▼ Journal of Research (Science), Bahauddin Zakariya University, Multan, Pakistan.
Vol.15, No.2, June 2004, pp. 159-179 ISSN 1021-1012

EVALUATION OF KJELDAHL DIGESTION METHOD

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Abstract: The evaluation of the Kjeldahl digestion method was investigated by comparing measured values of total nitrogen, phosphorus and potassium using three salt and catalyst mixture in Standard Kjeldahl digestion method and Salicylic acid Modification method with certified values of plant material as well as comparison was made for determination of total nitrogen from steam distillation method verses the Technicon Auto-analyzer, and phosphorus Ascorbic acid/Molybdate method verses Molybdate/ Metavanadate method on the Technicon Auto-Analyzer. The 1 g salt/catalyst mixture recovered less nitrogen than the 2.5 g in the standard Kjeldahl method due to the lower temperature and incomplete digestion in both plant and soil samples. The 2.5 g catalyst mixture partially recovered nitrate in the standard Kjeldahl method and the salicylic acid modification fail to recover all nitrate in plant material. Use of 2.5 g salt catalyst mixture and selenium appears to promote nitrogen losses in salicylic acid modification method but not in the standard Kjeldahl method of digestion for soil samples. No interference of selenium or copper was observed in Nitrogen and Phosphorus on colorimetric determination. The standard kjeldahl method with 2.5 g of salt/catalyst mixture of sodium sulphate cupper sulphate (10:1) in 5 ml sulphuric acid were found suitable for determination of total Nitrogen, phosphorus and potassium.

The steam distillation and the Technicon Auto-Analyzer technique measure similar amounts of ammonium nitrogen. However, the Technicon Auto analyzer technique is easier, rapid, high degree of reproducibility, precise, accurate, reliable and free from human error. The amount of phosphorus measured by the Ascorbic acid/Molybdate method was more accurate than by the Molybdate/ Metavanadate method on Technicon Auto-Analyzer.

Keywords: Auto Analyzer Technicon, Total Nitrogen, Total Phosphorous, Total potassium, Salt catalyst mixture, Standard Kjeldahl Digestion, Steam distillation.

INTRODUCTION

Kjeldahl [1883] developed a method involving the conversion of organic nitrogen into ammonium by boiling with sulphuric acid and distilling with alkali to liberate ammonia for determination by titration. The Kjeldahl process with certain modifications has been widely used for plant and soil analysis ever since. A few other methods of determining total nitrogen like the sulphuric acid and phosphoric acid mixture method of Honda [1962], the rapid chromic acid method of Flowers and Bremner [1991a,b], and Sharma and Sud [1980] have been published but the most generally accepted is the Kjeldahl method.

The Kjeldahl procedures generally employed for the determination of total N involves two steps [Nelson and Sommers 1973]: i) digestion of the sample to convert organic and inorganic forms of N to ammonium-N and ii) determination of ammonium-N in the digest.

The total nitrogen recovery during digestion is influenced by the quantity of sample and sample size. Semi-micro Kjeldahl digestion methods require a small quantity of sample, 50-200 mg plant material or 100-500 mg soil. Material must be finely ground before analysis. If plant material is ground to less than 40 mesh, a sample quantity of at least 100 mg is recommended to ensure that a representative sub sample is analyzed. Use of a 50 mg sample required that the plant material be ground to less than 80 mesh. To obtain reproducible results during semi-micro Kjeldahl analyses of soils, it is normally necessary to grind the samples to less than 100 mesh [Nelson and Sommers 1980].

A second factor which influences the total nitrogen recovery is pretreatment of the sample to include nitrate nitrogen. Pretreatment of samples to include nitrate in total N determination is necessary because the routine Kjeldahl process fails to estimate nitrate and nitrite nitrogen. Three modifications of the Kjeldahl method have been used to recover nitrate and nitrite nitrogen in total Nitrogen analysis:-

(1) The salicylic acid modification introduced by Cope [1916] where the sample is pre-heated with salicylic acid and sodium thiosulphate.

(2) The alkaline reduction modification of Davisson and Parsons [1919] in which Devarda's alloy and alkali are used to reduce nitrate and nitrite to ammonia which is then collected in sulphuric acid and added to the digest.

(3) The permanganate-reduced Iron modification of Olsen [1929] in which potassium permanganate is used to oxidize nitrite to nitrate-Nitrogen which is then reduced to ammonium by reduced iron. The most popular method in use is the salicylic acid modification.

In the salicylic acid modification of the Kjeldahl method, the sample is treated with salicylic acid dissolved in concentrated sulphuric acid. The nitro compounds formed by the reaction of salicylic acid with nitrate in acid medium are reduced to the corresponding amino compounds by heating the mixture with sodium thiosulphate or Zn dust before digestion. The identity of the nitro compounds formed in this procedure have not been fully established, but work by Stalcup and William [1955] indicated that the main product of nitration is 5-nitrosalicylic acid and that small amounts of 3-nitrosalicylic acid are also formed. Although this method has been used extensively for total N analysis of soils containing nitrate and nitrite serious doubts have existed about its ability to recover nitrate quantitatively and its applicability to undried soils [Bremner 1965]. According to Piper [1947], nitration of salicylic acid cannot be applied successfully to undried soil samples. However, Bremner and Mulvaney [1982] suggested that good recovery of nitrate and nitrite can be obtained with the salicylic acid modification method in soils containing 0.6 ml of water per gram of soil. The salicylic acid method of pre-digesting was slightly modified using sodium sulphate-catalyst mixture instead of potassium sulphate-catalyst mixture with selenium by Khan [1994] to facilitate the measurement of nitrogen, phosphorus and potassium in the same digest. The determination of nitrogen, phosphorus and potassium are frequently required on the same plant materials for plant nutritional studies. It is thus desirable that analyses be made on a single digest to save time, labor and cost of analyses.

Several digestion procedures have been described which allow the determination of multi-element nutrients on the same digestion solution. The most popular methods of digestion for multi-element analyses are:-

i) The dry ashing method of Ward and Johnston [1960] and Varley [1966]
ii) The mixed acid (HNO_3-$HClO_4$-H_2SO_4) method of Jackson [1958]
iii) Hydrogen peroxide method of Lindner [1944] and Thomas *et al*. [1967]
iv) The H_2SO_4-Se method of O' Neill and Webb [1970]
v) The H_2SO_4, H_2O_2 Li_2SO_4 and Se method of Parkinson and Allen [1975]
vi) Kjeldahl method of digestion.

Van Lierop [1976] conducted a study comparing the efficiency of several digestion procedures based on the conventional Kjeldahl digestion procedure. The digest contained 0.5 g sodium sulphate salt per ml of sulphuric acid, with various additions of hydrogen peroxide or perchloric acid to aid clearing and digestion for multi-element analyses. He concluded that a wet-digestion technique using hydrogen peroxide and perchloric acid as oxidative agent in sulphuric acid medium was the most advantageous among dry ashing and conventional Kjeldahl procedures for multipurpose analyses. But he admitted that the amount of ammonium recovered by the suggested method was lower than by the Kjeldahl method. Similar amounts of phosphorus were recovered by all digestion procedures. However, he could not measure phosphorus by the Kjeldahl procedure method because of selenium precipitation. Potassium, calcium and magnesium recoveries were less by the dry ashing method than with the Kjeldahl and hydrogen peroxide methods. Smith [1979] compared peroxidation digestion (H_2SO_4-H_2O_2-Li_2SO_4-Se), the mixed acid digestion (HNO_3-H_2SO_4-$HClO_4$) and dry ashing procedures and reported that the mixed acid digestion method generally yielded higher Ca, Mg, and P values than dry ashing and peroxidation. The K contents of plant tissue estimated by both wet digestion techniques were not significantly different but were lower by the dry ashing method. Novozamsky *et al*. [1983] determined N, P, K, Ca and Mg in plant material using a mixture of sulphuric acid and hydrogen peroxide, or a sulphuric acid-selenium, salicylic acid and hydrogen peroxide method. The measured values of all the elements were higher in the latter method. Alfonso and Nelson [1991] determined N, P, and K in sugar cane leaf by the Kjeldahl method using all combinations of 3 different amounts of catalyst mixture of sodium sulphate (2.5 g, 5 g and 10 g) and four digestion times (0.75 hr, 1 hr, 1.5 hr and 2 hrs). He concluded that the amount of salt catalyst mixture has influenced the recovery of nitrogen and potassium, while the period of digestion affected recovery of nitrogen, phosphorus and potassium.

These previous investigations show that dry ashing and sulphuric acid and peroxide methods recover low amounts of nutrients. The mixed acid method recovers low amounts of ammonium nitrogen. The Kjeldahl digestion method is influenced by the time and catalyst mixture quantity. Use of selenium as catalyst can cause interference or nitrogen losses.

Bal [1925] found that the total nitrogen values obtained by Kjeldahl analysis of clay soils significantly increase with pre-treating with water before digestion. Bremner and Harada [1959] showed that inclusion of a pretreatment with water had no effect in soils high in clay and clay fixed ammonium. Later Bremner and Keeney [1965] found that hydrogen fluoride pretreatment in Kjeldahl analysis of soils is an effective method for the inclusion of clay fixed ammonium.

A third factor which influences the total nitrogen recovery as reported by Nelson and Sommers [1980] is the acid mixture used for digestion. Concentrated sulphuric acid is normally the acid specified for digestion, although sulphuric/ phosphoric acid mixtures have been used in semi-micro-Kjeldahl procedures [Honda 1962, Skjemstad and Reeve 1976]. Batey *et al*. [1974] advocated use of an H_2SO_4-$HClO_4$ mixture for Kjeldahl analysis of plant materials, although the procedure has not been widely adopted. Other investigators have recommended use of hydrogen peroxide to assist in clearing the digests [Thomas *et al*. 1967, Parkinson and Allen 1975] thereby decreasing digestion times. However, Nelson and Sommers [1973] found that sulphuric acid-hydrogen peroxide digestion procedures (no potassium sulphate or catalysts were used) produced poor recoveries of total N from plant materials (only 84% of the total N found by the standard procedure). In addition, Hambelton and Noel [1975] reported that hydrogen peroxide treatment resulted in excessive foaming and loss of sample from digestion tubes. There appears to be little advantage in the use of other acids or oxidants in conjunction with sulphuric acid for Kjeldahl digestion. However Hach *et al.* [1985] reported that results with a peroxymonosulphuric acid method are accurate, fast, and comparable to standard Kjeldahl methods.

A fourth factor is the addition of salt to raise the temperature. Various salts, such as potassium sulphate, sodium sulphate and phosphorus pentoxide have been added to Kjeldahl digestion mixtures to raise the boiling point of sulphuric acid. Digestion temperature increases from 332 (C with no salts to 371 (C when potassium sulphate is added at 1 g ml-1 sulphuric acid. High temperature digestion ensures recovery of nitrogen in compounds which do not decompose at the boiling point of concentrated sulphuric acid and markedly reduces the time required for complete digestion of samples. Some recently published semi-micro-Kjeldahl methods do not specify use of salts in the digestion. However, Nelson and Sommers [1973] found that digestion mixtures without potassium sulphate gave significantly lower total N recoveries from plant materials than did mixtures with potassium sulphate.

The amount of salt specified per ml sulphuric acid in semi-micro-kjeldahl procedures varies widely. High salt to acid ratios (more than 0.8 g potassium sulphate ml-1 H2SO4) give high digestion temperatures and reduced times for complete digestion of the sample. However, sample handling difficulties increase with high salt ratios because mixtures tend to bump and splatter during digestion and digests are often difficult to dissolve prior to ammonium analysis. Furthermore, samples containing high salt concentrations may solidify during digestion, resulting in loss of nitrogen from the digests. Use of lower salt to acid ratios promotes easier digestion and analysis of digests, however, more time is required for complete sample digestion. Adding 0.33-0.5 g potassium sulphate per ml of sulphuric acid results in a digestion mixture which is easy to handle and which gives good recoveries of total N from soil and plant material [Nelson and Sommers 1980, Bremner and Mulvaney 1982].

A fifth factor which influences the total N recovery is the use of catalysts. Wilfarth [1885] used copper sulphate and mercury oxide catalyst to hasten the reaction. Lauro [1931] introduced selenium as a catalyst and it has remained one of the most efficient means of hastening the reaction. Since that time copper, mercury and selenium have been the major catalysts used in the Kjeldahl digestion process to shorten the digestion time. Copper, generally in conjunction with limited amounts of selenium, is the most widely used catalyst for Kjeldahl analysis of soils. Mercury is more generally specified as the catalyst for total N determination of plant materials but it was reported by Singh et al. [1984] that use of mercury catalyst causes significant interference due to precipitation of mercury in the colorimetric determination by the Technicon Autoanalyzer. In addition to interference, mercury is also hazardous to health. Ashton [1936] compared the effect of selenium and copper sulphate on the time of digestion and found that selenium is effective in reducing digestion time. However, some early investigations suggest that selenium in the digest promotes volatile losses of nitrogen [Davis and Wise 1933]. A number of investigations have not confirmed that selenium promotes N loss during digestion and have established the applicability of selenium as a catalyst in routine Kjeldahl determinations on biological materials [Bremner 1965, Nelson and Sommers 1972, 1973, Bremner and Mulvaney 1982]. In addition to interference and causing losses in nitrogen recovery, selenium is also hazardous to health.

A final factor which influences recovery is the digestion time. All the modifications of the Kjeldahl method recognize that digests must be at least "clear" if quantitative recovery of N in the sample is to be obtained. There are large differences in the boiling period after clearing which is recommended for various semi-micro-Kjeldahl procedures. For digestion of plant materials, an after-boil period of 0-150 min has been specified. Nelson and Sommers [1973] found that a 1 hour after-boil period was required for maximum recovery of N from a variety of plant materials.

Relatively long periods of digestion after clearing are required to recover fixed ammonium from soil samples. Bremner [1965] observed that a 5 hour after-boil period was required for some soils when a low K2SO4: H2SO4 ratio was used. With the high K2SO4:H2SO4 ratio a shorter period of boiling was required after clearing of digests. Nelson and Sommers [1972] found that a 3 hour after-boil period was required for maximum recovery of N in soils studied. Nelson and Sommers [1980] and Bremner and Mulvaney [1982] recommended at least 3 hour after clearing to ensure complete recovery of nitrogen.

The Kjeldahl procedure and most modifications for determining total nitrogen in soils and plants published prior to 1950 specified the use of macro-Kjeldahl flasks (350-800 ml) for digestion and distillation. The introduction of aluminum blocks for Kjeldahl digestion in the early 1970s is a most noteworthy development. Nelson and Sommers [1972], Schuman *et al*. [1973], Gallaher *et al*. [1976], Douglas *et al.* [1980] and Bremner and Breitenbeck [1983] have described the use of block digester techniques for soil and plant digestion.

Ammonium in digests of plants, soils and animal wastes can be measured by steam distillation, colorimetric methods and the ammonium electrode system. These methods have various degrees of sophistication, sensitivity, cost of system and reagents, rapidity, accuracy and precision.

The distillation methods are simple, accurate and they are not affected by various organic and inorganic substances that often interfere with colorimetric method of determining inorganic nitrogen [Bremner 1965a,b, Bremner and Keeney 1965, Keeny and Nelson 1982].

The determination of ammonium in the Kjeldahl digest of soil by the ammonia electrode has been reported by Bremner and Tabatabai [1972]. These electrodes on first consideration appeared to be quite attractive when compared with manual distillation or colorimetric methods. In theory this approach should be simpler, more rapid and less expensive. However, specific ion electrodes are expensive and the cost of suitable meter electrode combinations easily exceeds the costs of distillation units or simple spectrophotometers.

The use of automated colorimetric analysis by the Technicon Auto-Analyzer for the determination of inorganic nitrogen in various Kjeldahl digest solutions is attractive because large numbers of samples can be analyzed quickly and with a high degree of reproducibility. In this method ammonium in the digest is separated from other digest constituents by continuous flow distillation or dialysis and determined by a procedure involving measurement of the color of the indophenol complex formed by the Berthelot reaction. Alternatively it may be enough to simply dilute and neutralize the digests before colorimetric determination. Khan [1994] found that dilution and dialysis measured equal quantities of nitrogen in plant digests by the Technicon Autoanalyzer. Similarly Wang and Oien [1986] concluded that simple dilution of Kjeldahl digests for determining nitrogen at room temperature on the Auto-analyzer by the indophenol method is satisfactory.

The development of the Technicon Auto-Analyzer has provided a system capable of automating many analyses. Thomas *et al.* [1967] produced a paper concerned with the development of a single digestion procedure combined with an automated system of analysis for N and P and spectrographic analysis for K in plant material. Phosphorus in the plant digest solutions may be determined spectrophotometrically by two methods: (1) Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to an intensely colored molybdenum blue by ascorbic acid. (2) In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdo phosphoric acid is formed. The concentration of P in solution is measured as the blue or yellow color. Apostolatos [1984] published a rapid procedure for determination of phosphorus from a Kjeldahl digest. Blakemore *et al*. [1987] described the Auto-Analyzer method as the most widely used in New Zealand for the determination of the main fractions of phosphorus. The automated method is labor conserving and can handle large numbers of samples on a routine basis with a high degree of reproducibility. It is also relatively more precise as each sample is treated in the same way. The human error in mixing and other operations is reduced to a great extent. The automated procedure can also tolerate slight turbidity in the extract which is sometimes unavoidable. Lennox [1979] reported that the automatic determination of phosphate was an excellent procedure as regards recovery and effectiveness. The detection limit for the automated step was 1µg P litre^{-1}. The concentration of K in the diluted digest solution is determined with a flame photometer [Collins and Polkinhorne 1952].

Keep in view the above facts a study was undertaken to measure the nitrogen, phosphorus and potassium content in certified reference plant material and total nitrogen in soil samples of different texture class. The samples were digested by two methods the standard Kjeldahl digestion method and salicylic acid modification method. Each digestion method was examined for three salt catalyst mixtures for digestion. Two analytical techniques of determination of nitrogen and phosphorus were compared for precision and accuracy.

MATERIALS AND METHODS

The study was conducted in the laboratory at Glasgow University, Glasgow, United Kingdom during 1993 on two plant materials and two soils of different texture class (Midelney and Dreghoran). The description of plant material is as fallow.

HAY POWDER

Certified reference material No BCR 129 (hay powder) with individual identification No. 0088 supplied by the Commission of the European Communities, Community Bureau of Reference was purchased from the Office of Reference Materials, Laboratory of the Government Chemist, Queens Road, Teddington, Middlesex TW11 0LY, UK.

CABBAGE POWDER

Certified reference material No. GBW 08504 (cabbage powder) supplied by Food Detection Science Institute Ministry of Commerce, Beijing, China was also obtained from the Laboratory of the Government of Chemist.

Table 1: Certified values for total N, P and K content of hay and cabbage powder.

Element	Hay Certified value (mg g^{-1})	Cabbage certified value (mg g^{-1})
Kjeldahl-N	34.20	28.0
Total N	37.20	-
Total P	2.36	3.4
Total K	33.80	14.5
Extractable nitrate	3.10	3.2

PROCEDURE FOR STANDARD KJELDAHL DIGESTION METHOD

Before use the plant material was shaken for 2 minutes as per instructions provided by the supplier for re-homogenization of the bottle contents. The samples were weighed on a boat made from aluminum foil. The boat was placed on the end of a pipette, pushed down to the end of a Tecator Kjeldahl tube and emptied. This was done to ensure that the entire sample reached the base of the tube. The boat was reweighed to allow correction for the small amount of sample remaining in the weighing boat.

Working in the fume cupboard a 5 ml aliquot of concentrated sulphuric acid and the catalyst salt mixtures were added. The tubes were gently shaken to mix well. The 40 tube rack was placed in the Tecator block digester and heated at 375 °C for 3 hours. Following digestion, the tubes were removed from the block digester and left to cool until they were able to be handled. The digest was first made up to approximately 30 ml with deionized water and shaken well to dissolve the entire digest. Then the digest was diluted to 100 ml in the graduated digestion tube.

After mixing, approximately 40 ml of the digest was stored in a plastic bottle for determination of the nitrogen content by steam distillation. The remaining portion of the digest was filtered through a Whatman No. 40 filter paper. The first 30 ml was discarded to wash the filter paper and the remaining 30 ml was collected and stored in a plastic capped glass vial for analysis on the Technicon Auto-Analyzer.

PROCEDURE FOR SALICYLIC ACID MODIFICATION DIGESTION METHOD

Same as above with modification that 25 g salicylic acid was dissolve in a litre of sulphuric acid which was used as a 5ml aliquot along with 0.5 g of sodium thiosulphate in each tube in place of sulphuric acid of standard method. The tubes were left stand overnight. The tubes were heated for 45 minutes at 135 °C and salt catalyst mixture was added. The tubes were shaken to mix well. The rest of the procedure is same.

EXPERIMENTAL DESIGN

Experiment consisted of the analysis of 2 plant materials with five replications using Two Digestion Methods and 3 catalyst mixtures, 3 blank for each catalyst. Fifteen tubes were allotted to each of the two plant materials and nine to blanks. Each tube was randomly placed into the holes of the block digester. The random number was allotted to each tube by a computer program.

DIGESTION METHODS

M1	Standard Kejldahl Digestion Method
M2	Salicylic Acid Modification Digestion Method.

SALT/CATALYST TREATMENTS

1) 1.0 g of sodium sulphate/copper sulphate mixture (10:1).
2) 2.5 g of sodium sulphate/copper sulphate mixture (10:1).
3) 2.5 g of potassium sulphate/copper sulphate /selenium (100:6:1).

DETERMINATION OF MAJOR PLANT NUTRIENTS IN THE DIGESTS

Nitrogen

Determination of the concentration of ammonium nitrogen in the digest was carried out by steam distillation and by the Technicon Autoanalyzer system.

Preparation of Working Standard Solution and Reagents

The standard stock solution (1000 N mg l^{-1}), reagents and working standards solutions were prepared for ammonium determination in plant. Working standards solution was prepared at 0 and 100 mg N l^{-1} for the analyses of plant material digests. The standards contained the same concentration of acid and catalyst mixture as the digests. The appropriate weight of catalyst mixture was added to 5 ml sulphuric acid and heated at 375 °C on the Tecator block digester until dissolved. The solution was cooled and diluted with 60 ml of deionized water. The diluted solutions were transferred into the volumetric flasks. The solutions were filtered through a Whatman filter paper No. 40 before transfer to the volumetric flask. An appropriate volume of 1000 N mg l^{-1} NH^{4+} stock solution was added and the volume made up to 100 ml.

Additional Reagents

a) Wash chamber solution: 50 ml of concentrated sulphuric acid was added to 800 ml of water in 1 litre bottle and made up to the mark.
b) Dilution / neutralizing solution: 3.6 g of sodium hydroxide was dissolved in a litre of deionized water.

Procedure

Filtered solutions were analyzed directly on a Technician Auto-Analyzer II system. Forty samples per hour were run with a dilution / neutralization step before the main manifold.

i) Sample wash solution 5 % v/v H_2SO_4, ii) Dilution ratio 20:1,
iii) Sample 0.1 ml min^{-1}, iv) Dilutent 2.0 ml min^{-1}
v) Air 0.8 ml min^{-1}

Phosphorus and Potassium

Determination of phosphorus concentration in the digests was carried out by two methods, the Ascorbic acid/Molybdate method and Molybdate/ Metavanadate method. The potassium was measured on flame photometer by diluting the digests. The detail method and procedure is available in thesis of [Amin 1995].

STATISTICAL ANALYSIS

The Minitab programme version 7.2 was used for statistical analysis of the data. The General Linear Model and Fisher LSD test was used for analysis of variance. A t test was used to compare results with the certified values and a paired t test was used to compare individual digests analyzed by two different analytical methods.

RESULTS AND DISCUSSION

EFFECT OF CALALYST MIXTURE ON STANDARD KJELDAHL DIGESTION METHOD AND SALICALIC ACID MODIFICATION

The amount of salt catalyst has affected handling and dissolution. The 2.5g salt catalyst mixture of sodium sulphate and kjeltab in salicylic acid method was difficult to handle due to solidification of digest on cooling compared with standard digestion Kjeldahl method. Which required more time for shaking to dissolve in water in block digestion tubes, however no bumping and splattering was observed during digestion. This effect was increased in soil sample as well and loss of nitrogen was measured in the salicylic acid modification method. It has been reported that high salt to acid ratios (more than 0.8 g potassium sulphate ml^{-1} H_2SO_4) give high digestion temperatures and reduced times for complete digestion of the sample. However, sample handling difficulties increase with high salt ratios because mixtures tend to bump and splatter during digestion and digests are often difficult to dissolve prior to ammonium analysis. Furthermore, samples containing high salt concentrations may solidify during digestion, resulting in loss of nitrogen from the digests. Use of lower salt to acid ratios promotes easier digestion and analysis of digests, however, more time is required for complete sample digestion. Adding 0.33-0.5 g potassium sulphate per ml of sulphuric acid results in a digestion mixture which is easy to handle and which gives good recoveries of total N from soil and plant material [Nelson and Sommers 1980, Bremner and Mulvaney 1982].

NITROGEN

Effect of Catalyst and Digestion Method on Total Nitrogen Recovery in Plants

Tables 2 to 5 show the effect of catalyst mixture on digestion methods on the determination of total nitrogen in hay and cabbage powder. For both hay and cabbage significantly lower amounts of total nitrogen were recovered by the treatment containing 1 g of catalyst mixture compared to other treatments of catalyst mixture in Standard Kjeldahl Method and Salicylic acid Modification. To test the reliability, the results were compared with the certified values of Kjeldahl nitrogen. As Tables show, recovered values of Kjeldahl nitrogen in hay and cabbage which were non significantly different from the certified values except in 1 g of catalyst mixture recovered less Kjeldahl nitrogen significantly than the respective certified values. However higher values were measured in rest of the treatment of both Methods.

Table 2: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Hay powder (CRM129) measured by Technicon Auto-Analyzer.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	33.5 a[1]	*[2]	35.3 bc	NS
$Na_2SO_4/CuSO_4$	2.5	35.1 bc	NS	35.3 bc	NS
K2SO4/CuSO4/Se	2.5	34.9 b	NS	35.7 c	*

Table 3: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Hay powder (CRM129) measured by Steam distillation titration.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	34.4 a[1]	NS[2]	35.4 bc	NS
$Na_2SO_4/CuSO_4$	2.5	35.2 bc	NS	35.7 c	*
$K_2SO_4/CuSO_4/Se$	2.5	34.6 ab	NS	35.2 bc	NS

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from a certified value (34.2 mg g^{-1}) using a t test, are indicated as follows. * 5% level.

Table 4: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Cabbage powder measured by Technicon Auto-Analyzer.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	28.1 a[1]	NS[2]	30.4 d	*
$Na_2SO_4/CuSO_4$	2.5	29.3 b	NS	29.7 c	NS
$K_2SO_4/CuSO_4$ /Se	2.5	29.2 b	NS	29.3 b	NS

Table 5: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Cabbage powder measured by Steam distillation titration.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	28.4 a[1]	NS[2]	30.9 c	*
$Na_2SO_4/CuSO_4$	2.5	28.3 a	NS	29.9 b	*
$K_2SO_4/CuSO_4/Se$	2.5	29.2 b	NS	29.7 b	*

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from a certified value (34.2 mg g^{-1}) using a t test, are indicated as follows. * 5% level.

The reason for the low recovery with 1 g catalyst in the standard Kjeldahl method may be incomplete digestion and the higher recovery of Kjeldahl nitrogen with respect of certified value in standard method with 2.5 g catalyst may be the partial recovery of nitrate. To confirm the nitrate content in the samples nitrate was extracted from samples. The nitrate content in hay and cabbage powder was 3.1 and 3.2 mg g^{-1}, respectively, which is approximately 10% of total Kjeldahl nitrogen (the same amount of nitrate has been reported by the material certifying agency). It is clear that a significant amount of nitrate is present in the powders. Nelson and Sommers [1973] evaluated a standard digestion procedure (1.1 g of catalyst mixture of 100 g potassium sulphate, 10 copper sulphate and 1 g selenium in 4 ml of sulphuric acid) and a Salicylic acid digestion procedure (1.1 g of above catalyst mixture in 4 ml of salicylic-sulphuric acid mixture of 5 g : 200 ml) on the recovery of nitrate nitrogen from plant material. They showed similar total N values for both methods for samples containing low levels of nitrate. However, the salicylic modification yielded higher total N values for samples containing 0.35 and 0.31% nitrate nitrogen. They further evaluated nitrate nitrogen recovery by both methods with added potassium nitrate and reported that recovery of added nitrate by the Salicylic acid method ranged from 95 to 100 % whereas for the standard method it was between 16 and 55% of the added nitrate-N. They also concluded that the standard method recovered 21-55% of the nitrate naturally present in the plant material.

The other reason for the higher recoveries in the study is the digestion method used for certification. The method used by the Commission of European Communities, Community Bureau of Reference for determining nitrogen in the certified plant material was described as follows:

The certified plant material was digested in a 50 ml Kjeldahl flask with 5 ml sulphuric acid, 3 ml H_2O_2 and 50 mg selenium as a catalyst, boiling over glass beads for 1.5 to 3.5 hr until clear; after completion of dissolution, dilution of the cooled digest to 50 ml with distilled water; mixing and filtering through filter paper; steam distillation of 10 ml of digest for 5 minutes in a Markham unit after addition of 15 ml NaOH; absorption of NH_3 in a 1% H_3BO_3 solution; verification of the alkalinity at the end of the distillation and finally titrating with acid. Nelson and Sommers [1973] reported that various methods using H_2O_2-H_2SO_4

digestion gave poor recoveries of N in plant materials (84% N was recovered by H_2O_2-H_2SO_4 with 1.1 g of catalyst mixture in 4ml of sulphuric acid). Similarly Van Lierop [1976] and Singh *et al.* [1984] measured higher nitrogen values by catalyst digestion than with hydrogen peroxide accelerated Kjeldahl digestion.

Effect of Catalyst and Digestion Method on Total Nitrogen Recovery in Soil

Tables 6 to 9 of soil samples show that significantly lower total nitrogen recovery by the standard Kjeldahl method with the treatment containing 1g of catalyst mixture compared to 2.5 g catalyst and Kjeltabs. The difference between 2.5 and Kjeltabs in standard method is non significant in both soils. The salicylic acid modification shows lower recovery of nitrogen than standard kjeldahl method. The effect of three catalysts in salicylic acid modification shows a downward trend of nitrogen recovery from 1 g to 2.5 g and Kjeltabs. The lowest nitrogen was recovered in treatment containing Kjeltabs in salicylic acid modification in both soils.

Table 6: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Midelney soil measured by Technicon AutoAnalyzer.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})	
Salt/ Catalyst	weight (g)	Standard Method	Salicylic Modification
$Na_2SO_4/CuSO_4$	1.0	7.19 ab[1]	7.23 bc
$Na_2SO_4/CuSO_4$	2.5	7.49 d	7.21 bc
$K_2SO_4/CuSO_4$/Se	2.5	7.38 cd	7.01 a

Table 7: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Midelney soil measured by Steam distillation titration.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})	
Salt/ Catalyst	weight (g)	Standard Method	Salicylic Modification
$Na_2SO_4/CuSO_4$	1.0	7.07 a[1]	7.05 abc
$Na_2SO_4/CuSO_4$	2.5	7.24 c	6.95 ab
$K_2SO_4/CuSO_4$/Se	2.5	7.15 bc	6.85 a

Table 8: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Dreghorn soil measured by Technicon Auto-Analyzer.

Catalyst Mixture		Total Nitrogen Content (mg g^{-1})	
Salt/ Catalyst	weight (g)	Standard Method	Salicylic Modification
$Na_2SO_4/CuSO_4$	1.0	2.15 c[1]	2.11 bc
$Na_2SO_4/CuSO_4$	2.5	2.24 d	2.07 ab
$K_2SO_4/CuSO_4$/Se	2.5	2.28 d	2.01 a

Table 9: Effect of catalyst mixture and Digestion Method on Total Nitrogen in Dreghorn soil measured by Steam distillation titration.

Catalyst Mixture		Total Nitrogen Content (mg/g)	
Salt/ Catalyst	weight (g)	Standard Method	Salicylic Modification
$Na_2SO_4/CuSO_4$	1.0	1.99 a[1]	1.97 a
$Na_2SO_4/CuSO_4$	2.5	2.13 b	1.98 a
$K_2SO_4/CuSO_4$/Se	2.5	2.15 b	1.97 a

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

The reason for the low nitrogen recovery with the 1 g catalyst mixture in standard method is incomplete digestion as for plant materials. The reason for downward trend in salicylic acid Modification method is the high salt content and selenium. Similar types of result were reported by Ashton [1936] and Bremner [1960].

Comparison of Steam Distillation with Technicon Auto-Analyzer Method

The manual steam distillation-titration method of determining ammonium nitrogen involves liberating ammonia with the flow of steam from the alkaline solution into the boric acid indicator mixture solution. The quantity of ammonium was estimated by titrating the indicator mixture with acid. The Technicon Auto-Analyzer method of determining ammonium nitrogen is based on the change in color developed by reacting with phenol under alkaline conditions and measured in a colorimeter. The complexing reagent is used to prevent interferences and to increase sensitivity. The methods were compared by using the Kjeldahl digests of certified hay and cabbage powders. No interference of selenium and copper was observed in the analysis of Technicon Autoanalyzer. Table 10 shows the means and standard deviations of the measured values of total nitrogen by the steam distillation method and the Technicon Auto-Analyzer in hay and cabbage.

Table 10: Comparison of steam distillation vs Technicon Auto-Analyzer.

Sample	Steam distillation		Technicon Auto Analyzer	
	Means	SD	Means	SD
	..(mg N g^{-1})...		....(mg N g^{-1})..	
Hay	34.90	0.90	35.00	0.80
Cabbage	29.40	1.10	29.20	0.90
Midelney	7.23	0.17	7.26	0.17
Dreghorn	2.08	0.10	2.09	0.10

The means of the measured values by both methods show similar amounts of nitrogen in hay and cabbage powders. The standard deviations of the steam distillation method and the Technicon Auto-Analyzer method are the same. Statistically there is no significant difference between the two analytical techniques by applying a paired t test on the data for hay and cabbage powders. Both methods achieve comparable ammonium values and although the time saved is confined to the second step of the final N determination it is substantial. The rate of estimating ammonium by steam distillation method was 12 samples per hour while the Technicon Auto-Analyzer analyzed 40 samples per hour. The Technicon Auto-Analyzer method is labor conserving and can handle a large number of samples on a routine basis with a high degree of reproducibility. It is also relatively more precise as each sample is treated in the same way.

PHOSPHORUS

Effect of Catalyst and Digestion Method on Total Phosphorus Recovery in Plants

Tables 11 to 14 show recovered values of Phosphorus in hay and cabbage which are non-significantly from the certified values at 5% however recovered values were slightly higher than certified values particularly in Metavanadate technique. Over all the effect of catalyst mixture and digestion method is non significant in phosphorus determination, however little contradictory effect was observed.

Similarly Alfonso and Nelson [1991] found that the amount of sodium sulphate and copper sulphate with Se and time of digestion did not affect the determination of total P in Kjeldahl digests of plant material.

Table 11: Effect of catalyst mixture and Digestion Method on Total phosphorus in Hay powder (CRM129) measured by Ascorbic acid/Molybdate Technique.

Catalyst Mixture		Total Phosporus Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	2.61 bc[1]	NS[2]	2.54 a	NS
$Na_2SO_4/CuSO_4$	2.5	2.65 c	NS	2.55 ab	NS
$K_2SO_4/CuSO_4$ /Se	2.5	2.66 c	NS	2.64 c	NS

Table 12: Effect of catalyst mixture and Digestion Method on Total Phosporus in Hay powder (CRM129) measured by Molybdate/Metavanadate Technique.

Catalyst Mixture		Total Phosphorus Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	2.69 a[1]	NS[2]	2.71 a	NS
$Na_2SO_4/CuSO_4$	2.5	2.76 a	NS	2.76 a	NS
$K_2SO_4/CuSO_4$/Se	2.5	2.70 a	NS	2.73 a	NS

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value (2.36 mg g^{-1}) using a t test are indicated as follows. * 5% level.

Table 13: Effect of catalyst mixture and Digestion Method on Total Phosporus in Cabbage powder measured by Ascorbic acid/Molybdate Technique.

Catalyst Mixture		Total Phosporus Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	3.89 a[1]	NS[2]	3.88 a	NS
$Na_2SO_4/CuSO_4$	2.5	3.86 a	NS	3.91 a	NS
$K_2SO_4/CuSO_4$ /Se	2.5	3.91 a	NS	3.85 a	NS

Table 14: Effect of catalyst mixture and Digestion Method on Total Phosphorus in Cabbage powder measured by Molybdate/Metavadate technique.

Catalyst Mixture		Total Phosphorus Content (mg/g)			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	3.95 a[1]	NS[2]	4.02 c	*
$Na_2SO_4/CuSO_4$	2.5	3.97 a	NS	3.75 b	NS
$K_2SO_4/CuSO_4$/Se	2.5	3.97 a	NS	3.96 b	NS

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value (3.4 mg g^{-1}) using a t test are indicated as follows. * 5% level.

Comparison of Ascorbic/Molybdate and Molybdate/Metavanadate Method

The Ascorbic acid / Molybdate method of phosphorus determination in which ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to an intensely colored molybdenum blue by ascorbic acid was compared with the Molybdate/Metavanadate method of phosphorus determination in which orthophosphate and, ammonium molybdate react under acid conditions to from a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The concentration of P in solution is measured as the blue or yellow color on the Technicon Autoanalyzer.

Table 15 shows the means and standard deviations of the measured values of phosphorus in hay and cabbage by the Ascorbic acid/ Molybdate method and the Molybdate/ Metavanadate method. The means of the measured values of phosphorus of hay and cabbage by both analytical methods were significantly higher ($P < 5\%$) than the certified values. Statistically there is also a significant difference between the two analytical techniques. The Ascorbic acid/ Molybdate method measured values are closer to the certified values than the Molybdate/ Metavanadate method. No interference effect of selenium and copper was observed by both methods. The standard deviation of the Ascorbic acid /Molybdate method for the cabbage powder was lower than for the Molybdate/ Metavanadate method.

Table 15: Comparison of Ascorbic acid/Molybdate Vs Molybdate/Metavanadate

Sample	Ascorbic acid		Metavanadate	
	Means ..(mg P kg^{-1})..	SD	Means ..(mg P kg^{-1})..	SD
Hay	2.60	0.07	2.72	0.07
Cabbage	3.88	0.09	3.94	0.18

Van Lierop [1976] modified the basic Kjeldahl method (0.5 g catalyst/ml of acid, 100:10:1 Na_2SO_4, $CuSO_4$, Se) by various additions of hydrogen peroxide and perchloric acid to aid clearing and digestion. He obtained good recovery of K Ca Mg and P for all wet digestions. Phosphorus could not be measured by the ascorbic acid-molybdate method where Cu and Se were used due to precipitation of the metals. So the high values of total phosphorus may be due to the presence of copper and selenium as catalysts in the digest. However, no precipitation was observed with any catalyst mixture in this study.

Thomas *et al.* [1967] reported comparisons of the dry ashed vanadate and the sulphuric acid peroxide ashed autoanalyzer method. Their results show that vanadate method values for phosphorus are higher than ascorbic acid. Due to different methods of digestion as well as analytical technique it is not clear whether the higher values of phosphorus are due to digestion method or analytical technique.

POTASSIUM

Effect of Catalyst and Digestion Method on Total Potassium Recovery in Plant

Tables 16 to 17 show recovery and effect of catalyst mixture in digestion methods on the determination of potassium in hay and cabbage powders respectively. The determination of potassium in the treatment using potassium sulphate/copper sulphate mixture was not possible. The recovered values are non-significantly different than certified values. The 1 g catalyst mixture values are significantly higher than 2.5 g catalyst mixture in both methods in both plant materials. The values are higher in salicylic acid method and the standard method.

Table 16: Effect of catalyst mixture on total potassium determination in Hay powder (CRM129).

Catalyst Mixture		Total Potassium Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	33.3 a[1]	NS[2]	36.4 c	NS[2]
$Na_2SO_4/CuSO_4$	2.5	32.7 a	NS	34.7 b	NS

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value (33.8 and 14.5 mg g^{-1}) using a t test are indicated as follows. * 5% level and NS indicates non-significant from certified value.

Table 17: Effect of catalyst mixture on total potassium determination in Cabbage powder.

Catalyst Mixture		Total Potassium Content (mg g^{-1})			
Salt/ Catalyst	weight (g)	Standard Method		Salicylic Modification	
$Na_2SO_4/CuSO_4$	1.0	14.2 b[1]	NS[2]	15.4 c[1]	NS[2]
$Na_2SO_4/CuSO_4$	2.5	13.6 a	NS	14.2 b	NS

1 Means with the same letter are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value (14.5 mg g^{-1}) using a t test are indicated as follows. * 5% level and NS indicates non-significant from certified value.

Alfonso and Nelson [1991] found that the amount of sodium sulphate and copper sulphate with Se (2.5 g, 5 g, 10 g per 20ml sulphuric acid) and digestion time (0.75 hr, 1 hr, 1.5 hr and 2 hrs) affected the determination of K in Kjeldahl digests of plant material. They reported significantly lower K in treatments containing 2.5 g catalyst during 0.75 hr digestion of plant material. Van Lierop [1976] recovered significantly higher K with a conventional Kjeldahl digestion method than with a dry ashing method. Smith [1979] estimated K content in three plant materials by three methods of digestion (mixed acid digestion; peroxide procedure; and dry ashing). He reported that the wet digestion methods recovered similar amounts of potassium but the dry ashing method recovered a smaller amount.

CONCLUSIONS

The results show that the recovered values of total nitrogen, phosphorus and potassium in plant and total nitrogen in soil as affected by method of digestion and catalyst mixture. The 1 g salt/catalyst mixture recovered less nitrogen than the 2.5 g in the standard method due to the lower temperature and incomplete digestion in both plant and soil samples. The 2.5 g catalyst mixture partially recovered nitrate in the standard method and in the salicylic acid modification fail to recover all nitrate in plant material. Use of 2.5 g catalyst mixture and selenium appears to promote nitrogen losses in salicylic acid modification but not in the standard kjeldahl method of digestion for soil samples. No interference of selenium or copper was observed in Nitrogen and Phosphorus on colorimetric determination. The standard kjeldahl method with 2.5 g of salt/catalyst mixture of sodium sulphate cupper sulphate (10:1) in 5 ml sulphuric acid were found suitable for determination of total N, P and K.

The steam distillation and Technicon Auto-Analyzer technique measure similar amounts of ammonium nitrogen. However, the Technicon Auto-Analyzer technique is easier, rapid, high degree of reproducibility, precise, accurate, reliable and free from human error. The amount of phosphorus measured by the Ascorbic acid/Molybdate method was more accurate than by the Molybdate/Metavanadate method on Technicon Auto-Analyzer.

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