Report on the Determination of Total Nitrogen in Tobacco[1]

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The time-honored Kjeldahl method for the determination of nitrogen has been under continuous study and revision since its inception in 1883. Recent studies and action by the Association of Official Agricultural Chemists has resulted in the adoption of a single official method in which mercury or its oxide is the only catalyst. Potassium sulfate (or anhydrous sodium sulfate) is added to increase the digestion temperature and thus reduce the digestion time required particularly for refractory compounds such as the alkaloids present in tobacco.

The Commitee decided that the A.O.A.C. method should be tested to determine its applicability to the analysis of tobacco. A detailed copy of the procedure was sent to each collaborator. The only deviation from the official procedure as modified by Davis and Miles were: 1. The amount of acid specified was related to the sample weight (35 ml. for 1 g., 37.5 ml. for 1.5 g. and 40 ml. for 2 g. of tobacco). 2. The mixed indicator methyl red-methylene blue could be used instead of methyl red. 3. The digestion time was one hour after clearing. 4. The analysts were given an option of using boric or standard acid for absorbing the ammonia.

Each collaborator was asked to analyze five tobacco samples in duplicate and report all values obtained on the "as received" basis. The samples were one each of burley, flue-cured, cigar filler, Maryland and Turkish tobacco which had been ground to pass a one millimeter sieve, equilibrated in air, mixed and sealed in screw-capped glass jars. Refrigerator tape was placed around the rim of the jar caps to reduce the possibility of change in moisture content between the time of bottling and analysis.

In addition to analyzing the samples by the A.O.A.C. method, the colaborators were asked to determine the total nitrogen in the samples by the procedure they normally used in their laboratory, to report the results of duplicate analyses and to submit a detailed description of the procedure used.

This was done to permit comparison of the results by the A.O.A.C. method with those obtained by different procedures which the colaborators found suitable for the analysis of tobacco.

Kjeldahl Procedure

Reagents

(a) Sulfuric acid—A.C.S., sp. gr. 1.84
(b) Mercuric oxide — reagent grade
(c) Potassium sulfate — A.C.S. granular
(d) Salicylic acid—reagent grade
(e) Sodium thiosulfate—$(Na_2S_2O_3.5H_2O)$ A.C.S.
(f) Zinc granules—A.C.S.
(g) Sodium hydroxide — thiosulfate solution—Dissolve 500 g. NaOH pellets (A.C.S.) and 40 g. $Na_2S_2O_3.5H_2O$ in water and dilute to 1 liter.
(h) Methyl red indicator — Dissolve 1 g. in 200 ml. alcohol or mixed indicator—dissolve

0.8 g. methyl red and 0.2 g. methylene blue in 500 ml. alcohol.

(i) Standard hydrochloric acid (or sulfuric acid)—0.1000 N. standardized against borax or sodium carbonate by A.O.A.C. procedure, or saturated boric acid solution.

(j) Standard sodium hydroxide—0.1000 N. standardized against potassium acid phthalate by A.O.A.C. procedure (not required if boric acid is used to trap NH_3).
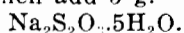
Apparatus

(a) Kjeldahl digestion flasks—Pyrex, 500 to 800 ml. capacity.

(b) Digestion apparatus—either gas or electrically heated and adjusted to give boiling conditions described under Note 1.

(c) Distillation apparatus — either gas or electrically heated with efficient trap and condenser.

Method

Kjeldahl Method for Nitrate-Containing Samples (For nitrate-free samples see Note 5)

Place weighed sample (1-2 g.) in digestion flask. Add volume of H_2SO_4 (containing 2 g. salicylic acid per 40 ml.) corresponding to weight of sample (Table 1.) Shake until thoroughly mixed and let stand 30 minutes or more with occasional shaking, then add 5 g.
$Na_2S_2O_3 \cdot 5H_2O.$
Shake, let stand five minutes, then heat carefully until frothing ceases. Turn off heat, add 0.7 g. HgO (or metallic Hg) and 15 g. K_2SO_4, and boil briskly[3] for one hour after solution clears.

Cool, add ca. 200 ml. H_2O, cool to about room temperature and add few Zn granules. Tilt flask and add carefully 50 ml. of the NaOH-thiosulfate solution without agitation. Immediately connect flask to distillation bulb on condenser. Place receiving flask containing 50 ml. of standard 0.1 N acid[4] so that condenser tube is im-

mersed in acid solution. Then rotate digestion flask carefully to mix contents. Heat until at least 150 ml. of distillate have been collected and titrate excess acid with standard base using methyl red or the mixed indicator. Correct for blank determination on reagents.

Results

The results obtained by the ten collaborators who used the A.O.A.C. procedure are shown in **Table 2.** Each value shown is an average of duplicate determinations. The intralaboratory standard deviation for each collaborator (s) was calculated from the difference between duplicates for the five sets of analyses by

$$s = \sqrt{\frac{(\text{sum of } d^2)}{2n}}$$

where d is the difference between duplicates and n is the number of pairs. The mean of the collaborator's averages is $\bar{x}$ and s_m is the interlaboratory standard deviation for each sample. The intra- and interlaboratory precision are good in all cases. The close agreement between the s_m values for the five samples indicates that neither the type of tobacco nor the nitrate content has much influence on the interlaboratory precision. The coefficients of variation for the five samples varied from 1.2 to 2.6 per cent, which is considered

good for samples with as low as 1.5 per cent nitrogen.

Of the ten collaborators, five chose to use a standard acid and five saturated boric acid to absorb the ammonia evolved. The average per cent nitrogen obtained for all five samples was slightly higher for those who used the standard acid. However, no statistically significant differences were found. The slightly higher results with standard acid seem to be in agreement with previous A.O.A.C. studies comparing the two ammonia absorbents. No difference in efficiency between the two absorbents has been noted in microanalysis. It may be that the

Table 1.—Volume of sulfuric acid for different weights of tobacco sample.

Sample wt. (g) for nitrate-containing samples	Vol. H_2SO_4 (ml.)
1	35
1.5	37.5
2	40
for nitrate-free samples	
1	20
1.5	22.5
2	25

Table 2.—Percentages of total Nitrogen found by the A.O.A.C. method[1]

Collab. No.	\ 1	2	Sample No. 3	4	5	s	Acid Absorbent Standard	Boric
0[2]	3.52	1.57	4.60	2.20	1.80	0.023		x
2	3.50	1.62	4.52	2.23	1.82	0.015	x	
3	3.40	1.53	4.53	2.16	1.73	0.046		x
4	3.35	1.53	4.47	2.06	1.71	0.021		x
5	3.47	1.59	4.60	2.19	1.76	0.007	x	
7	3.30	1.49	4.48	2.15	1.69	0.015		x
9	3.46	1.56	4.51	2.14	1.75	0.028	x	
11	3.40	1.52	4.45	2.07	1.73	0.050	x	
13	3.40	1.53	4.46	2.14	1.68	0.021		x
14	3.36	1.54	4.46	2.12	1.69	0.030	x	
$\bar{x}$	3.42	1.55	4.51	2.15	1.74	(0.026)		
s_m	0.070	0.036	0.055	0.054	0.045			

[1] Each value is the average of duplicates.

[2] One tenth specified amounts of sample and reagents used.

[3] Heaters should be adjusted to bring 250 ml. H_2O from 25° C. to a rolling boil in four to six minutes. To test heater, preheat for 10 minutes if gas or 30 minutes if electric and add three or four boiling chips to flask to prevent superheating.

[4] 25 ml. saturated H_3BO_3 may be used in place of standard 0.1 N HCl and the ammonia titrated with standard 0.1 N HCl (or H_2SO_4) using mixed indicator.

[5] For nitrate-free samples, use volume of acid noted in Table 1 and omit the salicylic acid and thiosulfate treatment.

Table 3.—Percentages of total Nitrogen[1] found by and details of each collaborator's procedure.

Collab. No.	Sample No.							
	1	2	3	4	5	s	Sample wt. g.	Diges- tion[2] hrs.	H_2SO_4 ml.	K_2SO_4 g.	Catalyst	Reducing agent	Trapping acid
2	3.25	1.53	4.28	2.06	1.70	0.103	1.4	½	30	7-8	Se	Salicylic acid	HCl
4	3.32	1.50	4.59	2.10	1.74	0.018	0.04	2	2	1	Cu-Hg	None	H_3BO_3
5	3.50	1.60	4.61	2.21	1.78	0.010	1.4	2	35	5	Cu-Hg	Salicylic acid	H_2SO_4
7	3.27	1.50	4.43	2.09	1.68	0.037	0.5	1	25	15	Hg	Salicylic acid	H_3BO_3
9	3.41	1.54	4.39	2.12	1.73	0.034	2	12	55	0	Hg-Se	Fe and Sali- cylic acid	HCl
11	3.48	1.55	4.56	2.20	1.75	0.012	1	1-1½	40	10	HgO	Salicylic acid	HCl
12	3.49	1.57	4.55	2.16	1.71	0.037	1	12	40	0	Se	Fe and Sali- cylic acid	HCl
13	3.54	1.52	4.58	2.18	1.74	0.026	1-2	1	40	14	Cu	Salicylic acid	H_3BO_3
14	3.29	1.46	4.22	2.04	1.64	0.066	0.1	2	6.5	2.5	Se	Fe	H_3BO_3
$\bar{x}$	3.39	1.53	4.47	2.13	1.72	(0.038)							
s_m	0.113	0.041	0.145	0.061	0.042								

[1] Each value is the average of duplicates.
[2] Digestion time after clearing.

concentration of the ammonia in the initial phase of the distillation is sufficiently greater in the macro determination to lead to a slight loss. Although hydrochloric acid must be used in any official procedure for tobacco, boric acid seems satisfactory for ordinary laboratory analyses.

The results obtained when the collaborators used their own methods and some of the details of the procedures are shown in **Table 3**. The intra- and interlaboratory

standard deviations, s and s_m values, are on the average a little higher than those for the A.O.A.C. method. Only two collaborators (No. 4 and 11) failed to obtain slightly better precision with the A.O.A.C. method than with their own. The $\bar{x}$ values for the A.O.A.C. method for all five samples were slightly higher than those obtained when the collaborators used their own procedures.

An attempt was made to evaluate

the collaborators' methods by ranking each method for each sample. The assumption was made that the highest value was the most nearly correct because it is known that for materials containing refractory compounds, the Kjeldahl nitrogen results tend to be low. By assigning a value of 1 to the method producing the highest value, 2 to the next highest, etc., and summing the rankings for the five samples, the five top methods in order of rank were those of collaborator 5, 11, 13, 12 and 9. The relative rankings were 6, 15, 16, 20, and 26. It is interesting to note that the method of collaborator 5 differed from the A.O.A.C. method only slightly. Copper and mercury catalysts were used instead of only mercury and about half as much potassium sulfate was added but the digestion time was doubled.

The data from this study are summarized in **Table 4**. It would appear (1) that the A.O.A.C. method is satisfactory for the determination of total nitrogen in tobacco; (2) that standard acid may be slightly better than boric acid and (3) that the digestion time should be increased to one and one-half hours to allow for the possibility that some digestion apparatus do not provide

Table 4.—Summary of Means, standard deviations and coefficients of variation for the two methods and five samples.

	Sample No.				
	1	2	3	4	5
Method			Means		
A.O.A.C.	3.42	1.55	4.51	2.15	1.74
Collaborators	3.39	1.53	4.47	2.13	1.72
		Standard Deviations			
A.O.A.C.	0.070	0.036	0.055	0.054	0.045
Collaborators	0.113	0.041	0.145	0.061	0.042
		Coefficients of Variation			
A.O.A.C.	2.05	2.32	1.22	2.51	2.59
Collaborators	3.33	2.68	3.24	2.86	2.44

the optimum amount of heat to the digestion flask. The method tested this year has been recommended as an official A.O.A.C. procedure.

Acknowledgment

Literature Cited

H. A. Davis and S. R. Miles, Jour. Assoc. Official Agric. Chemists, **39**, 550 (1956).

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