How fresh is the fish? – Ion chromatography provides the answer!

Fish and the products made from them are very nutritious foodstuffs. They provide a lot of protein, fat, numerous vitamins (A, B and D vitamins), minerals (potassium, phosphorus, etc.) and trace elements (e.g. iodine and selenium in sea fish). The fat content of the different types of fish varies greatly. In general, fish fats have a high proportion of unsaturated essential fatty acids, which, just like the fish's high protein content (approx. 20%), favors rapid decomposition. This is why maintaining the freshness of the fish throughout the whole production chain: fishing grounds – processing – shop – customer presents a major challenge to the fishing industry.

As spoiled fish no longer has any commercial use, reliable methods for checking the freshness are of immense importance for both producers and consumers.

From an analyst's point of view there are three groups of substances that are particularly interesting as «freshness indicators»:

- Total content of volatile N-bases (total volatile nitrogenous bases – TVB-N)
- Content of trimethylamine N-oxide (TMAO)
- Content of biogenic amines (in fish primarily histamine, putrescine and cadaverine)

Within the EU the maximum permitted TVB-N content in different sea fish is governed by the Council Directive 95/149/EC. TVB-N concentrations in the range 5...100 mg/100 g sea fish can be determined. However, the titration method used is time-consuming and determines only a sum parameter.

Various components contribute to the «spoiling parameter» for fish. This is firstly the microbiological degradation of TMAO (which serves to regulate the osmotic pressure in fish) to trimethylamine (TMA) and ammonia. Further substances, which are also formed during the degradation of fish protein by microorganisms, are summarized under the collective term biogenic amines. These are histamine, putrescine and cadaverine. As histamine is one of the substances that trigger allergies, its exact determination is of great general interest.

In order to be able to determine the «freshness indicators» in sea fish accurately, the Cantonal Laboratory in Schaffhausen, Switzerland (Ms. Oechslin, Ms. Steil) has developed a new ion chromatographic method. This procedure allows the simultaneous determination of dimethylamine (DMA), trimethylamine (TMA), trimethylamine N-oxide (TMAO), histamine, putrescine and cadaverine and can be used for nitrogen concentrations of 5 to 1500 mg/kg fish.

Following this, Sigma-Aldrich Chemie GmbH (formerly Fluka) in Buchs, Switzerland, and Metrohm Ltd. in Herisau have cooperated to produce an analysis kit for testing 10 sea fish samples (Fluka no. 53851). In addition to the necessary IC precolumn, this kit also includes the reagents for the eluents, the buffer and the standard solutions.

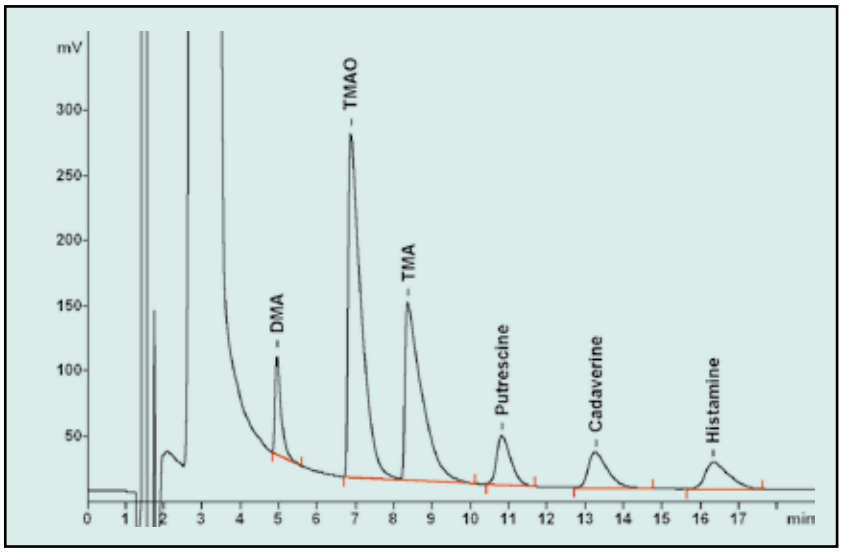
Up to now the following fish have been successfully analyzed: cod, monkfish, plaice, lemon sole and rosefish. With halibut and tuna the evaluation of the components cadaverine and histamine is affected by interfering peaks. In this case the eluent would have to be adjusted accordingly.

Description of the ion chromatographic analysis method

5 g sea fish is mixed with 50 mL acetate buffer pH = 4.8 and homogenized with a dispersing instrument. The sample solution is filtered first through a 5 µm and then through a 0.45 µm filter and subsequently injected automatically using an IC Sample Processor. Three spiked samples are prepared in the same way by adding 300, 500 or 700 µL of a mixed amine standard to the fish prior to sample preparation. The system and the IC method are checked using various standard solutions as well as a sample solution that was spiked after sample preparation. The determination of the amine concentrations is always carried out by calibration with external standards (7-point calibration).

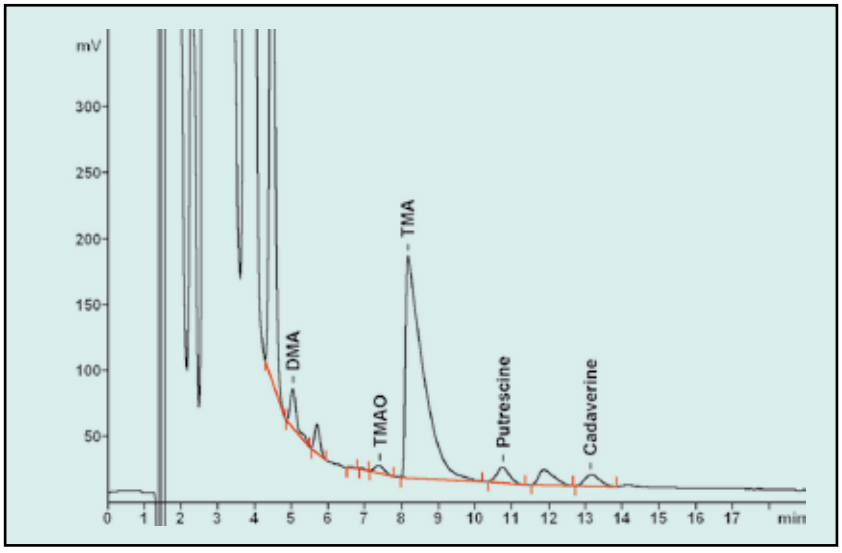
In order to prevent changes resulting from degradation processes, the samples should be stored in the cold before the analysis and, if possible, be processed on the same day as the sample was taken.

The IC measurements were made in the Application Laboratory of Metrohm Ltd. (Ms. Seifert) using an automated Advanced IC System. Work was carried out with a Metrosep C 2 – 150 (6.1010.220) separation column and Metrosep C 2 Guard (6.1010.200) precolumn. The eluent was 6 mmol/L HNO_3 at a flow rate of 1.0 mL/min. The standard solutions were each injected twice, the samples five times.



Standard chromatogram with the six amines.

Standard 3:	DMA-N	2.08 ppm
	TMAO-N	13.6 ppm
	TMA-N	9.98 ppm
	Putrescine-N	2.39 ppm
	Cadaverine-N	2.18 ppm
	Histamine-N	2.06 ppm



Chromatogram obtained for the analysis of fresh plaice.

Results:	DMA-N	10.5 mg/kg fish
	TMAO-N	3.85 mg/kg fish
	TMA-N	130 mg/kg fish
	Putrescine-N	7.12 mg/kg fish
	Cadaverine-N	5.38 mg/kg fish

Conclusion

The ion chromatographic method presented here allows the simultaneous and rapid determination of the different «freshness indicators» and is thus ideally suitable for the quality control of sea fish.