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## Determination of Nitrogen in Vegetable Oils<sup>1</sup>

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THE quality of edible oils has improved steadily during recent years. In seeking further improvement, new means of measuring and testing oil are needed in order to evaluate the effect of laboratory and plant processing procedures. It has been recognized for some time that nitrogen compounds occur in nature along with glycerides and accompany the oil as it is separated and processed for use. The removal of these nitrogen compounds and other non-glyceride materials is a major purpose of present-day refining practice. Free acids in oils can be determined, even in very small amounts, with relative ease and accuracy and this determination is now in use to control refining operations. It would be of material assistance if a similar satisfactory method for measuring the nitrogen content of vegetable oils could be found.

The difficulty of determining nitrogen in vegetable oil results from the exceedingly small amounts of nitrogen present. The efficiency of present refining methods is so high that the amount of nitrogen left in oil after treatment is of the order of 1 to 10 mg. of nitrogen per kg. of oil, or 1 to 10 p.p.m. In attempting to use the conventional Kjeldahl procedure for measuring such small quantities of nitrogen, the well known difficulty of measuring small differences between large numbers is encountered. Thus, with the A.O.C.S. Official Method Aa-5-38, using 25 ml. of

concentrated sulfuric acid and appropriate quantities of other reagents, about 0.1 mg. of nitrogen is found in the blank even though the best commercial A.C.S. grade reagents are used. Since 25 ml. of acid will decompose properly only about 1.0 gram of oil, the nitrogen present in the sample will be from 0.001 to 0.01 mg. while about 0.1 mg. of nitrogen will be present from the reagents used. Modifications of the Kjeldahl method, such as increasing the weight of the oil sample, using very dilute acid and alkali, or adopting micro-Kjeldahl techniques, cannot change the unfavorable ratio between the nitrogen content of the reagent blank and the amount of nitrogen to be determined.

The Nessler reagent provides a means of determining, with somewhat limited accuracy, extremely small amounts of nitrogen in the form of ammonia. Here again, however, the conversion of nitrogen compounds in the oil to ammonia by the usual Kjeldahl procedure would introduce so much extraneous nitrogen that Nesslerization would not be successful.

This paper describes the development and application of a method for concentrating and separating the nitrogenous compounds, and gives in detail the precautions required. Since most of the nitrogen compounds expected to be present would be basic in character, conditions of acid hydrolysis were sought under which the nitrogen compounds present in a large amount of oil could be converted to products soluble in a relatively concentrated aqueous phase while at the same time the major portion of the glycerides would remain insoluble.

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In broad outline the procedure developed was to hydrolyze an alcoholic solution of the sample with concentrated hydrochloric acid on a steam bath. After hydrolysis, ether and water were added and the aqueous phase transferred as completely as possible to a Kjeldahl digestion flask. The usual macro-Kjeldahl digestion and distillation (1) were carried out with the volume of distillate limited to a total of 90 ml. This was made up to volume and aliquots were measured visually by a standard Nessler procedure (2), or titrated directly with standard acid.

Visual comparison of the Nessler tubes and the small quantities of nitrogen measured did not permit a high degree of precision. Results which checked each other within 10% were regarded as satisfactory.

### Experimental

Since phosphatides would be expected to comprise a major portion of the nitrogen compounds in oil, the effect of acid hydrolysis under various conditions was studied on crude soybean lecithin. As shown in the first part of Table I, the ammonia in the distillate was

TABLE I.  
Studies on Hydrolysis of Crude Soybean Lecithin.

Treatment	Nitrogen found
Titrated:	%
Without hydrolysis, Kjeldahl digestion only.....	0.74
Without hydrolysis, Kjeldahl digestion only.....	.75
Hydrolysis, 8 hr., 1 ml. HCl.....	.69
Hydrolysis, 8 hr., 1 ml. HCl.....	.70
Hydrolysis, 8 hr., 1 ml. HCl, 25 grams oil*.....	.72
Hydrolysis, 8 hr., 1 ml. HCl, 100 grams oil*.....	.72
Hydrolysis, 8 hr., 5 ml. HCl, 100 grams oil*.....	.71
Hydrolysis, 8 hr., 5 ml. HCl.....	.73
Hydrolysis, 8 hr., 5 ml. HCl.....	.74
Hydrolysis, 8 hr., 5 ml. HCl, oil phase.....	.013
Nesslerized:	
Without hydrolysis, Kjeldahl digestion only.....	.74
Without hydrolysis, Kjeldahl digestion only.....	.73
Hydrolysis, 2 hr., 5 ml. HCl.....	.72
Hydrolysis, 2 hr., 5 ml. HCl.....	.71
Hydrolysis, 2 hr., 5 ml. HCl, 100 grams oil*.....	.70
Hydrolysis, 2 hr., 5 ml. HCl, 100 grams oil*.....	.71

Note: Sample weight approximately 2 grams for direct digestion, 5 grams for hydrolyses.

\* Oil added to crude lecithin before hydrolysis.

measured by acid titration, while in the latter part samples were Nesslerized. The Kjeldahl digestion was made only on the aqueous phase except in one instance where the oil phase was digested and found to contain less than 2% of the total nitrogen. Table I shows that even in the presence of 100 grams of oil, hydrolysis with from 1 to 5 ml. of concentrated acid on the steam bath for 2 to 8 hours will quantitatively convert the nitrogen of phosphatides to water-soluble form.

The conditions for hydrolysis were then studied on samples of crude extracted and screw-pressed soybean oils. Table II shows that on 100-gram samples, fairly complete recovery of nitrogen is obtained when crude extracted oil is hydrolyzed for 2 hours with 5 ml. of concentrated hydrochloric acid. In crude screw-pressed oil more than 80% of the nitrogen is recovered. Apparently 1 ml. of acid for this same length of time is not sufficient, but if the time is lengthened to 8 hours, 1 ml. may be sufficient. The poor results obtained on direct digestion of the crude extracted oil in Table II were attributed to the difficulty of obtaining representative samples from the cloudy, unfiltered oil.

In carrying out this work dealing with small quantities of nitrogen, several details which reduced the

TABLE II.

Nitrogen in Crude Soybean Oil. Comparisons of Direct Digestion With Hydrolysis. Study of Time of Hydrolysis and Amount of Acid.

Weight of oil	Hydrolysis time	Acid used	Nitrogen found
Grams	Hours	Ml.	%
Crude extracted:			
100.....	2	1	0.019 .019
100.....	8	1	.026
100.....	2	5	.025 .025 .025 .026
100.....	5.5	5	.023
100.....	6	5	.026
100.....	8	5	.024
100.....	2	6	.024
1.....	....*	....*	.033 .027 .023
Crude screw-pressed:			
100.....	2	5	.019 .019 .023
1.....	....*	....*	.022 .021

\* Direct digestion.

blank and improved reproducibility were discovered. For example, nitrogen-free water left uncovered in the laboratory quickly became contaminated. At first, the acid hydrolysis on the steam bath was carried out with rubber stoppers and air condensers. The nitrogen in the blank was appreciably reduced when the rubber stopper was replaced by a ground-glass joint. Nitrogen impurities in the reagents used in the Kjeldahl method could be greatly reduced by combining the potassium sulfide and sodium hydroxide solutions, adding nitrogen-free water, and distilling off an equivalent amount of water. The combined alkali-sulfide reagent was then added to the acid-digested sample before distillation. This procedure lowered the blank from 0.16 mg. to 0.11 mg. of nitrogen. The distillations were made in the customary banked racks with metal condensers. All of the ammonia must be kept in a volume of less than 100 ml. to avoid unnecessary dilution. This permitted a volume of distillate of about 75 ml. Before starting distillation, nitrogen-free water was always distilled through the condensers until no color could be found by the Nessler reagent in the distillate.

A check was made on completeness of nitrogen recovery. After finishing the distillation of a sample, two successive portions of 100 ml. each of nitrogen-free water were distilled through the same condensers and the nitrogen determined separately in each portion. Table III shows that small but measureable amounts of nitrogen are not recovered by distillation of 75 ml. The amount not recovered is, however, less than 1% of the sample used except in the case of the blank and is proportional to the nitrogen originally present in the sample. The distillation of 75 ml. was regarded as adequate.

The final method adopted as satisfactory for determining nitrogen in oil is as follows:

**Apparatus.** Digestions and distillations are made in 800-ml. Kjeldahl flasks on conventional multi-unit, electrically heated, digestion and distillation equipment.

**Reagents.** The reagents are of ACS grade or special low-nitrogen reagents when available on the market.

TABLE III.  
Nitrogen Retention in Kjeldahl Stills.

Sample No.	Total nitrogen	Nitrogen recovered			
		First 100 ml.	Second 100 ml.	Total	
	Mg.	Mg.	Mg.	Mg.	%
1.....	19.00	0.128	0.012	0.140	0.74
2.....	24.00	.144	.028	.172	.72
3.....	25.00	.144	.024	.168	.67
Blank.....	.12	.016	.000	.016	13.3

All distilled water is purified by distilling in an all-glass apparatus from dilute sulfuric acid.

**Procedure.** A sample of oil is weighed, containing, if possible, at least 0.1 mg. of nitrogen, with 5 mg. of nitrogen a desirable amount. If this quantity is 2 grams or less, the usual Kjeldahl digestion may be completed, using about 25 ml. of concentrated sulfuric acid for each gram of oil. For larger amounts of oil, a preliminary acid hydrolysis is necessary. For 100 grams of oil, a 1-liter, round-bottom flask with standard taper joint is used and there is added a total of 5 ml. of concentrated hydrochloric acid and approximately 200 ml. of ethyl alcohol. This mixture is refluxed on the steam bath for 2 hours with an air condenser. After the mixture has cooled, 100 ml. each of water and ethyl ether are added, the mixture shaken, and the aqueous phase removed with a separatory funnel. The oil phase is washed successively three times with 50 ml. of water, and the combined aqueous portion washed once with 100 ml. of petroleum ether. The aqueous portion is transferred to a Kjeldahl flask, 10 ml. of sulfuric acid added, and heated until charring begins. The remainder of the acid (a total of about 30 ml. for 100 grams of oil), potassium sulfate, and mercury are then added and digestion completed. Heating is continued for 45 minutes after the solution clears. After dilution with 150 ml. of water, the alkali-potassium sulfide mixture

TABLE IV.  
Nitrogen Content of Crude Extracted Soybean Oil After Various Treatments (100-Gram Samples Except Where Otherwise Indicated).

Treatment	Not water washed	Water washed		
		Once	Twice	Three times
	%	%	%	%
None.....	0.019	0.00044	0.00026	0.00019
	.018	.00043	.00022	.00019
Refined and bleached.....	.0010 <sup>1</sup>	.00016 <sup>1</sup>	.00014 <sup>1</sup>	.00010 <sup>1</sup>
	.0008 <sup>1</sup>	.00008 <sup>1</sup>	.00010 <sup>1</sup>	.00008 <sup>2</sup>
Refined, bleached, and deodorized.	.00007	.00003	.00004	.00005
	.00007	.00006	.00002	.00002

<sup>1</sup> 50-gram sample. <sup>2</sup> 25-gram sample.

is added and the sample distilled into 15 ml. of water until the total volume of distillate is 90 ml. This is made to 100 ml. in a volumetric flask and portions removed for Nesslerization. The procedure of the American Public Health Association is followed.

## Discussion

The application of this method for nitrogen determination is illustrated in Tables IV and V. In Table IV, the nitrogen content of a soybean oil is followed as the oil undergoes standard laboratory refining, bleaching, and deodorizing treatments. Portions of the sample were water-washed before refining with a consequent marked reduction in nitrogen content. In 100-gram samples, 0.1 mg. of nitrogen represents 0.0001%, and from Table IV it is apparent that the method loses much of its precision when less than 0.1 mg. of nitrogen is present. No great significance can be attached to the differences in nitrogen content shown by any of the deodorized samples. Better results would have been obtained with larger samples of soybean oil, but sufficient amount of the sample was not available for further determination. Table V

TABLE V.  
Nitrogen Content of Various Commercial Edible Oils.

Oil	Sample weight	Nitrogen found	
		Mg.	%
	Grams		
Soybean.....	100	0.01	0.00001
	400	.09	.00002
Cottonseed.....	100	.03	.00003
	500	.14	.00003
Corn.....	100	.13	.00013
	100	.14	.00014
	500	.56	.00011 <sup>1</sup>

<sup>1</sup>An additional 0.00004% of nitrogen was found by rehydrolyzing the oil phase.

shows the nitrogen content of soybean, cottonseed, and corn oils sold as salad oils on the open market. Nitrogen is present and can be measured even in these highly refined oil. As shown in Table V, 2 hours may not be sufficient time to completely hydrolyze and recover all the nitrogen in samples of 500 grams or over. The possibility of using portions of the aqueous extract for the determination of phosphorus has not been completely investigated.

## Summary

Hydrolysis with alcoholic hydrochloric acid quantitatively converts the nitrogen of crude soybean lecithin to a water-dispersible form. This alcoholic-acid hydrolysis also converts the nitrogen in large amounts of crude soybean oil to a water-dispersible form which can then be separated and digested with a minimum amount of sulfuric acid by the Kjeldahl procedure. With careful control of both reagents and blank determinations, as little as 0.1 mg. of nitrogen can be measured with a precision of about 10%. The details of the method are given and it is applied to a series of soybean oils after various treatments and to three kinds of salad oils.

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