**Traceability**

**Project 6.3 - Valid**

# Volatile amines as criteria for chemical quality assessment

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*Draft to be reviewed by SeaFoodPlus experts (without English correction)*

## Abstract

Volatile amines are the characteristic molecules responsible for the fishy odour and flavour present in fish several days after the catch and they are commonly used as criteria for assessing the fish quality. The nature and the formation of the volatile amines are discussed. Ammonia is present in freshly caught fish, during chilled storage it is formed by endogenous and bacterial enzymes, it is a poor indicator of fish freshness and cannot be considered as an effective marker of fish spoilage. DMA is present at very low concentration in freshly caught, about 0.2 mg/100g, it is formed from TMAO, under TMAO-ase action, an endogenous enzyme which is mainly present in gadoids; DMA-N can be considered as an effective marker of fish freshness of many white fish and it can be used to monitor the quality of frozen-stored gadoid fish. In a very fresh fish the amount of TMA-N is low, ≤ 2 mg/100g, it arises from the bacterial reduction of TMAO. TMA-N is an excellent indicator for the spoilage of gadoid fish, it is useful as a rapid means of objectively measuring the eating quality of many demersal fish specially on the medium-later phases of spoilage but it cannot be used as an freshness indicator (constant level during the first days of iced storage). TVB represents the sum of ammonia, DMA, TMA and others basic nitrogenous compounds volatile under the analysis conditions. In freshly caught fish its content is generally superior to 10 mg/100g and does not exceed 15 mg/100g except for pelagic fish. TVB-N is an indicator of spoilage of some fish species such as red fish, flat fish, gadoids, hake and Atlantic salmon, legal requirements in directive 91/493/EEC have been established for the limits of this indicator in the fish muscle for several species. However TVB-N cannot be used as an freshness indicator (constant level during the first days of iced storage) and des not reflect the mode of spoilage. The volatile amines being essentially formed by degradation of TMAO, their production is linked to the initial concentration of TMAO in the muscle which depends on the species, season, fishing ground and depth of living. The influence of processing such as chilling, ice storage, slurry ice, freezing, cooking, canning and packaging including modified atmosphere packaging is discussed.

The methods of determination of volatile amines are presented, the first one are specific methods which are used by many laboratories. Some more recent techniques are used in research laboratories and many new rapid methods have been described or are under development. About the methods two main conclusions can be withdrawn : 1) With regard to quality control methods of spoilage, as the volatile amines are produced by enzymatic reaction, their level increases in the chain, even under chilling condition, so it is important to perform the analysis very quickly after the sampling and the analysis result should be express clearly, i.e. TVB in mgN/100g, with the reference of the used method and details about the sampling (nature, date, place); 2 ) With the regard to the validation methodology: In the fish commercialisation chain, it is important to have the possibility of checking quickly the

quality, however classical laboratory methods, that cannot be considered as rapid techniques, are still used in Europe. For TVB, the AOAC Official method "Volatile bases in fish, ammonia ion selective electrode method" which is more rapid and easier to perform than our reference method could be compare to the EU official procedure in the aim to facilitate the controls in the chain. For TMA-N, specific sensors and probes recently developed and others being developed in other European projects, based on the selective identification of this molecule, must be validated to assess the possibility of having them as quality control tools for the fish industry.

## Contents

## Volatile amines as criteria for chemical quality assessment

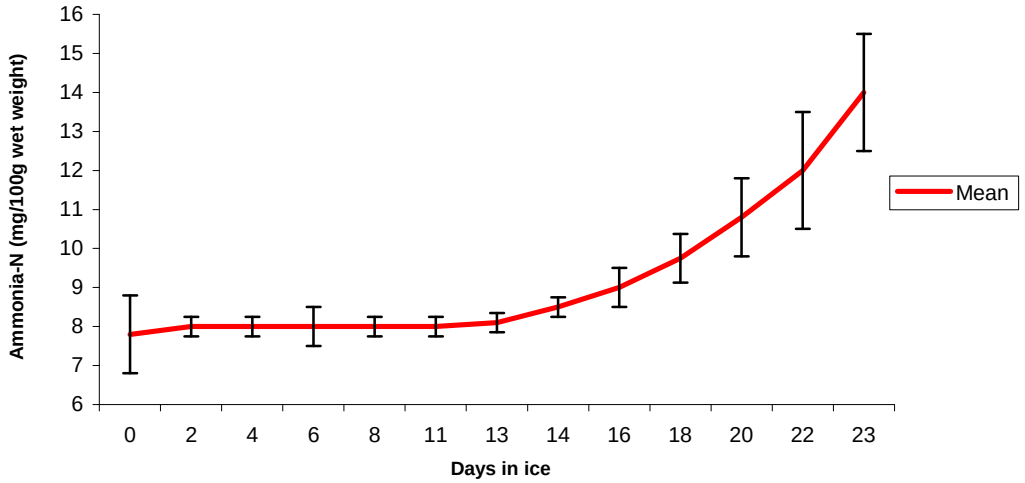
Volatile amines are the characteristic molecules responsible for the fishy odour and flavour present in fish several days after the catch and together with the sensory parameters they are the most common criteria for assessing the fish. The significance and the use of this criteria have been underlined by numerous authors and synthesis about the suitability of volatile amines as freshness/spoilage of seafood have been published by Oehlenschläger (1997 a, b) and in FAO Fisheries Technical Paper 348 (Huss, 1995).
The designation "volatile amines" regroups mostly three molecules, ammonia, dimethylamine (DMA) and trimethylamine (TMA). DMA and TMA result of the degradation of trimethylamine oxide (TMAO) a fish typical molecule which has an important role in osmoregulation, DMA is mostly produced by endogenus enzymes and TMA by bacterial enzymes (Huss, 1995). The total volatile basic nitrogen or total volatile bases noted TVB-N or TVB or TVN consists mainly of a mixture of ammonia, DMA and TMA plus amines from the decarboxylation of amino acids (Garcia-Garribo and Luque de Castro, 1997) and other nitrogen compounds that become volatile when made alkaline (Pedrosa-Menabrito and Regentrein, 1990). The results of analysis are given in nitrogen equivalent, ammonia-N, DMA-N, TMA-N and TVB-N.

### 1 - The different volatile amines – nature and formation

#### 1.1 - ammonia-N

In freshly caught fish ammonia is present in muscle at an average concentration of 10 mg ammonia-N/100 g wet weight, with low variation, between 7 to 15 mg/100g (Oehlenschläger, 1997a). It is formed by bacterial deamination of proteins, peptides and amino-acids. It can also be produced by an autolytic breakdown of adenosine monophosphate (AMP) in chilled seafood products (Huss, 1995). It remains relatively constant during the initial phase of iced storage. A small decrease of the ammonia content can be observed in this first phase in particular in small sized fishes, it is due to leaching effects. After, at day 7-12 depending of the fish species an increase is noted, the increase is variable during the whole remaining storage, a large scattering of the concentration is generally observed.
However if ammonia is considered as a poor indicator of fish quality it has been described as an excellent indicator of squid quality according to LeBlanc and Gill (1984). More recently, Paarups et al (2002) noticed the evolution of NH3 during iced storage, nevertheless they proposed another amine, agmatine as a freshness indicator.
***Ammonia-N is a poor indicator of fish freshness and cannot considered as an effective marker of fish spoilage***

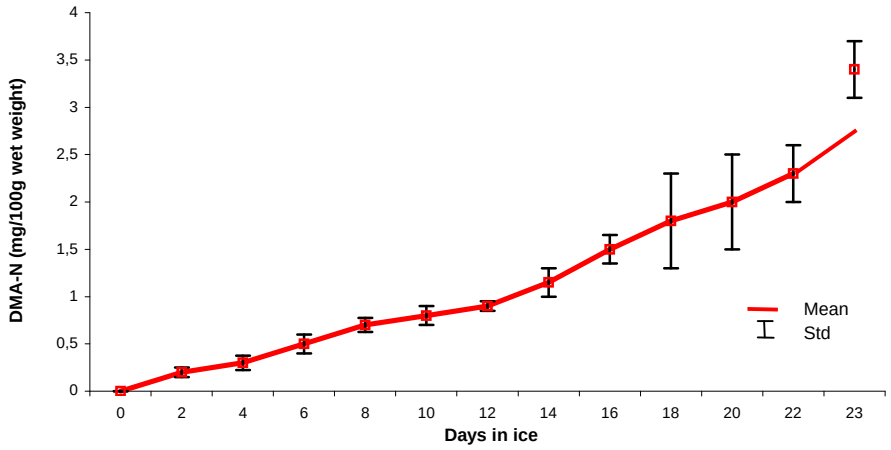
**Figure 1: Develoment of ammonia-N in muscle during iced storage of cod (according to Oehlenschläger, 1997 b)**




### 1.2 - DMA-N

In freshly caught fish DMA-N is present at a very low concentration, about 0.2 mg DMA-N/100g with a variation in the range 0.1 to 0.4 mg/100g (Oehlenschläger, 1997 a). Its development is different in fishes which contain the enzyme TMAO-ase or TMAO demethylase from species in which a lack of this enzyme as been observed because this enzyme converts TMAO into equimolar quantities of DMA and formaldehyde (FA) (Huss, 1995).
In fishes containing TMAO-ase such as gadoids (cod, haddock, whiting) DMA-N increases slowly and continuously in the first week of iced storage until about 1mg/100g. After the concentrations start to scatter and the standard deviation (SD) is much increasing. As example the figure 2 shows the result of DMA-N development obtained during an experimental iced-storage of cod (*Gadus morhua*). The suitability of DMA-N for freshness evaluation has been shown for cod, haddock and whiting because it remains below 0.5 mg/100 g when the fish is assessed as being optimal fresh by organoleptic analysis and during this first stage it is increasing slowly with a very low SD, but in fish species which lack the enzyme TMAOase no DMA is formed during iced storage (Oehlenschläger, 1992, 1997b). DMA content has been proposed as an index of frozen storage deterioration (Castell et al., 1974).

**Figure 2 : Develoment of DMA-N in muscle during iced storage of cod (according to Oehlenschläger, 1997 b)**



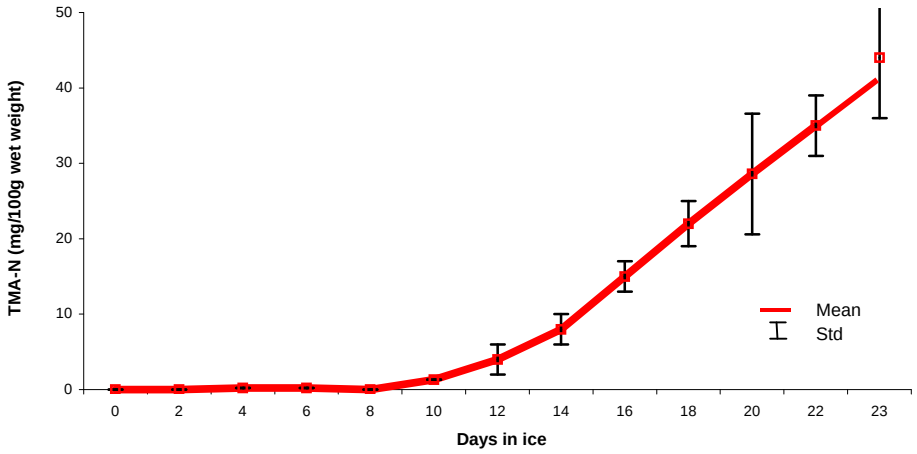

***DMA-N can be considered as an effective marker of fish freshness however its use is limited to those fish species which contain the enzyme TMAO-ase such as cod (*Gadus morhua*), haddock (Melanogrammus aeglefinus) and whiting (Merlangius merlangus) and it can be used to monitor the quality of frozen-stored gadoid fish.***

### 1.3 - TMA-N

Trimethylamine arises from the bacterial reduction of TMAO (Malle et Al., 1986). It is a pungent volatile amine which gives the typical "fishy" odour of spoiled seafood (Gill, 1992). In a very fresh fish the amount of TMA-N is low: near zero for albacore (Pérez-Villareal B. and Pozo R., 1990) about 2 mg/100g wet weight for cod (Dyer W.J., Mounsey Y.A., 1945) an average of 2 mg/100g for some fish species from the North-East Atlantic (Oehlenschläger, 1996).
TMA remains on its initial level, inferior to 3 mg/100 g a low concentration during about 10 days for cod in iced storage until the onset of microbial action starts (Oehlenschläger, 1997 b). After this first stage of apparent stability TMA-N increases rapidly until the fish is spoiled (Figure 3).
TMA is produced by a small numbers of "specific spoilage" bacteria which do not always represent a large proportion of the total bacterial flora. An advantage of the TMA-N analysis over the bacterial counts is that the TMA-N analysis can be performed more quickly and often reflect more accurately the degree of spoilage. The correlation between TMA-N level or TMA index [log (1 + TMA-N)] and eating quality has been describe in some cases for hake and other species (Pérez-Villarreal B. and Howgate, P., 1987; Huss,1995). It has been described as a good index of quality for many fish species (Gill, 1990; Baixas-Nogueras et al., 2002,2003)."

The mean disadvantage of TMA-N analysis is that this does not reflect the earlier stages of spoilage and are only reliable of certain fish species (Pedrosa-Menabrito and Regenstein, 1990, Huss, 1995, Oehlenschläger, 1997 a,b).
***TMA-N is an excellent indicator for the spoilage of gadoid fish, it is useful as a rapid means of objectively measuring the eating quality of many demersal fish specially on the medium-later phases of spoilage but it cannot be used as an freshness indicator (constant level during the first days of iced storage).***

**Figure 3 : Develoment of TMA-N in muscle during iced storage of cod (according to Oehlenschläger, 1997 b)**



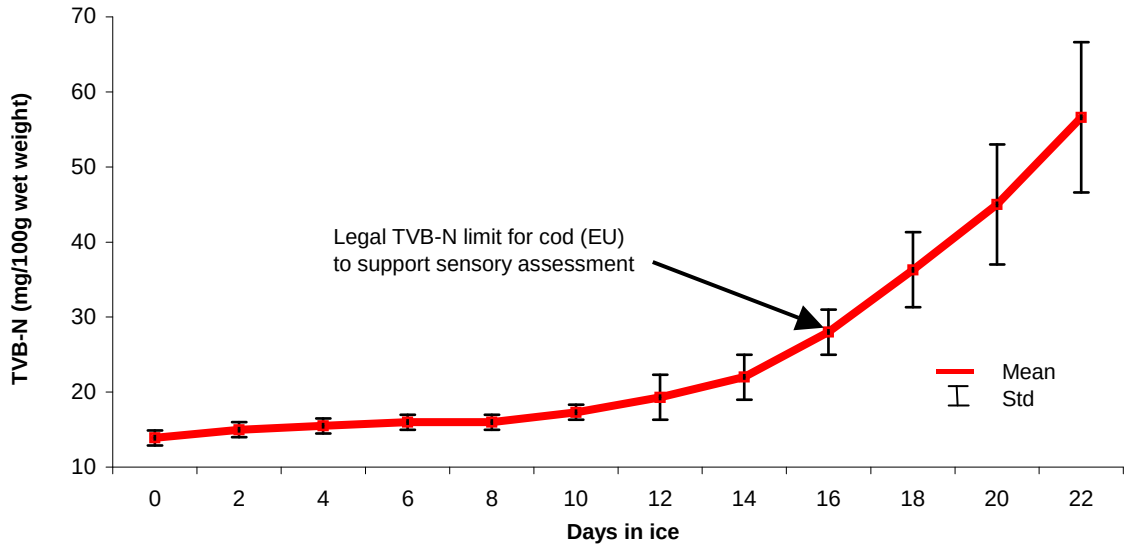

### 1.4 - TVB-N

TVB is one of the most widely used parameter to evaluate fish quality. It represents the sum of ammonia, DMA, TMA and others basic nitrogenous compounds volatile under the analysis conditions. In freshly caught fish TVB-N content is generally superior to 10 mg/100g and does not exceed 15 mg/100g except for pelagic fish, 16-18 mg/100g for sardine (El Marrrakchi et al., 1990), 18-20 mg/100g for mackerel (Malle et al., 1983), about 30 mg/100 g for albacore tuna (Pérez-Villarreal and Pozo, 1990). The TVB-N content increases slightly during the first days of storage, this slight increase may reflect the amines production by autolytic processes (Figure 4). Nevertheless in some experiments on small fishes as plaice or whiting during the first week of iced storage a decrease of TVB-N content has been observed, the some volatile amines, mainly ammonia, are leached out by the melting ice (Oehlenschläger, 1997 b). After the early days of iced storage the TVB-N content increases with a larger scattering of the values mostly produced by spoilage bacteria.
TVB analyses reflect only stages of advanced spoilage of fish, they are considered unreliable for the evaluation of the fish freshness in the early stage of storage and they don't reflect the mode of spoilage, bacterial or autolitic (Oehlenschläger, 1992, 1997a, b, Nunes et al;, 1992, Huss, 1995, Baixas-Nogueras et al. 2002). TVB is considered as useful parameter although it does not fully satisfy sanitarians and is therefore subject to certain valid criticisms (Malle and Poumeyrol, 1989).

***TVB-N is an indicator of spoilage of some fish species such as red fish, flat fish, gadoids, hake and Atlantic salmon, legal requirements in directive 91/493/EEC have been established for the limits of this indicator in the fish muscle for several species. However TVB-N cannot be used as an freshness indicator (constant level during the first days of iced storage) and does not reflect the mode of spoilage.***

TMA-N and TVB-N cannot replace the organanoleptic examination, because for most fish species their content in the flesh are relatively low during the edible storage period and after the bacterial population has grown, in the later phase of spoilage, when the fish is near to rejection, increasing amounts of TMA-N and TVB-N are found. They cannot be used as universal quality indicators with a specific set of criteria and standards applicable to all fish species (Ababouch et al., 1996). Nevertheless they may help the for spoilage determination of some fish species and they are still the best chemical indicators that are relatively simple to analyse.

**Figure 4 : Develoment of TVB-N in muscle during iced storage of cod (according to Oehlenschläger, 1997 b)**




## 2 - Factors of variation

### 2.1 - Fish species – depth of living

The volatile amines being essentially formed by degradation of TMAO, their production is linked to the initial concentration of TMAO in the muscle which depends on the species, season, fishing ground (Huss, 1995). More recently Kelley and Yancey (1999) have shown the influence of the **depth of living**, the TMAO content increased significantly with the depth at which the animal was caught - in order of shallow < bathyal < abyssal. In general the highest TMAO-N content is found in skates (85-340), squids (30-466 mg/100g) (Kelley and Yancey, 1999) and dogfish (175-217 mg/100g) (Oehlenschläger, 1996). Gadoids, hakes and redfish have somewhat less (60-120 mg/100g) while flatfish and pelagic fish have the least, normally herring, mackerel or horse mackerel do not exceed 30 mg/100g (Huss, 1995 & Oehlenschläger, 1996).

To confirm a doubt after an organoleptic examination, TVB-N and TMA-N can be used as chemical parameters to reject spoiled fish belonging to Pleuronectidae (except halibut), Merluccidae and Gadidae families, and also for red fish and salmon. They are useful for the measurement of quality of cephalopods such as squid following LeBlanc and Gill (1984) and scallops (Ruiz-Capillas et al., 2001). But they are not good spoilage indicators of herring (Huss, 1995), albacore tuna (*Thunnus alalonga*) and skipjack *(Katsuwonus pelamis)* (Pérez-Villarreal and Pozo, 1990, Tilve-Jar et al., 1997) neither for black skipjack (*Euthynnus lineatus*) (Mazorra-Manzano, 2000). TVB-N and TMA-N values did not follow any pattern with sensory scores of shrimps, probably due to leaching effect (Lakshmanan et al, 1988). On another hand, even in fatty fish as sardines or mackerel the volatile amines contents increase during spoilage (El Marrakchi et al., 1990, Civera, et al., 1993, Ababouch, 1996, Bennour et al., 1991, Malle and Poumeyrol, 1989), some authors found no correlation between TVB-N and TMA-N an freshness for sardine (Nunes et al, 1992) and the values depend on the storage condition, chilling temperature or melting ice (Civera et al., 1993). No definite standards has been adopted for the pelagic fatty fish, nether for ray fish, cephalopods, scallops and crustacean.

## 2.2 - Processing

### Chilling / ice storage / gutting / filleting

The contact with ice such as flake ice can produce a loss of the volatile compounds, in particular ammonia which is highly water soluble. Depending of the size of the fish, volatile amines are more or less washed away with water when the fish is kept in melting ice or "strongly" washed, the leaching can be important in small fish and shrimp as well as in scallops (Whittle et al., 1990, Ruiz-Capillas and al., 2001). Aubourg (2001a) has shown throughout the storage period of horse mackerel, lower values of TVB-N and TMA-N were obtained for the fillet samples than for the whole fish ones (figure 5). The lower formation of volatile amines in fillets could bee explained by the absence of viscera and head in which micro organisms able to convert TMAO in TMA are known to be concentrated (Whittle et al., 1990). Smith at al., 1980, have shown that gutted fish had lower TMA-N formation than whole fish.

**Figure 5 a: TMA-N measured during the chilled storage of whole and fillet samples of horse mackerel (adapted from Aubourg, 2001)**

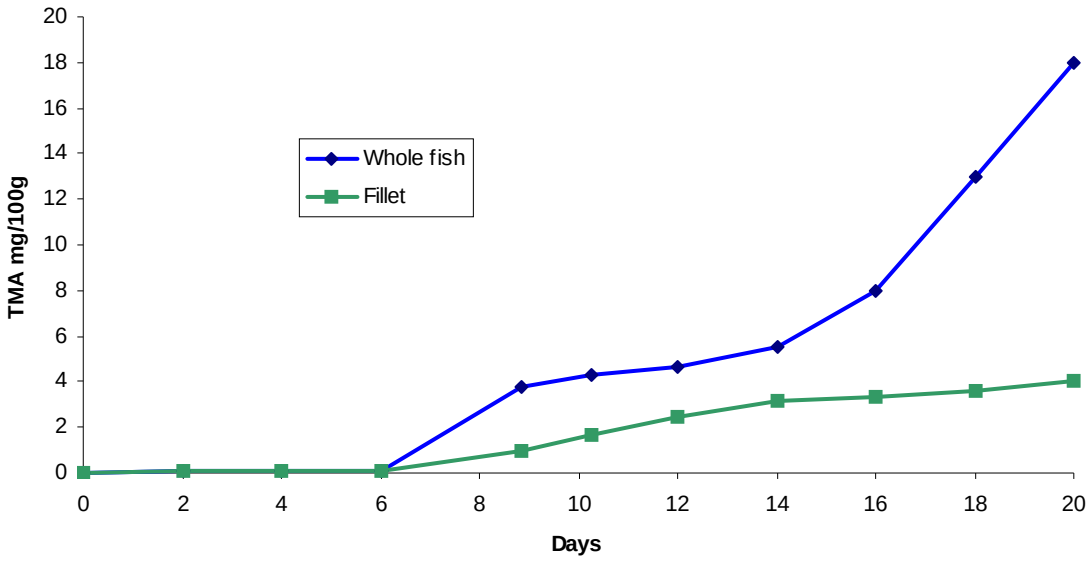
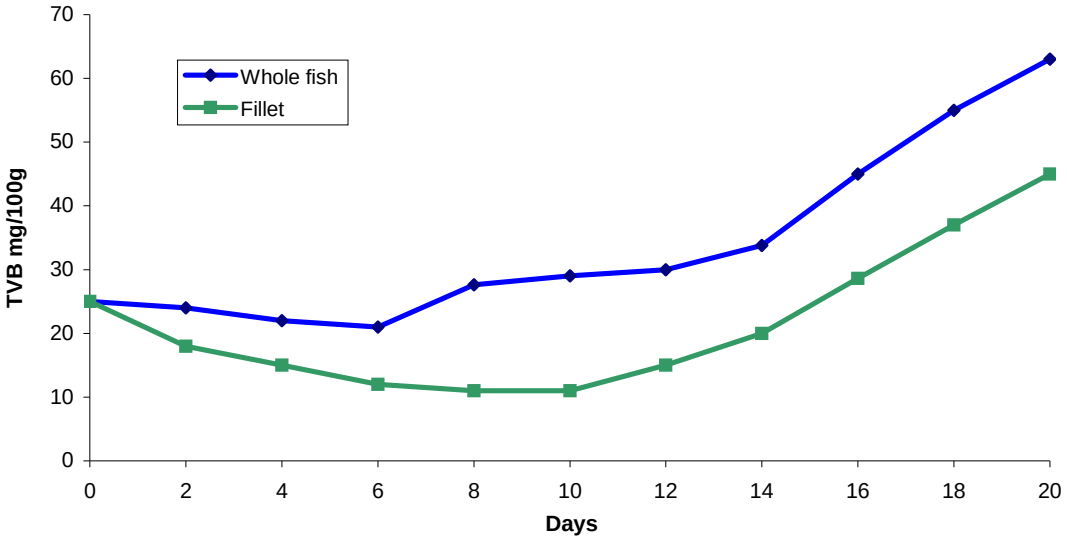


**Figure 5 b: TVB-N measured during the chilled storage of whole and fillet samples of horse mackerel (adapted from Aubourg, 2001)**




Slurry ice, also known as fluid ice, slush ice or liquid ice is a biphasic system consisting of small spherical ice crystals surrounded by seawater at subzero temperature now, it is more and more used for the preservation of seafood products because it allows an extended shelf-life. Rodríguez et al., 2004 have shown a low production of volatile bases in hake muscle stored in slurry ice in comparison with storage in flake ice (fig 6). Hake stored 22 days in slurry ice never reached the legal limit of 35 mg/100 g set for TVB-N and 5 mg/100 g for TMA even it was classified C (to be rejected). Volatile amines, TMA and TVB is not a good indicator of spoilage when the fish is stored in slurry ice.

**Figure 6 a: Comparative evolution of TMA-N content in hake muscle during storage in either flake ice or slurry ice (adapted from Rodriguez et al., 2004)**

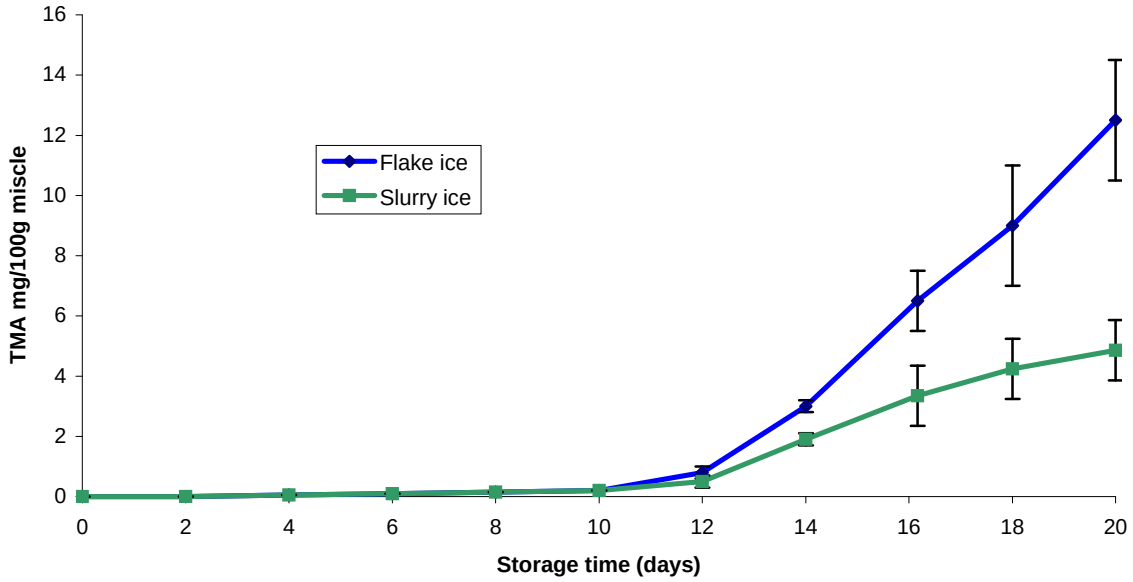
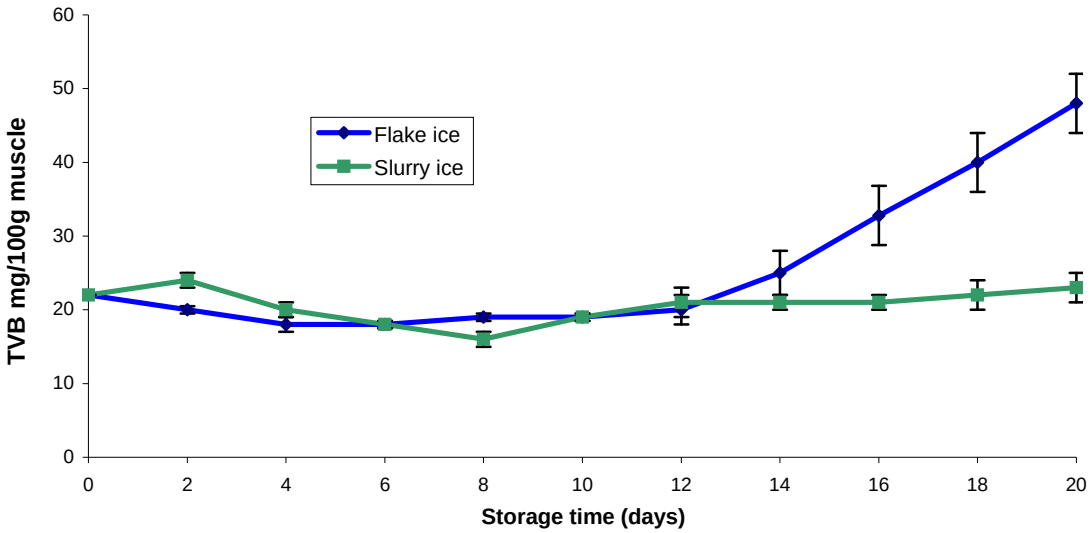



**Figure 6 b: Comparative evolution of TVB-N content in hake muscle during storage in either flake ice or slurry ice (adapted from Rodriguez et al., 2004)**




## Freezing

For fish belonging to gadoid family DMA-N is produced in frozen storage with formaldehyde which induces toughening of the flesh. The amount of protein denaturation is linked to the amount of DMA produced during a frozen storage, the production of DMA depends on the sub-zero temperature of storage and DMA can be used to monitor the quality of frozen stored gadoid fish. (de Koning and Mol, 1992, Huss, 1995, Sotelo et al., 1995). Gallardo et al. (1991) also noted production of DMA-N in hake and kinglip during a frozen storage, but it may not be a useful index of frozen albacore storage (Price et al; 1992). At classical frozen storage temperature (-18°C or below), no significant changes in TMA and TVB are observed (Mackie and Thompson, 1974, Sotelo et al., 1995).

**Frozen-thawed fish:** The longer the fillets are kept frozen, the slower the TMA and TVB formation in the fillets during iced storage is observed, TMA and TVB are not good indicators to evaluate spoilage of thawed whole cod, cod fillets and ocean perch fillets kept in ice (Magnússon and Martinsdóttir, 1995).

**Cooking -canning**

Cooking and canning increase the content of TVB-N, a gradual increase of volatile amines has been observed by comparing the raw material and the final canned product (Yeannes et al., 1983, Aubourg, 2001b, Quitral-Robles et al., 2003), the increase is linked to the processing conditions (Gallardo et al;, 1990). High values of TVB-N (mean 137 mg/100 g) have been found in commercial olive oil cans of bluefin tuna (*Thunnus thynnus*), and bonito (*Sarda sarda*) (Martin de la Hinojosa et al., 1996). Since TVB is not a good quality indicator of fresh tuna and the increase depends on the processing, TVB cannot be used as a quality parameter of canned tuna. Even it as been used as quality indicator for canned sardine it can not be considered as a good parameter (Bacetti et al. 1994).

***TVB-N cannot be used as quality criteria of canned fish***

**Packaging - Modified atmosphere Packaging**

Volatile amines levels are higher in packaged samples (Ruiz-Capillas et al., 2001), there is no leaching as in the samples kept in ice. In modified atmosphere packaging the $CO_2$ increased contributes in a slightly lower production of TMA and TVB (Pastoriza et al., 1994, 1996, de la Hoz et al., 2000).

## 3 - Methods of determination of volatile amines

Several methods have been proposed for the determination of volatile amines, the first ones were specific, they provided results for one single substance only. The most recent procedures allow the simultaneous determination of several parameters such as DMA, TMA and TVB.

### 3.1 - Specific methods

**DMA**

A classical procedure to determine DMA in fish is the **colorimetric method** described by Dyer and Mounsey (1945) using dimethyldithiocarbamate. Other methods include gas-liquid chromatography using the procedure of Keay and Hardy, 1972.

**TMA**

The most common method for TMA analysis is based on the reaction of TMA with picric acid in benzene or toluene to form a coloured complex, this method has been described in 1945 by Dyer, and after some modifications were proposed (Tozawa et al., 1971, Murray and Gibson, 1972); now it is an official AOAC method [**AOAC 971.14**]. This is a method which involves several time-consuming steps using toxic reagents. At the same time, it has been reported that some overestimation of the total TMA values can occur with this method compared to more specific GLC procedures (see 3.2 Multiparameter methods).Others methods based on **Conway micro diffusion** and titration (Conway, 1962), or **steam distillation** of an acid extract and titration (Stockemer and Kruse, 1985, Malle and Tao, 1987) are also used in control laboratories. An **enzymatic methodology** a been developed for the routine screening of large number of samples (Wong and Gill, 1987), bacterial sensors were also tested (Gamati et al., 1991).

**TVB**

TVB can be determined by distillation methods, a **direct water vapour distillation** method (Antonacopoulos, 1968, Antonacopoulos and Vyncke, 1989) or a water vapour **distillation of an acidic extract** made with aqueous perchloric or trichloracetic acid (Codex method-

FAO/WHO, 1968, Billon et al., 1979, Stockemer and Kruse, 1985). The **microdiffusion** of an acidic extract (Conway and Byrne,1933, Conway, 1962) is also used. These methods have been studied, modified, and tested by many authors and compared in several collaborative trials including three WEFTA studies (Botta et al., 1984, Vyncke et al., 1987, Antonacoupolos and Vyncke, 1992, Vyncke, 1996), an literature review has been published by Oehlenschläger in 1995.

The Commission decision of 8 March 1995 has specified the analysis methods to be used: a **reference procedure** involving preliminary deproteination with perchloric acid followed by a water vapour distillation of the acidic extract (see annex 1) and three others routine methods that may be used, the microdiffussion method described by **Conway and Byrne (1933**), the direct distillation method (**Antonacopoulos**, 1968) and the distillation of an extract deproteinesed by trichloacetic acid (**Codex** Alimentarius Committee on Fish and Fishery Products, 1968). The sample must consist of about 100 g. of flesh taken from at least three different points and mixed together by grinding.

### 3.2 - Multiparameters methods

A colorimetric assay has been proposed by Castell et al (1974) for the simultaneous determination of DMA and TMA. At present, **gas chromatography** techniques (Lundstrom and Racicot, 1983, Pérez-Martin et al., 1987, Kruse and Stockemer, 1989, Veciana-Noguès et al., 1996, Oh et al., 1997, Oetjen and Karl, 1999, Béné et al., 2001), and **high performance liquid chromatography** methods (Gill and Thomson, 1984, Fiddler et al., 1991, Monser and Greenway, 1996, Ozogul et al., 2002) are most widely adopted techniques for volatile amines determination. The major advantages of the chromatographic techniques are higher sensitivity and specificity, and the simultaneous determination of several substances. However, these methods have some inherent problems related to the difficulty in handling low-molecular-mass amines due to their high water solubility and volatility, and also to the requirement for expensive specialised equipment and experienced technical staff. Some other methods in use are based on **ion selective electrodes**; they have the great advantage of on-site portability and their application doesn't need extensive training requirements (Chang et al., 1976, Pivarnik et al., 1998, 2001, Ellis et al., 2000), a collaborative study showed that the ammonia electrode method for the determination of volatile bases in seafood reported as ammonia gives consistent precision performance (Ellis et al., 2000). Now ammonia ion selective electrode method is an AOAC official method for the determination of volatile bases in fish [**AOAC 999-01**]. Recently **flow injection/gas diffusion** with spectrophotometric detection methods have been developed (Zhi *et al*., 1995, Sadok *et al*., 1996, Garcia-Garrido and Luque, 1997, Ruiz-Capillas and Horner 1999, Baixas-Nogueras et al., 2001, Adhoum *et al*. 2003) as well as **Chemiluminescence measurement** (Cobo et al.,1997), ion mobility spectrometry (Karpas et al., 2002) and **capillary electrophoretic method** with indirect UV detection (Timm and Jorgensen 2002). Loughran and Diamond (2000) demonstrated that sensitive measurements of increasing TVB-N levels in fish headspace are possible by monitoring changes in the colour of paper disks impregnated with the $Li^+$-dye complex using reflectance UV–Vis spectroscopy. A TMA probe based on a sensitive membrane on piezoelectric quartz crystal has been described by Zao et al. (2002) as a new tool of rapid measurement. All these new procedures were performed in specialised laboratories.

## 4 - Legal requirements

In the European directive on fish hygiene (91/493/EEC) it is specified that if the sensory examination reveals any doubt to the freshness of the fish, TVB-N may be used as chemical index. The Commission decision of 8 March 1995 has fixed the TVB-N limit values for certain

categories of fishery products (see table1) and specified the analysis methods to be used (see annex 1).

| Fish species | | TVB-N limits mg/100g muscle |
|---|---|---|
| *Sebastes sp.*<br>*Helicolenus dactyloperus*<br>*Sebastichthys capensis* | Redfish belonging to *Sebastes* genus<br>Blackbelly rosefish<br>False jacopever | 25 |
| Species belonging to the Pleuronectidae* family (with the exception of halibut *Hippoglossus sp.*) | Righteye flounders: dab, plaice, Greenland halibut, lemon sole, flounder, witch, with the exception of Atlantic and Pacific halibuts | 30 |
| Salmo salar<br>Species belonging to the Merluccidae family<br>Species belonging to the Gadidae family | Atlantic salmon<br>Merluccid hakes<br>Cods, haddocks, whitings, pouting, saithe, pollocks, pollack, tusk, lings, forkbeard | 35 |

* The pleuronectiformes order (flatfishes) regroups Pleuronectidae (righteye flounders), Scophthamidae (turbot, brill, megrim), Bothidae (lefteye flounders: scaldfish) and soleidae(soles)

Table 1 : TVB-N limit values for certain categories of fishery products
(Commission decision of 8 March 1995)

## 5 - Conclusion

Some volatile amines, characteristic molecules responsible for the fishy odour and flavour can be used as criteria for assessing the fish quality. DMA which is present at very low concentration in freshly caught, can be considered as an effective marker of fish freshness of many white fish and it can be used to monitor the quality of frozen-stored gadoid fish. TMA which arises from the bacterial reduction of TMAO is an excellent indicator for the spoilage of gadoid fish, it is useful as a rapid means of objectively measuring the eating quality of many demersal fish specially on the medium-later phases of spoilage but it cannot be used as an freshness indicator (constant level during the first days of iced storage). TVB represents the sum of ammonia, DMA, TMA and others basic nitrogenous compounds volatile under the analysis conditions. In freshly caught fish its content is generally superior to 10 mg/100g and does not exceed 15 mg/100g except for pelagic fish. TVB-N is an indicator of spoilage of some fish species such as red fish, flat fish, gadoids, hake and Atlantic salmon, legal requirements in directive 91/493/EEC have been established for the limits of this indicator in the fish muscle for several species. However TVB-N cannot be used as an freshness indicator (constant level during the first days of iced storage) and des not reflect the mode of spoilage. The fish treatments such as chilling, ice storage, slurry ice, freezing, cooking, canning, packaging , modified atmosphere influence the volatile amine content. TVB-N cannot be used as quality criteria of canned fish.

About the methods of determination of volatile amines, two main conclusions can be withdrawn :

* With regard to quality control methods of spoilage,

- The volatile amines are produced by enzymatic reaction, their level increases in the chain, even under chilling condition, so it is important to perform the analysis very quickly after the sampling or to prepare the acidic extract that can be kept about one week at +4°C.

- The analysis result should be express clearly, i.e. TVB in mgN/100g, with the reference of the used method and details about the sampling (nature, date, place).

* With the regard to the validation methodology

In the fish commercialisation chain, it is important to have the possibility of checking quickly the quality, however classical laboratory methods, that cannot be considered as rapid techniques, are still used in Europe. For TVB, the AOAC Official method "Volatile bases in fish, ammonia ion selective electrode method" which is more rapid and easier to perform than our reference method could be compare to the EU official procedure in the aim to facilitate the controls in the chain. For TMA-N, specific sensors and probes recently developed and others being developed in other European projects, based on the selective identification of this molecule, must be validated to assess the possibility of having them as quality control tools for the fish industry.

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