Comparison of Kjeldahl and Combustion Methods for Determining Protein Contents of Some Food Using Data from Proficiency Testing

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ABSTRACT

The Kjeldahl method which relies on the conversion of protein nitrogen into the ammonium ion by boiling sulfuric acid in the presence of a catalyst and the combustion method, which relies on the combustion of the testing material and measurement of the resulting elemental nitrogen are normally used for the measurement of protein in some food are milk powder, flour and feeding stuffs. This paper describes part of a study to provide a definitive comparison of the results of the two methods. The preliminary work reported here is based on historical data collected from a proficiency testing project. Data used in this study were taken from Taiwan Accreditation Foundation (TAF), a proficiency testing scheme organized by The Department of Science Service (DSS), Bangkok, Thailand. A range of testing material types were offered for the determination of nitrogen in this scheme. Many laboratories using the Kjeldahl or combustion methods participated in these rounds and data from 5 rounds were considered. For each round the results of the participants were segregated according to method used. Preliminary studies showed no discrimination between identifiable variations within the two methods under consideration, so the results of all variants of the Kjeldahl method were lumped together, as were those of all variants of the combustion method. The results of this empirical study showed that overall there was a clear bias between the methods, with the combustion method normally providing a slightly higher result than the Kjeldahl method. This finding is consistent with previous studies and with the generally accepted explanation, namely that nonprotein forms of nitrogen are converted into elemental nitrogen in the combustion method. However, the bias between the methods can vary significantly, among both food types and individual examples of a particular type. The methodology of observing for bias between Kjeldahl and combustion methods is critically discussed.

Keywords : Protein Contents / Protein Analysis / Proficiency Testing

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Introduction

Methods currently used for the determination of the protein content of foodstuffs depend on the determination of nitrogen. An empirically determined factor is subsequently used to convert the nitrogen content into the protein content. Until recently the Kjeldahl method was used almost universally to determine nitrogen in foods. Consequently, the empirical nitrogen factors for various foods were themselves determined by the application of the Kjeldahl method.

The Kjeldahl method, devised in 1883, relies on the conversion of protein nitrogen into the ammonium ion by boiling sulfuric acid in the presence of a catalyst. The reaction mixture is made alkaline and the ammonia separated by distillation and determined by acid–base titrimetry. In the over 10 years there has been a tendency for the Kjeldahl method to be replaced in food laboratories by instrumental realisations of the combustion method, which relies on the combustion of the test material and measurement of the resulting elemental nitrogen. Modern instrumentation has transformed this venerable method—it was devised in 1831—into a cost-effective alternative.

A number of studies suggest that the combustion method usually provides a result higher than the Kjeldahl method by about 1.5% relative. (Richard, L.E. et al., 1997; Jan-Ake, P., 2008; Koch, M., 2009) The difference is probably due to the near-complete conversion of non-protein forms of nitrogen into elemental nitrogen in the combustion method: in the Kjeldahl method, nitrates, nitrites and some nitrogenous compounds are converted into the ammonium ion incompletely or not at all. As a consequence of this difference between the methods, it is now necessary to determine whether currently accepted nitrogen factors can be safely used with the results produced by the combustion method for determining the nutritional value of a food or in the enforcement of food legislation.

This paper describes part of a study to provide a definitive comparison of the results of the two methods. The preliminary work reported here is based on historical data collected from a proficiency testing project.

Protein determination

There are several factors that make a definitive study of possible bias between competing analytical methods more difficult than normally realised.

(a) The comparison needs to be carried out at various concentrations of the analyte, because a bias between the results of the methods might vary with concentration.

(b) It needs to be carried out with a set of test materials, the matrices of which span the scope of the study, in case the bias depends on matrix type.

(c) Results need to be replicated many times to improve the precision of the mean results and so that significance tests can be carried out if they are needed.

(d) Most importantly (although possibly the least-recognized factor) method comparison requires an interlaboratory study.

This last measure is necessary if the effects of laboratory bias are to be avoided. (In principle, the use of matrix certified reference materials could achieve the same result in a single laboratory, but there are considerable complications in that strategy, not least that the certification will usually have been carried out by using one or both of the methods under consideration.)

Laboratory biases make a substantial, quite often the major, contribution to overall uncertainty of an analytical measurement. This can be seen clearly in the results of any interlaboratory comparison. Some biases between laboratories using nominally the same method arise because of deliberate variations in the procedure. For instance there are many minor variants of the Kjeldahl method, involving use of different catalysts, heating times, volumes of sulfuric acid, and masses of test portion. There are also uncontrolled laboratory effects resulting from, for example, biased calibration procedures and variant realizations of the method protocol in different laboratories. If a single laboratory attempts a method comparison, the bias between the results of the methods will be contaminated by the laboratory biases for the two methods. These biases will be different and therefore not cancel out, except sometimes in the special case where two similar versions of a single method are under comparison. Repeatability errors and run biases within laboratory can be reduced by averaging run-to-run replicated results, but individual laboratory biases can be reduced to insignificance only if results from a sufficiently large group of laboratories are collected and averaged.

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 analyst concentrations and matrices, and results from many laboratories. When two or more well-defined methods have been used by a substantial number of participants, a comparison is possible (Lowthian, P.J. et al., 1996). There is a theoretical objection to such usage of results: the laboratory subsets are disjoint and elective, that is, each laboratory chooses and uses only one method. In principle, differences between means of subsets might be due to the nature of the laboratories in the subsets rather than the methods they employ. However, it is difficult to think of plausible circumstances in which the results considered in the present study could be thus affected.

Data used in this study were taken from Taiwan Accreditation Foundation (TAF), a proficiency testing scheme organized by The Department of Science Service (DSS), Bangkok, Thailand. A range of test material types are offered for the determination of protein in this project. Many laboratories using the Kjeldahl or combustion methods participate in these rounds.

Data from 5 rounds were considered. For each round the results of the participants were segregated according to method used. Preliminary studies showed no discrimination between identifiable variations within the two methods under consideration, so the results of all variants of the Kjeldahl method were lumped together, as were those of all variants of the combustion method. A typical set of z- score results for a round is shown in Figure 1. The subsets can be plausibly regarded as roughly normally distributed samples that are contaminated with a small proportion of outliers, so the use of z-score test, which down weights the influence of outliers, is appropriate and indeed essential in this type of study.

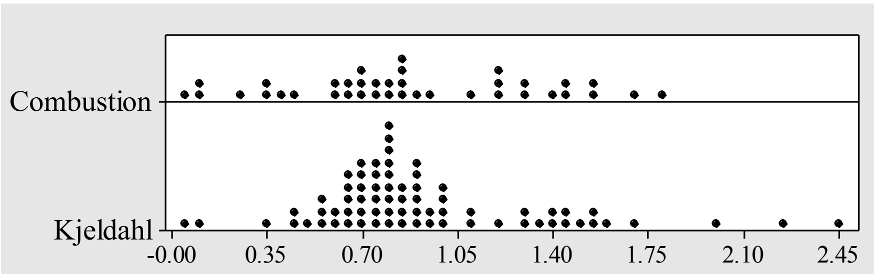

Figure 1 A typical dataset with z-score results separated according to analytical method. Each point represents the normally trend.

The summary statistics for all of the individual rounds are shown in Table 1. The means and standard deviations are robust estimates obtained by using the algorithm A (ISO13528, 2005) follow by ISO 13528 standard.

Table 1 Robust statistics for individual foodstuffs (% protein).

Food type	TAF round	Kjedahl mean (K)	Kjeldahl sd	Kjeldahl n	Combustion mean (C)	Combustion sd	Combustion n	Bias (C-K)
Feeding stuffs	1301	18.893	0.297	85	18.992	0.334	12	0.029
Feeding stuffs	1201	27.676	0.465	70	27.669	0.466	14	-0.007
Feeding Stuffs	1101	17.913	0.286	71	17.937	0.327	15	0.024
Wheat flour	1001	10.684	0.230	23	10.700	0.275	11	0.016
Milk powder	0901 (part a)	24.496	0.342	19	24.516	0.455	6	0.020
Milk powder	0901 (part b)	24.064	0.343	19	24.084	0.491	6	0.020

The bias estimates given for each material are the combustion mean minus the Kjeldahl mean. The 6 individual biases is shown in Table 1 and %bias in Table 2. The finding showed that feeding stuff with Kjeldahl method had a better result than any food type of this proficiency testing project. The null hypothesis for a normality test states that the population is in normal distribution. The alternative hypothesis states that the population is non-normal distribution. Inspection of Figure 2 suggests that the probability of Kjeldahl method have individually different biases, and oneway analysis of variance confirms that impression with a low level of significance (*p-value* < 0.005) while Figure 3 shows the probability plotted as a normally and linear (*p-value* < 0.478) in combustion method.

Table 2 Bias statistics for some food group.

Food type	Mean (%protein)	Mean bias	n	Bias (%)
Feeding Stuffs	25.596	0.015	267	0.006
Wheat flour	10.692	0.016	34	0.047
Milk powder	24.290	0.020	50	0.040

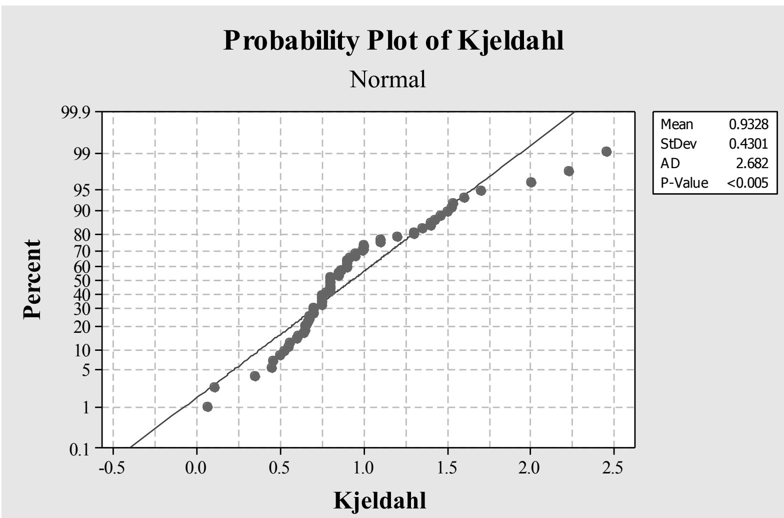

Figure 2 A probability plot of Kjeldahl method. Each point represents a TAF round.

While the principal focus of this study is bias between the methods, it is useful to compare the precisions obtained by them. The between-laboratory standard deviations are given in Table 1 and expressed for comparison as percent coefficient of variance (%CV) in Figure 4. This shows the Kjeldahl resulted in a tight cluster centred at about 2.5 %CV and the combustion resulted centred slightly higher at about 2.9%. A noteworthy feature is that the results were strikingly more disperse for the combustion method, although no explanation for that finding is apparent from the data.

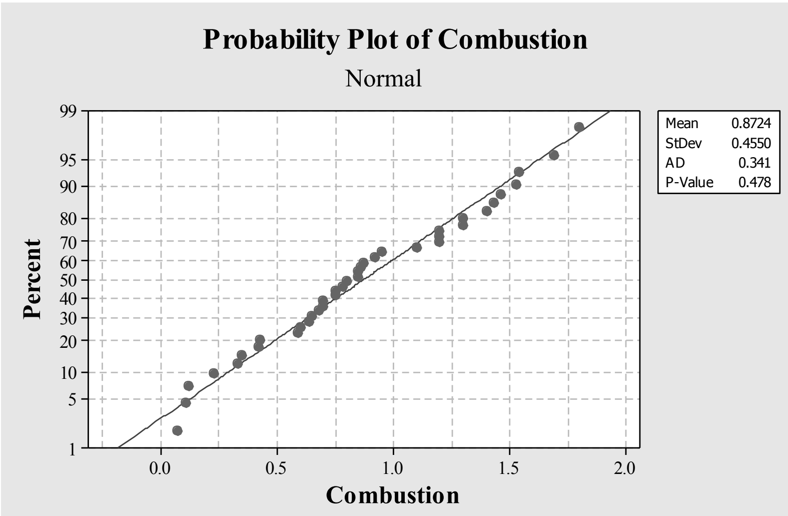

Figure 3 A probability plot of combustion method. Each point represents a TAF round.

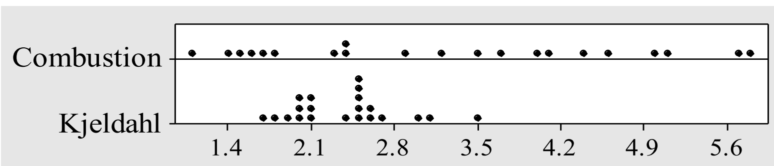

Figure 4 A percentage coefficient of variance separated by analytical method. Each point represents a TAF round.

Conclusions

The results of this empirical study showed that overall there was a clear bias between the methods, which the combustion method normally provided a slightly higher result than the Kjeldahl method. This finding is consistent across many research studies and with the generally accepted explanation, that non-protein forms of nitrogen are converted into elemental nitrogen in the combustion method that hidden on measurement of uncertainty. The higher protein food had low bias than lower protein food. However, the bias between the methods could vary significantly especially, feeding stuffs and milk powder examples of a particular type. Moreover, there may be a variation in bias with the concentration of the analyst and finding measurement of uncertainty of the methods must be observed. Further studies with designed experiments are needed to throw light on these findings.

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