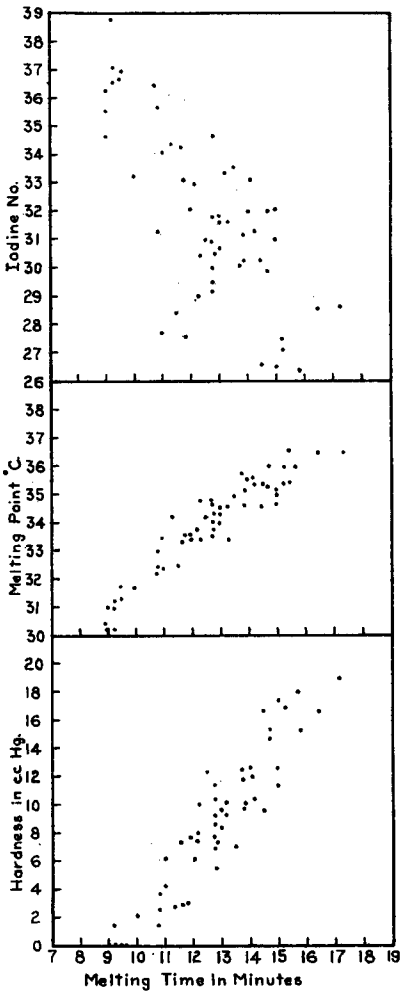


FIGURE 3

one end, are inserted through holes in the rubber stoppers.

The selection of 45° C. as the temperature at which the melting point determinations are carried out was made after comparing the results of tests in which the values of nine different fat samples were determined for each of several temperatures ranging from 36° C. to 60° C. A graphical representation of the results is shown in Figure 2. Each curve represents an individual sample; each point on the curve was located by plotting the temperature at which the bath was held against the melting time of the sample at that temperature. At temperatures above 45° C. the differences between the melting rates of the various samples were not great. Below 45° C. some samples did not form a clear transparent liquid on melting.

The index of hardness, determined by the method of Gallup (3), is expressed as the number of cc. of mercury required to force a plunger 5 mm. in diameter through a butter fat disk 6 mm. in thickness held at a temperature of 20° C. Values for duplicate samples varied as much as 10% from the mean; for this reason the average of either two or three samples was taken as the index.

Iodine number was determined by the Hanus method and melting point by the alcohol-water method; both are official A.O.A.C. methods (1).

### Origin of Butter Fat Samples

The butter fat samples used were extracted from butter churned from the cream produced by individual cows in the college herd. The cows were selected from four different breeds and were fed various rations; the butter fat samples, therefore, varied widely in physical properties, due probably to differences in composition. Determinations made on 61 samples were used in comparing the four constants studied by the authors.

### Discussion of Results

The graphs in Figure 3 show the results of plotting index of hardness, melting point and iodine number as ordinates against melting time as abscissa for each individual sample.

In Figure 3 hardness and melting point are each shown to be directly proportional to melting time; an inverse relationship, not as definite as the two previously mentioned, is shown to exist between iodine number and melting time. These correlations are not surprising, for the constants compared are measures of the same or similar properties. The scattered points obtained upon plotting iodine number against melting time indicate that a chemical property other than degree of unsaturation is a factor in determining the consistency of butter fat.

By use of these data, indexes of hardness and melting points of butter fat samples may be estimated from the melting time values.

### Summary

1. A method for the determination of the melting time of butter fat samples has been devised.

2. Definite correlations between hardness and melting time and between melting point and melting time have been shown.

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# SOYBEAN PHOSPHATIDES*

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### Introduction

THE present investigation is concerned with the phosphatides extracted from soybeans by hot ethyl alcohol. Evidence will be given on the existence of two types of phosphatides not related to cephalin and lecithin, as well as on the nature of the extracted lecithin complex.

In a previous investigation (1) it was found that the phosphatides which separate from the expressed oil upon standing, after purification by repeated solution in ether and precipitation by acetone, still contained much less phosphorus than that calculated from the soybean lecithin formula (2).

Formerly it was believed that lecithin was the only phosphatide in plants. Later, the presence of cephalin was shown. The soybean investigations of Levene and Rolf (2) and Suzuki and Yokoyama (3) indicated that the cephalin and lecithin are quite similar to the cephalin and lecithin isolated from egg yolk. Levene and Rolf also separated another compound insoluble in alcohol and acetone from soybean phosphatides, similar to cuorin. They believed that this substance resulted from the partial hydrolysis of ceph-

*The experimental data present in this paper were taken from the thesis submitted by Robert S. McKinney to the Graduate School of American University in 1936 in the partial fulfillment of the requirements for the degree of Doctor of Philosophy.

alin and lecithin. Schulze and Winterstein (4) have shown that phosphatides other than cephalin and lecithin can be extracted from plant material by ethyl alcohol at 60° C. Sullivan and Near (5) in their investigation of wheat germ phosphatides found that the nitrogen-phosphorus ratio was greater than 1:1 (cephalin and lecithin ratios) and they believed that a di-amino phosphatide was present in the mixture. Recent work of Rosenbusch (6) and Notthohm and Mayer (7) has shown that the phosphorus and nitrogen content of certain plant phosphatide fractions is notably less than the phosphorus and nitrogen content of egg lecithin, and the latter investigators found about 26% of carbohydrates (calculated as glucose) in wheat phosphatides.

In view of the growing importance of commercial "lecithin" preparations in this country derived principally from soybeans, it became of interest to make a further study of the phosphatides extracted from these beans at 60° C. by ethyl alcohol.

## Experimental

One hundred grams of soybean meal previously extracted with petroleum ether were extracted in a large Smalley tube for about four hours with hot 95% ethyl alcohol. The alcoholic extract was evaporated on the steam bath with the aid of a rapid stream of carbon dioxide gas. After cooling, the residue was dissolved in several volumes of ethyl ether and left overnight in the refrigerator. During this time the solution became turbid and a small precipitate separated. Neither the turbidity nor the precipitate could be removed from the solution by filtration or centrifuging. The solution was concentrated by heating on the steam bath. This treatment resulted in the formation of a gelatinous precipitate. Ether was again added, but stirring and warming for a considerable period of time did not redissolve the precipitate. After decanting the solution from the precipitate the latter was washed several times with ether and completely freed from solvent and moisture. It will be designated as the ether insoluble precipitate.

The solutions and washings were combined and concentrated to a small volume. To this solution several hundred cubic centimeters of acetone were added while stirring. The resulting brownish-yellow precipitate was filtered, washed and freed from solvent. It will be known as acetone precipitate No. 1. A small portion of it was reserved for analysis. The remainder was dissolved in a small quantity of ether and again precipitated by the addition of acetone. The solvent-free product will be called acetone precipitate No. 2. The two acetone solutions were evaporated and their respective residues numbered one and two. The phosphorus and nitrogen content of the five fractions were determined and the results are given in Table 1.

TABLE I

| Fractions | Phosphorus (Pregl) Per cent | Nitrogen (Kjeldahl) Per cent |
|---|---|---|
| Ether insoluble precipitate | 0.067 | 0.70 |
| Acetone precipitate No. 1 | 1.89 | 1.27 |
| Acetone soluble residue No. 1 | 0.48 | 0.12 |
| Acetone precipitate No. 2 | 2.19 | 1.06 |
| Acetone soluble residue No. 2 | 1.25 | 1.14 |

*Ether insoluble precipitate:* This fraction amounted to 3.1 grams and equivalent to 2.5% of the original soybeans, which contained about 20% of oil. The atomic ration of the phosphorus to nitrogen was 1 to 23. The small quantity of phosphorus indicates that most of the nitrogen present was not in a phosphatide combination. The substance gave a strong test for carbohydrates and a negative Biuret test for proteins. No further attempts were made to identify the source of the nitrogen in this fraction.

*Acetone precipitate No. 1:* This fraction, which weighed 3.3 grams, was equivalent to 2.6% of the original soybeans. The atomic ratio of phosphorus to nitrogen was about 2 to 3. This indicates that the fraction in addition to the monoamino compounds contained a considerable quantity of diamino phosphatides.

*Acetone soluble residue No. 1:* This fraction, which weighed 1.2 grams, amounted to 0.97% of the original soybeans. It gave no response to tests for either carbohydrates or proteins. The atomic ratio of phosphorus to nitrogen was about 2 to 1, which indicated the presence of a monoamino-diphosphatide. Being soluble in alcohol as well as in acetone differentiated it from cuorin (8) of heart muscle and from the compound separated by Levene (2) from crude soybean lecithin.

*Acetone precipitate No. 2:* The fraction, which weighed 2.7 grams, amounted to 2.2% of the original soybeans. The atomic ratio of phosphorus to nitrogen was 1 to 1, which indicated monoamino-mono-phosphorus compounds. The percentages of phosphorus and nitrogen, however, are about half of those required, for example, by the formula assigned to lecithin (2).

*Acetone soluble residue No. 2:* This fraction, which weighed 0.6 grams, was equivalent to about 0.5% of the original soybeans. The atomic ratio of phosphorus to nitrogen was 1 to 2, which indicated the presence of a diamino-phosphatide. Unlike sphingomyelin, this substance was soluble both in ether and acetone.

From the results of the analysis of these fractions, it would seem that soybeans contain, besides cephalin and lecithin, two other types of phosphatides, namely, monoamino-diphosphorus and diamino-monophosphorus compounds, which are extracted from the beans by 95% ethyl alcohol at 60° C.

In order to make a further investigation of the phosphatides, it was necessary to get them in a larger quantity. A 240 gram portion of ground soybeans was extracted as previously described, first by petroleum ether and then with hot ethyl alcohol to isolate the phosphatides. As previously described the ethyl ether soluble portion of the phosphatides was treated with a large volume of acetone. The resulting precipitate was separated, dissolved in a small quantity of ether and reprecipitated two more times. After removal of the solvent and moisture an analysis of the precipitate showed that it contained 1.965% of phosphorus, which is about half of that calculated for a mixture of cephalin and lecithin. The final acetone insoluble precipitate was only partly soluble in cold ethyl alcohol. The alcohol insoluble fraction, which was soluble in ether, after removal of all solvent and moisture contained 2.30% of phosphorus, and this is about half of the phosphorus content of cephalin. An excess of a saturated methyl alcohol solution of cadmium chloride was added to this ethyl alcohol solution of the phosphatides. The precipitate which formed was removed by filtration and discarded, as it had been previously investigated by Levene (2) and Suzuki (3). The residue which was obtained by the evaporation of the alcoholic filtrate was fractionated into three parts—(1) water soluble, (2) water insoluble, but

soluble in alcohol, and (3) insoluble in both water and alcohol. These fractions were found to be free from nitrogen and phosphorus compounds.

*Water soluble substances:* This fraction was dissolved in water and the cadmium precipitated with hydrogen sulphide. The cadmium sulphide was separated by filtration and air was passed through the cooled filtrate until the hydrogen sulphide was removed. The hydrochloric acid liberated by the hydrogen sulphide reaction with cadmium chloride was removed by means of silver carbonate in the usual manner. The solution which had been freed from silver salts gave a strong Molisch test for carbohydrates. A test made with Benedict's reagent indicated the presence of a considerable quantity of monosaccharides. The osazone was prepared according to the directions of Mulliken (9). The reaction began at once and within two minutes a heavy yellow crystalline precipitate had settled from the solution. This was filtered, washed with several small portions of cold water, and recrystallized from 50% ethyl alcohol. The dried osazone melted at 201° C. The rapid rate of the formation and precipitation of this osazone is noteworthy because no monosaccharide known to us reacts with this speed. The alkaline-iodine procedure as described by Lothrop and Holmes (10) was applied to a portion of the sugar solution and the oxidation which took place indicated that the sugar was an aldose. The amount of the oxidation, which was several times that occurring with glucose, indicated that this sugar is quite labile.

Analysis of the osazone was made by Dr. J. R. Spies of the Bureau of Entomology and Plant Quarantine, using the micro-Dumas method, with the following results:

Anal. Calcd. for $C_{19}H_{24}O_5N_4$ (388.2); Nitrogen 14.43 Found (1) 14.48; (2) 14.79. The analysis indicates that the water-soluble monosaccharide is probably a heptose.

Dr. C. B. Purvis, National Health Institute, Washington, D. C., measured the optical activity of the aqueous solution of this sugar and obtained a specific rotation of + 10.5°. The solution was evaporated to a small volume under reduced pressure at 50° C. The concentrated yellow syrupy solution was diluted with water and heated to 70° C. for two hours with decolorizing carbon. After filtration the solution was again concentrated as already described. The syrup was diluted with small quantities of water and methyl alcohol and placed in a desiccator under reduced pressure. After concentration the solution was set aside for several months, during which it was occasionally stirred, but no crystals formed. Diluting, concentrating, and allowing the solution to stand, as previously described, was repeated several times over a period of six months but without results. The solution was then completely evaporated and the residue weighed. It was dissolved in a measured quantity of water and now found to show no discernible optical activity. This would seem to indicate that mutarotation had occurred or that this very labile sugar had suffered some decomposition during the purification process.

*Alcohol soluble substances:* This water insoluble fraction gave a Molisch test which indicated the presence of carbohydrates. A molecular weight determination by the Rast camphor method made on the solvent free material gave a value of 787. On account of its insolubility in water the substance was believed to be a glucoside, as a polysaccharide having this molecular weight would probably be soluble in water. An experiment was made by mixing 40.5 mg of the substance with 50 cc. M/25 acetic acid (pH 4.5) which contained 20 mg of emulsin, and allowing the reaction to continue for three hours. During this time, a granular precipitate separated and the solution became clear. The precipitate was removed by filtration and the filtrate was boiled with Fehling's solution. The precipitated cuprous oxide was filtered, washed, and dried. It weighed 6.7 mg. The precipitate previously removed from the solution gave a strong test for carbohydrates, indicating that the emulsin in the three hours treatment had not completely hydrolized this beta-glucoside.

*Alcohol insoluble fraction:* This brown gum-like substance, after the complete removal of alcohol and moisture, melted at about 103°. It responded to no tests for carbohydrates. A portion was dissolved in cold 15% potassium hydroxide solution and upon shaking lathered profusely. Acidification with hydrochloric acid produced an amorphous precipitate. Another portion was used to make a molecular weight determination by the Rast method, which gave a value of 616. The neutralization equivalent of the substance was found to be 307.5, which indicated that it was a dibasic acid with a molecular weight of 615.

The difference between the molecular weights of the dibasic acid and the beta-glucoside is 172, which is approximately the molecular weight of the monosaccharide of the water-soluble fraction, when allowance has been made for the water of hydrollysis.

An explanation of the origin of these three compounds can now be offered. It is believed that the beta-glucoside was originally attached to the lecithin, and that the cadmium chloride used to precipitate the lecithin resulted in the hydrolysis of the combination. Although it is commonly understood that lecithin forms an addition product with cadmium chloride, the results of this investigation make it appear probable that a cadmium salt is formed, the cadmium combining with the phosphoric acid radical, and that the resulting hydrochloric acid causes the hydrolysis of the lecithin-beta glucoside combination. Furthermore, the hydrochloric acid would react with the free hydroxyl of the choline radical, giving a chloride, with the result that this compound would give analytical results like those for a lecithin-cadmium chloride addition compound.

It. appears that a part of the beta-glucoside underwent hydrolysis and this would account for the monosaccharide and dibasic acid found. If this assumption is correct the dibasic acid should contain a hydroxyl group in view of the fact that the glucosides upon hydrolysis give monosaccharides and hydroxy compounds. Consequently a portion of the dibasic acid was treated in the cold with an excess of acetyl chloride. After allowing the reaction to continue for one-half hour, the resulting product was isolated in the usual manner. It gave a saponification value of 310.4. The saponification equivalent is 180.7 and four times this value gives 723, the estimated molecular weight of the acetylated di-hydroxy dibasic acid. From this value it was calculated that the molecular weight of the original acid was 639, which is in fair agreement with the values prevously obtained by two other methods.

There are two positions in the lecithin molecule where the beta-glucoside with its two carboxyls and one free hydroxyl group could be attached. It could be joined by an ester linkage between the hydroxy and the third hydrogen of the

phosphoric acid in the lecithin molecule, or the beta-glucoside could be attached in a salt formation, being united by a carboxyl of its dibasic acid with the free hydroxyl of the choline group of the lecithin molecule.

The hydrolysis of the lecithin-beta-glucoside by cadmium chloride in an alcoholic solution at ordinary temperatures would indicate that the second assumption is probably correct, particularly as it would not be expected that an ester would be split under these conditions.

A weighed quantity of this lecithin complex was dissolved in neutral ethyl alcohol and titrated with a tenth normal alkali solution in the usual manner. Only one acid group reacted, and that slowly, with the alkali and this must belong to the phosphoric acid; otherwise the treatment with cadmium chloride, as previously mentioned would not have resulted in the hydrolysis of the lecithin-beta-glucoside combination. Also, it appears that the second carboxyl of the dibasic acid is linked with the second hydroxyl in a lactone formation.

## Summary

The fractionation of the hot ethyl alcohol extract of the oil-free soybean meal yielded: (a) nonprotein nitrogenous compounds containing no phosphorus; (b) monoamino-diphosphorus compounds; (c) diamino-monophosphorus compounds; (d) monoamino-monophosphorus compounds containing only half as much nitrogen and phosphorus as lecithin and cephalin.

These monoamino-monophosphorus compounds, which are insoluble in acetone, were fractionated into: (a) a compound insoluble in cold ethyl alcohol, about 50% of which is probably cephalin; (b) a compound soluble in cold ethyl alcohol, which seems to be a lecithin-beta-side appears to consist of a monosaccharide in combination with a dibasic dihydroxy acid.

It is suggested that the use of cadmium chloride in the separation of vegetable lecithin may result in the formation of a cadmium chloride salt of lecithin rather than an addition compound as formerly believed.

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## Application

Application for Referee Certificate. Mr. Vincent S. Skinner of Shilstone Testing Laboratory, Houston, Texas, has applied for a Referee Certificate reading on the analysis of all cottonseed products.

# ABSTRACTS

## Oils and Fats

***Edited by***
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**Pigments associated with the fatty tissues of plants and animals.** I. M. Heilbron and A. E. Gillam. *Nature* **139**, 612-5, 657-60 (1937). A review.

**An important source of fat: the fat of grape pomace.** J. Maille de Girves. *Bull. assoc. chim.* **54**, 140-6 (1937).—Grape pomace contains on the av. 8.45% of fatty material in the form of oil from the seeds and solid fats from the stems and pulp. It is green in color, has a distinct odor, d. 0.917, m. p. 51°, $n_D$ 1.4691, acid no. 26.80, sapon. no. 182, I no. 118, acetyl no. 28.6. It contains about 5% unsaponifiable matter. The mech. sepn. of the seeds from the pomace is difficult and costly, but a simple machine has been designed for removing the stems, the fat content of which is very low. Soap prepd. from the fat retains the green color, and may prove useful in the natural silk industry. In the case of a fat scarcity grape pomace may become an important raw material in wine-producing countries. (*Chem. Abs.*)

**The field of fats. XXIX. Thiocyanogen iodine and its addition to unsaturated fat acids.** H. P. Kaufmann and G. H. Oetringhaus. *Ber.* **69B**, 2670-6 (1936). I(SCN) was prepd. and its existence substantiated. The I(SCN) addn. product of elaidic acid was prepd. This when refluxed with alc. $NaHCO_3$, the I splits off to yield thiocyanostearic acid. Alc. KOH, removed the (SCN) group also and gave 10-oxostearic acid. Oleic acid reacted similarly but gave poor yields. Similar reactions occurred with erucic acid.

**XXX. Diene syntheses in the field of fats. .2. Composition of Chinese wood oil.** H. P. Kaufmann and J. Baltes. *Ibid.* 2676-9. The compn. of the oil could not be detd. from diene, I and (SCN) nos.

**XXXI. 3. Oiticica oil.** *Ibid.* 2679-83. The oil had a diene no. of 60.8, SCN no. 75.3; it contd. 70.0% α-licanic acid, 15.2% unsatd. nonconjugated acids, 9.9% satd. acids, 0.4% unsapon. 4.5% glycerol residue. (*Chem. Abs.*)

**Bromine value of some fatty oils.** Eiichi Yamaguchi, Takashi Matsumura and Tomo-o Takagi. *Waseda Applied Chem. Soc. Bull.* **13**, No. 4 (29), 7-11 (Abstracts (in English) 61) (1936).—In detn. of Br value by the method of P. Becker a ground-glass disk with rounded and turned-up edges was substituted for the glass plate in order to effect an even coating of oil film. Br values of olive, Tsubaki, peanut, rapeseed, Shoshi, tallow, lard, whale, sardine and liver oils agreed well with Wijs I values. Sesame, soy, linseed, whale, blubber and hydrogenated soy oils varied 2 to 6 units from the Wijs value, castor oil 7 to 8, polymerized sardine oil, 20, and Japanese and Chinese wood oils more than 40 to 70 units. (*Chem. Abs.*)

**The oxidation of butterfat. I. The catalytic effect of light.** V. C. Stebnitz and H. H. Sommer. *J. Dairy Sci.* **20**, 181-196 (1937). The authors' results