Headspace Gas Chromatography-Mass Spectrometry and Electronic Nose Analysis of Volatile Compounds in Canned Alaska Pink Salmon Having Various Grades of Watermarking

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ABSTRACT: Volatiles in canned pink salmon, produced from different degrees of skin watermarked raw material and stored for 2 and 9 mo, were characterized and compared using static headspace gas chromatography analysis coupled to a mass spectrometer (SHGCMS). Sulfur-containing compounds comprised 30% to 50% of the total volatiles and tended to decrease with increasing degrees of skin watermarking, and dimethyl sulfide was the most abundant compound of this class of molecules. A few alcohols, aldehydes, ketones, and furans were also identified. Forward stepwise general discriminant analysis (FSGDA) was used to investigate prediction models based on degree of skin watermarking. The 2- and 9-mo models using SHGCMS showed 92.5% and 93.75% correct classifications, respectively. The ability of the Cyranose 320, a hand-held electronic nose (EN), to differentiate these grades of watermarking in the canned samples was also tested. EN analysis using FSGDA resulted in models with 90% and 92.5% correct classifications for the 2- and 9-mo samples, respectively. Overall, results indicate that the watermarking grades studied are not readily distinguishable from each other by either method of analysis.

Keywords: pink salmon, electronic noses, seafood quality, headspace analysis

Introduction

Wild salmon fisheries contribute significantly to the economy of Alaska. In 2003, the commercial salmon harvest was estimated at 350000 metric tons (MT) of fish (ADFG 2004). During the past 3 y, pink salmon catches have ranged between 130000 to 200000 MT of fish per year (ADFG 2004). Salmon are anadromous fish that undergo drastic physiological and biochemical changes during spawning migration (Reid and others 1993). Fish do not feed and metabolic degeneration occurs as they approach full sexual maturity (Ando 1985). Changes such as migration of lipid-soluble pigments from the muscle into the skin and gonads (Kitahara 1983; Reid and others 1993), increase in flesh pH (Huynh and Mackey 1990), decrease in protein and lipid content (Ando and others 1985; Huynh and Mackey 1990), and decrease in blood cholesterol levels (Idler and Tsuyuki 1958) have been observed. Muscle quality is greatly impacted by these biochemical changes and late-run salmon often have less desirable texture and flavor. Flesh softness, poor taste, and the development of a distinct "late" odor tend to decrease the value of the product (Huynh and Mackey 1990). It is known by seafood processors in Alaska that moderate to heavy watermarking produces an off-odor in the canned product, often defined as stale or musty (Huynh and Mackey 1990). Therefore, seafood processors in Alaska-grade salmon according to its degree of skin watermarking.

A major challenge for the Alaska salmon industry has been to improve the inconsistent quality of its products (ASITF 2003). This requires processors to become more diligent in assessing quality and applying new technology that can provide the product consistency demanded by the consumers. A number of studies have been reported on the use of electronic noses (EN) to assess seafood quality. A semiconductive metal oxide sensor sensitive to different trimethylamine (TMA) concentrations for development of a fish freshness sensor resulted in the ability to detect 50 ppm of TMA with excellent selectivity (Egashira and others 1990). An EN based on metalloporphyrin-coated quartz microbalance was used to evaluate freshness of stored cod fillets and showed the presence of volatile amines, alcohols, and sulfides (di Natale and others 1996). Luzuriaga and Balaban (1999) successfully correlated electronic nose (EN) results of raw farmed Atlantic salmon fillets with storage time and sensory scores. Korel and others (2001) were able to find good relationships between microbial load, sensory scores, and EN results for quality assessment of tilapia.

The objectives of this study were to use static headspace gas chromatography analysis coupled to a mass spectrometer (SHGC-MS) to characterize and compare the volatiles in canned pink salmon, after 2 and 9 mo of storage, produced from fish showing various grades of skin watermarking. These results were further evaluated using general discriminant analysis to determine whether it was possible to build predictive models for degree of skin watermarking based on the composition of the volatiles for both storage times. Another aim was to test the ability of a hand-held EN to differentiate between grades of skin watermarking in canned salmon and to correlate sensor readings with results from SHGCMS.

Materials and Methods

Salmon sampling and canning

Fresh Alaska pink salmon (*Oncorhynchus gorbuscha*) were collected from seafood processing plants on Kodiak Island during

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summer 2002. All fish used in this study were less than 36 h postmortem. Fish were sampled the same day and from the same fishing vessel. The salmon were graded based on the Alaska Seafood Marketing Inst. skin color evaluation guides (ASMI 2004). However, instead of using the 7-grade system (A, B, C, D, E, F, and G) as suggested by ASMI (2004), fish were graded and combined into 4 main grading categories of skin watermarking and labeled A, BC, DE, and FG. Fish weight averaged from a minimum of 1.0 kg to a maximum of 2.5 kg. Fish were immediately eviscerated and steaks of about 215 g were cut and placed in a 307 × 200.25 (8.73 cm width × 5.12 cm height) 2-piece cans with no added salt. The cans were vacuum-sealed, retorted, and cooled in our pilot plant using standard industry practices (NFPA 1982). Eighteen cans were produced for each of the 4 grades of watermarking. In addition, a commercially canned batch was provided by a local seafood processor. During commercial processing, salmon is canned with approximately 1% salt added. The fish in the commercial cans were described by the processor as dark and represented watermarking grades DE or FG. No additional information about origin or catch date was provided.

Static headspace gas chromatography analysis coupled to a mass spectrometer (SHGCMS)

At 2 and 9 mo of storage at 25 °C, the headspaces of the liquors of 10 and 8 cans, respectively, were analyzed for each grade of watermarking and the commercial sample coded 'I'.

The liquid phase from the canned salmon was drained into a clean beaker. Two 10-g portions of the aqueous phase were immediately poured into 20-mL hypovials and crimp-sealed with a Teflon/silicon septum (replicates). The SHGCMS methodology was adapted from methods described by McLachlan and others (1999) and by Girard and Nakai (1991). An HP 7694 (Agilent Technologies, Wilmington, Del., U.S.A.) static headspace autosampler with a 44-sample capacity was used with the following conditions: oven temperature, 75 °C; loop temperature, 85 °C; transfer line temperature, 105 °C; loop capacity, 3 mL; loop fill time, 0.3 min; pressurization time, 0.3 min; vial equilibration time, 15 min; injection time, 1 min; vial pressure, 10 psi; shaker mode, fast; GC cycle, 30 min.

Volatile separation and identification was accomplished using a gas chromatograph model GC6890 interfaced with a mass spectrometer MS5973 (Agilent Technologies) and fitted with a dB-WAX capillary column of 30 m × 0.25 mm × 0.25 mm film thickness (J&W Scientific, Folsom, Calif., U.S.A.). Helium was used as carrier gas at 1 mL/min at an average velocity of 37 cm/s in constant flow mode. Injector temperature was 100 °C. Oven programming was as follows: initial temperature, 58 °C; hold for 5 min; increase temperature at 20 °C/min to 110 °C to give a total run time of 7.6 min. The MS was operated in electron impact mode under the following conditions: temperature of interface 240 °C; source temperature, 230 °C; quadrupole temperature, 150 °C; solvent delay, 1.35 min; acquisition rate, 3.99 scans/s. Positive identifications were based on comparison of GC retention times and mass spectra of unknowns with those of authentic standard compounds (Sigma-Aldrich, St. Louis, Mo., U.S.A.) analyzed under identical conditions. Tentative identifications were based on matching spectra of unknowns with those found in the NIST'98 mass spectral data library (Agilent Technologies).

EN analysis

A Cyranose 320 (Cyrano Sciences, Inc., Pasadena, Calif., U.S.A.) was used to analyze the headspace of canned pink salmon having different grades of watermarking. This hand-held electronic nose has 32 conducting polymer sensors, each coated with a unique carbon black film, as described by Van Deventer and Mallikarjunan (2002). The instrument was conditioned in air for 6 min before testing the headspace for each batch of cans (Cyrano Sciences 2000). The settings in the software program (PCnose 6.9, Cyrano Sciences) used for the Cyranose 320 were as follows: 10-s baseline purge at medium pump speed, 10-s sample draw at medium speed, 2 s for snout removal, 0 s 1st sample gas purge, 5 s 1st air intake purge at high speed, 30 s 2nd sample gas purge at high speed, and 0 s 2nd air intake purge. The substrate heater was set at 35 °C and digital filtering was active. Of the 32 sensors, 4 (sensors 5, 6, 23, and 31) were deselected due to their sensitivity to water (Cyrano Sciences 2001).

Cans were held at room temperature (25 °C) and tested after 2 and 9 mo of storage. For the 2-mo samples, 10 cans of each watermarking grade and the commercial sample were tested. At 9 mo, 8 cans of each grade plus the commercial lot were tested. For data processing, a canonical algorithm for discriminant analysis, auto-scaling, and normalization 1 settings were used. The Cyranose 320 was equipped with a 5.1-cm stainless-steel, luer-lock blunt-end needle for drawing the headspace samples immediately after opening each can. The needle was positioned horizontally approximately 0.5 cm above the sample surface and moved gently side to side during the 10-s sample draw. After all samples were tested, the data were cross-validated for the training set (Cyrano Sciences 2000). Results for each sensor are expressed as changes in resistance ($\Delta R/R$).

SHGCMS statistical analysis

SHGCMS results were evaluated using factorial analysis of variance with Statistica 6.1 (StatSoft Inc., Tulsa, Okla., U.S.A.). For tests of statistical significance between classes and storage time, unequal N Tukey's test for significant differences ($P < 0.05$) was used. In addition, SHGCMS data were subjected to forward stepwise general discriminant analysis (FSGDA) using the Multivariate Exploratory Techniques module from Statistica 6.1. Using the watermarking grading system (A, BC, DE, FG, and I-Commercial) as grouping variable, this procedure ranked each gas chromatography-mass spectrometry (GC-MS) peak on the basis of the magnitude of its contribution to the discriminatory power of the system. Thus, a number of peaks were selected for each dataset (2 and 9 mo of can storage) and a 2-dimensional canonical plot was obtained using the 1st 2 canonical roots, together with the classification matrix and the calculated squared Mahalanobis-distances (M^2-distances).

EN statistical analysis

The software PCnose 6.9 used multivariate discriminant analysis to calculate interclass Mahalanobis-distances (M-distances), which include the responses for all sensors. Results are expressed in a classification matrix as % correct identification along with a 2-dimensional canonical plot using the 1st 2 canonical roots. To select the most responsive sensors and to eliminate sensors with low or null contributions to the model, FSGDA was performed using the Multivariate Exploratory Techniques module from Statistica 6.1. Using the watermarking grading system (A, BC, DE, FG, and I-Commercial) as grouping variable, this procedure ranked each sensor response based on the magnitude of its contribution to the discriminatory power of the system (Van Deventer and Mallikarjunan 2002). Thus, a number of sensors were selected for each dataset (2 and 9 mo of can storage), and a 2-dimensional canonical plot was obtained using the 1st 2 canonical roots, together with the classification matrix and M^2-distances.

Results and Discussion

SHGCMS

Watermarking in pink salmon is used by seafood processors to grade the quality of the canned salmon. Figure 1 depicts pink salm-

on showing 3 distinct degrees of watermarking, which were labeled according to the ASMI grading system (ASMI 2004). Some morphological changes are readily noticeable such as increase in snout length and development of a hump in the dorsal area. It is not possible to notice the dramatic change in skin color in the black-and-white images. Grade A fish had glossy silver scales, whereas grade D showed significant skin darkening and early development of hump and snout, and grade G showed pronounced enlargement of hump and snout and severe darkening of skin and belly cavity.

A representative chromatogram of grade A canned pink salmon at 2 mo of storage showed 13 peaks in the 7.5-min run (Figure 2). Table 1 shows the results in peak area percentages for the compounds quantified for the 2- and 9-mo cans and the results of the post hoc test for significant differences ($P < 0.05$). One of the noticeable differences between the 2- and 9-mo samples was a chromatographic signal identified as unknown 2. This compound was below detection limits in the 2-mo samples. However, this peak occurred at 1.8 min just after peak number 5 in cans stored for 9 mo. This compound may be a marker to detect aging in canned salmon. A variety of sulfur-containing compounds such as methanethiol, dimethyl sulfide, and carbon disulfide were identified in all samples, supporting previous research (Girard and Nakai 1991; Milo and Grosch 1996). Sulfur-containing compounds made up 30% to 50% of the total volatiles and tended to decrease with increasing degrees of watermarking. Moreover, 9-mo samples experienced a significant ($P < 0.05$) increase in the S-containing compounds for grade BC and in the commercial batch (Table 1). Sulfur-containing amino acids such as methionine and cysteine are, most likely, the precursors of these small-molecular-weight sulfur-containing volatiles identified.

Several alcohols, aldehydes, and ketones were also identified, and most have already been reported as headspace constituents of fish products (Girard and Nakai 1991; Zhang and Lee 1997; Prost and others 2004). Aldehydes, ketones, and alcohols have also been identified in freshly harvested seafood, and as suggested by Lindsay (1990) are derived from specific oxygenase activity on polyunsaturated fatty acids. Propanal showed a significant ($P < 0.05$) increase and doubled in concentration for all groups during storage (Table 1). Acetaldehyde showed a marked increase with more severe degrees of watermarking from 4% to a maximum of 8% of the total volatiles. Methyl-isobutyl-ketone (MIK) ranged from 1% to 3.5% of the total volatiles and was detected only in grade A and commercially canned samples. This compound may be used as marker for grade A pink salmon, produced from fish showing no skin watermarking and commanding the highest market prices. However, additional studies to confirm this observation are needed. In addition, aging did not affect levels of MIK in the samples. Acetone levels ranged from 6% to 9% of the total volatiles and showed an increase to about 12% to 13% during aging for pink salmon samples grade DE and FG.

Two furans were present at small concentrations accounting for less than 5% of total volatiles, regardless of watermark level or storage period. Furans can be formed by acid catalysis of 1,4-diketones through intramolecular addition (March 1985). In case a methyl- or ethyl- substituent is present in positions 1- or 4- in 1,4-diketones, then methyl- or ethyl- substituted furans at position 2 are formed (March 1985). Thus, it is possible that both 2-methyl furan and 2-ethyl furan are formed during the thermal treatment required for

Figure 1—Alaska pink salmon having different grades of skin watermarking (adapted from ASMI 2004).

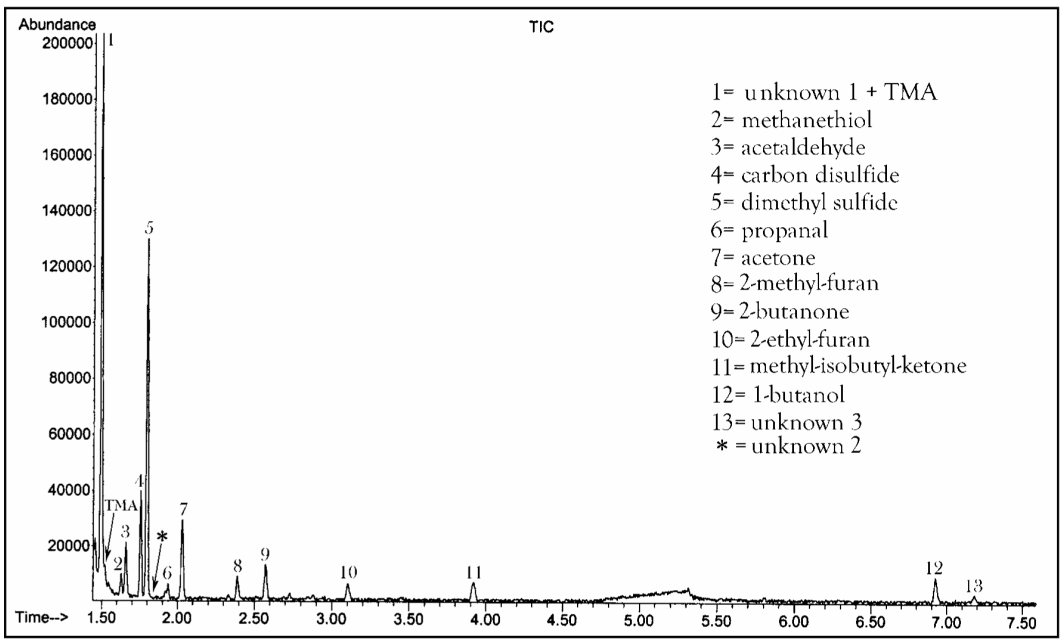

Figure 2—Total ion count chromatogram of grade A canned pink salmon at 2 mo of storage (* = not present at 2 mo of storage).

Table 1—Chemical constituents (peak area %) of canned salmon produced from watermarked pink salmon after 2 and 9 mo of storage[a,b]

Can age	2 Mo old				
Degree of watermarking	A	BC	DE	FG	I
Sulfur-containing compounds	46.32 ^{AB} (12.01)	43.40 ^{B} (8.71)	30.64 ^{C} (10.11)	27.91 ^{C} (7.23)	53.56 ^{A} (10.29)
Aldehydes (A)	4.96 ^{A} (2.19)	7.04 ^{A} (2.36)	8.97 ^{ABC} (2.68)	8.48 ^{AB} (1.88)	6.77 ^{A} (2.59)
Ketones (K)	9.35 ^{A} (2.89)	10.58 ^{A} (1.80)	11.54 ^{AD} (2.9)	9.20 ^{A} (1.79)	12.10 ^{ABD} (3.11)
A & K	14.27 ^{A} (4.85)	17.62 ^{AB} (4.06)	20.50 ^{B} (5.37)	17.68 ^{AB} (3.56)	18.87 ^{AB} (5.47)
Unknown 1 + TMA[c]	31.67 ^{AB} (8.19)	30.44 ^{A} (6.70)	38.88 ^{BC} (7.48)	44.52 ^{C} (9.93)	23.04 ^{D} (5.56)
Methanethiol[d]	1.99 ^{A} (0.56)	2.88 ^{AB} (0.57)	3.44 ^{BC} (1.09)	3.94 ^{C} (1.59)	2.21 ^{AD} (0.91)
Acetaldehyde[c]	4.06 ^{AC} (2.03)	5.95 ^{ABC} (1.94)	7.56 ^{B} (2.33)	7.19 ^{B} (1.77)	6.18 ^{BC} (2.38)
Carbon disulfide[c]	3.12 ^{AC} (1.70)	2.64 ^{A} (0.93)	4.65 ^{AC} (1.96)	7.20 ^{B} (4.29)	5.49 ^{BC} (3.50)
Dimethyl sulfide[c]	41.21 ^{AB} (12.45)	37.88 ^{A} (8.97)	22.55 ^{C} (10.83)	16.77 ^{C} (4.94)	45.86 ^{BD} (11.89)
Unknown 2	BDL	BDL	BDL	BDL	BDL
Propanal[c]	0.91 ^{AD} (0.53)	1.10 ^{AD} (0.56)	1.41 ^{AD} (0.85)	1.29 ^{AD} (0.75)	0.63 ^{A} (0.34)
Acetone[c]	5.69 ^{AC} (1.52)	8.40 ^{BC} (1.65)	9.22 ^{B} (2.38)	7.38 ^{AB} (1.44)	6.89 ^{AC} (1.88)
2-Methyl-furan[c]	0.96 ^{AC} (0.30)	1.38 ^{BE} (0.30)	1.25 ^{AB} (0.37)	1.25 ^{AB} (0.39)	0.80 ^{C} (0.24)
2-Butanone[c]	2.4 ^{ABC} (0.92)	2.19 ^{AB} (0.29)	2.31 ^{ABC} (0.66)	1.82 ^{B} (0.46)	2.88 ^{ACE} (0.81)
2-Ethyl-furan[c]	1.94 ^{AC} (0.91)	2.19 ^{AC} (0.50)	2.71 ^{A} (1.25)	1.98 ^{AC} (0.80)	0.50 ^{B} (0.02)
Methyl-isobutyl-ketone[c]	1.74 ^{A} (0.62)	BDL	BDL	BDL	2.33 ^{B} (0.55)
1-Butanol[c]	1.96 ^{AD} (0.81)	1.52 ^{A} (0.47)	1.52 ^{A} (0.41)	2.13 ^{AB} (0.59)	1.51 ^{AC} (0.40)
Unknown 3	2.87 ^{ACD} (1.15)	3.53 ^{AB} (0.76)	4.49 ^{B} (1.98)	4.54 ^{B} (2.26)	2.19 ^{ACD} (0.73)

(continued on next page)

industrial sterilization of the cans. Trimethylamine and a co-eluting unknown compound ranged from 10% to 20% of the total volatiles and showed a significant ($P < 0.05$) increase with increasing degree of watermarking. These latter 2 compounds also showed a significant decrease of about 20% as the cans were aged.

SHGCMS prediction models using FSGDA

According to Alasalvar and others (2004), discriminant function analysis is a pattern-recognition tool used to maximize multidimensional distances between groups of observations. The reported M-distances (or M^2-distances) represent the distance between 2 points in a multidimensional space, which is defined by 2 or more correlated variables (Alasalvar and others 2004). The minimum level required for a database predictive model is a value of 90% correct classification, but higher percentages may be required for specific applications (Van Deventer and Mallikarjunan 2002). In addition, a model showing high percent recognition during cross-validation will yield M^2-distance values higher than 25 or M-distances higher that 5 (Van Deventer and Mallikarjunan 2002). Results from FSGDA obtained with Statistica 6.1 for the 2- and 9-mo studies are shown in Figure 3 and 4, respectively. The FSGDA for 2-mo-old cans, at step number 9 (final step) using a *P* value of 0.05, removed 5 GC peaks from the model. The compounds included in the 2-mo model were carbon disulfide, dimethyl disulfide, 1-butanol, propanone, TMA and the co-eluting unknown 1, MIK, 2-methyl furan, and 2-ethyl furan. The M^2-distances for this model ranged from 6.07 to 82.24 (Table 2), with cross-validation showing a total of 92% correct classifications (Table 3). FSGDA for 9-mo-old cans, at step number 10 (final step) using a *P* value of 0.05, also removed 5 peaks from the model. The compounds included in this model were carbon disulfide, dimethyl disulfide, methyl mercaptan, 1-butanol, propanal, MIK, unknown 2, unknown 3, and 2-ethyl furan. The M^2-distances for this model ranged from 11.45 to 131.98 (Table 2), with cross-validation showing a total of 93.75% correct classifications (Table 3).

No overlapping occurred with samples FG or commercial samples (I), and both sample groups showed 100% correct classification in either storage time (Table 3). Samples coded A overlapped with samples coded BC in both storage periods. BC samples showed 95% and 100% correct classifications for the 2- and 9-mo storage samples; however grades lower (A) or higher (DE) than BC were incorrectly classified as BC, showing overlap between those classes (Table 3). The lowest percentages of correct classification occurred with DE samples at 75% and 87.5% for the 2- and 9-mo models, respectively. M^2-distances clearly show that the differences detected in the proportion of the volatiles present in the headspace of the canned samples do not allow clear distinction of grade A salmon from salmon graded as BC and DE (Table 2). On the other hand,

Table 1 *(continued)*—Chemical constituents (peak area %) of canned salmon produced from watermarked pink salmon after 2 and 9 mo of storage[a,b]

Can age	9 Mo old				
Degree of watermarking	**A**	**BC**	**DE**	**FG**	**I**
Sulfur-containing compounds	56.17 ^{AD} (9.70)	58.69 ^{D} (7.76)	36.61 ^{BC} (7.92)	31.99 ^{C} (4.24)	65.57 ^{D} (19.54)
Aldehydes (A)	6.65 ^{A} (1.80)	7.45 ^{A} (1.88)	11.03 ^{BC} (1.87)	11.33 ^{C} (3.01)	6.45 ^{A} (3.06)
Ketones (K)	12.20 ^{ABD} (2.66)	10.86 ^{AD} (1.99)	15.32 ^{BC} (2.75)	16.36 ^{C} (3.15)	13.35 ^{CD} (5.99)
A & K	18.84 ^{AB} (4.26)	18.32 ^{AB} (3.71)	26.35 ^{CD} (4.01)	27.69 ^{D} (5.28)	19.79 ^{BC} (8.93)
Unknown 1 + TMA[c]	10.13 ^{E} (3.19)	10.69 ^{E} (1.65)	18.88 ^{D} (10.06)	18.83 ^{D} (8.48)	BDL
Methanethiol[d]	3.18 ^{BCD} (0.73)	4.19 ^{CE} (0.65)	6.22 ^{F} (1.32)	5.22 ^{EF} (1.10)	2.82 ^{ABC} (1.09)
Acetaldehyde[c]	4.27 ^{C} (1.05)	4.95 ^{C} (1.38)	7.86 ^{B} (1.80)	7.9 ^{B} (2.48)	5.28 ^{AC} (1.99)
Carbon disulfide[c]	2.71 ^{AC} (1.11)	2.86 ^{AC} (0.69)	4.25 ^{AC} (1.51)	5.40 ^{BC} (2.01)	5.15 ^{BC} (3.41)
Dimethyl sulfide[c]	50.29 ^{BD} (10.58)	51.65 ^{BD} (8.51)	26.14 ^{C} (9.48)	21.38 ^{C} (4.97)	57.60 ^{D} (14.23)
Unknown 2	6.05 ^{A} (1.58)	5.08 ^{A} (2.87)	8.53 ^{C} (3.92)	11.97 ^{B} (1.61)	8.47 ^{C} (3.10)
Propanal[c]	2.38 ^{B} (1.22)	2.50 ^{B} (0.82)	3.17 ^{CB} (1.02)	3.43 ^{C} (1.14)	1.59 ^{D} (0.77)
Acetone[c]	7.45 ^{AB} (1.32)	8.37 ^{BC} (1.67)	12.07 ^{D} (2.48)	13.58 ^{D} (2.69)	8.18 ^{BC} (2.90)
2-Methyl-furan[c]	1.47 ^{BE} (0.34)	1.38 ^{BE} (0.36)	1.91 ^{D} (0.43)	1.73 ^{DE} (0.37)	1.20 ^{ABC} (0.46)
2-Butanone[c]	3.05 ^{CD} (0.87)	2.50 ^{ABCD} (0.35)	3.25 ^{D} (0.46)	2.78 ^{ACD} (0.65)	3.31 ^{DE} (1.3)
2-Ethyl-furan[c]	2.00 ^{AC} (0.53)	1.66 ^{C} (0.44)	2.62 ^{A} (0.73)	2.35 ^{AC} (1.14)	0.21 ^{B} (0.16)
Methyl-isobutyl-ketone[c]	1.69 A (0.38)	BDL	BDL	BDL	2.75 ^{B} (1.07)
1-Butanol[c]	3.39 ^{B} (2.42)	2.47 ^{ABCD} (0.41)	3.17 ^{BD} (0.79)	2.33 ^{ACD} (0.59)	2.15 ^{ACD} (0.66)
Unknown 3	1.94 ^{ACD} (1.48)	1.71 ^{CD} (0.56)	1.93 ^{ACD} (1.03)	3.11 ^{ABC} (2.14)	1.29 ^{D} (0.93)

[a]BDL = below detection limit; I = Commercial canned pink salmon; (SD) = standard deviation of the mean; TMA = trimethylamine.
[b]Different superscript letters in a row indicate significant differences ($P < 0.05$) between samples.
[c]Match retention time and mass spectra of standard and also NIST'98 mass spectral data library.
[d]Compound identified by NIST'98 mass spectral library at a match quality above 95%.

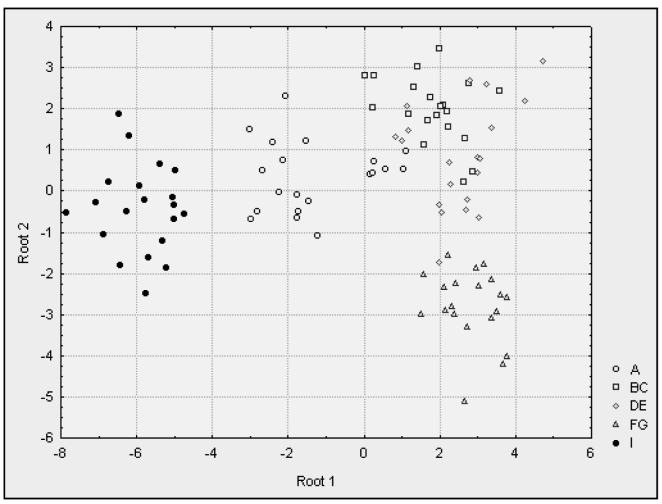

Figure 3—Canonical projection plots of static headspace gas chromatography analysis coupled to a mass spectrometer (SHGCMS) data for different skin watermarking grades of canned salmon stored for 2 mo derived from forward stepwise general discriminant analysis (FSGDA).

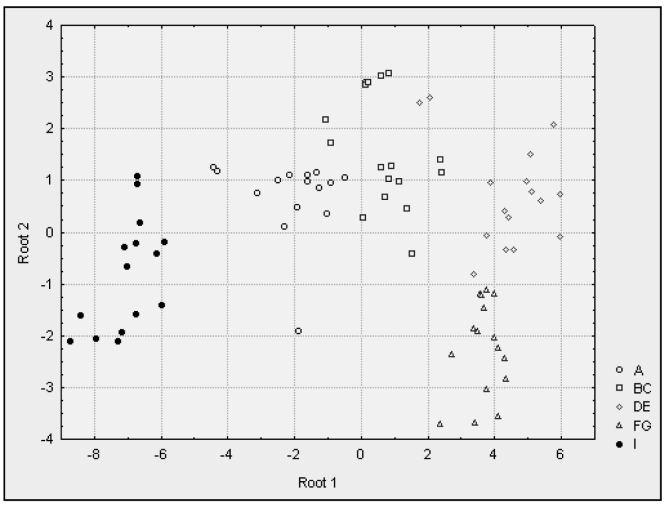

Figure 4—Canonical projection plots of static headspace gas chromatography analysis coupled to a mass spectrometer (SHGCMS) data for different skin watermarking grades of canned salmon stored for 9 mo derived from forward stepwise general discriminant analysis (FSGDA).

Table 2—Mahalanobis distances (M-distances) and squared Mahalanobis distances (M^2-distances) for 2- and 9-mo samples[a]

	M-Distances EN data PCnose 6.9		M^2-distances EN data FSGDA		M^2-distances SHGCMS data FSGDA	
Mo	2	9	2	9	2	9
A to BC	3.21	2.76	18.62	40.86	17.11	11.45
A to DE	3.21	4.39	18.97	64.96	23.55	43.54
A to FG	1.25	5.81	19.69	90.32	30.49	42.01
A to I	1.94	8.34	21.58	115.68	28.43	31.23
BC to DE	3.44	1.96	38.12	10.29	6.07	17.02
BC to FG	3.74	3.80	46.63	30.63	24.28	23.32
BC to I	4.00	5.86	46.75	39.40	66.68	65.40
DE to FG	2.89	2.73	2.99	17.94	15.92	11.16
DE to I	3.74	4.67	47.35	24.99	73.54	131.98
FG to I	1.41	3.43	48.39	7.26	82.24	115.75

[a]EN = electronic nose; FSGDA = forward stepwise general discriminant analysis; SHGCMS = static headspace gas chromatography analysis coupled to a mass spectrometer.

grade A (bright fish) was readily distinguishable from grade FG (dark fish), and this is in agreement with the reported sensory differences found in canned bright and dark chum salmon (Huynh and Mackey 1990). Although the commercial batch contained watermark grade DE or FG fish, the canned products were distinguishable from all watermarking grades used in the FSGDA model for the SHGCMS data (Table 3). However, the M^2-distances and the 2-dimensional canonical plots show that the commercial batch was closest to grade A canned salmon stored for 2 and 9 mo (Figure 3 and 4; Table 2). Differences in processing 454-g cans of pink salmon at the commercial facility versus the 227-g cans in our pilot plant may attribute to the discrepancies observed in using the model.

EN prediction models using PCnose

The analysis performed with PCnose includes the response for all sensors except those highly sensitive to water vapors (Cyrano Sciences 2000). For the 2-mo samples, there was considerable overlap in the canonical plot (Figure 5) as a result of small differences in M-distances, which ranged from 1.2 to 4.0 between any given grades (Table 2). Therefore, cross-validation results from the classification matrix showed low correct classification at 54%, and a significant overlap between watermarked grades occurred (Table 4; Figure 6). A better separation was observed in the canonical plot for the 9-mo samples (figure not shown), particularly between Grade A and Grade DE and between the commercial lot (I) and Grades A, BC, and DE. The M-distances for this model ranged from 4.7 to 8.3 (Table 2) with cross-validation showing 62.5% correct classification (Table 4). The watermarked grades for cans stored for 9 mo showed an identification primarily of 1 grade higher or lower than expected for the training set (Table 4).

The percent correct classification determined with PCnose 6.9 for both storage time models was very low, and neither model can be used to predict watermarked grade of Alaska pink salmon. Van Deventer and Mallikarjunan (2002) reported excellent discrimination for food packaging taints using the Cyranose 320 and the PCnose software with a cross-validation value of 100%. In their study, 3 levels of retained solvents in a 2-layer polymer film, used for food packaging, were compared. In contrast, we investigated the ability of the Cyranose 320 to discriminate between 5 classes of canned salmon for each of the 2 intervals of time studied. It is definitely harder to achieve cross-validation of 100% when trying to discriminate a larger number of sample classes involving the chemical complexity of food versus pure chemical compounds. Following Van Deventer and Mallikarjunan (2002) suggestions, we carried out further investigations using FSGDA to try to increase the percent correct classifications of the models by selecting the most discriminatory sensors for the 2- and 9-mo storage studies.

EN prediction models using FSGDA

Results from multivariate discriminant analysis obtained with Statistica 6.1 for the 2- and 9-mo studies are shown in Figure 6 and 7, respectively. FSGDA for 2-mo-old cans, at step number 17 (final step) using a *P* value of 0.05, removed all but 10 sensors from the model. The sensors included in the 2-mo cans model were sensor numbers 8, 12, 14, 15, 16, 20, 23, 24, 26, and 31. The M^2-distances for these differences ranged from 2.99 to 48.39 (Table 2), with cross-validation showing 90% correct identification (Table 5). FSGDA for 9-mo cans, at step number 8 (final step) using a *P* value of 0.05, removed all but 8 sensors from the model. In this case, the sensors included in the model were sensor numbers 3, 5, 6, 10, 16, 24, 27, and 30. The M^2-distances for these differences ranged from 7.26 to 115.68 (Table 2), with cross-validation showing 92.5% correct identification (Table 5). It is clear that using the response from the selected sensors drastically increased percent correct classification in both models. In the 2-mo model, 1 grade A can was misclassified as grade B can, whereas cans DE and FG showed considerable overlap. In the 9-mo model, 1 BC can was misclassified as a DE can, and 1 DE can was misclassified as a BC can. In addition, 1 I can was misclassified as an FG can. Although the commercial batch contained watermark grade DE fish at the processing plant, the canned products (except 1 can) were distinguishable from all watermarking grades used in the FSGDA model for the EN data (Table 5). However, the M^2-distances and the 2-dimensional canonical plots show that the commercial batch was closest to grade A canned salmon stored for 2 mo and closest to grade FG cans stored for 9 mo (Figure 6 and 7; Table 2).

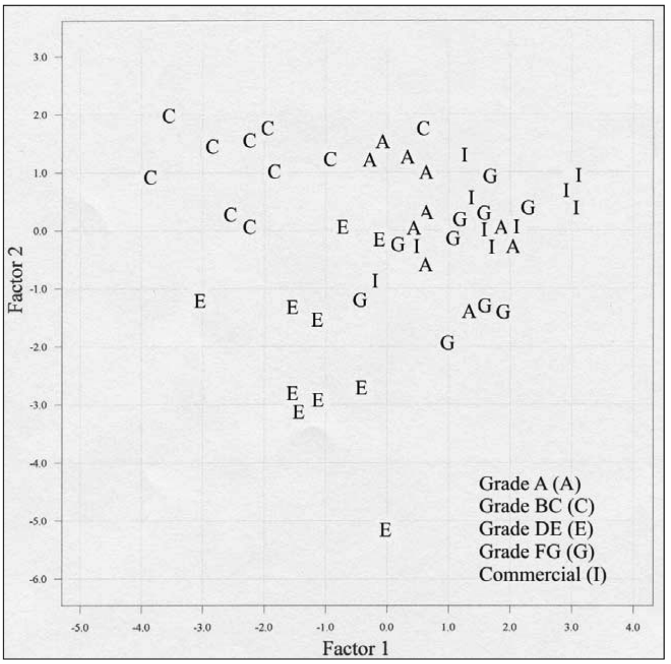

Figure 5—Canonical projection plots of electronic nose (EN) data for canned salmon stored for 2 mo derived from PCnose 6.9.

Correlation of SHGCMS and EN data

As suggested by Alasalvar and others (2004), Pearson correlation coefficients were calculated to determine if any univariate correlation existed between EN sensor responses and SHGCMS. We tested the correlation between the peaks and the sensors selected by FSGDA for both, the 2- and 9-mo models. Several correlations between sensors and volatiles were significant ($P < 0.05$); however r values ranged from a high of 0.6 to a low of –0.6 for either the

Table 3—Classification matrix for 2- and 9-mo samples from SHGCMS data using FSGDA[a]

	% correct		A		BC		DE		FG		I	
Mo	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**
A	90	81.25	18	13	2	3	0	0	0	0	0	0
BC	95	100	0	0	19	16	1	0	0	0	0	0
DE	75	87.50	0	0	4	2	15	14	1	0	0	0
FG	100	100	0	0	0	0	0	0	20	16	0	0
I	100	100	0	0	0	0	0	0	0	0	20	16
Total	92	93.75	18	13	25	21	16	14	21	16	20	16

[a]FSGDA = forward stepwise general discriminant analysis; SHGCMS = static headspace gas chromatography analysis coupled to a mass spectrometer.

Table 4—Classification matrix for 2- and 9-mo samples from EN data using PCnose 6.9[a]

	% correct		A		BC		DE		FG		I	
Mo	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**
A	50	75	5	6	1	2	0	0	3	0	1	0
BC	90	37.5	0	2	9	3	1	3	0	0	0	0
DE	60	50	2	0	1	3	6	4	1	1	0	0
FG	10	62.5	5	0	0	1	2	2	1	5	2	0
I	60	87.5	1	0	0	0	1	1	2	0	6	7
Total	54	62.5	13	8	11	9	10	10	7	6	9	7

[a]EN = electronic nose.

Table 5—Classification matrix for 2- and 9-mo samples from EN data using FSGDA[a]

	% correct		A		BC		DE		FG		I	
Mo	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**	**2**	**9**
A	90	100	9	8	1	0	0	0	0	0	0	0
BC	100	87.5	0	0	10	7	0	1	0	0	0	0
DE	80	87.5	0	0	0	1	8	7	2	0	0	0
FG	80	100	0	0	0	0	2	0	8	8	0	0
I	100	87.5	0	0	0	0	0	0	0	1	10	7
Total	90	92.5	9	8	11	8	10	8	10	9	10	7

[a]EN = electronic nose; FSGDA = forward stepwise general discriminant analysis

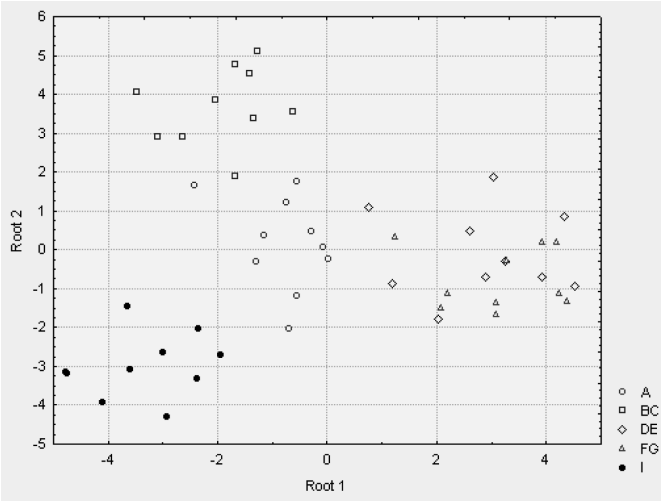

Figure 6—Canonical projection plots of electronic nose (EN) data for different skin watermarking grades of canned salmon stored for 2 mo derived from forward stepwise general discriminant analysis (FSGDA).

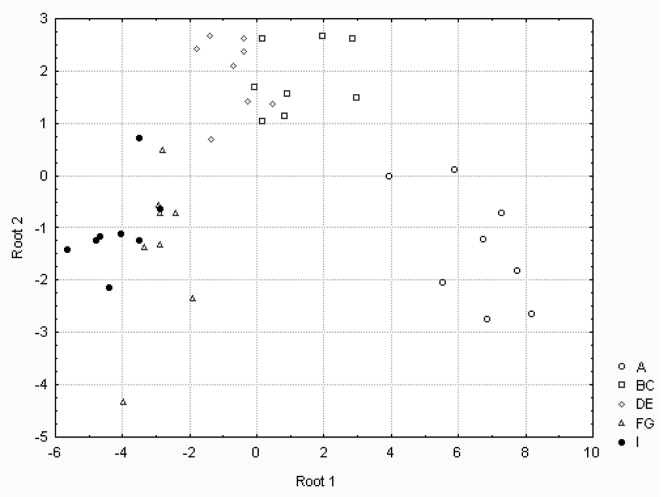

Figure 7—Canonical projection plots of electronic nose (EN) data for different skin watermarking grades of canned salmon stored for 9 mo derived from forward stepwise general discriminant analysis (FSGDA).

2- or the 9-mo datasets. If the calculated value of the Pearson correlation coefficients between 2 variables is zero, then no linear correlation is likely to exist between the variables. Therefore, the responses of the group of sensors investigated showed weak positive (or negative) linear correlations with SHGCMS peaks (Alasalvar and others 2004). Furthermore, the compounds that showed correlations values close to –0.6 or 0.6 presented similar correlation values for all sensors included in the analysis, suggesting that the sensors of the EN tested show low specificity for the group of volatiles found in canned pink salmon.

We did not pursue further investigations using nonlinear (multivariate) correlations as suggested by Alasalvar and others (2004) because even though our results indicate that it is possible to distinguish between bright and dark fish, the intermediate grades (BC and DE) show considerable overlap with grades A and FG. Alasalvar and others (2004) were able to use Pearson correlation, followed by the more sophisticated canonical correlation analysis, to determine very strong correlations between specific sensors in the e-NOSE 4000 model and volatiles of varieties of hazelnuts quantified using dynamic headspace GC-MS analysis. In our study it is apparent that the watermarking grades are not readily distinguishable from each other by either method of analysis (Table 2) and that there are many more differences in the proportion of the volatiles rather than in the quality (Table 1).

Future studies

Further research is needed to determine the ability of the proposed models to correctly classify "unknown" samples. Additionally, other electronic nose systems and alternative EN sensor types should also be tested. More research in volatile compounds of canned salmon using GC-MS is needed, and future studies should include the use of gas chromatography coupled to olfactometry (GC-O) for determining possible odor compounds responsible for the "late odor" previously reported by Huynh and Mackey (1990).

Conclusions

We report for the 1st time, the use of SHGCMS and EN for differentiating grades of watermarked fish in canned salmon. Headspace analysis revealed a few significant differences in the profile of the volatiles in canned salmon produced from fish with different watermarking grades. Additionally, differences were also detected between samples analyzed at 2 and 9 mo of can storage. Results from Cyranose 320 analysis using the software provided by the manufacturer of the instrument did not grant good discrimination between watermarking grades of salmon at either storage time. However, higher discrimination was achieved at both storage times using FSGDA. Overall, results indicate that the watermarking grades studied are not readily distinguishable from each other by either method of analysis and that there are many more differences in the proportion of the volatiles rather than in the quality. Future studies will build on this initial database for predicting chemical quality attributes in commercially canned salmon.

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