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Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products

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Abstract

In this article the results on the content of total nitrogen (crude protein) obtained using the following three methodologies were compared: the Kjeldahl method (most commonly used and well-known), the Dumas method (total combustion method) and NIR (Near InfraRed) for rapid on-line analysis. The analysis involved the samples of turkey meat and diverse meat products (pate, sausages, hot dog). The obtained results revealed the precision rate below 2% for the Kjeldahl method, whereas precision rate for the Dumas method ranged within an interval 2-4%. NIR method proved most rapid in determining protein content; however, its precision ranged between 3% and 6%. Potential statistically significant differences between the results on crude protein content in meat and meat products obtained by different methodologies were determined using analysis of variance. The Kjeldahl method, because of its high precision and very small values of intervals of variation, have made it the major method for the estimation of protein in foods. The Dumas method for the quantitative determination of organic nitrogen was at least as precise as the Kjeldahl method, but considerably faster. NIR method has high relative standard deviation and is particularly useful for rapid on-line analysis of the protein content.

Keywords: Kjeldahl method, Dumas method, NIR method, crude protein

1. Introduction

Elementary life consists of nitrogen compounds in a great diversity of combinations and linkage forms. Food and feed and their protein content are of prime importance. However, nitrogenous substances such as fertilizers, or the raw and end products of processing industries, also play a role in the total concept of elementary life. Analysis of increasing precision and easier handling are required for the nitrogen concentrations in numerous and diverse samples [1].

Proteins are polymers of amino acids and differ from each other according to the type, number and sequence of amino acids. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of energy, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize [2].

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Out of 21 amino acids found in proteins of animal origin, the human body can synthesize only 11. The synthesis of remaining 10 amino acids in the body is negligible or nearly impossible, so the intake has to be through food in order to meet the needs of the organism [3].

Food analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the pro ns in foods. In order to make such findings, we have to choose the method which will ensure the required quality of information obtained. In this sense, objective approach to the concept of quality includes analytical testing and precise measuring of selected (representative) qualities, properties, characteristics of products, as well as appropriate data processing. The obtained results are quality indicators and are expressed in appropriate measuring units [2,4]. From the perspective of nutrition and technology proteins present the most important ingredients in meat and meat products, therefore it very important to know their exact concentration. Nitrogen is one of the building blocks for proteins and therefore our basis for life. The precise nitrogen analysis is of vital importance, not only for the determination of raw protein in food and feed but also in connected areas like soil fertility, fertilizers as well as biological and industrial materials [1]. To this end we compared three most widely used analytical methods for determination of overall protein concentration: the Kjeldahl method, the Dumas method and NIR method in order to determine the quality of analytical information they provide.

2.Material and Methods

The analyses of total crude protein was carried out in four different types of samples: raw turkey meat, pate (type of the sample – cooked sausage), sausage (type of the sample – fermented, dry) and hot dog (type of the sample – finely minced boiled sausage). Before measuring, the samples were well homogenised by grinding in a meat grinder (grid diameter Ø=4mm). The homogenised samples were placed in plastic containers fully loaded and hermetically sealed, for the food not to spoil and change its composition.

A total of 12 probes from the selected samples of meat and meat products were analysed as soon as possible - immediately after their homogenisation [5].

The principle of the Kjeldahl method: The digestion of the samples is carried out by concentrated sulfuric acid, by using copper (II) sulfate as a catalyst, whereby organic matter is oxidized to carbonic acid. Nitrogen, that is released in the form of ammonium, forms ammonium sulphate with a sulfuric acid. Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia, and other organic matter to CO_2 and H_2O. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH_4^+) which binds to the sulfate ion (SO_4^{2-}) and thus remains in solution: N(food) $\rightarrow$ $(NH_4)_2SO_4$.

Digestion [2, 6]: Organic matter + $H_2SO_4 \rightarrow CO_2$ +H_2O + $(NH_4)_2SO_4$ + SO_2

Under the influence of base on the created ammonium sulfate, ammonia is liberated and distilled into an excess of boric acid solution of known molarity and followed by titration with hydrochloric acid used for determination of ammonia bound with boric acid. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas [2, 7]:

Neutralization: $(NH_4)_2SO_4 + 2\ NaOH \rightarrow 2NH_3 + 2H_2O + Na_2SO_4$

The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion [2, 8]:

$NH_3 + H_3BO_3$ (boric acid) $\rightarrow NH_4^+ + H_2BO_3^-$ (borate ion)

The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.

Titration: $H_2BO_3^- + H^+ \rightarrow H_3BO_3$

Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor [9]: Protein [%]= 6.25 x N [%].

All the reactions that take place during the destruction of organic matter with sulfuric acid are still unknown [8]. Most probably sulfuric acid acts dehydrating at first, then oxidizing, whereby the carbon gradually oxidizes into carbon dioxide, hydrogen and water, and an appropriate amount of sulfuric acid is reduced into sulfur dioxide. Nitrogen is reduced to ammonia by the formed sulfur dioxide, as well as some carbon compounds formed as intermediates during organic matter destruction.

The principle of the Dumas method: The principle of Dumas method for nitrogen determination is based on the quantitative combustion digestion of the sample at approx. 900ºC in excess oxygen. The sample is burnt and the organic elements are oxidized. The combustion gases (O_2, CO_2, H_2O, N_2 and nitrogen oxides NO_x) are collected and passed through several traps. All gases are eliminated except nitrogen and nitrogen oxides. The bound nitrogen is transferred into molecular nitrogen and nitric oxides [10]. The analysis gases are transferred with CO_2 as a carrier gas via a catalytic post combustion zone onto a reduction zone. At this point, the conversion of the nitric oxides into nitrogen at hot tungsten takes place. Furthermore, the excess oxygen is bound. After a two stage drying phase, the gas mixture flows to the thermoconductivity detector via an electronic flow controller. A connected PC calculates the nitrogen concentration in the sample from the TCD signal of the N_2 in the CO_2 and from the sample weight. The content of crude protein is calculated by multiplying the measured nitrogen quantity by the appropriate factor (6.25) and is expressed in percent [11].

The principle of the NIR method: The analyser is based on near infrared transmittance technology, which can be used for simultaneous and accurate determination of several parameters such as moisture, protein and fat content [12]. From a tungsten-halogen lamp housing on the back of the instrument, light is guided through an optical fibre into the monochromator inside the instruments. The monochromator provides monochromatic light in the spectrum from 850 nm to 1050 nm. Through an optical fibre, light is guided to the collimator lens system, which is placed over the sample cup in the sample cup room. After the light is transmitted through the sample, unabsorbed light reaches the detector. The detector measures the amount of light and sends the result to the digital signal processor that communicates with the PC, calculating the result. Rotation of the sample cup between sample scans (called sub-samples) allows you to analyse various parts of the sample. The sub-samples are chosen from one or two concentric circles in the sample cup, giving a more representative result from an inhomogeneous sample. Calibration based on a high number (minimum 30 samples) and therefore robust for the different variations in the product [13].

3.Results and Discussion

Based on the obtained results displayed in Table 1 it may be concluded that the relative standard deviation (RSD), i.e. the precision of the Kjeldahl method is very stable [14]. In all the types of samples it was lesser than 2%. In the analysed samples RSD ranged between 1.54% (turkey meat) and 1.87% for sausage samples. RSD for hot dog was 1.61%, and for pate samples 1.84%.

The precision rate for the Dumas method in all the analysed samples was higher than for the Kjeldahl method, and it ranged between 2% and 4%, which is in accordance with other authors [15]. In the turkey meat samples the RSD values were the lowest - 2.16%, but for hot dog it was 2.57%, and for pate 3.70%. The RSD value for sausage samples was the highest and it was 3.97%.

The results displayed in Table 1 for the NIR method lead to a conclusion that the RSD values are by far the highest and range from 3.32% (turkey meat) to 5.74% (pate). For sausage samples it was 4.42%, and for hot dog 5.13%.

The obtained values for variation interval (VI) lead to a conclusion that the lowest value for all the analysed samples are for Kjeldahl method and they range from 0.42 (pate samples) and 1.08 (turkey meat samples). In Dumas method variation interval for all types of samples has higher values and they range from 0.88 (hot dog) and 1.82 (sausage).

Variation interval is the highest in the results on crude protein content obtained by NIR method and they range from 1.78 (hot dog), even 2.76 (turkey meat).

Table 1. The crude protein content, relative standard deviation and variation interval (span) for the samples of turkey meat, pate, sausages and hot dogs according to methods of analysis.

Method / Type of samples	Crude protein [%]		
	Kjeldahl method	**Dumas method**	**NIR method**
TURKEY MEAT (n = 12)	24.70 ± 0.38 (RSD=1.54%) VI= 1.08	25.04 ± 0.54 (RSD=2.16%) VI= 1.60	24.43 ± 0.81 (RSD=3.32%) VI= 2.76
PATE (n = 12)	11.03 ± 0.18 (RSD=1.63%) VI= 0.42	10.80 ± 0.40 (RSD=3.70%) VI = 1.30	11.15 ± 0.64 (RSD=5.74%) VI= 2.10
SAUSAGE (n = 12)	14.44 ± 0.27 (RSD=1.87%) VI= 0.78	14.87 ± 0.59 (RSD=3.97%) VI= 1.82	15.09 ± 0.64 (RSD=4.24%) VI= 1.90
HOT DOG (n = 12)	13.02 ± 0.21 (RSD=1.61%) VI= 0.70	12.86 ± 0.33 (RSD=2.57%) VI= 0.88	13.06 ± 0.67 (RSD=5.13%) VI= 1.78

VI – variation interval (span) = max. value – min. value

Table 2. Statistically significant difference between measured crude protein content depending on the method obtained by analysis of variance.

t-test	Method		
	Kjeldahl - Dumas	**Kjeldahl - NIR**	**Dumas - NIR**
Risk level Type of sample	P=0.05	P=0.05	P=0.05
Turkey meat	DINSS	DINSS	*
Pate	DINSS	DINSS	DINSS
Sausage	DINSS	DINSS	DINSS
Hot dog	DINSS	DINSS	DINSS

DINSS- difference is not statistically significant * difference is statistically significant

Based on the statistical analysis (Table 2) it may be concluded that statistically significant difference (*$p<0.05$) occurred only in analysing crude protein content in turkey meat using the Dumas and NIR methods. No statistically significant difference was noticed comparing the results of all other test methods.

4.Conclusions

The Kjeldahl method is widely used internationally and is still the standard method for comparison against all other methods. Its universality, high precision and very low variation interval have made it the major method for the estimation of protein in foods.

The disadvantage of this method is that the use of concentrated sulfuric acid at high temperatures poses a considerable hazard, as does the use of some of the possible catalysts. The technique is time consuming to carry-out [2, 16].

The Dumas method for the quantitative determination of organic nitrogen was at least as precise as the Kjeldahl method, but considerably faster (under 4 minutes per measurement, compared to several hours for the Kjeldahl), which makes it of great interest for research and industrial applications [1, 2, 10]. It doesn't need toxic chemicals or catalysts. Many samples can be measured automatically and it is easy to use. The basic disadvantage of Dumas method is that the high initial costs and the small sample size make it difficult to obtain a representative sample. It can be concluded that both methods are in good agreement considering that the variation interval and the results of analysis of variance for the materials assayed, both techniques led to comparable precision [17]. For some samples (turkey meat, sausages) the Dumas method gave slightly higher values than the Kjeldahl procedure but the means were not significantly different. Also, it was concluded that the Dumas procedure can be replaced with the advantage of the Kjeldahl procedure in animal nutrition laboratory analysis [18, 19].

The results obtained by NIR method on examining crude protein content have the highest standard deviation, which means higher impact of the random errors on individual measurement results, i.e. lower quality of measuring process. NIR is particularly useful for rapid on-line analysis of protein content. It also requires little sample preparation and is nondestructive. Its major disadvantages are its high initial cost and the need for extensive calibration [2, 12, 13].

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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