GLYCERIDE STRUCTURE AND PHOSPHOLIPID OF SOME CHENOPODIACEAE SEEDS

By

SEHAM FIAD* Ph.D. **MAHASSEN H. ABBASSY**** Ph.D. **FOUAD OSMAN*** Ph.D.

INTRODUCTION

Seeds of some plants belonging to family *Chenopodia ceae* have been investigated in relation to both food chemistry and pharmaceutical industry. The first report on the lipid fraction of the seeds of this family has been given by (Fiad, Osman and Shoeb, 1967).

Allen et al. (1964) have found seventeen lipids in the neutral oil extracted from *Spinecea oleracea* leaves. It was noticed that 3—hexadecacenoic acid was concentrated mainly in the phosphatidyl glycerol in the 2—position (Allen and Haverkale 1964, 1965). Also hexadecatrienoic acid was found concentrated in the monoglactosyl diglyceride fraction ((Allen, 1964).

Gas liquid chromatography (GLC) is the most recent and accurate method for determining the fatty acid composition of fats and oils. The pancreatic lipase hydrolysis technique has also been used enabling the computation of the available glycerides (Mattson and Sanary 1961, 1963).

In this study, the glyceride structure, the fatty acid composition and the phospholipid fraction have been studied in oil sample extracted from the seeds of some species of the Chenopodiaceae namely : *Spinacea oleracea* L. (Spinach), *Beta vulgaris* L. var. rapa (beet root) and *Beta vulgaris* L. var. sicla (leaf beet).

EXPERIMENTAL

Triglycerides and fatty acids : The oil was extracted from the crushed seeds with n-hexane under atmosphere of nitrogen and the triglycerides were isolated according to the method of Quinlin and

* Fats and Oils Department, National Research Centre, Dokki, Cairo, Egypt.

** Girl's College, Ain Shams University, Cairo, Egypt.

Weiser (1958). The triglyceride (0.5 g.) was subjected to panceratic lipase hydrolysis at pH 8.0 and 40 °C. to attain two thirds hydrolysis only (Osman et al. ; Fiad and Osman 1968). The hydrolysate was fractionated by preparative thin layer chromatography (TLC) where the 2—monoglyceride were isolated (Osman et al., 1968). The fatty acids of the neutral triglycerides as well as those of the 2—monoglycerides were methylated then quantitatively analysed using GLC technique (Osman et al., 1973). The apparatus used was a Perkin-Elmer F-154 provided with a flame ionisation detector. The column was packed with 20% ethylene glycol succinate on chromosorb w and worked at 190°C., using purified nitrogen as carrier gas.

The triglycerides of the oils were computed from the fatty acids in the 2—position and from those in the 1, 2, 3—position (Coleman, 1961).

Phospholipids : Crushed seeds were extracted throughly with methanol : chloroform (1:3) under atmosphere of nitrogen. The phospholipid fraction was then isolated from the extract by chromatography (Kuchmak and Dugan, 1964) using silicic acid column (50 g. 100 mesh Malin Krodt), purified chloroform was used to remove any trace of the neutral triglycerides. The column was then eluted with chloroform : methanol (S:1) to isolate the phosphatides. The course of column chromatography was monitored by TLC on silica gel G using as a solvent system, petroleum ether : ethyl ether : acetic acid (85:15:1) and iodine vapours as detecting agent. The spots were identified by using standard phospholipid samples, the mixed phosphatides of each sample was interesterified with methanol—HCl to determine the phosphatidyl fatty acids (Osman et al., 1969).

RESULTS AND DISCUSSION

Fatty Acids and Glycerides :

The molar percentages of fatty acids present in the triglycerides and of the 2—monoglycerides obtained by pancreatic lipase hydrolysis are represented in Table 1. The results indicated clearly that the oil of spinach seed is quite different in its fatty acids from those of the two *Beta* varieties. *Spinacea oleracea* seed oil is composed of six individual fatty acids. It contained considerable amounts of lower fatty acids (La = 1.9%, La 0 = 2.9% and M = 2.9%), while it is devoid of stearic acid. The two *Beta* varieties were devoid of the lower fatty acids, they contain only the four fatty acids : palmitic, stearic, oleic and linoleic. However, there is an increase in the percent of both palmitic and oleic acids from spinach to leaf-beet oil samples Table 1, from 16.2—32.3 and 28.8—47.4%, respecively. Linoleic acid represents the main fatty acid in spinach oil (47.3%) and also it represents a large amount in case of beet-root (30.4%) on the other hand, linoleic acid reported considerable amount (15.6%) in case of leaf-beet oil sample. As for stearic acid which was represented only in the two *Beta* varieties, it reported close value (4.1 and 4.7%, respectively).

Regarding the 2—position of spinach oil sample, it was mainly acylated by linoleic acid where it reached up to 53.1%. Also oleic acid was well represented in the 2—position of spinach oil (18.8%). However La, La O, M and P acids were represented also in considerable amounts in 2—position of spinach (5.0—8.8 for palmitic and myristic acids, respectively). On the other hand, oleic acid is the main fatty acid acylated in 2—position for both *Beta* varieties (45.9, 62.2%, respectively). However, linoleic acid is also well represented in beet-root (38.8%) 2—position, while palmitic acid was reported 26.1% in 2—position of leaf-beet oil sample.

The number of glycerides computed for *Spinacea oleracea, Beta vulgaris* V. *rapa* and *Beta volgaris* v. *sicla* were 36, 38, glyceride. It is evident that the difference in the number and percentages of individual glycerides is due to a corresponding difference in the quality and quantity of the fatty acids especially those acylated in the 2—position.

The computed glycerides, including the positional isomers, for the three oil samples under investigation were shown in Table 2. It is apparent from Table 2 that beet-root and leaf-beet oils are devoid of lauric, lauroleic and myristic glycerides in which these acids are acylated in the inner position of the glyceride moiety. On the other hand, 50% of the number of the total glycerides of spinach oil were that of La, LaO and M—glycerides (22.7%). Stearic acid glycerides were not represented in spinach oil sample, while it was represented in both two *Beta* varieties in relatively considerable amounts (2.9, 4.9%). As palmitic acid glycerides it reached up to 26.1% in case of leaf-beet glycerides. The oleic acid glycerides reported by large amounts in the three oil samples with a maximum concentration of 62.2% in case of leaf-beet. Also trimonoene glyceride is shown in considerable amounts in both *Beta* varieties till 10.0% in leaf-beet. However, linoleic acid glycerides reported by considerable amount in both spinach and beet-root oil samples (53.1 and 38.8%, respectively), while in leaf-beet oil it represented only 6.8%. The simple tridience glyceride percent was proportional to its percent in the total 2—

monoglycerides in their respective oil samples and reported 10.4, 2.7 and 0.3% for spinach, beet-root and leaf-beet triglycerides, respectively Table 2.

When the results were grouped as the main four triglyceride categories, namely, GS_3, GS_2U, GSU_2 and GU_3, the difference between the three oil samples of *Chenopodiaceae* became more evident.

	GS_3	GS_2U	GSU_2	GU_3
Spinacea oleracea	0.9	11.9	39.2	48.0
Beta vulgaris v. *rapa*	1.2	14.5	43.3	41.0
Beta vulgaris v. *sicla*	5.0	25.8	44.3	24.9

The figures indicated that the two main glyceride categories in case of the first two oil samples were the diunsaturated (GSU_2) and triunsaturated (GU_3) which recorded 39.2, 48.0% and 43.3, 41.0 for spinach and beet-root, respectively. On the other hand in leaf-beet oil, the main glyceride category is GSU_2 and reported 44.4%. However, leaf-beet oil showed relatively high value of trisaturated glycerides 5.0% compared to almost about 1% in the other two oil samples.

Phospholipids :

The fatty acid constitution of the phospholipid fractions of the three oils under investigation are almost beyond expectation and showed wide deviation from those of the corresponding triglycerides Table 1. In case of spinach the lower fatty acids which are well represented in the triglycerides have disappeared completely from the phospholipid fraction. Also stearic acid which was not reported in the triglycerides of spinach was represented by 1.3% in its phospholipid fraction, on the contrary in beet-root and leaf-beet it was disappeared from their phospholipid fractions although it reported 4.1 and 4.7% in their respective triglycerides. However, palmitic acid constitutes the main fatty acid in the three phospholipid fractions instead of oleic or linoleic or both (not less than about 70%, both) as the main fatty acid in their triglycerides.

Figure 1 shows a schematic representation of the individual phospholipids in the three samples as qualitatively fractionated by T.L.C. using a solvent system chloroform-methanol (65:2 v/v).

Lecithin which has been assumed to be the characteristic plant phosphatide showed corresponding spot in all samples under investigation. The density and area of the spots reveal that it is the major phospholipid constituent (R_f, 0.38).

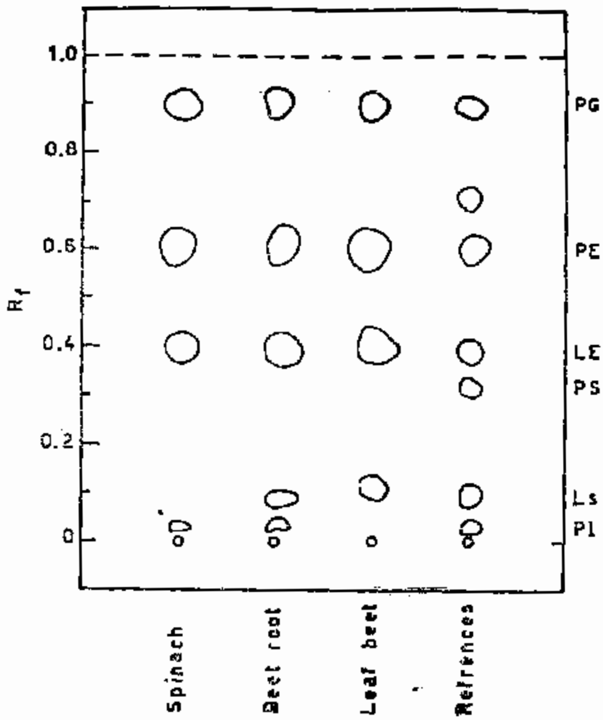

Fig. 1 — Diagramatic Representation of Phospholipid Samples

PI = Phosphatidyl Inositol

Ls = Lysolecithin

PS = Phosphatidyl Serine

LE = Lecithin

PE = Phosphatidyl Ethanolamine

PG = Phosphatidyl Glycerol

Lysolecitin is found in a minor quantity only in the phospholipids of both beet-root and leaf-beet oils (R_F, 0.09). It is reported that lysolecithin is present in some food plants such as rice, millet, barely and wheat.

Phosphatidyl inositol with R_f, 0.03 was found in small quantities in both spinach and beet-root phospholipid fractions and seem to be absent from leaf-beet.

Phosphatidyl ethanolamine as well with R_F, 0.6, also phosphatidylglycerol was reported in the three samples (R_F, 0.9).

As a general conclusion the three oils studied follow the 1,3—random and the 2—random distribution pattern. These oils may suggest the possibility of their utilization as potential oils for human consumption.

SUMMARY

The fatty acid composition, the triglyceride structure and also the fatty acids found in the phosphatides of three seed-oils namely : *Spinacea oleracea, Beta vulgaris* v. rapa and *Beta vulgaris* v. sicla belong to the family Chenopodiaceae were determined. Also the phosphatides were fractionated on thin-layer chromatography for the three seeds.

REFERENCES

Fiad, S. ; Osman, F. and Shoeb, Z.E. ; Grasas Y Aceites, 18, 6 (1967).

Allen, C.F. ; Good, P. ; Davis, H.F. and Flower, S.D. ; Biochem. Biophys. Res. Commun., 15, 424-50 (1964).

Haverkale, F. and Van Deenen, L.M. ; Biochim. Biophys. Acta 106 (1), 78-92 (1965).

Mattson, F.H. and Valpenheim, R.A. ; J. Lipid Res., 2, 58, (1961).

Sanary, P. and Desnuelle, P. ; Biochim. Biophys. Acta 21, 439 (1956) ; J. Lipid Res., 4, 369, (1963).

Quinlin, P. and Weiser, H.J. ; J.A.O.C.S., 35, 325 (1958).

Osman, F. ; Subbarm, M.R. and Achaya, K.T. ; Fette Seifen Anstrichmittel, 70, 69, (1968).

Fiad, S. and Osman, F. ; Chem. J. of U.A.R. (Submitted for Publication).

Osman, F. ; Ashour, A.F.A. and Gad, A.M. ; Planta Medica, 24, 53, (1973).

Coleman, M.H. ; J.A.O.C.S. 38, 685, (1961).

Kuchmak, M. and Dugan, L.R. ; J.A.O.C.S. 40, 737, (1964).

Osman, F. ; Ashour, A.E. and Gad, A.M. ; Fette. Seifen. Anstrichmittel, 71, 264-6, (1969).

TABLE 1

Fatty acid composition of tri, 2—monoglycerides and phospholipids of Chenopodiaoeae seed-oils.

Sample	Spinacea oleracea			Beta vulgaris (v. sicla)			Beta vulgaris (v. rapa)		
Fatty acid	T.G.	M.G. 2—	Phosph.	T.G.	M.G. 2—	Phosph.	T.G.	M.G. 2—	Phosph.
Lauric (La)	1.9	5.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lauro-oleic (LaO)	2.9	8.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Myristic (M)	2.9	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palmitic (P)	16.2	5.0	47.3	21.2	12.4	50.8	32.3	26.1	38.9
Stearic (S)	0.0	0.0	1.3	4.1	2.9	0.0	4.7	4.9	0.0
Oleic (O)	28.8	18.8	22.1	44.3	45.9	32.4	47.4	62.2	33.3
Linoleic (Li)	47.3	53.1	29.3	30.4	38.8	16.8	15.6	6.8	27.8

T.G. = Triglycerides　　2—M.G. = 2—Monoglycerides
Phosph. = Phospholipids.

TABLE 2

Glyceride structure of Chenopodiacea seed oils.

Glyceride	Spinacea oleracea	Beta vulgaris v. rapa	Beta vulgaris v. sicla	Glyceride	Spinacea oleracea	Beta vulgaris v. rapa	Beta vulgaris v. sicla
P La P	0.3	0.0	0.0	S S S	0.0	0.0	0.0
O La O	0.7	0.0	0.0	O S O	0.0	0.6	0.8
Le La Le	1.1	0.0	0.0	Le S Le	0.0	0.2	0.2
P La O	0.8	0.0	0.0	P S S	0.0	0.1	0.2
P La Le	1.1	0.0	0.0	P S O	0.0	0.6	1.4
O La Le	1.7	0.0	0.0	P S Le	0.0	0.4	0.6
P La O P	0.4	0.0	0.0	S S O	0.0	0.1	0.2
O Lao P	1.0	0.0	0.0	S S Le	0.0	0.1	0.1
Le Lao Le	1.7	0.0	0.0	O S Le	0.0	0.6	0.8
P Lao O	1.2	0.0	0.0	P O P	0.9	3.00	7.8
P Lao Le	1.6	0.0	0.0	S O S	0.0	0.1	0.1
O Lao Le	2.6	0.0	0.0	O O O	2.1	8.6	10.0
P M P	0.4	0.0	0.0	Le O Le	3.7	3.2	2.5
O M O	1.0	0.0	0.0	P O S	0.0	1.1	2.0
Le M Le	1.7	0.0	0.0	P O O	2.8	10.2	17.6
P M O	1.2	0.0	0.0	P O Le	3.6	6.2	8.8
P M Le	1.6	0.0	0.0	S O O	0.0	1.9	2.2
O M Le	2.6	0.0	0.0	S O Le	0.0	1.2	1.2
P P P	0.2	0.8	3.3	O O Le	5.6	10.4	10.0
S P S	0.0	0.0	0.1	P Le P	2.5	2.5	0.9
O P O	0.6	2.3	4.2	S Le S	0.0	0.1	0.0
Le P Le	1.0	0.9	1.0	O Le O	6.0	7.3	1.1
P P S	0.0	0.3	0.8	Le Le Le	10.4	2.7	0.3
P P O	0.7	2.8	7.4	P Le S	0.0	1.0	0.2
P P Le	1.0	1.6	3.6	P Le O	8.0	8.6	2.0
S P O	0.0	0.5	1.0	P Le Le	10.2	5.2	1.0
S P Le	0.0	0.3	0.5	S Le O	0.0	1.6	0.2
O P Le	1.5	2.8	4.2	S Le Le	0.0	1.0	0.1
P S P	0.0	0.2	0.6	O Le Le	16.0	8.8	1.0