

THE DETERMINATION OF NITROGEN IN ORGANIC COMPOUNDS.

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THE Kjeldahl method of decomposing organic matter by digestion with strong sulphuric acid having been subjected to so many modifications, a series of comparative determinations was undertaken to find, if possible, the most advantageous form of the process and to test its applicability to different types of organic substances. The results of the work upon food materials and physiological products were recently published.¹ A similar study with a few other natural products and some pure compounds has since been concluded.

The digestion of the sample with sulphuric acid was carried out in different ways, the usual method of distillation being used throughout. Usually 0.5 or 1 gram² of the substance, according to its nitrogen content, was taken for each determination and transferred to the flask by means of pure filter-paper. About 0.5 gram of cellulose was thus introduced with the sample.

The methods of digestion, compared in the series with pure compounds, were:

(1) The Kjeldahl-Willfarth method as adopted by the Association of Official Agricultural Chemists.³ The sample was treated with sulphuric acid and mercury, boiled until colorless and potassium permanganate added in small portions until the solution remained permanently colored.

(2) As in (1), except that the potassium permanganate was omitted.

(3) As in (2), except that the boiling was continued for two hours after the liquid had become colorless.

(4) The Gunning process as adopted by the Association of Official Agricultural Chemists.⁴ About 20 cc. of sulphuric acid and 10 grams of potassium sulphate were used and the boiling continued until the solution became colorless.

(5) As in (4), but boiled two hours longer.

¹ This Journal, 26, 367.

² Of the peppers and tobacco mentioned below, portions of 1 to 2 grams were taken.

³ U. S. Dept. Agr., Bureau of Chemistry, Bull. 46.

⁴ *Loc. cit.*

PERCENTAGES OF NITROGEN FOUND BY MODIFICATIONS OF KJELDAHL, METHOD.

Substance.	Theoretical.	WILLFARTH MODIFICATION.				GUNNING MODIFICATION.			DYER MODIFICATION.			
		Boiled till colorless.				Boiled till colorless.			Boiled till colorless.			
		Time of boiling. Hrs. Min.	KMnO ₄ used. (1) ¹ .	KMnO ₄ omitted. (2).	Boiled 2 hours longer. (3).	Time of boiling. Hrs. Min.	(4).	Boiled 2 hours longer. (5).	Time of boiling. Hrs. Min.	(6).	Boiled ½ hour longer. (7).	Boiled 2 hours longer. (8).
Benzamide.....	11.59	3 : 0	11.57	11.41	11.50	4 : 0	11.45	11.54	1 : 0	11.54	11.58	11.58
Betaine hydrochloride	9.15	1 : 0	{ 7.11 9.02	6.20	8.92	1 : 0	9.11	9.13	0 : 15	7.42	9.12
Acetanilide	10.32	1 : 35	10.14	10.04	10.19	1 : 45	10.11	10.24	0 : 50	10.07	10.31	10.31
Naphthylamine	9.80	3 : 40	9.45	9.38	9.49	2 : 20	9.57	9.62	0 : 40	9.66	9.74	9.75
Diphenylamine	8.30	2 : 30	7.85	7.66	7.76	3 : 0	7.74	7.84	0 : 45	7.75	8.30	8.32
Cinchonine sulphate..	7.75	1 : 00	7.64	5.00	7.56	2 : 15	6.65	6.91	0 : 30	6.03	6.57	7.65

¹ The number in parenthesis at the head of each column is that of the method, outlined above, by which the percentages of nitrogen shown in that column were found.

(6) The Dyer modification¹ of Gunning's method. The substance was heated with sulphuric acid and mercury until frothing subsided, after which 10 grams potassium sulphate were added and the solution boiled until colorless.

(7) As in (6), but boiled one-half hour after colorless.

(8) As in (6) and (7), but boiled two hours after colorless.

The results obtained are shown in the accompanying table.

These results taken in connection with those previously reported show that it is not safe in the analysis of any class of nitrogenous substances to stop the digestion as soon as the liquid becomes colorless, as is authorized by the methods of the Association of Official Agricultural Chemists.

The association recommends² the Arnold-Wedemeyer modification of the Gunning method for the determination of nitrogen in peppers in order to insure complete ammonification of the alkaloid nitrogen present. The Arnold-Wedemeyer process differs from that of Dyer simply in that the former uses mercury, copper and potassium sulphate in the digestion, while the latter omits the use of copper, thus reducing the number of reagents and securing a colorless solution. In order to find whether the use of copper gives a higher result, the two processes were compared on black and white pepper, tobacco and two of the cinchona alkaloids with the following results:

Sample	Percentage of nitrogen found.	
	Dyer process (using no copper). Digestion continued 2 hours after colorless or 2½ to 3 hours in all.	Arnold-Wedemeyer process (using copper). Digestion continued 3 hours.
Black pepper	2.32	2.31
White pepper	1.83	1.87
Tobacco	3.14	3.14
Cinchonine sulphate	7.65	7.64
Impure quinine	8.28	8.22

Taking these results in connection with those obtained by Dyer³ at atropine, morphine, quinine, strychnine and pyridine, it seems safe to conclude that the use of copper is not necessary either in the case of pepper or of other substances containing nitrogen in the form of alkaloids.

¹ *J. Chem. Soc.*, 67, 811 (1895).

² U. S. Dept. Agr., Bureau of Chemistry, Bull. 65.

³ *Loc. cit.*

In every case thus far studied the conversion of nitrogen to ammonia had appeared to be complete when the sample was boiled for two hours after colorless, or about three hours in all, with sulphuric acid, mercury and potassium sulphate. Very recently, however, comparative determinations made by one of us (M. J. F.) on some samples of bituminous coal yielded higher results when the solutions, obtained as just described, were further treated with potassium permanganate. The average results for six similar coals were: 1.40 per cent. nitrogen by boiling with sulphuric acid, mercury and potassium sulphate for three hours; 1.59 per cent. by treating in the same manner and then adding permanganate. One or two of the samples were treated by the Arnold-Wedemeyer method. The results were no higher than those obtained by the Dyer method without permanganate.

SUMMARY OF RESULTS.

The experiments here described, taken in connection with other work recently published, lead to the following conclusions:

(1) So many substances yield nitrogen compounds which are colorless in hot sulphuric acid that the disappearance of color from the digesting solution gives little indication of the extent to which ammonification has taken place. When either mercury or potassium sulphate was used alone it was always necessary to digest the solution after it had become colorless in order to secure all of the nitrogen as ammonia. The temperature of the boiling solutions and the total time of boiling are fully as important as the disappearance of color.

(2) Proteids and simple amides, even of aromatic acids, yield their nitrogen as ammonia without especial difficulty. When treated by either the Willfarth or the Gunning method, about 98 to 99 per cent. of the total nitrogen present has usually been converted to ammonia by the time the solution becomes colorless. By continuing the boiling for at least two hours longer or by the careful use of potassium permanganate, most of the remaining nitrogen can be obtained. The complete ammonification of the nitrogen of proteids and amides is more readily and certainly accomplished by the use of both mercury and potassium sulphate, as recommended by Dyer, the boiling being continued for at least one-half hour after the solution becomes colorless, or for at least one hour from the time the potassium sulphate is added.

(3) Aromatic amines also yield colorless solutions before all of the nitrogen has reached the form of ammonia. The amount of nitrogen lost by using mercury or potassium sulphate alone and stopping the digestion as soon as the solution becomes colorless, is greater with naphthylamine than with aniline (acetanilide), and greater with diphenylamine than with naphthylamine. Boiling for two hours longer with either of the reagents alone does not always secure complete ammonification. This is most readily accomplished by using both mercury and potassium sulphate, as in the case of proteids.

(4) Many alkaloids and certain of the so-called "nitrogenous extractives" of plant and animal substances, such as betaine and creatine, yield solutions in sulphuric acid which readily become colorless on boiling after addition of mercury, but less readily yield their nitrogen as ammonium sulphate. In such cases entirely erroneous results are obtained, if ammonification is assumed to be complete when the solution becomes colorless. The use of permanganate to complete the decomposition does not always yield correct results. Potassium sulphate alone sometimes gives much better results than are obtained by the use of mercury alone, probably because the boiling-point of the digesting liquid is higher and a longer time is usually required to obtain a colorless solution. Boiling for one hour with both mercury and potassium sulphate appears to be sufficient for the "nitrogenous extractives," but not for all alkaloids.

(5) Very resistant substances such as alkaloids, coal, etc., should be boiled with sulphuric acid, mercury and potassium sulphate for at least two hours after the solution becomes colorless, and for not less than three hours in all. No advantage has been found in the use of copper in addition to the reagents mentioned, but with the coals tested slightly higher results were obtained by the careful use of permanganate at the end of the three hours' boiling. On account of the danger of loss of nitrogen through too violent a reaction of the permanganate, it is advisable to make the determinations in duplicate, using permanganate in one case and omitting it in the other.

(6) For all samples in which the nitrogen exists essentially as proteids or albuminoids and related compounds, including the called "nitrogenous extractives," and other amides and amino-compounds, it is only necessary to use mercury and potassium sul-

phate, as recommended by Dyer, and to boil for one-half hour after the solution becomes colorless, or in the case of quickly decolorized samples, for at least one hour from the time the potassium sulphate is added.

(7) The Dyer modification appears to combine the advantages of the other methods (Kjeldahl-Willfarth, Gunning and Arnold-Wedemeyer) now recommended by the Association of Official Agricultural Chemists for the determination of nitrogen in organic compounds.

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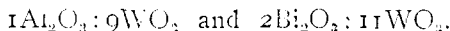
DERIVATIVES OF COMPLEX INORGANIC ACIDS.

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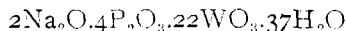
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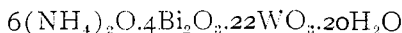
IN A previous communication¹ from this laboratory, attention was called to complexes of certain sesquioxides with tungstic acid; for example, the aluminico-tungstates and the bismuthico-tungstates, described by Balke and Smith, in which there exist the following ratios between sesquioxide and tungstic acid:



The literature on complexes reveals the fact that the sesquioxides which, in the past, have been combined with tungstic and molybdic acids are those of phosphorus, arsenic and antimony, corresponding to the chloride type RCl_3 . It might well be expected that bismuth oxide, corresponding to the same chloride type, would enter into combination with tungstic acid in ratios similar to those found among the complex phosphorico-, arsenico- and antimonico-tungstates and molybdates. This surmise proved correct, for among the many derivatives of complex acids containing those oxides, synthesized by Wolcott Gibbs, appear the analogues of phosphorus, more particularly the compound



with which



¹ This Journal, 25, 1229.