

DETERMINATION AND CHARACTERIZATION OF GLUCOSINOLATE  
CONSTITUENTS IN RAPESEED MEAL (BRASSICA NAPUS, FRENCH  
VARIETY, LESIRA 145)

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ABSTRACT

Total glucosinolates were determined in rapeseed meal (Brassica napus, Lesira 145). The meal extracted from seeds by using hot hexane contained the highest amounts of glucosinolates i.e. 74.9  $\mu\text{mole/g}$  while the meal after oil extraction with pre-pressing included 25.80  $\mu\text{mole/g}$  total glucosinolates. Moreover, very slight residual content of total glucosinolates i.e. 5.6  $\mu\text{mole/g}$  remained in the meal after oil extraction from seeds by using solvent mixture (chloroform : methanol : water).

Glucosinolates of rapeseed meal batches were isolated and purified on mini-column ion exchange. Then, treated by sulphatase enzyme to remove their sulphate groups. The desulphoglucosinolates were derivatized to volatile compounds with trimethylchlorosilane (TMCS) and n-methyltrimethylsilyl-trifluoroacetamide (MSTFA) followed by using chromatographic techniques for characterization.

GLC technique was found to be fruitful for estimation and identification of aliphatic glucosinolates in the meals under investigation i.e. 2-OH-3-butenyl-(progoitrin), 3-butenyl-(Gluconapin), 4-pentenyl-(Glucobrassicinapin), 2-OH-pentenyl-and allyl-(Singrin) were identified in meals extracted by different methods. In addition, p-OH-benzyl (sinalbin) and 4-OH-3-indolyl (Glucobrassicin) glucosinolate compounds were recognized in meals extracted with pre-pressing and hot hexane.

The results indicated that HPLC technique identified both aliphatic and aromatic glucosinolate constituents. The indolyl-glucosinolates i.e. 4-OH-3-indolyl-, 4-methoxyindole-, 3-indolyl methyl- and 1-methoxyindole glucosinolates were characterized. Furthermore, 2-phenylethyl-, p-OH-benzyl and benzyl-(Glucotropaeolin) were identified.

## INTRODUCTION

Rapeseed is known to be considered as a potential source of protein since its meal contained about 35% protein (Ballester et al., 1970)., Also, El-Nockrashy et al.(1977) stated <sup>that</sup> there are some factors which encouraged the use of protein in human nutrition e.g. its high concentration of essential amino acid contents specially lysine and sulphur amino acids, leading to high nutritional value. However the main problem involved in the use of defatted rapeseed meal includes the presence of thioglucosides and the products of their enzymatic hydrolysis which yield undesirable and toxic factors such as isothiocyanates and glucosinolate compounds (Sehovic et al., 1980). Fenwick and Curtis(1980) reported that rapeseeds contain a thioglucoside glucohydrolase enzyme(E.C.3:2:3:1) which under suitable conditions works on the hydrolysis of glucosinolates. The decomposition products in the meal reduces its palatability and limits its usefulness as an animal foodstuff, Pearson et al.(1981) have shown that the substances derived from glucosinolates the depress action of trimethyl amine oxidase (TMA) present mainly in liver and kidneys. Also, Butler et al.(1982) stated that such compounds have a deleterious effect on the biliary system .

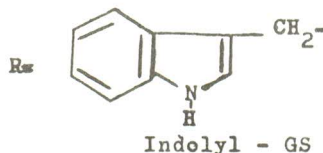
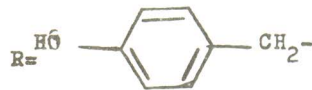
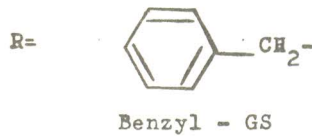
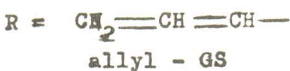
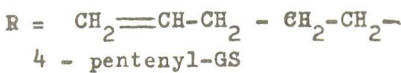
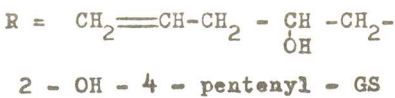
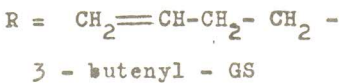
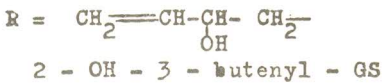
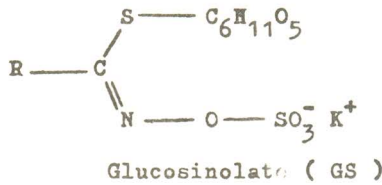
The major fractions compounds in glucosinolates of rapeseeds were gluconapin (3-butenyl -Gs), progoi trin (2-OH-3-butenyl-Gs), indolyl and p-OH-benzyl glucosinolates, as shown in Chart(1), (Tapper and Gibbon, 1967). Appelqvist (1972) found that there were quantitative and qualitative differences between glucosinolates in different varieties of rape e.g. the meal derived from Brassica napus seeds contained large amounts of the glucosinolates, progoitrvn, which on treatment with myrosinase enzyme gave a number of the different products, the ratio between the products dependent upon the condition used. The presence of such compounds limits potentiality of rapeseed meal and hence such efforts have been directed towards elimination or reduction of these compounds from rapeseed meal for employing it as animal foodstuff, (Rauchberger et al. 1979).

European communities have encouraged the move towards rapeseed cultivars to produce new rapeseed varieties with low glucosionlates content i.e. less than 35 umole/g of seed, Heaney et al. (1988). Most determination indicate only the total glucosinolate content, without information about the nature or relative proportions of the individual components, Heaney et al.(1984) . Very few methods provide

qualitative and quantitative data about the nature of glucosinolate contents. Daun McGregor (1983) and Spinks et al.(1984).

Thies (1977) introduced ion-exchange chromatographic method to remove impurities followed by glucosinolate determination. A further improvement was also introduced by Thies (1980) using sulphatase enzyme to remove the sulphate group from the glucosinolate compounds to facilitate subsequent derivatization.

The present work is mainly concerned with the separation, identification and determination of the individual components of glucosinolates in rapeseed meal (Lesira 145) with some new modification and extension of other published methods. At the same time, different extraction methods of rapeseed oil were compared regarding the glucosinolates content in the produced meal.



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Chart ( 1 ) : Basic structure of different glucosinolate ( GS ) compounds in rapeseed as proposed by Tapper and Gibbon ( 1967 ) .

### MATERIALS AND METHODS

#### Material:

Rapeseeds (Brassica napus) French variety, Lesira 145 , was obtained from the Agric. Res. Center, Oil and Crops Dept., Giza. Different methods for oil extraction from the seeds were applied to extract the oil i.e. with hot hexane, solvent mixture (Chloroform: methanol : water, 2: 1:0.8) and pre- pressing method for comparative study.

#### Chemicals:

- I. DEAE-Sephadex A-25, G-25 and sulphatase type H-1 were obtained from Sigma Chemical Company, U.S.A.
- II. Benzyl glucosinolate (Glucotropaeolin), Trimethylchlorosilane (TMCS) and n-methyl-trimethylsilyl trifluoroacetamide (MSTFA) were obtained from Canola Council of Canada, Winnipeg.

#### Standard preparations:

- I. Benzyl glucosinolate (tetramethyl ammonium salt), 24.1 mg of the solid was weighed and made up to 50 ml. to prepare a 1 umole/ml. standard solution as mentioned by Daun and McGregor (1983).
- II. The preparations of mini-column ion exchange of DEAE-Sephadex A-25 was carried out according to Thies (1977).

#### Methods of analyses:

- I. Oil-extracted meals were dried overnight at 45°C and ground. Moisture content was determined according to Croft (1979).
- II. Inactivation of myrosinase, followed by extraction and purification of glucosinolates were carried out according to the procedure described by Underhill and Kirkland (1971).
- III. Removal of sulphate group of the glucosinolates with sulphatase enzyme "desulphoglucosinolates" according to the method of Thies (1980).
- IV. Derivatization of the desulphoglucosinolates was carried out as described by Daun and McGrgor (1983) using 100 ul of pyridine mixed with 100 ul TMCS.

#### V. Separation of the derivatized desulphoglucosinolates by GLC

The desulphoglucosinolate (TMS derivative) containing eluate (2ul) was injected on to the OV-7 column of GLC Apparatus using Hewlet Packard HP-5890-A with flame ionization detector (FID) supplied with integrator and computer control under the following conditions :

Column temp. 280°C, detector and injection at 280°C, Flow rates: helium 30 ml/min, hydrogen 50 ml/min. and air 500 ml/min.

Quantitation of TMS-derivatives was carried out by the relative response of benzyl-TMS derivative as internal standard according to the procedure described by Daun and McGregor (1983).

#### VI. Separation of the derivatized desulphoglucosinolates by HPLC

The desulphoglucosinolates (TMS derivative) containing 5 ul eluate was injected on to the column of HPLC using a Perkin Elmer Sigma 3B system. Oven temp. 30°C and flow rate 1.5 ml/ min. The eluted desulphoglucosinolates were monitored with a Perkin Elmer Lc-75 spectrophotometric detector as described by the method of Minchinton et al (1982).

### RESULTS AND DISCUSSIONS

Three meal batches were extracted with pre-pressing, hot hexane and solvent mixture (chloroform: methanol: water) from rapeseeds (*Brassica napus*) French variety; Lesira 145. Total glucosinolates were determined in the extracted meals.

In general, meal extracted from rapeseed by using hot hexane contained relatively the highest amount of glucosinolates i.e. 74.9 umole/g while the meal after oil extraction with pre-pressing contained 25.80 umole/g. total glucosinolates. Moreover, very slight residual concentration of total glucosinolates content remained in the meal after oil extraction from crushed seeds through solvent mixture. Such value reached 5.6 u mole/g.

The variations in the extraction efficiency of different solvents for glucosinolates isolation from crushed rapeseeds could be explained mainly on the basis of polarities of both

solvents and these compounds. It is understandable that more polar solvent mixture (chloroform: methanol: water) will be more efficient in extraction of such polar compounds. However, water has been shown to be the most effective polar solvent in leaching glucosinolates from rapeseeds, Rauchberger et al. (1979).

Consequently, glucosinolates residues in the obtained meal after extraction with the non-polar solvent, hexane, will be relatively high. It seems that, using solvent mixture is very reasonable in rape oil extraction since that total glucosinolates content of the meal were reduced to low levels.

The glucosinolates constituents of the meals under investigation were analyzed using GLC procedures, table(1) and the elution peaks are shown in fig. (1).

The results indicated that desulphoglucosinolates of the extracted meals by using pre-pressing and hot hexane methods possessed 3-butenyl-, 4-pentenyl-, 2-OH-3-butenyl-, 2-OH-4-pentenyl-, allyl- p-OH-benzyl- and 4-OH-indolyl methyl glucosinolates. Also, it was noticed that 2-OH-3-butenyl- (Progoitrin) and 3-butenyl- (Gluconapin) were the major fractions. However, allyl - (Singrin) and p-OH- benzyl- (Sinalbin) were the minor fractions, table (1).

On the other hand, the extracted meal with solvent mixture, contained the abovementioned compounds but the 2-OH-4-pentenyl was the predominant one. Moreover, p-OH-benzyl and 4-OH-3-indolyl glucosinolates were not detected in the meal.

It is observed that GLC technique was useful to determine the aliphatic glucosinolate fractions. Such results are in agreement with that reported by Heaney and Fenwick (1980).

The derivatized desulphoglucosinolates (TMS) of the extracted meals were analyzed by using HPLC and the obtained results are shown in table (2) and figures (2,3 and 4). Such results indicate that the identified compounds 2-OH-3-butenyl-, 4-methoxy indol- and 4-OH-3-indolyl- glucosinolates are the major compounds in glucosinolates of rape-

Table (1): Glucosinolate constituents of rapeseed meals extracted by different methods (measured by GLC).

Glucosinolate fractions	RT	Trivial name	Amount ( $\mu$ mole/g) in the whole seed,		
			Pre-pressing	Hot hexane	Solvent * mixture
Allyl -	8.0	Singrin	0.3	0.7	0.2
3-butenyl -	8.5	Gluconapin	8.3	24.4	1.4
4-Pentenyl -	9.2	Glucobrassicinapin	2.2	6.3	0.4
2-OH-3-butenyl -	9.7	Progoitrin	13.1	39.9	1.6
2-OH-4-Pentenyl -	10.3	--	2.1	4.3	2.2
P.OH-benzyl -	13.5	Sinalbin	0.1	0.7	0.0
4-OH-3-indolyl methyl	16.5	Glucobrassicin	1.7	4.6	0.0

\* Solvent mixture = Chloroform : methanol : water (2 : 1 : 0.08).

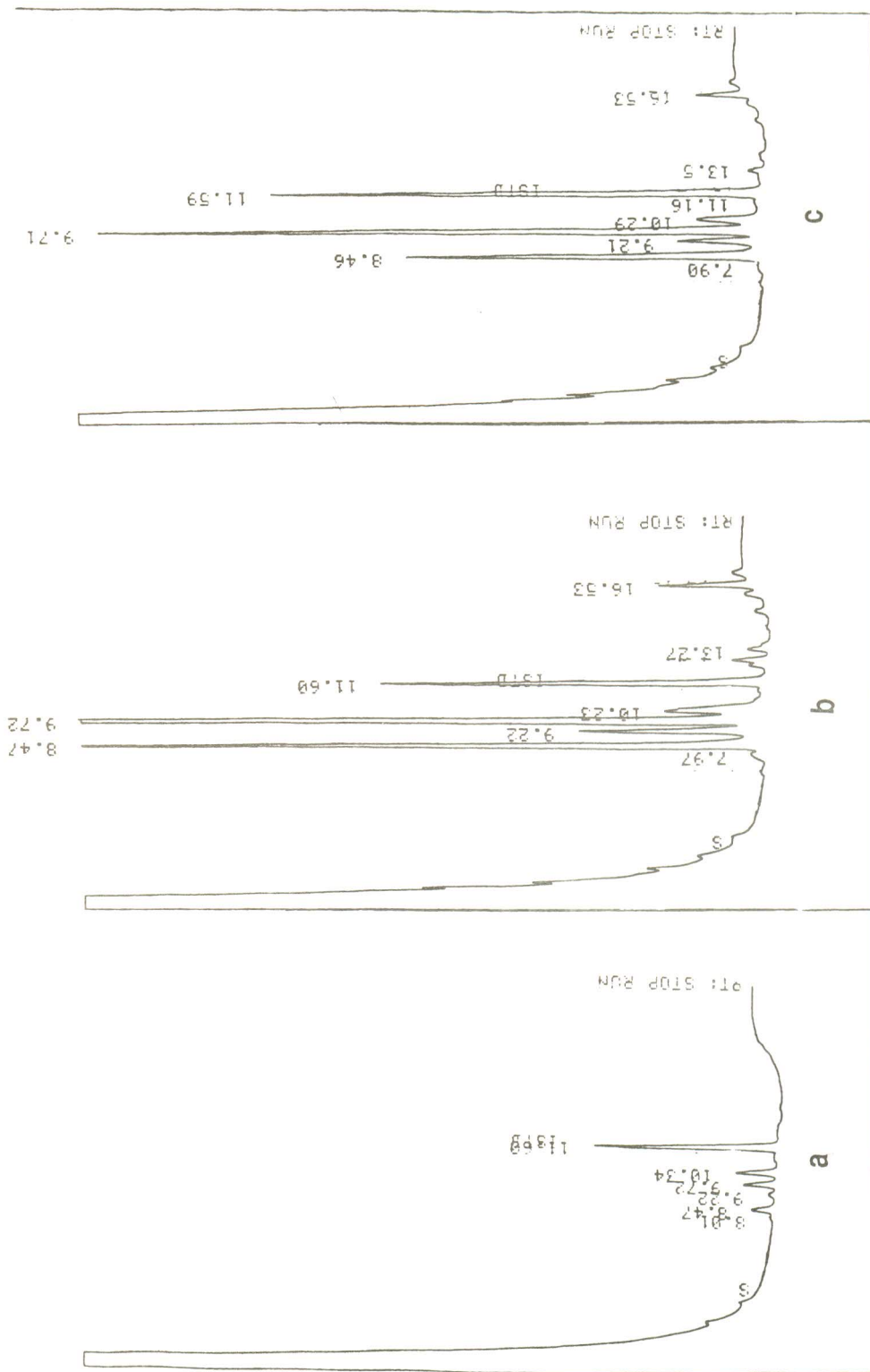


Fig. (1): GLC chromatograms of glucosinolates in rapeseed meals: a) meal extracted with solvent mixture.

b) meal extracted with hot-hexane and c) meal extracted with pre-pressing method.

\* RT = 11.68 min., ISTD, Benzyl GS as internal standard.



**Table (2):** Glucosinolate constituents of rapeseed meals extracted by different methods (measured by HPLC).

Glucosinolate fractions	Retention time (RT), (minutes)		Travel name	Area %	
	Pre-pressing	Hot hexane		Pre-pressing	Hot hexane
Unknown	---	---	-----	---	13.37
2-OH-3-butenyl	9.88	3.65	Progolitrin	27.81	---
Unknown	10.58	---	---	0.58	---
P-OH-benzyl	12.93	---	Sinalbin	2.13	12.33
Allyl	10.98	---	Singrin	1.99	---
3-Butenyl	---	---	Glucanapin	---	36.26
4-OH-3-indolyl	14.62	14.4	---	16.22	14.64
Unknown	---	15.68	---	---	---
Benzyl	15.92	19.30	Glucotropaeolin	8.09	22.62
4-Pentenyl	---	18.80	Glucobrassicinapin	---	7.71
2-Phenylethyl	19.25	---	---	3.42	---
4-Methoxy-indole	19.75	---	---	22.61	---
Unknown	21.47	23.85	---	0.62	2.46
Unknown	---	---	---	1.19	---
1-Methoxyindole	22.13	---	---	0.30	---
Unknown	24.70	---	---	0.81	---
Unknown	29.07	---	---	14.24	---
Unknown	---	28.83	---	---	3.97
3-Indolyl methyl	---	20.88	Glucobrassicin	---	---
Unknown	---	28.90	---	---	1.59
				100.00	99.99
				---	7.57
				---	99.99

\* Solvent mixture = Chloroform : methanol : water (2 : 1 : 0.8)

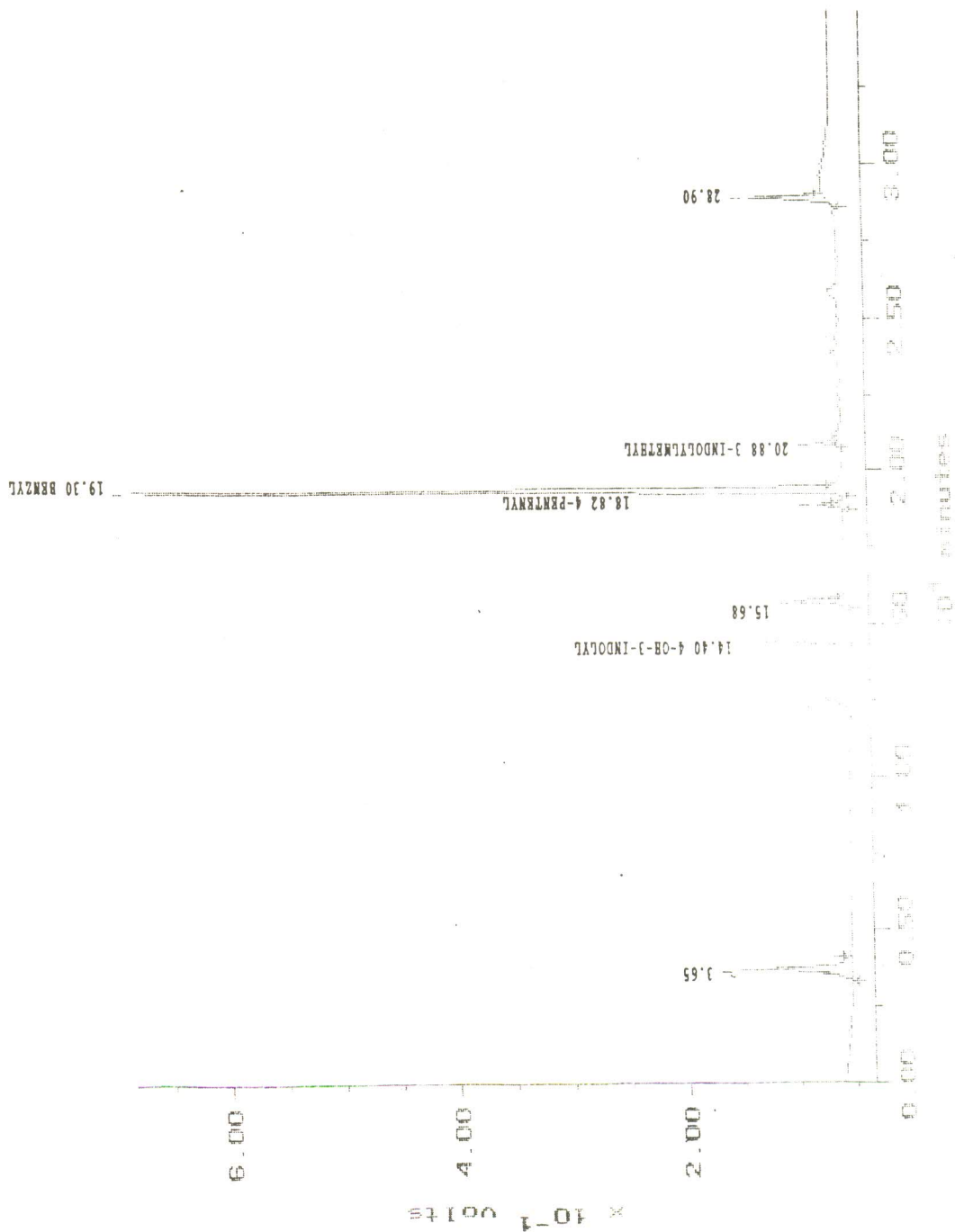


Fig. (2): HPLC chromatogram of glucosinolates in rapeseed extracted with solvent mixture.

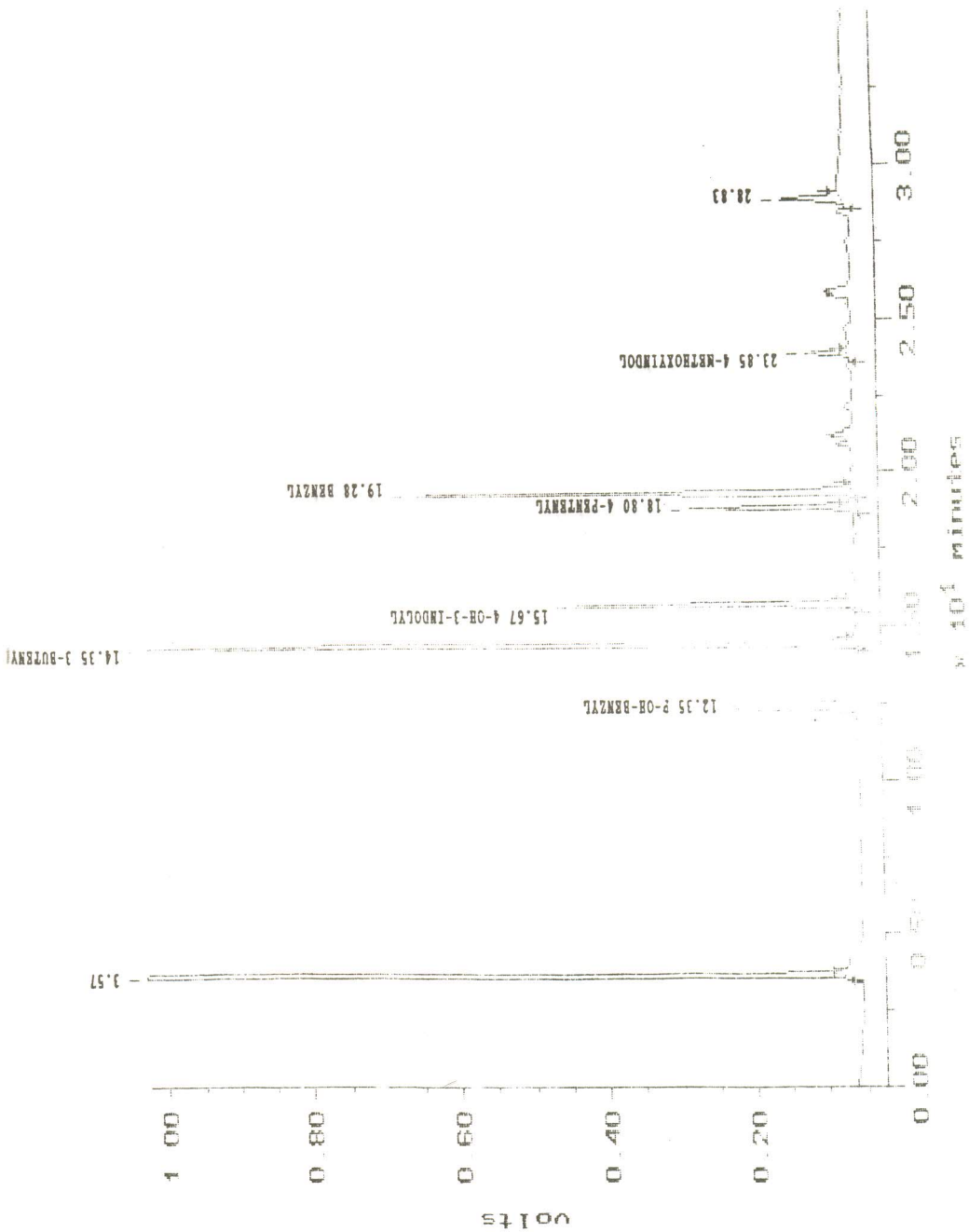


Fig. (3): HPLC chromatogram of glucosinolates in rapeseed extracted with hot-hexane.

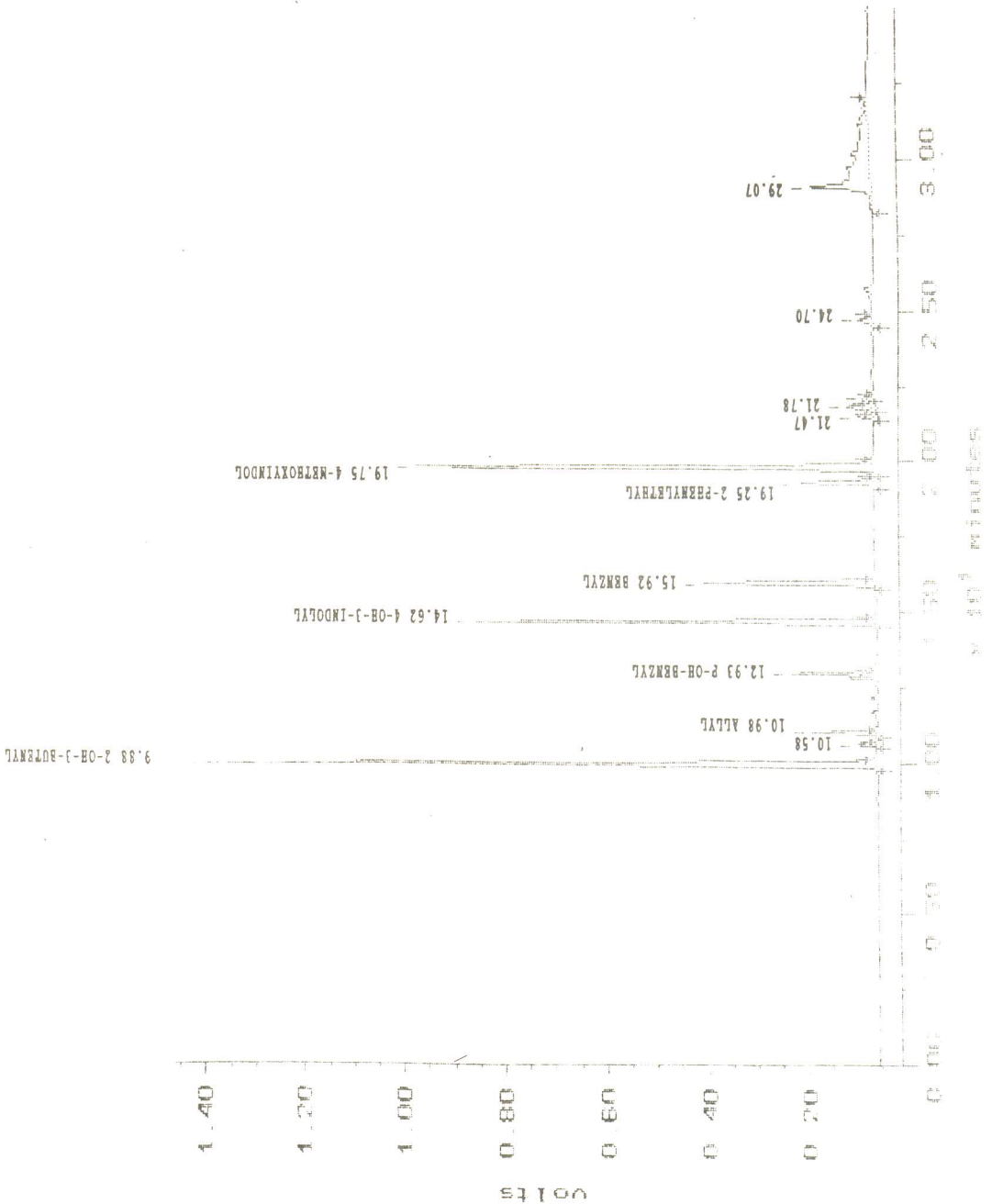


Fig. (4): HPLC chromatogram of glucosinolates in rapeseed extracted with pre-pressing method.

seed meal extracted by pre-pressing method. On the other hand 3-butenyl-, benzyl-, 4-OH-3-indolyl- and p-OH-benzyl glucosinolates were found as predominant ↗

Compounds in glucosinolates of rapeseed meal extracted by hot hexane. However, the benzyl- glucosinolate was the major compound in glucosinolates of rapeseed extracted with mixture of solvents. Furthermore, the latter minor compounds were identified as allyl-, 2-phenylethyl-, 1-methoxyindol-, 4-pentenyl-, and 3-indolyl methyl glucosinolates. The obtained results are in accordance with the data acquired by Minchinton et al. (1982).

As illustrated in table (2) the total identified aromatic glucosinolates ranged from 52.07 % to 72.84%. On the other hand aliphatic glucosinolates varied from 1.73% to 43.97% , respectively, by using different methods for extraction of rapeseed meal.

It is unquestionable from the obtained results that benzyl-, 4-OH-3-indolyl-, 3- indolyl methyl-, 4-methoxy indole- and 1-methoxy indole- were readily eluted, identified and determined by HPLC technique.

Such estimation of these constituents and improvement in the method might be as an advantage comparing with that of GLC technique. Such results are in agreement with that reported by Truscott et al. (1983).

Recently, new breeds of rapeseed varieties were raised by plant breeders containing relatively low levels of aliphatic glucosinolates, but their level of indolyl glucosinolates remained almost constant . Consequently, the measurement of the latter compounds in the new breeds of rapeseeds became more important. Such indolyl compounds were successfully identified and determined during this experiment by HPLC technique.

Finally, it could be concluded that the application of GLC and HPLC techniques were found to be useful for identification and estimation of aliphatic and aromatic glucosinolates. Therefore, a valuable information were obtained about the qualitative and quantitative constituents of glucosinolate of rapeseed meal by the abovementioned techniques.

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تقدير وتشخيص مكونات الجلوكوسينولات فى كسب بذور اللفت  
( الشلجم - ليزرا ١٤٥ )

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يهدف هذا البحث الى دراسة تأثير طرق استخلاص الزيت من بذور اللفت (الشلجم) الصنف الفرنساوى ( ليزرا ١٤٥ ) على مادة الكلوكوسينولات فى الكسب الناتج وكذلك تقدير المكونات المختلفه لهذه الماده باستخدام طرق التحليل الكروماتوجرافى المختلفه .

تم استخلاص الزيت من بذور الشلجم باستخدام الضغط الهيدرولىكى ، والهكسان الساخن ومخلوط المذيبات ( كلوروفورم - ميثانول - ماء ) ، وأوضحت النتائج أن الكسب الناتج بعد استخلاص الزيت بالهكسان الساخن احتوى على أعلى نسبة من الجلوكوسينولات (٧٤ر٩ ميكرومول / جم) ، بينما الكسب الناتج بعد استخلاص الزيت بالعرض الهيدرولىكى احتوى على ٢٥ر٨٠ ميكرومول/جم . اما الكسب الناتج بعد استخلاص الزيت باستخدام مخلوط المذيبات احتوى على أقل نسبة من هذه الماده السامه ( ٦ر٥ ميكرومول/جم) .

أجريت عملية التنقيه باستخدام المبادلات الأيونيه ثم عملت هذه المواد الجلوستيوليتيه بانزيم السلقاتيز لنزع مجاميع السلفات وحضر مشتق السليل **Silyl** ثم قدرت مكونات الجلوكوسينولات باستخدام جهازى الكروماتوجرافى الغازى ( **IGLC** ) والسائل **HPLC** .

أوضحت نتائج التحليل الكروماتوجرافى الغازى احتواء مكونات الجلوكوسينولات فى عينات الكسب المختلفه على المركبات : ٢- هيدروكسى - ٣ - بيوتينيل - (بروجوتيرين) ٣ - بيوتينيل - (جلوكونابين) ، ٤ - بنتينيل ( جلوكوبراسكانابين) ٢ - هيدروكسى بنتينيل ، والسيل - (ستجزيين) . بالاضافه الى تلك المركبات احتوى الكسب الناتج من الاستخلاص بالعرض والهكسان الساخن على مركبى : ٤ هيدروكسى - ٣ - اندولييل - (جلوكوبراسكين) ومركب باراهيدروكسى بنتيل ( سينالابين) .

أظهرت النتائج أنه باستخدام جهاز التحليل الكروماتوجرافى السائل **HPLC** تم التعرف على كلا من المكونات الجلوكوستيوليه الأليفاتيه والعطريه فى عينات الكسب المختلفه حيث تم التعرف على مركبات الأندوليل : هيدروكسى - ٣ - اندولييل - ، ٤ - ميثوكسى أندول ، ٢ - أندوليل - ميثيل - ( جلوكوبراسكين) ، ١ - ميثوكسى أندول ، - ، - ، واطافه الى ذلك تم التعرف على المركبات الجلوكوستيوليينه : ٢ فينيل ائينيل - ، وباراهيدروكسى بنزيل ، بنزيل ( جلوكوتروبايولين) .