Turk J Zool
24 (2000) 113–120

Comparision of Methods Used for Determination of Total Volatile Basic Nitrogen (TVB-N) in Rainbow Trout *(Oncorhynchus mykiss)*

Fatih ÖZOĞUL, Yeşim ÖZOĞUL
The University of Hull, International Fisheries Institute, Hull, ENGLAND

Received: 29.01.1999

Abstract: TVB-N (total volatile basic nitrogen) concentration in fish muscle was used as an index of freshness quality to compare the spoilage of rainbow trout wrapped in cling film and in aluminium foil. TVB-N levels in different muscle tissue and in the whole fish were measured every two days for a period of 16 days at 4-6°C. In addition, three different methods of determining of TVB-N were evaluated in terms of sensitivity, reliability, speed, and cost. Regardless of the method of analysis, there was an increase in the level of TVB-N during storage, especially after 7-9 days. In general, the trend of increase was similar in each method, but semi-steam distillation yielded the highest concentration of TVB-N. Results obtained also showed that TVB-N in the belly flesh sample was the highest, whereas the lowest TVB-N value was obtained with the dorsal flesh for each method. If the high cost can be tolerated, flow injection method is recommended for determination of TVB-N. It was also found, however, that TVB-N can not be used as an index of spoilage but as an indicator of advanced spoilage in the sample.

Key Words: TVB-N, rainbow trout, Conway micro-diffusion method, semi micro steam distillation, flow injection gas diffusion

Gökkuşağı Alabalığında (*Oncorhynchus mykiss*) Toplam Uçucu Temel Nitrojen (TVB-N)'nin Belirlenmesinde Kullanılan Metodların Karşılaştırılması

Özet: TVB-N (toplam uçucu teme nitrojen)'in konsantrasyonu, hem aliminyum folyaya hemde ince seffaf plastige sarılı alabalığın kasında tazeliğinin göstergesi olarak miktarı ölçülüp karşılaştırıldı. Örnekler her iki günde 16 gün boyunca 4-6°C depolanan balığın çeşitli yerlerinden ve ayrıca tüm balığı temsil etmesi için homojen bir örnek alınarak TVB-N miktarı ölçüldü. Buna ek olarak, TVB-N miktarını belirlemek için kullanılan 3 method hassaslık, güvenilirlik, hızlılık ve fiyat yönünden değerlendirildi. Kullanılan metodlara bakmaksızın, TVB-N miktarı özellikle 7-9 gün sonra hızlı bir şekilde yükseldi. 3 metoddan elde edilen sonuçlar, TVB-N miktarının balığın bel kısmında en yüksek olduğu, dorsal kısmında ise en düşük olduğu göstermiştir. Eğer Flow injection metodunun yüksek fiyatı tölerans edilirse, TVB-N'in belirlenmesi için bu metod tavsiye edilir, bununla beraber TVB-N'nin balığın bozulmasının göstergesi olarak kullanılamayacağı fakat ileri düzeydeki bozulmanın göstergesi olarak kullanılabileceği bulunmuştur.

Anahtar Sözcükler: TVB-N (toplam uçucu nitrojen), gökkuşağı alabalığı, Conway micro-diffusion method, semi micro steam distillation, flow injection gas diffusion.

Introduction

Much attention has been given to finding a rapid and reliable method of fish freshess assessment. A variety of methods have been used to measure the postmortem changes in sensory quality, chemistry and microbiology.

One of the chemical methods makes use of the total volatile basic compounds (TVB) in fish, which contains mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA), the levels of which increase with spoilage by either bacterial or enzymic degradation. Changes in TVB content during spoilage are very similar to those of TMA, except that the initial values are much higher. One component of TVB, ammonia, is present even in very fresh fish.

The present investigation was undertaken to assess three methods for the determination of TVB-N in terms of sensitivity, reliability as an index of spoilage and speed and cost of determination.

Materials and Methods

Fish sample

Rainbow trout were obtained from a local fish farm and had been kept in ice for less than 1 hour on arrival. The fish were wrapped individually, some in cling film and others in aluminium foil, and stored in a refrigerator at 4-6°C. Every two days, two fish, one wrapped in cling film and the other in aluminium foil, were taken out of the stock for analysis of TVB-N.

Chemical analysis

Three different parts of the fish were taken as samples, namely, the ventral, dorsal and lateral parts. The fourth sample consisted of the whole fish to represent all parts of one particular fish. Four groups of 10 grams of muscle were used to prepare an extract for analysing the TVB-N according to Derrick (1). Ten grams of fish flesh were homogenised in 90ml of 6% (w/v) TCA (trichloroacetic acid) solution. The solution was centrifuged at 3000 rpm for 10 minutes and the homogenate filtered through Whatman No. 1 filter paper. The extract collected was stored in a freezer at -20˚C until it was required for analysis.

Methods for estimating TVB-N

Three methods were used to determine TVB-N: the Conway micro-diffusion method (2), semi micro steam distillation (3) and flow injection gas diffusion (4).

Results and Discussion

For fish wrapped in cling film (A) and aluminium foil (B), the TVB-N content of each part of body muscle taken from the lateral, dorsal and ventral parts of the fish were compared with a sample of muscle homogenate representing the whole fish for all of the methods (Figs. 1, 2, 4, 5, 7, 8). In all samples, the TVB-N concentration increased slowly during the first 7 days for fish wrapped in cling film (A) but approximately 9-10 days for fish wrapped in aluminium foil (B). Regardless of the mehod used, the ventral part of fish (A) and (B) had the highest TVB-N value, whereas the TVB-N value was the lowest in the dorsal part of fish throughout the trial. The whole fish sample had the values expected according to values for ventral and dorsal samples.

The quality and storage life of fish may decrease if they have not been gutted. Fish contain many bacteria in the digestive system and strong digestive enzymes are produced during the feeding periods, which will be able to cause rapid autolysis post-mortem during the later stage of storage. This may give rise to a strong off-flavour, which might be related to the breakdown of protein and the production of nitrogenous volatile materials, especially in the ventral area, or even cause belly-burst (5). This would explain why the ventral part of fish had the highest TVB-N value of any of the fish samples.

The results obtained from the same fish extracts are shown in Figures 10 and 11 for Conway micro diffusion (CM-D), semi-micro steam distillation (S-MSD), flow injection gas diffusion (FIGD). The trends in the results for fish A and B with CM-D and FIGD were similar throughout the storage periods. The highest TVB-N values for fish A and B were obtained with semi-micro steam distillation. In both cases, the variation was highly significant ($P<0.005$). This may be due to the secondary deamination which may have taken place during the steam distillation of the alkalised fish extract producing extraneous ammonia. It was not the product of normal spoilage activity. This was likely to occur since the amount of alkali added to extract was increased to obtain reproducible results.

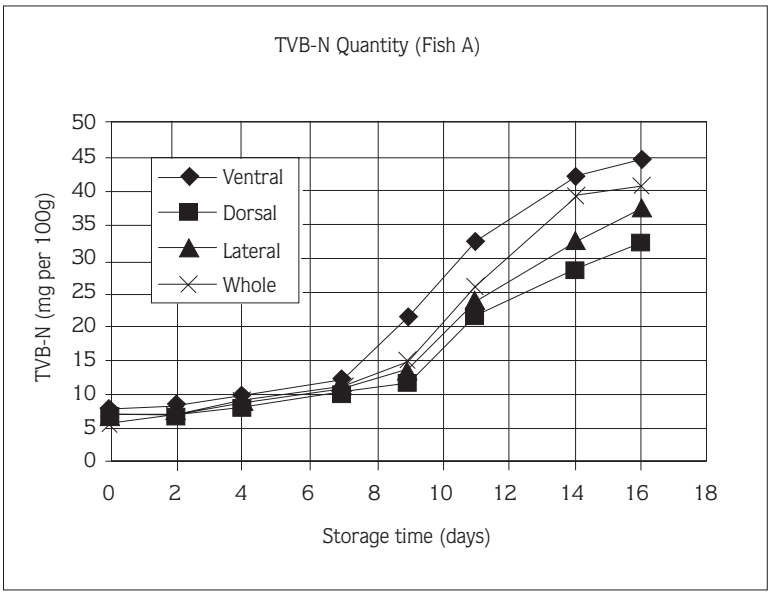


Figure 1. Total volatile base content in various parts of fish wrapped in cling film at 4-6˚C (Conway micro-diffusion method).

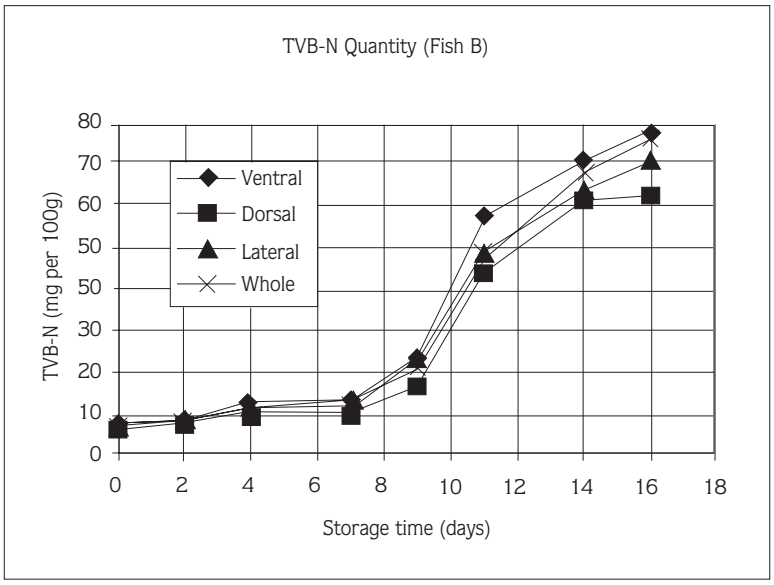


Figure 2. Total volatile base content in various parts of fish wrapped in aluminium foil stored at 4-6°C (Conway micro-diffusion method).

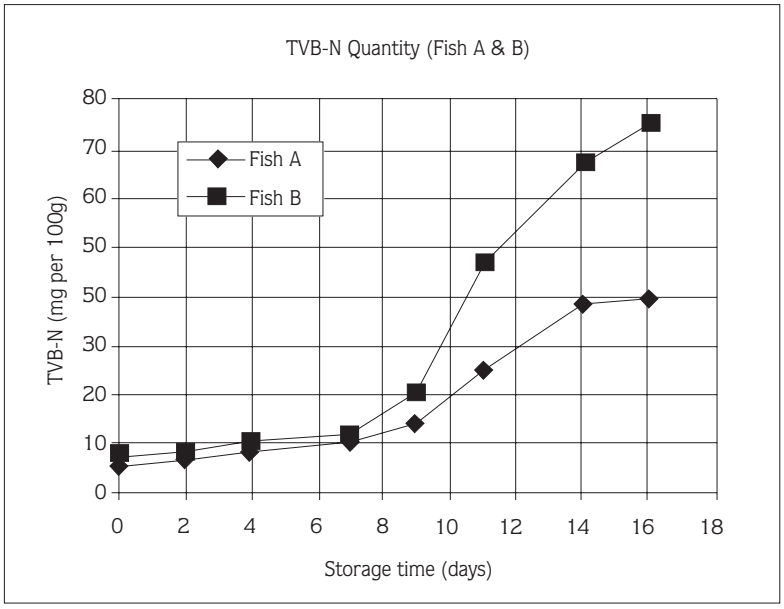


Figure 3. Comparison of TVB-N values in fish wrapped in aluminium foil (B) and in cling film (A) stored at 4-6°C (Conway micro-diffusion method).

The results obtained from all methods showed that there was a significant difference ($P<0.05$) in TVB-N levels between trout wrapped in aluminium foil (B) and trout wrapped in cling film (A), which suggests that the spoilage bacteria mainly responsible for volatile base production are more active in aerobic than in anaerobic conditions (Figs. 3, 6, 9).

Connell (6) suggested that the recommended level of TVB-N for rejection is 20mg N/100g flesh for fatty fish, which would include trout. Oehlenschlager (7) concluded that when the concentration of TVB-N exceeded 30mg/100g flesh, the fish should be considered unfit for consumption. Hence, TVB-N can be used only as an indicator of fitness for consumption rather than as an index of freshness throughout the storage life of fish. On this basis, fish wrapped in cling film (A) would have been rejected at day 10 or 11 by the FIGM and CM-D methods of TVB-N determination (Figs. 3 and 5) but at day 7 by the S-MSD method (Fig. 4). Fish wrapped in aluminium foil (B) would have been rejected at day 9 by the FIGM and CM-D methods of TVB-N determination, but at day 6 by the S-MSD method.

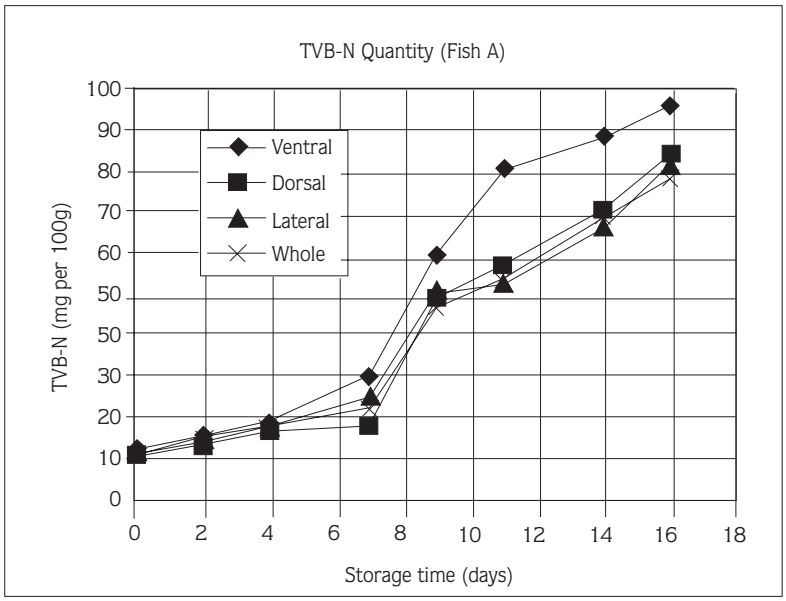


Figure 4. Concentration of TVB-N (mg N/100g) in various parts of fish wrapped in cling film stored at 4-6°C (semi micro steam distillation method).

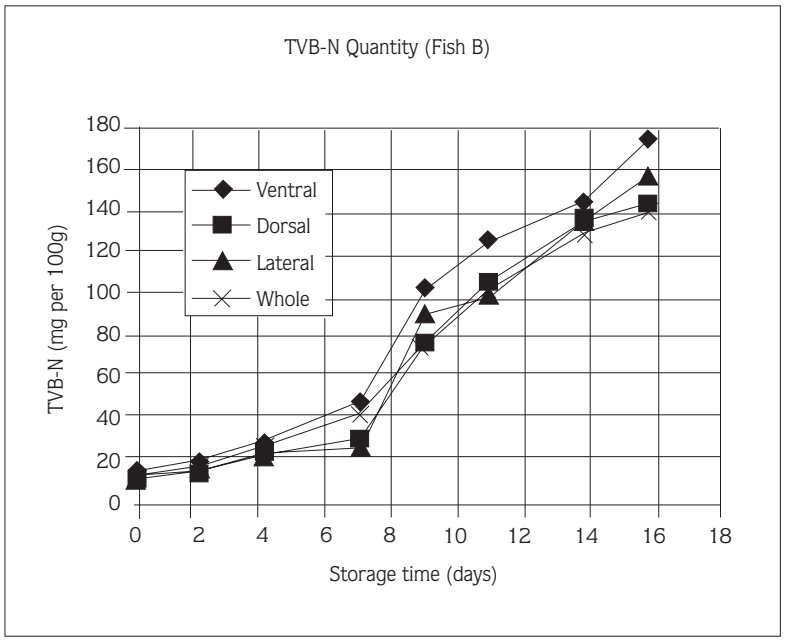


Figure 5. Concentration of TVB-N (mg N/100g) in various parts of fish wrapped in aluminium foil stored at 4-6°C (semi micro steam distillation method).

The TVB-N level did not increase much during the early stages of deterioration, which is the main difficulty in analysing the value. They only rise as a result of the bacterial activity during later stages of deterioration. Therefore, they are not a reliable indicator of early changes in quality. In addition, it was reported that TVB-N rose only slowly during the chilled storage of most freshwater species. Such as trout and pike, principly due to negligible TMA-O levels. Therefore, it is of little use for freshwater species.

Experiments have been undertaken with some freshwater species to determine TVB-N. Karnicki and Lima Dos Santos (8) observed that the initial TVB value for fresh *Haplochromis* was 51.8 mg TVB-N/100 g, which is much higher than that usually found in fresh fish. For instance, Meynel (9) found only approximately 7mg TVB-N/100g in fresh *Haplochromis* ssp. The same range of TVB values has also been found for fresh tilapia and other freshwater tropical fishes. The TVB value increases due to autolytic and bacterial spoilage during the storage of the fish.

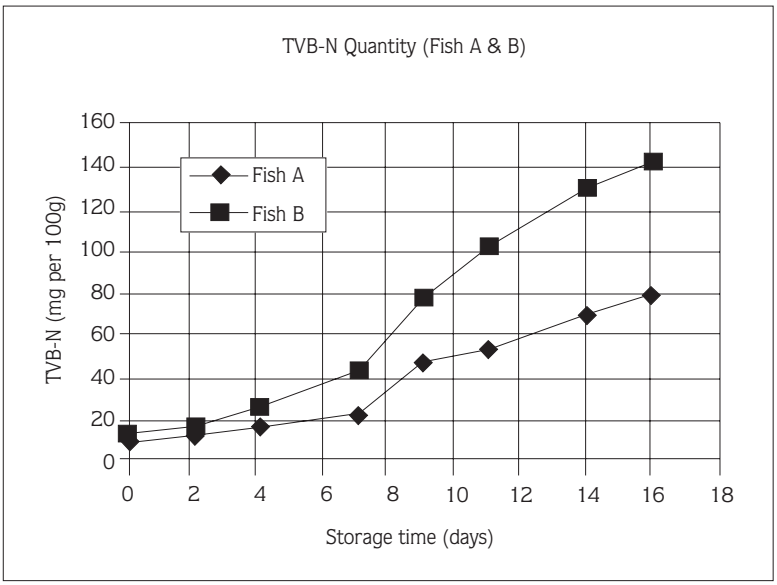


Figure 6. Comparison of TVB content in trout wrapped in aluminium foil (B) and in cling film (A) stored at 4-6°C (semi micro steam distillation method).

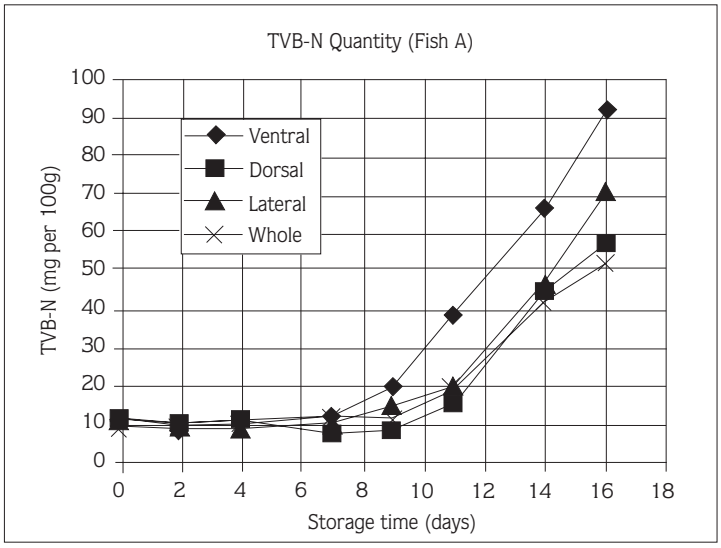


Figure 7. TVB-N values for trout wrapped in cling film (A) stored at 4-6°C (flow injection/gas diffusion).

It was found that during storage of *Haplochromis* at room temperature, the TVB-N value increased within one day from 51.8 to 91.4mg TVB-N/100g flesh (8). However, when *Haplochromis* was stored in ice, the TVB-N value, instead of increasing, had a tendency to decrease, falling from 51.8mg TVB-N/100g at the beginning of the experiment, to 16.3 mg TVB-N/100g in ice and 20. 6 mg TVB-N/100g in chilled lake water after 10 days of storage. This could be due to the fact that volatile bases were leached out during the storage period.

Conversely, Yamanaka (10) studied saury pike and found the TVB-N content to be 5.5mg TVB-N/100g flesh during a storage period of 0 days at 5°C. After day 11, the TVB-N content reached 64.1mg TVB-N/100g flesh. Estimation of TVB in nile perch fillets gave an initial value of 48.5mg TVB-N/100g, and rose to 60.2mg TVB-N/100g flesh when stored for two days at ambient temperature. These results could not have been subject to the leaching effect, because fillets were stored in tightly closed plastic bags (8).

TVB-N is present in even very fresh fish. TVB-N increases to approximately 50-70mg nitrogen in cod held for 20-25 days in melting ice. These values can be taken as the upper limits. Lower values would be set for good and passable quality fish; for instance, 35-40mg TVB nitrogen/100g are considered limits for cod. Higher values than these are regarded as too spoiled for use. A value of no higher than 30 mg TVB-N/100 g has been

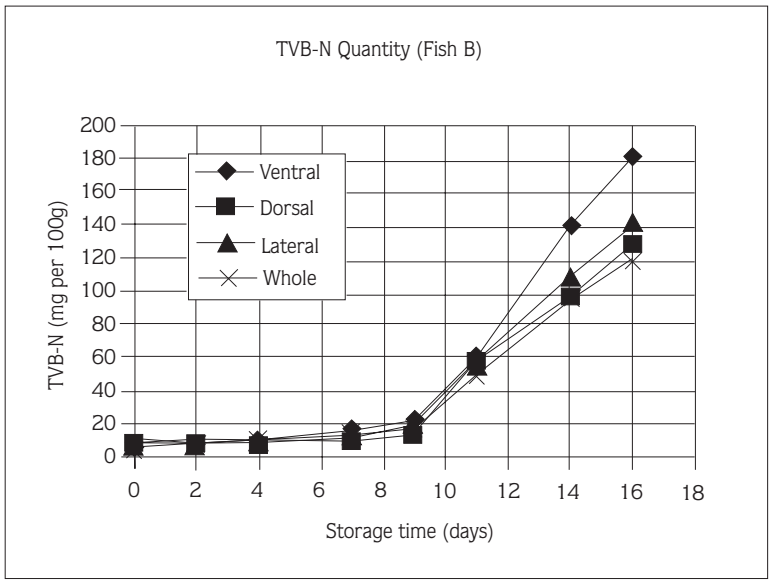


Figure 8. TVB-N values for trout wrapped in aluminium foil stored at 4-6°C (flow injection/gas diffusion).

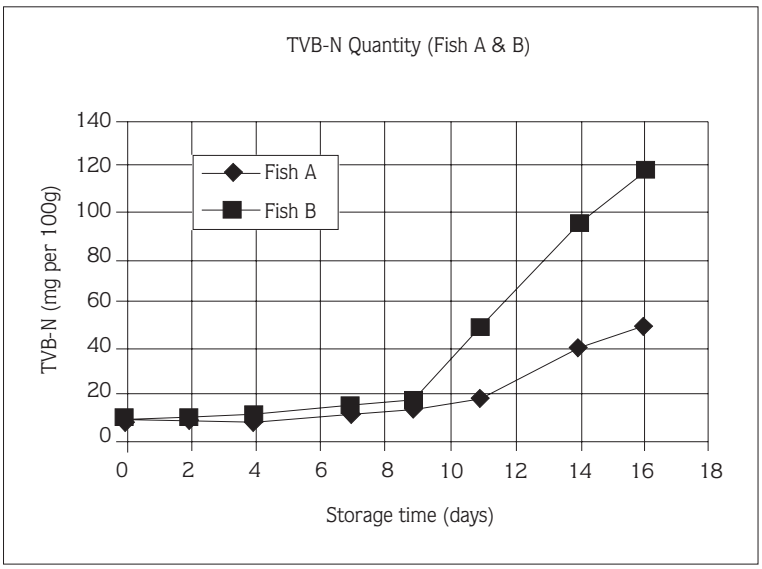
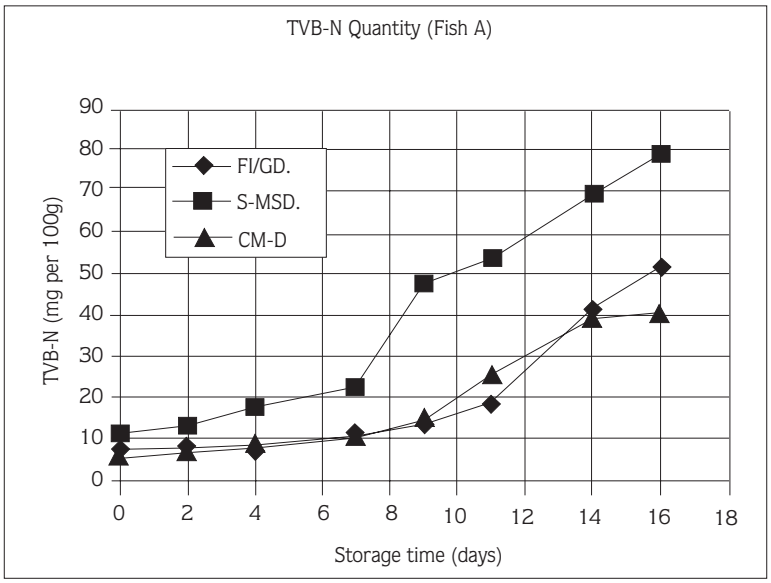


Figure 9. TVB-N values for fish both wrapped in aluminium foil and in cling film and stored at 4-6°C (flow injection/gas diffusion).

specified for frozen tuna and swordfish; no greater than 100-200 mg TVB-Ng for salted and dried fish; and no greater than 20 mg TVB-N/100g for the raw material used in various canned products (6).

Oehlenschlager (7) studied cod and haddock for determination of freshness. He reported that TVB-N can not be used as a freshness indicator. In the first 8-12 days of ice storage (0°C), the concentration of TVB-N remained constant or decreased slowly. Nevertheless, after 12 days of storage, TVB values began to increase rapidly with time, reaching 30mg/100g at day 15-20. However, in this experiment which was conducted using refrigerated storage (4-6°C) the TVB-N value increased slowly in the first 7-9 days of the storage period, after which it increased rapidly. It was concluded that when the concentration of TVB-N exceeded 30mg/100g flesh, the fish should be considered unfit for consumption. Hence, TVB-N can be used only as an indicator of fitness for consumption rather than as an index of freshness throughout the storage life of fish.

In sum, TVB-N is insensitive to "freshness", which means that it can not be used as a freshness indicator. However, it is sensitive in terms of "fitness" for human consumption, and it is also a good spoilage indicator (11).



Figurel 10. Comparison of methods of assessing freshness quality {Key: FI/GD=Flow injection gas diffusion, S-MSD= semi-micro steam distillation, CM-D= Conway micro-diffusion}.

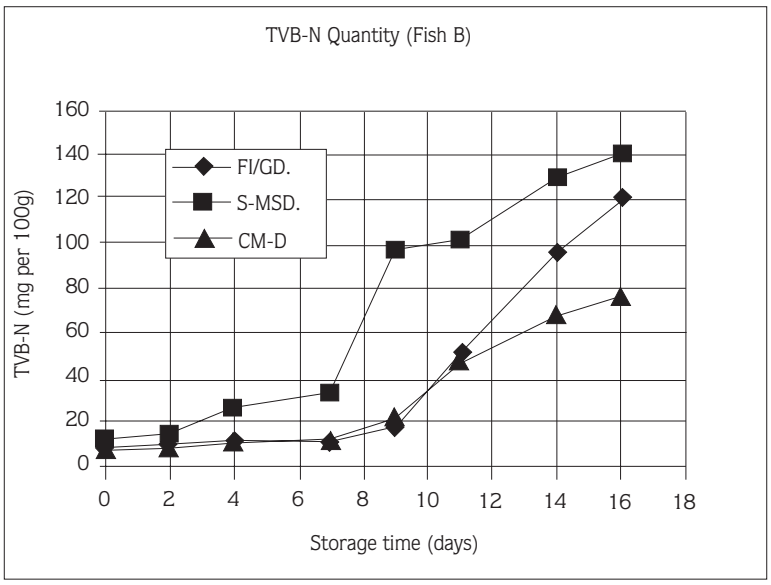


Figure 11. Comparison of methods of assessing freshness quality {Key: FI/GD=Flow injection gas diffusion, S-MSD= semi-micro steam distillation, CM-D= Conway micro-diffusion}.

The fish is considered spoiled (12) when TVB exceeds 30 mg nitrogen per 100 g fish. The level of spoilage can also be detected by sensory assessments.

In the comparison of three methods (the Conway micro-diffusion method, semi micro steam distillation and flow injection gas diffusion), it was found that all methods were sensitive only after 10 days of storage. The reliability of semi micro steam distillation and flow injection gas diffusion depends on contamination level and type. On the other hand, the Conway micro-diffusion is reliable if it is used by experienced personnel. Flow injection gas diffusion is the quickest of the methods. In terms of cost, both semi micro steam distillationand flow injection gas diffusion are quite expensive because of equipment and running cost.

Acknowlagement

We wish to express our gratitude to Mr. W. F. A Horner for his advice and to Dr. S. Derrick for assistance throughout the research at the International Fisheries Institute of Hull University.

References

1. Derrick, S., Post harvest technology practical-Biochemical indices of fish freshness. University of Hull. HIFI, unpublished manuscript, 2, (1995).
2. Conway, E.J., Microdiffusion Analysis and Volumetric Error. Grosby, Lockwood and Sons Ltd., London, (1968).
3. Brodin, V. Guidelines for the methodology of Qualpoiss Project. University of Hull: HIFI, Unpublished manuscript, 10, (1995).
4. Sadok, S., Uglow, R., Haswell, S.J., Determination of Ninhydrin Positive Substances. In Sea-water and Hemolymph. In Analyst, August vol. 120., 2097-2099, (1995).
5. Huss, H. H. Quality and Quality Changes in Fresh Fish. Roma: Food and Agriculture Organisation (FAO) of the United Nations, 51-92 (1995).
6. Connell, J.J., Control of Fish Quality. Published by Fishing News Books. 3rd edition, 122-150, (1990).
7. Oehlenschlager, J., Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In Quality Assurance in the Fish Industry, Huss, H.H. (editor). Elsevier Science Publishers B. V., Netherlands, 339-351, (1992).
8. Karnicki, Z., Lima Dos Santos, C., Storage Life of Chilled African Fish Species. In Storage Lives of Chilled and Fish and Fish Prdoucts. Aberdeen, UK, 169-175, (1985).
9. Meynell, P. Y., Effect of pre-freezing handling procedures on the quality of Chisawasawa (Lethrinops and Haplochoromis spp.) from Lake Malawi, Trop. Sci., 2, 211, (1979).
10. Yamanaka, H., Polyamines as potential indices for freshness and decomposition of saury pike (Cololabis saira) and scallop (patinopecten yessoensis). In Chilling and Freezing of New Fish Products, Torry Research Station, Aberdeen., 123-129, (1990).
11. Horner, W. F. A., Final report to directorate general for fisheries DG XIV commission of the European Community. (a study on the health quality of fish products). University of Hull: HIFI, unpublished manuscript, 9-57, (1997).
12. Connell, J.J., Control and Fish Quality. Fishing News (Books) Ltd., Farnham, Survey, UK, (1975).