Factors Influencing the Determination of the Acetone Insolubles of Commercial Lecithins

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COMMERCIAL soybean lecithins are regarded as mixtures of phospholipides in soybean oil (4). These components can be separated by extraction with acetone. The insoluble portion (about 65% by weight) consists primarily of a mixture of lecithin, cephalin, inositol-phosphatides, and sugars (3). The acetone soluble portion (about 35%) contains soybean oil, fatty acids, sterols, and various hydrolysis products formed during the extraction and processing operations. This fortunate phenomenon of insolubility in acetone of the phospholipide constituents forms a basis for the analysis of commercial lecithins. Commercial products therefore list their phosphatidic contents as percentage of acetone insolubles (A. I. %).

Prior to 1946 it was assumed that producers of commercial lecithins were all using similar analytical procedures for the assaying of their products, but no definite Official Methods were available for the standardization of these techniques. This situation was remedied by the inclusion of "Analysis of Lecithin" in the "Report of the Committee on Analysis of Commercial Fats and Oils—October 1946" (2). The Lecithin Subcommittee at that time presented procedures and recommended for adoption, methods for the determination of moisture, benzene insolubles, and acetone insolubles. The Official and Tentative Methods of the American Oil Chemists' Society (1) have assigned tentative method numbers Ja 2-46, 3-46, and 4-46 to these procedures.

The method (Ja 4-46) proposed for the determination of acetone insolubles requires the removal of the acetone soluble portion by extraction with acetone, the removal of the solvent, and the drying and weighing of the residue. The quantity of phospholipides expressed as percentage of acetone insolubles (A. I. %) is calculated as the difference from 100% of the sum of the percentages of acetone solubles, moisture, and benzene insolubles. It is apparent that errors in the determination of any of these values are reflected in the resultant sum and difference. The question arises therefore as to why the insoluble residue from the acetone extraction of the original sample is not dried and weighed directly. This procedure would result in a saving of analysts' time in that it would eliminate the necessity of double weighings of extra beakers, the time involved in evaporating 100 ml. of acetone, and the accurate determination of moisture and benzene insolubles. In order to check the accuracy of a direct weighing procedure, data have been compiled from the analysis of many samples, using both the A.O.C.S. Method (1) and the modified direct weighing method.

Experimental

Most samples were run in duplicate and cover a range of concentration of acetone insolubles and moistures. The two sets of values (direct and indirect) were obtained on each sample. The procedure followed was that described in Tentative Method Ja 4-46 (1). Samples were weighed into centrifuge tubes which had been previously tared with a stirring rod. Three ml. of petroleum ether (A.O.C.S. Spec. H 2-41) (1) were added to dissolve the material and then 15 ml. of cold acetone added from a buret. After chilling the mixture in ice water, cold acetone (0-5°C.) was added to bring the liquid level to the 50-ml. mark. The tubes were centrifuged to clarify the solution, and the upper acetone layer was decanted into a previously dried and tared 250-ml. beaker. The residue was washed with another 50-ml. portion of cold acetone, centrifuged, and the clear acetone layer added to the first portion. The acetone was evaporated from both the solution in the beaker and the insoluble residue in the centrifuge tube on a steam bath. Beaker and tube were dried in an oven at 105 ± 2°C. for 1 hour, cooled to room temperature in a desiccator, and weighed. Percentage of acetone insoluble (A. I. %) was calculated as follows:

a) Acetone Insolubles % = A. I. × 100/Wt. Sample

b) Acetone Insolubles % = $100 - [(A.S. \times 100/\text{Wt. Sample}) + B + C]$

A.I. = Weight of Acetone Insoluble residue
A.S. = Weight of Acetone Soluble residue
B = % Moisture
C = % Benzene Insolubles

Lecithins analyzed in the course of this investigation were found to contain less than 0.15% benzene insolubles so, for most practical purposes, this figure can be eliminated from the calculations.

TABLE I

Comparative Analyses of Lecithins

Sample No.	Weight of Sample	Acetone Insolubles		Weight Accounted for (A.I. & A.S.)	H_2O	
		Direct Weighing	Indirect Weighing		Analyzed	By Difference
	gms.	%	%	*gms.*	%	%
1	2.0001	64.55	63.96	1.9903	1.0	0.49
	2.0001	64.25	63.86	1.9865	1.0	0.68
2	2.0003	63.64	63.45	1.9863	0.8	0.70
	2.0001	63.63	63.60	1.9829	0.8	0.86
3	2.0004	65.27	65.10	2.0004	0.2	0.00
	2.0001	65.19	65.02	2.0000	0.2	0.00
4	2.0174	44.08	43.35	1.8638	8.3	7.68
	2.0262	44.07	43.43	1.8702	8.3	7.80
5	2.0187	46.88	46.71	1.8378	9.0	9.05
	2.0477	47.14	46.97	1.8541	9.0	9.68
6	1.9997	63.81	63.51	1.9838	1.0	0.80
	1.9995	63.98	63.74	1.9824	1.0	0.85
7	2.0002	64.37	64.15	1.9846	0.7	0.78
	1.9998	64.44	64.32	1.9844	0.7	0.77
8	2.0003	64.79	64.56	1.9948	0.5	0.28
	2.0003	64.79	64.53	1.9953	0.5	0.25
9	2.0069	64.25	64.10	1.9999	0.4	0.35
	2.0046	64.24	64.08	1.9978	0.4	0.34
10	2.0001	52.52	51.65	1.8453	8.5	7.74
11	2.0000	52.44	51.90	1.7287	14.0	13.6

Data presented in Table I are values obtained from routine laboratory analyses on several plant and commercial lecithins. Comparison is made between values from the two methods for the determination of acetone insolubles. It will be noted that, while values by the direct weighing method are slightly higher than those of the "by difference" method (Ja 4-46), they are essentially in agreement and well within experimental precision for this procedure.

Comparison of initial sample weights with the sum of the weights of the A. I. and A. S. fractions also shows excellent agreement when the moisture content of the original sample is taken into consideration. If the assumption is made that the difference between the initial weight of the sample and the sum of the weights of the two fractions is due to the moisture content of the original, then this system will provide a faster and more accurate measurement of this value. The last two columns of Table I list values for the moisture content of the original lecithin as determined by distillation (Ja 2-46) and by subtracting from 100 the sum of the percentages of acetone soluble and insoluble residues. It is obvious from these data that the latter method will yield accurate values irrespective of the composition of the sample.

In order to compare the relative ease and rate of drying to uniform weight of the acetone soluble and acetone insoluble residues, two samples of each type of material were placed in a drying oven at 105 ± 2°C. Beakers and tubes were removed after 15-minute intervals, cooled in a desiccator, and weighed. Data obtained in this series indicated that the acetone soluble extract residue reached constant weight in about 15 minutes whereas 45 to 60 minutes is needed for the acetone insoluble residue.

During the major part of this work some concern was expressed over the possible solubility of the phosphatides in acetone, and it was thought that presaturation of the acetone wash solution with purified acetone insoluble materials might result in truer values. Evaporation of 100-ml. aliquots of acetone, filtered after contact with dry purified phosphatides, showed that the acetone had absorbed 0.046 grams/100 ml. at 35°C. and 0.031 grams/100 ml. at 5°C. Loss of 0.031 gram of acetone insoluble material due to solubility in the acetone wash solution would result in an error of 1.5% in the determination.

The error would be the same in both the direct and indirect weighing procedures. It is suggested therefore that acetone previously saturated with purified phosphatides be used for extraction and washing of the samples. The solution was prepared by allowing acetone to remain overnight in a refrigerator (4°C.) in contact with an excess of oil-free phosphatides. The cold acetone was filtered before use. Table II lists values obtained from analysis of several samples of lecithin using C. P. and presaturated acetone. In the case of analyses in which the presaturated acetone was used, no analogy can be drawn between the direct and indirect weighing methods as the acetone soluble extract residue would contain extraneous acetone insolubles.

It will be noted from these data that while values from determinations using presaturated acetone are increased by only about half the postulated amount, duplicates are in excellent agreeement. Values for acetone insolubles are raised 0.7 to 0.8% over those in which C. P. acetone was used. It has been realized

TABLE II

Effect of a Presaturated Acetone Extraction Solution on the Acetone Insolubles Determination

Sample No.	Acetone Washing Solution	Acetone Insolubles	
		Direct Weighing	Indirect Weighing
		%	%
1	C.P.	64.55	63.96
	C.P.	64.25	63.86
	A.I. saturated	64.98	.....
	A.I. saturated	64.97	.....
2	C.P.	63.64	63.45
	C.P.	63.63	63.60
	A.I. saturated	64.39	.....
	A.I. saturated	64.44	.....
6	C.P.	63.81	63.51
	C.P.	63.98	63.74
	A.I. saturated	64.66	.....
	A.I. saturated	64.81	.....
7	C.P.	64.37	64.15
	C.P.	64.44	64.32
	A.I. saturated	65.15	.....
	A.I. saturated	65.10	.....

that the quantity of petroleum ether used to dissolve the original sample might also contribute to loss of acetone insolubles by effecting the solubility of these materials in the acetone washes. Previous experience in this laboratory indicated that reclaimed acetone from distillation of the acetone soluble solution always gave lower values when reused in the acetone insoluble determination than fresh acetone. Solubility of acetone insoluble compounds in the reclaimed acetone was thought to be due to contamination with petroleum ether. In order to test this premise a series of samples was analyzed, using different initial amounts of petroleum ether to dissolve the sample. Data are presented in Table III.

TABLE III

Effect of Petroleum Ether Solvent on the Acetone Insolubles Determination

Amount of Petroleum Ether	Acetone Insolubles		Difference (1)-(2)
	Direct Weighing (1)	Indirect Weighing (2)	
ml.	%	%	%
0	65.14	64.66	0.48
1	64.89	64.34	0.55
2	64.62	64.12	0.50
3	64.14	63.49	0.65

It will be noted from the above data that the increase in the amount of petroleum ether used for putting the sample into solution is inversely proportional to the decrease in acetone insoluble value. Difference between values by the two methods remain constant irrespective of the quantity of petroleum ether used.

Table IV presents data accumulated from analysis of three commercial lecithins. Comparison is made between the A.O.C.S. Tentative Method Ja 4-46 and a modified method in which petroleum ether is eliminated as the solvent, acetone presaturated with acetone insoluble compounds used as the extractant, and the acetone insoluble residue weighed directly.

The data in Tables III and IV indicate that the elimination of factors responsible for solubilizing acetone insoluble materials will result in higher, more uniform, and truer values in the assaying of lecithin. The modified method is responsible for increases of 1.23 to 1.52% in the acetone insoluble values of these samples.

TABLE IV

Comparison of Tentative A.O.C.S. and Modified Method for the Determination of Acetone Insolubles

Sample No.	Official Tentative A.O.C.S. Method		Modified Method No Pet. Ether, A.I. Sat. Acetone Direct Weighing
	Direct Weighing	Indirect Weighing	
	%	%	%
A	62.56	62.66	63.93
	62.71	62.85	64.09
	63.12	62.83	64.33
B	64.15	64.43	65.32
	63.87	64.04	65.24
	63.98	63.64	65.24
C	65.03	65.46	66.31
	64.83	65.22	66.62
	65.14	65.04	66.62

Attempts were made to recombine dry acetone insolubles, soybean oil, and soybean fatty acids to produce synthetic commercial lecithins of known composition. Difficulty was encountered in reblending these materials and the final products did not truly resemble normal commercial lecithins. When these synthetic mixtures were analyzed by the above methods, differences between the calculated and found values for acetone solubles and acetone insolubles varied from 1.72 to 2.64%. It is unfortunate that synthetic mixtures could not be prepared to test the accuracy of the two procedures. Drying and heating of the acetone insoluble residue apparently causes changes in solubility through resinification and structural changes.

Summary

Data are presented from numerous analyses of commercial lecithins for the comparison of values obtained from the determination of acetone insolubles by a direct weighing method against those by the A.O.C.S. Tentative Method Ja 4-46. Discrepancies introduced into these methods by the use of petroleum ether as a solvent and those due to the solubility of acetone insoluble compounds in the acetone extract and wash solution are evaluated. A proposal is made for the consideration of a direct weighing procedure for the acetone insolubles content of commercial lecithins whereby petroleum ether is eliminated, the acetone extract solution saturated with pure phosphatides before use, and the insoluble residue dried and weighed.

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Formation of Trans Isomers During the Hydrogenation of Glyceride Oils

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WITH the development of the infrared spectrophotometric method for the determination of trans isomers in fats (1, 2), workers in the field have shown renewed interest in the natural occurrence and synthesis of these high-melting glycerides (3, 4, 5, 6, 7). Lemon (8) developed curves showing the formation and disappearance of trans double bonds during the hydrogenation of vegetable oils. The present paper is in part an extension of this study to the animal fats.

TABLE I–

Hydrogenation—Soybean Oil

200°C.								
PSI H_2	% Ni. Cat.	Time Min.	I No.	FAC M.P.	Soft. Pt.	% Lino-leic	% Lino-lenic	% Trans isomers
10	1	240	78	96	93	1.28	0.00	54
225	0.05	38	76	118	98	5.37	0.13	44
700	0.05	3	79	122	109	12.30	0.64	30

Conditions which favor selective hydrogenation of glyceride oils also favor the development of trans isomers. For example, Table I shows the effect of using high hydrogen pressure for the partial hydrogenation of soybean oil (9). Although all three of these samples were hardened to approximately the same iodine number, the first contains the largest percentage of trans double bonds.[1] It also has the least number of polyunsaturated chains, the lowest melting point, and the shortest spread between melting and softening points. The third sample was very firm, as the result of its relatively high content of fully saturated fatty acid chains.

TABLE II

Hydrogenation—Soybean Oil

200°C.								
PSI H_2	% Ni. Cat.	Time	I No.	FAC M.P.	Soft. Pt.	% Lino-leic	% Lino-lenic	% Trans isomers
1100	0.05	30 sec.	100	135	115	28.4	3.5	18
550	0.05	45 sec.	100	130	111	27.8	3.2	19
225	0.05	80 sec.	100	123	106	25.3	2.8	25
30	0.05	8 min.	99	....	....	23.5	1.2	32
0	0.05	57 min.	99	83	....	21.3	1.0	39

Table II illustrates further the direct relationship which exists between selectivity of hydrogenation and formation of trans isomers (9). As the hydrogen gas pressure is increased, the content of polyunsaturates at a given iodine number increases, but the percentage of trans linkages formed decreases. The last sample of this series was hardened by bubbling hydrogen through the oil while maintaining a vacuum of 28 inches. Notice that it actually melts higher than the sample hardened at 30 PSI, which was too soft to be

[1] The contents of trans isomers in these samples and in all others described in this paper were determined by measuring absorptions in chloroform at 10.3 microns in the infrared (1). These absorptions were compared with that of pure elaidic acid, prepared by the isomerization of oleic acid with selenium (10, 11).