

Rapid Gas Sensor Measurements To Determine Spoilage of Capelin (*Mallotus villosus*)

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Changes in volatile compounds of capelin were monitored by gas sensor measurements, sensory evaluation, and TVB analysis. Capelin was stored at 5 and 0 °C for 8 days, and the effect of 0.2% added acetic acid was studied at 5 °C. An instrument with electrochemical gas sensors was developed to monitor the volatile compounds of capelin during spoilage such as alcohols, amines, and sulfur compounds. The results of the gas sensor measurements showed good reproducibility, and the responses of selected sensors correlated well with classical TVB measurements. GC analysis of sulfur compounds was done simultaneously to identify and determine the relative abundance of these compounds in the headspace of capelin during storage, and a descriptive sensory evaluation scheme was developed.

Keywords: Gas sensors; volatile compounds; fish spoilage; sensory analysis

INTRODUCTION

Capelin catches in Iceland have increased during the last few years. Most of the raw material is used in fish meal production, but some of the high-quality capelin in Iceland is frozen for export to Japan. Increasing demand for high-quality fish meal requires better quality control during meal production. The trend is to use more controlled conditions such as cooling of the raw material aboard the vessels and in the storage tanks. However, preservatives that inhibit the growth of microorganisms often are used to prolong the shelflife of the raw material in the storage tanks. The classical spoilage profile is altered when preservatives such as acetic acid are used, and a detailed description and understanding of the spoilage processes occurring are not known.

Quality evaluation of raw material for fish meal production is based on a chemical measurement of total volatile bases (TVB) that form during spoilage of fish. The fish meal industry classifies the raw material according to TVB values. Capelin meal of highest quality requires raw material with less than 50 mg of TVB-N/100 g. A rapid and inexpensive method that could replace TVB measurements would facilitate quality control and classification of the raw material and allow for more frequent measurements to be taken during processing.

The application of chemical gas sensors for instruments to evaluate fish freshness in a rapid and nondestructive way is appealing for on-line quality evaluation of fish. Gas sensors can be used for the rapid detection of volatile spoilage compounds in capelin as an alternative to and more rapid method than the classical TVB measurement. Volatile compounds in the headspace of fish that can be detected by gas sensors are low molecular weight microbial metabolites, such as ammonia, trimethylamine (TMA), ethanol, hydrogen sulfide, methyl mercaptan, and sulfides. These components are present in the headspace above fish during spoilage at the ppm level (Herbert *et al.*, 1975; Ahmed

and Matches, 1983; Kamiya and Ose, 1984). Some of these compounds have been suggested as indicators of spoilage (Connell, 1980; Oehlenschläger, 1992; Lerke and Huck, 1977; Kelleher and Zall, 1983; Human and Khayat, 1981; Lindsay *et al.*, 1986; Strachan and Nicholson, 1992), and they contribute to spoilage odors such as fishy, stale, rotten, and putrid.

Sensor arrays have been used in field applications for the identification and quantification of chemical gases and mixtures. Sensor arrays have also been used for the characterization of various beverages (Gardner *et al.*; 1992; Shurmer and Gardner, 1992). For quality estimation of ground meat Winquist *et al.* (1993) used an instrument with MOSFET sensors (metal oxide field effect transistors). The detection of fish freshness by gas sensors has been studied by using metal oxide sensors (Ólafsson *et al.*, 1992; Nanto *et al.*, 1992; Egashira *et al.*, 1990) and by amperometric gas sensors (Schweiser-Berberich *et al.*, 1994; Ólafsdóttir *et al.*, 1995, 1996).

Commercial instruments or "Electronic Noses" with different gas sensors are now available on the market for the food industry; however, their validation for specific usage is lacking. The instruments have been tested by various research groups and industry, and the main concern is the sensitivity of these instruments to humidity. This is a problem when analyzing food with high water activity. In general, the type of sensors that are used for these instruments are metal oxide sensors, organic polymer sensors, SAW (surface acoustic wave sensors), MOSFET (metal oxide field effect transistors), piezoelectric sensors, and electrochemical sensors.

The objective of this study is to monitor changes in volatile compounds during storage of capelin and investigate the effect of different storage temperatures and added acetic acid on the development of volatile compounds during storage. An experimental instrument with gas sensors has been developed with a closed sampling container, an array of eight electrochemical gas sensors, and a PC with a measurement program. Electrochemical sensors were selected for the experimental instrument, because they are less sensitive to humidity than many of the other sensors that are used in electronic noses. The results of measurements with

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the gas sensor instrument will be compared to classical TVB measurements, sensory analysis, and gas chromatography.

MATERIALS AND METHODS

Materials. Capelin was harvested in late February 1996 south of Iceland and three groups of samples (20 kg each) were prepared within 24 h. Samples were stored in plastic containers (40 L) for 9 days at 0–2 and 4–5 °C, and one group was stored at 4–5 °C with added 0.2% acetic acid. In this report the three series will be referred to as 0, 5, and 5 °C with acid, respectively. Two sets of 1 kg samples were analyzed each day with electrochemical gas sensors, gas chromatography, TVB measurements, and sensory analysis. Samples were drawn, and sensory analysis and measurements with the gas sensor instrument were done immediately. Samples for TVB analysis were put in polyethylene bags and analyzed within 2 h. Samples for GC analysis were put in vacuum bags (Multiseven 80HS (25 × 40 cm) WIPAK, Finland) and analyzed after 30 min.

Measurements with the Gas Sensor Instrument. The gas sensor instrument consists of a glass container (5.2 L) closed with a plastic lid, an aluminum sensor box (dimensions: 16 × 12 × 10 cm) fastened to the lid, and a PC computer running a measurement program. The sensor box contains different electrochemical gas sensors (CO, H₂S, NO, NO₂, and SO₂ (Dräger) CO, SO₂, and NH₃ (City Technology, Portsmouth, Britain)) and a temperature sensor (PT 100). Electronics, an A/D converter, and a microprocessor to read the measurements and send them to the PC are also in the box. A miniature fan (1 W; Nitec) is positioned in the container to ensure gas circulation (Ólafsdóttir *et al.*, 1997).

The measurement technique for the analysis of volatile compounds in capelin with the gas sensor instrument is based on a static headspace system, analyzing direct headspace of fish stored in the closed glass container during sampling at room temperature. The temperature of the samples from the 0 °C storage reached 5–7 °C during measuring and the temperature of the samples from the 5 °C storage increased to 8–9 °C during measuring. Approximately 1 kg of capelin was put into the glass container and the lid put on. Measurements were taken every 10 s for 20 min. In the data analysis, the reported value (current) is calculated as follows: the final measurement (average of the last three measurements of the 20 min measurement cycle) minus the initial measurement (the average of 18 measurements before the sample is introduced into the container).

The sensitivity of the gas sensors toward sulfur compounds was estimated by measuring the headspace of 25 mL of varying concentrations of dimethyl disulfide and dimethyl sulfide diluted in water (12.5, 62.5, and 250 ppm) in the 5.2 L glass container.

TVB Measurements. Total volatile bases (TVB as mg of N/100 g of whole fish) were measured according to Antonacopoulos (1968) with a Struer automatic distillation unit.

Sensory Analysis. A traditional freshness grading system (Botta, 1995) for capelin was developed by two members of the IFL sensory panel by describing the characteristic odor of the capelin samples each day of sampling.

Gas Chromatography. Direct headspace analysis of volatile sulfur compounds from capelin by gas chromatography was done on days 4, 5, 6, and 8. At the time of sampling, the capelin (1 kg) were put in plastic bags (Multiseven 80HS, WIPAK, Finland) equipped with a septum and sealed. The plastic bags were kept at room temperature for 30 min before samples were withdrawn from the bags by piercing through the septum with an airtight syringe. Headspace samples (1 mL) were injected splitless onto a HP-1 (cross-linked methylsilicone gum) capillary column (30 m; 0.32 mm i.d.; 4.0 μm film thickness) that was maintained at 50 °C for 4 min and then programmed to increase 10 °C/min to 170 °C. The helium carrier gas flow rate was 32 mL/min. The gas chromatograph (HP5890) was equipped with a FPD sulfur sensitive detector.

The temperature of the injector was 75 °C, and the detector temperature was 250 °C.

Tentative identification of compounds in capelin was based on comparison with authentic standards. The standards selected were liquid samples of DMDS (dimethyl disulfide) and DMS (dimethyl sulfide) (standard analytical grade samples from Merck) and compressed gas mixture of hydrogen sulfide (H₂S) and air (50 ppm ± 5 ppm) (EURO-GAS, Bramley, Surrey GU5 0EG, England). Semiquantitative information on compounds in capelin was obtained by measuring DMDS and DMS diluted in water. A 25 mL aliquot of each concentration (12.5 and 62.5 ppm) was put in a Petri dish, which was then put in a plastic bag equipped with a septum and sealed and allowed to equilibrate at room temperature for 30 min. The overall trend in the change of concentrations of the components in the capelin samples was observed by the intensity of the detector signal, and quantification was based on comparison to the external standards (DMS and DMDS).

RESULTS AND DISCUSSION

Measurements with the Gas Sensor Instrument.

The sensitivities of the electrochemical sensors have been tested toward three classes of compounds that are present in high levels in the headspace of fish during spoilage. These classes are short chain alcohols, sulfur compounds, and amines (Lindsay *et al.*, 1985). The CO and SO₂ sensors from Dräger and the NH₃ sensor from City Technology were most sensitive toward the standard compounds tested and also showed the most characteristic responses to the capelin headspace (Ólafsdóttir *et al.*, 1997). These sensors were therefore selected to demonstrate the results in this report. The three sensors selected, the CO, NH₃, and SO₂ sensors, all showed similar response curves toward the compounds that they were sensitive for. The response time of the sensors was around 60 s, but the liquid–gas system needs time to equilibrate. To allow time for equilibration, the measurements were continued for 20 min to observe the sensor signal, but more than 90% of the final value was reached after 5–7 min. This indicates that measurement time could be shortened considerably. With a shorter exposure time of the sample, the sensors would need less time to recover from each measurement. Approximately 20–25 min was needed to allow the sensors to recover and reach the initial value, after the exposure of large amount of samples such as spoiled capelin samples.

Typical Response Curves of the Sensors to Capelin Headspace. In Figures 1–3 the CO sensor was selected to show the characteristic responses of the sensors during 20 min (1200 s) of measuring capelin samples for each day of storage at 0, 5, and 5 °C with acid. The intensity of the response curves increases with days as expected, because as the fish spoils the degradation components in the headspace become more concentrated.

Figures 1 and 2 show that the CO sensor could not detect a difference between the first two days at either storage temperature (0 and 5 °C), but an increase in the response was noticed already between the first two days and day 4 at both temperatures. The spoilage is much more rapid in samples stored at 5 °C than at 0 °C, as reflected by the higher sensor signal in the 5 °C samples (note different values on the y-axes in Figures 1 and 2). In Figure 3 the effect of added acid can be seen and the intensity of the signal is much lower than in the other two series, reflecting a lower concentration of microbially formed volatile degradation compounds.

The characteristic response curves of the SO₂ and NH₃ sensors to capelin headspace were similar, and

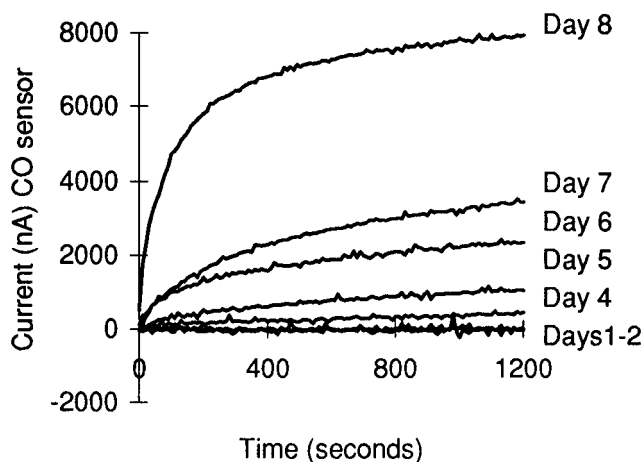


Figure 1. Response curves (current in nanoamperes) of the CO sensor to capelin headspace during storage at 0 °C for 8 days.

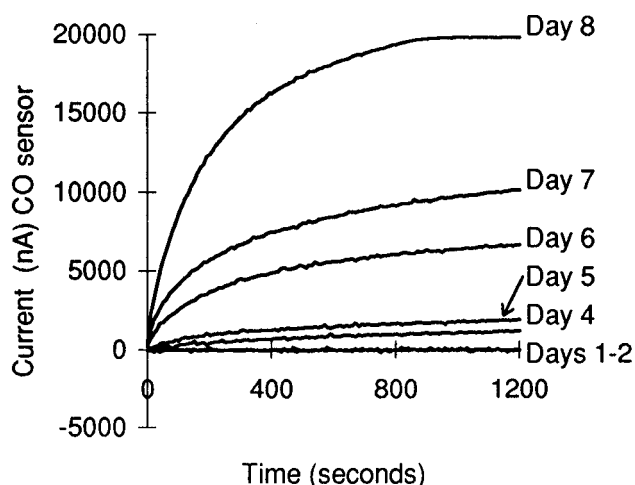


Figure 2. Response curves (current in nanoamperes) of the CO sensor to capelin headspace during storage at 5 °C for 8 days.

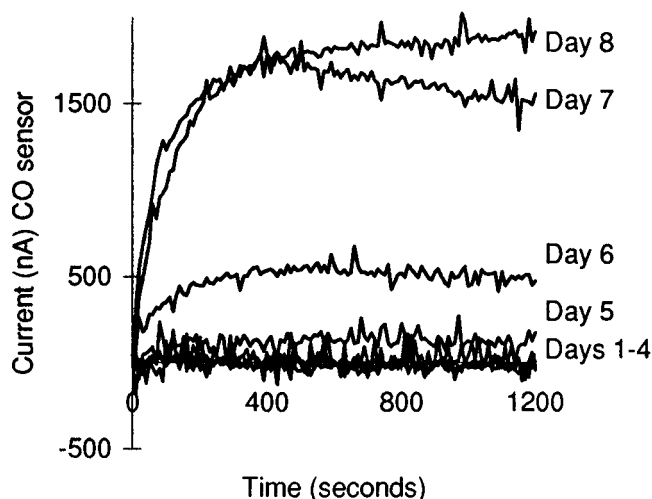


Figure 3. Response curves (current in nanoamperes) of the CO sensor to capelin headspace during storage at 5 °C with added acid for 8 days.

increased signal with days was observed as expected. The same overall trend in sensor response was observed for the three groups of samples, and the responses of the SO₂ and NH₃ sensors were very low to the headspace of the capelin samples with added acetic acid during the storage time.

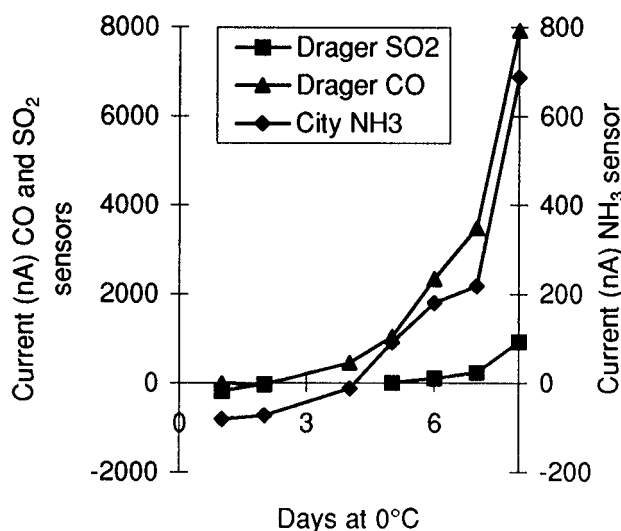


Figure 4. Responses (current in nanoamperes) of CO (▲), SO₂ (■), and NH₃ (◆) sensors to capelin headspace during storage at 0 °C for 8 days.

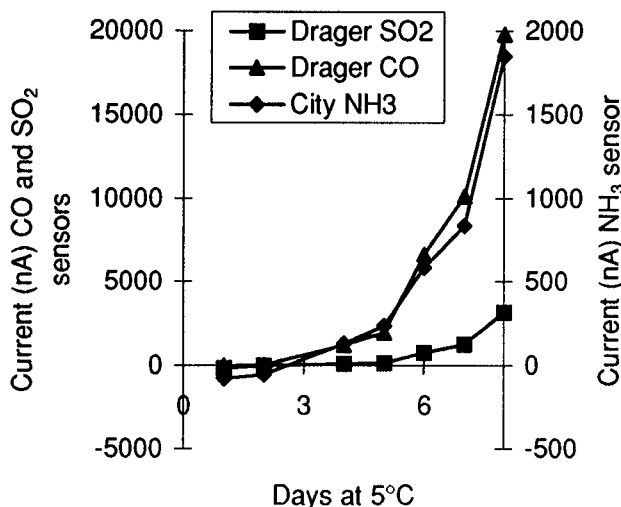


Figure 5. Responses (current in nanoamperes) of CO (▲), SO₂ (■) and NH₃ (◆) sensors to capelin headspace during storage at 5 °C for 8 days.

Comparison of the Responses of the CO, NH₃, and SO₂ Sensors to the Headspace of Capelin Stored under Different Conditions for 8 Days.

Figures 4–6 sum up the information obtained in the response curves of the CO sensor in Figures 1–3 in addition to the information on the responses of the NH₃ and SO₂ sensors to capelin headspace during storage at 0, 5, and 5 °C with added acid. The overall trend is the same at the different storage conditions and it is evident that the spoilage rate is most rapid at 5 °C, as noted by the highest intensity of the sensor signals (Figure 5). The added acetic acid slows down the spoilage rate or the formation of volatile compounds, and the intensities of the sensors are lowest in that sample (Figure 6).

The increase in the response of the NH₃ and CO sensors occurs between days 2 and 4 in both the 0 and 5 °C capelin series. The response of the SO₂ sensors appears to increase later in the storage on days 8 and 6 in the 0 and 5 °C samples, respectively, and then a sudden increase appears.

TVB Measurements Compared to Gas Sensor Measurements. Figure 7 shows the results of TVB measurements in the three series of capelin stored at

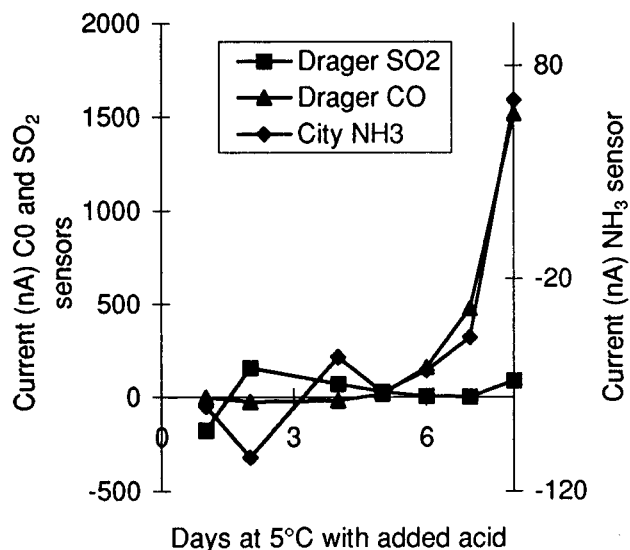


Figure 6. Responses (current in nanoamperes) of CO (\blacktriangle), SO_2 (\blacksquare) and NH_3 (\blacklozenge) sensors to capelin headspace during storage at 5 °C with added acetic acid for 8 days.

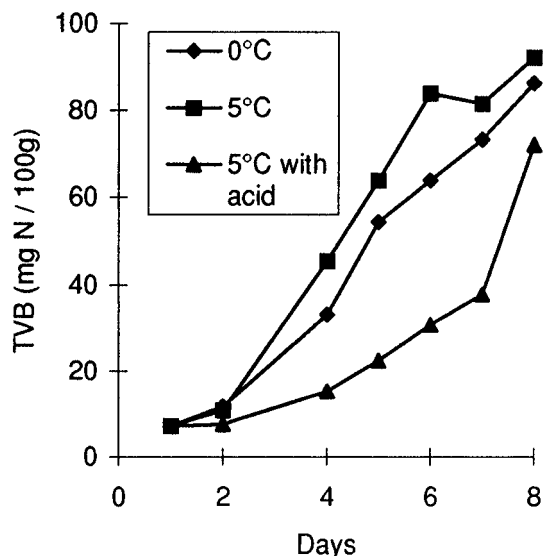


Figure 7. Results of TVB measurements in capelin stored at 0 °C (\blacklozenge), 5 °C (\blacksquare), and 5 °C with added acid (\blacktriangle) for 9 days.

0, 5, and 5 °C with added acid. The TVB values in the capelin series kept at 5 °C increased more than in the capelin series kept at 0 °C, as expected. Capelin stored at 0 °C reached a TVB value of about 50 mg of N/100 g in 5 days, while capelin stored at 5 °C reached the same TVB value in 4 days and the 5 °C series with added acid reached that value after 7 days of storage. During the last two days of the experiment the TVB values for the 5 °C samples were lower than was expected and the curve appears to level off. The usual trend is that TVB values increase exponentially with days during the spoilage of fish (Connell, 1980).

Correlation (using Microsoft Excel Version 7 for Windows 95) between TVB measurements of capelin and the response of the NH_3 and CO sensor, respectively, to capelin headspace in the 0 °C samples is higher for the NH_3 sensor ($r_{0^\circ\text{C}} = 0.98$), than for the CO sensor ($r_{0^\circ\text{C}} = 0.92$). This is in agreement with previous experiments carried out at the IFL (Ólafsdóttir *et al.*, 1997). The NH_3 sensor is very sensitive toward amines and is measuring the same components as the TVB analysis. The CO sensor is not sensitive toward amines

Table 1. Linear Regression between the CO Sensors from Dräger, the NH_3 Sensors from City, and the SO_2 Sensor from Dräger in the Two Sets of Instruments A and B

	CO	NH_3	SO_2
r (corr coeff)	0.994	0.973	0.993
m (slope)	1207	1465	0.3
b (intercept)	-104	45	13

but is responding to components that are being produced at a similar rate as the amines. These components are, for example, alcohols such as ethanol that forms during spoilage. The correlation is high between the CO and NH_3 sensor for capelin under these experimental conditions. However, based on their unlike sensitivities toward different compounds, it is useful to maintain both sensors in the instrument to be applicable for fish under different conditions.

Figure 7 shows that during the last few days of storage the responses of the sensors increase more than the TVB values. An explanation may be that the sensors are not very selective and can detect a range of different very volatile degradation compounds forming at advanced stages of spoilage. This increase in the response of the sensors during advanced spoilage results in lower correlation between the sensors and TVB values in the 5 °C series ($r_{5^\circ\text{C}} = 0.89$ for the NH_3 sensor and $r_{5^\circ\text{C}} = 0.90$ for the CO sensor) since spoilage is more advanced at higher temperatures. The correlation was done until day 7 in the 0 and 5 °C series and through day 8 in the 5 °C with acid series, respectively, to take into account the nonlinear trend in the increase of the sensor signals at advanced spoilage. In the 5 °C with acid series the correlation for the NH_3 and CO sensors to TVB analysis is very high ($r_{5^\circ\text{C}} \text{ with acid} = 0.97$ for both sensors). This is because of the slow production of volatile compounds in the samples because of the inhibiting effect of the acetic acid on the growth of microorganisms. The TVB values appear to increase at a similar rate or even slightly more than the sensor signals.

Calibration of Gas Sensor Instruments. During this study, two sets of instruments with gas sensors were used, instruments A and B. In this report, data from instrument A have been selected to represent the results of the experiments. The instruments showed very similar results, and comparisons were done between identical sensors in each instruments. The results of linear regression between the identical CO sensors, the two NH_3 sensors, and the SO_2 sensors from the two instruments for all the data in this study are shown in Table 1.

The correlation is high between the sensors, and for comparison of data from the two instruments, the slope of each sensor can be used for calibration. The slope is highly significant for all the sensors ($p < 0.0001$), but the intercept is not significant.

Measurements using standard compounds have shown that the sensitivities of identical sensors are different (Ólafsdóttir *et al.*, 1997) and new sensors have to be calibrated. Different standards are used for each sensor and ethanol was selected to represent alcohols to calibrate the CO sensor, dimethyl disulfide for sulfur compounds to calibrate the SO_2 sensor, and TMA for amines to calibrate the NH_3 sensor.

The sensitivity (nA/ppm) of the sensors was obtained as the slope of the calibration curve of varying concentration of dimethyl disulfide and dimethyl sulfide (12.5, 62.5, and 250 ppm of each component in a mixture).

Table 2. Descriptive Analysis of the Odor of Capelin during Storage at 0, 5, and 5 °C with Acid

days	0 °C	5 °C	5 °C with acid
1	little odor at first, but characteristic cucumber or fresh capelin odor soon develops, metallic-like	heavy, sweet, cucumber-, melon-, capelin-like odor, metallic	characteristic capelin odor, strong cucumber, acetic acid
2	strong, fresh, characteristic capelin odor, strong cucumber, fresh grass-like; metallic odor from the gills	melon-like sweet odor, slightly stale	cucumber and melon odors and weak acetic acid
4	no change in odor (heavy capelin, cucumber, melon odor)	stale, hay-like, ammoniacal, metallic (oxidized)	sweet and sour, melon
5	melon-like, sweet odor, slightly stale	rotten, putrid	sweet and sour, slightly grass-like
6	heavy, sweet, hay-like, ammoniacal, metallic (oxidized), stale	rotten, putrid	heavy, sweet and sour
7	heavy, sweet, hay-like, ammoniacal, metallic (oxidized), stale, sour	rotten, putrid	heavy, sweet and sour
8	rotten, putrid	rotten, putrid	heavy, sweet and sour
9	rotten, putrid	rotten, putrid	heavy, sweet and sour

Table 3. Relative Concentration of Sulfur Compounds Analyzed by GC-FPD in Capelin Stored at 0, 5, and 5 °C with Added Acetic Acid

	H ₂ S			CH ₃ SH			DMDS		
	0 °C	5 °C	5 °C + acid	0 °C	5 °C	5 °C + acid	0 °C	5 °C	5 °C + acid
4 days	—	—	—	—	++	—	—	—	—
5 days	—	—	—	++	+++	—	+	+	—
6 days	—	+	—	++	++++	—	++	++	—
8 days	+	++	—	+++	++++	—	+	++	—

Sensitivities of the CO, NH₃, and SO₂ sensors to the standards were 15.2, 0.91, and 0.12 nA/ppm, respectively. The actual concentration of the components in the headspace of the samples is not known, but standards of known concentration will give an estimate of the level of the components in fish.

Sensory Analysis. Table 2 shows the descriptive sensory evaluation of the odor of capelin stored at 0, 5, and at 5 °C with added acid during 8 days of storage. The characteristic fresh capelin odor was very reminiscent of cucumbers in the beginning and the TVB value was around 10 mg of N/100 g.

The identity of the compound that contributes to this characteristic cucumber-like fresh capelin odor is 2,6-nonadienal (Ólafsdóttir *et al.*, 1997) and is a lipid-derived oxidation product. The fresh capelin-cucumber-like odor became more sweet and melon-like with time and diminishes as the classical spoilage odors appear. During the following days (4th–5th day) the first spoilage odors appeared and stale odor was first noticed on day 4 in the 5 °C series and on day 5 in the 0 °C series. TVB values had then reached levels around 40–50 mg of N/100 g. After 6 and 8 days of storage in the 5 and 0 °C series, respectively, the odor of the capelin was putrid and the TVB values had increased to about 80 mg of N/100 g.

The classical spoilage odors that developed in the 0 and 5 °C series were not present in the series with added acid. The inhibiting effect of the acetic acid on the growth of microorganisms alters the spoilage pattern in these samples. The influence of the acetic acid on the odor was noticed on days 2 and 3 and after that the odor became more bland and sweet and sour-like.

Gas Chromatography. Table 3 shows the results of the analysis of sulfur compounds in the headspace of capelin samples. Sulfur compounds were not detected in the samples with added acid, but their presence in the 0 and 5 °C series was associated with the onset of spoilage in these samples. On days 4 and 5 methyl mercaptan (CH₃SH) was first detected in the 5 and 0 °C series, respectively, and this coincides with the development of stale odor (see Table 2). The further increase in the level of sulfur compounds was accompanied with rotten and putrid odors on days 8 and

6 in the 0 and 5 °C series, respectively. The increasing level of sulfur compounds analyzed by GC can be compared to the results of the gas sensor measurements. The SO₂ sensor is sensitive toward sulfur compounds, and the sensor shows little response until days 8 and 6 in the 5 and 0 °C series, respectively, and then a sudden increase appears at the same time as putrid and rotten odors were noticed by sensory evaluation (see Table 2).

The results of the measurements of standard samples of DMDS and DMS can be used to estimate the level of sulfur compounds in the capelin samples. For estimation of relative concentration of sulfur compounds in capelin samples in Table 3, intensities of detector signals in the samples were expressed as ++, +, and +++++ and corresponded to the area of 0.5, 5, and 50 ppm of standards, respectively.

CONCLUSIONS

The spoilage in the capelin series kept at 5 °C was most rapid, as expected. Capelin stored at 0 °C reached a TVB value of about 50 mg/100 g in 5 days, while capelin stored at 5 °C reached the same TVB value in 4 days, and the 5 °C series with added acid reached that value after 7 days of storage.

The characteristic fresh capelin odor was very reminiscent of cucumbers in the beginning when the TVB value was around 10 mg of N/100 g. The cucumber odor became more sweet and melon-like with time. During the following days (4th–5th day) the first spoilage odors appeared and stale odor was first noticed on day 4 in the 5 °C series and on day 5 in the 0 °C series. TVB values had then reached levels around 40–50 mg of N/100 g. After 6 and 8 days of storage in the 5 and 0 °C series, respectively, the odor of the capelin was putrid and the TVB values had increased to about 80 mg of N/100 g.

The responses of the three sensors (CO, NH₃, and SO₂) to capelin headspace increase at different stages of spoilage, and it is suggested to use only these three selective gas sensors to simplify the data handling.

The responses of the CO and NH₃ sensors correlate well with the classical TVB measurements. This is in agreement with earlier experiments at the IFL (Ólafsdóttir

dóttir *et al.*, 1995, 1997). Earlier results have shown that the NH₃ sensor is very sensitive to amines, but the CO sensor is not and therefore must be responding to components that are being produced at a rate similar to that of the amines.

On days 4 and 5 methyl mercaptan (CH₃SH) was first detected in the 5 and 0 °C series, respectively, and this coincides with the development of stale odor (see Table 2). The further increase in the level of sulfur compounds was accompanied by rotten and putrid odors on days 8 and 6 in the 0 and 5 °C series, respectively. The increase in the response of the SO₂ sensor occurs at the same time as stale odors are first detected, and sulfur compounds are detected at the same time by gas chromatography.

It can be concluded that the results from the gas sensor instrument can be used to predict TVB values in capelin. The results reported herein indicate that rapid measurements of volatile compounds with the gas sensor instrument operated at room temperature can be useful to indicate the freshness stage of capelin and classification of the raw material can be achieved. Future research aims at improving the sampling system of the gas sensor instrument and continuing storage experiments and collecting information for a data base for application of the instrument in the fish meal industry.

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