

NITROGEN CONTENTS IN FOOD: A COMPARISON BETWEEN THE KJELDAHL AND HACH METHODS

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Abstract

The objective of the present work is to compare the nitrogen contents measured by the fast method of Hach and the traditional Kjeldahl method, allowing for the influence of the variation in structure and macronutrient in the food content. Foods with a wide range of protein and moisture contents were selected. The nitrogen content of 25 food samples was measured with both methods.

Some studies have compared the effectiveness of the Hach methods with that of the Kjeldahl methods. This study emphasizes the evaluation of the digestion stage of both methods. In order to carry out an analytical quality control and to determine the accuracy and precision of both methods, two reference samples, elaborated by the Institute of Nutrition of the University of Mahidol, Thailand, were also analyzed. One was based on fish flour and the other on a mix of cereal and soybean products. The variance and linear regression between both methods were analyzed. The Tukey multiple comparison test was used to compare the means when significant differences were found in the variance analysis (Statgraphics). The correlation coefficient between the results obtained in both methods was 99%, suggesting that the Hach method can be used as an alternative to the Kjeldahl method. Despite its being slightly less accurate and precise than the Kjeldahl method, the Hach method exhibits some advantages, such as a lower consumption of reagents, a smaller sample size and mainly, the shorter time required.

Resumen

El objetivo del presente trabajo fue comparar los valores obtenidos con el método tradicional Kjeldahl para determinar nitrógeno en alimentos, con aquellos determinados con el método rápido de Hach, considerando la influencia de la variabilidad existente en los alimentos respecto a matriz alimentaria y contenido de nutrientes. Se seleccionaron alimentos con un amplio rango de contenido acuoso y proteínas. Se determinó el contenido de nitrógeno por ambos métodos en 25 muestras de alimentos.

Varios estudios han comparado la efectividad del método de Hach con la del método de Kjeldahl. Este estudio se centra en la evaluación de la etapa de digestión de ambos métodos. Con la finalidad de realizar un control de la calidad analítica y determinar exactitud y precisión en ambos métodos, dos muestras de referencia elaboradas por el Instituto de Nutrición de la Universidad de Mahidol, Tailandia, se analizaron simultáneamente, una de las cuales era una harina de pescado y la otra una mezcla de cereales y productos de soja. La variabilidad entre los dos métodos fue del 1%. Se realizaron los análisis de varianza y regresión con el método lineal. El test de comparación múltiple de Tukey fue usado para comparar los

promedios cuando se encontró varianza significativa con el análisis de varianza (Statgraphics). El coeficiente de correlación entre ambos métodos fue del 99 %, lo que sugiere que el método de Hach puede ser utilizado como un método alternativo al método de Kjeldahl, con las ventajas que este presenta: menos consumo de reactivos, sensibilidad para detectar pequeñas diferencias entre muestras y principalmente por el menor tiempo empleado para su determinación, a pesar de presentar exactitud y precisión ligeramente menores que los del método de Kjeldahl.

Introduction

The notion that the nutritional value of foodstuffs depends on their chemical composition dates from the end of the 19th century. Atwater, in 1894, stated that “*this information is essential to improve the family budget*”. At the same time this knowledge made the development of the first concepts about the relation between diet and public health possible. McCance and Widowsen proposed in 1940 that “*A knowledge of the chemical composition of foods is the first essential in the dietary treatment of disease or in any quantitative study of human nutrition*” [1-2]. Currently, the importance of information about the composition of food products has been reassessed due to its wide range of applications related to diet and nutrition programs, nutritional value labeling, nutritional education, international trade, promotion of new crops and transformation and consumption of new edible species, among others [3].

Availability of reliable data about the composition of food is essential. Therefore, the use of exact analytical techniques, better still if they are fast and economical, is required. Techniques that can be used for several types of food should be chosen over those suitable for specific foods only.

The Kjeldahl technique is the method commonly used for protein analysis in food products [4]. Products are first digested, a rather time consuming process which requires a number of reagents. Digestion is carried out with concentrated sulfuric acid in the presence of an inorganic catalyst, which accelerates reduction of all organic nitrogen present into an ammonium salt. The second step is separation of the ammonium formed using distillation and the capture of the ammonium with a weak acid (boric acid). The third step is quantification of the ammonium by titration with a strong acid (sulfuric acid).

Even though the original Kjeldahl method, developed more than 100 years ago [5], has undergone numerous modifications, it is still time consuming and complicated. Research on modifications of the method was first focused on the search for new catalysts [6] to increase the rate of decomposition; since that time many oxidants and catalysts have been studied [7]. Hydrogen peroxide has been successfully used to decompose organic samples. Miller and Miller [8] reported the importance of precarbonization and multiple additions of peroxide. Florence and Milner reported the need to heat the digestive mixture after clarifying for full nitrogen recovery [9].

More recent studies have focused on quantification of the released ammonium using colorimetric methods [10,11]. Furthermore, a great variety of devices have been developed to simplify, automate and/or enhance performance of nitrogen measurement by the Kjeldahl method [12].

The importance of the measurement of raw protein content in food is evidenced throughout the numerous studies involving several laboratories that are permanently being carried out in order to compare or update methodology for the analysis of children's food [13], dairy products [14], meat and meat products [15,16,17], animal feed [18], as well as soybean products [19], among others.

The method developed by Hach et al. [1] differs from the former in that it uses a fast digestion procedure with sulfuric acid and hydrogen peroxide without the need to add any catalyst (either salt or metal), followed by a colorimetric assay, for which the sample is previously treated with the Nessler reagent.

The aim of the present study is to compare the nitrogen values from proteins in food by using the Kjeldahl and Hach methods, allowing for the influence of variables existing in foodstuffs regarding their structure and nutrient content.

Materials and methods

The nitrogen content of 25 food samples was analyzed with both the Kjeldahl and the Hach methods. Food was divided into: a) cereals, legumes and derivatives; b) vegetables and derivatives; c) fruit and derivatives; d) oil and fat; e) meat and derivatives; f) dairy products and derivatives, as proposed in the Database of Food Composition by LATINFOODS [20].

Sample preparation

The 25 samples analyzed showed variable characteristics regarding structure as well as macronutrient content (Table 1). All samples were analyzed in triplicate.

Two certified reference samples were included in order to verify the data obtained with both techniques and to check the quality of the analyses and laboratory performance: one was based on fish meal (F-2) and the other on cereals and soybean (CS-2). Both samples were produced and provided by ASIANFOODS, APFAN (Asia Pacific Food Analysis Network) and the Nutrition Institute of the University of Mahidol, Thailand, and coordinated by LATINFOODS for an inter-laboratory assay [21].

Determinations with both techniques were carried out simultaneously to avoid possible modifications of the foods. Fresh products were processed immediately after they were obtained, whereas dry foods were kept refrigerated under controlled temperature and humidity conditions (4 ± 2 °C and $60 \pm 4\%$, respectively). According to their nature, samples were prepared as follows:

Meat products: 4 samples of 500g buttock steak were obtained from a supermarket. Samples were cut into smaller pieces after stripping off external visible fat and then ground and homogenized in a domestic food processor. Caution was exercised to include juice to minimize losses; samples of about 40-50 g were obtained by the "quarter method".

Dairy Products:

- * Milk: 4 boxes of powdered whole milk (400g each) were used. Samples were homogenized and then the size was reduced by the “quarter methods”
- * Yogurt: 4 containers, each with 170 g of whole natural yogurt, were homogenized with a whisk and small fractions were weighed for analysis.
- * Cheese (semihard Gouda type): small uniform pieces of about 50g each were cut from 1 kg of product and weighed for digestion.

Vegetables, Fruit and Dried Fruit: All samples were cut up after peeling and then homogenized in a domestic food processor.

Grains: Samples were ground in a coffee mill and then homogenized by passing through a sieve (size 14).

Flour and protein isolates: These products were also homogenized by passing through a sieve (size 14).

Soybean-based Drink: Soybean juice was stirred until completely homogeneous, after which small fractions were taken with a pipette.

Table 1. Nitrogen content of foodstuffs using the Kjeldahl and Hach method

Source	Nitrogen g / 100 g of dry product		Nitrogen g / 100 g of wet product	
	Hach ⁽¹⁾	Kjeldahl ⁽¹⁾	Hach ⁽¹⁾	Kjeldahl ⁽¹⁾
<u>Cereals, legumes and derivatives</u>				
Pretreated Soybeans	8.19	8.01	7.74	7.57
Commercial corn meal	1.64	1.78	1.44	1.56
Commercial wheat	2.54	2.64	2.18	2.26
Okara ⁽²⁾	6.68	6.20	6.21	5.76
Lupine	7.03	7.12	6.06	6.14
Lentils	4.46	4.74	3.90	4.15
Commercial Soybean Drink	1.47	1.65	0.17	0.19
<u>Vegetables and derivatives</u>				
Pumpkin	2.79	3.15	0.35	0.40
Potato	3.12	3.24	0.44	0.46
<u>Fruit and derivatives</u>				
Avocado	1.75	1.90	0.36	0.39
Banana	0.88	0.93	0.21	0.22
Walnut	2.79	2.95	2.67	2.83
<u>Meat and derivatives</u>				
Sausage	7.03	7.32	2.36	2.46
Beef	15.71	15.53	3.66	3.61
Pork	15.53	16.70	3.46	3.78
Chicken	15.64	16.04	3.11	3.27
Fish	15.21	15.50	3.07	3.14
<u>Dairy Products</u>				
Milk	5.92	6.73	0.55	0.63
Yogurt	6.70	8.25	0.69	0.75
Cheese (semihard Gouda type)	7.80	7.59	4.54	4.42

Table 1. Nitrogen content of foodstuffs using the Kjeldahl and Hach method (cont.)

Source	Nitrogen g / 100 g of dry product		Nitrogen g / 100 g of wet product	
	Hach ⁽¹⁾	Kjeldahl ⁽¹⁾	Hach ⁽¹⁾	Kjeldahl ⁽¹⁾
<i>Protein Isolates</i>				
Lactalbumin	16.01	16.44	14.75	15.14
Casein	15.01	15.01	13.49	13.49
Corn gluten	12.58	13.02	11.37	11.77
Wheat gluten	17.15	16.80	15.64	15.32
Soybean isolate	17.06	16.46	14.40	13.90

1) No significant statistical difference could be observed between both methods.

2) Insoluble residue of soybeans after extraction with water.

Measurement of water contents:

Humidity was measured according to the AOAC procedures [22]. Samples were placed at 105°C, monitoring weight variations until stable.

Nitrogen Assay:

Nitrogen assay was carried out on non-dried samples. The results were expressed as g N/100 g of food; N/100 g dry matter was calculated by using water content values.

Reagents

1.-Hach method: nitrogen-free concentrated sulfuric acid (95-98%), hydrogen peroxide (30% w/v), Nessler reagent (Hach Co., catalog N° 21194), polyvinyl alcohol solution (0.1 g/l by dilution from PVA 20 g/l (Hach Co. catalog N° 21100-14), demineralizing solution (Hach Co., catalog. N° 23766-26) and ammonium sulfate standards for calibration curve (Hach Co., catalog. N° 22204).

2.-Kjeldahl method: nitrogen-free concentrated sulfuric acid (95-98%), 0.05 N sulfuric acid, NaOH (50% w/w), boric acid (4% w/v), indicator: methyl red (1% in ethanol) + bromocresol green (1% in ethanol) (1:3 ratio) and digestion catalyst: anhydrous Na₂SO₄, CuSO₄, selenium (100:10:1 ratio).

Equipment

1.- Sample Digestion

1.1.-Hach method: 21400-I Digesdahl Digestion Apparatus, composed of a digestion flask, fractioning column, capillary funnel and aspirator (Hach Co., Loveland, CO, USA).

1.2.-Kjeldahl method: 425 Büchi Digestion equipment.

2.- Nitrogen assay

2.1.-*Kjeldahl method*: sample weights ranged from 0.4 g (protein concentrates and isolates) to 2 g (semimicro scale). Samples contained between 40 and 100 mg of protein; they were weighed in an analytical balance with a sensitivity of 0.001 g, and placed in a digestion flask. 3.3 g of catalyst and 10 ml concentrated H₂SO₄ were added to each flask. Digestion was carried out at 430°C until the solution was completely clear. Digestion time was between 90 and 120 min, depending on the sample. Once digestion was complete, the sample was distilled in a distillation flask (glass trap) and a condenser into a highly alkaline solution by addition of 10 ml of 50% (w/v) NaOH and 0.1% phenolphthalein. Released ammonium was caught in 10 ml boric acid. The titer of the ammonium borate formed was measured by addition of 0.05 N sulfuric acid, using a methyl red and bromocresol green indicator.

2.2.-*Hach method*: Each sample was weighed (0.10 - 0.50; micro scale) in an analytical balance with a sensitivity of 0.001 g and placed in a digestion flask. 4 ml of H₂SO₄ were added and the mixture was heated at 430°C for 5 min. Then 15 ml 30% H₂O₂ was added through the capillary funnel, maintaining the temperature stable. Once vapors within the digestion flask had disappeared and the digestion was complete the mixture was carefully washed with distilled water through a capillary funnel.

The digestion was complete after 8- 10 minutes. When the digestion flask reached room temperature, the volume was brought to 100 ml with distilled water. A 0.5 ml aliquot of the digest was withdrawn and mixed with 24.5 ml of a 0.1 g/l polyvinil alcohol solution and 1.00 ml of Nessler's reagent. The solution was poured into a flow-through spectrophotometer cell and the absorbance recorded on a at 450 nm. The absorbance was converted into concentration by means of a linear calibration curve (percentage of nitrogen or percentage of protein). The nitrogen content of the reagents was determined and a reagent blank correction applied.

Statistical Analysis

The nitrogen contents obtained through both methods (percentage of N per g of dry product) were analyzed with a single variable variance analysis (ANOVA). The main effects and interactions among effects were tested using the F-test. The Tukey multiple comparison test was used to compare the means when significant differences were found in the variance analysis (Statgraphics; P < 0.05 was used as criterion of significance). A linear regression model was used in order to establish the existence of a relationship between both methods and the degree of correlation between them (correlation coefficient) and the total variability ratio, which is expressed by the straight line or the random incidence of the determinations (determination coefficient).

The basic criterion adopted for the analysis of the results obtained from the reference samples (CS-2 and F-2) was the Z-score of the means of the assays carried out for each sample, which was calculated as follows:

$$Z_{score} = \frac{\bar{X} - v_{cert}}{\sigma_{cert} / \sqrt{n}}$$

in which

\bar{X} = mean value of the determinations

v_{cert} = certified value for the reference sample, according to the manufacturer

n = number of independent determinations

σ_{cert} = standard deviation of the reference sample (value given by the manufacturer)

The criterion to decide whether the values obtained were satisfactory was a Z-score within the 95% reliability interval (-2, 2).

Results and discussion

The average nitrogen content of the different food categories measured with the Kjeldahl and the Hach method is given in Table 1. The results show that the highest variation between both methods is found in samples with high humidity, such as milk, yogurt and soybean-based juice.

In 64% of the samples assayed, the Kjeldahl method gave slightly higher nitrogen values than the Hach method; this is coincident with results published by Watkins et al. [23]. These findings suggest that a more complete digestion is achieved with the Kjeldahl method, which takes a digestion time 9 times longer than the Hach method. However, no statistically significant differences could be found between both methods for the 25 samples analyzed.

Fig. 1 shows the linear regression of the nitrogen content between both methods with the following equation:

$$y \text{ (Hach)} = a + b \times \text{(Kjeldahl)}$$

It can be observed that the dispersion among the results is minimal; the average standard deviation is 0.19, which means that the results for both methods are similar. The relation between both is direct and exhibits a very tight correlation ($r_c = 0.996$); 99.22% of the total variability between the methods can be expressed with the straight line, which indicates a low random incidence in the relationship between the two methods.

The experimental results allow to affirm that nitrogen contents obtained for the different food categories measured with the Hach method are comparable to those obtained by using the Kjeldahl method, as has been reported previously by Hach et al. [1]. This assumption is also supported by the results from the two reference samples.

Table 2 shows the values for the protein content ($N \times 6.25$) obtained analytically (N) with both methods for the two reference samples (CS-2 and F-2), the consensus values and the corresponding Z-score.

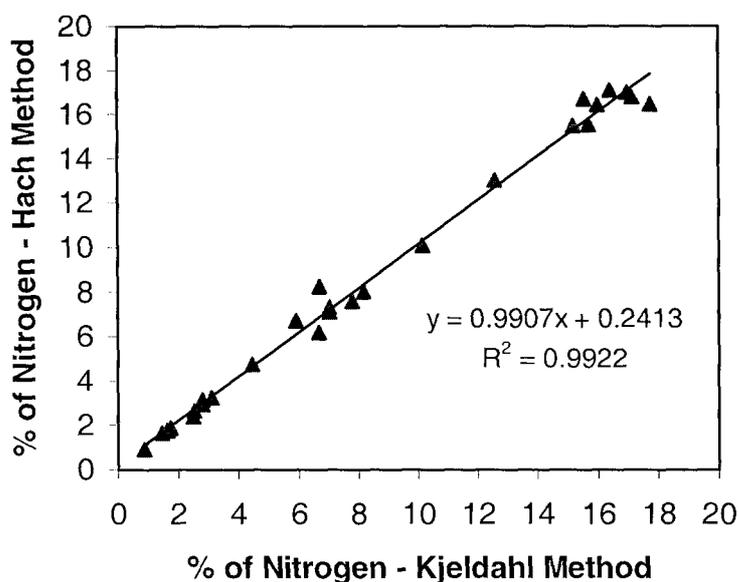


Fig. 1. Comparison between the Hach and Kjeldahl methods after linear regression.

Table 2. Protein content ($N \times 6.25$) of the reference samples CS-2 and F-2 obtained with the Kjeldahl and Hach methods.

Reference Sample	Protein content* (g /100 g sample)			Z-score**	
	Hach	Kjeldahl	Consensus value	Hach	Kjeldahl
CS-2 (cereal standard)	14.06 ± 0.39	14.49 ± 0.3	14.55 ± 0.51	-1.660	-0.200
F-2 (fish meal standard)	59.60 ± 0.74	59.25 ± 0.58	59.27 ± 1.34	0.426	-0.026

* $X \pm SD$: mean \pm standard deviation; $n = 3$

** Comparison between the values obtained with each method and the true values

The results show that the Kjeldahl method is more accurate than the Hach method, and that the mean values for the reference with the former method give a better estimate of the consensus values.

The lower accuracy of the Hach method could be due to the fact that during rapid digestion of the organic material, certain nitrogenated components are not fully oxidized [1].

Precision, i.e., the variability of the mean related to frequent performance of a given method, is usually estimated by the percent variation coefficient (%VC). According to our results, the %VC for the Hach method = 2.80 and 1.24 for samples CS-2 and F-2, respectively, whereas corresponding values for the Kjeldahl method are 2.10 and 0.97%, which means the latter method is more accurate.

Despite the difference in accuracy between the two methods and the lower precision found for the Hach method, the Z-score values for both samples obtained by the two methods are within the previously established reliability interval (-2, 2), which means that results of both methods are satisfactory with a 95% reliability. This also means that the operational laboratory conditions were good and provides confirmation of the results obtained.

Conclusions

A high correlation was found between the values of protein contents obtained by the method proposed by Hach and the traditional method of Kjeldahl in 25 samples of different foodstuffs.

Clear and diaphanous samples were obtained in significantly less time with the Hach method in all the samples analyzed during digestion.

Experimental results obtained with standard samples show that the precision and accuracy of the Hach method are slightly smaller than those of the Kjeldahl method. Nevertheless, it can be concluded that the Hach method is a good alternative to the more laborious Kjeldahl method for the analysis of protein nitrogen in foods. In fact, the Hach method exhibits considerable advantages, such as a remarkable reduction of the digestion time (9 fold), lower consumption of reagents and a smaller sample size, and consequently, a reduction in cost and requirements in equipment and supplies. An additional advantage is the possibility to use part of the sample digested with the Hach method for assay of minerals as proposed by Watkins et al. [22], because no catalysts are used during digestion.

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