

Analytical, Nutritional and Clinical Methods

Validation of a flow-injection-gas diffusion method for total volatile basic nitrogen determination in seafood products

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Abstract

A potentiometric flow-injection method, using a gas-diffusion cell, is described for the determination of total volatile basic nitrogen (TVB-N) in seafood products. The method was based on the change of the potential of a tungsten oxide electrode when volatile basic compounds, liberated from the fish extract sample, diffuses via a permeable membrane into a phosphate buffer acceptor stream and locally shifts the pH. After being optimized the method was validated in terms of linearity, precision sensitivity and recovery.

Linear calibration graph was obtained in the range 1–20 mg of N per L with a detection limit ($S/N = 3$) of 0.02 mg N per L. The precision (relative standard deviation, $n = 10$) of this method for within and between-days is better than 1.2% and 2.2%, respectively. The method was applied for the analysis of TVB-N in four fish species (sardine, red mullet, mackerel and hake) and gave results in close agreement with those obtained by the official method. Comparison of the results using student *t*-test and variance ratio *F*-test showed there was no significant difference between the methods compared. Thus the proposed method is simple, easy to handle and reliable instrument for quality control of seafood products with a high throughput (40 samples h^{-1}).

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1. Introduction

Trimethylamine (TMA-N) and total volatile basic nitrogen (TVB-N) are important characteristics for the assessment of quality in seafood products and appear as the most common chemical indicators of marine fish spoilage. It was indeed established that a postmortem reduction of trimethylamine oxide by bacterial enzymes may leads to a dramatic increase in TMA-N (Boskou & Debevere, 1997; Krzymien & Elias, 1990; Veciana-Nogues, Albalá-Hurtado, Izquierdo-Pulido, & Vida-Carou, 1996). This process is accompanied by a significant production of ammonia and other basic nitrogenous compounds such as methylamine and dimethylamine which are collectively known as TVB-N.

To date many analytical methods have been developed for quantitative measurements of TVB-N and TMA-N concentrations. They include steam distillation (Malle & Tao, 1987), colorimetry (AOAC, 1995, Chap. 35; Dyer, 1945), photometry (Zhi, Rios, & Valcàrcel, 1995), high performance liquid chromatography (Malle & Valle, 1996), gas chromatography (Fiddler, Doerr, & Dan gates, 1991; Veciana-Nogues et al., 1996), capillary electrophoresis (Timm & Jorgensen, 2002), semi-conducting metal oxides sensors (Hammond et al., 2002; Zhao, Wei, & Chen, 2000; Zhao et al., 2002), selective electrode (Mitsubayashi et al., 2004) as well as flow injection/gas diffusion (FIGD) with spectrophotometric (Baixas-Nogueras, Bover-Cid, Vidal-Carou, Veciana-Nogues, & Mariné-Font, 2001; Sadok, Uglow, & Haswell, 1996) or potentiometric detection (Adhoum et al., 2003).

Among all these methods, steam distillation and the colorimetric method of Dyer (1945) are the most known and

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widely used procedures for TVB-N and TMA-N determinations. The current reference method (AOAC, 1995) for TMA-N determination is based on the reaction of TMA with picric acid to form a colored complex. This method suffers from two main limitations: it is time consuming and requires the use of relatively large volumes of generally toxic organic solvent. The official method adopted by the European Community for TVB-N measurement (Official Journal, 1995) involves the rather laborious extraction of volatile bases by perchloric acid solution followed by steam distillation of the extract and subsequent titration against HCl standard. Although accurate, this method is tedious and time consuming (2–3 samples h^{-1}).

In the few last years, several FIGD methods (Adhoum et al., 2003; Baixas-Nogueras et al., 2001; Sadok et al., 1996) have been developed to overcome these drawbacks.

In a previous paper (Adhoum et al., 2003), we described a FIGD system coupled to a potentiometric detector and applied to the measurement of TMA-N in seafood products. However, no data were reported on its suitability for TVB-N determination.

In this paper, we report on the feasibility of FIGD-potentiometric determination of TVB-N in seafood products. The proposed method was studied in terms of linearity, precision, sensitivity and recovery and compared with the official steam distillation procedure.

2. Experimental

2.1. Reagents and solutions

All reagents used were of analytical-reagent grade and were provided by Prolabo (France). Sodium hydroxide solution (1 M) and potassium dihydrogen phosphate solution (10^{-2} M) were prepared by dissolving the corresponding salt in distilled water. A standard ammonium stock solution (1000 mg L^{-1}) was prepared by dissolving the appropriate amount of solid NH_4Cl in distilled water. Working standard solutions in the range 1–50 mg L^{-1} were freshly prepared by appropriate dilution of the stock solution.

2.2. Instrumentation

The flow injection equipment employed here is similar to that previously described by the present authors (Adhoum et al., 2003). The reagent streams were propelled by variable-speed peristaltic pump (Gilson Minipuls, anachem, Luton Bedfordshire, UK) using silicone rubber tubings (0.8 mm i.d.). A low pressure injection valve (Rheodyne 5020, Anachem, Luton, UK) with variable sample loop volume was used to inject sample extracts into NaOH stream. The evolved volatile bases diffuse via a Teflon micro-porous membrane, inserted in a gas diffusion cell, into a phosphate buffer acceptor stream and locally shift the pH. This is monitored with a potentiometric flow cell using a tungsten oxide electrode and an AgCl/Ag reference

electrode. The potential difference between the electrodes was measured using a Linseis chart recorder (Bioblock scientific, Germany).

The tungsten oxide electrode was prepared by polishing tungsten wire (1 mm) with a grit paper and then washed with acetone. The wire was then heated in an oven at variable temperatures (200–750 °C) during different exposure times (from 5 to 120 min). The obtained wire was soaked in sodium hydroxide solution (1 mM) for 15 min before use.

2.3. Preparation of fish samples

Four different fish species (sardine (*Sardina pilchardus*), mackerel (*Scomber scombrus*, L.), red mullet (*Mullus barbatus*) and hake (*Merluccius merluccius*)) were purchased from local tunisian market and immediately delivered to our laboratory to be treated to obtain the fish extract.

The fish samples were headed, eviscerated, thoroughly washed to remove the blood and carefully filleted. Ten grams of each fish fillets were homogenized with a pestle and mortar for 1 min. Then 50 ml of 6% perchloric acid were added and the extract was homogenized for a further 2 min. After that, the mixture was centrifuged at 14,000 rpm for 10 min to obtain a clear solution ready for analysis.

2.4. Determination of TVB-N by the official method

TVB-N was determined based on an adaptation of the current official European steam-distillation method (Official Journal, 1995). The method is based on the extraction of TVB using alkaline solution and the titration of the recovered ammonia as follows: 10 ml of fish extract and few drops of phenolphthalein indicator were placed in the distillation flask. Then 3.5 ml of 20% sodium hydroxide solution was added, the apparatus immediately sealed and the end of the steam distillate collected in a flask containing 20 ml of 4% boric acid and few drops of Tashiro indicator (methyl red/methylene blue 2:1). The steam distillation procedure was continued until 250 ml of distillate had been collected. The obtained basic solution was titrated against 0.01 M hydrochloric acid to the end point indicated by a green to gray color change. The TVB-N content was determined after blanc correction that has been determined by the steam distillation of 6% perchloric acid sample.

3. Results and discussion

3.1. Optimization of TVB-N detection

To obtain maximum sensitivity for the proposed FIGD method, a number of experimental parameters were optimized such as heating temperature and exposure time of the tungsten electrode, pH and concentration of phosphate buffer acceptor stream, concentration of NaOH donor

stream as well as flow rate and injected volume. The optimization was performed using the univariate method at mass concentration of 10 mg N/L.

The effect of oven temperature and exposure time of tungsten wire was studied in the range 200–750 °C and by varying the time of exposure between 5 and 120 min. The sensitivity of the electrode was found to increase upon increasing the temperature between 200 and 450 °C and reached a maximum at about 500 °C when the color of tungsten wire became bluish-violet. This indicates the formation of a stoichiometric oxide $WO_{2.9}$ (Lassner, 1996). At this optimum temperature, the peak height increased with increasing exposure time up to 30 min. However, a significant decrease of electrode sensitivity was observed when exposure times were over 60 min. As a result, the electrode was prepared by heating at 500 °C during 30 min for all subsequent experiments.

Similarly, optimum values of all other parameters were investigated and the results are shown in Table 1.

On the basis of these results all further experiments were performed under the optimized conditions.

3.2. Linearity of electrode response

The linearity of the developed method was investigated by measuring the electrode responses of three sets of standard aqueous solutions of ammonium in the range 1–20 mg of N/L over three different days. For every set of samples, calibration curves showed a linear dependence of the peak height of the flow injection signal with the logarithm of ammonium concentration. Typical peaks obtained with TVB-N standards are illustrated in Fig. 1. Least squares analysis gave determination coefficient values (r^2) higher than 0.99 in the considered concentration range. Cochran's C test was applied for checking the homogeneity of variances and Fisher test was used to examine the significance of the slope.

The equation of the line ($\Delta E = 43.32 \log[\text{TVB}] + 3.43$) was used for the determination of TVB in real samples.

3.3. Precision and sensitivity

The intra- and inter-days precision of the proposed method was evaluated as the relative standard deviation

Table 1
Studied operating parameters and their optimal values

Variables	Range studied	Optimal value
Donor stream concentration NaOH (M)	10^{-5} –2	0.5
Acceptor stream concentration Phosphate (M)	10^{-6} – 10^{-2}	5×10^{-6}
Acceptor stream pH	3–11	4
Heating temperature of tungsten wire (°C)	200–750	500
Exposure time (min)	10–120	30
Injection volume (μL)	100–1000	290
Flow rate (mL min^{-1})	0.45–1.45	0.8

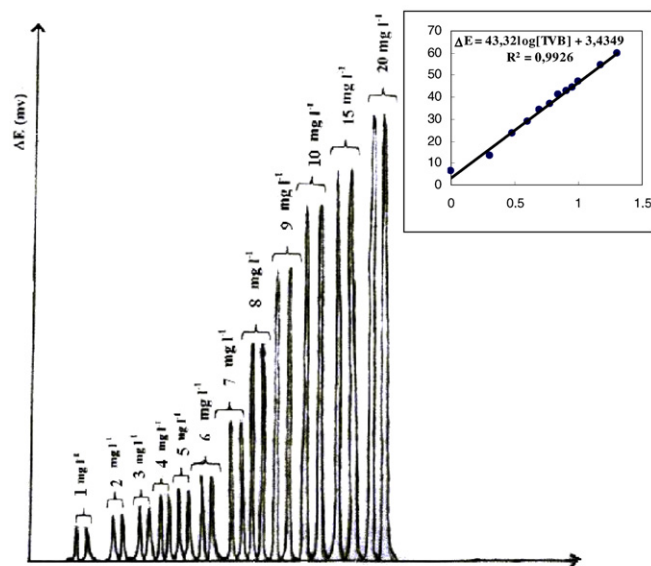


Fig. 1. Typical potentiometric response of the tungsten oxide electrode to TVB-N standards using the optimum operating conditions.

(RSD) of 10 repeated determinations of 10 mg N/L standard solution of ammonium. The precision was found to be satisfactory with an average of intra- and inter-days RSD values of 1.2% and 2.2%, respectively.

Table 2

Recovery of the FIGD method determined by analyzing spiked fish extract samples

Samples	Initial content (mg N/100 g)	Amount spiked (mg N/100 g)	Amount found	Recovery (%)	RSD (%)
Sardine	40.50	2.25	42.75	100	0.73
	40.50	4.50	45.00	100	1.1
Mackerel	22.20	1.11	23.32	101	1.2
	22.20	2.22	24.50	103.6	0.95
Hake	11.05	0.55	1.60	100	1.3
	11.05	1.10	12.19	103.6	1.2
Red mullet	58.20	3.38	62.10	103.4	1.8
	58.20	5.62	64.35	102.13	1.6

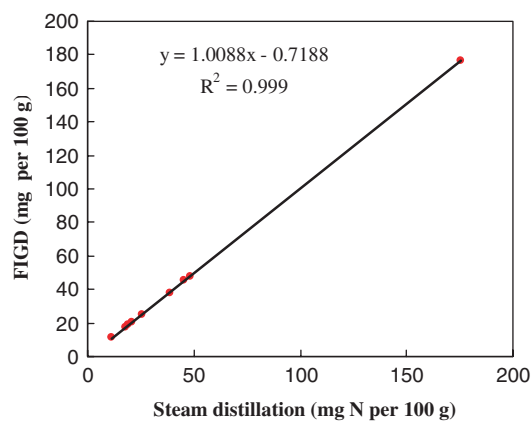


Fig. 2. Correlation between FIGD and the official steam distillation method for the determination of TVB-N.

Table 3
Determination of TVB-N in fish samples using the proposed FIGD procedure compared statistically with the official method

Samples		Proposed FIGD method ^a (mg N/100 g)	RSD (%)	Official method ^a (mg N/100 g)	RSD (%)	<i>t</i> -test ^b	<i>F</i> -test ^c
Sardine	1	18.64	1.8	19.20	3.5	1.32	0.025
	2	45.52	0.7	45.64	2.1	0.13	0.48
	3	47.60	1.3	48.16	2.5	1.62	0.19
Mackerel	1	20.33	1.6	20.85	2.8	1.11	0.15
	2	38.25	0.9	39.05	2.5	1.25	0.61
Hake	1	11.20	1.7	11.50	3.1	0.81	0.37
	2	17.50	1.3	18.00	2.7	1.12	0.21
Red mullet	1	25.02	1.4	25.50	2.8	0.38	0.15
	2	176.28	1.1	175.38	2	0.82	0.98

^a The presented concentrations are the average of six determinations.

^b Tabulated *t*-value for significance level $p = 0.05$ and $n = 10$ is 2.23.

^c Tabulated *F*-value for significance level $p = 0.05$ and $f_1 = f_2 = 5$ is 5.0.

The limit of detection (LD) was obtained from a signal-to-noise ratio of 3 and was found to be lower than 0.02 mg N/L (i.e. 0.01 mg N per 100 g of fish). Apparently, this value is one order of magnitude lower than that found with FIGD associated to spectrophotometric detection (Baixas-Nogueras et al., 2001) and two order of magnitude lower than the official steam distillation method which has a detection limit of about 5.6 mg N per 100 g of fish. Thus the present method gives very sensitive assay and allows quantification of 500-fold smaller amounts of TVB-N.

3.4. Application and accuracy

FIGD method was applied for the analysis of TVB-N in several samples of sardine, mackerel, red mullet and hake. The accuracy of the proposed assay was evaluated by adding known amounts of ammonium standards to the fish extract and determining the recovery. The results of these measurements are given in Table 2. As can be seen, the calculated mean recoveries were excellent in all cases, ranging between 100% and 103.4%. Based on Cochran's test we verified that accuracy did not depend on TVB content. In addition, we checked with Student's *t* test that there was no significant difference between the mean recovery found and the theoretical value of 100%.

The accuracy of the proposed procedure was further investigated by comparing the found values with those estimated by the steam distillation official method. The correlation of the analytical results between the two methods is fairly good as shown in Fig. 2. A highly significant linear regression was obtained ($y = 1.0088x - 0.7188$) with a regression coefficient of 0.999, indicating an excellent agreement between two methods. Moreover, the results obtained were compared statistically by student's *t*-test and variance ratio *F*-test (Table 3). The experimental values were below the theoretical values in either test, indicating that there was no significant difference between the compared methods.

These results show that the proposed method is applicable for TVB-N determination in seafood products. Indeed,

it offers interesting assets such as rapidity, simplicity, reproducibility, higher sensitivity when compared with the official steam distillation method.

4. Conclusion

We have developed and validated a simple potentiometric FIGD method for the determination of TVB-N in seafood products. The proposed method is rapid (40 samples h⁻¹), reliable and highly sensitive. Moreover, it has the advantages of low cost, simplicity and flexibility. Therefore, it should be a useful alternative for routine analytical control of fish freshness.

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