IODINE VALUE OF FATTY ACIDS FROM PLANT PHOSPHATIDES.*

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While much work has been done on animal lecithins and phosphatides, we find that, as yet, vegetable phosphatides have been very little investigated. Especially is this true of the fatty acids which may be secured by the hydrolysis of plant phosphatides.

Palmitic, stearic, and an unsaturated acid were found in various phosphatide preparations secured from seeds of Lupinus Albus by Njegovan (6). The iodine-value of the fatty acids from the various preparations varied greatly. Grafe and Magistris (3) using a preparation secured from Pisum arvense unicolor, secured flakes of a fatty acid which corresponded to palmitic acid. The most recent work along this line is that of Levene and Rolf (4) (5), who worked with phosphatide preparations secured from soy beans and identified stearic, palmitic, linolenic, linolic, and aleic acids.

It was felt desirable to determine whether or not the fatty acids secured from the phosphatides of various seeds are the same.

As there are a considerable number of methods of hydrolysis (2), (3), (7) it was deemed desirable to try out several of them on a sample of commercial lecithin and select the most satisfactory one for this problem. The methods and the iodine value of the resulting fatty acids are shown in Table I.

The samples were all refluxed over a flame, using 200 cc. of sulfuric or hydrochloric acid, and the heating was continued until the fatty acids separated and formed a clear layer on top. The sulfur dioxide gas was bought in cylinders and the carbon dioxide was generated from marble, using C. P. hydrochloric acid.

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TABLE I.

EFFECT OF VARIOUS METHODS OF HYDROLYSIS ON THE IODINE VALUE OF A LECITHIN SAMPLE.

TYPE OF HYDROLYSIS	IODINE VALUE	REMARKS
6% H_2SO_4.....	50.13	Fatty acid layer quite black.
6% H_2SO_4 + mossy tin.....	49.43	Fatty acids a light cream color.
6% H_2SO_4 + SO_2 gas.....	50.89	Fatty acids brown.
6% H_2SO_4 + CO_2 gas.....	49.86	Fatty acids brown to black.
10% HCl.....	51.24	Fatty acids dark.
10% HCl + mossy tin.....	52.54	Fatty acids light brown.
10% HCl + CO_2 gas.....	52.50	Fatty acids brown to black.
10% HCl + SO_2 gas.....	55.41	Fatty acids dark.
10% HCl + SO_2 gas.....	56.40	Fatty acids dark.

It is at once apparent that the use of sulfuric acid as a hydrolysing agent considerably lowers the iodine value, presumably either by oxidation or addition reactions at the double bonds; conversely hydrochloric acid in the presence of a strong reducing agent as sulfur dioxide seems to give considerably higher values and it was accordingly selected as the method to be used in the following work.

PREPARATION OF PHOSPHATIDE SAMPLES.

Since this is a preliminary study, no attempt was made to fractionate the phosphatides and the samples studied are the acetone insoluble fractions from the various seeds.

The seeds were ground thoroughly, then dried over night on a steam plate at about 95°C. They were then placed in an enclosed continuous extractor and extracted first with 95 per cent alcohol, then with ether, and finally again with alcohol. The alcohol and ether were then evaporated at a low temperature and the residue extracted with ether. The ether was then concentrated and acetone added in excess. The precipitate was then separated and twice more stirred up with ether and precipitated by the addition of acetone. Finally the precipitate was washed three or more times with acetone to insure complete removal of fats and the last traces of solvents allowed to evaporate, after which samples for phosphorus, nitrogen and hydrolysis were immediately weighed out so as to prevent any long contact with the air.

METHODS OF ANALYSIS.

Total phosphorus determinations were made by digesting the samples with HNO_3 and H_2SO_4 and the phosphorus determined according to the method of the Association of Official Agricultural Chemists (1).

Total nitrogen was determined in the usual manner as described under Kjeldahl method, official, in the methods of the Association of Official Agricultural Chemists (1).

Hydrolysis was effected by heating the samples with 200 cc of 10 per cent HCl and bubbling in SO_2 gas until the fatty acids formed a clear layer on top. The samples were then cooled and the liquid siphoned off from the solidified fatty acids. These were then washed several times with boiling water and finally dissolved in ether and filtered. Aliquots were then removed from the ether extract (kept at 20°C.) to be used for iodine determinations and to determine the amount of fatty acids in solution. The latter were determined by evaporation of the ether.

Iodine number. The Hanus method, according to the Association of Official Agricultural Chemists (1) was used.

ANALYTICAL RESULTS.

PHOSPHATIDE SAMPLES (Acetone Insoluble).

SOURCE	PERCENT NITROGEN	PERCENT PHOSPHORUS	IODINE NUMBER OF THE FATTY ACIDS
Wheat (Poole).....	0.99	1.26	81.49
Corn (Leaming).....	1.14	1.65	65.30
Soybeans (Manchu)....	0.70	0.495	92.48
Oats (Fulghum).....	1.61	0.535	88.80

SUMMARY AND CONCLUSION.

1. Phosphatide material prepared in the same manner from different seeds varies widely in its nitrogen and phosphorus content, which fact indicates the desirability of fractionating the material for further study.

2. There is considerable range in the iodine values showing that there are either varying amounts of unsaturated fatty acids in the seeds, or that different ones are present.

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LITERATURE CITED.

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1920. Official and Tentative Methods of Analysis, 1920 Ed.
2. DAUBNEY, C. G., AND MACLEAN, I. S.
1927. The Carbohydrate and Fat Metabolism of Yeast. IV. The Nature of the Phospholipins, BioChem. Jour. 21: 373–385.
3. GRAFE, V., AND MAGISTRIS, H.
1926. Zur Chemie and Physiologie der Pflanzenphosphatide., Biochem. Z. 176: 266–290.
4. LEVENE, P. A., AND ROLF, I. D.
1925. Plant Phosphatides. I. Lecithin and Cephalin of the Soybean. Jour. Biol. Chem. 62: 759–766.
5. ———.
1926. Plant Phosphatides. II. Lecithin, Cephalin and So-called Cuorin of the Soy Bean. Jour. Biol. Chem. 68: 285–293.
6. NJEGOVAN, V.
1911. Bertrage zur Kenntnis der pflanzlichen Phosphatide. Z. Physiol. Chem. 76: 1–26.
7. SCHULZE, E.
1908. Uber die zur darstellung von Lecithin und anderen Phosphatiden aus Pflanzen samen verwendbaren Methoden. Z. Physiol. Chem. 55: 338–351.

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