Sonderdruck aus „ARCHIV FÜR LEBENSMITTELHYGIENE", 47. Jahrgang, Heft 5, Septermber/Oktober 1996, Seiten 110–112
Verlag M. & H. Schaper, Kalandstraße 4, 31061 Alfeld (Leine) - Druck: Dobler-Druck GmbH & Co. KG, Alfeld (Leine)

Comparison of the official EC method for the determination of total volatile bases in fish with routine methods

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The determination of total volatile bases (TVB) is probably the oldest objective chemical method for assessing the spoilage of fishery products. It was proposed about a century ago and was already published as standard method for the inspection of fish in Germany in the first years of this century (KÖNIG, 1910). This simple method is still widely used.
A European Union directive on fish hygiene specifies that if the organoleptic examination reveals any doubt as to the freshness of the fish, inspectors may use TVB as chemical check (EU, 1991).
The European Commission Decision of 8 March 1995 fixes the TVB limit values for three categories of fishery products and specifies the analysis methods to be used. The reference method involves steam distillation of an extract deproteinised by perchloric acid (PCA). For routine work, three other techniques are also allowed i. e. microdiffusion according to CONWAY (1947), direct distillation with magnesium oxide (ANTONACOPOULOS, 1968) and distillation of an extract deproteinised by trichloracetic acid (TCA) (Codex Alimentarius, 1968).
The aim of the present work was to compare the EC-reference method (PCA method) with the direct method (MgO method) and the TCA extraction method, using two types of apparatus: the compact Antona still and a semi-automatic Kjeldahl distilling unit (Kjeltec).

Material and methods

Fish

Samples of cod (Gadus morhua), plaice (Pleuronectes platessa) and Dover sole (Solea solea) of varying degree of freshness were used.

Apparatus

Antona still: unit consisting of 2 l round-bottom flask with side arm and stopcock (steam generator) and reaction vessel insert (ANTONACOPOULOS, 1960). Semi-automatic Kjeldahl distilling unit: Kjeltec type T 1002 (Tecator, Höganäs, Sweden).

Procedure

PCA method: 10 g of fish is blended with 90 ml of PCA 6 %. Fifty ml of filtrate is made alkaline with 6.5 ml sodium hydroxide 20 % and distilled for 10 min (Antona still) or 5 min (T 1002).
TCA method: 10 g of fish is homogenised with 20 ml TCA 7.5 %. Six ml sodium hydroxide 10 % is added to 25 ml of extract and distilled as in the PCA method. MgO method: 2 g of magnesium oxide is added to 10 g of minced fish. Distillation takes 12 min in the Antona still (ANTONACOPOULOS, 1968) or 6 min in the T 1002.
Titration: with 0.01 N sulphuric acid against mixed indicator for ammonia titrations (Merck).

Results and discussion

Control of TVB distillation with ammoniumchloride

The recovery of volatile nitrogen was tested on both apparatus: 50.00 mg ammoniumchloride-N were distilled over and titrated. Twenty-five analyses were carried out over a period of three months. Distillation time was 10 min. Small but significant systematic errors of 0.5 % (Antona still) and 1.5 % (T 1002) were noted (table 1). In practice, however, this is negligible. The standard deviations were low and did not differ significantly (F-test), indicating the precision of the two methods to be similar.

Control of distillation rate

According to the EC method, a distillation rate of 100 ml in 10 min should be maintained. This appeared to be possible only with the Antona still. Without modifications, the Tecator unit has a markedly higher rate of 200 ml. No attempts were made to modify this apparatus by fitting a valve for steam regulation as indicated in earlier work (ANTONACOPOULOS and VYNCKE, 1989). Instead, distillation time was reduced to 5 min.
The reproducibility of the distillation rate with the Antona still was tested on ten occasions over a period of three months. The mean volume was 100.6 ml with a standard deviation of 8.6 ml (confidence interval of 95-107 ml), which is satisfactory. With T 1002, these figures were 100.5, 1.0 and 100-102 ml respectively, indicating a higher reproducibility with this semi-automatic device. Taking into account the results of the recovery tests (table 1) however, this seems to be of less importance.

Table 1: **Recoveries of 50 mg distilled volatile nitrogen**

Still	mgN	standard deviation	coefficient of variation (%)
Antona	49.76	0.43	0.86
T 1002	49.24	0.48	0.97

Table 2: **Corresponding TVB limits (mg N/100g) for three determination methods**

Reference method (PCA)	TCA	MgO
25	27	23
30	32	28
35	37	33

Comparison of methods

The PCA and MgO methods were first compared using both apparatus. Twenty samples of fish (cod, plaice, sole) of varying degree of freshness were used. No differences were noted between the fish species. There was a high correlation for both the PCA extraction method ($r = 0.987$) (fig. 1) and the MgO method ($r = 0.977$) (fig. 2). Moreover, both slopes did not differ significantly indicating both techniques to have the same sensitivity. TVB values determined with T 1002 were about 7 % lower than those obtained with Antona.
A further series of 40 comparative tests between the MgO and PCA methods was carried out with the Antona still. The results (fig. 3) indicate an excellent relationship between both methods ($r = 0.991$).
Trichloracetic acid (TCA) is often used instead of PCA as a deproteinising agent. Correlation between both extraction methods was high ($r = 0.981$; $n = 30$) (fig. 4). In the TVB range 25–35 mg TCA values were 5–7 % higher than PCA data, indicating probably a more pronounced degradation of proteins and peptides.
Good correlations between different methods were also found by other authors. The microdiffusion method, one of the EC routine methods, was reported to be in good agreement with the MgO method (WITTFOGEL, 1960; BROOKER and MAHNKE, 1966; DAVIDOVICH and GIANNINI, 1984). BOTTA et al. (1984) tested six methods, including the MgO and TCA methods and concluded that all were very similar in their suitability as an index of spoilage.
The EC has set limits for three categories of fish: 25, 30 and 35 mg N/100 g. Table 2 shows the corresponding TVB limits for the three methods using the Antona still.
The precision of the different techniques was tested by calculating the standard deviation of the duplicates ($n = 30$) in the TVB range 25–40 mg. The results were: 1.20 for PCA, 1.45 for TCA and 1.50 for MgO. A Hartley test performed on the variances showed no significant differences, indicating the reproducibility of the three methods to be similar. These standard deviations however were higher than that obtained with ammoniumchloride (0.43; table 1) showing the fish sample itself to influence distillation.
It should be emphasized that the different correlations and TVB-limits (table 2) cited here could change more or less when using different apparatus or procedures. In early work with TCA extracts e. g., markedly lower results were obtained when using a Markham Kjeldahl still (VYNCKE, 1971). It is imperative that procedures should be standardised and followed accurately, also for routine methods. This was also stressed by other research workers (REHBEIN and OEHLENSCHLÄGER, 1982, 1990).

Conclusions

There was an excellent correlation both between the three methods investigated and the two types of distillation apparatus used, showing that the routine methods involving MgO or TCA can be used instead of the official reference method with PCA. This is especially true for the direct distillation method according to Antonacopoulos which necessi-

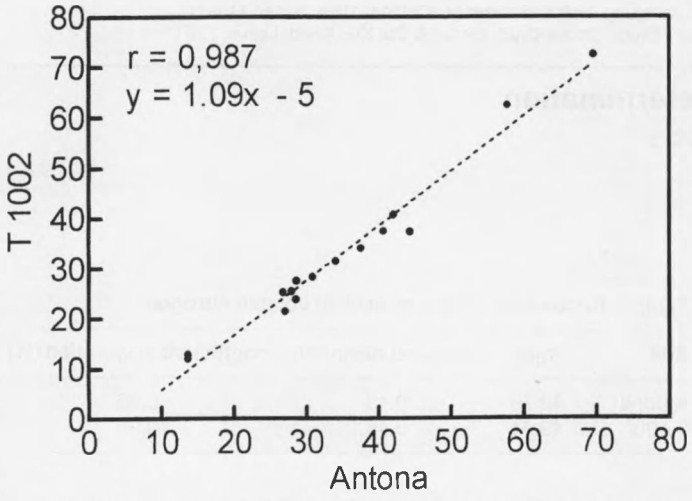

Fig. 1. Comparison of distillation methods (T 1002 and Antona-still) with PCA-extract

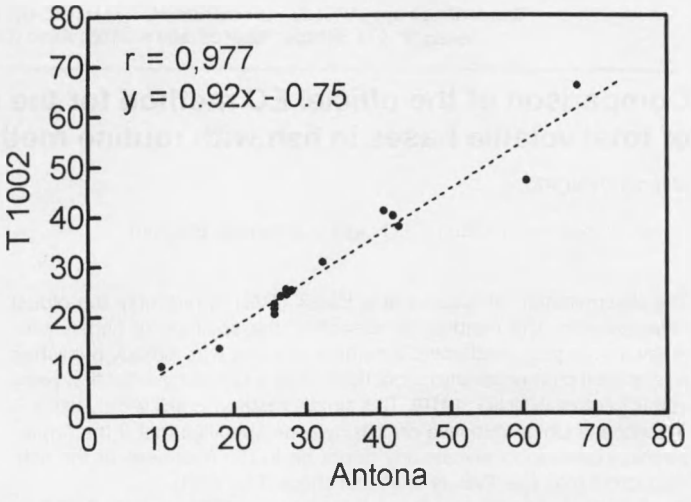

Fig. 2. Comparison of distillation methods (T 1002 and Antona-still) with MgO

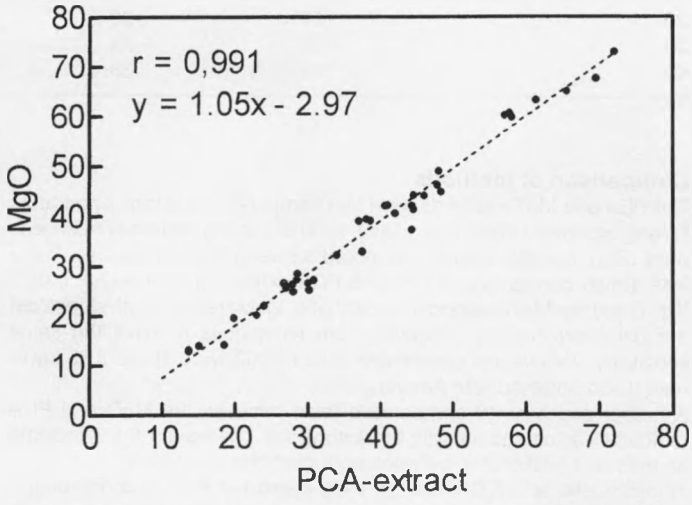

Fig. 3. Correlation between PCA and MgO methods with Antona-still

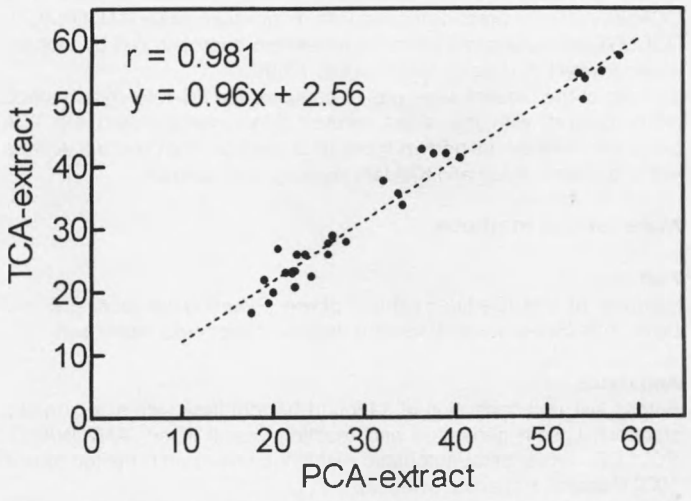

Fig. 4. Correlation between PCA and TCA methods with Antona-still

tates less analytical steps than the other techniques. This method should be recommended as a quick routine method. It should be recalled however that in case of doubt or in the event of dispute regarding the results of analysis performed by one of the routine methods only the reference method may be used to check the results.

Summary

The EC reference method for the determination of total volatile bases (TVB) in fish, involving preliminary deproteinisation with perchloric acid, was compared with two routine methods i. e. direct distillation of fish after addition of magnesium oxide and use of trichloracetic acid instead of perchloric acid. Furthermore, two types of distillation units were used i. e. a compact still consisting of a round-bottom flask (steam generator) with inserted reaction vessel (Antona still) and a semi-automatic Kjeldahl distilling unit (Tecator T 1002). There was an excellent correlation both between the three methods studied and distillation apparatus used, showing that the routine methods can be used instead of the official reference method. The direct distillation method which necessitates less analytical steps is recommended as a quick routine method.

Zusammenfassung

Die Referenzmethode der Europäischen Kommission zur Bestimmung des flüchtigen basischen Stickstoffes (TVB) in Fisch, wobei vorherige Enteiweissung mit Perchlorsäure ausgeführt wird, wurde mit zwei Routinemethoden verglichen, Direkt-Destillation unter Zugabe von Magnesiumoxid und Benutzung von Trichloressigsäure statt Perchlorsäure. Dabei wurden zwei Destillationstypen benutzt eine kompakte Destillationseinheit bestehend aus Rundkolben (Wasserdampfentwickler) mit Reaktionsgefäß-Einsatz (Antona-Gerät) und eine halbautomatische Kjeldahl Destillationseinheit (Tecator T 1002). Eine sehr gute Korrelation zwischen den drei überprüften Methoden und zwischen den zwei Destillationsstellen wurde festgestellt. Die Routinemethoden können damit statt der offiziellen Referenzmethode benutzt werden. Die Direktdestillation, die weniger arbeitsaufwendig ist, wird für die Routinepraxis empfohlen.

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