ACKNOWLEDGMENT

This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Project number 57550601). H. Machida of Meito Sangyo Co. Ltd., Hachioji, Tokyo, supplied the lipases.

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[Received September 25, 1984]

♣Cottonseed Oil Estimation by Pulsed Nuclear Magnetic Resonance Technique

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ABSTRACT

Seed asymmetry and moisture associated with the seeds are known to affect seed oil estimation by pulsed nuclear magnetic resonance (NMR) technique employing free induction decay or single spin echo (SE) pulse sequence. Using *Gossypium* (cottonseeds) as experimental material, it is shown that transverse relaxation times (T_2) of seed oil, in different varieties of seeds, measured in vivo, are not the same. The mean T_2 value of tetraploid seeds is found to be significantly higher than that of diploids. The effect of T_2 variation and other problems on oil estimation by the free induction decay and SE methods can be avoided by using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence to monitor the signal intensities of a certain number of selected echoes and processing them to yield the extrapolated signal intensity at zero time. The oil content values thus estimated are found to agree well with those obtained by Soxhlet method. The agreement between the two methods might depend upon the presence of gossypol and other pigments present in the samples. Neither delinting nor dehydrating the seeds is necessary in the present method. Even with the CPMG sequence, use of individual echoes is not recommended, as the T_2 variations give rise to erroneous values.

INTRODUCTION

Pulsed nuclear magnetic resonance (NMR) provides a quick and convenient method for the determination of solid fat content in partially crystallized fats (1), seed oil in oil bearing seeds (2,3) and oil and water determinations in emulsions (4). Recently, this technique has been used to estimate the moisture content in paddy seeds (5,6). A detailed study on *Gossypium* has substantiated our earlier contention (7) that among the various pulse sequences available in NMR spectroscopy, the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (8-10) is by far the best so far as the effects of seed orientation, seed asymmetry and seed moisture are concerned. The various aspects investigated were: (i) measurement of transverse relaxation times (T_2) of oil protons; (ii) the effect of T_2 variation on oil estimation; (iii) the effect of lint and moisture on oil estimation, and (iv) the estimation of oil content by CPMG pulse sequence and comparison with chemically estimated values.

BASIC PRINCIPLE

The basic principles involved in the different pulse sequences have been described elsewhere (7,8). In the simple, two-pulse spin echo sequence, the amplitude of the echo at 2τ is given by:

$$A = M_0 \text{ EXP } [-(2\tau/T_2) - 2\gamma^2 G^2 D\tau^3/3] \quad [1]$$

where γ = magnetogyric ratio of protons; D = diffusion coefficient; G = magnetic field gradient; τ = pulse separation between 90 and 180 pulses; T_2 = transverse relaxation time of the protons, and M_0 = proportionality constant.

The departure from simple exponential decay due to diffusion (second term) becomes pronounced for large values of τ. Moreover, the shape and amplitude of the echo are highly influenced by magnetic field instabilities. The effect of diffusion can be arbitrarily minimized by applying the pulse sequence $(90)_x$-τ-$(180)_y$-2τ-$(180)_y$-2τ-$(180)_y$..... etc., and reducing the pulse spacing to less than a msec. Such a pulse train is known as CPMG sequence. Under these conditions, a series of spin echoes with decreasing amplitudes are formed and, neglecting the diffusion term, the echo amplitude corresponding to any echo can be represented by

$$A = M_0 \text{ EXP } (-2n\tau/T_2) \quad [2]$$

n being the echo number. The nondestructive estimation of oil content in seeds consists essentially of evaluating the

signal intensity from the oil protons present in the seeds and comparing it with that from a known amount of pure seed oil under identical instrumental settings. On the basis of equation 2, two samples with the same number of protons but with different T_2 values would yield the same signal intensity only at the initial time t = 0 (t = 2nτ). Thus, in order to carry out a precise estimation of oil content in seeds, the signal intensity at t = 0 would have to be known. It is not possible to measure this signal intensity directly because of the finite pulse width. Moreover, at very low t values, i.e., for the earlier echoes, the protons of the solid matrix of the seeds and those of associated moisture also would contribute to the intensity of the signal, as reported earlier (7). As these protons have short T_2 values, one can expect this contribution to decay according to equation 2 so that it can be neglected for the later echoes. However, measurements at a single echo position would not suffice as, the ratio of the signal intensities at different echoes would be different because of the possible variation in T_2 values among the different varieties of seeds, and between the seed and the oil, as is experimentally shown in the present work.

Oil Estimation-Extrapolation Method

These two conflicting requirements could be reconciled by measuring the signal intensities corresponding to a number of appropriately spaced echoes at which the contribution of the protons of the solid matrix and moisture would have decayed to an insignificantly small value. The procedure adopted in the present study for the non-destructive estimation of seed oil consisted of applying a CPMG pulse sequence to the seed sample, measuring the signal amplitudes of seven different echoes and fitting these amplitudes to equation 2 by the method of least squares, so as to yield the values of the parameters M_0 and T_2. This value of M_0, which is effectively the signal intensity at t = 0, was then compared with that measured under identical instrumental conditions from a known amount of oil extracted from seeds of SRT-1 cotton. The percentage oil content in seeds was then calculated by using the relation given below. This method will hereafter be called the extrapolation method.

$$\text{Percentage oil content in seeds} = 100(M_s/W_s)(W_o/M_o) \quad [3]$$

where M_o and M_s = signal intensities at t = 0 of the oil and seed samples, respectively, and W_o and W_s = weight of the oil standard and the dry weight of seed samples, respectively.

MATERIALS AND METHODS

Seed Samples

To investigate the effects of lint and moisture on oil estimation of cottonseeds, samples, each weighing about 3 gm, were taken from 10 different varieties (4 diploid and 6 tetraploid). A known amount of SRT-1 oil served as the control. To start with, each sample had a certain amount of lint associated with it. The samples were subjected to various treatments indicated below, and oil estimation was carried out at each of the following stages: (i) Undelinted—original sample having a lint and loose fiber content of about 4 to 30%; (ii) Delinted—the above sample after removal of lint as per the procedure described later; (iii) Dried—the above sample after drying at 95-100 C for 16 hr, and (iv) Hydrated—the above sample after exposure to an atmosphere of saturated water vapor for 40 hr after which the sample was maintained at 37 C for 4 hr to get rid of any free water that might be associated with the seeds.

For transverse relaxation time measurements, and to study the variation in oil content within replicates, delinted cottonseeds, with three replicates for each variety, each replicate weighing about 5 gm, were used. The delinting of the seeds was performed by exposing the seeds to concentrated hydrochloric acid fumes inside a desiccator for 8 hr, after which the lint could be removed simply by brushing the seeds. This provides a quick and convenient method to delint the seeds, in contrast to the conventional methods. These samples were analyzed for their oil content, by NMR method, using one of the seed varieties itself as a control. The oil content in control samples was determined against a known amount of SRT-1 oil as absolute standard. The oil content also was estimated individually by Soxhlet extraction technique using petroleum ether (B.P.:60 to 80 C) as solvent, so that a direct comparison could be made between the NMR and Soxhlet methods. Each set of triplicates was processed under identical conditions for oil estimation by Soxhlet method.

TABLE I

Transverse Relaxation Times of Oil Protons in Delinted Cottonseeds Measured In Vivo

Seed variety		Transverse relaxation times in msec				
		1	2	3	Mean	s.d.
Diploid						
1.	Gaorani 22	44.7	47.1	53.1	48.3	4.3
2.	Western 1	38.4	41.1	42.6	40.7	2.1
3.	V-797	46.6	41.8	43.2	43.9	2.5
4.	G-1	50.6	51.8	52.4	51.6	0.9
Tetraploid						
5.	Khandwa 2	48.2	54.1	53.3	51.9	3.2
6.	Sea Island	64.5	59.8	54.4	59.6	5.0
7.	Varalaxmi	72.5	73.8	70.4	72.2	1.7
8.	Laxmi	49.6	51.2	52.6	51.1	1.5
9.	Hybrid 4	55.6	52.3	55.0	54.3	1.8
10.	320-F	47.2	49.3	48.4	48.3	1.0
11.	SRT-1	84.1	84.5	88.7	85.8	2.6
Oil						
12.	SRT-1 oil	96.1	94.3	95.5	95.3	0.9

1, 2 and 3 denote replicate samples.

Pulsed NMR Spectrometer

A coherent, pulsed, NMR spectrometer, fabricated at Jozef Stefan Institute, Ljubljana, Yugoslavia, was operated at a nominal frequency of 16 MHz. The full volume of the r.f. coil, measuring 24 × 24 mm, was filled with the seed sample. The 90 pulse width was 37 μsec, and pulse spacing between any two adjacent 180 pulses was kept at 0.5 msec. The echo amplitudes at 16, 32, 48,..... 112 msec were measured. The spectrometer has a hardwired program to process these amplitudes to yield the values of the transverse relaxation time and the extrapolated signal intensity at t = 0. The amplitude of any desired echo could be averaged over 10 or more repetitions and recorded on a four-digit display. In the present work, each signal intensity measured was an average of 10 repetitions. All NMR measurements were carried out at 26 C.

RESULTS AND DISCUSSION

Transverse Relaxation Time—Variation Among Varieties

The transverse relaxation times of oil protons in seeds and that of SRT-1 oil are shown in Table I. This table illustrates that for each variety the variation in T_2 values, within the three replicates, is quite small. However, one way analysis of variance of the data (11) reveals that the mean T_2 values

are significantly different, with $p < 0.001$ for each of the three groups, viz., diploid, tetraploid and all samples pooled together. It may be pointed out that the mean T_2 values of tetraploid (70.5 msec) and diploid (46.1 msec) cottonseeds are also different at the same level of significance. The variation in T_2 values, which has important bearing on oil estimation, can be attributed to a variety of causes, such as differences in fatty acid composition, inhomogeneous distribution of oil in the seeds, inhomogeneity in internal magnetic field, etc. It is interesting to note that only in the case of SRT-1 seeds does the T_2 value approach that of oil, probably because the oil has been extracted from the same variety.

Oil Estimation—Use of Individual Echoes

The significance of the variation in the T_2 values lies in the fact that NMR signal intensity depends both on the number of oil protons and their T_2 value. Thus, for oil estimation, the use of the echo amplitude at a single echo would result in different values for different echoes, as illustrated in Figure 1. The oil contents shown in this figure have been calculated by comparing the echo amplitudes of certain selected echoes of the signal from the sample subjected to the CPMG sequence, with the corresponding amplitudes observed in a known amount of SRT-1 oil. The oil content progressively decreases for later echoes because the T_2 values of the oil protons in the seed (also shown in the figure) are less than that of oil. For the SRT-1 variety of seeds whose T_2 value is close to that of oil, the variation is within experimental error.

Extrapolation Method—Effect of Lint and Moisture

Table II, depicting the results of oil content measurement, shows that neither the presence of lint nor the associated moisture has any effect on the estimated values of oil content. The last column indicates that even in artificially hydrated seeds the values are in excellent agreement with those of the corresponding dried samples. The oil contents are consistently higher only for those samples which had a water content greater than 22% (last 4 varieties in the table). The water content of seeds under normal storage conditions is well below this value; thus, this method can be used without drying the seeds.

Extrapolation Method—Comparison with Soxhlet Extraction Technique

It now remains to establish a one to one correspondence between the values obtained by the extrapolation method and those obtained by Soxhlet extraction. First, the linearity between the amount of oil and the signal intensity obtained by the present method is shown in Figure 2 for a sample of SRT-1 oil. In order to get comparable signal intensities from the seed sample and the corresponding oil control, it is necessary to use much smaller volumes of oil, resulting in the filling factor being reduced with a concomitant reduction in the signal intensity. This is likely to be one of the major sources of error in the absolute estimation of oil content by any NMR method using an oil sample as control. This can be circumvented by using a sample of seeds itself as control so that the filling factor does not differ greatly. The oil content of this control sample can be estimated carefully either by NMR or chemical method, and used to calculate the oil content of other samples.

The results of such a study using the Sea Island variety of seeds as control are shown in Table III. The corresponding values obtained by Soxhlet extraction method also are shown in Table III. The standard deviations in the table refer to the variation from replicate to replicate rather than due to the methods themselves. In general, the NMR

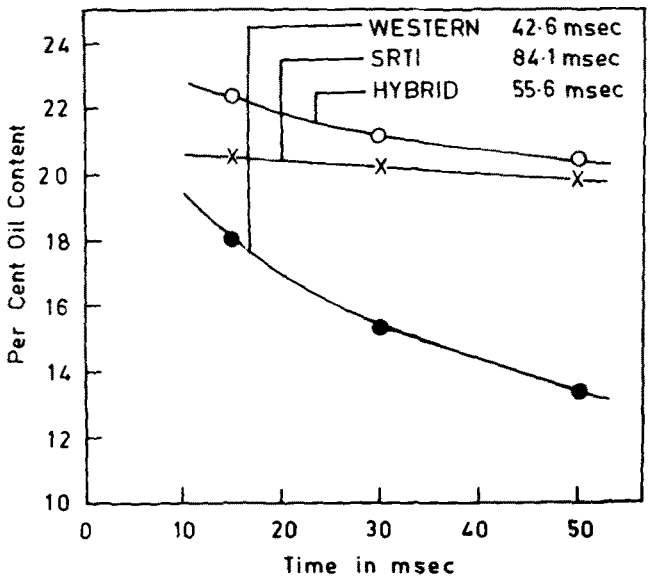

FIG. 1. Variation in oil content as estimated by using the signal intensities of individual echoes. The varieties of cottonseeds and the corresponding T_2 values also are indicated.

TABLE II

Oil Content in Undelinted, Delinted, Dried and Hydrated Samples of Cottonseeds as Estimated by CPMG Technique Using SRT-1 Oil as Control

Seed variety	Per cent water[a]	Per cent oil content[b] 1	2	3	4
Diploid					
1. Gaorani 22	13.8	11.2	11.5	11.7	11.0
2. Western 1	14.5	12.5	12.6	12.5	11.9
3. V-797	18.3	11.3	11.4	11.4	11.1
4. G-1	24.1	17.4	18.2	18.2	18.4
Tetraploid					
5. Khandwa 2	14.3	20.9	21.8	22.0	21.9
6. Sea Island	17.2	19.8	19.9	19.7	19.8
7. Varalaxmi	22.8	20.3	20.7	21.0	22.0
8. Laxmi	27.2	17.9	17.4	17.1	18.3
9. Hybrid 4	28.3	23.8	24.4	24.3	26.8
10. 320-F	30.8	12.1	12.4	12.5	14.5

1 = undelinted; 2 = delinted; 3 = dried; 4 = hydrated.
[a]Water content of the hydrated samples on basis of dry weight.
[b]Oil content calculated on basis of dry weight of the seeds.

method has been reported to be more precise and reproducible (12), which is in accordance with our experience.

It can be seen from Table III that the agreement between the two methods is good except in a few cases. It was observed earlier that oil extracted from the samples by the Soxhlet method did not all have the same color. Some of the samples yielded light brown oil; others were dark brown. Analysis of the data on the basis of the color revealed that a deviation of more than 1% between the two methods is restricted to those samples which yielded dark brown oil. The mean difference in oil content between the extrapolation and Soxhlet methods, along with their 99% confidence limits, are shown in Table IV. It can be seen that the agreement is best for samples with light brown oil. The SRT-1 oil used as the absolute standard also is light brown.

Thus, the small but significant discrepancy observed for the samples with dark brown oil indicates the importance

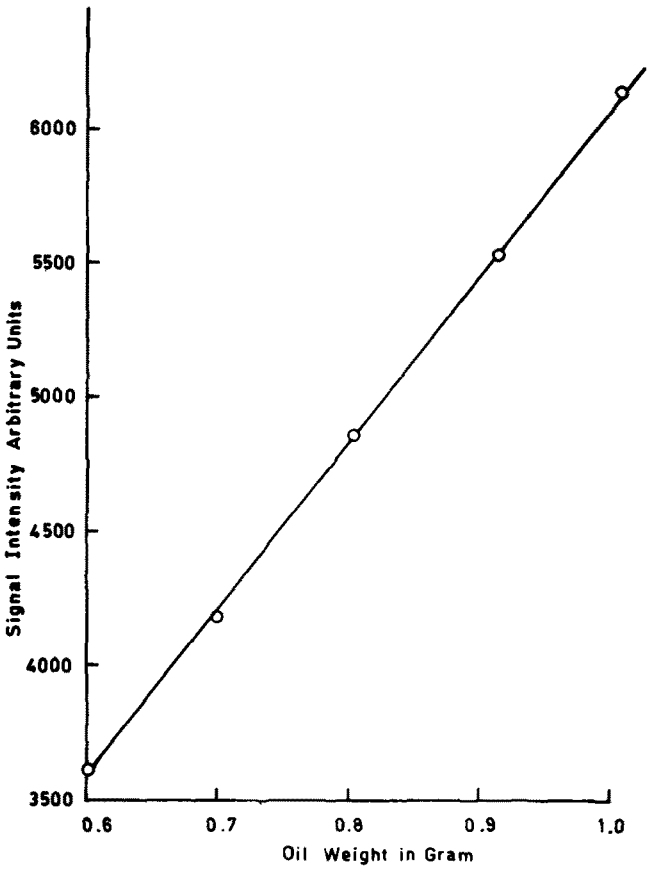

FIG. 2. Linearity between the amount of SRT-1 cottonseed oil and NMR signal intensity, as estimated by extrapolation method.

of the nature of the oil used as a standard. Generally, it is preferable to use the oil from one of the varieties themselves as the standard. However, it is unlikely that oil from different varieties of the same crop will have identical properties. In fact, it has been reported that the reliability of solid-content determination by wide-line NMR depends on the reliability of the oil chosen for reference (13). One of the parameters which is different for different varieties of seeds is the transverse relaxation time as shown in Table I. However, as the extrapolation method takes into account the variations in the T_2 values, this cannot be the reason for the observed discrepancy. We believe that the variation is due to varying amounts of gossypol present in the different samples, as most of the pigments in cottonseed oil are reported to be of the gossypol type (15). Even though the maximum gossypol content in cottonseeds is only about 2%, it can be shown that a difference of just 1% in gossypol content can cause as much as 3% variation in NMR signal intensities. This is due to the fact that the proton density per unit mass of gossypol is only about half that of fatty acids. The details will be published separately.

Other Methods

Infrared reflectance spectroscopy (IRS) has been used for rapid measurement of oil, protein and moisture in commercial samples of cottonseeds (16). But NMR has been reported to be more precise and reproducible (12). Seed oil estimation carried out by the free induction decay method, which involves measurement of the free induction decay signal of solid and liquid, has been reported for mustard, sunflower and soybean seeds (2,3). In the earlier method (2), the seeds had to be dried, and moreover it was not applicable to asymmetric seeds. Even in the latter method

TABLE III

Comparison of NMR and Soxhlet Methods of Cottonseed Oil Estimation

Seed variety	Method	Per cent oil content[a] 1	2	3	Mean	s.d.
Diploid						
1. Gaorani 22	NMR	13.5	14.4	10.9	12.9	1.8
	Soxhlet	14.1	14.9	11.9	13.6	1.6
2. Western 1	NMR	12.1	11.7	12.0	11.9	0.2
	Soxhlet	13.6	13.1	13.7	13.5	0.3
3. V-797	NMR	13.1	12.5	12.1	12.6	0.5
	Soxhlet	13.8	12.3	12.0	12.7	1.0
4. G-1	NMR	19.5	18.9	19.8	19.4	0.5
	Soxhlet	21.3	19.8	21.8	21.0	1.0
Tetraploid						
5. Khandwa 2	NMR	22.5	22.6	21.9	22.3	0.4
	Soxhlet	21.8	22.2	20.0	21.3	1.2
6. Sea Island[b]	NMR	20.0	21.4	21.1	20.8	0.7
	Soxhlet	21.6	21.1	21.9	20.8	0.4
7. Varalaxmi	NMR	20.4	21.4	20.9	20.9	0.5
	Soxhlet	20.7	19.6	19.2	19.8	0.8
8. Laxmi	NMR	19.8	19.8	18.3	19.3	0.9
	Soxhlet	20.0	20.0	17.7	19.2	1.3
9. Hybrid-4	NMR	24.6	23.5	24.3	24.1	0.6
	Soxhlet	23.7	22.8	23.5	23.3	0.5
10. 320-F	NMR	14.7	14.6	13.3	14.2	0.8
	Soxhlet	14.4	14.8	12.8	14.0	1.1

[a]Oil content calculated on basis of dry weight of seeds.

[b]The NMR values of oil content for this variety were determined using SRT-1 seed oil as control. This variety was used as the standard for the other samples.

TABLE IV

Difference in Oil Content between NMR and Soxhlet Methods

Basis of classification		Difference Mean	s.d.	Confidence limits[a]
Samples with light brown oil	(17)	−0.05	0.69	−0.74 to 0.64
Samples with dark brown oil	(13)	−0.38	1.29	−1.98 to 1.22
All samples	(30)	−0.20	0.99	−0.86 to 0.46

Numerals in parentheses indicate number of samples.

[a]99% confidence levels.

(3), correlation coefficients are not very good particularly for nonspherical, asymmetric seeds such as sunflower. In fact, Van Boekel (14) has reported that correct results were obtained with methods that use only the signal of the liquid phase. On the other hand, the present extrapolation method based on T_2 decay, which has been used for both dry and moist cottonseeds, characterized by their big size and asymmetry, and which measures only the liquid signal, can be expected to be better. The value of 0.05 with a s.e. of 0.17 obtained in the present studies for the mean difference in oil content between the two methods (NMR and Soxhlet) is remarkably good in view of the fact that these samples, on the average, have only about 17% oil content. The agreement can be expected to be much better for seeds with higher oil content, such as mustard, sunflower and groundnut.

This method also has been used by others to estimate both oil and water content in oil bearing seeds simultaneously (17). A very similar method, but based on longitudinal relaxation times, has been used for oil and water determination in O/W emulsions (4). It suffers from the disadvantage that the pulse sequence involved would take a much longer time than the present method.

ACKNOWLEDGMENTS

S. S. Narayanan of the Central Institute for Cotton Research, Nagpur, provided the SRT-1 variety of cottonseeds and seed oil. L. V. Chowdhary provided technical assistance in the chemical estimation of seed oil.

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[Received August 14, 1984]

♣Determination of Volatile Sulfur Compounds in Canola Oil

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ABSTRACT

A simple and sensitive method for the quantitative determination of volatile isothiocyanates in canola oil has been developed. The method is based on the specific absorbance of isothiocyanates in the infrared region. The results obtained were confirmed by gas liquid chromatography using a flame photometric detector. The various volatile isothiocyanates isolated from the oil were allyl isothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate and 2-phenethyl isothiocyanate. Their identities were confirmed by mass spectroscopy and by retention times. The recoveries of sulfur from volatile sulfur compounds by this method ranged from 93.6% to 101.1% when compared to the amount determined by gas liquid chromatography. The coefficients of variability of volatile sulfur compounds in canola oils ranged from 1.7% to 3.2%. The sulfur content represented by the volatile sulfur compounds comprised 21.7% of the sulfur determined by the Raney nickel method for crude oil, 36.6% for refined oil and 22.7% for refined, bleached and deodorized oil.

INTRODUCTION

The rapeseed varieties presently grown in Canada belong to the *Brassica napus* and *B. campestris* species, and most of these varieties are low in erucic acid and glucosinolates. These cultivars and the oil and meal derived from them are referred to by the Canadian industry as canola, canola oil and canola meal. The specifications for canola are an oil which is low in erucic acid (<5%) and a meal which contains no more than 3 mg glucosinolate per gram of moisture free, oil free meal.

Sulfur compounds in canola oil have been implicated as hydrogenation catalyst poisons. Though the chemical nature of sulfur compounds in canola oil is not fully understood, they are believed to be the hydrolysis products of the glucosinolates present in the canola seed. An earlier study (1) showed that as little as 5 mg/kg of sulfur greatly affected the hydrogenation. Hence, there is a need for rapid, sensitive and comparatively simple methods for the quantitation of sulfur compounds in canola oil. Some of the methods available to determine volatile sulfur compounds in canola oil are the gas chromatographic technique reported by Daun and Hougen (2) and George and Töregård (3).

In this study, at least nine sulfur-containing compounds were found in crude canola oil. Four of these were identified and were determined quantitatively by gas liquid chromatography and by a method using infrared absorption spectroscopy as reported by Ashley and Leigh (4), Caldow and Thompson (5), and Leiber, Rao and Ramachandran (6).

MATERIALS AND METHODS

Canola oil samples used were commercially extracted crude, refined, bleached and deodorized oils. For reference compounds allyl, n-butyl, heptyl and 2-phenethyl isothiocyanates were purchased from Eastman Kodak Co., Rochester, New York. 5-Vinyloxazolidinethione was supplied by the National Research Council, Saskatoon, Canada.

For separation of the volatile sulfur compounds, 500 g samples of canola oil were placed in a 1-liter Parr pressure reaction apparatus series 4500. Nitrogen gas was bubbled through the inlet at a rate of 60 bubbles per min. The oil was heated from 25 C to 200 C at a rate of 5 C/min and kept at 200 C for 2 hr. The gas carrying the volatiles was led into a cold trap cooled with liquid nitrogen. The other end of the trap was connected to a vacuum pump. After 2 hr of heating at 200 C, the trap was disconnected and the volatiles were dissolved in appropriate solvents. The final volume was made up to 10 ml. For chromatographic analysis, HPLC grade n-hexane was used. Analytical grade carbon tetrachloride was used for infrared analysis.

Gas chromatography of the volatile sulfur compounds was done using a Shimadzu model GC-8A gas chromatograph equipped with a flame photometric detector and a 394 nm filter for operation in the sulfur mode (2,3). The flame photometric detector has a very high sensitivity for sulfur compounds. Columns (1.5 m × 3.2 mm OD) were packed with FFAP on 100/120 mesh chromosorb WAW DMCS (1:19, w/w) and EGSS-X on 100/120 mesh gas chrom P (1:99, w/w). These columns were used for the analysis of isothiocyanates. The FFAP column was kept at 100 C for 7 min and then programmed at 10 C/min to 200 C. The EGSS-X column was programmed at 5 C/min from 60 C to 200 C. The injection and detection temperature was 200 C, and the nitrogen flow rate was 50 ml/min. The flow rates for hydrogen and air were kept at 50 ml and 60 ml/min, respectively. Two μl of hexane extract was injected. It was found that better separations were achieved with the FFAP column.