.

Seasonal stability sets

1988 data - to test single season calibrations

1989 data - to test single season and two season calibrations.

Appendix 2

STATISTICAL SUMMARY OF REFERENCE DATA FOR THE TRAINING SETS

Values for each range are: TN - % (w/w)

TSN - % (w/w);

HWE - 1°/kg

The mean value for each range and the standard deviation from the mean (SD) are also tabulated for each training set.

Appendix 2

TABLE 1: SPRING MALTING BARLEY TRAINING SETS

TRAINING SET	RANGE	MEAN	SD
TN			
1987 Calibration	1.27-1.90	1.60	0.170
1987 Prediction	1.32-1.81	1.58	0.133
1988 Prediction	1.27-1.83	1.51	0.164
1989 Prediction(A)	1.29-1.82	1.56	0.133
1989 Prediction(B)	1.26-1.82	1.55	0.141
2 Season Calibration	1.07-1.90	1.53	0.225
2 Season Prediction	1.13-1.86	1.53	0.187
3 Season Calibration	1.07-2.53	1.66	0.341
3 Season Prediction	1.13-2.42	1.57	0.233

1989 Prediction Set (A) was used for STN3 (see Appendix 5).
1989 Prediction Set (B) was used for STN4 and STN5(see Appendix 5).

TRAINING SET	RANGE	MEAN	SD
TSN			
1987 Calibration	0.51-0.81	0.65	0.083
1987 Prediction	0.53-0.78	0.65	0.069
1988 Prediction	0.51-0.80	0.63	0.077
1989 Prediction	0.55-0.77	0.66	0.055
2 Season Calibration	0.51-0.81	0.65	0.083
2 Season Prediction	0.52-0.80	0.65	0.077
3 Season Calibration	0.49-0.87	0.67	0.101
3 Season Prediction	0.52-0.84	0.66	0.082
HWE			
1987 Calibration	290-312	304	6.104
1987 Prediction	291-312	304	6.043
1988 Prediction	291-312	304	6.060
1989 Prediction	290-312	302	6.685
2 Season Calibration	290-318	304	7.330
2 Season Prediction	294-315	305	6.284
3 Season Calibration	285-319	302	8.988
3 Season Prediction	287-315	303	7.255

TABLE 2: WINTER MALTING BARLEY TRAINING SETS

TRAINING SET	RANGE	MEAN	SD
TN			
1987 Calibration	1.18-2.58	1.83	0.346
1987 Prediction	1.42-2.23	1.80	0.229
1988 Prediction	1.44-2.39	1.87	0.261
1989 Prediction	1.28-2.57	1.89	0.350
2 Season Calibration	1.18-2.58	1.87	0.314
2 Season Prediction	1.42-2.41	1.85	0.285
3 Season Calibration	1.18-2.58	1.88	0.367
3 Season Prediction	1.40-2.42	1.88	0.285
TSN			
1987 Calibration	0.52-0.92	0.70	0.101
1987 Prediction	0.57-0.82	0.67	0.070
1988 Prediction	0.59-0.91	0.73	0.090
1989 Prediction	0.52-0.79	0.63	0.087
2 Season Calibration	0.52-0.92	0.72	0.099
2 Season Prediction	0.58-0.90	0.70	0.080
3 Season Calibration	0.46-0.92	0.69	0.128
3 Season Prediction	0.47-0.91	0.67	0.111
HWE			
1987 Calibration	274-312	295	9.842
1987 Prediction	274-304	291	9.098
1988 Prediction	283-304	292	7.385
1989 Prediction	277-310	294	8.369
2 Season Calibration	274-312	292	9.199
2 Season Prediction	274-312	292	8.749
3 Season Calibration	274-312	292	10.171
3 Season Prediction	278-305	292	7.816

TABLE 3: SPRING MALTING AND FEED BARLEY TRAINING SETS

TRAINING SET	RANGE	MEAN	S.D.
TN			
1987 Calibration	1.27-1.90	1.60	0.166
1987 Prediction	1.32-1.83	1.58	0.135
1988 Prediction (A)	1.27-1.83	1.52	0.169
1988 Prediction (B)	1.07-1.83	1.43	0.207
1989 Prediction (A)	1.29-1.87	1.56	0.152
1989 Prediction (B)	1.26-1.87	1.55	0.160
2 Season Calibration	1.07-1.90	1.52	0.221
2 Season Prediction	1.13-1.86	1.52	0.186
3 Season Calibration	1.07-2.53	1.72	0.376
3 Season Prediction	1.13-2.44	1.65	0.293

1988 Prediction set (A) and 1989 Prediction set (A) were used for STN7 (see Appendix 5).
1988 Prediction set (B) and 1989 Prediction set (B) were used for STN8 (see Appendix 5).

TRAINING SET	RANGE	MEAN	S.D.
TSN			
1987 Calibration	0.48-0.81	0.65	0.083
1987 Prediction	0.52-0.78	0.64	0.069
1988 Prediction	0.44-0.80	0.61	0.087
1989 Prediction	0.48-0.77	0.62	0.076
2 Season Calibration	0.44-0.81	0.63	0.087
2 Season Prediction	0.48-0.80	0.63	0.079
3 Season Calibration	0.44-0.87	0.66	0.113
3 Season Prediction	0.48-0.85	0.65	0.092
HWE			
1987 Calibration	287-312	302	6.204
1987 Prediction	287-312	303	5.703
1988 Prediction	288-318	304	8.619
1989 Calibration	287-312	299	7.911
2 Season Calibration	287-318	304	7.572
2 Season Prediction	287-315	304	6.672
3 Season Calibration	271-319	297	12.122
3 Season Prediction	274-315	298	10.556

TABLE 4: WINTER MALTING AND FEED BARLEY TRAINING SETS

TRAINING SET	RANGE	MEAN	SD
TN			
1987 Calibration	1.18-2.65	1.87	0.303
1987 Prediction	1.42-2.23	1.80	0.211
1988 Prediction	1.40-2.51	1.87	0.271
1989 Prediction	1.28-2.57	1.89	0.321
2 Season Calibration	1.18-2.65	1.87	0.350
2 Season Prediction	1.40-2.42	1.84	0.243
3 Season Calibration	1.18-2.65	1.89	0.348
3 Season Prediction	1.40-2.42	1.86	0.265
TSN			
1987 Calibration	0.47-0.92	0.67	0.098
1987 Prediction	0.53-0.82	0.66	0.069
1988 Prediction	0.46-0.91	0.67	0.122
1989 Prediction (A)	0.47-0.79	0.61	0.094
1989 Prediction (B)	0.46-0.79	0.60	0.097
2 Season Calibration	0.46-0.92	0.68	0.125
2 Season Prediction	0.50-0.91	0.67	0.093
3 Season Calibration	0.37-0.92	0.64	0.152
3 Season Prediction	0.40-0.91	0.63	0.135

1989 Prediction set (A) was used for WTSN5 (see Appendix 5).
1989 Prediction set (B) was used for WTSN6 and WTSN7 (see Appendix 5).

TRAINING SET	RANGE	MEAN	SD
HWE			
1987 Calibration	265-312	290	11.456
1987 Prediction	274-304	289	8.943
1988 Prediction	265-310	287	12.035
1989 Prediction	266-310	288	11.802
2 Season Calibration	265-312	289	11.541
2 Season Prediction	265-312	289	10.424
3 Season Calibration	258-312	286	14.015
3 Season Prediction	265-312	287	12.456

Appendix 3

REPRODUCIBILITY OF REFERENCE METHODS

Constituent	R_{95}: Malt Analysis	R_{95}: Malting & Malt Analysis
TN(%)	0.083 (0.030)	0.14 (0.049)
TSN(%)	0.061 (0.022)	0.11 (0.039)
HWE (l°/kg)	3.48 (1.24)	7.73 (2.76)

Figures in parenthesis are comparable to SE (R_{95}/2.8)

Appendix 4

REGRESSION RESULTS

Tables 1-3: Spring malting barleys

Tables 4-6: Winter malting barleys

Tables 7-9: Spring malting and feed barleys

Tables 10-12 Winter malting and feed barleys

The correlation coefficient (R) indicates how closely the data fit to a straight line equation. A value of 1.0 is a perfect fit.

The standard error of prediction (SEP) indicates the predictive capability of each calibration. The SEP values can be compared with $R_{95}/2.8$ values from Appendix 3.

The wavelengths used in calibration equations are detailed in Appendix 5.

The figures in bold type are for the equations which gave the lowest SEP for each of the seasonal subsets.

Appendix 4

REGRESSION RESULTS

Tables 1-3: Spring malting barleys

Tables 4-6: Winter malting barleys

Tables 7-9: Spring malting and feed barleys

Tables 10-12 Winter malting and feed barleys

The correlation coefficient (R) indicates how closely the data fit to a straight line equation. A value of 1.0 is a perfect fit.

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The wavelengths used in calibration equations are detailed in Appendix 5.

The figures in bold type are for the equations which gave the lowest SEP for each of the seasonal subsets.

Appendix 4

TABLE 1: CALIBRATIONS DEVELOPED USING SPRING MALTING BARLEYS FROM THE 1987 HARVEST

CONSTITUENT	CALIBRATION	1987 CALIBRATION		1987 PREDICTION		1988 PREDICTION		1989 PREDICTION	
EQUATION	R	SE	R	SE	R	SE	R	SE	
TN (%)	STN1	0.877	0.0823	**0.800**	**0.0804**	0.826	0.0950	0.625	0.106
	STN2	0.885	0.0803	0.761	0.0863	**0.927**	**0.0634**	0.784	0.0846
	STN3	0.889	0.0790	0.696	0.0956	0.706	0.119	**0.824**	**0.0771**
TSN (%)	STSN1	0.761	0.0541	**0.779**	**0.0460**	0.849	0.0414	0.406	0.0511
	STSN2	0.702	0.0594	0.653	0.0519	**0.892**	**0.0353**	**0.626**	**0.0436**
HWE(1°/kg)	SHWE1	0.855	3.05	**0.770**	**3.22**	0.842	3.39	**0.859**	**3.52**
	SHWE2	0.847	3.12	0.686	3.67	**0.867**	**3.13**	0.729	4.70

TABLE 2: CALIBRATIONS DEVELOPED USING SPRING MALTING BARLEYS FROM THE 1987 AND 1988 HARVEST

CONSTITUENT	CALIBRATION EQUATION	2 SEASON CALIBRATION R	2 SEASON CALIBRATION SE	2 SEASON PREDICTION R	2 SEASON PREDICTION SE	1989 PREDICTION R	1989 PREDICTION SE
TN (%)	STN4	0.933	0.0824	**0.867**	**0.0932**	0.678	0.106
	STN5	0.927	0.0858	0.847	0.0993	**0.789**	**0.0884**
TSN (%)	STSN3	0.791	0.0510	**0.844**	**0.0414**	0.518	0.0479
	STSN4	0.722	0.0578	0.704	0.0548	**0.583**	**0.0454**
HWE (1°/kg)	SHWE3	0.882	3.47	**0.903**	**2.71**	0.744	4.55
	SHWE4	0.872	3.61	0.863	3.18	**0.772**	**4.33**

TABLE 3: CALIBRATIONS DEVELOPED USING SPRING MALTING BARLEYS FROM THE 1987, 1988 AND 1989 HARVESTS

CONSTITUENT	CALIBRATION EQUATION	3 SEASON CALIBRATION		3 SEASON PREDICTION	
		R	SE	R	SE
TN (%)	STN6	0.937	0.120	0.837	0.127
TSN (%)	STSN3	0.878	0.0494	0.748	0.0541
HWE (l°/kg)	SHWE5	0.869	4.48	0.798	4.34

Only one calibration equation was developed for each parameter

TABLE 4: CALIBRATIONS DEVELOPED USING WINTER MALTING BARLEYS FROM THE 1987 HARVEST

CONSTITUENT	CALIBRATION EQUATION	1987 CALIBRATION R	1987 CALIBRATION SE	1987 PREDICTION R	1987 PREDICTION SE	1988 PREDICTION R	1988 PREDICTION SE	1989 PREDICTION R	1989 PREDICTION SE
TN(%)	WTN1	0.959	0.101	**0.926**	**0.0868**	0.922	0.0989	0.900	0.156
	WTN2	0.941	0.119	0.923	0.0882	**0.931**	**0.0938**	0.899	0.157
	WTN3	0.965	0.0928	0.925	0.0871	0.921	0.0995	**0.919**	**0.141**
TSN (%)	WTSN1	0.858	0.0529	**0.813**	**0.0396**	0.804	0.0547	**0.762**	**0.0577**
	WTSN2	0.692	0.0774	0.679	0.0510	**0.807**	**0.0544**	0.716	0.0622
HWE (l°/kg)	WHWE1	0.908	4.21	**0.921**	**3.55**	**0.859**	**3.64**	0.735	5.77
	WHWE2	0.887	4.62	0.896	4.03	0.849	3.79	**0.838**	**4.67**

TABLE 5: CALIBRATIONS DEVELOPED USING WINTER MALTING BARLEYS FROM THE 1987 AND 1988 HARVESTS

CONSTITUENT	CALIBRATION EQUATION	2 SEASON CALIBRATION		2 SEASON PREDICTION		1989 PREDICTION	
		R	SE	R	SE	R	SE
TN(%)	WTN1	0.944	0.105	**0.939**	**0.0982**	0.900	0.156
	WTN4	0.938	0.111	0.925	0.108	**0.964**	**0.0952**
TSN (%)	WTSN3	0.861	0.0505	**0.893**	**0.0359**	0.701	0.0636
	WTSN4	0.882	0.0469	0.877	0.0382	**0.750**	**0.0589**
HWE(l°/kg)	WHWE1	0.903	3.99	**0.914**	**3.58**	0.735	5.77
	WHWE3	0.904	3.98	0.755	5.73	**0.923**	**3.29**

TABLE 6: CALIBRATIONS DEVELOPED USING WINTER MALTING BARLEYS FROM THE 1987, 1988 AND 1989 HARVESTS

CONSTITUENT	CALIBRATION EQUATION	3 SEASON CALIBRATION		3 SEASON PREDICTION	
		R	SE	R	SE
TN(%)	WTN5	0.960	0.104	0.954	0.0861
TSN (%)	WTSN1	0.814	0.0755	0.864	0.0530
HWE(l°/kg)	WHWE4	0.854	5.35	0.849	4.13

Only one calibration equation was developed for each parameter.

TABLE 7: CALIBRATIONS DEVELOPED USING SPRING MALTING AND FEED BARLEYS FROM THE 1987 HARVEST

CONSTITUENT	CALIBRATION EQUATION	1987 CALIBRATION R	1987 CALIBRATION SE	1987 PREDICTION R	1987 PREDICTION SE	1988 PREDICTION R	1988 PREDICTION SE	1989 PREDICTION R	1989 PREDICTION SE
TN(%)	STN7	0.911	0.0691	**0.862**	**0.0689**	0.882	0.0813	0.873	0.0767
	STN8	0.826	0.0947	0.787	0.0834	**0.943**	**0.0699**	**0.927**	**0.0620**
TSN(%)	STSN5	0.740	0.0560	**0.699**	**0.0492**	0.876	0.0424	0.619	0.0610
	STSN6	0.723	0.0576	0.591	0.0555	**0.904**	**0.0375**	0.406	0.0710
	STSN7	0.736	0.0564	0.642	0.0527	0.850	0.0463	**0.649**	**0.0591**
HWE(1°/kg)	SHWE6	0.807	3.69	**0.726**	**3.93**	0.921	3.42	0.748	5.32
	SHWE7	0.703	4.44	0.721	3.95	**0.960**	**2.48**	**0.823**	**4.55**

TABLE 8: CALIBRATIONS DEVELOPED USING SPRING MALTING AND FEED BARLEYS FROM THE 1987 AND 1988 HARVESTS

CONSTITUENT	CALIBRATION EQUATION	2 SEASON CALIBRATION		2 SEASON PREDICTION		1989 PREDICTION	
		R	SE	R	SE	R	SE
TN(%)	STN8	0.925	0.0849	0.889	0.0848	0.927	0.0620
TSN(%)	STSN8	0.769	0.0560	0.758	0.0511	0.566	0.0640
	STSN9	0.805	0.0520	0.668	0.0571	**0.692**	**0.0561**
HWE(1°/kg)	SHWE6	0.869	3.76	0.819	3.83	0.748	5.32

Only one calibration equation was developed for TN and HWE.

TABLE 9: CALIBRATIONS DEVELOPED USING SPRING MALTING AND FEED BARLEYS FROM THE 1987, 1988 AND 1989 HARVEST

CONSTITUENT	CALIBRATION EQUATION	3 SEASON CALIBRATION R	3 SEASON CALIBRATION SE	3 SEASON PREDICTION R	3 SEASON PREDICTION SE
TN(%)	STN9	0.951	0.117	0.923	0.113
TSN(%)	STSN10	0.814	0.0661	0.819	0.0526
HWE(1°/kg)	SHWE11	0.915	4.92	0.886	4.90

Only one calibration equation was developed for each parameter.

TABLE 10: CALIBRATIONS DEVELOPED USING WINTER MALTING AND FEED BARLEYS FROM THE 1987 HARVEST

CONSTITUENT	CALIBRATION EQUATION	1987 CALIBRATION R	1987 CALIBRATION SE	1987 PREDICTION R	1987 PREDICTION SE	1988 PREDICTION R	1988 PREDICTION SE	1989 PREDICTION R	1989 PREDICTION SE
TN(%)	WTN6	0.926	0.111	0.936	0.0738	0.907	0.115	0.927	0.122
TSN(%)	WTSN5	0.726	0.0679	**0.631**	**0.0534**	0.629	0.0959	0.398	0.0871
	WTSN6	0.629	0.0769	0.508	0.0593	**0.791**	**0.0759**	**0.603**	**0.0789**
HWE(1°/kg)	WHWE5	0.848	6.14	**0.859**	**4.58**	0.740	8.25	**0.807**	**7.12**
	WHWE6	0.781	7.23	0.763	5.71	**0.818**	**7.05**	0.767	7.75

Only one calibration equation was developed for TN.

TABLE 11: CALIBRATIONS DEVELOPED USING WINTER MALTING AND FEED BARLEYS FROM THE 1987 AND 1988 HARVESTS

CONSTITUENT	CALIBRATION EQUATION	2 SEASON CALIBRATION R	2 SEASON CALIBRATION SE	2 SEASON PREDICTION R	2 SEASON PREDICTION SE	1989 PREDICTION R	1989 PREDICTION SE
TN(%)	WTN7	0.917	0.138	0.885	0.113	0.928	0.121
TSN(%)	WTSN6	0.781	0.0789	**0.703**	**0.0656**	0.603	0.0789
	WTSN7	0.760	0.0823	0.631	0.0718	**0.706**	**0.0701**
HWE(l°/kg)	WHWE7	0.867	6.89	**0.743**	**6.97**	0.767	7.74
	WHWE8	0.838	6.37	0.610	8.26	**0.872**	**5.91**

Only one calibration equation was developed for TN.

TABLE 12: CALIBRATIONS DEVELOPED USING WINTER MALTING AND FEED BARLEYS FROM THE 1987, 1988 AND 1989 HARVESTS

CONSTITUENT	CALIBRATION EQUATION	3 SEASON CALIBRATION R	3 SEASON CALIBRATION SE	3 SEASON PREDICTION R	3 SEASON PREDICTION SE
TN(%)	WTN8	0.859	0.179	0.845	0.141
TSN(%)	WTSN8	0.797	0.0925	0.757	0.0884
HWE(l°/kg)	WHWE9	0.816	8.20	0.840	6.72

Only one calibration equation was developed for each parameter.

Appendix 5

WAVELENGTHS USED IN THE CALIBRATION EQUATIONS

Second derivatives, generated from sequential (gap=0), 20nm segments, were used in all cases.

Appendix 5

TABLE 1: SPRING BARLEY CALIBRATION EQUATIONS

CONSTITUENT	CALIBRATION EQUATION	WAVELENGTHS
TN	STN1	1688+1730+1970
	STN2	1688+1736+1824
	STN3	1688+2350+2072
	STN4	1440+1654+1944
	STN5	1442+1654+1888
	STN6	1262/1772+1218
	STN7	1686+1160+1264
	STN8	1778+1854+1738
	STN9	1776+1388+1266
TSN	STSN1	1394+2254+1646
	STSN2	2144+1740+1150
	STSN3	2140+1234+1898
	STSN4	2144+1266+1736
	STSN5	2144+1262+1420
	STSN6	1260+1176+1686
	STSN7	2140+1262+1762
	STSN8	2136+1744+1892
	STSN9	1690+2312+2248
	STSN10	2250+1690+2154
HWE	SHWE1	2284+1684+1352
	SHWE2	2284+1210+2366
	SHWE3	1654/1706+2362
	SHWE4	1706+1158/1284
	SHWE5	1396+1738+1922
	SHWE6	1388/1676+1738
	SHWE7	1734+1844/1704
	SHWE8	1842/1764+1738

Appendix 5

TABLE 2: WINTER BARLEY CALIBRATION EQUATIONS

CONSTITUENT	CALIBRATION EQUATION	WAVELENGTHS
TN	WTN1	1690+2356+1952
	WTN2	1688+1260+1352
	WTN3	1688+1262+1352
	WTN4	1690+1734+2050
	WTN5	1690+1734+2044
	WTN6	1690+1734+2052
	WTN7	1690+1732+2054
	WTN8	1840/1764+1738
TSN	WTSN1	1766+1656+1370
	WTSN2	1692+2358+1886
	WTSN3	1766+1274+1650
	WTSN4	1766+1850+1656
	WTSN5	1294+1762/1232
	WTSN6	1740+2236+1886
	WTSN7	1740+2238+1930
	WTSN8	1692+1896+1360
HWE	WHWE1	1688+1160/2042
	WHWE2	1764+1228+1598
	WHWE3	1746+1666+2360
	WHWE4	1760+1642+2098
	WHWE5	1688+2234+1394
	WHWE6	2026+1460+2380
	WHWE7	1686+1232+1416
	WHWE8	1686+1230+1282

WMG/TB/hgca.wmg/20th December 1990