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PRACTICAL SECTION FOR GROWERS

Background and Objectives

The Martin Pea Tenderometer has been the standard instrument for measuring vining pea maturity since the 1950's. It is used to assess harvest date and forms the basis for payment to the grower, which for vining peas is approximately £50 million per annum. However, the Tenderometer is difficult to standardise, regular cross checks with the PGRO Master and Industry instruments are needed and TR readings do not necessarily relate to the quality of the finished product.

Alcohol Insoluble Solids (AIS) are sometimes used as measurement of quality of peas for export, but this is a laboratory test and is unsuitable for field use. Despite the limitations given above, Tenderometer Readings (TR) are widely accepted by growers as the basis for crop payment.

Near Infra-Red Spectroscopy (NIR) has been used for many years as a quick method for measurement of protein and moisture content of grain, and it has other applications in the food industry. NIR is already used commercially in some other European countries to measure AIS and is thus important to growers of crops for export. Work was begun by Campden & Chorleywood Food Research Association (CCFRA) in 1994 to assess a NIR method for quality and maturity determination for peas and the results were promising.

This is a potentially significant development for the vining pea crop, but it is vital that work meets the needs of growers as well as processors and that any new system of maturity assessment is acceptable to both parties. A collaborative project was begun with CCFRA in 1996.

Funding from HDC enabled PGRO to represent growers interests by evaluation of a fixed wavelength (13) NIR InfraAlyser 260 manufactured by Bran & Luebbe and to compare measurements of NIR with TR using the PGRO Master Tenderometer, for fresh samples of whole vining peas. This was undertaken in 1996 and 1997 to cover different seasons and with a range of varieties at different maturities.

Similar NIR instruments were sited at two processing factories for the 1996 and 1997 season and data for a large number of fresh pea samples was collected.

In 1997 comparisons were made with a full scanning Bran & Luebbe instrument to confirm which wavelengths are important for NIR calibration.

From the same pea samples in 1996 several were blanched and quick-frozen at PGRO and sub-samples of these were de-frosted and NIR scanned with another full wavelength NIR scanning instrument at CCFRA. AIS determinations were also carried out on sub-samples of the quick frozen peas. In 1997 de-frosted samples were scanned with the full wavelength NIR instrument at PGRO. AIS determinations of samples from PGRO and other sites were again carried out at CCFRA.

In addition to the HDC project a sensory evaluation for quality of the defrosted vining pea samples was carried out at CCFRA.

The aims of the project were:-

- to identify the correct wavelengths needed for NIR analysis of vining peas;
- to obtain a large and representative set of data over seasons and sites throughout the UK so that a robust calibration for NIR/TR and NIR/AIS can be produced.
- to investigate the response of different varieties.
- the relationship between NIR and Sensory Quality were also investigated (not funded by HDC)

Summary of results

Results from the Bran & Luebbe fixed filter (13 wavelength) instrument InfrAlyser 260 at PGRO (work funded by HDC) and at some UK processors, from the full scanning instruments at PGRO and CCFRA and data from an EU project, all confirm that Near Infra-Red Spectroscopy (NIR) can be used to calibrate Tenderometer Reading (TR) for fresh vining peas and Alcohol Insoluble Solids (AIS) for defrosted frozen peas.

- A list of important wavelengths for vining peas required for a dedicated NIR filter instrument for use in factory or field has now been identified for calibration and prediction of TR and AIS. The wavelengths available in the Bran & Luebbe InfrAlyser 260 are suitable for TR and AIS prediction.

- There is excellent correlation between AIS, another quality parameter, with NIR. Results for combined data for all sites, varieties and seasons for predicted versus measured AIS is given in Fig. 12.

- The relationship between NIR and TR is good, particularly with measurements using a Master Tenderometer. A graph of combined data for measured TR v predicted is shown in Fig. 11. Errors are slightly higher than for AIS but this is likely to be due to poor reliability of the current Tenderometer instruments rather than NIR itself.

- A substantial amount of data has now been collected from several UK sites and in different seasons. This has enabled a robust reliable calibration to be made for TR and AIS before the test is introduced. Growers can now be reassured that the system is reliable and will cover seasonal variability of the crop. The next stage will be commercial validation and in 1998 some factories will run the NIR system alongside TR measurement. A procedure for NIR tests for vining peas has been documented by Bran & Luebbe.

- Calibrations for single varieties for TR and AIS produced better statistics (Standard Error of calibrations and Multiple Correlation Coefficients) than those derived from mixed variety calibrations. However there are fewer samples in single variety calibration sets and this may be the reason for apparent improved calibration results. For practical application the prediction must be based on a multi-variety calibration.

- Sensory attributes of skin firmness, flesh firmness, mealiness and in 1996 only, sweetness showed promising calibration and prediction results for NIR. These may involve the use of spectral data which include wavelengths above 1700 nm, higher than those in the fixed filter instruments. However extra wavelengths can be added to existing instruments.

Figure 11. NIR predicted TR versus actual TR 1996 &1997 all data

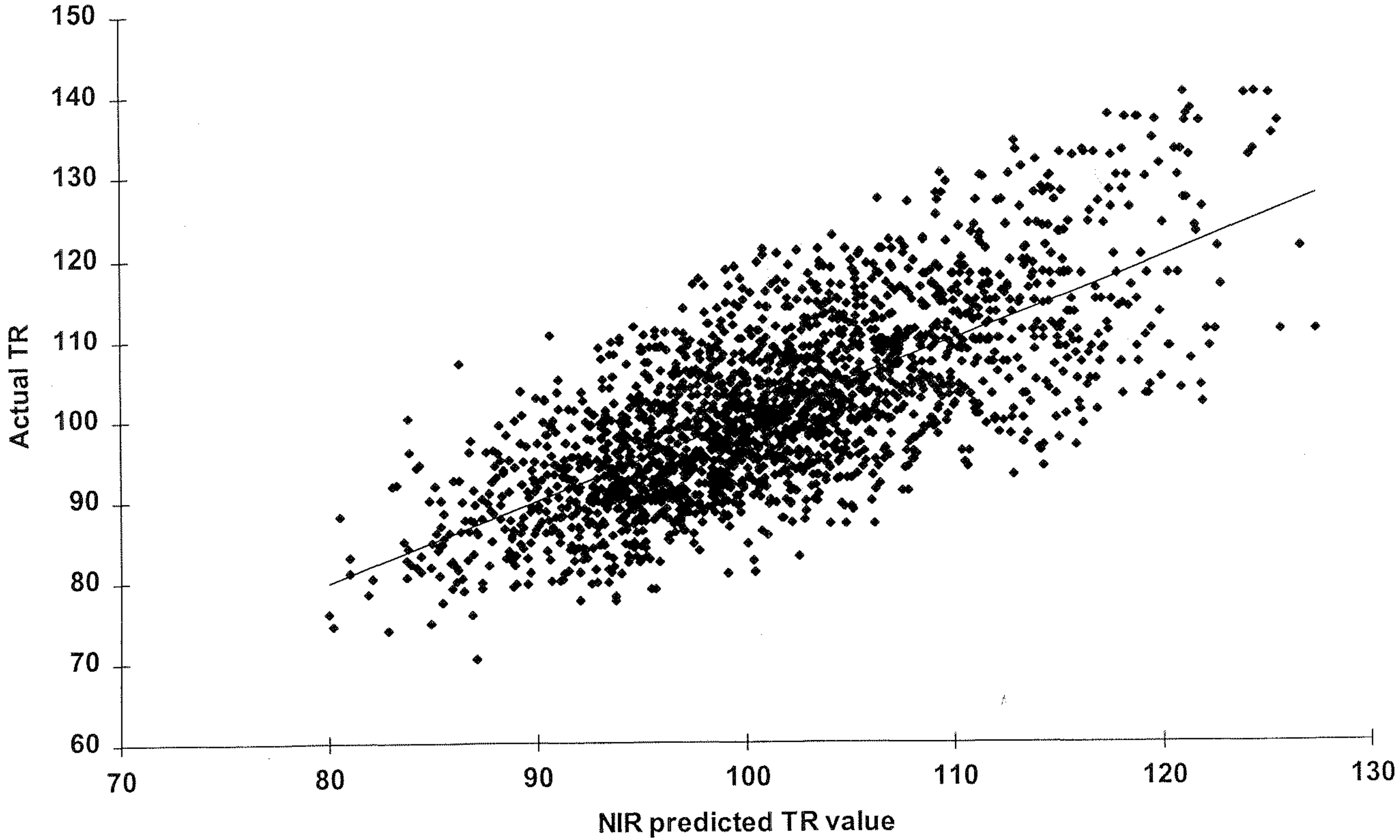
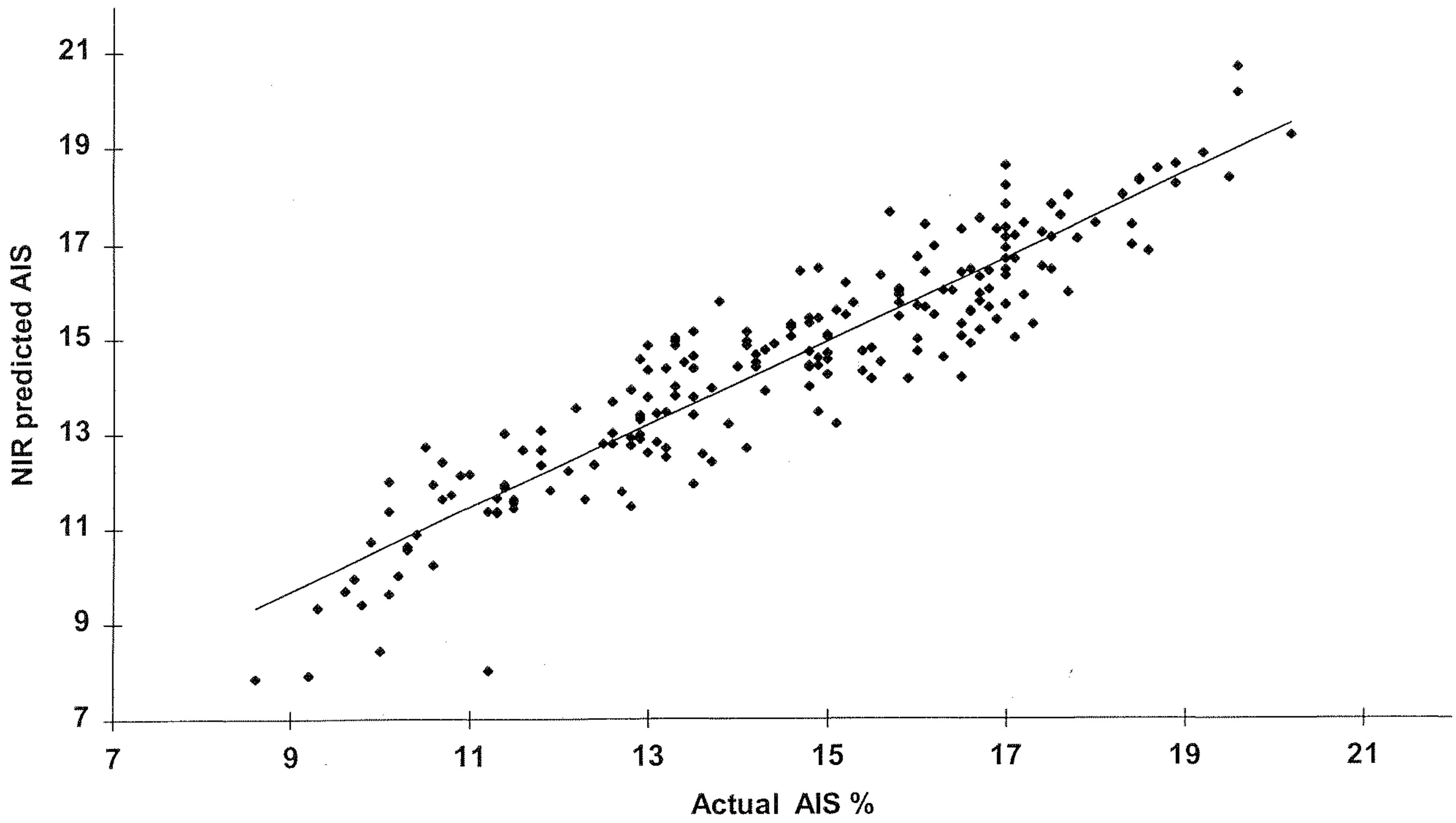


Figure 12. NIR predicted AIS versus actual AIS % defrosted frozen peas 1996 & 1997 all data



Future action

Growers need to be reassured that any new system is reliable and will not adversely affect their payment. In 1998 an NIR system will be run alongside TR in some factories to demonstrate this. The NIR calibration will fit the practical range of TR values and all varieties. However AIS, another quality parameter measured for quick-frozen vining peas shows an excellent correlation with NIR which is a quicker method, and it is possible that NIR/AIS could replace the old Tenderometer system. There are commercial advantages to growers and processors for NIR as a quick measurement of a vining pea quality parameter for a quick frozen, rather than a perishable fresh pea product as the basis of payment in the future. There are also advantages for a system which avoids the necessity for Tenderometer standardisation and cross-checks.

Meanwhile growers will still need Tenderometers to assess the correct time of harvest, but there is a possibility of a hand-held portable NIR instrument in the future. A prototype is being developed by research workers at VTT Electronics in Finland.

SCIENCE SECTION

Introduction

The Martin Pea Tenderometer has been the standard instrument for measuring vining pea maturity since the 1950's. It is used to assess harvest date and form the basis for payment to the grower, which for vining peas is approximately £50 million per annum. However, the Tenderometer is difficult to standardise, regular cross checks with the PGRO Master and Industry instruments are needed (PGRO, 1996) and TR readings do not necessarily relate to the quality of the finished product.

The Tenderometer grids are difficult to replace, stones can cause grid deformation and maintenance costs are high, and in addition it is possible that Masters have drifted over the years.

Near Infra-Red (NIR) Spectroscopy is a non-destructive technique for measuring the attributes of food materials. NIR absorbance spectra reflect the organic constituents in a food product both qualitatively and quantitatively and contain information about the conformation of the chemical components such as proteins and polysaccharides. NIR has been used for many years as a quick method for measurement of protein and moisture content of grain and it has other applications in the food industry (Scotter, 1990; Scotter & Legrand, 1994; Scotter, 1995). NIR is already used commercially in France (UNILET, personal communication) and in some other European countries to measure AIS thus it is important to growers of crops for export. A brief literature review and a list of references to NIR investigations is given in Appendix I.

Work was begun by the Campden & Chorleywood Food Research Association (CCFRA) - 1994 to assess a NIR method for quality and maturity determination for peas for UK processors and pea breeding companies (the CCFRA NIR Pea Club) and CCFRA were also partners in an EU COPERNICUS project in collaboration with Hungarian research workers). Results were promising and indicated that NIR can be related to TR but had a better correlation with AIS. Unlike the Tenderometer, NIR standardisation would be by objective physical measurement.

This is a potentially significant development for the vining pea crop, but it is vital that work meets the needs of growers as well as processors and that any new system of maturity assessment is acceptable to both parties. Thus a collaborative project was begun in 1996.

Funding from HDC enabled PGRO to represent growers interests by evaluation of a fixed wavelength NIR instrument with a rotating cup drawer manufactured by Bran & Luebbe and to compare measurements of NIR with TR for a large number of fresh samples of whole vining peas for a range of varieties and maturities in 1996. Presentation methods using 1 or 2 layers of whole peas in the drawer were also compared

The project was extended in 1997 so that another seasons data could be included to give a more robust calibration. In both years similar instruments were sited at two processing factories.

The most important wavelengths involved in the NIR calibrations for TR and AIS were to be identified with a full scanning instrument at CCFRA in 1996 and were confirmed at PGRO in 1997 when a full wavelength instrument was compared with the fixed (13) wavelength InfraAlyser 260.

The objective of the second part of the project, funded by PGRO was to calibrate NIR for quick-frozen samples from PGRO, CCFRA and industry sites against AIS determinations and sensory methods of quality evaluation carried out at CCFRA. These data were statistically analysed by CCFRA.

In summary the aims were:

- To identify the correct wavelengths needed for an NIR instrument for vining peas.
- To produce a robust calibration for NIR/TR and NIR/AIS.
- To assess varietal effects.

In the longer term the ideal would be for the grower to have a hand-held portable NIR instrument calibrated using data generated in the experiments. This would offer an easy method of monitoring maturity for harvest stage in the field.

Materials & methods

In 1996 and 1997 vining peas were grown using normal crop husbandry at Sacrewell Farm, Thornhaugh on a sandy loam soil and at sites in South Lincolnshire on a silt loam. The main varieties used in this study covered a range of different types and were Avola, Waverex, Scout, Bikini, Puget and in addition Tristar in 1997.

Plots were sown at Thornhaugh and in addition produce from PGRO variety trials was used. A few samples of other varieties were also included.

Sowing dates in both years were from mid-March until mid-May.

In both years a Bran & Luebbe InfrAlyser 260, a 13 fixed wavelength filter instrument with a rotating cup drawer designed to hold a double layer of peas, was installed at PGRO. In 1997 a full wavelength (1100 - 2500 nm at 2 nm intervals) instrument Bran & Luebbe 1A500 was also used to investigate whether inclusion of extra wavelengths in the region 1700nm and 2200nm would be required.

a) NIR/TR (1996 and 1997)

A plot of peas was hand-harvested and vined. The vined peas were washed (but not graded) and well mixed and four A1 cans were filled.

Three cans were used for three tests with the PGRO Master Tenderometer, and a mean TR reading was recorded.

Peas from one can were placed as a single layer on tissue placed on a tray and the peas were covered with a layer of tissue. The peas were rolled and blotted to remove surface moisture. Peas were spooned to fill a cup to overflowing with a double layer and any excess peas were carefully scraped off so that the pea sample surface was not above the edge of the cup. The cup was placed in the drawer which was closed and the peas were scanned. After scanning with the fixed wavelength NIR instrument the peas were discarded. A second cup full of peas were taken from the tissue-dried sample and scanned. The cup was cleaned with 10% industrial methylated spirits in water. In 1997 peas were also scanned with the full wavelength instrument.

This procedure was repeated for a range of varieties, mainly Avola, Scout, Bikini, Waverex, Puget and also Remus, Novella, Jaguar and Ambassador in 1996 and in 1997 for Jaguar, XPF 357, Celebration, Colana, Pacha, Samish, WAV 663, Paso, Salsado, Gemini, Balmoral and CMG 297 from sites at Thornhaugh on a fine sandy loam soil.

In 1996 the Tenderometer readings for these samples were from 85 to 164. In 1997 readings ranged from 83 to 155. In both years the TR readings were mostly within the 95-125 range. Altogether 500 samples of fresh peas were scanned with the NIR fixed wavelength instrument in 1996, and 148 samples were scanned with both instruments in 1997 and measured for TR with the PGRO Master Pea Tenderometer.

In 1996 samples (2 bags) of 200g of peas from the same plot were blanched and quick-frozen at PGRO. These were selected to cover a range of varieties and maturities and were used for AIS tests at CCFRA and for full scanning using the CCFRA full scan machine.

In 1997 samples from the same plot were also quick-frozen for full and fixed wavelength scanning at PGRO, and for AIS tests at CCFRA. For selected main varieties, an extra 3 bags were blanched and quick-frozen, for a range of maturities 90 - 140 TR reading to give 28 samples altogether for organ oleptic (sensory) quality appraisal, to be carried out at CCFRA.

In all cases the time between vining and TR measurements, NIR scans or quick-freezing was less than 60 minutes.

b) NIR/AIS (1996 and 1997)

In 1996 determination of Alcohol Insoluble Solids (AIS) using the AOAC official method 1990, modified using a stronger ethanol solution of 80% was carried out at CCFRA for 153 of the quick-frozen samples from PGRO, and in 1997, 100 samples were analysed.

In 1996, 44 of these samples were scanned with the CCFRA full wavelength NIR instrument over the range 1100 nanometers (nm) - 2500 nm and in 1997, 116 samples were scanned with the fixed and full wavelength instruments Bran & Luebbe instruments at PGRO. The frozen samples were left to thaw and then blotted with tissue to remove surface moisture before scanning.

c) NIR calibrations for TR & AIS related to variety

Separate calibrations for TR and AIS were made for individual varieties: Avola, Scout, Bikini, Waverex in 1996, and in addition to these, Tristar in 1997.

d) Presentation method single v. double layer (1996 only)

At PGRO, 10 samples each of Span, Avola, Waverex were used. The TR readings for these varieties were in a narrow range for this test: Span from 88-92; Avola from 111-114, and Waverex from 93-99. The peas were blotted dry as before and scans using the Bran & Luebbe fixed wavelength instrument were carried out for two presentation methods of peas in the cup.

A) one layer of peas well packed
B). cup filled to overflowing with a double layer and levelled as in the method for (a).

e) NIR/Sensory attributes (1996 and 1997)

Sensory appraisal was calibrated against NIR for a small number of samples (27) at CCFRA in 1996. In 1997 more samples were evaluated including 28 from PGRO, others were generated at CCFRA or were from Industry sources. The Sensory attributes assessed are shown in Tables 4 and 5.

RESULTS AND DISCUSSION

1996, <u>Bran & Luebbe (13 wavelength) filter instrument, NIR scan data</u>

a) **NIR/TR** **& b) NIR/AIS**

A correlation analysis of all 13 wavelengths (WL's) with all others was carried out on the PGRO data. As expected all wavelengths were too highly correlated to carry out a regression analysis. The WL absorbance values at 1320 nm were subtracted from all the other WL values for each sample to alleviate the Y axis shift in the data. A further correlation analysis revealed that the wavelength data was not so correlated as to obviate the regression analysis. The data now consisted of 12 WL's (1320 equals zero).

A best subsets regression was performed for TR and AIS. This algorithm selects the best 1-5 combinations of the 11 wavelengths used. Thus:

Best 5 equations using one wavelength
Best 5 equations using two wavelengths

and so on up to 11 WL's.

The data from the best subsets was examined to determine the combination of WL's which gave the best regression equation. A regression analysis was then carried out to obtain the Multiple Correlation Coefficient (MR) and Standard Error of Calibration or Prediction (SEC or SEP) values as well as the residuals to identify outlying samples in the regression.

a) NIR (fresh pea)/TR

The following results were obtained for TR for fresh pea samples:

TR	MR = .824	SEC = 8.5
WL's	1128, 1188, 1212, 1254, 1276	

All samples were used.

The data for Tenderometer was then split randomly to provide calibration and test sample sets. The following regression statistics were generated from the calibration set of 289 samples of which 41 were missing values:

TR	MR = .811	SEC = 8.94
WL's	1128, 1212, 1154, 1188, 1254	

(These wavelengths were selected from a best subsets regression as being the best for regression of this data set).

The calibration was then used to predict the TR values of fresh peas from the test set NIR data and the following prediction statistics were generated:-

TR	MR = 0.808	SEP = 8.74

These TR calibration results based on 5 wavelengths give an error similar to that provided by the full wavelength NIR instrument calibrations for previous work at CCFRA. Results were also comparable to a similar fixed filter instrument in a processing factory.

NIR predicted versus measured Tenderometer (TR) values for 1996 are shown in Fig. 1 (PGRO Master Tenderometer) and Fig. 2 (CCFRA, PGRO and Processor Companies).

Figure 1. PGRO Master Tenderometer TR fresh peas versus NIR predicted value 1996 (Bran & Luebbe)

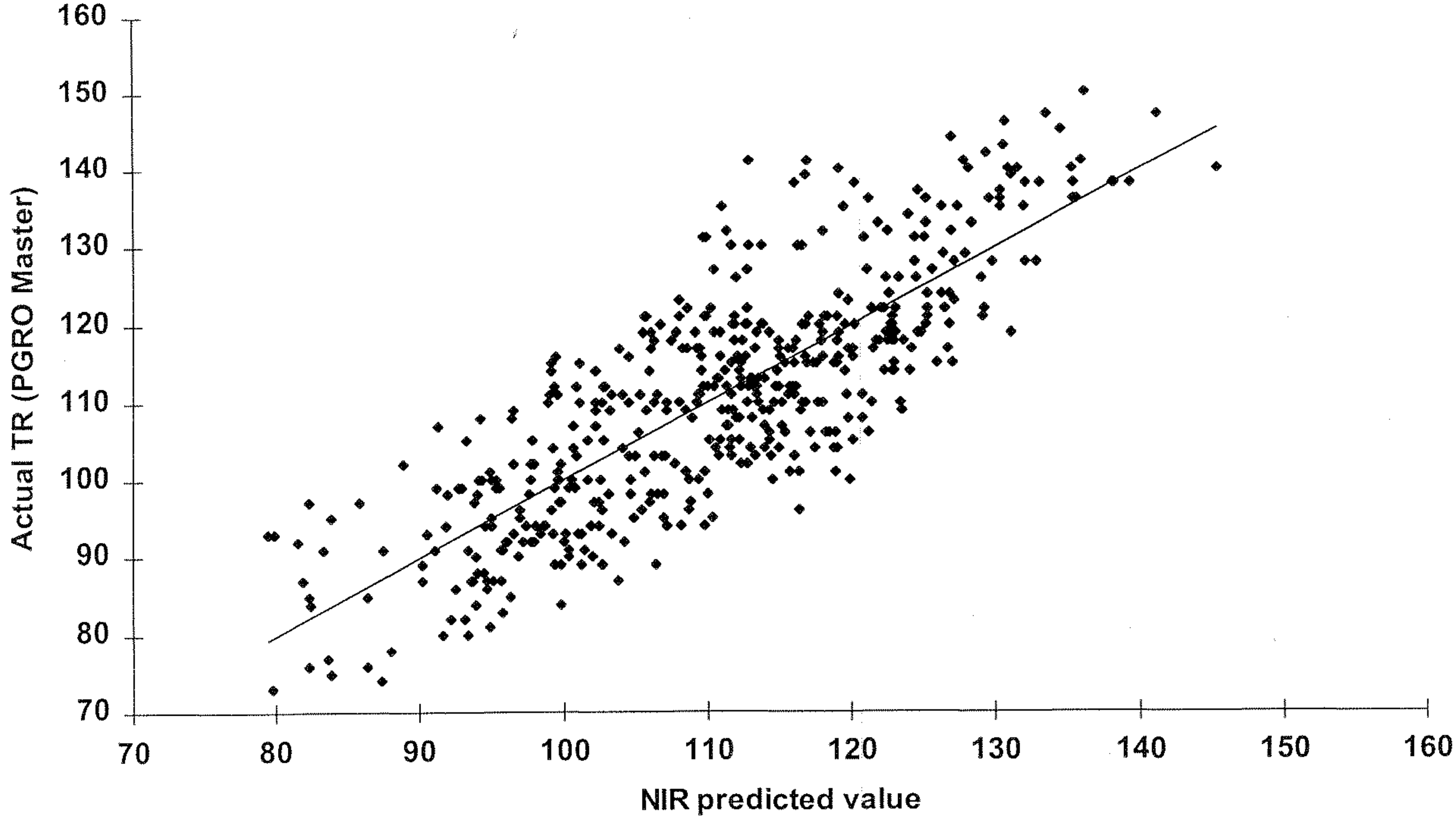
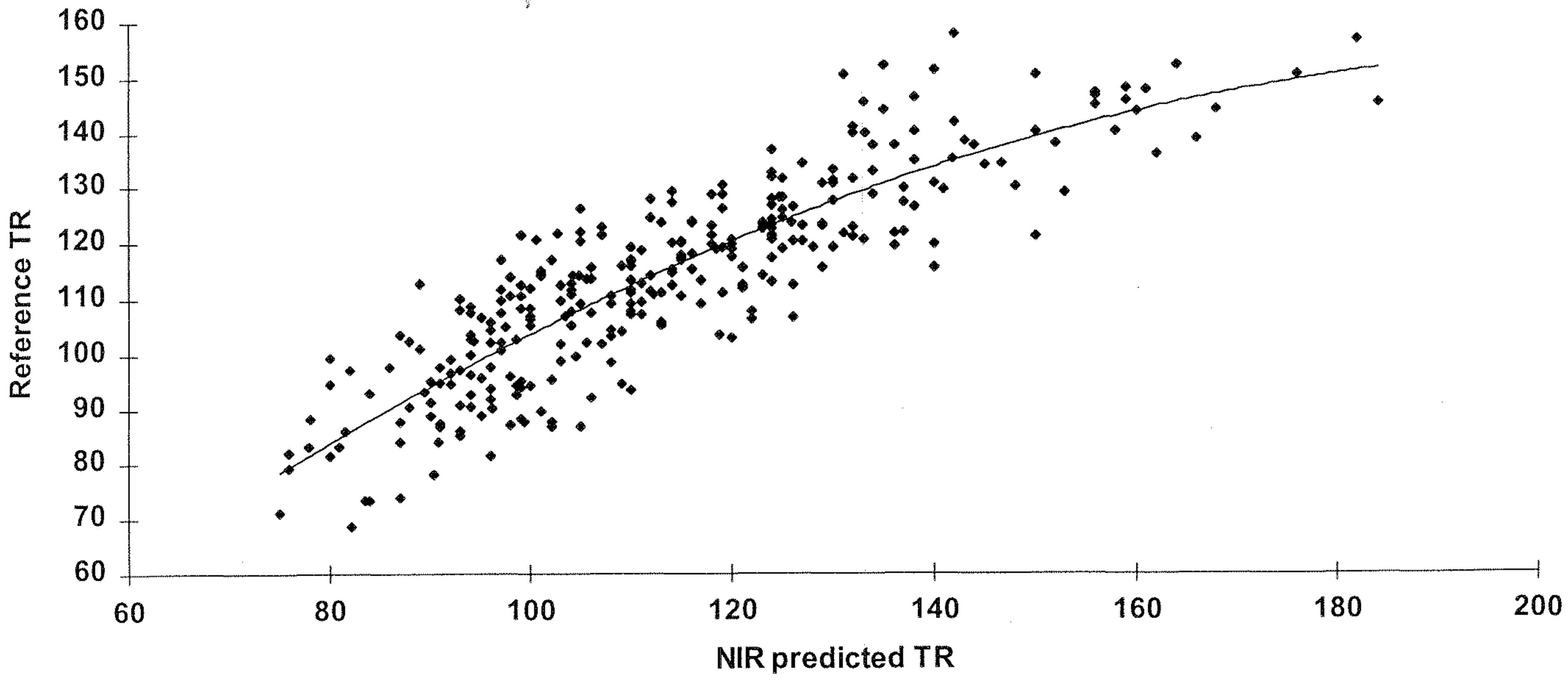


Figure 2. CCFRA, Industry, PGRO, EU project : Reference tenderometer values versus NIR predicted values for peas 1996



b) NIR/AIS

The following results were obtained for AIS based on 153 values:

AIS	MR = .849	SEC = 1.11
WL's	1128, 1188, 1212, 1254, 1276	

Nine samples were omitted, since they proved to be extreme outliers in the first regression analysis.

As with the TR calibrations the samples were split randomly into a calibration and a prediction on test set. The calibration generated from the calibration set had the following statistics:

AIS	MR = .909	SEC = 0.99
WL's	1128, 1138, 1154, 1212, 1238, 1254, 1262, 1276, 1670	

This calibration was selected as the best from a best subsets regression compilation. Nine samples were also omitted when the calibration was computed because they were identified as extreme outliers.

The calibration was then used to predict the AIS values from the test set NIR data and the following prediction statistics were generated:

AIS	MR = .803	SEP = 1.15

These results obtained are in broad agreement with CCFRA and EU project results for NIR/TR and NIR/AIS.

NIR predicted (thawed frozen peas) versus measured AIS values for 1996 are shown in Fig. 3 (PGRO data) and Fig. 4 (CCFRA, PGRO and Processor Companies.

Thus satisfactory calibrations were obtained for TR and AIS but some wavelengths identified using the full scanning equipment at CCFRA were not present in the Bran & Luebbe filter instrument.

Figure 3. CCFRA actual AIS % defrosted frozen peas versus NIR predicted 1996

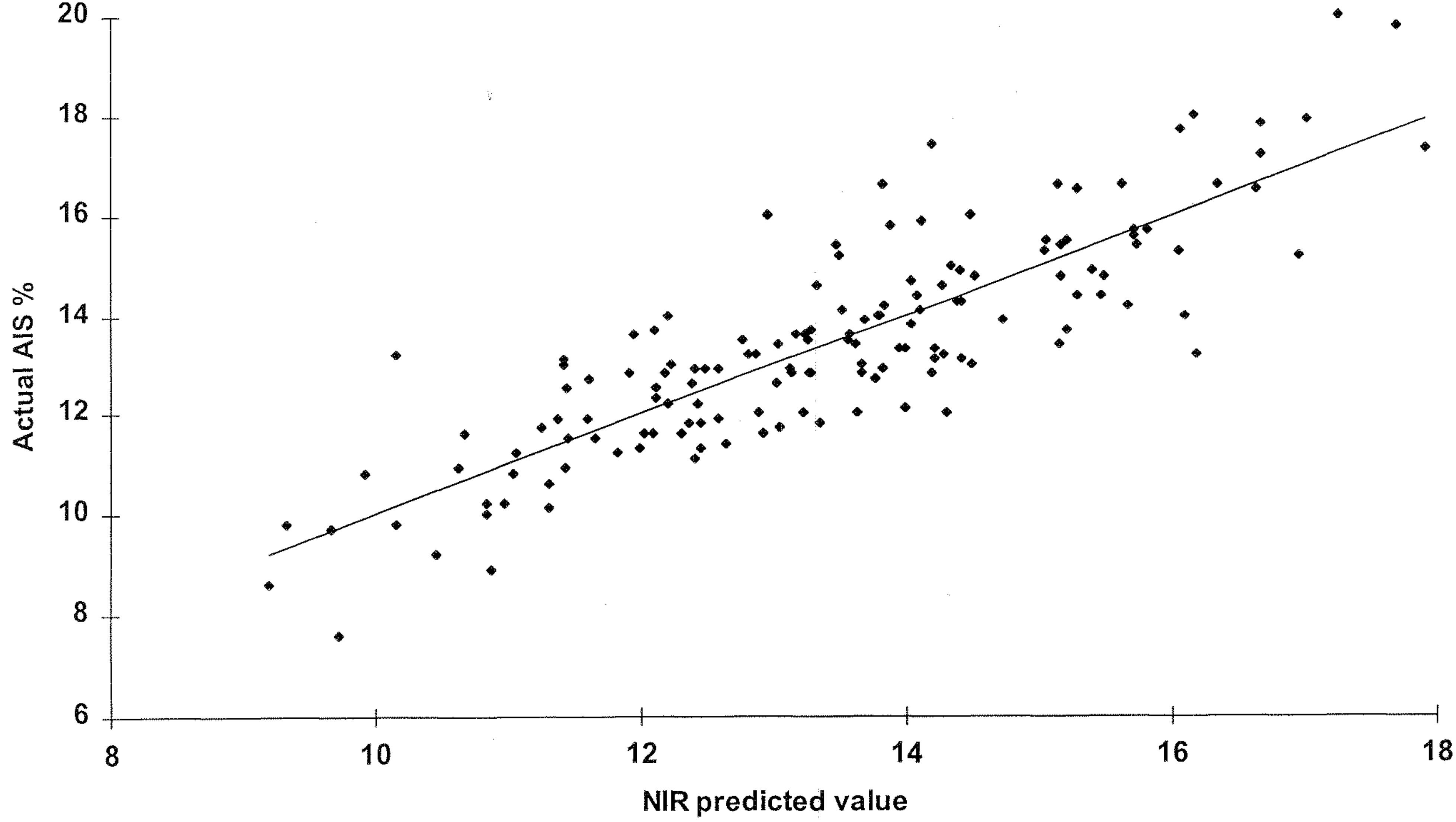
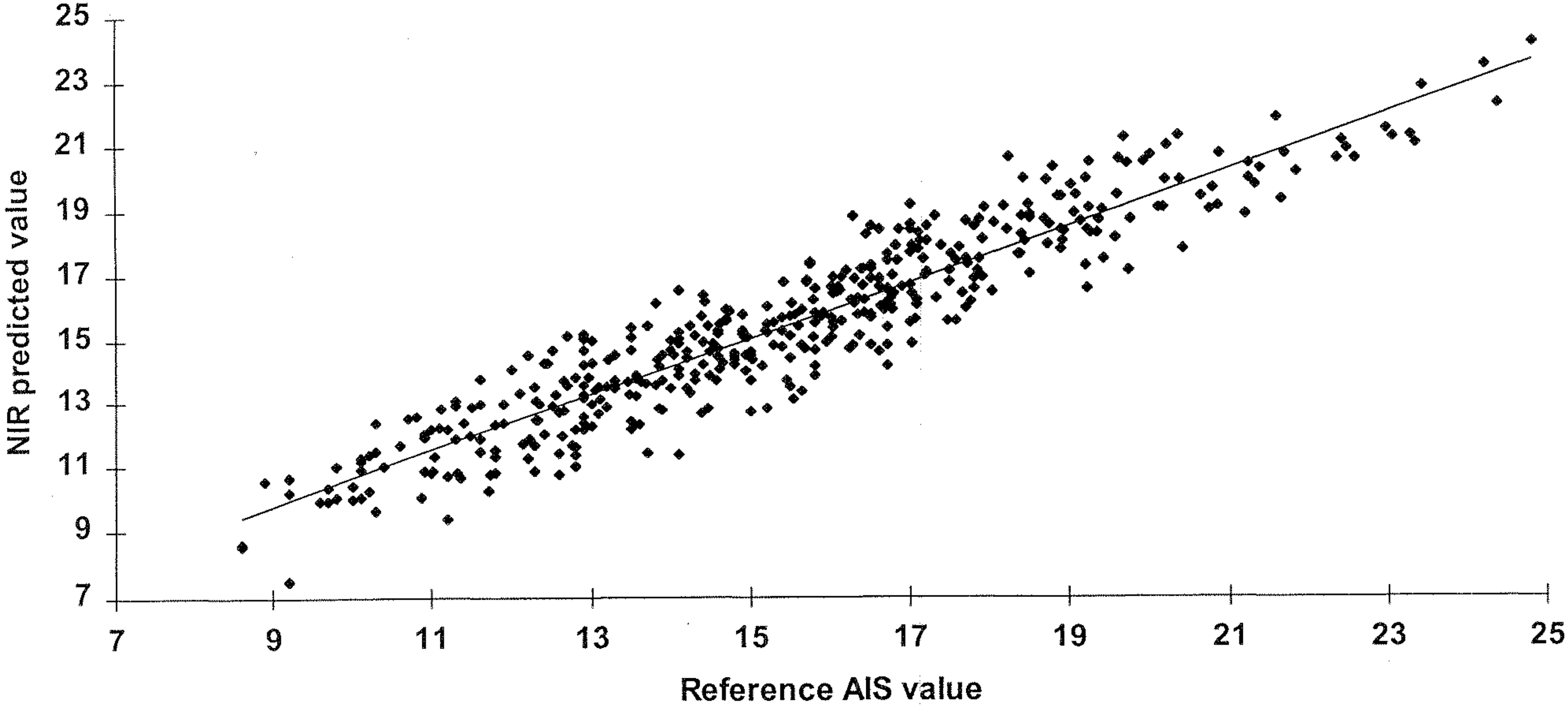


Figure 4. CCFRA, Industry, PGRO,EU project : Reference value AIS versus the NIR predicted value for peas 1996



1996, CCFRA full wavelength instrument NIR scan data frozen peas/TR fresh peas and AIS

a) NIR (thawed frozen peas)/TR

Based on 44 samples the following regression statistics were generated:

MR = .879 SEC = 7.87
WLs 1210, 1178, 2076 nm
2nd Derivative transformation

These statistics are very similar to the results obtained for the EU project and CCFRA NIR work. No calibration test was performed because there were too few samples to split the data set into a calibration and prediction (test) set (please see Appendix IV for explanation).

b) NIR (thawed frozen peas)/AIS

For the calibration equation results noted below, 41 samples were used for AIS. Samples 623, 677, 794 were removed from the analysis because they were identified as outliers.

The regression results were as follows:

MR = .932 SEC = 0.87
WLs 1708, 1328, 2324 nm
2nd Derivative transformation

Again no calibration validation was performed because too few samples were scanned.

Thus the wavelengths involved in the NIR calibrations for TR on fresh peas and AIS of the frozen samples were identified.

Only one wavelength (1178) was common to the fixed filter instrument at PGRO and the full wavelength EU/CCFRA calibrations.

For AIS, all three wavelengths are similar to those obtained in the EU/CCFRA calibrations.

1997

a) NIR fresh peas/TR & b) NIR thawed frozen/AIS

An extra year's data was needed to produce a more robust calibration and Fig. 5 shows the 1997 measured PGRO Master Tenderometer TR values for fresh peas. Figure. 6 shows CCFRA AIS values for frozen peas versus predicted values for the 144 samples.

Figure 5. PGRO Master Tenderometer TR fresh peas versus NIR predicted value 1997 (Bran & Luebbe)

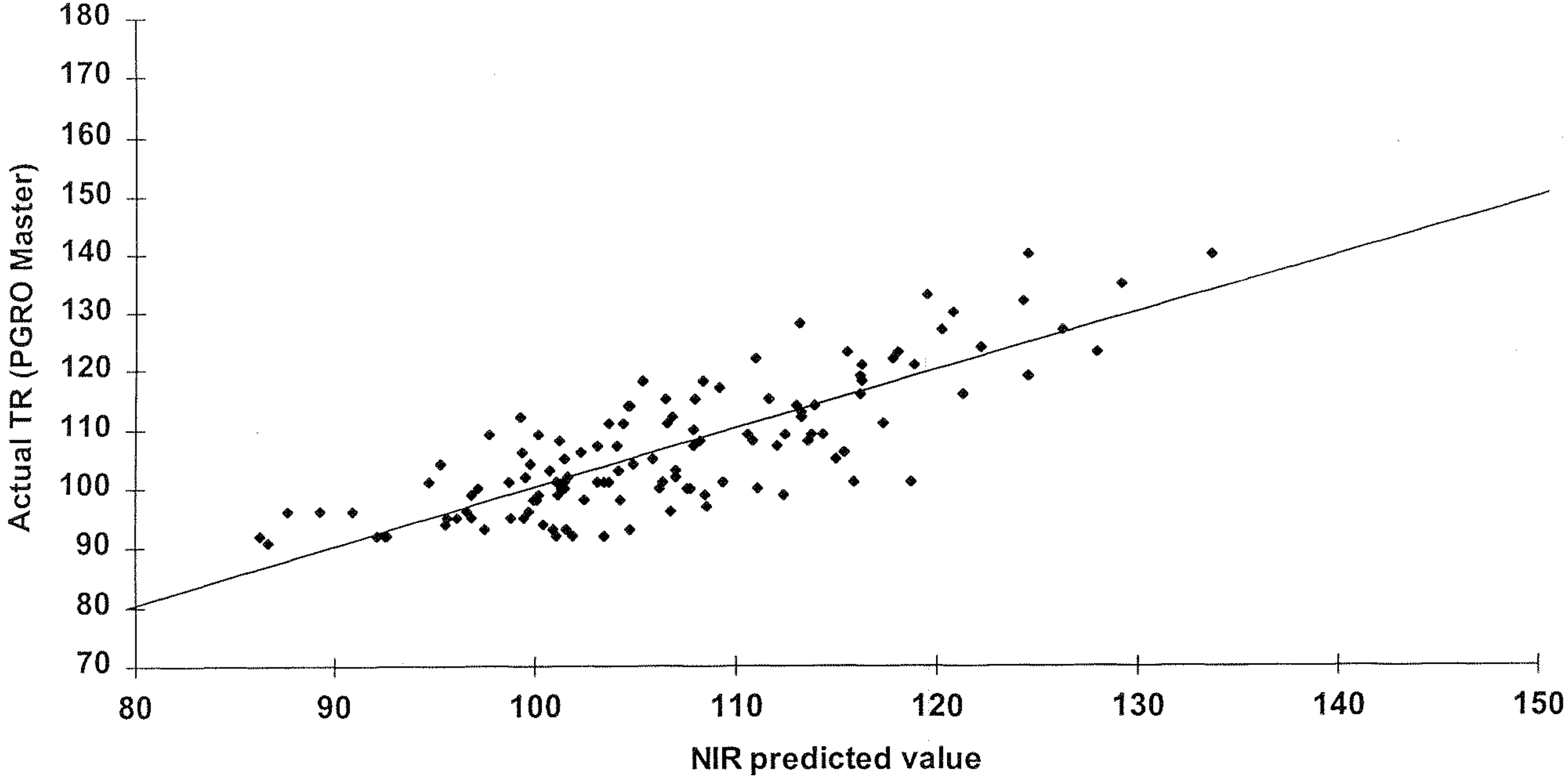
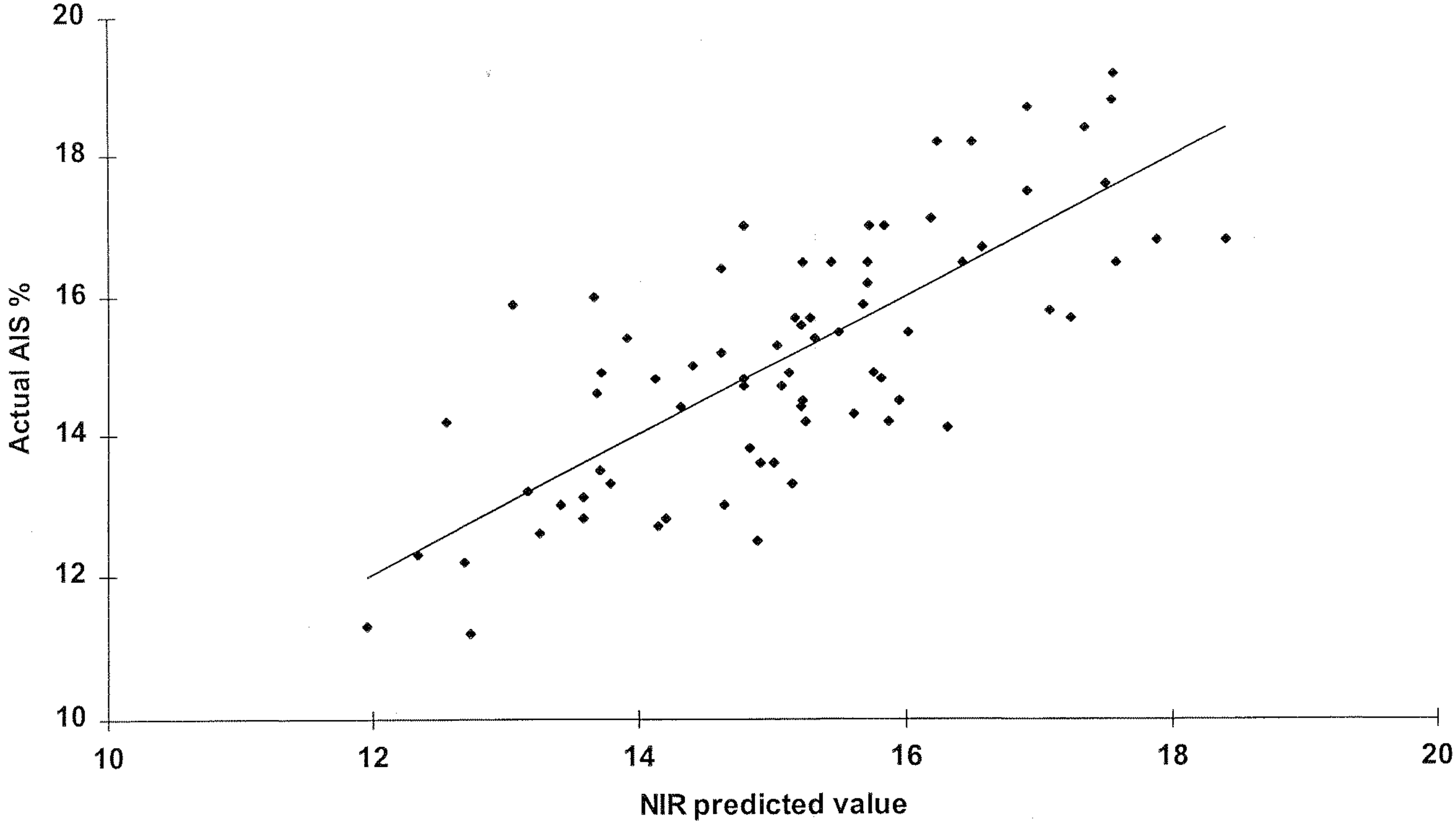


Figure 6. CCFRA actual AIS % defrosted frozen peas versus NIR predicted value 1997



1994 - 1997

a) NIR (fresh peas)/TR & b) NIR (thawed frozen)/AIS

The extra data produced in 1997 was to further develop robust calibrations.

Calibration and test statistics (PGRO (1996-1997); CCFRA and Industry (1994 - 1997) are compared for 1994 - 1997. Comparison of the results for 94, 95 and 96 with those for the whole four years indicated there was no appreciable increase in errors when the fourth year samples were included in the calibration and test sets.

Table 1 records the individual year calibrations and the combined years calibrations for comparison. The trend from this table is that the errors in both TR and AIS increase slightly as the number of samples in the calibration set increase.

The 1995 calibrations provide the lowest errors, however, for both TR and AIS.

Table 1. TR and AIS calibrations for each of the four years plus a combined years calibration.

	1994 CAL	1995 CAL	1996 CAL	1997 CAL	All years CAL	PRED
TR						
Samples	30	48	105	94	175	75
SEC(P)	6.83	5.63	6.87	7.04	8.23	8.66
R	0.953	0.901	0.897	0.9	0.852	0.818
WLs	**2118**	**1774**	**2412**	**1274**	**2406**	-
	1144	**2398**	**1210**	**1630**	**2284**	-
	1244	**1328**	**1346**	**1128**	**1344**	-
	-	**1726**	**2076**	**1162**	**2114**	-
	-	-	**1330**	**1588**	**1200**	-
AIS						
Samples	30	48	105	94	175	75
SEC(P)	1.16	0.637	1.44	1.15	1.21	1.33
R	0.951	0.956	0.804	0.867	0.888	0.837
WLs	**1528**	**1594**	**2262**	**1232**	**2286**	-
	1700	**1614**	**1704**	**1142**	**1178**	-
	1334	**2246**	-	**2114**	**1342**	-
	-	**1698**	-	**2084**	**1234**	-

Key

CAL	-	Calibration	PRED	-	Predicted
SEC(P)	-	Standard Error of Calibration (Prediction)	WLs		Wavelengths
R	-	Multiple correlation coefficient			

Graphs combining data for 1996 and 1997 NIR Predicted v Measured Tenderometer values & NIR Predicted v. AIS % are shown in Figs.11 and 12 respectively.

Figure 11. NIR predicted TR versus actual TR 1996 &1997 all data

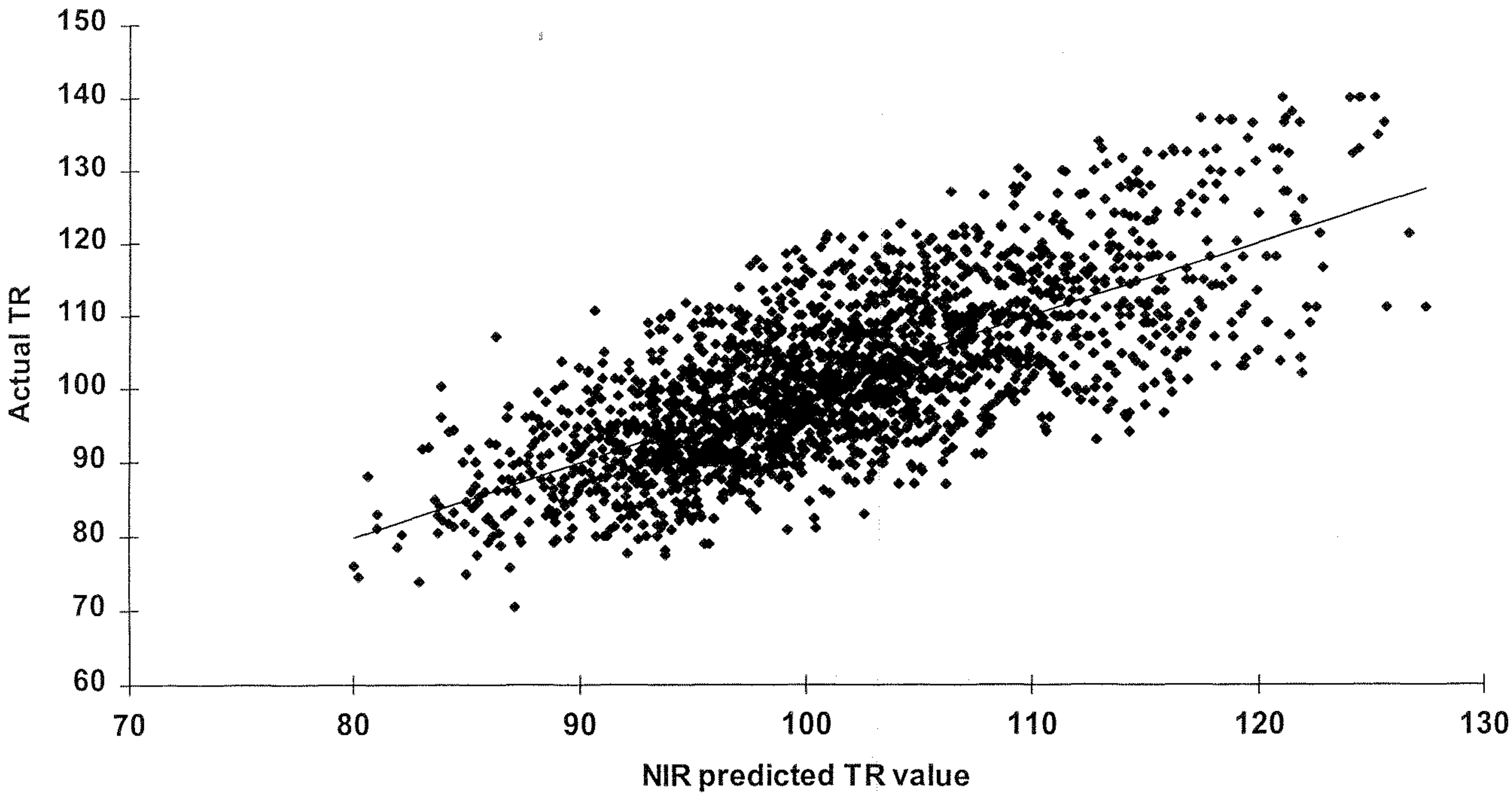
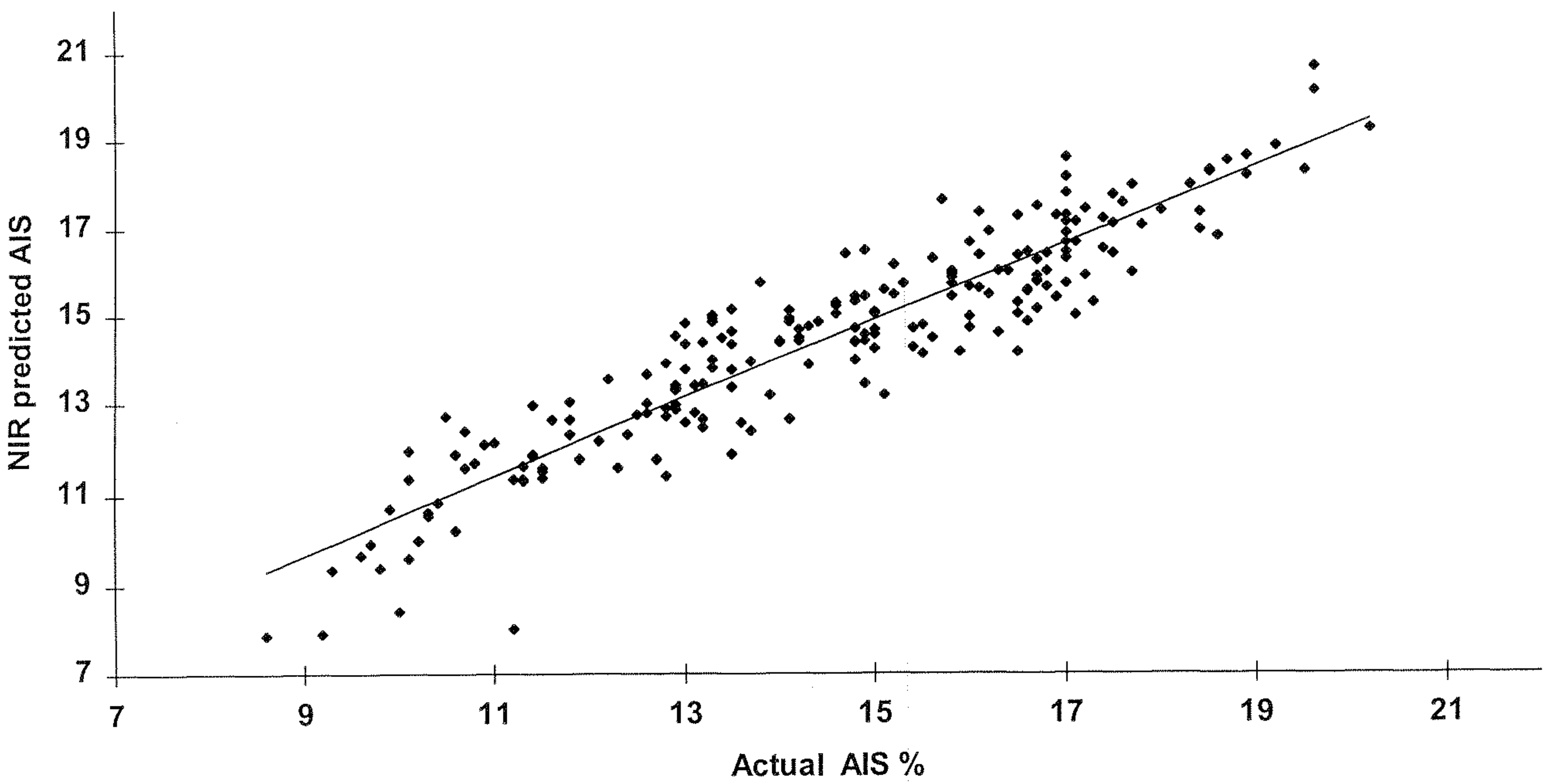


Figure 12. NIR predicted AIS versus actual AIS % defrosted frozen peas 1996 & 1997 all data



Figures 7 and 8 show comparison of NIR predicted TR and AIS, using the fixed wavelength spectrum i.e. eliminating wavelengths above 1700nm, for four years 1994 - 1997, data from CCFRA, PGRO and Processors.

Some data originally suggested that wavelengths of 1700 nm and above may be important for AIS and TR calibrations, and these were not present in the Bran & Luebbe 13 fixed wavelengths filter instrument being tested. However, tests in 1997 with a full spectrum verses the fixed wavelength filter instrument showed that the filter instrument was quite satisfactory to make AIS and TR measurements.

Figure 7. NIR predicted TR versus actual TR fixed wavelength - CCFRA, Industry, PGRO data 1994 -1997

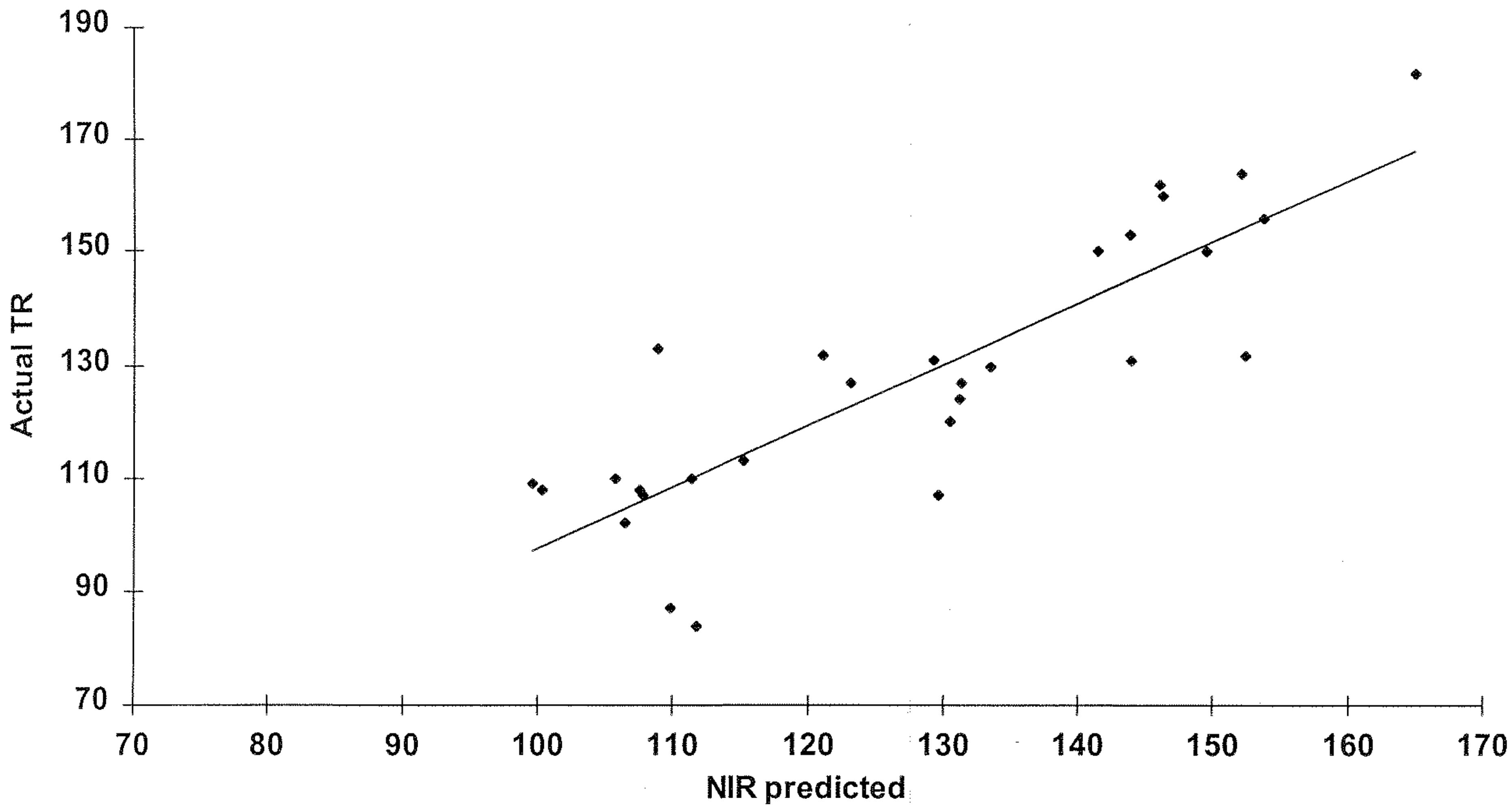
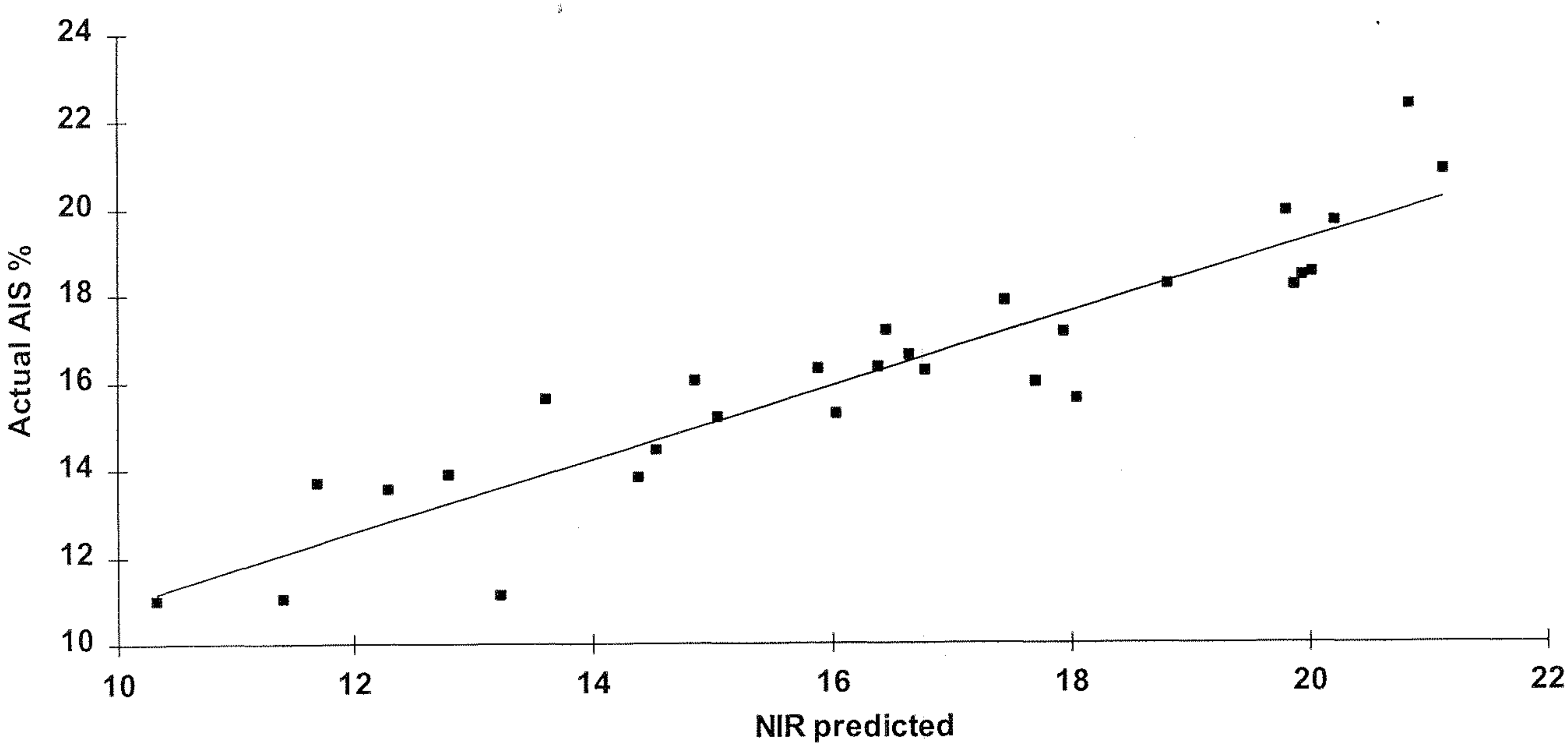


Figure 8. NIR predicted AIS % versus actual AIS % fixed wavelength - CCFRA, Industry, PGRO data 1994-1997



c) **Effect of variety: NIR calibration and test statistics for TR and AIS.**

Calibrations and statistics for TR and AIS were calculated for all samples from 2 years of PGRO work and 4 years of the CCFRA co-ordinated work. These are shown in Table 2.

Table 2. NIR Calibration and test statistics for TR and AIS results for all four years samples.

	TR Results		AIS Results	
	Calibration Set	Test Set	Calibration Set	Test Set
Samples	175	75	175	75
SEC or SEP	8.36	9.05	1.27	1.34
R	0.834	0.800	0.876	0.837

TR Wavelengths (nm) 2282, 2114, 1344, 1200
AIS Wavelengths (nm) 2286, 1178, 1342, 1234

Note: These are the optimum number of wavelength terms for the calibrations to provide the best test statistics.

The individual variety calibrations are detailed in Table 3.

Table 3. TR and AIS Results from NIR separate variety calibrations

TR variety results

Variety	Number of samples	SEC	R	WL1	WL2	WL3	WL4
Avola	43	7.55	0.892	1154	2412	1238	2122
Scout	36	4.85	0.958	1702	1774	2302	1722
Waverex	33	6.54	0.867	2110	1154	1232	-
Bikini	31	6.36	0.934	1750	1126	1246	1152
Tristar	23	5.06	0.938	1236	1162	-	-

AIS variety results

Variety	Number of samples	SEC	R	WL1	WL2	WL3	WL4
Avola	43	0.93	0.904	1234	2282	1184	2016
Scout	36	1.19	0.88	1272	2264	-	-
Waverex	33	0.66	0.953	1492	1126	1154	1230
Bikini	31	1.07	0.925	1338	1184	2044	-
Tristar	23	0.73	0.918	1284	1536	2472	2038

Key SEC - Standard Error of Calibration
R - multiple correlation coefficient
WL - wavelength (nm)

Several observations may be made when comparing the variety calibrations with the overall calibrations for TR and AIS

(i) Comparing the standard errors of calibration (SECs), in all cases the errors for the variety calibrations are lower than those for the complete sample set calibration.

This does not necessarily mean that single variety calibrations are more accurate, since there are many more samples in the overall calibrations than in the variety ones. More samples introduce more variation, so one would need more variety samples to demonstrate any real differences.

(ii) The overall calibrations show small increases in error when the calibration is tested with an independent set of samples (See Table 2).

(SEC for TR = 8.36, SEP = 9.05)
(SEC for AIS = 1.27, SEP = 1.34)

This suggests that they are good stable calibrations, based on several years data and several varieties.

(iii) Of the 34 wavelengths selected for the individual variety calibrations, 20 are within the region used by the filter instrument. Of the 8 wavelengths selected for the overall calibrations, 5 are within the range of the filter instrument.

Combined data for varieties Avola, Scout, Waverex, Bikini, Tristar from all sites were calibrated and give graphs of NIR predicted v actual. TR (all Tenderometer) (Fig. 9) or v. measured AIS (Fig. 10)

Figure 9. NIR predicted versus actual TR - five varieties 1998

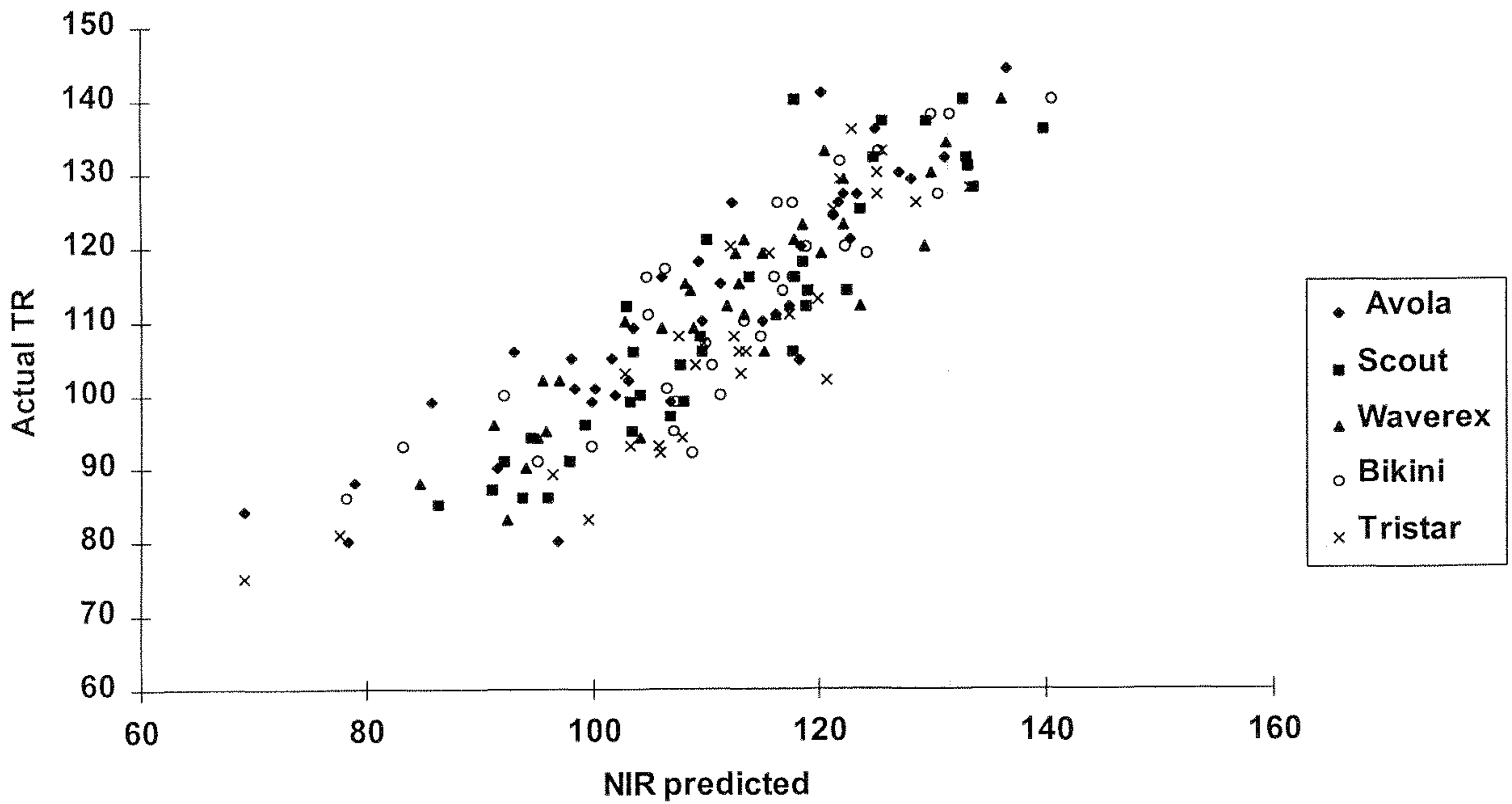
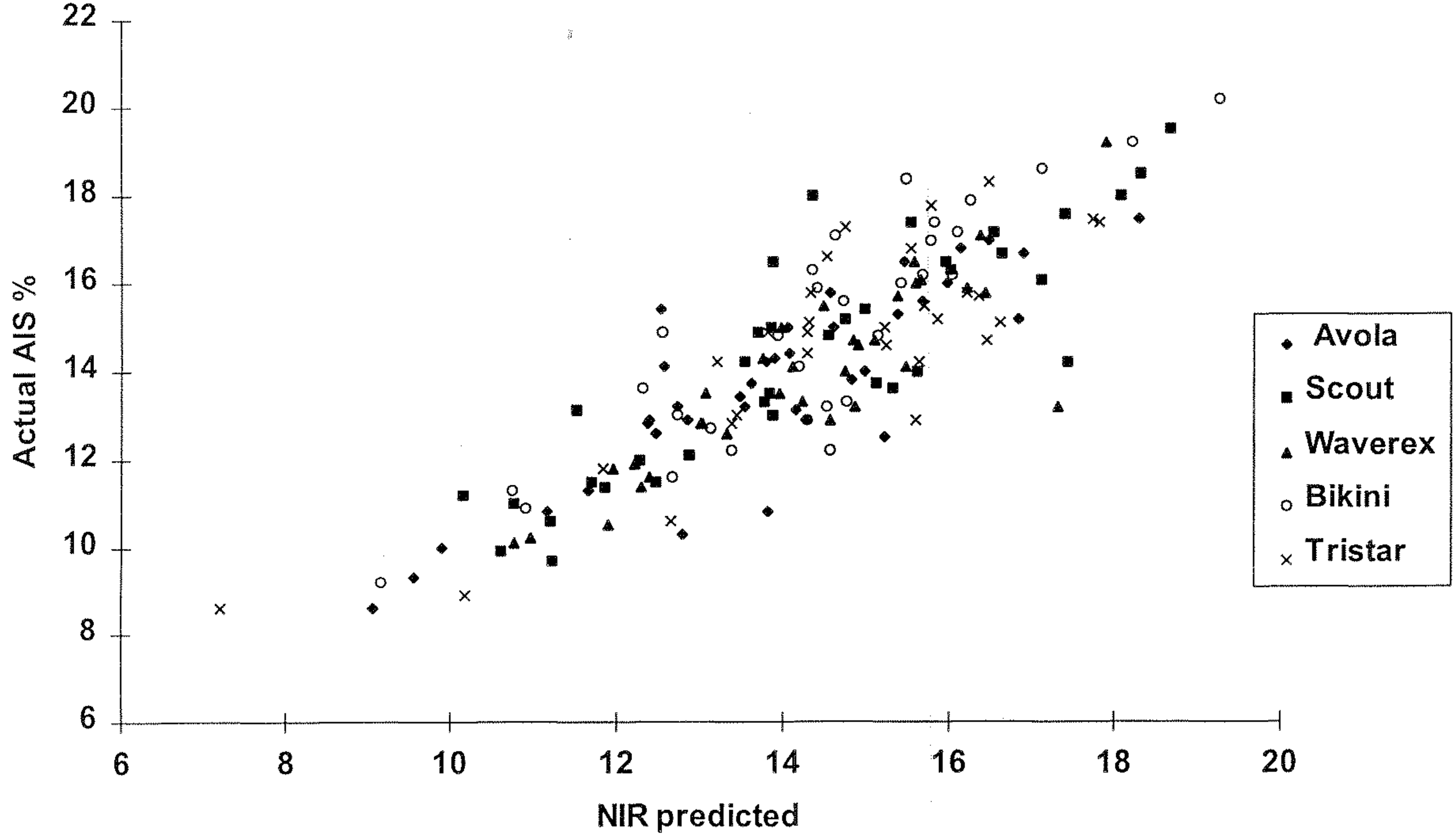


Figure 10. NIR predicted versus actual AIS % - five varieties 1998



d) **Presentation method single (A) v double (B) layer in rotating cup for Span, Avola, Waverex (1996 only)**

Overall the results confirmed that the use of a double layer of peas provides better spectroscopy data than one layer at most wavelengths.

e) **NIR Calibrations relating to sensory attributes (for information, not funded by HDC). Samples were provided by PGRO, RVP, Salvesens and CCFRA are listed in Appendix 3**

Table 4 records the calibration results for sensory attributes for 1996 and the calibration and test results for 1997, when sufficient data had been collected to provide both calibration and test sample sets. Table 5 details the wavelengths selected within the calibrations. The three sensory characteristics which produced useful calibration results (as noted in the 1996 report) were also shown to produce promising calibration and test statistics in 1997. These are: skin firmness, flesh firmness and mealiness. NIR showed good calibration and prediction results for sweetness in 1996, but not in the very different 1997 season.

Table 4. NIR calibration and prediction results for sensory attributes (1996 and 1997 compared), with wavelengths used in the calibration.

Sensory attribute	1996 R	SEC	Samples	1997 R	SEP	Samples Cal/test
Brightness	**0.835**	0.31	27	0.47	0.51	66/30
Uniform colour	0.616	0.70	27	-0.44	1.02	66/30
Green	0.618	0.36	27	-0.64	0.58	66/30
Yellow	0.594	0.60	27	-0.71	0.90	66/30
Khaki/Brown	0.677	0.32	27	0.39	0.64	66/30
Grey	**0.871**	0.32	27	0.27	0.60	66/30
Skin firmness	**0.933**	0.27	27	**0.81**	0.44	66/30
Flesh firmness	**0.846**	0.35	27	**0.91**	0.54	66/30
Mealiness	**0.907**	0.72	27	**0.88**	0.97	66/30
Sweet	**0.859**	0.33	27	0.66	0.64	66/30
Strength (flavour)	**0.852**	0.49	27	0.54	0.74	66/30
Stale (flavour)	**0.858**	0.71	27	0.68	0.95	66/30
Sour (flavour)	0.594	0.60	27	0.28	0.77	66/30

Note: A calibration with a lower 'R' than 0.8 is unlikely to perform satisfactorily.

Key
R - Multiple correlation coefficient SEC(P) - Standard Error of Calibration (Prediction)
Cal - calibration set test - test set

Table 5. Calibration wavelengths (nm) used for 1997 sensory attribute results

Sensory attribute	WL1	WL2	WL3	WL4	WL5
Brightness	2458	1234	2042	-	-
Uniform colour	2470	2008	2110	2448	-
Green	2206	2096	2416	-	-
Yellow	2444	2204	2096	2414	-
Khaki/Brown	2404	2038	2122	-	-
Grey	2084	2270	2106	2440	-
Skin firmness	1186	2334	2118	2020	1350
Flesh firmness	1746	1172	1908	1346	-
Mealiness	1658	2286	1184	2404	-
Sweet	1550	2030	1228	-	-
Strength (flavour)	2284	1184	-	-	
Stale (flavour)	1612	1598	-	-	
Sour (flavour)	2410	2450	1992	1800	

The results show that sensory characteristics are also amenable to NIR measurement but this may require the use of spectral data in the region above 1700 nm, higher than those used in the Bran & Luebbe filter instrument.

CONCLUSION

Results from the Bran & Luebbe fixed filter instrument InfrAlyser 260 on loan to PGRO, instruments at some UK processors, a full scanning instrument at PGRO, the full scanning instrument at CCFRA and from partners in a EU project all confirm that Near Infra-Red Spectroscopy (NIR) can be used to calibrate TR (in fresh peas) and AIS (defrosted quick-frozen vining peas).

- A list of important wavelengths from vining peas required for a dedicated NIR filter instrument for use in factory or field has now been identified for calibration and prediction of TR and AIS. The wavelengths available in the Bran & Luebbe InfrAlyser 260 are suitable for TR and AIS prediction.
- There is excellent correlation between the quality parameter AIS with NIR. NIR spectroscopy is a quicker method of determination and is used on defrosted quick-frozen peas. It is possible that NIR/AIS may provide a more satisfactory basis for payment in the future.
- The relationship between NIR and TR is good, particularly where predictions involve a Master Tenderometer, although errors are slightly higher than for AIS. This is likely to be due to poor reliability of the current Tenderometer instrument rather than NIR itself. The calibration will fit the practical range of TR values and uses data from all sites, seasons and varieties.
- A substantial amount of data has now been collected from several UK sites and in different season's. This has enabled a robust reliable calibration to be made for TR and AIS before the test is introduced. Growers can be reassured that the system is reliable and will cover seasonal variability in the crop. The next stage will be commercial validation and in 1998 some factories will run the NIR system alongside TR measurement. A procedure for NIR tests for vining peas has been documented by Bran & Luebbe.
- Calibrations for single varieties for TR and AIS produced better statistics (Standard Error of Calibration and Multiple Correlation Coefficient) than those derived from mixed variety calibrations. However there are fewer samples in single variety calibration sets and this may be the reason for apparent improved calibration results. For practical application the prediction must be based on a multi-variety calibration.
- Sensory attributes skin firmness, flesh firmness, mealiness and in 1996 only, sweetness showed promising calibration and prediction results for NIR. The use of special data which include wavelengths above 1700 nm, higher than those used in the fixed filter instrument used in the experiment. However extra wavelengths can be added to existing instruments.
- Growers will still need Martin Tenderometers to assess the correct time of harvest, but there is a possibility of a hand-held portable NIR instrument in the future. A prototype is being developed at VTT Electronics in Finland and a commercial partner is being sought.

GLOSSARY

NIR	Near Infra-red
TR	Tenderometer reading
AIS	Alcohol Insoluble Solids expressed as a %
WL	Wavelength
nm	nanometre
MR	Multiple Correlation Coefficient
SEC	Standard Error of Calibration
SEP	Standard Error of Prediction
CD	Coefficient of Determination
ANOVAR	Analysis of Variance

APPENDIX I

LITERATURE REVIEW

NIR spectroscopy is an established method for determination of constituents of cereals, such as protein and moisture. The work begun on cereals was extended to legumes and other crops and is now in routine use by feed compounders. In the late 1980's Davies *et al.* at the Food Research Institute, Norwich developed tests for protein, starch and lipid in dried pea flour. The aim of this work was to develop NIR analysis as a plant breeding aid. These studies were carried out on pea flour samples. Tkachuh *et al.* 1987, analysed protein in ground and whole peas but were less successful with whole peas. Williams *et al.* In 1985 used NIR to determine methionine content but again on ground samples.

Martens and Martens and Kjolstad *et al.* At the Norwegian Food Research Institute have related NIR to sensory quality of vining peas. They used homogenised peas and measured diffuse reflectance with a Technicon Infra Analyser 400.

Davies, AMC., Coxon, D.T., Gavrel, G.M., and Wright, D.J. (1984). Determination of starch and lipid in pea flour by near infrared reflectance analysis. The effect of pea genotype on starch and lipid content. *J. Sci Food Agric* **36**, pp 49-54.

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APPENDIX II

1996 Data for PGRO samples for AIS frozen peas and TR fresh peas. Some of these frozen samples (44) were defrosted and scanned with the CCFRA full wavelength NIR instrument.

PGRO Code	Variety	AIS %	TR Reading (Fresh)
500	Avola	12.9	116
503	"	14.0	110
506	"	13.6	112
509	Jaguar	12.0	107
512	"	13.0	110
515	"	12.3	104
518	Avola	12.9	117
524	"	16.3	110
527	"	10.8	105
530	"	11.6	114
539	Jaguar	14.3	120
542	Avola	13.3	123
545	"	13.7	117
548	"	12.5	110
551	Bikini	12.2	105
554	"	11.3	100
557	Scout	11.2	94
560	"	10.0	86
563	"	10.1	88
566	Avola	12.8	117
569	"	12.6	119
572	"	11.9	112
575	Waverex	10.2	102
578	Jaguar	15.4	121
581	"	13.7	110
584	"	16.6	112
587	Avola	13.5	121
590	"	14.6	126
593	"	13.2	115
596	"	15.2	132
599	"	12.6	119
602	"	11.8	114
605	Scout	13.3	108
608	Bikini	14.1	110
611	Scout	17.7	93
614	Bikini	12.8	111
617	Scout	12.8	105
620	"	11.8	94
623	"	18.0	104
626	Waverex	12.9	117

PGRO Code	Variety	AIS %	TR Reading (fresh)
632	Scout	12.0	112
635	Bikini	17.9	138
638	"	14.2	119
641	"	14.9	118
644	Scout	13.0	128
647	"	13.8	105
650	"	15.4	118
653	Avola	12.0	116
656*	"	13.2	94
659	Waverex	12.7	126
662	"	14.0	135
665	Scout	15.5	123
668	"	15.6	132
671	Puget	11.1	89
674	"	11.6	94
677	"	17.4	104
680	"	14.4	110
683	"	15.3	120
686	Bikini	12.8	102
689	"	8.0	98
692	"	14.1	110
698	"	13.0	107
701	"	13.3	115
704	"	14.8	122
707	Puget	15.7	118
710	"	16.5	126
713	"	16.5	135
716	"	7.6	74
719	Waverex	11.7	97
722	"	9.2	93
725	Scout	11.8	89
728	"	13.4	94
731	Bikini	13.5	115
734	"	14.4	127
737	"	20.0	150
740	Avola	13.6	107
743	"	11.3	106
746	"	13.1	114
749	Puget	9.8	78
752	Avola	13.2	105

PGRO Code	Variety	AIS %	TR Reading (fresh)
755	Avola	10.3	109
758	"	12.1	103
761	"	16.0	116
764	Waverex	11.6	99
770	Avola	14.8	119
773	"	12.9	112
776	Scout	13.2	122
779	Avola	14.8	122
782	Puget	11.5	85
788	"	14.4	115
791	"	13.1	106
794	Scout	13.2	140
797	Waverex	14.6	117
800	"	13.9	123
803	"	12.8	121
806	Avola	16.6	137
809	"	14.2	140
812	"	15.0	131
815	Puget	13.5	89
830	Scout	12.7	84
833	Avola	9.8	87
836	Puget	13.9	103
842	Scout	12.0	94
845	Avola	9.7	91
848	Puget	17.3	145
851	Scout	14.0	101
854	Avola	13.1	96
857	Scout	17.8	100
860	"	11.7	97
863	Bikini	15.4	116
866	"	16.6	119
872	Scout	14.9	121
875	Avola	12.5	107
878	Waverex	11.5	99
881	Puget	11.6	100
884	"	13.6	93
887	Bikini	17.2	136
890	"	17.8	136
893	Puget	14.3	113
896	"	15.3	117

PGRO Code	Variety	AIS %	TR Reading (fresh)
899	Avola	13.6	122
902	Scout	19.8	138
905	Waverex	12.2	111
908	"	12.8	99
911	Scout	18.0	120
914	"	15.5	112
917	"	15.7	120
920*	"	10.9	91
923*	"	15.8	113
926*	Waverex	10.8	95
929*	"	10.9	100
932*	Scout	11.2	92
935*	"	11.9	95
938	"	15.2	137
941	"	14.7	121
944*	Waverex	11.9	98
947*	"	13.0	108
950	Scout	10.6	83
953	Bikini	8.6	75
956*	Scout	15.9	121
959*	Waverex	17.7	140
962*	"	16.6	138
965	Bikini	8.9	81
968	Scout	11.4	93
971	Bikini	10.2	87
974	Scout	13.4	103
977	"	13.2	116
980	Puget	12.9	92
983	Bikini	11.6	88
986	"	12.8	103
989	Puget	13.4	96
992	Scout	14.0	122
995*	Ambassador	16.0	105

* from S. Lincs site.
All other samples from Thornhaugh

APPENDIX II (continued)

1997 Data for PGRO samples - Tenderometer reading (fresh peas) & AIS (quick frozen) by variety, 1997

PGRO Code	Variety	TR Reading (Fresh)	AIS %
504	Avola	101	15.4
510	"	90	11.3
511	"	91	11.7
512	"	101	13.2
513	"	104	12.6
514	"	115	16.5
515	"	113	16.4
516	"	115	15.2
517	"	107	13.3
519	"	102	14.2
520	"	111	14.8
521	"	105	17.0
522	"	108	13.8
528	"	122	18.2
529	"	109	15.3
530	"	132	17.5
550	"	121	15.6
527	"	107	13.6
585	"	95	13.3
590	"	91	12.3
594	"	114	14.6
599	"	90	13.0
605	"	112	18.5
610	"	109	12.5
611	"	101	19.0
524	Bikini	93	12.7
525	"	99	13.2
526	"	92	12.8
533	"	116	16.2
534	"	108	14.3
544	"	117	16.7
545	"	127	19.2
546	"	127	15.7
553	"	95	14.7
554	"	98	14.5
555	"	94	14.1
570	"	116	16.8

PGRO Code	Variety	TR Reading (Fresh)	AIS %
571	Bikini	109	15.4
572	"	111	16.5
586	"	123	17.6
595	"	140	20.2
600	"	164	25.4
622	"	92	14.8
623	"	95	13.0
630	"	118	18.2
632	"	104	14.2
633	"	103	13.6
636	Paso	100	14.7
641	"	96	19.6
642	"	109	14.9
551	Puget	93	16.1
552	"	92	14.6
567	"	121	20.3
569	"	102	14.9
583	"	97	14.2
584	"	96	14.4
593	"	124	18.8
542	Scout	100	13.5
543	"	112	15.5
556	"	114	16.5
557	"	109	15.7
558	"	99	14.8
565	"	95	15.0
574	"	105	15.8
578	"	101	17.0
579	"	100	15.2
588	"	123	16.5
591	"	135	16.8
592	"	140	19.5
596	"	112	17.0
601	"	114	15.4
604	"	101	16.2
631	"	86	12.8
566	"	95	14.4
647	"	128	18.0
576	Tristar	83	14.9
597	"	106	15.5
603	"	93	14.9
606	"	130	18.3
609	"	155	18.7

PGRO Code	Variety	TR Reading (Fresh)	AIS %
617	Tristar	130	18.4
539	Waverex	108	19.3
540	"	100	12.2
541	"	101	13.1
559	"	113	14.8
560	"	111	13.3
562	"	96	12.8
563	"	101	18.7
575	"	92	11.2
580	"	103	19.7
582	"	107	13.6
616	"	110	13.9
624	"	129	17.1
643	"	103	13.0
561	"	111	14.5
648	"	119	15.8
637	Wav 663	98	14.9
607	XPF 357	88	15.9
612	XPF 357	96	14.8
614	XPF 357	96	16.0

APPENDIX III

TENDEROMETER READINGS AND ALCOHOL INSOLUBLE SOLIDS RESULTS FOR SAMPLES SUPPLIED FOR SCANNING AT CCFRA 1997

Variety	CCFRA				PGRO		RVP		Salvesens				Total Number of Samples
	Larkstoke		Smiths		Thornhaugh		East Anglia		East Anglia		Scotland		
	TR	AIS	TR	AIS	TR	AIS	TR	AIS	TR	AIS	TR	AIS	
Avola	88	9.3	110	13.2	90	11.6	100	13.2			120	16.0	
	101	10.8	118	13.4	101	15.4	102	12.5			127	16.8	
	112	12.9	126	14.2	121	15.6	109	13.1			127	16.7	
	136	15.3	141	14.4	132	17.5	116	13.7					
							129	17.0					20
Scout	85	9.9	86	10.6	95	15.0	86	11.4	137	17.6			
	99	11.5	91	11.5	114	16.5	99	17.4					
	106	13.0	100	12.1	128	18.0	107	13.7					
	112	14.9	116	13.6	140	19.5	112	14.0					
			137	16.3			131	14.2					19
Waverex	102	11.4	94	10.5	96	12.8	83	13.5					
	111	13.5	110	12.6	111	13.3	112	15.0					
	114	14.3	109	12.8	119	15.8	119	14.1					
	133	16.0	115	13.2	129	17.1							15
Bikini	107	12.2	95	11.6	99	13.2			86	14.9	92	15.9	
	113	13.3	119	14.8	116	16.2			93	15.6	101	16.3	
	133	16.0	126	14.8	127	19.2			104	17.0	116	17.1	
			138	17.2	140	20.2			116	17.4	120	18.6	
											132	18.4	20
Tristar	89	10.6	93	11.8	83	14.9	92	15.1	104	15.8	103	16.8	
	113	14.2	106	13.0	93	14.9	108	14.6	108	17.3	107	16.6	
	120	15.0	133	15.8	106	15.5	111	15.1					
	125	15.2			130	18.3	128	17.4					
	125	14.7											20
Total Number of Sample	20		20		20		17		7		10		

APPENDIX IV

Summary of Approach to NIR data analysis

A Bran & Luebbe 13 filter instrument, with a rotating cup was stationed at PGRO during the 1996 pea season and 500 fresh samples were scanned. CCFRA was supplied with the NIR data by Bran & Luebbe. Accompanying the NIR data were Tenderometer (TR) and AIS data as well as information about the pea sample varieties.

In addition NIR filter instrument spectra were provided of 10 sub-sample scans for three pea varieties, using two types of presentation method.

44 pea samples were also scanned at CCFRA on the full wavelength range NIR spectrometer, after thawing. AIS and TR data were provided with these samples also.

This data noted was statistically analysed at CCFRA.

Regression analysis

Sufficient pea sample scans were provided to carry out a full regression analysis of the NIR data and the field and laboratory measurements. This means essentially, establishing how well the NIR responses for the samples at each wavelength 'pattern-match' or correlate with the field or laboratory data.

The relationship between the NIR data and the laboratory or field data is expressed in the regression analysis by a regression equation which, when NIR response values are inserted in the equation, will provide an NIR predicted value for, in this case the AIS or TR data. This is the first step in the calibration process. As noted 'NIR predicted values' are available from the calibration equation.

These are not, however, sufficient to establish how well the equation will predict the AIS or TR values for samples which are not used to form the calibration equation. The next step, then, is to use the NIR response data from a new set of samples, which represents the same kind of variability associated with the calibration set of samples, e.g. range of TR and AIS, pea variety etc.

This new 'Test' or 'Prediction' set will now provide NIR prediction values, generated by the calibration equation, which will give a more realistic picture of the accuracy and precision of the calibration's ability to predict new sample values for TR and AIS.
The key statistics that are generated by the regression analysis are:

i. The MR or Multiple Correlation coefficient. When the value approaches 1.00, this demonstrates a good matching of the NIR and Lab or field data. An MR of less than .85 indicates that the equation is likely not to be a very good predictive equation.

ii. The Standard Error of Calibration (SEC) or Prediction (SEP). This describes the error range for the NIR prediction of the lab or field value; thus an SEC of 0.97 for an AIS regression equation means that, at a confidence limit of 95%, the predicted AIS value for 95% of the samples will lie within plus or minus 0.97% of the measured value.

iii. The range of the measured value, say AIS is also important, so a further statistic is often provided, the CD or Coefficient of Determination, which expresses the Standard Error (SEC or SEP) as a percentage of the AIS range, for example. Thus an apparently small SEC or SEP will be truly represented in relation to the range.

The regression approaches to the filter and scanning instrument data are essentially similar, but the two types of data require different mathematical pre-treatments.

Mathematical pre-treatments of spectral data regression analysis

NIR spectra contain chemical bond information in the form of reflectance value changes which relate to the concentration of the chemical in the material being analysed. The spectral reflectance values also, however, represent responses due to mirror like reflections from the sample which contain no chemical information, but influence the overall position of spectra on the vertical axis. In general, this non-chemical spectral data interferes with regression analysis, so mathematical pre-treatments are applied to the spectra to alleviate these effects and maximise the chemical spectral information. The commonest pre-treatments are first and second derivatives.