

# Multiple Compound Quality Index for Cold-Smoked Salmon (*Salmo salar*) Developed by Multivariate Regression of Biogenic Amines and pH

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Production of biogenic amines during chill storage of 12 lots of cold-smoked salmon was studied. These data allowed for a multiple compound quality index to be developed by multivariate regression (partial least square regression). The quality index was based on concentrations of cadaverine, histamine, putrescine, and tyramine and pH and showed good correlation with sensory assessments. Biogenic amines were indicators of spoilage rather than casual agents of spoilage off-flavors. Four different biogenic amine profiles were found at the time of spoilage in cold-smoked salmon. These were the results of differences in the spoilage microflora. Histamine was detected above regulatory limits but below toxic levels. Measurements of salt and dry matter for calculation of water phase salt could be substituted by rapid water activity measurements.

**Keywords:** *Agmatine; cadaverine; putrescine; histamine; tyramine; partial least squares regression; spoilage; water activity*

## INTRODUCTION

Spoilage of cold-smoked salmon has been the subject of several studies in recent years. Microbial activity is found to be responsible for off-flavors produced at spoilage (Truelstrup Hansen et al., 1996; Joffraud et al., 1998; Leroi et al., 1998). A wide variety of bacterial species can be isolated from cold-smoked salmon, and some are potential spoilage organisms, i.e., *Shewanella putrefaciens*, *Aeromonas* spp., *Brochothrix* spp. (Leroi et al., 1998); *Lactobacillus* spp., Enterobacteriaceae, *Photobacterium phosphoreum* (Truelstrup Hansen, 1995). Despite this, spoilage reactions limiting product shelf life remain poorly understood, and no objective index for spoilage or indicator of quality has been identified.

Numerous individual chemical measurements are used as indices of spoilage in cold-smoked salmon i.e., acetic acid, ethanol, hypoxanthine, trimethylamine, and total volatile bases (Truelstrup Hansen, 1995). Unfortunately, none of these parameters fulfill the general requirements for chemical indicators of quality proposed by Fields et al. (1968). Indices of quality relying on several chemical compounds are suggested for tuna (Mietz and Karmas, 1977; Veciana-Nogués, 1997). These studies use the concentration of biogenic amines with equal weights reflecting an assumption of equal importance for the different biogenic amines in the index, which do not necessarily give the best correlation with sensory quality.

Salmon has a high content of nonprotein nitrogen, in which amino acids constitute a high proportion. Concentrations of arginine, histidine, lysine, and tyrosine range from 20 to 150 ppm in wild and farmed *Salmo salar* (Cowey and Daisley, 1962; Espe et al., 1993). At

the time of sensory rejection, cold-smoked salmon harbor high numbers of bacteria of different genera possessing decarboxylase activity. Consequently, production of histamine and other biogenic amines during storage of cold-smoked salmon is possible. Cantoni et al. (1993) found up to 258 ppm of various biogenic amines, i.e., cadaverine, histamine, putrescine, and tyramine, in this product. However, the potential of biogenic amines as objective indices of spoilage has not been evaluated in cold-smoked salmon.

The objective of the present study was to develop a multiple compound quality index for cold-smoked salmon based on biogenic amine production and changes in other chemical and microbiological parameters during storage at 5 °C. Vacuum-packed and sliced products were stored and analyzed for chemical, microbiological, and sensory changes. Products from three smokehouses were analyzed in initial storage trials to elucidate the potential of different spoilage indices. Data from additional storage trials were then used to develop the multiple compound quality index. Chemical changes analyzed include agmatine, cadaverine, histamine, putrescine, tyramine, spermidine, spermine, and pH. Multivariate data analysis in the form of partial least squares regression (PLSR) was used to correlate essential information from 11 variables of chemical and microbiological analyses to sensory changes.

## MATERIALS AND METHODS

**Samples of Cold-Smoked Salmon.** Sliced vacuum-packed cold-smoked salmon from three smokehouses were studied. Salmon used was Norwegian farmed *S. salar*. Two distinct lots from each smokehouse from 1997 (97-1 to 97-6) were frozen and transported to our institute where each lot was thawed overnight and stored at 5 ± 1 °C until 1–2 weeks after sensory spoilage was evident. The temperature was continuously monitored by temperature loggers (Tinytag, Gemini Ltd., UK). One-and-a-half years later, two additional lots of cold-smoked

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salmon (98-1 to 98-6) were taken from the smokehouses, and the experiments were repeated. Information on salting, drying, and smoking processes used in the three smokehouses was collected (Table 1). At appropriate intervals during storage, samples were withdrawn for chemical, sensory, and microbiological analyses. On each sampling occasion, two packs were taken for microbiological analysis followed by chemical analysis except at the time of spoilage in which three to four packs were used. In addition, two different packs were taken for sensory analysis.

**Chemical Analysis.** Direct electrodes were used to measure pH in three places per pack, and means were calculated (PHC2431, PHC2441, PHM250, Radiometer, Copenhagen, Denmark). From each pack, 50 g representing all slices in a pack was homogenized and used for chemical analysis. Two grams was used for water activity measurements using an AquaLab CX2 (Decagon Devices, Inc., Pullman, Washington). Biogenic amines in 15 g of homogenate were extracted with 30 mL of 7.5% (w/v) trichloroacetic acid. The extracts were analyzed for agmatine (Agm), cadaverine (Cad), histamine (His), putrescine (Put), tyramine (Tyr), spermidine (Spd), and spermine (Spm) by HPLC on a reverse-phase column followed by postcolumn derivatization using  $\alpha$ -phthalaldehyde. The fluorescence intensity was measured at 354/430 nm. (Seiler and Knödgen, 1980; Luten et al., 1992). Nitrite was analyzed on fresh samples as previously reported (Dalgaard and Jørgensen, 1998).

**Sensory Evaluation.** Panels of five to eight persons experienced in seafood evaluation were used for the sensory evaluations. At every evaluation, a sample from each production lot stored at  $-30^{\circ}\text{C}$  was thawed and used as reference sample (fresh). A three-class evaluation scheme was used: class 1, no off-flavors, equal to the reference sample; class 2, slight off-flavors but not spoiled; and class 3, clearly recognizable off-flavors. A lot was classified as spoiled when 50% or more of the panel members determined the samples to be in class 3.

**Microbiological Analysis.** From each pack, 25 g of cold-smoked salmon was taken aseptically, representing all slices in the pack, diluted in 0.1% (w/v) peptone saline (0.85% NaCl), and homogenized for 60 s in a Stomacher 400 Lab Blender. A psychrotrophic bacterial count was performed on spread plates of modified Long and Hammer's medium (L&H) (van Sprooken, 1974), which were incubated at  $15^{\circ}\text{C}$  for 5–7 days. Pour plates of nitrite actidione polymyxin agar at pH 6.7 (NAP6.7) were used to determine numbers of lactic acid bacteria (LAB) (Davidson and Cronin, 1973). Enterobacteriaceae were enumerated in pour plates of 5 mL of tryptone soya agar, which after 2 h at  $20$ – $25^{\circ}\text{C}$  were overlaid by 12–15 mL of violet red bile glucose agar (TSA/VRBG). Typical Enterobacteriaceae colonies were counted after 2 days of incubation at  $30^{\circ}\text{C}$  (Truelstrup Hansen et al., 1996).

**Water Phase Salt and Water Activity in Cold-Smoked Salmon.** The most important safety parameter in cold-smoked salmon production is the amount of water phase salt in the finished product (Huss et al., 1995). Unfortunately, salt analysis is time-consuming and laborious. Therefore, the relationship between water activity ( $a_w$ ) and water phase salt was evaluated. Forty samples of cold-smoked salmon were collected from 10 different smokehouses. These were homogenized in a domestic food processor and analyzed in duplicate for salt content (AOAC, 1995a), dry matter (AOAC, 1995b), and water activity using an AquaLab CX2 (Decagon Devices, Inc., Pullman, Washington). Water phase salt was calculated from salt content and percentage of dry matter of each sample.

**Data Analysis.** Chemical and microbiological data ( $X$  variables) were used to predict results obtained from sensory analyses ( $Y$  variable), i.e., percentage of panelists rejecting the sample (%-class 3). The multiple compound quality index was developed by PLSR on the chemical, microbiological, and sensory data. Microbial counts were analyzed as nontransformed and log-transformed, but this did not affect the results. Chemical and microbial data were centered and scaled by subtracting the mean and dividing by the standard deviation of the concerned variable across all

samples. Full cross validation (leave one out) was used to test the performance of the PLSR model, and the root-mean-square error of cross validation (RMSECV) was used for assessing the influence of simplifying the model by reducing the number of original variables. Unscrambler version 6.1 (CAMO A/S, Trondheim, Norway) was used for multivariate data analysis. One-way ANOVA for repeated sampling was used to test differences between fresh and spoiled samples. For these calculations, Statgraphics Plus (version 7, Manugistics, Inc., Rockville, MD) was used.

## RESULTS AND DISCUSSION

**Development of a Multiple Compound Quality Index.** A PLSR model was calculated on the basis of the original 11  $X$  variables. A PLSR model with only one latent variable described 70% of the variation in the sensory data. The second latent variable only accounted for an additional 4% point of the total variation. Full cross validation of the one latent variable PLSR model resulted in a RMSECV of 14.8%-class 3. For reduction of the model, original variables were eliminated. Special emphasis was paid to eliminate the time-consuming microbiological parameters and to obtain a robust chemometric model. Two simple models could be obtained with little or no impact on RMSECV. The first cold-smoked salmon quality index, CSS-QI<sub>5v</sub> (eq 1) incorporated pH, cadaverine, histamine, putrescine, and tyramine, and the second CSS-QI<sub>3v</sub> (eq 2) used pH, tyramine, and histamine as original variables into only one latent variable. These models had an RMSECV of 15.0 and 14.8%-class 3, respectively, whereas the average standard deviation for the sensory analysis was 12%-class 3. Hence, the PLSR models could substitute for the sensory analysis, as it had errors of prediction similar to the standard error of the sensory data. The multiple compound quality indices have the following notations:

$$\text{CSS-QI}_{5v} = 200 - (31 \times \text{pH}) + (0.06 \times \text{Tyr}) + (0.06 \times \text{Cad}) + (0.04 \times \text{Put}) + (0.15 \times \text{His}) \quad (1)$$

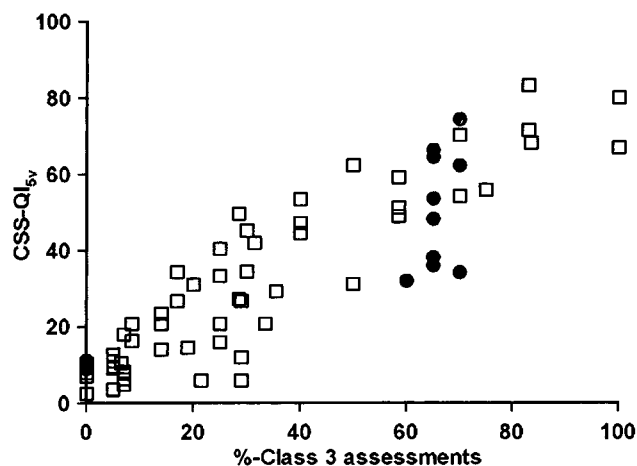
$$\text{CSS-QI}_{3v} = 298 - (47 \times \text{pH}) + (0.10 \times \text{Tyr}) + (0.23 \times \text{His}) \quad (2)$$

where the response variable, CSS-QI is %-class 3 assessments, and concentrations of biogenic amines are in ppm. The coefficients of histamine in eq 1 and eq 2 were 2–3 times higher than the coefficients of the other biogenic amines. Thus, the production of histamine in cold-smoked salmon was more important than other biogenic amines for prediction of %-class 3 assessments. CSS-QI<sub>5v</sub> and CSS-QI<sub>3v</sub> were successfully validated on data from storage trials of lot 97-1 to 97-6 (Figures 1 and 2). The correlation between CSS-QI<sub>5v</sub> ( $r^2 = 0.79$ ) and CSS-QI<sub>3v</sub> ( $r^2 = 0.79$ ) and sensory data was high as compared to indices of Mietz and Karmas (1977),  $r^2 = 0.34$ , and Veciana-Nogués et al. (1997),  $r^2 = 0.63$  (Figures 3 and 4). The classical biogenic amine quality index of Mietz and Karmas (1977) developed for tuna has been suggested as a quality index for cold-smoked salmon (Cantoni et al., 1993). Recently, Veciana-Nogués et al. (1997) proposed another quality index based on biogenic amines for tuna. These quality indices were less applicable for quality assessment of chill stored vacuum-packed cold-smoked salmon than CSS-QI. This was due to elimination of irrelevant biogenic amines, adjustment of the coefficient used, and inclusion of pH in the quality indices. CSS-QI was intended to correlate with the

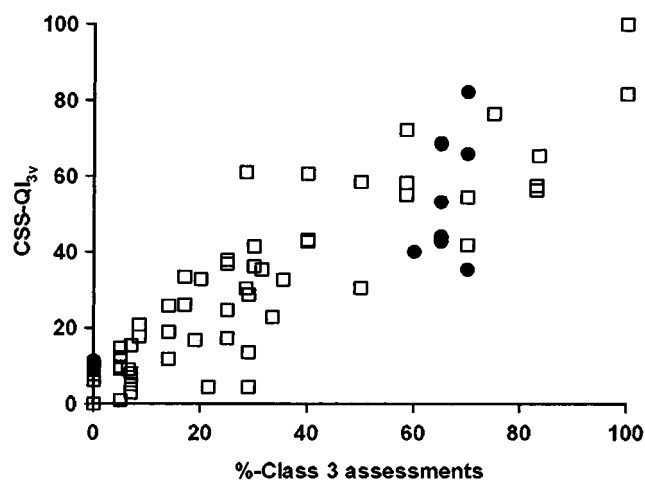
Table 1. Processing, Product, and Spoilage Characteristics of Sliced Vacuum-Packed Cold-Smoked Salmon Stored at 5 °C

lot no.	A				B				C			
	97-1	97-2	98-1	98-2	97-3	97-4	98-3	98-4	97-5	97-6	98-5	98-6
smokehouse												
process	brine injection NaCl				brine injection NaCl, sucrose				dry salting NaCl, nitrite, sucrose			
salting	3–4 h, 26 °C, no humidity control				no separate drying process				6–12 h, 27 °C, 50% relative humidity			
drying	4 h, 26 °C, no humidity control				4–7 h, 21–22 °C, no humidity control				6–12 h, 27 °C, 65% relative humidity			
smoking												
product												
initial pH	6.09 ± 0.02 <sup>a</sup>	6.09 ± 0.01	6.14 ± 0.04	6.01 ± 0.02	6.11 ± 0.01	6.07 ± 0.04	6.13 ± 0.07	6.00 ± 0.04	6.08 ± 0.02	6.11 ± 0.05	6.16 ± 0.03	6.11 ± 0.03
NaCl (% WPS)	5.4 ± 0.4	5.0 ± 0.5	4.9 ± 0.2	4.1 ± 0.5	7.5 ± 0.6	5.9 ± 0.4	4.2 ± 0.3	4.2 ± 0.6	7.9 ± 1.3	5.6 ± 0.5	3.9 ± 0.5	4.9 ± 0.7
NaNO <sub>2</sub> (ppm)	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	16 ± 7	22 ± 10	8 ± 5	12 ± 8
shelf life (weeks)	4–5	4.5–5	4–5	4.5–5.5	8.5–9	7–8	3–4	5.5–6.5	5–6	4.5–5	4–4.5	5.5–6.5
characteristics at time of spoilage												
pH	6.10 ± 0.01	6.23 ± 0.05	6.06 ± 0.04	5.98 ± 0.03	5.95 ± 0.08	5.64 ± 0.2	5.70 ± 0.18	5.63 ± 0.03	6.06 ± 0.08	6.18 ± 0.02	5.90 ± 0.06	5.99 ± 0.19
biogenic amines (ppm)												
agmatine	234 ± 107	220 ± 118	29 ± 26	121 ± 25	18 ± 7	2 ± 1	88 ± 24	32 ± 30	142 ± 179	270 ± 90	25 ± 13	2 ± 1
cadaverine	265 ± 72	251 ± 63	135 ± 69	345 ± 95	36 ± 11	101 ± 27	152 ± 70	131 ± 135	168 ± 170	277 ± 33	178 ± 66	303 ± 140
histamine	135 ± 73	190 ± 130	3 ± 3	96 ± 20	19 ± 27	4 ± 2	102 ± 15	50 ± 41	108 ± 118	240 ± 64	10 ± 6	16 ± 10
putrescine	11 ± 4	3 ± 1	11 ± 9	28 ± 16	31 ± 16	8 ± 6	7 ± 3	40 ± 34	33 ± 32	32 ± 18	190 ± 64	383 ± 32
tyramine	137 ± 63	228 ± 23	180 ± 10	235 ± 15	202 ± 21	128 ± 42	82 ± 29	158 ± 74	108 ± 102	235 ± 40	223 ± 33	335 ± 31
PBA <sup>b</sup>	I	I	II	I	IV	II	I	II	I	I	III	III
sensory attributes												
off-flavors	sour, <sup>c</sup> bitter, fishy, rancid	sour, faecal, rancid	sour, faecal	sour, faecal	rancid, sour	sour, chemical	sour	sour	sour, faecal	sour, faecal	sour, faecal	sour, faecal
texture	pasty, sticky	soft, sticky	soft, sticky	soft, sticky	soft	soft	soft	soft	soft	soft	soft	soft
microflora, log <sub>10</sub> (CFU/g)												
TPC	6.9 ± 0.1	7.6 ± 0.2	7.3 ± 0.3	7.6 ± 0.2	8.2 ± 0.4	8.7 ± 0.3	7.8 ± 0.4	8.5 ± 0.1	6.9 ± 0.3	7.2 ± 0.2	8.3 ± 0.1	8.5 ± 0.2
LAB	6.8 ± 0.2	7.5 ± 0.1	7.6 ± 0.1	7.8 ± 0.2	8.1 ± 0.4	8.5 ± 0.3	8.6 ± 0.1	8.7 ± 0.2	6.8 ± 0.3	7.1 ± 0.2	8.4 ± 0.1	8.4 ± 0.3
Enterobacteriaceae	4.1 ± 1.0	6.7 ± 0.6	6.1 ± 0.2	6.4 ± 0.8	<3.0	6.5 ± 0.5	3.4 ± 1.0	6.1 ± 1.5	<3.0	4.2 ± 1.6	5.9 ± 1.4	6.5 ± 0.8

<sup>a</sup> Average ± standard deviation of 3 or 4 individual packs, lots 97-1 to 98-6, respectively. <sup>b</sup> Profile of biogenic amines (PBA). <sup>c</sup> Attributes responsible for spoilage are indicated in italics.



**Figure 1.** Correlation between the multiple compound quality index CSS-QI<sub>5v</sub> (eq 1) based on cadaverine, putrescine, histamine, tyramine, and pH and percentage of class 3 sensory assessments of cold-smoked salmon. Samples were from storage trial of lot 97-1 to 97-6 (circles) and 98-1 to 98-6 (squares),  $r^2 = 0.79$ .

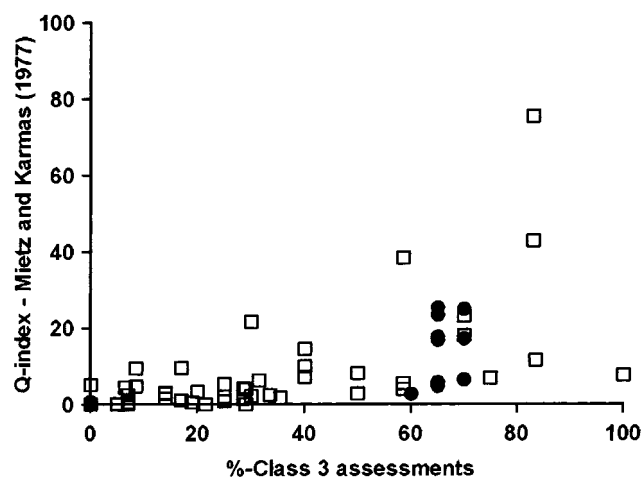


**Figure 2.** Correlation between the multiple compound quality index CSS-QI<sub>3v</sub> (eq 2) based on histamine, tyramine, and pH and percentage of class 3 sensory assessments of cold-smoked salmon. Samples were from storage trial of lot 97-1 to 97-6 (circles) and 98-1 to 98-6 (squares),  $r^2 = 0.79$ .

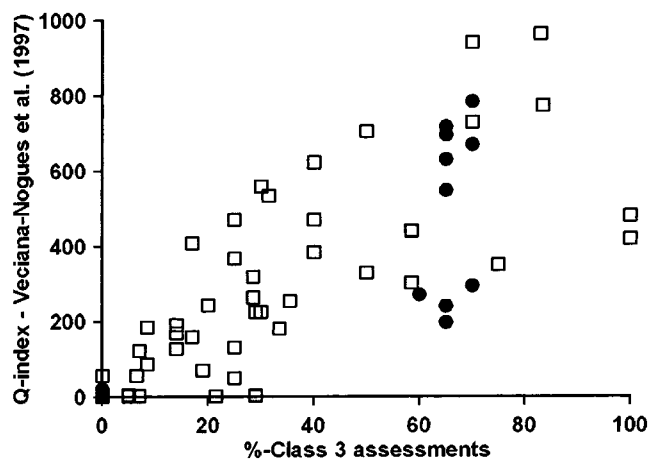
sensory score and not the storage time. Therefore, CSS-QI can substitute sensory assessment in discrepancies in trading, as chemical analysis is easier to compare between parties. Further studies are needed to determine whether CSS-QI can be used on cold-smoked salmon from other countries that might use different raw material and processing technologies. In addition, such studies will determine if CSS-QI can be reduced from five variables to only three variables, which this study indicates.

#### Production of Biogenic Amines during Storage.

Concentrations of agmatine, cadaverine, histamine, putrescine, and tyramine in freshly produced products were low, usually below 2 ppm. In contrast, spoiled samples could reach concentrations of 300 ppm of the individual biogenic amines (Table 1). Production of various biogenic amines and ratios between them varied considerably between the different production lots (Table 1). The concentrations of agmatine ( $P = 0.0008$ ), cadaverine ( $P < 0.0001$ ), histamine ( $P = 0.0009$ ), putrescine ( $P < 0.0001$ ), and tyramine ( $P < 0.0001$ ) increased during chill storage, while the concentration of spermi-



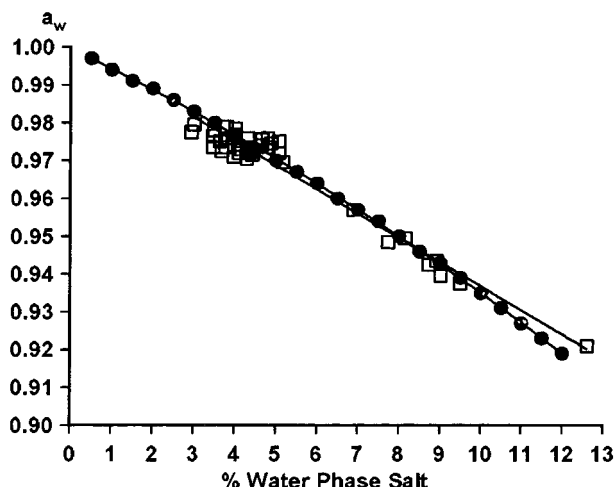
**Figure 3.** Correlation between the quality index of Mietz and Karmas (1977) and the percentage of class 3 assessments of cold-smoked salmon. Samples were from storage trial of lot 97-1 to 97-6 (circles) and 98-1 to 98-6 (squares),  $r^2 = 0.34$ . The data point (100; 142) was out of range.



**Figure 4.** Correlation between the quality index of Veciana-Nogués et al. (1997) and the percentage of class 3 sensory assessments of cold-smoked salmon. Samples were from storage trial of lot 97-1 to 97-6 (circles) and 98-1 to 98-6 (squares),  $r^2 = 0.63$ .

dine ( $P < 0.0001$ ) decreased and the concentration of spermine ( $P > 0.1$ ) remained constant. Agmatine concentrations peaked at the spoilage time but decreased with additional storage of some lots. Although this was the general trend, four different profiles of biogenic amines (PBA) were found (Table 1). PBA I consisted of high levels ( $\geq 100$  ppm) of agmatine, cadaverine, histamine, and tyramine but low levels ( $\leq 50$  ppm) of putrescine. PBA II consisted of high levels of cadaverine and tyramine but low levels of agmatine, histamine, and putrescine. PBA III consisted of high levels of cadaverine, putrescine, and tyramine but low levels of agmatine and histamine. PBA IV consisted of high levels of tyramine but low levels of agmatine, cadaverine, histamine, and putrescine (Table 1). Production of biogenic amines results from microbial activity (Silla Santos, 1996; Taylor, 1986). These findings suggest that highly variable and complex microbial activities occur during spoilage of chill stored vacuum-packed cold-smoked salmon. Further studies of the spoilage flora will show how these four PBA relate to the scenarios for spoilage microflora development (Truelstrup Hansen, 1995; Truelstrup Hansen et al., 1996, 1998; Paludan-Müller et





**Figure 5.** Correlation between water activity and water phase salt of cold-smoked salmon (squares) and data from Chirife and Resnik (1984) on salt in water solutions (circles).

al., 1998; Leroi et al., 1998; Truelstrup Hansen and Huss, 1998).

**Sensory Importance of Biogenic Amines.** Concentrations of biogenic amines correlated well with sensory analysis but are not necessarily the casual agents of spoilage off-flavors. In fact, preliminary experiments at our institute showed that these biogenic amines could not be the casual agents of the spoilage flavors in cold-smoked salmon. As much as 700 ppm of cadaverine, putrescine, histamine, or tyramine added to fresh cold-smoked salmon homogenates showed no sign of off-flavors. This was supported by published flavor threshold values, in which the odor detection threshold of cadaverine in water has been estimated to be 20–190 ppm, which is similar to values determined for putrescine in water, i.e., 15–22 ppm (Wang et al., 1975; Holluta, 1960; Amore et al., 1975). These values are most likely higher in cold-smoked salmon, e.g., the odor detection threshold for putrescine was 5-fold higher in 2% soybean suspension as compared to water. The recognition taste threshold for histamine and tyramine in water is higher than concentrations found in spoiling cold-smoked salmon, i.e., 1100–2200 ppm and 270–340 ppm, respectively (Wieser and Berlitz, 1975). Although biogenic amines are not responsible for spoilage off-flavors, concentrations of biogenic amines correlated with the development of spoilage off-flavors in this study and others (Mietz and Karmas, 1977; Sims et al., 1992).

**Water Phase Salt and Water Activity in Cold-Smoked Salmon.** Water phase salt and water activity of cold-smoked salmon ranged from 3 to 12.6% water phase salt (WPS) and 0.923–0.980  $a_w$ , respectively. Data from vacuum-packed cold-smoked salmon compared well with the results of NaCl in water by Chirife and Resnik (1984), which suggest that WPS is determining water activity in this product (Figure 5). Chirife and Resnik's data fitted a quadratic equation (eq 3,  $r^2 = 0.99$ ). In cold-smoked salmon, however, a straight line fitted data just as well (eq 4,  $r^2 = 0.95$ ) as a quadratic equation (eq 3,  $r^2 = 0.95$ ).

$$a_w = 1 - (0.005 \times \text{WPS}) - (1.3 \times 10^{-4} \times \text{WPS}^2) \quad (3)$$

$$a_w = 1.006 - (0.0064 \times \text{WPS}) \quad (4)$$

Measurement of water activity can therefore substitute the laborious analysis of salt content and dry

matter used for verification of the salting process of cold-smoked salmon.

**Shelf Life.** Shelf life of the chilled products was determined by the sensory panel to be between 3 and 9 weeks (Table 1). Lot 97-1 was rejected because of soft texture and off-flavors. The remaining lots were rejected only because of off-flavors, although these developed a soft texture during the storage period (Table 1). The spoilage off-flavors and shelf life detected in this study were similar to those reported by others (Truelstrup Hansen et al., 1995, 1996, 1998; Paludan-Müller et al., 1998).

**Microbiological Growth and Spoilage Microflora.** Microbial counts of cold-smoked salmon showed a variable initial contamination ranging from  $10^2$  to  $10^5$  CFU/g on L&H (results not shown). During the first two weeks of storage, bacteria in the product grew and reached counts of  $10^7$ – $10^8$  CFU/g on L&H, which remained at this level until spoilage several weeks later (Table 1). Although this was the general pattern, variation in composition of the microflora between lots was observed. Production of biogenic amines during storage revealed four different profiles of biogenic amines (Table 1). Products with PBA I were characterized by a total psychrotrophic count (TPC) of  $10^{6.9}$ – $10^{7.8}$  CFU/g and a LAB count equal to TPC. Colony morphology on L&H revealed a mixed flora of pinpoint and big opaque colonies likely to be LAB and Gram negative bacteria, respectively. Enterobacteriaceae counts on TSA/VRBG were low,  $\leq 10^4$  CFU/g, and not important for the microbial activity except maybe for lots 97-2 and 98-2, which had a TSA/VRBG count of  $5 \times 10^6$  CFU/g. Total psychrotrophic counts of cold-smoked salmon with PBA II or III were  $10^7$ – $10^{8.5}$  CFU/g consisting of pinpoint colonies. LAB counts were equal to TPC. In comparison to PBA I, the Enterobacteriaceae counts of PBA II and III were high,  $10^6$ – $10^{6.5}$  CFU/g. Consequently, spoilage microflora of PBA II and III consisted of LAB (90–99%) and Enterobacteriaceae (1–10%). Cold-smoked salmon of lot 97-3 (PBA IV) had LAB counts equal to TPC and no detectable Enterobacteriaceae,  $< 10^3$  CFU/g. In this lot, LAB dominated the microflora ( $\geq 99\%$ ). Spoilage microflora of PBA I, PBA II and III, and PBA IV corresponded well with the scenarios for spoilage microflora development in cold-smoked salmon, as proposed by Truelstrup Hansen (1995). However, more detailed studies are required to determine the specific spoilage organisms responsible for biogenic amine production in cold-smoked salmon.

## CONCLUSION

A multiple compound quality index developed from a combination of biogenic amines and pH can be used to determine the sensory quality of cold-smoked salmon. This multiple compound quality index fulfills the criteria put forward by Fields et al. (1968) for single compound quality index, i.e., the index should be low in fresh cold-smoked salmon and increase during spoilage, be produced by the dominating spoilage microflora, and be as reliable as sensory assessment. The suggested multiple compound quality indices (eq 1 and eq 2) rely on measurements that may be carried out by direct electrode (pH) and biosensors for biogenic amines (Chemnitius et al., 1992) and are therefore of practical use for quality inspection.

Production of biogenic amines in cold-smoked salmon during chill storage is unlikely to result in histamine

poisoning in humans as indicated by epidemiological data (Bartholomew et al., 1987; Scoging, 1998; Smart, 1992). Some samples exceeded histamine defect action levels of 50 ppm laid down by the FDA for *Scombridae* and 100–200 ppm by EU regulations for *Scombridae* and *Clupeidae*, but no samples reached toxic levels of 500 ppm (FDA Compliance Policy Guide Sec. 540.525; Directive 91/493/EEC; Taylor, 1986).

#### ACKNOWLEDGMENT

We thank Gerda Breil, Jonas Nordahl, Lene Tokkesdal, and Janne Nielsen (Danish Institute for Fisheries Research) for technical assistance and Bo Jørgensen (Danish Institute for Fisheries Research) and Brian Himelbloom (University of Alaska—Fairbanks) for valuable comments to the manuscript.

#### LITERATURE CITED

- Amoore, J. E.; Forrester, L. J.; Buttery, R. G. Specific anosmia to 1-pyrroline: the spermous primary odor. *J. Chem. Ecol.* **1975**, *1*, 299–310.
- AOAC. Salt (chlorine as sodium chloride) in seafood. Volumetric method. *Off. Methods Anal.* **1995a**, *16*, 937.09.
- AOAC. Moisture in meat. Air drying. *Off. Methods Anal.* **1995b**, *16*, 950.46.
- Bartholomew, B. A.; Berry, P. R.; Rodhouse, J. C.; Gilbert, R. J. Scombrototoxic fish poisoning in Britain: feature of over 250 suspected incidents from 1976 to 1986. *Epidemiol. Infect.* **1987**, *99*, 775–782.
- Cantoni, C.; Moret, S.; Comi, G. Indici batteriologici e chimici per valutare la qualità del salmone affumicato. *Ind. Aliment.* **1993**, *32*, 842–845.
- Chemnitz, G. C.; Suzuki, M.; Isobe, K.; Kimura, J.; Karube, I.; Schmid, R. D. Thin-film polyamine biosensor: substrate specificity and application to fish freshness determination. *Anal. Chim. Acta* **1992**, *263*, 93–100.
- Chirife, J.; Resnik, S. L. Unsaturated solutions of sodium chloride as reference sources of water activity at various temperatures. *J. Food Sci.* **1984**, *49*, 1486–1488.
- Cowey, C. B.; Dailsey, K. W. Study of amino acids, fresh or as components of protein, and of some B vitamins in the tissues of the atlantic salmon, *Salmo salar*, during spawning migration. *Comp. Biochem. Physiol.* **1962**, *7*, 29–38.
- Dalgaard, P.; Jørgensen, L. V. Predicted and observed growth of *Listeria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. *Int. J. Food Microbiol.* **1998**, *40*, 105–115.
- Davidson, C. M.; Cronin, F. Medium for the selective enumeration of lactic acid bacteria from foods. *Appl. Microbiol.* **1973**, *26*, 439–440.
- Espe, M.; Lied, E.; Torrissen, K. R. Changes in plasma and muscle free amino acids in atlantic salmon (*Salmo salar*) during absorption of diets containing different amounts of hydrolysed cod muscle protein. *Comp. Biochem. Physiol.* **1993**, *105A*, 555–562.
- Fields, M. L.; Richmond, B. S.; Baldwin, R. E. Food quality as determined by metabolic by-products of microorganism. In *Advances in Food Research*; Chicester, C. O., Mrak, E. M., Stewart, G. F., Eds.; Academic Press: New York, 1968.
- Holluta, J. Geruchs- und Geschmacksbeeinträchtigung des Trinkwassers—Ursachen und Bekämpfung. *GWF, Gas Wasserfach* **1960**, *101*, 1018–1023.
- Huss, H. H.; Embarek, P. K. B.; Jeppesen, V. F. Control of biological hazards in cold-smoked salmon production. *Food Control* **1995**, *6*, 335–340.
- Joffraud, J.-J.; Leroi, F.; Chevalier, F. Development of a sterile cold-smoked fish model. *J. Appl. Microbiol.* **1998**, *85*, 991–998.
- Leroi, F.; Joffraud, J.-J.; Chevalier, F.; Cardinal, M. Study of the microbial ecology of cold-smoked salmon during storage at 8 °C. *Int. J. Food Microbiol.* **1998**, *39*, 111–121.
- Luten, J. B.; Bouquet, W.; Seuren, L. A. J.; Burggraaf, M. M.; Riekwel-Booy, G.; Durand, P.; Etienne, M.; Gouyou, J. P.; Landrein, A.; Ritchie, A.; Leclercq, M.; Guinet, R. Biogenic amines in fishery products: standardization methods within EC. In *Quality Assurance in the Fish Industry*; Huss, H. H., Jakobsen, M., Liston, J., Eds.; Elsevier: Amsterdam, 1992.
- Mietz, J. L.; Karmas, E. Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. *J. Food Sci.* **1977**, *42*, 155–158.
- Paludan-Müller, C.; Dalgaard, P.; Huss, H. H.; Gram, L. Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5 °C. *Int. J. Food Microbiol.* **1998**, *39*, 155–166.
- Scoging, A. Scombrototoxic (histamine) fish poisoning in the United Kingdom: 1987 to 1996. *Comm. Dis. Public Health* **1998**, *1*, 204–205.
- Seiler, N.; Knödgen, B. High-performance liquid chromatographic procedure for the simultaneous determination of the natural polyamines and their monoacetyl derivatives. *J. Chromatogr.* **1980**, *221*, 227–35.
- Silla Santos, M. H. Biogenic amines: their importance in foods. *Int. J. Food Microbiol.* **1996**, *29*, 213–231.
- Sims, G. G.; Farn, G.; York, R. K. Quality indices for canned Skipjack tuna: correlation of sensory attributes with chemical indices. *J. Food Sci.* **1992**, *57*, 1112–1115.
- Smart, D. R. Scombroid Poisoning—a report of seven cases involving the Western Australian salmon *Arripis truttaceus*. *Med. J. Aust.* **1992**, *157*, 748–751.
- Taylor, S. L. Histamine food poisoning: toxicology and clinical aspects. *Crit. Rev. Toxicol.* **1986**, *17*, 91–128.
- Truelstrup Hansen, L. Quality of Chilled, Vacuum-Packed Cold-Smoked Salmon. Ph.D. Thesis. Danish Institute of Fisheries Research, Department of Seafood Research, Technical University of Denmark, Lyngby, Denmark, 1995.
- Truelstrup Hansen, L.; Huss, H. H. Comparison of the microflora isolated from spoiled cold-smoked salmon from three smokehouses. *Food Res. Int.* **1998**, *31*, 703–711.
- Truelstrup Hansen, L.; Gill, T.; Huss, H. H. Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. *Food Res. Int.* **1995**, *28*, 123–130.
- Truelstrup Hansen, L.; Gill, T.; Røntved, S. D.; Huss, H. H. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. *Food Res. Int.* **1996**, *29*, 181–188.
- Truelstrup Hansen, L.; Røntved, S. D.; Huss, H. H. Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. *Food Microbiol.* **1998**, *15*, 137–150.
- van Spreckens, K. J. A. The Suitability of a Modification of Long and Hammer's Medium for the Enumeration of more Fastidious Bacteria from Fresh Fishery Products. *Arch. Lebensmittelhyg.* **1974**, *25*, 213–219.
- Veciana-Nogués, M. T.; Marine-Font, A.; Vadal-Carou, M. C. Biogenic Amines as Hygienic Quality Indicators of Tuna. Relationships with Microbial Counts, ATP-Related Compounds, Volatile Amines, and Organoleptic Changes. *J. Agric. Food Chem.* **1997**, *45*, 2036–2041.
- Wang, L. C.; Thomas, B. W.; Warner, K.; Wolf, W. J.; Kwolek, W. F. Apparent odor thresholds of polyamines in water and 2% soybean flour dispersions. *J. Food Sci.* **1975**, *40*, 274–276.
- Weiser, H.; Belitz, H.-D. Zusammenhänge zwischen Struktur und Bittergeschmack bei Aminosäuren und Peptiden. *Z. Lebensm. Unters. Forsch.* **1975**, *159*, 65–72.

Received for review August 23, 1999. Accepted March 29, 2000. The European Commission, FAIR-project CT95-1207 has supported this work.