IAFMM

international association of fish meal manufacturers

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RECOMMENDED METHOD OF ANALYSIS

FOR DETERMINATION

OF CRUDE PROTEIN IN FISH MEAL

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1. General

It is useful to know the crude protein content of fish meal. The nitrogen figure (Kjeldahl) is multiplied by the factor 6.25 to obtain the crude protein content.

2. Principle

The method is based on the conversion of organic nitrogen to inorganic nitrogen (digestion according to Kjeldahl); the ammonium sulphate thus formed is diluted and made alkaline with sodium hydroxide and the ammonia distilled over, caught in a known amount of sulphuric acid solution and determined titrimetrically.

3. Reagents

All reagents shall be of analytical reagent quality. Distilled water shall be used or water of at least equivalent purity.

Sulphuric acid conc. – nitrogen-free.

Potassium sulphate anhydrous.

Mercuric Oxide.

Zinc filings.

Sodium hydroxide solution, 40 per cent (by weight).

Sulphuric acid, 0.25 N.

Sodium hydroxide, 0.25 N.

Sodium thiosulphate solution (prepared by dissolving 80g $Na_2S_2O_3.5H_2O$ per litre of water).

Indicator: Dissolve 2g of methyl red and 1g of methylene blue in 1000 ml of 95% (v/v) ethanol.

Tryptophan (melting point 282° C).

Sucrose (nitrogen-free).

Litmus paper.

4. Apparatus

a. Distillation apparutus. To avoid loss of ammonia a fitted dropping funnel is recommended for the addition of the sodium hydroxide. The distillation apparatus must also have a device which prevents traces of alkali mixing with the distillate. Capacity of the Kjeldahl flask 800 or 1,000 ml.

b. Electrical heaters are preferred but gas burners may be used provided caution is exercised.

5. Method

A. Digestion

About 1g of the sample is accurately weighed to the nearest 1mg and put into the digestion flask. To this are added 15g dry potassium sulphate and the catalyst: 0.7g mercuric oxide. Finally 25ml of conc. sulphuric acid are added and the mixture is swirled around carefully.

In the beginning heat is applied carefully, since dehydration, charring and frothing may need attention and turning of the flasks. The heat is then increased till the liquid boils evenly (the addition of glass beads is recommended). Care must be taken to ensure that no organic matter remains adhering to the walls of the flasks. To prevent the organic substance from decomposing on the walls and so to avoid nitrogen loss, the walls of the flask must not be overheated.

If the substance tends to froth it is recommended that the mixture of sample + sulphuric acid + catalyst is allowed to finish frothing before the potassium sulphate is added.

As soon as the liquid has cleared, boiling is continued for at least two hours.

B. Distallation of the ammonia

After cooling, dilution of the liquid is carried out by carefully adding about 250 to 350ml distilled water. The sulphate must all dissolve and the acid solution must be well cooled. A little zinc is added. If a dropping funnel is used, the distillation flask is connected to the vertical condenser.

The bottom end of the condenser, which might have to be extended with a suitable fitting, must dip at least 2cm into exactly 35ml of 0.25N sulphuric acid solution which fixes the ammonia. Add a few drops of indicator.

Forty per cent sodium hydroxide solution is carefully run into the digestion flask so as to form a layer below the cooled acid solution in an amount large enough to make the mixture strongly alkaline, 120ml should be sufficient for this purpose. This can be checked by the addition of a few drops of phenolpthalein – the mixture must remain red up to the end of the distillation. 25ml of $Na_2S_2O_3 5H_2O$ solution must be mixed with the NaOH solution before addition to the flask.

If a dropping funnel is not used, the NaOH solution, together with $Na_2S_2O_3 5H_2O$ solution, is added to the acid solution by careful layering just before the distillation flask is connected to the condenser, to avoid mixing of the alkaline phase at the bottom with the acid phase at the top.

The flask is heated in such a way that about 150ml of liquid is distilled within 30 min. At the end of this time, check the pH of the distillate at the tip of the condenser using litmus paper. If the reaction is alkaline, repeat the analysis allowing longer distillation time. During the distillation the receiving flask which catches the distillate must be shaken occasionally and the colour of the indicator checked. If the colour changes, the method must be repeated with smaller fish meal samples.

C. Titration of the distillate

The excess sulphuric acid is back-titrated with 0.25N sodium hydroxide. The indicator turns green at the end point.

D. Blank test

To test the reagents for purity, a blank run should be made (distillation and titration) with the addition of 2g of nitrogen-free sucrose.

6. Check Test

In order to test the whole process including digestion, perform a check test by determining the nitrogen content of a known organic compound, for example tryptophan plus 1g of sucrose.

7. Calculation

$$\frac{(\text{ml } H_2SO_4 - \text{ml NaOH}) - \text{ml blank}}{\text{Wt. of fish meal (g)}} \times 0.3502^* \times 6.25 = \text{Protein content (\%)}$$

$$^* \; 0.3502 = \frac{\text{molecular weight of N [14.01]} \times \text{normality of sodium hydroxide [0.25]}}{10}$$

Only applied when sulphuric acid and sodium hydroxide are of equal normality.

8. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.40%.

Footnote

If the acid digest, on cooling, becomes solid, the test should be repeated and the heating or the slope of the Kjeldahl flask should be adjusted to prevent loss of sulphuric acid.

SOME NOTES ON THE ORDINARY KJELDAHL DETERMINATION OF PROTEIN IN FISH MEAL

by

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Abstract

An attempt is made to clarify why the ordinary Kjeldahl determination sometimes does not give consistent results. The importance of correct heating during the digestion time as well as the efficiency of some catalytic agents are examined.

Introduction

Determinations of crude protein in fish meal and other fish products have been carried out for many years at our laboratory, and like most other similar laboratories we have used the Kjeldahl method of digestion, distillation and titration.

Although the reproducibility of this analysis is normally regarded as good, we get, from time to time, greatly different results from duplicates, and sometimes Kjeldahl determinations made on the same product by different laboratories give differing results too. As a whole the precept used is almost the same everywhere, but small differences in the procedure occur with regard to the heating apparatus for digestion and the catalytic agent used. At our laboratory we have used in the course of time both gas heaters and two different kinds of electrical heaters for the digestion of the samples. The first of these electrical heaters had hot plates with cast light-metal bowls which fit the flasks. The electrical heaters used today are Gallenkamp's * heating units with open, glowing metal spirals and heat regulation of every sample on a scale from 0 to 100.

When the Gallenkamp * units came into use it was supposed that the heating effect of the metal-spirals was about the same, if the same adjustment on the scale was used. Apparently there was nothing wrong; the sulfuric-acid mixture was boiling, and the flasks were filled with mist. However, there were difficulties from the beginning, when these heaters were used. It took too long (several hours) to clarify some samples and it was necessary to intensify the heating, often resulting in evaporation of the sulfuric acid.

The catalytic agent we have used for many years is a mixture of potassium sulfate,copper sulfate, and mercury sulfate in the ratio of 100:6:7.5. The amount of mercury in the samples has been about 0.5 g.

In recent years some laboratories have recommended other catalysts, for example selenium + copper (2) or only copper (3). It has also been recommended that hydrogen peroxide be added to the sample (4).

* Mention of commercial companies does not necessarily imply endorsement.

Methods and materials

In an attempt to improve the whole procedure at our laboratory the effect of the heating units was examined and corrected and, further, the efficiency of some catalysts was examined.

Examination and correction of the heaters

According to the AOAC (5), heaters for Kjeldahl-digestion must be adjusted to make 250ml of water boil from 25°C in 5 minutes. This is a strong heat effect and probably maximum with the apparatus used, if excess evaporation of the sulfuric acid is to be avoided.

In agreement with this principle the effect of all metal spirals in our Gallenkamp-units was examined and corrected to a level just beneath the point on the scale where the sulfuric acid evaporated. Every metal spiral was adjusted to a special value on the scale from 0 to 100.

To establish whether there are appreciable differences in results if different heat sources are used for digestion of samples, some analyses were made on herring meal (Table1).

TABLE 1

KJELDAHL DETERMINATIONS WITH DIFFERENT HEAT SOURCES FOR DIGESTION OF THE SAMPLES (NORMAL Hg-CATALYST)

Type of heater	HOURS OF BOILING			number of samples	% crude protein (mean values)	standard error of the mean values
	to clarify the fluid	after clarification	total			
Extr. herring meal. 2.5% fat						
gas heaters	2-3	4	6-7	8	82.55	0.24
hot plates	10	4	14	6	81.48	0.59
Gallenkamp*						
units not adjusted	1.5-29	4	5.5-33	6	82.75	0.36
- - adjusted	0.3	1.7	2	6	83.61	0.08
herring meal 9.7% fat						
Gallenkamp*						
unit not adjusted	1-4	4	5-8	6	70.42	0.23
- - adjusted	0.4	1.6	2	6	71.28	0.10

Standard error of the mean = $\frac{s}{\sqrt{n}}$, where $s = \sqrt{\frac{\geqq (x - x)^2}{n - 1}}$

The table shows that it is possible to digest the samples in a shorter time, if the heaters all produce the same maximum effect. There is a difference of several hours' boiling time between adjusted and non-adjusted heat sources. The reason is that when using non-adjusted apparatus it is necessary to lower the heating level – if not, the sulfuric acid evaporates in some of the samples. Therefore, more time is needed to clarify the sample. According to previous practice, the samples were boiled for another four hours. This is not necessary if all samples are heated with the same maximum effect. They then become green and clear in half an hour or less.

The results agree more closely too if the heaters produce the same maximum effect (the standard error of the mean values varied from 0.59 to 0.08), just as the level of percent crude protein is a little higher with adjusted heaters.

Examination of the efficiency of some catalytic agents

Herring meal with 9.7% fat was analysed using adjusted Gallenkamp* units for digestion of the samples. Various catalysts were tried in connection with five different digestion times varying from 1.5 to 3.5 hours.

TABLE 2

KJELDAHL-DETERMINATION WITH DIFFERENT CATALYSTS AND DIGESTION TIMES

Catalyst	hours of boiling	% protein (maximum mean values)	standard error of the mean values
0.6 g $CuSO_4$ +0.55 g HgO	2	71.28	0.10
0.6 g " +0.2 g selenium	2	71.13	0.11
0.6 g " +0.1 g "	3	71.33	0.12
0.6 g " +0.1 g " +5ml 30% H_2O_2	3	70.89	0.21
0.6 g "	2.5	70.96	0.10
0.6 g " +5ml 30% H_2O_2	3	71.40	0.14
1.0 g "	3.5	71.36	0.13
1.0 g " +5ml 30% H_2O_2	3	70.93	0.17

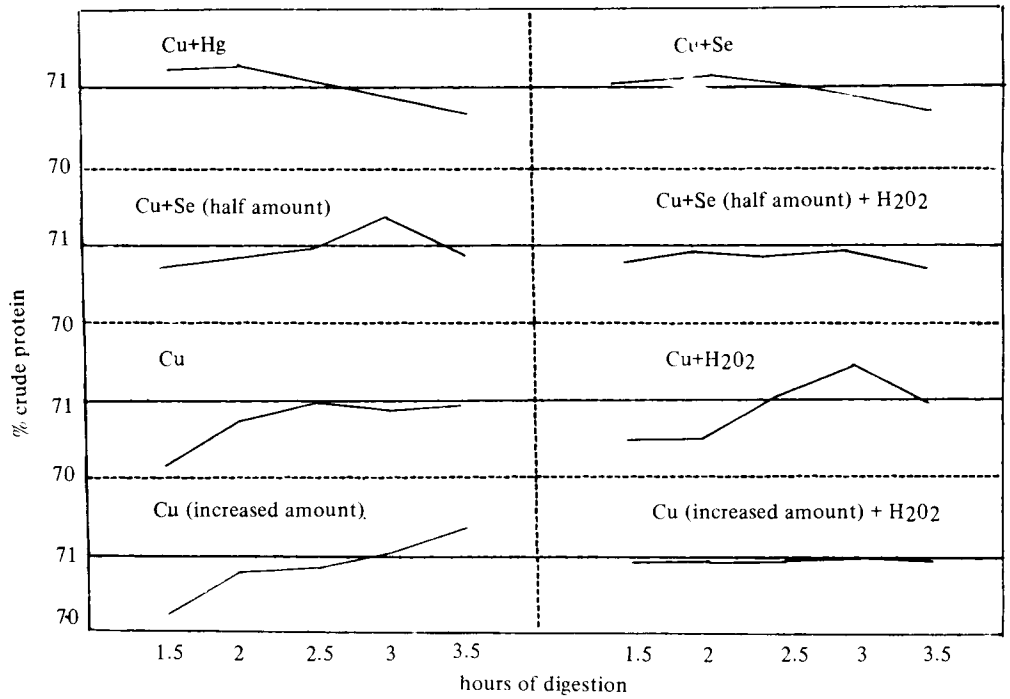
Table 2 shows an extract of 240 analyses and each mean value represents six determinations. Only the maximum main values at certain digestion times are mentioned in the table. The following figure 1 is a graphic representation of all the results of this experiment.

There are no big differences to be seen. Mercury and selenium (0.2g) produce their maximum values in less time than other catalysts (2 hours), but if the samples are digested a little longer (3 – 3.5 hours) on an adjusted heater, especially copper in increased amounts is able to give results as good as those obtained with mercury.

The use of hydrogen peroxide has improved the result in only one case, and perhaps addition of this agent has a tendency to give less confident results.

FIG. 1 KJELDAHL DETERMINATIONS USING DIFFERENT DIGESTION TIMES AND CATALYSTS, ADJUSTED HEATERS

Herring meal, 9.7% fat.



Conclusion

From our examination of heat sources and catalysts used for Kjeldahl determinations it looks as if most of our former difficulties were due to unequal heating of the samples during the digestion. Probably it is not possible to make accurate Kjeldahl determinations without using adjusted heaters, which give all samples the same maximum heat effect. Gas burners such as ordinary Bunsen burners do not seem sufficiently adjustable, and, also, they can be influenced by draughts.

The catalyst tests showed that it is possible to avoid mercury in the Kjeldahl digestion. However, when, for example, copper is used as the only catalyst, a slightly longer digestion time is required.

(1) Osborn, R.A., and Krasnitz, A., Journal of the Association of Official Agricultural Chemists, 1933, vol. 16, page 107-113 and Journal of the Association of Official Agricultural Chemists, 1934, vol. 17 page 339-342.

(2) Kvaelstofbestemmelser i kødvarer efter Kjeldahl, Proteinbestemmelse, blad 3, 1971, Slagteriernes Forskningsinstitut, Roskilde.

(3) Meddelelse fra korn- og foderstofafdelingen, 6. argang, nr. 1 1973. Forskningsinstituttet for Handels- og Industriplanter, Kolding.

(4) Instruktionsbog for TECATOR Kjeldahl-analyseapparatur, 1974, TECATOR AB, Kronborggatan 6, Helsingborg.

(5) Official Methods of Analysis of The Association of Official Agricultural Chemists, 10th Edition 1965. page 16.