Determination of Crude Protein Content in Diverse Standard Reference Materials and Infant Formulas Using the Dumas Combustion Method

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Abstract

Crude protein was determined through total nitrogen content by the combustion-based Dumas method for diverse standard reference materials (SRMs), infant formulas, and spike recoveries in accordance with a Level 2 validation study. For the SRM studies, results were collected using both the digestion-based Kjeldahl method and the Dumas method. For SRM 1849a, with a certificate of analysis (COA) declared protein content of 13.225 ± 0.056 g/100 g, the Kjeldahl and Dumas methods detected 12.75 ± 0.03 and 13.12 ± 0.01 g/100g, respectively. Other SRMs were chosen in a range of protein content from 7.25 ± 0.18 to 66.92 ± 0.61 g/100g. For each SRM, results using the Dumas method were found to have improved precision and accuracy compared to results from Kjeldahl analyses. Further Dumas analyses with spike recoveries were performed on milk-based, soy-based, and liquid infant formulas with RSDs ranging from 0.10 to 2.25% and average spike recoveries ranging from 98.96 to 103.73%. Dumas analyses were also performed in triplicate on a wide variety of food, medical food and infant formulas, and protein supplements, in powder, liquid, and composite matrices, ranging from 2.5 to 75% protein content. These results were compared to their respective declarations as a model for a screening method used in conjunction with subsequent Kjeldahl analysis for violations.

Introduction

One of the primary missions of the Nutrient Analysis Branch (NAB) in the U.S. Food and Drug Administration is to test and verify the declared nutritional claims of domestic and import products.[1] Commodities that are routinely tested at NAB include infant formulas, medical foods, foods, and, increasingly, dietary supplements. Among the many nutrients analyzed within these products is protein, a class of macromolecules polymerized from amino acids, and necessary for the development, maintenance, and repair of bodily tissues and enzymes.[2] As a desired nutrient, protein has historically been subject to economic adulteration such as the dilution of milk with water and the substitution of chalk within flour.[3] Because protein dilution in processed foods is easy to conceal through the addition of filler solids or solvent liquids, powdered and liquid foods such as infant formulas, medical foods, and, more recently, protein supplements are highly vulnerable to bad actors and require vigilance and verification of the actual protein content.

Since its beginnings in the 19th century,[4,5] protein analysis has been and remains a challenging field primarily due to the inherent variability in source, composition, structure, and size of protein molecules.[2] As the methods of protein analysis have developed, there have been two primary directions: to determine either the “true” protein content[6,7] or the approximate “crude” protein content within a given sample.[4-6]

With regard to the first option, determining "true" protein content, the protein chain must first be systematically disassembled before the resultant amino acids can be quantified. However, diverse treatments are required to properly hydrolyze all of the constituent amino acids;[7] such treatments may result in added time, waste, and potential error. For this reason, quantitation of true protein is not routinely performed at NAB. The second option of approximating the total protein through crude protein may be acquired either spectrally, e.g., infrared spectroscopy, through colorimetry via binding agents, or through the determination of total nitrogen content of a given sample [4-6] with the use of Jones Factors.[8] The two most widely used techniques for total nitrogen content determination are the digestion and titrimetric-based Kjeldahl method,[4] and the combustion-based Dumas method.[5]

The Kjeldahl method of total nitrogen determination, which is currently employed at NAB for regulatory samples, is based on the heavy metal-catalyzed acid digestion of proteins such that all nitrogen within the sample is converted into ammonium sulfate.[4] The acid digest is subsequently treated with base, liberating the nitrogen into the form of gaseous ammonia, which in turn is streamed into a standardized aqueous acid solution. Back titration with a boric acid titrant is directly correlated to the amount of ammonia taken into the acid solution, and, hence, the total nitrogen content of the original sample.[5] The advantages of this method are its reliability, relative simplicity, and the availability of relatively low-priced instrumentation and supplies. The disadvantages of the method are the generation of copious amounts of caustic and toxic waste, general safety risks associated with pressurized caustic substances, and a certain loss in precision from the open system in the detection of the analyte gas.[6]

By comparison, the Dumas method is dependent on the total combustion of sample into gaseous products and the subsequent catalytic conversion of nitrous oxide gases (NO_x) into nitrogen gas.[5] The main advantages are that the system is much cleaner, safer, and more precise because of the closed system employed. The primary disadvantages for the Dumas method are the initial cost in instrumentation, and the more complex automation which requires greater upkeep. A central weakness in both the Kjeldahl and Dumas methods, however, is inherent in their quantitation of nitrogen as they are both able to be fooled by the addition of non-proteinaceous, nitrogen-rich molecules, e.g., melamine, or ammonium species.[9] For this LIB we will compare results between these two methods for the purpose of validating the use of the Dumas method according to OFVM's Guidelines for the Validation of Chemical Methods for a single lab validation (i.e., Level Two Validation),[10] as well as extending the method across a greater degree of matrices than is covered by the original AOAC method.[11]

Experimental

The following equipment and reagents are listed as a guide; substitutions may be made.

Equipment

(a) Leco TruMac N-Analyzer
(b) Velp Scientifica UDK 152 Distillation & Titration Unit
(c) Sartorius CPA 124S mass balance (attached to Leco TruMac)
(d) Mettler AT261 DeltaRange mass balance
(e) Foss Tecator 2000 digestor/block heater with controller
(f) Consumables
    1) Ceramic weigh boats (Leco)
    2) Nickel inserts for weigh boats (Leco)
    3) 250 mL test tubes (Foss)

Reagents

(a) Dumas Method Reagents
    1) LecoSorb (Solid sodium hydroxide) (Leco)
    2) Anhydrone (Magnesium perchlorate) (Leco)
    3) Catalyst (Platinum/Rhodium catalyst on aluminum oxide substrate) (Leco)
    4) Copper filings, copper sticks (Leco)
    5) UHP Helium
    6) UHP Oxygen
    7) Compressed Air
    8) Glass wool
    9) Steel wool
(b) Kjeldahl Method Reagents
    1) Sulfuric Acid
    2) Sodium Hydroxide solution, 40% (w/w)
    3) Kjeltabs ($CuSO_4$, K_2SO_4)
    4) Kjel-Sorb (saturated boric acid with indicator)
(c) Standards
    1) Ethylenediaminetetraacetic acid (EDTA) (Leco)
    2) Nicotinic Acid, ≥99% (Sigma Aldrich)
    3) Hydrochloric Acid 0.1 N (ERA)

(d) Standard Reference Materials

1) 1849a Infant Formula/Medical Food (NIST)
2) 2387 Peanut Butter (NIST)
3) 3234 Soy Flour (NIST)
4) 3252 Protein Drink Mix (NIST)
5) 3290 Dried Cat Food (NIST)
6) 1548a Typical Diet (NIST)
7) 3233 Fortified Breakfast Cereal (NIST)

Sample Preparation

All SRMs were stored per certificate specifications, e.g., SRM 1849a Infant Formula/Medical Food and SRM 2387 Peanut Butter were stored at -80 °C, to prevent both spoilage and the addition of moisture to the standards. All SRMs came up to room temperature prior to analysis.

All non-SRM samples (e.g., infant formulas, protein bars, etc.) were homogenized prior to analysis, and stored according to sample type. Powders were stored at room temperature, composites stored in refrigerator or freezer, and liquids stored in refrigerator. All samples came up to room temperature prior to analysis.

All powder standards were kept in a desiccator at room temperature. All aqueous standards were kept sealed in their original containers, and wrapped with parafilm.

Procedure

Dumas Method

1. A calibration curve was created by weighing and analyzing EDTA standards (Figure 1) in triplicate at masses of 0.010, 0.025, 0.050, 0.100, 0.250, 0.500, 0.750, and 1.000 g into tared ceramic weigh boats which were placed into the Leco TruMac N-Analyzer via automation.

2. Prior to each use, the system was evaluated for leaks and for general operation, and two wake-up blanks were performed.

3. For all sample batch analyses, three empty ceramic weigh boats were analyzed in the instrument for blank analysis. Following the blank analysis, three 0.500 g EDTA samples were placed into tared ceramic weigh boats and analyzed in the instrument for drift calibration.

4. One 0.500 g Nicotinic acid sample (Figure 1) was collected as an independent calibration verification (ICV) sample.

5. Samples were weighed into tared ceramic weigh boats such that the total nitrogen content was within the limits of the calibration curve, typically in the range of 0.500-2.000 g.

6. For all liquid samples, a nickel liner was inserted into the ceramic weigh boat prior to taring.

7. Continuous calibration verifications (CCV) were made after every 10 sample runs, and at the end of a sample batch.

8. For all spikes, a sample was first weighed into a tared ceramic weigh boat and its mass recorded under sample mass; the weighed boat was re-tared, and an aliquot of EDTA standard was added to the tared sample and its spike mass recorded in the sample name, e.g., 'Protein Sample 0.1234 g EDTA'. Analysis then proceeded as normal.

Kjeldahl Method

1. Prior to use, the UDK system was set to a cleaning cycle, all bulk reagents were refilled, and the functionality of the water flow to and from the condenser was verified.

2. Samples were weighed and placed in individual 250 mL glass test tubes. Samples were weighed out such that the total nitrogen content would be within the limits of the titrant, typically in the range of 0.5-2.0 g.

3. 10.0 mL of concentrated sulfuric acid and 1 Kjeltab were added to each test tube. Test tubes were placed and secured in a block steam heater set for 400 °C, and the samples digested overnight.

4. After cooling, the test tube digests were collected and sequentially run through the UDK system in the order of blank analysis, reagent blank analysis, and samples. SRM 1849a was used for CCV for the analysis.

5. Following each analysis, the test tube was manually removed and replaced by the analyst with the subsequent sample digest.

Calculations

Percent crude protein (% Protein) was determined by multiplying the observed percent nitrogen (% Nitrogen) by the appropriate protein factor (Jones Factor) (Table 1).

% Protein = (% Nitrogen) x **(Jones Factor)**

Results and Discussion

We began our investigation with Dumas and Kjeldahl treatments of SRM 1849a for infant formula and medical foods due to NAB's historic interest in infant formula and medical food analysis, and its possession of years-worth of crude protein data from prior Kjeldahl analyses (Figure 2a). This data collection revealed the extent of variability in results for the standard reference material over the years ranging from 11.07 to 13.80% protein. While this range is likely due to an aggregation of minor error sources, it served as our point of comparison for the results from the Dumas analyses. Both intensive and periodic samples of SRM 1849a were collected and evaluated using the Dumas method over a two-year period (Figure 2b) to compare to the five-year timeline of Kjeldahl results.

Though these Dumas results over time exhibited their own variability, they were both more precise and provided closer agreement to the COA for SRM 1849a, suggesting the day-to-day greater precision and accuracy that could be gained with use of the Dumas method (Table 2, Figures 2-3). Indeed, one data point for the Dumas method for SRM 1849a closely matched the mean of the prior Kjeldahl results, but was itself rejected as an outlier within the Dumas dataset per Grubbs' test. Further testing with SRM 1849a involved the possible use of non-UHP oxygen for combustion as a cost-saving step (Figure 2, circled portion): all contemporaneous measurements experienced an approximate two percent increase in perceived nitrogen content, and blanking the increase did not sufficiently correct the higher error observed. This increase was attributed to gaseous impurities in the tank, either nitrogen or argon, that became incorporated within the sample as part of the combustion process and registered on the detector as nitrogen. Changing back to UHP oxygen for the combustion produced nitrogen measurements in agreement with COA.

Observing a clear difference between the Dumas data points and the historical Kjeldahl results, it was appropriate to consider the possible effect of Jones factors in both the Kjeldahl and Dumas methods. Many scientists have observed that Dumas results tend to report greater protein amounts than Kjeldahl, but theories differ as to the exact cause of the higher bias, e.g., a conversion of inorganic nitrogen containing species in the Dumas method or the presence/absence of wet chemical error in the Kjeldahl/Dumas method. While this debate is beyond the scope herein, some have argued for secondary

factors to be used in order to convert Dumas results into Kjeldahl results.[6a] However, the authors of this bulletin have found little to no evidence that this call has been implemented either by industry, government,[12] greater academia, or in any applicable AOAC method.[11] Similarly, the National Institute of Standards and Technology (NIST) regularly compiles Kjeldahl and Dumas data into their protein findings, using identical factors for the two analyses.[13]

Continuing to use identical Jones factors between the two methods, six more SRMs of various protein compositions ranging from 7.25 ± 0.28 to 66.92 ± 0.61 g/100 g were chosen based on samples to be analyzed at NAB, e.g., protein content ≥ 10 g/100 g. Each SRM was analyzed by the Dumas and Kjeldahl methods, and the results of these analyses are presented in Table 2. Overall comparisons of Dumas and Kjeldahl results for the diverse matrices revealed closer and more consistent agreement of the Dumas results with COA values than did the Kjeldahl results (Table 2, Figures 4-5). The difference between mean Dumas results and COA values ranged from 0.05 to 3.5%; the observed difference between mean Kjeldahl results and COA ranged from 0.18 to 4.69%. The analyses of SRM 3234 (Figure 5) by Kjeldahl and Dumas methods displayed the greatest deviation in mean results from a COA value; however, these two methods consistently matched each other, albeit with greater precision in the Dumas data points. While the differences in the data between these methods were subtle, they revealed the ability of the Dumas method to meet or excel beyond the official Kjeldahl method of analysis.

Having observed high precision and accuracy of Dumas results in the diverse SRMs, the next step was to investigate the precision of Dumas results within commercial matrices. In view of the mission of NAB, a series of analyses on various infant formulas was conducted. Guidelines for the Validation of Chemical Methods for the OFVM's programs were followed for the single lab validation (i.e., Level Two Validation) of the method.[10] Milk-based and soy-based powdered infant formulas and various liquid infant formulas (i.e., milk-based, soy-based, and amino acid-based) were investigated to ensure breadth of coverage. Three of the liquid samples were formed from commercial powders and reconstituted per the product instructions. For each sample, excellent precision was observed with repeatability relative standard deviation (RSD_r) values of ≤ 2.25% (Table 3). As a further check on the precision and accuracy of the system, a series of EDTA spikes was conducted (Table 4), with recoveries for powder samples ranging from 99-100%, and for liquid samples ranging from 102 to 104%.

The excellent results for infant formulas encouraged a cursory analysis into the suitability of the Dumas method with other food and dietary supplement matrices (Table 5). Dumas results for these samples were routinely collected in triplicate, and this has been found to be highly useful as a good quality assurance practice. Three main sample subsets were selected to ensure a diverse and comprehensive array of

products typically analyzed at NAB: protein supplements, infant formulas/medical foods, and processed foods. Different states of samples were also investigated, i.e., powder, liquid, or solid composite. Judging from the results obtained, these sample states had little effect on the precision of the analysis; however, sample homogeneity was found to be the greatest positive contributor to precision in the analysis.

Dumas results for some samples revealed what would be considered as violations, either in deficiency or excess in crude protein levels. These results were checked via the Kjeldahl method, and in each case the violation and its extent were confirmed (Table 6). Indeed, this agreement between two orthogonal methods was taken to be an inherent benefit from a regulatory standpoint: when two substantially differing chemical pathways consistently provide similar responses, there is more confidence in the results, especially regarding violative samples. Again, these methods are ideal for violations based on insufficient protein, and both can be fooled by bad actors incorporating non-proteinaceous nitrogen-rich sources.[9]

Having demonstrated the usefulness of both methods, a final series of examinations considered the quality of life differences between them. These qualitative aspects included: sample throughput/efficiency, waste generation, and general safety. While the Kjeldahl method, as currently practiced at NAB, relies on a manual sample transfer system, many of the observations derived from its use may also apply to automated systems as the initial digestion step is common to both. In general, however, our findings correlated strongly with others in the field that the Dumas method was faster, cleaner, and safer than the Kjeldahl method.[6c, 9]

For sample throughput comparisons between the Dumas and Kjeldahl methods, the primary determinant of speed and efficiency was the initial digestion step. Though some analysis times for automated Kjeldahl systems cite sample throughput in terms comparable to Dumas analysis, e.g. 12 minutes for the first sample and 3 minutes for subsequent samples on the automated Kjeldahl [6a,c] versus 6 and 3 minutes for the automated Dumas,[6c] the digestion step for the Kjeldahl significantly is not included within such figures.[6c] The high temperatures and pressures required for this sulfuric acid digestion require time in order to come up to 400 °C, maintain the temperature, and then cool down the digest and glassware. By comparison, the combustion of the sample in the Dumas method is by necessity factored into the sample analysis time. As an illustrative example, samples by their nature have unknown amounts of protein despite having a declaration. Using either method, a given sample aliquot may exceed the titrimetric limit/calibration curve and require a re-run. Assuming a still-running batch, this re-run time will add a second weigh-out and digestion time for the Kjeldahl method in addition to the three-minute

analysis, e.g., 1-4 hours, depending on the digestor; for the Dumas, the re-run time will add a second weigh-out time and a three-minute run time, e.g., 4 minutes.

The second quality of life aspect considered was cleanliness, and the difference in waste generation between the Kjeldahl and Dumas methods was significant. For the manual and automatic Kjeldahl, both the initial digestion process and the titrimetric process become waste generators. The digestion process employs toxic heavy metals such as copper or mercury to catalyze the reaction, and, coupled with the caustic acids and bases, gallons of environmentally toxic waste are generated within relatively few sample batches. By contrast, the toxic waste from the Dumas method is the spent sodium hydroxide and magnesium perchlorate powders, and is solely derived from the scrubber system; over 900 sample analyses were performed to generate enough waste to fill a 500 mL waste container. Further waste from the Dumas method, e.g., ash, glass wool, and rusted steel, are considered universal waste and can be disposed in a trash can.

Cleanliness of the procedure also impacts the safety, the third quality of life issue considered in the study. The copious waste generated in the Kjeldahl method must be disposed, leading to hidden costs in waste removal, transport, and costs to workers' health operating around the waste. Prior to the generation of waste are the digestion and titration steps which have their own hazards. The initial digestion common to both manual and automated systems employs neat sulfuric acid which is pressurized and heated to 400 °C. During the titration step, manual burns are a potential hazard with the manual systems, while exposure to chemical fumes, and dangers with glass that has been alternately heated and cooled under pressure are common to both manual and automated methods. In comparison, the Dumas method instrumentation uses pressurized air, oxygen, and helium gas cylinders, common to any scientific lab.

Conclusion

The Dumas analysis offers an analysis of crude protein that compliments the currently used Kjeldahl method. The Dumas analysis meets or excels the current methodology in terms of accuracy and precision while offering clear advantages with regards to cleanliness and safety over the Kjeldahl method. Application of the technique across diverse standard reference matrices, commercial infant formula sample matrices, and diverse food and dietary supplement matrices showed the wider application possible for the method than is currently employed by the AOAC method. Furthermore, the use of the Dumas method in addition to the Kjeldahl method provides orthogonal support for the determination of violative samples.

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Table 1. Specific (Jones) factors for converting observed % Nitrogen to %Protein[8a,b]

Protein Origin	Factor
General	6.25
Amino Acid	1.2
Barley	5.38
Castor Bean	5.3
Millets/Oats	5.83
Milk/Whey	6.38
Rice	5.95
Rye	5.83
Soybean	5.71
Peanuts	5.46
Wheat (Kernel)	5.83
Wheat (Bran)	6.31
Wheat (Endosperm)	5.7

Table 2. Comparison of Certificates of Analysis, Dumas results, and Kjeldahl results for diverse matrices

Matrix	SRM	COA (g/100g, μ ± U)	Dumas (μ ± S.E.)	Kjeldahl (μ ± S.E.)
Infant formula/Medical Food	1849a	13.225 ± 0.056	13.12 ± 0.01	12.75 ± 0.03
Peanut Butter	2387	22.2 ± 0.5	21.90 ± 0.08	21.73 ± 0.15
Soy Flour	3234	53.37 ± 0.36	50.06 ± 0.09	49.38 ± 0.25
Protein Drink Mix	3252	66.92 ± 0.61	63.97 ± 0.15	62.23 ± 0.63
Dried Cat Food	3290	32.77 ±0 .30	31.24 ± 0.04	30.38 ± 0.14

Typical Diet	1548a	18.08 ± 0.42	15.06 ± 0.06	Not performed
Fortified Breakfast Cereal	3233	7.25 ± 0.18	7.30 ± 0.18	7.068 (n=1)

Table 3. Replicate measurements of crude protein according to the Dumas method in diverse infant formula matrices and comparison to declarations

Matrix	Amount Found (g/serving)				Ave	S.D.	%RSD	Declared Amt	% Declared
Milk-Based Powder									
SRM 1849a	13.11	13.07	13.07	---	**13.08**	0.02	**0.16**	13.225	**98.92**
M.P. 1	2.24	2.18	2.20	2.17	**2.20**	0.03	**1.37**	2	**109.97**
M.P. 2	2.51	2.51	2.47	2.51	**2.50**	0.02	**0.79**	2.1	**119.10**
M.P.3	2.40	2.39	2.40	2.40	**2.39**	0.01	**0.60**	2.1	**113.81**
	2.36	2.39	---	---					
Soy-Based Powder									
S.P.1	2.59	2.60	2.59	2.59	**2.59**	0.004	**0.17**	2.5	**103.70**
S.P.2	2.61	2.61	2.61	2.61	**2.61**	0.003	**0.10**	2.5	**104.40**
S.P.3	2.57	2.56	2.56	2.57	**2.57**	0.01	**0.25**	2.45	**104.73**
	2.56	2.57	---	---					
Liquids									
Milk-Based Liquid									
SRM 1849a (Reconstituted)	13.11	13.07	13.07	---	**13.08**	0.08	**0.62**	13.225	**99.65**
L.1	2.51	2.52	2.51	2.50	**2.51**	0.01	**0.25**	2.5	**100.38**
L.2	2.40	2.39	2.40	2.40	**2.39**	0.04	**1.90**	2.07	**101.39**
	2.36	2.39	---	---					
Amino Acid-Based Liquid									
L.3	16.79	17.01	17.52	16.65	**16.99**	0.38	**2.25**	16	**106.21**
Soy-Based Liquid									
L.4	2.49	2.46	2.42	2.50	**2.47**	0.04	**1.90**	2.5	**98.73**

Table 4. Spike recoveries according to the Dumas method in diverse infant formula matrices

Matrix	Spike Level	Spike Recovery (%)			Ave	Replicates
Milk-Based Powder						
SRM1849a	50%	100.40	100.34	99.77	**100.17**	3
	100%	99.69	99.74	99.78	**99.74**	3
	200%	100.21	100.37	100.08	**100.22**	3
M.P.3	50%	100.28	99.73	99.03	**99.68**	3
	100%	99.76	99.89	97.22	**98.96**	3
	200%	100.00	99.98	100.13	**100.04**	3
Soy-Based Powder						
S.P.3	50%	100.07	99.85	100.04	**99.99**	3
	100%	99.98	100.01	100.03	**100.00**	3
	200%	100.04	100.00	99.93	**99.99**	3
Liquid						
SRM 1849a (Reconstituted)	50%	106.33	104.34	101.66	**103.73**	4
		102.60	---	---		
L.2	50%	102.73	103.93	101.54	**102.73**	3
	100%	102.24	104.06	103.52	**103.27**	3
	200%	102.03	101.29	102.32	**101.88**	3

Table 5. Comparison of Dumas results to declared values in diverse sample matrices

Matrix	State	Protein (g/100 g) ($\mu \pm \sigma$)	Declared	Dumas Mean (n=3)	% Declared
Infant Formula (A)	Powder	14.41 ± 0.08	13 g	14.41 g	111%
Infant Formula (B)	Powder	22.45 ± 0.14	2.3 g	2.35 g	102%
Infant Formula (C)	Powder	11.74 ± 0.02	22 g	22.45 g	102%
Lysine Supplement	Powder	67.15 ± 0.41	1.50 g	1.98 g	132%
Protein Shake Mix (A)	Powder	85.52 ± 0.08	24 g	27.37 g	114%

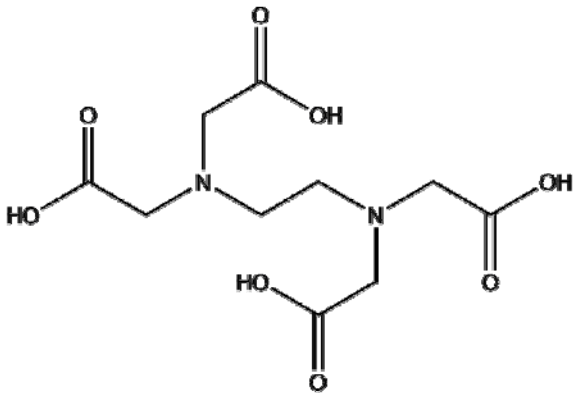
Protein Shake Mix (B)	Powder	70.39 ± 0.06	24 g	24.29 g	101%
Whey Protein Drink Mix	Powder	37.98 ± 0.10	26 g	12.57 g	48.4%*
Blue Corn Flour	Solid	7.672 ± 0.037	8 g	7.67 g	95.9%
Dried Taro	Solid	51.62 ± 0.07	23 g	51.62 g	224%*
Nut Bar	Solid	10.00 ± 0.26	7 g	4.00 g	57.1%*
Peanut Butter	Solid	22.35 ± 0.47	7 g	7.17 g	102%
Protein Bar (A)	Solid	22.98 ± 0.22	10 g	9.65 g	96.5%
Protein Bar (B)	Solid	35.24 ± 0.21	21 g	21.24 g	101%
Frozen Turkey Meal	Composite	7.033 ± 0.101	22 g	17.93 g	81.5%
Protein Tea Drink	Liquid	2.232 ± 0.001	12 g	10.64 g	88.7%
Protein Shake	Liquid	3.216 ± 0.007	10 g	10.00 g	100%

*Check analyses performed using Kjeldahl method on UDK a

Table 6. Comparison of Dumas and Kjeldahl results in sample matrices where declaration exceeded ± 20%

Matrix	Declared	Dumas Mean (n=3)	Dumas % Declared	Kjeldahl Mean (n=2)	Kjeldahl % Declared
Whey Protein Drink Mix	26 g	12.57 g	48.4%	12.64 g	48.6%
Dried Taro	23 g	51.62 g	224%	49.75 g	216%
Nut Bar	7 g	4.00 g	57.1%	4.28 g	61.1%

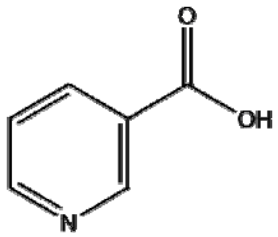
Figure 1. Standards with molecular weight and percent nitrogen content



Ethylenediaminetetraacetic acid (EDTA)

Molecular Weight: 292.24 g/mol

Percent Nitrogen content: 9.59% at 100% purity
(Leco standard COA at 9.56 ± 0.02)



Nicotinic Acid

Molecular Weight: 123.11 g/mol

Percent Nitrogen content: 11.38% at 100% purity
(Sigma-Aldritch COA at ≥ 99.5% purity)

Figure 2. Comparison of Kjeldahl vs. Dumas results for SRM 1849a

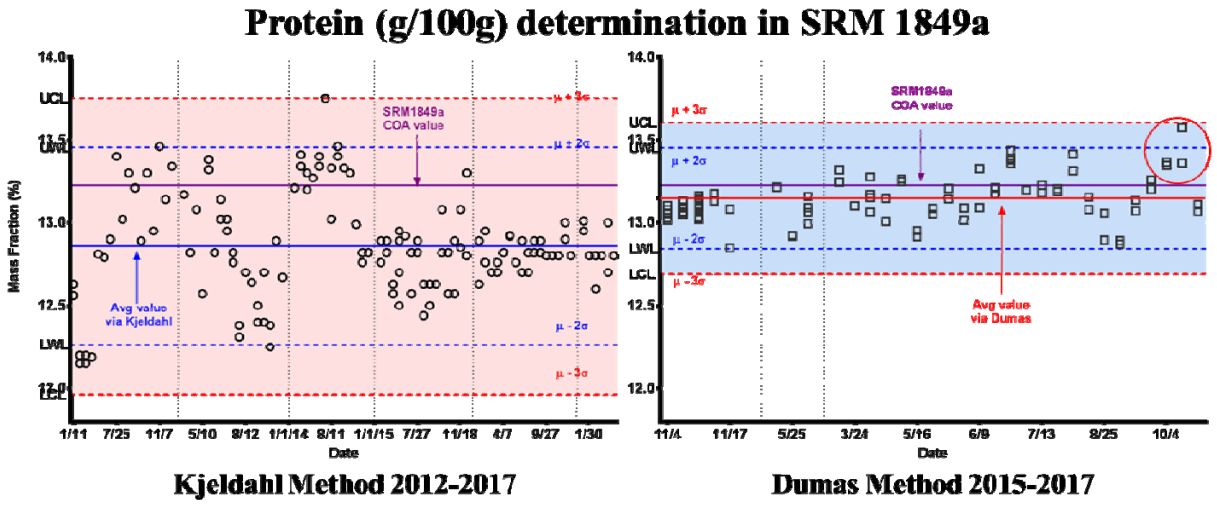


Figure 3. Precision and accuracy comparison of Dumas vs. Kjeldahl methods in SRM 1849a

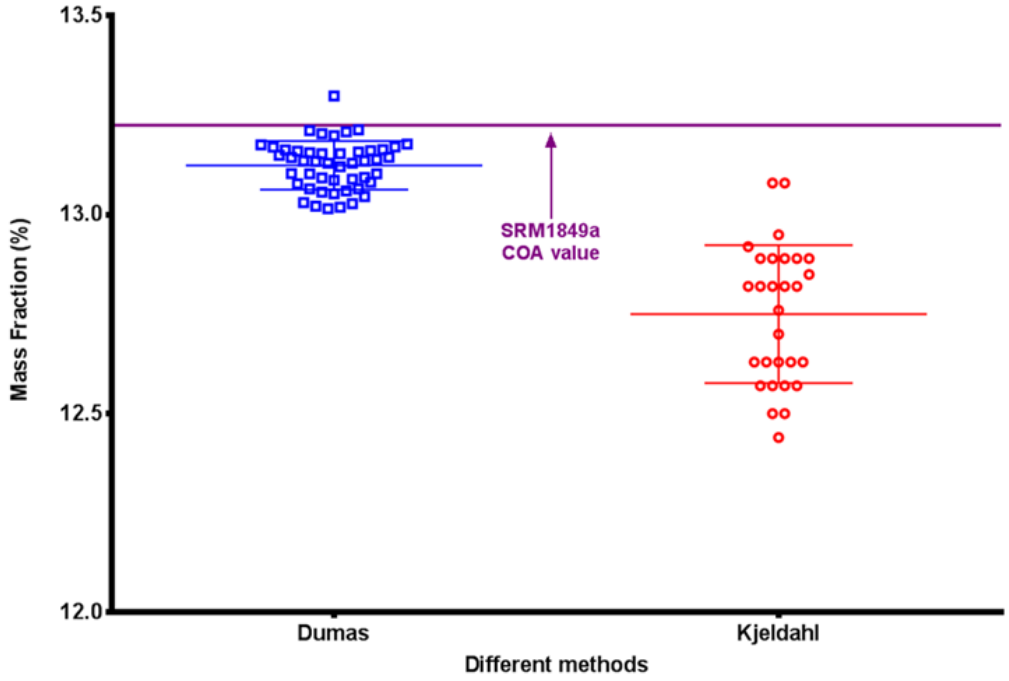


Figure 4. Precision and accuracy comparison of Dumas vs. Kjeldahl methods in SRM 3252

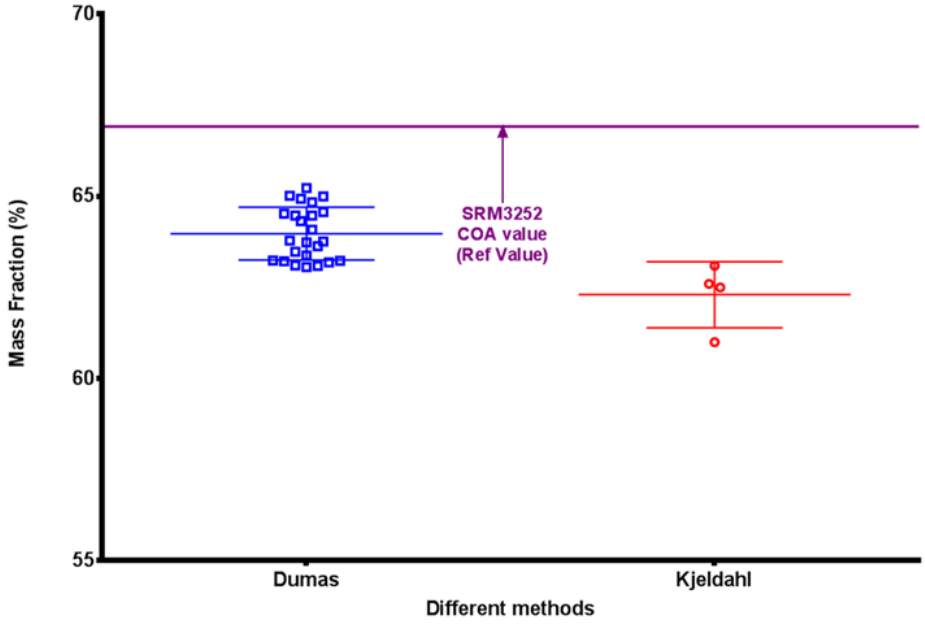
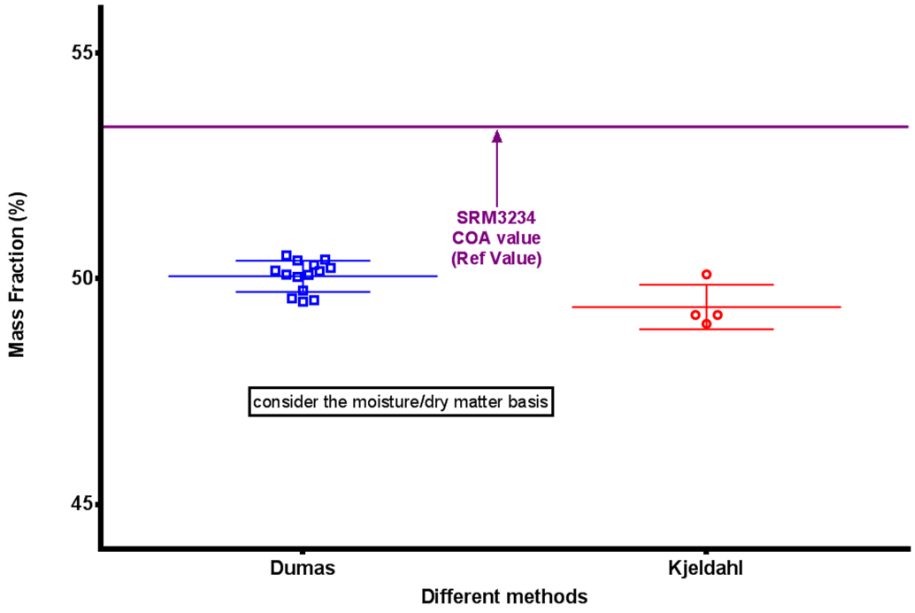


Figure 5. Precision and accuracy comparison of Dumas vs. Kjeldahl methods in SRM 3234


55
50
45
Mass Fraction (%)
SRM3234
COA value
(Ref Value)
consider the moisture/dry matter basis
Dumas
Kjeldahl
Different methods