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Characterization of Volatile Compounds in Chilled Cod (*Gadus morhua*) Fillets by Gas Chromatography and Detection of Quality Indicators by an Electronic Nose

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Volatile compounds in cod fillets packed in Styrofoam boxes were analyzed during chilled storage (0.5 °C) by gas chromatography (GC)−mass spectrometry and GC−olfactometry to screen potential quality indicators present in concentrations high enough for detection by an electronic nose. *Photobacterium phosphoreum* dominated the spoilage bacteria on day 12 when the fillets were rejected by sensory analysis. Ketones, mainly 3-hydroxy-2-butanone, were detected in the highest level (33%) at sensory rejection, followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%), and a low level of esters (<1%). The electronic nose's CO sensor showed an increasing response with storage time coinciding with the production of ethanol and 2-methyl-1-propanol that were produced early in the storage, followed by the production of 3-methyl-1-butanol, 3-methyl-butanal, 2,3-butandiol, and ethyl acetate. Lipid-derived aldehydes, like hexanal and decanal, were detected in similar levels throughout the storage time and contributed to the overall sweet odors of cod fillets in combination with other carbonyls (3-hydroxy-2-butanone, acetaldehyde, 2-butanone, 3-pentanone, and 6-methyl-5-heptene-2-one).

KEYWORDS: Volatile compounds; quality indicators; SSO; gas chromatography; electronic nose; cod fillets

INTRODUCTION

The use of electronic nose based on different sensor technologies has been suggested for the rapid detection of quality-related volatile compounds for various food products (*1*, *2*) including monitoring the quality and spoilage processes in fish (*3*−*6*). Gas sensors that are commonly used in electronic noses are nonselective toward individual compounds but show sensitivity toward certain classes of compounds. This property induces their potential for monitoring quality and the onset of spoilage associated with varying levels of different classes of volatile compounds produced in fish during storage (*7*). The progression of characteristic odors in fish during chilled storage caused by microbial growth is well-documented (*8*) and has been associated with the formation of volatile compounds produced by the main spoilage organisms (*9*−*11*). Both single compounds and a combination of compounds representing the different changes occurring during storage have been suggested as indicators for freshness and spoilage (*12*−*14*).

Alcohols, aldehydes, ketones, amines, and sulfur compounds have been identified in different seafood products during chilled storage and related to the growth of specific spoilage organisms (SSOs) (*15*, *16*). However, because of the interaction of the microorganisms and the complexity of the dynamic spoilage changes, the levels of the volatile compounds may vary and they are often not detected until the products are overtly spoiled. Storage temperature, packaging, and the inherent composition of available nutrients in the fish influence the growth and spoilage potential of the dominating SSO. The SSOs in chilled fish are mainly Gram-negative, psychrotrophic bacteria like *Pseudomonas* spp. and *Shewanella* spp. (*17*). Pseudomonads typically cause sweet, malty, fruity, and onionlike odors contributed by alcohols, carbonyls, esters, and sulfur compounds (*9*, *10*), while *Shewanella putrefaciens* can produce more potent odors related to high levels of sulfur compounds (*11*) and fishy odors because of the reduction of TMAO to trimethylamine (TMA) (*17*). *Photobacterium phosphoreum* is also of interest as SSOs in chilled fish and has been identified as an active TMA producer in iced cod and in cod fillets (*18*). Oxidative processes occurring during the storage of fish will also result in the accumulation of both saturated and unsaturated aldehydes that contribute to the development of rancid cold store flavors (*19*, *20*).

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The information on the identity and quantity of volatile compounds present in the headspace during storage of fish is essential when selecting sensors in an array for quality monitoring of fish (*7*). It should be clearly stated that in many cases, the most abundant volatiles may have minimal odor significance. However, they may be indicative for the degradation processes occurring in the products such as the production of metabolites by the SSOs. Therefore, the potential ability of the electronic nose to monitor quality is not necessarily directly related to detecting the most influential aroma active compounds contributing to off odors, since these may be present in too low concentrations.

The aim of the study was to screen the most abundant volatile compounds produced by SSOs that could be used as quality indicators for chilled fillets and to evaluate the spoilage odor to characterize the spoilage potential of the SSO. This study was done in parallel to extensive storage studies on cod fillets (*21*) where the SSOs were monitored and the sensory shelf life was determined. Herein are the results from gas chromatography analysis of cod fillets stored in Styrofoam boxes under chilled conditions (0.5 °C), and a comparison was made with electronic nose analysis, total volatile basic nitrogen (TVB-N), microbial analysis, and pH measurements. The results obtained will be useful to guide the future development of the electronic nose technique based on selecting sensors that are sensitive to the indicator compounds identified in chilled cod fillets.

MATERIALS AND METHODS

The fish was processed by a conventional process 3 days after catch as described earlier (*21*). The process included mechanical filleting, skinning, and packing of fillets in Styrofoam (EPS, expanded polystyrene) boxes (160 mm × 400 mm × 263 mm) lined with a plastic bag. The fillets were stored at 0.5 °C until analyzed. Gas chromatography analysis [gas chromatography−mass spectrometry (GC-MS) and gas chromatography−olfactometry (GC-O)], electronic nose, TVB-N, and pH measurements were performed on days 4, 7, 10, 12, and 14 after catch.

GC-MS Measurements. The headspace from approximately 500 g of fish (1−2 fillets) in a glass container (2.3 L, Ø 17 cm) was collected by an air pump sampling (ALPIN-2, Air sampler, METEK) by sweeping volatiles from the surface of the fish. Aqueous heptanoic acid ethyl ester solution (10 μL/L) was used as an external standard, using an amount of 1 mL in a 25 mL beaker (Ø 3.5 cm) located in the glass sampling container. Quantification of volatiles PAR (peak area ratio) was based on comparison of peak area to the peak area of the external standard. The sampling time was 2 h at room temperature (RT) (20−22 °C) using a flow rate of approximately 100 mL/min. Duplicate headspace samples were collected on 250 mg of Tenax 60/80 (Alltech, IL) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, United Kingdom). Volatiles were thermally desorbed [automated thermal desorber (ATD) 400, Perkin-Elmer, Buchinghamshire, United Kingdom] from the sampling tubes prior to separation on a DB-5ms column (30 m × 0.25 mm i.d. × 0.25 μm, J&W Scientific, Folsom, CA) using a GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA). Helium was used as a carrier gas, and the temperature program used was as follows: 50 °C for 7 min, 50−120 °C at 5 °C/min and from 120 to 220 °C at 10 °C/min. The injector temperature was 250 °C, and the detector temperature was 280 °C. The mass detector ion range was 35−300 *m*/*z*.

GC-O Measurements. Samples were prepared by weighing 100 ± 2 g of fish fillets and 100 ± 5 g of saturated aqueous solution of NaCl into a 250 mL round-bottom flask and blended manually. Saturated NaCl solution (200 ± 5 g) was prepared as a reference sample. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 1 mL of 10 μL/L aqueous solution of the standard to the 200 g of fish and $NaCl_{sat}$ solution to evaluate the extraction based on the flame ionization detector (FID) response. The sample was purged at RT with nitrogen at about 100 mL/min for 2.5 h. Volatiles were collected on 150 mg of Tenax in a Pasteur pipet. Each sample was prepared in duplicate. Volatiles were extracted from the Tenax traps with 1 mL of diethyl ether. The sample was concentrated by passing nitrogen over the solution leaving a small amount of sample (20−30 μL), and 1 μL was then injected splitless onto the column. Measurements were performed on a GC (HP 5890, Hewlett-Packard) with the same type of column and the same conditions as for the GC-MS measurements. The end of the column was split 1:1 between FID and an ODO-1 olfactory detector outlet (SGE International Pty. Ltd., Australia). Nitrogen bubbled through water to add moisture was used to drive the sample to the sniffer port. Two trained panelists with former experience in describing seafood odors sniffed the effluent. The description of each odor and its duration in time were recorded, and the intensity was evaluated using a category scale (*22*) with a description of intensity of odor at each score: 0, none; 0.5, thresholds or just detectable; 1, slight; 2, little; 3, moderate; 4, strong; and 5, very strong. GC-O analyses were done on days 4, 10, and 17.

Identification of volatiles was done by matching retention indices (RI) of ethyl esters, calculated according to Van den Dool and Kratz (*23*) (i.e., RI of ethyl pentanoate is 500) and mass spectra of samples with authentic standards [C_3−C_{10} ethyl esters, TMA, hexanal, and butanone, from Sigma-Aldrich Chemical Co. (St. Louis, MO); acetaldehyde, 3-methyl-butanal, 1-penten-3-ol, and dimethyl disulfide from Merck-Schuchardt (Hohenbrunn, Germany)]. Tentative identifications were based on the MS library data in the HP GCD ChemStation software (Hewlett-Packard Co, 1997) and manually checked against literature sources and the database Flavornet (*24*).

Electronic Nose Measurements. The electronic nose instrument (FreshSense, Bodvaki-Maritech, Kópavogur, Iceland) is based on four electrochemical gas sensors: CO, H_2S, SO_2 (Dräger, Luebeck, Germany), and NH_3 (City Technology, Portsmouth, Britain). The measurements were performed at RT as described earlier (*5*). Approximately 500 g of fish fillets was weighed and tempered for 30 min in the sampling container (2.3 L, Ø 17 cm). The measurement time was 5 min, and the temperature of the fillets reached 8−12 °C before the measurements started. All measurements were done in duplicate.

TVB-N and pH Measurements. The TVB-N content was measured by the steam distillation method described by Malle and Poumeyrol (*25*). The pH was measured in 5 g of mince moistened with 5 mL of deionized water. The pH meter was calibrated using buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25 °C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Microbial Analysis. Fillets were aseptically minced, assessing two pooled fillets for each sample. Minced flesh (25 g) was mixed with 225 mL of cooled maximum recovery diluent (MRD, Oxoid) in a stomacher for 1 min. Successive 10-fold dilutions were done as required. Total viable psychrotrophic counts (TVC, 15 °C, 4−5 days) were evaluated by spread-plating aliquots onto modified Long & Hammer's medium; counts of H_2S-producing bacteria and presumptive pseudomonads were evaluated on spread-plated iron agar (15 °C, 4−5 days) and modified CFC medium (22 °C, 3 days), respectively (26). Counts of *P. phosphoreum* were estimated by using the PPDM−Malthus conductance method (*27*), as described by Lauzon (*28*).

Data Analysis. Statistical analysis was carried out with the Number Cruncher Statistical Software (NCSS) 2000, using analysis of variance. In case of statistical significance, the Duncan's multiple range was performed. An effect was considered significant at the 5% level.

RESULTS AND DISCUSSION

Characteristic Spoilage Volatiles and Dominating SSO in Cod Fillets. TMA, 2-methyl-1-propanol (isobutanol), and 3-hydroxy-butanone (acetoin) were detected in the highest amount in cod fillets, and their levels were increased with storage time (**Table 1**). However, dynamic changes in their levels were noticed, which could be related to the growth of the spoilage bacteria. An initial decline of the pH (**Figure 1**) could be explained by the postmortem changes in chilled fish, which are initially dominated by autolytic activity. Proliferation of the microflora follows these changes (**Figure 2**) and

Table 1. Volatile Compounds Associated with Spoilage in Cod Fillets during Storage in Styrofoam Boxes at 0.5 °C for 17 Days[a]

compounds	RI[b]	PAR[c] day 4	day 7	day 10	day 12	day 14	day 17	odor description[d]	odor score[e]	ID means[f]
acetaldehyde	<165	2.8 ± 0.8	1.4 ± 0.4	0.9 ± 0.6	0.8 ± 0.5	5.8 ± 4.4	7.5 ± 6.4			MS, 1
ethanol	<165	17.4 ± 20.7		13.1 ± 3.8						MS, 1
TMA	<165				91.6 ± 28.5	922.2 ± 1064.2	1721.4 ± 47.6	TMA-like, dried fish	3.0	MS, 1, 2
2-methyl-1-propanol/ pentane[g]	<165	14.0 ± 1.4	85.4 ± 36.5	64.4 ± 13.2	35.0 ± 2.8	934.6 ± 1096.8	451.9			MS
dimethyl sulfide	165	4.5								MS
acetic acid	194			6.3 ± 4.2	14.2 ± 13.0	11.4 ± 4.3	15.5			MS, 3
2-butanone	194	14.3 ± 10.2	8.1 ± 1.1	9.0	-	24.0 ± 13.9				MS, 1, 3
ethyl acetate	200				0.6	6.6 ± 1.4	258.9 ± 330.0			MS, 3
3-methyl-butanal	240			1.7	1.3 ± 0.2	2.6	1.2	sweet, caramel, fish fillet	1.5–3.0	MS, 1, 2, 3
1-penten-3-ol	265	2.3	0.4	0.8 ± 0.1	1.2 ± 0.0	2.5 ± 1.7				MS, 1, 3
3-pentanone	276	3.7 ± 3.2	8.5 ± 5.2	8.2 ± 3.9	13.6 ± 6.2	23.5 ± 17.7	34.6 ± 18.0	sweet, caramel	1.5–2.0	MS, 2, 3
3-hydroxy-2-butanone	294	2.6 ± 1.3	16.8 ± 20.8	90.3 ± 51.6	95.3 ± 5.6	259.2 ± 177.5	341.8 ± 129.3	sweet, sour	1.5–2.0	MS, 2, 3
3-methyl-1-butanol	317			2.7 ± 2.4	8.5 ± 1.0	18.3 ± 6.2	31.6 ± 5.6			MS, 3
2-methyl-1-butanol	320					5.3 ± 2.7				MS, 3
dimethyl disulfide	329						1.2	onion like	1.5–2.5	MS, 1, 2
2,3-butandiol	378			0.9 ± 1.2	4.0 ± 4.4	0.7	21.3 ± 30.1			MS
ethyl butanoate	400						13.9 ± 2.2	sickenly sweet, vomit	2.3	MS, 1, 2, 3
hexanal	401	1.9	2.1 ± 2.0	3.2 ± 1.2	2.0 ± 0.1	8.7 ± 6.2				MS, 1, 2, 3
unknown	460–478							onion, mushroom	3.0–4.8	2
heptanal	500		2.4	1.7 ± 0.8	0.7 ± 0.2	2.9 ± 2.2	3.5 ± 1.8	earthy, boiled potato	2.0–3.0	MS, 2, 3
dimethyl trisulfide	564							rotten, sulfur, cabbage	2.5	2, 3
6-methyl-5-hepten-2-one	587	0.8 ± 0.2	0.9 ± 0.2	1.9 ± 0.4	1.0 ± 0.1	6.1 ± 4.1	5.4 ± 1.8	spicy, flowery	1.5	MS, 2
octanal	606			1.4 ± 0.6						MS, 3
2-ethyl-1-hexanol	633		2.0 ± 2.1	2.3 ± 1.0	1.8 ± 0.2	1.4	2.5 ± 1.1			MS
nonanal	709		4.3 ± 3.7	6.3 ± 1.1	3.7 ± 0.6	10.2 ± 4.4				MS, 3
decanal	807	2.3 ± 0.2	2.5 ± 1.9	3.5 ± 0.4	2.4 ± 0.3	8.0 ± 4.2	14.9 ± 5.7	fresh, floral	1.5	MS, 2, 3
undecanal	910		0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1		2.0 ± 1.1	sweet, candy	1.5	MS, 2

[a] PAR of compounds analyzed by GC-MS, odor descriptions, and odor scores based on GC-O analysis. [b] RI: calculated RI based on ethyl esters on DB-5ms capillary column. [c] PAR: average of duplicate analysis ± standard deviation when detected in both samples or "–" if not detected. [d] Odor evaluated by GC-O. [e] GC-O odor scores (average value of two panelists) giving the range of scores on days 4, 10, and 17. [f] Identification means: MS, mass spectra; 1, authentic standards; 2, odor identification; and 3, RI references (Flavornet). [g] Coeluting peaks.

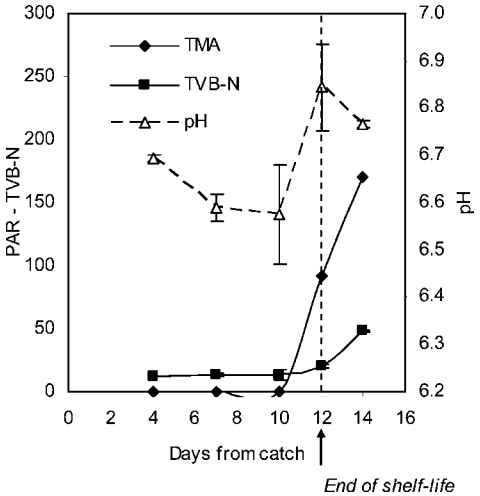

Figure 1. Changes in pH (-△-), development of TMA (-◆-) measured by GC-MS (PAR), and TVB-N (mg N/100 g) (-■-) in cod fillets packed in Styrofoam boxes during storage at 0.5 °C. The vertical line indicates the end of shelf life determined by sensory analysis.

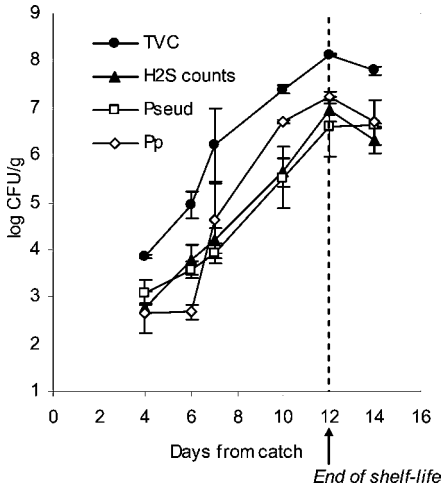

Figure 2. Counts (log CFU/g) of total viable psychrotrophic bacteria (TVC) (-●-), H_2S-producing bacteria (-▲-), presumptive *Pseudomonas* spp. (-□-), and *P. phosphoreum* (-◇-) in chilled cod fillets packed in Styrofoam boxes during storage at 0.5 °C. The vertical line indicates the end of shelf life determined by sensory analysis.

development of microbial metabolites contributing to spoilage changes as seen by increased pH value coinciding with the detection of TMA and higher TVB-N values on day 12 (**Figure 1**). In the parallel study on sensory and microbiological changes of packed cod fillets (*21*), the shelf life was determined as 12 days by sensory analysis using the Torry scheme (*8*). TMA and acetoin, which were present in the highest concentration on day 12 (**Table 1**), have earlier been associated with dominating *P. phosphoreum* growth (*18*). Increasing levels of acetoin were detected on day 7 coinciding with a rapid *P. phosphoreum* growth reaching counts of log 7.2 CFU/g at sensory rejection. *P. phosphoreum* was identified as the main SSO in the cod fillets based on its high counts throughout the storage time and was found in the highest levels (12.6%) at the end of shelf life, while *Pseudomonas* spp. and H_2S-producing bacteria represented 4.9 (log 6.6 CFU/g) and 7% (log 7 CFU/g), respectively, of the total microflora at sensory rejection (**Figure 2**).

Characteristic Odor of Cod Fillets Detected by GC-O. The overall odor of the fillets was observed as a mild and sweet odor that became sour, fruity, and stale during storage, and much less potent odors developed on the fillets than are generally observed on whole fish. Characteristic odor development during storage of the fillets could be explained by the odor description and odor scores for the individual compounds analyzed by GC-O (**Table 1**). The GC-O scores were generally low but increased with storage time in agreement with the increased level of the compounds measured by GC-MS. The values and ranges of the GC-O odor scores detected on days 4, 10, and 17 are shown in **Table 1**. The most potent odor described as spicy, flowery, sweet, onion, and mushroom-like was detected at RI 460−478 in all samples during storage, with increasing odor scores with time of 3 (moderate) to 4.8 (very strong). This complex odor is most likely contributed by coeluting compounds in low concentrations, which were not detected by GC-MS.

Aldehydes appeared to influence the characteristic mild and sweet odors of the fillets throughout the whole storage time. 3-Methyl-butanal and heptanal had the highest odor scores of the aldehydes (**Table 1**). On the basis of the odor description of heptanal (earthy and boiled potato-like), it is likely that low levels of methional and 4-heptenal, which are known to coelute at similar retention times (4-heptenal, heptanal, and methional; RI = 497, 500, and 502, respectively) (*6*), may have influenced the odor. However, these compounds were not detected by GC-MS. The odors of decanal and undecanal were perceived as fresh/floral and sweet/candy, respectively (**Table 1**). The straight chain aldehydes have been reported to exhibit green, fatty, soapy, and tallowy odors, and hexanal is characterized by a green odor (*24*). The branched chain aldehyde, 3-methyl butanal, was perceived as a sweet, caramel, and fish filletlike odor by GC-O (**Table 1**). The corresponding alcohol 3-methyl-1-butanol and also 2-methyl-1-propanol exhibit alcoholic and fruity odors (*24*). However, their flavor thresholds are high as compared to the carbonyls and their odors were not detected by GC-O; therefore, the alcohols probably did not contribute much to the overall odor of the fillets (**Table 1**). The high levels of acetoin suggested that this compound could contribute to the onset of spoilage odors in cod fillets (**Table 1**). The odor of acetoin has been described as buttercream-like (*24*), but a mild, sweet−sour-like odor was detected by GC-O (**Table 1**). A sweet, caramel-like odor was detected by GC-O for 3-pentanone. The GC-O analyses indicated that the ketones in combination with 3-methyl-butanal and aldehydes such as hexanal, nonanal, decanal, and undecanal contributed to the overall characteristic sweet, caramel, and flowery odor of the cod fillets. Because aldehydes generally have low odor thresholds, their odor impact was greater than the alcohols and the ketones although their overall levels were less (**Table 1**). However, these compounds do not cause potent spoilage odors, and in fact, the aldehydes, like decanal, were detected in lower levels at sensory rejection on day 12 than on day 10 ($p < 0.05$).

The presence of TMA on day 12 overpowered the odor and contributed to the sensory rejection of the fillets. TMA is a potent odorant with a characteristic fishy, dried fish, ammonia-like odor as detected by GC-O and received high odor scores. Ethyl acetate was first detected on day 12 and probably also influenced the overall potent spoilage odor development in combination with the other compounds detected on day 12. The odors of esters and sulfur compounds were detected by GC-O only at advanced spoilage on day 17. A sickenly sweet odor detected by GC-O on day 17 was identified as ethyl butanoate. The formation of esters suggested the activity of *Pseudomonas fragi* (*9*). At the end of the shelf life on day 12, the counts of *Pseudomonas* spp. reached log 6.6 CFU/g (**Figure 2**) and an increasing level of ethyl acetate was seen on day 14 and much higher levels on day 17 at overt spoilage (**Table 1**). Esters are known to contribute to sweet and fruity spoilage odors in seafood at advanced stages of spoilage (*9*, *15*). Similarly, ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 3-hydroxy-2-butanone, ethyl acetate, and butanoic acid ethyl ester were the most abundant volatiles in the headspace of haddock stored in ice associated with the growth of *Pseudomonas* spp. in an earlier study on packed haddock fillets stored under chilled conditions (*29*). *Pseudomonas* species have also been found responsible for the formation of volatile sulfides, alcohols (3-methyl-1-butanol and 1-penten-3-ol), and ketones (2-butanone) contributing to the stale and putrid off odors in fish because of amino acid and lipid degradation (*9*, *10*).

Odor of fresh cod fillets is typically characterized by a very mild and pleasant, marinelike odor. Species specific odors of fresh fish are contributed by long chain alcohols and carbonyl compounds such as 1,5-octadien-3-ol and 2,6-nonadienal, respectively, that are derived from polyunsaturated fatty acids (*30*). These compounds are more pronounced on the skin than in the muscle and were not detected in the fillets using the sampling conditions herein. In boiled cod, 3-methyl-butanal in combination with acetaldehyde, methional, 1,5-octadiene-3-one, 2,6-nonadienal, and 2,4-decadienal were determined as character impact odorants and the malty flavor of 3-methyl butanal was suggested to be mainly responsible for the malty off flavor defect of boiled cod by Milo and Grosch (*31*).

The change in the pH value on day 12 may have influenced the overall odor perception, and synergistic effects may have occurred. For example, TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from autoxidation of polyunsaturated fatty acids (*32*). The possible influence of other compounds present in low levels such as the unsaturated autoxidatively derived aldehydes should not be overlooked, but the sampling techniques used in our study were not sensitive enough to allow detection of these compounds.

Volatile Compounds as Potential Quality Indicators Detected by GC-MS. *Alcohols, Carbonyls, Esters, and Acids.* The dynamic changes in the levels of the most abundant alcohols, esters, acids, and carbonyls are demonstrated in **Figures 3** and **4**. Ethanol was detected in high levels initially, followed by an increase in 2-methyl-1-propanol, 3-methyl-1-butanol, 2,3-butandiol, 3-methyl-butanal, and ethyl acetate (**Figure 3**). The early detection of ethanol and 2-methyl-1-propanol is of interest to monitor the initial changes related to the loss of freshness before the obvious spoilage signs appear. However, their levels did not increase continuously with time.

The variation in GC analysis for duplicate samples was high for the very volatile compounds such as acetaldehyde and ethanol (**Table 1**), most likely because of their high break-through volume on the Tenax and also the fact that in some cases compounds with RI < 165 coeluted and made both identification and quantification difficult. This was the case for ethanol, TMA, and dimethyl sulfide as well as 2-methyl-1-propanol, which was also identified as pentane in the MS library database (**Table 1**).

The initial production of ethanol in spoilage of fish has been related to the utilization of carbohydrate sources, while the formation of branched chain alcohols and aldehydes such as 2-methyl-1-propanol, 3-methylbutanol, and 3-methyl-butanal probably originate from degradation of valine and leucine,

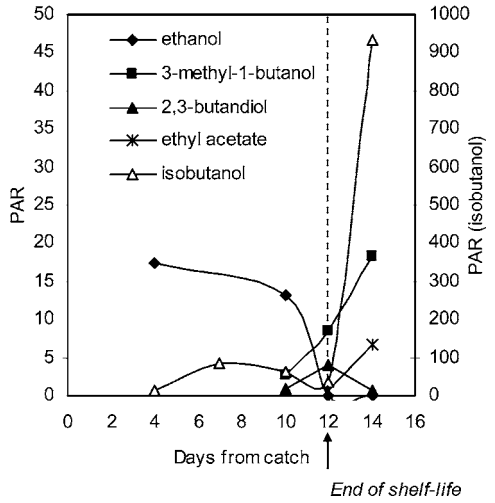

Figure 3. PAR for alcohols [ethanol (-◆-), 3-methyl-1-butanol (-■-), 2,3-butandiol (-▲-), and isobutanol (-△-)] detected in highest concentration and ethyl acetate (-×-) in cod fillets packed in Styrofoam boxes during storage at 0.5 °C. The vertical line indicates the end of shelf life determined by sensory analysis.

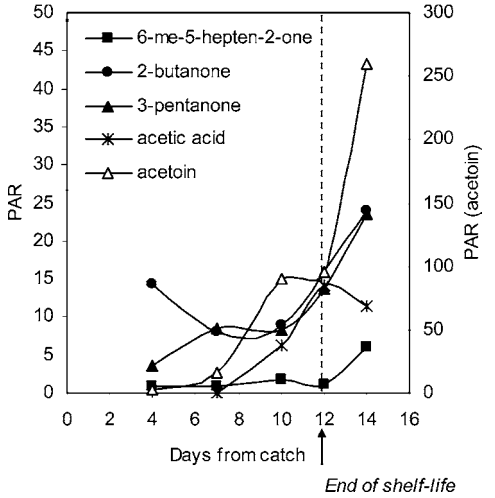

Figure 4. PAR for ketones [6-methyl-5-hepten-2-one (-■-), 2-butanone (-●-), 3-pentanone (-▲-), and acetoin (-△-)] and acetic acid (-×-) in cod fillets packed in Styrofoam boxes during storage at 0.5 °C. The vertical line indicates the end of shelf life determined by sensory analysis.

respectively (*33*). 3-Methyl-1-butanol was first detected on day 10, and significant continuous increases ($p < 0.05$) were seen with time.

The formation of acetic acid and a decline in the ethanol level were observed on day 10 followed by the detection of ethyl acetate at the end of the shelf life on day 12. Much higher levels of 3-methyl-1-butanol, 2,3-butandiol, 2-methyl-1-propanol, TMA, ethyl acetate, and acetoin were detected on days 14 and 17 as compared to day 12 when the end of sensory shelf life was reached (**Table 1**). The late development of the spoilage indicators is in agreement with studies on spoilage indicators for cultured and wild sea bream stored in ice for 23 days (*14*). TMA, 3-methyl-1-butanol, 1-penten-3-ol, piperidine, methanethiol, dimethyl disulfide, dimethyl trisulfide, and acetic acid were suggested as spoilage indicators, and the increase in the levels of most of the compounds was detected around day 10 of storage; however, methyl mercaptan and dimethyl trisulfide appeared to accumulate later when the products were already spoiled.

Table 2. PAR of Main Classes of Compounds Identified by GC-MS in Cod Fillets Packed in Styrofoam Boxes during Storage at 0.5 °C for 17 Days

	PAR[a]					
class	day 4	day 7	day 10	day 12	day 14	day 17
			quality indicators			
alcohols	34 (29)	88 (45)	84 (28)	51 (15)	963 (39)	507 (15)
aldehydes	7 (6)	13 (6)	19 (6)	11 (3)	38 (2)	27 (1)
ketones	21 (18)	34 (18)	109 (36)	110 (33)	313 (13)	382 (11)
acids			6 (2)	14 (4)	11 (<1)	15 (<1)
amines				92 (27)	922 (37)	1721 (51)
esters				1 (<1)	7 (<1)	273 (8)
sulfur compounds	5 (4)					1 (<1)
			miscellaneous			
alkanes	3 (3)	4 (2)	6 (2)	3 (1)	4 (<1)	3 (<1)
aromatics	17 (15)	39 (20)	40 (13)	22 (7)	80 (3)	76 (2)
other	5 (4)	5 (3)	3 (1)	12 (4)	97 (4)	351 (10)
unknown	26 (22)	10 (5)	36 (12)	17 (5)	28 (1)	44 (1)
total volatiles	118	194	306	335	2464	3402

[a] PAR and percentage of total volatiles each day shown in parentheses.

Ketones. The formation of microbially derived acetoin was characteristic for the spoilage of chilled cod fillets as discussed above. Levels of acetoin increased earlier than TMA; therefore, it is more useful to monitor the loss of freshness as an early indicator of spoilage. Acetoin can be formed from carbohydrate sources via pyruvate and diacetyl; however, the mechanism of its formation in fish is not well-known, but acetoin and diacetyl have been suggested as early indicators of spoilage in beef (*34*). Acetoin is a common metabolite of anaerobic bacteria like lactic acid bacteria (LAB), which often predominates in products such as vacuum-packed smoked salmon, but direct counts of LAB were not done in this study. It is possible that microaerophilic conditions may have developed in the Styrofoam boxes, especially in the center of the fillets' bulk, and *P. phosphoreum*, which is a facultative anaerobe, can well-tolerate those conditions. The concentration of the microbially derived acetoin was much higher than the lipid-derived ketones detected like 2-butanone, 3-pentanone, and 6-methyl-5-heptene-2-one that were present in cod fillets throughout storage, but no obvious increase occurred until after the end of shelf life (**Figure 4**).

Aldehydes. The straight chain lipid-derived aldehydes, hexanal, heptanal, nonanal, decanal, and undecanal were detected in the cod fillets throughout the storage time. Their levels did not appear to increase until after the end of shelf life was reached (**Table 1**). Therefore, they did not appear to be useful as indicators of spoilage during chilled storage of the cod fillets, but aldehydes have been suggested as indicators of spoilage in fatty species (*20*). Oxidation of fatty acids contributes to the rancid odors of fish with the formation of aldehydes such as hexanal, 2,7-heptadienal, and 2,4,7-decadienal (*35*). The unsaturated aldehydes were not detected by GC-MS in this study using the surface stripping sampling of the volatiles.

Main Classes of Compounds as Quality Indicators in Cod Fillets during Storage. *Ketones, Amines, Alcohols, Acids, Aldehydes, and Esters.* The sum of the PAR for all of the volatile compounds detected in cod fillets by GC-MS showed that the total amount of volatiles increased with storage, and alcohols, ketones, and TMA (amines) were present in the highest amount (**Table 2**). On day 4, the alcohols were the most abundant of the volatiles (29%), mainly contributed by ethanol. Again, on day 7, the alcohols were still the most abundant volatiles (45%) and 2-methyl-1-propanol was detected in the highest level. The ketones (36%) increased considerably on day 10 with the

development of acetoin, and acid (2%) was first detected on day 10. At sensory rejection on day 12, the ketones were detected in the highest level (33%) followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%), and a low level of esters (ethyl acetate) (<1%). Esters and acids were not detected during early storage, and sulfur compounds were only detected in low levels (**Table 2**) as expected since these compounds are typically produced at advanced spoilage of seafood (*7*, *9*, *10*, *13*−*15*). TMA was the most abundant volatile compound at advanced spoilage on day 17, and alcohols and ketones were also in high levels, but in addition, the level of esters had increased considerably comprising 8% of the total PAR (**Table 1**).

Miscellaneous Classes of Compounds. Although alcohols, aldehydes, ketones, acids, sulfur compounds, esters, and acids are primarily of interest as spoilage indicators of fish, other classes of compounds were also present in the headspace, which may have an impact when measuring the total headspace with electronic noses. The concentration of the straight chain alkanes (nonane, decane, and undecane) appeared to be similar throughout storage. Additionally, numerous branched chain alkanes were identified by the MS library database, but their RIs were not confirmed; therefore, they were classified with the group "unknown", which represented compounds that remained unidentified. The alkanes will not influence the responses of the electrochemical sensors of the electronic nose and are not considered of interest as quality indicators since they are not aroma active.

Compounds classified as "others" appeared to increase with storage time. Among these compounds were some odorous compounds such as 3-methyl-piperidine (RI, 604). This compound was tentatively identified and has not been reported before in cod. However, piperidine was suggested earlier as a quality indicator of sea bream (*14*) and has been found in low levels in fish associated with 1,5-diaminopentane (cadeverine) (*36*).

The aromatics detected were mainly benzene derivatives that were only tentatively identified. Styrene (RI, 481) and chloroform (RI, 212) were most abundant and were found to increase with storage. Styrene was also tentatively identified in wild and cultured sea bream packed in polystyrene boxes during chilled storage (*14*). The odor of styrene is described as kerosene-like and has been associated with off odors in surimi-based products related to the growth of yeasts (*37*). However, it is speculated that both styrene and chloroform could originate from the Styrofoam boxes; however, this was not confirmed. Tainting from the packaging may be of concern when monitoring spoilage changes with an electronic nose, if the sensors are sensitive to the respective compounds. Therefore, it is very important to analyze the total headspace by GC and understand the origin of the suggested quality indicating compounds prior to the selection of suitable sensors for monitoring the relevant spoilage changes.

Electronic Nose Analysis of the Main Classes of Compounds in Cod Fillets during Storage. The results of the electronic nose measurements showed that the CO sensor was most sensitive to monitor the changes in volatiles during storage (**Figure 5**). The response of the CO sensor was higher than the other sensors' responses (H_2S, SO_2, and NH_3), and a significant increase ($p < 0.05$) was first observed between days 7 and 10; thereafter, a continuous increase occurred. Earlier studies have shown that the main classes of spoilage indicator compounds present in the headspace of whole fish can be estimated based on the individual sensor responses (*5*). Selected standard compounds representing the main classes of compounds causing

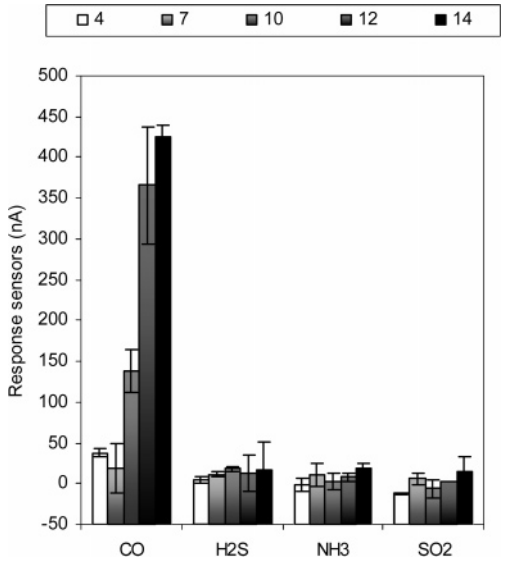

Figure 5. Response of the electronic nose sensors (CO, H_2S, NH_3, and SO_2) toward cod fillets during storage in Styrofoam boxes at 0.5 °C on days 4, 7, 10, 12, and 14 after catch.

spoilage odors showed that the CO sensor is sensitive to alcohols, aldehydes, and esters. The NH_3 sensor is sensitive to amines, and the H_2S and SO_2 sensors can detect sulfur compounds. The comparison of the electronic nose measurements and the GC analysis has some drawbacks related to the different sampling techniques used. Therefore, the results are only a semiquantitative approach to screen the major changes in the composition of the volatiles, and the electronic nose responses can be partly explained by comparison to the compounds identified in the highest concentration by the GC-MS.

The increasing PAR for alcohols, aldehydes, and esters (**Table 2**) could explain the increasing CO sensor response with time based on its sensitivity toward these classes of compounds. It is of interest that higher responses were observed for the CO sensor in an earlier study of haddock fillets at the end of shelf life when stored under identical conditions as the cod fillets. This can be explained by higher levels of alcohols and esters produced by the SSO in the haddock fillets (*29*). Although *P. phosphoreum* was also identified as the main SSO in the haddock fillets, high counts of *Pseudomonas* spp. were observed at sensory rejection. The pseudomonads are not able to reduce TMAO to TMA (*38*) and may have contributed to the higher levels of alcohols because of their metabolism and need for hydrogen acceptors, hence favoring the production of alcohols. The dynamic spoilage changes and development of volatiles are thus dependent on the combination of the dominating SSO.

The NH_3 sensor's response increased first on day 14 although not significantly because of the high standard deviation. The high standard deviations for replicate samples for both the electronic nose and the GC analysis were partly caused by the influence of the temperature on the very volatile compounds during sampling (*32*). Although high levels of TMA were detected by GC-MS on day 12 in agreement with high TVB-N levels (48.5 mg N/100 g), the NH_3 sensor was not sensitive enough to detect this. Higher responses for the NH_3 sensor were observed in earlier studies for both shrimp (*39*) and whole capelin (*4*), but in these products, TVB-N levels were higher than in cod fillets.

The ketones, mainly acetoin, increased continuously with storage (**Figure 4**) and appeared to be promising indicators for

cod fillets packed in Styrofoam boxes. However, the detection of the high level of acetoin produced in cod fillets was not achieved by the electronic nose since none of the sensors is sensitive to ketones. Improvements should be made to include selective sensors in the electronic nose for the detection of ketones and acids to monitor spoilage of cod fillets in addition to a more sensitive sensor for the detection of TMA. Other sensors such as metal oxide sensors (MOS) have been shown to be useful for monitoring fish quality and the detection of spoilage volatiles, including ketones, in a vacuum-packed smoked salmon (*40*).

The low response of the H_2S and SO_2 sensors suggested that the H_2S-producing bacteria were not important in the development of putrid spoilage odors in packed cod fillets in agreement with the GC-MS analysis. Dimethyl sulfide, which was tentatively identified, was detected on day 4 suggesting that this compound was most likely not associated with microbial spoilage but rather reflected the feeding conditions as has been suggested by others (*41*). Sulfur compounds such as hydrogen sulfide and methyl mercaptan, which are produced by microbial degradation of fish constituents (*11*), were not detected by GC-MS, but GC-O analysis on day 17 indicated that dimethyl trisulfide was present in the spoiled fillets (**Table 2**). Earlier studies using the FreshSense electronic nose have shown an increasing response of the H_2S and SO_2 sensors at advanced spoilage of whole capelin and redfish (*4*, *5*), but response to fish fillets is generally low (*29*).

It is suggested that selective sensors for the detection of ketones, amines (TMA), alcohols, acids, aldehydes, and esters could be used for monitoring spoilage changes of various fish products because similar volatile compounds emerge during chilled storage (*7*, *13*−*15*). The CO sensor was useful for detecting incipient spoilage in the Styrofoam-packed chilled cod fillets and reflected the characteristic odor changes, which were dominated by mild, sweetlike odors. Acetoin and TMA were most promising as quality indicators in chilled cod fillets since these compounds were produced in the highest and increasing amounts during storage coinciding with the growth of *P. phosphoreum*, which was identified as the dominating SSO under these conditions. Because of the complexity of the spoilage processes caused by the diversity of the dominating microflora and their different spoilage potential, it is likely that fixed values to determine the end of shelf life or the quality of fish fillets based on electronic nose responses will have to be developed for each product and the respective storage conditions.

ABBREVIATIONS USED

GC-MS, gas chromatography−mass spectrometry; GC-O, gas chromatography−olfactometry; ATD, automated thermal desorber; RI, retention index; TMA, trimethylamine; TVB-N, total volatile basic nitrogen; PAR, peak area ratio; SSO, specific spoilage organisms.

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