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Determination of ammonia in dressed thornback ray (*Raja clavata* L.) as a quality test

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Summary

The sensory determination of ammonia in raw, steamed and boiled ray wings using a scoring system appeared to be a valuable method of quality assessment. The borderline of acceptability was reached after 10 (±1) days in ice. A combination of the steaming and boiling tests (with addition of acetic acid and salt) was useful to confirm definite spoilage or to indicate the approach to the borderline of acceptability.

The chemical determination of ammonia appeared to be a useful objective method in addition to the organoleptic tests.

Thornback ray was borderline at a concentration of 60–70 mg N. From the other related parameters determined (urea, pH, redox potential, total bacterial count, α-amino nitrogen) only pH appeared to be of value.

Introduction

The occurrence of a high level (1 to 2.5%) of urea in the muscle, blood and organs is a characteristic of the Elasmobranchs (sharks, rays, skates). During spoilage, this urea breaks down with formation of ammonia due to the activity of bacterial urease (Simidu & Oisi, 1951). The often rapid development of ammonia in cartilaginous fish causes problems to the fish trade of countries such as Belgium where those species are popular.

In a previous paper (Vyncke, 1970) the determination of the ammonia content as an objective quality assessment method for several fish species and crustaceans was evaluated and appeared to be useful for spurdog (*Squalus acanthias* L.) and spotted dogfish (*Scylliorhinus canicula* O.). This work was continued on ray. Thornback ray (*Raja clavata* L.) which together with the common skate (*Raja batis* L.) is the most commonly landed species in Western Europe was chosen for the present experiments. Besides the chemical determination, special attention was paid to the sensory assessment of ammonia.

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Materials and methods

Fish

Thornback rays from the Southern North Sea weighing 2.5 to 3.5 kg and 2 (±1) days old at landing were prepared on the premises of a wholesale trader by the method usually adopted for this fish species, i.e. severing the wings from the trunk and skinning them. They were washed by dipping them for 30 sec in tap water.

Laboratory methods

Ammonia: with the accelerated microdiffusion method described previously (Vyncke, 1968a).

Urea: with urease according to Conway (1962) but using the modified ammonia determination.

pH: measured directly in minced fish with glass electrode.

Redox potential: measured in expressed fish fluid (Vyncke, 1968b) with a combined platinum-calomel electrode after 5 min stirring under a stream of nitrogen.

α-Amino nitrogen: with 2, 4, 6-trinitrobenzene-1-sulfonic acid according to Satake *et al.* (1960) on a 7.5% trichloracetic acid extract (4 g of fish per 200 ml).

Total bacterial count: *c.* 5 g of muscle aseptically cut from the central part of five ray wings and blended for 1 min in a sterile Waring blendor beaker after addition of a tenfold of sterile water. An appropriate dilution series was made and the resulting suspension inoculated on Petri dishes containing Plate Count Agar (Difco); incubation time was 72 hr at 20°C.

Differentiation of organisms on the basis of urease activity: fifty colonies taken at random from the plates used for the total bacterial count were first cultured on nutrient agar (Difco) slants in test tubes at 20°C for at least 4 days. The bacteria were then inoculated on urea agar base (BBL Cockeysville, Maryland, U.S.A.) slants in test tubes, according to Christensen (1946) but incubating for 1 day at 20°C.

Organoleptic tests: the degree of intensity of the odour of ammonia was graded on raw, steamed and boiled rays by a taste panel consisting of 3–4 members of laboratory staff experience on quality research on Elasmobranchs. The following scale was used:

5 – not present
4 – just recognizable
3 – slight
2 – moderate
1 – strong.

The raw fish was allowed to warm up to room temperature before the test.

Steaming was carried out in Pyrex dishes with loose lids over a boiling water bath for 30 min; about 200 g of boneless fish taken from five wings were used. The dishes were then placed in a thermostatic water bath kept at 60°C. Ammonia was assessed immediately upon removing this lid.

Boiling was performed in a litre beaker containing a solution of 0.15% acetic acid and 1% sodium chloride in water. The beaker was covered with a watch glass. The fish (200 g) was introduced when the liquid was boiling and cooked for 10 min. The sample was then removed and put in a Pyrex dish with loose lid and further assessed as in the steaming test.

General appearance, taste and texture were also examined.

Procedure

The ray wings were divided into two batches. A first batch was iced immediately and stored at 0°C. In order to enhance spoilage and for the sake of comparison, a second batch was kept for 15 hr in a room at 15°C before being iced and stored at 0°C.

At five time intervals after catching (Figs 1 and 2) five wings from each batch were taken for objective and organoleptic tests, which were carried out on pooled samples.

Besides the determination of ammonia and its precursor, urea, pH and redox potential were also measured as these parameters are linked to the bacterial ammonia production; α-amino nitrogen was also determined as ammonia can also be formed by deamination of amino acids. Ammonia being freed by bacterial action, total viable count and urease-producing organisms were also assessed.

For the organoleptic tests, the bacteriological analyses and the determination of redox potential, whole pieces of muscle were cut from the wings. The rest of the flesh was minced in an electric meat grinder and thoroughly mixed. The mince was used for the chemical determinations.

The experiments were repeated five times during the period January–May.

Results and discussion

The average results of the five experiments are reported graphically in Figs 1 and 2. The range of experimental data is also indicated.

Exposing the fish to a temperature of 15°C for 15 hr markedly changed the spoilage pattern as measured by the different subjective and objective methods. This treatment decreased shelf life of the rays by 2 to 3 days. Using the mentioned grading system for raw and steamed fish the taste panel judged the rays to be of acceptable commercial quality up to a score of 3. The panel however agreed that in actual commercial practice this score could be 0.5 units higher or lower according to consumer acceptance. Score 3 was reached after

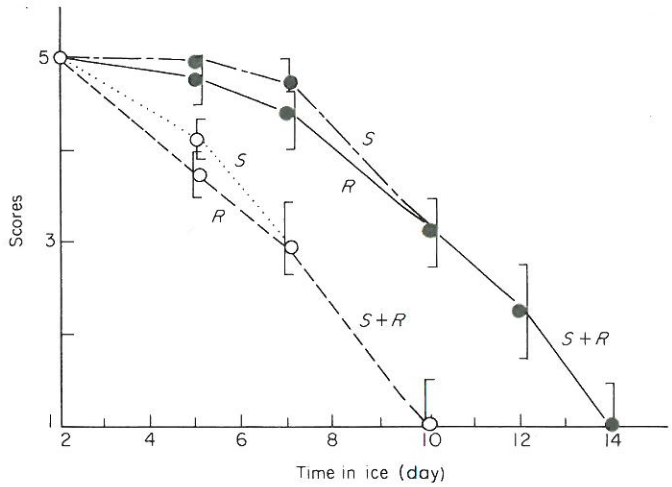

Figure 1. Evolution of organoleptic scores during storage of thornback ray in ice ●, immediately iced; ○, iced after 15 hr exposure to 15°C; *R*, raw odour score; *S*, steamed odour score.

9 to 11 days (average 10) for the immediately iced fish and after 6 to 8 days (average 7) for the 15°C fish.

When comparing the scores of the raw and steamed fish (Fig. 1), the latter ones appeared to be higher during the first 6 or 7 days of storage. One could conclude this test to be less sensitive. However, it should be emphasized that a certain amount of blood is present at the surface of skinned rays. As Elasmobranch blood usually has a higher urea content than the muscle (Simidu, 1961) and is readily attacked by bacteria present at the surface of the fish, the ammonia thus formed in an early stage of storage is in fact not a good indicator of spoilage and can easily be removed by washing; the fish flesh itself is still unchanged.

From a theoretical view-point, the steaming test could suffice. However, as assessing the raw odour score does not require special preparations and is rapid, it should also be carried out, increasing the reliability of the whole organoleptic judgment. On the other hand, when no ammonia is detected on the raw rays, the steaming test can be omitted.

After a series of screening experiments it was decided to add a boiling test to the procedure. This test is nearer to actual consumer practice, where a certain amount of vinegar is usually added to the cooking water to bind small amounts of ammonia. The boiling test was performed daily as soon as the score (raw and steamed) had reached about 3. Ammonia could not be detected in the boiled ray until the product was really unacceptable. This occurred 1 or 2 days after reaching score 3 on the raw or steamed rays. For quality control (e.g. in cases of dispute) a combination of steaming and boiling tests could be very useful, the latter indicating either that the borderline of acceptability has approached (when negative, the raw and steaming scores being around 3) or had been passed (when positive).

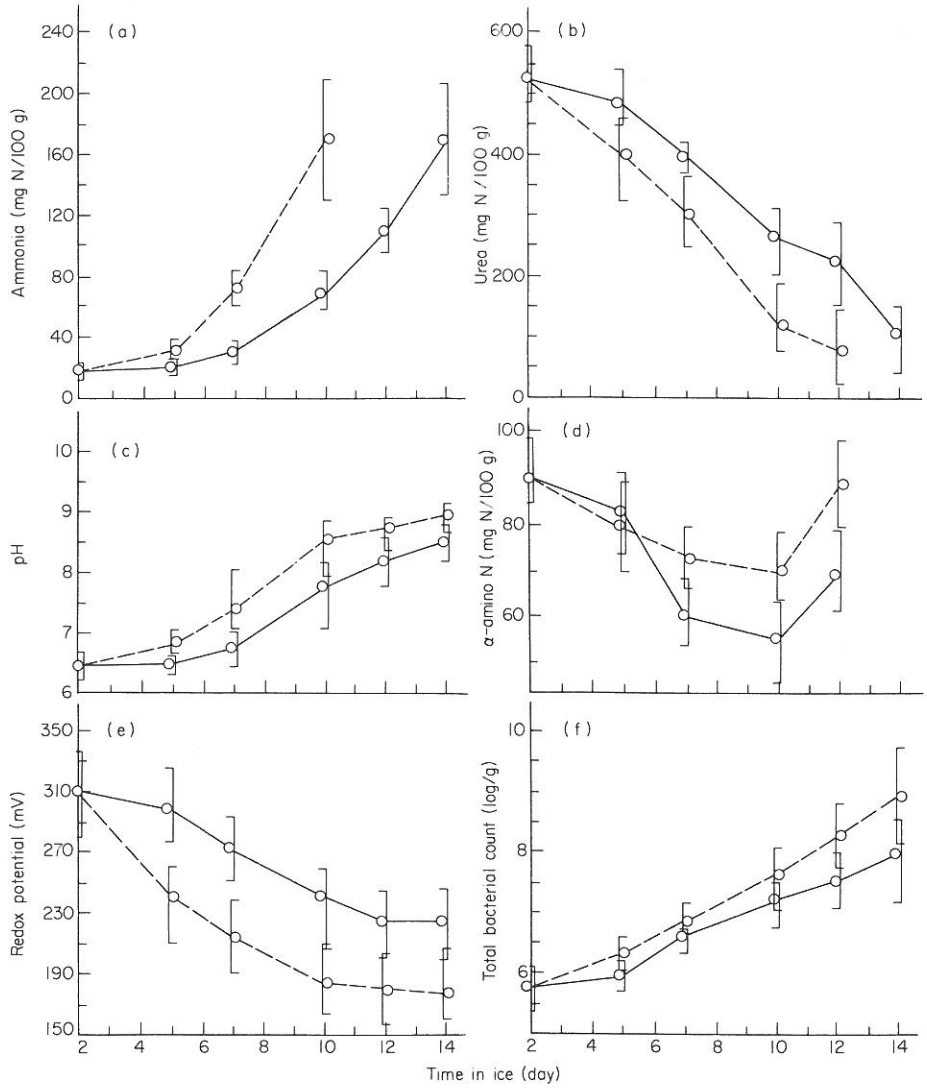

Figure 2. Evolution of (a) ammonia, (b) urea, (c) pH, (d) α-amino nitrogen, (e) redox potential and (f) total bacterial count, during storage of thornback ray in ice. ——, Immediately iced (0°C); – – –, iced after 15 hr exposure to 15°C.

The other organoleptic characteristic appeared to be less valuable with skinned ray wings. Other odours and flavours normally associated with fish spoilage could also be detected but were dominated by the development of ammonia and hence of less importance. No important discolorations were observed except at the end of the shelf life when the wings became yellowish. Texture became softer both in the raw and cooked rays but only when approaching the limit of acceptability.

When comparing the graphs giving the evolution of the concentration of

ammonia and the organoleptic scores (Figs 1 and 2), there appeared to be a good relationship between both parameters. A slight odour (score 4–4.5) was detected at a concentration of 30–40 mg of ammonia–N.

The borderline of acceptability could be set at 60–70 mg which is higher than the values noted earlier for dogfish, i.e. 55–60 mg (Vyncke, 1970) and also above the limit of 30 mg quoted by James & Olley (1971) for shark.

The concentration of the main precursor of ammonia, urea, decreased sharply during storage. When comparing the figures for urea and ammonia however it can be concluded that the leaching effect is much stronger than the breakdown to ammonia. This is further confirmed by the fact that the 15°C curve runs parallel to the 0°C-curve when it would be expected to diverge.

Owing to the development of ammonia, pH also increased significantly. From a value of about 8 onwards however the progress was distinctly slowed down. As the levelling-off effect occurred only when the ray was practically unacceptable, pH appeared to be a good complementary quality assessment method. The limit of acceptability could be set between 7.2 and 7.8, values superior to 8 indicating definite spoilage.

The determination of α-amino nitrogen did not allow to conclude that ammonia is also formed in significant amounts by deamination. The concentration in fact depends upon the rate of formation of new amino acids by the breakdown of proteins and peptides, their deamination (and other reactions) and leaching by the melting ice. Nevertheless, this test showed the activity of the bacterial exopeptidases to be greater than that of the deaminases when spoilage was enhanced either by natural causes (growth of bacteria during storage in ice) or artificially (temperature influence). This could be assessed by the fact that at the end of the storage period, when spoilage was pronounced, the content of α-amino nitrogen increased significantly. Moreover, the 15°C samples showed a higher value after a few days' storage. As many amino acids show an activating effect on urease by binding heavy metals, thereby protecting the urease sulphydrylgroups (Pinter, Tashovski & Karas, 1954), the relative increase of α-amino nitrogen probably also furthered urea breakdown.

Bacterial activity was clearly reflected by the evolution of the redox potential making the medium more reducing with progressing spoilage. Exposing the fish to a temperature of 15°C markedly decreased the potential. Values of 230–250 mV indicated the onset of spoilage, but some discrepancy was observed between 0 and 15°C experiments in this respect, rendering redox potential measurements a doubtful objective quality method.

Total bacterial count increased regularly during spoilage. It can be noticed that the initial load was already rather high, ranging from log 5.3 to 6.2 (average 5.7). It should be stressed that rays have a large amount of very viscous mucus on the surface and that contamination of skinned ray wings with bacteria from this slime is difficult to avoid. For the same reason the total viable count was high at the borderline of acceptability, ranging from log 6.5 to 7.8

From investigations by Liston (1957) the bacterial population of skin and gills of skate (*Raja* spp.) appeared to be composed principally of Gram-negative rods of the *Pseudomonas* and *Achromobacter* genera and was in fact similar to the population of other fish species of the North Sea. No further differentiation of the microbial flora was carried out except for the organisms showing urease activity.

Table 1. Differentiation of bacteria on the basis of urease activity (in % of total count)

Temperature	Storage time (day)				
	0	5	7	10	12
0°C	20	25	29	36	44
15°C	20	31	42	45	62

Although the culture medium used probably did not react on all urease producing bacteria present, it was considered to give a good estimate of the urease activity. The results reported in Table 1 indicated the percentage of micro-organisms showing urease activity to increase significantly during spoilage. This was also confirmed by the higher numbers of the 15°C experiment, where spoilage was enhanced from the beginning of the storage period. The changes in pH and redox potential are at least partially responsible for the changes in microbial flora.

It should further be stressed that urease is known to occur in over two hundred species of bacteria (Sumner & Somers, 1953) emphasizing the high risks of ammonia development in Elasmobranchs.

The present experiments showed the chemical determination of ammonia to be a useful objective test in addition to the organoleptic assessment of this compound in ray. From the other related parameters studied, especially pH appeared to be of value. An ammonia content exceeding 60 mg and a pH higher than 7.2 should be regarded as indicating rays of suspect quality.

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