96. Phosphatides from Oil Seeds

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All oil-producing seeds contain phosphatides, but so far only a few of these seeds have been examined carefully with regard to their phosphorus-containing glycerides. There are many publications about the phosphatides of soya beans, and a few other phosphatides have been also examined (e.g. rape seed), but there seems to be very little known about the phosphatides of other important oil seeds, especially those of groundnuts, cottonseed, linseed or sunflower seed. For this reason, experiments have been carried out to extract these phosphatides from the raw materials, or to separate them from the sludge of commercially manufactured oils. As it is well known that the phosphatides of the vegetable kingdom are always mixtures of choline and aminoethanol phosphatides, a separation of these two groups was carried out, and the percentage ratio determined.

Oil seeds are usually crushed in expeller presses or similar machines, and the crude oil thus obtained contains a certain amount of phosphatides which separate out, with other impurities, after the oil has been kept in storage for some time. These sludges have been one source of raw material for the extraction and separation of the phosphatides, although only a certain percentage, usually no more than half of the phosphatides present in the seeds, is found in the oil; another, and sometimes a very large amount, of these phosphatides remains in the press cake. It seems that the phosphatides are distributed in the oil-containing seed in two different forms: in one form they can be regarded as 'free' phosphatides, and here they are dissolved in the oil and therefore are pressed out together with the oil and later settle out in the sludge; in the other case they seem to be 'physically' linked, usually to the proteins and sugars. It is very well known that egg yolk for instance contains most of its lecithin in a combined form which cannot be extracted with the ordinary solvents, light petroleum, benzene, trichlorethylene etc.; only by the use of alcohol can the lecithin be freed from the proteins and later be extracted without any difficulty. The same phenomena occur in seeds. The phosphatides remaining in the press cakes cannot be extracted with any of the ordinary solvents in absence of alcohol. For this reason the cakes were exhaustively extracted with a mixture of alcohol and benzene (20 : 80). Such a mixture is necessary because of the presence of large quantities of phosphatides of the kephalin type, which are not soluble even in boiling alcohol, and require a solvent in which the phosphatides can be dissolved later. In previous cases [Rewald, 1936; 1937 *a, b*] this particular mixture gave very satisfactory results.

The extraction of the phosphatides was always carried out in the same way. The sludge usually contains a high amount of moisture, besides large quantities of oil and impurities (husks, etc.). This sludge is treated with a large quantity of acetone, which removes not only the water but also the oil, sterols, pigments, etc. The remaining paste is dried and dissolved in light petroleum. It always takes some time before the very fine powder has settled out and the clear solution can be filtered. This solution contains the phosphatides and a certain amount of oil. It is treated with an excess of acetone, and the flocculated phosphatides are separated and dried in a vacuum. This procedure has to be repeated once or twice. The phosphatides must be stored in a vacuum desiccator and the adherent solvent evaporated very carefully. Phosphatides in a pure state absorb moisture very quickly and decompose in a short time if exposed to the open air.

These phosphatides are not absolutely pure. They still contain a certain amount of carbohydrate, mostly disaccharides, which are very difficult to remove. It is interesting that these mixtures of phosphatides and sugars are easily soluble in nearly all of the fat solvents in which sugars are otherwise absolutely insoluble. This sugary matter can be mostly removed by prolonged shaking of the solution in light petroleum with water, but the emulsions formed and the losses are so great, that the preparation of sugar-free phosphatides not only takes a very long time, but also needs rather large quantities of material. The final purification was not carried out in the following cases, which probably accounts for the somewhat low phosphorus figures observed.

The extraction of the phosphatides from the finely ground oil cakes was carried out as follows. The cake was first extracted with light petroleum, to remove any oil still present. This oil, as expected, contained only traces of phosphatides. The oil-free powder was then repeatedly boiled, under reflux, with a mixture of alcohol and benzene. The solvent was evaporated, and the dry residue extracted with light petroleum. As is always the case when alcohol is used, this residue contained large quantities of sugary matter. The clear solution was then treated with acetone, which, as in the previous case, flocculated the phosphatides. The purification by redissolving in light petroleum, etc. was carried out twice.

The phosphatides, extracted from the sludge or the press cake, were then separated into the two main fractions, lecithin and kephalin, by using boiling alcohol, which dissolves the lecithin but not the kephalin. Occasionally a third fraction could be separated, soluble in hot alcohol but insoluble in cold; it could not be determined whether this fraction is a pure compound or a mixture, but it was found that it could not be resolved further by treating it many times with boiling alcohol.

The appearance of the phosphatides proves that they must contain a large amount of kephalin in addition to lecithin, since they are mostly somewhat hard solids, whilst lecithins are soft and pasty. This is confirmed by analysis, which in most cases indicate that only one-third of the total phosphatides belong to the lecithin type.

The results of the experiments can be seen from the tables.

Table 1 gives the percentages of phosphorus found in the lecithin and kephalin separated from the sludges of the different oils pressed from the seeds. It will be seen that the lecithin figures are on the low side, which may be due to the presence of carbohydrates; the kephalin figures are in most cases more normal. Only the linseed and the sunflower seed products are out of range, but linseed is a drying oil and it must be borne in mind that decomposition may have occurred, which might well influence the solubility.

Table 1. *Phosphatides in seed oil sludges*

Oil	Lecithin (alcohol-soluble)	Kephalin (alcohol-insoluble)	Soluble in hot, insoluble on cold, alcohol
	% P	% P	% P
Groundnut	3.64	4.26	3.52
Sesame	3.29	4.34	3.90
Cottonseed	3.52	4.00	—
Linseed	3.35	3.00 (?)	—
Sunflower seed	3.08	2.94 (?)	—

Table 2 shows the data for the phosphatides extracted from the cakes. It is remarkable that nearly always the same amount (about 0.5 %) of phosphatides remains in the cake. The quantities of oil which remain in the cake are 6–8 %, and the phosphatides are only present in small amounts in these oils. For comparison linseed itself was also treated in the same way; here the quantity of oil is naturally high, but the alcohol-benzene extract is nearly the same, and the phosphatide isolated also has the same composition (3.71 and 3.93 % P).

Table 2. *Phosphatides in oil press-cakes*

Seed-cake	Light petroleum extract		Alcohol-benzene extract	Acetone-insoluble	
	Extract %	P content %		P %	Lecithin equivalent in cake %
Groundnut	8.43	Traces	1.34	3.77	0.57
Cottonseed	7.19	0.20	1.31	3.59	0.58
Linseed	6.24	0.41	3.82	3.71	0.41
Linseed meal (not pressed)	38.00	0.02	2.81	3.93	—

Table 3 gives the distribution of lecithin and kephalin in the phosphatides.

Table 3. *Ratio of lecithin and kephalin in seed phosphatides*

	Lecithin %	Kephalin %
Groundnut	35.7	64.3
Sesame	52.2	40.6*
Cottonseed	28.8	71.2
Linseed	36.2	63.8
Sunflower	38.5	61.5

* 7.2% soluble in hot, insoluble in cold, alcohol.

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REFERENCES

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