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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.

TABLE I

Relationship Between Concentration and Specific Absorbance of Three Standard Isothiocyanates

Compound	Conc. mg/ml	Conc. -N=C=S mg/ml	Absorbance	Specific absorbance number per unit -N=C=S
Allyl isothiocyanate				
	0.84	0.492	0.144	0.293
	1.52	0.890	0.272	0.306
	1.81	1.060	0.351	0.331
	4.50	2.636	0.760	0.291
Butyl isothiocyanate				
	1.40	0.706	0.218	0.309
	2.30	1.160	0.275	0.237
	2.36	1.190	0.345	0.290
	3.40	1.715	0.550	0.321
Phenethyl isothiocyanate				
	1.20	0.427	0.150	0.351
	2.66	0.946	0.290	0.316
	4.85	1.725	0.600	0.347
	10.00	3.558	1.380	0.374

TABLE II

Linear Regression and Correlation Coefficients for the Calculated -N=C=S Concentrations of Three Standard Isothiocyanates

Standard isothiocyanate	Linear regression	Correlation
Allyl isothiocyanate	Y = 0.282 X +0.023	0.997
Butyl isothiocyanate	Y = 0.335 X −0.053	0.954
Phenethyl isothiocyanate	Y = 0.384 X −0.046	0.999
Combined	Y = 0.372 X −0.066	0.979

X = Calculated -N=C=S concentration. Y = Specific absorbance number.

The GC-MS analyses were performed on a VG Micromass 12000 mass spectrometer (VG Micromass Ltd., England) interfaced with a Hewlett Packard GC.5790 equipped with data system PDP 11/224. The glass capillary column (30 m × 0.5 mm) was programmed from 30 C to 200 C. Ions were produced with a 70 eV electron beam, and a mass range of 50-200 was scanned.

A Beckman model 4230 infrared spectrophotometer with sodium chloride cells of 0.2 mm pathlength was used for the determination of volatile sulfur compounds. Standard solutions of three different isothiocyanates were made in carbon tetrachloride and the absorbance measured at 2105 cm^{-1} against pure carbon tetrachloride as the blank. Carbon tetrachloride was used because it does not absorb around 2105 cm^{-1}. Table I lists the concentration of different isothiocyanates vs absorbance. Although different isothiocyanates are used, the absorbance is due to the -N=C=S radical, the calculated value of which is given in the second column of Table I. The specific absorbance number per unit of -N=C=S is given in the fourth column. The combined linear regression and correlation are given in Table II. The phenyl isothiocyanate gave the highest correlation coefficient (0.999). The volatile material obtained from the oil samples was dissolved in carbon tetrachloride, and the solution was concentrated using a rotary evaporator at room temperature. The final volume was made up by using a graduated vial (No. 13233, Pierce Chemical Co., Illinois, U.S.A.). The absorbance at 2105 cm^{-1} was measured, and the amount of -N=C=S and sulfur was calculated from the established average specific absorbance for the -N=C=S radical.

The sulfur content in canola oil was determined with the commonly used Raney nickel method (7). It is known that this method does not measure all of the sulfur contained in canola oil (8).

The sulfur contained in canola oil may occur in a variety of forms, volatile and non-volatile organic compounds (8) and possibly inorganic forms such as sulfates and sulfides (9). Sulfur content is usually expressed as mg/kg S.

RESULTS AND DISCUSSION

Samples of volatiles obtained from canola oil were analyzed on the FFAP column (Fig. 1) and EGSS-X column, and nine major and some minor peaks were obtained. Four of the major peaks corresponded in retention time to allyl, 3-butenyl, 4-pentenyl and 2-phenethyl isothiocyanate. Methanol extracts of canola oil injected directly into the EGSS-X column gave similar peaks. 5-Vinyl oxazolidine-2-thione was not found in any of the extracts, in contrast to the findings of Daun and Hougen (2). It is possible that this compound decomposed during the initial heating process. The mass spectra of the eluted isothiocyanates gave peaks corresponding to the molecular ions m/e 99 for allyl-, m/e 113 for 3 butenyl-, m/e 127 for 4-pentenyl- and m/e 163 for 2-phenethyl isothiocyanate and m/e 72 corresponding to the $^+CH_2$-N=C=S ion. The 3-butenyl isothiocyanate also had a prominent peak for R^+ ion m/e 55 corresponding to the butenyl ion $CH_2 = CH\text{-}CH_2\text{-}CH_2^+$. Peak no. 1, although not identified by mass spectrometry, probably corresponds to vinyl isothiocyanate on the basis of retention time.

The response of sulfur compounds in the flame photometric detector has been discussed by several workers (10, 11). Although it is generally believed that the response is proportional to the square of the sulfur concentration, Sugiyama (10) reported that the response may vary from 1-1.8th power of the sulfur concentration. Hence, calibration curves using standard isothiocyanates were used in

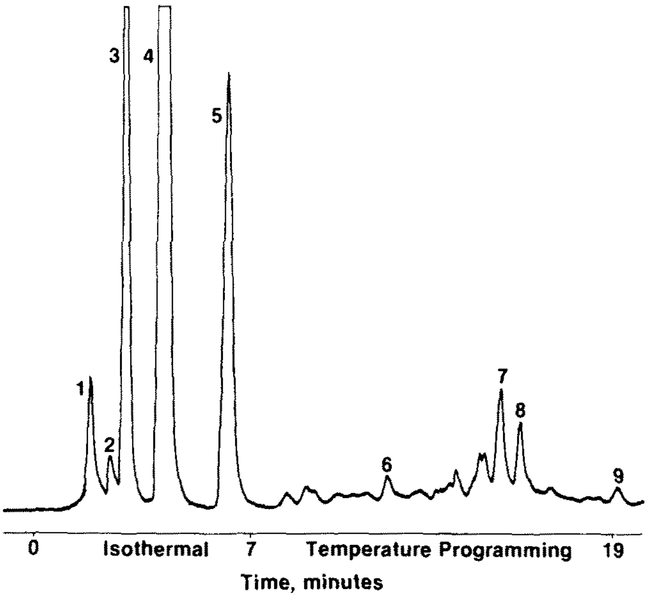

FIG. 1. Gas liquid chromatography of volatile sulfur compounds from canola oil on FFAP column. Identity of peaks: (2) allyl isothiocyanate, (4) 3-butenyl isothiocyanate, (5) 4-pentenyl isothiocyanate, (6) heptyl isothiocyanate (internal standard), (9) phenethyl isothiocyanate, (1,3,7,8) unknown peaks.

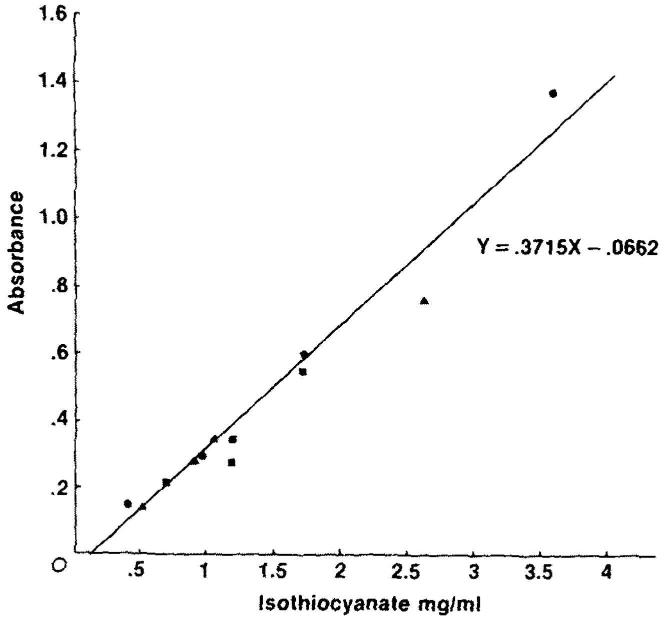

FIG. 2. Plot of infrared absorption of three isothiocyanates vs -N=C=S concentration. Allyl isothiocyanate (▲); butyl isothiocyanate (■); phenethyl isothiocyanate (●).

this study. The flame photometric detector is based on the emission of S_2 species at 394 nm in a hydrogen/air flame. Quantitative determination of sulfur compounds can be done by using one standard sulfur compound for intensity calibration (11). However, in this study, two standard compounds were used for calibration purposes, allyl isothiocyanate for low molecular weight sulfur compounds and phenethyl isothiocyanate for high molecular weight compounds. Peaks one to five were measured using allyl isothiocyanate at a temperature of 100 C. Peak no. 9 is not eluted at this temperature. Hence peak nos. 6-9 were measured using phenethyl isothiocyanate programmed from 100-200 C at a rate of 10 C/min. When programming conditions were used, peak nos. 1-5 were not well separated. Therefore, two calibration curves were used for the analysis.

Of all the volatile sulfur compounds identified by gas chromatography, 3-butenyl isothiocyanate amounted to more than 75% of the total. Levels of aromatic sulfur compounds were comparatively low (only phenethyl isothiocyanate was found).

The aromatic isothiocyanates have an intense absorption band in the wavelength range of 2000-2200 cm^{-1}. Aliphatic isothiocyanates have an intense absorption near 2100 cm^{-1}, but band splitting is more marked than with aromatic isothiocyanates. The volatiles consisting of aromatic and aliphatic compounds had a strong absorption at 2105 cm^{-1}. Use of carbon tetrachloride as solvent for infrared isothiocyanate determination has been reported by several authors (4,5,6). The curve relating concentration and absorption (Fig. 2) consisted of one aliphatic unsaturated sulfur compound, one saturated aliphatic compound and one aromatic sulfur compound. Since the volatiles are a mixture of unknown isothiocyanates, the combined average specific absorbance unit is a better representation than the specific absorbance unit of one single isothiocyanate. The amounts of sulfur calculated from the volatile sulfur compounds by the infrared method and gas chromatographic method were close (Table III). Analysis of variance and confidence limits for the regression line is given in Table IV. These data show a correlation coefficient of 0.996 for the regression line and a slope very close to 1 (Fig. 3). Although there is a high degree of precision, a probable weak point is the use of three pure isothiocyanates to estimate the -N=C=S in the infrared method. The specific absorbance numbers per unit -N=C=S ranged from 0.24 to 0.37. The sulfur content of canola oil as determined by the Raney nickel method and by the infrared method is listed in Table V. Sulfur content as determined by the infrared method represented 21.7% to 36.6% of the sulfur determined by the Raney nickel method.

In conclusion, nine volatile sulfur compounds were found in canola oil. All of these were present in both crude and refined oils. Bleached and deodorized oil contained only 3-butenyl and 2-phenethyl isothiocyanates. Volatile

TABLE IV

Analysis of Variance and Confidence Limits for Regression Line

Source	Degrees of freedom	SS	MS
Due to regression	1	0.5954	
Deviation from regression	22	0.0049	.0002
Total	23	0.6003	

Regression points		95% Confidence limits for the mean of Y for given X		
X	Y			
0.250	0.240	0.229	–	0.251
0.470	0.469	0.463	–	0.475
0.650	0.640	0.630	–	0.650
0.000	0.023 (for individual Y values)	−0.012	–	0.060

Regression line: $Y = .0234 + .9491\ x$.

Correlation coefficient = 0.9959.

X = values for GLC assay. Y = values for IR assay.

TABLE III

Content of Volatile Sulfur Compounds in Canola Oil as Determined on 8 Replicates by Gas Chromatographic and Infrared Methods (Expressed as mg/kg S)

	Crude mg/kg		Refined mg/kg		Refined, bleached and deodorized mg/kg	
	(GC)	(IR)	(GC)	(IR)	(GC)	(IR)
Range	0.605 – 0.666	0.598 – 0.646	0.506 – 0.542	0.517 – 0.544	0.239 – 0.246	0.248 – 0.254
Mean	0.638	0.623	0.526	0.532	0.244	0.251
SD	2.27×10^{-2}	1.83×10^{-2}	1.18×10^{-2}	9.07×10^{-3}	2.75×10^{-3}	2.66×10^{-3}

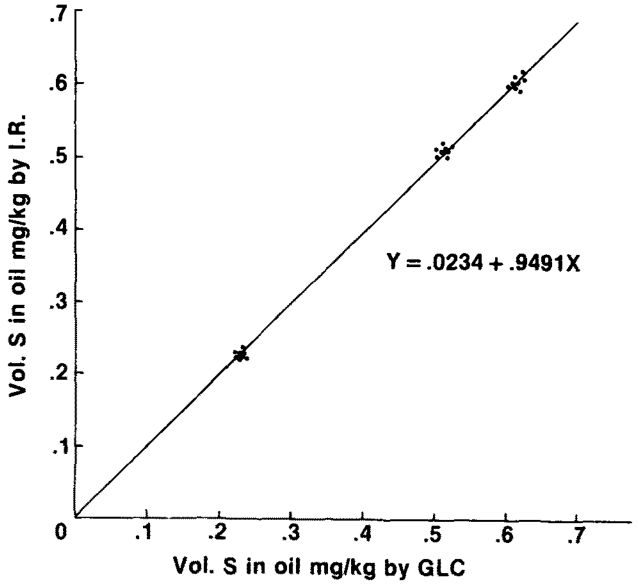

FIG. 3. Relationship of volatile sulfur content in canola oil as determined by gas chromatography and infrared spectroscopy.

TABLE V

Sulfur in Single Samples of Canola Oil as Determined by the Raney Nickel Method and the Infrared Method

Sample	Raney nickel method (mg/kg S)	Infrared method (mg/kg S)	Sulfur in volatile S compounds as % of Raney nickel S
Crude	2.86	0.62	21.7
Refined	1.45	0.53	36.6
Refined, bleached and deodorized	1.10	0.25	22.7

Each value represents the mean of 8 determinations.

sulfur compounds have been reported (8,12) to be mainly responsible for the poisoning effect on hydrogenation catalysts. The canola industry usually relies on the use of the Raney nickel method to measure the sulfur content. However, it is evident that the sulfur contained in the volatile sulfur compounds represents only a relatively small portion of the sulfur measured by the Raney nickel method. The infrared procedure described in this paper can be used to determine the volatile sulfur content of canola oil.

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