NIR and NMR Soybean Composition Database

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Abstract

Our NIR and NMR data acquired since 2002 with cutting edge instrumentation and novel NIR calibrations will allow the development of an *Internet Database of Soybean Composition* for 15,000 Soybean Accessions from the National Soybean Collection at the University of Illinois, containing the data obtained from over 80,000+ spectroscopic NIR and NMR measurements for: *proteins, amino acids, oils, fatty acids, isoflavones, carbohydrates, fiber, moisture* in intact soybean seeds both in bulk and single soybean seed samples.

This novel **Soybean Composition Database** will also include more than 12,000 NIR measurements on soybeans from the **International Soybean Germplasm Collection**, such as those received from Peking at the National Soybean Collection.

XLS files of our novel spectroscopic data are currently available for *all 80,000+* NIR and FT-NMR measurements—such data will be made available from an ultra-fast and secure supercomputer server utilizing the current version of the *Scientific-Linux* OS-based software.

1. FT-IR and FT-NIR Microspectrometers

A microspectrometer is defined as a combination of a spectrometer and a microscope that has both spectroscopic and imaging capabilities. Such an instrument is capable, for example, of obtaining visible images of a sample using a CCD camera, and chemical images with an NIR detector. Chemical images are then employed for sophisticated quantitative analyses. The results reported in this chapter for soybean seeds and embryos were obtained with FT- IR and -NIR spectrometers made by the PerkinElmer Co. (Shelton, CT, USA). The FT-NIR (NTS model) spectrometer was equipped with an integrating sphere accessory for diffuse reflectance. The FT- IR or -NIR spectrometers were, respectively, attached to microscopes for the IR region (Spotlight 300) or NIR region (NIR Autoimage), as illustrated in Fig. 3.2.1 and Fig. 3.2.2. Each spectrometer has an internal desiccant compartment to remove the water vapor and the carbon dioxide from air that may interfere with the spectrum of a sample. Apart from the improved resolution and acquisition time, these instrument models, offer increased sensitivity and also allow the transfer of spectra to different instruments of similar design. The two microspectrometers are each equipped with two cassegrain imaging objectives and a third cassegrain before the NIR detector in order to improve focus and sensitivity, as shown in Fig. 3.2.3.

Introduced in 2002 by
the PerkinElmer Co.
(Shelton, CT, USA) for
high-resolution studies

Employed for our mid-IR
Microspectroscopy and
Chemical Imaging
investigations of thin
sections of soybean seeds
and embryos

Fig. 1.1. FT-IR Microspectrometer (Spotlight 300) introduced by the PerkinElmer Co.

Introduced in 2002 by PerkinElmer Co. (Shelton, CT, USA) for high-resolution studies

Employed for our NIR Microspectroscopy and Chemical Imaging investigations of soybean seeds and embryos

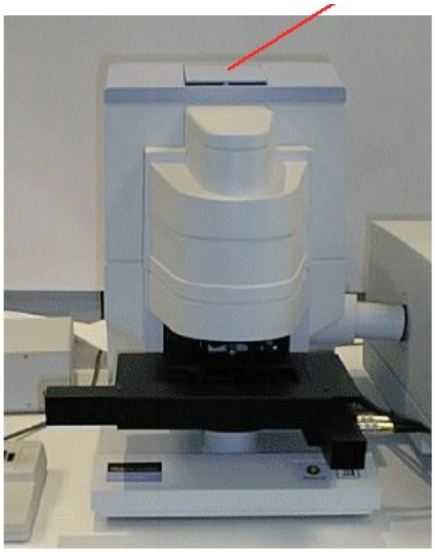


Fig. 1.2. FT-NIR Microspectrometer (AutoImage) made by the PerkinElmer Co.

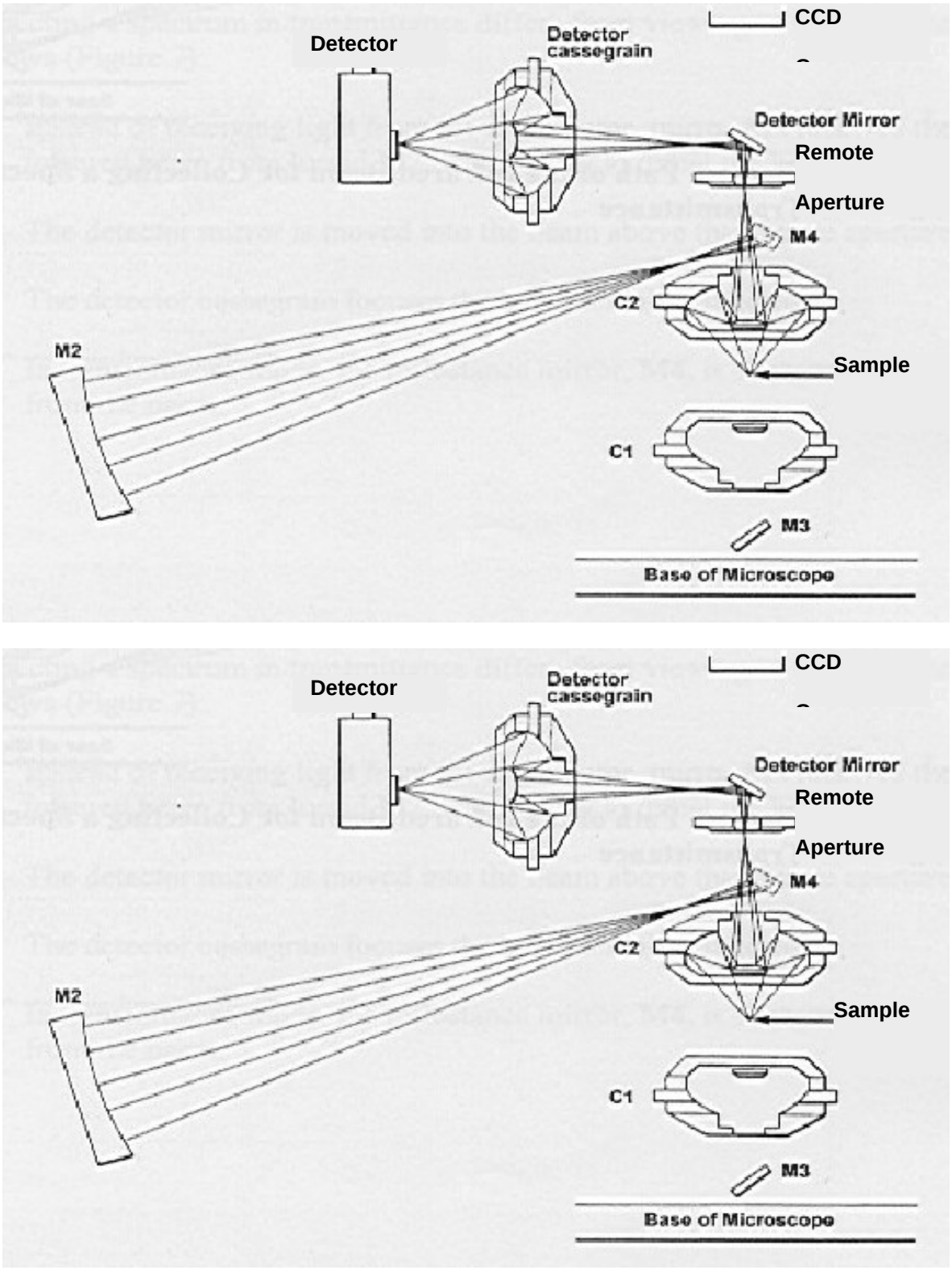


Fig. 1. 3. A simplified diagram of the reflection mode of operation for the AutoImage FT-NIR Microspectrometer, manufactured by the PerkinElmer Co.

Fig.1.4. The FCS Alba™ Microspectrometer System manufactured by ISS Co., (Urbana, Illinois, USA). The inverted, epi-fluorescence microscope shown in the figure is the Nikon TE-300 –special Model, that has available *both* a back illumination port and a left-hand side port. The PC employed for data acquisition, storage and processing is located behind the instrument, as is the laser illumination source (not visible in the figure).

2.1 High-Resolution NMR Method for Oil Determination

The technique applied to obtain the oil content in soybean embryos was simple one-pulse, High-Resolution (HR) NMR (11). The HR-NMR technique was explained in Section 3.4 of Chapter 1x. A Varian U-400 NMR instrument was employed for oil measurements; the selected 90 deg pulse width was 19.4 μs and the ^{1}H NMR signal absorption intensity was recorded with a 4 s recycling interval to avoid saturation.

2.1.1 Oil Determination in Somatic Soybean Embryos by ^{1}H High-Resolution Nuclear Magnetic Resonance

High-resolution ^{1}H NMR measurements of oil were carried out with an U400 MHz NMR spectrometer as described in Section 3. A complete, nanoliter (nL) range oil calibration is reported here for soybean somatic embryos with a measurement precision of ~ $\pm$0.1 % oil (calculated as a percentage of oil from the total, wet embryo weight). These are the first reported results of oil determination by high-resolution NMR in soybean somatic embryogenic cultures. From a 105-sample lot investigated, 89 were considered valid for further growth and selection. A summary of our is presented in Tables 4.3.1 to 4.3.4.

The corresponding quantity of oil in the embryos was calculated from a linear fitting of the HR-NMR data in our standard plot of soybean oil shown in Fig.4.3.1 with the following equation:

$$x = \frac{y + 0.0092}{0.0029},$$

where ***y*** was the normalized value of the oil peak integral from the experimental NMR spectrum of each sample (Fig.2.1.). The ratios of a chemical group proton signal other than water protons, with respect to the water proton signal--and the wet mass of the sample-- were then compared with the oil standard plot in order to estimate the quantity of oil present in soybean embryos.

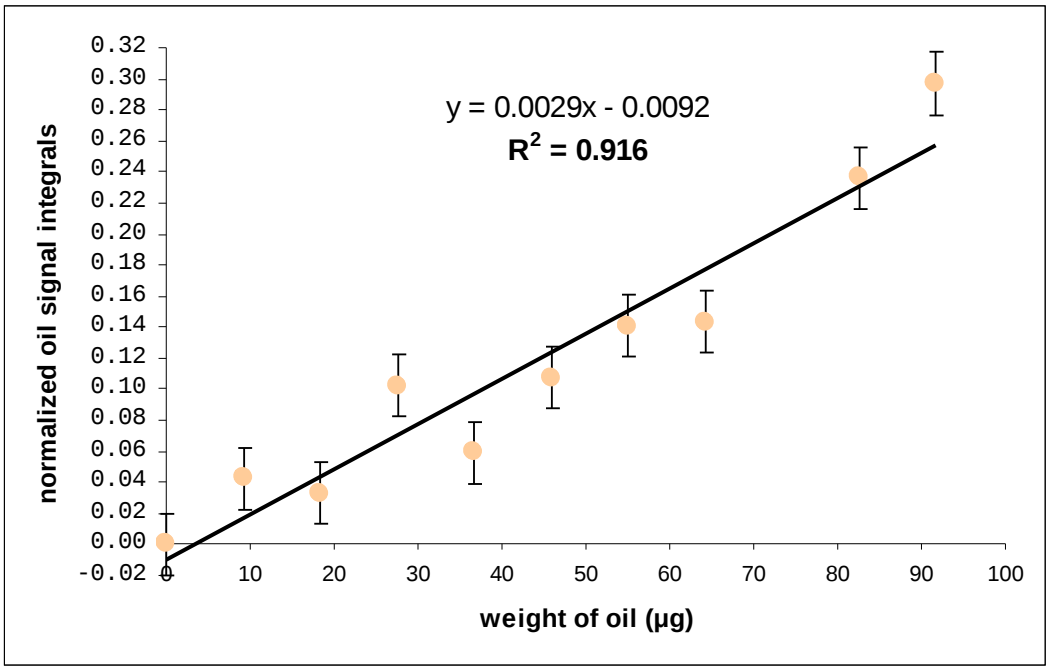


Fig. 2.1. The soybean oil standard plot for 400 MHz ^{1}H NMR measurements on the Varian U400. The probe was a Nalorac 5 mm QUAD for high-resolution

3. Soybean Correlation Data

The 99% linear correlation between the ^{13}C SS-NMR and the corresponding Dual DA-NIRS oil and protein measurements on the same samples of well-defined soybean accessions from the USDA Soybean Germplasm Collection at UIUC, suggests that both techniques are suitable for the non-destructive, practical determination of both oil and protein content of soybean flours.

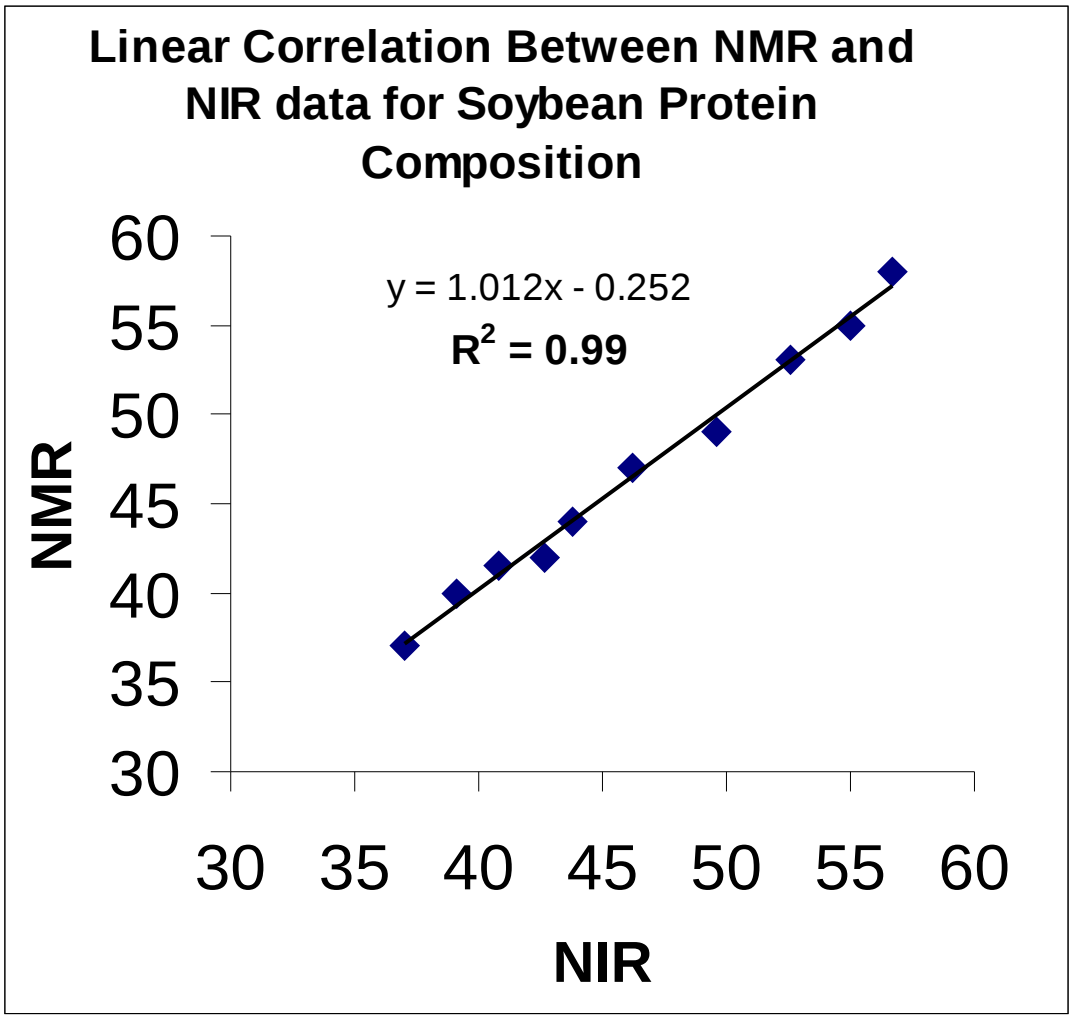


Fig. 3.1. VACP ^{13}C SS-NMR Measurements of Protein Content in Soybean Flours and their direct, Linear Correlation with the Corresponding NIR Data.

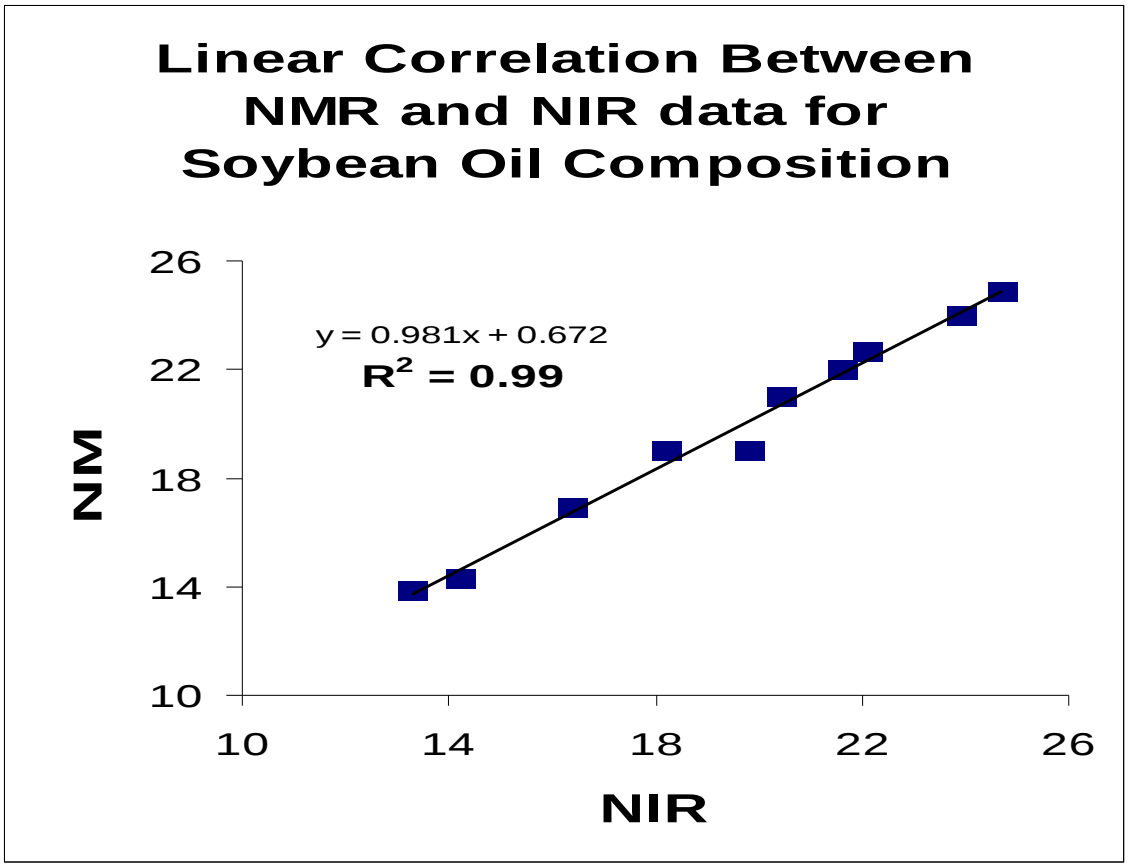


Fig. 3.2. 1PDNA ^{13}C SSNMR of Oil Content of Soybean Flours and their direct linear correlation with the corresponding NIR data.

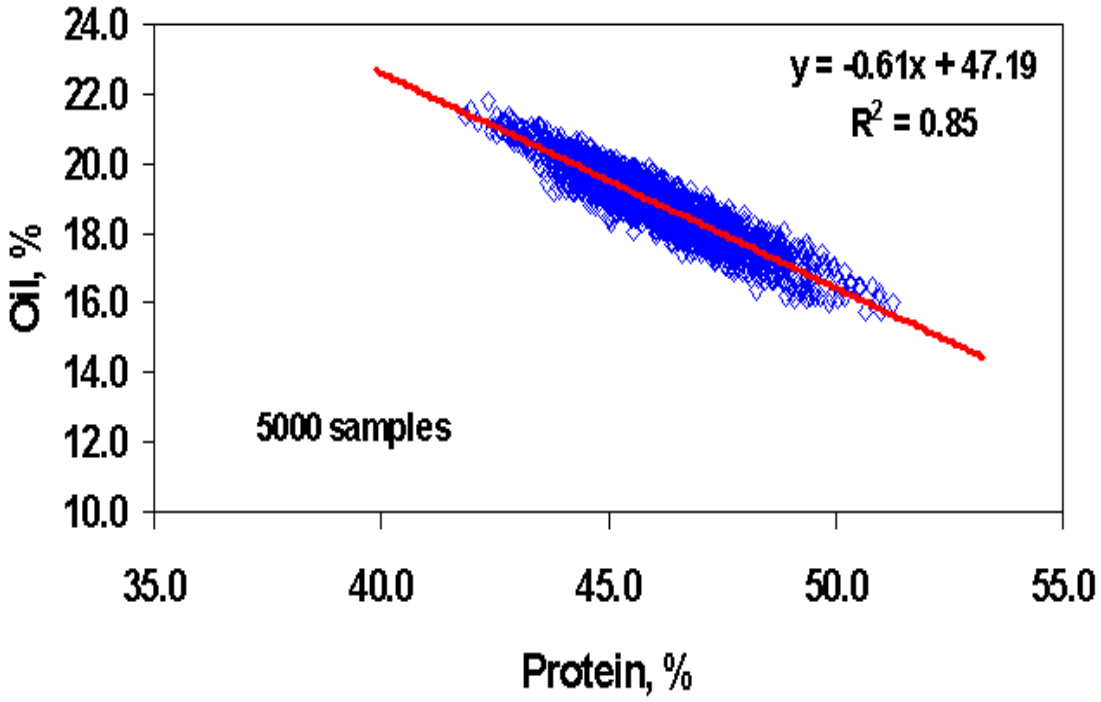


Fig.3.3. Protein-Oil Inverse Correlation of 5,000 Soybean Samples of Experimental Lines at UIUC.

4. EXCEL Data Tables of Soybean Accessions Composition

Table 4.1. VACP ^{13}C SS-NMR Measurements of Protein Content of Soybean Flours and Their Direct Correlation with the NIR Data (as shown in Section 2.6).

Soybean Seed ID	**M2123**	**W2101**	**W2403**	**M309**	**M285**	**96-960A2-2687**	**W1228**	**96-959A6-1447**	**LG00-13523**	**LG00-13251**
% protein by NIR	37.0	39.1	40.8	42.7	43.8	46.2	49.6	52.6	55.0	56.7
% protein by NMR	37.1	40.0	41.5	42.0	44.0	47.0	49.0	53.1	55.0	58.0

Table 4.2. **Range of constituents for 66 selected soybean samples selected as standards for Fatty Acid NIR Calibrations (data is courtesy of the USDA Peoria Laboratory). (Soybean lines are identified as: Stoneville 1999, MG V–VIII. USDA Germplasm, National Research Center, Urbana, IL.)**

Simple Statistics	**Dry Protein**	**Dry Oil**	**Moisture**	**%16:0**	**% 18:0**	**% 18:1**	**% 18:2**	**% 18:3**
Mean	47.61	17.11	5.86	11.91	3.24	21.19	55.42	8.24
Stdev	2.03	1.21	0.17	0.70	0.49	2.51	2.08	1.03
Max	53.74	19.32	6.19	14.10	4.35	27.63	60.74	10.93
Min	43.80	13.70	5.48	10.54	2.33	14.24	51.09	6.32

Table 4.3. HR-NMR results for the Control (0 mM EMS) Soybean Embryo Group.

EMS solution conc. (mM)	Sample ID	Number of transients	Embryo mass (mg)	Normalized oil signal integral in the 3 ppm-0 ppm region	Quantity of oil in embryos (nL/mg)	Percentage of Oil in wet embryos (%)
0	6-29-0A2-10-5	500	9.80	0.0357	18	1.7
0	6-23-0A2-10-1	500	10.90	0.0269	14	1.3
0	7-11-0A2,0C2,8	500	10.79	0.0255	13	1.2
0	5-14-4HR-W0	500	7.82	0.0232	12	1.1
0	6-19-0A2-10-15	500	8.90	0.0196	11	1.0
0	4-3-0A6	500	20.52	0.0162	10	0.9
0	6-19--0B2-11-13	500	10.30	0.0153	9	0.8
0	6-19-0B1-10-15	500	11.20	0.0141	9	0.8
0	7-25-0A5,0B8,0C7	500	21.01	0.0119	8	0.7
0	6-26-0B5-12-4	500	14.80	0.0083	6	0.6
0	6-23-0B3-9-15	500	21.50	0.0072	6	0.6
0	6-26-0B1-12-4	500	24.80	0.0052	5	0.5
0	6-26-0B4-12-4	500	26.70	0.0040	5	0.5
0	6-23-0A3-11-2	500	19.50	0.0010	3	0.3

Table 4.4. HR-NMR results for the 1 mM EMS group.

EMS solution conc. (mM)	Sample ID	Number of transients	Embryo mass (mg)	Normalized oil signal integral in the 3 ppm-0 ppm region	Quantity of oil in embryos (nL/mg)	Percentage of Oil in wet embryos (%)
1	4-3-1A10,11,1C7	500	7.61	0.0598	27	2.5
1	6-23-1A2-10-15	500	6.80	0.0451	21	1.9
1	6-23-1B4-10-15	500	6.70	0.0290	15	1.4
1	7-11-1A8,1B7	500	14.79	0.0254	13	1.2
1	7-25-1A10,1B2,6,7	500	10.91	0.0241	13	1.2
1	7-25-1C3,9	500	12.56	0.0214	12	1.1
1	6-19-1A5-10-5	500	11.40	0.0192	11	1.0
1	4-3-1A5,6,11-1B10	500	18.58	0.0137	9	0.8
1	6-19-1A7-11-2	500	11.80	0.0127	8	0.7
1	4-3-1A5-9-17-s	500	57.82	0.0067	6	0.6
1	6-19-1A12-11-13	500	11.90	0.0065	6	0.6
1	6-19-1A6-11-2	500	12.60	0.0013	3	0.3

Table 4.5. Average values of oil content (wet %) in somatic embryogenic cultures of soybean samples, measured by 1PULSE ^{1}H NMR experiments, and variation range.

Embryo Oil Average Values				
0 mM EMS Oil, %	**1 mM EMS Oil,%**	**3 mM EMS Oil, %**	**10 mM EMS Oil,%**	**30 mM EMS Oil,%**
0.8	1.1	0.7	1.2	0.9
Ranges				
3-17	3-25	4-11	4-17	3-25

Table 4.6.

Mean values and variances of protein and oil for 17 Bulk Soybean groups.

Soybean groups and instruments used	Sample number	P-mean	P-var	O-mean	O-var
Germplasm98, DA7000	400	46.2	13.55	19.6	2.23
MAPIII97-mix, F3, DA7000	496	43.8	5.53	21.4	1.50
MAPIII98-mix, F3, DA7000	529	45.1	6.56	20.1	1.73
MAPIII97-Bell, F3, IM9100	707	47.3	3.84	19.7	1.25
MAPIII97-Fisher, F3, IM9100	704	46.7	3.85	20.0	1.03
MAPIII97-EG1000, F3, IM9100	703	43.0	3.80	21.9	0.95
OPMAP98, F3, IM9100	379	44.5	6.19	20.8	2.54
Germplasm99, ZX-50	961	44.9	2.61	19.4	0.96
99ProSel-F3, ZX-50	72	51.2	3.69	16.7	0.28
99ProSel-F4, ZX-50	206	49.1	4.40	17.7	0.90
OPMAP99-Hume, F3, ZX-800	495	47.2	6.83	17.9	2.76
OPMAP99-Bell, F3, ZX-800	490	47.0	6.91	17.7	3.07
99ProGen, F5, ZX-800	362	49.3	6.31	16.9	1.71
2000YLDMAP-Hume, F6, ZX-800	380	42.9	1.68	22.0	0.55
2000YLDMAP-MIV, F6, ZX-800	380	42.0	2.70	22.4	0.75
2000 ProSel and BCPro, F8, ZX-800	79	51.3	3.67	16.5	1.14
2000 ProSel, F9, ZX-800	24	54.6	0.99	14.2	0.27

Note 1: P-mean, P-var, O-mean, O-var represent protein mean, protein variance, oil mean, oil variance, respectively.

5. NIRS Basic Results

Figure 5.1. Pure Component FT-NIR Spectra of Major Soybean Constituents: Protein, Oil, Moisture

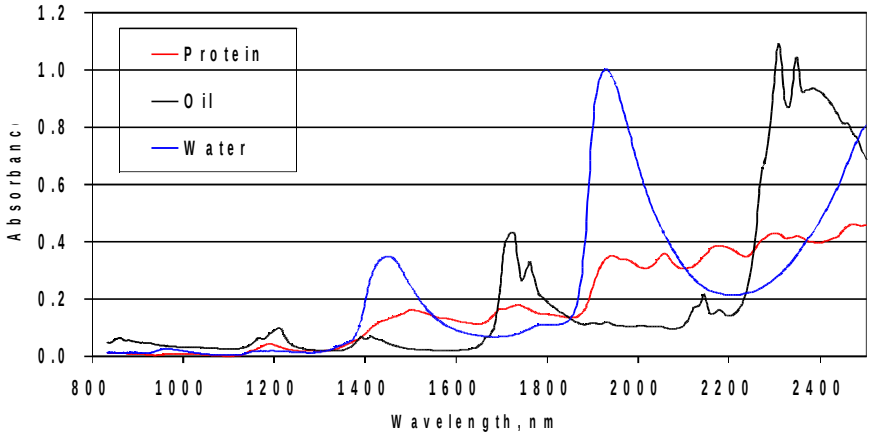


Figure 5.2.

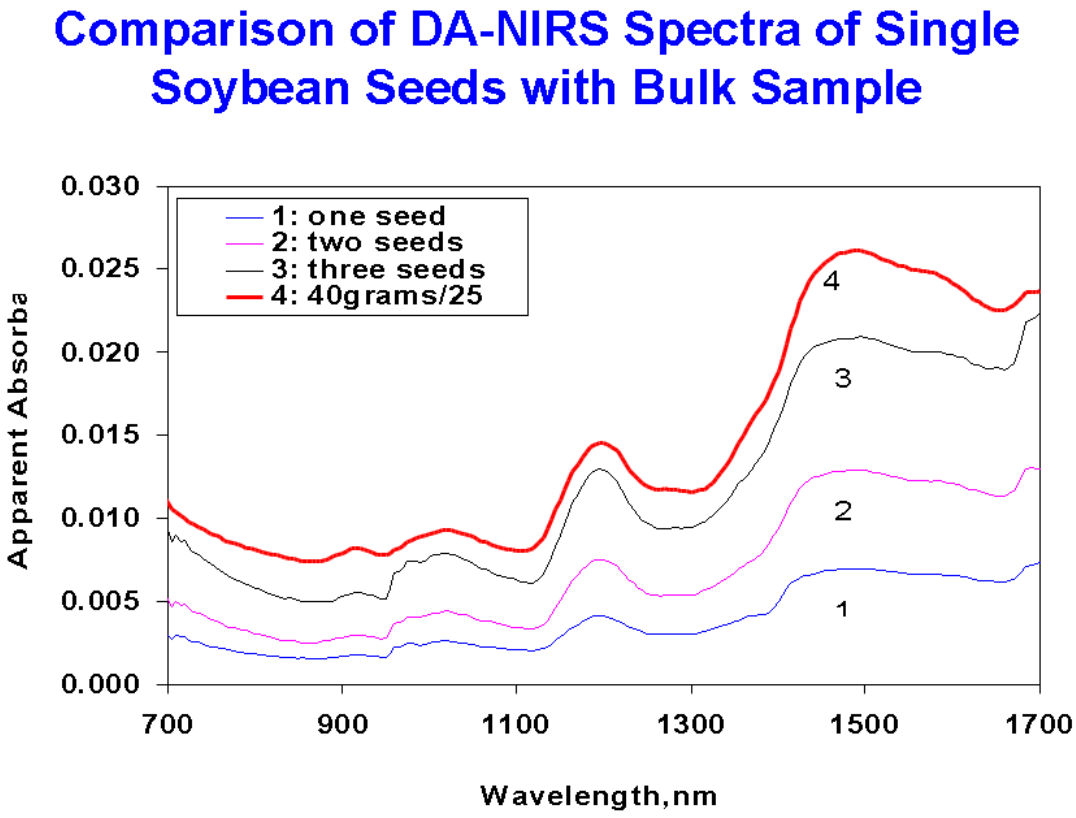


Figure 5.3. NIR Predicted vs Reference Protein Values by Nicolet Antaris Single Seed Calibration

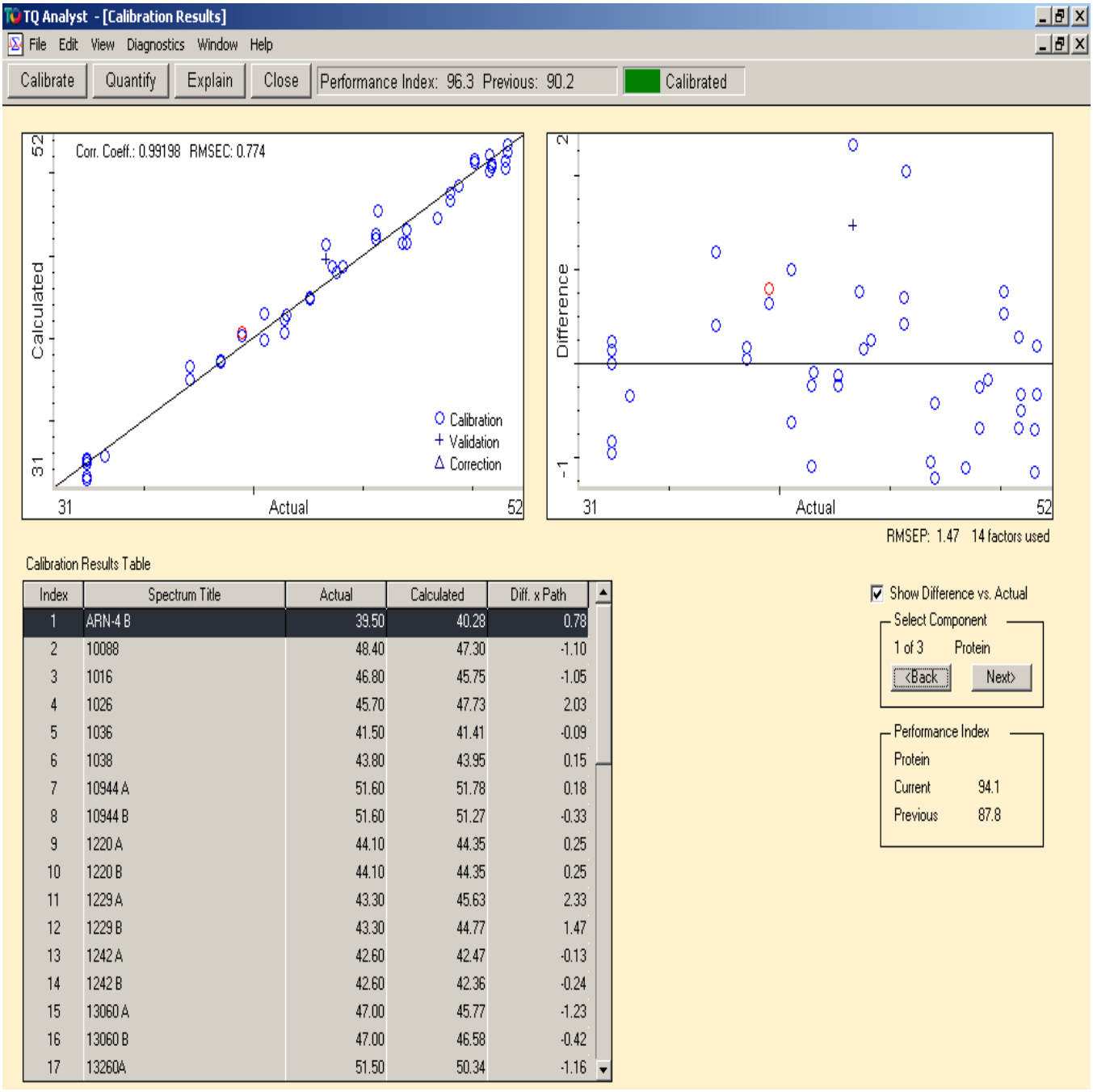
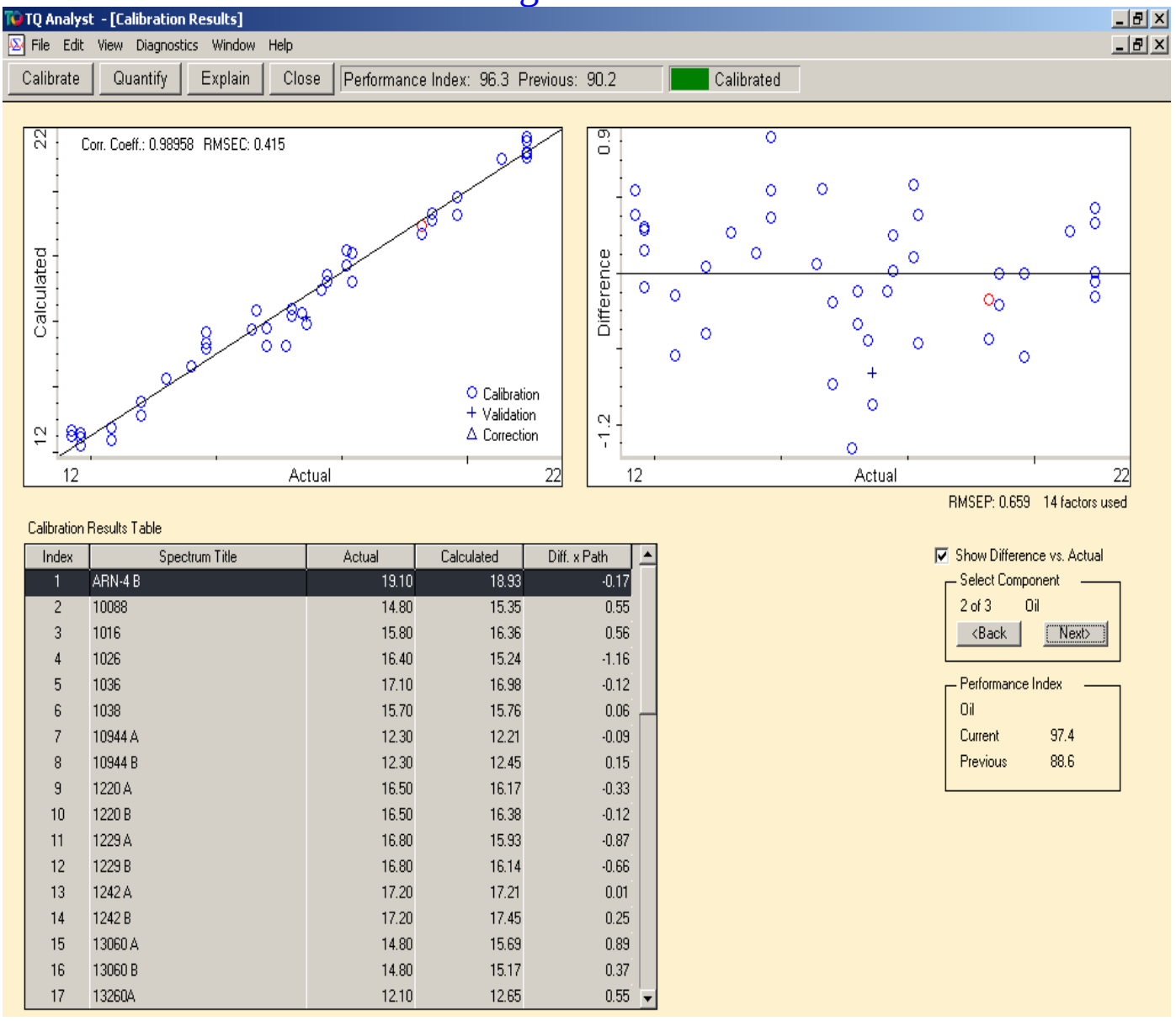


Figure 5.4. NIR Predicted vs Reference Oil Values by Nicolet Antaris Single Seed Calibration



Index	Spectrum Title	Actual	Calculated	Diff. x Path
1	ARN-4 B	19.10	18.93	-0.17
2	10088	14.80	15.35	0.55
3	1016	15.80	16.36	0.56
4	1026	16.40	15.24	-1.16
5	1036	17.10	16.98	-0.12
6	1038	15.70	15.76	0.06
7	10944 A	12.30	12.21	-0.09
8	10944 B	12.30	12.45	0.15
9	1220 A	16.50	16.17	-0.33
10	1220 B	16.50	16.38	-0.12
11	1229 A	16.80	15.93	-0.87
12	1229 B	16.80	16.14	-0.66
13	1242 A	17.20	17.21	0.01
14	1242 B	17.20	17.45	0.25
15	13060 A	14.80	15.69	0.89
16	13060 B	14.80	15.17	0.37
17	13260A	12.10	12.65	0.55

Table 5.1.

Correlation Coefficients (R) and Standard Errors of Cross Validation (SECV) for Single Seeds Analysis on the DA-7000 Instrument

Component	Number of Factors	R	SECV
Protein	15	98.5%	1.1
Oil	16	98.5%	0.5
Moisture	16	99.0%	0.3

Table 5.2.

Correlation Coefficients (R) and Standard Error of Cross Validation (SECV) for Single Seed Analysis on the Nicolet Antaris FT-NIR Instrument

Component	Number of Factors	R	SECV
Protein	14	99.2%	0.77
Oil	14	99.0%	0.42
Moisture	11	99.1%	0.27

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Table 5.3.

Correlation Coefficients (R) and Standard Errors of Cross Validation (SECV) for Single Seeds Analysis on the DA-7000 Instrument

Component	Number of Factors	R	SECV
Protein	15	98.5%	1.1
Oil	16	98.5%	0.5
Moisture	16	99.0%	0.3

Figure 5.5.

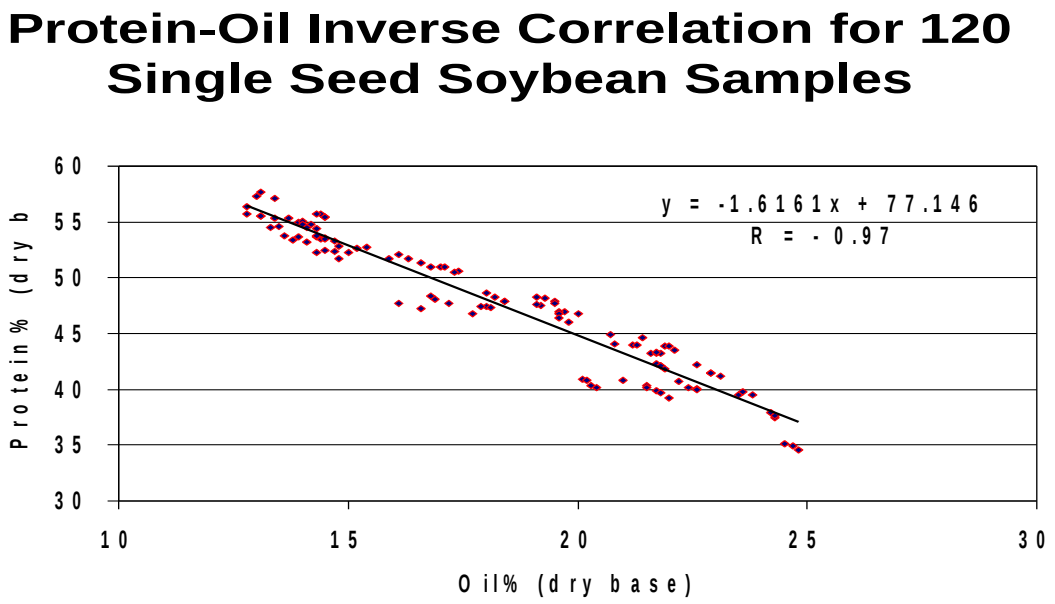


Figure 5.6.

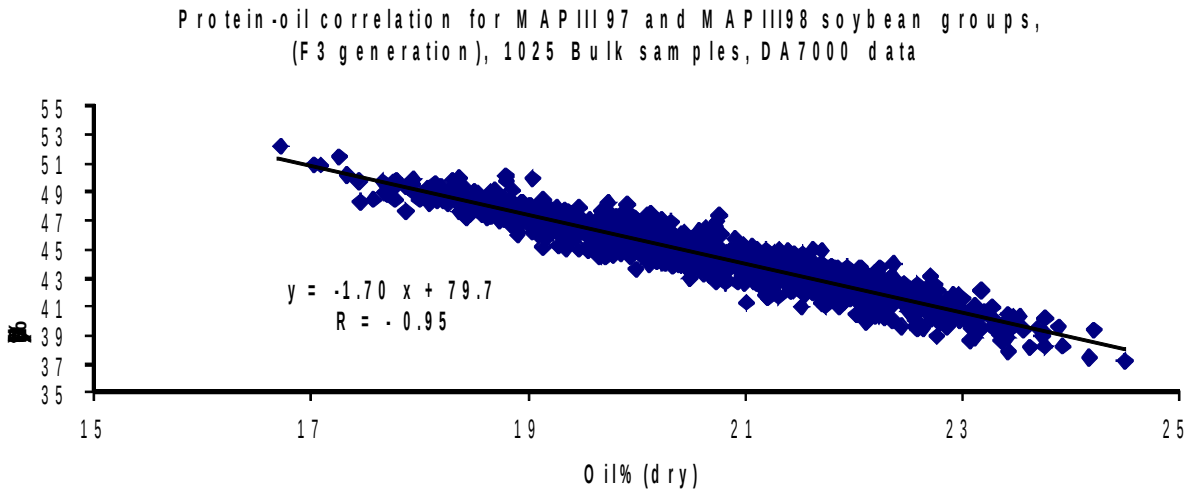


Figure 5.7.

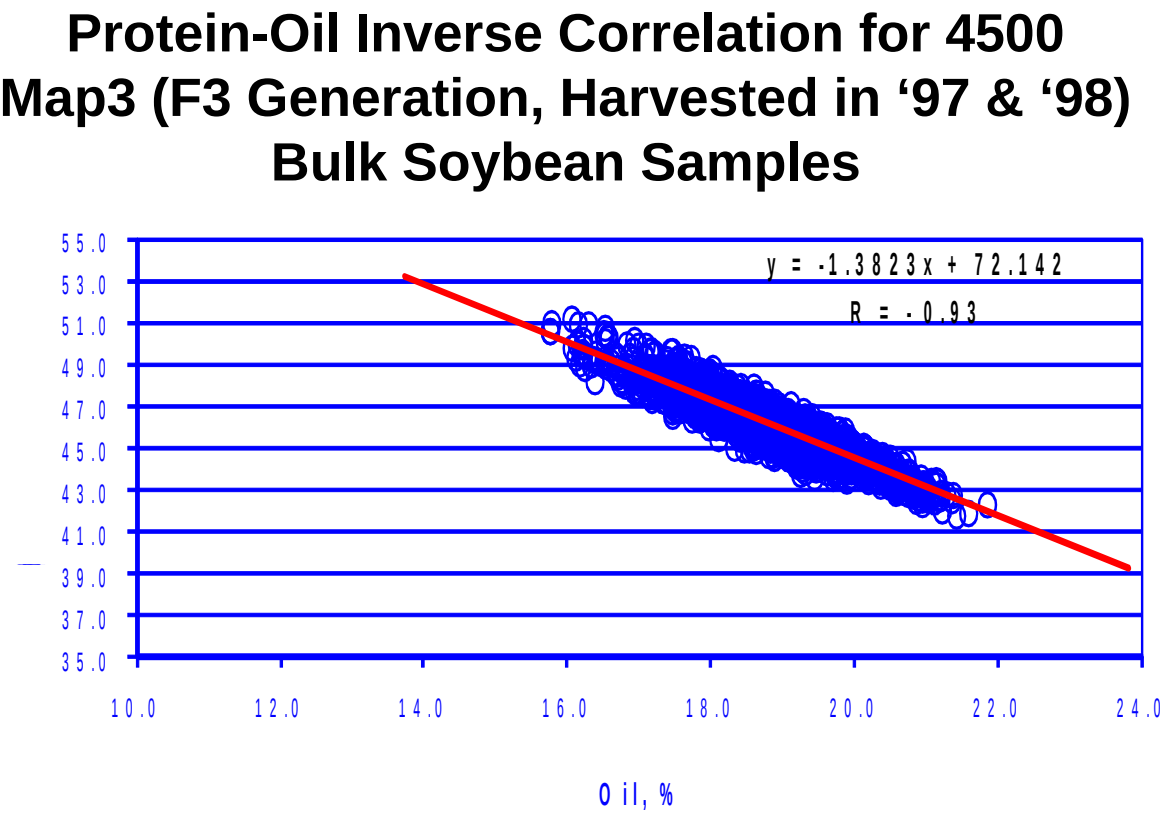


Figure 5.8.

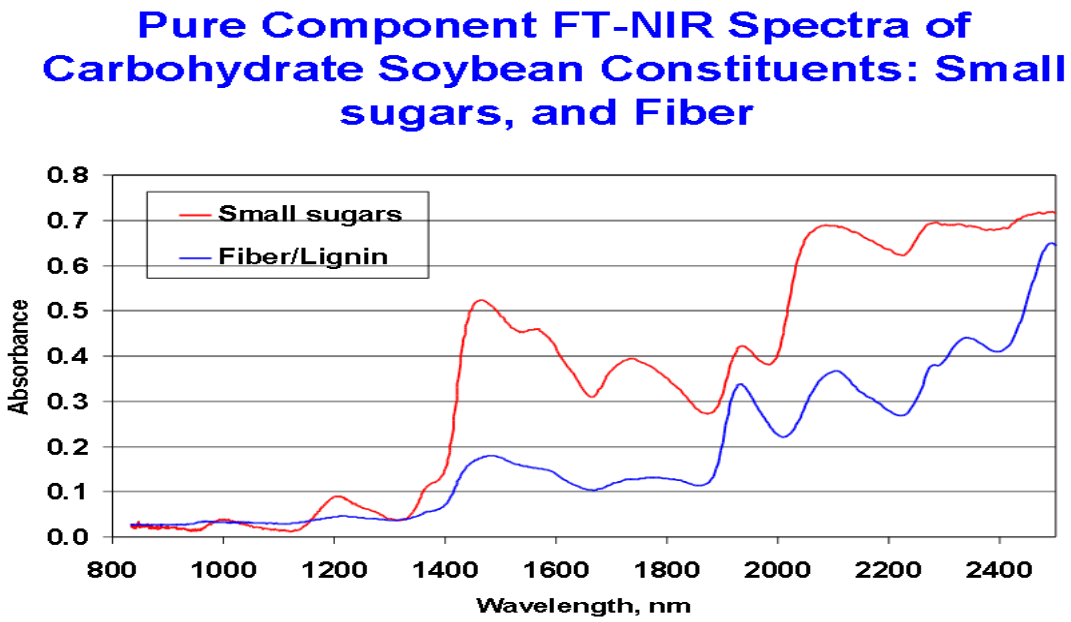
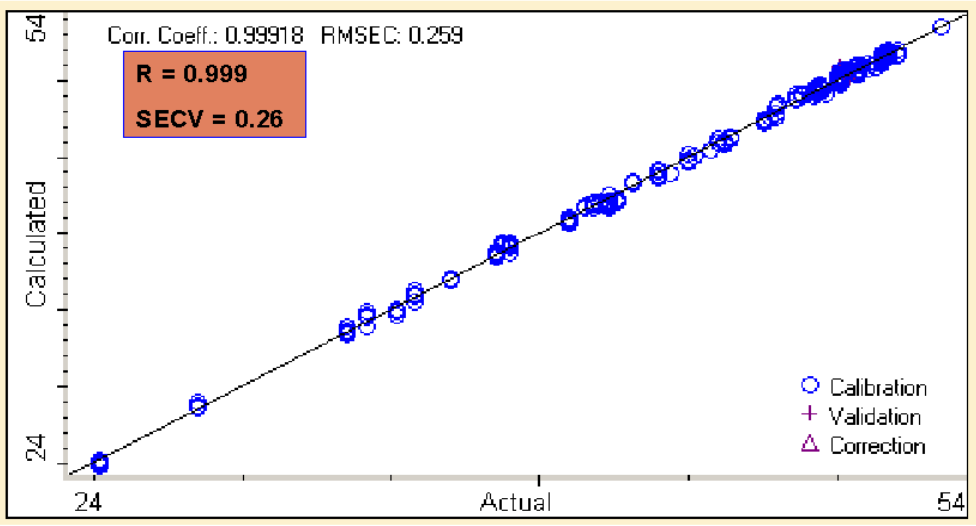


Figure 5.9.

Protein Calibration for Bulk Soybean Analysis on the Spectrum One NTS FT-NIR Instrument

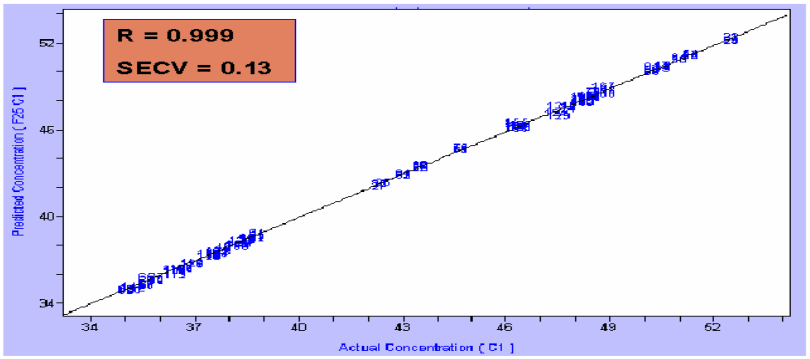


65 calibration standards, 20 grams for each standard, 8.9mm NIR beam size

Source: Soybean NIR Database, UIUC

Figure 5.10.

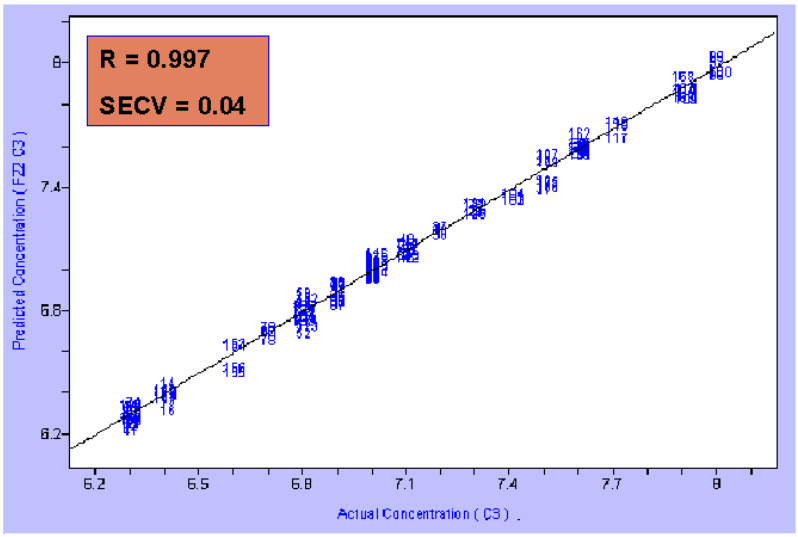
Protein Calibration for Bulk Soybean Analysis on the DA-7000 Diode Array NIR Instrument



Source: Soybean NIR Database, UIUC

Figure 5.11.

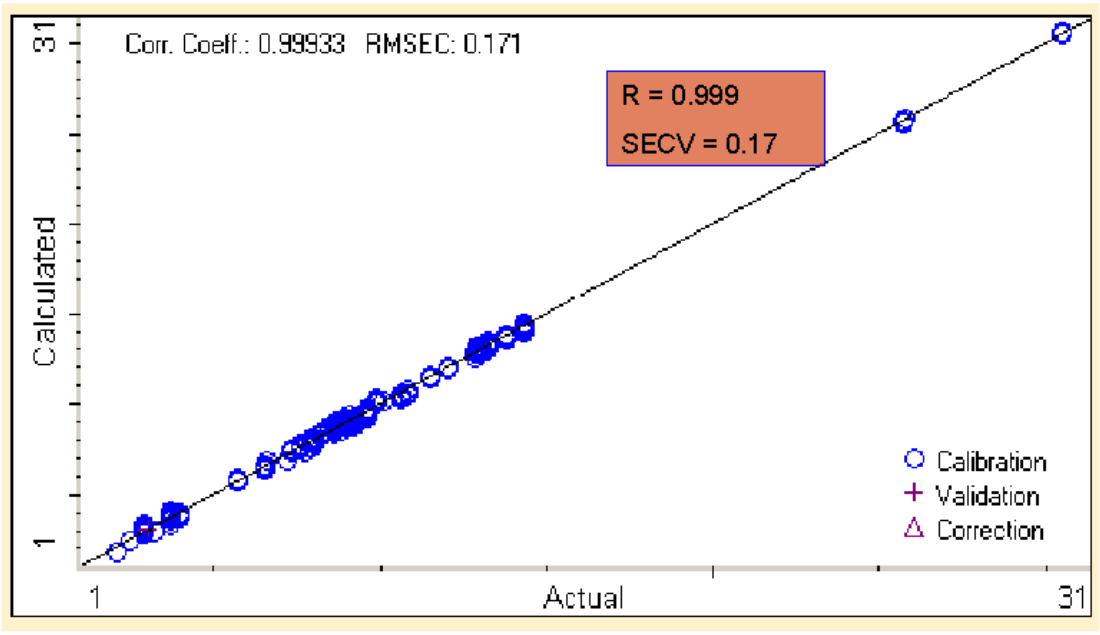
Moisture Calibration for Bulk Soybean Analysis on the DA-7000 Diode Array NIR Instrument



Source: Soybean NIR Database, UIUC

Figure 5.12.

Moisture Calibration for Bulk Soybean Analysis on the Spectrum One NTS FT-NIR Instrument



65 calibration standards, 20 grams for each standard, 9mm NIR beam size

Source: Soybean NIR Database, UIUC

Table 5.4.

Calibration Results for Protein and Oil Analysis with the Perten DA-7000, Dual Diode-Array DA-NIR Instrument

Components	Protein		Oil	
	Bulk Sample	Single Seeds	Bulk Sample	Single Seeds
SECV	0.1	1.1	0.1	0.5
R	99.9%	98.5%	99.9%	98.5%

Source: Soybean NIR Database, UIUC

Table 5.5.

Bulk Soybean Calibration with 65 Standards for Protein, Oil, and Moisture Analysis

Developed with Data from the SpectrumOne NTS FT-NIR Spectrometer

Component	Number of Factors	R	SECV	SEP
Protein	13	99.9%	0.26	0.33
Oil	15	99.9%	0.13	0.23
Moisture	15	99.9%	0.17	0.30

R : Correlation Coefficient
SECV: Standard Error of Cross Validation
SEP: Standard Error of Prediction

Source: Soybean NIR Database, UIUC

Table 5.6.

Detrimental Effects of Light Scattering on the Accuracy of NIR Analysis for Whole Soybeans

(measured with the FT-NIR, SpectrumOne NTS Spectrometer)

	R		SECV	
Component	No MSC	MSC	**No MSC**	**MSC**
Protein	99.5	99.9	**0.63**	**0.26**
Oil	99.3	99.9	**0.29**	**0.13**
Moisture	99.8	99.9	**0.26**	**0.17**

R: Correlation coefficient
SECV: Standard Error of Cross Validation
(Tested with 65 bulk whole soybean standards)

Source: T. You, 2006

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6. AMINO ACID COMPOSITION OF SOYBEAN SEEDS: NIR CALIBRATIONS

Table 6.1.

Correlations of Amino Acids with Crude Protein in Soybeans

A.A.	R^2	R
ASX	0.634	0.796
THR	0.005	0.071
SER	0.578	0.760
GLX	0.690	0.831
PRO	0.480	0.693
GLY	0.628	0.792
ALA	0.619	0.787
VAL	0.640	0.800
ILE	0.631	0.794

A.A.	R^2	R
LEU	0.644	0.802
TYR	0.536	0.732
PHE	0.474	0.689
HIS	0.499	0.706
LYS	0.652	0.807
ARG	0.687	0.829
MET	0.548	0.740
CYS	0.498	0.706
M+C	0.538	0.733

Table 6.2.

Amino Acids **Highly-Correlated** with the Dry Protein Content:

- Histidine: **R = 0.93**
- Arginine: **R = 0.90**
- Glx: **R = 0.88**
- Valine: **R = 0.87**
- Leucine: R = *0.85* → *borderline*

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and the imino acid Proline: R = **0.87**

Figure 6. 1.

Histidine: His vs. % Dry Protein

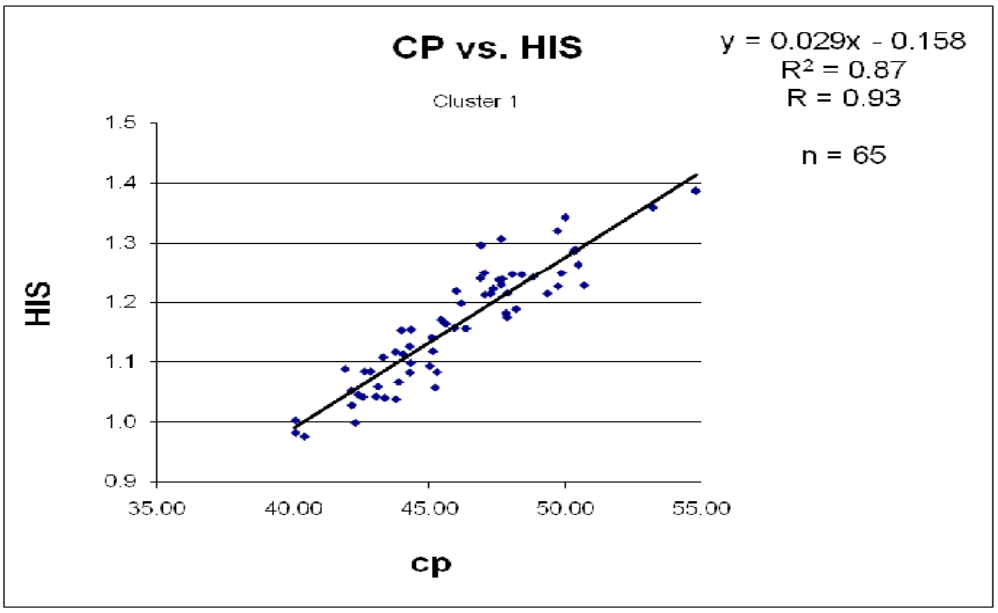


Figure 6.2.

Glutamine plus Glutamic Acid, "GLX", as Total Dry Weight % vs. Dry Soybean Protein %

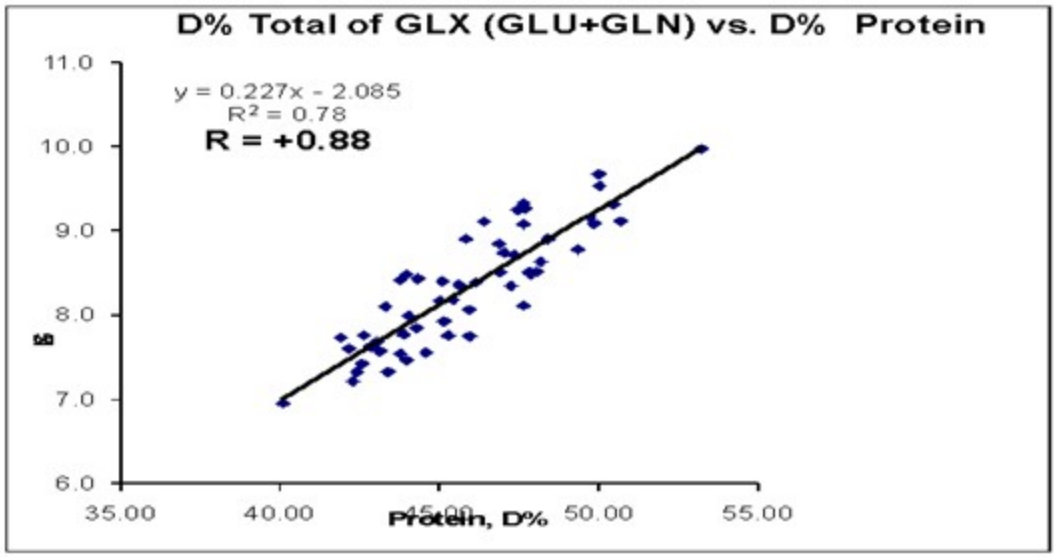


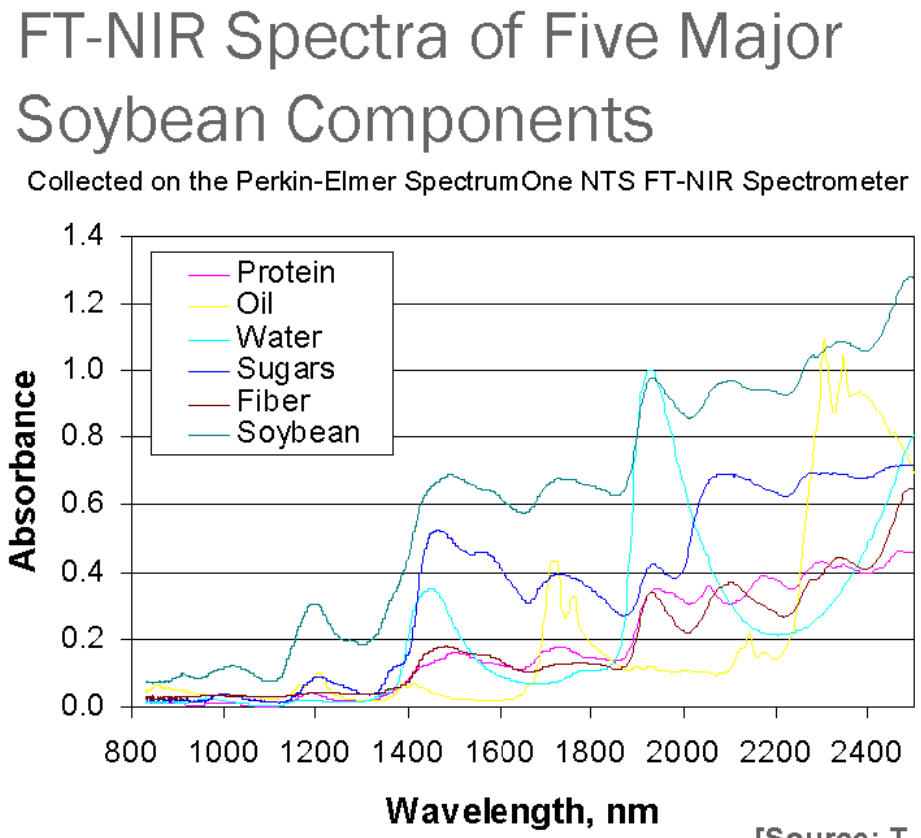
Table 6.3.

VIP-Data **Example:**

10 Data Points **TOTAL: 3,816 data-points**

Prot	Moist	Oil	DP	DP calc	DO calc
35.57	11.10	20.50	40.01	40.01	23.06
36.91	10.00	20.76	41.00	41.01	23.07
41.75	10.41	17.06	46.60	46.60	19.04
41.44	10.30	10.02	46.20	46.20	11.17
41.10	10.16	18.60	45.75	45.75	20.70
36.71	9.12	20.60	40.40	40.39	22.67
38.03	10.84	20.24	42.65	42.65	22.70
39.52	10.44	19.34	44.13	44.13	21.59
40.17	9.04	18.80		44.16	20.67
40.67	10.25	18.30	45.31	45.31	20.39

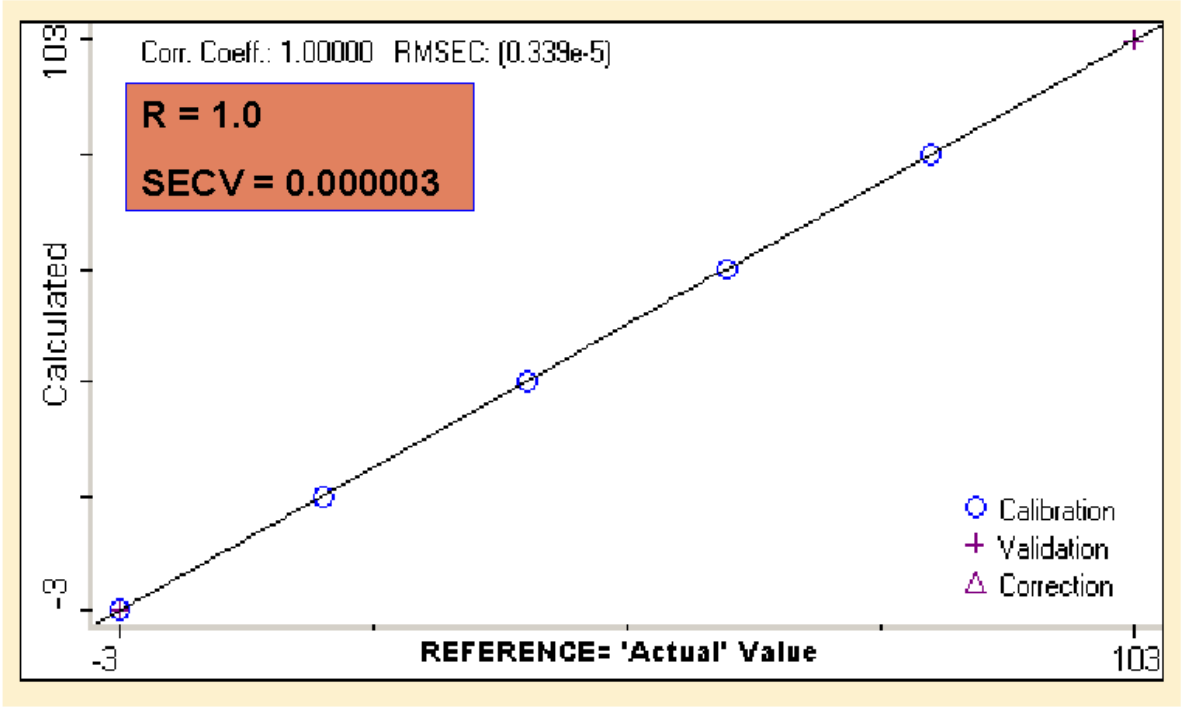
Figure 6.3.



[Source: T. You, 2006]

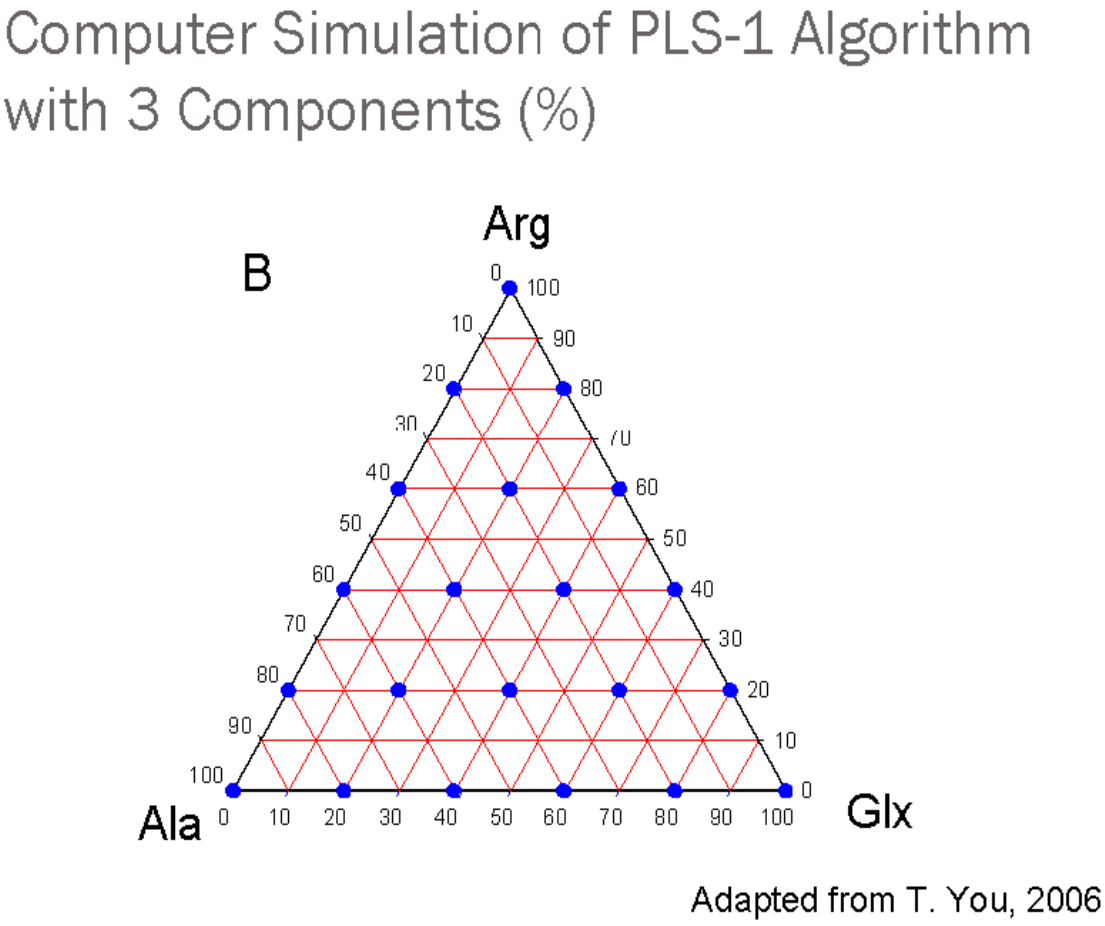
Figure 6.4,

Predicted vs. Reference Concentrations of Component C1 for our PLS-1 Simulation Study



Source: T. You, 2006

Figure 6.5. Matrix NIR Analysis setup for three AA components.

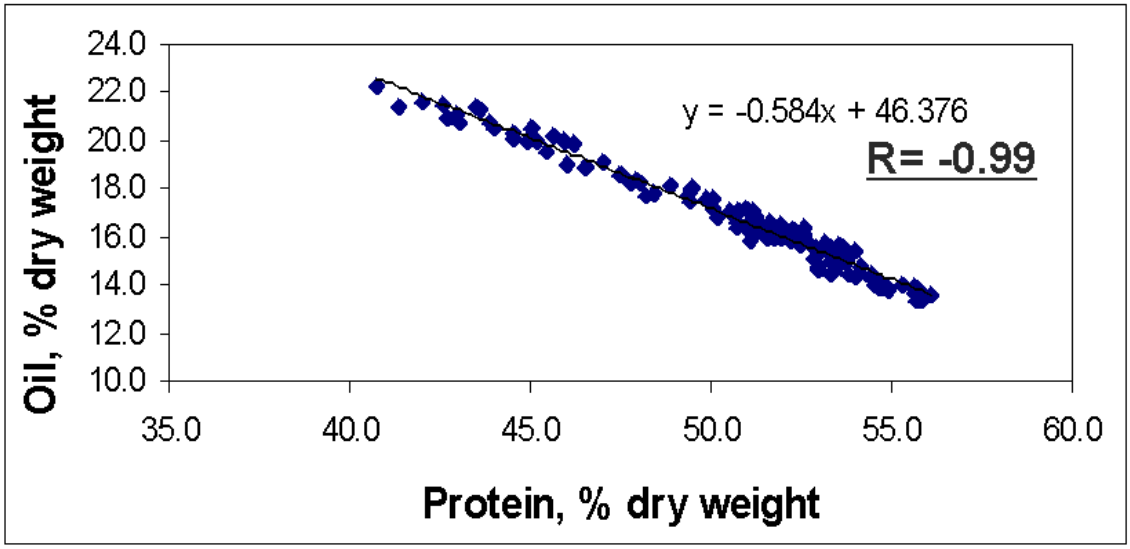


7. NEW SOYBEAN SEED NIR CALIBRATIONS

Figure 7.1.

A new and Improved Set of 124 Bulk Soybean Standard Samples:

Protein-Oil Inverse Correlation for the Year 2003 Calibration Standard



* 124 Standard samples were selected with a wide range of Protein and Oil concentrations that were uniformly distributed in 0.5% concentration steps for Protein, and in 0.2% steps for Oil. Source: Soybean NIR Database, UIUC

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Near Infrared Microspectroscopy, Fluorescence Microspectroscopy, Infrared Chemical Imaging and High-Resolution Nuclear Magnetic Resonance Analysis of Soybean Seeds, Somatic Embryos and Single Cells

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Novel Techniques for Microspectroscopy and Chemical Imaging Analysis of Soybean Seeds and Embryos

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