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SULFUR COMPOUNDS IN RAPESEED OIL

by

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ABSTRACT

Seven sulfur compounds were found in industrially produced rapeseed oils. Four of these were identified as glucosinolate hydrolysis products, namely, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, phenethyl isothiocyanate, and 5-vinyl-2-oxazolidinethione. The identity of these compounds was established by comparison with the pure compounds using gas chromatography, thin-layer chromatography, and mass spectroscopy. Three compounds were not identified; it is possible that these may be previously unreported glucosinolate hydrolysis products.

A number of industrially produced rapeseed oils at different stages of extraction and refining were analyzed for their contents of sulfur compounds. Six of the seven compounds revealed were quantitatively determined by gas chromatography using a sulfur-sensitive flame photometric detector. Phenethyl isothiocyanate was not determined because of interference in this method from one of the unidentified sulfur compounds. The total sulfur contents of the oils, as calculated by the sum of the individual sulfur compounds, agreed well with the total sulfur contents as earlier determined by a Raney nickel procedure.

In expelled, extracted, and crude oils, the contents of 3-butenyl isothiocyanate were approximately the same (0.7 - 4.3 ppm S) and were not substantially different in these oils from *B. napus* and *B. campestris*. The ratio of 3-butenyl isothiocyanate to 4-pentenyl isothiocyanate was approximately the same in the oils as in the meals. The amounts of oxazolidinethione in expelled, extracted, and crude oils ranged from 2.0 to 26.1 ppm S. The highest contents of oxazolidinethione were found in oils which contained large amounts of gums. The presence or absence

of gums did not seem to be related to the content of the other sulfur compounds. The unidentified compounds were present in much larger quantities in oils from *B. campestris* (0 - 3.7 ppm) than in oils from *B. napus* (0 - 1.2 ppm). Expelled oils contained substantially larger amounts of the unidentified compounds than extracted oils. Crude oil extracted from a low-glucosinolate rapeseed (*B. napus*, cultivar Bronowski) contained only traces of each sulfur compound.

Industrial degumming, refining, and bleaching removed most of the oxazolidinethione and substantial amounts of the unidentified compounds from the oils. The content of 3-butenyl- and 4-pentenyl isothiocyanate was not lowered by these procedures. Deodorization left only traces of sulfur compounds in the oils.

The quality and moisture content of rapeseed had a marked effect on the amounts of sulfur compounds in oils extracted in the laboratory. Traces of at least one sulfur compound were found in oils extracted from seeds of low moisture content (4%). Most of the sulfur compounds found in industrially extracted oils were found in oils extracted in the laboratory from seeds with moisture content of 8 - 10% or more. Relatively large amounts of sulfur compounds, including epithiobutanes, were found in oils extracted from seeds with moisture contents of 10.5% or more. Epithiobutanes, which are formed on hydrolysis of 2-hydroxy-3-butenyl glucosinolate were not found in the industrial rapeseed oils studied. Oils extracted from a low-glucosinolate rapeseed (*B. napus*, cultivar Tower) contained smaller amounts of sulfur compounds than oils from the high-glucosinolate seeds (*B. napus*, cultivar Oro and *B. campestris*, cultivar Span). Oil extracted from green or frost-damaged seed

contained lesser amounts of sulfur compounds than oil extracted from sound, mature seed. Oil extracted from heated seed contained relatively large amounts of sulfur compounds, similar to oil from sound seed.

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INTRODUCTION

Rapeseed contains a number of sulfur-containing glucosides known as glucosinolates. When rapeseed is damaged, i.e., by crushing, enzymes are released which catalyze the hydrolysis of the glucosinolates to form glucose, sulfate, and a number of organic compounds, most of which contain sulfur. Many of these compounds are toxic and their presence in rapeseed meal has hampered its utilization as a protein source for animals or humans.

Although glucosinolates and their hydrolysis products are associated primarily with the meal, there has been some concern over the possible extraction of a portion of the glucosinolate hydrolysis products with the oil. Difficulties in hydrogenating rapeseed oil have been ascribed to the presence of glucosinolate hydrolysis products, especially those containing sulfur, which might poison the hydrogenation catalyst (1). A "sulfurous" component has also been noted as contributing to the unpleasant odor associated with heated rapeseed oil (2). The presence of small amounts of these compounds in the oil may even be of nutritional significance, especially since an adverse effect has been demonstrated in humans consuming very small amounts of these compounds (3). Although some studies have been carried out on the total sulfur content of rapeseed oil, there is very little information available on the nature of the sulfur compounds present. Information on the types and quantities of sulfur compounds present in rapeseed oil would be of interest to industrial processors as well as to researchers carrying out studies on the quality of rapeseed oil and its products such as shortening and margarine.

The objectives of this study were (a) to identify the sulfur compounds present in rapeseed oil, (b) to determine the effect of industrial extraction and refining on the sulfur compounds of rapeseed oil, and (c) to study some seed conditions which may influence the quantity of sulfur compounds obtained in rapeseed oil.

Before presenting the experimental work relating to the above objectives, it was considered useful to review the literature related to the chemistry of glucosinolates and to the occurrence of sulfur and sulfur compounds in rapeseed oil and to their effect on the processing of rapeseed oil.

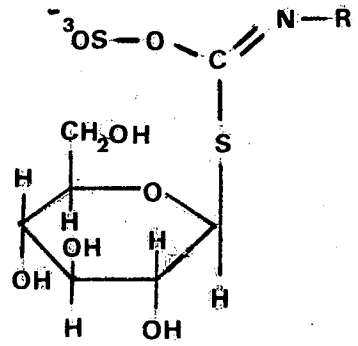
LITERATURE REVIEW

Chemistry of Glucosinolates

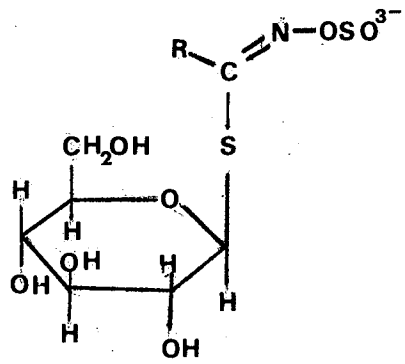
Many toxic constituents which have been isolated from various plants occur in the form of glycosides. Often the toxic effect is apparent only after the glycoside has been hydrolyzed, releasing a sugar and a toxic aglycon. Scission of the glycosidic bond most often is mediated by enzymes which usually occur in the same plant material but which also may be produced by external sources such as bacteria and fungi.

Structure and nomenclature of glucosinolates. The first use of pungent-tasting plants such as mustard, radish, and cabbage for food or medicine cannot be documented. It is known that the ancients used these plants as potherbs, condiments, and remedies (4). A crystalline precursor of the pungent principles of black mustard was isolated by Bussy in 1840 (5). The compound, allyl glucosinolate, was named sinigrin after *Sinapis nigra*. Sinalbin (*p*-hydroxybenzyl glucosinolate) had earlier been isolated from *Sinapis alba* but was not recognized as a glucosinolate until after Bussy's work.

In 1897 Gadamer (6) proposed a structure (Fig. 1a) for the compounds; this was revised in 1956 by Ettlinger and Lundeen (7) who confirmed their structure (Fig. 1b) a year later by synthesis (8). The nomenclature for glucosinolates has been somewhat confusing. In the early literature glucosinolates were referred to as "mustard oil glucosides." Sinigrin and sinalbin are trivial names which were given to allyl glucosinolate and *p*-hydroxybenzyl glucosinolate before much was known about their structure. Progoitrin was named because it is the precursor of goitrin



a. Gadamer (1897)



b. Ettlinger and Lundeen (1956)

Sinigrin, R = Allyl

Sinalbin, R = *p*-Hydroxybenzyl

Figure 1. Structure of glucosinolates.

(trivial name for D-5-vinyl-2-oxazolidinethione). Early workers in the field adopted a trivial system which attaches the prefix "gluco" to a part of the Latin name of the plant from which the glucosinolate was first isolated, i.e., gluconapin, gluconasturtiin. Kjaer (4) cited the need for a more systematic nomenclature with a chemical basis. Ettlinger and Dateo (9) in 1961 recommended the adoption of the suffix glucosinolate to which is prefixed the chemical description of the organic aglycone; i.e., sinigrin becomes allyl glucosinolate, sinalbin becomes *p*-hydroxybenzyl glucosinolate. This system is becoming popular although many authors still use the trivial names. Glucosinolates are still referred to as thioglucosides. The former term is preferred since it is more specific.

Occurrence of glucosinolates. Glucosinolates are found mostly in members of the Cruciferae, Capparidaceae, and Resedaceae although they also have been noted in species of Tropaeolaceae, Salvadoraceae, Caricaceae, Limnanthaceae, Moringaceae, Plantaginaceae, and Euphorbiaceae and perhaps in Phytolaccaceae and Aquifoliaceae (4). Approximately 50 different glucosinolates have been found. Table 1 lists some foodstuffs which contain glucosinolates (10). Table 2 gives the structural formulae of the glucosinolates found in rape (*B. napus* and *B. campestris*).

Biosynthesis of glucosinolates. Kjaer (16) was the first to point out the apparent relationship between glucosinolates and the α-amino acids. Evidence at the present time indicates that glucosinolates are derived from the α-amino acids through a chain lengthening process involving acetate. The immediate source of the sulphur atom in glucosinolates is not known, but Wetter and Chisholm (17, 18) using radiotracer techniques,

Table 1. Glucosinolates in Cruciferae

Plant	Glucosinolates present
For Food	
Brassica oleraceae cabbages, kale, brussel sprouts, cauliflower, broccoli, kohlrabi	sinigrin, glucobrassicin, progoitrin, gluconapin, neoglucobrassicin
Brassica campestris turnips	progoitrin, gluconasturtiin, D-2-hydroxy-4-pentenyl glucosinolate [a]
Brassica napus rutabaga	progoitrin, glucobrassicin, neoglucobrassicin
Lepidium sativum garden cress	glucotropaeolin
Raphanus sativus radish	4-methylthio-3-butenyl glucosinolate, glucobrassicin
For Condiments	
Amoracia lapathifolia A. rusticana horseradish	sinigrin, gluconasturtiin
Brassica carinata Ethiopian rapeseed	sinigrin
B. juncea Indian or brown mustard	sinigrin
B. nigra black mustard	sinigrin
Sinapis alba white mustard	sinalbin
Sinapis arvensis charlock	sinigrin
For Feed as Processed Seed Meal	
Brassica campestris rape, turnip rape, Polish rape, rubsen, naverte	gluconapin, progoitrin, glucobrassicanapin, glucoalyssin, glucoraphanin
Brassica napus rape, Argentine rape, winter rape	progoitrin, gluconapin, glucobrassicanapin, gluconasturtiin, glucoiberin, sinalbin
Crambe abyssinica crambe, Abyssinian kale	*epi*-progoitrin, sinigrin, gluconapin, gluconasturtiin

[a] The symbols D and L are used in this work when referring to configuration although many authors use the system (R,S) of Cahn *et al.* (120) when referring to glucosinolate structure.

Table 2. Glucosinolates found in rape

Trivial name	Structure of 'R' group[a]	*B. napus*[b]	*B. Campestris*[b]	Reference
Sinigrin	Allyl	*		(11)
Gluconapin	3-Butenyl	*	*	(12)
Glucobrassicanapin	4-Pentenyl	*	*	(12)
Glucoalyssin	4-Methylsulfinylbutyl		*	(12)
Glucoraphanin	5-Methylsulfinylpentyl		*	(12)
Glucoiberin	3-Methylsulfinylpropyl	*		(12)
Gluconasturtiin	2-Phenylethyl	*	*	(12)
Sinalbin	p-Hydroxybenzyl	*		(12)
Glucoerucin	4-Methylthiobutyl	*	*	(13)
Glucoberteroin	5-Methylthiopentyl	*	*	(13)
Progoitrin	2-Hydroxy-3-butenyl	*	*	(12)
	2-Hydroxy-4-pentenyl	*	*	(14)
Glucobrassicin[c]	3-Indolylmethyl	*	*	(15)
Neoglucobrassicin[c]	N-Methoxy-3-indolylmethyl	*	*	(15)
Glucocheirolin	3-Methylsulphonylpropyl	*		(11)

[a] "R" refers to the glucosinolate structure given in Fig. 1b.

[b] Glucosinolates occur in the species as designated *.

[c] These glucosinolates have not been found in the seed. The other glucosinolates occur in all parts of the plant.

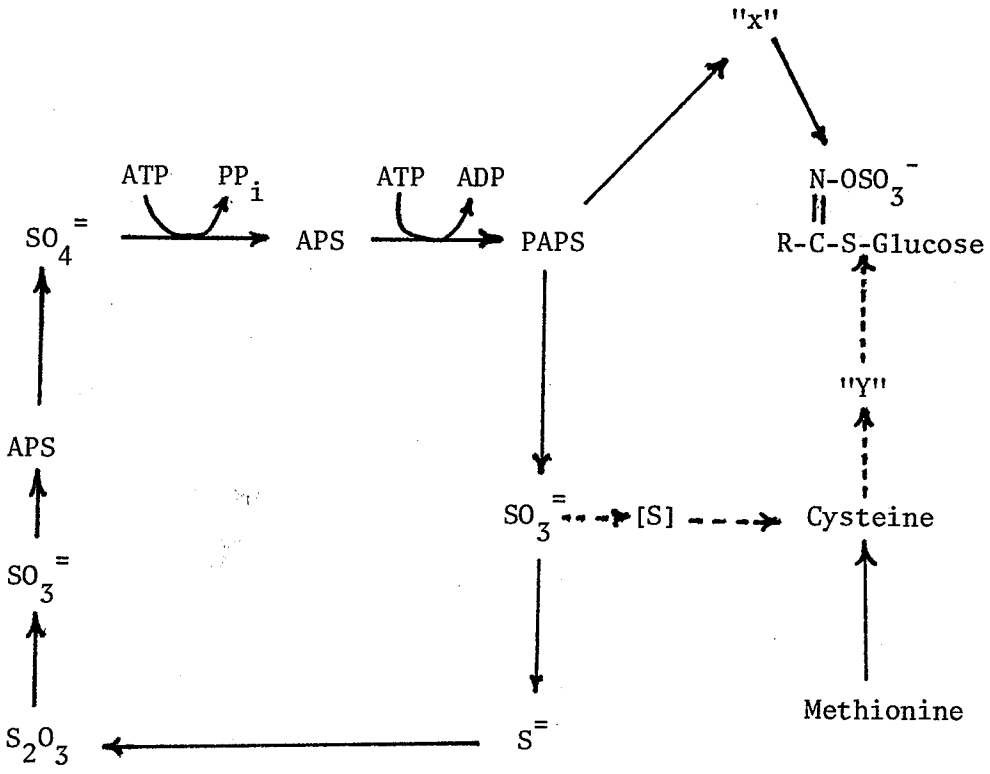
found that $SO_4^=$, SO_2, $S_2O_5^-$, and $S^=$ were all incorporated into glucosinolates. Sulfur from cysteine, methionine, homomethionine, and 1-thioglucose was also incorporated, with preference being given to sulfur from cysteine and methionine. Wetter (18) had earlier postulated a pathway for sulfur incorporation (Fig. 2). Figure 3 combines the results from several studies (19, 20, 21) to indicate current views on glucosinolate biosynthesis. The main areas of uncertainty seem to be the identity of the sulfur carrier ("XS") and the step at which the hydroxy group is incorporated in the formation of goitrin. Josefsson (20) emphasizes the importance to plant breeders of a more complete investigation of glucosinolate biosynthesis.

Glucosinolates are found in all parts of cruciferous plants. Root material and seeds seem to be the richest sources (4). In *Crambe abyssinica* seed, the glucosinolates are concentrated in the cotyledon and hypocotyl (22). In rapeseed, air-classification studies have shown higher glucosinolate levels in endosperm fractions than in the pericarp (23).

Hydrolysis of glucosinolates. Tissues which contain glucosinolates also contain the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1.). This enzyme mediates the hydrolysis of the thioglucosyl bond of glucosinolates. In rapeseed, myrosinase is deposited in special cells, idioblasts (4), and only after the cell walls have been broken does the enzyme come into contact with the glucosinolate.

There has been some confusion concerning the action of myrosinase. Gaines and Goering (24) claimed to have separated two myrosinase fractions, one having sulfatase activity and the other thioglucosidase activity.

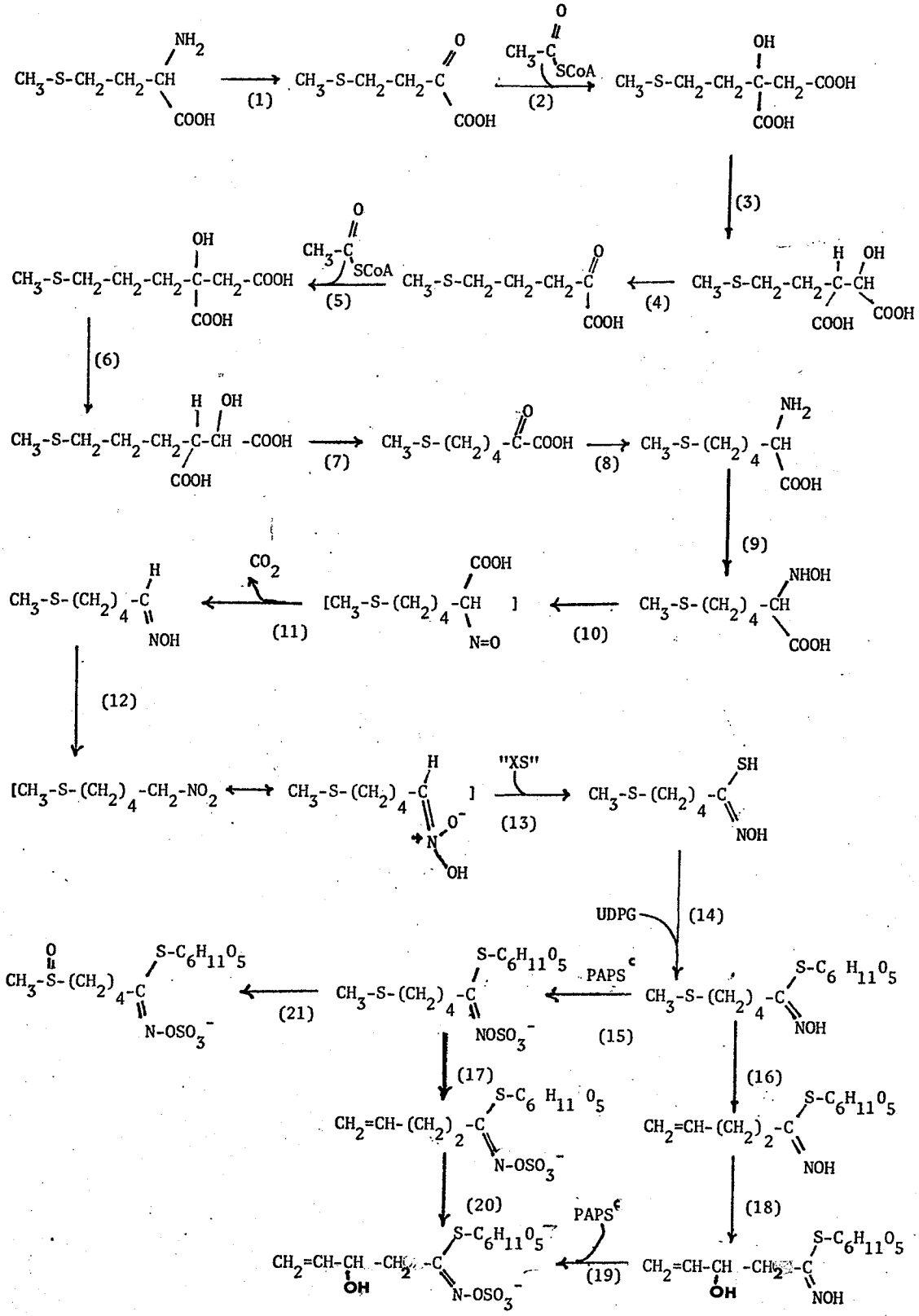
Figure 2. Utilization of sulphur in the biosynthesis of glucosinolates (18).



APS Adenosine-5'-phosphosulfate

PAPS 3'-phosphoadenosine-5'-phosphosulfate

Figure 3. Biosynthesis of rapeseed glucosinolates[a,b]



[a] Steps (1)-(8) after Chisholm and Wetter (19); steps (10)-(20) after Josefsson (20); step (21) after Chisholm (21).

[b] Biosynthesis of C_3,C_5 and aromatic glucosinolates is presumed to follow analogous pathways.

[c] 3'-phosphoadenosine-5'-phosphosulfate.

Calderson *et al.* (25) were unable to repeat this work. Tsuro *et al.* (26) found two fractions, both of which had dual activity. Recently, gel electrophoresis has been used in several studies of myrosinase. Using this technique myrosinase has been separated into several isozymes some of which are activated by ascorbate (27, 28, 29, 30). The question of myrosinase action is still under investigation.

Enzymatic hydrolysis of glucosinolates has been explained as an "enzymatic Lossen rearrangement" (8, 31). The reaction produces glucose, bisulfate, and several compounds formed by rearrangements of the organic aglucon (Fig. 4). The variation in enzymatic hydrolysis products has been investigated by several authors (32, 33, 34). In general, it has been found that the formation of isothiocyanates rather than nitriles is favored by dilution of the meal with water, increasing the hydrolysis temperature above 60°C, increasing the pH above 5 (this effect is variable between seeds of the same species from difference sources), heating of dry seed meal, and by storage of the seed at temperatures above 5°C.

The isothiocyanates formed by enzymatic hydrolysis of progoitrin from rapeseed or *epi*-progoitrin from crambe seed spontaneously react in the presence of water to form D- or L-5-vinyl-2-oxazolidinethione (35). Lanzani and Jacini (36) studied the chemical stability of oxazolidinethione. They found that heat or metallic catalysts caused oxazolidinethione to form a polymer or a thiazolidone (Fig. 5). Lanzani *et al.* (37) found that both the oxazolidinethione and the thiazolidone were formed directly from progoitrin in the rumen of sheep.

Other hydrolysis products from these thioglucosides have been identified as D- and L-1-cyano-2-hydroxy-3-butene (32) and 2L- and 2D-1-cyano-2-hydroxy-3,4-epithiobutanes (38). The configurations of the

Figure 4. Enzymatic hydrolysis of glucosinolates

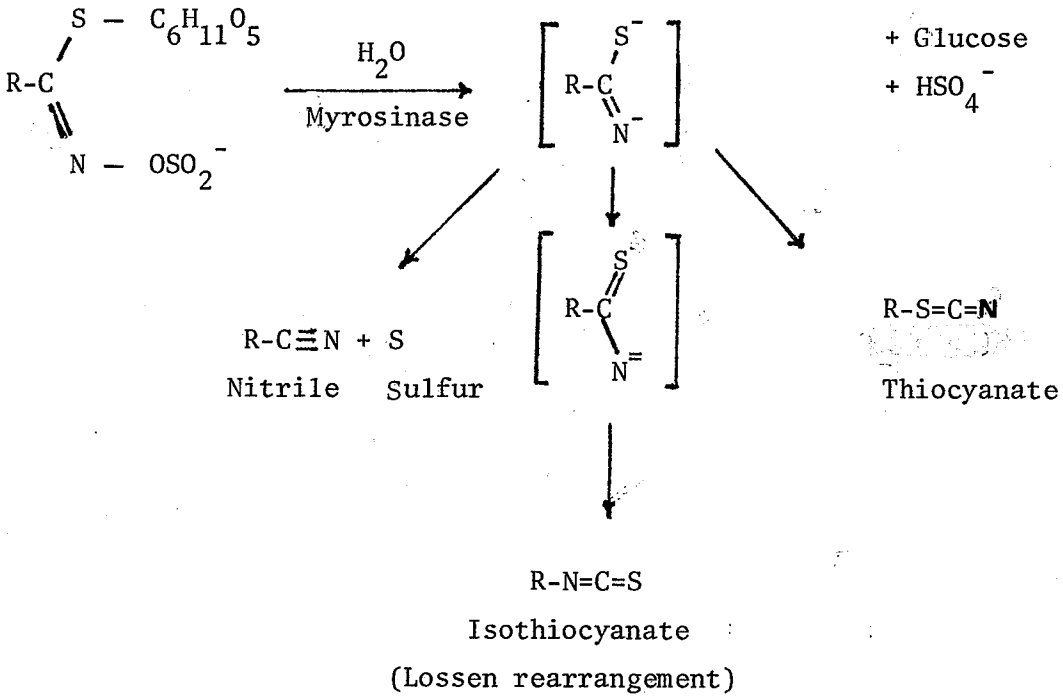
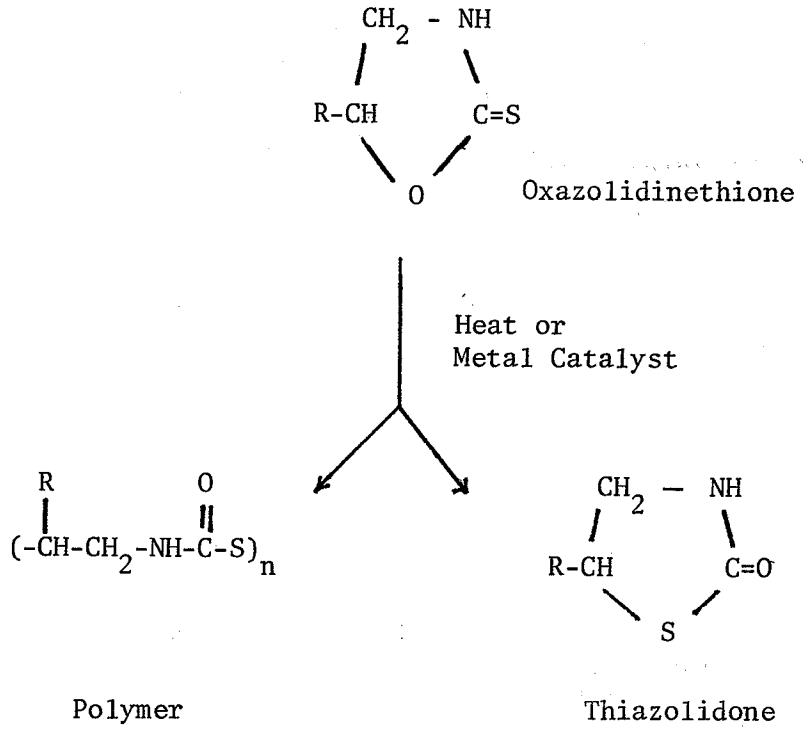


Figure 5. Chemical transformation of oxazolidinethione

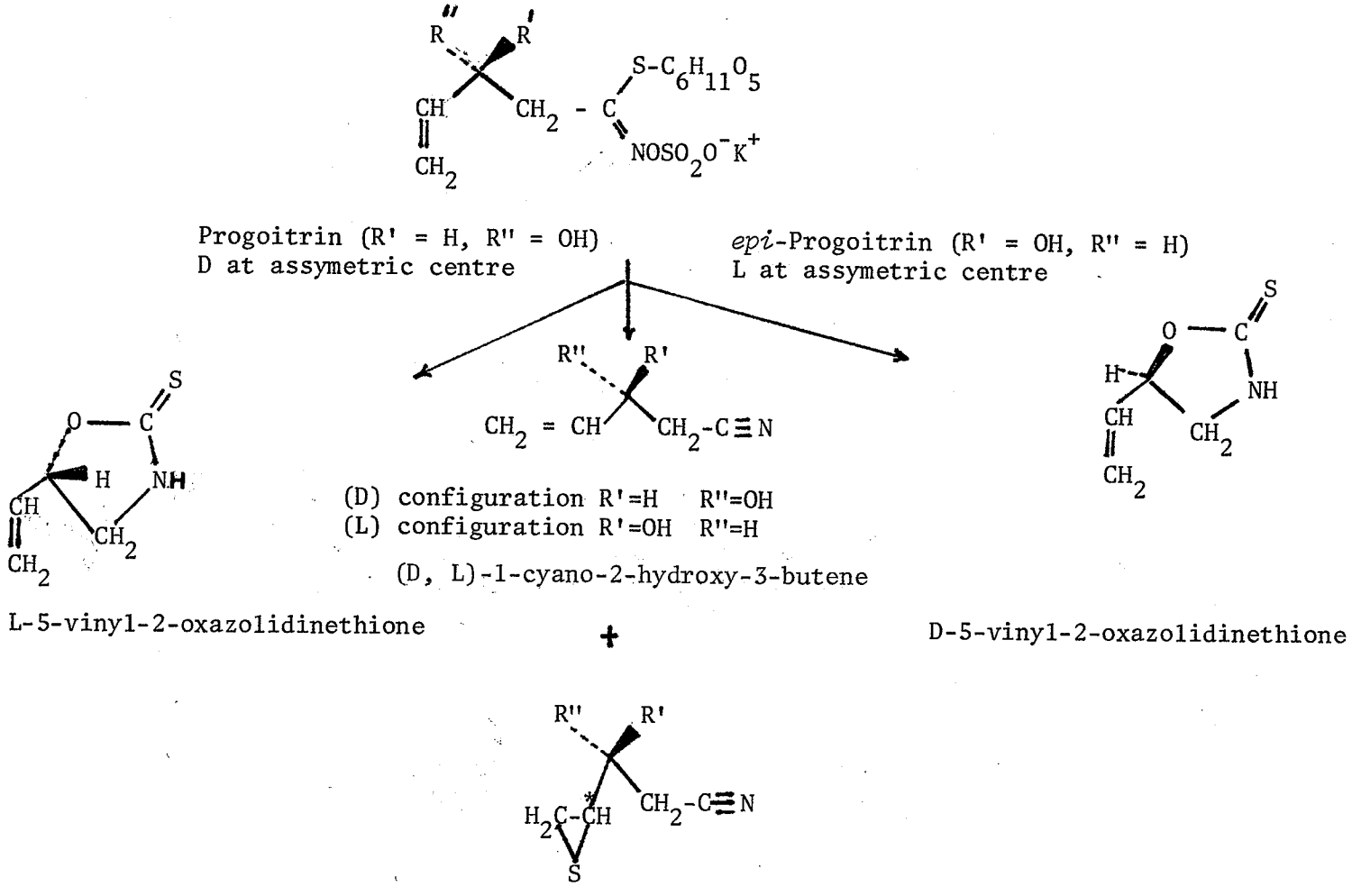


hydrolysis products are shown in Figure 6. Recently, a protein has been isolated, which acts in conjunction with myrosinase to specify the formation of epithiobutanes (39).

Enzymatic hydrolysis of other glucosinolates has also been shown to lead to complex mixtures of products. Allyl glucosinolate has been shown to hydrolyze to form allyl isothiocyanate or allyl cyanide depending on whether the conditions of hydrolysis are acidic or basic (40). Saarivirta (41) has shown that enzymatic hydrolysis of benzyl glucosinolate (from *Lepidium sativum*) gives a mixture of benzyl cyanide, benzyl thiocyanate, benzyl isothiocyanate, and benzylamine depending on the reaction conditions. Kawakishi *et al.* (42) have studied the decomposition of *p*-hydroxybenzyl glucosinolate from *Sinapis alba*. They found that enzymatic hydrolysis of this glucosinolate led to a complex mixture of products (Fig. 7). Jirousek (43) isolated an organic polysulfide fraction from cabbage and other *Brassica* plants and further isolated a "trithionen" (1,2-dithiocyclopenta-4-ene-3-thione) from this polysulfide fraction (44). A relationship to glucosinolates was not established but the "trithionen" compound was found to be goitrogenic.

Glucosinolates have also been hydrolyzed non-enzymatically by a number of reagents (40). Treatment of allyl glucosinolate (sinigrin) with one equivalent of silver nitrate resulted in the detachment of glucose and the formation of silver sinigrinate. Treatment with potassium methoxide gave 1-thio-D-glucose. Treatment with acid gave a carboxylic acid and D-glucose. Reduction gave a primary amine and 1,5-anhydro-D-sorbitol. Barium hydroxide selectively liberated sulphate presumably by hydrolysis of the ester linkage (6). Austin, Gent and Wolff (45) found that 2-hydroxy-3-butenyl glucosinolate reacted with ferrous ammonium sulfate at pH 5.3

Figure 6. Hydrolysis of progoitrin and *epi*-progoitrin (38)

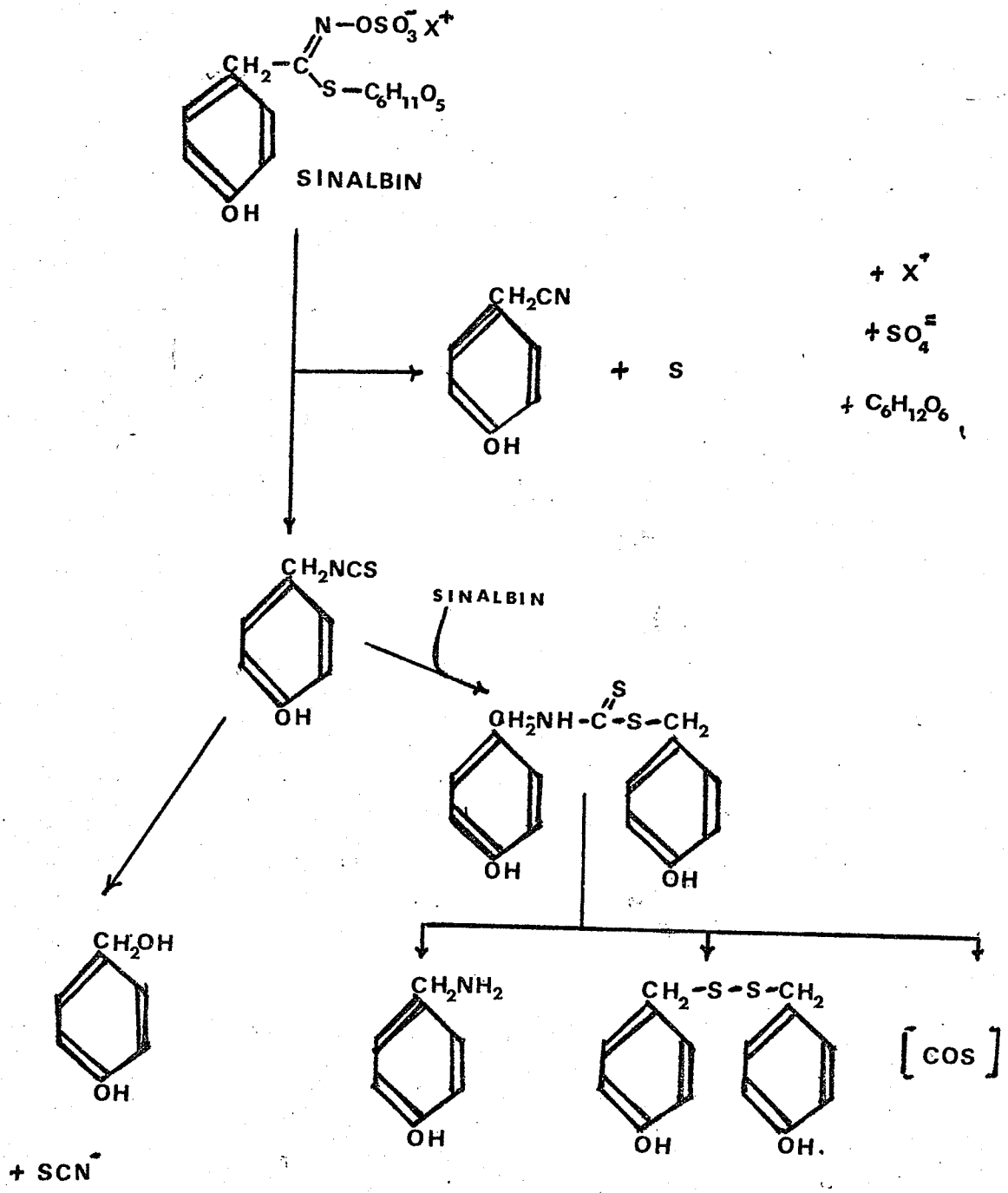


(2D) configuration R' = H R'' = OH
(2L) configuration R' = OH R'' = H

(2D, 2L)-1-cyano-2-hydroxy-3-4-epithiobutane

* assymetric centre.

Figure 7. Decomposition pathway for sinalbin (42).



to produce D-1-cyano-2-hydroxy-3-butene and D-1-cyano-3-hydroxypent-4-ene thionamide. Youngs and Perlin (40) cleaved allyl glucosinolate with ferrous sulfate at 95°C to form allyl cyanide, bis(β-D-glucopyranosyl) disulphide and other sugars. They also noted the formation of 1-cyano-3-butene and 1-cyano-4-pentene from rapeseed meal treated in a similar manner.

Analysis of glucosinolates. Due to the large number of different glucosinolates which may be present in a single plant species, and due to the complex mixture of products which may result from hydrolysis of any one glucosinolate, the analytical chemistry of glucosinolates is very complex.

A compilation of early analytical techniques may be found in the review by Stoll and Jucker (46), while a more recent review of the analysis of isothiocyanates may be found in the monograph by Ashworth (47). Recently, different methods for the determination of glucosinolates have been compared (48, 49, 50).

Most analytical techniques for glucosinolates rely on determination of products of glucosinolate hydrolysis but there are a few methods available for the separation and determination of intact glucosinolates. Matsuo (51) discussed several solvent systems applicable to the separation of glucosinolates by thin-layer chromatography. Björkman (52) described a method for the separation, isolation and quantitation of relatively large quantities of glucosinolates by ion-exchange chromatography on columns of DEAE-Sephadex A-25 and arginine coupled to Sephadex G-10. More recently, Lanzani *et al.* (53) have separated rapeseed glucosinolates on silicic acid columns. Underhill and Kirkland (14) described

a method for the determination by gas chromatography of the trimethylsilyl derivatives of many of the glucosinolates found in rapeseed.

Analytical techniques involving the products of glucosinolate hydrolysis may be used for determination of the total glucosinolates present in plant material, or for analysis of the individual glucosinolates. Glucose and sulfate are common hydrolysis products of all glucosinolates, and analysis of these gives a convenient estimate of total glucosinolates. McGhee *et al.* (54) used a gravimetric sulphate determination for determining the total glucosinolate content of crambe meal. These workers also determined total glucosinolates by the difference between total sulfur and amino acid sulfur, by an argentimetric titration (formation of the silver-glucosinolate complex), and by the loss of weight and sulfur content of the meal on extraction with hot water. The total glucosinolate content of rapeseed meal has been estimated by extraction of the glucosinolates with methanol-acetone-water, purification by chromatography on acid alumina, and quantitation by measuring the glucose by the phenol sulphuric acid reaction (Dr. F. W. Hougen, Department of Plant Science, University of Manitoba, personal communication). Lein and Sebödn (55) quantitatively determined glucosinolates in a single cotyledon of rapeseed by determining the amount of glucose released on hydrolysis with myrosinase. The glucose was determined by enzymatic oxidation followed by colorimetry. More recently (56) this method has been adapted for rapid screening of plant material for glucosinolate content by utilizing test paper impregnated with the enzymes and chromogens necessary for glucose determination.

When glucosinolates are hydrolyzed with myrosinase, at near neutral pH, the predominant organic aglucon product is an isothiocyanate. Isothiocyanates react with ammonia to form thioureas:

$$R\text{-}N{=}C{=}S \quad + \quad NH_3 \quad \rightarrow \quad RNH\text{-}\overset{\overset{S}{\|}}{C}\text{-}NH_2$$

It was through the formation of these compounds that the structure of many glucosinolates was first elucidated (57, 58). Wetter (59) determined the volatile isothiocyanates formed on hydrolysis of glucosinolates in rapeseed by steam distilling the isothiocyanates into a solution of ammonia and silver nitrate. The silver reacted quantitatively with the thioureas to form Ag_2S. Excess silver was determined by titration with KCNS. Later (60) he described a spectrophotometric method for the determination of oxazolidinethione formed from hydrolysis of 2-hydroxy-3-butenyl glucosinolate in rapeseed meal. Appelqvist and Josefsson (61, 62) described a modification of Wetter's method in which the seed meal enzymes were inactivated by boiling buffer solution instead of dry heat; hydrolysis was carried out at pH 7 instead of pH 4, and isothiocyanates and oxazolidinethiones were extracted with isooctane. Isothiocyanates were converted to thioureas, and thioureas and oxazolidinethiones were determined by ultra-violet spectrophotometry. Recently, an inter-laboratory study was carried out using a similar method (63).

Thioureas and oxazolidinethiones have been shown to catalyze the reduction of iodine by azide. Szewezuk *et al.* (64) described a method for the determination of oxazolidinethione in rapeseed meal utilizing iodine reduction. Isothiocyanates did not interfere and the sensitivity was similar to spectrophotometric methods. Kurzawa and Krzymien (65) measured the total glucosinolate content of a half-rapeseed utilizing the iodine-azide reaction catalyzed by the thioureas formed by the reaction of isothiocyanates and methylamine.

Other methods for the determination of total glucosinolate content include a polarographic determination of sulphide ion produced by alkaline hydrolysis of glucosinolates (66) and a gravimetric bioassay method utilizing grasshoppers fed wafers of rapeseed meal (67).

Determination of individual glucosinolates by separation and determination of their hydrolysis products was first reported by Kjaer and Rubinstein (58) who separated thioureas formed from isothiocyanates by paper chromatography. More recently, Rutkowski *et al.* (11) have developed a method whereby glucosinolates extracted from rapeseed were separated into "volatile" and "non-volatile mustard oils" which were converted into thioureas and separated by paper chromatography employing two different solvent systems. The spots were eluted from the paper and the thioureas were quantitatively determined by spectrophotometry.

For routine analysis, however, separation of individual thioureas by paper chromatography is not suitable. Youngs and Wetter, in 1967, published a rapid method by which the major isothiocyanates formed on hydrolysis of rapeseed glucosinolates were determined by gas-liquid chromatography (20). Oxazolidinethiones were determined by spectrophotometry. The method was reported to be suitable for breeding programs since only 5-20 mg of seed were required per analysis and one operator could perform as many as 24 determinations per day.

When glucosinolates are hydrolyzed under uncontrolled conditions, i.e., autolysis, isothiocyanates may not be the only hydrolysis products (Figs. 6 and 7). Originally the products were analyzed by techniques which were laborious and gave no information as to individual components of a single chemical species. For example, although the oxazolidinethione and thionamide can each be analyzed by ultra-violet spectrophotometry, only

the total quantity of 1-cyano-2-hydroxy-3-butene and related epithiobutanes was estimated by an infra-red procedure (38). Daxenbichler *et al.* (68) devised a rapid gas chromatographic procedure for analysis of the products formed from random hydrolysis of progoitrins in rapeseed and crambe meals. Lo and Hill (69) applied this method to the determination of glucosinolate hydrolysis products in blood, urine, and intestinal contents in rats.

Toxicity of glucosinolates. The first indication of glucosinolate toxicity was the observation by Chesney *et al.* (70), in 1926, of enlarged thyroid glands in rabbits fed mainly on cabbage. Since then many studies have been carried out, often presenting conflicting evidence on the toxicity of cruciferous foods. Daxenbichler *et al.* (38) have suggested that the wide variation observed in the response of animals in feeding trials with rapeseed meals may be due to the wide variation in the type of products resulting from hydrolysis of progoitrin as some of these products may be more toxic than others. These studies have been reviewed recently (10, 71, 72, 73) and reviews have appeared on goitrogens in human nutrition (74), in animal feeds (75), in milk (76) and in rapeseed (77).

Unhydrolyzed glucosinolates do not produce goitre. The goitrogenic properties are associated with the glucosinolate hydrolysis products. 5-Vinyl-2-oxazolidinethione has similar antithyroid potency as the antithyroid drug n-propylthiouracil (74). These antithyroid drugs interfere with iodine uptake into the thyroid gland and the binding of iodine to tyrosine in the thyroid gland (3, 78). Isothiocyanates have been shown to react with amino acids to produce thiohydantoins which have an effect similar to oxazolidinethiones (78). Nitriles and isothiocyanates both

are detoxified in the liver (79). One of the end products of detoxification is thiocyanate ion which competitively inhibits uptake of iodine by the thyroid. Langer and Greer (80) have shown that isothiocyanates are capable of interfering with iodination of amino acids in an *in vitro* system. In many feeding trials involving foods containing isothiocyanates, the goitrogenic effect was noted only if the diet was low in iodine (81).

Glucosinolate hydrolysis products have also been implicated as the cause of other toxic effects in animals. Perhaps the most prominent of these effects is hepatic lesions which have been observed in rodents (82, 83) and in chickens (84, 85). It has been shown that the nitriles formed on autolysis of glucosinolates are more toxic than the oxazolidinethiones and that the nitrile compounds are likely the causative agent in the hepatic lesions (83).

Although rapeseed meals have equal or slightly better protein quality than soybean meal, the presence of glucosinolates in rapeseed meals has hindered their use in animal feed or human food. It was hoped that heat-treatment to inactivate myrosinase would lower the toxicity of the meal (86, 87). The effectiveness of this procedure is questionable, however, since it has been shown that many bacteria, including some commonly found in the gut, have enzymes which hydrolyze glucosinolates (88). Studies have been made on the removal of glucosinolates from meals by physical (89) and chemical procedures (90), as well as on the preparation of detoxified protein concentrates (91, 92) and flour (93), similar to the products obtained from soybeans. Probably the most effective means of overcoming the glucosinolate problem lies in the development of glucosinolate-free seed (94).

Occurrence of Sulfur and Sulfur Compounds in Rapeseed Oil

Total sulfur content of rapeseed oil. There is relatively little data available on the total sulfur content of crude and processed rapeseed oils. Table 3 gives a summary of several studies. Wide variation in the sulfur content of extracted and crude oils has been noted. Daun (99) observed that the sulfur content of oils depended on the moisture content of the seed. Oil extracted from seed containing more than about 10% moisture contained relatively large amounts of sulfur. Industrial refining techniques have been shown to decrease the sulfur content of the oils so that the final deodorized oil contains only a trace of sulfur (Table 3).

Sulfur compounds in rapeseed oil. There is almost no information available concerning the sulfur compounds present in rapeseed oil. Grzybowska (100) adapted the method of Astwood (101) to the determination of 5-vinyl-2-oxazolidinethione in rapeseed oil. The oxazolidinethione was extracted from the oil with water, and reextracted from the water solution with ether. The ether extract was evaporated to dryness and the residue was extracted with water. The water extract was washed with petroleum ether and the oxazolidinethione was estimated spectrophotometrically. No oxazolidinethione was found in rapeseed oil. Furthermore, oxazolidinethione (5 ppm) added to rapeseed oil was found to decompose rapidly. Grzybowska suspected that the decomposition products were disulfides formed by oxidation of oxazolidinethione by peroxides in the oil.

Zeman and Zemanova (95) used steam distillation according to Wetter (59) to determine the isothiocyanate content of rapeseed oils. Expelled oils contained 0.01-0.02% isothiocyanates while extracted oils contained

Table 3. Sulfur content of processed rapeseed oils

Author, date and reference	Sulfur content of oil (ppm)					
	Expelled	Extracted	Crude	Refined	Bleached	Deodorized
Von Fellenberg, 1945 (95)	23-28	31		13		
Kucera and Hejtmanek,[a] 1957 (96)			500	50	250	5
Zeman and Zemanova,[b] 1967 (97)	40	600				
Franzke *et al.*, 1972 (98)			9-45	0.6-2.0		
Daun, 1973 (99)	19-25	10-57	17-31	2-9	1-4	<1

[a] The oil was bleached before refining.

[b] Calculated from results for isothiocyantes.

0.18-0.46% isothiocyanates (Table 3).

Jacini (G. Jacini, Experimental Station for Fats and Oils, Milan, Italy, personal communication) found that methanol would extract virtually all the oxazolidinethione from rapeseed oils. A methanol extract from 10 kg of oil was concentrated, buffered to pH 7, extracted with petroleum ether to eliminate traces of oil, and finally extracted with ether to obtain the sulfur compounds. The concentrated ether solution was fractionated by thin-layer chromatography, the spots were eluted, and oxazolidinethiones were determined by ultra-violet spectrophotometry. The oxazolidinethione content of a crude "Canbra" oil was 0.5-1.0 ppm.

Effect of Sulfur and Sulfur Compounds on the Processing of Rapeseed Oil

Although there is some evidence that sulfur or sulfur compounds present in rapeseed oil may contribute to an undesirable odor of the heated oil (2), the major problem associated with sulfur compounds in rapeseed oil is the role of the sulfur in poisoning the hydrogenation catalyst.

Catalyst poisoning. The most common sulfur-containing catalyst poisons encountered are H_2S, CS_2, SO_2, and COS, all of which may occur as impurities in the hydrogen used in the hydrogenation process. Generally, active nickel catalysts, as used in these processes, will be completely deactivated by 3-5 g of sulfur per 100 g of catalyst. Inactive-type catalysts, which are sometimes used for special products, will be deactivated with 0.5-1.0 g of sulfur per 100 g of catalyst (102).

Babuchowski and Rutkowski (103) studied the effect of several sulfur-containing compounds on the hydrogenation of rapeseed oil on a laboratory scale (Table 4). They found that allyl isothiocyanate and 5-vinyl-2-oxazolidinethione were very potent inhibitors of hydrogenation, causing

Table 4. Effect of sulfur compounds on the hydrogenation of rapeseed oil

Compound	Amount present in oil		Inhibitory effect (%)[a]
	ppm	ppm Sulfur	
Methionine	0.6	0.0128	5.3
	1.2	0.0257	10.1
	6.0	0.128	17.0
	12.0	0.257	23.0
Cystine	0.6	0.0161	3.8
	1.2	0.0322	7.5
	6.0	0.161	15.8
	12.0	0.322	20.7
Cysteine	0.6	0.0158	3.0
	1.2	0.0317	10.0
	6.0	0.158	12.8
	12.0	0.317	20.3
Allyl isothiocyanate	2	0.646	23.8
	4	1.29	42.0
	20	6.46	51.8
	40	12.9	63.4
5-Vinyl-2-oxazolidinethione	1	0.248	10.5
	2	0.496	17.0
	10	2.48	36.6
	20	4.96	54.4

[a] Percent inhibition is based on a comparison of the iodine value of the oil plus sulfur compound compared to the iodine value of a control sample hydrogenated under the same conditions.

50% inhibition when present in the oil at the 20 ppm level. For all compounds studied inhibition was approximately proportional to the total amount of sulfur present. Addition of 10 ppm phosphorus in the form of rapeseed- or soybean lecithin gave almost complete inhibition. On an industrial scale, it is generally believed that the presence of 5-7 ppm sulfur causes a significant reduction in the rate of hydrogenation (Dr. B. Weinberg, Department of Industry, Trade and Commerce, Ottawa, personal communication).

One of the reasons for hydrogenating oils is to produce a product of higher melting point. The melting point of an oil is raised not only by reducing the degree of unsaturation, but also by increasing the proportion of *trans* isomers present in the oil. *Trans* isomers of unsaturated fatty acids melt at much higher temperatures than the naturally occurring *cis* isomers. Hydrogenation with active catalysts produces about 80% *trans* isomers. Partially poisoned catalysts can cause a change in the proportion of *trans* isomers present in the hydrogenated product compared to the product obtained from non-poisoned catalysts. Rao *et al.* (104) studied the hydrogenation of peanut oil containing 0.1% or less allyl isothiocyanate. They found that as the allyl isothiocyanate level was raised from 0.01%, hydrogenation and *trans*-isomerization were suppressed. They presumed this effect to be due to a decrease in the number of catalytic sites available for hydrogenation and isomerization.

Since most processors desire a hydrogenated fat with a high level of *trans* isomers, the presence of sulfur is undesirable in oils, even at levels which would not seriously inhibit the overall hydrogenation. Preliminary hydrogenation (105) or bleaching with spent catalyst (106) has been shown to be effective in removing inhibitors from sulfur-containing

oils. Niewiadomski and Drozdowski (107) have even considered molecular distillation as a means of preparing rapeseed oil free from hydrogenation inhibitors.

Teasdale (108) has stated that millions of kilos of rapeseed oil have been successfully hydrogenated in Canada using only 0.03-0.08% of active nickel catalyst. He suggested that if it is necessary to hydrogenate a rapeseed oil which is high in sulfur content, the processor should consider either using larger amounts of catalyst or removing the sulfur by deodorization or by bleaching with expended catalyst.

EXPERIMENTAL

Estimation of Purity of Isothiocyanate Standards

The reaction of Ag^+ reagents with thioureas yields N-cyano compounds and silver sulfide according to the equation:

$$R - NH - \overset{\overset{S}{\|}}{C} - NH_2 + 2\ Ag^+ \rightarrow RNH - C \equiv N + Ag_2S + 2H^+$$

This reaction is the basis for the determination of isothiocyanates by many procedures (47). In most of these procedures, the reaction mixture is filtered and the unused silver salt in the filtrate determined by titration according to Volhard (109).

A sample of isothiocyanate was weighed into a 16 x 125 mm test tube. Sample sizes were 100 mg for butyl isothiocyanate and 200 mg for phenethyl isothiocyanate and heptyl isothiocyanate. Ten ml of a solution of concentrated NH_4OH in ethanol (1:1) was then added. The tube was stoppered with a teflon-lined cap and was heated in a water bath at 45°C for 16 hr. The solution was quantitatively transferred to a 50-ml beaker and 10.0 ml of 0.5N $AgNO_3$ was added with stirring. The solution was left for 30 min and then was filtered into a 250-ml conical flask. The filter paper was washed with 10 ml of distilled water which was added to the flask along with 5 ml of 6N HNO_3 and 1 ml of saturated ferric alum solution. The silver salt in the flask was titrated with 0.1N KCNS to the first appearance of a permanent pale orange color.

Preparation of Thiourea Derivatives from Pure Isothiocyanates

Approximately 500 mg of isothiocyanate (allyl isothiocyanate, butyl isothiocyanate, heptyl isothiocyanate, and phenethyl isothiocyanate,

Technical Grade, Eastman Organic Chemicals) were placed in a 16 x 125 mm test tube along with 10 ml of concentrated NH_4OH in ethanol (1:1). The tube was sealed with a teflon screw cap and heated at 45°C in a water bath for 16 hr. The tube contents were then transferred to a 50-ml beaker along with 20 ml of H_2O. The beaker was placed in a refrigerator (4°C) for 16 hr or until crystals formed. The crystals were separated by filtration and purified by recrystallization from H_2O. The identities of the thioureas formed were confirmed by melting point and by infra-red spectrophotometry and mass spectroscopy.

Preparation of Thiourea Derivatives from Rapeseed Meal

Approximately 5 g of seed (*B. campestris*, cultivar Echo) was heated for 16 hr at 118°C. The seed was then finely ground in a Braun coffee grinder and the oil was removed by soxhlet extraction with petroleum ether (Skelly F, b.p. 36-58°C, Skelly Oil Co., Kansas City, Mo.). The seed meal was placed in a 50-ml centrifuge tube and mixed with 10 ml of phosphate buffer (pH 7) and 100 mg of myrosinase enzyme [prepared according to Lein (110)]. The mixture was stoppered and allowed to react for 1 hr and then was extracted with 20 ml of methylene chloride. The methylene chloride extract was separated from the meal by filtration and was dried with anhydrous sodium sulfate. The dry solution was transferred to a 50-ml pear-shaped flask and the methylene chloride was removed by rotary evaporation at a temperature of 40°C or less. The residual oily liquid was treated with 5 ml of concentrated NH_4OH in ethanol (1:10). The flask was stoppered and heated at 45°C in a water bath for 16 hr. The resulting solution was used as a source of thioureas derived from rapeseed isothiocyanates.

Preparation of Thiourea Derivatives from Rapeseed Oil

Approximately 500 g of rapeseed oil (*B. campestris*, cultivar Span, caustic refined) was added to a 2-1 conical flask along with 500 ml of concentrated NH_4OH in methanol (1:5). The flask was placed in a 45°C water bath and the two phases were mixed by a stirrer for 4 hr. The mixture was then transferred to a separatory funnel and allowed to separate for 48 hr. After the two phases had fully separated, the oil phase was removed and discarded. The methanol phase was washed with four portions of 500 ml of "Skelly F" petroleum ether. The methanol solution was then transferred to a 1-1 flask and evaporated to dryness by rotary evaporation. The residual 1 ml of oily material was used as a source of thiourea derivatives from rapeseed oil.

Thin-layer Chromatography of Thioureas

Thin-layer chromatography (TLC) of thiourea derivatives of isothiocyanates was performed on Bakerflex TLC media (Silica gel, $1B_2$ or $1B_2F$). Sheet sizes of 5 x 20 cm and 20 x 20 cm were used. Gelman ITLC media were tried originally but these failed to give adequate separation. The Bakerflex sheets gave almost identical separation with or without activation at 120°C for 1 hr. Spotting and developing procedures were as described in Gelman technical bulletin 17 (111). Spot sizes were 2 µl and the sample concentrations or the number of repetitive spottings were adjusted so that 5-20 µg of each thiourea was present. For preparative work samples of up to 50 µl were placed across the origin as a streak by applying 2-µl spots at 5 mm intervals.

The mobile phases used were chloroform:methanol (10:1), not earlier reported, which gave good separation, and the upper phase of ethyl acetate:

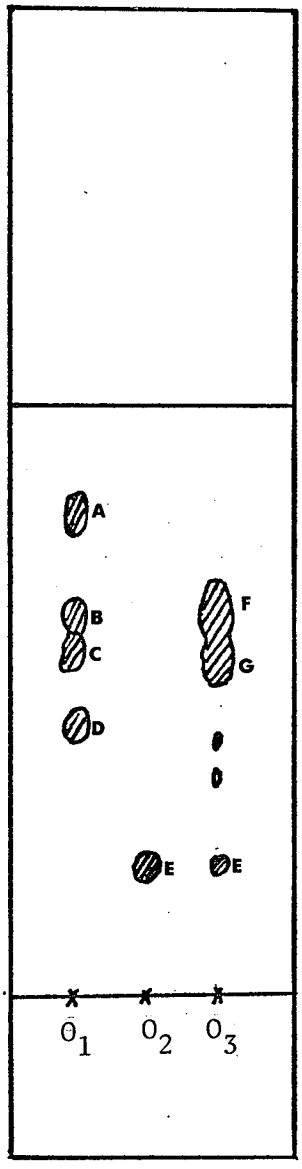
chloroform:water (30:30:40) according to Ashworth (47). The chloroform: methanol system (Fig. 8 and Table 5) was useful in separation of oily material from the thioureas isolated from rapeseed oil prior to their further separation by the ethyl acetate:chloroform:water system (Fig. 9 and Table 5). The chloroform:water system also separated oxazolidine-thiones from thioureas while the ethyl acetate:chloroform:water system gave a good separation of individual aliphatic and aromatic thioureas.

The compounds on the chromatograms were visualized by a number of spray reagents (Table 6). On plates treated with a fluorescent binder, thioureas and oxazolidinethiones were observed under ultra-violet light as dark spots on a fluorescent background.

Samples which were separated in preparation for mass spectroscopy were applied to the plate in a streak. After developing, the bands were located by spraying the plate through a slitted card. The unreacted portions of a band were transferred to a dispo-pipette and the compound was eluted with 50-100 µl of methanol. The eluates were collected in micro-centrifuge tubes and evaporated to about 10 µl.

Gas Chromatographic Determination of Sulfur Compounds in Rapeseed Oil

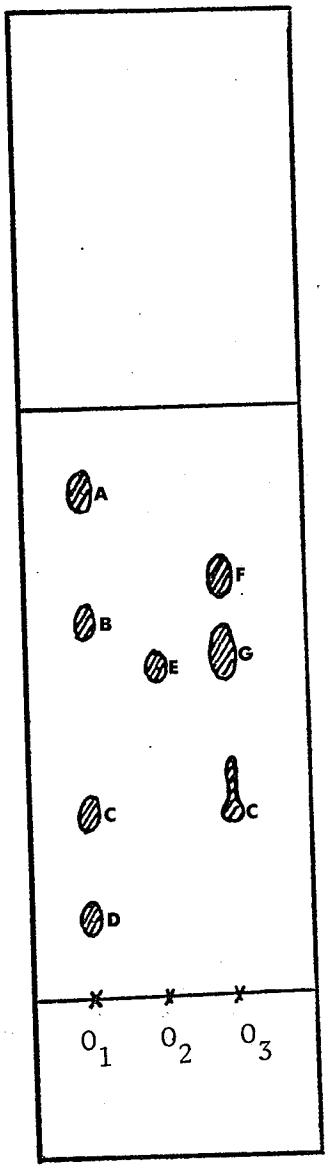
Gas chromatographic conditions. Gas chromatography (GLC) was carried out with a Varian model 1400 gas chromatograph which had been fitted with a Tracor flame photometric detector (FPD). The FPD was designed to detect the emission spectra of molecular species in a flame (114). The FPD is very sensitive and can be made specific for various elements, especially sulfur and phosphorus, although specificities for halogens, nitrogen, silylated compounds, and various metal chelates have been reported (114). Wavelength selection has normally been achieved by the use



A Allyl thiourea
B Butyl thiourea
C Phenethyl thiourea
D Heptyl thiourea
E 5-Vinyl-2-oxazolidinethione
F 3-Butenyl thiourea
G 4-Pentenyl thiourea

O_1 Mixture of pure thioureas
O_2 5-Vinyl-2-oxazolidinethione
O_3 Thioureas from rapeseed meal

Figure 8. Thin layer chromatogram of thioureas. Solvent system chloroform:methanol (10:1).



A Heptyl thiourea
B Phenethyl thiourea
C Butyl thiourea
D Allyl thiourea
E 5-Vinyl-2-oxazolidinethione
F 4-Pentenyl thiourea
G 3-Butenyl thiourea

O_1 Mixture of pure thioureas

O_2 5-Vinyl-2-oxazolidinethione

O_3 Thioureas from rapeseed meal

Figure 9. Thin layer chromatogram of thioureas. Solvent system ethyl acetate:chloroform:water (40:40:60). Aqueous phase.

Table 5. R_f values for thin-layer chromatograms of thioureas

Compound	Methanol: Chloroform 1:10	Ethyl Acetate: Chloroform: Water 40:40:60
Allyl thiourea	0.45	0.86
3-Butenyl thiourea [a]	0.57	0.71
Butyl thiourea	0.59	0.64
4-Pentenyl thiourea [a]	0.62	0.58
Phenethyl thiourea	0.64	0.31
Hepty1 thiourea	0.82	0.13
5-Vinyl-2-oxazolidinethione	0.18	0.56

[a] The identities of 3-butenyl- and 4-pentenyl isothiocyanate in the mixture of thioureas was confirmed by preparative TLC coupled with mass spectroscopy.

Table 6. Spray reagents used for visualization of sulfur compounds on TLC plates

Name of Reagent	Preparation and Use	Specificity	Ref.
Iodine	Plate exposed to iodine vapors in chamber. Brown spots.	Organic compounds	112
Silver nitrate	NH_4OH added to 0.1M $AgNO_3$ to dissolve first precipitate. Spray. Black spots. Plate eventually blackens.	Many sulfur compounds	47
Grote's reagent	Na nitroprusside treated with $NH_2OH.HCl$ + $NaHCO_3$, then with Br_2. Blue spots. Unstable.	Divalent sulfur doubly linked to a single non-metallic element, eg., C=S	113
Iodine-azide-starch	Spray with 1% starch, then 1% iodine, then 1% NaN_2. White spots on blue background	Thiols, sulfide ion, thioureas, oxazolidinethiones	47
$FeCL_3$-$K_3Fe(CN)_6$	5% $FeCL_3$ + 5% $K_3Fe(CN)_6$ (1:1). Blue spots on yellow background	Substances reducing Fe^{+3}	47

of monochromators although the Tracor unit uses filters with maximum transmission at 526 nm for phosphorus and at 394 nm for sulfur. Emission for sulfur has been found to be due to S_2 in cool flames (less than 1000°C) and to CS in hotter flames. Also, the detector's response to sulfur has been reported to vary with different sulfur compounds (115). With cool flames, simple sulfur compounds such as H_2S are reported to produce a response proportional to the square of the sulfur concentration (114).

It was found to be necessary to make some modifications to the gas chromatograph assembly. The FPD was mounted on the lid of the gas chromatograph and it was necessary for the sample to pass through the lid to reach the detector. When the column oven was 200°C, the temperature of the 8-cm tube between the oven and the detector was as low as 120°C and samples were observed to be condensing in this area. An auxiliary heating unit with a thermocouple was installed around the tubing leading through the lid, thus allowing control and monitoring of the temperature in that region. With the detector mounted on the lid of the gas chromatograph, it also became necessary to use a column of flexible material such as teflon, rather than glass or metal.

Samples of volatile liquids were injected directly on-column, while samples of oil were injected onto a pre-column insert. The insert was constructed from a 15-cm length of 3 mm OD glass tubing which was flared slightly at one end. The tubing was filled with Chromosorb W (acid-washed, DMCS treated, 60/80 mesh) which was secured with plugs of silanized glass wool. The insert was placed into the injection port of the gas chromatograph with the flared end nearest the injection septum. It was secured with a 1/8" stainless steel Swagelok nut, two 1/8" silicone

rubber o-rings, and a reversed Swagelok back ferrule. The column was attached to the insert by a similar connection. One insert was found to last for 8 injections of 2-2.5 µl of rapeseed oil. After 8 injections the chromatograph peaks became noticeably broadened. The insert could be changed in about 10 min, without extinguishing the flame.

Operation of the FPD was as described in the operation and service manual (116) except that the oxygen-air mixture was connected to the "hydrogen inlet" and the hydrogen to the "oxygen air inlet" according to Burgett and Green (117). This modification gave improved baseline stability and allowed injection of larger amounts (> 10 µl) of volatile solvents without extinguishing the flame. The detector and auxiliary heater were maintained at 200°C. The injection port was maintained at 220°C. The gas flows were as follows: nitrogen, 40 ml/min; hydrogen, 50 ml/min; air, 50 ml/min; and O_2, 10 ml/min. The electrometer attenuation and range were 128 x 10^{-9} for isothiocyanates, and 32 x 10^{-9} for oxazolidinethiones.

The signal from the detector was monitored by an Infrotonics model CRS 100 integrator. The integrator converted the signal to a logarithmic output before passing it on to a Honeywell Lab/Test recorder. Peak areas were recorded by the integrator and computations were carried out with the aid of a Hewlett-Packard Model 9100A electronic calculator.

Three chromatographic columns were used. Isothiocyanates were determined on a column of 6' x 1/8" teflon tubing packed with FFAP on 60/80 mesh Chromosorb W, AW, DMCS (1:10) and on a column of 6' x 1/8" teflon tubing packed with Apiezon L on 60/80 mesh Chromosorb W, AW, DMCS (3:97). Oxazolidinethiones were determined on a column of 6' x 1/8" teflon tubing packed with EGSS-X on 100/120 mesh Gas-Chrom Q (1:99). The FFAP column

was conditioned first at 120°C overnight with no gas flow and then again overnight at 200°C with a reduced flow. The other columns were conditioned at 200°C overnight with reduced flow.

Industrial samples of rapeseed oil. Samples of rapeseed oil (5 g) were weighed into 20 x 70 mm shell vials. To each sample was added 25 µl of methylene chloride containing approximately 5 µg of sulfur as heptyl isothiocyanate. The samples were shaken for 30 sec on a "Delux mixer" (Scientific Products, McGaw Park, Ill.).

A 2.5-µl sample of oil was then injected with a 25-µl syringe (Hamilton) onto the precolumn of the gas chromatograph. The column temperature was programmed from 100°C to 175°C at 8°C/min. The FFAP column and the Apiezon L column were both used for qualitative studies. The FFAP column was used for all quantitative work. The sulfur content of each of the peaks was calculated according to the formula

$$[S_x] = K\left[\frac{S_{Heptyl}}{W}\right] \times \left[\frac{A_x}{A_{Heptyl}}\right] C$$

where: $[S_x]$ is concentration of unknown (ppm S),

S_{Heptyl} is heptyl isothiocyanate added (µgm S),

W is weight of sample (g),

A_x is peak area of unknown,

A_{Heptyl} is peak area of heptyl isothiocyanate,

K is 0.85 for peaks eluting before heptyl isothiocyanate, and 1.29 for peaks eluting after heptyl isothiocyanate,

C is 0.60 for peaks eluting before heptyl isothiocyanate, and 0.66 for peaks eluting after heptyl isothiocyanate.

The derivation of the formula and the constants K and C is discussed in the section on standardization.

After the determination of isothiocyanates, the 5-g sample of oil was shaken on a "Delux mixer" for 30 sec with 5 ml of absolute methanol. After the phases had separated, a 2-µl sample of the methanol phase was injected with a 5-µl syringe (SGE) directly onto the EGSS-X column. The column temperature was programmed from 120°C to 210°C at 10°C/min. The sulfur content of the peaks corresponding to oxazolidinethione was determined according to the formula

$$[S_{OZT}] = \frac{K\ [A_{OZT}]^{C}}{W}$$

where: $[S_{OZT}]$ is concentration of oxazolidinethione (ppm S),

$[A_{OZT}]$ is peak areas of oxazolidinethione (integrator counts),

W is weight of sample (g),

K is 3.09×10^{-3} and C is 0.73.

The derivation of the formula and the constants K and C is discussed in the section on standardization.

Laboratory extracted samples of rapeseed oil. Six samples of seed (20 g) were placed in a desiccator in which the desiccant had been replaced by water. The desiccator was sealed and left at room temperature (22°C). At intervals of 48 hr, two sub-samples of each seed sample were taken - 2 g for moisture determination and 2g for determination of sulfur compounds in the oil. Moisture was determined by loss of weight after drying the seed at 103°C for 16 hr. The oil was quantitatively removed by first crushing and extracting the seeds in steel tubes after the "Swedish extraction method" of Troeng (118), and then completing the extraction by the Soxhlet method. The 2-g sample of seed was weighed into a 60-ml stainless steel tube. Three ball bearings (3/4" diameter) and

50 ml of "Skelly F" petroleum ether were then added and the tube was stoppered with a fluorosilicone rubber stopper. The tube was shaken longitudinally in a shaking machine at 200 cycles/min (4 cm displacement) for 3 hr. After shaking, the extract was filtered through Whatman No. 2^V fluted filter paper. The filtrate was collected in 150-ml beakers designed for use with the Goldfisch extraction apparatus (Laboratory Construction Co., Kansas City, Mo.). The filter paper containing all the seed meal was transferred to the Goldfisch extraction apparatus in the place of thimbles and the meal was extracted for a further 3 hr using the solvent in the filtrate as the extractant. After extraction, the solvent was evaporated in a fume hood and the residual oil samples were transferred to 5-ml vials for storage. Recovery of oil by this method was 97-100%.

To each oil sample was added 5 µl of methylene chloride containing approximately 1 µgm sulfur as heptyl isothiocyanate. The sample was then extracted with 1 ml of anhydrous methanol for 30 sec on a "Delux mixer" shaker. After separation of the phases 2 µl samples of the methanol phase were injected onto the FFAP column and onto the EGSS-X column for determination of sulfur compounds as described.

Determination of Oxazolidinethione in Rapeseed Oils by Ultra-Violet Spectrophotometry

The method was basically that of Grzybowska (100). A sample of oil (10 g) was weighed into a 125 x 16 mm test tube. Water (10 ml) was added and the tube was shaken longitudinally for 30 min in a shaking machine at 200 cycles/min (4 cm displacement). After shaking, the phases were allowed to separate and the oil phase was discarded. The aqueous phase

was extracted 4 times with 10-ml portions of "Skelly F" petroleum ether and finally with 10 ml of methylene chloride. The methylene chloride extract was removed and dried with anhydrous sodium sulfate; its ultraviolet absorption spectrum was recorded between 230 nm and 260 nm. The "corrected optical density" (COD) value for oxazolidinethione was obtained according to the formula

$$COD = OD_{245\ nm} - 1/2\ (OD_{255\ nm} + OD_{235\ nm}) \qquad (60)$$

The oxazolidinethione concentration was computed by comparing with a standard curve. The standard curve (Fig. 9) was made by analyzing known quantities of D-5-vinyl-2-oxazolidinethione (obtained by courtesy of Mr. Trevor Smith, Department of Animal Science, University of Manitoba) in methylene chloride.

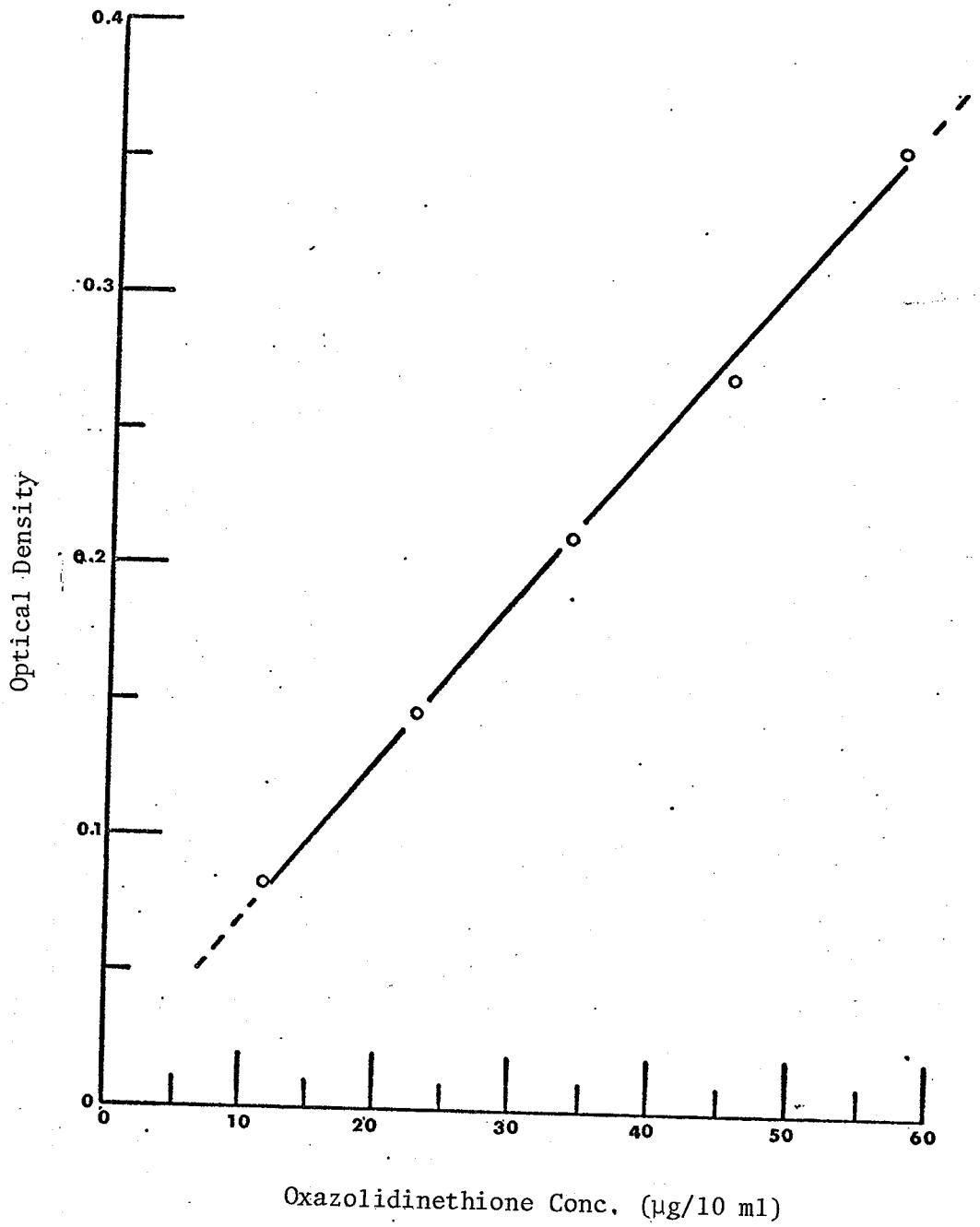


Figure 10. Calibration curve for the spectrophotometric determination of oxazolidinethione.

RESULTS AND DISCUSSION

This chapter is divided into four main sections. The first section deals with the identification of the sulfur compounds found in rapeseed oil. The second section discusses the quantitative determination of the sulfur compounds found in rapeseed oil and is divided into two subsections. The first subsection deals with the calibration of the flame photometric detector while the second section deals with the attempted determination of oxazolidinethione in rapeseed oil by ultra-violet spectrophotometry. The third section deals with the effect of industrial extraction and refining on the sulfur compounds in rapeseed oil. The fourth section deals with the effect of seed quality and moisture content on the amount of sulfur compounds present in laboratory extracted samples of rapeseed oil.

Identification of Sulfur Compounds in Rapeseed Oil

The sulfur compounds found in rapeseed oil were considered likely to be hydrolysis products of glucosinolates since many of these compounds are relatively non-polar and hence are oil soluble. The methods used in analyzing these compounds were, as far as possible, adaptations of earlier methods devised for the analysis of glucosinolate hydrolysis products in meals. For example, the FFAP column was used by Youngs and Wetter (13) for analysis of isothiocyanates, while the EGSS-X column was used by Daxenbichler *et al.* (68) for analysis of epithiobutanes, thioamides, and oxazolidinethiones.

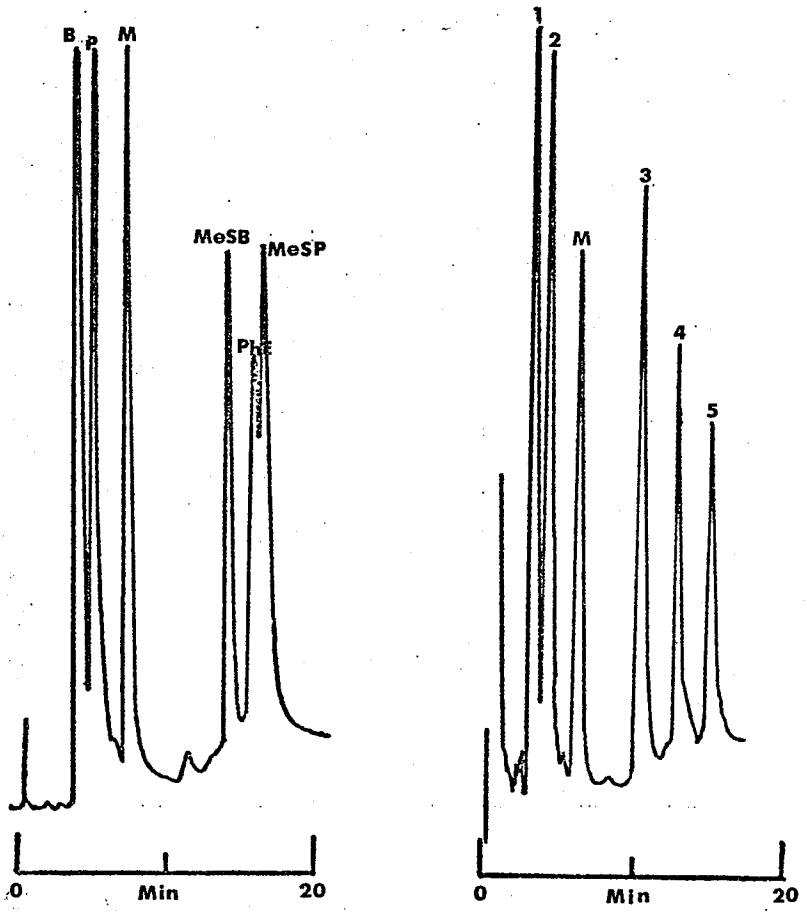
The flame photometric detector, operated in the sulfur sensitive mode, provided a convenient means for registering gas chromatographic peaks

specifically due to sulfur compounds. Where possible, samples of oil for analysis by gas chromatography were injected onto a pre-column where the volatile components were swept onto the analytical column leaving the non-volatile oil behind. When samples of rapeseed oil were injected into the gas chromatograph, five major peaks were observed for both the polar (FFAP) and non-polar (Apiezon L) columns (Figs. 11 and 12). A comparison of retention times (Table 7) indicates that peaks 1 and 2 from the oil correspond with 3-butenyl isothiocyanate and 4-pentenyl isothiocyanate, respectively. Peaks 3, 4, and 5, assumed to be the same for the two different columns, did not correspond to any of the isothiocyanates formed from hydrolysis of rapeseed glucosinolates. These peaks also did not correspond with the retention times for the episulfides formed from autolysis of 2-hydroxy-3-butenyl glucosinolate; a pure sample of the *threo* form of episulfide (obtained by courtesy of Mr. T. Smith, Department of Animal Science, University of Manitoba) was found to elute much later than any of the sulfur compounds in the oil.

For analysis of oxazolidinethiones on the EGSS-X column it was not possible to inject oil samples onto the pre-column insert since the high temperatures of over 200^{o}C quickly damaged the silicone o-rings which held the insert in place, usually resulting in detachment of the column from the insert during temperature programming. Instead, methanol extracts were prepared from the oil and were injected directly onto the column. It had been observed (Dr. G. Jacini, Experimental Station for Fats and Oils, Milan, Italy, personal communication) that methanol would almost completely extract the polar sulfur compounds from rapeseed oil.

When a sample of methanol extract from rapeseed oil was injected onto the EGSS-X column, four peaks were found with retention times of 11.4, 12.1, 13.6 and 14.5 min (Fig. 13). The first two of these peaks corresponded in

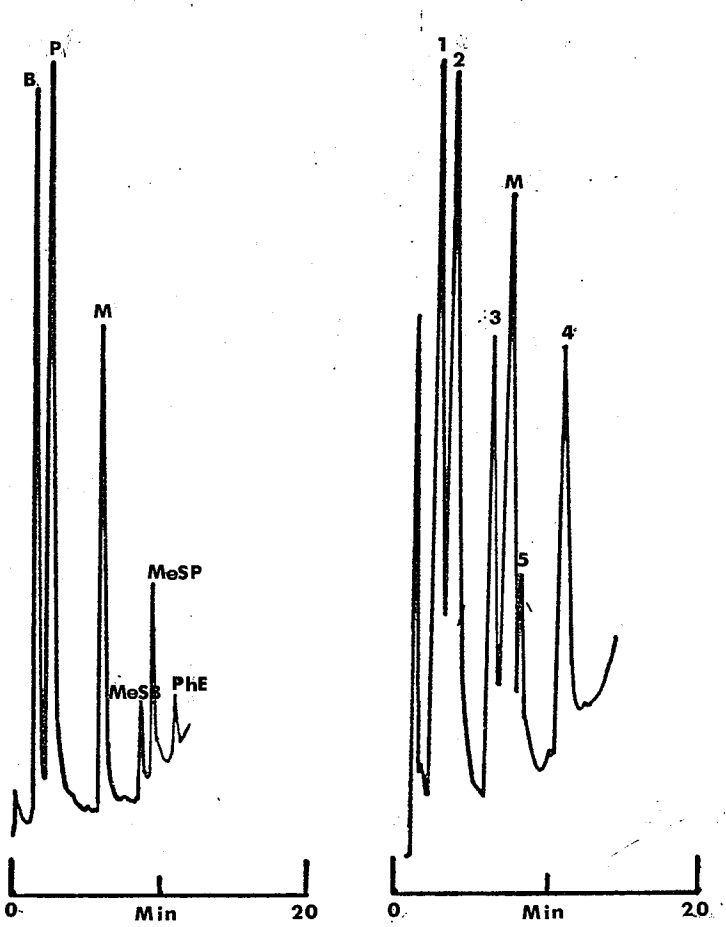
Peak		Compound
B	1	3-Butenyl isothiocyanate
P	2	4-Pentenyl isothiocyanate
M		Heptyl isothiocyanate (Internal Standard)
MeSB		4-Methylthiobutyl isothiocyanate
PhE		Phenethyl isothiocyanate
MeSP		4-Methylthiopentyl isothiocyanate
	3	Unidentified
	4	Unidentified
	5	Unidentified



Echo Seed Extract Span Oil

Figure 11. Gas chromatograms of sulfur compounds from Echo seed and Span oil on FFAP column.

Peak		Compound
B	1	3-Butenyl isothiocyanate
P	2	4-Pentenyl isothiocyanate
M		Heptyl isothiocyanate (Internal Standard)
MeSB		4-Methylthiobutyl isothiocyanate
PhE		Phenethyl isothiocyanate
MeSP		5-Methylthiopentyl isothiocyanate
	3	Unidentified
	4	Unidentified
	5	Unidentified



Echo Seed Extract Span Oil

Figure 12. Gas chromatograms of sulfur compounds from Echo seed and Span oil on Apiezon L column.

Table 7. GLC relative retention times for sulfur compounds from Echo seed and Span oil.

Peak Label[a]	Compound[c]	Relative Retention Times[b]			
		FFAP		Apiezon L	
		Echo seed	Span oil	Echo seed	Span oil
1	3-Butenyl isothiocyanate	0.50	0.51	0.31	0.31
2	4-Pentenyl isothiocyanate	0.68	0.68	0.47	0.47
M	Heptyl isothiocyanate	1.00	1.00	1.00	1.00
MeSP	4-Methylthiobutyl isothiocyanate	2.00		1.61	
PhE	Phenethyl isothiocyanate	2.30		2.26	
MeSP	5-Methylthiopentyl isothiocyanate	2.41		1.96	
3	Unidentified		1.61		0.81
4	Unidentified		1.96		1.56
5	Unidentified		2.26		1.13

[a] Peak labels refer to peak labels in Figures 11 and 12.

[b] Heptyl isothiocyanate = 1.00.

[c] Identification of the compounds in the echo seed extracts based on published chromatograms (Youngs and Wetter 13)

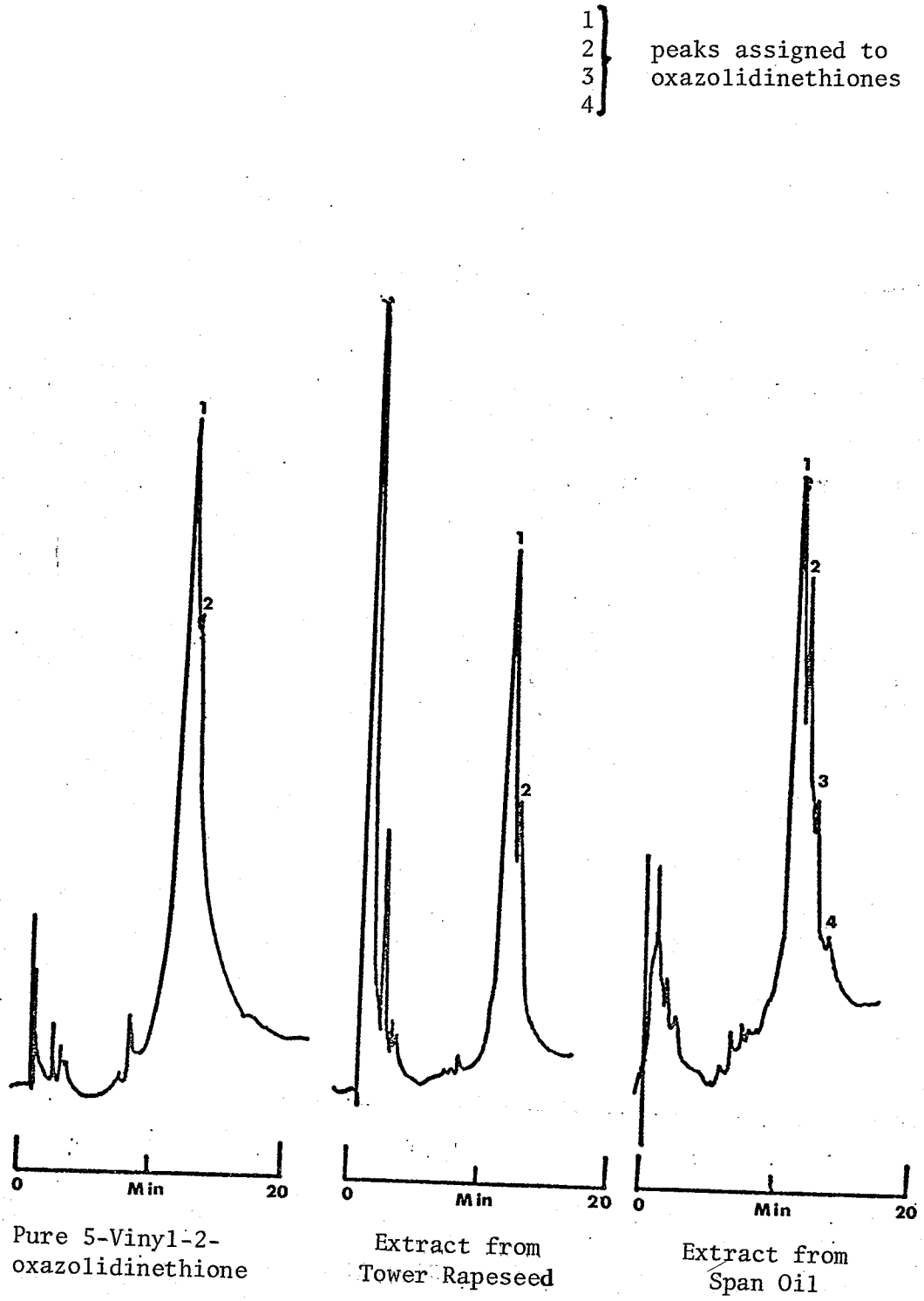


Figure 13. Gas chromatograms of oxazolidinethiones on EGSS-X column.

retention time with two peaks obtained by chromatography of pure 5-vinyl-2-oxazolidinethione. Only one peak would normally be expected; it is possible, however, that the oxazolidinethione had partially isomerized, either on formation, or on standing, or on injection into the gas chromatograph, to form thiazolidones (36, 37). The third and fourth peaks were possibly due to a similar rearrangement of 5-allyl-2-oxazolidinethione formed on a hydrolysis of 2-hydroxy-4-pentenyl glucosinolate which has been reported to occur in rapeseed (14). In this study, further references to oxazolidinethiones, as determined by gas chromatography, refer to the sum of all four peaks.

The gas chromatographic studies described above indicated that rapeseed oil contained 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, and 5-vinyl-2-oxazolidinethione. Further confirmation of the identity of these compounds was obtained by a study of the thiourea derivatives. Most of the isothiocyanates formed on hydrolysis of glucosinolates have been identified by their thiourea derivatives (57, 58). Although these thiourea derivatives have most often been analyzed by paper chromatography (58), thin-layer chromatography was used in this work because of its greater speed, sensitivity, and suitability for preparative work.

Thiourea derivatives were prepared from the isothiocyanates from rapeseed meal as well as from the oil. For starting material, the meal from *B. campestris*, cultivar Echo was chosen. Echo is a high-glucosinolate rapeseed which yields large quantities of isothiocyanates upon hydrolysis and extraction. The oil from *B. campestris*, cultivar Span was chosen because it contained substantial amounts of all the sulfur compounds that had been found in rapeseed oil by gas chromatography. A

refined Span oil was used since the final volume of the ammoniacal methanol-extracted material from this oil was only about 1 ml as opposed to 10 ml from crude oils. Since the crude oil contained only slightly larger quantities of sulfur compounds than the refined oil, the concentration of sulfur compounds in the extract from the refined oil was much greater. Gas chromatography of the residual oil after extraction with the ammoniacal methanol indicated that 90% or more of the sulfur compounds were removed by the extraction procedure.

Thin-layer chromatograms of thioureas from the oil (Fig. 14) showed four spots which had reacted with the spray reagents sensitive to sulfur (Table 6). These four spots corresponded to four of the spots on thin-layer chromatograms of thioureas from the meal (Fig. 15 and Table 8), indicating the presence in the oil of 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, phenethyl isothiocyanate, and 5-vinyl-2-oxazolidinethione. Phenethyl isothiocyanate had not been observed by gas chromatography but it could have been obscured by one of the unknown compounds.

In order to confirm these tentative identifications, large samples of thioureas from both the seed and the oil were fractionated by TLC on a preparative scale. The individual thioureas obtained were analyzed by mass spectroscopy. Molecular ions were obtained at 130 AMU for 3-butenyl thiourea, 144 AMU for 4-pentenyl thiourea, and 180 AMU for phenethyl thiourea which confirmed the expected molecular weights for these compounds. The mass spectra for compounds from the seed were similar but not identical to the mass spectra for the compounds from the oil. The differences were probably due to impurities in the samples. It was not possible to obtain a mass spectrum for oxazolidinethiones from the oil, possibly because these compounds decomposed during the concentration procedures.

A = Phenethyl thiourea
B = 4-Pentenyl thiourea
C = 3-Butenyl thiourea
D = 5-Vinyl-2-oxazolidinethione

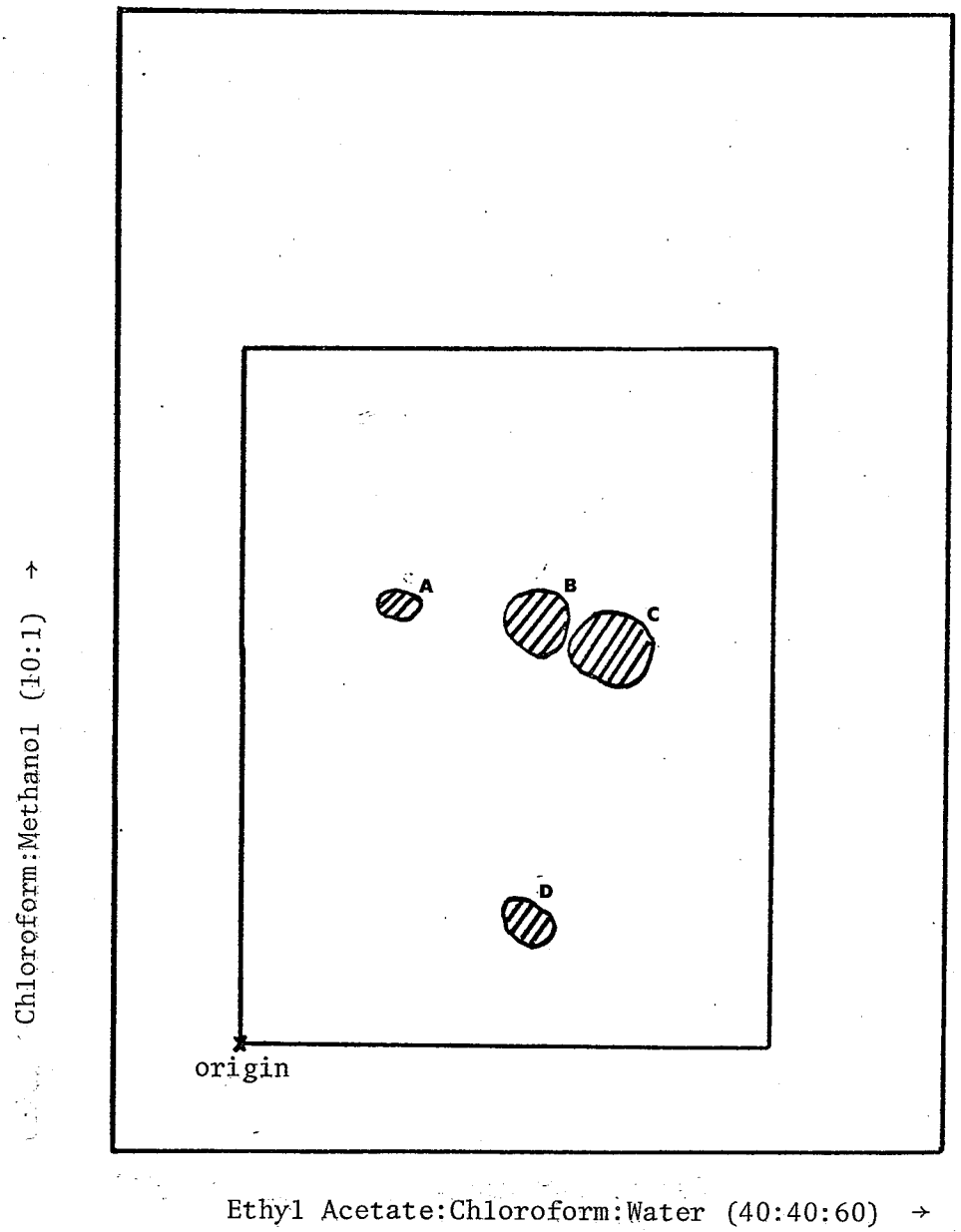


Figure 14. Thin layer chromatogram of thioureas prepared from Span oil.

A = Phenethyl thiourea
B = 4-Pentenyl thiourea
C = 3-Butenyl thiourea
D = 5-Vinyl-2-oxazolidinethione

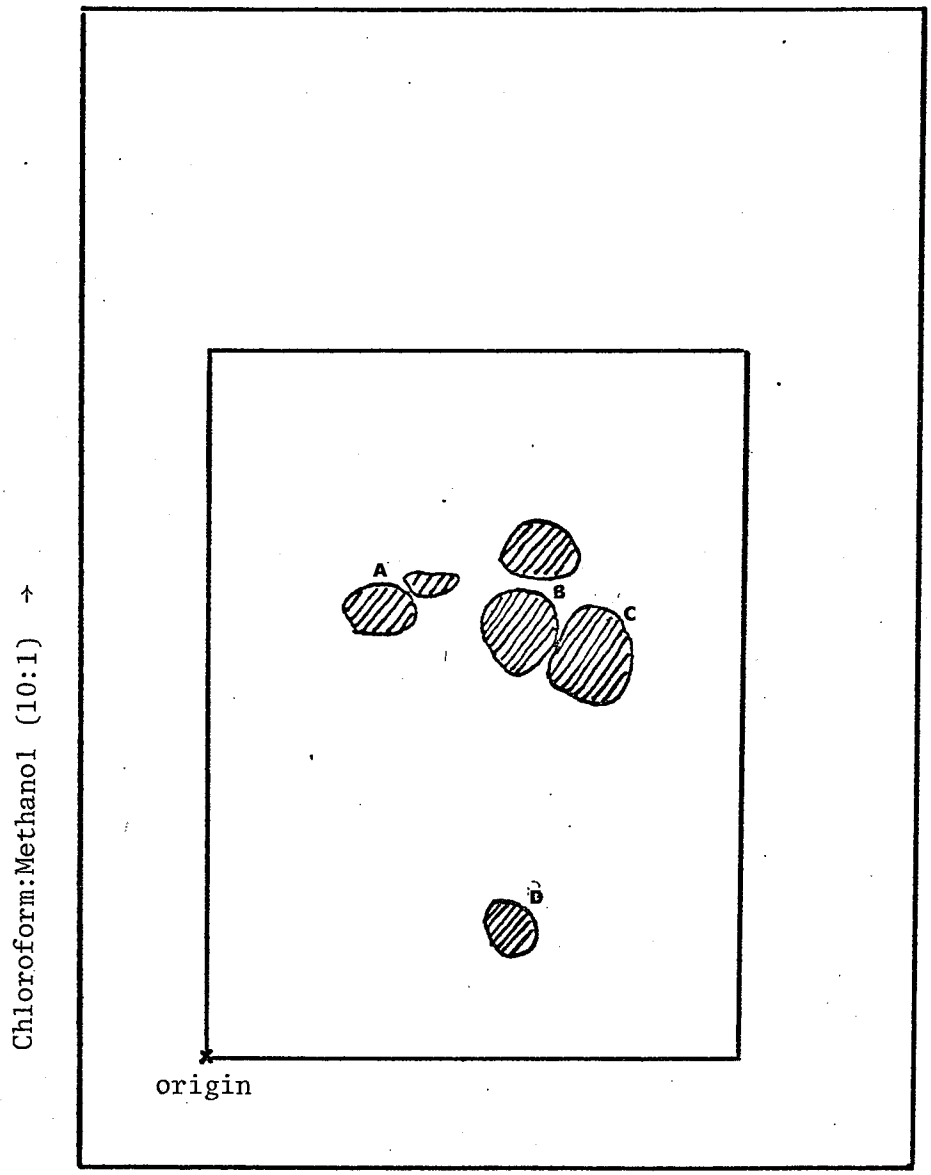


Figure 15. Thin layer chromatogram of thioureas prepared from Echo seed meal.

Table 8. R_f values from two-dimensional TLC of thiourea derivatives from Span oil and Echo seed meal.

Spot[a]	Compound	Methanol:Chloroform (1:10)		Ethyl Acetate:Chloroform:Water (40:40:60)	
		Span Oil	Echo Seed Meal	Span Oil	Echo Seed Meal
A	Phenethyl thiourea	0.63	0.63	0.34	0.30
B	4-Pentenyl thiourea	0.60	0.61	0.58	0.56
C	3-Butenyl thiourea	0.57	0.57	0.73	0.71
D	5-Vinyl-2-oxazolidinethione	0.18	0.17	0.57	0.55

[a] As in Figures 14 and 15.

An attempt was made to isolate some of the unidentified compounds by preparative gas chromatography for a possible identification by subsequent mass-spectroscopic analysis. The moisture content of a 100-g sample of rapeseed (*B. campestris*, cultivar Span) was raised to 15% in a humid atmosphere. The seed was crushed and extracted with methanol. The methanol was evaporated and the resulting oily material was partitioned between methylene chloride and water. The methylene chloride layer was dried and reduced to about 1 ml in volume. A gas chromatogram of this extract with the FFAP column using the flame ionization detector, is shown in Figure 16. The peaks marked 3, 4, and 5 correspond in relative retention with the unidentified peaks found in industrial samples of rapeseed oil. Attempts to collect peaks No. 3 and 5 for further examination were unsuccessful, possibly because these compounds condensed before they reached the collection trap.

Quantitative Determination of Sulfur Compounds in Rapeseed Oil

Sulfur compounds were determined in rapeseed oil by gas chromatography. An attempt to determine oxazolidinethione by ultra-violet spectrophotometry was not successful. 3-Butenyl- and 4-pentenyl isothiocyanate and the unidentified compounds designated 3, 4, and 5 in Figure 12 were determined by direct injection of the oil onto a pre-column connected to the FFAP column. Oxazolidinethiones were determined by injection of methanol extracts directly onto the EGSS-X column. Phenethyl isothiocyanate was not determined because its chromatographic retention coincided with that of the unidentified peak designated number 5.

Because of the low concentration of sulfur compounds in the oil, and because of the presence of many interfering compounds, it was not possible

M Heptyl isothiocyanate (Internal Standard)

3, 4, 5 Unidentified compounds, numbered as in Figure 11.

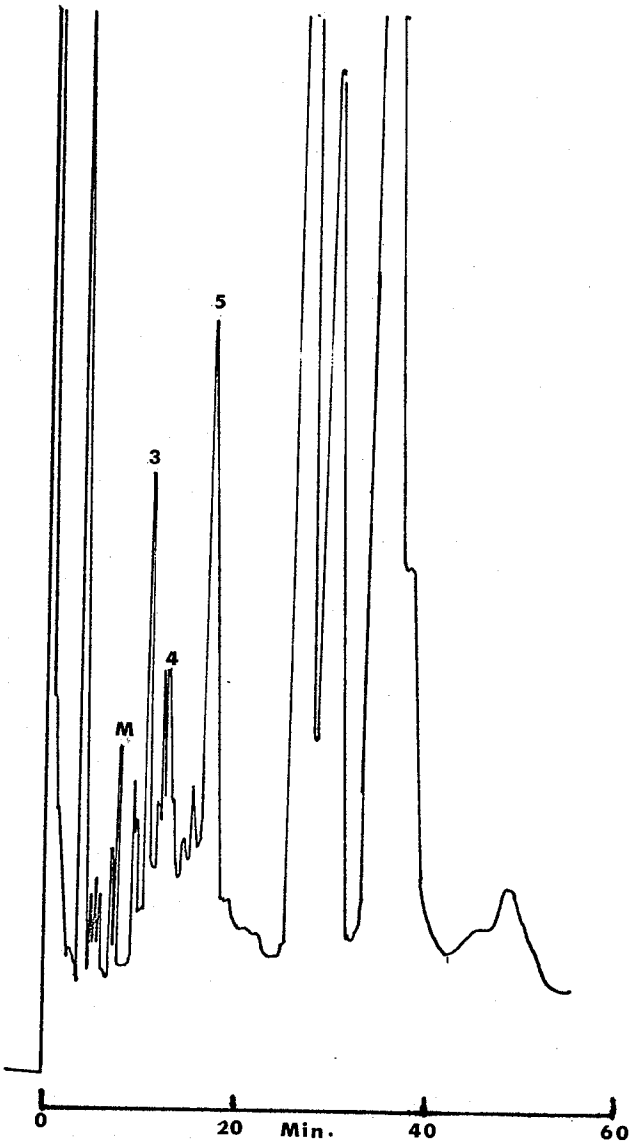


Figure 16. Gas chromatogram of extract from autolyzed Span seed on FFAP column (flame ionization detector).

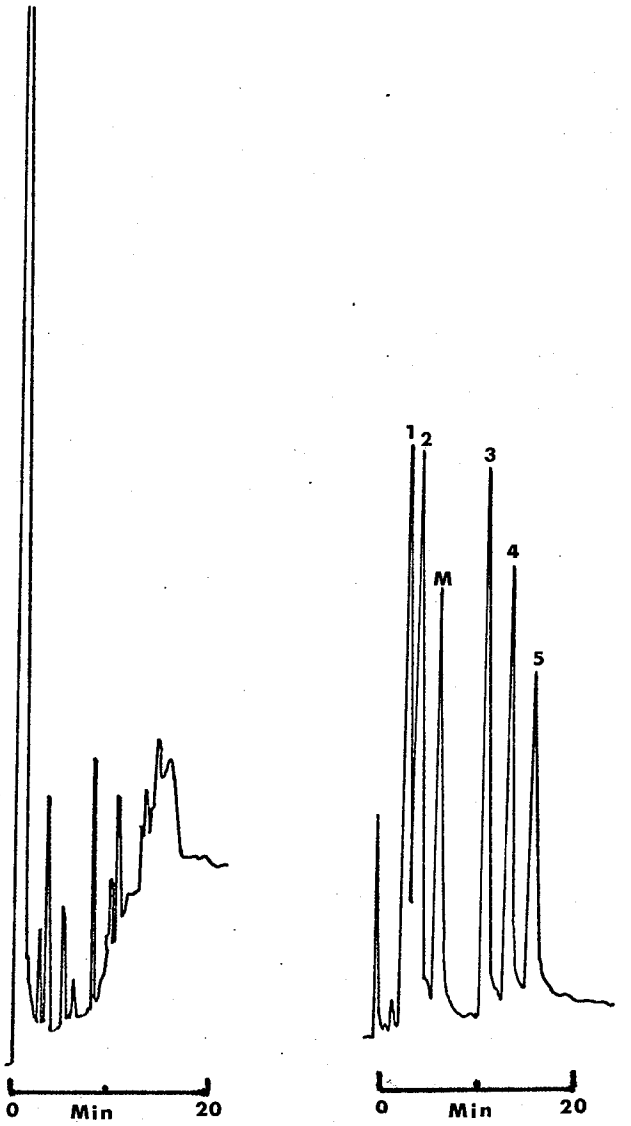
to use a flame ionization detector for the quantitative gas chromatographic determination of sulfur compounds in rapeseed oil. The use of the flame photometric detector, however, enabled sulfur compounds to be determined, even in crude oils in which interfering compounds completely masked the appearance of sulfur compounds with the flame ionization detector. Figure 17 shows gas chromatograms of the same sample of crude Span oil with the FFAP column using the flame ionization detector and the flame photometric detector. None of the peaks seen with the flame ionization detector correspond with any peaks seen with the flame photometric detector.

Calibration of the flame photometric detector for sulfur compounds. The flame photometric detector does not give a linear response with concentration and responses are different for different compounds; therefore, calibration of the flame photometric detector is desirable for each compound to be analyzed. Since some of the compounds found in rapeseed oil are of unknown composition, and since pure standards of the identified compounds 3-butenyl- and 4-pentenyl isothiocyanate were not obtained, it was not possible to calibrate the flame photometric detector for each of the sulfur compounds found in the oil.

In preparing the calibration curves, butyl isothiocyanate was used as a substitute for 3-butenyl- and 4-pentenyl isothiocyanate; this was not likely to result in much error since these three compounds are similar in structure and molecular weight. Phenethyl isothiocyanate was used as a substitute for the unidentified compounds because it has approximately the same gas chromatographic retention time as these and may have similar characteristics.

1
2
M
3
4
5

Sulfur compounds, numbered as in Figure 11.



Flame Ionization Detector

Flame Photometric Detector

Figure 17. Gas chromatograms of crude Span oil on FFAP column using flame ionization and flame photometric detectors.

Also, since it was found to be very difficult to inject reproducible volumes of oil into the gas chromatograph, heptyl isothiocyanate was used as an internal standard for the direct gas chromatographic analysis of oil samples.

The purity of the compounds used as standards was checked by determination of their sulfur contents by reaction with Ag^+ ion. The purities, ± 1 SD, indicated by these analyses were as follows: butyl isothiocyanate, 99.8 ± 0.5%; heptyl isothiocyanate, 99.3 ± 0.5%; and phenethyl isothiocyanate, 98.9 ± 0.7%. These compounds each showed only one peak when analyzed by gas chromatography on an FFAP column using either a flame ionization detector or a flame photometric detector. These compounds also each showed only one spot when converted to thioureas and analyzed by thin-layer chromatography.

Solutions of the three reference isothiocyanates were prepared in a commercial soybean oil (Crisco). The concentration of heptyl isothiocyanate was 1 ppm sulfur and the concentrations of butyl isothiocyanate and phenethyl isothiocyanate ranged from 0.2 ppm S to 10 ppm S. The solutions were analyzed by gas chromatography using the FFAP column and the flame photometric detector. A good fit to a straight line was found when the ratios of the peak areas (butyl isothiocyanate:heptyl isothiocyanate and phenethyl isothiocyanate:heptyl isothiocyanate) were plotted against the ratios of the respective sulfur concentrations on a log-log scale (Fig. 18). This fit suggested the parabolic relationships

$$\frac{[\,S_{Bu}\,]}{[\,S_{He}\,]} = 0.85 \left[\frac{A_{Bu}}{A_{He}}\right]^{0.60} \qquad r = 0.991,\ S^2 = 0.08 \text{ and}$$

$$\frac{[\,S_{PhE}]}{[\,S_{He}\,]} = 1.29 \left[\frac{A_{PhE}}{A_{He}}\right]^{0.66} \qquad r = 0.999,\ S^2 = 0.01$$

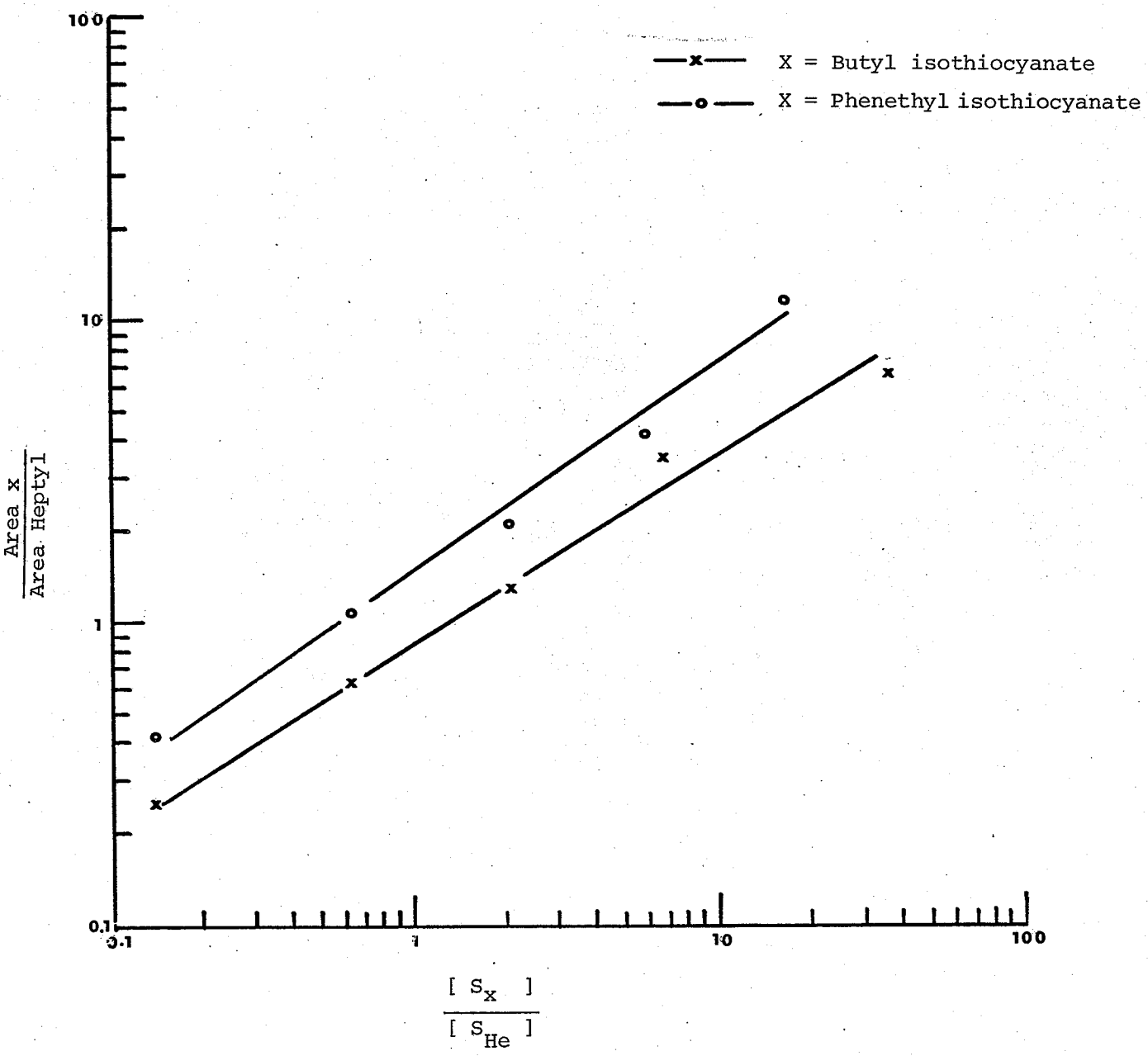


Figure 18. Calibration of flame photometric detector.

where $[S_{Bu}]$, $[S_{PhE}]$, and $[S_{He}]$ are the sulfur concentrations for butyl isothiocyanate, phenethyl isothiocyanate, and heptyl isothiocyanate, respectively, and A_{Bu}, A_{PhE}, and A_{He} are the peak areas for butyl isothiocyanate, phenethyl isothiocyanate, and heptyl isothiocyanate, respectively. The coefficients for the formulae as well as the correlation coefficient (r) and the standard error (S^2) were obtained by determining the least squares fit for the straight lines formed by plotting the logarithms of the ratios of the concentrations against the logarithms of the ratios of the peak areas.

In order to test their accuracy, these formulae were used in the determination of the isothiocyanate contents of rapeseed meal and compared with results from a conventional method of analysis. Extracts of rapeseed meal were made according to the method of Youngs and Wetter (20). Small samples of seed were crushed in the presence of water and the enzyme myrosinase. The isothiocyanates released on hydrolysis were extracted with methylene chloride for determination by gas chromatography. Analyses of these extracts using the flame ionization detector were made according to the method of Youngs and Wetter (13) while for determination by the flame photometric detector, the same extracts were diluted approximately 20 times before gas chromatographic analysis. Also, for samples analyzed by the flame photometric detector,heptyl isothiocyanate was used as an internal standard instead of butyl isothiocyanate as used in the standard method. The results, except for some of the lower values (Table 9) indicate a good agreement between the two detectors, although the flame photometric detector consistently gave somewhat higher values than the flame ionization detector.

Table 9. GLC determination of the isothiocyanate contents of rapeseed meals using two different detectors.

Seed	Isothiocyanate Content (mg/g oil-free meal)									
	B[a]		P		MeSB		PhE		MeSP	
	FID	FPD	FID	FPD	FID	FPD	FID	FPD	FID	FPD
Oro	2.1	2.3	0.6	0.7	0.01	0.04	0.05	0.15	tr	0.03
Tower	0.3	0.7	0.03	0.06	ND	0.02	ND	0.05	ND	0.02
Span	2.1	2.2	1.8	2.7	0.03	0.18	0.09	0.50	0.06	0.09
Green	1.0	1.7	0.4	1.0	0.01	0.06	0.06	0.12	tr	0.04
Heated	1.8	2.1	0.5	0.6	tr	0.07	0.04	0.22	tr	0.04
Frost-damaged	1.3	2.0	0.9	1.1	tr	0.09	0.05	0.15	tr	0.03

[a]B = 3-Butenyl isothiocyanate
MeSB = 4-Methylthiobutyl isothiocyanate
MeSP = 5-Methylthiopentyl isothiocyanate

P = 4-Pentenyl isothiocyanate
PhE = Phenethyl isothiocyanate

FID Flame ionization detector
FPD Flame photometric detector

The flame photometric detector was calibrated for oxazolidinethione by making 2.0-µl injections of methanol solutions of pure 5-vinyl-2-oxazolidinethione. The solutions varied in composition from 1.5 to 15 ppm sulfur. When the oxazolidinethione content (ppm S) was plotted against the peak area on a log-log scale (Fig. 19) a straight line plot was obtained. This suggested the parabolic relationship,

$$[S_{OZT}] = 6.2 \times 10^{-4} \ [\text{Peak Area}]^{0.73} \qquad r=0.9997, \ S^2=0.00008$$

where $[S_{OZT}]$ is the oxazolidinethione concentration (ppm S) and the peak area is measured in integrator counts. The coefficients for the formula as well as the correlation coefficient (r) and the standard error (S^2) were obtained by determining the least squares fit for the straight line formed by plotting the logarithms of the concentrations against the logarithms of the peak areas.

In order to assess the recovery of oxazolidinethione from an oil by extraction with methanol, known quantities of oxazolidinethione were added to 1-g samples of a commercial soybean oil (Crisco). These samples were extracted with 1 ml of absolute methanol and 2.0-µl samples of the methanol phase were analyzed for oxazolidinethione by gas chromatography using the flame photometric detector. The mean recovery of oxazolidinethione from the oil by this single extraction was 91.5% (Table 10). This recovery factor was included in the calculation of the oxazolidinethione content of industrial oils.

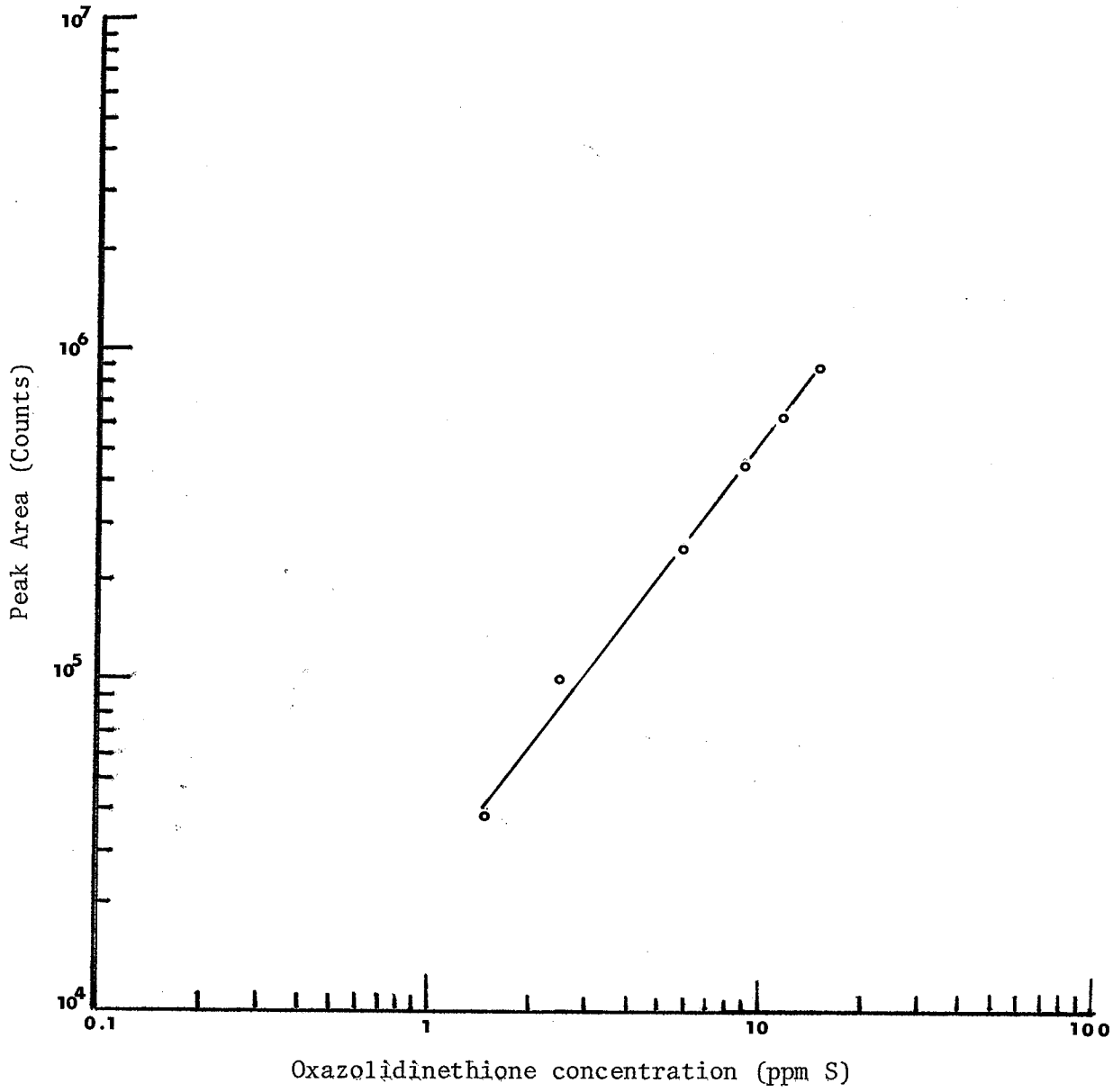


Figure 19. Calibration of flame photometric detector for oxazolidinethione.

Table 10. GLC determination of oxazolidinethione added to soybean oil and extracted with methanol.

Oxazolidinethione Added (ppm S)	Oxazolidinethione Found (ppm S)	Recovery[a] (%)
11.8	10.9	92.5
8.9	8.1	91.0
5.9	5.3	90.0
3.0	2.7	90.0
1.5	1.4	93.5

[a]The mean recovery was 91.5 ± 1.5% (± SD).

Attempted determination of oxazolidinethione in rapeseed oil by ultra-violet spectrophotometry. In order to obtain information on the oxazolidinethione content of rapeseed oil which could corroborate the information obtained by gas chromatography, an adaptation of the method of Grzybowska (100) was attempted.

Known quantities of 5-vinyl-2-oxazolidinethione were added to samples of a commercial soybean oil (Crisco). The oxazolidinethione was extracted and determined by the ultra-violet spectrophotometric method as described. The results indicated that approximately 72% of the oxazolidinethione was recovered and accounted for by this method.

The method was applied to crude and refined rapeseed oil without success. The ultra-violet absorption could not readily be determined because of interference from colored substances.

Effect of Industrial Extraction and Refining on the Sulfur Compounds in Rapeseed Oils

A number of oil samples representative of different processing steps were obtained from industrial processing plants, most of which used the prepress-solvent method of extraction. For reasons of confidentiality, the identity of the processors has not been given. These samples included four sets of oils from known seed varieties processed in cooperation with the Rapeseed Association of Canada, i.e., from the low erucic acid cultivars Span (*B. campestris*), Oro (*B. napus*), and Zephyr (*B. napus*), and from the high erucic acid cultivar Echo (*B. campestris*). These cultivars are among the most commonly grown varieties in Western Canada.

The samples were analyzed for individual sulfur compounds by the methods described and for total sulfur by the Raney nickel procedure (99). Results for the four special sets of oils are shown in Tables 11, 12, 13, and 14, and in Figure 20. Table 15 gives a summary of all the samples studied including some samples not recorded in Tables 11, 12, 13, and 14. All results are indicated as ppm sulfur since the molecular structure of some of the compounds is not known.

There were large differences in the patterns of sulfur compounds between oils extracted from *B. napus* and *B. campestris*. In oils from *B. campestris*, approximately equal amounts of 3-butenyl- and 4-pentenyl isothiocyanate were found while oils from *B. napus* contained much more 3-butenyl isothiocyanate than 4-pentenyl isothiocyanate. The molar ratios, 3-butenyl isothiocyanate:4-pentenyl isothiocyanate, ranged from 0.9 to 1.2 for oils from *B. campestris* and from 2.5 to 3.5 for oils from *B. napus*. These ratios are similar to the ratios observed for the respective glucosinolates of the meals. The amounts of 3-butenyl isothiocyanate were similar in the expelled, extracted, and crude oils from all the cultivars.

Larger amounts of the unidentified compounds (peaks 3, 4, and 5) were found in oils from *B. campestris* than from *B. napus*. Also, larger amounts of these compounds were found in expelled oils than in extracted oils.

There was no noticable difference in the oxazolidinethione content in oils from *B. campestris* and *B. napus*. This was surprising since *B. napus* meals contain several times as much oxazolidinethiones as *B. campestris*

Table 11. Effect of processing on the sulfur compounds in Span oil.

Processed Oil Sample	Content of Sulfur Compounds (ppm S)[a]						Total, by Summation	Total, by Raney Nickel
	1	2	3	4	5	OZT		
Expelled	2.1	2.3	3.2	2.0	3.7	5.8	19.1	25
Extracted	4.3	4.4	0.4	0.4	0.4	13.1	23.0	33
Refined	1.7	1.9	2.8	1.6	1.2	2.0	11.2	9
Bleached	2.1	2.0	0.3	trace	0.9	trace	5.3	4
Deodorized	trace	trace	0	0	0	0	trace	1

[a]Compounds 1-5 correspond to peaks 1-5 in Figure 11. OZT corresponds to oxazolidinethiones in Figure 13.

Table 12. Effect of processing on the sulfur compounds in Echo oil.

Processed Oil Sample	Content of Sulfur Compounds (ppm S)[a]						Total, by Summation	Total, by Raney Nickel
	1	2	3	4	5	OZT		
Expelled	2.0	1.9	2.8	1.5	2.5	12.5	23.0	21
Extracted	0.8	0.7	1.0	0.8	0.9	6.7	10.0	10
Refined	0.7	0.7	0.1	0.1	0.1	2.5	4.2	4
Bleached	0.8	0.8	trace	trace	0.1	2.2	3.8	3
Deodorized	0	0	0	0	0	trace	trace	1

[a]Compounds 1-5 correspond to peaks 1-5 in Figure 11. OZT corresponds to oxazolidinethiones in Figure 13.

Table 13. Effect of processing on the sulfur compounds in Oro oil.

Processed Oil Sample	Content of Sulfur Compounds (ppm S)[a]						Total, by Summation	Total, by Raney Nickel
	1	2	3	4	5	OZT		
Expelled	1.3	0.4	0.8	trace	1.2	12.2	14.9	19
Extracted	2.5	0.9	0.3	trace	trace	19.9	23.3	57
Crude	1.6	0.5	0.6	trace	1.2	13.6	17.5	31
Degummed	1.5	0.6	trace	trace	trace	7.7	9.8	16
Refined	1.2	0.6	trace	trace	trace	5.3	7.1	7
Bleached	1.4	0.5	trace	trace	trace	1.3	3.2	5
Deodorized	trace	0	0	0	0	0	trace	1

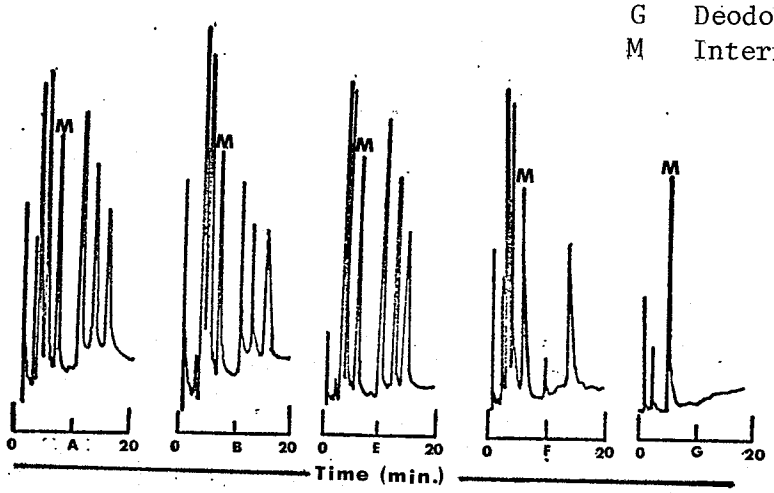
[a] Compounds 1-5 correspond to peaks 1-5 in Figure 11. OZT corresponds oxazolidinethiones in Figure 13.

Table 14. Effect of processing on the sulfur compounds in Zephyr oil.

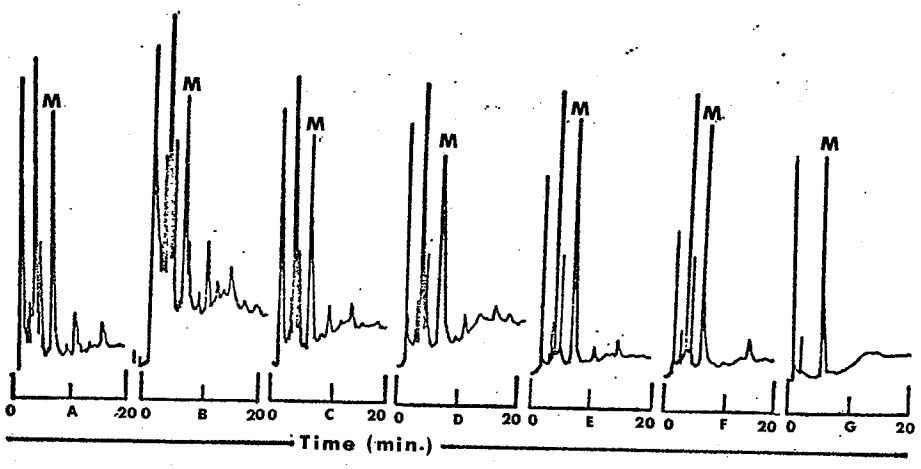
Processed Oil Sample	Content of Sulfur Compounds (ppm S)[a]						Total, by Summation	Total, by Raney Nickel
	1	2	3	4	5	OZT		
Crude	1.0	0.4	trace	trace	trace	9.1	10.5	10
Bleached	0.3	0.1	trace	trace	trace	0	0.4	1
Deodorized	0	0	0	0	0	0	0	1

[a] Compounds 1-5 correspond to peaks 1-5 in Figure 11. OZT corresponds to oxazolidinethiones in Figure 13.

A Expelled Oil
B Extracted Oil
C Crude Oil
D Degummed Oil
E Refined Oil
F Bleached Oil
G Deodorized Oil
M Internal Standard



Span Oil



Oro Oil

Figure 20. Effectiveness of processing in removing sulfur compounds from rapeseed oil. Chromatograms of rapeseed oils on FFAP column as in Figure 11.

Table 15. Summary of the effect of processing on the sulfur compounds in rapeseed oil.

Processed Oil Sample	No. of Samples	Content of Sulfur Compounds (ppm S)[a]					
		1	2	3	4	5	OZT
Expelled							
B. napus	1	1.3	0.4	0.8	trace	1.2	12.2
B. campestris	2	2.0-2.1	1.9-2.3	2.8-3.2	1.5-2.0	2.5-3.7	5.8-12.5
Extracted							
B. napus	1	2.5	0.9	0.3	trace	trace	19.9
B. campestris	2	0.8-4.3	0.7-4.4	0.4-1.0	0.4-0.8	0.4-0.9	6.7-13.1
Crude							
B. napus	6	0.9-1.6	0.4-0.5	trace-0.6	trace	0-1.2	2.0-13.6
B. campestris	6	0.7-1.3	0.7-1.1	0-0.7	trace-1.4	0-1.3	7.6-26.1
Degummed							
B. napus	1	1.5	0.2	trace	trace	trace	7.7
B. campestris	1	0.5	0.6	trace	trace	trace	3.0
Refined							
B. napus	3	0.4-1.2	trace-0.6	trace-0.1	trace	trace-0.2	trace- 5.3
B. campestris	3	0.7-1.8	0.7-1.9	trace-2.8	trace-1.6	0.1-1.2	2.0-13.9
Bleached							
B. napus	2	0.3-1.4	0.1-0.5	trace	trace	trace	0- 1.3
B. campestris	2	1.6-2.1	1.7-2.0	trace-0.3	trace	trace-0.9	trace- 2.2
Deodorized							
B. napus	2	trace	0	0	0	0	0
B. campestris	4	trace	trace	0	0	0	trace

[a]Compounds 1-5 correspond to peaks 1-5 in Figure 11. OZT corresponds to oxazolidinethiones in Figure 13.

meals. It is possible that more oxazolidinethione is formed in the meal than is found in the oil and that the amounts found in the oil represent values for saturated solutions. It was also surprising that any oxazolidinethione was found in the oils at all since Grzybowska (100) found that oxazolidinethione added to the oil was rapidly destroyed; she presumed that this was through oxidation by peroxides in the oil. In the present study, oxazolidinethione added to a commercial soybean oil (Crisco) remained stable for at least several days. Possibly the antioxidants added to the soybean oil and the natural antioxidants in crude rapeseed oils prevented oxidation of the oxazolidinethione.

Degumming, refining and bleaching almost completely removed the oxazolidinethiones from rapeseed oil. These processes did not significantly reduce the amounts of the two identified isothiocyanates in the oil although they did remove much of the unknown compounds. Isothiocyanates would thus seem to be the sulfur compounds most likely to remain in the refined and bleached oils and to possibly be responsible for catalyst poisoning in hydrogenation. Perhaps some form of refining with ammonia would aid in the removal of the isothiocyanates as thioureas. Deodorization almost completely removed all the sulfur compounds from the oils.

Comparison of the total amount of sulfur found by the Raney Nickel method and by summation of the individual sulfur compounds showed good agreement between the two methods for most of the oils, particularly for the refined and bleached oils. The widest differences between the total sulfur contents, as determined by the two methods, occurred in samples of crude and extracted oils which also contained large amounts of gum and sediment. It is possible that some of the sulfur compounds in these oils

became irreversibly bonded to gum components or that these oils contained large amounts of sulfur-containing polymers such as are formed on breakdown of oxazolidinethione (36). It is also possible that rapeseed oil gums may contain small amounts of intact glucosinolates.

A sample of crude oil from the low-glucosinolate cultivar Bronowski (*B. napus*) was also analyzed. This sample contained only 3 ppm total sulfur by analysis by the Raney nickel method and traces of each of the sulfur compounds by gas chromatography. This result indicates that the introduction of low-glucosinolate cultivars of rapeseed should result in a reduction of the amounts of sulfur compounds found in the oil.

Effect of Seed Quality and Moisture Content on the Amount of Sulfur Compounds in Rapeseed Oil

It may be assumed that glucosinolates in the seed, because of their polar character, are insoluble in the oil and that the isothiocyanates and related compounds formed upon hydrolysis of the glucosinolates are preferentially soluble in the oil. Mustakas (119) showed that the glucosinolates in mustard seed were not hydrolyzed when the seed was crushed unless the seed contained at least 13% moisture. Daun (99) showed that rapeseed oil extracted from seed with greater than 15% moisture contained relatively large amounts of sulfur; the amounts of sulfur were less in oils extracted from low-glucosinolate seeds than in oils from high-glucosinolate seeds. He also found sulfur in oils extracted from green, "heated" and frost-damaged seed. No sulfur was found in oils extracted from sound rapeseed at moisture levels of less than 10%.

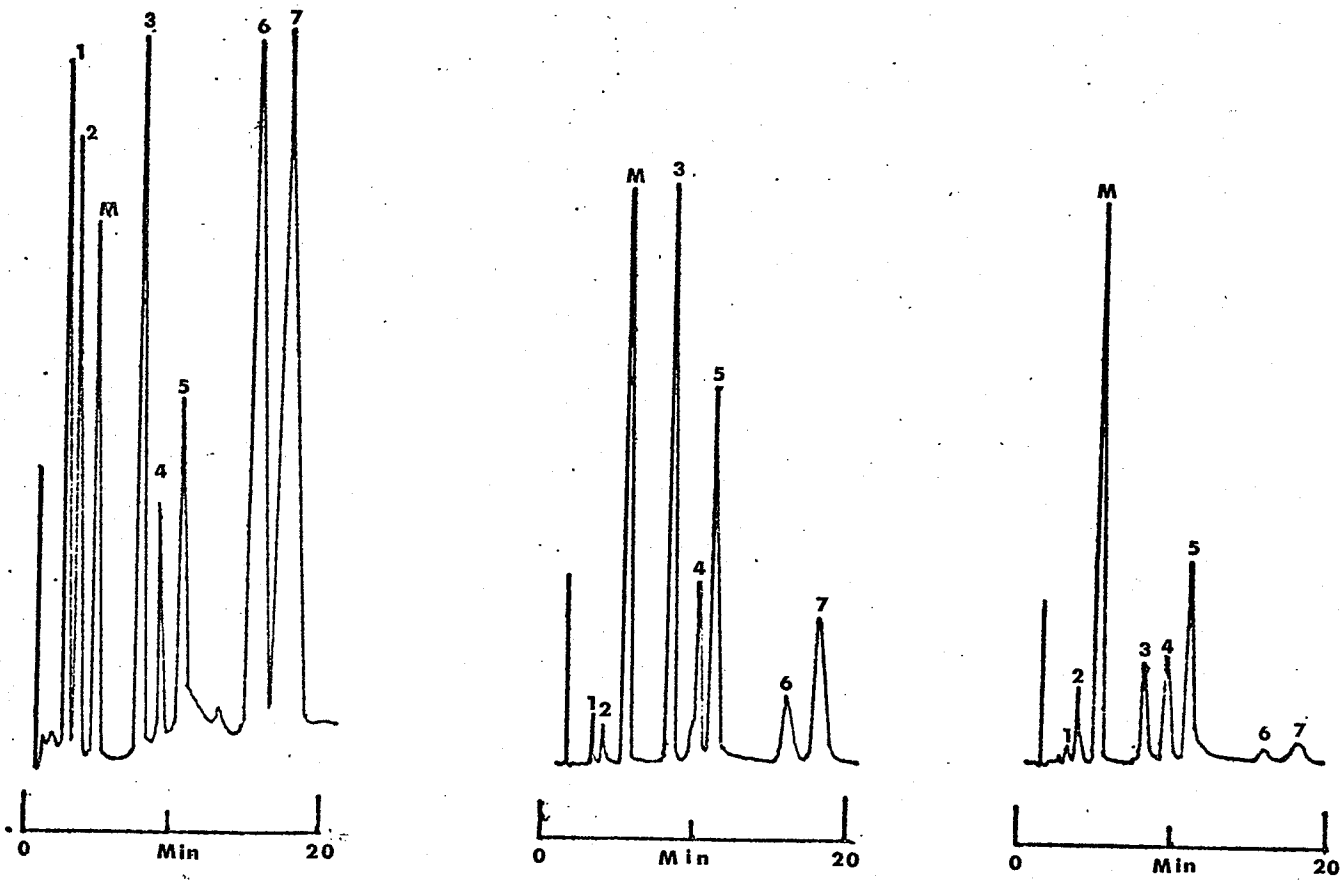
Oil samples used in this study were prepared in the laboratory from the high-glucosinolate cultivars Span (*B. campestris*) and Oro (*B. napus*)

and from the low-glucosinolate cultivar Tower (*B. napus*). These cultivars are currently being grown in Western Canada. Samples were also obtained from seed of lower quality classified as green, heated, and frost-damaged seed (obtained by courtesy of Mr. I. Levi, Grain Research Laboratory, Canadian Grain Commission, Winnipeg).

Oil samples were extracted in the laboratory from seed at different moisture levels as described. Each oil sample was extracted with methanol and the methanol extract was analyzed for sulfur compounds by gas chromatography. Chromatography of the sulfur compounds was done with methanol extracts rather than with the whole oils because of the large number of samples involved. Chromatography of the methanol extracts could be carried out without frequent changing of the pre-column inserts, thus saving considerable time. Studies with industrial rapeseed oils showed that 60-90% of the sulfur compounds were extracted with methanol, the amount varying with the compound studied. This extraction efficiency was considered adequate for a semi-quantitative analysis. For a more quantitative analysis of certain samples, the whole oil was used.

Figure 21 shows typical chromatograms of the methanol extracts with the FFAP column. Peaks 1 to 5 are the same as those found in industrial oils (Fig. 11). Peaks 6 and 7 were not observed in the industrial oil samples. Peak 6 eluted with the same retention time as a pure sample of (*threo*)-1-cyano-2-hydroxy-3,4-epithiobutane. Based on this evidence and on the report of Daxenbichler *et al.* (68), peaks 6 and 7 were tentatively identified as the *threo* and *erythro* forms of 1-cyano-2-hydroxy-3,4-epithiobutane.

1 = 3-Butenyl isothiocyanate
2 = 4-Pentenyl isothiocyanate
M = Heptyl isothiocyanate (Internal Standard)
3 = Unidentified
4 = Unidentified
5 = Unidentified
6 = *threo*-1-cyano-2-hydroxy-3,4-epithiobutane
7 = *erythro*-1-cyano-2-hydroxy-3,4-epithiobutane (tentative)



Span (11.7% H_2O)

Oro (11.3% H_2O)

Tower (10.8% H_2O)

Figure 21: Gas chromatograms of sulfur compounds in oil from laboratory-extracted rapeseed.

The results of the study (Table 16) indicate that traces of sulfur compounds are present in oils extracted from seeds of even very low moisture content. All of the sulfur compounds found in industrial oils were also found in laboratory-extracted oils from seeds with moisture contents higher than 10%. Oils with relatively large amounts of sulfur compounds only occurred when the moisture level of the seed exceeded 10.5 - 11.5%. This last observation is in agreement with the earlier observations by Mustakas (119) and Daun (99).

Considerably smaller amounts of sulfur compounds were found in oils extracted from low-glucosinolate rapeseed than from high-glucosinolate rapeseed, again in agreement with the earlier work by Daun (99).

Only small amounts of sulfur compounds were found in oils extracted from green seed and from frost-damaged seeds. This was not in agreement with the earlier results by Daun (99), who found substantial amounts of sulfur in oils extracted from these seed samples. Possibly, this could be due to sulfur compounds which do not appear in the gas chromatographic analysis, or the Raney nickel procedure used in the earlier study may have been subject to analytical error due to the small sample size or to some unknown interference. Heated seed was found to release an oil with relatively large amounts of sulfur compounds at a somewhat lower moisture level than undamaged seed.

The total amounts of sulfur compounds present in oils from the various seed samples did not increase consistently with increasing moisture content. This effect was possibly due to the loss of variable amounts of sulfur compounds by evaporation along with the petroleum ether solvent after extraction. This inconsistency could also have been caused by small amounts of water which were observed in some of the oils; this water presumably originated in the seed and was extracted along with the

Table 16. Effect of seed quality and moisture content on the amount of sulfur compounds in rapeseed oil.

Seed Type	H_2O %	Content of Sulfur Compounds (ppm S)[a] 1	2	3	4	5	6	7	8	Total
B. napus Cv. Oro	4.3					tr.[b]				tr.
	5.6				tr.	tr.				tr.
	8.4	tr.	tr.		tr.	tr.				tr.
	10.5	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
	11.3	13	11	31	6	9	19	31	10	130
	13.0	tr.	3	7	7	12	tr.	tr.	tr.	19
	14.4	1	tr.	2	2	1	16	9	3	34
	19.6	tr.	tr.	12	4	9	27	26	8	86
B. campestris Cv. Span	4.3			tr.	tr.	tr.				tr.
	6.1			tr.	tr.	tr.				tr.
	9.4		tr.	tr.	tr.	tr.				tr.
	10.9		1	3	3	5	tr.	tr.	tr.	12
	11.7	23	41	35	16	17	7	9	4	153
	13.4	3	24	53	39	48	20	28	12	227
	19.9	3	10	13	30	117	4	8	3	188
	20.5	tr.	3	12	76	114	7	10	3	226
B. campestris Cv. Tower	3.9					tr.				tr.
	5.6					tr.				tr.
	8.6	tr.	tr.		tr.	tr.				tr.
	10.6	tr.	tr.		tr.	tr.				tr.
	10.8	7	1	13	3	4	tr.	tr.	tr.	28
	12.6	tr.	tr.	tr.	9	3	tr.	4	tr.	12
	14.0	tr.	tr.	tr.	tr.	tr.	tr.	3	tr.	3
	18.8	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
Green[c]	4.2					tr.				tr.
	5.7				tr.	tr.				tr.
	7.9				tr.	tr.				tr.
	10.2				tr.	tr.				tr.
	10.4	2	2		2	tr.				6
	10.5	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
	14.2	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
	18.4	tr.	tr.	tr.	tr.	9	tr.	tr.	tr.	9
Heated[c]	4.0					tr.				tr.
	5.6			tr.	tr.	tr.				tr.
	7.9			tr.	tr.	tr.	tr.	tr.	tr.	tr.
	9.2			tr.	tr.	3	3	4	tr.	10
	10.4	6	5	14	13	5	25	29	10	107
	11.6	tr.	3	10	3	5	15	17	6	59
	12.5	tr.	tr.	6	7	11	tr.	tr.	tr.	24
	16.4	3	4	13	15	9	33	43	4	124
Frost damaged[c]	4.6					tr.				tr.
	6.9				tr.	tr.				tr.
	9.0				tr.	tr.				tr.
	10.7				tr.	9			tr.	9
	11.4	3	6		tr.	9			tr.	18
	12.9	1	4		tr.	3			tr.	8
	19.5	tr.	tr.		tr.	8			tr.	8

[a] Compounds 1-7 refer to compounds 1-7 in Figure 21. Compound 8 refers to oxazolidinethione as in Figure 13.

[b] Trace.

[c] These samples were unknown varieties of normal high-glucosinolate content.

oil. The water could increase the polarity and decrease the extraction efficiency of the methanol used to extract the sulfur compounds from the oil.

It was notable that the amount of 3-butenyl- and 4-pentenyl isothiocyanate in the laboratory-extracted oils was lower in proportion to the other sulfur compounds in comparison to the industrially-extracted oils. Furthermore, the ratio of 3-butenyl- to 4-pentenyl isothiocyanate in the laboratory-extracted oils was different from the ratio observed in industrial oils. These differences might be due to the different procedures used in the laboratory extraction and in the industrial extraction.

Despite the above differences, some useful conclusions may be drawn from the laboratory extraction study which may be applicable to industrial processing. Firstly, oil from green or frost-damaged seed may not contain as much sulfur as was originally reported; secondly, the introduction of low-glucosinolate rapeseed may result in oils with lesser amounts of sulfur compounds; and thirdly, processors may find a substantial reduction in the amounts of sulfur compounds present in crude oils if careful attention is paid to the moisture content of the seed and meal during crushing and extraction.

SUMMARY AND CONCLUSIONS

Identification of Sulfur Compounds in Rapeseed Oil

Seven sulfur compounds were found in samples of industrially processed rapeseed oils. Four of these compounds were identified as the glucosinolate hydrolysis products 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, phenethyl isothiocyanate and 5-vinyl-2-oxazolidinethione. The identity of the other three sulfur compounds was not established. It is possible that these compounds are previously unreported glucosinolate hydrolysis products. Epithiobutanes and thionamides, which have been reported to be formed on hydrolysis of 2-hydroxy-3-butenyl glucosinolate, were not observed in industrial oil samples although epithiobutanes were found in samples of oil extracted in the laboratory. The establishment of the identity of the four glucosinolate hydrolysis compounds found in the oil was based on the following information.

3-Butenyl- and 4-pentenyl isothiocyanate. The compounds in the oil gave a positive response with the flame photometric detector and their thiourea derivatives responded to spray reagents specific for sulfur compounds. The compounds in the oil had the same retention times as reference peaks of 3-butenyl- and 4-pentenyl isothiocyanate in a rapeseed meal extract when analyzed by gas chromatography on a polar (FFAP) and a nonpolar (Apiezon L) column. The thiourea derivatives of the compounds from the oil has the same R_f values as the reference spots of thiourea derivatives of 3-butenyl- and 4-pentenyl isothiocyanate in a rapeseed meal extract when analyzed by thin-layer chromatography. The mass spectra of the thiourea derivatives of the compounds from the oil gave the correct molecular ions (M = 130 AMU and M = 144 AMU) and were similar to the mass spectra

of the thiourea derivatives of 3-butenyl- and 4-pentenyl isothiocyanate from a rapeseed extract.

5-Vinyl-2-oxazolidinethione. The compound in the oil gave a positive response with the flame photometric detector and responded to spray reagents specific for sulfur compounds. The compound in the oil had the same retention time as a reference sample of 5-vinyl-2-oxazolidinethione when analyzed by gas chromatography (EGSS-X column). The compound also had the same R_f value as the reference sample when analyzed by thin-layer chromatography.

Phenethyl isothiocyanate. The thiourea derivative of the compound in the oil responded to spray reagents specific for sulfur compounds and had the same R_f value as the thiourea derivative of pure phenethyl isothiocyanate when analyzed by thin-layer chromatography. The mass spectrum of the thiourea derivative of the compound in the oil gave the correct molecular ion (M = 180 AMU) and was similar to the mass spectrum of pure phenethyl thiourea. Phenethyl isothiocyanate was present in trace amounts only and it was not seen on gas chromatograms, possibly because it was masked by other sulfur compounds.

Effect of Industrial Extraction and Refining on the Sulfur Compounds in Rapeseed Oil

The amounts of sulfur compounds present in a number of industrially extracted and refined rapeseed oils were determined by gas chromatography using a flame photometric detector. The total amount of sulfur present as determined by summation of the individual sulfur compounds agreed well with the total as determined by the Raney nickel procedure.

The amounts of 3-butenyl isothiocyanate (0.7 - 4.3 ppm) were not substantially different in the expelled, extracted, and crude oils. There was little difference also between *B. napus* and *B. campestris* with respect to these oils. The ratio of 3-butenyl isothiocyanate to 4-pentenyl isothiocyanate was approximately the same as in the seed meal. The unidentified compounds were present in greater quantities in oils from *B. campestris* (0 - 3.7 ppm S) than in oils from *B. napus* (0 - 1.2 ppm S) and were more predominant in the expelled oils than in the extracted oils. The oxazolidinethione content of expelled, extracted, and crude oils ranged from 2.0 to 26.1 ppm S. The oxazolidinethione content was highest in oils containing substantial amounts of gum. This relationship to gum content was not observed for the other sulfur compounds. A sample of crude oil from the low-glucosinolate rapeseed cultivar Bronowski (*B. napus*), which contained only 3 ppm total sulfur, showed traces of each of the sulfur compounds.

Degumming, refining, and bleaching were effective in removing the oxazolidinethiones and the unidentified compounds from the oil but did not significantly reduce the content of 3-butenyl- and 4-pentenyl isothiocyanate. Deodorization almost completely removed all sulfur compounds.

The results from this study indicate that present refining methods are capable of removing most of the sulfur compounds from rapeseed oils. Isothiocyanates, which are not removed by refining and bleaching, may be the sulfur compounds responsible for difficulties encountered in hydrogenating rapeseed oils. The introduction of low-glucosinolate rapeseeds may lead to the production of crude oils with substantially lower contents of sulfur compounds.

Effect of Seed Quality and Moisture Content on the Amount of Sulfur Compounds in Rapeseed Oil

Sub-samples of various seed types were prepared with moisture contents ranging from 4 to 20%. The oil was extracted from these sub-samples and the sulfur compounds in the oils were determined by gas chromatography.

Traces of sulfur compounds were observed in all oil samples from seed with moisture levels below 10.5%. Appreciable amounts of sulfur compounds were found in the oil when the moisture content of the seed had reached 10.5 - 11.5%. The amounts of sulfur compounds present in oils extracted from the low-glucosinolate rapeseed cultivar Tower (*B. napus*) were substantially lower than in oils extracted from the high-glucosinolate rapeseed cultivars Oro (*B. napus*) and Span (*B. campestris*).

Approximately the same amounts of sulfur compounds were found in oils from "heated" and sound seed but lesser amounts were found in oils from green or frost-damaged seed. This appears contrary to previous results obtained by the Raney nickel method (99). The present results suggest that seed quality may not be of great importance in determining the amount of sulfur compounds in the oil. The most important factors appear to be the total glucosinolate content and the moisture content of the seed.

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