Technical

Use of Gas Liquid Chromatography for Monitoring the Fatty Acid Composition of Canadian Rapeseed[1,2]

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ABSTRACT

Since 1972, Canadian rapeseed has been changing from high erucic acid types to low erucic acid types. In 1970, the Canadian Grain Commission instituted a program to monitor the fatty acid composition of rapeseed at the farm level, in railway carlot shipments and in export cargo shipments. Initially, in order to process up to 20,000 samples per year, a combined extraction and methylation procedure was developed in which methyl esters were analyzed within 5 min by manual injection on a nonpolar column. Since conversion to low erucic acid rapeseed types has been completed, other details of fatty acid composition have received more attention. In the system presently used, the rapeseed oil methyl esters are analyzed on a mixed-phase column which gives good resolution of all of the major fatty acids. Through the use of a microcomputer-controlled autosampler, 50 samples can be analyzed per day. Reports are generated giving complete fatty acid composition as well as estimates of iodine value and saponification value.

INTRODUCTION

The development of rapeseed as a modern agricultural commodity has been closely linked to the development of gas liquid chromatography (GLC) as an analytical tool—especially as used for determining fatty acid composition. Much of the early work was carried out at the National Research Council's Prairie Regional Laboratory at Saskatoon. This work included the development of synthetic polyester liquid phases including butanediolsuccinate (1). A varietal study of fatty acid composition (2) carried out by these workers eventually led to the development of low erucic acid types of rapeseed.

Gas chromatography proved to be an ideal tool for plant breeders. The high sensitivity of the technique coupled with a GLC throughput time of ca. 10 min for a chain length analysis (C16-C22) enabled Downey and Harvey (3) to develop a technique for analyzing the fatty acid composition in a single cotyledon excised from a rapeseed. When the fatty acid composition was suitable, the second cotyledon was grown to a mature plant. This technique, known as the "half-seed technique" was widely used in the development of both high and low erucic acid varieties of rapeseed.

Prior to 1970, rapeseed oil was different from the other major vegetable oils in that it contained large amounts of long-chain (> C18) fatty acids. Potential nutritional problems associated with the presence of long-chain fatty acids were highlighted at the International Conference on Rapeseed in 1970 (4). The implication of these findings, coupled with the rapid the rapid increase in rapeseed oil utilization in Canada, led the Canadian government to suggest that the Canadian rapeseed industry convert as soon as possible to low erucic acid varieties. The Canadian Grain Commission's Grain Research Laboratory (GRL) played a key role in this voluntary conversion by monitoring the fatty acid composition (in particular the erucic acid content) of rapeseed grown in Canada. This monitoring program provided the Canadian oilseed industry with information on the introduction of low erucic acid rapeseed (LEAR) varieties. The details of the conversion to LEAR varieties have been discussed recently (5) and are summarized in Figure 1. The purpose of this report is to discuss the methodology used in this monitoring study and to show the development of techniques and equipment used for fatty acid monitoring at the GRL from 1970 to the present.

SCOPE OF THE MONITORING PROGRAM

On a routine basis, the Grain Research Laboratory carries out surveys on grains and oilseeds as they move from the farm to the export market. In the case of rapeseed, these surveys include an annual new crop survey (up to 500 samples), a survey of railcars unloaded at export terminal elevators (quarterly composites), and each cargo of Canadian rapeseed which leaves an export terminal (up to 400 samples, depending whether different loading portions or composites are tested).

For monitoring the conversion to LEAR, a greater emphasis was placed on the railway carlot survey. In the early 1970s, grain companies were required to designate the type of seed being shipped by rail. Aside from the routine sampling (one carlot out of 5), the erucic acid monitoring program, at times, included analysis of every carlot designated "LEAR" by the shipper, every carlot designated high erucic acid rapeseed (HEAR) by the shipper, and every 5th carlot designated "OTHER" by the shipper.

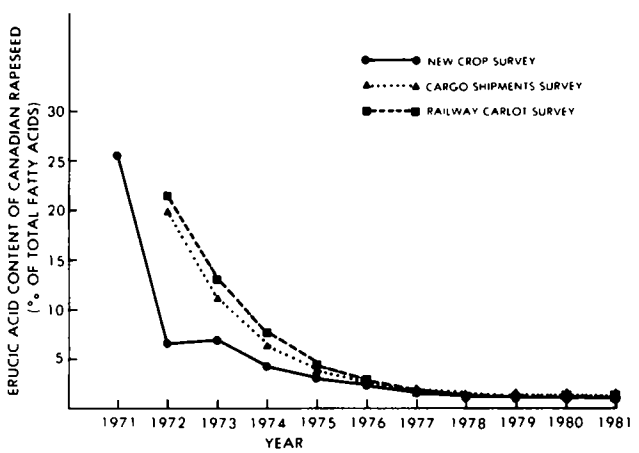


FIG. 1. Average levels of erucic acid in surveys of Canadian rapeseed (1971-82).

[1] Presented at the Symposium on Gas Chromatography—State of the Art, at the 73rd AOCS meeting, Toronto, 1982.
[2] Paper No. 518 from the Grain Research Laboratory.

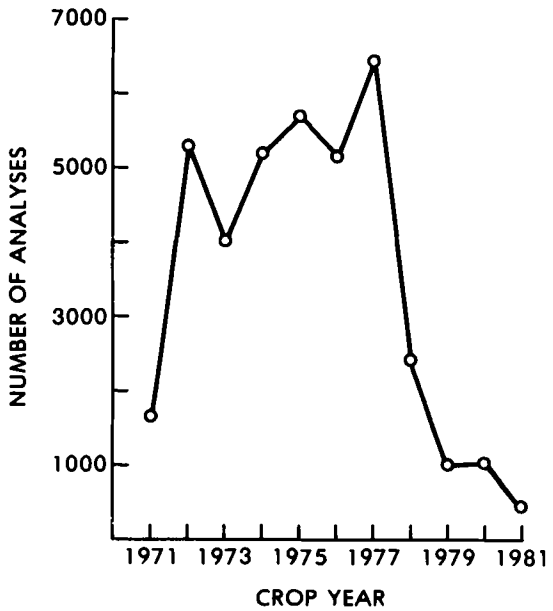


FIG. 2. Number of samples handled annually in the GRL rapeseed monitoring program (1971-81).

This amounted to as many as 5000 samples per year.

The total number of samples handled per year in the rapeseed monitoring program is shown in Figure 2. The number of individual samples analyzed decreased as the conversion to LEAR neared completion, since by that time essentially the same information could be obtained by analyzing individual cargo samples and railcar composite samples. Removal of emphasis on erucic acid monitoring also meant that more time could be spent testing for other quality factors such as glucosinolates, complete fatty acid composition of both rapeseed and other Canadian oilseeds, fiber content, chlorophyll content and free fatty acids.

METHODS USED FOR PREPARING METHYL ESTERS

In terms of accuracy and precision, monitoring programs allow some leeway in the interest of throughput of samples compared to regulatory programs. Sample throughput, however, cannot become a dominating factor as it often is in breeding programs. Since the Canadian Grain Commission's monitoring program was designed to become a regulatory program if necessary, the original methodology used was borrowed from Plant Products and Quarantine Division of Agriculture Canada. This group had developed a procedure for officially determining erucic acid in pedigreed rapeseed (6).

Although this official procedure gave accurate results in interlaboratory collaborative studies (6), it was lengthy, requiring 8 hr to prepare a 40-sample batch, and used large quantities of reagents. Obviously, a more rapid procedure was required for a program which tested many thousands of samples annually. A faster procedure was adopted which was based on procedures already in use in rapeseed breeding programs (7). In this method the oil was simultaneously extracted and transesterified as follows: (a) Ten g of seed were ground in a high speed grinder (8). (b) The ground seed was mixed and ca. 0.25 g was transferred into a 16 x 125 mm test tube. (c) Three mL hexane and 1 mL 0.4N $NaOCH_3$ in methanol were added. (d) The sample was mixed on a Vortex mixer and allowed to stand for 10 min. (e) Water (10 mL) was added, and one microliter of the hexane layer was injected into the GC.

Although this procedure had a slightly higher statistical error compared with results of the official procedure (Table I), the statistical errors were not sufficiently large to be of concern. Using the rapid method, the large sample size (10 g) is maintained while the total volume of solvents involved (excluding water) was reduced from 57 mL to 4 mL. The time required to prepare a batch of 40 samples was reduced from 8 hr to ca. 2 hr. A later modification to the rapid procedure involved neutralization of the hexane phase with HCl and $NaHCO_3$ in order to reduce the deterioration of GLC column packings and avoid base-catalyzed modification of the methyl esters. More recently, because the throughput of samples has lessened, 20 μL of oil, prepared as above or more routinely extracted from seed during the preparation of oil-free meal for glucosinolate or fiber analysis, is esterified, and the methyl esters are diluted to 5 mL in hexane before GLC analysis. Starting from seed, a technician can prepare 40 samples in 4 hr, excluding an overnight evaporation of solvent from the extracted oil.

TABLE I

Comparison of Long and Rapid Procedures for Preparing Methyl Esters from Rapeseed (Paired t-test)

Erucic acid range (%)	Mean difference (%) long-rapid	Standard deviation	T-value	Degrees of freedom
0-5.0	0.054	0.106	4.089	64
5.1-10.0	0.270	0.189	4.521	9
> 10.0	0.392	0.655	2.931	23

For routine samples, such as cargo survey samples, a 10-g sample may be ground and analyzed for chlorophyll by reflectance spectroscopy (8). A subsample (1 g) is analyzed for protein by the Kjeldahl method. Oil is extracted from a further 4 g subsample on a Goldfisch extraction unit (6 hr) to provide meal for glucosinolate and fiber analysis and to provide oil for fatty acid composition and free fatty acid determinations. This sample preparation flow provides an efficient use of time and resources. Oil content is routinely determined using broadband NMR spectroscopy.

GAS CHROMATOGRAPHIC ANALYSIS

Column Packings

To reduce analysis time to a minimum, no effort was made originally to separate fatty acids by degree of unsaturation. During the initial transition period, when total C22 fatty acids ranged from 3 to 30%, the amount of C22:0 fatty acid (0.2-0.3%) was of little consequence. In any case, this amount was relatively constant and could be subtracted from results. Fatty acids were separated on a nonpolar column such as UCW 98 (6).

As the transition from HEAR to LEAR neared completion, the monitoring program was shifted from determination of erucic acid to determination of total fatty acid composition and particularly to linolenic acid. The availability of highly polar, heat-stable cyanosilicone liquid phases made the rapid separation of methyl esters according to unsaturation possible.

Unfortunately, none of the individual polar liquid phases produced a good separation of linolenic acid from both C20:0 and C20:1 fatty acids during rapid analysis. It was necessary to correct for the presence of C20 fatty acids included in the linolenic analysis using the regression shown in Figure 3. This, of course, added considerably to the analysis time.

Resolution of the C18:3 and C20 fatty acids was made possible by use of a mixed phase (3% SP-2310/2% SP-2300) released by Supelco. With temperature programming,

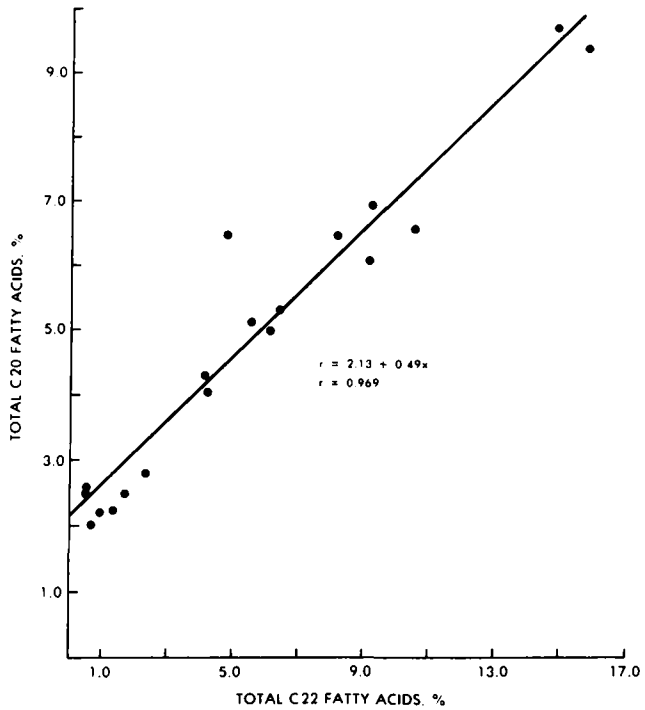


FIG. 3. Relationship between C20 fatty acids and C22 fatty acids in rapeseed (C22 < 10%).

routine separations were achieved in 25 min as shown in Figure 4.

Equipment

Originally, analyses were carried out using two gas chromatographs each with two columns. Two operators were required to inject samples and standards, manually calculate results from the digitally integrated peak areas, and continually to adjust flow settings to give correct results for standard samples. A major improvement to this system was the addition of an integration system with area percent calculation.

More recently, as a result of increased requirement for manhours in other monitoring programs, an automatic sampler and a computing integrator were purchased. Using the autosampler on a 24-hr basis, one operator responsible for sample preparation and GC-autosampler operation can analyze 50 samples per day. The results are presented in a notebook page format using Basic programming and include fatty acid composition and the calculated iodine value and saponification value (Fig. 5). At the present time, the main handicap in the system is that it must accommodate two different routine analyses (fatty acid composition and TMS-glucosinolates).

Accuracy and Precision

Accuracy and precision of the monitoring program for erucic acid was determined by means of a check sample program coordinated by Plant Products and Quarantine Division of Agriculture Canada. This program was established in 1973. In 1976, Barrette observed that the "six laboratories in the program have shown an average standard deviation of 0.15% on five monthly check samples over a 10-month period for C22 fatty acid analysis" (6). In 1980 (9), the average standard deviation was reduced to ±0.1% and the range of C22 fatty acids in the program had been changed in 1977 from 3-4% to 0.3-2%. The performance of the GRL in this program in recent years is outlined in

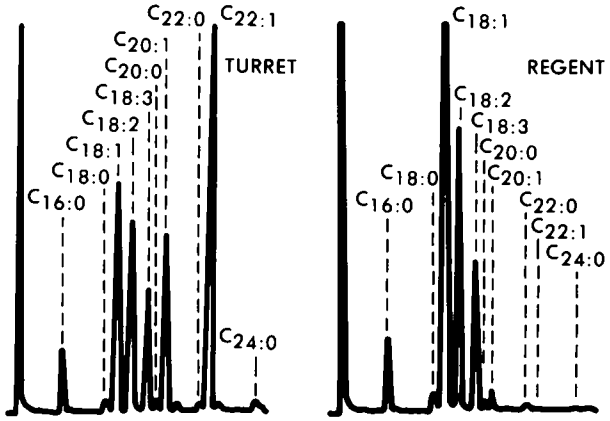
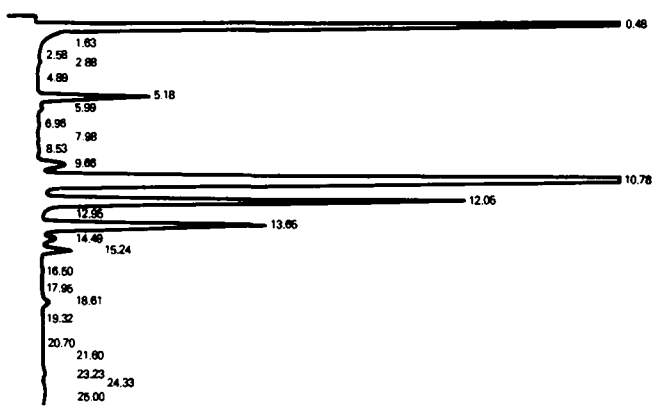


FIG. 4. Gas liquid chromatograms showing fatty acid methyl ester separation on a 2.5 m × 2 mm column of GP 3% SP-2310/2% SP 2300 on 100/120 Chromosorb W AW (glass column). Conditions: N_2 at 25 mL/min, 185 C for 8 min, 4 C/min to 220 C, hold for 8 min.

GAS LIQUID CHROMATOGRAPHIC ANALYSIS
FOR FATTY ACID COMPOSITION



REGENT
MARCH 23, 1981

FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS)

NO.	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0
3	3.75	1.68	60.76	20.18	11.32	0.60	1.17	0.34	0.06	0.16

C22 TOTAL = 0.39387
IODINE VALUE = 117.365
AVERAGE MOLECULAR WEIGHT TG = 882.38
SAPONIFICATION VALUE = 190.757
ERUCIC ACID (C22:1) = 0.06

FIG. 5. Report format for fatty acid methyl esters in rapeseed using computing (HP 3388A) integrator.

Table II.

The standard mixtures of fatty acid methyl esters supplied by Plant Products, made up of saturated fatty acids only, were found to be inadequate for polar column separations. At present, the standard mixture #62 from Nu Check Prep which contains 2% erucic acid is being used. There is need for a further standard mixture with 0.5% or less erucic acid, as well as a similar standard mixture containing 50% erucic acid for high erucic acid seed testing.

TABLE II

Grain Research Laboratory Results for Plant Products' Erucic Acid Check Sample Series: June 1978-February, 1982

Expected result (exp)	GRL result (GRL)	Difference exp−GRL	No. of tests	Standard deviation
0.32	0.35	-0.03	13	0.06
0.37	0.37	0.00	18	0.07
1.31	1.27	+0.04	13	0.14
0.70	0.76	-0.06	13	0.10
1.44	1.38	0.06	11	0.18
1.73	1.68	0.05	22	0.12
1.56	1.52	0.04	14	0.12
0.83	0.71	0.12	14	0.10
1.18	1.18	0.00	11	0.15
0.31	0.43	-0.12	10	0.08

Aside from the above collaborative study, several check samples are used to show the accuracy of GRL methods (Table III). A fairly high precision of 0.1-0.2% for C22 fatty acids has been achieved for the certified Candle and Regent varieties, but the more heterogeneous Span sample has a larger variation. The somewhat larger variation in linolenic acid results is only ca. ±5% of the value for that fatty acid.

FUTURE DEVELOPMENTS

The above discussion has shown how the GRL monitoring program has developed. "State of the art" is not stationary, however, and managers of programs utilizing "state of the art" technology must keep aware of new developments and updated procedures and equipment.

At the GRL, future developments will probably include automatic data processing and capillary GC. The first steps in automatic data processing have been taken by the purchase of a tape drive unit for the integrator. This unit will allow storage of up to 98 analyses on tape for future processing. Eventually the integrator will be interfaced to a more sophisticated system such as that used at Svalöf AB in a similar seed-testing program (10).

The use of capillary columns on a routine basis will depend on two factors. The first is the lifetime and durability of the column in terms of number of analyses. Fused silica columns may meet the requirements of handling hundreds of samples, but the cost/sample of present systems is still relatively high (11). The second factor is the development or availability of a reliable capillary injection system for autosampling. The introduction of capillary gas chromatography at the GRL will take place in the near future.

ACKNOWLEDGMENTS

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TABLE III

Check Samples for Fatty Acid Composition (GRL Internal Series)

	Span			Candle			Regent		
	N	C22:1 % of total fatty acids[a]	C18:3 % of total fatty acids[a]	N	C22:1 % of total fatty acids[a]	C18:3 % of total fatty acids[a]	N	C22:1 % of total fatty acids[a]	C18:3 % of total fatty acids[a]
Oct '81–Mar '82	19	3.02 (0.24)	10.11 (0.23)	15	1.74 (0.16)	13.15 (0.21)	16	0.09 (0.09)	10.05 (0.18)
Jan '81–Oct '81	–	– –	– –	36	1.72 (0.18)	13.25 (0.58)	39	0.07 (0.04)	10.44 (0.35)
Aug '80–Dec '80	36	2.96 (0.53)	10.8 (0.50)	–	– –	– –	28	0.10 (0.12)	10.7 (0.69)

[a]Bracketed values are standard deviations.