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Quantification of TMA & DMA by SPME-GC-MS for the freshness assessment of fish

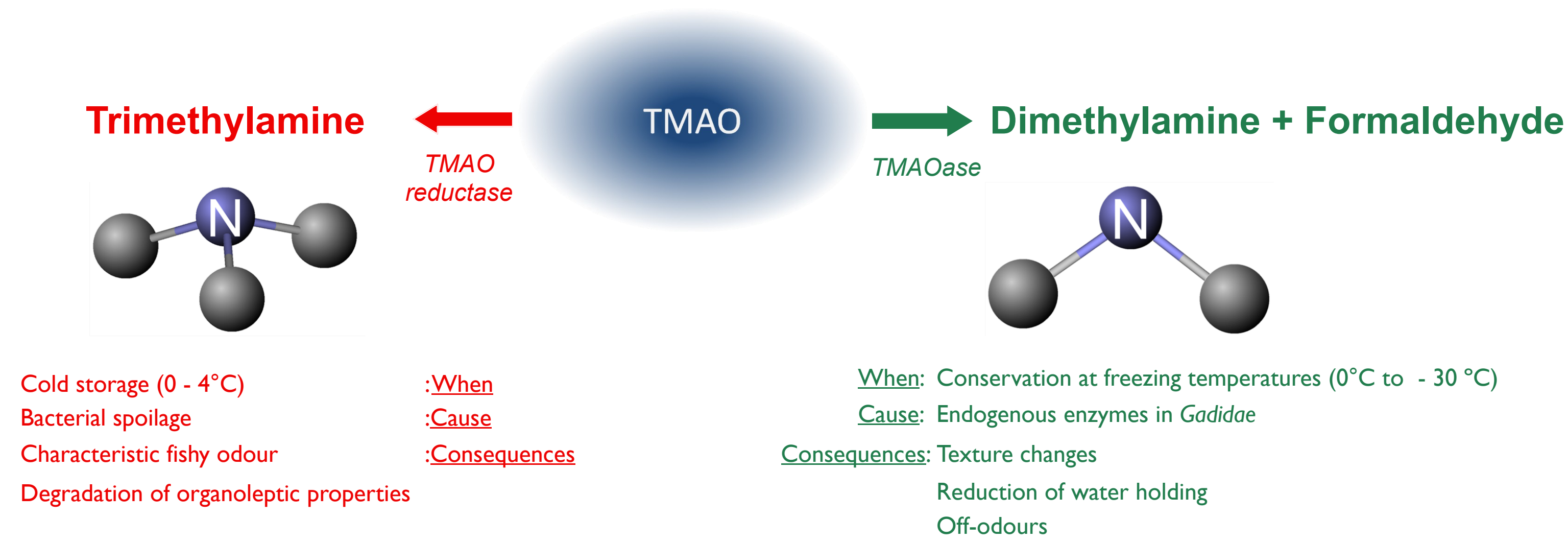
Alexandre DEHAUT, Simon DUTHEN & Guillaume DUFLOS

Introduction :

Nowadays, the freshness of fish is assessed by numerous physical, sensory or chemical methods.

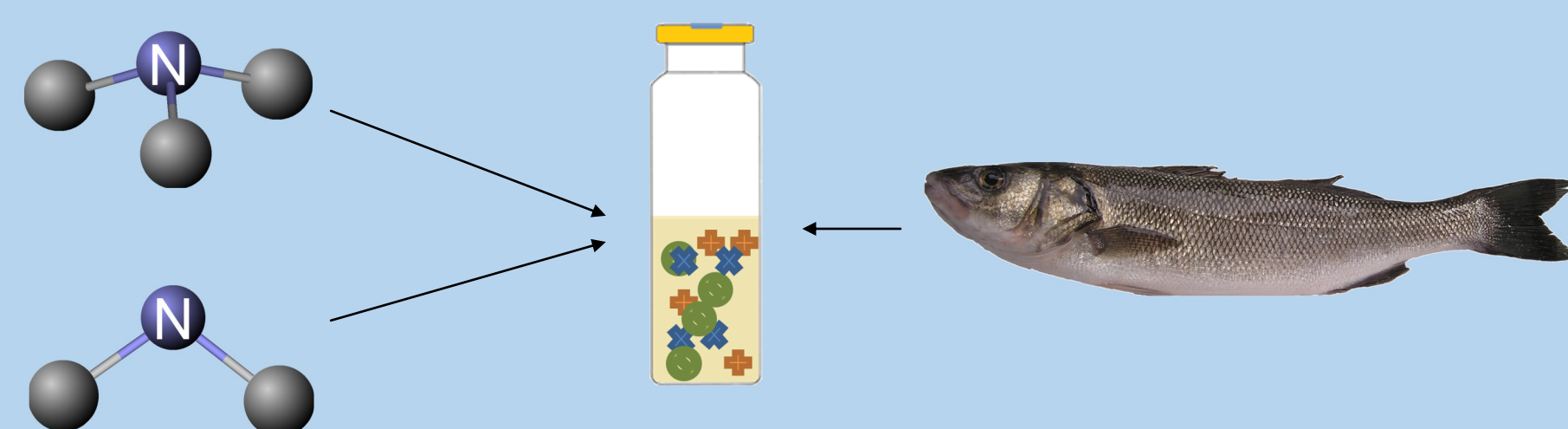
All of these techniques possess biases (subjectivity of the judges, working only on whole fishes, etc.).

The aim of this work is to follow the progress of the alteration of trimethylamine-N oxide (TMAO) with a new SPME-GC-MS approach. TMAO is a specific molecule of the flesh of marine fishes.

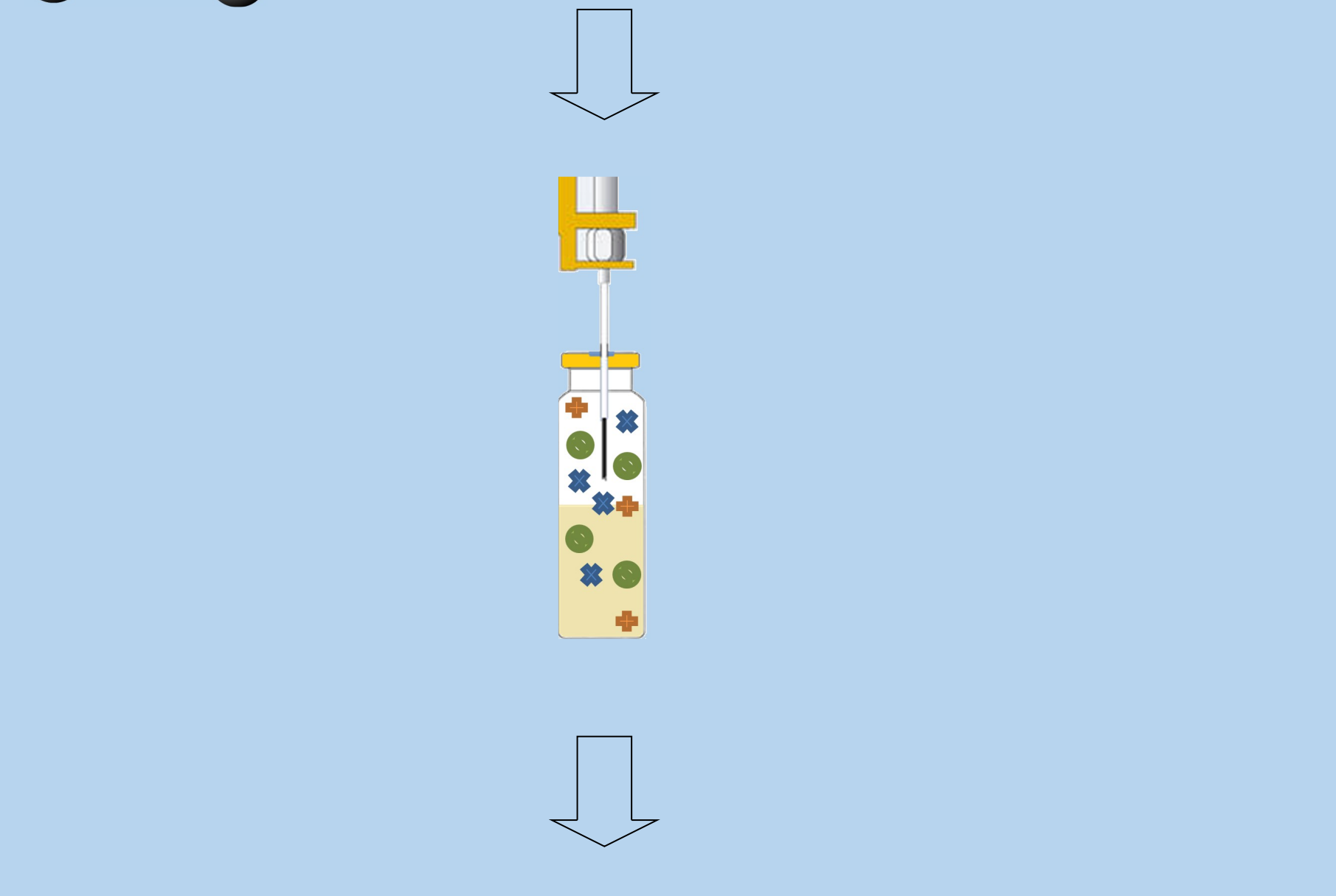


Materials and methods:

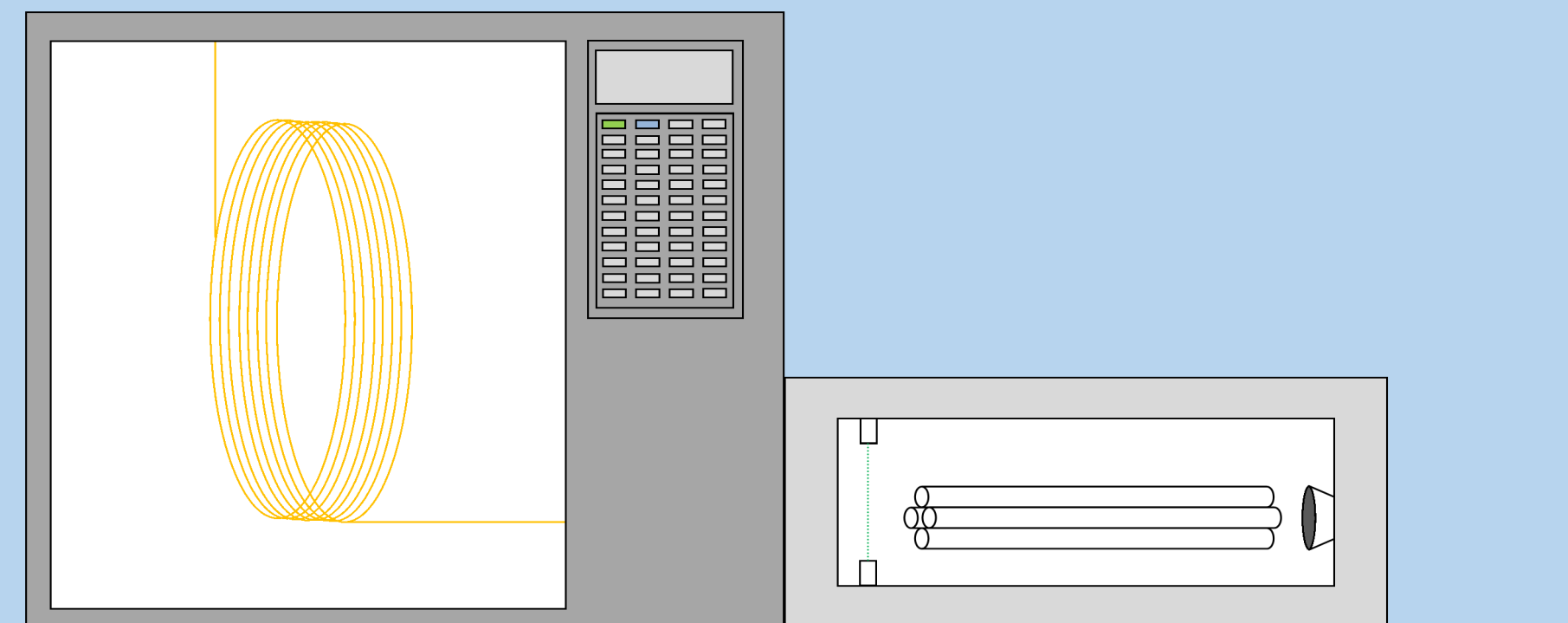
Simple sample preparation
 (Pure molecules or grinding of the flesh in a saturated saline solution and addition of KOH)



Extraction and collection
 of the basic volatiles with a CAR/PDMS SPME fibre



Separation and identification
 of the basic volatiles with a GC-MS device (Shimadzu Q2010-plus) equipped with a Stabilwax-DB column (Restek, France)



Results:

I) Optimisation of the sample preparation

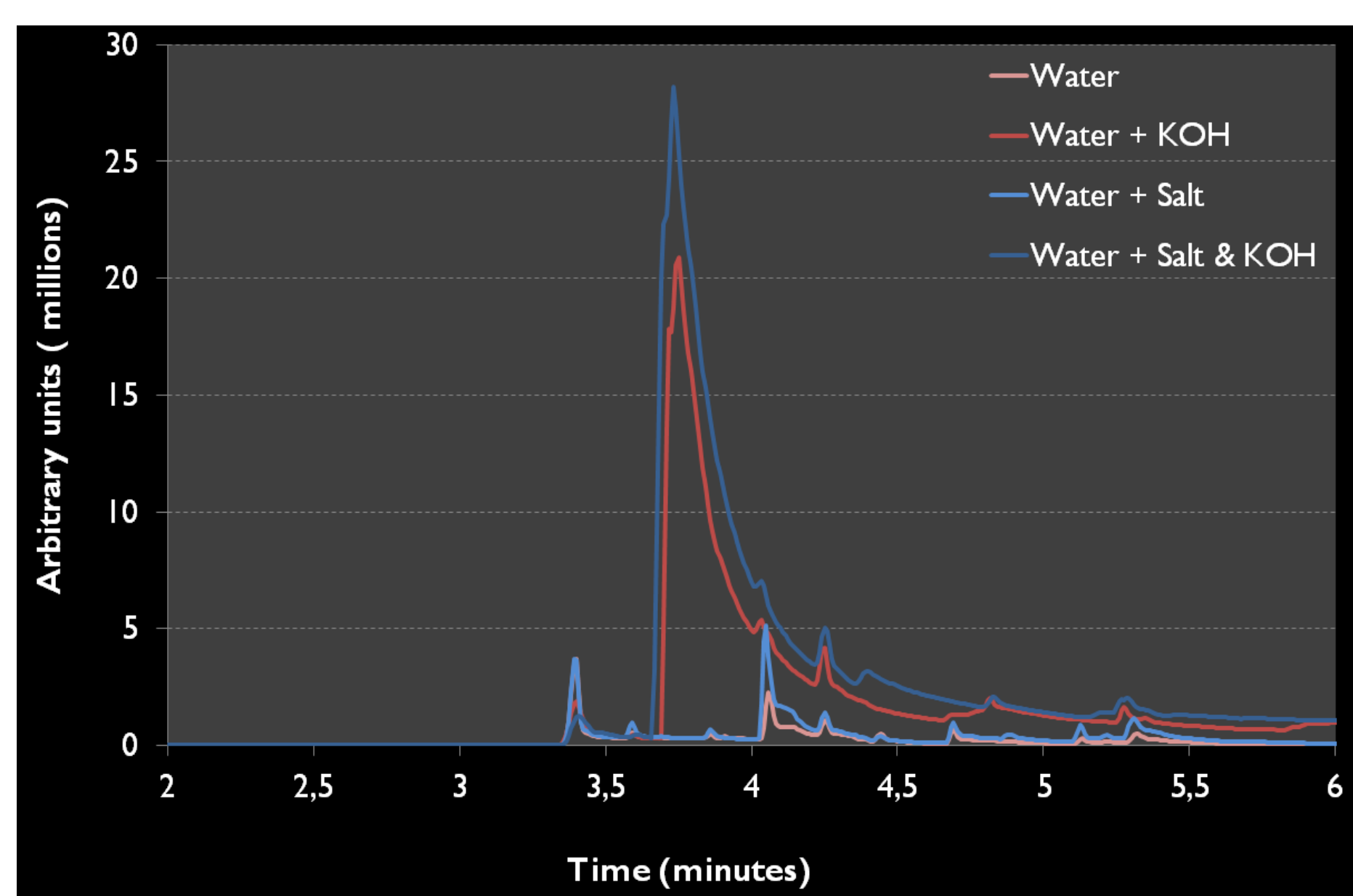


Figure 1 : Chromatograms obtained with different sample preparations (from 2 minutes to 6 minutes). The sample consists of the flesh of a whiting (*Merlangius merlangus*) kept several months at -20°C. Red curves correspond to the chromatograms of samples prepared with bidistilled water. Bleu curves correspond to the samples prepared with saturated saline solution (30% NaCl w:w). Darker curves correspond to the samples, where a KOH pellet was added to improve the pH of solution.

➤ Improvement of the signal when KOH & NaCl are added in the solution.

➤ Higher areas obtained with an extraction of 30 minutes at 35°C.

II) Optimisation of the separation conditions

Three major parameters were identified as key factors to improve the resolution of the peaks : Temperature of the injector (150°C & 210°C), mode of injection (splitless & split of 5) and initial temperature of the column (45°C & 60°C).

An experimental design was set up. Two levels (low and high) were applied for each parameters. Two variables were assessed: tailing factor (TF) and full width at half maximum (FWHM). They characterize the resolution of the peak.

Table 1: ANOVA tests were validated thanks to normality and homoscedasticity tests. Results of ANOVA tests were achieved on the two experimental designs (TMA & DMA) to assess the optimized parameters for getting peaks with the best resolution. For each variable (FWHM and TF) the probability of the significance of the three parameters (TI, TC & S) were given. Probabilities tagged with " * " mean that results are significantly different between the two levels (low and high).

	Data with Trimethylamine		Data with Dimethylamine	
	FWHM	TF	FWHM	TF
T°C of injector (TI)	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***
T°C of the column (TC)	0.0053 **	0.74	< 0.0001 ***	< 0.0001 ***
Split mode (S)	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***
TI x TC	0.0036 **	0.05 *	< 0.0001 ***	< 0.0001 ***
TI x S	< 0.0001 ***	< 0.0001 ***	0.0007 ***	< 0.0001 ***
TC x S	0.07	0.94	0.0012 **	< 0.0001 ***
TI x TC x S	0.77	0.0023 **	0.0003 ***	< 0.0001 ***

➤ Injection at 210°C, split of 5 and an initial temperature of the column at 60°C displays peaks of TMA with the best significant markers of resolution.

III) Specific detection of DMA and TMA in a spoiled sample.

After using total ion chromatogram (TIC) from 33 to 200 m/z a single ion monitoring (SIM) approach was performed.

It allows to follow the detection of specific ions markers of TMA (58 & 59 m/z) and DMA (44 & 45 m/z).

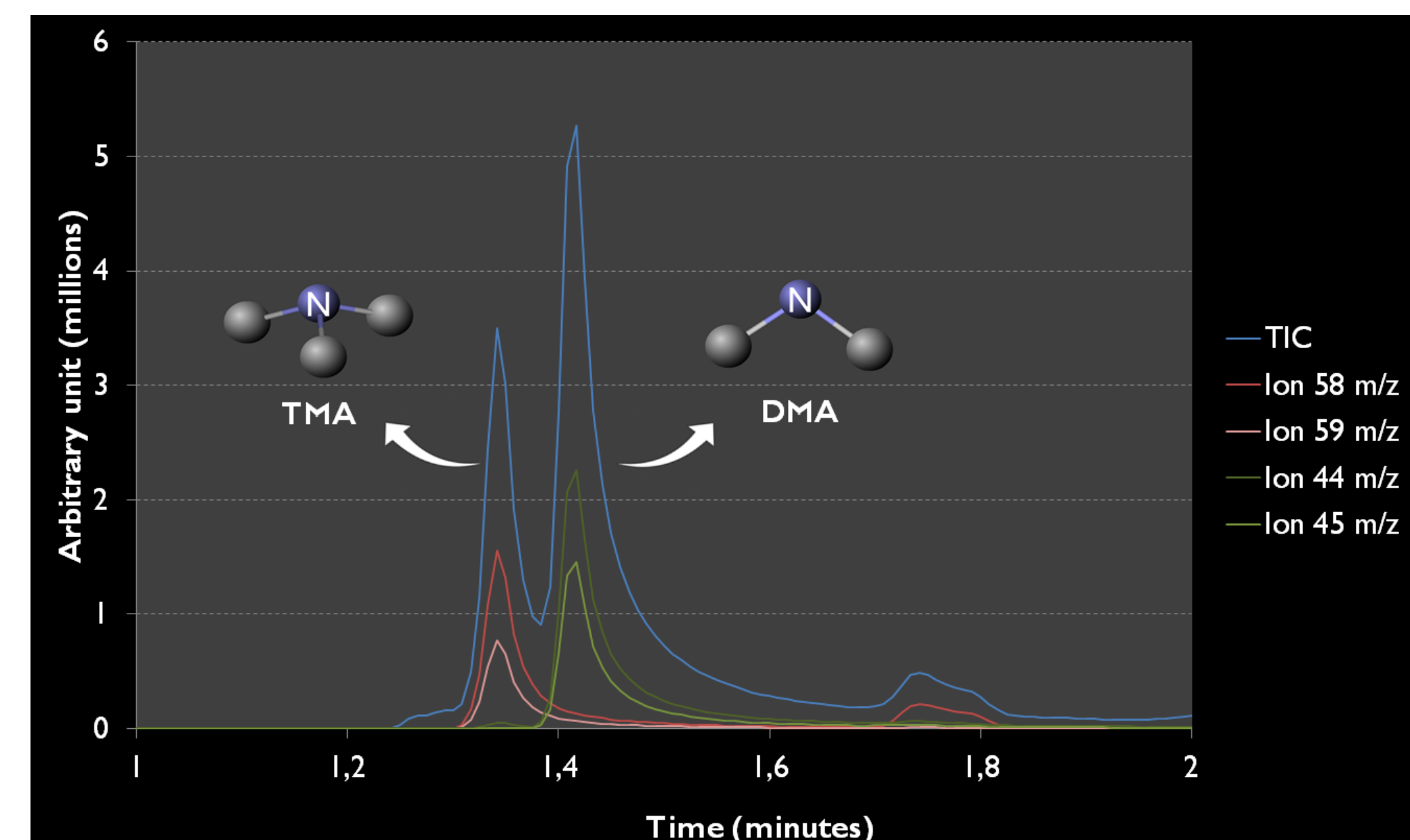


Figure 2 : Total and single ions chromatograms of a sample prepared from the flesh of a whiting (*Merlangius merlangus*) kept several months at -20°C with KOH and saturated saline solution. The blue curve corresponds to the total ions chromatogram. The red curves correspond to the specific ions of TMA : 58 m/z (darker) and 59 m/z (lighter). The green curves correspond to the specific ions of DMA : 44 m/z (darker) and 45 m/z (lighter).

➤ Efficient and specific detection of TMA & DMA with the SIM method.

Conclusions and Perspectives:

➤ Specific detection of TMA and DMA is now effective with optimised parameters of extraction and separation.

➤ Screening of potential internal standards.

➤ Development of a method of quantification (determination of LOD & LOQ).

➤ Application of the method to a study of spoilage of fishes.

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