Maintaining Consistency of Results for Analytical Data from Laboratories Supporting Canola Plant Breeding Programs[1]

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Introduction

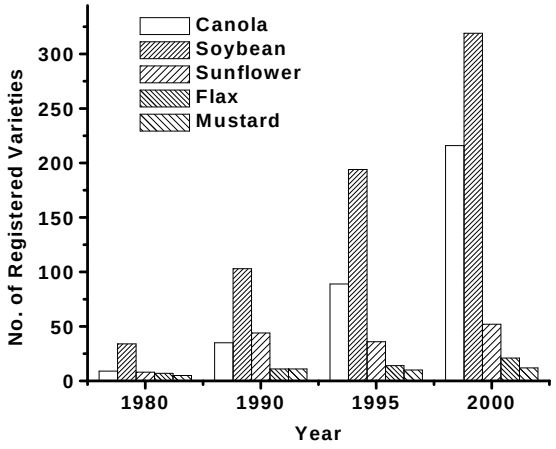

Figure 1. Varieties of canola and rapeseed registered in Canada.

In Canada, varieties of canola must be registered with the Canadian Food Inspection Agency in order to be sold by name. Part of the registration process involves evaluation of data collected on candidate cultivars in both private and public tests. In recent years, the number of varieties registered for production in Canada has increased dramatically (Figure 1). This is a direct result of plant breeders' rights legislation that resulted in a proliferation of private plant breeding laboratories. The increase in the number of plant breeders participating in the process was further complicated by the increasing complexity of the canola and rapeseed testing system. In addition to the regular *B. napus* and *B. rapa* canola lines, there are tests within each species for specialty oil type canolas, herbicide tolerant canolas which may be conducted in up to five different growing zones across western Canada. The canola public cooperative report for the past year's trials included a total of 149 pages of information (Table 1).

Table 1. Increase in complexity of the Canadian variety testing system for canola and rapeseed. Summary of the public cooperative test over the past 25 years.

Year	Entry Sources	Entries		Sites[a]	Data Pages
		B. napus	B. rapa		
1975	3	10	9	26	17
1985	5	11	7	30	56
1995	11	48	30	21 (10)	69
2000	13	72	1	30 (14)	149

[a]Brackets indicate number of sub-tests. In 2000 there were another 979 private trials

The compositional data required for evaluating candidate cultivars grown in both the public and private tests comes from a number of different laboratories. In order to ensure that this data is consistent between laboratories and meets standards for repeatability, the WCC/RRC

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requires that these laboratories participate in a certification program administered by the Canadian Grain Commission's Grain Research Laboratory.

When the program started in 1994, data was required on oil content and protein content as components of the merit score system developed under the rules of the WCC/RRC (Western Canada Canola/Rapeseed Recommending Committee Incorporated, 93). The rules also establish minimum requirements (i.e. maximum levels) for glucosinolates and erucic acid in canola and, more recently, total saturated fatty acids. Chlorophyll analysis has been established as a supplemental test. The analytical data for a candidate cultivar is not used on an absolute basis but rather is reported relative to analytical data for check varieties grown in the same location. It is still important, however, that the results be within acceptable standards for accuracy and precision. This will ensure that coefficient of variability for analytical data from one laboratory is comparable to that for other laboratories.

The objectives of the certification program were to;achieve consistency in information received from different laboratories; assist laboratories to develop precise and accurate methods and ;evaluate new methodologies which might be used in plant breeding programs.

This report summarizes the process and the success of the program between the years 1995 and 2000.

The Certification Process

Unlike certification under ISO or other organizations, the process to become certified to present data in support of registration is relatively inexpensive and easy. There are no laboratory visits or audits. Laboratories simply must demonstrate their ability to perform up to an analytical standard on a set of samples provided. Those that do not perform to standard are assisted to make the necessary modifications to their process to bring them into conformity. Laboratories are required to establish an ongoing internal check sample program and to provide the data from that program when required. While this is rather simple, the drawback is that the certification is very narrow. Even so, several laboratories that do not wish to submit data in support of registration have participated in recent years, possibly as a means of providing supporting evidence to a more rigorous certification.

The annual cost of the program to participating laboratories is either $500 or $700 depending on whether one or two sets of data will be submitted. The funds are used to hire a summer student who administers the project and who also carries out research on projects related to the analysis of canola and rapeseed.

The general timeline for the process is as follows:

March/April	Laboratories inform the GRL of their desire to participate in the program
May/June	Methods and samples sent to laboratories
August/September	Results sent to GRL
September/October	Laboratories informed of results .

Table 2. Origins of laboratories participating in the 2000 study.

Area	Participants (2000)
Canada	18
Ontario (3), Manitoba (5), Saskatchewan (7), Alberta (4)	
USA	3
Europe	5
France(1), Germany(1), Sweden (1), Denmark (2)	

When a laboratory joins the program, it is assigned a random number which it keeps while it is participating in the program. In reporting, laboratories are referred to by random number only. In 2000, a total of 26 different laboratories participated in the program. These laboratories were

located in Canada, the United States and in Europe.

It is notable that, the distribution of laboratories in 2000 fits the requirements for round robin studies under ISO.

Sampling Scheme

The sampling scheme is designed to cover the range of the analysis normally encountered. In addition, samples are selected in pairs about one unit of repeatability apart so that laboratories will have difficulty detecting the blind duplicates.

For oil and protein content 12 randomly numbered samples are sent to each laboratory (6 blind duplicates covering the expected range of about 39% to 50% oil. The strong inverse relationship between oil and protein has made it possible to cover both these analyses with one set of samples. In addition, each laboratory receives tow reference samples with results from the Grain Research Laboratory. Usually these are samples used by the GRL as internal checks. One reference protein sample is also sent for those laboratories using combustion analyzers to be certain that their instrument is calibrated properly.

For chlorophyll, five blind duplicate numbered samples covering range of interest (10 mg/kg to 75 mg/kg) are sent. Since chlorophyll is an optional test, many laboratories do not participate and many of those who do are not well equipped to carry out the analysis.Similar to oil and protein, the glucosinolate and fatty acid composition samples consist of 12 samples (6 blind duplicates covering the expected range of 0.2 to 1.0% erucic acid, 6% to 7.5% total saturates and 10μM/g to 20μM/g glucosinolates). It is sometimes difficult to include good pairs of samples but if possible each range will include 2 samples with close values. In addition, 2 reference samples with GRL results for fatty acid composition and total glucosinolates are included. Beginning in 2001, samples of high oleic acid canola will be included and it is anticipated that samples of *B. juncea* canola will be included in future years.

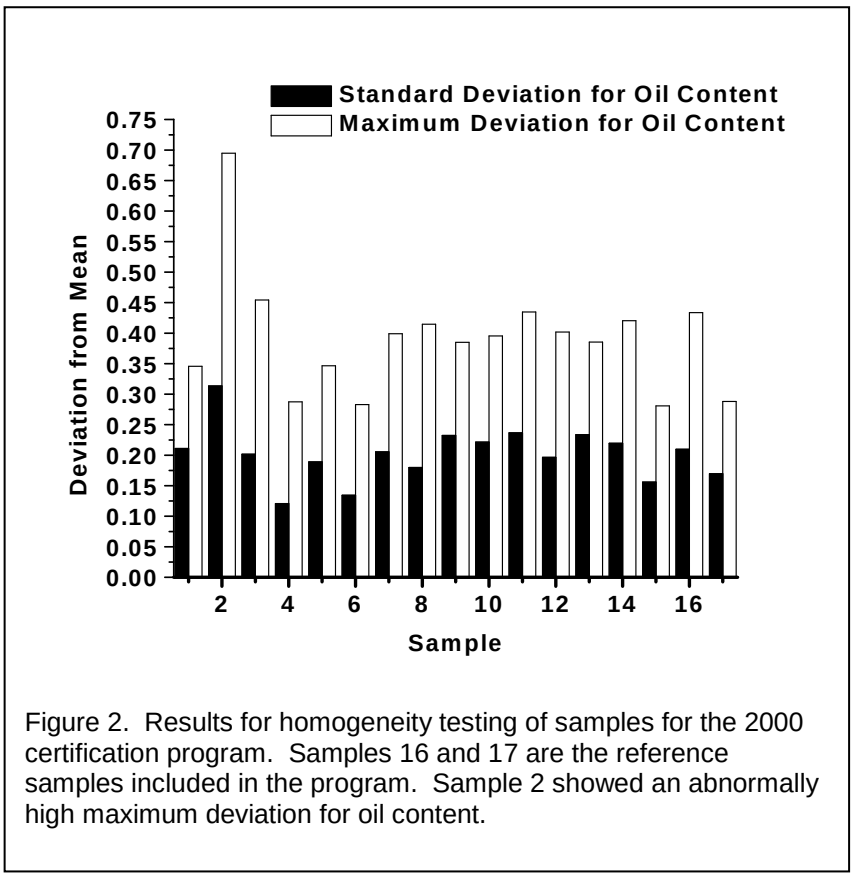

Figure 2. Results for homogeneity testing of samples for the 2000 certification program. Samples 16 and 17 are the reference samples included in the program. Sample 2 showed an abnormally high maximum deviation for oil content.

Prior to sending samples to the laboratories, they are tested for homogeneity by analyzing a representative number from each batch by NIR spectroscopy.(Figure 2) If a significant number of outliers are found, each sample in the batch must be tested before sending to the laboratories.

In addition to the samples, each laboratory is provided with a diskette containing files that are to be used for reporting results. Submission of electronic data is preferred as this reduces the

risk of data error. The files (in Excel format) include tables for entering the laboratory name and the analytical data beside the sample numbers provided (Table 3). In addition, each laboratory is required to provide information on their analytical procedures and is requested to provide a copy of their standard operation procedures for each method (one time unless significant changes are made). This latter information is valuable in assisting laboratories who have problems and also in determining whether new techniques are suitable for the program.

Laboratories are also provided with those parts of the rules of operation of the WCC/RRC that detail the methods that are acceptable for participation. If a laboratory wants to use a different method, it is up to them to provide data that confirms that this method meets the requirements of precision and accuracy within the program.

Statistical Analysis

At the time the program was being developed, there were no clear-cut guidelines for evaluating the performance of individual laboratories in this type of program. Most protocols were set up to evaluate methods rather than laboratories and although in the 1994 evaluation year such tests as the Dixon's test and Cochran test were used to identify outliers, a better method was clearly needed.

A method published in *Quality Assurance of Chemical Measurements* by J.K. Taylor (87) seemed to offer the best approach to this situation. Taylor's method provides a means of comparing different laboratories using a relatively small number of samples. The precision of the individual laboratories is compared to the expected precision (be equal to or less than) using a χ^2 test. Laboratories with probabilities greater than 0.05 accepted. Laboratories with probabilities in the range 0.05 to 0.01 (outriders) are warned and results from laboratories with probabilities less than 0.01 (outliers) are not accepted.

The first step in the statistical process is to decode the samples and arrange them in pairs (Table 4). Absolute differences between pairs of results are then estimated. The absolute difference for the results for an individual pair of blind duplicates is then compared to the mean for all pairs for that particular sample. Values in excess of 3.0 are considered outliers and require investigation. Often the investigation shows an error in data entry, sometimes a transposed number (34 rather than 43). Laboratories are informed of these errors, as avoiding them constitutes a part of good laboratory practice. Where the error is not due to data entry, the laboratory may be asked to check the results and finally to repeat the analysis. Where the laboratory has several outliers, it will be required to repeat the analysis on a new set of samples and efforts will be made to find the reason for the problem and rectify it.

Provided that there are no outliers, an estimate of the standard deviation is made using the mean difference across all samples (Nelson, L. S., 75) (Table 6). From the standard deviation, an estimate of the repeatability (R) is made where R=2.8 times the standard deviation. Based on this, differences larger than R should occur only once in 100 times. The repeatability achieved by each laboratory is tested against the desired level using a χ^2 test against the constant variance derived from the desired repeatability. Significant levels are selected to accept achieved variance less than or equal to the desired variance. Laboratories with probabilities greater than 0.05 accepted. Laboratories with probabilities in the range 0.05 to 0.01 (outriders) are warned and results from laboratories with probabilities less than 0.01 (outliers) are not accepted.

The values for S_b for individual samples should compare favorably with those for S_b(pooled). Where there is a significant difference, outliers in the laboratory means for may be found due to biased measurements. During the years of the program attempts have been made to assist laboratories to adjust biases. Initially this was handled graphically. For example, in 1996 a plot of oil content results, (Figure 3) showed a number of results outside of what would be expected based on a 95% confidence limit. When results for individual laboratories were plotted against the same overall regression line (Figure 4), it is possible to determine where a bias or slope adjustment is necessary for a laboratory to come closer to the mean.

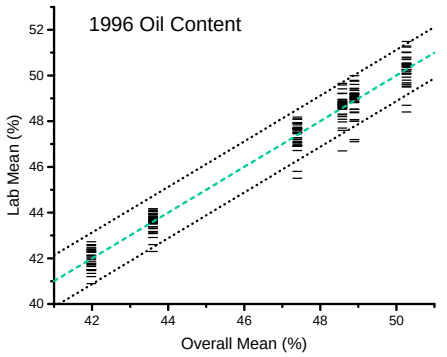

Figure 3. Variation between and within laboratories for oil content determinations.

the adjustments necessary to bring their data close to the mean data. Laboratories must have shown that they have good precision before making any accuracy adjustments.

Since 1999, a tabular method for correction of slope and bias has been used (Table 7). This allows laboratories to easily calculate

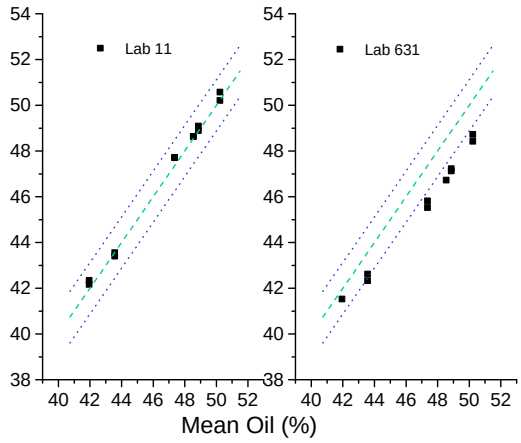

Figure 4. Graphical analysis of oil content results from two laboratories. Laboratory 631 requires a bias adjustment.

Success of the Program

The success of the program can be measured by the number of laboratories successfully qualifying without the need for repeat analyses and by the overall measurement of repeatability (R) for the various tests in the program (Table 8). For oil content, which is a test carried out by all laboratories, the overall mean R has been acceptable, even though the number of laboratories submitting NIR data has increased. (Note that while 26 laboratories participated in 2000, 8 laboratories submitted 2 sets of data of which one was NIR). Protein content was originally a difficult test for many laboratories and the repeatability measurement was set higher than that specified in the standard method. Some of this error was the result of laboratories using combustion analysis equipment with whole, rather than ground, seed samples which resulted in unacceptable precision. In recent years, the repeatability has improved. The lower weighting currently given to protein in the assessment of cultivars does not justify lowering the repeatability limit from the current level.

Repeatability measurements for glucosinolates and fatty acid composition should be discounted somewhat for 1995 and 1996 since blind duplicates were not issued in those years and laboratories were allowed to submit their own duplicate analyses. Difficulties in fatty acid analysis have also been attributed to the use of small whole seed samples, a practice common in selection programs but which is not suitable for achieving accuracy in testing larger bulk samples. Chlorophyll determinations are difficult for many laboratories. The extraction method requires the use of absolute alcohol that may be difficult to find. Also, sample size is extremely critical for this determination ((Daun, J. K. and Symons S., 2000). The evaluation of results has been complicated by laboratories attempting to submit results from determinations made on NIR instruments which are not, or which cannot be calibrated for determining chlorophyll.

Method Development

The funding for the program has also allowed the summer student associated with the program since 1997 to carry out research projects related to the methods. These projects have resulted in the development of a standard operating procedure for total glucosinolate determination by glucose release (1997), an improved understanding of the determination of saturated fatty acids (1998), and an understanding of the role of fatty acid composition in the errors in oil content determination (1999) and an evaluation of different methods for oil content determination, including those based on supercritical fluid extraction and gas

chromatography (2000). In 2001, the research will focus again on glucosinolates with the objective of finding methods for rapid assessment of glucosinolates in canola quality *B. juncea.* The results from these research projects are presented at the annual meeting of the Canadian Section of AOCS and reports are also made available to participants of the program. In addition, results submitted both by approved methods and by NIR instruments have allowed this methodology to be evaluated and now accepted as a routine procedure.

Acknowledgements

Barry Misener and Bert Siemens, Canadian Grain Commission assisted with preparing the samples. Project coordinators included Tricia Chornick, May Tiodin and Kerrie Vescio who were students at the University of Manitoba while assisting with this work.

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Table 3. Example of a reporting sheet for oil and protein.

WCC/RRC Certification 2000

Oil and Protein

Lab. No. 989 *(Place your Laboratory Number in Cell B4)*

Sample No.	Oil Content (% Dry Basis)	Protein Content (% Dry Basis)
OP513	44.08	25.63
OP164	44.05	25.79
OP969	43.11	24.53
OP287	43.04	24.45
OP196	45.02	24.51
OP674	44.94	24.72
OP925	47.14	23.18
OP621	46.79	23.25
OP614	49.34	21.14
OP689	49.25	21.25
OP59	51.99	19.17
OP390	51.97	19.34

Reference No.	Reference Oil	Reference Protein
Legend	47.3	23.6
Parkland	44.0	27.0

Value for reference protein mixture: 5.665 %N
(Theoretical)

Table 4. Raw data for oil content from 2000 certification program.

Level		1		2		3		4		5		6	
Lab	Method	OP513	OP164	OP969	OP287	OP196	OP674	OP925	OP621	OP614	OP689	OP59	OP390
46	Ex	42.22	42.47	42.32	42.31	44.09	44.03	45.62	45.89	48.17	48.41	50.69	50.63
113		42.57	42.58	42.59	42.80	44.42	44.17	46.76	46.25	48.29	47.95	50.87	50.46
146		42.98	43.17	43.11	43.42	45.27	44.78	46.86	47.19	49.67	49.52	52.81	52.65
225	CW	43.51	43.16	43.61	43.38	44.76	44.39	46.68	46.28	48.89	48.61	51.27	51.15
263	P	43.66	43.41	44.15	43.82	45.33	45.28	47.62	47.51	49.86	49.69	52.86	52.72
348	NIR	43.11	43.23	43.17	43.28	44.77	44.72	46.62	46.37	48.95	48.48	51.36	50.94
382	CW	43.91	43.87	43.87	43.60	45.39	45.39	46.99	47.35	49.32	49.25	51.24	51.47
542	CW	44.35	44.32	43.79	43.79	45.31	45.45	46.90	46.92	49.36	49.37	51.44	51.34
571	Ex	42.44	42.43	43.00	42.79	44.25	44.32	46.07	45.65	48.69	48.17	50.90	50.51
608	CW	43.00	42.73	42.90	42.92	45.02	44.50	46.45	46.17	48.25	48.31	51.10	50.93
620	CW	43.63	43.74	43.35	43.68	45.16	45.12	47.16	46.71	49.03	48.51	50.98	51.19
631	NIR	44.19	43.26	43.56	43.69	45.51	45.69	45.96	46.88	49.28	49.00	51.69	51.15
730	Ex	45.00	44.39	44.44	44.49	45.75	45.55	47.59	47.52	49.52	49.77	51.80	51.75
744	NIR	43.49	43.44	43.52	43.61	45.19	45.23	46.86	46.80	49.28	49.14	51.84	51.79
830	NIR	43.76	43.50	43.98	43.77	45.60	45.67	47.48	47.28	49.46	48.99	51.73	51.49
835	CW	43.30	43.50	43.70	43.70	45.20	45.00	47.20	47.10	49.70	49.40	51.90	51.90
899	P	43.27	42.84	43.55	43.27	45.44	44.63	46.94	46.78	49.31	48.60	52.38	52.07
899R	P	43.08	43.17	43.35	43.47	45.08	44.82	46.66	46.66	49.30	49.14	52.04	52.31
932	NIR	43.52	43.60	43.74	43.79	45.18	45.20	46.80	46.88	48.70	48.90	51.36	51.50
944	P	42.90	42.60	43.19	43.10	44.71	44.52	46.55	46.95	48.87	48.80	51.60	51.50
989	NIR	44.08	44.05	43.11	43.04	45.02	44.94	47.14	46.79	49.34	49.25	51.99	51.97
1005	NIR	44.14	44.74	44.15	44.44	45.63	45.74	48.70	47.47	50.05	49.64	52.76	51.94
1024		43.17	43.47	43.76	42.87	45.11	44.73	46.53	46.55	48.65	48.51	52.17	51.81
1067	Ex	42.99	43.27	42.59	42.62	44.52	44.01	46.61	46.55	48.37	48.35	50.31	50.37
1067B	NIR	44.18	44.32	44.82	44.68	46.03	46.06	47.55	47.32	49.18	49.25	51.25	51.13
225A	NIR	43.11	43.19	43.47	43.68	44.89	44.99	46.80	45.97	48.65	48.40	51.30	51.95
225B	NIR	44.09	44.22	44.66	44.84	45.10	45.20	47.59	47.50	49.28	49.46	51.29	51.37
542A	NIR	44.50	44.44	43.97	43.95	45.45	45.40	47.09	46.90	49.41	49.26	51.55	51.44
608A	NIR	43.80	43.88	43.80	44.07	45.62	45.87	47.52	47.67	49.04	49.28	52.23	51.97
744A	Ex	43.37	43.33	43.8	43.76	44.72	44.76	46.75	46.18	48.62	48.42	51.07	50.86
744B		43.24	43.35	43.66	43.67	45.04	45.17	46.90	46.86	49.09	48.97	51.47	51.39
803A	NIR	43.71	43.29	43.92	43.81	45.48	45.31	47.28	47.09	49.02	49.10	51.74	51.64
803		43.58	43.23	44.03	44.02	45.28	45.49	47.17	47.10	49.26	49.10	51.56	51.75
932A	P	43.50	43.35	43.60	43.65	45.17	45.00	47.10	47.00	48.85	48.80	51.37	51.32

Table 5. Ratios of the differences between individual blind duplicates to the mean value for all differences for that sample. Values in excess of 3.0 are considered outliers.

Ratios

Level Lab	Method	1	2	3	4	5	6	Mean
46	CW	1.15	0.06	0.32	0.95	1.05	0.28	0.69
113	CW	0.05	1.30	1.34	1.79	1.49	1.89	1.33
146	CW	0.87	1.93	2.62	1.16	0.66	0.74	1.26
225	CW	1.61	1.43	1.98	1.40	1.23	0.55	1.35
263	Extn	1.15	2.05	0.27	0.39	0.74	0.64	0.81
348	Extn	0.55	0.68	0.27	0.88	2.06	1.93	1.10
382	Extn	0.18	1.68	0.00	1.26	0.31	1.06	0.75
542	Extn	0.14	0.00	0.75	0.07	0.04	0.46	0.23
571	Extn	0.05	1.30	0.37	1.47	2.28	1.79	1.25
608	NIR	1.24	0.12	2.78	0.98	0.26	0.78	1.02
620	NIR	0.50	2.05	0.21	1.58	2.28	0.97	1.28
631	NIR	**4.27**	0.81	0.96	**3.23**	1.23	2.48	2.30
730	NIR	2.80	0.31	1.07	0.25	1.09	0.23	0.95
744	NIR	0.24	0.57	0.19	0.20	0.63	0.21	0.33
830	NIR	1.19	1.27	0.39	0.71	2.09	1.09	1.12
835	NIR	0.92	0.00	1.07	0.35	1.31	0.00	0.62
899	NIR	1.97	1.74	**4.33**	0.56	**3.11**	1.43	2.08
899R	NIR	0.41	0.75	1.39	0.00	0.70	1.24	0.69
932	NIR	0.37	0.31	0.11	0.28	0.88	0.64	0.44
944	NIR	1.38	0.56	1.02	1.40	0.31	0.46	0.89
989	NIR	0.14	0.43	0.43	1.23	0.39	0.09	0.49
1005	NIR	2.72	1.80	0.63	**4.29**	1.81	**3.77**	2.67
1024	NIR	1.38	**5.53**	2.03	0.07	0.61	1.66	1.61
1067	NIR	1.28	0.19	2.73	0.21	0.09	0.28	0.74
1067B	NIR	0.64	0.87	0.16	0.81	0.31	0.55	0.56
225A		0.37	1.30	0.53	2.91	1.09	2.99	1.63
225B		0.60	1.12	0.53	0.32	0.79	0.37	0.59
542A	P	0.28	0.12	0.27	0.67	0.66	0.51	0.45
608A	P	0.37	1.68	1.34	0.53	1.05	1.20	0.96
744A	P	0.18	0.25	0.21	2.00	0.88	0.97	0.85
744B	P	0.50	0.06	0.70	0.14	0.53	0.37	0.38
803A	P	1.95	0.70	0.91	0.65	0.36	0.48	0.83
803		1.57	0.02	1.10	0.27	0.72	0.90	0.76
932A		0.69	0.31	0.91	0.35	0.22	0.23	0.44

Table 6. Differences and calculated standard deviations (Sd), repeatability (R) and χ^2 (Chi2) for oil content determination in 2000 series. Tested against R=0.6% for oil content. Value S_w is an estimate of the overall standard deviation within a sample and r is an estimate of the overall repeatability within a sample.

Level Lab	Method	1	2	3	4	5	6	Mean R	Sd	R	Chi2
46	Ex	0.25	0.01	0.06	0.27	0.24	0.06	0.15	0.18	0.49	3.65
113		0.01	0.21	0.25	0.51	0.34	0.41	0.29	0.34	0.95	*13.79*
146		0.19	0.31	0.49	0.33	0.15	0.16	0.27	0.32	0.90	*12.24*
225	CW	0.35	0.23	0.37	0.40	0.28	0.12	0.29	0.34	0.96	*14.11*
263	P	0.25	0.33	0.05	0.11	0.17	0.14	0.18	0.21	0.58	5.08
348	NIR	0.12	0.11	0.05	0.25	0.47	0.42	0.24	0.28	0.78	9.29
382	CW	0.04	0.27	0.00	0.36	0.07	0.23	0.16	0.19	0.53	4.34
542	CW	0.03	0.00	0.14	0.02	0.01	0.10	0.05	0.06	0.17	0.41
571	Ex	0.01	0.21	0.07	0.42	0.52	0.39	0.27	0.32	0.89	*12.09*
608	CW	0.27	0.02	0.52	0.28	0.06	0.17	0.22	0.26	0.73	8.03
620	CW	0.11	0.33	0.04	0.45	0.52	0.21	0.28	0.33	0.91	*12.70*
631	NIR	0.93	0.13	0.18	0.92	0.28	0.54	0.50	0.59	1.64	***40.92***
730	Ex	0.61	0.05	0.20	0.07	0.25	0.05	0.21	0.24	0.68	6.97
744	NIR	0.05	0.09	0.04	0.06	0.14	0.05	0.07	0.08	0.23	0.84
830	NIR	0.26	0.20	0.07	0.20	0.48	0.24	0.24	0.29	0.80	9.72
835	CW	0.20	0.00	0.20	0.10	0.30	0.00	0.13	0.16	0.44	2.95
899	P	0.43	0.28	0.81	0.16	0.71	0.31	0.45	0.53	1.49	***33.59***
899R	P	0.09	0.12	0.26	0.00	0.16	0.27	0.15	0.18	0.50	3.73
932	NIR	0.08	0.05	0.02	0.08	0.20	0.14	0.10	0.11	0.31	1.50
944	P	0.30	0.09	0.19	0.40	0.07	0.10	0.19	0.23	0.63	6.09
989	NIR	0.03	0.07	0.08	0.35	0.09	0.02	0.11	0.13	0.35	1.89
1005	NIR	0.59	0.29	0.12	1.22	0.41	0.82	0.58	0.68	1.90	***55.06***
1024		0.30	0.89	0.38	0.02	0.14	0.36	0.35	0.41	1.15	***20.13***
1067	Ex	0.28	0.03	0.51	0.06	0.02	0.06	0.16	0.19	0.53	4.25
1067B	NIR	0.14	0.14	0.03	0.23	0.07	0.12	0.12	0.14	0.40	2.46
225A	NIR	0.08	0.21	0.10	0.83	0.25	0.65	0.35	0.42	1.17	***20.71***
225B	NIR	0.13	0.18	0.10	0.09	0.18	0.08	0.13	0.15	0.42	2.66
542A	NIR	0.06	0.02	0.05	0.19	0.15	0.11	0.10	0.11	0.32	1.55
608A	NIR	0.08	0.27	0.25	0.15	0.24	0.26	0.21	0.25	0.69	7.20
744A	Ex	0.04	0.04	0.04	0.57	0.20	0.21	0.18	0.22	0.61	5.58
744B		0.11	0.01	0.13	0.04	0.12	0.08	0.08	0.10	0.27	1.11
803A	NIR	0.43	0.11	0.17	0.18	0.08	0.11	0.18	0.21	0.59	5.37
803		0.34	0.00	0.21	0.08	0.16	0.20	0.16	0.19	0.54	4.50
932A	P	0.15	0.05	0.17	0.10	0.05	0.05	0.10	0.11	0.31	1.50
		0.22	0.16	0.19	0.28	0.23	0.22	0.22	0.25	0.70	
	S_w	***0.25***	***0.18***	***0.21***	***0.32***	***0.26***	***0.25***		.@ 0.10 x* = 9.90		
	R	***0.70***	***0.51***	***0.60***	***0.91***	***0.73***	***0.69***		.@ *0.05 x* = 11.78*		
									.@ 0.01 x* = 15.90		

Table 6. Table of means and calculation of the values for S_x standard deviation between laboratories within a sample, S_b overall precision for a sample (RMS mean of S_x and S_w) and S_b(pooled) overall precision for all samples.

Level	Method						
Lab		1	2	3	4	5	6
46	CW	42.35	42.32	44.06	45.76	48.29	50.66
113	CW	42.58	42.70	44.30	46.51	48.12	50.67
146	CW	43.08	43.27	45.03	47.03	49.60	52.73
225	CW	43.34	43.50	44.58	46.48	48.75	51.21
263	Extn	43.54	43.99	45.31	47.57	49.78	52.79
348	Extn	43.17	43.23	44.75	46.50	48.72	51.15
382	Extn	43.89	43.74	45.39	47.17	49.29	51.36
542	Extn	44.34	43.79	45.38	46.91	49.37	51.39
571	Extn	42.44	42.90	44.29	45.86	48.43	50.71
608	NIR	42.87	42.91	44.76	46.31	48.28	51.02
620	NIR	43.69	43.52	45.14	46.94	48.77	51.09
631	NIR	43.73	43.63	45.60	46.42	49.14	51.42
730	NIR	44.70	44.47	45.65	47.56	49.65	51.78
744	NIR	43.47	43.56	45.21	46.83	49.21	51.81
830	NIR	43.63	43.88	45.63	47.38	49.22	51.61
835	NIR	43.40	43.70	45.10	47.15	49.55	51.90
899	NIR	43.06	43.41	45.04	46.86	48.96	52.23
899R	NIR	43.13	43.41	44.95	46.66	49.22	52.18
932	NIR	43.56	43.77	45.19	46.84	48.80	51.43
944	NIR	42.75	43.15	44.62	46.75	48.84	51.55
989	NIR	44.07	43.08	44.98	46.97	49.30	51.98
1005	NIR	44.44	44.29	45.69	48.09	49.85	52.35
1024	NIR	43.32	43.32	44.92	46.54	48.58	51.99
1067	NIR	43.13	42.61	44.27	46.58	48.36	50.34
1067B	NIR	44.25	44.75	46.05	47.44	49.22	51.19
225A		43.15	43.58	44.94	46.39	48.53	51.63
225B		44.16	44.75	45.15	47.55	49.37	51.33
542A	P	44.47	43.96	45.43	47.00	49.34	51.50
608A	P	43.84	43.94	45.75	47.60	49.16	52.10
744A	P	43.35	43.78	44.74	46.47	48.52	50.97
744B	P	43.30	43.67	45.11	46.88	49.03	51.43
803A	P	43.50	43.86	45.40	47.18	49.06	51.69
803		43.40	44.02	45.38	47.14	49.18	51.65
932A		43.43	43.63	45.09	47.05	48.83	51.35
Mean		43.5	43.6	45.1	46.9	49.0	51.5
S_x		0.576	0.552	0.462	0.500	0.450	0.566
S_b		0.367	0.368	0.290	0.269	0.259	0.360
S_b(Pooled)			0.293				

Table 7. Table of differences showing tabular corrections for slope and bias from 2000 certification series for oil content.

Level	Method							Mean
Lab		1	2	3	4	5	6	Bias
46	Ex	-1.20	-1.32	-1.06	-1.21	-0.76	-0.95	*-1.08*
11A	NIR	1.03	1.09	1.70	1.01	1.41	1.81	*1.34*
11	NMR	-0.45	-0.31	-0.19	-0.19	-0.18	0.14	*-0.20*
113		-0.97	-0.94	-0.82	-0.46	-0.93	-0.94	*-0.84*
146		-0.47	-0.37	-0.09	0.06	0.55	1.12	0.13
225	CW	-0.21	-0.14	-0.54	-0.48	-0.30	-0.40	-0.35
263	P	-0.01	0.35	0.19	0.60	0.73	1.18	*0.51*
348	NIR	-0.38	-0.41	-0.37	-0.47	-0.33	-0.46	-0.40
382	CW	0.34	0.10	0.27	0.21	0.24	-0.25	0.15
542	CW	0.79	0.15	0.26	-0.05	0.32	-0.22	0.21
571	Ex	-1.11	-0.74	-0.83	-1.10	-0.62	-0.90	*-0.89*
608	CW	-0.68	-0.73	-0.36	-0.65	-0.77	-0.59	*-0.63*
620	CW	0.14	-0.12	0.02	-0.03	-0.28	-0.52	-0.13
631	NIR	0.18	-0.01	0.48	-0.54	0.09	-0.19	0.00
631R	NIR	-0.02	0.21	-0.19	0.74	-0.07	-0.63	0.00
730	Ex	1.15	0.83	0.53	0.59	0.60	0.17	0.64
730A	NIR	0.84	-0.30	-1.13	-0.36	-1.30	-0.12	-0.40
730R	NIR	-0.08	-0.08	0.09	-0.13	0.16	0.21	0.03
744	NIR	-0.08	-0.08	0.09	-0.13	0.16	0.21	0.03
830	NIR	0.08	0.24	0.51	0.42	0.18	0.00	0.24
835	CW	-0.15	0.06	-0.02	0.19	0.50	0.29	0.15
899	P	-0.49	-0.23	-0.08	-0.10	-0.09	0.62	-0.06
899R	P	-0.42	-0.23	-0.17	-0.30	0.17	0.57	**-0.06**
932	NIR	0.01	0.13	0.07	-0.12	-0.25	-0.18	-0.06
944	P	-0.80	-0.49	-0.50	-0.21	-0.21	-0.06	-0.38
989	NIR	0.52	-0.56	-0.14	0.00	0.25	0.37	0.07
1005	NIR	0.89	0.65	0.57	1.12	0.80	0.74	*0.80*
1005R	NIR	0.87	1.05	0.99	1.31	1.24	1.19	*1.11*
1024		-0.23	-0.32	-0.20	-0.42	-0.47	0.38	-0.21
1067	Ex	-0.42	-1.03	-0.85	-0.38	-0.69	-1.27	*-0.77*
1067B	NIR	0.70	1.11	0.93	0.47	0.17	-0.42	*0.49*
225A	NIR	-0.40	-0.06	-0.18	-0.58	-0.52	0.02	-0.29
225B	NIR	0.61	1.11	0.03	0.58	0.32	-0.28	0.40
542A	NIR	0.92	0.32	0.31	0.03	0.29	-0.11	0.29
608A	NIR	0.29	0.30	0.63	0.63	0.11	0.49	0.41
744A	Ex	-0.20	0.14	-0.38	-0.50	-0.53	-0.64	-0.35
744B		-0.25	0.03	-0.01	-0.08	-0.02	-0.18	-0.09
803A	NIR	-0.05	0.23	0.28	0.22	0.01	0.08	0.13
803		-0.14	0.39	0.26	0.17	0.14	0.04	0.14
932A	P	-0.12	-0.01	-0.03	0.09	-0.22	-0.26	-0.09

bias adjustment suggested
slope adjustment suggested

Table 8. Summary of performance of laboratories in the certification program, 1995 through 2000.

Year	Laboratories Participating	Met Standard	Mean R	Year	Laboratories Participating	Met Standard	Mean R
Oil Content Required R 0.6%				**Protein Content Required R 1.0%**			
1995	18	13	0.89	1995	16	16	1.03
1996	21	20	0.64	1996	17	17	0.70
1997	20	19	0.66	1997	17	17	0.94
1998	26	21	0.71	1998	24	24	0.79
1999	26	19	0.94	1999	25	25	0.74
2000	34	29	0.70	2000	30	28	0.74
Glucosinolates Required R 3.0 uM/g				**Erucic Acid Required R 0.4%**			
1995	13	11	2.69	1995	16	15	0.19
1996	17	17	1.40	1996	17	16	0.13
1997	15	14	1.77	1997	16	14	0.15
1998	19	17	3.65	1998	19	16	0.46
1999	25	22	3.00	1999	19	19	0.24
2000	24	24	1.63	2000	21	21	0.21
Chlorophyll Required R 5 mg/kg				**Saturated Fatty Acids Required R 0.5%**			
1995	9	7	8.10	1995	16	14	0.15
1996	14	9	7.51	1996	17	16	0.20
1997	8	7	3.80	1997	15	13	0.54
1998	10	7	3.90	1998	19	18	0.39
1999	15	15	5.58	1999	20	20	0.25
2000	16	15	6.09	2000	21	20	0.53