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Metrology for protein content measurement in food

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Abstract:
Suggestions for establishing metrological traceability and respect metrological traceability chain scheme for protein content measurements in food are presented. The most common methods for determining protein content in organic and inorganic substances are reviewed. Particular attention is given to the Kjeldahl method. A method is proposed for ensuring metrological traceability of the Kjeldahl method results through the use of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris base) and hydrochloric or sulfuric acid solutions, whose concentrations are traceable to a primary method—constant current coulometry. A constant current coulometry measurement system has been developed at the Ukrainian DI UMTS and is described in this paper.
Keywords: protein content, Kjeldahl method, metrological traceability, reference material, coulometry

I. INTRODUCTION

Protein is an essential component of a healthy diet because it plays critically important roles in nearly every biological function and structure in the human body [1]. Reliable evaluation of protein content in food enables the design of balanced daily, weekly, monthly, and yearly diets to support human health. Accurate protein determination is also necessary to ensure food safety and fair trade in the food market.

Protein content in food is typically determined using several well-established analytical methods – Dumas method, Nessler reagent method, Biuret method, Wiedemann method, Berthelot's method (alkali-phenol reagent), Kjeldahl method, Folin-Ciocalteu method, dye binding method, Lowry method, direct alkaline distillation, near-infrared reflectance (NIR), modified Berthelot reaction, modified Lowry method, Bradford method (coomassie blue dye-binding method), BCA (bicinchoninic acid method), 3-(4-carboxybenzyl)quinoline-2-carboxaldehyde method, and amino acid analysis [2,3].

Industrial and research laboratories primarily use the Kjeldahl, Dumas, NIR, or amino acid analysis methods, as described in ISO, ASTM, AOAC, and AACC standards [4–10]. The Kjeldahl method is the most commonly used due to its reliability, affordability, and ease of application.

Reliable measurements in industrial and research laboratories require evidence of metrological traceability. This paper explains details of the Kjeldahl method for protein content measurement in food.

II. PURPOSE

This paper compares various methods for measuring protein content in food. The main goal is to determine the optimal approach to ensure reliable traceability to SI units for protein content measurements in food.

III. METHODS OVERVIEW

The following methods are described in ISO, ASTM, AOAC, and AACC standards and are widely used to measure protein content in food.

A. The Kjeldahl method

The Kjeldahl method is a wet-chemistry (digestion-based) technique used to determine protein content by measuring the total nitrogen. It involves three main steps: digestion, distillation, and titration. In the digestion step, all organic nitrogen is converted into ammonium (NH_4^+). During distillation, ammonia (NH_3) is released from ammonium sulfate. In the titration step, the amount of ammonia is quantified by titrating with a standardized acid solution—typically hydrochloric acid (HCl) or sulfuric acid (H_2SO_4)—to calculate the nitrogen content.

B. The Dumas method

The Dumas method is a combustion-based technique for determining protein content by measuring total nitrogen. A small sample (~100-500 mg) is combusted at high temperatures (~900-1000 °C) in the presence of pure oxygen. The organic matter is completely burned, producing CO_2, H_2O, and nitrogen-containing gases (primarily N_2, NO_x, and NH_3). These gases pass through traps that remove CO_2 and H_2O and convert all nitrogen species to molecular nitrogen (N_2), which is then measured using a thermal conductivity detector (TCD). Protein content is calculated based on the nitrogen content.

C. The near-infrared spectroscopy (NIR)

NIR is a non-destructive technique that estimates protein content by analyzing how near-infrared light is absorbed by a sample. NIR operates in the 780–2500 nm wavelength range, detecting overtones and combinations of molecular vibrations, particularly N–H, C–H, and O–H bonds. Proteins contain N–H bonds (from amino groups and peptide bonds), which absorb specific NIR wavelengths. The absorption intensity correlates with protein concentration.

D. The amino acid analysis (AAA)

Amino acid analysis quantifies individual amino acids after complete hydrolysis of the sample. The sample is treated to break all proteins into their constituent amino acids. These are separated using chromatographic techniques, and their concentrations are determined using UV-spectroscopy, spectrofluorometry, or mass spectrometry. The total amino acid content is then converted into a protein content value.

IV. DISCUSSION

All of the described methods – Kjeldahl, Dumas, NIR, and AAA – can be used to determine protein content in food. Considering the advantages and disadvantages presented in Tables 1-4, the Kjeldahl method appears most promising and continues to be widely used in the industrial and research laboratories.

Table 1: Advantages and disadvantages of Kjeldahl method

Advantages	**Disadvantages**
Applicable to most food types	Poison reagents
High reproducibility and accuracy	Long period of time per measurement
Easy to apply	High electricity consumption
Commercially available equipment	
Cheap	

Table 2: Advantages and disadvantages of Dumas method

Advantages	**Disadvantages**
Applicable to most food types	Expensive
High reproducibility and accuracy	High electricity consumption
Commercially available equipment	
Easy to apply	
Short period of time per measurement	

Table 3: Advantages and disadvantages of NIR method

Advantages	**Disadvantages**
Non-destructive	Requires adjustment using CRMs/RMs
Applicable to most food types	Low reproducibility and accuracy
Easy to apply	Sensitive to moisture
Cheap	Sensitive to particle size
Commercially available equipment	Sensitive to sample homogeneity
Low electricity consumption	
Short period of time per measurement	

Table 4: Advantages and disadvantages of AAA method

Advantages	**Disadvantages**
Applicable to most food types	Difficult to apply
Commercially available equipment	Expensive
Low electricity consumption	Long period of time per measurement
High sensitivity	
High reproducibility and accuracy	

In any measurement, a key issue is ensuring metrological traceability. In the widely used Kjeldahl method, traceability is ensured through the use of a protein mass fraction CRM. The using of Tris as a RM for the mass fraction of protein in grain estimation by Dumas method is described in the IUPAC Technical Report [11], and may be applicable for the Kjeldahl method. A reliable titrant during the titration of the ammonium solution is also needed. The titrant is typically a solution of hydrochloric or sulfuric acid, whose concentration is determined using a certified reference material (CRM) of disodium carbonate. Disodium carbonate CRM is widely employed for the standardization of acid titrants. Its value is characterized using a primary method—constant current coulometry. Constant current coulometry can also be used to directly measure the concentration of titrants (hydrochloric or sulfuric acid solutions), but this is generally not practical. In the Kjeldahl method, the consumption of titrant is high, making direct coulometric determination expensive. Moreover, constant current coulometry requires the use of only high-purity reagents due to its stringent method requirements. Impurities from low-quality reagents can damage the measuring system (e.g., platinum electrodes, gel membranes) and lead to increased uncertainty in determining the titration end-point. By using disodium carbonate CRM, the Kjeldahl method can provide traceability to SI units while remaining cost-effective. A schematic of the metrological traceability chain is shown in Fig. 1.

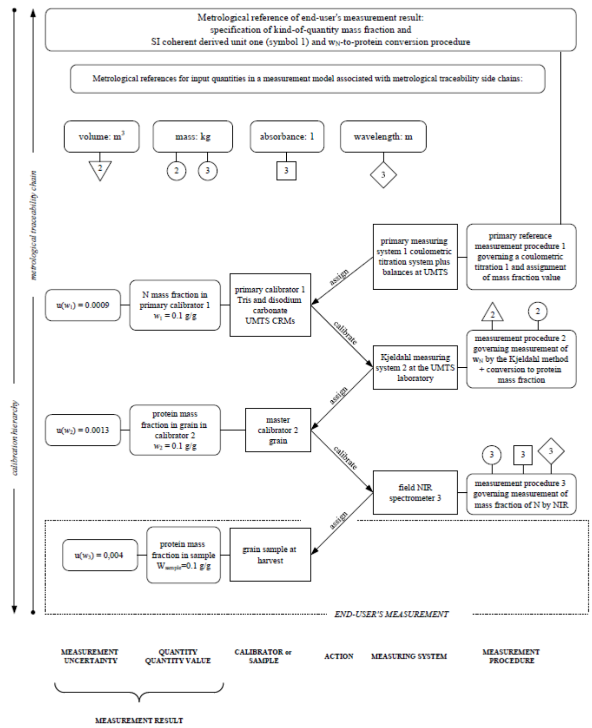

Figure 1. Metrological traceability chain of a measurement result of mass fraction of protein in food. (Measurement uncertainties are given as standard measurement uncertainties in mass fraction units.)

Constant current coulometry is one of the key electrochemical methods used in the National Metrology Institutes (NMIs) and Designated Institutes (DIs), which are responsible for ensuring accurate measurements at the highest metrological level and for establishing national primary standards. This precise method is based on Faraday's laws. According to Faraday, the amount of material deposited or evolved during electrolysis is directly proportional to the current and time, i.e., to the quantity of electricity passing through the solution (first law). The quantity of the product also depends on the equivalent mass of the substance being electrolyzed (second law).

Based on these principles, a measurement system for implementing the constant current coulometry method has been developed at UMTS [12], Ukrainian DI for metrology in chemistry. The system is shown in Figure 2. This development was carried out in collaboration with the Institute of Electrodynamics of the National Academy of Sciences of Ukraine (IED) and the Scientific-Production Center "Energoimpuls". Using this system, UMTS assays Tris and disodium carbonate.

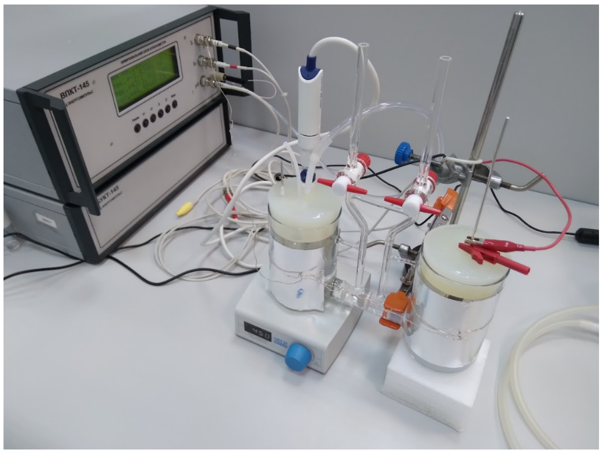

Figure 2. Constant current coulometry system developed at UMTS

V. SUMMARY

Certified reference materials (CRMs) are essential for measurement laboratories using the Kjeldahl method to determine protein content in food. By using Tris and disodium carbonate CRMs, the Kjeldahl method remains cost-effective while enabling traceability to SI units, thereby supporting food safety and fair trade in the food market.

Constant current coulometry can be used to certify CRMs that support the Kjeldahl method. A corresponding constant current coulometry measurement system was developed at the UMTS.

REFERENCES

[1] "Protein and amino acid requirements in human nutrition", Report of a Joint WHO/FAO/UNU Expert Consultation, Geneva, Switzerland, 2002.

[2] P. Sáez-Plaza, T. Michałowski, M.J. Navas, A.G. Asuero, S. Wybraniec, "An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish", Critical Reviews in Analytical Chemistry, vol. 43, 2013, pp. 178–223.

[3]M. Fountoulakis, H.-W. Lahm, "Hydrolysis and amino acid composition analysis of proteins", J. Chromatogr. A, vol. 826, 1998, pp. 109–134.
[4]"ISO 8968-1:2014 | IDF 20-1:2014 Milk and milk products — Determination of nitrogen content Part 1: Kjeldahl principle and crude protein calculation", Geneva, Switzerland, 2014.
[5]"ISO 16634-1:2008 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 feeding stuffs", Geneva, Switzerland, 2008.
[6]"ISO 12099:2017 Animal feeding stuffs, cereals and milled cereal products — Guidelines for the application of near infrared spectrometry", Geneva, Switzerland, 2017.
[7]"ISO 13903:2005 Animal feeding stuffs — Determination of amino acids content", Geneva, Switzerland, 2005.
[8]"ASTM D3590-17 Standard Test Methods for Total Kjeldahl Nitrogen in Water", ASTM International, West Conshohocken, USA, 2017.
[9]"AOAC 992.15-1996 Crude protein in meat and meat products", AOAC international, Rockville, USA, 1996.
[10]"39-00.01 Near-Infrared Methods —Guidelines for Model Development and Maintenance", AACC International, St. Paul, USA, 1999.
[11]P. De Bièvre, R. Dybkaer, A. Fajgelj, D. Brynn Hibbert, Metrological traceability of measurement results in chemistry: Concepts and implementation (IUPAC Technical Report), Pure Appl. Chem., vol. 83, No. 10, 2011, pp. 1873–1935.
[12]O.O. Lameko, A.V. Petrenko, V.G. Melnyk, O.D. Vasylenko, O.O. Melnykov, "Development of apparatus construction principles for the implementation of the amperostatic coulonometry method", Technical Electrodynamics, vol. 6, 2024, pp. 83–91