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# AN INVESTIGATION INTO THE PRESENCE OF DEGRADATION PRODUCTS FROM GLUCOSINOLATES IN RAPESEED OIL

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## SUMMARY

The results are presented of an investigation into the presence in rapeseed oil of low boiling (upto 250°C) nitrogen and sulphur containing compounds, formed by the degradation during processing of the glucosinolates present in rapeseed.

By the use of GLC, fitted with nitrogen and sulphur selective detectors, the presence of ten compounds has been tentatively established in the crude oil. The amounts of these compounds have been estimated, using an internal standard and direct injection of the oil, for each of the various refining stages. It was found that the level of these compounds fell below the detection limit ( 1 ppm) after the deodorising step and that the refined oil contained no detectable level of these 'split products' from the glucosinolates. A comparison between the amount of sulphur containing compounds found and the total sulphur content of the oil indicated that the majority of the sulphur present was contained in compounds which failed to pass through the GLC column.

## INTRODUCTION

When the virtual removal of erucic acid from rapeseed oil failed to completly solve the problem of 'antinutrional' effects, obtained in feeding trials with the oil, the search for other possible constituents which could cause pathological damage was intensified.

Since most rapeseed varieties contain glucosinolates, it was natural to look at these and see if they and or their 'split products' could have any of the observed effects.

The commercial process for the production of oil from rapeseed generates enough heat to partially destroy the thioglucosidase enzyme present but not sufficient to completly inactivate it. If enzymic hydrolysis of glycosinolates occurs before, or heat degradation occurs during extraction then the hydrolytic products can enter the oil.

These products can be detrimental, not only nutritionally, but also in the processing of the oil since some of the compounds formed contain sulphur which have an adverse effect on many of the refining stages - especially hydrogenation.

Normally only one or two glycosinolates are present in any large amounts in rapeseed, although as many as nine of these compounds have been found in certain cruciferae. Each of the characterized glycosinolates differs from the others only in the nature of the R group, fig. 1. The diagram also illustrates how the glucosinolates can breakdown under normal processing conditions.

With regard to the total sulphur content of rapeseed oil found at each of the various processing stages there is considerable variation in the literature. This is probable due to the variation in seed type, method of processing and method of analysis. However, it does seem that the lower, the glucosinolate level, in the original seed the lower is the subsequent sulphur content in the oil.

The formation of a particular isothiocyanate, thiocyanate, nitrile etc., depends considerable on the conditions of degradation. Fig. 2 lists some of the possible 'split products' likely to be found in the crude oil. Depending on the conditions these compounds can undergo numerous rearrangement, degradation and polymerisation reactions.

At Karlshamns oil processing factory we have paid particular attention to analyzing the amounts of these split products remaining in the oil after each refining step. However in order to achieve this we had to develop a simple/rapid method for the quantitative determination of the majority of these compounds.

EXPERIMENTAL

Intial attempts to investigate the volatile sulphur compounds present in crude rapeseed oil by direct injection into a gas chromatographic column fitted with a flame photometric detector (FPD), proved unsuccessful in terms of its use for quantitative determinations. However by using the FPD detector, a 2 meter 6% FFAP column and programing from 50-250°C at 6%/min, it was possible to obtain some useful qualitative information. The chromatogram shown in fig. 3 is typical of those obtained. Only four sulphur containing compounds were detected at the >1 ppm level, which is in contrast to the results obtained by Daun and Hougen in J.A.O.C.S. 54 (1977) where up to seven compounds were observed under not too disimilar conditions. The differences were thought to be due to the higher chromatographic temperatures used in the present study but after tentative investigation this was shown not to be the case. The four compounds were subsequently identified, using reference samples and mass spectrometry as: Butenyl isothiocyanate, Pentenyl isothiocyanate, 1-cyano-3,4 Epithiobutane and 2-Phenylethyl isothiocyanate. Other compounds which theoretically could be present such as 4-methylthiobutyl isothiocyanate and 5-vinyl-2-oxyolidinethione were not detected.

Since 'split products' from glocosinolates known to be present in rapeseed nearly always contain nitrogen, whilst only sometimes sulphur, it was decided to further concentrate the investigation to the low boiling (0-250°C) nitrogen compounds.

The Perkin Elmer thermionic (rubidium silicate bead) nitrogen selective detector proved very useful for analysing these nitrogen compounds in rapeseed oil. The P.E. 3920 model also had the advantage of a pre-column, which enabled the direct injection of the oil into the column via a silinated glass wool packing; this reduces breakdown of the stationary phase and minimises reconditioning.

In order to establish the best operating conditions the various parameters involved were investigated using those chemicals

which could be obtained relatively pure. As an internal standard 1-cyano-octane was found suitable. Increasing the 'bad' temperature produced a decrease in selectivity untill a constant response was obtained, however sensitivity increased with a rise in bead heating. Fortunately the relative response with respect to the internal standard of the compounds tested remained fairly constant over a wide bead (temp) setting. A decrease in hydrogen flow over the bead increased selectivity which whilst the effect on sensitively was more complicated. Down to 3.5 ml/min $H_2$ the relative response was constant, however below this flow rate there was a large divergence of the response by the compounds compared to 1-cyano octane. A hydrogen flow of about 4 ml/min and a bead 'temperature' setting of 600 gave a selectivity of nitrogen compounds to hydrocarbons of $>$ 2,000:1.

Because of the difficulty in obtaining samples of pure reference materials it was only possible to test the linearity range on a limited number of chemicals. For butenyl nitrile, pentenyl nitrile, butyl isothiocyanate (probable butenyl and pentenyl ITC'S as well), butyl hydroxy nitrile and 1-cyano octane the range is $4x10^{-9}$ g to $4x10^{-7}$ g injected. With epizulphides A and B not only is the response much less but the linearity range is smaller at $9x10^{-9}$ to $3x10^{-7}$ g.

Having obtained the best conditions and parameters for running samples of oil, suitable chromatogram could be obtained at each of the processing steps, and using the internal standard a calculation of the amount of each split product obtained.

By using the different solubilities of the chemicals in water, petroleium spirit and acetonitrile it was possible to obtain three fractions which were run through a GLC/ mass spectrophotometer and the corresponding peaks obtained with the nitrogen selective detector identified. A typical chromatogram obtained with the thermionic detector from an ether extract of enzymatically degraded glucosinolates in crushed rapeseed at pH 7 is shown in fig. 4, with the appropriate peaks identified.

All that is required to obtain semi quantitative determinations is to add about $10x10^{-6}$ g 1-cyano octane internal standard dissolved in methyl acetate to 1 gm of the oil, after mixing 0-5 ul of the oil is injected into the GLC's precolumn. It is possible to determine as low as $0.05x10^{-6}$ g of each substance per gram of sample, with a relative standard deviation of about 10%. As low as $1x10^{-9}$ g/g can be detected but with considerable loss in accuracy.

The accuracy of the method has been verified by recovery studies on several of the split products. Known amounts were added to rapeseed oil/meal together with the internal standard and analysed by injecting 2 µl of the oil or a methyl acetate extract into the gas chromatograph.

Recoveries of 80-100% were found for non sulphur containing compounds whilst only 60-70% for the two episulphides tested. The poor results with the latter compounds might be due to the impurity of the compounds and/or their breakdown on the column/ injector.

## RESULTS AND DISCUSSION

The correlation between the sulphur content of the identified sulphur compounds and the total sulphur of the oil, as determined by the oxygen flask combustion method (Bladh), is given by table 2, fig. 6. There is no apparent correlation, however the level of sulphur does decrease roughly as the amount of identified sulphur compounds decreases. This confirms work by Lanzoni et al. La Rivista Italiana Delle Sostanze Grasse Vol III 1975, who were only able to account for 25% of the sulphur present in terms of isothiocyanates and VOT. It would seem that the bulk of the sulphur present is in the form of relatively high molecular weight compounds. The possibility of the presence of a small amount of elemental sulphur must also be considered in relation to the known degradation of glucosinolates, although the FPD failed to indicate an amount over 2 ppm.

The results obtained for the level of degradation products found in crude rapeseed oil by (a) solvent extraction and (b) pressing as well as the levels in the gum-residue (lecithin residue) are presented graphically in fig. 7. Each 'analysis range' is the result of five determinations. The bulk of the split products occur in the lecithin residue, however there are considerable more of the degradation products in the pressed-out oil than the solvent extracted. A similar graphical representation has been made for the analysis of the rapeseed oil during processing, fig. 8 and a typical chromatogram is shown in fig. 5. The results are the range obtained for at least ten analysis of different factory processed rapeseed oils with varying levels of glucosinolates in the original seed.

The crude rapeseed oil contains nearly all the split products from glucosinolates in amounts from not detetable (ND i.e. $<0.1$ ppm) to approx 40 ppm. However during processing these compounds are removed, with the largest drop occuring during the deodorising step. The removal in the deodorizer is to be expected since most of these compounds are either readily steam volatile or are broken down under the conditions.

The results, with regard to the isothiocyanates, are in general agreement with those obtained by Franzke et al. Die Nahrung 19, Ihg. Heft. 7 (1975). Vot was only found in the extracted oil and no glucosinolates were detected in either oil.

We can conclude by saying that no evidence has been found for the presence of substantial amounts of low boiling (i.e. 0-250$^{o}$C) nitrogen or sulphur compounds in the refined rapeseed oil which could account for any 'antinutritional effect' of the oil when consumed. Since it was not possible to account for all the sulphur present, the oil could still contain a high boiling sulphur compound(s) having a negative nutritional effect.

FIGURE 1.

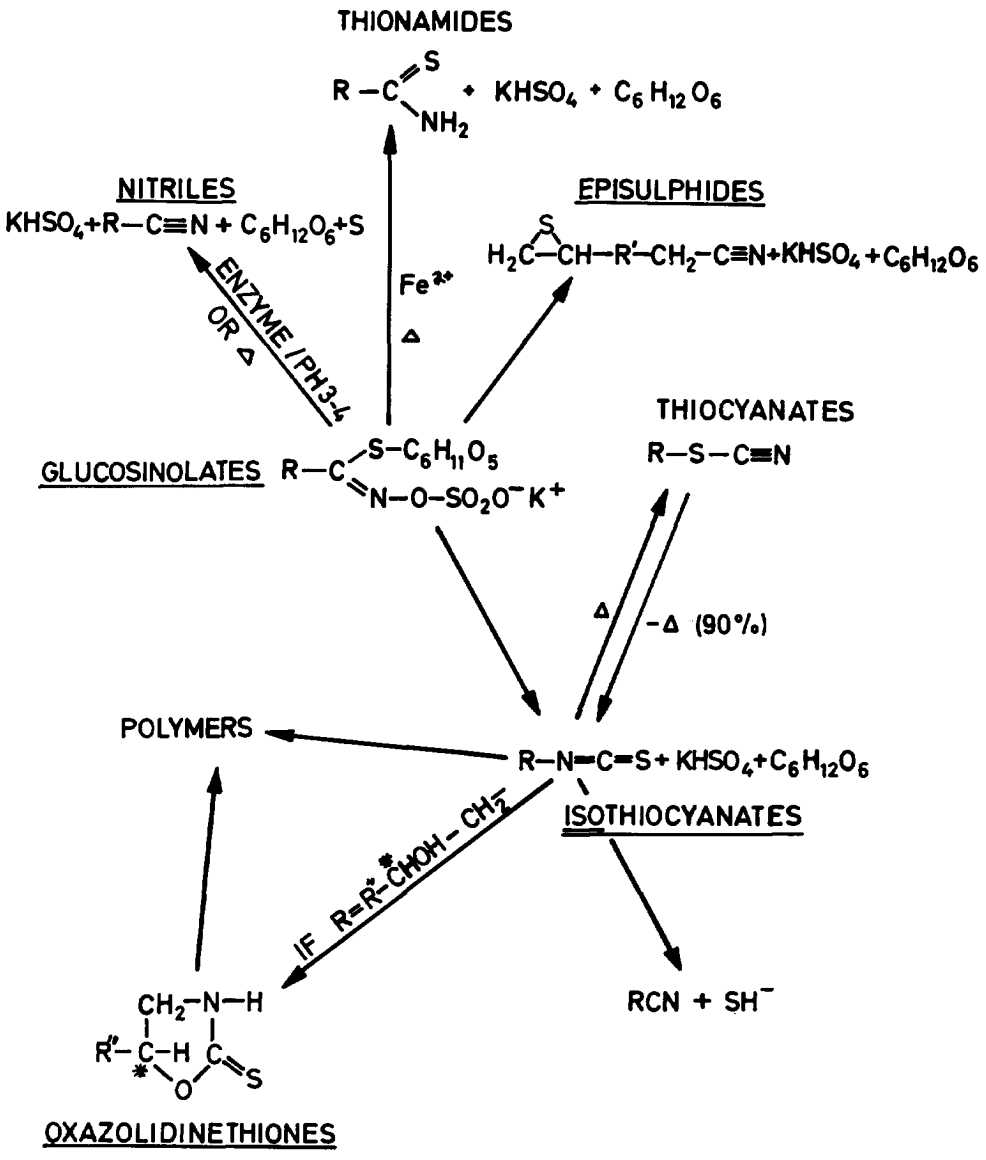

THIONAMIDES
R–C(=S)–NH2 + KHSO4 + C6H12O6
NITRILES
KHSO4+R–C≡N + C6H12O6+S
EPISULPHIDES
H2C–CH–R'–CH2–C≡N+KHSO4+C6H12O6
ENZYME / PH3-4
OR Δ
Fe2+
Δ
GLUCOSINOLATES
R–C(–S–C6H11O5)=N–O–SO2O⁻K⁺
THIOCYANATES
R–S–C≡N
Δ
–Δ (90%)
POLYMERS
R–N=C=S + KHSO4+C6H12O6
ISOTHIOCYANATES
IF R=R'*–CHOH–CH2
RCN + SH⁻
OXAZOLIDINETHIONES

FIGURE 2. SOME IDENTIFIED DEGRADATION PRODUCTS FROM GLUCOSINOLATES FOUND IN RAPESEED

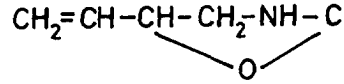
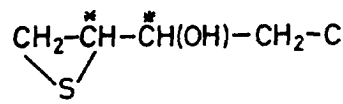
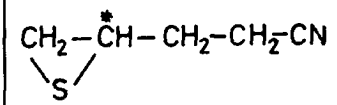
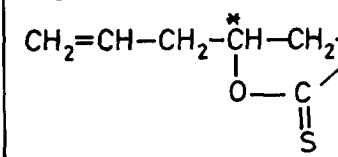
| CHEMICAL NAME | FORMULAE | ABBREVIATION |
|---|---|---|
| ALLYL ISOTHIOCYANATE | $CH_2=CH-CH_2\cdot NCS$ | AITC |
| 1-CYANO-3-BUTENE | $CH_2=CH-(CH_2)_2\cdot CN$ | CB |
| 1-ISOTHIOCYANATE-3-BUTENE | $CH_2=CH-(CH_2)_2\cdot NCS$ | BITC |
| 1-ISOTHIOCYANATE-4-PENTENE | $CH_2=CH-(CH_2)_3\cdot NCS$ | PITC |
| 1-CYANO-4-PENTENE | $CH_2=CH-(CH_2)_3\cdot CN$ | CP |
| 2-PHENYLETHYL-ISOTHIOCYANATE | $C_6H_5\cdot CH_2-CH_2\cdot NCS$ | PEI |
| (S)-1-CYANO-2-HYDROXY-3-BUTENE | $CH_2=CH-\overset{*}{C}H(OH)-CH_2-CN$ | CHB |
| (S)-1-CYANO-2-HYDROXY-4-PENTENE | $CH_2=CH-CH_2-\overset{*}{C}H(OH)-CH_2-CN$ | CHP |
| 5-VINYL-2-OXAZOLIDINETHIONE |  | VOT |
| [2S] [3R]-1-CYANO-2-HYDROXY OR [3S]-3,4-EPITHIOBUTANE |  | EpsA. EpsB. |
| 1-CYANO-3,4-EPITHIOBUTANE |  | CE. |
| ALLYL OXAZOLIDINETHIONE |  | AOT |

FIGURE 3: FPD CHROMATOGRAM

2 µl MEBRA RAPESEED OIL

COLUMN: GLASS 2m LONG. i. d. 2mm. PACKED WITH 6% FFAP ON ANAKROM 100/110 MESH.

CARRIER GAS: $N_2$ AT 15 mls/ min.

COLUMN TEMPERATURE: PROGRAMMING BETWEEN 50 AND 200°C AT 6 °/min.

INJECTOR TEMPERATURE: 210° C

DETECTOR TEMPERATURE: 265° C

PAPER SPEED: 10 mm/ min

1. BUTYL ISOTHIOCYANATE
2. 3-BUTENYL ISOTHIOCYANATE
3. 4-PENTENYL ISOTHIOCYANATE
4. 1-CYANO-3,4-EPITHIOBUTANE
5. 2-PHENYLETHYL ISOTHIOCYANATE

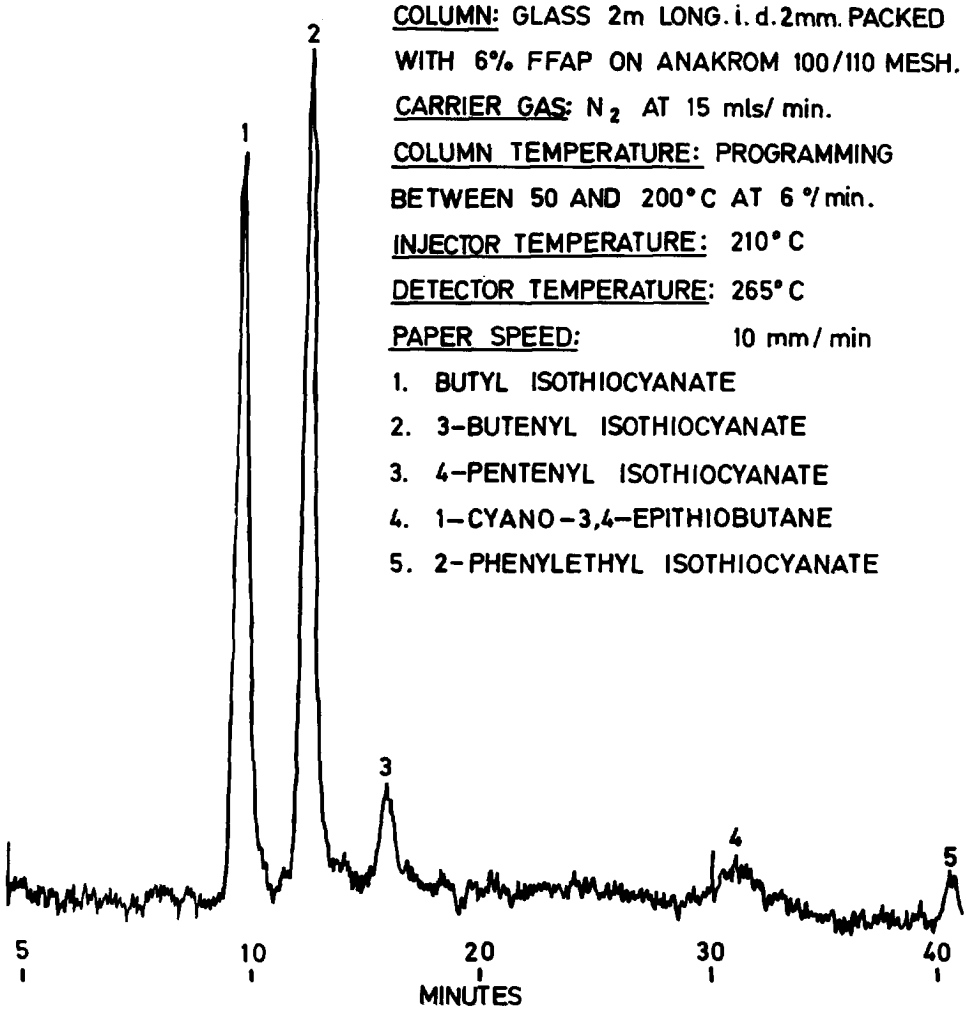


FIGURE 4

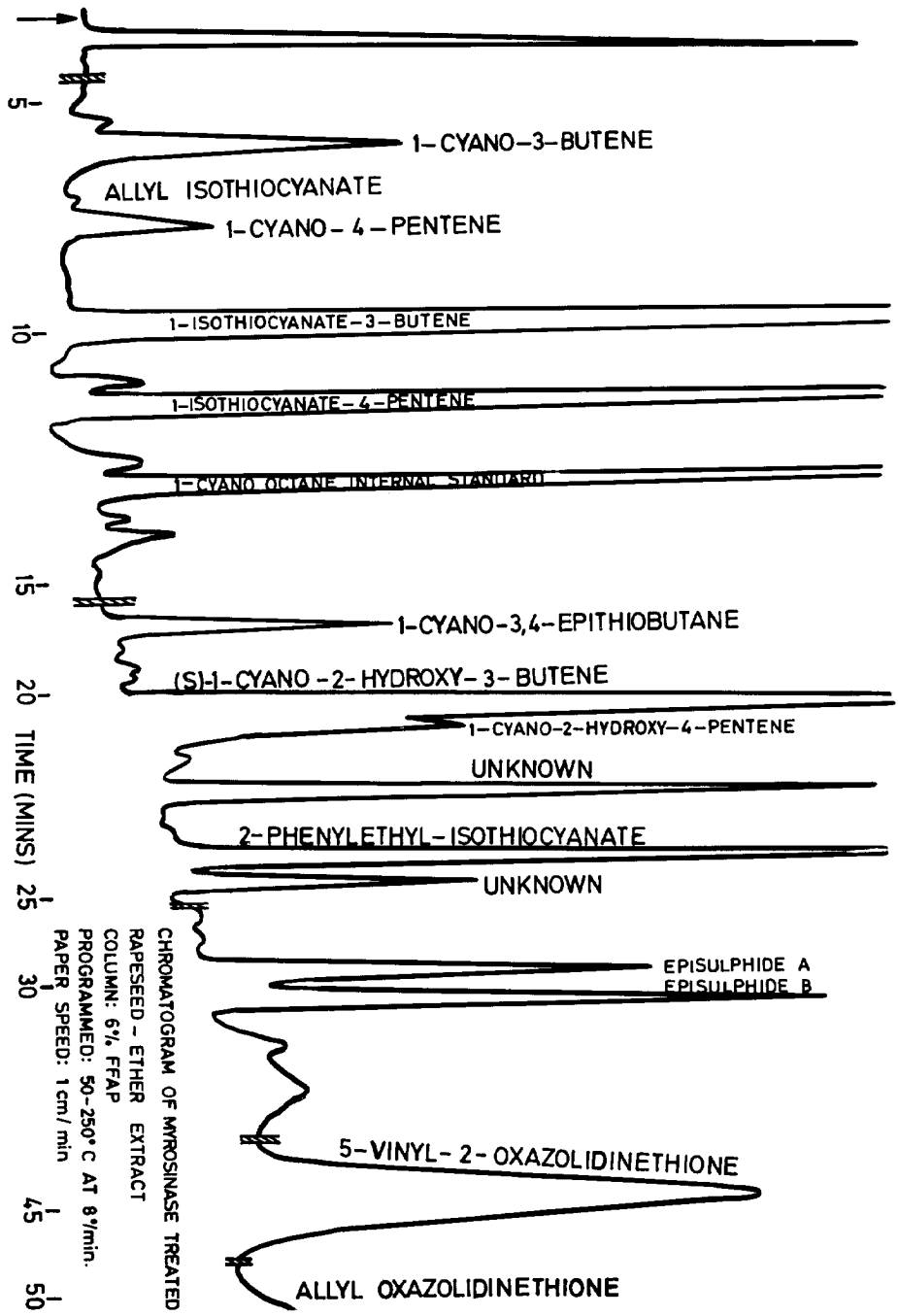

1-CYANO-3-BUTENE
ALLYL ISOTHIOCYANATE
1-CYANO-4-PENTENE
1-ISOTHIOCYANATE-3-BUTENE
1-ISOTHIOCYANATE-4-PENTENE
1-CYANO OCIANE INTERNAL STANDARD
1-CYANO-3,4-EPITHIOBUTANE
(S)-1-CYANO-2-HYDROXY-3-BUTENE
1-CYANO-2-HYDROXY-4-PENTENE
UNKNOWN
2-PHENYLETHYL-ISOTHIOCYANATE
UNKNOWN
EPISULPHIDE A
EPISULPHIDE B
5-VINYL-2-OXAZOLIDINETHIONE
ALLYL OXAZOLIDINETHIONE
5
10
15
20
25
30
45
50
TIME (MINS)
CHROMATOGRAM OF MYROSINASE TREATED
RAPESEED - ETHER EXTRACT
COLUMN: 6% FFAP
PROGRAMMED: 50-250° C AT 8°/min.
PAPER SPEED: 1 cm/min

FIGURE 5: CHROMATOGRAM OF RAPESEED OIL

COLUMN: GLASS 2 m LONG. i. d. 2 mm. PACKED WITH 6% FFAP ON ANAKROM 100/110 MESH.

CARRIER GAS: NITROGEN 20 ml / min

COLUMN TEMP: PROGRAMMING BETWEEN 50–250°C AT 16 °/min

INJECTOR TEMP: 250° C

DETECTOR TEMP: 265° C

PAPER SPEED: 1 cm / min

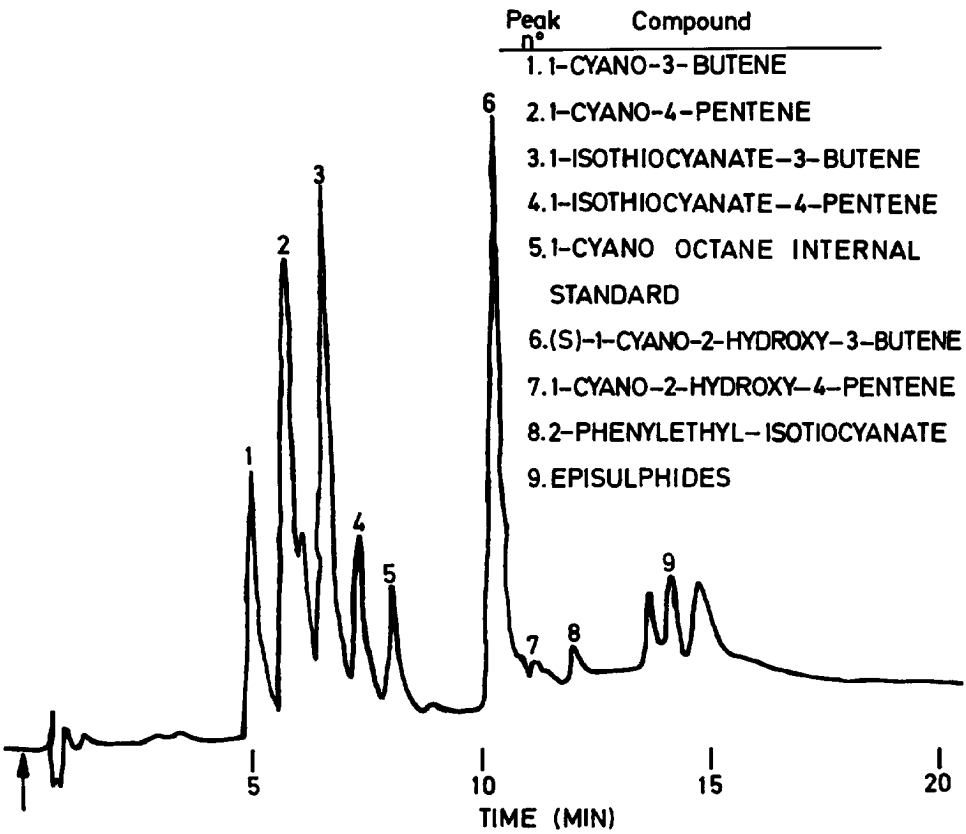


FIGURE 6. A COMPARISON OF THE TOTAL SULPHUR CONTENT OF RAPESEED OIL, AND THE SULPHUR IN IDENTIFIED COMPOUNDS

| Sample | S in g x $10^{-6}$/g | |
|---|---|---|
| | Total S | Compound S |
| CRUDE MEBRA | 15 | 4 |
| CRUDE LOBRA OLGA | 17 | 4 |
| CRUDE LOBRA GULLIVER | 27 | 4 |
| CRUDE ERGLU | 31 | 8 |
| BLEACHED MEBRA | 12 | 2 |
| BLEACHED LOBRA OLGA | 13 | 6 |
| BLEACHED LOBRA GULLIVER | 14 | <1 |
| BLEACHED ERGLU | 13 | 4 |
| DEOD. MEBRA | 9 | <1 |
| DEOD. LOBRA OLGA | 6 | <1 |
| DEOD. LOBRA GULLIVER | 11 | <2 |
| DEOD. ERGLU | 9 | <1 |

FIGURE 7: RESULTS OF ANALYSES OF RAPESEED OIL OBTAINED BY EITHER SOLVENT EXTRACTION OR PRESSING

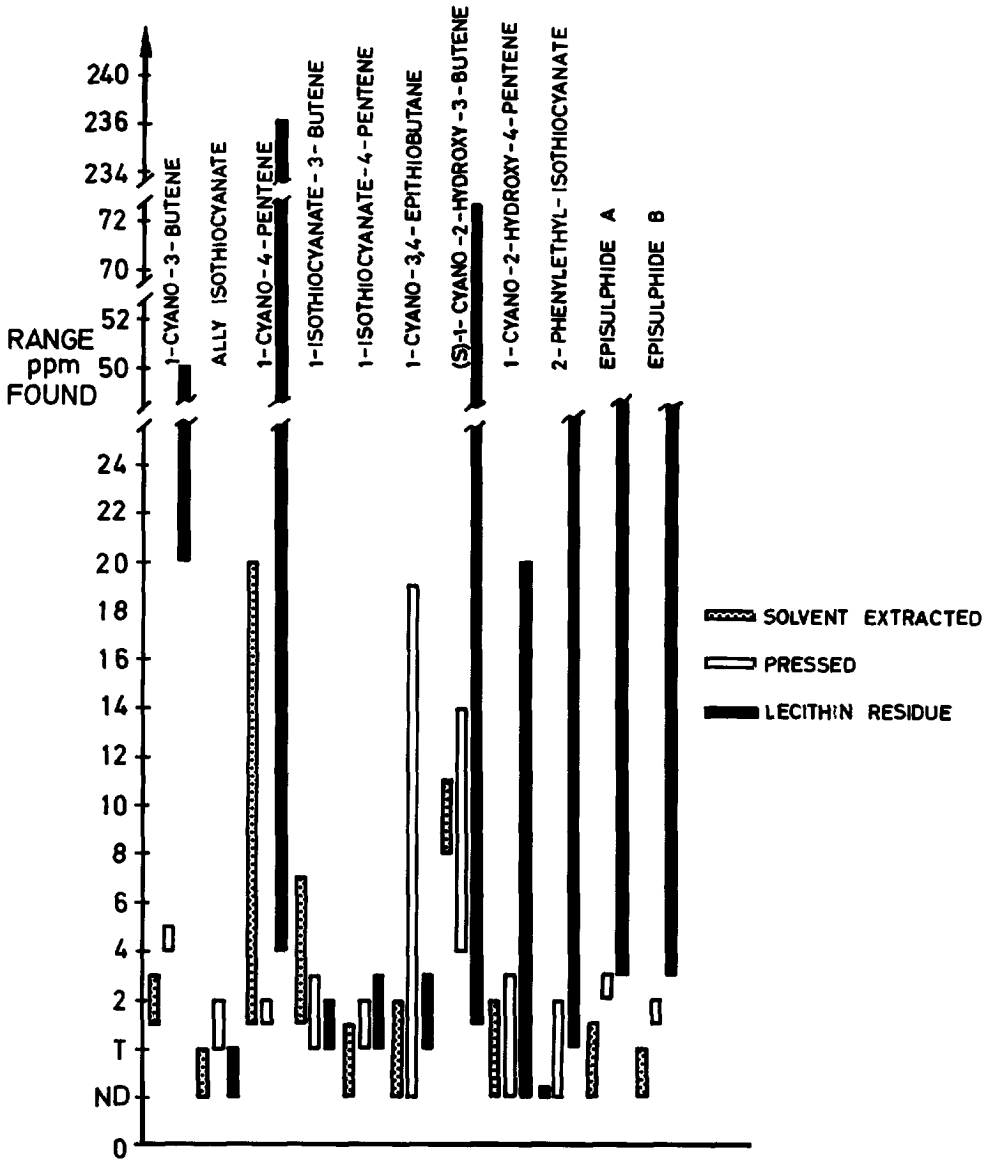


FIGURE 8: RESULTS OF ANALYSES OF RAPESEED OIL FOR SPLIT PRODUCTS DURING SEVERAL PROCESSING STAGES