95. The Component Fatty Acids of some Vegetable Seed Phosphatides

By Thomas Percy Hilditch and Youssef Abdel Hamid Zaky, *From the Department of Industrial Chemistry, University of Liverpool*

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The component fatty acids of phosphatides present in soya beans and in rape seeds were determined [Hilditch & Pedelty, 1937] by the method of ester-fractionation. In both cases linoleic acid was found to be the predominating component, although all the acids present in the respective seed glycerides were also observed, to a greater or less extent, in the corresponding seed phosphatides; palmitic acid formed a greater proportion of the phosphatide than of the corresponding seed glyceride acids in both instances studied. A review of earlier observations on the composition of vegetable phosphatides, notably by Levene & Rolf [1925; 1926], by Suzuki & Nishimoto [1930] and Suzuki & Yokoyama [1931], was given in Hilditch & Pedelty's communication.

Specimens of phosphatide concentrates prepared from four other vegetable oils, groundnut, cottonseed, sunflower and linseed, have been supplied to us by Messrs J. Bibby and Sons, Ltd., of Liverpool, to whom we express our cordial thanks and appreciation. We have accordingly been able to obtain data concerning the component fatty acids present in these four seed phosphatides, of which a summary is here presented.

EXPERIMENTAL

Phosphatides from groundnuts

The phosphatide concentrate supplied was an emulsion consisting of approximately 50 % phosphatides, 25 % oil (glycerides) and 25 % water. The water and some mucilage was separated by extraction of the emulsion with 5 vol. of ether, and the dry mixture of phosphatides and glycerides was then boiled with five times its weight of acetone and the solution cooled at 0° overnight. This treatment was repeated several times until practically no material soluble in acetone at 0° remained and all the glycerides had been removed. After removing all traces of acetone from the final product in a vacuum at room temperature the groundnut phosphatides then formed a granular brownish coloured powder (sap. equiv. 288.23 i.v. (Wijs) 52.0). Although free from glycerides, the material still contained considerable proportions of non-fatty matter, and had P 2.7 %, N 1.2 % (molar ratio P/N 1.02 : 1).

The phosphatides (110 g.) were hydrolysed by refluxing with alcohol containing 0.5 % H_2SO_4 for 3 hr., after which sufficient KOH was added to neutralize the H_2SO_4 and give a 5 % solution of the alkali and the mixture was again refluxed for 3 hr. [cf. Hilditch & Pedelty, 1937]. On acidification of the recovered soaps, about 5 % of the product separated in the form of an amorphous non-fatty powder similar to that encountered in the case of soya bean phosphatides [Hilditch & Pedelty, 1937]; the liberated fatty acids were freed from residual traces of this substance by solution in light petroleum (B.P. 40–60°). The mixed fatty acids (59.7 g.) were separated by lead salt separation from alcohol into 'solid' (26.3 %) and 'liquid' (73.7 %) acids, each group being then converted into methyl esters which were fractionally distilled in a vacuum through an electrically heated and packed column with the results given in Table 1.

Table 3. *Methyl esters of acids from cottonseed phosphatides*

From 'solid' acids				From 'liquid' acids			
No.	g.	Sap. equiv.	i.v.	No.	g.	Sap. equiv.	i.v.
S 1	3.29	277.3	0.6	L 1	3.48	272.8	52.4
S 2	3.43	280.2	0.6	L 2	3.83	289.4	120.4
S 3	4.73	280.2	7.0	L 3	3.94	291.5	141.0
S 4	1.85	338.2	8.8	L 4	5.18	294.1	146.6
				L 5	6.06	294.0	145.0
				L 6	6.22	294.8	145.0
				L 7	5.27	295.9	145.3
				L 8	6.04	296.1	143.5
				L 9	5.45	297.0	143.0
				L 10	7.30	297.1	140.0
				L 11	6.94	(462.5)*	(53.0)*
13.30				59.71			

* Sap. equiv. of esters, freed from unsaponifiable matter, S 4, 322.0; L 11, 318.7. The residue of 11 was lost owing to enemy action; the sap. equiv. and i.v. used were obtained in a separate distillation of a small portion of the methyl esters from the total phosphatide acids. These values may therefore be not wholly accurate, and the actual proportion of unsaturated C_{20-22} acids present may be somewhat different from that shown in Table 4.

Table 4. *Component acids of cottonseed phosphatides*

	'Solid' acids S (24.5%)	'Liquid' acids L (75.5%)	Total	Component acids	
				% (wt.)	% (mol.)
Palmitic	13.48	3.34	16.82	17.3	18.9
Stearic	7.03	—	7.03	7.3	7.1
Arachidic	2.73	—	2.73	2.8	2.5
Hexadecenoic	—	1.46	1.46	1.5	1.7
Oleic	1.09	18.56	19.65	20.3	20.0
Linoleic	—	43.07	43.07	44.4	44.2
Unsaturated C_{20-22}	—	6.21	6.21	6.4	5.6
Unsaponifiable	0.17	2.86	3.03	—	—

Phosphatides from sunflower seed

The quantity of sunflower seed phosphatides available was small, and only 40 g. of purified material were obtained for component acid analysis. The fatty acids obtained by hydrolysis of the phosphatides gave, on lead salt separation from alcohol, 32.9 % 'solid' and 67.1 % 'liquid' acids. Fractionation of the corresponding methyl esters gave the data summarized in Table 5.

Table 5. *Methyl esters of acids from sunflower seed phosphatides*

From 'solid' acids				From 'liquid' acids				CNS. V
No.	g.	Sap. equiv.	i.v.	No.	g.	Sap. equiv.	i.v.	
S 1	2.74	278.2	15.6	L 1	3.61	290.4	129.6	73.4
S 2	2.66	353.0*	18.6	L 2	3.66	294.0	153.3	88.4
				L 3	3.50	297.7	155.8	90.5
				L 4	3.00	364.1*	130.0	—
5.40				13.77				

* Sap. equiv. of esters, freed from unsaponifiable matter, S 2, 314.2; L 4, 303.8.

The thiocyanogen values of the 'liquid' esters did not indicate the presence of linolenic acid, and the data calculated for the component acids are therefore as shown in Table 6.

The component acids of the sunflower seed oil (glycerides) corresponding with the phosphatides examined were also determined, with the final results given in Table 7.

The oil had sap. equiv. 295.0, i.v. (Wijs) 138.2 and contained 1.0% of free fatty acids (as oleic).

Table 6. *Component acids of sunflower seed phosphatides*

	'Solid' acids S (32.9%)	'Liquid' acids L (67.1%)	Total	Component acids	
				% (wt.)	% (mol.)
Palmitic	11.37	2.71	14.08	14.7	16.1
Stearic	4.87	—	4.87	5.1	5.0
Arachidic	9.10	—	9.10	9.5	8.6
Oleic	5.71	12.69	18.40	19.3	19.2
Linoleic	—	43.87	43.87	45.9	46.3
Unsaturated C ₂₀₋₂₂	—	5.28	5.28	5.5	4.8
Unsaponifiable	1.85	2.55	4.40	—	—

Table 7. *Component acids of sunflower seed oil*

	'Solid' acids S (6.8%)	'Liquid' acids L (93.2%)	Total	Component acids	
				% (wt.)	% (mol.)
Palmitic	2.79	2.79	5.58	5.6	6.2
Stearic	2.13	—	2.13	2.2	2.1
Arachidic	0.92	—	0.92	0.9	0.8
Oleic	0.90	23.95	24.85	25.1	24.8
Linoleic	—	65.68	65.68	66.2	66.1
Unsaponifiable	0.06	0.78	0.84	—	—

Other workers [Baughman & Jamieson, 1922; Branke & Gutt, 1935] have given the component acids (% wt.) of sunflower seed oil as follows:

	Baughman & Jamieson	Branke & Gutt
Palmitic	3.9	1.6
Stearic	2.9	6.9
Arachidic	—	—
Oleic	34.2	26.6
Linoleic	59.0	64.9

Phosphatides from linseed

Unfortunately the amount of phosphatides from this interesting source was extremely small (20 g.). The acids obtained after hydrolysis of the phosphatides furnished, after lead salt separation from alcohol, 2.6 g. (20.4%) 'solid' and 10.3 g. (79.6%) 'liquid' acids. The 'solid' acids were converted into methyl esters, which had sap. equiv. 286.1 (freed from unsaponifiable, 285.4), i.v. 6.6. The methyl esters of the 'liquid' acids were fractionally distilled in the usual way with the following results:

No.	g.	Sap. equiv.	i.v.	CNS. V.
L 1	2.30	286.2	126.4	86.7
L 2	3.38	293.0	154.2	104.8
L 3	3.12	295.7	156.8	106.7
L 4	1.41	480.0*	131.9	71.9
	10.21			

* Esters in L 4, freed from unsaponifiable matter, sap. equiv. 304.0.

The esters of unsaturated C₁₈ acids present in these fractions were calculated, from the iodine and thiocyanogen values, as mixtures of methyl oleate, linoleate, and linolenate; the thiocyanogen values for pure methyl linoleate and linolenate were taken respectively as 91.8 and 154.7 [Hilditch & Murti, 1940]. The final results for the linseed phosphatide component acids are given in Table 8.

Table 8. *Component acids of linseed phosphatides*

	'Solid' acids S (20.4 %)	'Liquid' acids L (79.6 %)	Total	Component acids	
				% (wt.)	% (mol.)
Palmitic	8.68	2.17	10.85	11.3	12.3
Stearic	10.19	—	10.19	10.6	10.4
Hexadecenoic	—	3.33	3.33	3.5	3.8
Oleic	1.47	30.64	32.11	33.6	33.1
Linoleic	—	19.53	19.53	20.4	20.4
Linolenic	—	16.64	16.64	17.4	17.4
Unsaturated C ₂₀₋₂₂	—	3.05	3.05	3.2	2.8
Unsaponifiable	0.06	4.24	4.30	—	—

DISCUSSION AND SUMMARY

Including the soya bean and rape seed phosphatides previously studied, the component fatty acids of the phosphatides from six different species of seeds have now been determined. The proportions of the various acids present, in the phosphatides and also in the corresponding seed glycerides (for which typical recorded analyses are included), are shown in Table 9.

Table 9. *Component acids of seed phosphatides and glycerides (wt. %)*

	Cotton- seed	Sunflower seed	Rape seed	Soya- bean	Ground- nut	Linseed
(i) Phosphatides						
Myristic	—	—	0.8	—	—	—
Palmitic	17.3	14.7	8.3	11.7	16.2	11.3
Stearic	7.3	5.1	—	4.0	2.8	10.6
Arachidic	2.8	9.5	—	1.4	—	—
C ₂₀ , C ₂₂ , C ₂₄	—	—	1.5	—	4.6	—
As C ₂₆	—	—	—	—	2.5	—
Hexadecenoic	1.5	—	2.1	8.6	—	3.5
Oleic	20.3	19.3	22.4	9.8	47.1	33.6
Linoleic	44.4	45.9	42.2	55.0	22.7	20.4
Linolenic	—	—	Trace	4.0	—	17.4
Unsaturated C ₂₀₋₂₂	6.4	5.5	—	5.5	4.1	3.2
Erucic	—	—	22.7	—	—	—
(ii) Glycerides						
	(a)	(b)	(c)	(d)	(e)	(f)
Myristic	1.4	—	—	0.3	—	0.2
Palmitic	23.4	5.6	2	9.8	8.3	5.4
Stearic	1.1	2.2	—	2.4	3.1	3.5
Arachidic	1.3	0.9	—	0.9	—	0.6
C ₂₀ , C ₂₂ , C ₂₄	—	—	1	—	6.6	—
Hexadecenoic	2.1	—	—	0.5	—	—
Oleic	22.9	25.1	17	28.9	56.0	18.8
Linoleic	47.8	66.2	29	50.7	26.0	24.2
Linolenic	—	—	—	6.5	—	47.3
Erucic	—	—	51	—	—	—

(a) Hilditch & Maddison [1940]; (b) present work; (c) Hilditch & Paul [1935]; (d) Hilditch & Jasperson [1939]; (e) Hilditch & Jasperson [1938]; (f) Griffiths & Hilditch [1934]; Hilditch & Murti [1940].

The data presented in this paper largely confirm but also extend the conclusions suggested by Hilditch & Pedelty [1937] from the two seed phosphatides then studied. In all six instances the range of fatty acids in the phosphatides is qualitatively the same as that in the corresponding glycerides, but the proportions of the component acids vary widely as between the two types of seed lipids. The features most general throughout the six phosphatides appear to be the consistent occurrence of a minor proportion (3–6 %) of highly unsaturated acids of the C₂₀ and C₂₂ series (which are not present in seed

glycerides), and the presence of more saturated acids in the phosphatides than in the glycerides.

The presence of appreciable, though small, amounts of the highly unsaturated acids of higher molecular weight than C_{18} (possibly arachidonic, possibly mixtures of polyethenoid C_{20} and C_{22} acids) seems to be a characteristic of all natural phosphatides from vegetable or animal sources.

The increased proportions of saturated acids in phosphatides as compared with glycerides from the same site is also common to both vegetable and animal lipids. In the six seed phosphatides the increase is chiefly in the proportion of palmitic acid (the characteristic saturated acid of all vegetable lipids), although stearic acid also occurs to a larger degree in some of the phosphatides as compared with the corresponding glycerides. Again it may be noticed that in cottonseed, the glycerides of which contain a comparatively high proportion of saturated acids, the total amount of the latter in the phosphatide acids is not materially increased whilst the proportion of palmitic acid has decreased (from 23 to 17 %).

Hexadecenoic acid, which is found as a minor component in all animal phosphatides, was observed to be similarly present in rape seed phosphatides and to a somewhat larger extent in soya bean phosphatides; but the extended study suggests that on the whole it is a definitely minor component of seed phosphatide acids. Indeed, no evidence of its presence was observed in the phosphatides from groundnuts and sunflower seed.

The most abundant components of the seed phosphatides are linoleic and oleic acids. In our earlier communication it was suggested that linoleic acid might be the most important component of plant phosphatide acids, exceeding in its proportions those of oleic or other acids, with the ratio of linoleic to oleic acids higher in the phosphatides than in the corresponding glycerides. In four of the six instances now available, linoleic is the most abundant acid and forms 42–55 % of the total fatty acids. In these cases it also occurs in considerably higher proportions than oleic acid; but only in rape seed is the amount of linoleic acid notably higher in the phosphatides than in the glycerides. In the soya bean it is somewhat higher, in cottonseed, linseed and groundnuts somewhat lower, and in sunflower seed it is much lower in the phosphatides than in the glycerides.

In cottonseed and groundnuts the relative proportions of linoleic and oleic acids are much the same in both glycerides and phosphatides. In sunflower seed and in linseed the ratio of linoleic acid to oleic acid in the phosphatides is definitely lower than in the glycerides. Linseed stands somewhat apart from the rest in that the relative proportions of linolenic, linoleic and oleic acids in the glycerides are reversed in the phosphatides; in the latter oleic acid is the most, and linolenic the least, abundant of the three acids. In the light of the further instances now investigated, the earlier suggestion that linoleic is the most important component of seed phosphatides must therefore be replaced by the statement that linoleic acid (identical with seed glyceride linoleic acid) has been found to be an invariable major component of seed phosphatides, and that it frequently, but not always, forms 45 % or more of the total fatty acids.

From the six instances now studied (which cover a fairly wide range of botanical families) the following generalizations may be tentatively suggested: (i) seed phosphatides contain characteristic, although minor proportions of highly saturated C_{20} and C_{22} acids which are not present in the corresponding glycerides; (ii) acids (notably palmitic) of the saturated series form a greater proportion of seed phosphatide than of seed glyceride fatty acids; (iii) all the acids present in any seed glyceride are also found in the corresponding seed phosphatide; (iv) linoleic acid is on the whole the most characteristic acid of seed phosphatides and, although in two instances out of the six investigated it amounts to only 20–25 % of the total fatty acids, in the majority it forms 45–55 % of the total phosphatide fatty acids.

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