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COMPARATIVE STUDY ON GLUCOSINOLATES, FATTY ACIDS AND STEROL COMPOSITION IN HIGH, MODERATE AND LOW ERUCIC ACID SEED VARIETIES.

BY

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ABSTRACT

Glucosinolate, fatty acid and sterol compositions of high, moderate and low erucic acid seed varieties (Midas, Kid and Cloza) were isolated, separated and determined. The identification of the formentioned components was performed by thin layer (TLC), gas liquid (GLC)) and high performance liquid chromatography (HPLC). The results of GLC technique indicate that, high erucic rape seed variety (Midas) has much greater amount of glucosinolates (80.6 μmol/g dry weight seed) and characterized by high percentage of 3-butenyl glucosinolate (77.8% of total glucosinolates) as compared to low erucic rape seed variety (Cloza) which contained low level of total glucosinolates (57.0 μmol/g. dry weight seed) and characterized by high percentage of 2-OH-3-butenylglucosinolate (36.4% of total glucosinolates). Four compounds of indolyle glucosinolates i.e. 4-OH 3-indolyl-; 3-indolyl methyl-.; 4-methoxyindol- and 1-methoxyindol were readily eluted and determined as minor constituents by HPLC after desulphation in all the studied varieties of rape seeds.

The fatty acid analysis indicated that the high erucic acid variety (Midas) had 18.3%, 14.5% and 34.8% erucic acid ($C_{22:1}$), eicosenoic ($C_{20:1}$) and oleic ($C_{18:1}$), respectively compared to 2.90%, 4.8% and 56.30% for the low erucic rape seeds variety (Cloza).

β-Sitosterol was the major sterol component of all rape seed varieties oils which ranged from 55.95% (Midas) to 62.98% (Kid). Campesterol and brassicasterol represented 20.25% (kid) to 33.33% (Cloza) and 11.25% (kid) to 13.74% (Cloza), respectively, while stigmasterol was present in small amounts ranged from 5.12% (kid) to 8.91% (Midas) of total sterols of oils.

INTRODUCTION

The seeds of rape plant are economically important as they are used both as food and plant oil sources. High contents of erucic acid in the oil and of glucosinolates in the meal are the reasons for some unfavorable qualities. Heaney and Fenwick (1980) confirmed that 3-butenyl-, 4-pentenyl-, 2-OH- 3-butenyl and 2 OH 4-pentenyl- were the major glucosinolates in the rape seeds. Beside, it

contained substantial amounts of indolyl glucosinolate and trace amounts of several other glucosinolates.

The problems concerning glucosinolates in food are related to their total amount, the type of glucosinolates present, and to the products produced from them by autolysis or other degradation. Carlson *et al.*, (1981) reported that, a number of glucosinolates may be regarded as potential toxicants occurred in small amounts in Cruciferous vegetables. A product from one of these, 2-OH 3- butenyl glucosinolate is thyrotoxics, other may be thyrotoxic or toxic to liver or kidney. Also, Pearson *et al.*, (1981) noticed that the substances derived from glucosinolates depressed the action of trimethyl amino oxidase enzyme present mainly in liver and kidney. Accordingly, European communities encouraged the move towards rape seed cultivars of low glucosinolate contents with less than 35 μ mol g^{-1} of seeds (Heaney *et al.*, 1988).

Fatty acid compositions of rape seed oil were investigated by several workers, Kramer *et al.*, (1983), Farag *et al.*, (1986) and Abd El Rahman and Mahmoud (1996).There are a general agreement that diets containing rape seed oil high in erucic acid content cause lipidosis and necrotic lesions of the myocardium in laboratory animal species, Jorgensen (1972) and Rocquclin *et al.*, (1973). However, Kramer *et al.*, (1983), mentioned that erucic acid ($C_{22:1}$) was poorly metabolized, and consequently triglycerides containing this acid could be accumulated in heart muscle, adrenal gland and ovarian tissues. So, the level of erucic acid in rape seed oil which is used for human consumption must not exceed 2% as recommended by Food and Drug Administration (1985).

Sterol composition was used as an aid for the identification of vegetable oils and to detect adulteration. The sterols fraction of rape seed oil have already been analyzed by several authors using GLC, Ackman (1983), Shabana *et al.*, (1990) and Mahmoud (1995). They reported that, brassicasterol ranged from 7.4% to 16.31%, campesterol ranged from 11.06% to 35.5%, stigmasterol ranged from 27.88% to 30.74% and ß-sitosterol ranged from 52.70% to 61.11% in sterol fraction of rape seed oil.

This paper was mainly concerned with a comparative study on the individual components of glucosinolates, fatty acids and sterols in three rape seed varieties (high, moderate and low erucic acid varieties).

MATERIALS AND METHODS

1-Materials:

Three different varieties of rape seeds (Midas, Kid and Cloza) were used during this investigation. They were introduced and cultivated by the oil crops Research section, Field crops Res. Inst. Agricultural Research Center, Giza, Egypt.

Pure standard fatty acids and sterols were obtained from Sigma chemical company (USA).

Glucosinolates standard, Trimethyl chlorosilane (TMCS) and n-methyl-n-trimethylsilyl trifluoracetamide (MSTFA) were obtained from Canola Counicl of Canada.

2. Methods:

2.1. Seed analysis:

Moisture content, total proteins, oil percentage, total carbohydrates and ash were determined according to the AOAC methods (1990).

2.2. Quantitative analysis of glucosinolates:

- Oil extracted meals were dried overnight at 45°C ground followed by Inactivation of myrosinase, extraction and purification of glucosinolates were carried out according to Underhill and Kirkland (1971).
- Preparation of desulphoglucosinolates was carried out according to the method of Thies (1980).
- Separation and determination of desulphoglucosinolates by GLC using Hewlett Packard HP-5890-A equppied with flame ionization detector (FID) integrator and computer under the following conditions: Coulmn, detector and injection temperatures were 280°C, 300°C and 290°C, respectively. Helium flow rate was 30 ml/min. The quantitation of TMS-derivatives was carricd out by the relative response of benzyl-TMS derivative as internal standard according to the procedure described by Daun and McGregor (1983).
- Separation of the derivatized desulphoglucosinolates by HPLC: Using Perkin Elmer Sigma 3B system 5 μl of the desulphoglucosinolate containing eluate was injected on to 250×4.6 mm Spherisorb ODS_2 column. Oven temp. 30°C and flow rate 1.5 ml/min, the solvent system employed were (a) distilled water and (b) 20% acetonitrile in water . The eluted desulphoglucosinolates were monitored with a Perkin Elmer LC-75 spectrophotometric detector at 230 nm as described by the method of Minchinton *et al.*, (1982).

2.3. Analysis of oils:

Specific gravity, refractive index. acid value, iodine value, peroxide value and unsaponifiable matter% were determined according to A.O.A.C (1990).

- Fatty acid methyl esters were analyzed using Hewlett Packard HP-5890, equipped with flame ionization detector under the following conditions: Column HP 20 M (Carbowax 20 M), carrier gas. Nitrogen 30 ml/min; injector temperature 250°C, detector temp. 300°C, column temp. programme: Initial temp. 210 °C for 0.5 min., rate 4 °C/min., final temp. 240 °C. Peak area and the percentage of each separated esters were calculated by an integrator, Helwett pakard model 3390 A.

- **Analysis of sterols:**

The unsaponifiable matter was fractionated by TLC according to the method described by Price and Parsons (1975). TLC fraction of sterols were analyzed using the forementioned GLC apparatus under the following conditions: Column HP-5 (Crosslinked 5% Ph. Me. silicone), injector and detector temperatures were 265 °C and 300 °C, respectively. Column temp. programme: initial temp. 180 °C, rate 3 °C/min and final temperature. 260 °C.

RESULTS AND DISCUSSION

Three rape seed varieties (Midas, Kid and Cloza) were subjected to chemical analysis. Data in Table (1) show that, the three varieties are considered as rich sources of oil and protein. Kid variety had higher crude oil percentage than the other ones (Cloza and Midas). Midas variety contained the highest in percent of crude protein (24.64%). It could be noticed that the differences between moisture, ash and carbohydrate contents among all varieties are very small. These results were within the range that reported by Marianchuk *et al.*, (1987) and DeClereq *et al.*, (1992).

Table (2) refers to the major physical and chemical properties of oils which considered as a good criterion for keeping quality and nutritive value. The refractive index serves as an indication of the degree of unsaturation. The presence of a high concentration of unsaturated fatty acids and great amount of long chain fatty acids in all varieties of rape seed which elucidate the high values of their refractive index, as reported by Parodi and Dunstan (1971). The acid values obtained were very low and this is expected since the samples under investigation were fresh and well dried. Data in Table (2) show that the iodine value of Cloza oil variety was higher "112.60" than that of kid "110.8" and Midas "106.7". Peroxide value found in all samples were relatively very small, such results clearly indicate that the effect of autoxidation was very little on the different isolated oils. The unsaponifiable matter percentage ranged from 1.27% (Cloza variety) to 1.35% (Kid variety). The obtained data were within the ranges obtained by Kramer *et al.*, (1983) and Farag *et al.*, (1986).

Table (1): Chemical composition of the investigated rape seed varieties:

Rape seed variety	The component %				
	Moisture	Protein	Oil	Total carbohydrates	Ash
Midas	7.69	24.64	39.95	22.74	4.98
Kid	7.69	20.41	43.53	23.80	4.57
Cloza	7.99	21.58	40.70	24.64	5.00

Table (2): Physicochemical properties of the investigated rape seed oils.

Rape seed variety	Refractive index at 25°C	Specific gravity at 25°C	Acid value	Iodine value	Saponification value	Peroxide value meq/kg	Unsaponifiable matter %
Midas	1.4635	0.9165	0.30	106.7	181.11	0.30	1.30
Kid	1.4631	0.9177	0.29	110.8	187.63	0.43	1.35
Cloza	1.4626	0.9167	0.37	112.60	188.18	0.42	1.27

Total glucosinolates were determined in the extracted meals using GLC technique, (Table 3). The lowest amount of the total glucosinolate was found in Cloza (low erucic variety), since it contained the value 57.0 μ mol g^{-1}. However, appreciable amount (68.3 glucosinolates) was presented in kid variety. While Midas (high erucic variety) contained the highest amount of total glucosinolates

Midas (high erucic variety) contained the highest amount of total glucosinolates (80.6 μ mol g^{-1}). Olsen and S'rensen (1980), demonstrated that the glucosinolate pattern varied considerably within varieties belonging to the same species in different parts of the same plant and during the development of the plant.

It seems that total glucosinolate contents (aliphatic plus indolyl) provide no information about the nature or relative properties of the individual components. Therefore, trimethylsilylation of glucosinolates of the different seed varieties under study were isolated by ion exchange chromatography, and determined quantitatively by GLC.

It was noticed that 3-butenyl glucosinolate was the major constituent (77.8 μ mol g^{-1}) in Midas (high erucic variety), also 4-pentenyl-, 2-OH-3 butenyl; 2-OH-4-pentenyl glucosinolates were detected in trace amounts, Table (3). On the other hand, 2-OH 3-butenyl glucosinolate was the major constituent followed by 3-butenyl glucosinolate in cloza (low erucic variety). Our finding are similar with those reported by Daxenbichler *et al.*, (1979) and Fenwick *et al.*, (1983).

Table (3): Total and individual glucosinolate (GS) compounds of different rape seed varieties by GLC analysis:

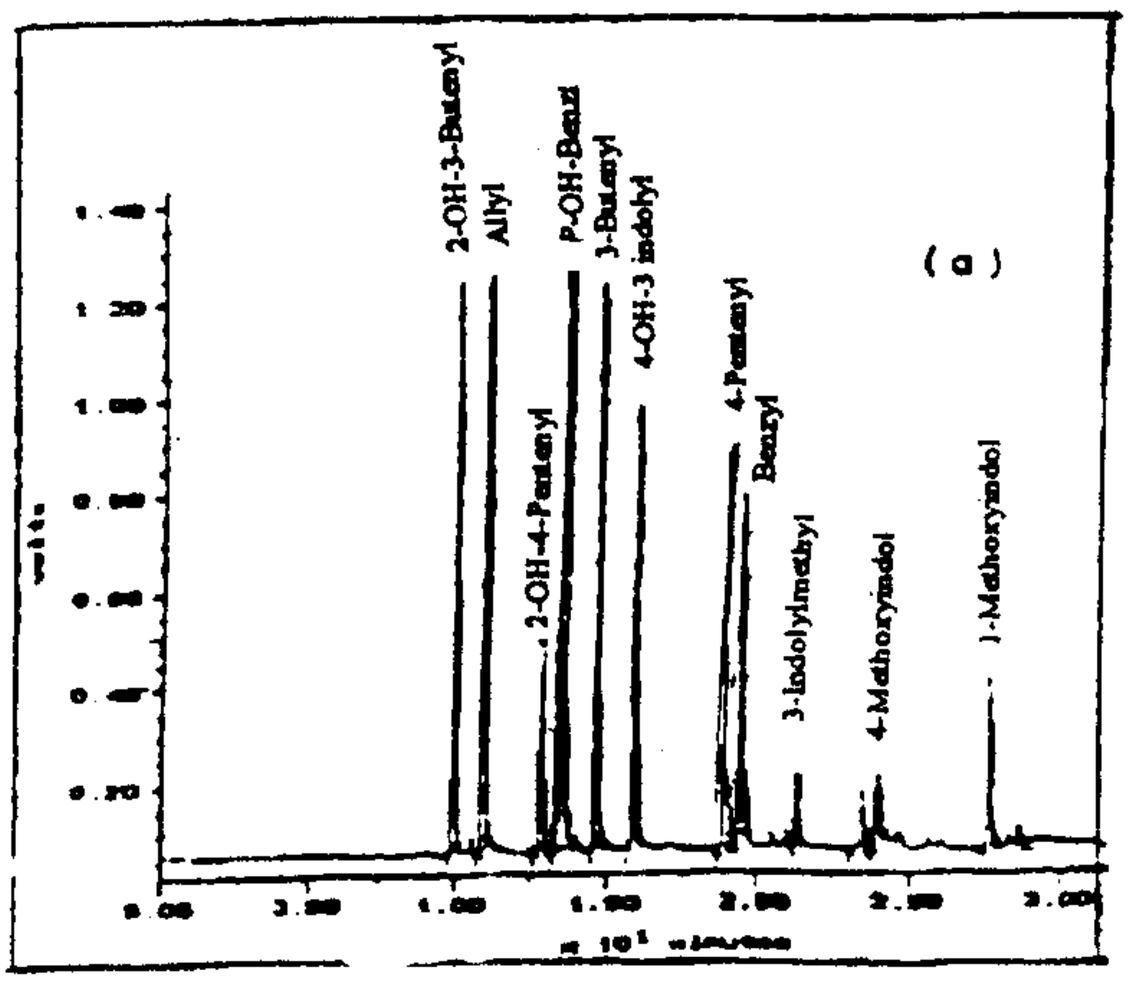
Rape seed variety	Total GS μ mol g^{-1}	Glucosinolates as μ moles/g seed *				
		3-butenyl GS	4-butenyl GS	2-OH3-butenyl GS	2-OH4-pentenyl GS	4-OH 3-Indolyl GS
Midas	80.6	77.8	1.0	0.4	0.6	0.8
Kid	68.3	17.0	3.6	40.9	3.8	3.0
Cloza	57.0	13.5	2.5	35.4	3.9	1.7

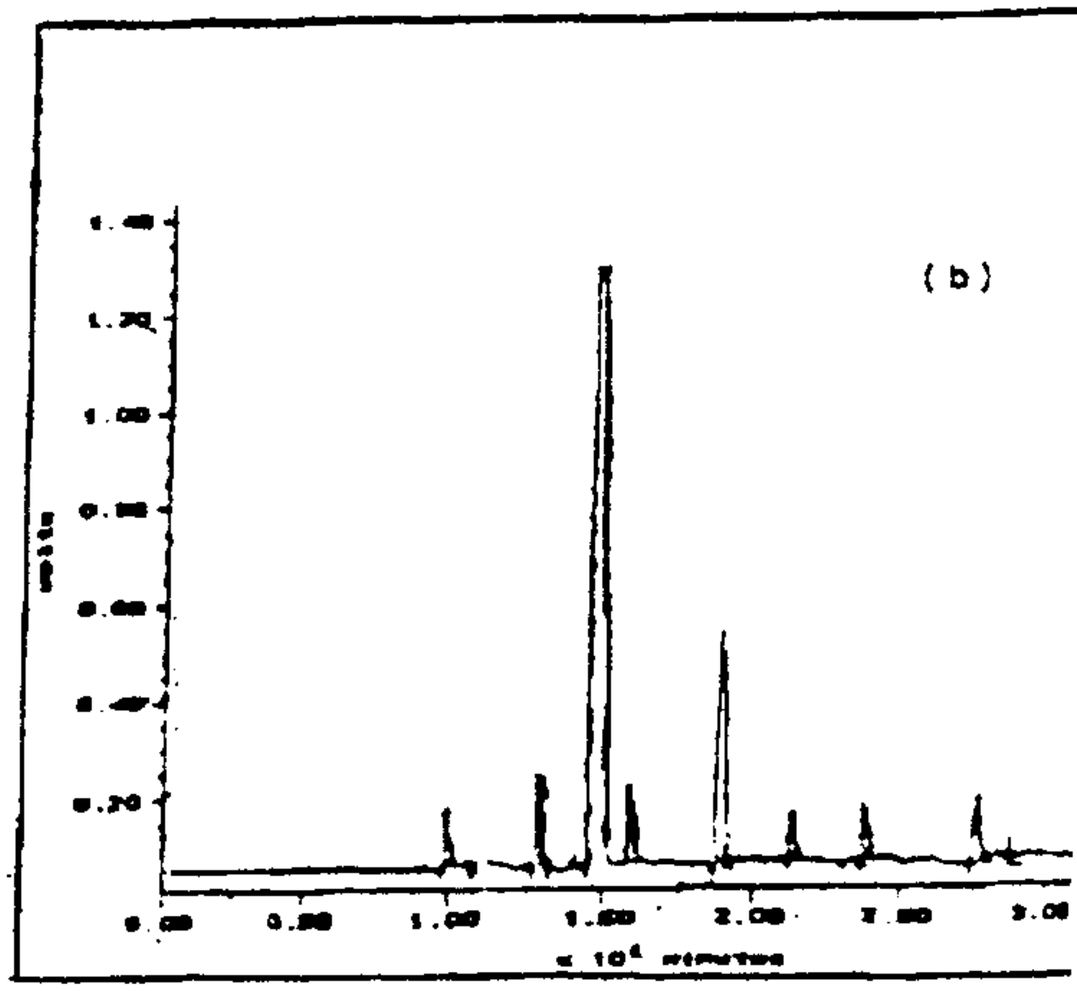
* The values of GS were based on dry basis.

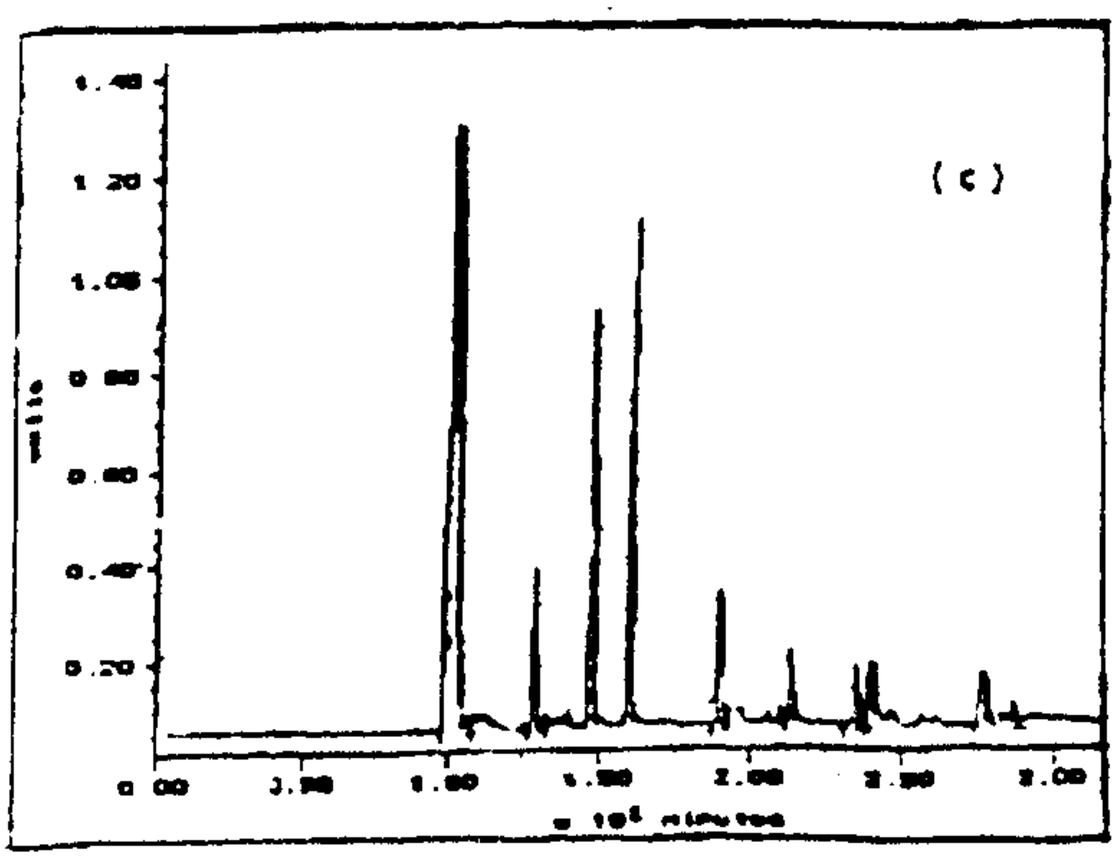
The derivatized desulphoglucosinolates of the extracted meals were analyzed using HPLC and the obtained data are shown in Fig (1). The present data revealed the existence of three major aliphatic glucosinolate i.e. 2-OH 4-pentenyl-, 3-butenyl and 4-pentenyl glucosinolates. Furthermore, other minor components, e.g., indolyl also contributes to the total glucosinolate values. The latter components (Fig 1) are 4-OH- 3indolyl-, 3-indolylemethyl-, 4-metoxyindole and 1-methoxy indole. Such results are in accordance with those obtained by Truscott *et al.*, (1983).

The fatty acid composition of rape seed oil varieties were analyzed by GLC as shown in Table (4) and Fig (2). The obtained results showed that the major constituents of unsaturated fatty acids in oils extracted from all rape seed varieties were C18:1 and C18:2. It is clear that oleic acid was the most prevalent unsaturated fatty acid since it ranged from 34.8% (Midas) to 55.80% (Cloza). Linoleic acid was the second major unsaturated acid, where its content ranged from 17.1% (kid) to 18.8% (Cloza).

Concerning the gadoleic acid (C20:1) and erucic acid (C22:1) content, the obtained data showed that Midas variety oil contains the highest amounts of







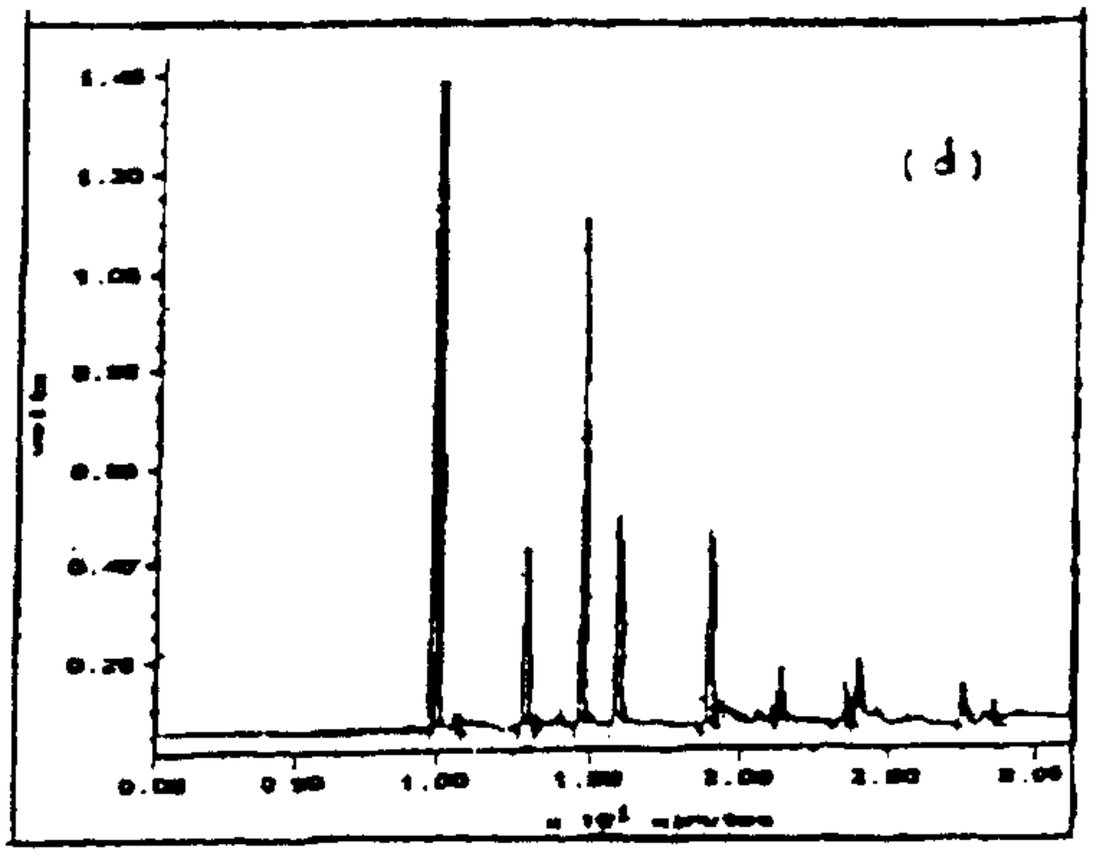


Fig (1): HPLC chromatograms of glucosinolate fractions in rapeseed meals.

a) authentic glucosinolates b) Midas c) Kid d) closa

Table (4): Fatty acid composition of different rape seed oil varieties.

Rape seed Variety	Fatty acids %									TU*/TS*
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	
Midas	4.3	1.60	34.8	17.6	8.5	0.6	14.5	0.5	18.3	13.4:1
Kid	4.5	1.80	47.3	17.1	8.8	0.6	9.1	0.4	10.4	12.7:1
Cloza	4.3	1.69	55.80	18.80	10.70	0.6	4.8	0.3	2.9	13.3:1

TU= Total unsaturated fatty acids.

TS = Total saturated fatty acids.

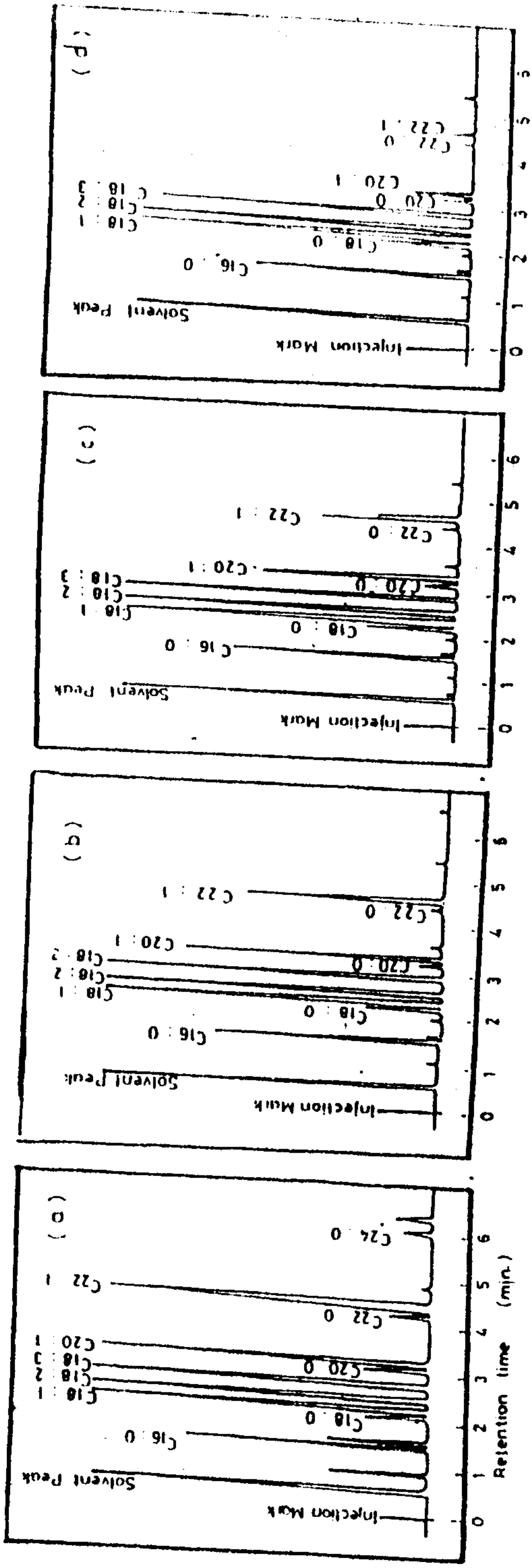


Fig (2):Fatty acid composition of rapeseed oils. a) authentic fatty acids b) Midas c) Kid d) closa

gadoleic and erucic acids (14.5% and 18.3%, respectively). While, Cloza variety oil contained little amounts of gadoleic and erucic acids (4.8% and 2.9%, respectively). A clear relationship between oleic, gadoleic and erucic acids may be originated in the seed oils of all varieties. In other words, a high oleic acid content in cloza variety 55.8% was accompanied by low level of gadoleic and erucic acids (4.8% and 2.9%, respectively). While Midas variety of high erucic acid variety contained 18.3% erucic acid and accompanied with a high level of gadoleic acid ($C_{20:1}$) reached 14.5% and low level of oleic acid (34.8%). Obviously, the elongation of oleic acid to erucic acid may be the main pathway of biosynthesis of the latter acid. Our conclusion agreed with that reported by Jönsson (1977) who suggested that the addition of two carbon atoms to the carboxyl group of oleic acid to from gadoleic acid, followed by second addition of another two carbon atoms to form erucic acid.

The obtained data in Table (5) showed that sterol content of the unsaponifiable matter ranged from 18.87% (Cloza) to 34.04% (kid). Kid variety was characterized by the highest level of total sterols, being 1.19 and 1.80 times as great as that in Midas and Cloza variety, respectively.

B-sitosterol was the major sterol component of all rape seed oil varieties which ranged from 55.95% (Midas) to 62.98% (kid). These findings were in harmony with those of Farag *et al.*, (1986) and Rady *et al.*, (1990). In addition, it could be observed that the percentage of campesterol and brassicasterol ranged from 20.25% (kid) to 23.33% (Cloza) and 11.25% (kid) to 13.74% (Cloza) of total sterols of oils, respectively. These results were in line with those obtained by Appelqvist *et al.*, (1981). The same data (Table 5) also indicated that the stigmasterol represented 5.12% (kid) to 8.91% (Midas).

Table (5): Sterols composition of polar fractions of different rape seed varieties:-

Rape seed Variety	% Sterols/ unsaponifiable	% Sterol / Total sterols			
		Brassica-sterol	Campe-sterol	Stigma-sterol	B-sito-sterol
Midas	28.72	13.46	21.70	8.91	55.95
Kid	34.04	11.25	20.25	5.12	62.98
Cloza	18.87	13.74	23.33	6.27	56.66

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مقارنة الجلوكوسينولات والأحماض الدهنيه والأستيرولات فى بذور الشلجم المرتفعه والمتوسطه والمنخفضة فى محتواها من حمض الأيريوسيك

أحمد على أحمد عبد الرحمن
قسم الأراضي والكيمياء الزراعية-كلية الزراعة بمشتهر-جامعة الزقازيق (فرع بنها)

يهدف البحث إلى دراسة إمكانية إيجاد علاقة بين محتوى بذور الشلجم من حمض الأيريوسيك ومحتواها ونوعية كل من الجلوكوسينولات والأحماض الدهنيه والأستيرولات.

ولتحقيق هذه الدراسة:
تم إختيار ثلاثة أصناف من بذور الشلجم (ميداس ، كيد، كلوزا) التى تتميز بمحتواها المرتفع والمتوسط والمنخفض من حمض الأيريوسيك.
أوضحت النتائج المتحصل عليها بإستخدام التحليل الكروماتوجرافى الغازى (GLC):

أولا:

١- الكسب الناتج بعد إستخلاص الزيت من بذور صنف Midas يحتوى على كمية مرتفعه من الجلوكوسينولات الكلية (٦ر٨٠ ميكرومول/جم) مقارنة بمثيلاتها من صنفى Kid و Closaحيث كانت ٣ر٦٨ ، ٥٧ ميكرومول/جم بذور جافة على الترتيب.

٢- يتميز الكسب الناتج من بذور صنف Midas بإحتوائه على نسبة مرتفعه من المركب ٣-بيوتينيل جلوكوسينولات ٨ر٧٧ ميكرومول/جم وهو المركب السائد من الجلوكوسينولات. بينما يتميز الكسب الناتج من بذور صنف Kid وصنف Closa بإحتوائه على نسبة مرتفعه من المركب ٢-هيدروكسى ٣-بيوتينيل جلوكوسينولات ٩ر٤٠ ، ٤ر٣٥ ميكرومول/جم بذره جافه على الترتيب.

٣- أوضحت نتائج التحاليل الكروماتوجرافى السائل HPLC أن جميع الأصناف تحتوى على كميات صغيره من مركبات الإندوليل لمادة الجلوكوسينولات (٤-هيدروكسى -٣-أندوليل،٣-إندوليل ميثيل ، ٤-ميثوكسي إندول، ميثوكسى إندول).

ثانيا:

الزيت الناتج من بذور صنف Midas (المرتفع فى محتواه من حمض الأيريوسيك ($C_{22:1}$) ٣ر١٨% من نسبة الأحماض الدهنيه الكلية) يحتوى على نسبة منخفضة من حمض الأوليك ($C_{18:1}$) ٨ر٣٤% ونسبة مرتفعة من الحمض الدهنى جادوليك ($C_{20:1}$) ٥ر١٤% وذلك بالمقارنة بصنف Closa (المنخفض فى محتواه من حمض الأيريوسيك) حيث كانت نسبة حمض الأوليك مرتفعه بلغت ٨ر٥٥% بينما كانت نسبة الحمض الدهنى جادوليك منخفضة بقيمه ٨ر٤%.

ثالثا:

أوضحت نتائج الـ GLC أن محتوى الزيوت الناتجة من الثلاثة أصناف متقاربة فى محتواها من مكونات الأستيرولات ووجد أن ال β-sitosterol هو السائد كما أن نسبة ال Brassicasterol متقاربة.