Acid Digestions Using the Hach Digesdahl® Digestion Apparatus

Sample Preparation for Protein and Elemental Analysis

Technical Information Series—Booklet No. 14

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I. INTRODUCTION

Acid digestion is an important sample preparation technique used for Total Kjeldahl Nitrogen and elemental analysis. Digestion may be defined as the process in which a complex substance is decomposed into volatile gases and simple salts which are soluble in dilute acid solution. This is accomplished by exposing the sample material to a combination of mineral acids or acid and hydrogen peroxide at an elevated temperature. Once the substance under analysis has been dissolved, chemical methods can be applied to measure the concentration of each element.

Research chemists at Hach Company have been studying the chemistry and mechanics of digestion for 14 years. Most of this work has focused on digestion using sulfuric acid and hydrogen peroxide, a modification of the Kjeldahl Method, which is suitable for determining a range of metals and nonmetals including nitrogen. Digestion was integrated into a total analytical system based on spectrophotometric analysis. Analytes determined include: Ag, Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, N, Ni, Pb, P, S, and Zn.

An early objective was to achieve quantitative recovery of Kjeldahl nitrogen in the digestion. It was discovered that standard natural samples and even certified reference materials could not be used as reliable means of assessing accuracy of the analysis. This stemmed from the fact that the accuracy of assay values are only as good as the laboratory method and technique used to generate the results. Also, there is a certain degree of uncertainty in composition of a heterogeneous natural substance. Analyses may determine the precision of sampling but not necessarily the precision of the analytical procedure.

Researchers were not aware of any formal standard system based on synthetic compounds. Kjeldahl laboratory standardization is frequently based on an ammonium salt, but this only assures the analyst the ammonia can be retained throughout the procedure. It does not prove the digestion is actually effective in converting organic nitrogen into ammonia. Consider the reaction complexity in the Kjeldahl digestion, where nitrogen in various structures and oxidation states must be fully reduced to (−3) while carbon is oxidized to carbon dioxide. There was clearly a need to establish a means of verification that the digestion was consistently recovering the nitrogen in the sample.

II. EXPERIMENTAL DEVELOPMENTS

Digesdahl Digestion Apparatus

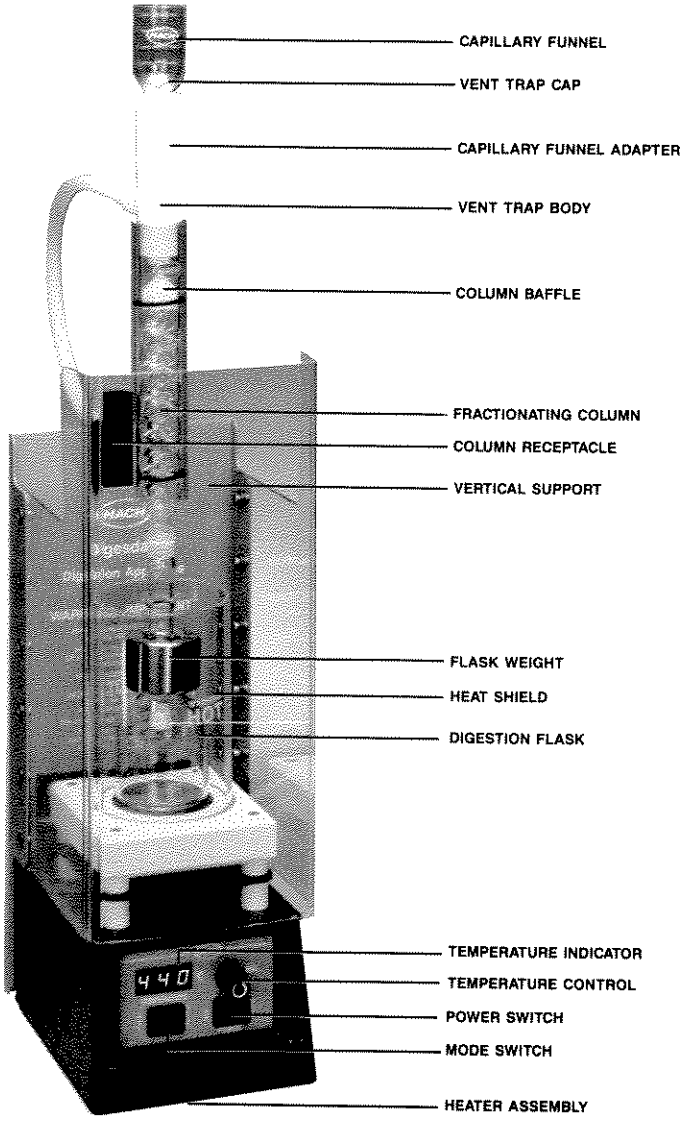


FIGURE 1. Digesdahl Digestion Apparatus

Digestion Flask

Initially, a 100-mL standard volumetric flask was used as the digestion flask. Later, a special flask with more consistent dimensions and a controlled flat bottom of uniform thickness was designed. This new design is important for consistent heat transfer so the temperature of the digestion mixture can be better regulated. A weight ring applied to the flask while on the heater provides stability.

Fractionating Column

The column or head serves two main functions. First, it provides a manifold for attachment of the fume evacuation hose and for support of the capillary funnel. Second, it acts as a fractional reflux condenser. Optimum reaction conditions require water to be rapidly boiled off while hydrogen peroxide (H_2O_2) and sulfuric acid (H_2SO_4) are retained. With proper condenser design, most of the H_2O_2, at a boiling point of 150 °C, is condensed and returned to the flask while steam is swept out the vent. The fractionating column of about 20-cm length has three rows of dimples, and is equipped with a baffle and a fume trap. The fractionating column is used for reagent addition and fume evacuation. A capillary funnel, inserted into the column, controls the rate of hydrogen peroxide addition during digestion. (See Figure 2.)

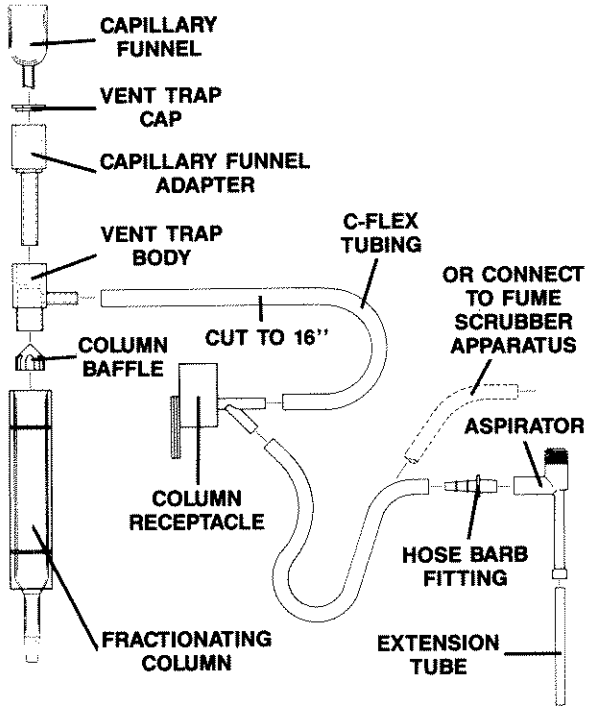


FIGURE 2. Vigreux Fractionating Column with Capillary Funnel

Capillary Funnel

At constant head pressure and temperature, a given liquid flows through a capillary column at a constant flow rate. This principle is applied to delivery of H_2O_2 through the capillary funnel. Although the liquid head changes somewhat, the flow rate averages 3 mL/minute.

Heater Assembly

The Digesdahl's heating apparatus is built around a 250-watt ceramic disc element. Initially, the controller was a proportional time on/off device. Recently, a thermostat was installed which uses a thermocouple to sense the temperature of the metal shell surrounding the ceramic element. The metal-clad element was found to provide better heat transfer to the flask than to the ceramic surface. The heating element and digestion flask are enclosed by a glass heat shield to aid in heat transfer to the sample.

Fume Evacuation

A water aspirator produces the ventilation air stream, and dissolves the exhaust gases from the digestion. Alternatively, a fan-driven fume scrubber has been constructed. It recirculates air back into the room after removing condensable gases in a stainless-steel condenser and absorbing the remaining components in a limestone-activated charcoal unit. (See Figure 3.) Use of a fume hood or safety shield is recommended.

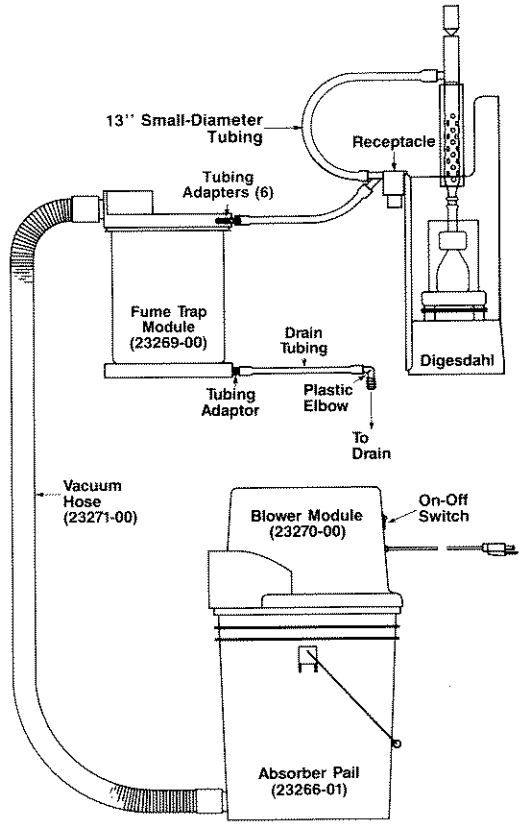


FIGURE 3. Fume Scrubber Apparatus

Reagents

Sulfuric acid (H_2SO_4) ACS, 95-98%
50% Hydrogen peroxide (H_2O_2), 50%
30% Hydrogen peroxide (H_2O_2) ACS, 29-32%

Being less concentrated, 30% H_2O_2 has less oxidizing strength than 50% H_2O_2, and is consequently a less effective reagent for this purpose. When using the 30% reagent, approximately 1/3 more volume is required to get the same results. Most research was done using the 50% reagent, and all the digestion times and volumes

reflect its use. When using 30% peroxide always run a digestion standard, either glycine p-toluenesulfonate or nicotinic acid p-toluenesulfonate, to check completion of the digestion. All recommended safety precautions apply to both strengths of the H_2O_2.

Procedure

Various procedure modifications were examined in the digestion with H_2SO_4 and H_2O_2. The following is an abbreviated procedure outline for the General Digestion Procedure and is not intended as stand-alone instructions. Refer to Hach's *Water Analysis Handbook* (literature code 8353) or the revised third edition of the *Digesdahl Digestion Apparatus Instruction Manual* (12-30-89-3ED, catalog number 23130-89) for the complete general digestion procedure. For a list of specific application procedure manuals, see section **VI. References** at the end of this booklet.

1. Measure or weigh the appropriate size of sample as specified in individual procedures. Transfer the measured sample to a digestion flask.
   Note: Never exceed 0.5 g of sample (dry weight). The maximum volume of a liquid sample is 40 mL.
2. Add concentrated sulfuric acid (specific gravity 1.84) and two or more silicon carbide boiling chips to the digestion flask. **Note:** Refer to specific procedures for recommended sulfuric acid volume. Never use less than 3 mL of concentrated sulfuric acid. **Note:** Always perform digestion behind a safety shield or in a closed fume hood. Safety glasses are mandatory.
3. Place the flask weight followed by the fractionating column with funnel on the flask. Place the flask on the Digesdahl and assemble the heat shield. Turn on the aspirator (mechanical or water). **Note:** Make sure there is suction to the fractionating column.
4. Heat for 3-5 minutes. **Note:** Do not boil sample to near or complete dryness.
5. Add 10 mL of 50% hydrogen peroxide to the funnel with a pour-out dispenser, pipet, graduated cylinder or Hach Hydrogen Peroxide Pump. **Note:** Visually confirm the presence of sulfuric acid in the flask before adding hydrogen peroxide. **Note:** If the digest does not turn colorless, add 5-mL increments of hydrogen peroxide until digest becomes clear.
6. Heat the sample for one minute after all hydrogen peroxide has entered the digestion flask. **Note:** Do not heat to dryness.
7. Take the flask off the heater and allow the flask to cool on the cooling pad. Remove the fractionating column from the digestion flask after the flask has cooled.
8. Dilute the digest to the 100-mL mark with demineralized water; mix. Complete the analysis by the appropriate analytical method.

III. RESULTS AND DISCUSSION

Conditions for Complete Kjeldahl Digestion Using Peroxide

Appropriate Sample Size

The scale of apparatus used dictates the maximum sample size. In the 100-mL digestion flask, <0.5 g of anhydrous material can usually be digested effectively. (As a routine practice 0.25 g of sample were used.) Samples that contain water may be scaled up by a proportional amount. Never exceed 0.5 g of sample (dry weight) or 40 mL of liquid sample.

More than 0.5 g (anhydrous) overloads the capacity of the flask, and is difficult to contain. Larger samples tend to form a charred mass which is hard to oxidize and requires an excessive volume of H_2O_2. There is no restriction on minimum quantity.

Sufficient Sulfuric Acid

The amount of concentrated sulfuric acid (specific gravity 1.84) must be sufficient to prevent the digestion from going to dryness. Too little acid may cause the sample to overheat, which may result in the loss of the analytes due to decomposition. **Any part of the flask bottom that becomes dry will overheat and may cause the flask to burst.** Use an amount that will leave about 2 mL of residual acid covering the bottom of the flask when the digestion is complete.

Sulfuric acid consumption depends on the anhydrous mass of material and the chemical composition of the substance. Use of 4 mL H_2SO_4 is suitable for many materials, but not all. The analyst must pay close attention to the amount of residual H_2SO_4 for the type of sample digested and adjust the amount of acid or sample accordingly. Never use less than 3 mL of concentrated sulfuric acid. Larger volumes of sulfuric acid may be used, but a large excess should be avoided because sample pH adjustment is required in most subsequent determinations.

Proper Digestion Solution Temperature

The technical literature contains several examples of ineffective use of H_2O_2 due to a combination of timing of the H_2O_2 addition and temperature. If the H_2O_2 is added to a cold digestion mixture followed by heating, the H_2O_2 decomposes before the digest reaches the proper temperature. Addition of H_2O_2 to a digest that is too hot volatilizes most of the H_2O_2 with little benefit. Furthermore, excessive temperature contributes to spray loss of sample as a fine mist.

The proper temperature on the metal-clad heating element as measured by the internal thermocouple is 440-480 °C. At this setting, the H_2SO_4 reaches 320 °C during preheat carbonization. During H_2O_2 addition, the solution temperature is maintained above 280 °C.

TABLE 1. Sulfuric Acid Residuals for a Variety of Sample Types*

Sample	Structure	H_2SO_4 Residual, mL
Starch	carbohydrate	2.32
Cyanoethylcellulose	carbohydrate	1.58
Soybean meal	protein	2.55
Linseed oil	fatty acid	1.21
Benzene	aromatic hydrocarbon	1.40
Paraffin	hydrocarbon	1.71
Motor oil	hydrocarbon	1.08
Polyethylene	hydrocarbon	0.67
Bunker C fuel oil	hydrocarbon	0.00
Sewage (25 mL)		3.44

*Experimental Conditions: 0.25 g sample, 4.00 mL H_2SO_4, four-minute preheat, 20 mL H_2O_2 at 3 mL/minute, two-minute postheat

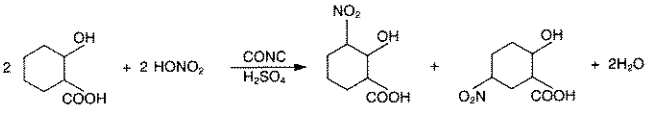
Carbonization Period

A carbonization period prior to the addition of H_2O_2 provides a reducing environment which helps convert organic nitrogen to ammonia. In the presence of oxidizable carbon compounds, sulfuric acid reacts to produce sulfur dioxide, which is the active reducing agent. The reaction is:

$$H_2SO_4 \longrightarrow H_2O + SO_2 + 1/2\ O_2$$

In nitroaniline, the nitro-N is fully recovered in as little as two minutes carbonization. Dinitrobenzene yielded about 85% recovery with the 4-minute preheat period. However, when 0.25 g of salicylic acid was added and carbonization was extended to 15 minutes, the recovery was about 98%. Inorganic nitrate was fully recovered using salicylic acid pretreatment in concentrated sulfuric acid according to the reactions:

$$2MNO_3 + H_2SO_4 \longrightarrow M_2SO_4 + 2HNO_3$$



It was interesting to find the amino-N in glycine was fully recovered regardless of whether there was a carbonization period or not. A preheat period of 2 to 5 minutes is recommended for routine digestions.

Adequate Peroxide Concentration for Sufficient Time

Researchers believe that H_2O_2 immediately reacts with H_2SO_4 at digestion temperature to give H_2SO_5 (peroxymonosulfuric acid) by the reaction:

$$H_2SO_4 + H_2O_2 \longrightarrow H_2SO_5 + H_2O$$

This is an extremely powerful oxidizing agent toward carbonaceous material. The objective is to maintain an adequate concentration of H_2SO_5 in the hot digestion mixture for a long enough time to complete oxidation of the carbonaceous material. Use the capillary funnel to meter the H_2O_2 into the flask at 3 mL/min.

The amount of peroxide that must be added for complete digestion can be determined by digesting a sample multiple times with incremental increases in the amount of peroxide added (i.e., 5 mL, 10 mL, 15 mL, 20 mL). Results of the analysis for the parameter of interest may then be graphed to determine the minimum amount of peroxide needed for optimum sample digestion.

The preferred and recommended peroxide reagent is 50% hydrogen peroxide. Research studies have shown that the best recovery and reproducibility are achieved using the 50% peroxide reagent. The 50% hydrogen peroxide reagent produces dependable results in research and actual applications. The optimal rate of peroxide addition has been determined as 3 mL/minute using the 50% reagent. This may not hold true if other strengths of hydrogen peroxide are used.

If 50% hydrogen peroxide is not available, 30% peroxide may be used as a last resort with caution. Because of the lesser strength, at least 1.67 times more volume must be used (i.e., 16.7 mL of 30% vs. 10 mL of 50% peroxide). Always run a digestion standard, either glycine p-toluenesulfonate or nicotinic acid p-toluenesulfonate, when using 30% peroxide to check completion of the digestion. All recommended safety precautions apply to both strengths of the hydrogen peroxide.

Containment of Sample

Loss of sample from the digestion flask can occur in two ways: 1) foaming or boiling over, and 2) mist or spray in the ventilation air stream. Foaming is a serious problem with certain samples types, so special techniques were developed. Some liquid samples, especially those containing sugars, cannot be digested by the standard procedure, which consists of placing a given

volume in the flask, adding H_2SO_4, and heating. Under those conditions, foaming cannot be controlled. Instead, the H_2SO_4 is heated in the flask and the liquid sample is added through the capillary funnel. Carbonization proceeds in a controlled manner. When all the sample has been added, the peroxide treatment begins and the digestion continues normally. Other ways to control foaming include early addition of H_2O_2 and reducing the temperature during carbonization.

Sample loss as spray or mist occurs when small droplets of liquid are swept out of the flask along with gases, and escape in the ventilation air stream. This loss was measured by passing the vent air stream through a glass, water-cooled condenser. The condensate was collected and analyzed. It was discovered that proper fractionating column design reduced spray loss to an insignificant level. Data is presented in Table 2.

Safety Considerations

Please read the entire *Digesdahl Digestion Apparatus Manual*, including the safety information, before using this apparatus. Read the Material Safety Data Sheet (MSDS) for all reagents.

Digestion

Procedures for the Digesdahl System vary slightly with sample type. The system uses a two-phase digestion process involving concentrated sulfuric acid and 50% hydrogen peroxide. The sulfuric acid dehydrates and chars the sample. The hydrogen peroxide is added via the capillary flow funnel to complete sample decomposition. The capillary funnel feeds hydrogen peroxide into the digestion flask at a rate of 3 mL per minute. This allows the analyst to control the amount of time the sample is exposed to the hydrogen peroxide by varying the volume of hydrogen peroxide used.

WARNING

Wear protective glasses and clothing. A strong acid (concentrated sulfuric acid) and a strong oxidant (50% hydrogen peroxide) are used in the digestion reaction. These chemicals can cause skin burns if splashed on the skin or permanent eye damage if they come in contact with the eyes. If the chemicals are hot, effects are considerably more severe. Immediately rinse any affected area with water and contact a physician.

TABLE 2. Effect of Fractionating Column Design on the Protein Loss Resulting from Mist Carryover in the Exhaust Stream

Column Design	Protein Loss, %*	Relative Loss, %†
Open	0.41	0.89
Vigreux without baffle	0.23	0.49
Helix packed column	0.10	0.22
Vigreux with baffle	0.05	0.11

*Digestion of 0.250 g of Ammonium-PTSA (Primary Standard, 46.26% crude protein) using 18 mL of 50% hydrogen peroxide

† $\frac{\%\ \text{Protein Loss} \times 100}{46.26\%}$

Use the Digesdahl Apparatus only behind a laboratory safety shield or in a closed fume hood. Use a water aspirator or Hach Fume Scrubber to remove fumes during digestion.

Some samples are more difficult to digest than others. In many cases, complete recovery of the analyte is achieved immediately upon clearing of the digestate (when the digest becomes colorless). However, resistant materials require several minutes of continued peroxide digestion after clearing to obtain 100% recovery. To ensure complete digestion, consider the variables described in the following paragraphs.

Sample Size

For solid or organic liquid samples, less than 0.5 grams of dry material can usually be digested. Routinely, use only 0.25 grams. Samples containing water can be increased by a proportional amount. The maximum sample volume for aqueous solutions or suspensions is 40 mL; there is no minimum sample size. When the percent solids exceeds 1% of the sample volume, reduce the maximum sample volume by this formula:

$$\text{mL sample} = \frac{40}{\%\ \text{solids.}}$$

Samples containing suspensions or a high percent of solids are very difficult to reproducibly sample. Mix or homogenize these samples thoroughly before sampling.

Chemicals

Concentrated sulfuric acid and hydrogen peroxide used in the digestion process should be handled correctly and with caution. Sulfuric acid is a strong acid and can cause burns if splashed on the skin and permanent damage if eye contact occurs. Hydrogen perox-

ide (30% or 50%) is a powerful oxidant and should never be stored near flammable materials. Like sulfuric acid, it can cause burns and eye damage. In case of eye or skin contact with either chemical, flush eyes and/or skin with water for 15 minutes. Remove contaminated clothing. Call a physician.

Both sulfuric acid and hydrogen peroxide are highly corrosive and should be cleaned up with water if spilled on instruments or a counter top. Read and observe all warnings on the reagent labels and MSDS.

Proper handling and storage procedures involving hydrogen peroxide should always address two major characteristics of the product: first, it is a strong oxidizing agent (corrosive), and second, it can decompose, releasing heat and oxygen. The chemical nature of hydrogen peroxide makes it an irritant to the skin, to mucus membranes and particularly to the eyes. It will cause chemical burns at industrial concentrations and may cause spontaneous combustion upon immediate or prolonged contact with combustibles.

Hydrogen peroxide decomposes to form water and oxygen. The natural decomposition rate of the normal industrial grade product is very low, but it will accelerate when contaminated by materials such as dust, metallic ions, or alkali. Accelerated decomposition from contamination will result in the significant production of oxygen and liberation of heat. These products will support combustion and will cause pressure bursts in confined spaces. Commercial grades of hydrogen peroxide contain small quantities of additives (termed "stabilizers") to prevent accelerated decomposition from occurring during normal product usage.

Please observe the following precautions for handling and storage of hydrogen peroxide.

Do store in a cool place away from direct sunlight (perferably in a refrigerator).

Do store in the original containers with closures as supplied and keep closed when not in use. (Be sure the containers are vented. Hach hydrogen peroxide bottles are shipped with a special permeable cap liner.)

Do wear gloves and safety glasses when handling the material.

Do use silicon carbide boiling chips when digesting liquid samples.

Do wash contaminated skin and body quickly with plenty of water. Remove contaminated clothing and wash well before using again. Wash regularly.

Do wash eyes with plenty of water if contaminated and do get medical advice quickly.

Do get medical advice without delay if the material is ingested.

Do flush all spillage with large amounts of water.

Do not store near heat sources or in contact with combustible or organic materials.

Do not allow contact with decomposition catalysts (metals, dust, alkali, etc.) for transfer or storage systems.

Caps on the reagent bottles are made with a special porous liner that allows venting of gas. The venting cap always must be used on the bottle of hydrogen peroxide. As a precaution, the reagent bottles are shipped in a plastic bag. If there is evidence of leakage during shipment, wear gloves when removing the bottle from the bag and rinse the bottle with water when removed from the bag. Rinse the bag before disposal.

Primary Standards

Several compounds were examined as candidates for digestion standards. Researchers decided the compounds chosen should meet the stringent criteria of "primary standards" so they could be synthesized anywhere in the world with the assurance that they contain, by definition, an absolute nitrogen content. This would provide every laboratory its own internal proof of accuracy.

The selection criteria for Kjeldahl primary standards:

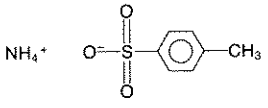
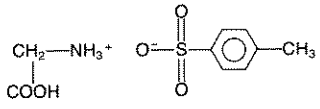
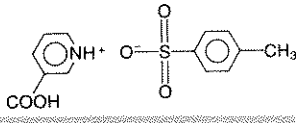
1) high molecular weight
2) recrystallization without water of hydration
3) nonhygroscopic
4) thermally stable
5) not air-oxidizable
6) water soluble
7) high purity
8) nitrogen content in useful range
9) degree of digestion difficulty
10) simple and economical, direct synthesis
11) free-flowing solid

Three primary standard compounds of varying digestibility (Ammonium-p-toluenesulfonic acid, [PTSA], Glycine-PTSA, and Nicotinic acid-PTSA) were found to be sufficient to prove the accuracy of the overall Kjeldahl nitrogen determination. Their physical properties are listed in Table 4. These p-toluenesulfonate salts were chosen because they are stable, nonhygroscopic materials that can be prepared and purified to primary standard quality, and the protein content is lowered to a practical range compatible with the method of analysis. For example, pure glycine (116.6% protein content) or pure nicotinic acid (71.12% protein content) could be used as a primary standard, but the nitrogen levels are too high to fit the working range of the analysis. The three selected standard compounds have digestibilities ranging from an index of zero for Ammonium-PTSA (no digestion required), to 10 for Nicotinic acid-PTSA, which is the most difficult material commonly encountered. Glycine-PTSA

TABLE 3. Candidate Compounds

Name/Formula	%N	% Protein (N × 6.25)	Digestion Comments
Ammonium Chloride NH_4Cl	26.18	163.7	Water Soluble Inorganic Salt: no digestion required
Ammonium Sulfate $(NH_4)_2SO_4$	21.20	132.5	Water Soluble Inorganic Salt: no digestion required
Ammonium Perchlorate NH_4ClO_4	11.92	74.51	Water Soluble Inorganic Salt: no digestion required
Ammonium-PTSA $C_7H_{11}O_3SN$	7.402	46.26	Water Soluble Organic Salt: may be digested but not necessary
Glycine $C_2H_{13}O_5SN$	18.66	116.6	Amino Acid: relatively easy to digest
Glycine-PTSA $C_9H_{13}O_5N$	5.665	35.40	Salt of Amino Acid: relatively easy to digest
Phenylalanine-PTSA $C_{16}H_{19}O_5SN$	4.151	25.95	Salt of Amino Acid: relatively easy to digest
Tryptophan-PTSA $C_{25}H_{28}O_8S_2N_2$	5.106	31.91	Salt of Amino Acid: moderately difficult to digest
THAM-PTSA $C_{11}H_{19}O_6SN$	4.775	29.84	Salt of a Primary Amine: moderately difficult to digest
Nicotinic Acid $C_6H_5O_2N$	11.38	71.11	Vitamin, Nitrogen Heterocyclic Compound: difficult to digest
Nicotinic Acid-PTSA $C_{13}H_{13}O_5SN$	4.743	29.64	Salt of the above: difficult to digest
Piperidine-PTSA	5.443	34.02	Nitrogen Heterocyclic Compound: difficult to digest

TABLE 4. Some Properties of Ammonium-PTSA, Glycine-PTSA, and Nicotinic Acid-PTSA

	Ammonium-PTSA	Glycine-PTSA	Nicotinic Acid-PTSA
Formula	$C_7H_{11}O_3SN$	$C_9H_{13}O_5SN$	$C_{13}H_{13}O_5SN$
Structure			
Molecular Weight	189.235	247.277	295.316
Melting Point	—	199	179
Percent Nitrogen	7.402	5.664	4.743
Percent Protein	46.261	35.403	29.643
Digestibility Index	0	3	10
Moisture Absorption			
at 25% Relative Humidity	0.04%	0.03%	0.00%
at 50% Relative Humidity	0.07%	0.047%	0.01%
at 90% Relative Humidity	0.14%	0.15%	0.08%

(digestibility index of 3) is intermediate, and is quite easily digested. All three compounds may be oven dried at 100 °C without thermal decomposition, although drying is unnecessary, because they do not absorb significant amounts of moisture, even at 90% relative humidity. Also, they are non-oxidizable, free-flowing, crystalline solids that do not stick on weighing paper or spatula, yet they are water-soluble and stable indefinitely.

DIGESTION INDEX

The digestion index is a numerical rating scale for classifying nitrogen compounds based on their resistance to decomposition. It was devised to optimize digestion conditions to obtain full nitrogen recovery in the least time. In a careful study of the minimal time required to digest a variety of materials, complete nitrogen recovery was achieved for many samples immediately upon clearing of the digest (when the digest becomes colorless). However, resistant or refractory materials such as nicotinic acid require several minutes of continued peroxide digestion after clearing to obtain 100% nitrogen recovery.

The arbitrary scale that was devised assigned zero to compounds not needing digestion (ammonium salts) and ten to nicotinic acid. All the substances digested can be assigned a number between "0" and "10," depending on the minimum peroxide reaction time required, relative to nicotinic acid. This provides an easy-to-use, reliable index of digestibility which is useful in forecasting the degree of treatment needed to bring about complete digestion. One may treat unknown samples

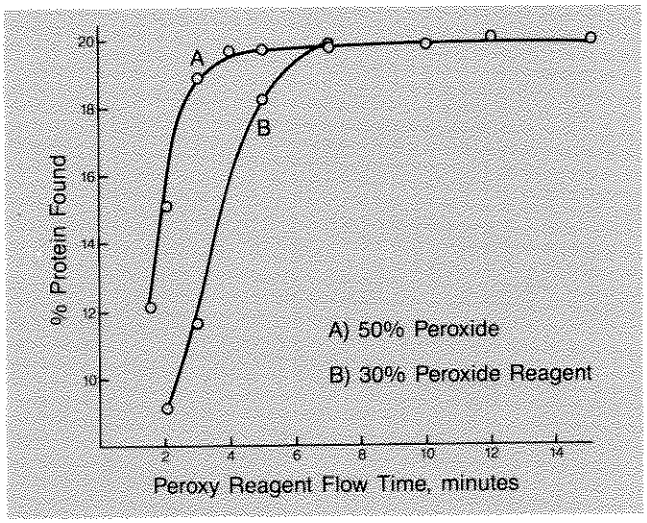


FIGURE 4. Nitrogen Recovery from a Nicotinic Acid-Succinate Sample

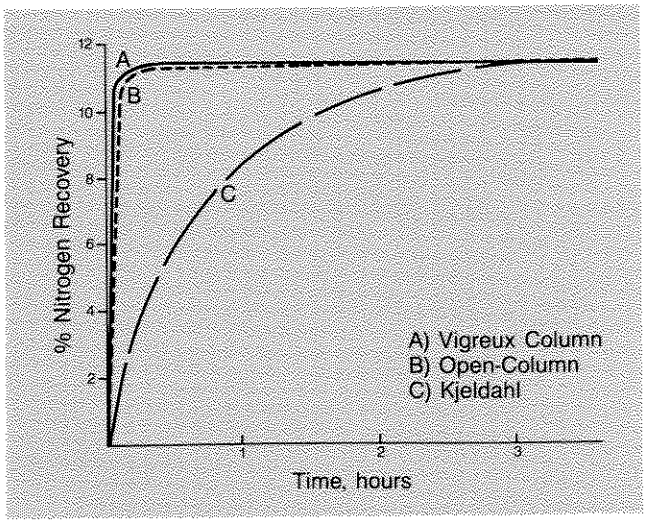


FIGURE 6. Nitrogen Recovery from Nicotinic Acid as a Function of Digestion Time for Kjeldahl and Digestion Systems

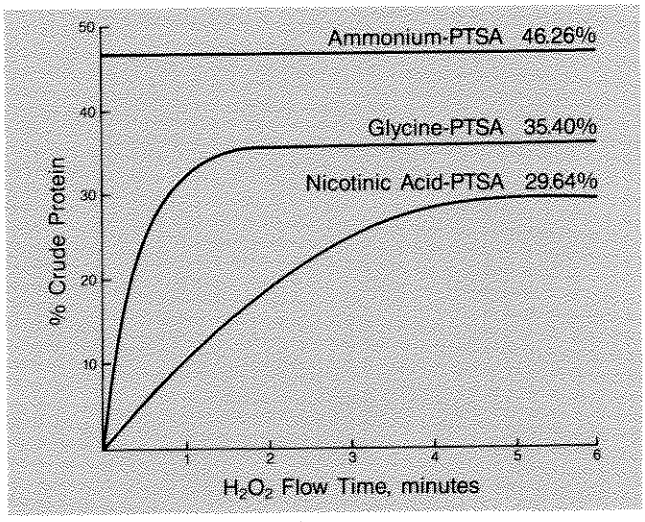


FIGURE 5. Crude Protein Recovery

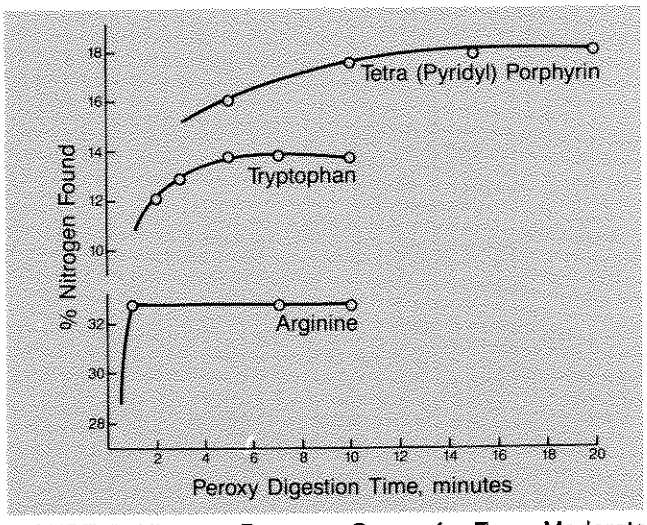


FIGURE 7. Nitrogen Recovery Curves for Easy, Moderate and Difficult to Digest Samples. Arrows indicate 99% Nitrogen Recovery.

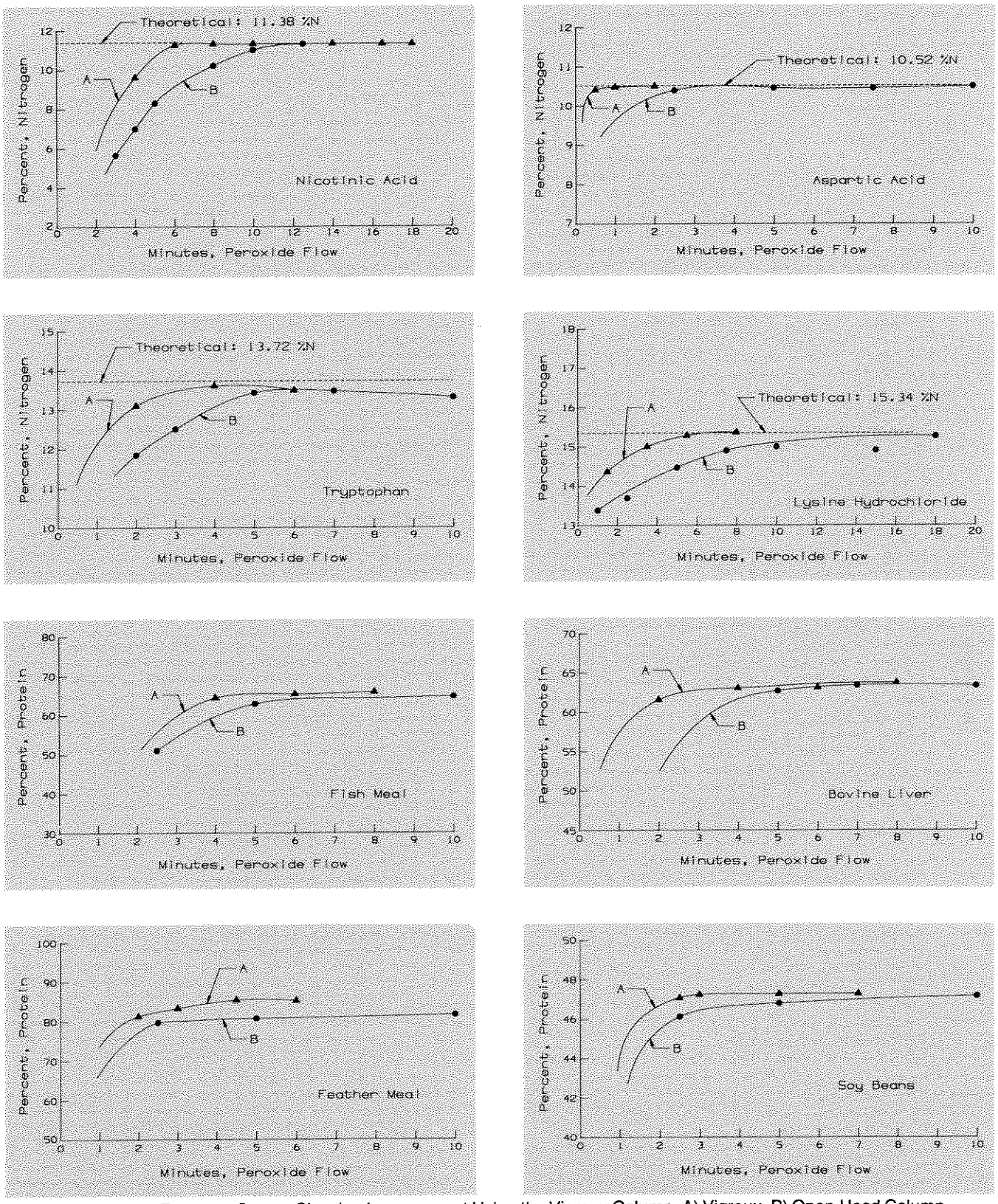


FIGURE 8. Nitrogen Recovery Curves Showing Improvement Using the Vigreux Column. A) Vigreux, B) Open-Head Column

with the maximum H_2O_2 volume to assure recovery, but if the digestion index is known the H_2O_2 treatment may be adjusted accordingly. Using more H_2O_2 than necessary does not cause any problem, it is simply less efficient.

A series of nitrogen recovery curves are presented in Figures 4-8.

IV. ANALYTICAL RESULTS

TABLE 5. Nitrogen Recovery with Continuous-flow Peroxy Digestion

Compound	Calculated, %N	Found, %N	Recovery, %
Arginine	32.16	32.71	101.7
Aspartic acid	10.52	10.48	99.6
Alanine	15.72	15.42	98.1
Phenylalanine	8.48	8.56	100.9
Serine	13.33	13.46	101.0
Nitroaniline	20.28	20.28	100.0
Valine	11.96	11.87	99.3
Acetanilide	10.36	10.41	100.4
Proline	12.17	11.95	98.2
Tryptophan	13.72	13.68	99.7
Methionine	9.39	9.41	100.2
Histidine	27.08	27.75	102.4
Pyridinium p-toluenesulfonate	5.58	5.61	100.5
Leucine	10.67	10.85	101.7
Tetraphenylporphyrin	9.11	9.10	99.9
Nicotinic acid	11.38	11.43	100.4
Lysine hydrochloride	15.34	15.27	99.6
Tetrapyridylporphyrin	18.11	18.05	99.7

Reference Materials	Certified, %N	Found, %N
NBS* Orchard leaves #1571	2.76 (0.05)	2.77
NBS Bovine liver #1577	10.60 (0.60)	10.40
AAFCO† Milk replacer #7925	3.28 (0.04)	3.28
AAFCO Cattle supplement #7926	8.84 (0.08)	9.26
AAFCO Swine feed #7928	2.52 (0.04)	2.53

*National Bureau of Standards
†Association of American Feed Control Officials

TABLE 6. Comparison of Protein Values Obtained Using Open-head, Vigreux, and Association of Official Analytical Chemists (AOAC) Kjeldahl Methods

[Results expressed as % Crude Protein (N × 6.25)]

Sample	Open Head (a)	Vigreux (b)	AOAC Kjeldahl (c)
Soybean meal	47.2	47.3	46.4
Fish meal	65.5	65.9	66.1
Feather meal	85.3	85.3	84.8
Bovine liver (NBS 1577)	67.1	63.8	66.3 (3.8) (NBS certified)
Orchard leaves (NBS 1571)	17.3	17.3	17.3 (0.3) (NBS certified)
Nicotinic acid	71.2	71.1	71.1 (calculated)
Tryptophan	85.2	85.4	85.7 (calculated)

(a) Open head, 4:1 peroxide reagent
(b) Vigreux fractionating head, 50% peroxide
(c) Section 7.015 Official Methods of Analysis, AOAC, 1984

TABLE 7. Crude Protein Results for Nicotinic Acid-PTSA (Primary Standard 29.64% Crude Protein) Using Vigreux Method

Eight Replicates Using 10-20 mL of 50% Hydrogen Peroxide
(Results in % Crude Protein)

29.60	29.69	29.36	29.93	Recovery	=	100.00%
				$\bar{x}$	=	29.63
29.44	29.44	29.69	29.93	s	=	0.22
				V	=	0.74%
				Range	=	0.57

TABLE 8. United States Environmental Protection Agency (USEPA) Sludge Sample Results: H_2SO_4—H_2O_2 Vigreux Method

(Concentrations expressed in mg/kg)

Parameter	Hach	USEPA*
Cadmium	19.1	19.1
Copper	1100	1080
Iron	18,700	16,500
Manganese	200	202
Nickel	191	194
Nitrogen (TKN)	25,400	25,200
Phosphorus	11,500	11,600
Zinc	1280	1320

*Average results from referee laboratories performing the analysis according to USEPA Reference Methods

TABLE 9. Results of H_2SO_4—H_2O_2 (Vigreux Method) Digestion and Spectrophotometric Analysis of BCR-Certified Sewage Sludge

[Concentrations expressed as μg/g and (uncertainty)]

Parameter	Hach		Certified BCR Value*	
Copper	720	(32)	713	(26)
Iron	45,150	(1,048)	43,100	(1,300)
Aluminum	12,180	(779)	11,400	(600)
Zinc	3,285	(187)	3,143	(103)
Manganese	431	(11)	449	(130)
Nickel	866	(5)	942	(22)
Phosphorus	19,960	(366)	20,040	

*Community Bureau of Reference, BCR No. 144

TABLE 10. Nitrogen Concentrations in Various Coals

(Results expressed as % Nitrogen, dry)

Coal	Rapid Dissolution Procedure* $\bar{x}$	Rapid Dissolution Procedure* range (n = 3)	CHN Analyzer $\bar{x}$	CHN Analyzer range (n = 2)
Pittsburgh No. 8	1.21	1.21-1.22	1.28	1.27-1.30
Illinois No. 6 (raw)	1.23	1.22-1.24	1.09	1.02-1.16
Illinois No. 6 (HNO-leached)	4.53	4.46-4.60	4.44	4.38-4.50
Dietz No. 1 & No. 2	1.02	1.01-1.02	0.86	0.82-0.90
Charming Creek	0.93	0.92-0.93	1.02	0.99-1.05
NBS 1632a	1.24	1.23-1.25	1.40	1.39-1.41
NBS 1635	1.62	1.62-1.63	1.72	1.71-1.74

*Three determinations on each of three digestions were performed

Mean values for each digestion were used for reporting the range and for calculating the sample average

TABLE 11. United States Department of Agriculture (USDA) Accreditation Test Data on the Hach System for Crude Protein

[Results expressed as % Crude Protein (N × 6.25)]

Ham, Smoked

Sample No.	USDA	Hach	Hach-USDA
16514	19.7	19.6	−0.1
16832	19.4	19.3	−0.1
16835	16.2	16.7	+0.5
16855	18.9	19.1	+0.2
16877	16.7	16.9	+0.2
16929	19.9	19.9	0.0
16989	18.4	18.6	+0.2
17052	20.4	20.4	0.0
17071	17.3	17.1	−0.2
17076	19.0	19.3	+0.3
17080	19.4	19.1	−0.3
17081	16.2	16.4	+0.2
17082	16.7	16.8	+0.1
17100	19.9	19.8	−0.1
17101	18.4	18.1	−0.3
17102	16.2	16.5	+0.3

	r	0.9902
	$\bar{x}$ (USDA)	18.29
	$\bar{x}$ (Hach)	18.35
$\bar{x}$ (Hach) −	$\bar{x}$ (USDA)	+0.06
	A	1.0680
	B	−1.3040

Ham, Water Added

Sample No.	USDA	Hach	Hach-USDA
15446	16.9	16.9	0.0
15505	19.7	19.8	+0.1
15670	13.2	14.0	+0.8
15671	17.1	17.0	−0.1
15797	18.4	18.6	+0.2
15811	16.2	16.1	−0.1
15816	15.8	14.9	−0.9
15863	15.5	15.1	−0.4
15865	15.9	16.0	+0.1
16026	15.2	14.4	−0.8
16031	17.6	16.5	−1.1
16036	18.3	17.5	−0.8
16040	16.2	15.2	−1.0
16054	16.5	16.4	−0.1
16055	15.9	15.7	−0.2
16181	18.9	18.1	−0.8

	r	0.9443
	$\bar{x}$ (USDA)	16.71
	$\bar{x}$ (Hach)	16.39
$\bar{x}$ (Hach) −	$\bar{x}$ (USDA)	−0.32
	A	0.9679
	B	0.8442

A = Slope B = Y-intercept

Pork Sausage

Sample No.	USDA	Hach	Hach-USDA
14708	14.6	14.6	0.0
15121	10.4	10.9	+0.5
15125	11.6	11.6	0.0
15131	15.7	15.8	+0.1
15163	13.8	14.0	+0.2
15203	14.6	15.6	+1.0
15221	15.1	15.0	−0.1
15330	10.8	10.9	+0.1
15332	12.8	12.9	+0.1
15360	12.1	12.5	+0.4
15423	14.3	14.2	−0.1
15438	14.9	14.6	−0.3
15439	13.5	13.9	+0.4
15483	14.1	14.9	+0.8
15667	12.0	12.6	+0.6
15669	11.5	10.8	−0.7

	r	0.9689
	$\bar{x}$ (USDA)	13.24
	$\bar{x}$ (Hach)	13.43
$\bar{x}$ (Hach) −	$\bar{x}$ (USDA)	+0.19
	A	0.9379
	B	0.6460

Cooked Sausage

Sample No.	USDA	Hach	Hach-USDA
14256	10.7	11.1	+0.4
14258	13.0	12.7	−0.3
14989	12.9	13.0	+0.1
14990	12.4	12.1	−0.3
14996	12.2	12.4	+0.2
15008	11.5	11.6	+0.1
15009	12.9	13.5	+0.6
15033	12.1	12.2	+0.1
15052	12.1	12.3	+0.2
15078	11.6	11.7	+0.1
15082	11.7	11.8	+0.1
15086	11.9	12.2	+0.3
15099	10.8	11.0	+0.2
15100	11.1	11.4	+0.3
15202	11.8	12.4	+0.6
15209	13.0	13.3	+0.3

	r	0.9426
	$\bar{x}$ (USDA)	11.98
	$\bar{x}$ (Hach)	12.17
$\bar{x}$ (Hach) −	$\bar{x}$ (USDA)	+0.19
	A	0.9655
	B	0.2323

Canned Ham

Sample No.	USDA	Hach	Hach-USDA
14435	17.9	17.6	−0.3
14980	17.9	18.0	+0.1
14982	17.5	15.8	−1.7
14984	18.6	18.5	−0.1
15066	18.5	18.3	−0.2
15067	18.3	17.8	−0.5
15070	17.7	17.5	−0.2
15072	17.6	17.3	−0.3
15074	18.1	17.8	−0.3
15077	18.3	18.6	+0.3
15095	19.3	18.6	−0.7
15175	17.4	17.5	+0.1
15337	17.6	17.2	−0.4
15339	18.0	18.2	+0.2
15340	17.8	18.0	+0.2
15346	18.9	18.2	−0.7

	r	0.7148
	$\bar{x}$ (USDA)	18.09
	$\bar{x}$ (Hach)	17.81
$\bar{x}$ (Hach) −	$\bar{x}$ (USDA)	−0.28
	A	0.5478
	B	8.3331

Hamburger

Sample No.	USDA	Hach	Hach-USDA
14312	17.6	18.0	+0.4
14782	18.0	18.2	+0.2
15113	18.4	18.1	−0.3
15115	19.3	19.1	−0.2
15118	20.6	20.9	+0.3
15127	19.2	19.0	−0.2
15130	17.7	17.7	0.0
15141	20.1	18.7	−0.4
15179	17.7	17.3	−0.4
15183	20.9	20.6	−0.3
15188	15.8	16.2	+0.4
15193	18.0	17.6	−0.4
15207	18.2	18.8	+0.6
15322	16.8	17.1	+0.3
15374	16.5	16.4	−0.1
15402	15.5	15.5	0.0

	r	0.9547
	$\bar{x}$ (USDA)	18.14
	$\bar{x}$ (Hach)	18.07
$\bar{x}$ (Hach) −	$\bar{x}$ (USDA)	−0.07
	A	1.0352
	B	-0.5667

V. APPLICATIONS

General Digestion Procedure

Refer to Hach's *Water Analysis Handbook*—Second Edition (literature code 8353) or the revised third edition of the *Digesdahl Digestion Apparatus Instruction Manual* (12-30-89-3ED, catalog number 23130-89) for the complete general digestion procedure. For a list of specific application procedure manuals, see section **VI. References** at the end of this booklet.

In the United States, request specific application procedures by the designated literature code numbers by calling 1-800-227-4224 or write:

HACH COMPANY
P.O. Box 389
Loveland, CO 80539

Outside the United States, contact the Hach office or distributor serving you.

Cereal Products

Examples: Grains, pasta, flour

Analytes: Protein, Ca, Cu, Fe, Mg, Mn, P, K, Zn

Procedure: General digestion

Request literature **3120.**

Cereal Products

Example: Corn starch

Analyte: Protein

Procedure: General Digestion

Request literature **3120.**

Fruits/Vegetables

Examples: Fresh, frozen and canned produce, processing samples

Analytes: Protein, Ca, Mg, P, Fe, Cu, Mn, Zn

Procedure: General digestion

Request literature **3120.**

Edible Oils

Examples: Corn oil, soybean oil, peanut oil

Analytes: Phosphorus

Procedure: General digestion, except split H_2O_2 addition—5 mL immediately upon heating and 5 mL later.

Request literature **3120.**

Meat/Poultry/Fish

Examples: Fresh or processed products, mechanically de-boned poultry

Analytes: Protein, Ca, Mg, P, bone-equivalent, fat

Procedure: Add 50 mL H_2O and 50 mL H_2SO_4. Stir until sample dissolves and fat separates*. Pipet 5 mL sample solution into digestion flask and use general digestion, except no additional H_2SO_4.

Request literature **3120.**

Meat/Poultry/Fish

Examples: Fresh meat used for processing

Analyte: Total extractable protein (salt soluble)

Procedure: Treat sample with 150 mL 10% NaCl solution for 2 minutes in a high-speed blender. Filter with Celite. Add 5 mL of the filtrate to the digestion flask. Add 5 mL of H_2SO_4 to the digestion flask. Heat to fuming (480 °C, 900 °F). Add 5 mL H_2O_2 at 3 mL/minute.

Request literature **3120.**

Dairy Products

Examples: Cheese, dry milk, dry whey, fluid milk and whey

Analytes: Protein, Ca, Mg, K, P

Procedure: General digestion

Request literature **3120.**

Dairy Products

Example: Cheese

Analyte: Fat

Procedure: Using the Hach Fat analysis glassware, add 50 mL H_2O, 2 mL pentadecane and 50 mL of H_2SO_4 to the sample. Stir until the sample dissolves and the fat separates*. Attach measuring stem, add 50% H_2SO_4 and read the fat content.

*The acid solution may be digested for protein and elemental analysis.

Request literature **3120.**

Beverages

Examples: Beer, wine, soft drinks, fruit and vegetable juices

Analytes: Protein, Ca, Mg, P, K, Fe, Mn, Cu

Procedure: Preheat 4 mL of H_2SO_4 for 1 minute (470 °C, 875 °F).

Add the sample through the capillary funnel. Add 10 mL of H_2O_2 at 3 mL/minute.

Request literature **3120.**

Animal Feed/Pet Food

Examples: Grains, forage, complete ration, protein supplements

Analytes: Protein, Ca, Cu, Fe, Mg, Mn, P, K, Zn

Procedure: General digestion

Request literature **3120.**

Fertilizer Solutions

Example: Nitrogen fertilizer solutions

Analyte: Total nitrogen (NH_4^+, NO_3, organic)

Procedure: Add 0.4 g salicylic acid and 4 mL H_2SO_4 to the sample and let stand for 10 minutes. Heat at 80 °C in a lab oven for 15 minutes. Use the general digestion, except no additional H_2SO_4.

Request literature **3201.**

Animal Waste

Example: Feces, urine, manure

Analytes: N, P, K

Procedure: General digestion

References: *Agricultural Analysis Handbook*, Hach catalog number 22546-08.

Watkins, et. al. *J. Assoc. Off. Anal. Chem.* **1987,** *70* (3).

Wastewater

Examples: Sewage, septage

Analytes: Ag, Al, Cd, Co, Cr, Cu, Fe, K, Mn, Ni, Pb, P, TKN, Zn

Procedure: Use 4 mL H_2SO_4 to digest the sample and heat to evaporate the water. Use the general digestion. Neutralize the digested sample with 8N KOH before analysis.

Request literature **8353** (*Water Analysis Handbook*).

Sludges

Example: Aerobic, anaerobic municipal sludge, dried sludge

Analytes: Ag, Al, Cd, Co, Cr, Cu, Fe, K, Mn, Ni, Pb, TKN, Zn

Procedure: Use the general digestion, except heat diluted digest for 15 minutes and filter.

Request literature **8353** (*Water Analysis Handbook*).

Fuels/Lubricants

Examples: Coal, grease, lubricating oil

Analytes: N, Ca, Cu, Cd, Cr, Fe, Ni, Zn

Procedure: Use the general digestion, except with 6 mL of H_2SO_4 and 20 mL of H_2O_2. Heat the diluted digest for 15 minutes and then filter for analysis.

References: Norton, et. al. *Fuel* **1987,** *66*.

Organic Chemicals

Examples: Aliphatic, cyclic and aromatic amines

Analyte: N

Procedure: General digestion

Reference: Treybig, et. al. *Anal. Chem.* **1983,** *55* (6).

VI. REFERENCES

Hach Procedure Manuals

Procedure Manuals	Catalog Number	Literature Code
Systems for Food, Feed, and Beverage Analysis	24309-00	3120
Plant Tissue Analysis Manual	23260-06	3118
Fluid Fertilizer Analysis Manual	23677-00	3201
Greenhouse Media Analysis	23260-09	3204
Hach *Water Analysis Handbook*	23196-00	8353
Digesdahl Digestion Apparatus Instruction Manual, 3rd edition	23130-89	
Agricultural Analysis Handbook	22546-08	

Technical Journals

Hach, C.C.; Brayton, S.V.; Kopelove, A.B. "A Powerful Kjeldahl Nitrogen Method Using Peroxymonosulfuric Acid"; *J. Agric. Food Chem.* **1985,** *33* (6).

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Norton, G.A.; Adams, N.S.; Markuszewski, R.; Brayton, S.V. "Rapid Dissolution Technique for Colorimetric Determination of Nitrogen in Coals"; *Fuel* **1987,** *66.*

Treybig, D.S.; Haney, P.L. "Colorimetric Determination Of Total Nitrogen in Amines with Selenium Catalyst"; *Anal. Chem.* **1983,** *55* (6).

Watkins, K.L.; Veum, T.L.; Krauge, G.F. "Total Nitrogen Determination of Various Sample Types: A Comparison of the Hach, Kjeltec and Kjeldahl Methods"; *J. Assoc. Off. Anal. Chem.* **1987,** *70* (3).

Chen, Y.S.; Brayton, S.V.; Hach, C.C. "Accuracy in Kjeldahl Protein Analysis"; *American Lab* **1988.**

Chen, Y.S.; Brayton, S.V.; Aspelund, T.G. "A Fast Method for Determination of Total Extractable Protein in Meat"; Manuscript in preparation.

About the Author

Scott Brayton, an honor graduate of Iowa State University with a B.S. degree in Chemistry, joined Hach Company's research program in 1975. Brayton's efforts in field research and commercial agricultural analysis were primarily responsible for the formation of the company's Agricultural Products Group. Manager of Research and Development for this group since 1977, Brayton has been dedicated to the development and support of products and practical testing procedures for analysts in agricultural research, education and agribusiness. He also served as Director of Chemical Research for Hach Company. Currently Coordinator and Instructor at the Ames Technical Training Center, he is a member of the American Chemical Society, the Institute of Food Technologists, and the Association of Official Analytical Chemists.

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