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An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control

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The Kjeldahl method was introduced in 1883 and consists of three main steps: sample digestion, distillation, and ammonia determination (titration being the primary method). The Kjeldahl method uses sulfuric acid, a variety of catalysts, and salts to convert organically bound nitrogen in samples to ammonium with its subsequent measurement (Sáez-Plaza et al., 2013). Today, this method is universally accepted and used in tens of thousands of laboratories throughout the world for nitrogen analysis in a wide variety of materials, such as foods, beverages, agricultural products, environmental samples, chemicals, biochemicals, and pharmaceuticals. However, successful analysis requires proper sampling and sample handling, which depend on the type of material. The Kjeldahl method has been validated and standardized for total (crude) protein estimation for a wide variety of food matrices, indirectly determined by their nitrogen content, and is the reference method adopted by many international organizations. The Kjeldahl procedure has several variants, based mainly on a sample size and apparatus required. A number of rapid and accurate instrumental methods have been gradually introduced that have some advantages compared to older techniques, if a large number of samples are to be run. Thus, extracted nitrogen from Kjeldahl can be determined by several other methods, i.e., spectrophotometric, potentiometric with ion selective electrode, FIA, ion chromatographic, and chemiluminescent methods. Quality control is essential for accurate and precise measurements of nitrogen by the Kjeldahl method. The importance of quality control in Kjeldahl analysis is stressed in this review. Despite some negative factors (i.e., it is hazardous, lengthy, and labor intensive), the Kjeldahl method and its variants with instrumental finish remain as accurate and reliable methods.

Keywords Kjeldahl method, sample preparation, working scale, nitrogen speciation, instrumental finish, quality control

INTRODUCTION

The Danish chemist Johan Kjeldahl (1849–1900) developed what today is well known as the Kjeldahl method for determining nitrogen in organic substances, originally used for determining the protein content in beer (Kjeldahl, 1883; Editor of *The Analyst*, 1885). Nitrogen in organic material is present in the form of amine groups ($-NH_2$) as constituents of aminoacids

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(proteins) and amino sugars. The Kjeldahl method indirectly quantifies the total protein content of foods by direct nitrogen measurement and subsequent multiplication by a conversion factor (Moore et al., 2010). The general conversion factor 6.25 (i.e., 100/16) is used for most foods because their non-protein nitrogen content is negligible (FIL/IDF, 2006; Mariotti et al., 2008). The determination of protein in food is a routine procedure for quality assurance and labeling (Buchi, 2012a, 2012b). The Kjeldahl method has three different steps: digestion, distillation, and titration (Saha et al., 2012). In this method, most organic nitrogen-containing samples are digested with sulfuric acid to ammonium sulfate; the ammonium is then liberated by raising the pH and measured by titration. Modifications of the basic design have been devised as shown in Figure 1, adapted from Fleck and Munro (1965). Details on the

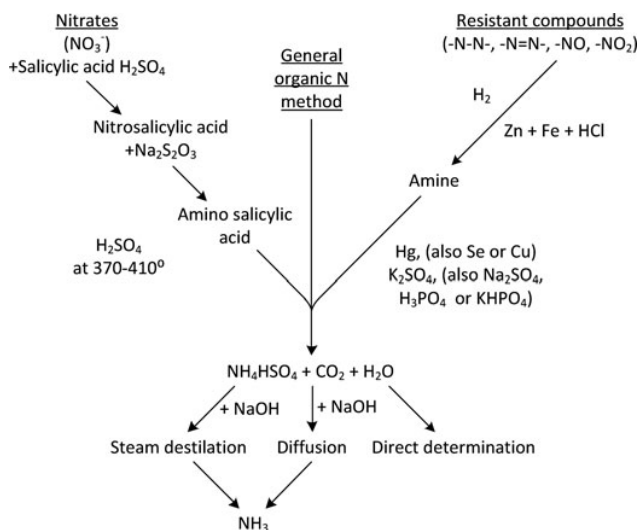


FIG. 1. Nitrogen determination by the Kjeldahl procedure and its modifications (adapted from Fleck and Munro, 1965).

chemistry of these steps can be found elsewhere (Bradstreet, 1965; McKenzie, 1994; Michałowski et al., 2013; Sáez-Plaza et al., 2013). The standard Kjeldahl method, which uses boric acid in the final step, is satisfactory for levels of nitrogen as low as 1.0 mg/L (Crowther and Evans, 1980). The Kjeldahl digestion procedure for total nitrogen determination is widely used for food, animal feed, and fertilizer quality control as well as for other agricultural materials (soils, plant materials), environmental samples (sediments, water and wastewater matrices), biological tissues, and pharmaceutical analysis (Chemat et al., 1998; Pasquini and de Faria, 1987; Sáez-Plaza et al., in press). The Kjeldahl method, which was first published in 1883, has been accepted, with modifications, as the standard method for the determination of nitrogen (protein) for decades (AOAC, 2001, 2011; Inacio et al., 2011; Moore et al., 2010; Rayment et al., 2012). Figure 2 shows the distribution of the articles compiled in the ISI Web of Knowledge under the "Title" entry "Kjeldahl" through the years. The number of ISI-compiled articles dealing with Kjeldahl as "Topic" raises the value to about 2150, and over 200 of them were published in the period 2010–2012.

The chemistry of nitrogen is complex due to the fact that nitrogen assumes several oxidation states (Sawyer et al., 2003). Nitrogen is one of the most important elements for plant nutrition. The compounds of nitrogen are of great worth in water resources, in the atmosphere, and in the life process of all plants and animals. Four forms of dissolved nitrogen are of greater interest: organic, ammonia, nitrite, and nitrate, ordered in an increasing state of oxidation. All these forms of nitrogen, as well as nitrogen gas (N₂), are mutually convertible, being components of the biological nitrogen cycle (Pehlivanoglou-

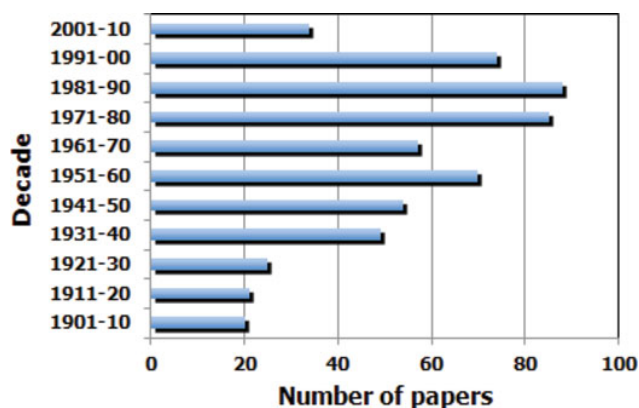


FIG. 2. Distribution by decades of articles under the "Title" entry "Kjeldahl" in the ISI Web of Knowledge (color figure available online).

Mantas and Sedlak, 2006; Worsfold et al., 2008). It is very important to ascertain the contribution (fractions) of different nitrogen species to the total nitrogen content (Prusisz et al., 2007).

The original method presented by Kjeldahl has been continuously improved. These developments have enhanced environmental and personal safety aspects, increased the speed and versatility of the method, and simplified the entire analytical procedure (Thiex et al., 2002). Proper sampling and sample handling are crucial to successful analysis and must be addressed individually for materials of different types (Lynch and Barbano, 1999; Simonne et al., 1993). The Kjeldahl procedure has several variants, mainly micro and macro, based primarily on sample size and apparatus required. In the original procedure, a large apparatus is required, frequently occupying an entire laboratory room, and using a relatively large analytical portion, which requires large amounts of acid. Micro Kjeldahl methods are much more commonly used as they produce a reduced amount of acid fumes and require less acid and catalyst mixture (Greenfield and Southgate, 2003). The micro Kjeldahl procedure is based on the same principles as the macro Kjeldahl procedure, but the apparatus used is scaled down (Horneck and Miller, 1998). The optimal reaction conditions for macro methods are not always applicable to micro methods (Campins-Falco et al., 2008). Emphasis needs to be given to semimicro digestions (Amin and Flowers, 2004; McKenzie, 1994). Appropriate choice of conditions will also obviate some of the difficulties with analysis of complex materials, and also result in saving of laboratory space with the more compact equipment involved (McKenzie, 1994).

Though the Kjeldahl procedure is hazardous, lengthy, and labor intensive, it has become the industry standard; it remains an accurate and reliable method and is used to standardize other methods (ISO, 2009a, 2011; Orlandini et al., 2009a; Orlandini et al., 2009b; Rayment et al., 2012). Semiautomated or fully

automated nitrogen (protein) analysis systems based on the classical Kjeldahl procedure (Rhee, 2001; Wright and Wilkinson, 1993) are preferable in order to cut cost and to save time when a large number of samples need to be analyzed. Like the Kjeldahl method, the combustion (Dumas) method is based on the determination of nitrogen (Sáez-Plaza et al., in press). The Dumas combustion method offers the advantages of shorter analysis time and no requirement to use hazardous materials (Rhee, 2001), and it is considered a useful alternative. Determination is based on the combustion of the sample material to release nitrogen gas. The liberated nitrogen can be quantitated by a variety of detection systems, including gas chromatography or thermal detection (Rutherford et al., 2008). These instruments, however, are expensive and require skilled operators (Unkovich et al., 2008).

However, a wide range of digestion blocks and distillation units are available today for the Kjeldahl process, e.g., from Foss Tecator (Persson et al., 2008), the KjelFlex K-360 by Buchi (Bufler and Amann, 2012), and RapidStill III from Labconco (1998). The required steps in the Kjeldahl procedure result in a time-consuming operation and therefore justify the need to develop automated methods, especially regarding distillation and determination procedures (Lima et al., 1999). A number of rapid and accurate instrumental methods have been gradually introduced that have some advantages compared to older techniques, e.g., spectrophotometric, potentiometric with ion selective electrode, FIA, ion chromatography, chemiluminescence, and others. The time required for the extraction of nitrogen from samples in a conventional Kjeldahl determination is clearly the rate-determining step for analysis of this kind (Domini et al., 2009; Mason et al., 1999). The desire to improve the efficiency of manufacturing processes is also driving the development of instruments that can monitor levels of sample components in-line.

The classical procedure often lacks the necessary precision and accuracy for detecting the low nitrogen levels (APHA, 2012) commonly encountered in some (e.g., marine) samples, even when large sample aliquots are utilized to increase the sensitivity. Many researchers have reduced the number of analytical steps by introducing direct chemical determinations of the ammonia formed in the digest. The choice for ammonia determination depends on sample volume, available facilities, and economics (Horneck and Miller, 1998). Today most laboratories implement quality assurance/quality control procedures to improve both laboratory efficiency and the quality of the data they generate. To support this improvement, it is important that officially approved methods are updated so that available technology can be used in the laboratory (Möller, 2009; Thiex et al., 2002).

Knowledge of the basics of the Kjeldahl method is fundamental to understanding this review properly. Readers are encouraged to refer to the first part of this report (Sáez-Plaza et al., in press).

SAMPLE PREPARATION: BOTTLENECK OF THE KJELDAHL PROCESS

Sample preparation still remains an essential part of chemical analysis (Feinberg et al., 1994), and it has been stressed that inadequate attention has been given to the preparation of samples (McKenzie, 1994; Navas et al., 2012a, 2012b). Settle and coworkers (1989) consider, as a result, that persons with experience in sample digestion have become an "endangered species." A representative sample should be sent to the laboratory and not damaged or changed during transport or storage (ISO, 2011). The sample must be stored in such a way that deterioration and change in its composition are prevented (Current and King, 2002; Namiesnik and Szefer, 2008; Wilde, 2005). Water samples may be preserved by addition of sulfuric acid (2 mL/L) and refrigeration at 4°C and should be analyzed as soon as possible (EPA Victoria, 2009) to minimize changes in composition due to contamination, sorption onto container walls, biological activity, and physicochemical coagulation processes (Badr et al., 2003). Plant materials must be clean and free of extraneous substances, including soil and dust particles and foliar spray residues, that may influence analytical results (Campbell and Plank, 1992).

Sampling also includes sample preparation, where the subdivision and grinding/homogenization of laboratory samples have an influence on measurement uncertainty (Ramsey, 1998; Ramsey and Ellison, 2007). Most frequently, standard sampling does not enter routine laboratory practice, and in collaborative studies performed for the validation of analytical methods, only well-homogenized samples are distributed (Möller, 2011). Sampling always makes a significant contribution to overall measurement uncertainty, but quantifying the measurement uncertainty of sampling is not an easy task (Asuero et al., 1988; Gonzalez et al., 2005; Herrador et al., 2005). Total sampling error is by far the dominant contribution to all analytical endeavors, often 100 times larger than the total analytical error (Möller, 2010).

As many analytical methods are rapid or automated, sample preparation is usually the most time-consuming part of a chemical analysis, especially when digestion is involved (Feinberg et al., 1993). Wet ashing still remains a very general method in analytical chemistry, although rapid, nondestructive methods exist. For example, matrix dissolution/mineralization is one of the drawbacks of Kjeldahl nitrogen determination in foods, requiring usually more than 2 hours, or about 1 hour using a heating block, whereas the measurement itself takes only a few minutes with automated distillation apparatus (Chemat et al., 1998; Domini et al., 2009; Feinberg et al., 1994). Nevertheless, the Kjeldahl procedure is used by standardization organizations as an official method in many countries (Moore et al., 2010). Various associations approve and have refined the Kjeldahl method (Table 1).

Sample preparation for Kjeldahl analysis should be carefully performed to avoid errors in the final result (Persson et al.,

TABLE 1
Scientific associations that approve Kjeldahl nitrogen determination

Scientific Association or Agency	Reference
AOAC International	AOAC, 2011: <i>Official Methods</i> of the AOAC
American Oil Chemists' Society	Berner and Brown, 1994
American Public Health Association (APHA)	APHA, 1998, 2005, 2012
American Society for Testing and Materials (ASTM)	ASTM International, 2001, 2002
Association of American Cereal Chemists	American Association of Cereal Chemists, 1995
European Commission	CEN/TF 2003, 2007; European Committee for Standardization, 2000, 2001; Sobiecka et al., 2007
International Dairy Federation (IDF)	International Dairy Federation, 1993
International Organization for Standardization (ISO)	ISO, 2001a, 2001b, 2005, 2006, 2009, 2011
U.S. Department of Agriculture	Holcombe et al., 1986; Labconco, 1998
U. S. Environmental Protection Agency (EPA)	U.S. EPA, 1979, 1983, 1990, 2001; O'Dell, 1993

2008; Tobiano, 2008; Wiles et al., 1998). This procedure must involve one or more treatments to homogenize the sample, i.e., the particle size of the sample should ideally be reduced to a size < 1 mm. Homogeneity of the analytical sample improves the reproducibility of the method and also offers the possibility of reducing the sample size used without sacrificing the quality of the final results (Persson et al., 2008). Uniform grinding and mixing are critical in obtaining accurate analytical results (Campbell and Plank, 1992). A good sample blender is necessary for sample preparation because the speed of the digestion is easily increased when samples with small particle sizes are used. Cereal grains are usually ground to a particle size distribution similar to that of flour prior to protein quantification (Van Camp and Dierckx, 2004). The moisture content and oil content of cereal grains and oilseeds contribute to the difficulty in grinding these types of samples (Bicsak, 1993). Since a loss of moisture can occur during this process, a simultaneous moisture determination should be performed in order to get accurate protein determination (Van Camp and Huyghebaert, 1996).

Digestion of samples with large particle size is not recommended because it requires a long time (Rhee, 2001). If the sample is homogeneous (e.g., powders and liquids) the material (0.15 g dry matter) can be digested in a small digestion flask (capacity 30–35 mL) using 0.8 g of catalyst mixture and 1 mL of concentrated sulfuric acid. If the sample is not homogeneous, e.g., meat products, it is preferable to dilute a macro digest (samples having a mass of about 1.5–2 g) to 100 mL and use 10 mL for the semimicro distillation (Pearson, 1976). Semisolid materials, e.g., meat products, can be conveniently weighed on folded filter paper and both the sample and paper dropped into the digestion flask. Similar paper should be included in the blank (Ó hAlmhain and Ó Danachair, 1974).

The samples for Kjeldahl analysis should be weighed on an analytical balance with an accuracy of 0.1 mg (Persson et al., 2008). The actual mass of the sample required for analysis depends on the degree of its homogeneity. Precise results cannot

be obtained for nonhomogeneous samples with small masses. For mg quantities, a very sensitive balance is required (± 0.00001 g), and scrupulous hygiene is necessary to prevent contamination (Unkovich et al., 2008). The smaller the sample size, the greater the need for fine grinding and high sample homogeneity. When dealing with homogeneous samples, mass is not as critical and can be optimized to give a suitable final titration volume, 2–20 mL. The analytical sample should ideally contain 30–140 mg N. The acid (HCl, H₂SO₄) concentration applied in the titrant should be approximately 0.1–0.2 N. It is important to know the moisture content of the sample in order to compare the results with those obtained in other laboratories. This can be achieved by correcting for the moisture content of the samples (referring the results to the mass of dry samples) and reporting results on a dry basis, or by analyzing pre-dried samples (Current and King, 2002).

Soil samples are collected from several points of the field down to a specific depth, e.g., 25 cm, so that the major nutrients are mostly accumulated on its surface. Samples are then stored in plastic bags in a refrigerator in order to prevent moisture loss and composition changes, as biological activity decreases at low temperature (Sen, 2003). Prior to analyses, soil samples are oven dried (e.g., in a forced-draft oven at 55°C or at 70°C in a gravity oven) for at least 12 hours, hand cleaned to remove foreign particles, and ground to pass through a 2 mm sieve (Hue et al., 2000). Many sophisticated techniques are available (e.g., Kriging, strip sampling) to deal with variability in the field. However, a zigzag sampling pattern or a variant of it is often adequate for obtaining a reasonably representative soil sample (Hue et al., 2000; Sabbe and Marx, 1987; Sheppard and Addison, 2008).

Grain, cereal, and feed samples are ground in a chopper mill to a fine powder (less than 1 mm or 20 mesh) before digestion. Coarse samples such as hay are ground first in a food blender before milling (Hach et al., 1987). The guidelines regarding sample masses for solid samples are valid for most of the liquid

samples that are tested for nitrogen content in food and feed. Liquid samples are thoroughly mixed before a subsample is removed with a syringe-type sampler (Current and King, 2002) or by submerging a container under the liquid surface (so-called grab samples). Large sample amounts need to be used when analyzing total Kjeldahl nitrogen (TKN) in water/wastewater where nitrogen content can be quite low (APHA, 2012). To obtain low detection limits, the titrant concentration of 0.01 M should be used. Analyzing the digest with use of a flow analysis technique, smaller sample amounts can be used (Anderson and Moller, 1995; Patton and Truitt, 2000).

Some problems associated with vegetables and fruits are related mainly to sample preparation, i.e., high moisture and fiber contents (Current and King, 2002). Moisture has also to be controlled in soil samples, which should not be dried because ammonia loss by evaporation may occur. Foods rich in proteins are rather easy to dissolve (Feinberg et al., 1994); acid decomposition is long, but relatively uncomplicated when the product contains little or no fats or tryptophan. Most of the recommendations that follow are taken from Persson and coworkers (2008). When dealing with meat and fish, samples lack homogeneity, and a large sample size is necessary in order to be representative. All sample components normally consumed should be included. Analyses of meat and meat products pose unusual problems due to the lack of homogeneity, various compositions of processed meat, and the limited storage time before deterioration begin (McGill, 1980). Drill-type devices are used to obtain a core from solid products such as cheese or frozen foods (Current and King, 2002). For cheese, the rind, a smear of moldy surface layer, is removed in order to provide a test sample representative of the cheese as consumed (ISO, 2011). More than 20 mL of concentrated acid is needed for samples high in fat or oil because more acid is required to effect complete digestion, avoid loss of nitrogen, and prevent caking (Rhee, 2001). The high fat or oil content in some sample types (meat, fish, oilseeds, petrochemicals) may cause foaming during digestion (such as soils with high organic content). A separation of fat from the rest of the sample occurs with dairy samples, and proper homogenization procedures should be applied. High contents of surface-active agents (in some wastewaters) may cause foaming during the initial period of digestion. Low nitrogen content samples make it necessary to use large sample volumes or masses (beverages, unpolluted waters, paper, pulp).

High fiber content samples may cause difficulties during the grinding step, especially where the fiber is strong (food choppers or the Knifetec mill are used). Boiling rods are used to prevent bumping when water must be initially evaporated from large sample volumes. The high mixture content of canned foods limits the use of conventional mills in sample preparation. Rubber, plastics, and polymers are usually difficult to grind in mills; moreover, the heat generated can melt the sample. A knife or scissors is normally used to reduce sample size of textiles, leather, paper, and pulp (Persson et al., 2008).

Chemistry of the digestion process is treated in detail in the first part of the review (Sáez-Plaza et al., in press).

MACRO, SEMIMICRO, AND MICRO: SIZE DOES MATTER

The total nitrogen recovery during digestion is influenced by the quantity or size of a sample. The major factor that influences the selection of a macro or semimicro Kjeldahl method to determine organic nitrogen is its concentration. In any case, the weighed quantity has to be adapted to the expected nitrogen content of the sample and the homogeneity reached (Janßen, 2005). The acid quantity including salt and catalyst has to be accommodated to this data (Sáez-Plaza et al., in press). The macro Kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen, but it requires a relatively large sample volume for low concentrations (APHA, 1998, 2012). Sample particle size, amount of sample assayed, and the digestion apparatus will contribute to the precision of determination (Jones, 1987). In the semimicro Kjeldahl method, applicable to samples containing high concentrations of organic nitrogen, the sample volume should be chosen to contain organic plus ammonia nitrogen in the range of 0.2 to 2 mg. As a means of determining nitrogen, the Kjeldahl macro method is a convenient and relatively simple procedure requiring no complicated equipment or techniques. However, the traditional macro (manual) Kjeldahl method is seldom performed in modern commercial laboratories because the numbers of samples per analysis are too large and laboratory health and safety regulations are becoming severe (Wiles et al., 1998).

Most often, the micro Kjeldahl methods involve homogeneous samples, relatively high nitrogen levels in the sample, and small sample sizes, normally less than 0.25 g. The installation considerations, initial expense, space requirements, and ongoing utility and reagent costs are much less with micro equipment (Labconco, 1998). Micro digestions can be used as indicated in EPA protocols, which can further reduce digestion reagents by as much as 10- to 50-fold (Anderson and Möller, 1995). This pollution prevention mechanism will reduce use of reagents and subsequent generation of waste. Problem troubleshooting for nitrogen determination with the micro Kjeldahl method has been briefly summarized by Vivek and coworkers (2008). Pregl-Parnas-Wagner is an example of glassware configuration utilized for Kjeldahl distillation available commercially (manufactured by Brand, Wertheim, Germany).

Semimicro Kjeldahl digestion methods for, e.g., soil, require a small quantity of sample, 50–200 mg plant material or 100–500 mg soil (Amin and Flowers, 2004). Material must be finely ground before analysis. A sample quantity of at least 100 mg is recommended to ensure that a representative subsample is analyzed when plant material is ground to less than 40 mesh. Use of a 50 mg sample requires that the plant material be ground to less than 80 mesh. To obtain reproducible results during semimicro Kjeldahl analyses of soils, it is normally

necessary to grind the samples to less than 100 mesh (Nelson and Sommers, 1980).

Many modifications of the classical Kjeldahl method have been devised to determine micro quantities of nitrogen in biological materials (Lang, 1958). In general, the specialized and unwieldy equipment required is not suited to routine analyses (Steyermark et al., 1951). Working with micro quantities is extremely time-consuming and requires special instruments and skillful handling, thus restricting its application to analytical laboratories (Campins-Falcó et al., 2008; Jacques and Peterson, 1987; Steyermark and McGee, 1960). Procedures for the Kjeldahl ultramicro determination of nitrogen have been described (Kuck et al., 1950) and used in biochemical research for some time. Although the micro method procedures are precise, they are time-consuming and, therefore, not recommended for plant breeding programs that need to analyze many samples in a short time (Vivek et al., 2008).

Much of the automated equipment, e.g., the Kjeldahl analyzer (Persson et al., 2008) is directed primarily towards macro Kjeldahl determinations. However, the only justification that one can see for macro rather than semimicro or micro determinations is the need for adequate sampling (McKenzie, 1994). Many of the samples involved are materials from plants, fertilizers, animal feeds, grains, dairy products, etc. However, there are ways of overcoming most of these sampling problems by appropriate homogenization treatment of the material before the actual sample is taken, as shown by Kirsten and coworkers (1984) in the case of the Dumas method.

Only macro Kjeldahl equipment, either a traditional system or block digester with steam distillation, is described in the official method. Micro Kjeldahl equipment is not suitable for Kjeldahl reference testing. This is because the accuracy and precision of the micro Kjeldahl system are inferior to that of macro Kjeldahl because of the use of small sample sizes, which magnifies sampling and analytical errors (Barbano et al., 1990; Lynch and Barbano, 1999). The traditional (manual) macro Kjeldahl method is the international reference method for determining total nitrogen of crude protein. However, with very little time to analyze large number of samples, commercial laboratories no longer use manual methods routinely. The generic Kjeldahl method has been automated to various degrees in various proprietary systems (Brayton, 1992; Egli, 2008; Labconco, 1998; Persson et al., 2008). Such systems are widely used even though throughput is still limited and safety concerns are significant (Moore et al., 2010; Wiles et al., 1998).

The amount of salt specified per mL of sulfuric acid in semimicro Kjeldahl procedures varies widely. High salt-to-acid ratios (more than 0.8 g potassium sulfate per mL H_2SO_4) give high digestion temperatures and reduced times for complete digestion of the sample (Amin and Flowers, 2004). However, difficulties in sample handling 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 (Persson et al., 2008; Sáez-Plaza et al., in press). 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 sulfate per mL of sulfuric acid results in a digestion mixture that is easy to handle and gives good recovery of total nitrogen from soil and plant material (Bremner and Mulvaney, 1982; Nelson and Sommers, 1980).

NITROGEN SPECIATION: BEYOND REPORTING OF ABSOLUTES

Nitrogen is the most important nutrient that affects the growth and development of plants (Masclaux-Daubresse et al., 2010); availability of nitrogen in soils largely controls plant productivity. Nitrogen, next to water, is considered to be the most important input for biomass production (Huang and Peng, 2004). Nitrogen may cause eutrophication in waterways, harmful effects on human health because of its presence in drinking water, or anthropogenic greenhouse effect increases through the emission of nitrous oxide (Lehmann and Solomon, 2008). The degree of environmental pollution is increasing with rapid industrial and economic growth. Since nitrogen has five valence electrons and may form compounds with oxidation states between + 5 and - 3, it has various chemical species. The nitrogen cycle also affects a variety of important biological and nonbiological processes in the environment (Kitamaki et al., 2003). In order to control these processes, it is essential to assess various nitrogen forms in soils, since microorganisms can access different nitrogen forms to different extents (Lehmann and Solomon, 2008). The estimation of the degree of environmental pollution also requires the determination of inorganic nitrogen species.

The majority of nitrogen occurrence in waters comes from rainfalls and leaching of agriculture soil. A small part of nitrogen originates from sewage and industry wastes. Nitrogen plays an extraordinary role in all biological processes taking place in surface, ground, and wastewaters (Praus, 2002), as shown above. One of the problems of high discharges of nitrogen into water is eutrophication, especially in zones identified as sensitive, such as natural freshwater lakes, other freshwater bodies, estuaries, and surface freshwater (Aneja et al., 2001; Cameron et al., 2003; Meseguer-Lloret et al., 2006). More than 90% of all nitrogen in soil is usually in organic form; a significant proportion of this organic nitrogen is suspected to be present as heterocyclic nitrogen, e.g., pyridine or pyrrole (Lehmann and Solomon, 2008).

Nitrogen in aquatic environments, i.e., drinking, ground, and surface waters, domestic and industrial water, saline water, and biosolids (municipal sewage sludge), comprises dissolved inorganic nitrogen (DIN, the sum of nitrate, nitrite, and ammonium), dissolved organic nitrogen (DON; the largest fraction is made up of amino acids and peptides, and it is often called amino N), and total particulate nitrogen, TPN, which is almost wholly organic

(Meseguer-Lloret et al., 2006). Traditionally, most studies have focused on DIN, although it is now known that DON frequently comprises the largest part (60–69%) of total dissolved N (TDN) in lakes, rivers, estuarine, and surface ocean waters (Worsfold et al., 2008). TDN includes all nitrogen species (organic and inorganic) dissolved in the sample. Knowledge of organic nitrogen is important for controlling the biological treatment of wastewater.

The Kjeldahl method provides the sum of DON (in the N(–3) oxidation state, except azide, azine, azo, hydrazone, nitro, nitroso, oxime, and semicarbozone functional groups) and ammonia contents and usually is called total Kjeldahl nitrogen (TKN) (ASTM, 2002; O'Dell, 1993). Ammonia concentration must be subtracted from total nitrogen to estimate DON. This problem can be accomplished by distilling away the NH_3 initially present in the sample prior to converting the DON to NH_3 (Pehlivanoglou-Mantas and Sedlak, 2006). The Kjeldahl method requires a relatively large volume of sample to quantify DON at concentrations encountered in the aquatic environment (e.g., the typical detection limit for a 1-L sample is $35 \mu\text{M}$ of N).

One approach to determine total nitrogen (inorganic plus organic nitrogen) is based on the reduction of nitrate and nitrite into ammonium followed by the Kjeldahl digestion of samples. The reduction can be carried out directly by Devarda's alloy (Stenholm et al., 2009) or by zinc after reaction with phloroglucinol (Amin and Flowers, 2004; Praus, 2002). Phloroglucinol forms nitro- and nitrosophloroglucinol, which are reduced by zinc into their amino derivatives. The mineralized samples are alkalined, and ammonia is distilled and determined.

In another approach, the alkalined water samples are digested by oxidation with peroxodisulfate at about 150°C and increased pressure conditions (Cornell et al., 2003; Davi et al., 1993; Ferree and Shannon, 2001; Halstead et al., 1999; Medina et al., 1994; Patton and Kryskalla, 2003; Valderrama, 1981). The samples can be heated in a microwave oven or in an electric heating block. Nitrates, produced by the digestion, are determined by conventional methods (Oms et al., 2000). Nitrogen compounds may also be catalytically ignited in an oxygen atmosphere at 1000°C forming NO, which reacts with ozone and chemiluminescent lighting in the range of 700 to 900 nm of the excited molecule NO_2^* to be detected (Badr et al., 2003; Cornell et al., 2003; Stenholm et al., 2009). Alkaline persulfate digestion methods have been widely applied lately for estuarine and marine water analysis in preference to Kjeldahl digestion methods. However, Kjeldahl digestion methods continue to be widely applied (Patton and Kryskalla, 2003) to freshwater analysis. National Pollution Discharge Elimination System (NPDES) and Safe Drinking Water Act (SDWA) compliance monitoring do not approve alkaline persulfate digestion methods. Nevertheless, an alkaline persulfate digestion method for total nitrogen determination is included in the 20th edition of *Standard Methods for the Examination of Water and Waste Water* (APHA, 1998).

Inorganic nitrogen is analyzed because of requirements regarding the quality of effluents from sewage and wastewater treatment plants to river water. This parameter is supposed to be the sum of nitrate, nitrite, and ammonium. This suggestion does not refer to industrial sewage containing other inorganic nitrogen compounds, such as cyanides and thiocyanides from coal carbonization (Praus, 2002).

The limit concentrations of nitrogen in drinking water are strictly regulated to guarantee water quality and protect the environment from adverse effects for humans (European Union, 1991; Ward et al., 2005; WHO, 2008, 2011). In waste/cleaned waters, the limits of nitrogen are also restricted in order to, e.g., avoid the eutrophication of surface waters. Nitrates occur in all types of water; in drinking water, their concentration must not exceed 50 mg/L for adults and 15 mg/L for babies (Dozier et al., 2008). The presence of nitrites in waters is most critical for ground and drinking waters, where it indicates fresh fecal pollution. Concentrations up to 0.5 mg/L in tap water are allowed. Nitrate is the most widespread agricultural contaminant in the United States, where 22% of the wells in agricultural areas have nitrate levels in excess of the drinking federal limit of 10 mg $\text{NO}_3^- \text{-NL}^{-1}$ and nearly 2% of the US population is exposed to elevated nitrate levels in drinking water wells (U.S. EPA, 1990, 2012; Yague and Quilez, 2010).

DON concentrations in water samples cannot be quantified directly. Analysis involves several steps: determination of the TDN concentration, determination of the DIN concentration, and finally subtraction of DIN concentrations from TDN concentrations (Figure 3). The difference method requires good analytical precision and accuracy of TDN and DIN analyses

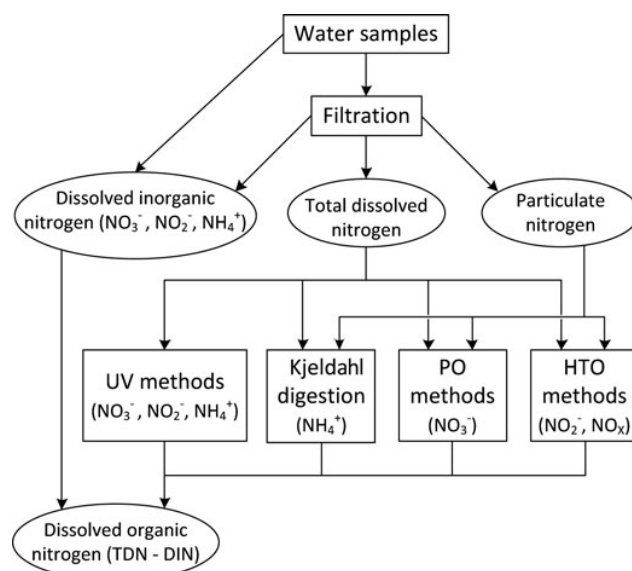


FIG. 3. Schematic diagram of the analytical methods for the determination of nitrogen in water (Nollet, 2007). PO: persulfate oxidation, HTO: high-temperature oxidation.

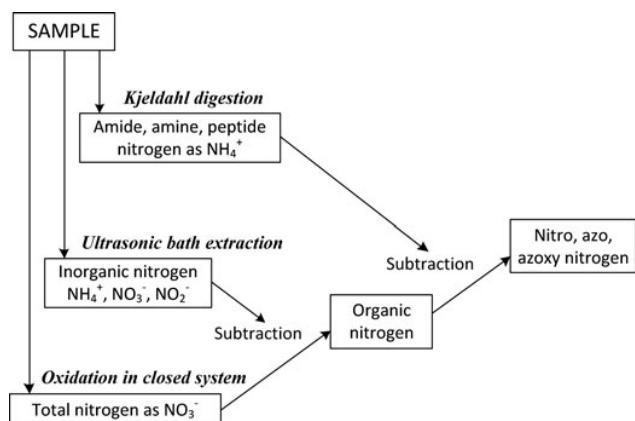


FIG. 4. Schematic chart of the nitrogen species analysis of potatoes (Prusisz et al., 2007).

(Badr et al., 2003; Nollet, 2007). TDN determination requires a preparatory digestion step, either chemical or by combustion. The main digestion methods available and in current use for total nitrogen determination in aqueous samples are: Kjeldahl digestion (Doval et al., 1997; Nozawa et al., 2005; Yasuhara and Nokihara, 2001), alkaline persulfate oxidation (Cornell et al., 2003; Valderrama, 1981), and high-temperature catalytic oxidation (Badr et al., 2003; Cornell et al., 2003; Stenholm et al., 2009).

DIN species (the sum of separate analyses for ammonium and nitrate plus nitrite) are analyzed using established colorimetric procedures (Oms et al., 2000). Several methods were re-

ported for the simultaneous determination of nitrogen species. One of these methods used cation-exchange and anion-exchange columns, together with switching valves. Other methods utilized bifunctional columns (Kitamaki et al., 2003). Nitrate, nitrite, and ammonium ions were separated by ion chromatography followed by direct UV detection of the anions and fluorimetric detection of ammonium ion.

A schematic chart showing all steps in estimating the contribution of different nitrogen species in potato samples (Prusisz et al., 2007) is given in Figure 4. A simplified flowchart showing the general methods for the preparation and analysis of samples for inorganic and organic nitrogen in soils and biosolids (Henry et al., 1999) is found in Figure 5.

AUTOMATED KJELDAHL ANALYSIS: QUICKER IS BETTER

The automation of chemical methods used routinely in research can lead to a considerable saving in time and labor and, thus, efficiency in carrying out a particular piece of work (Davidson et al., 1970; Feinberg, 1999). Automation makes it possible to avoid direct handling of dangerous reagents (Pansu and Gautheyrou, 2006), such as boiling sulfuric acid or concentrated soda. Ferrari (1960) succeeded in automating the Kjeldahl nitrogen procedure, describing the new concept of continuous nitrogen determination. The automated macro Kjel-Foss analyzer was introduced in 1973, with which one can routinely perform 20 analyses/hour (Oberreith and Neil, 1974). Various degrees of automation are available for the Kjeldahl method, including automated digestion and distillation followed by manual titration; fully automated digestion, distillation, and titration;

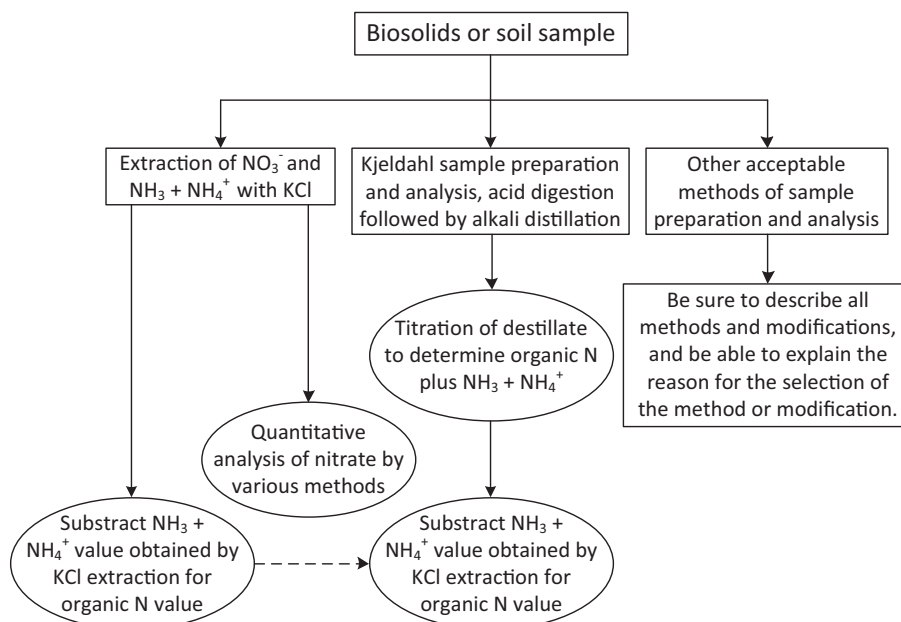


FIG. 5. Simplified flowchart for analysis of biosolids and solids (Henry et al., 1999).

and the use of block digesters and autosamplers for the unattended analysis of a maximum of 60 samples per batch. Semiautomated equipment is available with digestion and distillation-determination units at macroscale and microscale from, for example, the manufacturers Bicasa, Büchi, Gerhardt, Skalar, Foss-Tecator, and Velp (Pansu and Gautheyrou, 2006; Pansu et al., 2001). Depending on the analysis procedure used, the scale of operation applied, and the degree of automation installed, the analysis time of the procedure could be further reduced, corresponding to frequencies of analysis up to 20 samples/hour.

Kjeldahl analysis has undergone three forms of automation (Owusu-Apenten, 2002):

1. The Kjel-Foss instrument mechanizes the entire micro Kjeldahl procedure (digestion, neutralization, distillation, and titration).
2. The Kjel-Tec techniques use digestion block in conjunction with apparatus for automated distillation and titrimetric analysis.
3. The final form of automation is the Technicon AutoAnalyzer Instrument, which uses continuous flow analysis (CFA).

The Kjel-Foss instrument performs the entire Kjeldahl procedure automatically. Automation reduces the analysis time from 3 hours to 6 minutes. The first analysis is completed in 12 minutes and succeeding analyses every 3 minutes. The sample throughput is 120–160 analyses per day (Owusu-Apenten, 2002). The Kjel-Foss instrument requires a reliable supply of electricity and tap water for installation and adequate drains and ventilation (Persson et al., 2008). A fume cupboard is not essential. Accuracy, precision, and economics of the Kjeldahl-Foss method were compared with those of the manual Kjeldahl method, neutron activation analysis, proton activation analysis, combustion analysis, and the Kjel-Tec method. Results of the Kjel-Foss and manual Kjeldahl methods were highly correlated (Williams et al., 1978).

The efficiency of antimony versus mercury oxide as catalyst has been compared in a collaborative study analyzing fish meal, using the Kjel-Foss instrument (Bjarno, 1980). The Kjel-Foss automatic procedure performed as well with an antimony-based catalyst as with the traditional mercury catalyst for most products analyzed. Results obtained by the Kjel-Foss Automatic procedure and the improved AOAC Kjeldahl methods for protein analysis of a wide range of meat and meat products with low (25%) fat, high (40%) fat, and dry sausage (50%) fat were comparable (McGill, 1980), validating the Kjel-Foss instrument for meat product analysis. The Kjel-Foss instrument has also been evaluated for meat analysis using the AOAC Kjeldahl method as reference, in another collaborative study (Suhre et al., 1982).

In the automated colorimetric analysis by the Technicon AutoAnalyzer ammonia in the digest is separated from other digest constituents by continuous flow distillation or dialysis (Khan and Flower, 1999) and determined by a procedure involving measurement of the color of the complex formed by

ammonia with indophenol (Berthelot reaction) or with ninhydrin (Ruhemann purple). Alternatively, it may be enough to simply dilute and neutralize the digests before colorimetric determination. The Technicon AutoAnalyzer has two reaction modules. The first module (heated in two stages at 380°–400°C and 300°–320°C) digests water-dispersible samples. The digest is then pumped to a second module (AutoAnalyzer Sampler II). Chromogenic reagents are added in quick succession before the flow stream passes to a delay coil to allow color formation (Owusu-Apenten, 2002).

The AutoAnalyzer has been applied to a variety of samples (plant material, feedstuffs, grain flour, instant breakfast, meat analogues, meat products, canned and processed foods) and the results obtained usually agreed with micro-Kjeldahl analysis (Owusu-Apenten, 2002). The AutoAnalyzer digestion module gives low nitrogen recoveries with animal feeds (corn grain, wheat, barley, rice, alfalfa, mixed feeds, feed concentrates), whereas the use of a Technicon block digester (or a Tectator heating block) followed by AutoAnalyzer Sampler II (for ammonia detection using the alkaline phenol reagent) led to a complete recovery of nitrogen from cattle supplement, swine ration, pig starter, and poultry ration (assorted strains of rice and cereal-legume mixtures) (Owusu-Apenten, 2002). 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 AutoAnalyzer by the indophenol method is satisfactory.

The development of the Technicon AutoAnalyzer has provided a system capable of automating many analyses. 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. The job of the analyst in automated analysis is limited to filling up the reagents, regulating the heating programs, and recovering the results at the end of the day (Pansu and Gautheyrou, 2006). Monitoring is simplified because an alarm goes off in the case of an accident.

SPECTROPHOTOMETRIC ANALYSIS FOR KJELDAHL NITROGEN: FROM YELLOW TO BLUE

As a way of circumventing the distillation step, improvements of the Kjeldahl procedure have emphasized the quantitation of ammonia by spectrophotometric procedures (Table 2). Colorimetric analysis simplifies Kjeldahl analysis and increases the sample throughput. Other benefits include increased sensitivity and a greater potential for automation. Reagents for colorimetric Kjeldahl-N analyses include (i) alkali-phenol reagent (indophenol reagent), (ii) ninhydrin (indanetrione hydrate) reagent, (iii) Nessler's reagent, and (iv) acetylacetone reagent. However,

TABLE 2
Spectrophotometric methods

Sample	Comments	Reference
<i>Ninhydrin</i>		
Beer or wine	A ninhydrin-based microwell assay utilized in place of the traditional Kjeldahl method. The assay only measures alpha amino acids and ammonia so other nitrogen sources are not detected.	Abernathy et al., 2009
Four ruminally fistulated dairy cows	A subsample of digesta pooled across sampling intervals subjected to analysis using ninhydrin assay, Kjeldahl procedure, and amino acid analyzer, to determine the flow of soluble non-ammonia N.	Choi et al., 2002
Soil hydrolysates	Total hydrolyzable N is measured by Kjeldahl digestion of the hydrolysate and diffusion of the digest with NaOH; diffusion is performed with MgO to determine hydrolyzable $\text{NH}_4^+\text{-N}$; (NH_4^+ + amino sugar)-N is recovered by diffusion with NaOH, after which amino acid-N is liberated by ninhydrin oxidation and recovered by diffusion with NaOH.	Mulvaney and Khan, 2001
Vegetation, fruits, and flowers	Some common methods available for measuring the protein content of vegetation are reviewed, providing recommendations to wildlife ecologists and primatologists. Methods or conversion factors are related and their results may be compared with those already published.	Conklin-Brittan et al., 1999
Soil extracts	Two soil extracts used for chemical indexes for N availability, 0.01 M NaHCO_3 and boiling 0.01 M CaCl_2 , were analyzed to learn more about the nature of the extracted organic matter and to determine if a specific fraction of the total soil extracted organic matter was being removed.	Michirina et al., 1982
Samples containing N	An assay of nitrogen in Kjeldahl digests by the ninhydrin colorimetric method adapted to AutoAnalyzer analysis.	Quinn et al., 1974
Serum proteins	A method for determining ammonia by means of indanetrione hydrate. The digestion procedure is modified for the analysis of proteins, rendering the spectrophotometric procedure more sensitive.	Jacobs, 1962
Serum	The reaction for the spectrophotometric determination on the microscale of nitrogen, protein hydrolysates, and heterocyclic compounds between indanetrione hydrate and ammonia is investigated.	Jacobs, 1960
<i>Berthelot (automated)</i>		
Hay, cabbage powder, soil	Measurement of the color of the indophenol complex formed by Berthelot reaction by using a Technicon AutoAnalyzer.	Amin and Flowers, 2004
Pulp and paper mill matrices	Single laboratory method evaluation and comparative study of digestion techniques and analytical methods for the determination of total phosphorus and total nitrogen. Total nitrogen and TKN methods included EPA Methods 351.2, 351.4, and 353.2.	Cook and Frum, 2004
Drinking, ground, and surface water; domestic and industrial waste; biosolids	Procedures for the determination of TKN and/or organic nitrogen. Digestion/distillation of the TKN from the sample followed by analysis using automated colorimetry.	U.S. EPA, 2001

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Seawater	An update of the Kjeldahl method is presented for the direct determination of dissolved organic nitrogen. Dissolved inorganic nitrogen is previously removed. The sample is then mineralized to ammonium, which is measured with a Technicon AutoAnalyzer.	Doval et al., 1997
Cereals, buckwheat, and pulses (small red bean, kidney bean, soybean)	Kjeldahl-digested materials of powdered whole kernels of 719 samples analyzed by automated colorimetric procedure (Technicon Auto-Analyzer).	Horino et al., 1992
Soil and plant nitrogen	Continuous flow system involving the use of a unique micro-distillation unit followed by colorimetric detection of the pure ammonium chloride distillate.	McLeod, 1992
Urine	Data for adapting a colorimetric ammonia methodology to a centrifugal analyzer adaptable to a clinical setting. Guidelines for utilizing the research mode of the Encore centrifugal analyzer to initialize parameters.	Geiger et al., 1987
Surface freshwater and organic wastes	Organic nitrogen compounds are converted to ammonium sulfate by a catalytic (red mercuric oxide) acid-sulfate digestion in a Technicon AutoAnalyzer II. There is no pH adjustment required following the digestion.	Lennox and Flanagan, 1982
Surface water	Nitrogen by the Kjeldahl method and phosphorus simultaneously determined on a single aliquot of sample. Batch digestion via a block heater using the traditional Kjeldahl acid reagent followed by colorimetric measurements via an AutoAnalyzer system designed to handle very acidic samples.	Crowther et al., 1980
Foods and biological samples used in nutrition research	Samples digested in a sulfuric-phosphoric acid mixture in glass tubes in a thermostated aluminum block using selenium as catalyst. The ammonia liberated determined colorimetrically in a Technicon AutoAnalyzer.	Hambraeus et al., 1976
Wastewater samples	An ultramicro technique for the digestion of organic nitrogen and phosphorus compounds. The digests are analyzed simultaneously for phosphate and ammonia using automated spectrophotometry.	Jirka et al., 1976
Assorted instant breakfasts and meat analogs	Limitations and special modifications of the method (effects of composition of calibration standards, heat of continuous digestion, nitrogen concentration) are studied, as well as the agreement between the Technicon AutoAnalyzer and a manual macro Kjeldahl method.	Vincent and Shipe, 1976
Various amino acids, glucosamine, casein, urea, and nicotinamide	Sensitivity was substantially increased as a result of optimizing reagent ratios and increasing the proportion of digested sample being analyzed. This eliminates the need for a specially built longer flow-cell and its related problems. Technicon AutoAnalyzer.	Kramme et al., 1973
Corn, millet, wheat, sorghum, formulated cereal foods, soya	A procedure to determine the nitrogen content in an Automatic Analyzer with accuracy and precision comparable to the traditional macro and micro Kjeldahl.	Uhl et al., 1971
Biological samples	Studies in the Automated Analyzer system (Technicon) for determining ammonia in aqueous solution. Conditions were most stable when the reaction coil was maintained at $22^{\circ} \pm 1^{\circ}\text{C}$ and dwell time in the coil was about 3 minutes. Effects of mineral content of test solutions, sampling rate, and sampler on results of analyses.	Davidson et al., 1970

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Different nitrogenous materials	An automated method to determine total nitrogen on liquid samples containing a wide range of nitrogen without changing the manifold, based on the separation of ammonia-nitrogen by distillation from the acid digest prior to the determination of the ammonia-nitrogen content.	Harwood and Huyser, 1970a
Petroleum	The procedure for trace nitrogen determination in petroleum was fully automated. Sulfuric acid extraction, continuous Kjeldahl digestion, and indophenol blue color development are employed.	Heistand, 1970
Urine	The NH_4^+ produced by acid digestion reacts with sodium phenate, and then with sodium hypochlorite. Nitrosophenol is formed and condenses with phenol to give indophenol, which ionizes in alkaline solution to give a stable blue-green color; Beer's law is valid.	Peaston et al., 1968
Natural water	Digestion of the sample with a sulfuric acid solution containing perchloric acid and selenium as a catalyst to convert amine and amide-type organic nitrogen to ammonium. The digested sample is then made alkaline with a sodium hydroxide solution and treated with an alkaline phenol solution followed by an alkaline hypochlorite solution.	Kammerer et al., 1967
Urea, nicotinamide ammonium sulfate	Report on the interrelationship between digestion temperature, solvent, and catalysts in the Technicon AutoAnalyzer digester system. Reasons for short digestion times, normally minutes, in which digestion is completed.	Marten and Catanzaro, 1966
Plant materials	A method for use with the Technicon AutoAnalyzer. Nitrogen is determined on an aliquot of solution after digestion by a micro Kjeldahl technique.	Varley, 1966
Animal feeds, flours, grains, beer, pharmaceutical products, others	A new technique, which was essentially the classic Kjeldahl wet chemical digestion applied in a different way. The basic of this new technique involved the use of a revolving glass helix.	Ferrari, 1960; Ferrari et al., 1965
Berthollet (manual) Pharmaceutical materials, foods, and excrements	The method involves decomposition of samples, followed by trapping and quantitative colorimetric determination of the resulting ammonia. A novel reactor was constructed to realize routine high throughput analyses of multiple samples.	Yasuhara and Nokihara, 2001
<i>Paspalum fasciculatum</i> , <i>Hyparrhenia rufa</i> , SRM 1547 Peach Leaves	Applicability of the colorimetric procedure to measuring nitrogen concentration. Advantage of eliminating the distillation and titration steps of the Kjeldahl method, many samples being run in a single day.	Bilbao et al., 1999
Dairy products, dry cereals, or cereal-based products and legumes	An application of a method for protein determination, where nitrogen in Kjeldahl digests is measured colorimetrically. Advantage of eliminating the distillation and titration steps of the Kjeldahl method; many samples being run in a single day.	Cioccia et al., 1995
Water and soil extracts	The manual method for analyzing ammonium (NH_4^+) studied with the goal of eliminating interferences from commonly found ions. Using sodium citrate as one component of the method, each component or parameter of the method was fixed to achieve the maximum sensitivity.	Willis et al., 1993

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Plant and fecal samples	Kjeldahl digestion procedures and the colorimetric method allows successfully analyzing total nitrogen over 900 plant and fecal samples.	Clifton and Clifton, 1991
Complex fermentation media	A fast and simple micro method; samples are digested with sodium selenite in sulfuric-phosphoric mixture plus hydrogen peroxide for one hour at 380°C.	Guebel et al., 1991
Diets, feces, urine, and animal carcasses	A colorimetric procedure for determining nitrogen that significantly reduces analysis time, chemicals, and laboratory space. Application to the determination of the quality of dietary casein in rats. Comparison of fecal and urinary nitrogen losses in humans, measured by the Kjeldahl and colorimetric methods.	Hevia and Cioccia, 1988
Sintered UO ₂ sample	Method for the determination of ammonium ions in the solution for the chemical quality control of UO ₂ fuel for nitrogen. The acid mixture used simplifies the problem of recovery of uranium from the waste generated during the analysis of nitrogen.	Rizvi and Natrajan, 1986
Soil	Modifications of the indophenol method for determination of Kjeldahl-N and exchangeable NH ₄ -N. Analytical results for NH ₄ -N in Kjeldahl extracts and 2 M KCl extracts compared with NH ₄ -N determinations by distillation and a measurement with ammonium ion-selective electrode.	Wang and Oien, 1986
Freshwater	Effect of temperature on the modified Berthelot reaction over the range 4.5°–55°C. The rate of color formation increases with increasing temperature but the sensitivity decreases significantly.	Stewart, 1985
Gorse	Improved determination of ammonia in Kjeldahl digests and acidic solutions with a buffered Berthelot reaction. Addition of trisodium citrate to acidic samples and disodium carbonate to an alkaline hypochlorite reagent does not affect the absorbance significantly.	Reay, 1985
Fresh and domestic wastewater	A convenient and reliable analytical procedure for ammonia and Kjeldahl nitrogen determination. The buffering system introduced permits examination without pH correction. The solutions are ready for photometric measurement after 45 min of color development at room temperature.	Scheiner, 1976
Natural water	A manual method for Kjeldahl nitrogen determinations sufficiently simple to be performed routinely. Copper and mercury when used as catalysts in the digestion procedures interfere with the final determination of ammonia and no catalyst is used in the digestion step of the present method.	Nicholls, 1975
Serum and other tissues	Conditions have been defined that enable the use of nitroprusside as a catalyst for the phenol-hypochlorite reaction when the latter is applied to measurement of nitrogen in Kjeldahl digests containing mercury.	Shahinian and Reinhold, 1971
Homogenates and tissue fractions derived from biological material	Direct estimation of ammonia in total micro Kjeldahl digests by means of formation of "phenol-indophenol" from ammonia and phenol in the presence of base, sodium hypochlorite, and sodium nitroprusside.	Mann, 1963

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Plants	Study of the nitrogen metabolism of plants by the phenol-hypochlorite reaction.	Russell, 1944
<i>Salicylate</i>		
Soils	The Westco SmartChem Discrete Analyzer is used for the determination of total Kjeldahl nitrogen in soils with salicylate and hypochlorite in a buffered alkaline solution (pH 12.4, 12.7) in the presence of sodium nitroferrocyanide.	Roper et al., 2011b
Ground, surface, and saline waters, and domestic and industrial wastes	The Seal AQ2 Discrete Analyzer is used for the determination of total Kjeldahl nitrogen with salicylate and hypochlorite in a buffered alkaline solution (pH 12.4, 12.7) in the presence of sodium nitroferrocyanide.	Roper et al., 2011a
Filtered water and whole water samples	Third-generation air segmented continuous flow analyzer (Alpkem RFA-300) was used to automate photometric determination of ammonium ions in resoluted digests with sodium salicylate/sodium nitroferrocyanide reagent.	Patton and Truitt, 2000
Drinking, ground, and surface water, domestic and industrial wastes	The procedure converts nitrogen components of biological origin (amino acids, proteins, and peptides) to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines.	O'Dell, 1993
Soil and plant digests	An automated colorimetric analysis for $\text{NH}_4^+\text{-N}$ is modified (color reaction between NH_4^+ and a weakly alkaline mixture of Na salicylate and a chlorine source in the presence of Na nitroprusside) for manual use, and compared with the usual distillation-titration technique.	Baethgen and Alley, 1989
Dry soil	Dichromate digestion parameters (duration, temperature, acids, and catalysts) optimized, detection methods compared (manual and automated titration, colorimetric absorbance of Cr(III) and Cr(VI), and automated colorimetry of Cr(VI)), adapted salicylate-indophenol colorimetry for N detection, and compared N digestion efficiency with Kjeldahl digestion.	Doyle and Schimel, 1998
Soil	Digest acidity can interfere strongly with ammonium analysis using salicylate-indophenol colorimetry. To optimize analysis of acidic digests, phosphate buffer is replaced with salicylate.	Doyle and Schimel, 1996
Plants and animals	A manual colorimetric method for analyzing ammonia or total Kjeldahl nitrogen eliminates irreproducibility due to variability in time between additions of reagents. The procedure uses trisodium phosphate as the buffer instead of a combination of disodium phosphate and sodium hydroxide. Ammonia is heated with salicylate and hypochlorite in an alkaline phosphate buffer to give an emerald green color, intensified by the addition of sodium nitroprusside.	Willis et al., 1996 Grace Analytical Lab, 1994
Urine and feces	The determination of nitrogen in Kjeldahl digests is simplified by using a manual spectrophotometric method with salicylate and hypochlorite, which agreed with the conventional micro Kjeldahl method.	Fukumoto and Chang, 1982

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Single distal half-seeds	Reexamination of the optimum conditions (reagent concentrations, temperature, pH, and incubation time) of the reaction of salicylate and hypochlorite with ammonia. At least a 40% improvement in sensitivity over other colorimetric procedures is achieved.	Nkonge and Balance, 1982
Natural water	Spectrophotometric determination of ammonia by means of a modified Berthelot reaction in which salicylate, dichloroisocyanurate, and complex cyanides are the principal reagents.	Krom, 1980
Unfiltered river water samples	Mathematical modeling and optimization for the determination of Kjeldahl nitrogen and total phosphorus with a Technicon AutoAnalyzer, consisting of one digestion stage, one or two neutralization stages, and one color reaction stage.	Emi and Müller, 1976
Rainwater, lake water, treated sewage effluent, sanitary canal	Samples continuously digested at 300°C with sulfuric acid and hydrogen peroxide to convert the organic nitrogen to ammonia, which is determined colorimetrically with sodium salicylate and sodium dichloroisocyanurate.	Elkei, 1976
Animal feeds	A semiautomated method (digestion of sample in a block digester and determination of ammonia by ammonia-salicylate reaction), studied collaboratively, along with the official final action Kjeldahl method.	Noel, 1976
Crop plants and soils	Report on the effects caused by adding K, Ca, Mg, Fe, Cu, Mn, Al, Zn, Ni, and Se salts to aqueous ammonia standards at interfering ion concentrations up to the maximum likely to be found in Kjeldahl digests. The effect of adding complexing agents to lessen the precipitation of certain of the interfering species under the alkaline conditions required for the colorimetric determination is assessed.	Pym and Milham, 1976
Plant materials	The automated method of Crooke and Simpson has been modified to permit micro Kjeldahl analysis, following manual digestion.	Bietz, 1974
Plant materials	Manual Kjeldahl digests could be automatically analyzed for ammonium by the salicylate-dichloroisocyanurate reaction in the presence of nitroprusside.	Crooke and Simpson, 1971
Nessler		
Potatoes	6290 ± 990 μg g ⁻¹ dry mass of potatoes sample.	Prusisz et al., 2007
Aqueous liquids, oils, solids	Hydrogen peroxide is used in the Digesdahl ^R ; the digestable being analyzed for ammonia N by direct Nesslerization.	Hach Company, 1999
Forest streams or rainwater	Very low levels (0.02 to 1.5 mg/L) are determined as a routine laboratory procedure with high recovery and excellent precision and accuracy.	Holcombe et al., 1986
	Rapid sulfuric acid/hydrogen peroxide procedure, without any salt or metallic catalyst, followed by a simple colorimetric determination with an improved Nesslerization method.	AOAC, 1984
Plant material and river sediments	Persulfate digestion technique (which yields NO ₃ -N as the sole nitrogen product) is more precise than the Kjeldahl-Nesslerization procedure.	Smart et al., 1983

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Freshwater	The persulfate method was more precise than the Kjeldahl method for standards of known concentration and samples; accuracy and recovery of nitrogen from the samples were the same.	Smart et al., 1981
Plant material	Nessler method is suitable for continuous-flow analysis when a protective agent is used to prevent the deposition of precipitates.	Ginkel and Sinnaeve, 1980
Ground and surface water, domestic and industrial waste effluents	This 3rd edition of <i>Methods for Chemical Analysis of Water and Wastes</i> contains the chemical analytical procedures used in U.S. EPA laboratories.	U.S. EPA, 1979
Biological samples	A method is presented for the determination of as little as 0.002 to 0.20 μg of nitrogen using Nessler reagent solution with colorimetry.	Morrison, 1971
Feeding stuffs	The material to be analyzed is subjected to a Kjeldahl-type oxidation, and the diluted digest subjected to direct Nesslerisation with an improved Nessler's reagent, to determine total nitrogen.	Williams, 1964
Lipoproteins	The addition of a small amount of potassium cyanide to Nessler's reagent avoids turbidity and precipitation during color development.	Minari and Zilversmit, 1963
Materials and solutions	Colorimetric micro Kjeldahl method for determination of nitrogen in materials in solutions, particularly suitable for routine determination.	Burck, 1960
	A new Nessler reagent is proposed for the direct Nesslerization of Kjeldahl digest.	Middleton, 1960
Whole milk	A direct Nesslerization method is satisfactory for the determination of micro Kjeldahl nitrogen comparable to the macro method.	Rao and Whitney, 1960
Sterilized milk	Direct Nesslerization method used for determining nitrogen distribution.	Murthy and Herreid, 1958
Milk	Rapid method for the determination of nitrogen by direct Nesslerization of digested sample was developed. The effect of the variables involved in the test is investigated and their tolerance established.	Hetrick and Witney, 1949
Amino acids, proteins, biological materials, vitamin, others	Importance of a period of digestion before treatment with hydrogen peroxide and of repeated additions of peroxide. Conditions to complete liberation of nitrogen. Colorimetric aspects of the method.	Miller and Miller, 1948
Wheat flour, special bread, barley	A mixture of catalysts based on the one used for Chiles (1928) is utilized in Kjeldahl oxidation to yield an extract suitable for the determination of nitrogen by direct Nesslerization.	Kitto, 1934
Blood, blood filtrates, urine, milk, grain feeds, tankage, and cottonseed meal	A protective colloid (gum arabic solution) prevents precipitation of the color in the Nesslerized solutions, making it possible to Nesslerize directly any of the commonly used Kjeldahl digestion mixtures.	Chiles, 1928
Urine, blood filtrate	The method described avoids the troublesome separation of silicon dioxide so common in the Folin-Denis procedure and also shortens the time of digestion.	Koch and McMeekin, 1924

(Continued on next page)

TABLE 2
Spectrophotometric methods (*Continued*)

Sample	Comments	Reference
Blood	A method for the determination of total and nonprotein nitrogen and of total solids in 15 to 30 drop quantities of human blood, developed for measurement of the Nesslerized nitrogen solutions.	Peters, 1919
Sewage	A series of parallel determinations were made to test the advantage of direct readings of Kjeldahl digestates over the distillation method.	Adams and Kimball, 1908
	Probably the first article in which Nesslerization of the distillate from a Kjeldahl determination was advocated as a mean to estimate nitrogen.	Drown and Martin, 1889

sample color, turbidity, and interactions between the sample matrix and test reagents could affect the results.

Under alkaline conditions, ammonia, sodium hypochlorite, and phenol react to form an intensely blue product, soluble in water ($\epsilon = 1-2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$). Berthelot first reported this reaction in the 19th century (Berthelot, 1859). The Berthelot reaction has found wide analytical use for the measurement of nitrogen, and the literature on its theory and application is extensive (Harwood and Huyser, 1970b; Lubochinsky and Zalta, 1954; Searle, 1984; Thomas, 1912; Van Slyke and Hiller, 1933). In this reaction, ammonia (NH_3) reacts with a hypochlorite (OCl^-) to form a monochloroamine, which, in turn, reacts with two phenols to form an indophenol (Kimble et al., 2006; Marczenko, 1986). The blue color is due to the indophenol anion, formed in alkaline medium. Undissociated indophenol, present in acid solution, is yellow. Indophenol reaction is pH and temperature dependent. The linear dynamic range for ammonia is 0.3–3 ppm, with a sensitivity of 0.3284 (absorbance unit/1 ppm NH_3). Copper, zinc, and iron salts were found to act as interferences. The intensity of the blue color is greatly increased by adding a little portion of acetone ($\sim 0.3 \text{ mL}$ of acetone per 50 mL of solution). The molar absorptivity at $\lambda_{\text{max}} = 625 \text{ nm}$ is 4.5×10^3 (Marczenko, 1986).

The phenol-hypochlorite reaction is more specific and less subject to interference than the Nessler reaction. In the presence of catalysts (Mn^{2+} , acetone, sodium nitroprusside) color development proceeds rapidly at room temperature to give a blue-colored reaction, the intensity of which is proportional to the ammonia content of the sample. Many variations of this reaction are possible, but the phenol-hypochlorite reaction has been shown to be less subject to interference than the hypochlorite-phenol reaction (Searcy et al., 1965). In the automated analysis of protein nitrogen, the ammonia-phenate-hypochlorite reaction has received a great deal of attention and is the basis for the Technicon automated Kjeldahl system (Catanzaro et al., 1965).

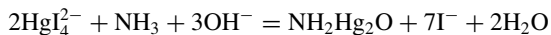
The phenate-hypochlorite assay has two weaknesses. It requires a highly alkaline pH for optimum sensitivity and is sensi-

tive to pH variation. Also, a number of metal ions interfere with color development even though chelating agents are included in the reagents (Kramme et al., 1973). In addition, it uses a carcinogenic reagent (phenol) (Pontes et al., 2009); the precision of the reaction is seldom better than about 10% in micromolar concentration range, and the blank measurements are typically high and unstable (Patton and Crouch, 1977), due to nitrogen contamination caused by the vapor from hoods and ventilation ducts of adjacent laboratories (Aminot et al., 1997), and the tap water that feeds the boiler of the Kjeldahl steam distillation system.

Because of the hazardous nature of phenol, sodium salicylate has been used in its place (Baethgen and Alley, 1989). This reagent is much less volatile, but reaction with ammonia is slower and the color yield appears to be more sensitive to reaction conditions (Fukumoto and Chang, 1982). The irreproducibility due to variability in time between additions of reagents is eliminated when trisodium phosphate is substituted in the buffer in place of the usual disodium phosphate and sodium hydroxide (Willis et al., 1996). The sensitivity of the colorimetric method on the Berthelot reaction with alkaline-phenolate-hypochlorite reagent was unsatisfactory. The use of the more sensitive color reaction between ammonia and a weakly alkaline mixture of sodium salicylate and dichloro-isocyanurate gave much better sensitivity (Nkonge and Balance, 1982). The color formed is stable and obeys Beer's law over a wide concentration range (Elkei, 1976).

The detection and estimation of extremely small quantities of nitrogen as ammonia can be made by use of potassium mercuric iodide in alkaline solution (Nessler, 1856), which produces a yellow color with small amounts of ammonia, orange with large amounts, and a brown precipitate when very large amounts are present. An article by Drown and Martin (1889) is probably the first in which Nesslerization of the distillate from a Kjeldahl determination was advocated as a mean to estimate nitrogen. Ammonia reacts with Nessler's reagent to form a colloidal complex ($\lambda_{\text{max}} = 430-460 \text{ nm}$). The linear range for

analysis extends to 75 mg (ammonia) mL⁻¹. A possible reaction scheme for Nesslerization is



Since the brown-orange sparingly soluble product forms a stable dispersion only at very low concentrations, protective colloids such as gum arabic, gelatin, and poly(vinyl alcohol) are added. Polyvinyl alcohol solution acts as a colloidal stabilizer and also improves the precision of the Nessler method. The molar absorptivity at $\lambda_{\text{max}} = 320 \text{ nm}$ is 6.8×10^3 , and at 400 nm, it is $\sim 5.1 \times 10^3$ (Marczenko, 1986).

Nessler's method is commonly used for determining ammonia directly in natural waters. Since calcium and magnesium present in water interfere by forming precipitates with the reagent, they are masked with tartrate. Hach and coworkers (1985) developed a commercial Nesslerization reagent for use in Kjeldahl analysis. The Nessler method is sensitive to numerous variables, including the age of the reagent. Optimal color reaction and stability require rigorous conditions; the colored complex does not form a molecular solution. The addition of potassium cyanide to the Nessler reagent avoids some of the difficulties such as the inhibition caused by minute amounts of some heavy metals or the precipitation of some cations with the alkali (Minari and Zilversmit, 1963). Thus, it is not suited for direct routine applications. It is also not sufficiently sensitive for analysis of low-level ammonia concentrations.

The indophenol or Nessler color reactions are carried out in alkaline medium. Precipitation of the metals used as catalysts for the digestion of organic compounds interferes with ammonia determination. For this reason, it is convenient to use a simple spectrophotometric method for the determination of ammonia in acidic Kjeldahl digest. The reaction of ammonia with β -ketoester is known to form 1,4-dihydropyridine derivative (Hantzsch reaction). This reaction has been exploited to develop a new spectrophotometric method for the determination of ammonia in Kjeldahl digest. The digest is reacted with acetylacetone-formaldehyde reagent in the presence of sodium acetate to give a yellow reaction product with maximum absorbance at 412 nm.

Prediluted digest reacts with a mixture of acetylacetone and formaldehyde, in the presence of sodium acetate (Devani et al., 1989). The yellow product (3,5-diacetyl-1,4-dihydrolutidine) is measured at 410 nm ($\epsilon = 1.4 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$). The linear dynamic range was 0.5–6.0 $\mu\text{g N}$ (per final reaction mixture) and shows levels of accuracy and precision equal to those of the micro Kjeldahl method (Devani et al., 1989). The reaction mixture has a pH of 5.5–6.0. Therefore, metal ions used in digestion apparently do not precipitate. The loss of ammonia by volatilization is also prevented. Thus, the proposed method is simple, rapid, precise, and accurate, and does not require basification of the digest and its distillation for ammonia determination (Devani et al., 1989). The reagents involved are stable, inexpensive, and readily available.

The reaction of ninhydrin with primary amino groups to form a purple dye was discovered by Ruhemann (1910). The colorimetric assay of Kjeldahl nitrogen using the ninhydrin reaction is sensitive and reliable as shown by Jacobs (1960, 1962). Ninhydrin reacts with amino acids in two stages: (i) the amino acid is oxidized to aldehyde and ammonia while ninhydrin is converted to hydrindantin and (ii) hydrindantin and one mole of ninhydrin reacts with ammonia to form Ruhemann's purple. The historical perspective, chemistry and mechanisms, applications, and research needs of the reaction of ninhydrin with primary amino groups to form Ruhemann's purple have been reviewed (Friedman, 2004). A reducing agent (e.g., hydrazine sulfate) added for ammonia determination is necessary to convert ninhydrin to hydrindantin. Results from the ninhydrin Kjeldahl assay closely agree with those from Kjeldahl analysis. The linear dynamic range for colorimetric Kjeldahl assay depends on the extent of sample dilution just before ninhydrin analysis ($A_{570} = 0.805/2.8 \text{ ppm}$ standard ammonium sulfate solution). Compared with other spectrophotometric methods, the ninhydrin assay is more resistant to interference from metal catalysts. The color is also formed at a buffered pH between 4.9 and 5.4. The assay of nitrogen in Kjeldahl digests by the ninhydrin colorimetric method is adaptable to AutoAnalyzer analysis. Automation provides rapid, accurate, and reproducible results (Quinn et al., 1974).

Recently, the most often used analytical reagent for the determination of ammonium ion and amino acids is *o*-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol (Kitamaki et al., 2003).

ION SELECTIVE ELECTRODE METHODS: THE SENSING PROBE

Although the known photometric methods for the determination of ammonia are accurate, they require a considerable amount of time, and only clear solutions can be processed. Cloudy solutions must first be clarified by time-consuming procedures. These problems do not exist with the ion-selective ammonium electrode (Bremner et al., 1972; Buckee, 1974; Electronic Instruments Ltd., 1974; Todd, 1973). The measurements can be easily performed in Kjeldahl digestion solutions without distillation.

The determination is based on the principle that the ammonium ion is released as ammonia gas upon addition of excess sodium hydroxide. The outer membrane of the electrode allows the ammonia to diffuse through it, and a combined glass electrode monitors the change in the pH value of the inner electrolyte solution. The known addition method (difference in potential before and after addition of a known amount of ammonium standard solution) provides more reproducible measurements than the direct method, and the shift in the electrode potential did not influence the results (Nubé et al., 1980).

The method was applied to the determination of total nitrogen in water (Campins-Falco et al., 2008), plant and soil samples (Wang and Oien, 1986), and a variety of other sample types (Table 3).

TABLE 3
Ion sensing electrode and probe

Sample	Comments	Reference
Irrigation water, residual water, tap water, river water	Nitrogen miniaturized Kjeldahl system; the ammonium content in the distilled solution is measured with an ammonium-selective electrode. Analysis time is reduced 4.8 times and the analysis costs 6.6 times compared with classical procedure.	Campins-Falco et al., 2008
Human blood serum	Development of an ammonia-sensitive material (a periodic array of highly charged colloidal particles embedded in a poly(hydroxyethyl acrylate) hydrogel) by coupling the Berthelot reaction to polymerized crystalline colloidal array technology.	Kimble et al., 2006
Urine, feces, protein standard	Investigation of the efficacy of the ammonia electrode for analysis of the nitrogen content of a large series of Kjeldahl digests. By using this electrode, two methods for the measurement of ammonium concentrations were compared, the direct method and the known-addition method.	Nubé et al., 1980
Soil	Nitrogen and exchangeable ammonium by colorimetry and ion selective electrode.	Wang and Oien, 1986
Forest vegetation	Ammonia electrode technique applied to micro Kjeldahl digest offers a four-fold saving in analytic time per sample and yields results comparable to those derived by the traditional method.	Powers et al., 1981
Coal and coke	The selective-electrode procedure described for determining nitrogen offers significant advantages over Kjeldahl distillation and titration. The Kjeldahl digest is diluted, neutralized, cooled, diluted to volume, adjusted to a standard temperature, and rendered alkaline prior to measurement with an ammonia-selective electrode.	Doolan and Belcher, 1978
Plants	Ammonium concentration is determined in semimicro Kjeldahl digests of plant samples with speed, precision, increased safety, and simplicity, in a small aliquot of the digest. Results agree closely with distillation and titration methods.	Eastin, 1976
Plant and soil samples	Samples digested in Al block digester and the total N determined with the ammonium electrode in a semiautomated reaction vessel assembly.	Gallaher et al., 1976
Freshwater	A commercially available ammonia probe was used in an automated continuous flow-through system to determine the ammonia N concentration in Kjeldahl digests. The ammonia probe with modified filling solution and polytetrafluoroethylene membrane is sufficiently stable for continuous use throughout a working day.	Stevens, 1976
Barley, malt, wort, and beer	Ammonia probe.	Buckee, 1974
Agricultural materials	Ammonium content of Kjeldahl digests of foodstuffs using the known-addition method.	Deschreider and Meaux, 1973b
Wine, natural mineral waters	Sensitivity to 0.05 mg NH ₄ ⁺ /L, without interference by mineral salts even in amounts of 4–5 g/L.	Deschreider and Meaux, 1973a
Meat products	Ammonia electrode.	Todd, 1973
Soils	The distillation-titration procedure is replaced by a manual ammonia probe measurement for the Kjeldahl digest. The method is rapid, simple, and precise, and it gives quantitative recovery of ammonium added to Kjeldahl digests of soils.	Bremmer and Tabataba, 1972
River and seawater	Ammonia is directly determined in the diluted destruction mixture, so that the time-consuming distillation procedure is avoided. The limit for precise measurements is around 0.5 ppm N, so that for seawater the method is not universally applicable.	Mertens et al., 1972

The working characteristics of the ion selective electrode are good, except for the potentiometric selectivity coefficients, namely the alkali metal ions that can be found in milk products in rather large amounts (Lima et al., 1999).

On first consideration, these electrodes appeared to be quite attractive (U.S. EPA, 1983) when compared with manual distillation or colorimetric methods. In principle 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 potentiometric method with ammonia is not suitable for low-level concentrations, amines interfere with it, and it is not applicable to some samples, such as seawater (Molins-Legua et al., 2006). Membrane life of the electrodes is only a few weeks (Verma et al., 2007; Vita, 1976).

FIA METHODS: DIFFUSION COMES IN

Flow injection analysis (FIA) is unquestionably an excellent tool for the automation of existing analytical methods. Continuous FIA based on gas diffusion separation (GD-FIA) is a useful non-chromatographic technique for the determination of volatile substances or those that can be selectively and quantitatively converted into gaseous species from a complex matrix, e.g., ammonium in Kjeldahl digests (Junsomboon and Jakmunee, 2008; Pasquini and de Faria, 1987; Su et al., 1997, 1998; Yao and Su, 1999), as shown in Table 4. Large variations in acidity are common for digested Kjeldahl samples, leading to several practical problems to deal with, since the dispersion coefficient of the FIA system is sample dependent (Tryzell and Karberg, 1997). Simple FIA methods are competitive with other expensive and chromatographic methods in terms of performance and are characterized by a high selectivity, good precision, promising sensitivity, favorable throughput, and simple experimental setup.

With a gas-permeable membrane in gas diffusion FIA, the volatile analyte from a donor stream is separated before it goes into a flow cell detector. Conventional spectrophotometry and ion-selective electrode potentiometry are mainly the techniques of choice for determination of the gaseous species trapped in the recipient solution. However, it is also possible to use a simpler and widely used general-purpose method such as conductivity, given its inherent selectivity. Nevertheless, high background conductivity of the recipient stream can cause an unacceptable noise level (Schulze et al., 1988). This drawback may be overcome by using an additional column of mixed-bed ion exchanger for final purification of the recipient stream or by applying a sophisticated electronic circuit with a differential conductimeter (Rohwedder and Pasquini, 1991).

Bulk acoustic wave (BAW) sensors (thickness shear mode or piezoelectric quartz crystal) have been mostly classified as a type of mass sensor or referred to as a microbalance (Su et al., 1997; Yao and Su, 1999). It is also a nonmass-effect sensor that gives sensitive response to changes in the physicochemical parameters of the medium such as the solution conductivity

and permittivity, showing in some cases that the nonmass-effect BAW sensor has an even more sensitive response than that of the mass-effect-based sensor. A nonmass-effect BAW detector provides an excellent technique for automatic and precise FIA of volatile substances in complex matrices, with reagents and equipment simpler and less expensive than those used in conventional methods. It can detect a slight change in the solution conductivity, even in the presence of excessive spectator electrolytes, with even better sensitivity and accuracy than those obtained in their absence. The precision range, detection range, sensitivity, selectivity, and throughput are comparable to the reported detection modes of spectrophotometry and potentiometry. The technique has also advantages over the conventional high-frequency impedance technique in terms of frequency stability, reproducibility, and precision.

A main problem in classical conductimetric detection is the double electric layer capacitance and faradaic impedance, which can change the effective potential applied to the electrodes of the detector cell and prevent accurate measurement. This limitation may be overcome by a multi-electrode technique or by applying electrodes with alternating potential, but the apparatus tends to be more complex. In the BAW impedance sensor (BAWIS), however, the double electric layer is eliminated, because the resonator supplies a high-frequency (9 MHz) alternating electric field to the electrode couple. Meanwhile, the small potential difference between the electrode couple is not enough to cause electrolysis. Moreover, it can detect a slight change in solution conductivity in the presence of an excess of a foreign electrolyte, and the sensitivity and accuracy are even better than those obtained in the absence of the foreign electrolyte.

Compared with the normal piezoelectric sensor, in which the quartz crystal is in direct contact with the solution, the BAWIS possesses the same sensitivity to the conductivity and permittivity of the solution and a better frequency stability, lower frequency-temperature coefficient, and much lower frequency dependence on the density and viscosity of the solution because only the electrode is immersed in the liquid and the crystal is out of contact with the solution (Su et al., 1997; Yao and Su, 1999).

ION CHROMATOGRAPHY: THE MULTI-SPECIES TECHNIQUE

Ion chromatography (IC) is a rapid, versatile, selective, and sensitive method for the determination of variety of anions and cations at trace and ultratrace levels. It has become a well-established technique for the determination of ammonium ion, and it has been applied for nitrogen analysis in diverse matrices, as shown in Table 5. The use of ion chromatography to determine total nitrogen as ammonium ion after Kjeldahl digestion can significantly improve the speed of analysis compared with the conventional method by eliminating the need for the distillation step (Michalski and Kurzyca, 2006; Verma, 2007). As only microliter volumes of samples are required for analysis, the mass of sample required for the digestion can be reduced, minimizing

TABLE 4
Flow injection methods (FIA)

Sample	Comments	Calibration range	Detection limit	Precision RSC (%)	Sample h-I	Reference
Cow and soy milks, chicken meat	A simple flow injection (FI) conductometric system with gas diffusion separation was developed for the determination of Kjeldahl nitrogen. The sample was digested according to the Kjeldahl standard method, and the digest was diluted and directly injected into the donor stream.	10–100 mg L ⁻¹ linear	1 mg L ⁻¹	0.3	35	Junsomboon and Jakmunee, 2008
Plant materials: soy, tobacco, pine, coffee, beans, rice, soy leaf, cotton, and peach standard reference materials	An improved flow-injection system with an AgCl (s) reactor is proposed for the spectrophotometric determination of total nitrogen in Kjeldahl digests. After sample injection, the established sample plug is alkalinized and allowed to flow through the mini-column where AgCl ions are displaced as the soluble Ag(NH ₃) ₂ ⁺ complex. Bromopyrogallol red and <i>o</i> -phenanthroline are then added and a colored ternary complex is formed under neutral conditions.	0.3–2.0 mg L ⁻¹ Ag ⁺ (linear)	0.2% w/w	< 2	150	Carneiro et al., 2000
Cow milk, infant powdered milk, yoghurt, cheese	A flow-injection analysis (FIA) system with a potentiometric tubular detector and a gas diffusion unit was developed for the determination of Kjeldahl nitrogen. The samples were digested by the Kjeldahl method and afterwards injected into the FIA system without prior treatment.	11–210 mg L ⁻¹	7 mg L ⁻¹	0.5	100	Lima et al., 1999
River and wastewaters	An FIA conductometric method for monitoring of ammonium ions is proposed. The results are comparable with those obtained by spectrophotometry (Nessler's reagent).		5.6 μmol L ⁻¹			Vicek and Kuban, 1999
Amino acids and blood proteins	An FIA technique was developed for determining nitrogen, carbon, or sulfite species. The method is based on the use of a bulk acoustic wave detector in combination with gas diffusion separation.	5–4000 μM (nonlinear)	1 μM	1	20–50	Yao and Su, 1999
Blood	A flow-injection system for the rapid and direct determination of both total ammonia and total carbon dioxide was developed. The trapped NH ₃ /CO ₂ in the acceptor was determined on-line by a bulk acoustic wave impedance sensor.	2.5–200 μmol L ⁻¹ (linear)	1.0 μmol L ⁻¹	1.05	20–65	Su et al., 1998
Vegetables	An FIA system with a potentiometric tubular detector and a gas diffusion unit was developed for the determination of Kjeldahl nitrogen. The samples once digested by the Kjeldahl method were afterwards injected into the FIA system without prior treatment.	0.076–110 millimol L ⁻¹ NH ₄ ⁺ (linear)	1.4 millimol L ⁻¹ , 25 mg L ⁻¹	< 3.5	40–60	Lima et al., 1997

(Continued on next page)

TABLE 4
Flow injection methods (FIA) (Continued)

Sample	Comments	Calibration range	Detection limit	Precision RSC (%)	Sample h-l	Reference
Kjeldahl digests	The method is based on diffusion of ammonia across a PTFE gas-permeable membrane from an alkaline (NaOH/EDTA) stream into a stream of diluted boric acid. The trapped ammonium in the acceptor is determined on-line by a bulk acoustic wave (BAW)-impedance sensor and the signal is proportional to the ammonium concentration present in the digests.	0.07–56 mgL ⁻¹ (nonlinear)	0.01 mg L ⁻¹	1.0	45–50	Su et al., 1997
Feed samples (protein content 14.7–60%)	By using standard solutions and multivariate calibration, the accuracy and precision of ammonium determination in Kjeldahl samples by FIA can be significantly improved. The method is based on partial least squares regression and on an extended number of calibration solutions covering the entire experimental domain for both ammonium and acid in the digest sample solutions.	40–400 mg L ⁻¹ (nonlinear; partial least squares analysis method)	—	0.8	—	Tryzell and Karberg, 1997
Waste and wastewater	Samples were digested and then placed on a flow injection autoanalyzer for ammonia determination.		0.1 mg L ⁻¹	< 8 (n = 15)		Anderson and Möller, 1995
Soils	An FIA system incorporating a gas-diffusion unit and a potentiometric detector was developed. The potentiometric detector is a PVC tubular selective electrode without inner reference solution, and with the sensor system composed of monactin in tris(2-ethylhexyl) phosphate.			< 3	80	Ferreira et al., 1996
Six samples of vegetable tissues	A differential conductivity meter was constructed for use in flow injection systems. The instrument employs a Wien bridge to generate a sinusoidal alternating electrical potential applied to a measurement bridge containing two twin conductance flow cells.	0.5–25 mmol dm ⁻³ (nonlinear)		< 1	90	Rohwedder and Pasquini, 1991
Vegetable tissues, animal feeds and fertilizers	A flow injection/conductometric method for determining ammonia in solutions obtained from Kjeldahl digestion. The method is based on diffusion of ammonia through a PTFE membrane from an alkaline (NaOH/EDTA) medium to a deionized water stream. The change in conductance of the deionized water stream is proportional to the ammonia concentration present in the digest.	14–112 mg L ⁻¹ (nonlinear)		1.0	100	Pasquini and de faria, 1987
Foliar material taken from various plants (21 samples)	The rapid simultaneous determination of nitrogen in a single acid digest of plant material based on the Berthelot method was adapted to FIA with a dual-channel manifold. Spectrophotometric measurement of a blue color is employed.	Linear or parabolic		0.75	200	Stewart and Ruzicka, 1976
					180	Stewart et al., 1976

RSC: Relative Standard Deviation

TABLE 5
Chromatographic methods

Sample	Comments	Calibration range	Detection limit	Precision RSD (%)	Reference
Brazilian oil reservoirs with different fixed ammonium	Ultrasonic batch for ammonia extraction and ion chromatography of Pontes et al. (2009) for fixed NH_4^+ determination.	100–800 $\mu\text{g L}^{-1} \text{NH}_4^+$	4.4 $\mu\text{g g}^{-1} \text{NH}_4^+$	11	Pontes et al., 2010
Total nitrogen in flue-cured tobacco (<i>Nicotiana tobacum</i> L.)	Total nitrogen and inorganic cations by high-performance ion chromatography with suppressed conductivity detector, in the presence of elevated levels of sulfuric acid found in digested samples.	0.05–50 $\text{mg L}^{-1} \text{NH}_4^+$	0.01 mg L^{-1}	0.56	Cai and Wang, 2009
Sediments, oil free sandstones, and soil certified reference materials (Beijing, China)	The total Kjeldahl nitrogen method is simplified by using a manifold connected to a purge-and-trap system immersed in an ultrasonic bath for simultaneous ammonia extraction from many previously digested samples.	30–800 $\mu\text{g L}^{-1} \text{NH}_4^+$	9 $\mu\text{g g}^{-1} \text{N}$	< 13	Pontes et al., 2009
Potatoes, certified reference materials, spinach leaves (NIST 1570a)	Amide and amine nitrogen content assessed after modified Kjeldahl digestion and determined as NH_4^+ . The total nitrogen quantified as NO_3^- after microwave-facilitated digestion.	upper linearity range: 130 $\text{mg L}^{-1} \text{NH}_4^+$	0.17 mg L^{-1}		Prusisc et al., 2007
Uranium alloy, uranium metal, sintered UO_2 pellets, sintered UO_2 microspheres	NH_4^+ determined by ion chromatography (cation exchange column) after matrix separation by Kjeldahl distillation.	0.02–1 $\text{mg L}^{-1} \text{N}$	4 $\text{ng g}^{-1} \text{N}$	3	Verma et al., 2007
Fountain water, irrigation ditch water, lake water, seawater	Ammonium derivatized as dansyl derivative, separated with a chromatographic column, and post-column mixed with peroxyoxalate and H_2O_2 to perform chemiluminescence detection.	0.027–750 $\text{mg L}^{-1} \text{NH}_4^+$	8 $\mu\text{g L}^{-1}$	2–8	Messeguer-Lloret et al., 2005
Certified materials, plant samples	Total N, K, Ca, and Mg were determined in plant samples by ion chromatography; a modified Kjeldahl method was used.				Masson and Andriu, 1996
Organic acids and some inorganic materials	Gas chromatographic method following Kjeldahl digestion.				Moldoveanu, 1988

the amount of reagent that needs to be used, e.g., the mercury(II) oxide or sulfate catalyst, and hence also disposed of (Jackson et al., 1991).

High-performance ion chromatography (HPIC) is a well-established multi-species technique, not only for the determination of non-metal elements, such as nitrogen, sulfur, and phosphorus as inorganic anion (Cai and Wang, 2009), but also for the determination of metal elements, such as inorganic cations after sample digestion, significantly improving the speed of the analysis compared with the conventional methods such as Kjeldahl digestion-distillation, dry combustion, atomic absorption spectroscopy (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), and inductively coupled plasma-mass spectroscopy (ICP-MS).

The chromatographic conditions selected for the determination of ammonium ion depend on the actual digestion procedure employed. Some procedures have been described to improve selectivity. The determination of low ppm levels of ammonium in samples digested using a potassium sulfate-sulfuric acid mixture could not be accomplished using a low-capacity cation-exchange column (IC-Pak Cation) owing to the presence of approximately 20,000 ppm of potassium in the final digest. A weakly basic anion-exchange resin column or high capacity cation exchange column has been used to separate ammonium from the other inorganic cations or/and aliphatic amines that can be present in water samples. A poly(styrene-divinylbenzene) cation-exchange column (Jackson et al., 1991) with dilute nitric acid as eluent and indirect conductivity detection was used for the ion chromatographic determination of ammonium in the presence of the elevated levels of sulfuric acid found in the digested sample.

A manifold connected to a purge-and-trap system immersed in an ultrasonic bath for simultaneous ammonia extraction from previously digested samples allows simplifying the total Kjeldahl nitrogen (TKN) method (Pontes et al., 2009). The ammonia collected in an acidic solution is converted to ammonium (NH_4^+) and finally determined by ion chromatography. Ammonia extraction by the ultrasonic-assisted purge-and-trap system is faster, simpler, and more sensitive than the classical Kjeldahl steam distillation method.

Some difficulties arise from using some catalysts and separation columns that are so expensive that few laboratories could afford the cost. It has been reported that the addition of hydrogen peroxide to the digesting sample can greatly improve the speed of sample digestion (Cai and Wang, 2009).

MISCELLANEOUS: THE CATCHALL

Chemiluminescent methods are much faster and more sensitive than the traditional Kjeldahl method, which is of limited usefulness in the clinical laboratory because it requires relatively large sample volumes. They have been applied to the estimations of small amounts of specific nitrogen-containing chemical substances in a wide variety of clinical and biological samples, providing fast, sensitive, accurate, and precise measure-

ments (Konstantinides et al., 1988; Skogerboe et al., 1990; Ward et al., 1980). The reagent mainly used is luminol combined with hypochlorite and FIA systems. The use of high-performance liquid chromatography (HPLC) to improve selectivity and sensitivity has been reported by using a dansylation derivatization procedure separated by HPLC and mixed with TCPO/ H_2O_2 in order to generate chemiluminescence (Meseguer-Lloret et al., 2006).

The direct estimation of the ammonia in the sulfuric acid digest may be carried out by a sensitive and specific enzymatic method with a minimum of technician time (Smit, 1979). The enzymatic determination is not inhibited by either mercuric ions or turbidity, since this does not influence the differential absorbance measurement. In Table 6 are compiled chemiluminescent procedures and some other methods applied to Kjeldahl nitrogen determination by unusual methods.

The manufacturer Wescan makes a very sensitive apparatus that can attain a detection limit of the order of ppm (linear response between 10 ppb and 1000 ppm). The analysis is carried out on diluted Kjeldahl digest and requires 2 minutes. The reaction is based on the diffusion of ammonia across a neutral Teflon membrane coupled to a sensor measuring the electrical conductivity (Pansu et al., 2001). Gas diffusion (by using pH change or heat) across a hydrophobic semipermeable membrane to separate the analyte of interest from the sample matrix may be used in place of manual or automated distillation in methods for analysis such as for ammonia, total cyanide, total Kjeldahl nitrogen, and total phenols (U.S. EPA, 2012). However, these procedures do not replace the digestion procedures specified in the approved methods and must be used in conjunction with those procedures.

NIR: THE NONDESTRUCTIVE TECHNIQUE

The use of near-infrared reflectance (NIR) spectrophotometry for the quantitation of proteins in cereals was first reported by Norris, who originally invented a moisture meter based on the NIR principle in 1964. The principle of near-infrared spectroscopy (NIRS) is to determinate the absorbance of a sample in the near-infrared region, which is caused by the vibration of the chemical bonds in the sample. NIR is an indirect method, which requires calibration of the equipment with reference values to determine a specific compound, e.g., soy protein in flour. The reference methodology for crude protein determination in milk is the Kjeldahl method (Etzion et al., 2004; Kaylegian et al., 2006). The advantage of using NIR lies in the great selectivity of the technique (Jarquin-Sanchez et al., 2011). Analyses can be performed with NIR in a few minutes without further manipulations, extraction, or use of chemical reagents and supply precise results (Picarelli et al., 1995). To perform robust NIRS calibration, a large and variable set of samples with a complete characterization determined by standardization methodologies is required (Fearn, 1986; Galvez-Sola et al., 2009).

NIR with diffuse reflectance is used for the direct and non-destructive determination of a large number of samples, with

TABLE 6
Miscellaneous

Sample	Comments	Reference
Soil extracts	A newly revised diffusion technique that allows correct and simultaneous determination of total N and (15)N at %, from aqueous solutions and Kjeldahl digests, with N concentrations down to sub-0.5 mg N L(-1) levels, tested under different conditions of (15)N isotope labeling.	Chen and Difter, 2008
Meat samples	The amount of added phosphorus was obtained by calculating the difference between the total phosphorus and protein bound phosphate, which were determined from the total nitrogen by the Kjeldahl method.	Jastrzebska, 2006
Spiked real water samples	Nitrogen compounds react with hypochlorite (to produce chloramines) in a continuous flow system, mixing with luminol; the chemiluminescence signal emitted at 425 nm decreases as nitrogen concentration increases. Linear interval 0.24.4 mg L ⁻¹ N, with the detection limit 0.07 mg L ⁻¹ N.	Meseguert-Lloret et al., 2006
Irrigation ditch, residual, and fountain waters	A method for the simultaneous determination of primary amino groups and ammonium ion is proposed. The method is based in solution derivatization with <i>o</i> -phthaldialdehyde/ <i>N</i> -acetyl-cysteine (OPA/NAC) and fluorescence measurement of the formed isoindols.	Lloret et al., 2005
Meat and meat protein	The amount of added phosphate is determined by the difference between the amount of total phosphate and protein bound phosphate, which is calculated from the concentration of nitrogen (Kjeldahl method) and from the known ratio of phosphate to protein; soluble phosphate is by capillary isotachopheresis.	Dusek et al., 2003
Kefalograviera cheese	The extent and characteristics of proteolysis in cheese were monitored during aging by Kjeldahl determination of soluble fractions (water-soluble nitrogen) and a number of additional analyses.	Katsiari et al., 2001
Serum, urine	Ultramicro method for the determination of the total nitrogen content of biological fluids and suspensions, based on a digestion in sulfuric acid and an enzymatic determination of the ammonia formed with glutamate dehydrogenase, with detection limit of 35 ng N per sample.	Smit, 1979
Urine, enteral and parenteral feeds, feces	Automated method of chemiluminescence analysis for use in clinical nutrition.	Grimble et al., 1988
Milk	Recovery of ammonia from ammonium sulfate solutions by a modified Conway microdiffusion procedure did not differ significantly from recovery by micro Kjeldahl distillation. Total and whey-protein nitrogen concentrations were determined by microdiffusion and microdistillation of Kjeldahl digests.	Lin and Randolph, 1978

savings of time and reagents. This technique provides rapid analysis of grain and powder samples. NIR has been frequently applied in the determination of organic compounds such as protein and fat with satisfactory results (Inácio et al., 2011). There

is growing interest in the use of near-range and/or midrange infrared (IR) diffuse reflectance (NIR and MIR) as nondestructive alternatives to chemical testing of soils (Rayment et al., 2012). The protein content of milk is usually analyzed with automated

TABLE 7
Selected applications of NIR

Sample	Comments	Reference
Bovine milk	Concentration of milk true protein is estimated by mid-infrared absorbance method in samples from bulk tank of dairy herd, correlating results obtained with those of the Kjeldahl method.	Botaro et al., 2011
Milk powder	The protein content was determined by the Kjeldahl method and near-infrared reflectance spectroscopy (NIRS) analysis. Principal component regression and partial least squares multivariate calibrations were used to predict the total protein.	Inacio et al., 2011
Tropical soils	Results obtained by means of the prediction models generated by NIRS correlate well with the Kjeldahl method for determining nitrogen, independently of the characteristics of each particular soil.	Jarquín-Sánchez et al., 2011
Sewage sludge	NIRS estimates the content of total nitrogen in sewage sludge samples with a high accuracy, supporting a quick predictive analysis, with low error of prediction. Elemental nitrogen (Dumas method) and Kjeldahl nitrogen were used for this estimation.	Galvez-Sola et al., 2009
Herbage from permanent meadows	NIRS accurately predicts total nitrogen, nitrogen trichloroacetic acid precipitated matter, and neutral-detergent insoluble nitrogen, but less precisely borate-buffer insoluble nitrogen and acid-detergent insoluble nitrogen (probably due to the accumulated errors for the different laboratory procedures involved).	Valdes et al., 2006
Milk and dairy products	Chemical and infrared methods for analysis of milk and dairy products. NIRS permits continuous inline monitoring of product and process factors, thereby giving better control and leading to greater product control and uniformity.	O'Sullivan et al., 1999
Feces	The procedure evaluates the accuracy and precision of NIR analysis in routine measurement of nitrogen compared with Kjeldahl method.	Picarelli et al., 1995
Soybeans	The accuracy in estimating nitrogen concentration (of four stages and/or parts of soybean plants) by NIR analysis based on two calibration methods, in comparison to Kjeldahl analysis, has been investigated.	Schaalje and Mündel, 1991
Samples of ammonium sulfate	Accurately weighed samples of ammonium sulfate converted to ammonia by the micro Kjeldahl procedure, distilled into standard acid, diluted to volume, and assessed by titration and transmission infrared analysis, to determine ammonium ion in aqueous solution by commercially available infrared filter instruments.	Van de Voort et al., 1986

analyzers based on the absorbance of mid-infrared (MIR) energy by the protein specific chemical group, amides (peptide bond), at a wavelength of about $6.7\mu\text{m}$ (Lynch et al., 2006), which allows estimating the concentration of crude protein (Barbano and Lynch, 2006).

Some applications are listed in Table 7, including the determination of true protein content (Botaro et al., 2011), total nitrogen in soils (Jarquín-Sánchez et al., 2011), foliar nitrogen in corn and soybean leaves, N content in sewage sludge (Galvez-Sola et al., 2009), the quantification of dif-

ferent chemical fractions of forages in a short period of time (Valdés et al., 2006), and the determination of N in feces (Picarelli et al., 1995). Many authors have reported success in using NIR and/or MIR spectra in conjunction with chemometrics to associate spectral information with sample properties obtained by conventional methods. Sophisticated mathematical approaches have greatly assisted NIR/MIR prediction of soil data, e.g., the artificial neural network calibration model (Anderson, 2007; Rayment et al., 2012; Wei et al., 2012).

KJELDAHL VERSUS DUMAS: THE GLOVES ARE OFF

The main advantage of Kjeldahl methods are their widely established use in crude protein estimation. Kjeldahl analysis gives accurate protein results no matter what the physical state of the sample (Owusu-Apenten, 2002). However, Kjeldahl methods require wet chemistry, the handling of concentrated sulfuric acid, and a heavy-metal catalyst (Bradstreet, 1965; Sáez-Plaza et al., in press). Moreover, the Kjeldahl analysis is labor and reagent intensive, time-consuming, and subject to numerous sources of errors when procedures are not strictly adhered to (Lynch and Barbano, 1999; Williams et al., 1998). In consequence, for routine analysis the Kjeldahl method is a tedious and time-consuming procedure requiring disposal of hazardous wastes (Patton and Kryskalla, 2003). Nevertheless, the Kjeldahl method has the advantage of requiring only simple cheap equipment, so that it can be used in places where infrastructure problems are limiting (Pansu et al., 2001). An alternative to save time is the use of devices that allow the digestion of many samples simultaneously, as well as the use of mechanically automated techniques (Barbarino and Lourenço, 2009; Watkins et al., 1987).

A number of articles have been published concerning the comparison between Kjeldahl and other nitrogen determination methods (Table 8). Researchers have tried to find alternatives to the Kjeldahl method either for specific samples or for a wide range of samples. Details are too lengthy to include here and should be consulted in the original articles. An alternative method for the determination of organic nitrogen is the alkaline peroxodisulfate digestion technique. This oxidizes all nitrogen in the sample using peroxodisulfate in a strongly alkaline environment under high pressure and temperature, as shown previously (Cornell et al., 2003; Ferree and Shannon, 2001; Patton and Kryskalla, 2003; Valderrama, 1981). In this sense, the Dumas combustion method deserves special consideration, however. A wide range of samples including foods and feedstuffs, cereals, grains, oilseeds, milk, cheese and dairy products, brewing substances, soils and plant materials, agricultural samples, and blood serum proteins have been analyzed by both Kjeldahl and combustion methods (Bellomonte et al., 1987; Buckee, 1974; Daun and deClercq, 1994; Etheridge et al., 1998; Jakob et al., 1995; Jung et al., 2003; Marco et al., 2002; Matejovich, 1995; Sader et al., 2004; Sarich et al., 1996; Simonne et al., 1997; Watson and Galliher, 2001), as shown in Table 8. A number of interlaboratory/collaborative studies concerning the Dumas versus Kjeldahl method (Table 9) have also been carried out. Wiles and coworkers (1998) have given a summary of previous comparisons of Dumas and Kjeldahl methods. Readers should refer to that article for such studies, of which no mention is made here.

Like the Kjeldahl method, the combustion method (Dumas, 1831; Tronimisdorff, 1832) is based on the determination of nitrogen. Modern advances in instrumentation have improved the capabilities of this venerable method, turning it into a useful alternative (Bicsak, 1993; Krotz and Giuzzi, 2011; Thompson

et al., 2002). This method normally involves an initial oxidation step followed by passage of gases through a reduction furnace to reduce NO_x to N_2 . The quantity of N_2 is usually determined using a thermal conductivity detector (Rutherford et al., 2008). The Dumas combustion method determines the total nitrogen including its inorganic fraction species and has a tendency to give higher protein values than the Kjeldahl method (Möller, 2009; Thompson et al., 2002). The Kjeldahl method does not recover fraction species like nitrite and nitrate and does not recover all organic nitrogen. Dumas techniques have the advantages of requiring less laboratory space, providing rapid analyses, requiring less chemical reactants, producing less noxious fumes and hazardous chemical waste (Bellomonte et al., 1987; Rutherford et al., 2008), and including all forms of nitrogen without lengthy pretreatments, as compared with the Kjeldahl method.

The Dumas combustion method instrumentation is commercially available (Krotz and Giuzzi, 2011; Krotz et al., 2008; Simonne et al., 1997) from a variety of suppliers (such as the LECO FP-428, LECO CHN 600, Carlo Erba NA-1500, and Thermo Scientific Flash 4000 elemental analyzer). The manufacturers' recommendations concerning sample size and analysis times vary considerably from instrument to instrument. In general, the sample size is proportional to the analysis time; time for analysis varies from 4 to 11 minutes, depending on the sample size and the instrument model (Bicsak, 1993). However, the equipment is costly to purchase and operate and requires considerable skill and experience for satisfactory use and maintenance. Sample variability may also be a concern with combustion techniques because of the small sample size commonly used for analysis (Kowalenko, 2001; Rutherford et al., 2008).

The Dumas combustion procedure has shown to give results for nitrogen content comparable to those obtained with the Kjeldahl procedure for soil and plant products, though slightly higher for most samples (Bremner, 1996; Simonne et al., 1997). It was concluded that the Dumas combustion procedure is capable of replacing the Kjeldahl procedure for routine animal nutrition laboratory nitrogen analyses (Etheridge et al., 1998). Both Kjeldahl and Dumas methods have equivalent variability when used for quantifying nitrogen content of soybean products (Jung et al., 2003).

Wiles et al. (1998), indicated (in 16 trials) that the Dumas method generally gave a slightly greater value, but there was no agreed relationship. However, there was not a difference in results between the two methods when applied to dairy products. Nitrogen values measured by Kjeldahl in one laboratory, for 20 Chilean fishmeals, correlated closely with the results by Dumas using one particular machine (LECO techniques) in a second laboratory, the latter giving a result of an average greater by 0.96% (Zaldivar, 1998).

Thompson et al. (2002) observed higher values for the Dumas method with both canned meat (0.014% N; relative increase 0.7%) and canned fish (0.045%; relative increase 2.0%).

TABLE 8
Comparison between Kjeldahl and other nitrogen determination methods

Sample	Methods	Reference
Tropical soils	NIRS (near-infrared reflectance spectroscopy) and Kjeldahl	Jarquín-Sánchez et al., 2011
Fish meal	Kjeldahl and Dumas methods	Kubota et al., 2011
Red wine, pinot noir	Dye binding (biconchonic acid assay) and micro Kjeldahl	Smith et al., 2011
Milk, cream	Sprint rapid protein analyzer (dye binding technique) and Kjeldahl methods	Amancharla and Metzger, 2010
Dairy products	Kjeldahl and Sprint protein analyzer	Zhao et al., 2010
Sewage sludge	NIRS and Kjeldahl methods	Galvez-Sola et al., 2009
Environmental samples	Inductively coupled plasma emission spectroscopy and Kjeldahl	Jaber et al., 2009
Human milk	NIR analysis and Kjeldahl method	Corvaglia et al., 2008
Fish protein (prepared surimi crabstick)	Lowry and Kjeldahl methods	Reed and Park, 2008
Rice	Kjeldahl and digital chroma methods	Sun et al., 2008
Feedstuffs	Combustion and Kjeldahl methods	Guo et al., 2007
Soy sauce	Combustion and Kjeldahl methods	Nozawa et al., 2007
Broiler litter	Kjeldahl and modification methods with a LECO-combustion (CHN-600)	Kpombrekou-A, 2006
Soils	Kjeldahl and PerkinElmer 2400 Series II-CHN Mode (dry combustion)	Pereira et al., 2006
Herbage samples from permanent meadows	Kjeldahl and NIRS methods	Valdes et al., 2006
Foods	Kjeldahl and Hach methods	Rossi et al., 2004
Concentrates, forages, excreta, and duodenal content	Kjeldahl procedure replaced by Dumas combustion	Sader et al., 2004
Soybean products	Kjeldahl and Dumas methods	Jung et al., 2003
Cow milk powder samples	Kjeldahl and spectrophotometric methods	Kamizake et al., 2003
Turbid waters	Alkaline peroxodisulfate digestion and Kjeldahl method	Maher et al., 2002
Animal feed	Kjeldahl and a combustion method	Marco et al., 2002
Cattle, pig, and turkey manure	Infrared techniques and Kjeldahl method	Kemsley et al., 2001
Wheat flour	Dumas and Kjeldahl methods	Sebecic and Balezovic, 2001
Agricultural samples	Dumas and Kjeldahl with automatic analyzers (under routine conditions)	Watson and Galliher, 2001
Pharmaceutical materials, foods, excrements	High-throughput analysis and Kjeldahl classic method	Yasuhara and Nokihara, 2001
Feedstuffs	Kjeldahl, Hach, and Dumas methods	Figenschou et al., 2000
Wild tropical vegetation	Kjeldahl and total ninhydrin protein	Conklin-Brittain et al., 1999
Feed, excreta, carcass, egg yolk, milk, urine	Kjeldahl and Dumas methods	Etheridge et al., 1998
Cheese and cheese fractions	Kjeldahl, spectrophotometric, and fluorimetric methods	Wallace and Fox, 1998
Cellulosic samples	LECO instruments and Kjeldahl method (catalyst not reported)	Sachen and Thiex, 1997
Foods	Dumas and Kjeldahl methods	Simonne et al., 1997

(Continued on next page)

TABLE 8
Comparison between Kjeldahl and other nitrogen determination methods (*Continued*)

Sample	Methods	Reference
Florida lakes	Persulfate following second derivative spectroscopy and Kjeldahl methods	Bachmann and Canfield, 1996
Swiss cheese proteolysis	Kjeldahl method and a spectrophotometric OPA assay	Rohm et al., 1996
Meat products	Dumas and Kjeldahl methods	Dautel et al., 1996
Liquid milk	Dumas instrument LECO FP-428 comparison	Jakob et al., 1995
Dairy products	LECO analyzers and Kjeldahl method(Cu catalyst)	King-Brink and Sebranek, 1993
Plant material	Dry combustion and Kjeldahl methods	Matejovic, 1995
Feces	NIR analysis and Kjeldahl method	Picarelli et al., 1995
Oilseeds	Combustion and Kjeldahl methods	Daun and deClercq, 1994
Leaves and petioles of shogoin turnips	Dumas and a modified micro Kjeldahl without salicylic acid.	Simonne et al., 1994
Plasma proteins	Automation-modified Dumas and Kjeldahl methods	Blondel and Vian, 1993
Soils low in nitrogen	Micro Kjeldahl and CHN combustion procedures	Wang et al., 1993
Fecal nitrogen	NIR analysis and Kjeldahl method	Benini et al., 1992
Urine	Chemiluminescence and Kjeldahl methods	Skogerboe et al., 1990
Samples of both animal and plant origin	LECO FP-228 N-determinator and Kjeldahl determination	Hansen, 1989
Urine	Pyrochemiluminescence and Kjeldahl methods	Konstantinides et al., 1988
Infant formulas and a variety of food commodities	Carlo Erba Dumas Analyzer and Kjeldahl (Cu catalyst) methods	Bellomonte et al., 1987
Plant tissue	Antek chemiluminescent system and Kjeldahl procedure	Jacques and Peterson, 1987
Feed materials (meals, grains, forages)	LECO-FP-228 nitrogen determination with copper catalyst Kjeldahl method	Sweeny and Rexroad, 1987
Retort waters and organonitrogen compounds	Chemiluminescence and Kjeldahl methods	Jones and Daughton, 1985
Nitrogen isotope ratios in organic matter	Kjeldahl and combustion methods	Minagawa et al., 1984
Skim milk powder (reference European milk protein standard)	Kjeldahl (Cu catalyst) method	Grieping et al., 1983
Fish meal	Kjeldahl and Lowry methods	Martone et al., 1980
Biological samples	Chemiluminescence and Kjeldahl methods	Ward et al., 1980
Wheat	Neutron activation analysis, proton activation analysis, thermal decomposition analysis, Kjeltec, and Kjel-Foss are compared.	Williams et al., 1978
Seawater	Semiautomated persulfate digestion method and simplified Kjeldahl method	Adamski, 1976
Feces	Dumas and Kjeldahl methods	Stitcher et al., 1969
Milk	Orange G dye and Kjeldahl methods	Dickinson et al., 1967
Milk	Orange G dye, formol titration, and Kjeldahl methods	Castillo et al., 1962
Milk	Orange G dye and Kjeldahl methods	Treece et al., 1959

Thompson et al. (2004) confirmed that the bias was small but varied with the product; it was significant for chicken test materials where the bias was $0.020 \pm 0.004\%$ N (relative increase of 0.70%) in an interlaboratory study of meat products involving 40

laboratories and 20 different test materials (sample preparations of pork, beef, chicken, and lamb).

The Dumas method gives values of N for fishmeals that are 1.1% greater and have better repeatability and reproducibility

TABLE 9
Collaborative/interlaboratory studies on Kjeldahl methods

Product	Analysis	Comments	Reference
Macaroni products	Crude protein	Combustion and Kjeldahl methods according to Japanese Agricultural Standard.	Hakoda et al., 2011
Meat	Protein	Rapid determination of protein in meat using the CEM Sprint™ Protein Analyzer.	Moser and Herman, 2011
Sheep and goat milk	Nitrogen	Reference method according to ISO 8968-1/2 IDF 20-1/2.	Orlandini et al., 2009a, 2009b; Polychroniadou, 2010; Hakoda et al., 2009
Macaroni products	Crude protein	Copper catalyst according to Japanese Agricultural Standard.	Hakoda et al., 2009
Fishmeal	Nitrogen content	Comparison between combustion (Dumas) and Kjeldahl methods: repeatability and reproducibility.	Miller et al., 2007
Soy sauce	Total nitrogen	Collaborative study.	Nozawa et al., 2005
Meat mixtures	Nitrogen	Kjeldahl and Dumas: testing for bias (interlaboratory study data).	Thompson et al., 2004
Animal feed, forage, grain, and oilseed	Crude protein	Block digestion with a copper catalyst and stem distillation into boric acid: collaborative study.	Thiex et al., 2002
Foods	Protein	Comparison between Kjeldahl and Dumas methods (data from a proficiency testing scheme).	Thompson et al., 2002
Hard, semihard, and processed cheese	Total nitrogen content	Collaborative study.	Lynch et al., 2002
Casein in milk	Nitrogen	Collaborative study.	Lynch et al., 1998
Dairy products	Protein	Kjeldahl and Dumas methods: interlaboratory study.	Wiles et al., 1998
Fish meals		Comparison between Kjeldahl and LECO techniques.	Zaldivar, 1998
Fishery samples/ standard substances	Nitrogen determination	Interlaboratory comparison based on "home methods."	Oehlenschläger, 1997
Skim milk powder	Total organic nitrogen	A dry reference value of 6.61%; a higher mean value for the Dumas method was found.	Wiles and Gray, 1996
Plant material		Dry combustion analyzer (CHN-600 LECO); interlaboratory proficiency test and results compared with the Kjeldahl method.	Matejovic, 1995
Oilseed meals	Protein nitrogen	Kjeldahl nitrogen and combustion results: collaborative study.	Berner and Brown, 1994
Barley, malt, and beer	Total nitrogen	Kjeldahl procedures and Dumas combustion.	Buckee, 1974
Meat, meat products		Mercury- and copper-based catalysts: collaborative study.	Price et al., 1994
Cereal grains, oilseeds		Kjeldahl and combustion method: collaborative study.	Bicsak, 1993

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TABLE 9
Collaborative/interlaboratory studies on Kjeldahl methods (*Continued*)

Product	Analysis	Comments	Reference
Meat and meat products		Used closely matched pairs; Kjeldahl assays performed with Hg catalyst. U.S. comparison.	King-Brink and Sebranek, 1993
Foods	Nitrogen	Mineralization by microwave digestion: interlaboratory study.	Suard et al., 1993
Milk	True protein content	Collaborative study.	Barbano et al., 1991
Soybean and oil	Protein	Interlaboratory study.	Hartwig and Hurburgh, 1991
Milk	Total nitrogen content	Collaborative study.	Barbano et al., 1990
Animal feedstuffs (seven samples in duplicate)		Kjeldahl analysis (Hg catalyst) and LECO.	Sweeney, 1989
Milk	Nitrogen content	Copper sulfate as catalyst: interlaboratory study.	Grappin and Horwitz, 1988
Cheese	Nitrogen	Copper sulfate as catalyst.	Florence and Harris, 1987
Animal feed		Comparison of HgO and CuSO ₄ /TiO ₂ as catalysts in manual digestion.	Kane, 1987
Animal feeds	Crude protein	HgO and CuSO ₄ as digestion catalyst: collaborative study.	Kane, 1984
Fishery samples/ standard substances	Nitrogen determination	Interlaboratory comparison.	Interlaboratory Study of Kjeldahl Methods, 1984
Fishery samples	Nitrogen determination	Comparative study.	Aitken and Smith, 1983a,b
Standard substances	Nitrogen determination	Comparative study.	Aitken and Smith, 1982
Wheat	Crude protein	Comparison of three methods.	Suhre et al., 1982
Fish meal	Crude protein	Collaborative study; Kjeldahl automated instrument.	Barlow et al., 1981
Fish meal		Kjeldahl automated analysis using an antimony-based catalyst.	Bjarno, 1980
Animal feeds, lysine monohydrochloride	Crude protein	Collaborative study/block digester.	Noel, 1979
Hard winter wheat			Miller et al., 1978
Animal feeds	Crude protein	Collaborative study; ammonia salicylate reaction; block digester.	Noel, 1976
Meat products	Nitrogen	Collaborative study; automated determination.	Gantenbein et al., 1974
Meat products	Nitrogen	Collaborative study.	Gantenbein, 1973
Animal feedstuffs (seven samples in duplicate)		Ten laboratories using Coleman 29A Nitrogen analyzers.	Ebeling, 1968

than the Kjeldahl method (Miller et al., 2007). The differences were mainly ascribed to incomplete recoveries of standards nitrogen (tryptophan, lysine-HCl) by the Kjeldahl method and not to the contribution from inorganic nitrogen, such as nitrate and nitrite. It is concluded that with fishmeal Dumas was the method of choice. However, the use of amino acids for recovery studies may not always be the best choice, as they are not always avail-

able in known purities and do not reflect the composition of the sample (Möller, 2011).

Fourteen laboratories participating in an interlaboratory study analyzed five test materials of macaroni product by Kjeldahl (Japanese Agricultural Standard) and combustion methods, with six different types of combustion instruments (Hakoda et al., 2011). Differences of means between both methods were

0.10–0.13% in the test materials; the differences were not significant.

The resolution of the Dumas-Kjeldahl bias question and their respective precision characteristics is a complex technical and statistical task. Nitrogen values obtained by using combustion have been reported to be higher than those from Kjeldahl analysis in some studies but not in others, although any bias is difficult to accurately quantitate because of the imprecision in the testing methodology (Lynch and Barbano, 1999; Wiles et al., 1998). Given sufficient observations, a statistical comparison between the two methods will almost inevitably reveal a significant bias. However, from a commercial perspective, such differences might be quite immaterial, though not always; an example of an Argentine/Malaysian conflict is given in the next section. The range of samples analyzed has been considerable, and, in most cases, the Dumas technique gives generally higher total nitrogen values than does the Kjeldahl method. However, the differences reported vary markedly, and little consensus has merged on an agreed relationship between the methods.

The Dumas, or nitrogen combustion, method is an attractive alternative to the widely used Kjeldahl assay (Beljkas et al., 2010). The Dumas combustion method is relatively faster, cheaper, easier to perform, safer, and more environmental friendly. It is now considered on equal terms with Kjeldahl analysis in the United States, Canada, and Western Europe (ISO, 2002, 2008, 2009b; Owusu-Apenten, 2002).

The dominance of the traditional Kjeldahl method for nitrogen measurements has recently come under threat by the challenge of safer, cleaner instruments employing the Dumas combustion principle (Williams et al., 1998). The Dumas method is replacing in some contexts the traditional Kjeldahl method as the method of choice for nitrogen analysis. However, the Kjeldahl method is used to standardize other methods, despite its drawbacks, and although the Dumas method is fast and does not use toxic chemicals or catalysts, its instrumental and operational costs are high. Besides being economical, the Kjeldahl method is easily performed on moist or liquid samples (Stevens et al., 2000). In addition, the sample size when applying the Kjeldahl method can range much more widely than with the combustion method, and, if necessary, can be increased to reduce sampling error. On the other hand, the Kjeldahl method with catalyst (Sahrawat et al., 2002) or without catalyst (Özer and Güçer, 2011) lends itself to the simultaneous analysis of many elements in a single digest. Digestion may be integrated in a total analytical system based in spectrophotometric analysis (Brayton, 1992).

Several instruments are available for the automated determination of total C, N, and S in plants and soils. These instruments provide rapid analysis of a large number of samples but are expensive and require skilled operators. In recent years, these combustion systems have also been coupled to isotope-ratio mass spectrometers so that both N and ^{15}N can be determined in a single sample (Ogawa et al., 2001; Unkovich et al., 2008). Often ^{15}N nitrogen is also measured starting from the Kjeldahl

distillates in the form of ammonium (Minagawa et al., 1984; Pansu and Gautheyrou, 2006). Diffusion techniques are usually preferred to steam distillation because they require less skilled operation and less specialized equipment, and there is also a lower risk of cross contamination (Chen and Difter, 2008). Thus, diffusion techniques have been described as a convenient and economical alternative to steam distillation for total N and ^{15}N analysis of Kjeldahl digests (Chen and Difter, 2008; Liu and Mulvaney, 1992; Stark and Hart, 1996; Stevens et al., 2000). However, this topic is beyond the scope of the present review.

QUALITY CONTROL: DON'T OVERLOOK THE OBVIOUS

A concern about the quality of analytical results has always been a constant leitmotiv through the years (Ortner, 2000). An up-to-date review of analytical methods is incomplete without the mention of quality control (Fleck, 1970). The final analytical result is always dependent on the quality of the total analytical process (Houba et al., 1996). It has been said that the utility of a method is great only when its limitations are clearly determined (Dakin and Dudley, 1914). Time is always a factor that should be taken into account in commercial analysis. Experiments are forever being carried out to find shortcuts without sacrificing accuracy (Lauro, 1931; Neill, 1962). The Kjeldahl method, like any other, is of real value only when performed under carefully standardized conditions that must be determined first if dependable results are to be obtained (Ballentine and Gregg, 1947; Möller, 2009). These conditions necessarily vary with the apparatus and reagents used, and with the material to be analyzed. Several steps, from sampling in the field to the final analysis report, may have influence on the quality: sampling procedure, physical pretreatment (i.e., drying, sieving, milling, or grinding), digestion procedures, chemical analysis, and calculations (Swyngedouw and Lessard, 2008; Wickstrom et al., 2004). Today, most laboratories implement quality assurance/quality control procedures to improve both laboratory efficiency and the quality of the data they generate (O'Dell, 1993; Thiex et al., 2002). However, too little attention has been paid to quality assurance/quality control (QA/QC) in sample preparation (Sneddon et al., 2006).

A number of factors can introduce errors into Kjeldahl measurements of the material to be analyzed (Moore et al., 2010), in part due to the various manipulations in the whole analytical process, i.e., the accuracy of sample weighing, purity of the reagents used, contaminated reagent or glassware, incorrect reagent blank compensation, lack of nitrogen recovery during the digestion (significance of errors in time and temperature), and concentration, volume, and temperature of the base and/or acid involved in the titration, (Barbano and Lynch, 1990; Chen et al., 1988; Lynch and Barbano, 1999; Möller, 2010). There are more than 20 factors that affect the recovery of nitrogen by the Kjeldahl process and its conversion to ammonia (Williams et al., 1998). In distillation the addition of steam, the steam effect, addition of sodium hydroxide, transport and capture of generated ammonia, design of the splash head, and temperature of

condensate and receiver solution all have a role to play (Möller, 2011). Ammonia is a pervasive contaminant; exposure of samples to air must be minimized given the propensity of acidic digests to trap and become contaminated by ammonia vapors in ambient laboratory air (U.S. EPA, 2001). Procedures used to minimize contamination during collection, subsampling, and analysis are thus important (Campbell and Plank, 1992; Holcombe et al., 1986).

Reasonable characterization of method performance requires special attention to each individual product's properties and a thorough investigation of appropriate sampling, sample handling, and sample preparation. Regular maintenance and cleaning of the Kjeldahl system is important (Holcombe et al., 1986). As the glass of the Kjeldahl flasks is seriously affected, their quality deteriorates rapidly (Pansu and Gautheyrou, 2006), and they need to be regularly replaced. Performance statistics of analytical methods are determined by conducting formal collaborative studies, which requires time, money, interest, and the availability of willing and competent participants (Hund et al., 2000; Lynch and Barbano, 1999). Sample preparation (16%), equipment failures (13%), human error (13%), and calibration (10%) were the top four general causes of poor analytical results in proficiency testing schemes as derived from the results of a recent survey (Ellison and Hardcastle, 2012). It is important that officially approved methods are updated so that available technology can be used in the laboratory (Thiex et al., 2002). Distillation and titration should be the reference method in any case because they avoid the matrix problem that may occur in other determination methods.

Many attempts have been made to optimize the procedure and to standardize methodology. The important variability of food matrices makes it difficult to define a common procedure applicable to all foods (Zorgati et al., 2000). Although it is possible to define large groups of matrices presenting similar behavior, exceptions are abundant. Although the Kjeldahl method has been standardized and published for a number of matrices, many of the AOAC Official Methods were published many years ago before inter- and intra-laboratory relative standard deviation calculations were routinely reported following method validation (Moore et al., 2010).

Since all nitrogen in the samples after digestion forms ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$, it can be used as standard to check the recovery of the distillation unit. Four procedures may be mainly used to verify the accuracy of the digestion process (Gonzalez et al., 1999):

1. Digestion of a standard substance with known nitrogen content, e.g., glycine, acetanilide, or nicotinic acid (APHA, 1998, 2012), to test completeness of digestion and evaluate nitrogen recovery (Chen et al., 1988; Lynch and Barbano, 1999; Ribadeau-Dumas and Grappin, 1989; Telleston, 1980) and with ammonium chloride to test for loss of nitrogen.
2. Digestion of certified reference samples; the use of reference materials with certified nitrogen content presents another ap-

proach to improve accuracy (Ribadeau-Dumas and Grappin, 1989; Staniszewska et al., 2008; Wiles and Gray, 1996).

3. Participation in proficiency testing schemes (Miller et al., 2007; Wiles et al., 1998).
4. Preparation of a reference sample within the laboratory (Swyngedoum and Lessard, 2008), verifying its nitrogen content versus other laboratories, and using this sample as internal reference in daily routine usage.

It is necessary to take into account (Hund et al., 2000; Thompson et al., 2002) that:

- The comparison should be carried out at various concentrations of the analyte, because a bias between the results of the methods might vary with concentration.
- The comparison needs to be made with a set of test materials, the matrices of which span the scope of the study, in case the bias depends on matrix type.
- Results need to be replicated many times in order to improve the precision of the mean results; significance tests should be carried out if they are needed.
- The comparison requires an interlaboratory study if the effects of laboratory bias are to be avoided. This factor is of the most importance, although not recognized enough.

In the absence of sufficient certified reference samples to cover the wide variety of food matrices, validation based on synthetic food samples is needed to define the digestion procedure (Zorgati et al., 2000). Nevertheless, speciation of the components used in the blend is really different from their actual chemical forms. This means that the chemical interactions that occur in real samples may be more complex than those obtained by a simple blending. Different standards were employed for each class of sample analyzed. Ideally, ammonium sulfate is the standard choice since all organic nitrogen is converted to this form. However, since some compounds are more readily digested than others, a standard having the same refractory properties as the unknowns is employed. Standards that have been employed include β -alanine for meat samples and 2-benzyl-2-pseudo-thiourea for milk samples (Kramme et al., 1973). Jacobs (1968) used a mixture of nicotinamide and urine as the standard in the analysis of urine, feces, and food. In other work, glycine was the standard of choice for urine and feces samples (Cox and Harmon, 1967). In many of these adaptations, the digestive system of the procedure was optimized for the specific sample class of interest. However, a difficulty arises when samples of unknown composition or mixtures of sample classes must be analyzed. In these instances, the digestive conditions and standards must be consistent. Tryptophan and lysine-HCl have been recommended as standards to check the recovery of N and adjust the use of catalyst, digestion temperature, and time to achieve at least 98% recovery (Miller et al., 2007). Arginine is taken as a model system for the comparative kinetic study between microwave and classical digestion; this amino acid is present

in a large variety of biological samples. It is also reputed to be difficult to digest because of the high nitrogen concentration of 32.18 g per 100 g (Chemat et al., 1998).

Three primary standard compounds of varying digestibility, ammonium p-toluensulfonic acid (PTSA), glycine-PTSA, and nicotinic acid-PTSA, were found to be sufficient to prove the accuracy of the overall Kjeldahl determination (Brayton, 1992). These p-toluensulfonate 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) could be used as a primary standard, but the nitrogen levels are too high to fit the working range of 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 encountered. Glycine-PTSA (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 spatulas, yet they are water soluble and stable indefinitely (Baker and Thompson, 1992; Brayton, 1992). An ammonium phosphate monobasic analytical standard for nitrogen determination according to the Kjeldahl method has been recently proposed (Bufer and Amann, 2012); its nitrogen content is traceable to NIST Standard Reference Materials (SRM).

Little work has been published on the development of comprehensive systems that could be used for all classes of samples (Kramme, 1973; Zorgati et al., 2000). Therefore, it is interesting to develop standardized procedures that could be employed without modification, regardless of the composition of the samples received for analysis by the research services laboratory.

Balances used for sample weighing should be calibrated at regular intervals. The equipment purchased must be used according to specifications. One way is to demand from the manufacturer that approved quality routines are used in the production and development of the equipment. It has been reported that on the basis of the uncertainty contributions, the most promising target for improvement is titration (Anglow et al., 1999), e.g., by improving the temperature control during titration or by improving the accuracy of the volume of titrant. The latter goal may be achieved by using more accurate titration equipment, or, at least partly, by increasing the number of replicate determinations.

The Kjeldahl method has been validated and standardized for total (crude) protein estimation for a wide variety of food matrices and has been adopted by AOAC International, AACC International, ISO, and AOAC (Moore et al., 2010). As protein determinations are often performed for trading purposes, it is important that both parties involved use similar analytical pro-

cedures as the basis for evaluation of results obtained from their test (Persson et al., 2008). The Argentine supplier of soymeal claims a protein content of 47.2% determined on the basis of the Dumas method, whereas the Malaysian importer of soymeal claims a protein content of 44.9%, determined on the basis of the Kjeldahl method; this may result in a conflict (Möller, 2009). This is also the reason the European Commission has recently confirmed the Kjeldahl method as its method for official control (Commission Regulation (EC) No. 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feeds).

Both economical and practical reasons are key factors that govern the choice of a method for crude protein determination. The global standard EN ISO 5983-2/AOAC 2001.11 has the advantage of broader applicability, shorter analysis times, and lower reagent costs versus the EN ISO 20483 method and a better status as reference method versus the Dumas method (Möller, 2009). It allows accreditation with full traceability (Möller, 2005) and easy estimation of the method uncertainty (Cheng et al., 2008) for a wide range of samples. The accuracy of the Kjeldahl method also depends directly on the accuracy of the nitrogen-to-protein factor applied, which is a subject of considerable debate. For example, mushrooms have high and variable nonprotein nitrogen content from chitin and chitosan compounds. It has been shown that use of Kjeldahl analysis with the 6.25 conversion factor overestimates the mushroom protein content by as much as 23% compared to amino acid analysis (Weaver et al., 1977).

Continuous monitoring of the processes is important; speed is essential, and, if possible, continuous recorded analysis is the ideal for samples (Anderson and Moller, 1995; Murphy, 1956). "Under these circumstances, chemical wet methods seem to be slow and cumbersome. In our enthusiasm for modernity, let us not forget that the chemical procedures have a very important role to play in their own right. When they are combined with the physical approach, the sensitivity, specificity, and speed are greater than when each method is left to its own peculiar limitations" (Murphy, 1956). The work that goes into the preparation of standards for such instrumental methods is considerable and is not justified for running a few samples. The basic methods used for standardization are, however, mostly chemical procedures, and they provide a check by an independent method, which is the only way to get data on the validity of any method (Möller, 2010). The question then, is not how to replace them (because that will not be an easy task), but how to use them more effectively in, e.g., our fight against fraud (Möller, 2010).

CONCLUDING REMARKS

Accurate food compositional analysis has been a key factor in helping the food industry to standardize both processes and its products, e.g., proteins. The determination of nitrogen in foods is critical for daily quality control of production and for specifications in contracts (Miller et al., 2007); many foods are traded on their protein content. Nitrogen is one of the most

important elements for plant nutrition. Results from the analysis of soil samples are important for decision-making in agricultural and environmental management (Wickstrom et al., 2004). It is therefore important that the quality of the data is good. Nitrogen determination is the most commonly used procedure for a protein assay and it has been the basis for various official and conventional methods currently in use for expressing total protein concentration of organic matters (Rhee, 2001). The Kjeldahl method is the reference method of nitrogen determination. In principle, organic nitrogen, but not nitrate or nitrite, is reduced to ammonium during Kjeldahl digestion. Therefore, ammonium determination according to the Kjeldahl method provides the content of organic nitrogen + ammonia (Patton and Kryskalla, 2003).

The areas of application of the Kjeldahl method involve analyses a wide range of samples, e.g., foods and samples of environmental or biological origin, raw materials, ingredients, soils, plants, cereals, oilseeds, and fertilizer products (Sáez-Plaza et al., in press; Saha et al., 2012). The measurement of various nitrogen species is of increasing importance as our knowledge about the impact of various forms of nitrogen on living organisms grows (Prusisz et al., 2007). Monitoring of the total nitrogen in soil is of particular interest for those concerned with plant growth (Mason et al., 1999). Nitrogen is considered to be one of major pollutants in wastewater. Total Kjeldahl nitrogen is a parameter often used to characterize nitrogen pollution potential (Lo et al., 2005). Ammonia is a significant parameter in most environmental studies. Total dissolved nitrogen (TDN) includes all nitrogen-containing species (organic and inorganic) dissolved in the sample. Dissolved inorganic nitrogen (DIN) includes nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) species dissolved in the sample. Concentrations of dissolved organic nitrogen (DON) are calculated as the difference between independent measurements of TDN and DIN.

Sample preparation demands skillful and well-trained staff; it's the major bottleneck in laboratory automation and an important part of the total analytical cost (Feinberg et al., 1994). Sample size is crucial when the nonhomogeneity of the sample increases due to the nature of the material and/or the fineness of grind (Bicsak, 1993). The size of the ground sample analyzed must represent the true sample as a whole. Quality control in both the field and laboratory is essential for accurate and precise nitrogen measurements. Sample collection, processing, subsampling, storage, atmospheric contaminants, and each analytical step are potential contributors to this problem (Holcombe et al., 1986). Moreover, the test should be carried out at different concentrations of the analyte, in the case when the bias is concentration dependent (Thompson et al., 2004). In addition, a variety of appropriate test materials should be studied in case the bias is matrix dependent. Quality is assured through calibration and testing of the analytical instruments and testing of the sample preparation (U.S. EPA, 2001).

Both macro and micro Kjeldahl methods have been developed for different sample sizes. One has to decide if the method

is being described as a macro method, with digestion flasks with containing 250, 500, or more mL, on a semimicro scale, or on a microscale with digestion flasks containing 30–50 mL (Janßen, 2005). The important variability of food matrices makes it difficult to define a common procedure applicable to all foods (Zoragati et al., 2000). The Kjel-Foss instrument does not basically alter the Kjeldahl analytical method of chemistry; however, it differs greatly in the speed at which an analytical method can be completed (Wall et al., 1975). The micro Kjeldahl method is qualified for homogeneous samples with high content of nitrogen and protein (Buchi, 2010). The analysis is carried out with a reduced amount of chemicals, which leads to shorter digestion and distillation times. Microscale chemistry is performed by using markedly reduced amounts of chemicals, safe and easy techniques, miniature labware, and high-quality skills (Campins-Falco et al., 2008; Skinner, 1998).

In this review, the main accent is put on the final step of nitrogen determination, involving other than classical Kjeldahl procedures. The analysis of extracted nitrogen is traditionally performed using a lengthy distillation/titration procedure; extracted nitrogen from the Kjeldahl procedure can be determined by several other methods. 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 (Meseguer-Lloret et al., 2005). The distillation methods are simple and accurate, not being affected by various organic and inorganic substances that often interfere with the colorimetric methods of determining inorganic nitrogen (Bremner, 1965a, 1965b; Bremner and Keeney, 1965; Keeney and Nelson, 1982). Gas diffusion is an approved alternative to manual distillation of ammonia samples prior to an instrumental finish (U.S. EPA, 2012). The ammonia electrode has found application in the determination of ammonium in the Kjeldahl digest of plants, soils, and various other samples. The ammonia electrode employs a hydrophobic gas-permeable membrane to separate the sample solution from an internal ammonium chloride solution; ammonia diffusing through the membrane changes the pH of the internal solution and is sensed by a pH electrode (U.S. EPA, 1983).

The minimum amount of nitrogen to be quantified is significantly improved when ammonia is directly determined in the digest by color-inducing compounds such as indophenol, Nessler reagent, or ninhydrin. Many laboratories utilize automated spectrophotometry to handle the large volume of analyses required (Crowther and Evans, 1980). The use of automated colorimetric analysis by the Technicon AutoAnalyzer 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.

Historically, Nesslerization has been the usual colorimetric method of measuring total Kjeldahl nitrogen. Nesslerization

requires precise timing and generates waste-containing mercury compounds that are detrimental to the environment and costly to dispose of properly (Willis et al., 1996). The Berthelot (indophenol) reaction is highly specific for ammonia and in the presence of a suitable catalyst, extremely high sensitivity is attained (Patton and Crouch, 1977). A variety of catalysts have also been explored as a means of increasing reaction kinetics. The most prevalently used catalyst is sodium nitroferricyanide(II) dehydrate (nitroprusside). Unfortunately, catalyzed Berthelot procedures are notoriously erratic in their response to ammonia. Blanks are typically high and unstable, and precision in the micromolar concentration is seldom better than 10%.

The spectrophotometric assay of Kjeldahl nitrogen using the ninhydrin reaction is sensitive and reliable. It can be utilized in place of the traditional Kjeldahl method for the determination of the protein content of beer or wine (Abernathy et al., 2009). Unfortunately, the color development by ammonia is influenced by various components used during the digestion step (e.g., catalysts). Ammonia reacts with acetylacetone-formaldehyde reagent in aqueous medium to yield a yellow complex having absorption maximum at 412 nm. The method is suitable for determination of nitrogen in acidic medium without interference from the usual catalysts employed for digestion in the Kjeldahl method (Devani et al., 1989). Laboratories whose volume of Kjeldahl nitrogen analysis does not justify purchase and maintenance of automated equipment may extract benefit from a rapid manual spectrophotometric method (Fukumoto et al., 1982).

The flow injection technique has been applied to automate the distillation/determination step of the Kjeldahl method (Lima et al., 1999; Pasquini and de Faria, 1987; Rohwedder and Pasquini, 1991; Su et al., 1997; Tryzell and Karlberg, 1997). The FIA method reduces analysis time, chemicals, and laboratory space and facilitates the determination of a large number of samples with the use of simple equipment. FIA methods applying standard colorimetric methods and FIA methods based on gas diffusion have been proposed for the determination of ammonium in Kjeldahl samples.

The use of ion chromatography to determine total nitrogen as ammonium ion after sample digestion improves the speed of the analysis compared with the conventional Kjeldahl method by eliminating the need for the distillation step. As only very small volumes of samples are required for analysis, the mass of a sample required for the digestion can be reduced, minimizing the amount of catalyst required, hence also reducing disposal costs. However, ion chromatography is expensive, and the instrument requires frequent maintenance to function properly. The analysis time is as high as 15 minutes for one sample, although other ionic constituents can be analyzed simultaneously, which is very advantageous (Ferree and Shannon, 2001).

Chemiluminescent procedures have also been developed for ammonium determination due to their high sensitivity, rapidity, simplicity, and feasibility.

Increased interest in quality and accreditation of laboratory systems has contributed to increased knowledge about uncertainty in digestion procedures and chemical analysis (Wickstrom et al., 2004). To verify that the digestion procedure is sufficient to digest organic nitrogen in the samples, primary nitrogen standards should be digested with each set of samples. Primary standards should be of known nitrogen content, high purity, and non-hygroscopic for easy storage (Baker and Thompson, 1992; Brayton, 1992; Holcombe et al., 1986). It is necessary to control all steps in the analytical chain (Houba et al., 1996). Under favorable circumstances, the results of proficiency tests, over a period of time, provide what is needed for method comparison: a variety of test materials, a range of analyte concentrations and matrices, and results from many laboratories. When two or more well-defined methods have each been used by a substantial number of participants, a comparison is possible (Lowthian et al., 1996).

As the industry changed from small shops to large corporations, the need for rapid methods became much greater because of the large-volume, high-speed production facilities that became necessary; consequently, industry has utilized rapid methods of analysis for many years. However, many of the rapid methods used in the past have not been comparable to "official methods" for accuracy and precision. Analysis by both rapid and time-consuming laborious "official methods" was often necessary (Sebranek, 1999, p. 161).

Foss product manager Camila Alvesson said: "The Kjeldahl method is still the primary reference method for protein analysis today and our aim is to bring it up to date with the latest possibilities, for example, with the use of network technology now so commonly used in our daily lives" (FOSS, 2009). Continuity in process monitoring is now a fundamental requirement of the modern food industry and has become increasingly important due to developing interest in computer-based automation and manufacturing (O'Sullivan et al., 1999).

Commercial companies are mentioned in this review solely for the purpose of providing specific information. The use of trade, product, or firm names in this review is for descriptive purposes only, and does not imply endorsement by the University of Seville or by the Technical University of Cracow.

REFERENCES

- Abernathy, D. G.; Spedding, G.; Starcher, B. Analysis of Protein and Total Usable Nitrogen in Beer and Wine using a Microwell Ninhydrin Assay. *J. Inst. Brew.* **2009**, *115* (2), 121–127.
- Adams, G. O.; Kimball, A. W. Studies of Direct Nesslerization of Kjeldahl Digestates in Sewage Analysis. *J. Am. Chem. Soc.* **1908**, *30* (6), 1034–1037.
- Adamski, J. M. Simplified Kjeldahl Nitrogen Determination for Sewater by a Semiautomated Persulfate Digestion Method. *Anal. Chem.* **1976**, *48* (8), 1194–1197.
- Aitken, A.; Smith, G. L. A Comparative Study of the Kjeldahl Method Applied to Fish Products, Part 1. Presented at the WEFTA Analytical Working Group Meeting, Ijmuiden, 1982; 8 pp.

- Aitken, A.; Smith, G. L. A Comparative Study of the Kjeldahl Method Applied to Fish Products, Part 2. Presented at the WEFTA Analytical Working Group Meeting, Hamburg, 1983a; 8 pp.
- Aitken, A.; Smith, G. L. A Comparative Study of the Kjeldahl Method Applied to Fish Products, Part 3. Presented at the WEFTA Analytical Working Group Meeting, Hamburg, 1983b; 10 pp.
- Amancharla, J. K.; Metzger, L. E. Evaluation of a Rapid Protein Analyzer for Determination of Protein in Milk and Cream. *J. Dairy Sci.* **2010**, *93* (8), 3846–3857.
- American Association of Cereal Chemists. *Approved Methods of Analysis*; American Association of Cereal Chemists: St. Paul, MN, 1995.
- Amin, M.; Flowers, T. H. Evaluation of Kjeldahl Digestion Method. *J. Res. (Science)* **2004**, *15* (2), 159–179.
- Aminot, A.; Kirkwood, D. S.; Kérousel, R. Determination of Ammonia in Seawater by the Indophenol-Blue Method. Evaluation of the ICES NUTS I/C Questionnaire. *Mar. Chem.* **1997**, *55* (1–2), 59–75.
- Anderson, S. Determination of Fat, Moisture, and Protein in Meat and Meat Products by Using the Foss Food Scan Near Infrared Spectrophotometer with Foss Artificial Neural Network Calibration Model and Associated Database: Collaborative Study. *J. AOAC Int.* **2007**, *90* (4), 1073–1083.
- Anderson, K. A.; Moller, G. Alternative Catalyst to Mercury for Kjeldahl Determination of Nitrogen in Water and Wastewater Samples. *J. AOAC Int.* **1995**, *78* (6), 1516–1519.
- Aneja, V. P.; Bunton, B.; Walker, J. T.; Malik, B. P. Measurement and Analysis of Atmospheric Emissions from Anaerobic Lagoons. *Atmos. Environ.* **2001**, *35* (11), 1949–1958.
- Anglow, O. T.; Petersen, I. M.; Kristiansen, J. Uncertainty of Nitrogen Determination by the Kjeldahl Method. *Accredit. Qual. Assur.* **1999**, *4* (12), 504–510.
- AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed.; AOAC: Arlington, VA, 1984; Sec. 33.056.
- AOAC. *Official Methods of Analysis, Method 2001.11: Determination of Crude Protein in Animal Feed, Forage (Plant Tissues), Grain, and Oil Seeds Using Block Digestion with a Copper Catalyst and Steam Distillation into Boric Acid*; AOAC International: Gaithersburg, MD, 2001.
- AOAC. *Official Methods of Analysis of AOAC International*, 18th ed. (Revision 4); AOAC: Gaithersburg, MD, 2011.
- APHA. *Standard Methods for the Examination of Water and Waste Waters*, 20th ed.; Clesceri, L. S.; Greenberg, A. E.; Eaton, A. D., Eds.; American Public Health Association: Washington, DC, 1998.
- APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; Eaton, A.; Clescery, L. S.; Greenberg, A. E., Eds.; American Public Health Association: Washington, DC., 2005.
- APHA. *Standard Methods for the Examination of Water and Waste Waters*, 22th ed.; Rice, E. W.; Baird, R. B.; Eaton, A. D.; Clesceri, L. S., Eds.; American Public Health Association: Washington, DC, 2012.
- ASTM International. *Standard Specification for Apparatus for Microdetermination of Nitrogen by Kjeldahl Method. Designation E-147-61*; American Society for Testing and Materials: West Conshohocken, PA, Reapproved 2001.
- ASTM International. *Standard Test Methods for Total Kjeldahl Nitrogen in Water. Designation: D3590-02*; American Society for Testing and Materials: West Conshohocken, PA, 2002.
- Asuero, A. G.; Gonzalez, G.; de Pablos, F.; Ariza, J. L. G. Determination of the Optimum Working Range in Spectrophotometric Procedures. *Talanta* **1988**, *35* (7), 531–537.
- Bachmann, R. W.; Canfield Jr., D. E. Use of an Alternative Method for Monitoring Total Nitrogen Concentration in Florida Lakes. *Hydrobiologia* **1996**, *323*, 1–8.
- Badr, E.-S.; Achterberg, E. P.; Tappin, A. D.; Hill, S. J.; Braungardt, C. B. Determination of Dissolved Organic Nitrogen in Natural Waters Using High Temperature Catalytic Oxidation. *Trends Anal. Chem.* **2003**, *22* (1) 819–827.
- Baethgen, W. E.; Alley, M. M. A Manual Colorimetric Procedure for Measuring Ammonium Nitrogen in Soil and Plant Kjeldahl Digests. *Commun. Soil Sci. Plant Anal.* **1989**, *20* (9–10), 961–969.
- Baker, W. H.; Thompson, T. L. Determination of Total Nitrogen in Plant Samples by Kjeldahl. In *Plant Analysis Reference for the Southern Region of the United States*, Southern Cooperative Series, Bulletin #368; The University of Georgia: Athens, GA, May 1992.
- Ballentine, R.; Gregg, J. R. Micro-Kjeldahl Determination of Nitrogen. *Anal. Chem.* **1947**, *19* (4), 281–283.
- Barbano, D. M.; Lynch, J. M. Kjeldahl Method for Determination of Total Nitrogen Content of Milk: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1990**, *73* (6), 849–59.
- Barbano, D. M.; Lynch, J. M. Major Advances in Testing of Dairy Products: Milk Components and Dairy Product Attribute Testing. *J. Dairy Sci.* **2006**, *89* (4), 1189–1194.
- Barbano, D. M.; Clark, J. L.; Dunham, C. E.; Fleming, J. R. Kjeldahl Method for Determination of Total Nitrogen-Content of Milk—Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1990**, *73* (6), 849–859.
- Barbano, D. M.; Lynch, J. M.; Fleming, J. R. Direct and Indirect Determination of True Protein-Content of Milk by Kjeldahl Analysis—Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1991**, *74* (2), 281–288.
- Barbarino, E.; Lourenço, S. O. Comparison of CHN Analysis and Hach Digestion to Quantify Total Nitrogen in Marine Organisms. *Limnol. Oceanogr. Methods* **2009**, *7*, 751–760.
- Barlow, S. M.; Bimbo, A.; Jensen, O. B.; Smith, G. L. International Collaborative Study of an Automated Method for the Determination of Crude Protein in Fish-Meals. *J. Sci. Food Agric.* **1981**, *32* (7), 732–736.
- Beljkaš, B.; Matic, A. J.; Milovanović, I.; Jovanov, P.; Mišan, A.; Šaric, L. Rapid Method for Determination of Protein Content in Cereals and Oilseeds: Validation, Measurement Uncertainty and Comparison with the Kjeldahl Method. *Accredit. Qual. Assur.* **2010**, *15* (10), 555–561.
- Bellomonte, G.; Costantini, A.; Giammarioli, S. Comparison of Modified Automatic Dumas Method and the Traditional Kjeldahl Method for Nitrogen Determination in Infant Food. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (2), 227–229.
- Benini, L.; Caliri, S.; Bonfante, F.; Guidi, G. C.; Brentegani, M. T.; Castellani, G.; Sembenini, C.; Bardelli, E.; Vantini, I. Near Infrared Reflectance Measurement of Nitrogen Faecal Losses. *Gut* **1992**, *33* (6), 759–762.
- Berner, D. L.; Brown, J. Protein Nitrogen Combustion Method Collaborative Study I. Comparison with Smalley Total Kjeldahl Nitrogen and Combustion Results. *J. Am. Oil Chem. Soc.* **1994**, *71* (11), 1291–1293.
- Berthelot, M. P. E. Violet d'Aniline. *Repert. Chim. Appl.* **1859**, *1*, 284.

- Bicsak, R. C. Comparison of Kjeldahl Method for Determination of Crude Protein in Cereal Grains and Oil Seeds with Generic Combustion Method: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1993**, *76* (4), 780–786.
- Bietz, J. A. Micro-Kjeldahl Analysis by an Improved Automated Ammonia Determination Following Manual Digestion. *Anal. Chem.* **1974**, *46* (11), 1617–1618.
- Bilbao, B.; Giraldo, D.; Hevia, P. Quantitative Determination of Nitrogen Content in Plant Tissue by a Colorimetric Method. *Commun. Soil Sci. Plant Anal.* **1999**, *30* (13–14), 1997–2005.
- Bjarno, O. C. Kjeld-Foss Automatic Analysis Using an Antimony-Based Catalyst: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1980**, *63* (3), 657–663.
- Blondel, P.; Vian, L. Dosage des Protéines par la Méthode de Dumas Modifiée Appliquée aux Préparations de Protéines Plasmatiques. *Ann. Pharm. Fr.* **1993**, *51* (6), 292–298.
- Botaro, B. G.; Cortinhas, C. S.; Mestieri, L.; Machado, P. F.; dos Santos, M. V. Prediction of Bovine Milk True Protein Content by Mid-Infrared Spectroscopy. *Cienc. Rural, Santa Maria* **2011**, *41* (8), 1472–1474.
- Bradstreet, R. B. *The Kjeldahl Method for Organic Nitrogen*; Academic Press: London, 1965.
- Brayton, S. V. *Acid Digestions Using the Hach Digesdahl[®] Digestion Apparatus: Sample Preparation for Protein and Elemental Analysis*; Technical Information Series—Booklet No. 14; Hach Company: Loveland, CO, 1992.
- Bremner, J. M. Inorganic Forms of Nitrogen. In *Methods of Soil Analysis*; Black, C. A., Ed.; American Society of Agronomy: Madison, WI, 1965a; Vol. 2, pp 1179–1237.
- Bremner, J. M. Total Nitrogen. In *Methods of Soil Analysis*; Black, C. A., Ed.; American Society of Agronomy: Madison, WI, 1965b; Vol. 2, pp 1149–1178.
- Bremner, J. M. Nitrogen-Total. In *Methods of Soil Analysis. Part 3, Chemical Methods*; Sparks, D. L., Ed.; Soil Science Society of America, American Society of Agronomy: Madison, WI, 1996; pp 1085–1121.
- Bremner, J. M.; Keeney, D. R. Steam Distillation Methods for the Determination of Ammonium, Nitrate and Nitrite. *Anal. Chim. Acta* **1965**, *32*, 485–495.
- Bremner, J. M.; Mulvaney, C. S. Nitrogen-Total. In *Methods of Soil Analysis. Part 2, Chemical and Microbiological and Properties*, 2nd ed.; Page, A. L.; Miller, R. H.; Kenney, D. R., Eds.; American Society of Agronomy, Soil Science Society of America: Madison, WI, 1982; pp 595–624.
- Bremner, J. M.; Tabatabai, M. A. Use of Ammonia Electrode for Determination of Ammonium in Kjeldahl Analysis of Soils. *Commun. Soil Sci. Plant Anal.* **1972**, *3* (2), 159–162.
- Büchi. *Nitrogen and Protein Determination in Milk According to the Micro-Kjeldahl Method*; Büchi Labortechnik AG: Flawill, Switzerland; Short Note, 2010.
- Büchi. *Nitrogen and Protein Determination in Dairy Products with Kjeldahl Tablets and Hydrogen Peroxide*; Büchi Labortechnik AG: Flawill, Switzerland; Short Note 079/ 2012a.
- Büchi. *Nitrogen and Protein Determination in Meat Products with Kjeldahl Tablets and Hydrogen Peroxide*; Büchi Labortechnik AG: Flawill, Switzerland; Short Note 080/ 2012b.
- Buckee, G. K. Estimation of Nitrogen with an Ammonia Probe. *J. Inst. Brew.* **1974**, *80* (3), 291–294.
- Butler, M.; Amann, N. An Overview of the Kjeldahl Method Including State-of-the Art Equipment and a New Traceable Standard for IR and OQ. *Analytix*, **2012**, *8*, Article 3.
- Burck, H. C. Kolorimetrische Micro-Kjeldahl-Methode mit direkter Neßlerisation zur routinemäßigen Stickstoffbestimmung (Nitro- und Nitrosogruppen ausgenommen). *Mikrochim. Acta* **1960** (2), 200–203.
- Cai; J. B.; Wang, S.-F. Simultaneous Determination of Total Nitrogen and Metal Elements in Tobaccos by High Performance Ion Chromatography. *J. Chin. Chem. Soc.* **2009**, *56*, 671–675.
- Cameron, K.; Madramootoo, C.; Crolla, A.; Kinney, C. Pollutant Removal from Municipal Sewage Lagoon Effluents with a Free-Surface Wetland. *Water Res.* **2003**, *37* (12), 2803–2812.
- Campbell, C. R.; Plank, C. O. Sample Preparation. In *Plant Analysis Reference Procedures for the Southern Region of the United States*, Southern Cooperative Series, Bulletin #368; University of Georgia: Athens, GA, May 1992; pp 1–12.
- Campins-Falco, P.; Meseguer-Lloret, S.; Climent-Santamaria, T.; Molins-Legua, C. A. Microscale Kjeldahl Nitrogen Determination for Environmental Waters. *Talanta* **2008**, *75* (4), 1123–1126.
- Carneiro, J. M. T.; Sartini, R. P.; Zagatto, E. A. G. Spectrophotometric Determination of Total Nitrogen in Plant Materials Using a Flow-Injection System with AgCl(s) Reactor. *Anal. Chim. Acta* **2000**, *416* (2), 185–190.
- Castillo, L. S.; Trimberger, G. W.; Henderson, C. R.; Herrington, B. L.; Turk, K. L. Comparison of Orange G Dye, Formol Titration, and Kjeldahl Methods for Milk Protein Determinations. *J. Dairy Sci.* **1962**, *45* (9), 1079–1082.
- Catanzaro, E. W.; Goldgraben, G. R.; Gasko, R. M. Automatic Determination of Low Level Kjeldahl Nitrogen. In *Automation in Analytical Chemistry*; Technicon Symposia; Mediad: White Plains, NY, 1965; p 241.
- CEN/TF. *Determination of Kjeldahl Nitrogen in Soil, Biowaste and Sewage Sludge*; European Standard, Working Document, 2003.
- CEN/TF. *Sludge, Treated Biowaste and Soil – Determination of Kjeldahl Nitrogen*; European Standard, Working Document, 2007.
- Chemat, Z.; Hadj-Boussaad, D. E.; Chemat, F. Application of Atmospheric Pressure Microwave Digestion to Total Kjeldahl Nitrogen Determination in Pharmaceutical, Agricultural and Food Products. *Analisis* **1998**, *26* (5), 205–209.
- Chen, R. R.; Difter, K. Diffusion Technique for 15N and Inorganic N Analysis of Low-N Aqueous Solutions and Kjeldahl Digests. *Rapid Commun. Mass Spectrom.* **2008**, *22* (11), 1727–1734.
- Chen, Y.-S.; Brayton, S. V.; Vanhatalo, A.; Hach, C. C. Accuracy in Kjeldahl Protein Analysis. *Am. Lab.* **1988**, June, 62–67.
- Cheng, S.; Gu, J.; Pang, J. Quantifying the Measurement Uncertainty for Protein Determinations in Soybeans Using the Kjeltac[™] 2300. *In Focus* **2008**, *32* (1), 24–27.
- Chiles, H. M. Direct Nesslerization of Kjeldahl Digestions. *J. Am. Chem. Soc.* **1928**, *50*, 217–221.
- Choi, C. W.; Ahvenjarvi, S.; Vanhatalo, A.; Tolonen, V.; Huhtanen, P. Quantification of the Flow of Soluble Non-Ammonia Nitrogen Entering the Omasal Canal of Dairy Cows Fed Grass Silage Based Diets. *Anim. Feed Sci. Technol.* **2002**, *96* (3–4), 203–220.
- Cioccia, A. M.; Gonzalez, E.; Perez, M.; Mora, J. A.; Romer, H.; Molina, E.; Hevia, P. Application of a Colorimetric Method to the

- Determination of the Protein Content of Commercial Foods, Mixed Human Diets and Nitrogen Losses in Infantile Diarrhoea. *Int. J. Food Sci. Nutr.* **1995**, *46* (1), 21–29.
- Clifton, K. E.; Clifton, L. M. A Field Method for the Determination of Total Nitrogen in Plant Tissue. *Commun. Soil Sci. Plant Anal.* **1991**, *22* (9–10), 851–860.
- Conklin-Brittain, N. L.; Dierenfeld, E. S.; Wrangham, R. W.; Norconk, M.; Silver, S. C. Chemical Protein Analysis: A Comparison of Kjeldahl Crude Protein and Total Ninhydrin Protein from Wild, Tropical Vegetation. *J. Chem. Ecol.* **1999**, *25* (12), 2601–2622.
- Cook, D. L.; Frum, N. L. Evaluation of Total Phosphorus and Total Nitrogen Methods in Pulp Mill Effluents. *Water Sci. Technol.* **2004**, *50* (3), 79–86.
- Cornell, S. E.; Jickells, T. D.; Cape, J. N.; Rowland, A. P.; Duce, R. A. Organic Nitrogen Deposition of Land and Coastal Environments: A Review of Methods and Data. *Atmos. Environ.* **2003**, *37* (16), 2173–2191.
- Corvaglia, L.; Battistini, B.; Paoletti, V.; Aceti, A.; Capreti, M. G.; Faldella, G. Near-Infrared Reflectance Analysis to Evaluate the Nitrogen Fat Content of Human Milk in Neonatal Intensive Care Units. *Arch. Dis. Child Fetal Neonatal Ed.* **2008**, *93* (5), F372–F375.
- Cox, J. L.; Harmon, B. G. Automated Determination of Kjeldahl Nitrogen in Urine and Feces. In *Automation in Analytical Chemistry 1966*; Technicon Symposia; Mediad: White Plains, NY, 1967; p 149.
- Crooke; W. M.; Simpson, W. E. Determination of Ammonium in Kjeldahl Digests of Crops by an Automated Procedure. *J. Sci. Food Agric.* **1971**, *22* (1), 9–10.
- Crowther, J.; Evans, J. Automated Distillation-Spectrophotometry Procedure for Determining Ammonia in Water. *Analyst* **1980**, *105*, 848.
- Crowther, J.; Wright, B.; Wright, W. Semiautomated Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Surface Waters. *Anal. Chim. Acta* **1980**, *119* (2), 313–321.
- Current, S. S. M.; King, J. W. Sampling and Sample Preparation for Food Analysis. In *Comprehensive Analytical Chemistry XXXVII*; Pawliszyn, J., Ed.; Elsevier: New York, 2002; Chap. 25, pp 869–894.
- Dakin, H. D.; Dudley, H. W. Some Limitations of the Kjeldahl Method. *J. Biol. Chem.* **1914**, *17* (2), 275–280.
- Daun, J. K.; deClercq, D. R. Comparison of Combustion and Kjeldahl Methods for Determination of Nitrogen in Oilseeds. *J. Am. Oil Chem. Soc.* **1994**, *71* (10), 1069–1072.
- Dautel, M.; Zimmermann, C.; Buschmann, R. Bestimmung des Proteingehalts in Eleischerzeugnissen nach der Dumas-methode. *Dtsch. Lebensm.-Rundsch.* **1996**, *92*, 390–393.
- Davi, M. L.; Bignani, S.; Milan, C.; Liboni, M.; Malfatto, M. G. Determination of Nitrate in Surface Waters by Ion-Exchange Chromatography after Oxidation of Total Organic Nitrogen to Nitrate. *J. Chromatogr. A* **1993**, *644* (2), 345–348.
- Davidson, J.; Mathieson, J.; Buyn, A. W. Use of Automation in Determining Nitrogen by Kjeldahl method, with Final Calculations by Computer. *Analyst* **1970**, *95* (2), 181–183.
- Deschreider, A. R.; Meaux, R. Specific-Ion Electrode Determination of the Total Nitrogen in Wine and the Ammonium Ion in Natural Mineral Waters. *Rev. Ferment. Ind. Aliment.* **1973a**, *28* (6), 238–244.
- Deschreider, A. R.; Meaux, R. Utilisation d'Une Electrode Ionique Spécifique pour le Dosage de l'Azote par la Méthode de Kjeldahl. *Analisis* **1973b**, *2* (6), 442–445.
- Devani, M. B.; Shishoo, C. J.; Shah, S. A.; Suhagia, B. N. Spectrophotometric Method for Microdetermination of Nitrogen in Kjeldahl Digest. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 953–956.
- Dickinson, F. N.; Gaunt, S. N.; Hankinson, D. J. Sources of Variation Affecting Relationship of Milk Protein Determination by Orange G Dye and Kjeldahl Methods. *J. Dairy Sci.* **1967**, *50* (11), 1841–1843.
- Domini, C.; Vidal, L.; Cravotto, G.; Canals, A. A Simultaneous Direct Microwave/Ultrasound-Assisted Digestion Procedure for the Determination of Total Kjeldahl Nitrogen. *Ultrason. Sonochem.* **2009**, *16* (4), 564–569.
- Doolan, K. J.; Belcher, C. B. Nitrogen Determination in Coal and Coke with an Ammonia Electrode. *Talanta* **1978**, *25* (7), 398–400.
- Doval, M. D.; Fraga, F.; Perez, F. F. Determination of Dissolved Organic Nitrogen in Seawater Using Kjeldahl Digestion after Inorganic Nitrogen Removal. *Oceanol. Acta* **1997**, *20* (5), 713–720.
- Doyle, A. P.; Schimel, J. P. Analysis of Kjeldahl Digests by the Salicylate Method: Optimizing pH and Buffering Improves Both Sensitivity and Precision. *Commun. Soil Sci. Plant Anal.* **1996**, *27* (11–12), 2549–2560.
- Doyle, A.; Schimel, J. P. Dichromate Digestion and Simultaneous Colorimetry of Microbial Carbon and Nitrogen. *Soil Sci. Soc. Am. J.* **1998**, *62* (4), 937–941.
- Dozier, M. C.; Melton, R. H.; Hare, M. F.; Hopkins, J.; Lesikar, B. J. *Drinking Water Problems: Nitrates*, B-6184, 3–08; AgriLife Extension, Texas A&M System: College Station, TX, 2008; 8 pp.
- Drown, T. M.; Martin, H. Estimation of Organic Nitrogen in Natural Waters by the Kjeldahl Method. *Chem. News J. Ind. Sci.* **1889**, *59*, 272–276. From: *J. Chem. Soc.*, Abstr. **1889**, *56*, 1035.
- Dumas, J.-B. Lettre de M. Dumas à M. Gay-Lussac, Sur les Procédés de l'Analyse Organique. *Ann. Chim. Phys.* **1831**, *2* (47), 198–215.
- Dusek, M.; Kvasnicka, F.; Lukáskova, L.; Krátká, J. Isotachophoretic Determination of Added Phosphate in Meat Products. *Meat Sci.* **2003**, *65* (2), 765–769.
- Eastin, E. F. Use of Ammonia Electrode for Total Nitrogen Determination in Plants. *Commun. Soil Sci. Plant Anal.* **1976**, *7* (5), 477–481.
- Ebeling, M. E. The Dumas Method for Nitrogen in Feeds. *J. Assoc. Off. Anal. Chem.* **1968**, *51* (1), 766–770.
- Editor of The Analyst. On Kjeldahl Method for the Determination of Nitrogen. *Analyst* **1885**, *10* (6), 127–128.
- Egli, H. Kjeldahl Guide, BÜCHI Labortechnik AG, Flawill, Switzerland, 2008.
- Electronic Instruments Ltd. *Use of Ammonia Probe in the Determination of Total Organic Nitrogen by the Kjeldahl Method*, Application Note; Chertsey, Surrey, UK: Electronic Instruments Ltd., 1974.
- Elkei, O. An Automated Method for the Determination of Low-Level Kjeldahl Nitrogen in Water and Waste Water. *Anal. Chim. Acta* **1976**, *86*, 63–68.
- Ellison, S. L. R.; Hardcastle, W. A. Causes of Error in Analytical Chemistry: Results of a Web-Based Survey of Proficiency Testing Participants. *Accredit. Qual. Assur.* **2012**, *17*, 453–464.
- Emi, P. E.; Müller, H.-R. Optimization of a Wet Chemical Continuous Flow Analysis Method Exemplified by the Determination of Kjeldahl Nitrogen and Total Phosphorus. *Anal. Chim. Acta* **1976**, *103* (2), 188–199.
- EPA Victoria (Australia). Sampling and Analysis of Waters, Wastewaters, Soils and Wastes. In *Industrial Waste Resources Guidelines*, EPA Victoria: Melbourne, 2009.
- Etheridge, R. D.; Pesti, G. M.; Foster, E. H. A Comparison of Nitrogen Values Obtained Utilizing the Kjeldahl Nitrogen and Dumas Combustion Methodologies (Leco CNS 2000) on Samples Typical of an

- Animal Nutrition Analytical Laboratory. *Anim Feed Sci. Technol.* **1998**, *73* (1–2), 21–28.
- Etzion, Y.; Linker, R.; Cogan, U.; Shmulevich, I. Determination of Protein Concentration in Raw Milk by Mid-Infrared Fourier/Transform Infrared Attenuated Total Reflectance Spectroscopy. *J Dairy Sci.* **2004**, *879*, 2779–2788.
- European Committee for Standardization. EN 13342: *Characterization of Sludges – Determination of Kjeldahl Nitrogen*; European Committee for Standardization: Brussels, 2000.
- European Committee for Standardization. EN 13654-1, *Soil Improvers and Growing Media – Determination of Nitrogen – Part 1 – Modified Kjeldahl Method, Nitrogen*; European Committee for Standardization: Brussels, 2001.
- European Union. *Council Directive 91/676/EEC of 12 December Concerning the Protection of Waters against Pollution Caused by Nitrates from Agricultural Sources*; Office for Official Publications of the European Communities: Brussels, 1991.
- Fearn, T. Some Statistical Comments on the Errors in NIR Calibration. *Anal. Proc.* **1986**, *23* (4), 123–125.
- Feinberg, M. H. Microwave-Assisted Digestion Technique for Kjeldahl Nitrogen in Foods. *Recent Research and Development in Agricultural and Food Chemistry*, **1999**, *3* (Part 1), 391–402.
- Feinberg, M. H.; Ireland-Ripert, J.; Mourel, R. M. Optimization Procedure of Open Vessel Microwave Digestion for Kjeldahl Determination in Foods. *Anal. Chim. Acta* **1993**, *272* (1), 83–90.
- Feinberg, M.; Suard, C.; Ireland-Ripert, J. Development of a Fully Automated Open Vessel Focused Microwave Digestion System. *Chemom. Intell. Lab. Syst.* **1994**, *22* (1), 34–47.
- Ferrari, A. Nitrogen Determination by a Continuous Digestion and Analysis System. *Ann. N. Y. Acad. Sci.* **1960**, *87*, 792–800.
- Ferrari, A.; Catanzaro, E.; Russo-Alesi, F. Nitrogen Analysis by a Continuous Digestion System. *Ann. N. Y. Acad. Sci.* **1965**, *130*, 602–620.
- Ferree, M. A.; Shannon, R. D. Evaluation of a Second Derivative UV/Visible Spectroscopy Technique for Nitrate and Total Nitrogen Analysis of Waste Water Samples. *Water Res.*, **2001**, *35* (1), 327–332.
- Ferreira, A. M. R.; Lima, J.; Rangel, A. Potentiometric Determination of Total Nitrogen in Soils by Flow Injection with a Gas-Diffusion Unit. *Aust. J. Soil Res.* **1996**, *34* (3), 503–510.
- Figenschou, D. L.; Marais, J. P.; de Figueiredo, M. A Comparison of Three Methods of Nitrogen Analysis for Feedstuffs. *S. Afr. J. Anim. Sci.* **2000**, *30* (Suppl. 1), 23.
- FIL/IDF. Comprehensive Review of Scientific Literature Pertaining to Nitrogen Protein Conversion Factors. *Bull. Int. Dairy Fed.*, *405*, 11 pp.
- Fleck, A. Total Nitrogen Determination. *Proc. Nutr. Soc.* **1970**, *29* (1), 81–85.
- Fleck, A.; Munro, H. N. The Determination of Organic Nitrogen in Biological Materials. *Clin. Chim. Acta* **1965**, *11* (1), 2–12.
- Florence, E.; Harris, W. M. Determination of Nitrogen in Cheese Using Copper Sulfate as a Kjeldahl Catalyst: Results of an Interlaboratory Study. *Analyst* **1987**, *112* (3), 317–320.
- FOSS. Kjeldahl Analysis Goes Online. New Kjeltec™ System Enhances Traceability and Efficiency in the Laboratory. *In Focus* **2009**, *33* (1), 4.
- Friedman, M. Applications of the Ninhydrin Reaction for Analysis of Amino Acids, Peptides, and Proteins to Agricultural and Biomedical Sciences. *J. Agric. Food Chem.* **2004**, *52* (3), 385–406.
- Fukumoto, H. E.; Chang, C. W. Manual Salicylate-Hypochlorite Procedure for the Determination of Ammonia in Kjeldahl Digest. *J. Assoc. Off. Anal. Chem.* **1982**, *65* (5), 1076–1079.
- Gallaher, R. N.; Weldon, C. O.; Boswell, F. C. A Semiautomated Procedure for Total Nitrogen in Plants and Soil Samples. *Soil Sci. Soc. Am. J.* **1976**, *40*, 887–889.
- Galvez-Sola, L.; Moral, R.; Moreno-Caselles, J.; Pérez-Murcia, M. D.; Pérez-Espinosa, A.; Bustamante, M. A.; Paredes, C. Effectiveness of Near Infrared Reflectance Spectroscopy in the Quick Evaluation of Nitrogen Content in Sewage Sludge. *Commun. Soil Sci. Plant Anal.*, **2009**, *40* (1–6), 726–735.
- Gantenbein, W. M. Collaborative Study of the Automated Determination of Nitrogen in Meat Products. *J. Assoc. Off. Anal. Chem.* **1973**, *56* (1), 31–35.
- Gantenbein, W. M.; Schermerhorn, J. L.; George Jr., E. Collaborative Study of the Automated Determination of Nitrogen in Meat Products. *J. Assoc. Off. Anal. Chem.* **1974**, *57* (4), 838–840.
- Geiger, J. W.; Davis, N. M.; Blakemore, W. S.; Long, C. L. A Method for Determining Total Nitrogen in Kjeldahl Digestion Solution Using a Centrifugal Analyzer. *J. Autom. Chem.* **1987**, *9* (2), 72–76.
- Ginkel, J. H.; Sinnavee, J. Determination of Total Nitrogen in Plant Material with Nessler's Reagent by Continuous-Flow Analysis. *Analyst* **1980**, *105* (12), 1199–1203.
- Gonzalez, A. G.; Herrador, M. A.; Asuero, A. G. Intra-laboratory Testing of Method Accuracy from Recovery Assays. *Talanta* **1999**, *48* (3), 729–736.
- Gonzalez, A. G.; Herrador, M. A.; Asuero, A. G. Practical Digest for Evaluating the Uncertainty of Analytical Assays from Validation Data According the LGC/VAM Protocol. *Talanta* **2005**, *65* (4), 1022–1030.
- Grace Analytical Lab. *Standard Operating Procedure for Total Kjeldahl Nitrogen (Lachat Method)*. Grace Analytical Lab: Chicago, IL, 1994; <http://www.epa.gov/glnpo/lmmb/methods/tknalr2.pdf>
- Grappin, R.; Horwitz, W. Determination of Nitrogen-Content in Milk by the Kjeldahl Method Using Copper Sulfate: Interlaboratory Study. *J. Assoc. Off. Anal. Chem.* **1988**, *71* (5), 893–898.
- Greenfield, H.; Southgate, D. A. T. *Food Composition Data. Production, Management and Use*, 2nd ed.; Food and Agriculture Organization of the United Nations, FAO: Rome, 2003.
- Grieping, B.; Gonska, H.; Muntan, H. *The Certification of the Content (Mass Fractions) of Nitrogen, Phosphorus, Chloride, Sodium, Potassium, Magnesium and Calcium and of the Kjeldahl Nitrogen Content in Skim Milk Powder*; Office for Official Publications of the European Communities: Brussels, 1983.
- Grimble, G. K.; West, M. F.; Acuti, A. B.; Rees, R. G.; Hunjan, M. K.; Webster, J. D.; Frost, P. G.; Silk, D. B. Assessment of an Automated Chemiluminescence Nitrogen Analyzer for Routine Use in Clinical Nutrition. *J. Parenter. Enteral Nutr.* **1988**, *12* (1), 100–106.
- Guebel, D. V.; Nudel, B. C.; Giuletti, A. M. A Simple and Rapid Micro-Kjeldahl Method for Total Nitrogen Analysis. *Biotechnol. Tech.* **1991**, *5* (6), 427–430.
- Guo, W. S.; Ren, L. P.; Zhou, Z. M.; Meng, Q. X. Difference of Nitrogen Contents, Determined by the Combustion and Kjeldahl Method in Response to Nitrate Nitrogen in Some Feedstuffs. *J. Anim. Feed Sci.* **2007**, *16* (Suppl. 2), 178–183.
- Hach, C. C.; Brayton, S. V.; Kopelove, A. B. A Powerful Kjeldahl Nitrogen Method Using Peroxymonosulfuric Acid. *J. Agric. Food Chem.* **1985**, *33* (6), 1117–1123.

- Hach, C. C.; Bodwen, B. K.; Kopelove, A. B.; Brayton, S. V. More Powerful Peroxide Kjeldahl Digestion Method. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (5), 783–787.
- Hach Company. *Digesdahl[®] Digestion Apparatus – Instruction Manual*; Hach Company: Loveland, CO, 1999.
- Hakoda, A.; Ii, Y.; Suzuki, T.; Yasui, A. Interlaboratory Study on the Determination of Crude Protein in Macaroni Products on JAS by Kjeldahl Method Using Copper Catalysts. *Food Sci. Technol. Res.* **2009**, *15* (5), 531–536.
- Hakoda, A.; Ii, Y.; Naito, S.; Suzuki, T.; Yasui, A. Determination of Crude Protein in Macaroni Products by the Combustion Method and Comparison with the Kjeldahl Method: Interlaboratory Study. *Food Sci. Technol. Res.* **2011**, *17* (3), 227–232.
- Halstead, J. A.; Edwards, J.; Soracco, R. J.; Armstrong, R. W. Potential for Chlorate Interference in Ion Chromatographic Determination of Total Nitrogen in Natural Waters Following Alkaline Persulfate Digestion. *J. Chromatogr. A* **1999**, *857* (1–2), 337–342.
- Hambraeus, L.; Forsum, E.; Abrahamsson, L.; Lönnerdal, B. Automatic Total Nitrogen Analysis in Nutritional Evaluation Using a Block Digester. *Anal. Biochem.* **1976**, *72* (1–2), 78–85.
- Hansen, B. Determination of Nitrogen as Elementary N, an Alternative to Kjeldahl. *Acta Agric. Scand.* **1989**, *39* (2), 113–118.
- Hartwig, R. A.; Hurburgh, C. R. Interlaboratory Comparison of Soybean Protein and Oil Determinations. *J. Am. Oil Chem.* **1991**, *68* (12), 949–955.
- Harwood, J. E.; Huyser, D. J. Automated Kjeldahl Analyses of Nitrogenous Materials in Aqueous Solutions. *Water Res.* **1970a**, *4* (8), 539–545.
- Harwood, J. E.; Huyser, D. J. Some Aspects of the Phenol-Hypochlorite Reaction as Applied to Ammonia Analysis. *Water Res.* **1970b**, *4* (7), 501–515.
- Heistand, R. W. Automated Kjeldahl Determination of Nitrogen in Petroleum. *Anal. Chem.* **1970**, *42* (8), 903–906.
- Henry, C.; Sullivan, D.; Rynk, R.; Dorsey, K.; Cogger, C. *Managing Nitrogen from Biosolids*; Northwest Biosolids Management Association: Washington, DC, 1999.
- Herrador, M. A.; Asuero, A. G.; González, A. G. Estimation of the Uncertainty of Indirect Measurements from the Propagation of Distributions by Using the Monte Carlo Method: An Overview. *Chemom. Intell. Lab. Syst.* **2005**, *79* (1–2), 115–122.
- Hetrick, J. H.; Whitney, R. M. Determination of Nitrogen in Milk by Direct Nesslerization of the Digested Sample. *J. Dairy Sci.* **1949**, *32* (2), 111–122.
- Hevia, P.; Cioccia, A. M. Application of a Colorimetric Method to the Determination of Nitrogen in Nutritional Studies with Rats and Humans. *Nutr. Rep. Int.* **1988**, *38* (6), 1129–1136.
- Holcombe, E. E.; Moore, D. G.; Fredriksen, R. L. *An Improved Method of Chemical Analysis of Low Levels of Nitrogen in Forest Streams or in Rainwater*; Research Note PNWRN-449; US Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, OR, 1986; 12 pp.
- Horino, T.; Fukuoka, T.; Hagio, T. Differences in Nitrogen and Mineral Contents in the Kernels of Cereals, Buckwheat and Pulses. *Jpn. J. Crop Sci.* **1992**, *61* (1), 28–33.
- Horneck, D. A.; Miller, R. O. Determination of Total Nitrogen in Plant Tissue. In *Handbook of Reference Methods for Plant Analysis*; Karla, Y. P.; Ed.; Soil and Plant Science Council; CRC Press: Boca Raton, FL, 1998.
- Houba, V. J. G.; Novazamsky, L.; van derLee, J. J. Quality Aspects in Laboratories for Soil and Plant Analysis. *Commun. Soil Sci. Plant Anal.* **1996**, *27* (3–4), 327–348.
- Huang, J.; Peng, S. Influence of Storage Methods on Total Nitrogen Analysis in Rice Leaves. *Commun. Soil Sci. Plant Anal.* **2004**, *35* (5–6), 879–888.
- Hue, N. V.; Uchida, R.; Ho, M. C. Sampling and Analysis of Soils and Plant Tissues. How to Take Representative Sampling, How the Samples Are Tested. In *Plant Nutrition Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture*; University of Hawaii at Manoa: Manoa, HI, 2000.
- Hund, E.; Massart, D. L.; Smeyers-Verbeke, J. Inter-laboratory Studies in Analytical Chemistry. *Anal. Chim. Acta* **2000**, *423*, 145–165.
- Inácio, M. R. C.; de Moura, M.; de F. V.; de Lima, K. M. G. Classification and Determination of Total Protein in Milk Powder Using Near Infrared Reflectance Spectrometry and the Successive Projections Algorithm for Variable Selection. *Vib. Spectrosc.* **2011**, *57* (2), 342–345.
- An Interlaboratory Study of Kjeldahl Methods. Presented at the WEFTA Analytical Working Group Meeting, Nantes, 1984, 21 pp. International Dairy Federation. *Milk - Determination of Nitrogen Content*; IDF Standard No. 20B: 1993. Parts 1 and 2; IDF: Brussels, 1993.
- ISO 8968-4, IDF 20-4. *Milk - Determination of Nitrogen Content - Part 4 - Determination of Non-Protein Content*; International Organization for Standardization: Genève, 2001a.
- ISO 8968-5, IDF 20-5. *Milk - Determination of Nitrogen Content - Part 5. Determination of Protein-Nitrogen Content*; International Organization for Standardization: Genève, 2001b.
- ISO. ISO 14891:2002 (IDF 185:2002). *Milk and Milk Products – Determination of Nitrogen Content. Routine Method Using Combustion According to the Dumas Principle*; International Organization for Standardization, Genève, 2002.
- ISO. ISO 5983-2. *Animal Feeds - Determination of Nitrogen Content and Calculation of Crude Protein Content - Part 2: Block Digestion/Steam Distillation Method*; International Organization for Standardization: Genève, 2005.
- ISO. ISO 20483:2006. *Cereals and Pulses - Determination of the Nitrogen Content and Calculation of the Crude Protein Content - Kjeldahl Method*; International Organization for Standardization: Genève, 2006.
- ISO. ISO 16634-1. *Food Products – Determination of the Total Nitrogen Content by Combustion According to the Dumas Principle and Calculation of the Crude Protein Content - Part 1: Oilseeds and Animal Feedingstuffs*; International Organization for Standardization: Genève, 2008.
- ISO. ISO 1871. *Food and Feed Products – General Guidelines for the Determination of Nitrogen by Kjeldahl Method*, International Organization for Standardization: Genève, 2009a.
- ISO. ISO 16634-2. *Food Products – Determination of the Total Nitrogen Content by Combustion According to the Dumas Principle and Calculation of the Crude Protein Content - Part 2: Cereals, Pulses and Milled Cereal Products*; International Organization for Standardization: Genève, 2009b.
- ISO. ISO/TC 34/SC 5N. *Milk and Milk Products – Determination of Nitrogen Content and Crude Protein Calculation – Part 1 & 2: Kjeldahl Principle*; International Organization for Standardization: Genève, 2011.

- Jaber, A. M. Y.; Mehanna, N.; Sultan, S. M. Determination of Ammonium Ion and Organic Bound Nitrogen by Inductively Coupled Plasma Emission Spectroscopy. *Talanta* **2009**, *78* (4–5), 1298–1302.
- Jackson, P. E.; Krol, J.; Heckenberg, A. L.; Mientjes, M.; Staal, W. Determination of Total Nitrogen in Food, Environmental and Other Samples by Ion Chromatography after Kjeldahl Digestion. *J. Chromatogr.* **1991**, *546*, 405–410.
- Jacobs, S. The Determination of Nitrogen in Organic Compounds by the Indanetrione Hydrate Method. *Analyst* **1960**, *85*, 257–264.
- Jacobs, S. The Quantitative Determination of Nitrogen by a Further Modification of the Indanetrione Hydrate Method. *Analyst* **1962**, *87* (1), 53–57.
- Jacobs, S. C. Assessment of Automated Nitrogen Analysis of Biological Fluids with Reference to the Kjeldahl Method. *J. Clin. Pathol.* **1968**, *21*, 218–219.
- Jacques, D. J.; Peterson, J. C. A Comparison of the Antek Chemiluminescent System and Kjeldahl Procedure for Determination of Total Nitrogen in Plant Tissue. *J. Plant Nutr.* **1987**, *10* (9–16), 1683–1688.
- Jakob, E.; Sievert, C.; Sommer, S.; Puhán, Z. Automated Determination of Total Nitrogen in Milk by the Dumas Method. *Z. Lebensm.-Unters.-Forsch.* **1995**, *200* (4), 239–243.
- Janßen, E. *Determination of Total Phosphorus, Total Nitrogen and Nitrogen Fractions*; Desk Study 16 in Project Horizontal, 2005, 47 pp.; <http://www.ecn.nl/horizontal>
- Jarquín-Sánchez, A.; Salgado-García, S.; Palma-Lopez, J.; Camacho-Chiu, W.; Guerrero-Peña, A. Análisis de Nitrógeno Total en Suelos Tropicales por Espectroscopía de Infrarrojo Cercano (NIRS) y Quimiometría. *Agrociencia* **2011**, *45* (6), 653–662.
- Jastrzebska, A. Determination of Sodium Tripolyphosphate in Meat Samples by Capillary Zone Electrophoresis with On-Line Isotachophoretic Sample Pre-treatment. *Talanta* **2006**, *69* (4), 1018–1024.
- Jirka, A. M.; Carter, M. J.; May, D.; Fuller, F. D. Ultramicro Semiautomated Methods for Simultaneous Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Waste Waters. *Environ. Sci. Technol.* **1976**, *10* (10), 1038–1044.
- Jones, B. M.; Daughton, C. G. Chemiluminescence vs. Kjeldahl Determination of Nitrogen in Oil Shale Retort Waters and Organonitrogen Compounds. *Anal. Chem.* **1985**, *57* (12), 2320–2325.
- Jones, J. B. Kjeldahl Nitrogen Determination—What's in a Name. *J. Plant Nutr.* **1987**, *10* (9–16), 1675–1682.
- Jung, S.; Rickert, D. A.; Deak, N. A.; Aldin, E. D.; Recknor, J.; Johnson, L. A.; Murphy, P. A. Comparison of Kjeldahl and Dumas Methods for Determining Protein Contents of Soybean Products. *J. Am. Oil Chem. Soc.* **2003**, *80* (12), 1169–1173.
- Junsomboon, J.; Jakmunee, J. Flow Injection Conductometric System with Gas Diffusion Separation for the Determination of Kjeldahl Nitrogen in Milk and Chicken Meat. *Anal. Chim. Acta* **2008**, *627* (2), 232–238.
- Kamizake, N. K. K.; Gonçalves, M. M.; Zaia, C. T. B. V.; Zaia, D. A. M. Determination of Total Proteins in Cow Milk Powder Samples: A Comparative Study between the Kjeldahl Method and Spectrophotometric Methods. *J. Food Compos. Anal.* **2003**, *16* (4), 507–516.
- Kammerer, P. A.; Rodel, M. G.; Hughes, R. A.; Lee, G. F. Low Level Kjeldahl Nitrogen Determination on the Technicon Autoanalyzer. *Environ. Sci. Technol.* **1967**, *1* (4), 340–342.
- Kane, P. F. Comparison of HgO and CuSO₄ as Digestion Catalyst in Manual Kjeldahl Determination of Crude Protein in Animal Feeds. Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1984**, *67* (5), 869–877.
- Kane, P. F. Comparison of HgO and CuSO₄/TiO₂ as Catalysts in Manual Kjeldahl Digestion for Determination of Crude Protein in Animal Feed: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (5), 907–911.
- Katsiari, M. C.; Alichanidis, E.; Voutsinas, L. P.; Roussis, I. G. Proteolysis in Reduced Sodium Kefalograviera Cheese Made by Partial Replacement of NaCl with KCl. *Food Chem.* **2001**, *73* (1), 31–43.
- Kaylegian, K. E.; Houghton, G. E.; Lynch, J. M.; Fleming, J. R.; Barbano, D. M. Calibration of Infrared Milk Analyzers: Modified Milk versus Producer Milk. *J. Dairy Sci.* **2006**, *89*, 2817–2832.
- Keeney, D. R.; Nelson, D. W. Nitrogen-Inorganic Forms. In *Methods of Soil Analysis*, 2nd ed.; Page, A. L.; Miller, R. H.; Keeney, D. R., Eds.; American Society of Agronomy: Madison, WI, 1982; pp 643–693.
- Kemsley, E. K.; Tapp, H. S.; Scarlett, A. J.; Miles, S. J.; Hammond, R.; Wilson, R. H. Comparison of Spectroscopic Techniques for the Determination of Kjeldahl and Ammoniacal Nitrogen Content of Farmyard Manure. *J. Agric. Food Chem.* **2001**, *49* (2), 603–609.
- Khan, M. A. Studies on the Measurements of Plants Nutrients in Farm Waste. *PhD Thesis*, University of Glasgow, Glasgow, Scotland, UK, 1994.
- Khan, M. A.; Flower, T. H. Elimination of Interferences of Organic Nitrogen Compounds by Dialysis Inclusion with Technicon Autoanalyzer II in Ammonium-N Determination. *Pak. J. Biol. Sci.* **1999**, *2* (3), 834–837.
- Kimble, K. W.; Walker, J. P.; Finefold, D. N.; Asher, S. A. Progress toward the Development of a Point-of-Care Photonic Crystal Ammonia Sensor. *Anal. Bioanal. Chem.* **2006**, *385* (4), 678–685.
- King-Brink, M.; Sebranek, J. G. Combustion Method for Determination of Crude Protein in Meat and Meat Products, Collaborative Study. *J. AOAC Int.* **1993**, *76* (4), 787–793.
- Kirsten, W. J.; Ternrud, I. E.; Hesselius, G. V. Automatic Simultaneous Determination of Nitrogen and Moisture in Grain with and without Weighings. *J. Agric. Food Chem.* **1984**, *32* (2), 279–284.
- Kitamaki, Y.; Jin, J.-Y.; Takeuchi, T. Simultaneous Determination of Inorganic Nitrogen Species by Macrocolumn Ion Chromatography. *J. Chromatogr. A* **2003**, *1003*, 197–202.
- Kitto, W. H. Rapid Determination of Nitrogen by a Kjeldahl-Nessler Process. *Analyst* **1934**, *59* (11), 733–735.
- Kjeldahl J. New Method for the Determination of Nitrogen. *Chem. News* **1883**, *48* (1240), 101–102; Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. *Z. Anal. Chem.* **1883**, *22*, 366–382; En ny Methode til Kvaeststoffbestemmelsei organiske Stoffer. *Medd. Carlsberg Lab.* **1883**, *2* (1), 1–27; Sur une Nouvelle Méthode de Dosage de l'Azote dans les Substances Organiques (French summary: *Résumé du CR Trav. Lab. Carlsberg*; separately pagged section) **1883**, *2* (Juni), 1–12.
- Koch, F. C.; McMeekin, T. L. A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia. *J. Am. Chem. Soc.* **1924**, *46* (9), 2066–2069.
- Konstantinides, F. N.; Boehm, K. A.; Radmer, W. J.; Storm, M. C.; Adderly, J. T.; Weisdorf, S. A.; Cerra, F. B. Pyrochemiluminescence: Real-Time, Cost-Effective Method for Determining Total Urinary Nitrogen in Clinical Nitrogen-Balance Studies. *Clin. Chem.* **1988**, *34* (12), 2518–2520.
- Kowalenko, C. G. Assessment of LECO CNS-2000 Analyzer for Simultaneously Measuring Total Carbon, Nitrogen and Sulphur in Soil. *Commun. Soil Sci. Plant Anal.* **2001**, *32* (13–14), 2065–2078.

- Kpoblekou-A, K. Relative Proportion of Inorganic and Total Nitrogen in Broiler Litter as Determined by Various Methods. *J. Sci. Food Agric.* **2006**, *86* (14), 2354–2362.
- Kramme, D. G.; Griffen, R. H.; Hartford, C. G.; Corrado, J. A. Automated Kjeldahl Nitrogen Determination—A Comprehensive Method for Aqueous Dispersible Samples. *Anal. Chem.* **1973**, *45* (2), 405–408.
- Krom, M. D. Spectrophotometric Determination of Ammonia: A Study of a Modified Berthelot Reaction Using Salicylate and Dichloroisocyanurate. *Analyst* **1980**, *105* (4), 305–316.
- Krotz, L.; Giuzzi, G. Achieving Rapid, Accurate, and Reliable Nitrogen Determination in Soils Using Dynamic Flash Combustion. *Am. Lab.* **2011**, *43* (8), 3 pp.
- Krotz, L.; Cicerci, E.; Giuzzi, G. Protein Determination in Cereals and Seeds. *Food Qual.* **2008**, *15* (4), 37–39.
- Kubota, T.; Oshida, T.; Yanai, K.; Inoue, Y.; Matsui, S.; Matsumoto, T.; Ishikuro, E.; Yasui, A. Improvement of the Conditions for the Determination of Total Nitrogen in Fish Meal in Kjeldahl Method and Its Comparison with Dumas Method. *Bunseki Kagaku* **2011**, *60* (1), 67–74.
- Kuck, J. A.; Kingsley, A.; Kinsey, D.; Sheehan, F.; Swigert, G. F. Kjeldahl Ultramicrodetermination of Nitrogen Applications in the Industrial Laboratory. *Anal. Chem.* **1950**, *22* (4), 605–611.
- Labconco. *A Guide to Kjeldahl Nitrogen Determination Methods and Apparatus*; Labconco Corporation: Kansas City, KS, 1998.
- Lang, G. A. Simple Microdetermination of Kjeldahl Nitrogen in Biological Materials. *Anal. Chem.* **1958**, *30* (10), 1692–1694.
- Lauro, M. F. Use of Selenium as a Catalyst in Determination of Nitrogen by the Kjeldahl Method. *Ind. Eng. Chem. Anal. Ed.* **1931**, *3* (4), 401–402.
- Lehmann, J.; Solomon, D. Nitrogen Speciation in Soils by Near-Edge X-Ray Absorption Fine Structure (NEXAFS) Spectroscopy and Scanning Transmission X-Ray Microscopy (STXM). In *Canadian Light Source Activity Report 2007*; Canadian Light Source Inc.: Saskatoon, Canada, 2008; pp 84–85.
- Lennox, L. J.; Flanagan, M. J. An Automated Procedure for Determination of Total Kjeldahl Nitrogen. *Water Res.* **1982**, *16* (7), 1127–1133.
- Lima, J. L. F. C.; Rangel, A. O. S. S.; Souto, M. R. S. Flow Injection System with Gas Diffusion for the Sequential Determination of Total Nitrogen and Phosphorus in Vegetables. *Fresenius' J. Anal. Chem.* **1997**, *358* (5), 657–662.
- Lima, J. F. C. C.; Delerue-Matos, G.; Vaz, M. C. Flow-Injection Analysis of Kjeldahl Nitrogen in Milk and Dairy Products by Potentiometric Detection. *Anal. Chim. Acta* **1999**, *385* (1–3), 437–441.
- Lin, R. H.; Randolph, H. E. Determination of Nitrogen in Milk by Microdiffusion of Kjeldahl Digests. *J. Dairy Sci.* **1978**, *61* (7), 870–873.
- Liu, Y. P.; Mulvaney, R. L. Diffusion of Kjeldahl Digests for Automated Nitrogen-15 Analysis by the Rittenberg Technique. *Soil Sci. Soc. Am. J.* **1992**, *56* (4), 1151–1154.
- Lloret, S. M.; Andres, J.; Legua, C. M.; Campins-Falcó, O. Determination of Ammonia and Primary Aminocompounds and Kjeldahl Nitrogen in Water Samples with a Modified Roth's Fluorimetric Method. *Talanta* **2005**, *65* (4), 869–875.
- Lo, K. V.; Wong, W. T.; Liao, P. H. Rapid Determination of Total Kjeldahl Nitrogen Using Microwave Digestion. *J. Environ. Sci. Health A* **2005**, *40* (3), 609–615.
- Lowthian, P.; Thompson, M.; Wood, R. Use of Proficiency Tests to Assess the Comparative Performance of Analytical Methods: The Determination of Fat in Foodstuffs. *Analyst* **1996**, *121* (7), 977–982.
- Lubochinsky, B.; Zalta, J. P. Microdosage Colorimétrique de l'Azote Ammoniacal. *Bull. Soc. Chim. Biol.* **1954**, *36*, 1363–1366.
- Lynch, J. M.; Barbano, D. M. Kjeldahl Nitrogen Analysis as a Reference Method for Protein Determination in Dairy Products. *JAOAC Int.* **1999**, *82* (6), 1389–1400.
- Lynch, J. M.; Barbano, D. M.; Fleming, J. R. Indirect and Direct Determination of the Casein Content of Milk by Kjeldahl Nitrogen Analysis. *JAOAC Int.* **1998**, *81* (4), 763–774.
- Lynch, J. M.; Barbano, D. M.; Fleming, J. R. Determination of Total Nitrogen Content of Hard, Semihard, and Processed Cheese by the Kjeldahl Method. *JAOAC Int.* **2002**, *85* (2), 445–455.
- Lynch, J. M.; Barbano, D. M.; Schweisthal, M.; Fleming, J. R. Precalibration Evaluation Procedures for Mid-Infrared Analyzers. *J. Dairy Sci.* **2006**, *89* (7), 2761–2774.
- Maher, W.; Krikowa, F.; Wruck, D.; Louie, H.; Nguyen, T.; Huang, W. Y. Determination of Total Phosphorus and Nitrogen in Turbid Waters by Oxidation with Alkaline Potassium Peroxodisulfate and Low Pressure Microwave Digestion, Autoclave Heating or the Use of Closed Vessels in a Hot Water Batch: Comparison with Kjeldahl Digestion. *Anal. Chim. Acta* **2002**, *463* (3), 283–293.
- Mann, L. T. Spectrophotometric Determination of Nitrogen in Total Micro-Kjeldahl Digests. Application of Phenol-Hypochlorite Reaction to Microgram Amounts of Ammonia in Total Digest of Biological Material. *Anal. Chem.* **1963**, *35* (13), 2179–2182.
- Marco, A.; Rubio, R.; Compano, I. R.; Casals, I. Comparison of the Kjeldahl Method and a Combustion Method for Total Nitrogen Determination in Animal Feed. *Talanta* **2002**, *57* (5), 1019–1026.
- Marczenko, Z. Nitrogen. In *Spectrophotometric Determination of Elements*; Ellis Horwood: Chichester, 1986; Chap. 35, pp 414–426.
- Mariotti, F.; Tomé, D.; Mirand, P. P. Converting Nitrogen into Protein—Beyond 6.25 and Jones' Factor. *Crit. Rev. Food Sci. Nutr.* **2008**, *48* (2), 177–184.
- Marten, J. F.; Catanzaro, G. Fundamental Studies in Automatic Nitrogen Detection. *Analyst* **1966**, *91* (1078), 42–47.
- Martone, C. B.; Grupkin, M.; Barassi, C. A.; Tiucco, R. E. Determination of Protein in Fish Meal. *J. Sci. Food Agric.* **1980**, *31* (8), 782–784.
- Masclaux-Daubresse, C.; Daniel-Vedele, F.; Dechornat, J.; Chardon, F.; Gaufichon, L.; Suzuki, A. Nitrogen Uptake, Assimilation and Remobilization in Plants: Challenges for Sustainable and Productive Agriculture. *Ann. Bot.* **2010**, *105* (7), 1141–1157.
- Mason, C. J.; Coe, G.; Edwards, M.; Riby, P. G. The Use of Microwaves in the Acceleration of Digestion and Color Development in the Determination of Total Kjeldahl Nitrogen in Soil. *Analyst* **1999**, *124* (11), 1719–1726.
- Masson, P.; Andrieu, M. M. Simultaneous Analysis of Nitrogen, Potassium, Calcium and Magnesium in Digested Samples by Ion Chromatography. *Analisis* **1996**, *24* (9–10), 380–382.
- Matejovic, I. Total Nitrogen in Plant Material Determined by Means of Dry Combustion: A Possible Alternative to Determination by Kjeldahl Digestion. *Commun. Soil Sci. Plant Anal.* **1995**, *26* (13–14), 2217–2229.
- McGill, D. L. Comparison of Automated Method and Improved AOAC Kjeldahl Method for Determination of Protein in Meat and Meat Products. *J. Assoc. Off. Anal. Chem.* **1980**, *64* (1), 29–31.

- McKenzie, H. A. The Kjeldahl Determination of Nitrogen: Retrospect and Prospect. *Trends Anal. Chem.* **1994**, *13* (4), 138–144.
- McLeod, S. Determination of Total Soil and Plant Nitrogen Using a Microdistillation Unit in a Continuous-Flow Analyzer. *Anal. Chim. Acta* **1992**, *266* (1), 113–117.
- Medina, H. L. de; Vargas, M. C. de; Marin, J.; Pirela, D. Determination of Total Nitrogen in Water Samples by Means of High Pressure Bombs and Ion Chromatography. *J. Chromatogr.* **1994**, *671*, 287–293.
- Mertens, J.; van der Winkel, P.; Massart, D. L. Determination of Organic Nitrogen in Natural Waters by Means of an Ammonia Probe. *Int. J. Environ. Anal. Chem.* **1972**, *3*, 159; *Int. J. Environ. Anal. Chem.* **1975**, *4* (1), 25–31.
- Meseguer-Lloret, S.; Molins-Legua, C.; Campins-Falcó, R. Selective Determination of Ammonium in Water Base on HPLC and Chemiluminescence Detection. *Anal. Chim. Acta* **2005**, *536* (1–2), 121–127.
- Meseguer-Lloret, S.; Molins-Legua, C.; Verdú-Andrés, J.; Campins-Falcó, P. Chemiluminescent Method for Detection of Eutrophication Sources by Estimation of Organic Amino Nitrogen and Ammonium in Water. *Anal. Chem.* **2006**, *78* (21), 7504–7510.
- Michalski, R.; Kurzyca, I. Determination of Nitrogen Species (Nitrate, Nitrite and Ammonia Ions) in Environmental Samples by Ion Chromatography. *Pol. J. Env. Stud.* **2006**, *15* (1), 5–18.
- Michałowski, T.; Asuero, A. G.; Wybraniec, S. The Titration in the Kjeldahl Method of Nitrogen Determination: Base or Acid as Titrant? *J. Chem. Educ.*, **2013**, *90* (2), 191–197.
- Michirina, B. P.; Fox, R. H.; Piekielek, W. P. Chemical Characterization of Two Extracts Used in the Determination of Available Soil Nitrogen. *Plant Soil* **1982**, *64* (3), 331–341.
- Middleton, K. R. New Nessler Reagent and Its Use in the Direct Nesslerization of Kjeldahl Digest. *J. Appl. Chem. (London)* **1960**, *10*, 281–286.
- Miller, B. S.; Pomeranz, Y.; Thompson, W. O.; Nolan, T. W.; Hughes, J. W.; Davis, G.; Jackson, N. G.; Fulk, D. W. Interlaboratory and Intra-laboratory Reproducibility of Protein Determination in Hard Winter Wheat by Kjeldahl and Near-Infrared Procedures. *Cereal Foods World* **1978**, *23* (4), 198–201.
- Miller, E. L.; Bimbo, A. P.; Barlow, S. M.; Sheridan, B. Repeatability and Reproducibility of Determination of the Nitrogen Content of Fishmeal by the Combustion (Dumas) Method and Comparison with the Kjeldahl Method: Interlaboratory Study. *J. AOAC Int.* **2007**, *90* (1), 6–20.
- Miller, G. L.; Miller, E. E. Determination of Nitrogen in Biological Materials. Improved Kjeldahl-Nessler Method. *Anal. Chem.* **1948**, *20* (5), 481–488.
- Minagawa, M.; Winter, D. A.; Kaplan, I. R. Comparison of Kjeldahl and Combustion Methods for Measurement of Nitrogen Isotope Ratios in Organic Matter. *Anal. Chem.* **1984**, *56* (11), 1859–1961.
- Minari, O.; Zilversmit, D. B. Use of KCN for Stabilization of Color and Direct Nesslerization of Kjeldahl Digests. *Anal. Biochem* **1963**, *6* (4), 320–327.
- Moldoveanu, S. Chromatographic Determination of Total-Nitrogen Following Kjeldahl Oxidation. *J. Chromatogr. Sci.* **1988**, *26* (1), 12–14.
- Molins-Legua, C.; Meseguer-Lloret, S.; Moliner-Martinez, Y.; Campins-Falcó, P. A Guide for Selecting the Most Appropriate Method for Ammonium Determination in Water Analysis. *Trends Anal. Chem.* **2006**, *25* (3), 282–290.
- Möller, J. Traceability in Modern Kjeldahl Analysis. *In Focus* **2005**, *29* (1), 4–5.
- Möller, J. Kjeldahl—Still Going Strong. *In Focus* **2009**, *33* (1), 14–16.
- Möller, J. Protein Analysis Revisited. *In Focus* **2010**, *34* (2), 22–23.
- Möller, J. *Measurement Uncertainty: “Top Down” or “Bottom Up”?* FOSS: Hilleroed, Denmark, 2011.
- Moore, J. C.; de Vries, W.; Lipp, M.; Griffiths, J. C.; Abernethy, D. R. Total Protein Methods and Their Potential Utility to Reduce the Risk of Food Protein Adulteration. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9* (4), 330–351.
- Morrison, G. Microchemical Determination of Organic Nitrogen with the Nessler Reagent. *Anal. Biochem.* **1971**, *43* (2), 527–532.
- Moser, C.; Herman, K. Method for the Rapid Determination of Protein in Meat Using the CEM Sprint™ Protein Analyzer: Collaborative Study. *J. AOAC Int.* **2011**, *94* (5), 1555–1561.
- Mulvaney, R. L.; Khan, S. A. Diffusion Methods to Determine Different Forms of Nitrogen in Soil Hydrolysates. *Soil Sci. Soc. Am. J.* **2001**, *65* (4), 1284–1292.
- Murphy, W. J. Why Bother with Chemical Methods? *Anal. Chem.* **1956**, *28* (4), 429.
- Murthy, L.; Herreid, E. O. Determination of Total Nitrogen in Stored Milk by Nesslerization and by the Macro-Kjeldahl Methods. *J. Dairy Sci.* **1958**, *41* (2), 314–315.
- Namieśnik, J.; Szefer, P. Preparing Samples for Analysis, the Key to Analytical Success. *Ecol. Chem. Eng.* **2008**, *15* (2), 168–244.
- Navas, M. J.; Jiménez-Moreno, A. M.; Bueno, J. M.; Sáez-Plaza, P.; Asuero, A. G. Analysis and Antioxidant Capacity of Anthocyanin Pigments. Part III An Introduction to Sample Preparation and Extraction. *Crit. Rev. Anal. Chem.* **2012a**, *42* (4), 284–312.
- Navas, M. J.; Jiménez-Moreno, A. M.; Bueno, J. M.; Sáez-Plaza, P.; Asuero, A. G. Analysis and Antioxidant Capacity of Anthocyanin Pigments. Part IV Extraction of Anthocyanins. *Crit. Rev. Anal. Chem.* **2012b**, *42* (4), 313–342.
- Neill, C. D. The Kjeldahl Protein Test. *Cereal Sci. Today* **1962**, *7*, 6–8, 10, 12.
- Nelson, D. W.; Sommers, L. E. Total Nitrogen Analysis of Soil and Plant Tissues. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 770–779.
- Nessler, J. Verhalten des Jodquecksilbers zu Ammoniak, und eine neue Reaktion auf Ammoniak. *Chem. Centr. Bl.* **1856**, *27*, *Neue Folge 1*, 529–541; On the Behavior of Iodide of Mercury to Ammonia, and a New Reaction for Ammonia. *J. Pract. Chem.* **1856**, *14*, 445–453: 463–466.
- Nicholls, K. H. Single Digestion Procedure for Rapid Manual Determinations of Kjeldahl Nitrogen and Total Phosphorus in Natural Waters. *Anal. Chim. Acta* **1975**, *76* (1), 208–212.
- Nkonge, C.; Balance, G. M. A Sensitive Colorimetric Procedure for Nitrogen Determination in Micro-Kjeldahl Digest. *J. Agric. Food Chem.* **1982**, *30* (3), 416–420.
- Noel, R. J. Collaborative Study of a Semiautomated Method for the Determination of Crude Protein in Animal Feeds. *J. Assoc. Off. Anal. Chem.* **1976**, *59* (1), 134–140.
- Noel, R. J. Determination of Crude Protein in Animal Feeds, Using Block Digestion Followed by Steam Distillation: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1979**, *62* (2), 290–291.
- Nollet, L. M. L. *Handbook of Water Analysis*, 2nd ed.; CRC Press: Boca Raton, FL, 2007.
- Nozawa, S.; Hakoda, A.; Sakaida, K.; Suzuki, T.; Yasui, A. Method Performance Study for the Determination of Total Nitrogen in Soy Sauce by the Kjeldahl Method. *Anal. Sci.* **2005**, *21* (9), 1129–1132.

- Nozawa, S.; Kasama, H.; Suzuki, T.; Yasui, A. Application of Improved Dumas Method to the Determination of Total Nitrogen in Soy Sauce. *Bunseki Kagaku* **2007**, *56* (3), 179–183.
- Nubé, M.; Van den Aarsen, C. O.; Giliams, J. P.; Hekkens, W. T. The Determination of Ammonium in Kjeldahl Digests Using the Gas-Sensing Ammonia Electrode. Comparison of the Direct Method with the Known Addition Methods. *Clin. Chim. Acta*, **1980**, *31* (3), 239–244.
- Ó hAlmhain, L.; Ó Danachair, D.. Filter-Papers as a Source of Error in Ammonia Determinations. *Analyst* **1974**, *99* (4), 211–213.
- Oberrieth, R.; Neil, H. M. Instrument Automates, Accelerates Nitrogen Determination. *Food Technol.* **1974**, *28* (6), 40–41, 43.
- O'Dell, J. W. *Determination of Total Kjeldahl Nitrogen by Semiautomated Colorimetry*; Method 351.2; U.S. Environmental Protection Agency: Cincinnati, OH, August 1993.
- Oehlenschläger, J. WEFTA Interlaboratory Comparison on Nitrogen Determination by Kjeldahl Digestion in Fishery Products and Standard Substances. *Inf. Fischwirtsch.* **1997**, *44* (1), 31–37.
- Ogawa, Y. D.; Nishikawa, M.; Nakasugi, O.; Ii, H.; Hirata, T. Determination of the Abundance of $\delta^{15}\text{N}$ in Nitrate Ion in Contaminated Groundwater Samples Using an Elemental Analyzer Coupled to a Mass Spectrometer. *Analyst* **2001**, *126* (7), 1051–1054.
- Oms, M. T.; Cerdà, A.; Cerdà, V. Analysis of Nitrates and Nitrites. In *Handbook of Water Analysis*; Nollet, L. M. L., Ed.; Marcel Dekker: New York, 2000; Chap. 12, pp 201–222.
- Orlandini, S.; Lattanzi, L.; Paggi, U.; Psathas, G.; Leray, O. Interlaboratory Collaborative Study on the Kjeldahl Reference Method for Nitrogen Determination in Goat Milk According to ISO 8968-1/2 IF 20-1/2. *Bull. Int. Dairy Fed.* **2009a**, *440*, 15–24.
- Orlandini, S.; Psathas, G.; Leray, O. Interlaboratory Collaborative Study on the Kjeldahl Reference Method for Nitrogen Determination in Sheep Milk According to ISO8968-172 IDF 20-1/2. *Bull. Int. Dairy Fed.* **2009b**, *440*, 2–14.
- Ortner, H. M. The Human Factor in Quality Management. *Accredit. Quality Assur.* **2000**, *5* (4), 130–141.
- O'Sullivan, A.; O'Connor, B.; Kelly, A.; McGrath, M. The Use of Chemical and Infrared Methods for Analysis of Milk and Dairy Products. *Int. J. Dairy Technol.* **1999**, *52* (4), 139–148.
- Owusu-Apenten, R. K. Kjeldahl Method, Quantitative Aminoacid Analysis and Combustion Analysis. In *Food Protein Analysis: Quantitative Effects on Processing*; Marcel Dekker: New York, 2002; Chap. 1, pp 1–45.
- Özer, E. T.; Güçer, Ş. Central Composite Design for the Optimization of Cd and Pb Determination in PVC Materials by Atomic Absorption Spectrometry after Kjeldahl Digestion. *Polym. Test.* **2011**, *30* (7), 773–778.
- Pansu, M.; Gautheyrou, J. *Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods*; Springer-Verlag: Berlin, 2006.
- Pansu, M.; Gautheyrou, J.; Loyer, J.-Y. *Soil Analysis, Sampling, Instrumentation and Quality Control*; Balkema, Swets & Zeitlinger: Lisse, Holland, 2001.
- Pasquini, C.; de Faria, L. C. Flow-Injection Determination of Ammonia in Kjeldahl Digests by Gas-Diffusion and Conductometry. *Anal. Chim. Acta* **1987**, *193*, 19–27.
- Patton, C. J.; Crouch, S. R. Spectrophotometric and Kinetics Investigation of the Berthelot Reaction for the Determination of Ammonia. *Anal. Chem.* **1977**, *49* (3), 464–469.
- Patton, C. J.; Kryskalla, J. R. *Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory – Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water*; Resources Investigations Report 03-4174; U.S. Geological Survey Water: Denver, CO, 2003.
- Patton, C. J.; Truit, E. P. *Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory: Determination of Ammonium plus Organic Nitrogen by a Kjeldahl Digestion Method and an Automated Photometric Finish That Includes Digest Clean Up by Gas Diffusion*; U.S. Geological Survey: Denver, CO, 2000.
- Pearson, D. *The Chemical Analysis of Foods*, Longman Group Ltd.: Harlow, U.K., 1976.
- Peaston, M. J. T.; Wilson, S.; Pemberton, J. An Automated Method for the Recovery of Urinary Nitrogen in Nitrogen Balance Studies. *Clin. Chem.* **1968**, *14* (9), 871–881.
- Pehlivanoglu-Mantas, E.; Sedlak, D. L. Wastewater-Derived Dissolved Organic Nitrogen: Analytical Methods, Characterization and Effects: A Review. *Crit. Rev. Env. Sci. Technol.* **2006**, *36* (3), 261–285.
- Pereira, M. G.; Espindula, J. A.; Valladares, G. S.; dos Anjos, L. H. C. Comparison of Total Nitrogen Methods Applied for Histosols and Soil Horizons with High Organic Matter Content. *Commun. Soil Sci. Plant. Anal.* **2006**, *37* (7–8), 939–943.
- Persson, J. A.; Wennerholm, M.; O'Halloran, S. *Handbook for Kjeldahl Digestion*; FOSS, Hilleroed, Denmark, 2008.
- Peters, A. W. A Method for the Determination of Total and Non Protein Nitrogen and of Total Solids in Fifteen to Thirty Drop Quantities of Human Blood, Developed for Measurement of the Nesslerized Nitrogen Solutions. *J. Biol. Chem.* **1919**, *39*, 285–298.
- Picarelli, A.; Greco, M.; di Giovambattista, F.; Ramazzotti, A.; Cedrone, C.; Corazziari, E.; Torsoli, A. Quantitative Determination of Faecal Fat, Nitrogen and Water by Means of a Spectrophotometric Technique: Near Infrared Reflectance Analysis (NIRA). Assessment of Its Accuracy and Reproducibility Compared with Chemical Methods. *Clin. Chim. Acta* **1995**, *234* (1–2), 147–156.
- Polychroniadou, A. Interlaboratory Collaborative Study on the Kjeldahl Reference Method for Nitrogen Determination in Sheep and Goat in Milk According to ISO 8968-1/2 – IDF 20-1/2. *Int. J. Dairy Technol.* **2010**, *63* (3), 473–475 (book review).
- Pontes, F. V. M.; Carneiro, M. C.; Vaitsman, D. S.; de Rocha, G. P.; da Silva, L. I. D.; Neto, A. A.; Montero, M. I. C. A Simplified Version of the Total Kjeldahl Nitrogen Method Using an Ammonia Extraction Ultrasound-Assisted Purge-and-Trap System and Ion Chromatography for Analyses of Geological Samples. *Anal. Chim. Acta* **2009**, *632* (2), 284–288.
- Pontes, F. V. M.; Carneiro, M. F.; Vaitsman, S. S.; Montero, M. I. C.; da Silva, L. I. D.; Neto, A. A. Application of a Purge-and Trap System for Fixed-NH₄⁺ Determination by Matrix Interference Free Ion Chromatography in Oil Reservoir Rocks. *J. Braz. Chem. Soc.* **2010**, *21* (6), 1126–1128.
- Powers, K. F.; Van Gent, D. L.; Townsend, R. F. Ammonia Electrode Analysis of Nitrogen in Microkjeldahl Digests of Forest Vegetation. *Commun. Soil Sci. Plant Anal.* **1981**, *12* (1), 19–30.
- Praus, P. Determination of Nitrogen in Water Technology. *Sbornik vedeckych prací Vysoke školy banske* **2002**, *48* (2), 35–42.
- Price, C. G.; Webb, N. B.; Smith, W. J.; Marks, H. M.; Yofle, A. M. Comparison of Mercury and Copper-Based Catalysts in the Kjeldahl

- Determination of Nitrogen in Meat and Meat Products: Collaborative Study. *J. AOAC Int.* **1994**, *77* (6), 1542–1546.
- Prusisz, B.; Jaskiewicz, L.; Pohl, P. High Performance Ion Chromatography Assessment of Inorganic and Organic Nitrogen Fraction in Potatoes. *Mikrochim. Acta* **2007**, *156* (3–4), 219–223.
- Pym, R. V. E.; Milham, P. J. Selectivity of Reaction among Chlorine, Ammonia and Salicylate for Determination of Ammonia. *Anal. Chem.* **1976**, *48* (9), 1413–1415.
- Quinn, J. R.; Boisvert, J. G. A.; Wood, I. Semi-Automated Ninhydrin Assay of Kjeldahl Nitrogen. *Anal. Biochem.* **1974**, *58* (2), 609–614.
- Ramsey, M. H. Sampling as a Source of Measurement Uncertainty: Techniques for Quantification and Comparison with Analytical Sources. *J. Anal. At. Spectrom.* **1998**, *13* (2), 97–104.
- Ramsey, M. H.; Ellison, S. L. R. *Measurement Uncertainty Arising from Samples: A Guide to Methods and Approaches*; EURACHEM/CITAC Guide; Eurachem, EUROLAB, CITAT, Nordtest and the RSC Analytical Methods Committee, 2007.
- Rao, M. B.; Whitney, R. M. An Improvement in the Direct Nesslerization Method for the Determination of Total Nitrogen in Stored Milk. *J. Dairy Sci.* **1960**, *43*, 563–564.
- Rayment, G. E.; Hill, R.; Greaves, A. T. Using Interlaboratory Proficiency Data to Guide NIR/MIR-calibrations. *Commun. Soil Sci. Plant Anal.* **2012**, *43* (1–2), 399–411.
- Reay, P. F. An Improved Determination of Ammonia in Kjeldahl Digests and Acidic Solutions with a Buffered Bethelot Reaction. *Anal. Chim. Acta* **1985**, *176*, 275–278.
- Reed, Z. H.; Park, J. W. Qualification and Quantification of Fish Protein in Prepared Surimi Crabstick. *J. Food Sci.* **2008**, *73* (5), C329–C334.
- Rhee, K. C. Determination of Total Nitrogen. In *Current Protocols in Food Analytical Chemistry*; Wiley: New York, 2001.
- Ribadeau-Dumas, B.; Grappin, R. Milk Protein Analysis. *Lait* **1989**, *69* (5), 357–416.
- Rizvi, G. H.; Natrajan, P. R. Determination of Nitrogen in UO₂ by Kjeldahl Spectrophotometry. *J. Radioanal. Nucl. Chem.* **1986**, *102* (2), 515–523.
- Rohm, H.; Tschager, E.; Jaros, D. Determination of Protolysis in Swiss Cheese: Comparison of the Kjeldahl Method and a Spectrophotometric OPA Assay. *Lebensm.-Wiss. Technol.* **1996**, *29*, 191–194.
- Rohwedder, J. J. R.; Pasquini, C. Differential Conductimetry in Flow Injection. Determination of Ammonia in Kjeldahl Digests. *Analyst* **1991**, *116* (8), 841–845.
- Roper, C.; Armour, C.; Steinbock, B. *Determination of Total Kjeldahl Nitrogen in Water Using the Seal AQ2 Discrete Analyzer*: NU-092-1.2, Bureau of Laboratories SOPs Results; Department of Environmental Protection: Tallahassee, FL, 2011a.
- Roper, C.; Armour, C.; Steinbock, B. *Determination of Total Kjeldahl Nitrogen in Soils Using the Westco SmartChem Discrete Analyzer*: NU-081-1.4, Bureau of Laboratories SOPs Results; Department of Environmental Protection: Tallahassee, FL, 2011b.
- Rossi, A. M.; Villarreal, M.; Juarez, M. D.; Sammán, N. C. Nitrogen Contents in Foods: A Comparison between the Kjeldahl and Hach Methods. *J. Argent. Chem. Soc.* **2004**, *92* (4/6), 99–108.
- Ruhemann, S. Triketohydrindene Hydrate. *Trans. Chem. Soc.* **1910**, *97*, 2025–2031.
- Russell, J. A. Colorimetric Estimation of Small Amounts of Ammonia by the Phenol Hypobromite Reaction. *J. Biol. Chem.* **1944**, *156*, 457–461.
- Rutherford, P. M.; McGill, W. B.; Arocena, J. M.; Figueredo, C. T. Total Nitrogen. In *Soil Sampling and Methods of Analysis*; Carter, M. R.; Gregorich, E. G., Eds.; CRC Press: Boca Raton, FL, 2008; Chap. 22.
- Sabbe, W. E.; Marx, D. B. Soil Sampling: Spatial and Temporal Variability. In *Soil Testing: Sampling, Correlation, Calibration, and Interpretation: Proceedings of a Symposium*; Brown, J. R., Ed.; SSSA Special Publication No. 21; Soil Science Society of America: Madison, WI, 1987; pp 1–14.
- Sachen, R. W.; Thiex, N. J. Effect of Sample Introduction and Atmospheric Blank on Determination of Nitrogen Crude Protein by Combustion. *J. AOAC Int.* **1997**, *80* (5), 14–19.
- Sader, A. P. O.; Oliveira, S. G.; Berchielli, T. T. Application of Kjeldahl and Dumas Combustion Method for Nitrogen Analysis. *Arch. Vet. Sci.* **2004**, *9* (2), 73–79.
- Sáez-Plaza, P.; Michałowski, T.; Navas, M. J.; Asuero, A. G.; Wybraniec, S. An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish. *Crit. Rev. Anal. Chem.*, **2013**, *43*(4), 178–223.
- Saha, U. K.; Sonon, L.; Kissel, D. E. Comparison of Conductimetric and Colorimetric Methods with Distillation-Titration Method of Analyzing Ammonium-Nitrogen in Total Kjeldahl Digests. *Commun. Soil Sci. Plant Anal.* **2012**, *43* (18), 2323–2341.
- Sahrawat, K. L.; Kumar, G. R.; Murthy, K. V. S. Sulfuric Acid-Selenium Digestion for Multi-element Analysis in a Single Plant Digest. *Commun. Soil Sci. Plant Anal.* **2002**, *33* (19–20), 3757–3765.
- Sarich, W.; Sanner, G.; Simon, C. Die Bestimmung des Gesamtslickstoffs in Düngemitteln mit dem macro N (modifiziertes Verfahren nach Dumas) im Vergleich zur Klassischen Kjeldahl-Distillation. *Agribiol. Res.* **1996**, *49*, 39–51.
- Sawyer, C. N.; McCarty, P. L.; Parkin, G. F. *Chemistry for Environmental Engineering and Science*, 5th ed.; McGraw-Hill: New York, 2003.
- Schaalje, G. B.; Mundel, H. H. Use of Residual Maximum-Likelihood to Evaluate Accuracy of Two NIRS Calibration Procedures, Relative to Kjeldahl, for Determining Nitrogen Concentration of Soybeans. *Can. J. Plant Sci.* **1991**, *71* (2), 385–392.
- Scheiner, D. Determination of Ammonia and Kjeldahl Nitrogen by Indophenol Method. *Water Res.* **1976**, *10* (1), 31–38.
- Schulze, G.; Liu, C. Y.; Brudowski, M.; Elsholz, O.; Frenzel, W.; Möller, J. Different Approaches to the Determination of Ammonium Ions at Low Levels by Flow Injection Analysis. *Anal. Chim. Acta* **1988**, *214*, 121–126.
- Searcy, R. L.; Simms, N. M.; Foreman, J. A.; Rergouist, L. M. A Study of the Specificity of the Berthelot Colour Reaction. *Clin. Chim. Acta* **1965**, *12* (2), 170–175.
- Searle, P. L. The Berthelot or Indophenol Reaction and Its Use in the Analytical Chemistry of Nitrogen. *Analyst* **1984**, *109* (5), 549–568.
- Sebecic, B.; Balenovic, J. Rapid Ecologically Acceptable Method for Wheat Protein Content Determination: Comparison of Results. *Dtsch. Lebensm.-Rundsch.* **2001**, *97* (6), 221–225.
- Sebranek, J. G. Rapid Methods for Compositional Analyses of Meat and Meat Products. In *New Techniques in the Analysis of Foods*; Tunick, M. H.; Palumbo, S. A.; Fratamico, P. M., Eds.; Kluwer Academic/Plenum Press, New York, 1998; pp 161–170.
- Sen, I. Spectroscopic Determination of Major Nutrients (N, P, K) of Soil. Master of Science Thesis, Food Engineering Department, Izmir Institute of Technology, Izmir, Turkey, September 2003.

- Settle Jr., F. A.; Diamonstone, B. I.; Kingston, H. M. An Expert-Database System for Sample Preparation by Microwave Dissolution 1. Selection of Analytical Descriptors. *J. Chem. Inf. Comput. Sci.* **1989**, *29* (1), 11–17.
- Shahinian, A. H.; Reinhold, J. G. Application of the Phenol-Hypochlorite Reaction to Measurement of Ammonia Concentrations in Kjeldahl Digests of Serum and Various Tissues. *Clin. Chem.* **1971**, *17* (11), 1077–1080.
- Sheppard, S. C.; Addison, J. A. Soil Sampling Handling and Storage. In *Soil Sampling and Methods of Analysis*, 2nd ed.; Carter, M. R.; Gregorich, E. G., Eds.; Canadian Society of Soil Science; CRC Press, Taylor & Francis: Boca Raton, FL, 2008; Chap. 4, 11 pp.
- Simonne, E. H.; Jones Jr., J. B.; Mills, H. A.; Smittle, D. A.; Hussey, C. G. Influence of Catalyst, Sample Weight, and Digestion Conditions on Kjeldahl Nitrogen. *Commun. Soil Sci. Plant Anal.* **1993**, *24*, 1609–1616.
- Simonne, E. H.; Mills, H. A.; Jones Jr., J. B.; Smittle, D. A.; Hussey, C. G. A Comparison of Analytical Methods for Nitrogen Analysis in Plant Tissues. *Commun. Soil Sci. Plant Anal.* **1994**, *25* (7–8), 943–954.
- Simonne, A. H.; Simonne, E. H.; Eitenmiller, R. R.; Mills, H. A.; Cresman III, C. P. Could the Dumas Method Replace the Kjeldahl Digestion for Nitrogen and Crude Protein Determinations in Foods? *J. Sci. Food Agric.* **1997**, *73* (1), 39–45.
- Skinner, J. *Microscale Chemistry: Experiments in Miniature*; Royal Society of Chemistry: Cambridge, UK, 1998.
- Skogerboe, K. J.; Labbe, R. F.; Rettmer, R. L.; Sundquist, J. P.; Gargett, A. M. Chemiluminescent Measurement of Total Urinary Nitrogen for Accurate Calculation of Nitrogen Balance. *Clin. Chem.* **1990**, *36* (5), 752–755.
- Smart, M. M.; Reid, F. A.; Jones, J. R. A Comparison of a Persulfate Digestion and the Kjeldahl Procedure for Determination of Total Nitrogen in Freshwater Samples. *Water Res.* **1981**, *15* (7), 919–921.
- Smart, M. M.; Rada, R. G.; Donnermeyer, G. N. Determination of Total Nitrogen in Sediments and Plants Using Persulfate Digestion. An Evaluation and Comparison with the Kjeldahl Procedure. *Water Res.* **1983**, *17* (9), 1207–1211.
- Smit, E. M. An Ultra-Micro Method for the Determination of Total Nitrogen in Biological Fluids Based on Kjeldahl Digestion and Enzymatic Estimation of Ammonia. *Clin. Chim. Acta* **1979**, *94* (2), 129–135.
- Smith, M. R.; Penner, M. H.; Bennett, S. E.; Bakalinsku, A. T. Quantitative Colorimetric Assay for Total Protein Applied to the Red Wine Pinot Noir. *J. Agric. Food Chem.* **2011**, *59* (13), 6871–6876.
- Sneddon, J.; Hardaway, C.; Bobbadi, K. K.; Reddy, A. K. Sample Preparation of Solid Samples for Metal Determination by Atomic Spectroscopy—An Overview and Selected Recent Applications. *Appl. Spectrosc. Rev.* **2006**, *41* (4), 1–14.
- Sobiecka, E.; van der Sloot, H.; Janssen, E.; Gawlik, B. M. *Project HORIZONTAL Validation Report on Kjeldahl Nitrogen. Validation of a Horizontal Standard for the Determination of Kjeldahl Nitrogen in Soils, Sludge and Treated Biowaste in a European Intercomparison Exercise*; EUR 23025EN-2007; European Commission: Luxembourg, 2007.
- Staniszewska M.; Koeber R.; Linsinger T.; Zeleny R.; Bernreuther A.; Ulberth F. *Certification of the Crude Protein, Fat, Lactose and Ash Content of Whole Milk Powder and the Crude Protein and Fat Content of Skim Milk Powder BCR-380R and BCR-685*; European Commission JRC Scientific and Technical Reports, EUR 23215 EN-2008; European Commission: Brussels, 2008.
- Stark, J. M.; Hart, S. C. Diffusion Technique for Preparing Salt Solutions, Kjeldahl Digests, and Persulfate Digests for Nitrogen-15 Analysis. *Soil Sci. Soc. Am. J.* **1996**, *60* (6), 1846–1855.
- Stenholm, A.; Holmström, S.; Ragnarsson, A. Total Nitrogen in Wastewater Analysis: Comparison of Devarda's Alloy Method and High Temperature Oxidation Followed by Chemiluminescence Detection. *J. Anal. Chem. USSR* **2009**, *64* (10), 1075–1081.
- Stevens, R. J. Semi-Automated Ammonia Probe Determination of Kjeldahl Nitrogen in Freshwaters. *Water Res.* **1976**, *19* (2), 171–175.
- Stevens, W. B.; Mulvaney, R. L.; Khan, S. A.; Holft, R. G. Improved Diffusion Methods for Nitrogen and ¹⁵Nitrogen Analysis of Kjeldahl Digests. *J. AOAC Int.* **2000**, *83* (5), 1039–1046.
- Stewart, B. M. Effect of Temperature on the Formation of Indophenol Blue in a Spectrophotometric Method for the Determination of Ammonia. *Water Res.*, **1985**, *19* (11), 1443–1445.
- Stewart, J. W. B.; Ruzicka, J. Flow Injection Analysis. Part V. Simultaneous Determination of Nitrogen and Phosphorus in Acid Digests of Plant Material with a Single Spectrophotometer. *Anal. Chim. Acta* **1976**, *82* (1), 137–144.
- Stewart, J. W. B.; Ruzicka, J.; Filho, H. B.; Zagatto, E. A. Flow Injection Analysis. Part III. Comparison of Continuous Flow Spectrophotometry and Potentiometry for the Rapid Determination of the Total Nitrogen Content in Plant Digests. *Anal. Chim. Acta* **1976**, *81* (2), 371–386.
- Steyermark, A.; McGee, B. Progress in Elemental Quantitative Analysis. *Microchem. J.* **1960**, *4* (3), 353–372.
- Steyermark, A.; Albert, H. K.; Aluise, V. A.; Huffman, E. W. D.; Kuck, J. A.; Moran, J. J.; Willits, C. O. Recommended Specification for Microchemical Apparatus. Micro-Kjeldahl Nitrogen. *Anal. Chem.* **1951**, *23* (3), 523–528.
- Stitcher, J. E.; Jolliff, C. R.; Hill, R. M. Comparison of Dumas and Kjeldahl Methods for Determination of Nitrogen in Feces. *Clin. Chem.* **1969**, *15* (3), 248–254.
- Su, X. L.; Nie, L. H.; Yao, S. Z. Determination of Ammonia in Kjeldahl Digests by Gas-Diffusion Flow-Injection Analysis with a Bulk Acoustic Wave-Impedance Sensor. *Talanta* **1997**, *44* (11), 2121–2128.
- Su, X. L.; Yu, B. S.; Yang, X. R.; Ne, L. H.; Yao, S. Z. Flow-Injection Determination of Total Ammonia and Total Carbon Dioxide in Blood Based on Gas-Diffusion Separation and with a Bulk Acoustic Wave Impedance Sensor. *J. Pharm. Biomed. Anal.* **1998**, *16* (5), 759–769.
- Suard, C. L.; Feinberg, M. H.; Ireland-Ripert, J.; Mourel, R. M. Interlaboratory Validation Analysis of the Mineralization by Microwave Digestion—Application to Kjeldahl Nitrogen in Foods. *Analisis* **1993**, *21* (6), 287.
- Suhre, F. B.; Corrao, P. A.; Glover, A.; Malanoski, A. J. Comparison of Three Methods for Determination of Crude Protein in Meat: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1982**, *65* (6), 1339–1345.
- Sun, J.-P.; Hou, C.-Y.; Feng, J.; Wang, X. Determination of the Protein Content in Rice by the Digital Chromatic Method. *J. Food Qual.* **2008**, *31* (2), 250–263.
- Sweeney, R. A.; Rexroad, P. R. Comparison of LECO FP-228 “Nitrogen Determinator” with AOAC Copper Catalyst Kjeldahl Method for Crude Protein. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (6), 1028–1030.

- Sweeney, R. A. Generic Combustion Method for Determination of Crude Protein in Feeds: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1989**, *72* (5), 770–774.
- Swyngedoum, C.; Lessard, R. Quality Control in Soil Chemical Analysis. In *Soil Sampling and Methods of Analysis*, 2nd ed.; Carter, M. R.; Gregorich, E. G., Eds.; Canadian Society of Soil Science; CRC Press, Taylor & Francis: Boca Raton, FL, 2008; Chap. 5, 19 pp.
- Telleson, J. R. Calibration in Kjeldahl Protein Analysis. *Cereal Food World* **1980**, *24* (2), 54–55.
- Thiex, N. J.; Manson, H.; Anderson, S.; Persson, J. A. Determination of Crude Protein in Animal Feed, Forage, Grain, and Oilseeds by Using Block Digestion with a Copper Catalyst and Steam Distillation into Boric Acid: Collaborative Study. *J. AOAC Int.* **2002**, *85* (2), 309–317.
- Thomas, P. Color Reaction of Ammonia. *Bull. Soc. Chim. Fr.* **1912**, *11*, 796.
- Thompson, M.; Owen, L.; Wilkinson, K.; Wood, R.; Damant, A. A Comparison of the Kjeldahl and Dumas Methods for the Determination of Protein in Foods, Using Data from a Proficiency Testing Scheme. *Analyst* **2002**, *127* (12), 1666–1668.
- Thompson, M.; Owen, L.; Wilkinson, K.; Wood, R.; Damant, A. Testing for Bias between the Kjeldahl and Dumas Methods for the Determination of Nitrogen in Meat Mixtures by Using Data from a Designed Interlaboratory Experiment. *Meat Sci.* **2004**, *68* (4), 631–634.
- Tobiano, P. Improving Protein Analysis Results by Kjeldahl Procedure. Paper presented at the 16th Annual ASA-IM SEA Feed Technology and Nutrition Workshop, Singapore, May 26–28, 2008.
- Todd, P. M. Use of Ammonia Electrode for Determination of Nitrogen in Meat Products. *J. Sci. Food Agric.* **1973**, *24*, 488.
- Treese, J. M.; Gilmore, L. O.; Fechheimer, N. S. A Comparison of the Orange G Dye and Kjeldahl Method for Determining Milk Protein. *J. Dairy Sci.* **1959**, *42* (2), 367–368.
- Tronimsdorff, N. *J. Pharm.* **1832**, *25*, St 2, 237–256.
- Tryzell, R.; Karlberg, B. Calibration Methods for Determination of Ammonium and Excess Acid in Kjeldahl Digests by Flow Injection Analysis. *Anal. Chim. Acta* **1997**, *343* (3), 183–190.
- Uhl, D. E.; Lancaster, E. B.; Vojnovich, C. Automation of Nitrogen Analysis of Grain and Grain Products. *Anal. Chem.* **1971**, *43* (8), 990–994.
- Unkovich, M.; Herridge, D.; Peoples, M.; Cadisch, G.; Boddey, B.; Giller, K.; Alves, B.; Chalk, P. *Measuring Plant-Associated Nitrogen Fixation in Agricultural Systems*; Australian Center for International Agricultural Research, ACIAR, Australian Government: Canberra, Australia, 2008.
- U.S. Environmental Protection Agency. *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020; Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency: Cincinnati, OH, 1979.
- U.S. Environmental Protection Agency. *Method 351.4 Potentiometric Ion Selective Electrode Method for Determining Total Kjeldahl Nitrogen*, EPA/600/4-79/020; Methods for Chemical Analysis of Water and Wastes; U.S. Environmental Protection Agency, March 1983.
- U.S. Environmental Protection Agency. *Estimated National Occurrence and Exposure to Nitrate/Nitrite in Public Drinking Water Supplies*; prepared by Wade Miller Assoc., under EPA contract no. 68-03-3514; U.S. Environmental Protection Agency: Washington, DC, 1990.
- U.S. Environmental Protection Agency. *Total Kjeldahl Nitrogen in Water and Biosolids by Automated Colorimetry with Preliminary Semi-automatic Digestion*; EPA-821-R-01-007; U.S. Environmental Protection Agency: Washington, DC, January 2001.
- U.S. Environmental Protection Agency. Editorial Note, *Fed. Regist.* **2012**, *77*, 29810.
- Valderrama, J. The Simultaneous Analysis of Total Nitrogen and Total Phosphorus in Natural Waters. *Mar. Chem.* **1981**, *10* (2), 109–122.
- Valdés, C.; Andrés, S.; Giráldez, F. J.; García, R.; Calleja, A. Potential Use of Visible and Near Infrared Reflectance Spectroscopy for the Estimation of Nitrogen Fractions in Forages Harvested from Permanent Meadows. *J. Sci. Food Agric.* **2006**, *86* (2), 308–314.
- Van Camp, J.; Dierckx, S. Proteins. In *Handbook of Food Analysis*, 2nd ed., Vol. 1, *Physical Characterization & Nutrient Analysis*; Nollet, L. M. L., Ed.; CRC Press: Boca Raton, FL, 2004; Chap. 7, pp 167–202.
- Van Camp, J.; Huyghebaert, A. Proteins. In *Handbook of Food Analysis*, Vol. 1, *Physical Characterization & Nutrient Analysis*; Nollet, L. M. L., Ed.; Marcel Dekker, New York, 1996; pp 277–310.
- Van de Voort, F. R.; Mills, B. L.; Paquette, G. A.; Grunfeld, E. Quantitative Aqueous Ammonium Ion Analysis by Transmission Infrared Spectroscopy. *J. Assoc. Off. Anal. Chem.* **1986**, *69* (6), 924–928.
- Van Slyke, D. D.; Hiller, A. Determination of Ammonia in Blood. *J. Biol. Chem.* **1933**, *102*, 499–504.
- Varley, J. A. Automatic Methods for the Determination of Nitrogen, Phosphorus and Potassium in Plant Material. *Analyst* **1966**, *91*, 119–126.
- Verma, P.; Rastogi, R. K.; Ramakumar, K. L. Determination of Trace Amounts of Nitrogen in Uranium Based Samples by Ion Chromatography (IC) without Kjeldahl Distillation. *Anal. Chim. Acta* **2007**, *596* (2), 281–284.
- Vincent, K. R.; Shipe, W. F. The Effect of Calibration Procedures on Accuracy and Precision of Automated Kjeldahl Nitrogen Analysis in Some Formulated Foods. *J. Food Sci.* **1976**, *41* (1), 157–162.
- Vita, O. A. The Determination of Bound Nitrogen in Uranium Hexafluoride with an Ammonia Electrode. *Anal. Chim. Acta* **1976**, *81* (1), 45–52.
- Vivek, B. S.; Krivanek, A. F.; Palacios-Rojas, N.; Twumai-Afriyie, S.; Diallo, A. O. *Breeding Quality Protein Maize Protocols for Developing QPM Cultivars*; CIMMYT: Mexico, DF, 2008.
- Vlcek, J.; Kuban, V. Gas Diffusion Flow Injection Determination of Ammonium Ions in River and Waste Waters by Conductimetry. *Collect. Czech. Chem. Commun.* **1999**, *64* (12), 1966–1974.
- Wall, L. L.; Gehrke, C. W.; Neuner, T. E.; Cathey, R. D.; Rexroad, P. R. Total Protein Nitrogen: Evaluation and Comparison of Four Different Methods. *JAOAC* **1975**, *85* (4), 811–817.
- Wallace, J. M.; Fox, P. F. Rapid Spectrophotometric and Fluorimetric Methods for Monitoring Nitrogenous (Proteinaceous) Compounds in Cheese and Cheese Fractions: A Review. *Food Chem.* **1998**, *62* (2), 217–224.
- Wang, D.; Snyder, M. C.; Bormann, F. H. Potential Errors in Measuring Nitrogen Content of Soils Low in Nitrogen. *Soil Sci. Soc. Am. J.* **1993**, *57* (6), 1533–1536.
- Wang, L.; Oien, A. Determination of Kjeldahl Nitrogen and Exchangeable Ammonium in Soil by the Indophenol Method. *Acta Agric. Scand.* **1986**, *36* (1), 60–70.
- Ward, M. H.; deKok, T. M.; Levallois, P.; Brender, J.; Gulis, G.; Nolan, B. T.; VanDerslice, J. Workgroup Report: Drinking-Water Nitrate

- and Health—Recent Findings and Research Needs. *Environ. Health Perspect.* **2005**, *113* (11), 1607–1614.
- Ward, M. W. N.; Owens, C. W. I.; Rennie, M. J. Nitrogen Estimation in Biological Samples by Use of Chemiluminescence. *Clin. Chem.* **1980**, *26* (9), 1336–1339.
- Watkins, K. L.; Veum, T. L.; Krause, G. F. Total Nitrogen Determination of Various Sample Types. A Comparison of the Halch, Kjeltex and Kjeldahl Methods. *J. Assoc. Off. Anal. Chem.* **1987**, *70* (3), 410–412.
- Watson, M. E.; Galliher, T. L. Comparison of Dumas and Kjeldahl Methods with Automatic Analyzers on Agricultural Samples under Routine Rapid Analysis Conditions. *Commun. Soil Sci. Plant Anal.* **2001**, *32* (13–14), 2007–2019.
- Weaver, K. C.; Kroger, M.; Kneebone, L. R. Comparative Protein Studies (Kjeldahl, Dye Binding, Aminoacid Analysis) of Nine Strains of *Agaricus bisporus* (Lange) Imbah Mushrooms. *J. Food Sci.* **1977**, *42* (2), 364–366.
- Wei, X.; Wu, D.; He, Y. Non-destructive Measurement of Crude Fat and Crude Protein Contents of Oats from Different Geographical Zones in China Using Visible and Near-Infrared Spectroscopy. *Afr. J. Agric. Res.* **2012**, *7* (11), 1703–1710.
- WHO. *Guidelines for Drinking-Water Quality, Third Edition Incorporating the First and the Second Addenda. Vol. 1. Recommendations*; World Health Organization: Genève, 2008.
- WHO. *Nitrate and Nitrite in Drinking Water (Background Document for Development of World Health Organization)*, WHO/SDE/WSH/07.01/16/Rev/1; World Health Organization: Genève, 2011.
- Wickstrom, T.; Ogner, G.; Remedios, G. Effects of Different Pretreatments (Sieving, Milling and Grinding) on Quality of Determination of Kjeldahl Nitrogen, pH, and Extractable Elements in Forest Soils. *Commun. Soil Sci. Plant Anal.* **2004**, *35* (3–4), 369–384.
- Wilde, F. D. Preparation for Water Sampling. In *National Field Manual for the Collection of Water-Quality Data*; U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9; U.S. Geological Survey: Reston, VA, 2005; Chap. 11; http://water.usgs.gov/owq/FieldManual/chapter1/Ch1_contents.html
- Wiles, P. G.; Gray, I. K. A Collaborative Trial for the Establishment of a Skim Milk Powder Reference Protein Standard. *Aust. J. Dairy Technol.* **1996**, *51* (1), 17–21.
- Wiles, P. G.; Gray, I. K.; Kissling, R. C. Routine Analysis of Proteins by Kjeldahl and Dumas Methods: Review and Interlaboratory Study Using Dairy Products. *J. AOAC Int.* **1998**, *81* (3), 620–632.
- Williams, P. C. The Colorimetric Determination of Total Nitrogen in Feeding Stuffs. *Analyst* **1964**, *89* (4), 276–281.
- Williams, P. C.; Norris, K. H.; Johnsen, R. L.; Standing, K.; Frictioni, R.; MacAffrey, D.; Mercier, R. Comparison of Physicochemical Methods for Measuring Total Nitrogen in Wheat. *Cereal Foods World* **1978**, *23* (9), 544–547.
- Williams, P.; Sobering, D.; Antoniszyn, J. Protein Testing Methods at the Canadian Grain Commission. In *Proceedings of the Wheat Protein Symposium, Saskatoon, SK, March 9 and 10, 1998*, University Extension Press: University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 12 pp.
- Willis, R. B.; Schwab, G. J.; Gentry, C. E. Elimination of Interferences in the Colorimetric Analysis of Ammonium in Water and Soil Extracts. *Commun. Soil Sci. Plant Anal.* **1993**, *24* (9–10), 1009–1019.
- Willis, R. B.; Montgomery, M. E.; Allen, P. R. Improved Method for Manual Colorimetric Determination of Total Kjeldahl Nitrogen Using Salicylate. *J. Agric. Food Chem.* **1996**, *44* (7), 1804–1807.
- Worsfold, P. J.; Monbet, P.; Tappin, A. D.; Fitzsimons, M. F.; Stiles, D. A.; McKelvie, I. D. Characterization and Quantification of Organic Phosphorus and Organic Nitrogen Components in Aquatic Systems: A Review. *Anal. Chim. Acta* **2008**, *624* (1), 37–58.
- Wright, W. D.; Wilkinson, D. R. Automated Micro-Kjeldahl Nitrogen Determination: A Method. *Am. Environ. Lab.* **1993** (2), 30–33.
- Yague, M. R.; Quilez, D. Response of Maize Field, Nitrate Leaching, and Soil Nitrogen to Pig Slurry Combined with Mineral Nitrogen. *J. Environ. Qual.* **2010**, *39* (2), 686–696.
- Yao, S. Z.; Su, X. L. Gas Diffusion Flow Injection Analysis with Bulk Acoustic Wave Detection and the Applications for Determining Nitrogen, Carbon, or Sulfite Species in Varieties of Complex Matrices. *J. AOAC Int.* **1999**, *82* (6), 1479–1487.
- Yasuhara, T.; Nokihara, K. High-Throughput Analysis of Total Nitrogen Content That Replaces the Classic Kjeldahl Method. *J. Agric. Food Chem.* **2001**, *49* (10), 4581–4583.
- Zaldivar, J. *Comparison between Kjeldahl and LECO Techniques for Measuring Protein Content of Fish Meals*; IFOMA Research Report 1998–4; International Fishmeal & Oil Manufacturers Association: St. Albans, UK, 1998.
- Zhao, D.; Jai, V.; Farkye, N. Y. Determination of True Proteins in Dairy Products: A Comparative Study between Kjeldahl and Sprint Protein Analyzer. *J. Dairy Sci.* **2010**, *93*, (Suppl. 1), 334.
- Zorgati, W.; Rutledge, D. N.; Feinberg, M. H. The Use of Synthetic Foods to Optimize Microwave-Assisted Digestion Procedures. *Analisis* **2000**, *28* (3), 245–252.