MINISTERIE VAN LANDBOUW
BESTUUR VOOR LANDBOUWKUNDIG ONDERZOEK
KOMMISSIE VOOR TOEGEPAST WETENSCHAPPELIJK ONDERZOEK
IN DE ZEEVISSERIJ (T. W. O. Z.)

(Voorzitter: F. LIEVENS, directeur-generaal)

THE AUTOMATIC DETERMINATION OF TOTAL NITROGEN IN COD MUSCLE

H. DEVRIENDT

ONDERWERKGROEP
„VISVERWERKENDE BEDRIJVEN - VOORVERPAKKING VIS" (I.W.O.N.L.)

Mededelingen van het Rijksstation voor Zeevisserij (C. L. O. Gent)
Publikatie nr 112-VB/VV (I.W.O.N.L.) 17, 1975

MINISTERIE VAN LANDBOUW
BESTUUR VOOR LANDBOUWKUNDIG ONDERZOEK
KOMMISSIE VOOR TOEGEPAST WETENSCHAPPELIJK ONDERZOEK
IN DE ZEEVISSERIJ (T. W. O. Z.)

(Voorzitter: F. LIEVENS, directeur-generaal)

THE AUTOMATIC DETERMINATION OF TOTAL NITROGEN IN COD MUSCLE

H. DEVRIENDT

ONDERWERKGROEP
„VISVERWERKENDE BEDRIJVEN - VOORVERPAKKING VIS" (I.W.O.N.L.)

Mededelingen van het Rijksstation voor Zeevisserij (C. L. O. Gent)
Publikatie nr 112-VB/VV (I.W.O.N.L.) 17, 1975

Paper presented at the Sixth Meeting of W. E. F. T. A.,
Ostend, September 1975

D/1975/0889/6

C.L.O. Offset - Repro - Fotografie

INTRODUCTION.

The new system of continuous-flow analysis adopted by Technicon in its AutoAnalyzer I for automatic determination of total nitrogen (1), comprises a module called the "digestor". The latter is made of a pyrex glass helix heated to 300-400°C, in which the digestion of organic substances by wet process takes place under the action of a mixture of concentrated acids. After digestion the nitrogen is determined as ammonia in an automated analytical system, by formation of an indophenol-blue colour. Sutton and Duthie (2) modified the new concept of continuous N-determination in order to investigate the nitrogen contents in fish muscle extracts. Intensive use of their technique however, didnot lead us to recoveries of 100 %, relative to manual Kjeldahl, but only of 90 %. Two reasons can be suggested for the low recovery in cod muscle compounds during automatic digestion. Probably an incomplete conversion of nitrogen into ammonium sulphate takes place or there may occur a loss of N from the $(NH_4)_2SO_4$- standard (3) (4).

It is apparent that a 100 % recovery can be obtained as a result of decomposition of the $(NH_4)_2SO_4$ without increasing digestion efficiency. This is not a reliable procedure to use however, since it depends on the operating conditions remaining constant. A better method is to use standards having very similar heat stability characteristics to the unknown (5) (6) (7) (8). In this paper an account is given of the use of freeze-dried cod muscle as standardising material in order to obtain more accurate total nitrogen (T. N.) determinations with the Sutton and Duthie technique. A number of cod samples has been analysed by each of three methods : manual Kjeldahl, AutoAnalyzer using as standard either ammonium sulphate or freeze-dried cod. A statistical comparison between the three analytical systems has been carried out.

EXPERIMENTAL.

Apparatus.

An AutoAnalyzer consisting of digestor, sampler, proportioning pumps, heating bath, colorimeter (625 nm filter) and recorder modules is assembled as shown in figure 1. The figure also gives details of the flow rates required in each line. Figure 2 shows the "chute" system adopted by Sutton and Duthie (2) to prevent precipitation of protein where the sample met the acid mixture before entering the revolving helix.

Reagents (2) (9).

digestion mixture :		
	sulphuric acid, concentrated	90 % v/v
	potassium sulphate	10 % w/v
	sodium sulphate	2.9 % w/v
	selenium dioxide	0.3 % w/v
sodium hydroxide reagent :		
	sodium hydroxide	35 % w/v
	EDTA disodium salt	4 % w/v
alkaline sodium phenate :		
	sodium hydroxide	20 % w/v
	phenol	25.7 % w/v
sodium hypochlorite solution :		5 % v/v
sulphuric acid solution :		20 % v/v

Sample preparation.

4-5 g of cod muscle are macerated in 100 ml of 5 % NaCl, adjusted to pH 7.0, using an Ultra-Turrax homogenizer. 20 g of the homogenate are accurately weighed out, 10 ml of concentrated H_2SO_4 added, mixed, and after cooling it is diluted to 100 ml and used for the T. N. -determination.

Preparation of standards.

Freeze-dried cod is pulverized in a mortar and stored in the freezer to prevent deterioration.

Standards are now prepared containing about 0, 5 ; 1 ; 1, 5 ; 2 and 2, 5 g of cod powder. To the weighed amounts 100 ml neutral 5 % sodium chloride solution are added and the mixture is macerated for 1 min. at full speed using an Ultra-Turrax homogenizer.

20 g of the homogenate are accurately weighed out into a Kjeldahl flask and digested for two hr with 13 ml of 98 % H_2SO_4 and 3 g Wieninger-selenium-mixture (Merck). Total nitrogen is estimated by a manual technique (10), using the steam distillation apparatus of Antonacopoulos (12). Another 20 g portion of the homogenate is weighed out and 10 ml of concentrated H_2SO_4 are added while mixing using a magnetic stirrer. After cooling the mixture is washed with water into a 100 ml standard flask and made up to the mark. The shelf life of this standard, stored by 5°C, is more than 30 days.

Procedure.

The operating conditions may be summerised as follows (2) :

sample/acid ratio : 1/3, 45 (see also Fig. 1)
sampling rate : 10 s. p. h. (i. e., a 20 s. p. h. cam with water between each sample)
helix speed : 4 r. p. m.
helix temperature : 485°C first bank, 310-330°C middle and last banks.

Reagents are passed through all the lines except the air lines to establish the baseline and the 0 % transmission control.

After a delay period of 1 hour (11) the digestion and determination of T. N. contents can be started.

Samples and standards are analysed in duplicate. The mean value of the optical density readings from the standards are used to prepare a calibration curve.

RESULTS AND DISCUSSION.

T. N. -determinations have been performed in duplicate for a number of cod samples, by each of three methods : traditional Kjeldahl, AutoAnalyzer using as standard either ammonium sulphate or freeze-dried cod. The results are shown in table 1, which gives also the average recoveries of nitrogen from the automated procedures relative to the manual method. Table 2 gives values of some statistical parameters of each method calculated from the results of table 1.

The statistical analysis of nitrogen contents obtained by each method, was made using Student's t-test and the F-test (13). The critical values for the two tests (table 3) were calculated from the parameters of table 2.

The results in table 3 show that there is no significant difference between the means of manual and automatic cod powder method but that the difference between the means of the manual and automatic $(NH_4)_2SO_4$-method is significant. From the critical values for the F-distribution it can be seen that there is no significant difference in the precision of the means obtained by the three different methods.

Table 1 - Results of T. N. -concentrations determined in cod muscle by a manual Kjeldahl technique and by the AutoAnalyzer using either ammonium sulphate or freeze-dried cod muscle as standard.

Sample	Manual	AA-$(NH_4)_2SO_4$	$100.\frac{AA}{M}$	AA-cod powder	$100.\frac{AA}{M}$
1	3.11-3.05%N	2.82-2.86%N	92.2%	2.96-3.08%	96.8%
2	3.14-3.08	2.86-2.80	92.3	3.00-3.02	96.9
3	3.08-3.06	2.89-2.87	93.8	2.96-2.94	96.1
4	3.12-3.10	2.88-2.86	92.3	3.03-3.01	97.2
5	3.25-3.27	2.90-2.96	90.0	3.10-3.18	96.3
6	3.19-3.19	2.92-2.94	91.7	3.13-3.15	98.5
7	3.14-3.18	2.84-2.90	90.9	3.03-3.09	96.9
8	3.05-3.11	2.84-2.80	91.4	3.05-3.01	98.4
9	3.14-3.08	2.82-2.88	91.6	3.02-3.10	98.4
10	3.10-3.12	2.86-2.80	91.0	3.07-3.01	97.9
11	2.80-2.88	2.59-2.51	89.8	2.81-2.71	97.1
12	3.07-3.05	2.70-2.90	92.8	3.01-3.13	100.3
13	3.10-3.08	2.82-2.72	89.6	3.04-2.92	96.4
14	2.66-2.62	2.49-2.49	94.3	2.67-2.67	101.1
15	2.94-2.92	2.69-2.69	91.8	2.90-2.90	99.0
16	3.06-3.08	2.72-2.76	89.3	2.96-3.00	97.1
17	2.95-2.93	2.71-2.75	92.9	2.93-2.97	100.3
18	3.09-3.09	2.80-2.76	90.0	3.01-2.97	96.9
19	3.31-3.27	2.99-2.95	90.3	3.21-3.17	97.0
20	2.87-2.85	2.71-2.65	93.7	2.92-2.86	101.0

Table 2 - Statistical parameters of each N-determining method.

Method	Arithmetic mean	Standard deviation	Relative stand. deviation
Manual	$\bar{x}_1 = 3.05$ %N	$s_1 = 0.0272$ %N	$v_1 = 0.89$ %
AA. $(NH_4)_2SO_4$	$\bar{x}_2 = 2.80$	$s_2 = 0.0427$	$v_2 = 1.53$
AA. cod powder	$\bar{x}_3 = 2.99$	$s_3 = 0.0429$	$v_3 = 1.43$

Table 3 - Critical values for Student's t-test and F-test.

t-test

$t_1 = \dfrac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{s_1^2 + s_2^2}{m - 1}}} = 6.81$	$t_{0.05} = 2.03$
$t_2 = \dfrac{\bar{x}_1 - \bar{x}_3}{\sqrt{\dfrac{s_1^2 + s_3^2}{m - 1}}} = 1.63$	degrees of freedom : 38

F-test

$F_1 = \dfrac{s_2^2}{s_1^2} = 1.57$	$F_{0.05}(20.20) = 2.12$
$F_2 = \dfrac{s_3^2}{s_1^2} = 1.58$	

m = number of cod samples.

SUMMARY.

A modification of a previously reported method for quantitative determination of total nitrogen in cod muscle extract, using the Technicon AutoAnalyzer digestor system, is described.

Freeze-dried cod muscle, made up as solutions and standardized by manual Kjeldahl analysis, was used for calibration instead of the ordinary ammonium sulphate liquid standard. 100 % recoveries, relative to the manual method, were obtained with the former, while only 90 % with the latter.

ACKNOWLEDGMENT.

The work described in this paper was carried out as part of the programme of the "Commissie voor Toegepast Wetenschappelijk Onderzoek in de Zeevisserij", Brussels and has been supported by grants from the "Instituut voor Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw", Brussels.

REFERENCES.

(1) Ferrari A., Ann. N.Y. Acad. Sci., 1960, 87, 792.

(2) Sutton A. & Duthie A., Automation in analitical chemistry 1967, volume II, Kawerau H., Editor, Mediad Inc., White Plains, New York, 1968, p. 333.

(3) Grunbaum B., Kirk P., Green L. & Kock C., Anal. Chem., 1955, 27, 384.

(4) Marten J. & Catanzaro G., The Analyst, 1966, 91, 1078, 42.

(5) McNeal J., Karasz A. & George E., Journal of the AOAC, 1970, 53, 5.

(6) Gantenbein W., Journal of the AOAC, 1973, 56, 1.

(7) Schmidhofer Th., Egli H., & Weber R., Alimenta, 1973, 12, 109-112.

(8) Uhl D., Lancaster E., & Vojnovich C., Anal. Chem., 1971, 43, 990.

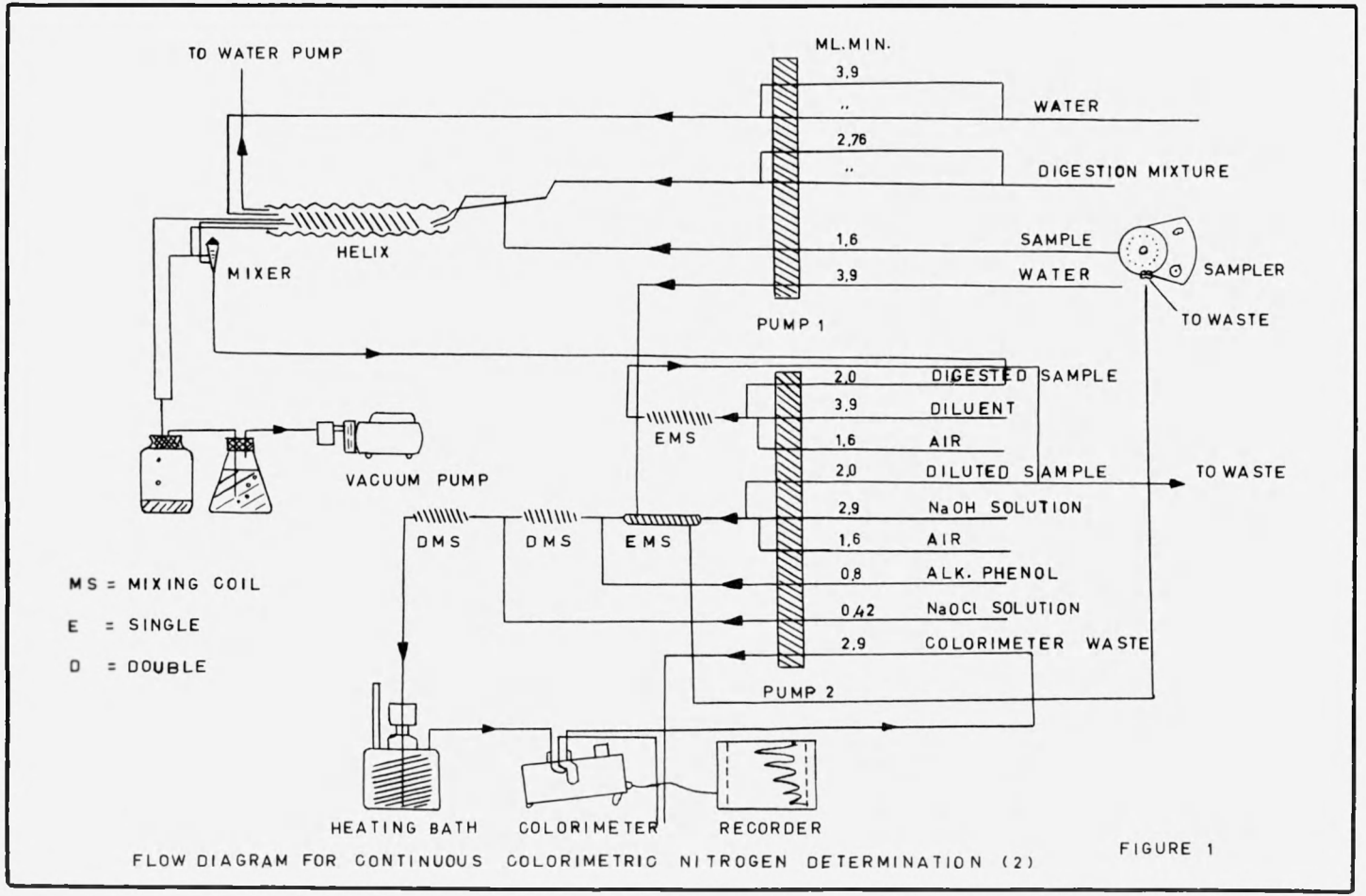
(9) Technicon AutoAnalyzer Methodology, N-3b.

(10) Vyncke W., Ministerie van Landbouw, 1969, publicatie 21, p. 65.

(11) Davidson J., Mathieson J., & Boyne A., The Analyst, 1970, 95, 181-193.

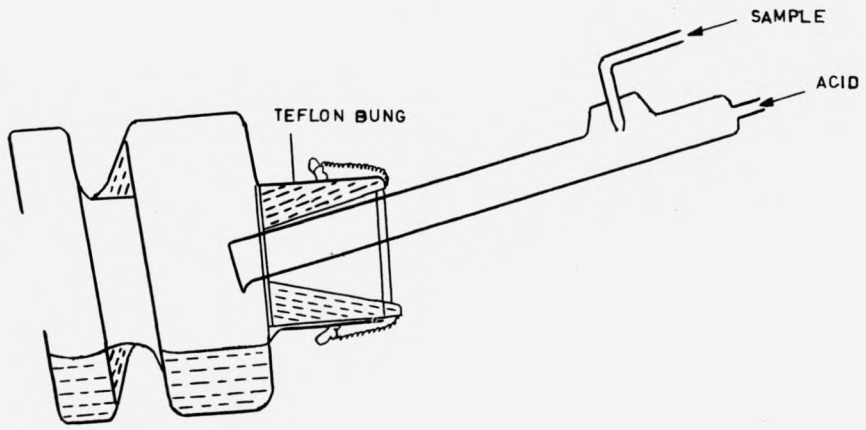
(12) Antonacopoulos N., Z. Lebensmittel-Untersuch., 1960, 113, 113.

(13) Eckschlager K., Errors, Measurement and Results in Chemical Analysis, Van Nostrand Reinhold Company, London, 1969.



FLOW DIAGRAM FOR CONTINUOUS COLORIMETRIC NITROGEN DETERMINATION (2)

FIGURE 1



CHUTE SYSTEM

FIGURE 2