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FATTY ACIDS COMPOSITION, NEUTRAL LIPIDS AND PHOSPHOLIPIDS FRACTIONATION IN EXTRACTED LIPIDS OF FOUR PLANT SEED VARIETIES

M. R. Rashwan and Salih A. Bazaid*
Food Science and Technology Dept. Faculty of Agric., Assiut Univ.,Assiut, Egypt.
*** Umm Al-Qura Univ., Makkah, Saudi Arabia.**

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ABSTRACT :

The study was carried out on the extracted lipids of four different varieties of plant seeds viz., *Sehouwia purpurea; Cassia senna; Cassia italica* and *Cassia holosericea* . The chemical characteristics and lipid classes composition of these lipids were studied.

The total lipids content of plant seeds ranged from 6.7 % to 29.87% , depending on the variety. The total lipids extracts were fractionated into neutral lipids, phospholipids and glycolipids by column chromatography. The neutral lipids varied from 95.62% to 96.28%, phospholipids from 2.04% to 2.78% and glycolipids from 1.06% to 1.6% . Thin layer chromatography (TLC) revealed that triglycerides constituted the major fraction of the neutral lipids , for all studied varieties and accounted from 90.78% to 92.70% . TLC showed the presence of seven fractions of phospholipids. The main component of phospholipids was phosphatidyl choline accounted from 36.72 % to 40.62 % of the total phospholipids .

The fatty acid composition of total lipids was carried out by gas liquid chromatography (GLC). The plant seed lipids contained high percentage of unsaturated fatty acids consisted mainly of oleic and linoleic acids. Therefore, the studied plant seeds could be used successfully as a source of unsaturated fatty acids, and offer a promising source of oil for nutritional purposes .

INTRODUCTION :

The shortage of oils and fats in several parts of the world has become more acute with the rapid increasing of population. Possible practical procedures to overcome this problem are the cultivation of new crops and/or plant breeding to increase the oil content of traditional oil crops .

The chemical composition of different seeds was affected by the variety of the seed and the environmental conditions of planting [1] .

Oil quality depends solely upon the fatty acid composition of the oil. The previous studies revealed the presence of palmitic, stearic, arachidic and behanic acid among the saturated fatty acids, and palmitoleic, hexadecadienoic, oleic, linoleic, linolenic, and erucic acid from the unsaturated fatty acids in the oil of gobhi sarson (*Brassica napus*)[2] .

Correlation studies revealed that erucic acid was negatively associated with oleic, linoleic and linolenic acids. It is suggested that the variation for fatty acids in gobhi sarson and nature of association amongst fatty acids can be exploited to develop genotypes with better oil quality [2].

Comparative physical and chemical characteristics of chinese sarson (*Brassica chinensis*) seeds oil and other mustard varieties indicated that the chinese sarson seeds are low in oil but high in protein content. Besides, the specific gravity and saponification values of Chinese sarson oil are slightly higher compared to other Brassicae seed oils . The oil contained more erucic acid and less linolenic acid than other varieties [3].

Lajolo et al.,[6] reported that the chemical composition, oil characteristics, and glucosinolate, sinapine, and phytic acid contents were studied in low glucosinolate *Brassica napus* varieties. Lipids (43- 45%) with an erucic acid content lower than 1% and proteins (18-20%) were the main components. The rape seed varieties introduced in Brazil showed adequate agronomic and chemical characteristics and still offer a promising source of oil and protein for nutritional purposes .

Therefore, the potential for utilizing some plant seeds for oils production appears to be favorable. This study was designed to shed light upon the lipids and phospholipids fractionation in the oils of four Saudian plant seeds. The fatty acid composition of the extracted lipids were studied as well .

EXPERIMENTAL :

Materials and Methods :

Materials :

Representative samples of four varieties of Saudian plant seeds namely: *Sehouwia purpurea*; *Cassia senna*; *Cassia italica* and *Cassia holosericea* were collected from Taif area, kingdom of Saudi Arabia. The seeds were crushed and dried in an oven at 40°C.

Analytical methods :

The crushed seeds (2g) were extracted with petroleum ether in a soxhlet apparatus at 40-60°C for 16 hrs. For quantitative determination of crude oil, according to AOAC method [5].

Total lipids of the ground seeds were extracted using a solvent mixture of chloroform methanol (2:1 v/v) according to the method described by Folch et al. [6].

Acidity, iodine, saponification, peroxide values and unsaponifiable matters were determined according to AOAC methods [5].

Fractionation of lipid classes:

The extracted lipids were fractionated by silicic acid column chromatography, using sequential elution with chloroform to obtain the neutral lipid. The glycolipids and phospholipids were eluted with acetone and methanol, respectively [7,8].

The eluted fractions were collected in preweighed flasks, solvents were removed using a rotary evaporator at 40°C. Each eluted fraction was determined gravimetrically as a weight percentage of the total lipids .

Fractionation of neutral lipids and phospholipids :

This was carried out on a thin layer of silica gel G plate using hexane : diethyl ether : acetic acid (80: 20:1 v/v) and chloroform: methanol: water (85: 10 : 5 v/v), respectively, according to the methods of Malins and Mangold and of Radwan [9,10] .

For quantitative analysis of neutral lipids and phospholipids, the chromatograms were scanned using Shimadzu TLC scanner (C-S-910). The area under each peak was measured by the triangulation method [11] . The percentage of each fraction was calculated with regard to the total area .

Estimation of fatty acids :

The methyl esters of fatty acid composition of total lipids of each variety were prepared as described by Rossell et al. [12] using 3% H_2SO_4 in absolute methyl alcohol .

A Perkin Elmer gas chromatograph (GC-4CM-Shimadzu), with a flame ionization detector was used in the presence of nitrogen as a carrier gas. The separation was carried out at 150-240°C (temperature rate 5°C min on a (3 M × 0.3 mm) glass column packed with silar 5 cp on chromo (80-100) mesh. Both the injector and detector temperatures were 270°C. The nitrogen, hydrogen and air flow rates were 20, 1 and 0.5 ml min, respectively. The chart speed was 5 mm min .

Peak identifications were established by comparing the retention times obtained with standard methyl esters. Quantitative results were obtained with the aid of an HP computing integrator .

RESULTS AND DISCUSSION :

Table(1) illustrates the chemical characteristics of plant seed lipids. The data showed that the chemical characteristics of the lipids varied slightly according to the variety of the seeds .

Table (1) : Chemical characteristics of plant seed lipids .

Characteristics	Seed varieties			
	Schouwia purpurea	*Cassia senna*	*Cassia italica*	*Cassia holosericea*
Iodine value	109	101	118	124
Acid number	0.9	0.4	0.6	0.4
Peroxide value	0.1	0.1	0.2	0.3
Saponification number	185	192	190	188
Unsaponifiable matter %	1.0	0.9	1.2	1.1

The iodine value of plant seed lipids were 109, 101, 118 and 124 in *Schouwia purpurea*; *Cassia senna*; *Cassia italica* and *Cassia holosericea* varieties, respectively. The extracted lipids showed relatively low acid numbers and peroxide values, indicating their high stability against deterioration. This clearly indicated that the studied plant seed lipids might have low levels of oxidative and lypolytic activities or could have high contents of natural antioxidants. As shown in the tabulated data, the saponification number and the unsaponifiable matter for all studied samples differed slightly .

Fractionation of Lipid Classes :

The relative percentages of the major lipid classes are presented in table (2).

Table (2): Total lipids and their classes from different varieties of plant seeds *

Consitatuents	*Schouwia purpurea*	*Cassia senna*	*Cassia italica*	*Cassia holosericea*
A- Total lipids content %	29.87	9.05	11.65	6.70
B- Lipid classes as % of total lipid :				
1- Neutral lipids	95.62	96.04	96.18	96.28
2- Phospholipids	2.78	2.66	2.04	2.62
3- Glycolipids	1.46	1.20	1.60	1.06
Column recovery	99.86	99.90	99.82	99.96

* On dry weight basis .

The neutral lipids represented 95.62%, 96.04 %, 96.18% and 96.28% of the total lipids of *Schouwia purpurea* ; *Cassia senna* ; *Cassia italica* and *Cassia holosericea* seed lipids, respectively.

However, phospholipids and glycolipids recorded(2.78% and 1.46%), (2.66% and 1.20%), (2.04% and 1.60%) and (2.62 %, and 1.06%) of the total lipids of the same studied varieties, respectively .

The qualitative and quantitative data of the individual lipid classes of the studied plant seed lipids are shown in table (3) and Figs (1 and 2) .

The tabulated data showed that the neutral lipids revealed the presence of seven fractions (Fig. 1).

Triglycerides constituted the major percentage of the neutral lipids, and accounted for 91.82% , 90.78% , 92.70% and 91.0% of *Schouwia purpurea*, *Cassia senna*, *Cassia italica* and *Cassia holosericea* respectively.

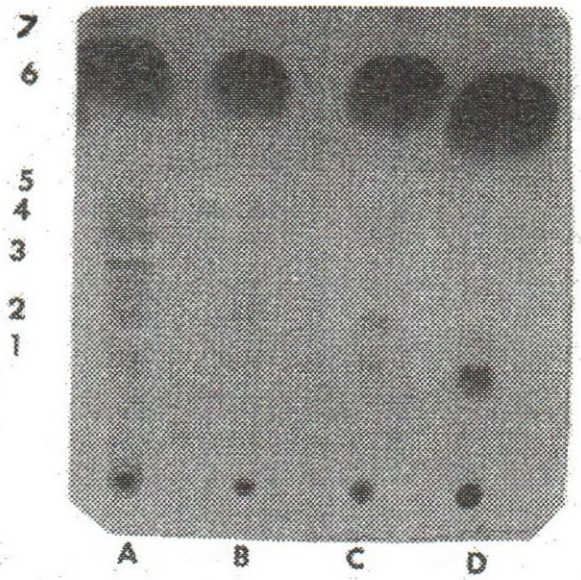

Fig. (1) : Thin layer chromatogram of neutral lipid fractions of plant seeds.

A= *Schouwia purpurea*
B=*Cassia senna*
C= *Cassia italica*
D=*Cassia holosericea*

1- Monoglycerides
2- 1,2 &2, 3 - diglycerides
3- sterols
4- 1,3 diglycerides
5- Free fatty acids
6- Triglycerides
7- Sterol esters + Hydrocarbons .

The data showed that the lowest percentage of triglycerides was found in *Cassia senna* lipids (90.78%), indicating that the lipid might had been hydrolyzed, which resulted in higher proportions of free fatty acids (3.62%).

Similar trend of results that reported in the literature concerning Brassica juncea lipids [13] .

Table (3): The densitometric analysis of the major lipid fractions of plant seed lipids .

Lipid	*Schouwia purourea*	*Cassia senna*	*Cassia italica*	*Cassia holosericea*
Neutral Lipids :				
Monoglycerides	0.62	0.78	0.50	0.44
1,2 & 2,3-diglycerides	1.24	1.02	1.68	1.82
Sterols	1.02	1.26	0.82	0.96
1,3 diglycerides	1.34	1.42	1.06	1.06
Free fatty acids	2.48	3.62	2.04	2.92
Triglycerides	91.82	90.78	92.70	91.00
Sterol esters+Hydrocarbons	1.48	1.12	1.20	1.80
Phospholipids :				
Phosphatidyl serine	5.62	6.45	5.44	5.82
Lyso-phosphatidyl choline	3.45	2.98	3.06	3.41
Phosphatidyl inositol	9.82	8.82	7.43	9.22
Phosphatidyl choline	36.72	38.04	40.62	37.33
Phosphatidyl ethanolamine	29.41	27.66	28.50	29.62
Unidentified	11.26	12.63	12.14	11.97
Phosphatidic acid	3.72	3.42	2.81	2.63

On the other hand, variations were detected in the minor lipid fractions among the different varieties of the studied plant seed lipids .

The phospholipid class showed seven fractions (Fig. 2). Six of these fractions were identified. The data indicated that the main component of phospholipids was phosphatidyl choline, amounting to 36.72%, 38.04%, 40.62% and 37.33% of the total phospholipids in the above studied samples, respectively. It was followed by phosphatidyl ethanol amine, unidentified, phosphatidyl inositol, phosphatidyl serine and Lysophosphatidyl choline, respectively .

However, rather slight variations in phospholipid components were observed among all the studied varieties .

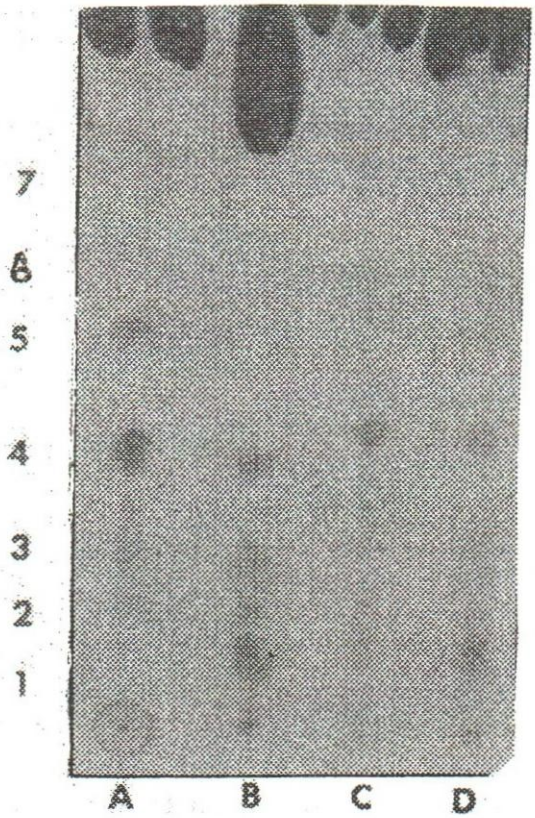

Fig (2) : Thin layer chromatogram of phospholipid fractions of plant seeds.

A= *Schouwia purpurea* B= *Cassia Senna*
C= *Cassia italica* D= *Cassia holosericea*

1- phosphatidyl serine 2-lyso-phosphatidyl choline
3- phosphatidyl inositol 4- phosphatidyl choline
5- phosphatidyl ethanolamine 6- unidentified
7- phosphatidic acid

Fatty acids composition :

The fatty acids composition of the total lipids are presented in Table (4).

The tabulated data showed that *schouwia purpurea* seed lipids contained high percentage of unsaturated fatty acids (53.417%) of the total fatty acids , consisted mainly of oleic acid (25.705%) followed by linoleic acid (15.474%) and linolenic acid (12.238%). The main saturated fatty acid was arachidic acid (18.059%).

Table (4) : Fatty acids composition of total lipids in some plant seeds (% of the total) .

Fatty acids %	Carbon Chain Length	*Schouwia purpurea*	*Cassia senna*	*Cassia italica*	*Cassia holosericea*
Hendeconic	C_{11} : 0	0.332	0.489	N.D.*	N.D.*
Lauric	C_{12} : 0	13.004	4.014	1.156	3.968
Trideconic	C_{13} : 0	0.784	N.D.*	0.129	N.D.*
Myristic	C_{14} : 0	1.988	1.264	0.491	0.663
Pentodeconic	C_{15} : 0	4.591	8.043	N.D.*	4.886
Palmitic	C_{16} : 0	7.829	40.424	29.812	18.856
Oleic	C_{18} : 0	25.705	41.773	29.323	20.011
Linoleic	C_{18} : 0	15.474	1.452	39.089	51.616
Linolenic	C_{18} : 0	12.238	N.D.*	N.D.*	N.D.*
Arachidic	C_{20} : 0	18.059	2.541	N.D.*	N.D.*
Total saturated fatty acids		46.583	56.775	31.588	28.373
Total unsaturated fatty acids		53417	43.225	68.412	71.627

*N.D. = Not detected

In addition, the data showed that total lipids of *Cassia holosericea* seemed to contain high levels of total unsaturated fatty acids (71.627%) followed by *Cassia italica* variety (68.412%), consisted mainly of linoleic acid (51.616% and 39.089%) followed by oleic acid (20.011% and 29.323%) respectively. The variation in unsaturated fatty acids in all studied samples may account for the differences in the iodine value among the varieties. The main saturated fatty acid was palmitic as its percent was 40.424%, 29.812% and 18.856% in *Cassia senna*, *Cassia italica* and *Cassia holosericea*, respectively. These trends of results are in agreement with those previously reported by Singh et al. [11].

From this study it could be noticed that the four varieties of Saudian plant seeds had a high unsaturated fatty acids content and they can be used successfully, as a source of these fatty acids, and offer a promising source of oil for nutritional purposes .

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دراسة تركيب الأحماض الدهنية ، تفريد مكونات الليبيدات المتعادلة والفوسفوليبيدات فى المستخلص الليبيدى لأربعة أنواع من البذور النباتية

محمد رشوان عبد العال رشوان* ، صالح على بازيد**

*قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة أسيوط – أسيوط – مصر

** جامعة أم القرى – مكة – المملكية العربية السعودية

أجريت الدراسة على الليبيدات المستخلصة من أربعة أنواع من البذور النباتية . تم دراسة الخصائص الكيميائية لليبيدات الكلية المستخلصة ، كذلك قسمت هذه الليبيدات إلى ثلاث أجزاء هى :

الليبيدات المتعادلة – الفوسفوليبيدات – الجليكوليبيدات ، وذلك باستخدام التحليل الكروماتوجرافى بالأعمدة . وإستخدمت كذلك طريقة التحليل الكروماتوجرافى بالطبقة الرقيقة (TLC) لتفريد مكونات الليبيدات المتعادلة والفوسفوليبيدات .

تم كذلك دراسة تركيب الأحماض الدهنية فى الليبيدات الكلية باستخدام التحليل الكروماتوجرافى الغازى (GLC) .

وقد أظهرت نتائج الدراسة ما يلى :

١ – تراوحت نسبة المحتوى الليبيدى فى البذور المختلفة من ٦,٧٪ – ٢٩,٨٧٪ .

٢ – تراوحت نسبة الليبيدات المتعادلة من ٩٥,٦٢٪ – ٩٦,٢٨٪ بالنسبة لليبيدات الكلية ، بينما كانت نسبة الفوسفوليبيدات من ٢,٠٤٪ – ٢,٧٨٪ ، والجليكوليبيدات من ١,٠٦٪ – ١,٦٠٪.

٣ – أظهرت نتائج التحليل الكروماتوجرافى بالطبقة الرقيقة وجود ٧ مكونات لليبيدات المتعادلة ، أكثرها الجلسريدات الثلاثية حيث تراوحت نسبتها من ٩٠,٧٨٪ –٩٢,٧٠٪ بينما تم تفريد الفوسفوليبيدات إلى ٧ مكونات أيضاً ، وكان أكثرها تركيزاً مركب الفوسفاتيديل كولين حيث كانت نسبته من ٣٦,٧٢٪ – ٤٠,٦٢٪ من التركيز الكلى للفوسفوليبيدات .

٤ – أوضحت نتائج التحليل الكروماتوجرافى الغازى أن الليبيدات المستخلصة من البذور موضع الدراسة تحتوى على نسبة مرتفعة من الأحماض الدهنية غير المشبعة وبصفة أساسية أحماض الأوليك واللينولينيك .

على ضوء هذه الدراسة يمكن التوصية بأنه يمكن استخدام البذور النباتية موضع الدراسة الحالية كمصدر للزيوت النباتية غير المشبعة حيث أن نسبة الزيوت بها مرتفعة وكذلك لإرتفاع محتوى هذه الزيوت من الأحماض الدهنية غير المشبعة ذات الأهمية من الناحية الغذائية .