Application of an Electronic Nose To Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production

G. Ólafsdóttir,* Á. Högnadóttir, E. Martinsdóttir, and H. Jónsdóttir

Icelandic Fisheries Laboratories, P.O. Box 1405, 121 Reykjavík, Iceland

An electronic nose was used as a rapid technique to monitor changes in the headspace gas above capelin (*Mallotus villosus*) during storage at 0 and 5 °C. At both temperatures, refrigeration was compared with an ice/seawater cooling system. At 5 °C, the effect of added 0.2% acetic acid was also tested. Electronic nose measurements were compared to measurements of total volatile bases (TVB), gas chromatography of volatile compounds, and sensory analysis. TVB analysis indicated less spoilage in seawater/ice systems than in refrigeration, but the other measurements indicated more spoilage in the seawater system compared to refrigeration. The possibility of using only a few sensors in the electronic nose to predict TVB was studied using partial least squares regression and a saturated generalized linear model. The results reported herein indicate that rapid electronic nose measurements, which require no sample preparation, can be used to predict the TVB value of the raw material stored under different conditions.

Keywords: *Electronic nose; total volatile bases; fish spoilage; fishmeal*

INTRODUCTION

Maintaining the freshness of the raw material used for fishmeal production is very important to ensure the nutritive value and quality of the meal. The rapid spoilage of capelin and insufficient cooling on board fishing vessels often result in poor quality of the raw material used for meal production. Recently, increased efforts have been put into maintaining the freshness of the raw material on board fishing vessels by cooling directly after catch. Various types of refrigerated sea-water (RSW) and chilled seawater (CSW) systems have been used on board fishing vessels (Dagbjartsson et al., 1982; Hiremath et al., 1982; Kolbe et al., 1985). In CSW systems ice is used to maintain the low temperature. The amount of ice needed depends on the temperature of the seawater, 0 and 5 °C, and the fish and also the amount and the thickness of the fish layers. Therefore, during summer months more ice is required; many boats are inadequately insulated, thus requiring more ice due to heat leakage into the hold during a trip. An increased spoilage rate has been reported for capelin stored in seawater systems compared to similar capelin held in ice (Shaw and Botta, 1975). One of the disadvantages to the use of recirculating RSW for the storage of fish is the faster than normal buildup of aerobic bacteria in the water, due mainly to the high level of nutrients leached from the fish.

The formation of volatile compounds as a result of microbial activity and lipid oxidation during storage of fish is well-known, and many volatile compounds have been suggested as indicators of spoilage. Very rapid spoilage of capelin during summer often leads to complaints because of malodors in the neighborhood of fishmeal factories. Bulk stored capelin with high stomach contents is easily solubilized because of high proteolytic activity in the gut (Aksnes and Brekken, 1988). This results in liberation of peptides and free amino acids, which bacteria utilize for their energy demand, resulting in the formation of foul-smelling microbial metabolites such as hydrogen sulfide, methyl mercaptan, and dimethyl disulfide from cysteine and methionine (Herbert and Shewan, 1975). High levels of ammonia and trimethylamine are also formed in addition to amines such as cadeverine, histamine, and putrecine from the breakdown of lysine, histidine, and arginine, respectively (Aksnes and Brekken, 1988). The formation of short-chain alcohols, ketones, aldehydes, aromatics, and acids also occurs as a result of microbial degradation of amino acids and lipid oxidation during fish spoilage (Lindsay et al., 1986). The combination of various low molecular weight volatile compounds contributes to the overall spoilage odors in fish.

Various methods are available to evaluate fish freshness (Ólafsdóttir et al., 1997c, 1998). Sensory analysis is most often used in the fish industry to evaluate the freshness of fish, and odor is one of the most important factors in the analysis. Total volatile bases (TVB) analysis is traditionally used in the fishmeal industry to evaluate the quality of raw material. Gas chromatography (GC) can be used to measure volatile compounds as indicators of freshness (Josephson et al., 1986) and spoilage of fish (Lindsay et al., 1986) by monitoring changes in the headspace. However, GC is a laboratory technique, and recently electronic noses have been introduced as an alternative rapid technique to supplement or replace traditional quality control techniques in the food industry (Bartlett et al., 1997). Electronic noses have been developed to mimic the human sense of smell. This artificial olfaction is generally based on nonselectivity of the sensors, and usually an array of a large number of sensors is utilized and the interpretation of the data requires the use of pattern recognition techniques (Craven et al., 1996). For quantitative analysis it is possible to use only a few sensors and to obtain optimal performance given that the selectivity of the sensors covers the different classes of compounds relevant for the particular application (Di-Natale et al., 1996). This approach was used in the

2354 *J. Agric. Food Chem.,* Vol. 48, No. 6, 2000

Ólafsdóttir et al.

development of the electronic nose FreshSense by the Icelandic Fisheries Laboratories and Element Sensor Systems. FreshSense is based on a closed, static sampling system and electrochemical gas sensors that are sensitive to the main classes of volatile compounds, namely, alcohols, carbonyls, sulfur compounds, and amines, which accumulate because of microbial growth during storage of fish (Ólafsdóttir and Fleurence, 1998).

Earlier work has shown that electronic nose measurements of capelin headspace correlate well with TVB measurements during storage at 0 and 5 °C and that acetic acid was effective in extending the shelf life of the capelin raw material (Ólafsdóttir et al., 1997a,b). This study was undertaken to confirm and investigate further the influence of different storage temperatures and added acetic acid and in addition the effect of adding an ice/seawater mixture to preserve capelin raw material during storage. The spoilage pattern of fish is altered when preservation techniques, such as seawater cooling systems, are used. Therefore, the fishmeal industry needs information regarding the validity of using traditional quality checks such as TVB for capelin stored in seawater systems. The application of an electronic nose (FreshSense) as a rapid technique to monitor volatile compounds in the headspace of capelin stored under different conditions was studied. Moreover, the potential to use electronic nose measurements instead of the traditional TVB method was examined, as was the possibility of using electronic nose measurements to predict TVB values of capelin stored under different conditions.

MATERIALS AND METHODS

Capelin was harvested in late February 1997 south of Iceland and transported by truck to the laboratory. The temperature of the capelin had reached 4 °C when the samples were prepared the following day (day1). Five groups of samples (20 kg each) were prepared and stored in 30 kg plastic barrels until analyzed. Samples were stored for 10 days both at $0-2$ °C and at $4-5$ °C, and the latter group was initially stored at 10 °C for 1 day to imitate temperature fluctuations that can occur during storage. At both temperatures, refrigeration was compared with an ice/seawater cooling system. At $4-5$ °C, the effect of added 0.2% acetic acid was also tested. One liter of acetic acid in water solution (62.5 g of 96% acetic acid) was prepared and added to the 30 kg of capelin and agitated carefully to achieve the 0.2% acetic acid capelin sample. The seawater was prepared by using tap water with 3.5% added salt (NaCl), and ice was added. The ratio by weight was 10% ice/15% seawater/75% fish. The ice and seawater mixture was put in the bottom of the barrel, the fish was put on top, and the mixture was carefully agitated so as not to cause any damage to the fish but ensuring that the ice was evenly distributed. In this paper the series will be referred to as 0 °C, 0 °C + sea, 5 °C, 5 °C + sea, and 5 °C + acid. Samples from each series were analyzed with the FreshSense electronic nose, GC, TVB measurements, and sensory analysis on days 1, 3, 6, 8, and 10. The temperature of each group was recorded every hour throughout the storage using an EBi 125 temperature recorder (Ebro electronic GmbH, Germany).

Electronic nose measurements were performed using an electronic nose called FreshSense, developed by the Icelandic Fisheries Laboratories (IFL) and Element Sensor Systems (Artorg 1, 550 Saudarkrokur, Iceland). The instrument consists of a glass container (5.2L) closed with a plastic lid, an aluminum sensor box fastened to the lid, and a personal computer running a measurement program. The sensor box contains nine different electrochemical gas sensors (Dräger, Germany, CO, H_2S, NO, NO_2, and SO_2; City Technology, U.K., SO_2, H_2S, NH_3A7AM, and NH_3) and a temperature sensor.

Electronics, an A/D converter, and a microprocessor to read the measurements and send them to the PC are also in the box. A fan is positioned in the container to ensure gas circulation. The measurement technique is based on a static headspace system as reported earlier by Ólafsdóttir et al. (1997a). One kilogram of capelin was analyzed; the measurement time was 20 min, which was sufficient time to allow the headspace gas to equilibrate in the 5.2 L container. In fact, 10 min was in all cases sufficient to reach the maximum value of the sensor's output. Therefore, shorter measurement times can be used for routine analysis in the fish industry.

TVB Measurements. Total volatile bases (TVB as milligrams of N per 100 g of muscle) were measured according to the method of Billon et al. (1979) with a Struer automatic distillation unit.

Sensory Analysis. Two members of the IFL sensory panel performed sensory analysis using a sensory classification scheme for capelin odor (fresh, flat, sweet, stale, and putrid) developed by Ólafsdóttir et al. (1997a,b). For multivariate analysis the classes were given scores from 1 to 5 with putrid as the highest score. Panelists at IFL are selected by a procedure described by Meilgaard et al. (1991). They have extensive experience in evaluating the freshness of various species of fish used for human consumption, and the Torry scheme (Shewan, 1953) is routinely applied. The limited number of panelists was considered to be sufficient for fish species such as the capelin that is not intended for human consumption. This is also the situation in the fish industry, where sensory analysis is commonly carried out by one or two trained personnel.

GC. Direct headspace analysis of volatile sulfur compounds from capelin was performed as reported earlier (Ólafsdóttir et al., 1997a). Headspace samples (1 mL) were injected splitless with a gastight syringe onto an HP-1 (cross-linked methyl silicone gum) capillary column (30 m; 0.32 mm i.d.; 4 μm film thickness) that was maintained at 50 °C for 4 min and then programmed to increase at 10 °C/min to 170 °C. The helium carrier gas flow rate was 32 mL/min. The gas chromatograph (HP5890) was equipped with an FPD sulfur sensitive detector. The temperature of the injector was 75 °C, and that of the detector was 250 °C.

Data Analysis. Multivariate analysis of the data was done using the software Unscrambler (Camo AS, Norway). Principal component analysis (PCA) for all of the samples was performed to study the main tendencies of variation among the measurement variables and to evaluate if the various analytical techniques applied were comparable to evaluate spoilage. PCA was also done to study the main trend in the data and to illustrate the effect of the different storage conditions on the spoilage level of capelin.

The essential relationships between TVB variables (Y block) and the electronic nose variables (X block) were studied by partial least squares (PLS) regression. PLS1 is an algorithm that relates a single TVB variable to the electronic nose variables by focusing the latter onto a few factors, which are used as regressors for Y (a vector of y values)**.** The algorithm optimizes several partial submodels by minimizing the lack-of-fit residuals for each of them (Martens and Martens, 1986).

A Generalized Linear Model with normal error was also fitted to the data using S-Plus, version 4.0 (Data Analysis Products Division, MathSoft, Inc., Seattle, WA) to predict TVB values with electronic nose data.

RESULTS AND DISCUSSION

Temperature and Time. The first sampling day (day 1) was within 48 h from catch and at that time the temperature of the capelin was 4 °C. Figure 1a shows the temperature history of the samples throughout the storage period starting on day 1. Unfortunately, the temperature monitor for the 5 °C + sea sample was damaged during the experiment, and the temperature data could not be retrieved for that sample. However, it is assumed that the ice must have slowed the increase

Electronic Nose To Predict TVB

J. Agric. Food Chem., Vol. 48, No. 6, 2000 **2355**
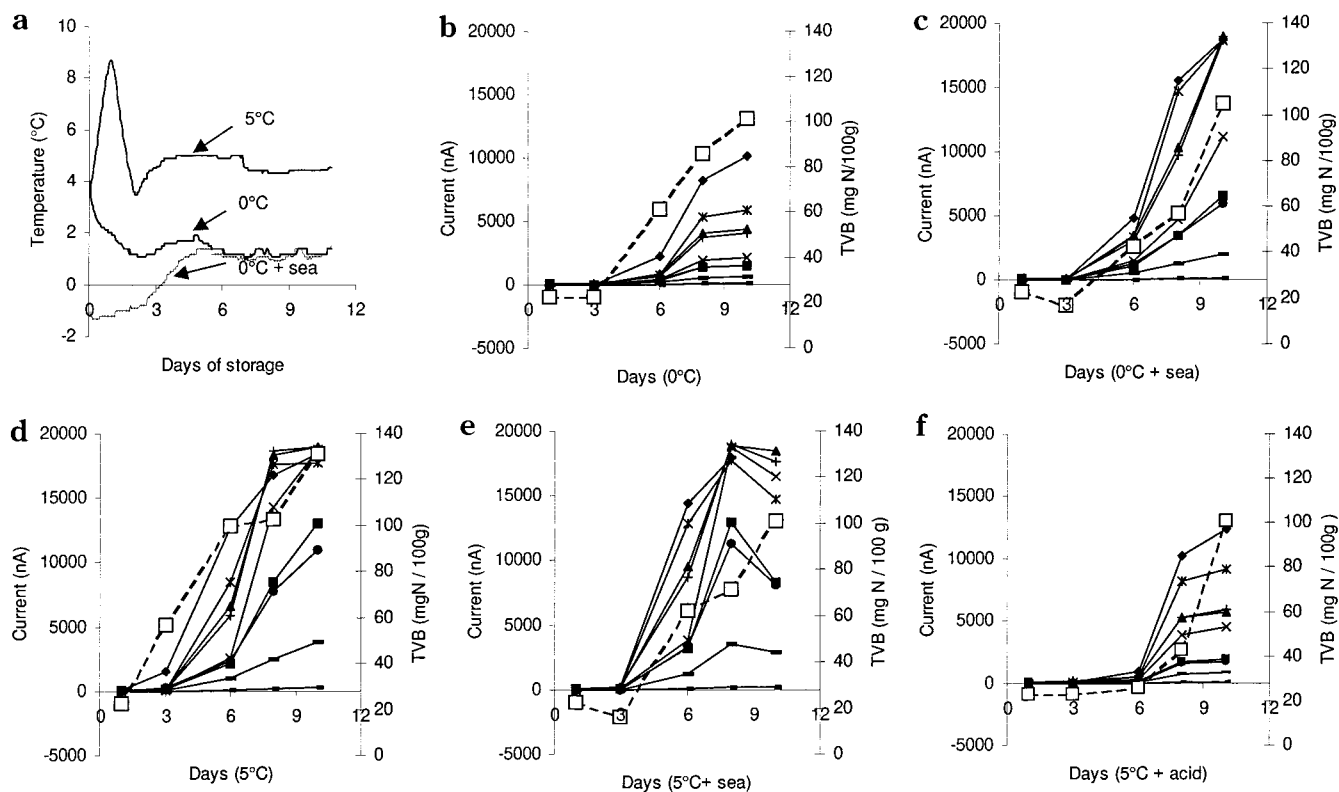
Figure 1. Temperature of samples (a) stored at 0 °C, 5 °C, and 0 °C + sea; TVB analysis and responses of electronic nose measurements of capelin samples stored under different conditions: (b) 0 °C; (c) 0 °C + sea; (d) 5 °C; (e) 5 °C + sea; (f) 5 °C + acid (TVB, □; CO, ◆; H_2Sa, ■; NO, ▲; NO_2, ×; SO_2, ∗; NH_3A7/AM, ●; H_2Sb, +; SO_2, - - -; NH_3b, —).

in temperature noticed in the other samples kept under the same conditions. On day 2 all of the ice had melted in the 5 °C + sea sample. The experimental conditions resemble CSW systems, where ice is not replenished during the storage time. On the other hand, when RSW systems are used, the effective cooling is throughout the storage period because cooling systems maintain the preset temperature.

Measurements of Capelin Headspace with Electronic Nose. Panels b, c, d, e, and f of Figure 1 show responses of the different gas sensors in the electronic nose to capelin during storage for 10 days under different storage conditions (0 °C, 0 °C + sea, 5 °C, 5 °C + sea, 5 °C + acid, respectively). The sensor responses indicate that the spoilage rates are different in these samples. The characteristic responses of individual sensors in all of the samples are all increasing with time. It is also evident from the graphs that the response increases logarithmically and then starts to level off, similar to traditional spoilage measurements.

The results show that on the same days, samples kept at 5 °C (Figure 1d) have considerably higher responses than those kept at 0 °C (Figure 1b). This is, however, not true for 5 °C + acid (Figure 1f), which has responses similar to those of the 0 °C sample because of the effect of the added acetic acid, which slows the spoilage rate as reported earlier (Ólafsdóttir et al., 1997a).

The results of the electronic nose measurements show that samples kept in ice/seawater have higher responses than those kept solely under refrigeration. This is especially clear for the 0 °C (Figure 1b) and 0 °C + sea (Figure 1c) samples, for which the difference in sensor responses is considerable, indicating increased spoilage in the seawater system. This is in agreement with the results of Shaw and Botta (1978), who reported an

increased spoilage rate for capelin stored in a seawater system. However, when the sensor responses during the first days of storage are studied more closely, it is evident that on day 3 no noticeable changes are detected in the headspace of the samples stored at 0 °C, neither with nor without added seawater. Furthermore, there is no change in response in the 5 °C + acid sample. The sensors are, on the other hand, detecting changes in the samples stored at 5 °C. A lower response of the sensors in the 5 °C + sea sample than in the 5 °C sample indicates less spoilage because of the cooling effect of the added ice in the beginning. The added ice is effective only in cooling the samples during the first days, and in the 5 °C + sea sample the ice had melted on the second day.

The results during later stages of spoilage, on the other hand, show a rapid increase in the sensor responses for the 5 °C + sea sample as compared to the 5 °C sample. On day 6 of storage the increased response of sensors in the samples with added seawater is observed at both temperatures. This is similar to earlier reports for herring indicating that seawater systems are equally as effective as ice only during the first days of storage (Smith et al., 1980). However, during later stages of storage a more rapid spoilage rate has been reported for herring and oil sardine kept in seawater cooling systems (Smith et al., 1980; Hiremath et al., 1982; Perigreen et al., 1975). In those studies the seawater system was comparable to iced storage only during the initial stages (up to 2 days) of storage for oil sardine and during 4–5 days of storage for herring. Results of sensory evaluation showed that off-flavors developed in the seawater-stored fish ~1 day earlier than in the iced fish. This was explained by the fact that seawater was not replenished at periodic intervals, and

it was noticed that the seawater began to develop off-odors at an earlier stage than the fish.

TVB Measurements. The results of the TVB analysis are also shown in Figure 1. TVB is lowest in the 5 °C + acid samples (Figure 1f) but highest in the samples stored at 5 °C, indicating that those samples have the highest level of spoilage. The TVB value in the samples stored in seawater is lower than for the samples stored under refrigeration at the same temperatures. This is contradictory to the results of the electronic nose, where the samples stored in seawater (0 °C + sea and 5 °C + sea) appeared to be more spoiled than the 0 and 5 °C samples, respectively. The low TVB values in seawater samples may be because volatile nitrogen compounds can possibly leak from the fish when stored in seawater or ice (Magnússon and Martinsdóttir, 1995), resulting in lower TVB values of the fish. This is in fact observed on day 3 for the 0 °C + sea and 5 °C + sea samples when the TVB values are lower than on day 1. Another explanation may be that less TVB is being produced in the seawater system. The contradictory results of the TVB analysis and electronic nose measurement could also simply be because of the different sample preparation. In the TVB method the whole fish is homogenized, whereas in the electronic nose measurement the fish is measured whole. Therefore, the high levels of spoilage compounds detected by the electronic nose are most likely originating from the seawater on the surface of the fish. As mentioned before, the seawater contains soluble nutrients that are easily metabolized by spoilage bacteria. On day 3 the 0 and 5 °C + acid samples have the same TVB values as initially, but the TVB value for the 5 °C sample has increased considerably, and this sample appears to have the most rapid spoilage during the first 3 days. The transfer of all the 5 °C samples to 10 °C on the first day has without doubt increased the spoilage rate of those samples as compared to their being kept at 5 °C throughout the storage time.

GC and Sensory Analysis. Table 1 shows the results of the measurements of the volatile sulfur compounds in capelin. No sulfur compounds were detected in the samples until the first putrid spoilage odors had appeared on day 6 in the 5 °C and 5 °C + sea samples. Methyl mercaptan is detected at highest levels in the 5 °C, 5 °C + sea, and 0 °C + sea sample, and hydrogen sulfide is in higher levels in the samples stored at 5 °C than in those stored at 0 °C. The volatile sulfides contribute to the spoilage odors because their odors are very potent and the odor threshold low. Hydrogen sulfide has a sulfury and boiled eggs character and an odor threshold of 5–40 ppb (Fazzalari, 1978). The odor of methyl mercaptan is described as rotten and cabbage-like, and the flavor threshold is very low (0.05 ppb) (Fazzalari, 1978). Dimethyl disulfide has a putrid, onion-like odor and an odor threshold of 12 ppb (Buttery et al., 1976). External standards dimethyl disulfide and dimethyl sulfide were used to give semiquantitative information on the level of the sulfur compounds in the samples as described in Ólafsdóttir et al. (1997a). The levels of sulfur compounds when detected in spoiled samples (0.5–15 ppm) were much higher than their flavor thresholds.

Table 1 shows that for all samples the progression of odor is from fresh to flat, progressing to sweet and/or stale odors, and ending with putrid odor. When fish turns putrid, the odor profile is very complex, and it was difficult to describe and distinguish among the various

Table 1. Sensory Classification and Relative Concentration of Sulfur Compounds Analyzed by GC-FPD in Capelin Stored for 10 Days at 0 °C, 0 °C plus Seawater, 5 °C, 5 °C plus Seawater, and 5 °C plus Acid

sample (°C/days)	relative intensities (peak areas) of S compounds				sensory classification
	H_2S	CH_3SH	DMS	DMDS	
0/1	0	0	0	0	fresh
0/3	0	0	0	0	flat
0/6	0	1×10^5	0	0	sweet
0/8	0	11.2×10^5	0	0.1×10^5	putrid
0/10	1.2×10^5	80.5×10^5	0.1×10^5	0.4×10^5	putrid
0+sea/1	0	0	0	0	fresh
0+sea/3	0	0	0	0	flat
0+sea/6	0	110×10^5	0	0.1×10^5	stale
0+sea/8	2.2×10^5	425×10^5	0.2×10^5	0.2×10^5	putrid
0+sea/10	5.7×10^5	769×10^5	0.1×10^5	0.8×10^5	putrid
5/1	0	0	0	0	fresh
5/3	0	0	0	0	flat
5/6	12.6×10^5	182×10^5	0.1×10^5	0.3×10^5	putrid
5/8	14.4×10^5	548×10^5	0.1×10^5	1.0×10^5	putrid
5/10	648×10^5	1160×10^5	0.3×10^5	0.7×10^5	putrid
5+sea/1	0	0	0	0	fresh
5+sea/3	0	0	0	0	sweet
5+sea/6	3.5×10^5	561×10^5	0.1×10^5	0.3×10^5	putrid
5+sea/8	139×10^5	996×10^5	0.2×10^5	1.2×10^5	putrid
5+sea/10	412×10^5	208×10^5	0.3×10^5	2.2×10^5	putrid
5+acid/1	0	0	0	0	fresh
5+acid/3	0	0	0	0	flat
5+acid/6	0	0	0	0	sweet
5+acid/8	0.1×10^5	1.1×10^5	0	0	stale
5+acid/10	43.0×10^5	65.6×10^5	0.1×10^5	0.1×10^5	putrid

odor notes. The sensory classification scheme was developed to classify capelin used for the production of high-quality fishmeal (TVB < 50 mg of N/100 g). Capelin will typically have TVB values around 80–100 mg of N/100 g to be classified as putrid. However, when standard fishmeal is produced, the raw material can exceed TVB 100 mg of N/100 g and in some cases sorting the raw material according to TVB values >100 mg of N/100 g is required. The TVB value of the capelin when it is classified as putrid is close to 100 mg of N/100 g for the samples that were not kept in seawater. On the other hand, samples that were kept in seawater at 0 and 5 °C had much lower TVB values (50–60 mg of N/100 g) when they were classified as putrid by sensory analysis. Therefore, the sensory scheme is not useful to grade such raw material. It was observed that the appearance of the samples stored in seawater was slightly different. The gills of the capelin kept in seawater lost their red color very early and were pale, because of the leaching out of slime and blood into the seawater. The 5 °C and 5 °C + sea samples had the most rapid spoilage; already on day 3 spoilage odor had developed, and both samples were classified as putrid on day 6. The 5 °C + acid sample had the slowest spoilage rate according to sensory analysis and was classified as putrid on day 10.

Data Analysis. The data from the various measurements used to monitor spoilage in capelin stored under different conditions was examined by principal component analysis (PCA). PCA describes the trend in the data and cannot be used to determine if there is a significant difference between samples. Figure 2 shows a PCA scores plot of all the samples and data from the electronic nose, sensory analysis, GC, and TVB. The x-axis is the first principal component, explaining 84% of the variance in the data. It can be seen that the first PC represents the spoilage of the samples. For each

Electronic Nose To Predict TVB

J. Agric. Food Chem., Vol. 48, No. 6, 2000 **2357**
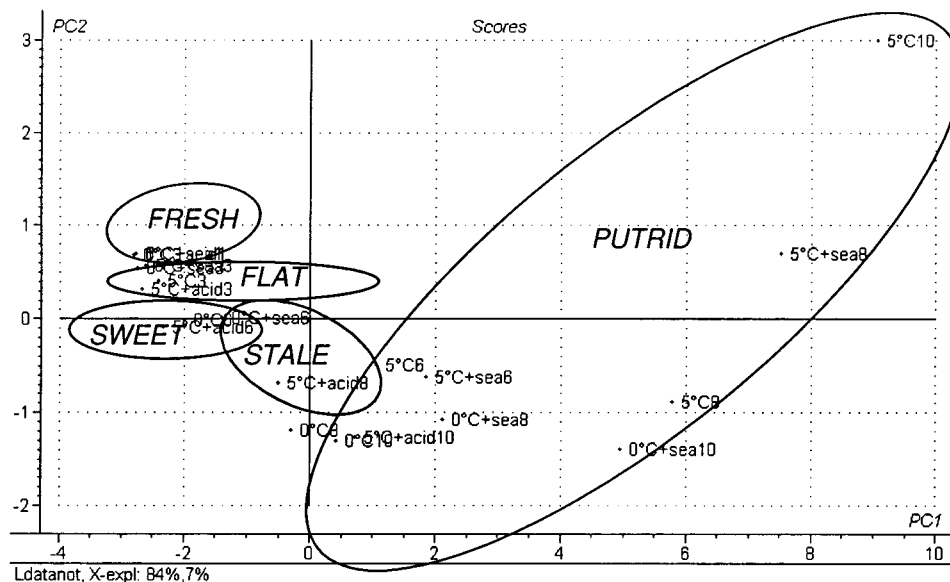
Figure 2. Scores plot for PCA of all the measurement variables [TVB, electronic nose (FreshSense), gas chromatography, and sensory analysis] and sample variables (0 °C, 0 °C + sea, 5 °C, 5 °C + sea, and 5 °C + acid).

group of samples the storage time increases from left to right. The days in the groups overlap, indicating that the samples kept in acid tend to spoil later than the other samples, the 5 °C samples spoil first, and the samples kept in seawater spoil sooner than the others. Samples of comparable freshness or quality are therefore located close to each other on the PCA plot and have been grouped together according to the sensory classification (fresh, flat, sweet, stale, and putrid). As mentioned before, it is clear that the sensory scale is not nearly wide enough and too many samples fall in the putrid category. Alternative schemes with more classes and detailed descriptions of spoilage odors may be more useful to classify raw material used for fishmeal production.

The loadings of all the variables were very similar in PC1, and because of its large explained variance it can be said that all of the different measurement methods are similar to monitor spoilage. After using PCA, error measures, and outlier detection, the 10th day measurement for the sample kept at 5 °C + sea was labeled an outlier and its results have been kept out of all calculations.

PLS regression and GLM were used to study the different types of spoilage measurements and to find a model to predict TVB from the electronic nose measurements. Also, the possibility of using fewer sensors in the electronic nose for this particular application was studied. The results of PLS regression are shown in Table 2. All of the sensors and two sets containing three of the highest responding sensors have been selected to predict TVB. The first set of sensors is CO Dräger, NO Dräger, and NH_3 A7AM, and the second set is CO Dräger, SO_2 Dräger, and NH_3 A7AM. The analysis was done using 15 samples (data from days 1, 3, and 6) and 24 samples (data from all days).

The root-mean-square error of prediction (RMSEP) indicates the accuracy of the model for prediction, and it is ~20 mg of N/100 g of fish for all samples (Table 2). The result when all sensors are used simultaneously is slightly better than in the case when only three sensors are used, but the results are fairly similar because the three sensors represent the whole array.

In Table 2 PLS regression parameters using only the

Table 2. PLS Results Using Two PCs, Giving the X Variables, Their Explained Variance, the Slope and Correlation of the Predicted vs Measured Plot of the Model, and the RMSEP, in Milligrams of N/100 g of Fish, for the Y Variable TVB

	X	expl X (%)	expl Y (%)	slope	corre-lation	RMSEP
all days	all sensors	96	75	0.9487	0.8354	19.3
	CO, NO, NH_3	98	69	0.8775	0.7512	23.4
	CO, SO_2, NH_3	99	67	0.8965	0.7518	23.3
days 1, 3, and 6	all sensors	100	82	0.8396	0.8836	11.5
	CO, NO, NH_3	99	93	1.0623	0.9459	7.6
	CO, SO_2, NH_3	99	94	1.2115	0.9426	8.9

samples from days 1, 3, and 6 are shown, as the TVB results did not agree well with electronic nose measurements in later stages of spoilage. The RMSEP is considerably lower in this case, and results using only three sensors are better than those using all sensors. This indicates that an instrument with only a few sensors could be used for this particular application. Furthermore, PLS was performed using only results from days 8 and 10, but a valid model could not be obtained.

In addition to PLS regression, a GLM with normal error was fitted to the data. Three gas sensor variables, CO, SO_2, and NH_3, that had the highest factor loadings in PLS analysis were selected as covariates. The variables CO, SO_2, and NH_3 are all pairwise highly correlated. A saturated model therefore includes only one of the three variables. Best fit was found using the NH_3 sensor. To predict TVB, the following explanatory variables were chosen: NH_3 and condition, as a factor with three levels of storage (condition 1 = 0 and 5 °C; condition 2 = 0 °C + sea and 5 °C + sea; and condition 3 = 5 °C + acid). Storage temperature as a factor of two levels, 0 and 5 °C, did not give a better fit to the data and is therefore excluded from the model.

After error distribution, link function, and different transformations of covariates were altered, the following model was chosen as an estimate of TVB given the storage condition and the response of the NH_3 sensor:

2358 *J. Agric. Food Chem.,* Vol. 48, No. 6, 2000

Ólafsdóttir et al.

$$TVB_i = \alpha_i + \beta_1 NH_3 + \beta_2 (NH_3)^2 + \epsilon_i$$

α_i represents the intercept for condition level i, with i = storage conditions (0 and 5 °C; 0 °C + sea and 5 °C + sea; 5 °C + acid), and ϵ_i the error term. α_i, β_1, and β_2 are parameters to be estimated. The results of the prediction of TVB are in Table 3. The reference level of the factor condition is chosen to be condition 1, which is storage in 0 and 5 °C. Thus, when TVB is predicted for 0 and 5 °C, the model is

$$\text{estimated TVB}_{0°C/5°C} = 43.15 + 0.02 NH_3 - [2 \times 10^{-6}(NH_3)^2]$$

The predicted values of TVB for the other storage conditions are 29.56 lower for 0 °C + sea/5 °C + sea and 13.68 lower for 5 °C + acid. Therefore, taking also into account storage in ice/seawater systems (0 °C + sea/5 °C + sea) and the use of acid (5 °C + acid) the models are

$$\text{estimated TVB}_{0°C+sea/5°C+sea} = 43.15 - 29.56 + 0.023157 NH_3 - [2 \times 10^{-6}(NH_3)^2]$$

$$\text{estimated TVB}_{5°C+acid} = 43.15 - 13.68 + 0.023157 NH_3 - [2 \times 10^{-6}(NH_3)^2]$$

Results from analysis of deviance are shown in Table 4. The terms are added sequentially one at a time. Deviance residuals divided with the estimated scale parameter (307.47) are approximately F-distributed $F(2,24)$ for condition and $F(1,24)$ for the other terms. All terms are highly significant, with $p < 0.001$, with NH_3 as the most important term and as the best single variable to use in a linear model. Explained variance, r^2, is 0.80.

If storage time is known, an additional continuous term, time, may be included in the model and β_3 becomes an additional parameter to be estimated.

$$TVB_i = \alpha_i + \beta_1 NH_3 + \beta_2 (NH_3)^2 + \beta_3 (\text{time}) + \epsilon_i$$

The results of the prediction of TVB and analysis of deviance, including time as a continuous explanatory variable, are shown in Tables 5 and 6. In this case r^2 = 0.88 and the most important term is time.

CONCLUSIONS

According to the results of sensory analysis, GC, and electronic nose measurements, the ice/seawater systems were as effective as refrigeration to maintain the freshness of capelin during the first days of storage. After prolonged storage (>6 days), an increased spoilage rate was observed in seawater/ice systems. The added ice was effective in cooling the samples only during the first 2−3 days of storage. The question remains if TVB is the most appropriate method to evaluate spoilage of fish stored in seawater/ice systems. TVB analysis indicated less spoilage in seawater/ice systems than in refrigeration, which is contradictory to the other measurements. GC and electronic nose measurements give more information than sensory analysis about the quantity and the complex combination of the volatile degradation compounds during later stages of spoilage. The electronic nose and TVB measurements give more

Table 3. Estimated Values, Standard Error, and Partial *t* Values for the Coefficients in a GLM Predicting TVB

coefficient	value ($\alpha_i, \beta_{1,2}$)	SE	*t* value
condition$_{0and5°C}$	43.15	6.43	6.71
condition$_{0°C+sea/5°C+sea}$	−29.56	7.95	−3.72
condition$_{5°C+acid}$	−13.68	9.79	−1.40
NH_3	2.3157×10^{-2}	3.671×10^{-3}	6.31
$(NH_3)^2$	2×10^{-6}	3×10^{-7}	−4.41

Table 4. Analysis of Deviance for the Terms in the Prediction Model for TVB[a]

term	degrees of freedom	residual deviance	*F* value
null	24	31397.53	
condition	22	28284.46	10.12
NH_3	21	12137.74	52.51
NH_3^2	20	6149.36	19.47

[a] *F* values equal to deviance residuals divided by the estimated scale parameter are all significant.

Table 5. Estimated Values, Standard Error, and Partial *t* Values for the Coefficients in a GLM Predicting TVB, Including Time as a Continuous Explanatory Variable

coefficient	value ($\alpha_i, \beta_{1,2,3}$)	SE	*t* value
condition$_{0and5°C}$	26.12	7.13	3.66
condition$_{0°C+sea/5°C+sea}$	−23.92	6.59	−3.63
condition$_{5°C+acid}$	−20.68	8.12	−2.55
NH_3	1.0×10^{-2}	4.7×10^{-3}	2.21
$(NH_3)^2$	-6.5×10^{-7}	3.8×10^{-7}	−1.73
time (days)	5.61	1.62	3.47

Table 6. Analysis of Deviance for the Terms in the Prediction Model for TVB Including Time as a Continuous Explanatory Variable[a]

term	degrees of freedom	residual deviance	*F* value
null	24	31397.53	
condition	22	28284.46	15.70
NH_3	21	12137.74	81.43
NH_3^2	20	6149.36	30.20
time	19	3767.71	12.01

[a] *F* values equal to deviance residuals divided by the estimated scale parameter are all significant.

possibilities than the sensory scheme used to grade the raw material after the capelin has turned putrid. The electronic nose monitors several classes of compounds and can possibly give more information than the traditional TVB analysis. In addition to the detection of amines by the NH_3 sensor, the CO and SO_2 sensors can detect the presence of volatile alcohols and sulfur compounds, respectively.

Better understanding of the spoilage processes in ice/seawater systems is needed to establish useful quality criteria. Further research should include investigations using GC techniques to identify and quantify the various volatile compounds contributing to spoilage in capelin stored under different conditions. Furthermore, the possibility of using rapid electronic nose measurements to predict the presence of the various quality-indicating compounds is of interest for the fish industry.

Results from multivariate analysis indicate that the electronic nose can be used to predict capelin quality as TVB and models using only three gas sensors gave similar or better results than prediction with nine sensors. TVB is very well predicted by a saturated GLM (r^2 = 0.80) with the values from only one gas sensor [NH_3 and $(NH_3)^2$] and storage conditions (0 °C/5 °C; 0 °C + sea/5 °C + sea; 5 °C + acid) as linear predictors. Storage time can also be included in the model, and a

Electronic Nose To Predict TVB

J. Agric. Food Chem., Vol. 48, No. 6, 2000 **2359**

better prediction of TVB is obtained ($r^2 = 0.88$). It is reasonable and can be expected that the NH$_3$ sensor gives the best prediction of TVB because it is selective and sensitive to volatile amines.

The electronic nose has potential as a rapid analysis tool to predict TVB of capelin raw material during fishmeal production. No sample preparation is required, and it is a rapid, easy-to-use technique. Data acquisition and analysis can be connected directly to information systems in fishmeal factories to be used in process control. The next step is to evaluate the performance of the electronic nose to measure the quality of the raw material during processing in fishmeal factories.

ABBREVIATIONS USED

CSW, chilled seawater; FPD, flame photometric detector; GC, gas chromatography; GLM, generalized linear model; IFL, Icelandic Fisheries Laboratories; PCA, principal component analysis; PLS, partial least squares regression; r^2, explained variance; RMSEP, root-mean-square error of prediction; RSW, refrigerated seawater; TVB, total volatile bases; SE, standard error.

LITERATURE CITED

Ahmed, A.; Matches, J. R. Alcohol production by fish spoilage bacteria. *J. Food Prot.* **1983**, *46*, 1055−1059.

Aksnes, A.; Brekken, B. Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. *J. Sci. Food Agric.* **1988**, *45*, 53−60.

Bartlett, P. N.; Elliot, J. E.; Gardner, J. W. Electronic Noses and Their Application in the Food Industry. *Food Technol.* **1997**, *51* (12), 44−48.

Billon, J.; Ollieuz, J. N.; Tao, S. H. Etude d'une novelle méthode de dosage de l'azoto basique volatil total pour lévaluation qualitative des produits de la peche. *RTVA, Rev. Tech. Vet. Aliment.* **1979**, *1149*, 13−17.

Buttery, R. G.; Guadagni, D. G.; Ling, L. C.; Seifert, R. M.; Lipton, W. Additional volatile components of cabbage, broccoli and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829−832.

Craven, M. A.; Gardner, J. W.; Bartlett, P. N. Electronic noses—development and future prospects. *Trends Anal. Chem.* **1996**, *15* (9), 486−493.

Dagbjartsson, B.; Valdimarsson, G.; Arason, S. Icelandic experience in storing fish in chilled seawater. In *Advances in the Refrigerated Treatment of Fish*; Proceedings of the International Institute of Refrigeration Conference, Boston, MA; IIR: Paris, France, 1982; pp 233−242.

DiNatale, C.; Brunink, J. A. J.; Bungaro, F.; Davide, F.; d'Amico, A.; Paolesse, R.; Boschi, T.; Faccio, M.; Ferri, G. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas. Sci. Technol.* **1996**, *7*, 1103−1114.

Fazzalari, F. A. *Compilation of Odor and Taste Threshold Value Data*; American Society for Testing and Materials: Philadelphia, PA, 1978.

Herbert, R. A.; Shewan, J. M. Precursors of the Volatile Sulphides in Spoiling North Sea Cod (*Gadus morhua*). *J Sci. Food Agric.* **1975**, *26*, 1195−1202.

Herbert, R. A.; Ellis, J. R.; Shewan, J. M. Isolation and identification of the volatile sulphides produced during chill-storage of North Sea Cod (*Gadus morhua*). *J. Sci. Food Agric.* **1975**, *26*, 1187−1195.

Hiremath, G. G.; Teutscher, F.; Nordheim, A. Experiments on Storage of Fish in Chilled Sea Water. *Myosore J. Agric. Sci.* **1982**, *16*, 329−337.

Josephson, D. B.; Lindsay, R. C.; Ólafsdóttir, G. Measurement of Volatile Aroma Constituents as a Means for Following Sensory Deterioration of Fresh Fish and Fishery Products.

In *Proceedings of an International Symposium*; University of Alaska Sea Grant Program, Anchorage, AK; Kramer, D. E., Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp 27−47.

Kamiya, A.; Ose, Y. Study of the odourous compounds produced by putrefaction of foods. V. Fatty acids, sulphur compounds and amines. *J. Chromatogr.* **1984**, *292*, 383−391.

Kolbe, E.; Crapo, S.; Hilderbrand, K. Ice Requirements for Chilled Seawater Systems. *Mar. Fish. Rev.* **1985**, *47* (4), 33−41.

Lindsay, R. C.; Josephson, D. B.; Ólafsdóttir, G. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In *Proceedings of an International Symposium*; University of Alaska Sea Grant Program, Anchorage, AK; Kramer, D. E., Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp 221−234.

Magnússon, H.; Martinsdóttir, E. Storage Quality of Fresh and Frozen-Thawed Fish in Ice. *J. Food Sci.* **1995**, *60*, 273−278.

Martens, M.; Martens, H. Partial Least Squares Regression. In *Statistical Procedures in Food Research*; Piggott, J. R., Ed.; Elsevier Applied Science Publishers: Amsterdam, The Netherlands, 1986; pp 293−359.

Meilgaard, G.; Civille, V.; Carr, B. T. *Sensory Evaluation Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 1991; pp 142−147.

Ólafsdóttir, G.; Fleurence, J. Evaluation of fish freshness using volatile compounds—classification of volatile compounds in fish. In *Methods to Determine the Freshness of Fish in Research and Industry*; International Institute of Refrigeration: Paris, France, 1998; pp 55−69.

Ólafsdóttir, G.; Martinsdóttir, E.; Jónsson, E. H. Rapid gas sensor measurements to predict the freshness of capelin (*Mallotus villosus*). *J. Agric. Food Chem.* **1997a**, *45*, 2654−2659.

Ólafsdóttir, G.; Martinsdóttir, E.; Jónsson, E. H. Gas sensor and GC measurements of volatile compounds in capelin (*Mallotus villosus*). In *Seafood from Producer to Consumer, Integrated Approach to Quality*; Luten, J. B., Börresen, T., Oehlenschläger, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1997b; pp 507−520.

Ólafsdóttir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I. M.; Henehan, G.; Nielsen, J.; Nilsen, H. Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **1997c**, 258−265.

Ólafsdóttir, G.; Verrez-Bagnis, V.; Luten, J. B.; Dalgaard, P.; Careche, M.; Martinsdóttir, E.; Heia, K. The need for methods to evaluate fish freshness. In *Methods to Determine the Freshness of Fish in Research and Industry*; International Institute of Refrigeration: Paris, France, 1998; pp 17−29.

Perigreen, P. A.; Pillai, S. A.; Surendran, P. K.; Govindan, T. K. Studies on preservation of fish in refrigerated sea water. *Fish Technol.* **1975**, *12*, 105−111.

Shaw D. H.; Botta, J. R. Preservation of Inshore Male Capelin (*Mallotus villosus*) Stored in Refrigerated Sea Water. *J. Fish. Res. Board Can.* **1975**, *32*, 2047−2053.

Shewan, J. M.; Mackintosh, R. G.; Tucker, C. G.; Ehrenberg, A. S. C. The Development of a Numerical Scoring System for the Sensory Assessment of the Spoilage of Wet White Fish Stored in Ice. *J. Sci. Agric.* **1953**, *6*, 183−189.

Smith, J. S. M.; Hardy, R.; MacDonald, I.; Templeton, J. The storage of herring (*Clupea harengus*) in ice, refrigerated sea water and at ambient temperature. Chemical and sensory assessment. *J. Sci. Food Agric.* **1980**, *31*, 375−385.