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Comparison of two methods for determining volatile basic nitrogen (TVN)

by

W. Vyncke
Proefstation voor Zeevisserij
Ministerie van Landbouw
Ostend, Belgium

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COMPARISON OF TWO METHODS FOR DETERMINING VOLATILE BASIC NITROGEN (TVN)

Abstract

The method of Lücke and Geidel (1935), involving distillation in the presence of magnesium oxide in a still devised by Antonacopoulos (1960), was compared with the method proposed by the Codex Committee on Fish and Fishery Products, in which proteins are first precipitated.

The reproducibility of both methods was very satisfactory, the Codex method being somewhat more precise (2 percent against 3.5 percent). The correlation coefficient was 0.99, indicating an excellent relationship. Fish species (cod, redfish and herring) did not influence TVN determinations. On the whole, the MgO_4 method proposed by Antonacopoulos (1968) offers more advantages than the Codex-method and can be highly recommended for objective quality control of fish.

COMPARAISON DE DEUX METHODES DE DETERMINATION DE L'AZOTE
BASIQUE TOTAL VOLATIL (TVB)Résumé

On a comparé la méthode de Lücke et Geidel (1935), par distillation en présence de peroxyde de magnésium (MgO_4) dans un appareil mis au point par Antonacopoulos (1960), et la méthode proposée par le Comité du Codex sur les poissons et les produits de la pêche, dans laquelle on précipite d'abord les protéines.

La reproductibilité des deux méthodes est très satisfaisante, la méthode Codex étant un peu plus précise (2 pour cent contre 3,5 pour cent). Le coefficient de corrélation est de 0,99, ce qui indique un excellent rapport. Pour les poissons étudiés (morue, rascasse du Nord, hareng), l'espèce n'a pas influé sur les dosages de l'azote basique volatil. Dans l'ensemble, la méthode au MgO_4 proposée par Antonacopoulos (1968) offre plus d'avantages que la méthode Codex et peut être vivement recommandée pour le contrôle objectif de la qualité du poisson.

COMPARACION DE DOS METODOS PARA DETERMINAR EL NITROGENO
BASICO VOLATIL (NBV)Extracto

El método de Lücke y Geidel (1935) de destilar en presencia de óxido de magnesio en un destilador ideado por Antonacopoulos (1960), se comparó con el propuesto por el Comité del Codex sobre Pescado y Productos Pesqueros, que comienza precipitando las proteínas.

Ambos métodos se pueden reproducir muy satisfactoriamente, siendo el del Codex algo más exacto (2 por ciento contra 3,5 por ciento). El coeficiente de correlación es de 0,99, lo que indica la excelencia de ésta. Las especies de pescado (bacalao, gallineta y arenque) no influyeron en las determinaciones del NBV. En general, el método del MgO_4 propuesto por Antonacopoulos (1968) es más ventajoso que el del Codex y puede recomendarse sin reservas para la determinación objetiva de la calidad del pescado.

INTRODUCTION

The determination of volatile basic nitrogen (TVN) is a simple and fairly reliable method for assessing the degree of freshness of fish. Two methods are commonly employed, viz. micro-diffusion according to Conway (1962) and distillation either directly or after removal of proteins. The micro-diffusion method is simple and precise but rather time-consuming, which makes it less suitable for routine quality control.

Among the many modifications of the distillation method, the Lücke and Geidel (1935) technique, which involves distillation of the volatile bases under standardized conditions in the presence of magnesium oxide, is commonly used in several countries, especially in Europe.

When using the steam-distillation unit devised by Antonacopoulos (1960), the original procedure can be greatly simplified and speed of analysis significantly enhanced. This technique has been used for more than seven years in our laboratory and is entirely satisfactory. The detailed procedure was only recently published (Antonacopoulos, 1968).

The Codex Committee on Fish and Fishery Products (FAO/WHO) has proposed a standard method, elaborated by Canada, in which the proteins of the fish are first precipitated with trichloroacetic acid and TVN steam-distilled in a micro-Kjeldahl apparatus, after liberation by sodium hydroxide (FAO/WHO, 1968).

It seemed worthwhile to make a comparative study of both methods. Reproducibility and correlation were determined and the most important factors (influence of fish species, distillation time, filtering and centrifuging) were investigated.

EXPERIMENTAL

The fish species tested were: cod (Gadus morhua L.), redfish (Sebastes marinus L.), and herring (Clupea harengus L.) of varying degrees of freshness. The distillation methods employed were: the procedures proposed by Antonacopoulos (1968) and by the Codex Committee (FAO/WHO, 1968) both of which were accurately followed. For convenience, the former is referred to as the "MgO₂ method" and the latter as the "Codex method".

RESULTS AND DISCUSSION

Reproducibility

The standard deviation was calculated from the duplicate analyses according to:

$$s = \sqrt{\frac{\sum \bar{d}^2}{2n}}$$

with \bar{d} = difference between duplicates and n = number of pairs.

The coefficient of variation was also computed. Six ranges of TVN were taken from 10 to 69 mg% N and 200 fish were analyzed in each group. The results for cod are reported in Table 1. It appears that the standard deviation is not the same in the six groups and this is true for both methods. Application of the Hartley Test (1950) showed them to be significantly different. The coefficients of variation, however, were fairly similar, indicating that both methods maintain the same relative reproducibility over a wide range.

The Codex method appears to be more precise. Both methods, however, can be considered as having very satisfactory reproducibility. With 95 percent probability, the standard errors are respectively about 3.5 and 2 percent for the MgO₂ and the Codex methods, when determinations are carried out in duplicate. The same reproducibility was obtained with redfish and herring, which indicates that the fish species (and the fat content) have no influence on the TVN determination.

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Table 1

Standard deviations (in mg% N) and variation coefficients (in brackets) of the MgO₂ and the Codex methods for TVN determination

Range (mg% N)	MgO ₂ method	Codex method
10 - 19	0.24 (1.6%)	0.33 (2.2%)
20 - 29	0.54 (2.2%)	0.39 (1.6%)
30 - 39	0.88 (2.5%)	0.35 (1.0%)
40 - 49	0.87 (1.9%)	0.44 (1.0%)
50 - 59	1.17 (2.1%)	0.59 (1.1%)
60 - 69	1.39 (2.1%)	0.74 (1.1%)

Correlation

The methods correlated very well: the correlation coefficient determined on 200 paired determinations equalled 0.99, and was highly significant. Results given by the MgO₂ method correspond to those of the Codex method by the function (regression line)

$$y = 5.30 + 0.93 x$$

where y and x are the TVN-values of respectively the Codex and the MgO₂ method.

Distillation time

In the Codex method, approximately 40 ml are distilled over in 7 min. This period appeared to be satisfactory, all TVN being distilled over in about 5 min. The amounts of distillate (40 ml) and of boric acid (10 ml) need not be measured accurately and the use of a volumetric flask of 50 ml is superfluous. An Erlenmeyer flask of 250 ml can be used directly.

In the MgO₂ method, approximately 120 ml are distilled over in 12 min. Most of the TVN comes over in 5 min. Magnesium oxide, being a weak base, has a minimal effect on protein breakdown; between 5 and 20 min, 0.5 mg% N (\pm 0.1) are distilled over per min; between 20 and 60 min, the rate is about 0.4 mg% N/min. These amounts are independent of the fish species and of the initial concentration of TVN and are very reproducible. Nevertheless, a definite distillation time with a 1 min tolerance should be observed. Any time between 5 and 20 min can be chosen, but the 12 min proposed by Antonacopoulos (1968) and also used at our laboratory, seem convenient.

Centrifuging and filtering

In the proposed Codex procedure, fish is homogenized with 7.5 percent trichloroacetic acid, filtered and centrifuged. Filtering alone was investigated to determine if it would not be sufficient without centrifuging. Using the statistical method of paired comparisons, centrifuged extracts gave on average 0.24 mg% N higher results, but this difference was not significant. Moreover, the reproducibility was not increased by centrifuging, the standard deviations remaining identical.

CONCLUSIONS

Although its reproducibility is somewhat lower, the MgO₂ method offers more advantages than the Codex procedure. It involves only four steps: weighing the fish sample, adding MgO₂ (not accurately), distilling and titrating. The Codex method on the other

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hand is carried out in nine phases: weighing the sample, adding exactly 200 ml of trichloroacetic acid solution, homogenizing, filtering, centrifuging, pipetting an aliquot into the distillation unit, adding a measured amount of sodium hydroxide solution, distilling and titrating.

The Codex-procedure, however, can be simplified by using an Erlenmeyer flask directly as titration vessel and omitting the centrifuge step.

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