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Oostende

DETERMINATION OF THE AMMONIA CONTENT OF FISH AS AN OBJECTIVE QUALITY ASSESSMENT METHOD

by

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During spoilage of fish, different volatile nitrogen bases are formed : ammonia, mono-, di- and trimethylamine and higher amines (histamine, tryptamine, etc.).

These volatile bases are generally determined together as total volatile basic nitrogen (TVN) which is considered to be a rather reliable method for the objective assessment of the degree of freshness of fish.

Individual compounds however can give an even more precise picture of the quality of the fish. Trimethylamine (TMA) is the only base which was studied extensively and which also appeared to be a good objective laboratory method for most fish species.

Besides TMA, ammonia is the most important base. It can be formed during spoilage by different reactions. Bacterial urease converts urea into ammonia and carbondioxide.

$$(NH_2)_2\,CO + H_2O \xrightarrow{\text{urease}} CO_2 + 2\,NH_3$$

This reaction is of importance especially in cartilaginous fish such as dogfish and ray where 1.5 to 2 % urea is found (1). Small quantities of ammonia are also formed by degradation of nucleic bases (2).

$$ATP \xrightarrow{\text{ATP-ase}} ADP \xrightarrow{\text{Myokinase}} AMP \xrightarrow{\text{AMP-desaminase}} IMP + NH_3$$

In a more pronounced state of spoilage, oxidation of amines by bacterial amino-oxidases leads to the formation of ammonia (3) :

$$RCH_2 - NH_2 + O_2 + H_2O \xrightarrow{\text{oxydases}} RCH : O + NH_3 + H_2O_2$$

In bony fish, however, ammonia is formed mainly by oxidative desamination of amino acids, which occur either free or are splitt off from proteins or peptides and related compounds (e.g. creatine). The responsible enzymes are bacterial desaminases (4) :

$$R - CH_2 - CH(NH_2) - COOH \xrightarrow[O_2]{\text{desaminase}} R\,CH_2 - CO - COOH + NH_3$$

It should further be noted that free ammonia occurs in the living fish as a result of normal desaminations during cell metabolism. Concentration varies between 5 and 25 mg N % in bony fish and 6 to 36 mg N % in cartilaginous fish (4).

Only a few studies were published on the value of the ammonia determination in fish and the results are rather inconclusive. Some authors (5) (6) (7) found a good relationship between the ammonia content and the state of freshness of several fish species. Others, however, reported less favourable results (8) (9).

For that reason, it was decided to study the evolution of ammonia during spoilage of fish and crustaceans. The experiments were carried out on four typical representatives of the bony fishes, i.e. cod *(Gadus morhua L)*, a lean roundfish, plaice *(Pleuronectes platessa L)*, a lean flatfish, redfish *(Sebastes marinus L)*, a semi-fatty fish and herring *(Clupea harengus L)*, a fatty fish. Two cartilaginous fish, spurdog *(Squalus acanthias L)* and spotted dogfish *(Scylliorhinus canicula L)*, and two crustaceans (common shrimp, *Crangon vulgaris Fabr.* and Norway lobster, *Nephrops norvegicus L*) were also investigated. It should be noted that favourable results were already obtained in previous work on spurdog and crustaceans (10) (11) (12).

Experimental

Fish and crustaceans:

— Cod *(Gadus morhua L)* of ca. 3 kg; 4 days old, period October-December.

— Plaice *(Pleuronectes platessa L)* of ca. 250 g; 3 days old, period October-December.

— Redfish *(Sebastes marinus L)* of ca. 1 kg; 7 days old, period February-April.

— Herring *(Clupea harengus L)* of ca. 165 g; 2 days old, period September-November (plain herring, 10-15 % fat).

— Spurdog *(Squalus acanthias L)* of ca. 800 g; 5 days old, period December-March.

— Spotted dogfish *(Scylliorhinus canicula L)* of ca. 800 g; 5 days old, period December-March.
— Common shrimp *(Crangon vulgaris Fabr.)*; 1 day old, period August-September.
— Norway lobster *(Nephrops norvegicus L)*; 4 days old, period July-August.

The redfish was caught in Icelandic waters, the other fish species in the Southern North Sea. Spurdog and spotted dogfish were beheaded and skinned before storage, as is usual in Belgian commercial practice. Shrimps were cooked on board and Norway lobsters on land, a few hours after arrival in the harbour.

Chemical methods.

— Ammonia : with the accelerated microdiffusion method described previously (11).
— Total volatile basic nitrogen (TVN) : according to Lücke and Geidel (13), but with the steamdistillation still of Antonacopoulos (14).
— Trimethylamine (TMA) : with Dyer's method (15) but on 2 ml of the distillate of the TVN-determination.

Procedure.

All fish species were divided into two batches. A first batch was iced immediately and stored at 0 °C. In order to enhance spoilage and at the same time to study the effect of temperature, a second batch was kept for 15 hours at 15 °C before being iced and kept at 0 °C (*)

Crustaceans were stored directly at two different temperatures : 0 and 20 °C. At regular intervals, samples of 5 to 10 fish according to fish species and 1 kg of crustaceans were taken from the different batches and analysed for TVN, TMA and ammonia. With cartilaginous fish, only ammonia was determined as TVN and TMA do not appear to be reliable objective quality determination methods (11) (12).

All experiments were repeated five times.

Results and discussion

As results of individual experiments were fairly similar, average values were calculated; they are reported graphically in figures 1 to 7.

(*) For convenience, this procedure is further called "15° C-experiment".

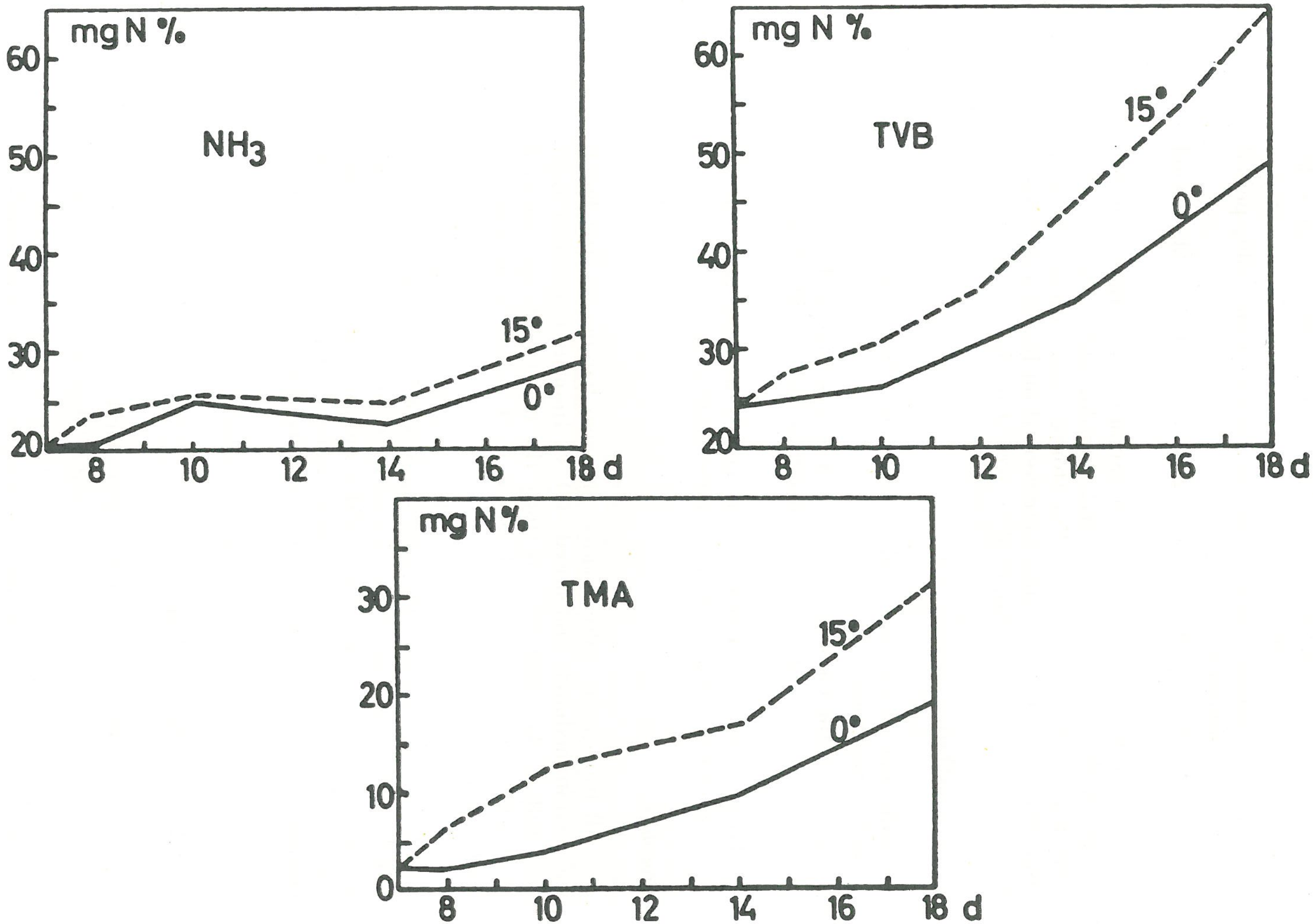

Fig. 1 — Evolution of ammonia, TVN and TMA in cod *(Gadus morhua L)*

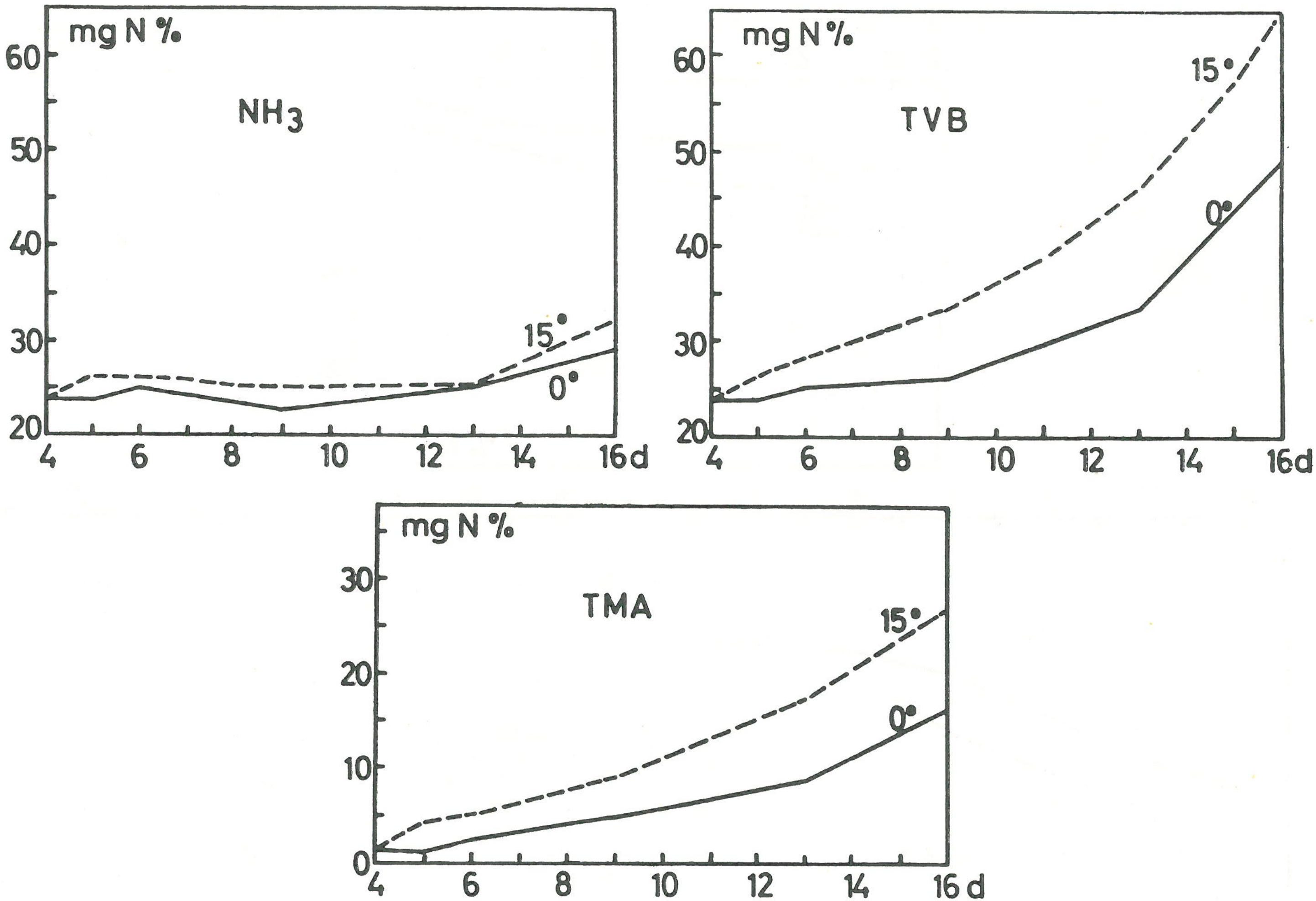

Fig. 2 — Evolution of ammonia, TVN and TMA in redfish *(Sebastes marinus L)*

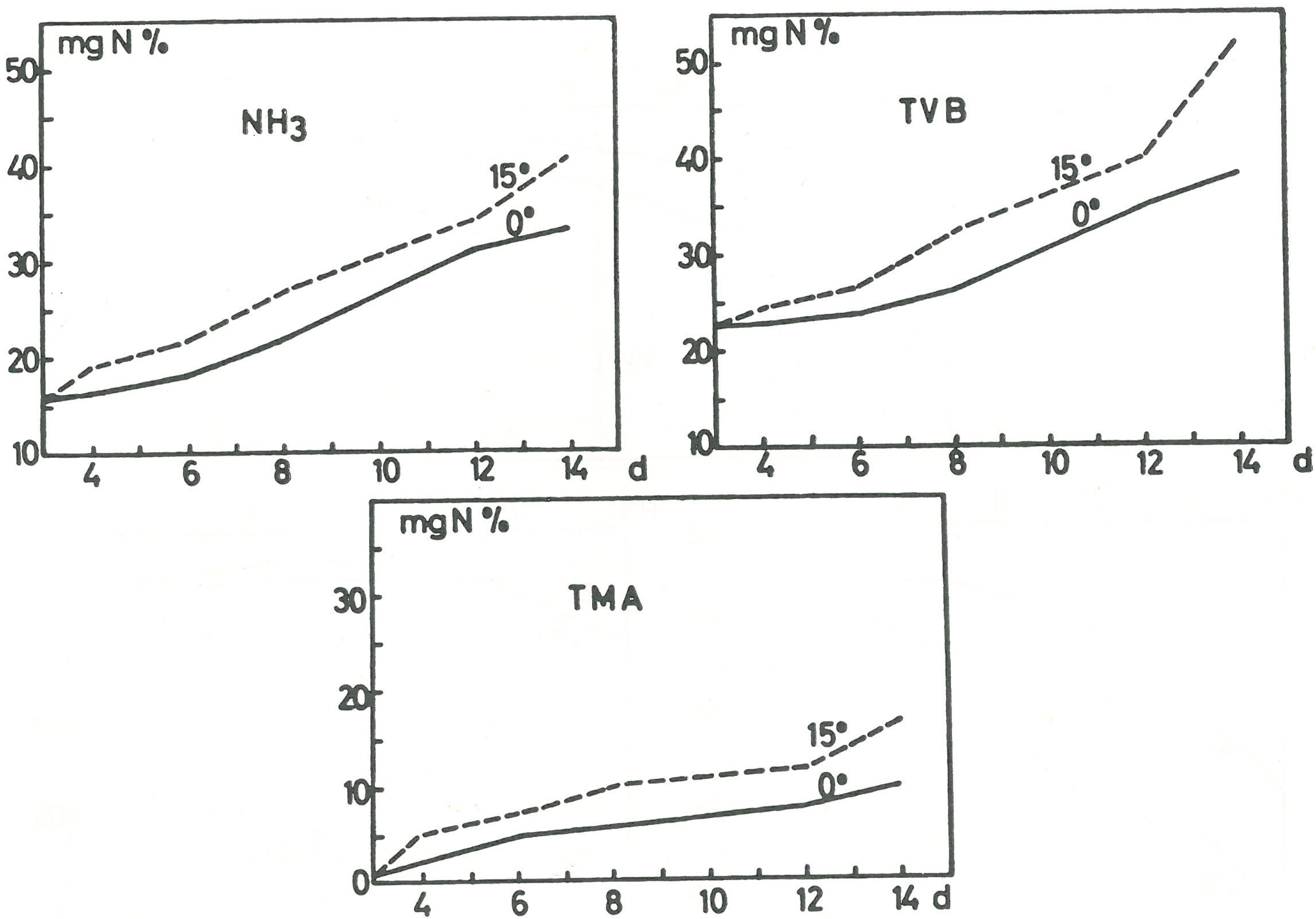

Fig. 3 — Evolution of ammonia, TVN and TMA in plaice *(Pleuronectes platessa L)*

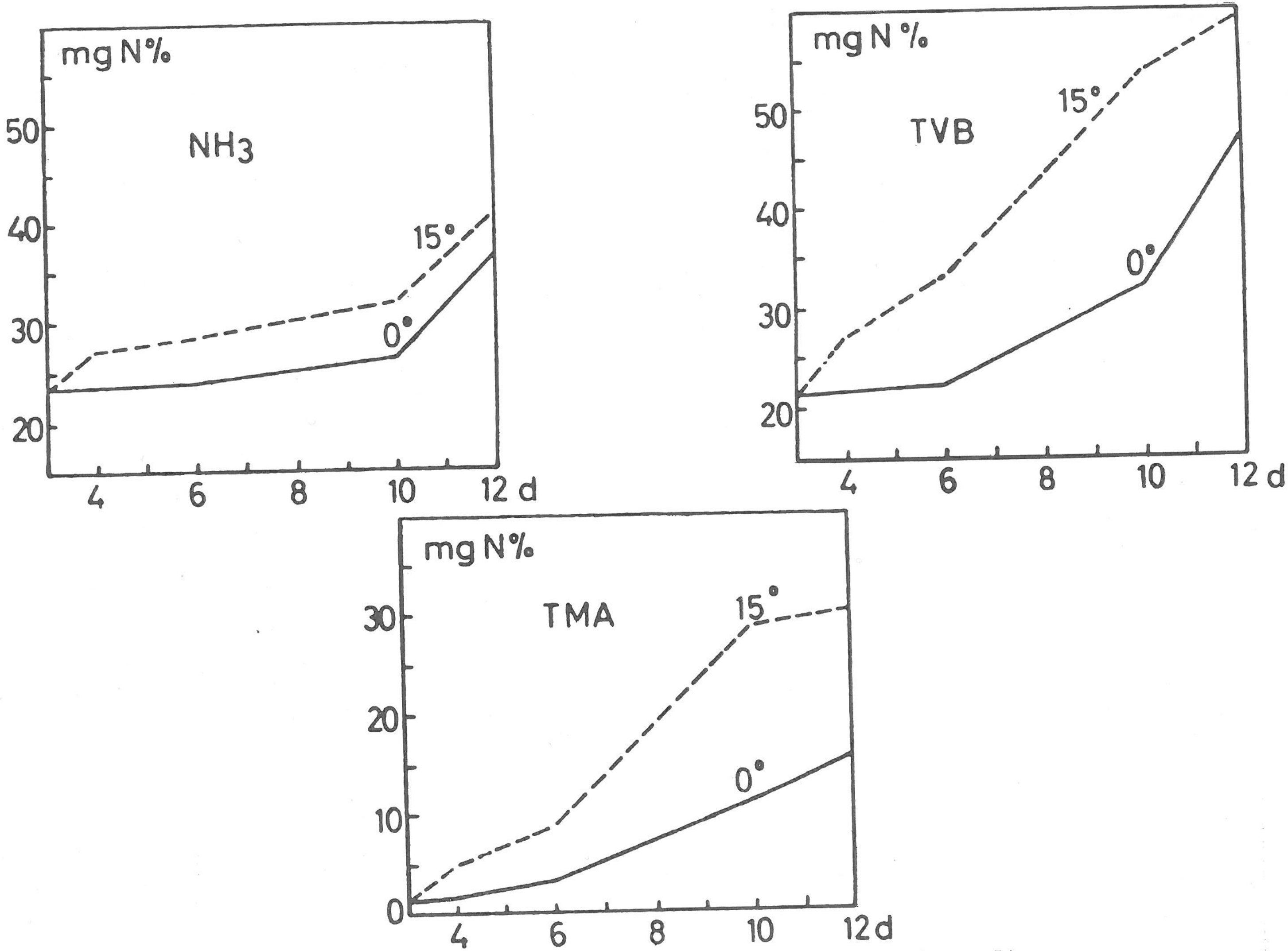

Fig. 4 — Evolution of ammonia, TVN and TMA in herring *(Clupea harengus L)*

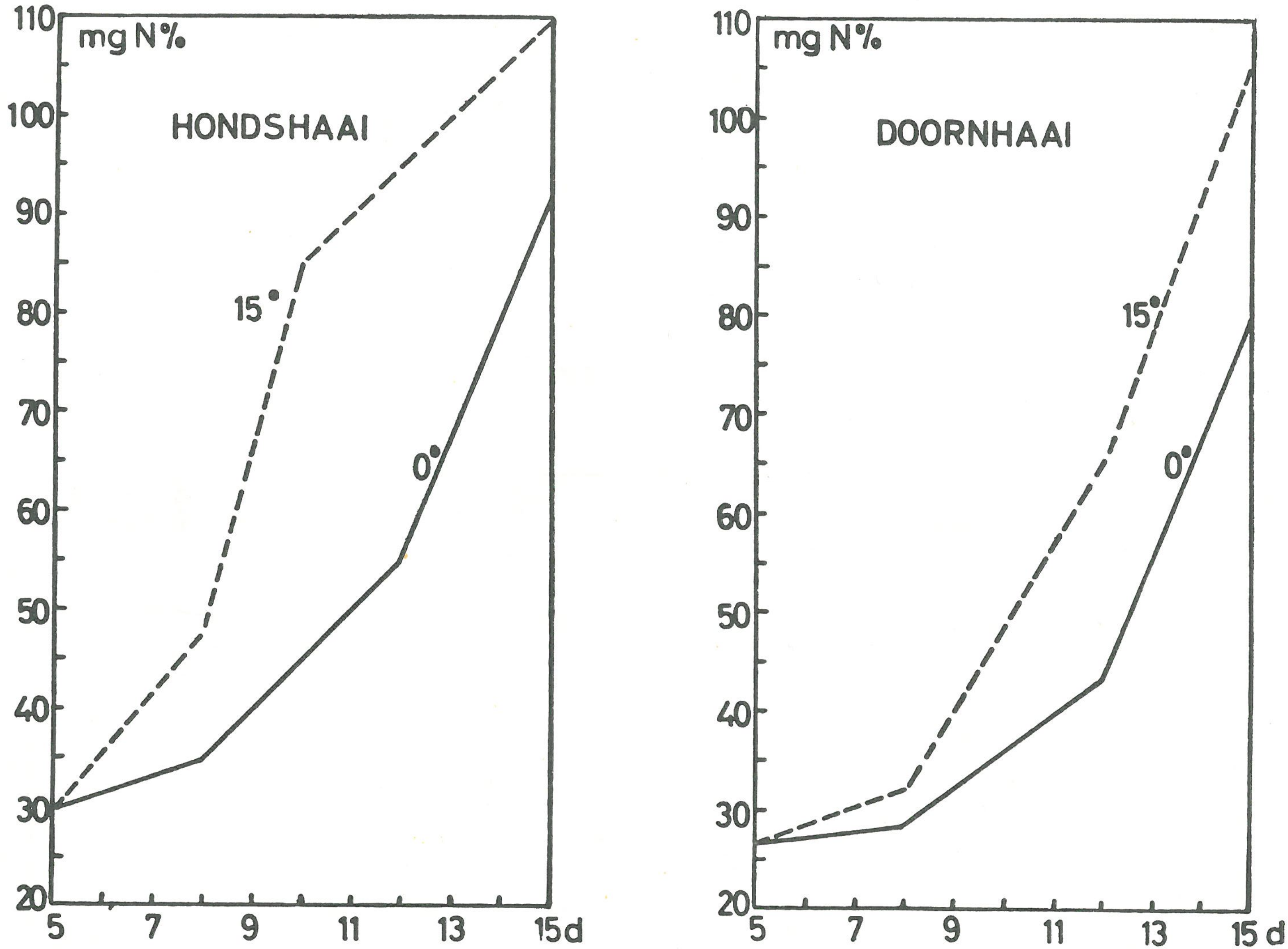

Fig. 5 — Evolution of ammonia, TVN and TMA in spurdog *(Squalus acanthias L)* and spotted dogfish *(Scylliorhinus canicula L)*

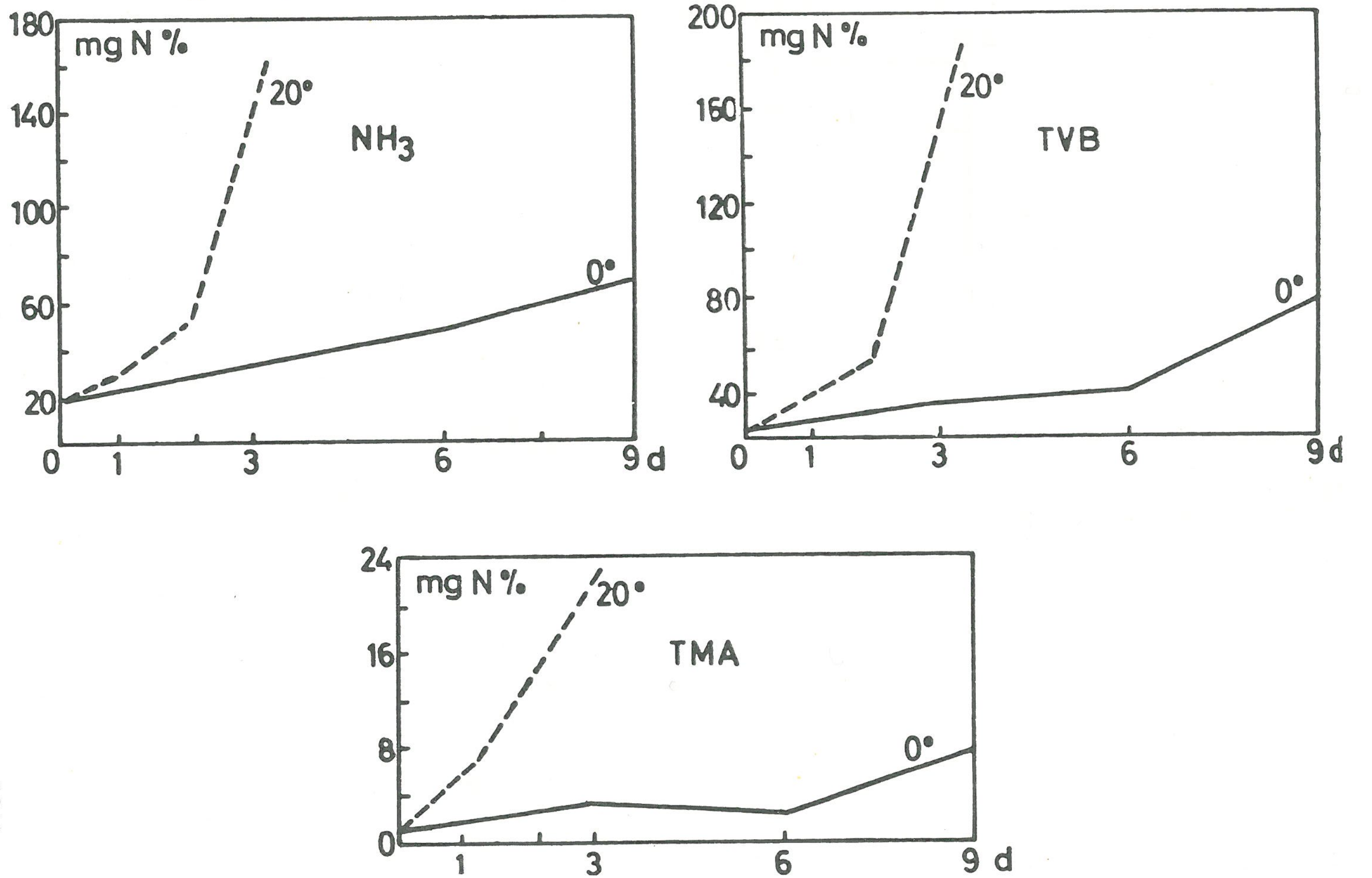

Fig. 6 — Evolution of ammonia, TVN and TMA in common shrimp *(Crangon vulgaris Fabr.)*

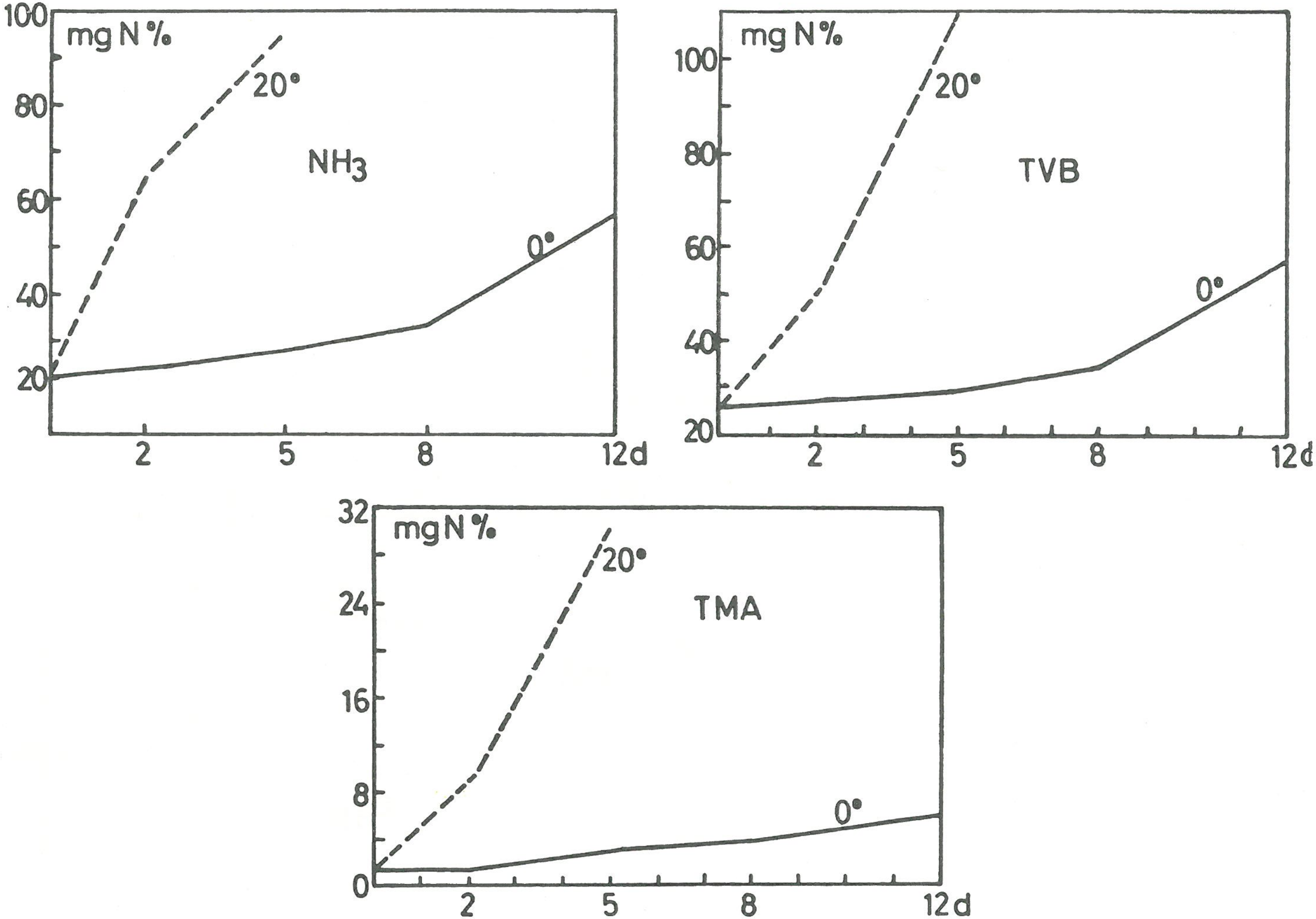

Fig. 7 — Evolution of ammonia, TVN and TMA in Norway lobster *(Nephrops norvegicus L)*

— *Bony fish*

With cod (fig. 1) and redfish (fig. 2) ammonia values remained practically at ca. 25 mg N % and only at the end of the storage period a slight increase to ca. 30 mg N % was noted. This indicates that bacterial desaminases have a marked activity only in the vicinity of the limit of acceptability of these fish. This limit was reached after respectively 15 and 12 days for the experiments at 0° and 15 °C with cod and after 14 and 12 days with redfish.

During the warming-up period of 15 hours at 15° C these desaminases were activated as shown by the increase of 3 to 4 mg N % ammonia content. During the following storage period in ice, however, there was no further increase and the difference of 3 to 4 mg N % remained at the same level.

TVN and TMA values on the other hand increased markedly and the spoilage curve of the 15 °C experiment was distinctly steeper. Both determinations showed a very similar pattern until the 13th or 14th day indicating that the increase in TVN during this period was almost entirely due to TMA formation. After this period, some ammonia was liberated as already mentioned.

Plaice presented a different picture (fig. 3). Ammonia increased at the same rate as TVN and the influence of temperature (15 °C experiment) could be clearly shown. TMA on the other hand was formed to a lesser extent; this test seems to be less sensitive than the TVN or ammonia determinations. The limit of acceptability was reached after 14 days at 0 °C; plaice which had been warmed up at 15 °C before ice storage was borderline after 11 days.

With herring (fig. 4) ammonia values remained at the same level the first 10 days of storage. After this time, when the fish was practically spoiled, a sharp increase of 10 mg N % was noted. With cod and redfish, this increase at the end of the storage period was only ca. 5 mg N %. The ammonia curve of the 15 °C experiment was distinctly higher than the 0 °C curve, indicating that herring has a greater sensitivity towards temperature influences than the other fish species examined. At 0 °C. herring was borderline after ca. 10 days. In the 15° experiment, the limit of acceptability was about 6 days.

The reasons for the different behaviour pattern of plaice in relation to ammonia formation during spoilage were not investigated any further as the present experiments were conducted in the first place in order to evaluate the possibilities of the ammonia determination as an objective freshness test. The earlier production of the base, however, is probably related to a modified microflora producing more active bacterial desaminases than in other fish species.

— *Cartilaginous fish.*

As mentioned, the most important part of the free ammonia in those fish is formed by hydrolysis of urea; bacterial urease catalyses the reaction. With spurdog, only a small production of ammonia was noted until the 8th day (fig. 5). After this period, however, the concentration increased sharply to reach values of respectively 105 mg (15 °C experiment) and 80 mg (0° experiment) after 15 days. The fish was posiled after about 13 days (0 °C) or 11 days (15 °C).

With spotted dogfish, ammonia increased at an earlier stage and reached higher values (110 and 92 mg). This was in good agreement with the organoleptic judgment : the limits of acceptability (respectively 12 and 9 days) were reached earlier than with spurdog. Furthermore, spotted dogfish appeared to be more sensitive to the influence of temperature : ammonia values of the 15 °C experiment increased at a faster rate than with spurdog.

— *Crustaceans.*

With shrimps (fig. 6) and Norway lobsters (fig. 7), TVN and ammonia curves showed practically the same pattern for both experiments (0 and 20°), the influence of temperature being very pronounced. As TVN consists mainly of ammonia and TMA, it was to be expected that TMA values would increase to a lesser extent. Data of figures 6 and 7 show that this was indeed the case. TMA determinations appeared to be a less sensitive objective quality assessment method for shrimps and Norway lobsters.

At 20 °C both crustaceans were spoiled after about 2 days; at 0 °C they were borderline after respectively 8 and 11 days for shrimps and Norway lobsters. It should be observed however that the shelf life of cooked shelfish also depends upon the cooking method (salt content, length of cooking, etc.). No precise data on this subject were available for these experiments.

Conclusions

Results of the experiments show that the value of the ammonia determination as an objective quality assessment method is rather limited for bony fish. With cod, redfish and herring, ammonia values increase only slightly during storage in ice and do not follow the organoleptic judgment. Only with plaice a better agreement was noted but even in that case, TVN determinations gave a better picture of the decrease in freshness.

Although the determination of ammonia is a poor objective quality test for bony fish — at least for the species under investigation — the method has its usefulness for more detailed spoilage studies and especially in these cases were the fish is not kept in „normal" conditions in ice (e.g. prepacked fish, fish treated with additives, etc.). The determination of ammonia generally gives a fair picture of the speed of desaminations and of proteolysis.

With cartilaginous fish, the ammonia content is in good agreement with the organoleptic judgment. The usefulness of the method should be emphasized when considering the fact that only a limited number of objective quality methods can be used for Elasmobranchs The limit of acceptability can be set at 55-60 mg N %.

Finally, for crustaceans, the determination of ammonia has practically the same value as the TVN determination and can be used as an alternative and confirmatory test. Crustaceans become borderline when the concentration of ammonia reaches 45-50 mg N %.

SUMMARY

In order to evaluate the possibilities of the determination of ammonia as an objective quality assessment method for fish and crustaceans a series of storage experiments was carried out on cod *(Gadus morhua L)*, redfish *(Sebastes marinus L)*, plaice *(Pleuronectes platessa L)*, herring *(Clupea harengus L)*, spurdog *(Squalus acanthias L)*, spotted dogfish *(Scylliorhinus canicula L)*, Norway lobster *(Nephrops norvegicus L)* and shrimp *(Crangon vulgaris Fabr.)*. Besides ammonia, total volatile basic nitrogen (TVN) and trimethylamine (TMA) were determined.

The experiments showed the value of the test to be limited for bony fish. With cod, redfish and herring, ammonia values increase only slightly during storage in ice and do not follow the organoleptic judgment. Only with plaice a better agreement was noted but even in that case, TVN determinations gave a better picture of the decrease in freshness.

With cartilaginous fish, however, the determination of ammonia is of value and is in good agreement with the organoleptic judgment. Finally, experiments on the two species of crustaceans showed that the determination of ammonia has about the same usefulness as the TVN test, and can be considered as an alternative method.

SAMENVATTING

Dosering van de ammoniak in vis als objektieve kwaliteitsbepalingsmetode

Om de waarde van de ammoniakdosering voor de objektieve kwaliteitsbepaling van visserijprodukten na te gaan werd een reeks bewaarproeven op kabeljauw *(Gadus morhua L)*, rode zeebaars *(Sebastes marinus L)*, schol *(Pleuronectes platessa L)*, haring *(Clupea harengus L)*, doornhaai *(Squalus acanthias L)*, hondshaai *(Scyllioshinus canicula L)*, Noorse kreeft *(Nephrops norvegicus L)* en garnalen *(Crangon vulgaris Fabr.)* uitgevoerd. Naast ammoniak werden de totale vluchtige basische stifstof (TVB) en het trimethylamine (TMA) bepaald.

Uit de proeven is gebleken dat de waarde van de metode bij beenvissen beperkt is. Bij kabeljauw, rode zeebaars en haring vertonen de ammoniakgehalten gedurende het bewaren van de vis weinig verschil en komen niet overeen met de organoleptische keuring. Alleen bij schol wordt een goede overeenkomst genoteerd, maar zelfs in dit geval geeft de TVB-bepaling een nog beter beeld van het kwaliteitsverloop.

Bij kraakbeenvissen is de ammoniakbepaling echter waardevol en komt goed met de organoleptische keuring overeen.

Voor schaaldieren tenslotte heeft de ammoniakdosering praktisch dezelfde waarde als de TVB-bepaling, en is dan ook een alternatieve metode voor deze laatste test.

RÉSUMÉ

Dosage de l'ammoniac dans le poisson comme méthode objective de détermination de la qualité.

Afin d'évaluer les possibilités du dosage de l'ammoniac comme méthode objective de détermination de la qualité de produits de la pêche, une série d'expériences de conservation a été effectuée sur le cabillaud *(Gadus morhua L)*, la rascasse du Nord *(Sebastes marinus L)*, la plie *(Pleuronectes platessa L)*, le hareng *(Clupea harengus L)*, l'aiguillat *(Squalus acanthias L)*, la roussette *(Scylliorhinus canicula L)*, la langoustine *(Nephrops norvegicus L)* et la crevette grise *(Crangon vulgaris Fabr.)*.

A part l'ammoniac, l'azote basique volatil total (AVT) et la triméthylamine (TMA) furent dosés.

Les expériences ont démontré que la valeur de la méthode est restreinte pour les poissons osseux. Dans le cabillaud, la rascasse du Nord et le hareng les taux d'ammoniac ne changent guère pendant la conservation et ne suivent pas l'examen organoleptique. Seule la plie donne une bonne corrélation, mais même dans ce cas, l'AVT donne une meilleure image de la diminution de fraîcheur.

Avec les poissons cartilagineux cependant, la détermination de l'ammoniac est à préconiser car elle concorde bien avec l'examen organoleptique.

Le dosage de l'ammoniac dans les crustacés enfin a pratiquement la même valeur que la détermination de l'AVT et peut être considéré comme une méthode alternative et confirmative de ce dernier test.

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