# Evaluation of Shelf Life of Superchilled Cod (*Gadus morhua*) Fillets and the Influence of Temperature Fluctuations During Storage on Microbial and Chemical Quality Indicators

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**ABSTRACT: Quality changes of aerobically packed cod fillets stored under superchilling and abusive temperature conditions were characterized by the growth of specific spoilage organisms (SSO) and the production of microbial metabolites measured by an electronic nose along with traditional sensory and chemical analysis (total volatile basic nitrogen [TVB-N], pH). A new process based on quick contact freezing and cold air blasting was used to achieve superchilling of fillets before chilled (0.5 °C) or superchilled (–1.5 °C) storage. *Photobacterium phosphoreum* dominated under temperature abusive conditions coinciding with high levels of TVB-N and increased electronic nose responses indicating increased levels of alcohols and aldehydes at sensory rejection. Dominating growth of *Pseudomonas* spp. in 1 batch was associated with the origin, the catching method, and the cooling conditions during processing. The superchilling process followed by superchilled storage (–1.5 °C) extended the sensory shelf life of the fillets for at least 3 d compared with traditional process, resulting in a total shelf life of 15 d. High content of TVB-N was observed in superchilled fillets at sensory rejection. *P. phosphoreum* counts were lower under superchilling conditions (6.0 to 6.8 log colony-forming units [CFU]/g), compared with the traditionally processed chilled fillets (7.2 log CFU/g). However, $H_2S$-producing bacteria appeared to grow steadily under superchilling conditions reaching counts as high as 7.6 log CFU/g at sensory rejection.**

**Keywords: superchilling, cod fillets, sensory analysis, specific spoilage organisms, electronic nose, TVB-N**

## Introduction

The extension of shelf life of chilled fish fillets is of importance to allow the transport of products to distant markets at lower cost. Superchilling has proven to effectively delay bacterial growth and prolong the shelf life of chilled fish (Huss 1995; Chang and others 1998). Various types of cooling systems have been used for superchilling (–4 °C to 0 °C) of seafood products including flake ice or slurry ice (Losada and others 2004; Zeng and others 2005) and subzero temperatures during storage (–2 °C) (Sivertsvik and others 2003). Chilling of fillets is traditionally done in the fish industry by immersing the fillets in ice/water or brine solutions (Lee and Toledo 1984). Subsequent chilling in a freezer after packaging has proven useful to store refrigerating capacity into the product (Magnussen and others 1998). However, if left too long, slow freezing can occur causing undesirable ice crystal formation, inducing tissue damage. A new technique, "Combined Blast and Contact" cooling (CBC) (Skaginn, Iceland), is based on superchilling by lowering the temperature of the fillets quickly to –1 °C. The cooling of the fillets is then based on the cooling capacity stored in the skin side surface layer, thus minimizing ice crystal formation in the fillets. The rate of freezing and the size of ice crystals are important factors influencing the survival of microorganisms.

*Shewanella putrefaciens* and *Pseudomonas* spp. are well established as the main SSO in fresh chilled aerobically stored fish caught in temperate waters (Huss and others 1997). The influence of high temperature during storage (10 °C to 15 °C) on the proliferation of the SSOs in fish model system has shown the importance of *S. putrefaciens* at high temperature and as a late spoiler (Lauzon 2000). *Photobacterium phosphoreum*, the main SSO in packaged cod fillets (Dalgaard 1995), was earlier identified as an important trimethylamine (TMA) producer in iced cod and cod fillets and known to originate from the intestines (Van Spreekens and Toepoel 1981). The knowledge on the effect of superchilled storage on the proliferation of SSOs and their production of metabolites in cod fillets is limited. As a result of delayed or altered microbial growth when new preservation techniques are used, the traditional spoilage signs may become distorted and therefore the commonly used quality indices may be of questionable value (Lindsay and others 1986). Detection of the main classes of volatile degradation compounds (alcohols, aldehydes, esters, sulfur compounds, and amines) produced during chilled storage of fish by an electronic nose has proven useful to determine the spoilage level of different fish species (Olafsdottir and others 2000, 2002; Di Natale and others 2001).

The aim of the experiments reported herein was to study the spoilage characteristics of CBC-processed cod fillets compared with traditionally processed fillets stored under superchilled and/or chilled conditions and the effect of abusive temperature on the de-

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velopment of specific spoilage organisms and their metabolites. Spoilage changes were monitored by sensory, microbial, and chemical analyses and by an electronic nose.

Characterization of the spoilage changes and the spoilage domain of the SSOs in superchilled cod fillets are needed to determine, which quality indicators are relevant for monitoring quality changes of cod fillets stored under superchilled and temperature abusive conditions. Furthermore, the knowledge can be applied to optimize the chilling conditions to extend the shelf life of cod fillets.

## Materials and Methods

### Preparation of samples

Three extensive storage studies were performed with products from 2 different fish factories in Iceland, which were interested in utilizing the new CBC technique. Characterization of the spoilage processes was therefore necessary before the investment in a new processing equipment could be justified. The raw material originated from different catching areas (Southwest and Northeast of Iceland), using different fishing gear (longline and bottom trawl) and varying processes in the factories.

**Traditional process Factory I—Experimental groups A, B, and D**. The fish was caught using a bottom trawl by the ice fish trawler Brettingur East off Iceland in the Berufjardarall catching zone in October 2003. The fish (3 to 3.5 kg each), selected from 1 haul (batch 1), was gutted on board and iced in tubs (400L) (ice/fish ratio 1:3). Landing was 3 d after catch at the processing plant. Approximately 170 fish (*post rigor*) from batch 1 were processed into fillets to prepare the sample groups. The traditional process in factory I involves mechanical filleting and skinning, and packing of fillets in styrofoam (EPS, expanded polystyrene) boxes (160 × 400 × 263 mm) lined with a plastic bag. Each box contained 8 to 12 fillets, an absorbing pad at the bottom and a cooling mat (230 × 160 mm; 146-g gel) placed on top. Temperature data loggers were inserted into selected boxes at the factory. The samples were transported by a refrigerated truck (4 °Cto 5 °C) to Reykjavik the same d and the samples arrived in the morning the following day (day 4 after catch) (groups A and B). Group A was then stored constantly at 0.5 °C, whereas group B was stored at 16 °C for 8 h on day 6 and then transferred back to the cold store (0.5 °C). Thirty fish were selected from the same batch at the factory to prepare sample group D that represented bad handling. Abusive temperature conditions were simulated by keeping the fish without ice in a tub in the reception area of the factory for approximately 8 h at 2 °C and then transferring the tub into the processing area (15 °C to 18 °C) until the following morning when the fish was filleted, skinned, packed, and transported to the laboratory. The same temperature conditions during storage were used as for group B.

**Traditional process Factory II—Storage groups H and I**. The fish was caught by longlining close to Sandgerði Southwest off Iceland in November 2003. The cod was gutted and iced on board the boat. Landing was in the afternoon the same day, and the fish was stored iced overnight. The fish was hand filleted the following morning and cooled by placing the fillets into aluminum pans with ice-water for 10 to 20 min before being mechanically skinned and packaged into plastic bags in styrofoam (EPS) boxes with a cooling mat on top. Eleven boxes (each containing 11 fillets) were then moved to a cooling room until shipped by refrigerated truck to the laboratory the same day. Temperature loggers were inserted into selected boxes at the laboratory. Two groups of samples were prepared (7 boxes for group H and 4 boxes for group I). Both sample groups were initially stored at 0.5 °C, but on the 4th d of storage, group I was removed from the cooler and stored overnight (16 h) at room temperature (RT, 18 to 20 °C) and then moved back to the cooler.

**Superchilling process Factory I—Experimental groups C, E, F, and G.** Group C was prepared from batch 1 in October 2003 (labeled "C new" indicating that this was the 1st trial with the new superchilling technique). After filleting, 80 fillets with skin on were immersed in an ice-water cooling solution containing 0.85% NaCl for 45 min. The temperature of fillets after brining was about 0.9 °C when further processed with the new superchilling CBC (Combined Blast and Contact) technique (Skaginn, Iceland). The CBC technique involves superchilling of the surface of the skin side of the fillets by moving them through a freezing tunnel on a teflon covered aluminum conveyor belt (–6.5 to –6.7 °C) and simultaneously blasting cold air (–8 °C) over the fillets. The transfer time for each fillet through the freezing tunnel (8.5 m) was about 8 min (rate of conveyor belt was approximately 1 m/min). The superchilling process facilitates handling of the fillets, in particular the removal of the skin, and effective cooling of the fillets resulted in a temperature approximately –0.9 °C when packed. Group C was transported to the laboratory, and arriving on day 4 after catch and then stored at 0.5 °C.

The 2nd trial with the new superchilling technique was done in December 2003. Three experimental groups (E, F, and G) were prepared from about 190 fish (batch 2). The fish was caught by the same trawler as batch 1, but ice slurry was used on board for cooling. Landing and processing were on day 1 after catch.

The fish was hand filleted and the fillets were cooled in ice water (0.85% NaCl) for 20 min before being superchilled by the new technique and skinned. Nine to 11 fillets were packed in each styrofoam box with a cooling mat put on top. The samples were transported by a refrigerated truck (4 °C to 5 °C) to the laboratory and arrived the following morning on day 2 post catch. The sample groups were stored under different conditions, group E at 0.5 °C, group F at –1.5 °C, and group G was initially stored at –1.5 °C but transferred to 0.5 °C on day 8 post catch.

### Sampling

At each day of sampling, 1 box containing 8 to 12 fillets was used for the various analyses. Three or 4 fillets were used for sensory analysis. The other 4 fillets were pooled into 2 samples and used for microbial analysis (total viable counts) [TVC] and SSO counts), chemical analysis of total volatile bases nitrogen (TVB-N), and pH. Four fillets were then used for the analysis of volatile compounds with the electronic nose. The 1st samples were measured on the day following processing, and duplicate samples were analyzed regularly until sensory rejection.

### Temperature monitoring

Selected boxes contained automatic temperature data loggers (Stow Away®, Onset Computer Corp., Bourne, Mass., U.S.A.) to monitor the temperature of fillets during storage. Temperature was recorded at 5-min intervals. The loggers were inserted underneath, in between, and above the fillets in 1 box for each temperature treatment, as well as on top of the box to follow the environmental temperature. Data are shown only from loggers located underneath the fillets.

### Sensory analysis

The Icelandic Fisheries Laboratories (IFL) sensory panel evaluated the freshness of the fillets to determine the shelf life of differently treated products. The selection, training, and monitoring of assessors was performed according to international standards (ISO 1993, 1994), including detection and recognition of tastes and odors and training in the use of scales. Sensory assessments were carried out by 8 to 12 assessors (age range, 30 to 55 years). Fish from each treatment was portioned (approximately 30 g) into an aluminum box and cooked in a steam oven (98 °C to 100 °C for 5 min), and a lid was put on each box

when removed from the oven. Each treatment was assessed in duplicate, and the samples were anonymously coded. A computerized system (FIZZ, Version 2.0, 1994–2000, Biosystèmes, Couternon, France) was used for data recording of cooked samples and further data processing. Average scores were calculated for each treatment, and significant differences between corresponding treatments were evaluated.

The Torry scheme (Shewan and others 1953) was used to assess the freshness of cooked pieces. The scheme is a detailed description of the characteristic flavor changes (odor and taste) and ranges from 10 = very fresh to 3 = very spoiled, with a rejection level at 5.5.

## Microbial analysis

Fillets were aseptically minced, assessing 2 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 to 5 d) 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 to 5 d) and modified CFC medium (22 °C, 3 d), respectively (Lauzon and others 2002).

Counts of *Photobacterium phosphoreum* were estimated by using the PPDM-Malthus conductance method (Dalgaard and others 1996), as described by Lauzon (2003).

## TVB-N and pH measurements

Total volatile basic nitrogen content (TVB-N) was determined by the direct steam distillation into boric acid using a Kjeldahl-type distillatory (Struer TVN). The acid was titrated with diluted $H_2SO_4$ solution as described by Malle and Poumeyrol (1989). The pH was measured in 5 g of mince moistened with 5 mL of deionized water. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25 °C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

## Electronic nose

Electronic nose measurements were performed using a gas sensor instrument called FreshSense (Maritech, Kópavogur, Iceland). The instrument is based on 4 electrochemical gas sensors: CO, $H_2S$, $SO_2$ (Dräger, Lübeck, Germany), and $NH_3$ (City Technology, Portsmouth, Britain). Duplicate analyses were done using approximately 500 g of fish fillets in a 2.3-L headspace sampling vial and a pump was used to circulate the headspace volatiles to the sensor chamber as described earlier (Olafsdottir and others 2002). The samples were tempered at RT for approximately 30 min until the temperature of the fillets reached 8 °C to 12 °C before recording the sensors' responses for 5 min.

## Data analysis

Multivariate analysis was performed using the Unscrambler Version 9.1 (CAMO Process, Trondheim, Norway). The main variance in the data set was studied using Principal Component Analysis (PCA). Partial Least Squares Regression (PLSR) models were used to explore the correlation of the sensory, microbial, TVB-N, pH, and electronic nose variables. The effect of temperature of fillets

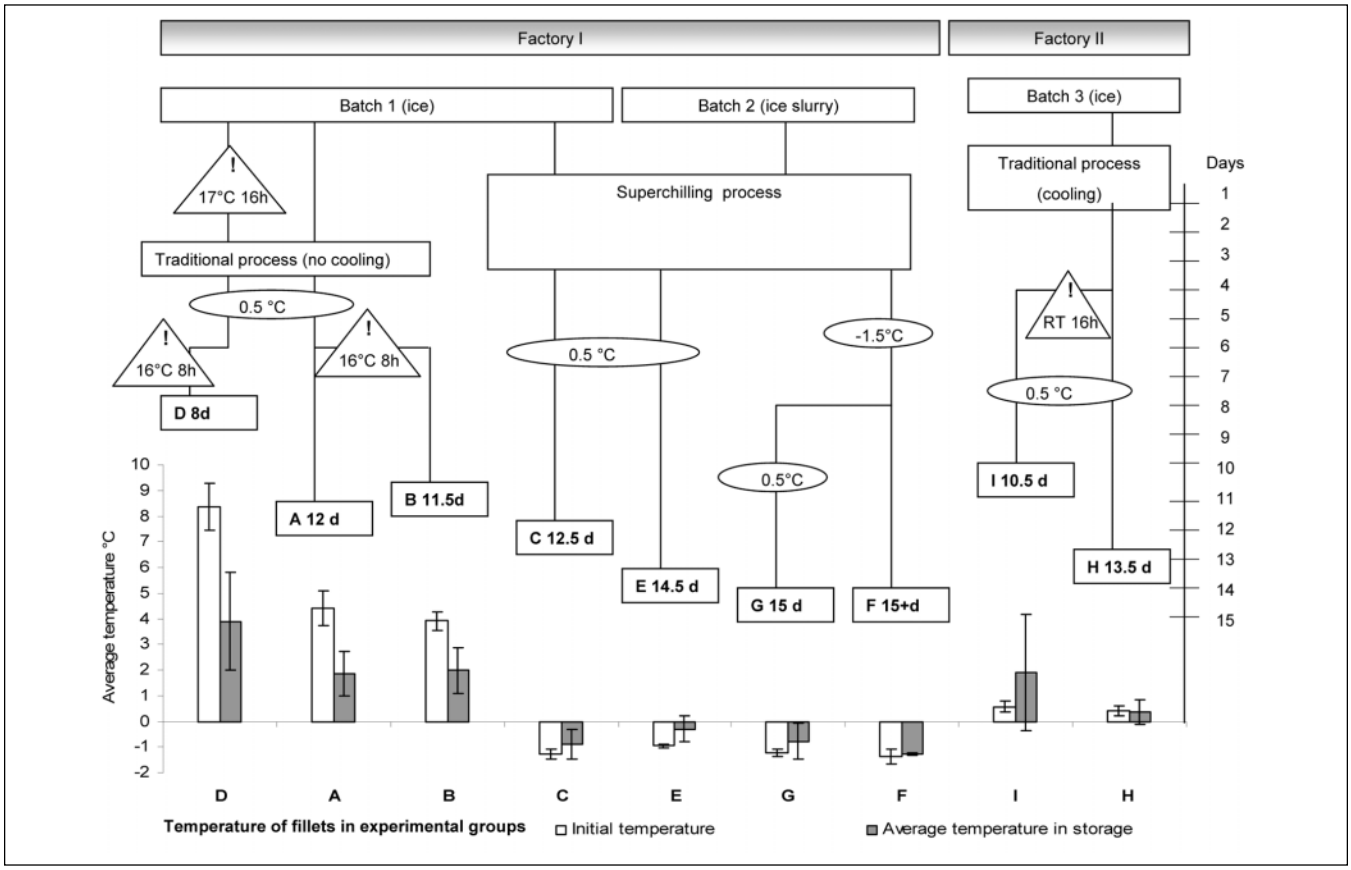


**Figure 1—Influence of processes, initial average temperature (1st d after packing), and average temperature of the fillets during storage on the shelf life of experimental groups from different factories processed from different batches. Values next to experimental groups labels (A, B, C, D, E, F G, H, and I) indicate the sensory shelf life (days from catch). Temperature conditions of each group are shown in circles and abusive temperature treatments in triangles (RT = room temperature).**

and time was also included as an independent variable by calculating, for all samples at each sampling day, the accumulative influence of temperature (T) and time (t): $T_{accumulative} = \Sigma (T - T_{min}) \times dt$; $T_{min}$ was defined as the minimum temperature of fillets in storage and time; dt is time interval between sampling days.

Soft independent modeling of class analogy (SIMCA), a pattern recognition method relying on independent modeling of defined classes by means of PCA (Wold 1976), was used to classify samples according to sensory quality (Torry score) based on the sensor responses, microbial, and chemical data. Average scores of assessors were used for the sensory data, and furthermore, average value of duplicate samples were used for all the measurement variables. Values were standardized to equal variance. Full leave-one-out cross validation was used in the validation method. The Jack-knife method (Martens and Martens 1999) was used to determine significant variables in X with a significance level of 5% ($P < 0.05$).

Microsoft Excel 97 was used to calculate means and standard deviations for all multiple measurements and to generate graphs.

Analysis of variance (ANOVA) was applied to the data using the Number Cruncher Statistical Software (NCSS 2000). Significant differences were determined by 1-way ANOVA and Duncan's Multiple-Comparison Test was used to determine the statistical difference between samples. An effect was considered significant at the 5% level ($P < 0.05$).

The bacterial growth data collected were fitted to determine maximum specific growth rates (/h) by using DMFit (http://www.ifr.ac.uk/safety/DMFit/), an inhouse program of IFR (UK), which is based on a reparameterized version of the model of Baranyi and Roberts (1994). DMFit is an Excel add-in program to fit curves in which a linear phase is preceded and followed by a stationary phase.

## Results and Discussion

### Effect of handling and temperature on shelf life determined by sensory analysis

Initial average temperature of the fillets varied based on the processing practice used (Figure 1). In a conventional process, the temperature of fillets will typically increase during filleting, skinning, and trimming. The process in factory I (batch 1) was a conventional process that did not include cooling of the fillets before packaging, resulting in a much higher initial temperature of the fillets (4 °C to 6 °C for groups A and B) than in factory II where cooling of the fillets was applied (0.5 °C for groups H and I) (Figure 1). The temperature fluctuations of the fillets that were simulated 1st by storing the raw material without ice until processed (group D), as seen by the highest initial temperature, and 2nd, by exposing the fillets to temperature fluctuations during storage to simulate possible scenarios of abusive conditions during transport (groups B, D, and I) are shown in Figure 2a. The superchilling process effectively cooled the fillets, and much lower initial temperature was observed (–1.0 °C to –1.4 °C) than for the traditionally processed groups (Figure 1).

The shelf life, determined by estimating the day of sensory rejection (Torry score of 5.5) from the sensory data (Figure 2b and 3b), was much shorter for the traditionally processed groups that were severely temperature abused (8 and 10.5 d for groups D and I, respectively), compared with the corresponding groups (A and H from batches 1 and 3) stored continuously at the same temperature with a shelf life of 12 and 13.5 d, respectively (Figure 1). The extended shelf life of group H was due to the lower initial temperature of batch 3 (Figure 1) compared with batch 1 because of the initial cooling of the fillets. It should also be pointed out that the processes differed in the initial handling because batch 3 was processed 1 d after catch, while batch 1 had been stored whole on ice for 3 d onboard the trawler before processing. The average storage temperature of groups A and B was similar, but the temperature abuse of group B (16 °C for 8 h) (Figure 2a) resulted in its slightly more rapid quality deterioration, as seen by a lower Torry score ($P < 0.05$) on day 7 compared with group A (7.6 and 8.0, respectively). However, the resulting shelf life was similar (11.5 to 12 d) for both groups (Figure 2b). Similar shelf life of 10 to 12 d was reported in an earlier study on conventionally processed cod fillets, filleted

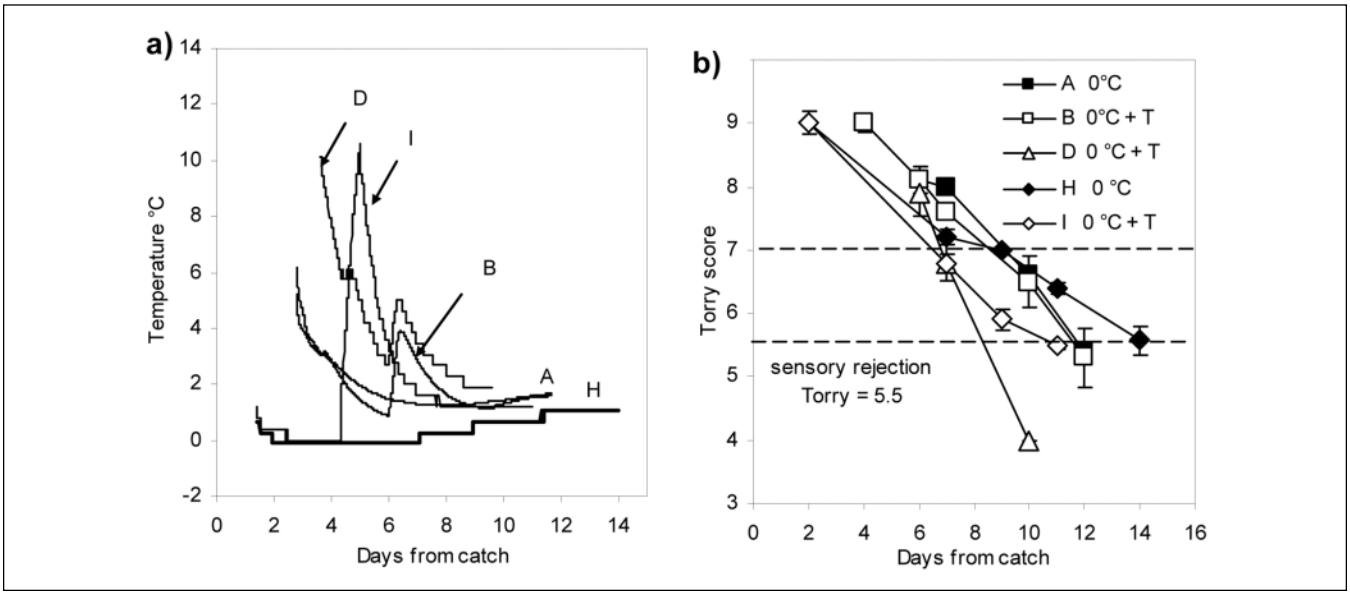


**Figure 2—(a) Temperature profiles of sample groups A, B, and D (batch 1); H and I (batch 3); (b) sensory analysis (Torry score) of cod fillets from storage studies of traditionally processed and temperature abused experimental groups from factory I (A = 0 °C [-■-]; B = 0 °C + post processing abuse [-□-]; D = 0 °C + pre- and post-processing abuse [-△-] and experimental groups from factory II (H 0 °C (-◆-); I = 0 °C + abused [-◇-]). Open data points indicate temperature abuse during storage and bars show the standard deviation. Dotted lines indicate limit of sensory rejection (Torry score = 5.5) and marketable shelf life (Torry score = 7.0).**

1 d after catch or a total shelf life of 11 to 13 d post catch (Magnússon and Martinsdóttir 1995).

The superchilling process (CBC) extended the shelf life of batch 1 slightly by 0.5 d (12.5 d for group C), while the 2nd trial (batch 2: groups E, F, and G) demonstrated that further storage of CBC-processed fillets under superchilled conditions (–1.5 °C) was most effective in prolonging shelf life, resulting in a shelf life for groups G and F of about 15 d compared with groups C and E with a shelf life of 12.5 and 14.5 d, respectively (Figure 1). Additional sensory analysis was performed (data not shown) to evaluate the texture of the superchilled fillets. Only little difference was observed between groups except on day 8 when the fillets stored at –1.5 °C (group F) exhibited softer texture ($P < 0.05$) than fillets stored at 0.5 °C (group E).

## Extension of marketable shelf life and effect of processing and storage techniques

It is of interest to compare the actual time after catch of the fillets when the sensory score was 7 because this score is more realistic to evaluate the marketability of the fillets (Figure 2b and 3b). At this stage (Torry score = 7), the fillets are described as neutral in odor and taste and have lost their characteristic initial fresh sweet taste (Shewan and others 1953). Shelf life extension of fresh fish can be reached by different processing and/or storage methods, but usually the neutral phase is extended rather than the earlier stage where freshness characteristics are important. It is therefore important to evaluate whether superchilling processing and storage is extending this earlier phase because this would provide an improved market value. For batches 1 and 3, the traditionally processed fillets had reached score 7 on day 9 (groups A and H) while the CBC-processed fillets (groups F and G) reached this score on day 11. Further storage of the superchilled (CBC) processed fillets resulted in a total shelf life of 12.5 d for group C and 14 d for group E (0.5 °C storage), while groups stored at –1.5 °C (F and G) had total shelf life of at least 15 d. This shows that CBC-processed fillets will have an extended marketable shelf life and storage under superchilled conditions (–1.5 °C) will prolong the time of marketability, providing the retailers maintain this low temperature. This could contribute to improved quality of fillets for consumers in distant markets.

## Microbial analysis

The initial microbiological quality of the raw material of the different batches varied when evaluated on the 1st sampling day, 1 d after packing in styrofoam boxes (Table 1). The initial total viable psychrotrophic counts (TVC) were lowest (3.9 log colony-forming units [CFU]/g) for traditionally processed fillets (batch 1, Factory I) and highest for the CBC-processed fillets in batch 2 (5 log CFU/g). A higher TVC (4.8 log CFU /g) was also observed for CBC-processed fillets (group C) from batch 1 compared with traditionally processed groups, which could be related to poorer hygienic handling of the former (contaminated chilling brine or processing equipment) as well as to the fact that the traditionally prepared fillets underwent a rinsing step while being skinned mechanically, hence slightly decontaminated. Also, a high TVC was observed for batch 2 although the raw material was processed 1 d after catch whereas batch 1 was processed 3 d after catch. It should be pointed out that because of the continuing process in the factory, an older batch had been processed earlier the same day as the experiment was carried out with batch 2. Apparently, the processing lines were not cleaned appropriately before the experimental groups were processed and therefore cross-contamination from the older raw material may have occurred. This was unfortunate, but emphasizes the need for thorough cleaning procedures between processing different batches. This may have influenced the outcome of the study, resulting in a shorter shelf life of the superchilled experimental groups than could have been obtained if hygienic conditions would have been optimal. These results emphasize the importance of studies performed with natural products and processes to accumulate information about the spoilage domain of SSOs, such as pseudomonads, $H_2S$-producers, and *P. phosphoreum* under actual conditions. As an example, the high TVC values initially, possibly caused by cross-contamination from older to newer raw material via uncleaned processing equipment, may have contributed to the establishment and further proliferation of $H_2S$-producing bacteria on CBC-processed fillets (batch 2).

Initial counts of SSOs showed that slightly higher levels of $H_2S$-producing bacteria and *P. phosphoreum* (*Pp*) were found in batch 1 (3 d post catch) than batch 2 (1 d post catch) and that batch 3 (1 d post catch) had the lowest counts of all (Table 1). Pseudomonads counts were found in highest numbers on the 1st sampling day for all batches. Comparison of bacterial development under normal chilled conditions (Figure 4a and 4b) explains the shorter shelf life of group A than group H, as best demonstrated by the maximum specific growth rate ($\mu_{max}$) of *Pp* being higher for A (0.377/h after a lag phase of 60 h) than H (0.055/h) (Table 2), probably due to higher initial *Pp* load (Figure 4b) and higher temperature of processed fillets for batch 1 (Figure 2a). Abusive temperature conditions (groups B, D, and I) triggered all SSOs evaluated as seen by high $\mu_{max}$ for *Pseudomonas* spp, *S. putrefaciens*, and *P phosphoreum* (Table 2). *P*ost-processing temperature abuse usually led to a lag phase (32 to 60 h) of SSOs before rapid growth, showing that such undesirable environmental conditions affected the physiological state of bacterial groups. This behavior could be attributed to the fact that bacteria need to readjust to current environmental conditions, independent to how beneficial these are to their development. Under abusive conditions (8 and 16 h at RT), *Pp* was the fastest-developing SSO (groups B and I: 0.153 and 0.442/h) and the longer abusive treatment (I) affected its growth rate tremendously. Similarly, the maximum specific growth rate of $H_2S$-producing bacteria was much higher after having undergone the longer treatment, doubling in value (groups B and I: 0.062 and 0.124/h). However, similar growth rates were observed for pseudomonads following either treatment (groups B and I: 0.085 and 0.089/h). Dominance of *Pp* (7.2 to 7.8 log CFU/g) over other SSOs was usually observed under chilled and abused conditions, representing 12.6% to 17.2% (A and H) and 16.2%, 24.0%, and 12.7% of the total microflora (B, I, and D, respectively). *Pp* load was considerable in group D (6.0 log CFU/g, representing about 10% of microflora) on the 1st sampling day (day 6) compared with that of group B (2.7 log CFU/g) analyzed on day 4 (Table 1). The apparent slower growth rate observed for group D (Table 2) reflected the declining growth during late exponential growth phase while approaching the stationary phase (Figure 4b). Extreme temperature abuse (groups I and D) obviously stimulated $H_2S$-producing bacteria over pseudomonads, as seen by a faster growth rate and the levels reached by the former (6.2 log CFU/g) compared with the latter (4.4 log CFU/g) on the last sampling day (group D). Interestingly, dominance of pseudomonads was only observed in spoiled fillets originating from the factory II (groups H and I, batch 3) where cod was caught by longline in the Southwest of Iceland. The predominance of pseudomonads may be related to the catching method and the raw material handling and can possibly be explained by the iron-binding capacity of the pseudomonads siderophores (Gram and Dalgaard 2002), while the *Shewanella putrefaciens* ($H_2S$-producer) may be favored by conditions associated with bottom trawl and possibly more damaged fish, that is, muscle with blood (rich in iron) as was noticed in batch 1.

High counts of pseudomonads in fillets from batch 3 are in agreement with recent studies on haddock fillets from the same factory (Olafsdottir and others 2005). In that study, *Pp* was the predominant

**Table 1—Overview of average storage temperature, shelf life estimation, and experimental data comparing initial (1st sampling day) and final values measured at or near sensory rejection for all experimental groups of cod fillets stored in styrofoam boxes at different temperatures**

| | Initial values[a] | | | Data at sensory rejection | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Traditional process and temperature abuse | | | | | Superchilling process | | | |
| | Batch 1 (A, B, C, D) | Batch 2 (E, F, G) | Batch 3 (H, I) | A 0 °C | B 0 °C + T | D 0 °C + T | H 0 °C | I 0 °C + T | C 0 °C | E 0.5 °C | F –1.5 °C | G –1.5/0.5 °C |
| Average temp of fillets during storage | | | | 1.9 ± 0.9 | 2.0 ± 0.9 | 3.9 ± 1.9 | 0.4 ± 0.5 | 1.9 ± 2.3 | –0.9 ± 0.6 | –0.3 ± 0.5 | –1.3 ± 0.0 | –0.8 ± 0.7 |
| Estimated shelf life (days)[b] | | | | 12.0 | 11.5 | 8.0 | 13.5 | 10.5 | 12.5 | 14.0 | >15.0 | 15.0 |
| Estimated shelf life (days)[c] | | | | 11.0 | 10.2 | 7.0 | >15.0 | 11.5 | 11.0 | 12.0 | 15.0 | 15.0 |
| Measurement d of data | | | | 12.0 | 12.0 | 8.0[d] | 14.0 | 11.0 | 12.0 15.0 | 15.0 | 15.0 | |
| Sensory analysis (Torry score) | 9.0 ± 0.2 | 8.6 ± 0.1 | 9.0 ± 0.2 | 5.4 ± 0.2 | 5.3 ± 0.5 | 4.0 ± 0.1[d] | 5.6 ± 0.2 | 5.5 ± 0.1 | 5.9 ± 0.4 | 5.4 ± 0.4 | 5.7 ± 0.2 | 5.6 ± 0.5 |
| Total viable counts (TVC) (log10 colony-forming units CFU/g) | 3.9 ± 0.0 (A,B)<br>4.8 ± 0.0 (C)<br>7.0 ± 0.1 (D) | 5.0 ± 0.2 | 4.2 ± 0.1 | 8.1 ± 0.1 | 8.0 ± 0.1 | 7.8 ± 0.1 | 8.0 ± 0.1 | 8.0 ± 0.1 | 7.9 ± 0.1 | 8.0 ± 0.1 | 8.0 ± 0.1 | 8.1 ± 0.5 |
| $H_2S$-producer counts | 2.8 ± 0.1 (A,B)<br>3.0 ± 0.0 (C)<br>3.4 ± 0.5 (D) | 2.3 ± 0.6 | 2.3 ± 0.7 | 7.0 ± 0.2 | 6.1 ± 0.1 | 6.2 ± 0.2 | 7.1 ± 0.0 | 6.6 ± 0.0 | 5.9 ± 0.4 | 7.4 ± 0.1 | 7.6 ± 0.2 | 7.5 ± 0.7 |
| % $H_2S$-producers/TVC | | | | 7.0% | 1.4% | 2.6% | 12.9% | 3.7% | 1.0% | 25.1% | 36.3% | 24.0% |
| *Pseudomonas* counts | 3.1 ± 0.3 (A,B)<br>3.5 ± 0.1 (C)<br>3.8 ± 0.0 (D) | 3.4 ± 0.3 | 2.8 ± 0.1 | 6.6 ± 0.6 | 6.4 ± 0.4 | 4.4 ± 0.1 | 7.5 ± 0.3 | 7.4 ± 0.1 | 5.8 ± 0.5 | 6.4 ± 0.1 | 6.5 ± 0.2 | 6.5 ± 0.4 |
| % *Pseudomonas* spp./TVC | | | | 4.9% | 3.0% | 0.04% | 37.5% | 25.4% | 0.2% | 2.5% | 2,9% | 2,4% |
| *P. phosphoreum* counts | 2.7 ± 0.4 (A,B)<br>3.1 ± 0.3 (C)<br>6.0 ± 0.3 (D) | 2.2 ± 0.7 | 1.3 ± 0.5 | 7.2 ± 0.1 | 7.2 ± 0.1 | 7.1 ± 0.4 | 7.2 ± 0.1 | 7.4 ± 0.1 | 6.8 ± 0.2 | 6.8 ± 0.1 | 6.0 ± 0.1 | 6.7 ± 0.1 |
| % *P. phosphoreum*/TVC | | | | 12.6% | 16.2% | 12.7% | 17.2% | 24.0% | 6.7% | 6.3% | 0.9% | 3.8 |
| Total volatile basic nitrogen (TVB-N) (mg N/100 g) | 12.2 ± 0.3 (A,B)<br>12.4 ± 1.2 (C)<br>18.0 ± 1.4 (D) | 12.1 ± 0.5 | 12.4 ± 0.9 | 48.5 ± 0.9 | 47.5 ± 0.1 | 94.2 ± 15.3[d] | 26.6 ± 0.6 | 33.6 ± 5.3 | 53.4 ± 0.4 | 55.8 ± 4.7 | 38.4 ± 5.0 | 38.7 ± 1.6 |
| pH | 6.70 ± 0.01 (A,B)<br>6.58 ± 0.06 (C)<br>6.55 ± 0.11 (D) | 6.78 ± 0.13 | 6.90 ± 0.03 | 6.85 ± 0.09 | 6.73 ± 0.04 | 6.85 ± 0.05 | 6.86 ± 0.03 | 6.91 ± 0.08 | 7.00 ± 0.04 | 6.91 ± 0.01 | 6.92 ± 0.06 | 6.98 ± 0.02 |
| CO sensor (nA) | 37 ± 5 (A,B)<br>42 ± 2 (C)<br>232 ± 70 (D) | 34 ± 20 | 14 ± 5 | 366 ± 72 | 439 ± 165 | 215 ± 65 | 98 ± 29 | 253 ± 22 | 158 ± 89 | 300 ± 156 | 136 ± 43 | 225 ± 108 |
| NH3 sensor (nA) | <10 | <10 | <10 | <10 | 23 ± 6 | 34 ± 13 | <10 | 33 ± 8 | 52 ± 13 | <10 | <10 | <10 |
| H2S sensor (nA) | <20 | <20 | <20 | <20 | 21 ± 24 | 20 ± 6 | 35 ± 8 | 22 ± 11 | <20 | 20 ± 3 | 28 ± 33 | <20 |

[a]Initial values on 1st d of sampling (days after catch): Groups A, B, C (day 4), group D (day 6), goups E, F, G, H, and I (day 2).
[b]Total shelf life, including days from catch, based on the sensory evaluation of cooked fish (Torry score = 5.5).
[c]Total shelf life, including days from catch, based on total volatile basic nitrogen [TVB-N] = 35 mg N/g.
[d]Values for sensory and TVB-N data for group D from measurements on day 10.

**Table 2—Maximum specific growth rate (/h) of different bacterial groups (*Pseudomonas* spp., *Photobacterium phosphoreum*, $H_2S$-producing bacteria) assessed by curve fitting using DMFit in cod fillets stored in styrofoam boxes under different temperature conditions**

| | Traditional process and temperature abuse | | | | | Superchilling process | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A 0 °C | B 0 °C + T | D 0 °C + | H 0 °C | I 0 °C + | C 0 °C | E 0.5 °C | F –1.5 °C | G –1.5/0.5 °C |
| Total viable counts (TVC) | 0.063 | 0.117 (28.4 h)[a] | 0.028 | 0.034 | 0.114 (49.9 h)[a] | 0.035 | 0.035 | 0.031 (48.9 h)[a] | 0.024 |
| *Pseudomonas* spp | 0.039 | 0.085 (38.8 h)[a] | 0.040 | 0.039 | 0.089 (32.1 h)[a] | 0.022 | 0.031 | 0.026 (29.5 h)[a] | 0.024 |
| $H_2S$ producers | 0.039 | 0.062 | 0.079 | 0.043 | 0.124 (59.5 h)[a] | 0.030 | 0.043 | 0.034 (38.9 h)[a] | 0.042 |
| *P. phosphoreum* | 0.377 (60.1 h)[a] | 0.153 (44.2 h)[a] | 0.035 | 0.055 | 0.442 (52.9 h)[a] | 0.042 | 0.072 (75.2 h)[a] | 0.059 (105.6 h)[a] | 0.034 |

[a]Estimated lag phase (h).
[b]High initial counts.

SSO based on their counts, but the characteristic sweet, fruity spoilage odors were associated with the growth of *Pseudomonas* spp. The study of Jørgensen and Huss (1989) showed on the contrary that $H_2S$-producers were the main SSO in aerobically stored cod fillets from different origin, but this can be explained by the different conditions in the factories because the origin of the $H_2S$ producers is mainly from the filleting plants (Van Spreekens and Toepoel 1981).

The CBC-superchilling process was efficient in cooling the newly processed fillets (groups C, E, F, and G) (Figure 3a), resulting in slower bacterial development (Figures 5; Table 2), which was further decreased under superchilled storage (groups F and G). The total superchilling effect of each group, ranking the most to the least as F, G, C, and E, is illustrated in Figure 1 and reflected by the corresponding *Pp* growth rates of 0.059 (lag phase of 105.6 h), 0.034, 0.042, 0.072/h (lag phase of 75.2 h), respectively (Table 2), exemplifying the slower growth of *Pp* caused by the degree of superchilling. *Pp* had therefore reached much lower counts (6.0 to 6.8 log CFU/g) than in traditionally processed fillets (7.2 log CFU/g) at sensory rejection (Table 1). This difference is probably explained by the sensitivity of this bacterium to sudden cooling/freezing, which can lead to a cold shock, damaging the cells and resulting in slower initial growth. An increased growth rate of *P. phosphoreum* was observed for group G between d 10 and 12 after the group had been transferred to higher storage temperature (Figure 5b).

Pseudomonads had the slowest growth rates (Table 2) and reached similarly low levels at sensory rejection (Table 1), indicating their sensitivity to superchilled conditions. $H_2S$-producing bacteria grew quite steadily in fillets from batch 2 (Figure 5d) and dominated (24% to 36%) other SSOs (Table 1). However, it cannot be confirmed whether this was due to the effect of the superchilling process on other SSOs, which may have decreased competition for $H_2S$-producing bacteria or to the cross-contamination from the older raw material occurring during processing. $H_2S$-producing bacteria were found at lower levels (5.9 log CFU/g) in superchilled fillets of batch 1 (group C), but in batch 2 the $H_2S$-producing bacteria appeared to tolerate well the superchilling conditions as discussed above.

## TVB-N analysis

The formation of TVB-N was delayed in the superchilled groups (Figure 6b) compared with the traditionally processed groups (Figure 6a). At sensory rejection different levels of TVB-N were found in the experimental groups (38 to 55 mg N/100 g) (Table 1). The highest TVB-N value (94 mg N/100 g) was measured for the severely abused group D (Table 1), but this value is overestimated because the measurement was performed on day 10 while the estimated value at sensory rejection (day 8) seen in Figure 6a is about 35 mg N/100 g. The influence of temperature abuse (group B) was seen as a more rapid production of TVB-N compared with samples stored constantly at low temperature from the same batch (group A). Similarly, higher TVB-N values were observed for the temperature

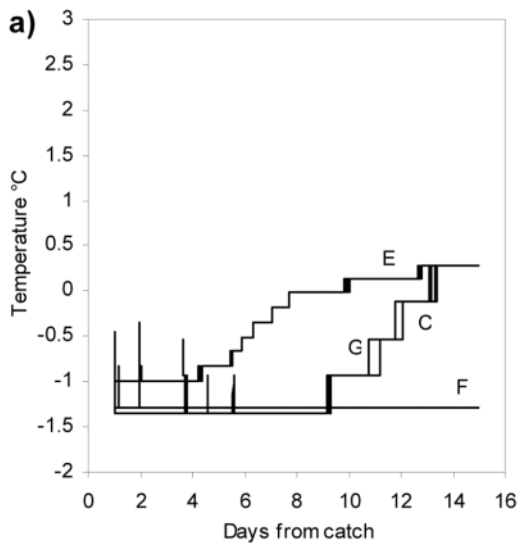


**Figure 3—(a) Temperature profiles of sample groups E, F, and G (batch 2) and C (batch 1) and (b) sensory analysis (Torry score) of cod fillets from superchilled experimental groups in factory I: C = 0 °C new (-■-) and E = 0.5 °C (-▲-); F = –1.5 °C (-●-); G = –1.5 °C / 0.5 °C (-○-). Dotted lines indicate limit of sensory rejection (Torry score = 5.5) and marketable shelf life (Torry score = 7.0) and bars show the standard deviation.**

abused group I compared with group H from the same batch, which was stored constantly at low temperature (Figure 6a).

The highest TVB-N values at sensory rejection for the superchilled groups (Figure 6b) were observed in groups C and E that were stored at 0.5 °C. Both $H_2S$-producing bacteria and *P. phosphoreum* are known to be able to produce TMA (Van Spreekens 1974; Jørgensen and others 1988; Dalgaard 1995). The higher levels and more rapid growth of *P. phosphoreum* in groups C and E stored at 0.5 °C compared with groups G and F stored at –1.5 °C (Figure 5b), suggest their importance in contributing to higher TVB-N values for groups C and E (53 and 56 mg N/100 g, respectively) compared with groups G and F stored at –1.5 °C (approximately 38 mg/100 g) (Table 1).

Based on the findings of Dalgaard (1995) that *P. phosphoreum* was a 30 times more active TMA producer than *S. putrefaciens* and cell counts of $10^7$ CFU/g of *P. phosphoreum* corresponded to 30 mg N/100 g TMA in packed cod fillets, it is likely that *P. phosphoreum* is very important in the formation of TVB-N at least in the traditionally processed groups where *P. phosphoreum* dominated and reached the highest counts of $>10^7$ CFU/g at sensory rejection. However, based on their lower counts in the superchilled groups (6.0 to 6.8 log CFU/g) (Table 1), the role of the $H_2S$-producers in contributing to TVB-N cannot be overlooked because their counts reached $>10^7$ CFU/g in the groups E, F, and G. According to Jørgensen and Huss (1989) in a study with fish juice inoculated with *S. putrefaciens* and Dalgaard (1995) who studied TMA production in packed cod, TMA was detected when counts of *S. putrefaciens* were about $10^7$ CFU/g and off odors when counts reached $10^8$ CFU/g. Similarily, Koutsoumanis and Nychas (2000) reported that higher counts ($10^8$ CFU/g) were observed in sterile fish inoculated with *S. putrefaciens* when TVB-N started to increase, followed by off odors detected by the sensory panel.

It has been pointed out that TVB-N and TMA often give ambiguous information about the quality of the products and levels only increase at late storage when spoilage signs are obvious (Oehlenschläger 1998). Varying levels of TVB-N have been found at sensory rejection in whole cod and vacuum packed fillets (Jørgensen and others 1988). TVB-N levels are also influenced by the storage method (Magnússon and Martinsdóttir 1995; Debevere and Boskou 1996; Guldager and others 1998; Lauzon and others 2002).

Despite this controversy, fixed TVB-N limit (35 mg N/100 g) for acceptability of consumption for gadoids as a confirmation of a prior sensory assessment have been set in EU regulations (European Union1995). Based on these limits, the shelf life of the experimental

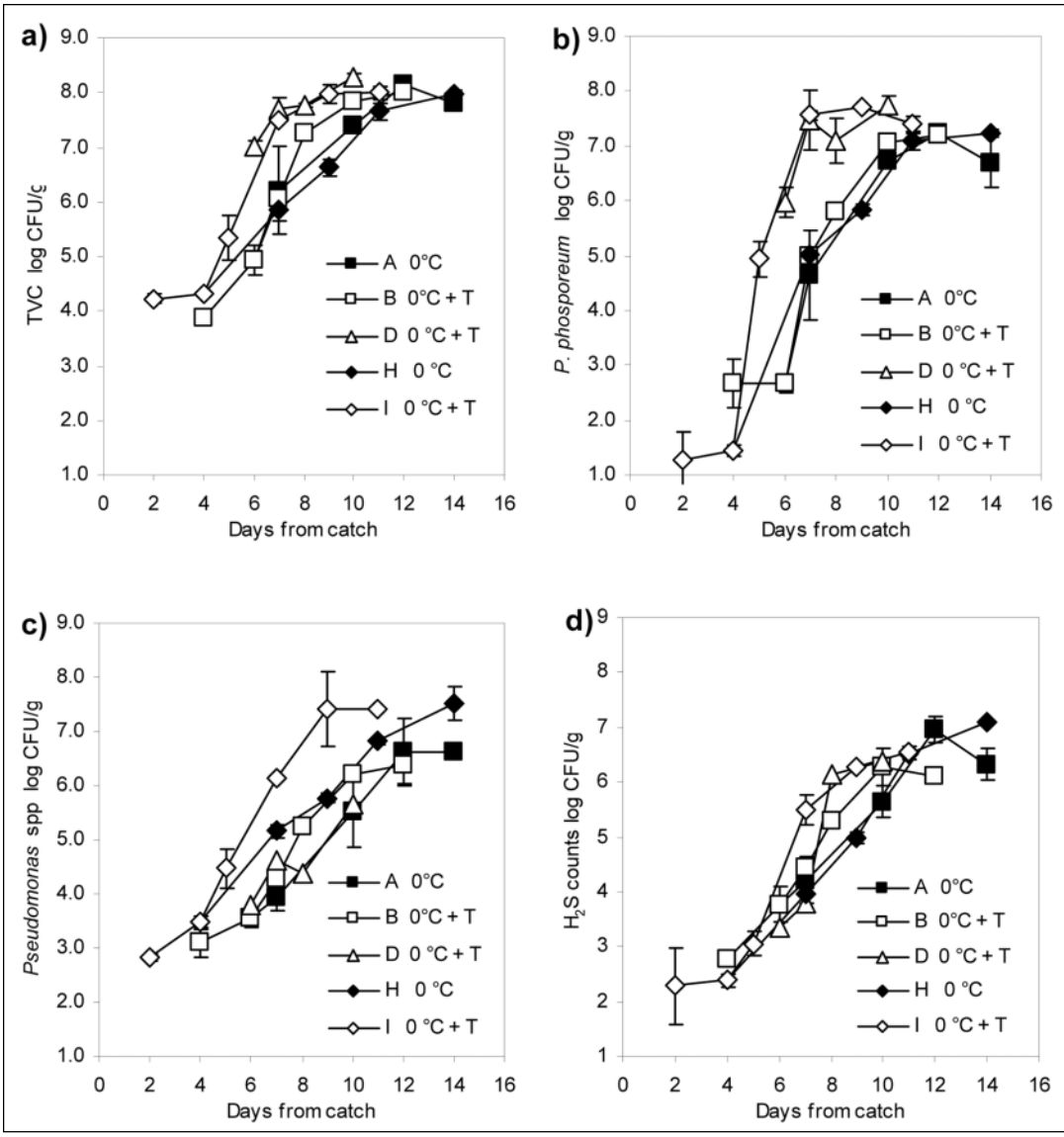


**Figure 4—Counts of (a) total viable psychrotrophic bacteria (TVC), (b) *Photobacterium phosphoreum* (Pp), (c) presumptive *Pseudomonas* spp., and (d) $H_2S$-producing bacteria in traditionally processed and temperature-abused experimental groups of cod fillets stored in styrofoam boxes: A = 0 °C (-■-), B = 0 °C + post processing abuse (-□-), and D = 0 °C + pre- and post-processing abuse (-△-) from batch 1. Groups H = 0 °C (-◆-) and I = 0 °C + abused (-◇-) from batch 3. Open data points indicate temperature abuse during storage and bars show the standard deviation.**

groups was evaluated (Figure 6). A slightly shorter shelf life was estimated than when using the sensory Torry score criteria of 5.5, for the traditionally processed and temperature abused experimental groups A, B, and D from batch 1 and the superchilled groups C and E. The estimated shelf life based on TVB-N criteria for the superchilled groups F and G was in agreement with shelf life according to the sensory rejection limit (Table 1). On the other hand, longer shelf life was estimated for sample groups H and I processed from batch 3 and they had not exceeded the TVB-N limit of 35 mg N/100 g at sensory rejection. Interestingly, the predominance of *Pseudomonas* spp. (7.4 to 7.5 log CFU/g) in these groups may explain the lower level of TVB-N because pseudomonads do not produce TMA (Castell and others 1959). However, considerable levels of TVB-N (27 to 34 mg N/100 g) were found at sensory rejection which can be accounted for by the high levels of *P. phosphoreum* or 17% to 24% of the psychrotrophic counts, although the pseudomonads were dominating (25% to 38%). Koutsoumanis and Nychas (2000) suggested pseudomonads level of $10^7$ CFU/g as a limit for the end of shelf life coinciding with a TVB-N level of about 26 mg N/g in a Mediterranean species.

## pH measurements

The effect of the different catching techniques and time elapsed from catching until processing is seen by the difference in the initial pH value (Table 1). Higher pH value ($P < 0.05$) was observed for batch 3 than batch 1 initially (6.90 and 6.70, respectively); this may be explained by the different catching methods, longline and bottom trawl, respectively, influencing the rate and the extent of the glycolytic changes and the resulting pH. Similar difference in pH for cod was observed for longline and gillnet by Esaiassen and others (2004) showing that initial pH for longline was higher than for gillnet. The age of the raw material when processed can also explain the difference in initial pH measured because of the autolytic postmortem changes (Huss 1995). Therefore, the lower pH value for batch 1 (6.70) can also be related to the fact that it was processed 3 d post catch while batch 3 was processed 1 d post catch (6.90). The difference in the initial pH value because of the effect of different catching methods is a drawback when using pH to monitor quality. However, changes are seen with time in some groups although the end value is not the same at sensory rejection. Increase in pH values ($P < 0.05$) were noticed with storage time for sample groups A,

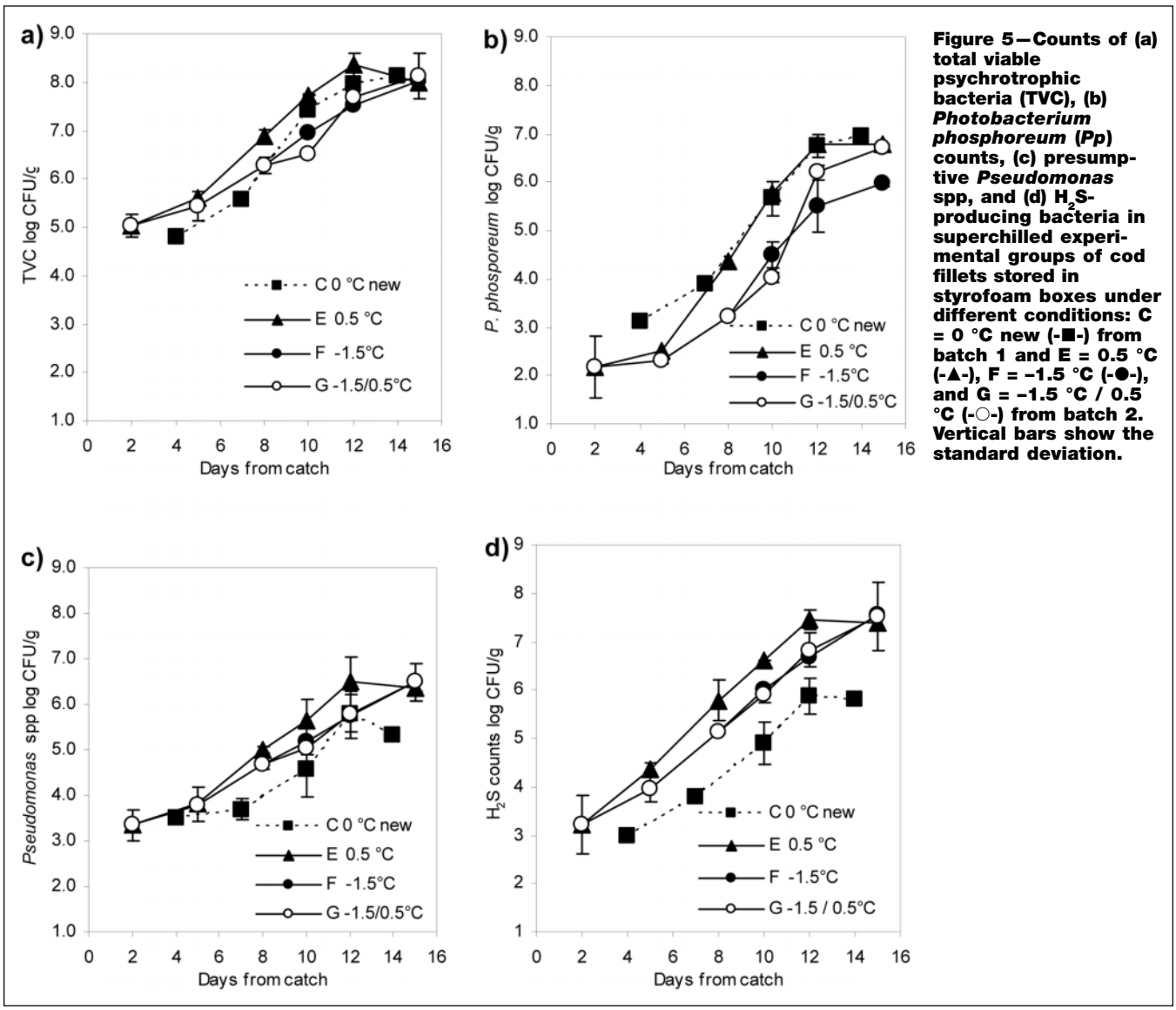


**Figure 5—Counts of (a) total viable psychrotrophic bacteria (TVC), (b) *Photobacterium phosphoreum* (*Pp*) counts, (c) presumptive *Pseudomonas* spp, and (d) $H_2S$-producing bacteria in superchilled experimental groups of cod fillets stored in styrofoam boxes under different conditions: C = 0 °C new (-■-) from batch 1 and E = 0.5 °C (-▲-), F = –1.5 °C (-●-), and G = –1.5 °C / 0.5 °C (-○-) from batch 2. Vertical bars show the standard deviation.**

D, the superchilled group C from batch 1, and sample group G from batch 3 (Table 1). The initial pH value of group D measured on day 6 post catch was the lowest (6.55), possibly because of the extent of the autolytic changes under high temperature storage (Huss 1995). A lower initial pH for group C (6.58) compared with the traditionally processed groups may be related to the chilling process. The highest increase in pH with storage time was observed for groups C and D with the lowest initial pH.

## Electronic nose measurements

**CO sensor—detection of alcohols, aldehydes, and esters.** Spoilage of the fillets was characterized by higher response of the CO sensor than the other sensors (Figure 7). The CO sensor can detect alcohols, aldehydes, and esters (Olafsdottir and others 2002), which are common metabolites of spoilage bacteria like *Pseudomonas* spp. (Miller and others 1973a, 1973b). Increase in CO sensor response was noticed earlier for the traditionally processed (Figure 7a) groups than for the superchilled groups (Figure 7b) showing that the spoilage process was slower at lower temperature, which was in agreement with the other data. Increased CO response was 1st observed for the temperature-abused groups D and I, followed by group B (seen as open data points in Figure 7a) in agreement with the TVB-N analysis. The superchilled group F had the lowest CO response (Figure 7b) suggesting the slowest spoilage rate for that group. The influence of higher temperature storage of group G is seen as slightly higher CO response on day 12 in agreement with microbial analysis, in particular *P. phosphoreum*, showing increasing counts (Figure 5b) and TVB-N measurements (Figure 6b).

The fact that the CO sensor responses correlated well with the TVB-N values for both the traditionally processed groups ($r^2 = 0.87$) and the superchilled groups ($r^2 = 0.83$) indicates that TMA and metabolites like alcohols and aldehydes were being produced simultaneously. Based on the higher counts of the *P. phosphoreum* and $H_2S$-producing bacteria (Figure 4 d) it is likely that these bacteria could be contributing to the development of metabolites that the CO sensor can detect. *S. putrefaciens* is known to produce alco-

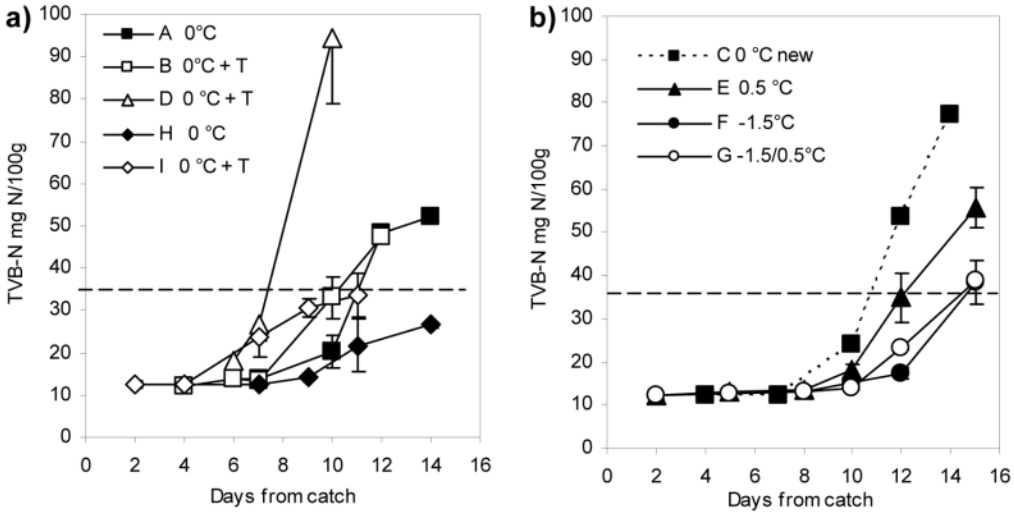


**Figure 6—Total volatile basic nitrogen (TVB-N) values in (a) traditionally processed and temperature abused experimental groups: A = 0 °C (-■-), B = 0 °C + post processing abuse (-□-), D = 0 °C + pre- and post processing abuse (-△-), H = 0 °C (-◆-), and I = 0 °C + abused (-◇-). (b) superchilled experimental groups: C = 0 °C (-■-), E = 0.5 °C (-▲-), F = –1.5 °C (-○-), and G = –1.5 °C / 0.5 °C (-●-). Open data points indicate temperature abuse during storage and bars show the standard deviation. Dotted line indicates the limit of acceptability TVB-N = 35 mg N/100 g.**

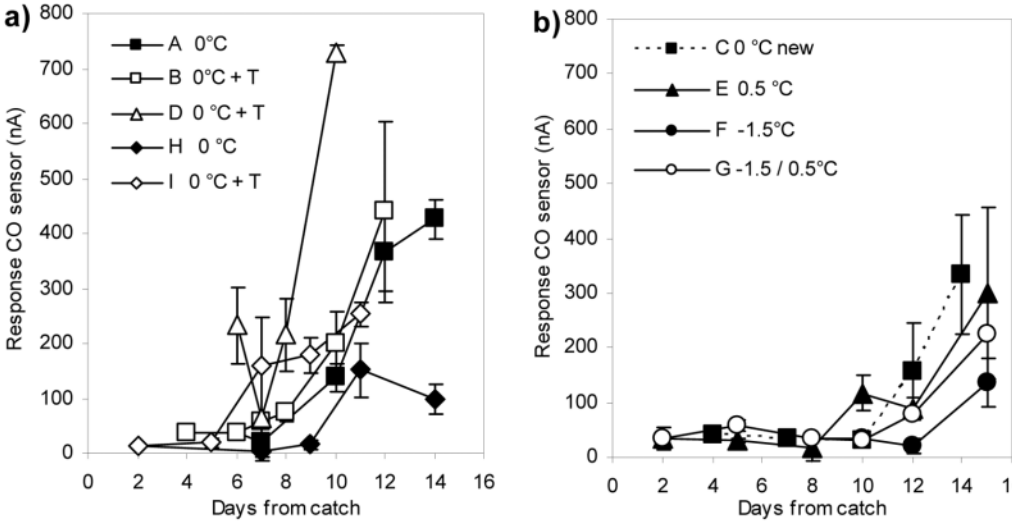


**Figure 7—Electronic nose CO sensor response (a) in traditionally processed and temperature abused experimental groups: A = 0 °C (-■-), B = 0 °C + post-processing abuse (-□-), and D = 0 °C + pre- and post-processing abuse (-△-) from batch 1. H = 0 °C (-◆-) and I = 0 °C a + abused (-◇-) from batch 3. (b) In superchilled experimental groups: C = 0 °C (-■-) from batch 1 and E = 0.5 °C (-▲-), F = –1.5 °C (-○-), and G = –1.5 °C / 0.5 °C (-●-) from batch 2. Open data points indicate temperature abuse during storage and bars show the standard deviation.**

hols like 3-methyl-butanol (Miller and others 1973b) and could contribute to the CO sensor response. It is likely that other volatile compounds not detected by the sensors may also contribute to the spoilage of the samples, in particular it should be mentioned that the CO sensor can not detect ketones, like acetoin, which is produced by *Photobacterium* spp. in fish during storage (Van Spreekens and Toepoel 1981). Large standard deviation observed for the CO sensor is explained by the effect of the varying temperature (8 °C to 12 °C) during measurements of samples influencing the volatility of the compounds measured and causing deviations.

**$NH_3$ sensor—detection of TMA and $NH_3$.** At the end of the storage time, increasing response was observed for the $NH_3$ sensor for sample groups B, D, and I stored under abusive temperature conditions and the superchilled group C (Table 1). The response of the $NH_3$ sensor was higher ($P < 0.05$) for the superchilled group C than the traditionally processed group A from the same batch, although the overall spoilage appeared to be slower in C. As discussed before, it is likely that *P. phosphoreum*, which is known to be an active TMA producer (Van Spreekens, 1974; Dalgaard 1995), may be contributing to the high TVB-N content and $NH_3$ sensor response under superchilled conditions. Offensive ammonia-like, dried fish odor was observed at sensory rejection of the superchilled fillets, but when tasted the spoilage was less noticeable indicating that odor and volatile compounds were probably limiting their shelf life.

**$H_2S$ and $SO_2$ sensors—detection of sulfur compounds.** Very low response of the $H_2S$ and $SO_2$ sensors were observed in all samples but slight increase was observed for the $H_2S$ sensor at the end of the storage time for traditionally processed and abused sample groups B, D, H, and I although not significant (Table 1). This suggests the influence of $H_2S$-producing bacteria under abusive temperature conditions as reported by Lauzon (2000). Slightly higher $H_2S$ sensor values were also noticed in the superchilled groups E and F, which can be explained by dominance of the $H_2S$-producers in these groups (25% and 36%, respectively) (Table 1). At advanced spoilage of whole fish on ice, onion-like, sulfide and rotten vegetable-like, putrid spoilage odors develop because of microbially produced sulfur compounds (Shewan and others 1953; Miller 1973a; Herbert and others 1975). Earlier studies using the FreshSense electronic nose have shown increasing response of $H_2S$ and $SO_2$ sensors at advanced spoilage of whole capelin and redfish (Olafsdottir and others 2000, 2002), but response to fillets of cod and haddock is generally much lower (Tryggvadóttir and others 2001). The overall insignificant response of the $SO_2$ sensor indicated that sulfides were not present or in very low levels in the samples, below the detection limit of the sensors (data not shown). Based on the low responses of the $H_2S$ and $SO_2$ sensors in this study, it appears that volatile sulfur compounds do not contribute significantly to the development of off-odors of fillets at sensory rejection.

## Correlation of microbial counts,TVB-N, pH, and electronic nose sensors and prediction of sensory quality

Partial least squares regression (PLSR) was used to study the correlation of the variables and their contribution to predict the Torry sensory score (Figure 8). A PLSR model based on all the samples ($N = 41$) and all the measured variables had a high correlation ($r^2 = 0.97$) with a root mean square error of prediction (RMSEP) of 0.29 (Table 3). Because of the low responses of the $NH_3$, $H_2S$, and $SO_2$ sensors, they explained less of the variance in the data than the other variables, as seen by their location in the middle of the plot (Figure 8). The same was observed for pH because the influence of the different initial pH values of the samples precludes the use of pH as a spoilage indicator as explained above. The contribution of the significant ($P < 0.05$) variables (TVC, SSO, TVB-N and CO sensor) to predict the sensory score was studied further by exploring different PLSR models (Table 3). A model based on the microbial variables had high correlation ($r^2 = 0.96$) and low prediction error (RMSE$P = 0.32$) indicating that microbial counts were well suited to predict the sensory quality of the overall data set. This was expected because the growth of the SSO characterized the spoilage pattern of the samples. A model based on the classical spoilage indicators TVC, TVB-N and pH had a lower correlation ($r^2 = 0.92$) and higher error (RMSE$P = .41$), indicating that the SSO gave better information about the sensory quality. PLSR model based on the electronic nose variables was inferior with much lower correlation ($r^2 = 0.70$) and higher error (RMSEP = 0.80).

Because of the significance of the CO sensor variable to predict

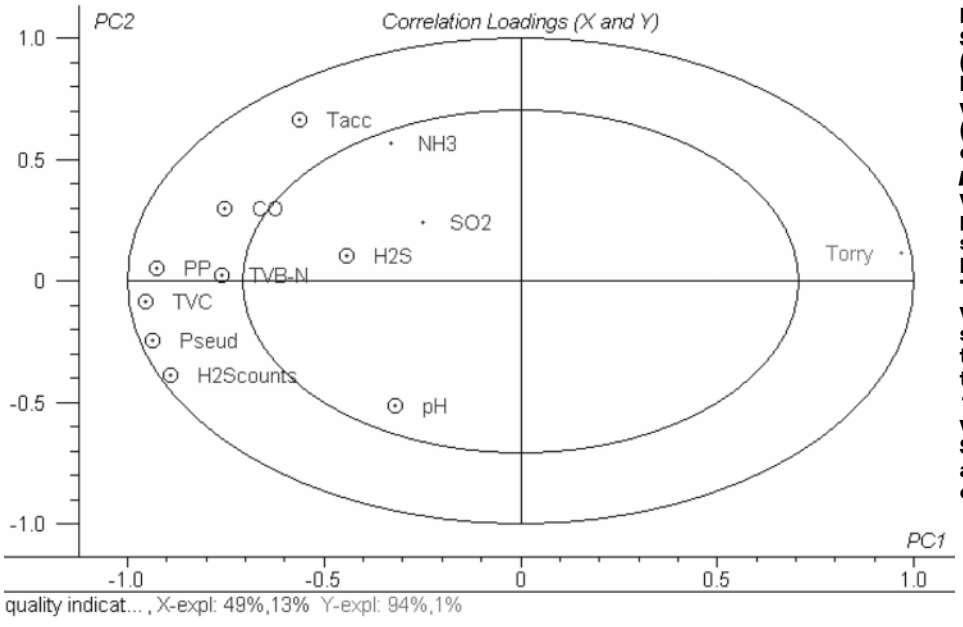


**Figure 8—Partial Least Squares Regression models (PLSR) correlation loadings based on all the measured variables: total viable counts (TVC), pseudomonads, $H_2S$ counts, *Photobacterium phosphoreum* (*Pp*), total volatile basic nitrogen (TVB-N), pH, $T_{acc}$ and electronic nose sensors (CO, $NH_3$, $SO_2$, and $H_2S$) as predictors (X) for the Torry scores as a response variable (Y) for the cod samples stored at different temperatures. The outer and the inner ellipses indicate 100% and 50% explained variance, respectively. Significant variables ($P < 0.05$) are symbolized with small circles.**

**Table 3—Correlation ($r^2$) and the error of prediction (root mean square error of prediction [RMSEP]) for different Partial Least Squares Regression (PLSR) models based on the measured variables (X): chemical total volatile basic nitrogen [TVB-N], pH), microbial total viable counts [TVC], Pseud, Pp, $H_2S$ counts), electronic nose (CO, $NH_3$, $H_2S$, $SO_2$) to predict the sensory Torry scores (Y) of differently treated cod fillets**

| | X | Nr of variables | Correlation $r^2$ | RMSEP | N |
|---|---|---|---|---|---|
| All variables measured | sensors (4), microbes (4), TVB-N and pH | 10 | 0.97 | 0.29 | 41 |
| Microbial counts | TVC, Pseud, Pp, $H_2S$ counts | 4 | 0.96 | 0.32 | 40 |
| Electronic nose | CO, $NH_3$, $H_2S$, $SO_2$ | 4 | 0.70 | 0.80 | 40 |
| Classical | TVB-N, TVC, pH | 3 | 0.92 | 0.45 | 41 |
| E-nose+ TVB-N + SSO | CO, TVB, Pseud, $H_2S$ counts, Pp | 5 | 0.97 | 0.30 | 41 |

the Torry score and the importance of TVB-N (Figure 8) it was of interest to study further a model including these variables in combination with the SSOs. The resulting PLSR model ($r^2$ = 0.97 and RMSEP = 0.30) showed that the overall spoilage pattern of the differently treated samples was similar as indicated by the arrow, tracking the increasing spoilage level of samples along PC 1 from right to left (Figure 9). The 2nd PC explained the influence of the temperature conditions on the spoilage pattern in the different experimental groups. The superchilled samples E, F, and G tended to be located lowest on the plot and were best described by the $H_2S$-producer counts. The traditionally processed groups H and I were placed in the middle and described by the *Pseudomonas* spp., whereas the traditionally processed groups with the highest temperature during storage were situated on the upper half of the plot showing that the temperature abused samples D6 and D7 were most affected and described by high *Photobacterium* counts and high values of the TVB-N and the CO sensor. Despite the differences in the spoilage rate of the groups explained by the influence of the different temperature conditions and initial handling on the dominating SSO in the samples (PC2), the main changes in their spoilage level was explained by their different location along PC 1 influenced by the SSO (*Pp, Pseud*, and $H_2S$ producers), the TVB-N value, and the CO sensor response. Therefore, it appears that these quality indicators could be used to classify both the traditionally processed and the superchilled samples.

## Classification based on sensory criteria

Classification of the differently treated samples based on sensory criteria (Torry score >7 and Torry score <7) using 5 selected variables (TVB-N, CO sensor, $H_2S$ producers, pseudomonads, and *P. phosphoreum*) was studied using SIMCA. The Torry score of 7 was selected because at this point the samples are at the last stage of acceptable marketable quality and it is of interest to determine whether samples are still acceptable for distribution. The results showed that 85% of the samples were correctly classified into respective classes (34 samples) while 15% were identified in both classes (6 samples). The samples that were wrongly classified in both classes were samples A10, C10, D7, H9, E10, and F12 that were located in the middle of the plot and not well-described by the variables (Figure 9). All these samples had sensory scores close to 7. For example, the superchilled samples (E10 and F12) with Torry scores <7 had at this point low values for the quality variables. This sug-

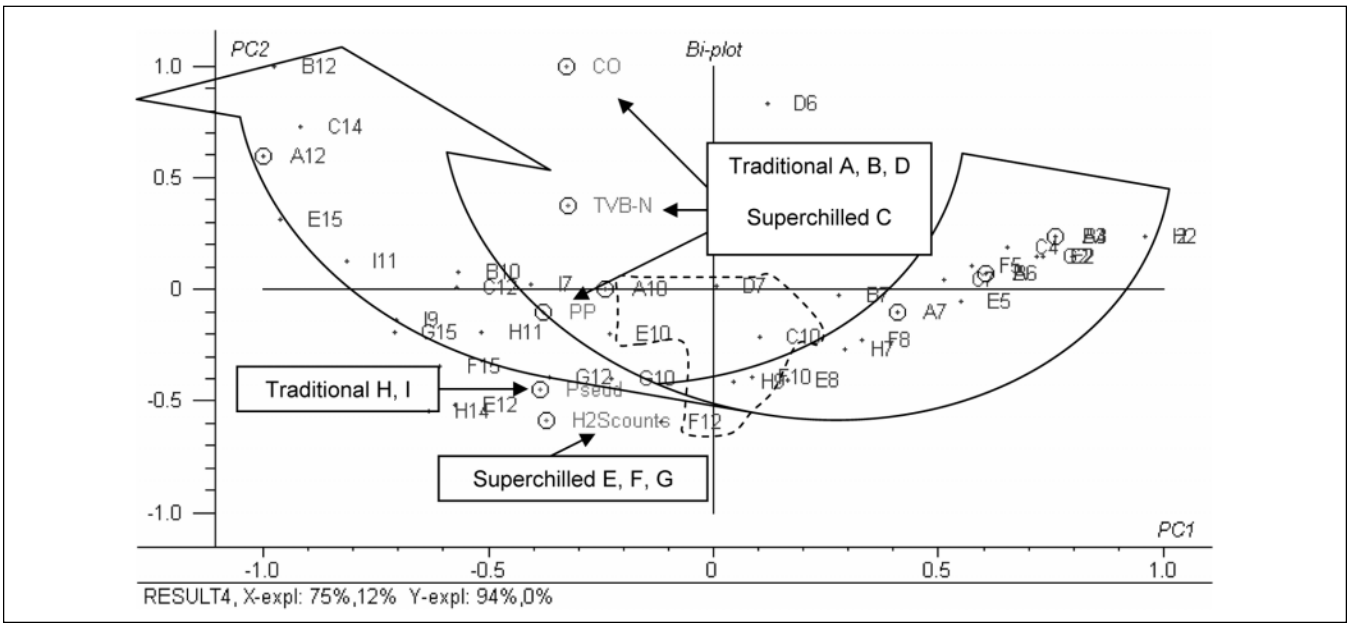


**Figure 9—Biplot for the principal components of a Partial Least Squares Regression models (PLSR) model ($r^2$ = 0.94; root mean square error of prediction [RMSEP] = 0.49) based on all the data (*N* = 41) of cod fillets stored at different temperatures and 5 variables: *Photobacterium phosphoreum*, pseudomonads, $H_2S$ producers, total volatile basic nitrogen (TVB-N), and the electronic nose CO sensor as predictors for the response variable (Torry score). Samples are labeled with letters indicating storage groups (A, B, C, D, E, F,G, H, and I) and storage days. The arrow shows the spoilage trend of the samples with increasing storage time and the samples encircled (dotted line) in the middle were badly described by the variables.**

gests that these quality indicators do not adequately describe the quality changes of the samples and other indicators are needed to describe better their sensory characteristics. Further studies should include more sensitive detection of the microbial metabolites and other oxidatively or enzymically derived degradation compounds contributing to the sensory rejection of chilled and superchilled fillets. The role of endogenous enzymatic degradation in the fillets may explain partly the quality deterioration (Ashie and others 1996) and should be studied further in particular in relation to superchilled conditions when microbial growth is delayed.

## Conclusions

Improper icing and handling of raw material, leading to higher temperature of the fillets, especially influenced the development of *P. phosphoreum,* but growth of $H_2S$-producing bacteria was also accelerated at higher temperatures contributing to a shorter shelf life of temperature abused cod fillets. Superchilling with the new CBC technique and storage at 0.5 °C resulted in an overall sensory shelf life of 12.5 to 14 d. When combined with superchilled storage at –1.5 °C, an extension in shelf life to at least 15 d was achieved.

Under superchilling conditions, the growth rate of all the bacterial groups was delayed. Higher levels of TVB-N were observed at sensory rejection for superchilled fillets stored under chilled conditions (0.5 °C) compared with samples stored at –1.5 °C.

Comparing the marketable shelf life (Torry score = 7) of all the groups tested revealed that the CBC-process increased the shelf life, while further storage of such fillets under superchilled conditions gave an additional extension. Such gain in shelf life is of high economical value because it would allow the distant transportation of fresh fillets by ship or truck, which is less costly compared with air freight.

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