Standardization of the Ammonia Electrode Method for Evaluating Seafood Quality by Correlation to Sensory Analysis

L. PIVARNIK, P. ELLIS, X. WANG, AND T. REILLY

ABSTRACT: Ammonia ion-selective electrode (ISE) measurements, reported as apparent ammonia, were correlated to expert sensory assessments of 6 different fish species stored on ice and at room temperature. Total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), and apparent ammonia showed the same development trend during storage. ISE measurements and TVB concentrations had a correlation of $r^2 = 0.92$. Sensory assessment, using a 1- to 100-mm line scale with values $>$ 50 considered unacceptable, resulted in an $r^2$ between sensory scores and ISE measurements of 0.78. Regression analysis predicted 19.6 mg/100 g of apparent ammonia in fish tissue at the sensory limit of 50, regardless of storage conditions. ISE measurements could be used in predicting borderline quality and decomposition.

Keywords: seafood quality, sensory analysis, ammonia, ion-selective electrode, total volatile bases

Introduction

QUALITY AND SPOILAGE PROFILES OF FIN-FISH HAVE BEEN the subject of intense research for many years in an effort to establish guidelines and action levels to control product quality and safety, and thereby promote consumer confidence. The effects of time and temperature abuse are clear and the types of compounds that reflect freshness and decomposition have been elucidated. Although a multitude of procedures have been developed to analyze seafood for many of these compounds, many of these techniques are complex, require expensive equipment, extensive training, and use of dangerous chemicals. Many are laborious and time-consuming (Ellis and others 2000). In response to the mandatory U.S. Food & Drug Admin. (FDA) 1997 seafood safety regulations (21 CFR 123, HACCP), rapid test kits have been developed by a number of companies that address some of the biological (Listeria) and chemical (histamine) hazards that are safety concerns in seafood processing facilities. While not normally considered safety concerns under the HACCP regulations, however, adulterated/decomposed products are still in violation of the Food, Drug and Cosmetic Act, and could reflect processing in an unsanitary manner. Sensory assessment by trained analysts has been the foundation of the regulatory system for purposes of final dispensation of questionable products, based on rejection of product when odors of decomposition are present (Staruszkiewicz 1994). Even though highly trained and capable of detecting decomposition or assessing quality, individual calibrated sensory experts are expensive to maintain and too few in number (Sakaguchi and Koike 1992; Bett and Dionigi 1997). More importantly, these experts are not the individuals who routinely make determinations as to the condition of the product at processing and distribution levels (Pivarnik and others 1998). The industry not only has difficulty training staff to be proficient and consistent in sensory assessment, but the need for continuous updating and recalibration, as well as the inevitable turnover in personnel, further complicate the problem. Chemical standards of seafood quality and decomposition are necessary to verify sensory judgments when disagreements arise, and whenever untrained individuals perform sensory evaluation. Therefore, a simple, rapid and validated procedure should be made available to both processors and regulators which reflect compounds that generally have been accepted as quality indicators, but whose measurements have been tedious and complex.

Current research, particularly in the area of biosensors, has been directed at developing rapid on-line and/or sensing methods for determining those compounds that reflect spoilage and/or freshness. But many of these techniques, while having great potential, are still far from implementation by both seafood industry and regulatory agencies. Other rapid methods designed to measure metabolites of bacterial action and compounds of biochemical/chemical degradation have been utilized to assess fish quality (Jacober-Pivarnik and Rand 1982; Ellis and others 1997). However, many of these biochemical methods also require laboratory training, extraction with dangerous chemicals, and/or sophisticated detection methods. Electronic “noses,” which have the potential for instantaneous information on quality, are expensive and have not been evaluated for use with seafood. Simple freshness/quality tests are being developed commercially, but are semi-quantitative, not yet available, or need more validation/verification (Conn 2001; Madden 2001).

Currently, total volatile base nitrogen (TVB-N) and trimethylamine (TMA) routinely have been used worldwide as indicators of fish quality and decomposition (Regenstein and Regenstein 1991; Huss 1995; Dullos and others 1999). The European Union has established guidelines as to use and measurement of TVB-N values for certain categories of fishery products (EU Commission 1995). Nonetheless, these methods are laborious, pose potential safety hazards, and therefore can only be performed in a laboratory with skilled analysts (Pivarnik and others 1998). Furthermore, the analytical procedures used to measure these volatile bases must be specifically stipulated, because results differ with the distillation procedures used to measure them (Pivarnik and others 1998).

A simple procedure was developed to help both industry and regulatory agencies routinely screen for quality attributes and indicators of decomposition, and was verified as to precision and accuracy through an inter-laboratory col-
Food Chemistry and Toxicology

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Prior to iced or room-temperature storage. With d for iced storage, samples were taken to obtain a full quality 7 fish, layered singly, per container. At predetermined time temperature conditions were stored under a hood with 5 to fish with a final ice layer. Fish stored under abusive or room-at 21 to 22 °C. Fish were iced in alternating layers of ice and were counted and divided into 2 groups. Each species was immediately transported on ice to the Food Science and Nut-
ticeps, scad) Lophius americanus (fluke, Paralichthys dentatus) whole mackerel (Scomber scombrus); whole, gutted tilefish (Lopholatilus chamaeleonticeps), and whole bluefish (Pomatotomus saliantax). Fish were obtained from local Rhode Island seafood processors, immediately transported on ice to the Food Science and Nutrition Research Center (FSNRC) at Univ. of Rhode Island (URI), U.S.A., for the storage trials. Upon arrival, the fish were counted and divided into 2 groups. Each species was aged in 2 ways: stored in ice at 4 °C and at room temperature at 21 to 22 °C. Fish were iced in alternating layers of ice and fish with a final ice layer. Fish stored under abusive or room-temperature conditions were stored under a hood with 5 to 7 fish, layered singly, per container. At predetermined time intervals, 4- to 8-h intervals for room temperature and 2 to 3 d for iced storage, samples were taken to obtain a full quality profile (from very fresh to clearly decomposed). “Day zero” represented the first-day samples, and were received by re-
searchers prior to iced or room-temperature storage. With the exception of whiting, 4 fish were sampled at each time point for sensory and chemical testing. The fish were filleted and 4 fillets were packaged in 3-mil vacuum pouches (Market Sales Co., Newton, Mass., U.S.A.), vacuum-sealed (~24 mm Hg) using a Super Vac (Smith Eqpt., Clifton, N.J., U.S.A.), then were frozen and stored at ~60 °C until testing could be accomplished. Vacuum pouches used were 10 × 10, 12 × 10, or 10 × 15, depending on the size of the fish species. Since whiting is small, 6 fish were sampled at each time interval and 2 packages of 6 fillets were packaged and stored. Packag-
es for each sampling interval were assigned a random 3-digit code. One package of vacuum-packaged frozen fish was used for sensory testing at the National Marine Fisheries Service (NMFS), Gloucester, Mass., U.S.A., and the other pack-

gave promising potential use of the ISE as a screening tool in lieu of the traditional TVB method, both at industry and reg-

ularly levels. The ISE method was validated through an AOAC International collaborative study as First Action Offi-

cial Method 999.01, “Volatile Bases in Seafood” (AOAC 2000; Ellis and others 2000).

Regardless of the method’s speed, ease of use, validated performance characteristics, or its apparent agreement with other methods of analysis, the ultimate acceptance and substantiation of the ISE method would be its correlation with expert sensory analysis. Sensory assessment would provide the final determinant for predictive ability and acceptability of an analytical technique in food (Poste and others 1991). Sensory analysis of seafood has always been part of seafood processing/distribution through government and/or indus-

try application (York 1990). While this “instrument” has con-
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ry arena, carried out by trained experts. Hence, the next obliga-
ty step toward validating the ammonia electrode for use as a regulatory and industrial analytical tool for deter-

mining acceptance/rejection of seafood was to correlate it to expert sensory analysis. The overall goal of this project, then, was to evaluate the relationship between sensory assessment and the ammonia ISE procedure on fish.

Materials and Methods

Initial sample preparation and storage
Six fish species were evaluated in this study: whole, gut-
ted/headless monkfish (Lophius americanus); whole whiting (silver hake, Merluccius bilinearis); whole summer flounder (fluke, Paralichthys dentatus); whole mackerel (Scomber scombrus); whole, gutted tilefish (Lopholatilus chamaeleon-
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searchers prior to iced or room-temperature storage. With

Expert sensory assessment
Vacuum-packaged, frozen fish samples were shipped via overnight mail to the U.S. Dept. of Commerce/National Oceanic and Atmospheric Admin. (USDC/NOAA) sensory laboratory in Gloucester, Mass. This laboratory fulfilled all the 1993 requirements mandated by the American Soc. of Testing Materials (ASTM STP 913) guidelines for physical design of sensory evaluation laboratories. Upon arrival, the samples were checked to ensure that the frozen integrity had been maintained, then placed in a ~80 °C freezer, and stored for no longer than 1 m before sensory evaluation of the raw product. Prior to a scheduled sensory session, samples were prepared in an odor-free area that was separated from the sensory testing facility. The vacuum-sealed samples were tempered at 4 °C for 12 h, and brought to room temperature in running water immediately before sensory evaluation. All packages were identified by random 3-digit codes and placed in booths.

Three expert inspectors, internationally calibrated and trained, who had achieved test scores of greater than 85% during past testing sessions, were chosen to conduct the sensory assessments on all samples. Each inspector had at least 10 y of practical experience and had been trained by attend-
ing a minimum of 3 international Free Trade Harmonization Workshops. According to statistical criteria agreed upon by FDA and NMFS in the U.S. and the Canadian Food Inspection Agency, each inspector (after training) was presented with 120 test samples, using a 100-mm line, and each had to achieve a score of 85% or better, which confers “expert” status (Reilly and York 1994). Each inspector also met Interna-
tional Standards (ISO) for sensory evaluation criteria (ISO 1994). A minimum of 3 sensory experts are required to achieve statistically valid results for quality and decomposi-
tion evaluation, and to ensure a high degree of acuity and re-

producibility (Poste and others 1991; Sims and others 1992). For each species, the analysts independently evaluated each sample for appearance, texture, and odor in the raw state.

Analysts evaluated each sample, using a standardized bal-

lot developed during international exercises involving har-

monization for product sensory standards and criteria, which USDC/NOAA seafood analysts had participated in de-
veloping. The ballot consisted of the 3-digit sample number, an unstructured 100-mm line scale, with a place to indicate whether the sample passed or failed for decomposition. Also included was a space to write any descriptive information for each sample. The line represented the degree of continuous deterioration of the sample, where 0 = “no deterioration” and 100 = “severe decomposition.” Samples obtaining sensory

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scores of greater than 50 were considered unacceptable, as agreed during an International Free Trade Agreement workshop and detailed during the harmonization workshops. Following independent evaluation, a panel leader collected Pass/Fail decisions, numerical data, and any terminology that described sensory characteristics.

Sample preparation for chemical analyses
Once the sensory assessment began on a given set of fish samples, chemical analyses were initiated. Frozen samples were thawed using cold running water. With the exception of whiting, 2 separate, composite fish-tissue homogenates for each sampling interval were prepared, using 2 fillets each. For whiting, the 2 homogenates contained 3 fillets each. Each homogenate was then sampled by duplicate extractions for ISE, TVB, and TMA determinations, resulting in 4 measurements.

Apparent ammonia by ISE
Apparent ammonia for all fish species was determined on all homogenates using the ISE procedure (AOAC 2000), as originally outlined by Pivarnik and others (1998). Briefly, 5 g of comminuted fish-tissue samples were blended with 95 mL of water for 2 min, and pH-adjusted with 2.0-mL alkaline ion-strength-adjuster (ISA) solution. The compounds contributing to the ammonia response were immediately determined using a precalibrated Orion Model 95-12 ammonia gas-sensing, ion-specific electrode, and an Orion Model 290A portable pH/ISE meter (Thermo Orion, Beverly, Mass., U.S.A.). The results were reported as apparent ammonia, due to data showing that the probe was detecting other volatile amines and not just ammonia (Pivarnik and others 1998). All results were reported as mg apparent ammonia per 100 g of fish tissue.

TVB and TMA analyses
The fish-tissue preparation and the sampling scheme are described above under “Materials and Methods.” Both TMA and TVB analyses were conducted on a single trichloroacetic acid (TCA) extraction. TVB concentrations were determined by distillation and titration, as specified by published procedures (Malle and Tao 1987; Malle and Poumeyrol 1989). Briefly, 50 g of comminuted fish were blended in 100 mL of 7.5% (w/v) TCA solution at high speed for 2 min. The homogenate was filtered through Whatman #1 or equivalent filter paper. A 25-mL aliquot of the TCA extract was pipetted into a distilling flask (250-mL Tector® digestion tube) and 10 mL of 10% NaOH was added. Steam distillation in a Tector Kjeltec Model 1002 distillation unit lasted until 75 mL of liquid was collected in a 125-mL Erlenmeyer flask containing 10 mL Kjeldahl indicator solution (4 g boric acid in distilled wa-

Statistical analysis
ISE results were statistically evaluated using regression analysis to determine the predictive ability of the ISE proce-

Table 1—Summarized descriptive statistics for expert sensory panel and ISE determinations

<table>
<thead>
<tr>
<th>Statistic</th>
<th>All samples</th>
<th>Samples when ISE &lt; 30 mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>107</td>
<td>91</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.54</td>
<td>0.78</td>
</tr>
<tr>
<td>Slope</td>
<td>0.29</td>
<td>0.165</td>
</tr>
<tr>
<td>Intercept</td>
<td>7.9</td>
<td>11.4</td>
</tr>
<tr>
<td>Predicted ISE (Y)</td>
<td>when sensory (X) = 50</td>
<td>22.3 19.6</td>
</tr>
<tr>
<td>95% CL</td>
<td>14.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Figure 1—Development pattern of volatile base (TVB-N), trimethylamine (TMA-N), apparent ammonia, and expert sensory analyses during storage of whole, gutted tilefish (Lopholatilus chamaeleonticeps) held on ice at refrigeration temperatures (4 to 5 °C) and at room temperature (21 to 22 °C).
dure, as compared to expert sensory assessment for all species and storage trials evaluated in this study. Regression analysis was also run to correlate TVB and TMA with apparent ammonia. Residual scatter plot and regression analyses were also used to evaluate the linear relationship between sensory and electrode data, and were used to support the removal of extremely spoiled samples from the final regression model (Bender and others 1982; Anderson 1987). All analyses were run using the NCSS 2000 6.0 software (Hintz 2000).

**Results and Discussion**

Figuress 1 to 6 illustrate the patterns of TVB, TMA, ISE and expert sensory scores for all 6 fish species in both temperature storage conditions. At both iced and ambient temperature storage, TVB-N, TMA-N, and apparent ammonia clearly showed the same development trend over the entire storage period for all species. The pattern of expert sensory scores also followed the same general trends of results obtained with the chemical tests. Of the species tested, results obtained by chemical tests on fluke were slightly slower to change, whereas sensory assessment showed a steep rate of decline (higher scores). As was previously reported in a similar study (Pivarnik and others 1998), the ISE measurements obtained during this project again mirrored TVB concentrations with a correlation of TVB with ISE at $r^2 = 0.92$ when all samples were included (Figure 7).

Figure 8 and 9 and Tables 2 to 3 illustrate the results of expert sensory assessment, as compared to apparent ammonia or ISE determinations. When all fish samples (N = 107) were incorporated into the statistical analysis scheme, the relationship between sensory and chemical determinations was marginal, having an $r^2$ of 0.54. An ISE value of over 22 corresponded to sensory failure (Figure 8, Table 1).

Table 2 shows the actual sensory and apparent ammonia values obtained at the sampling interval, first identified by the sensory analysts as unacceptable. Clearly, many of the

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**Figure 2**—Development pattern of volatile base (TVB-N), trimethylamine (TMA-N), apparent ammonia, and expert sensory analyses during storage of whole summer flounder (fluke, *Paralichthys dentatus*) held on ice at refrigeration temperatures (4 to 5 °C) and at room temperature (21 to 22 °C).

**Figure 3**—Development pattern of volatile base (TVB-N), trimethylamine (TMA-N), apparent ammonia, and expert sensory analyses during storage of whole whiting (silver hake, *Merluccius bilinearis*) held on ice at refrigeration temperatures (4 to 5 °C) and at room temperature (21 to 22 °C).
Table 2—Apparent ammonia values at first detected sensory failure (≥50) for tilefish, fluke, whiting, mackerel, bluefish, and monkfish held on ice and at room temperature

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Interval</th>
<th>Storage type</th>
<th>Apparent ammonia (mg/100 g)</th>
<th>Actual sensory score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilefish</td>
<td>8 days</td>
<td>Iced</td>
<td>24.9</td>
<td>69.7</td>
</tr>
<tr>
<td></td>
<td>28 h</td>
<td>Room temperature</td>
<td>20.6</td>
<td>67.7</td>
</tr>
<tr>
<td>Fluke</td>
<td>11 days</td>
<td>Iced</td>
<td>19.3</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>22 h</td>
<td>Room temperature</td>
<td>18.6</td>
<td>50.3</td>
</tr>
<tr>
<td>Whiting</td>
<td>13 days</td>
<td>Iced</td>
<td>22.8</td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>22 h</td>
<td>Room temperature</td>
<td>23.9</td>
<td>65.0</td>
</tr>
<tr>
<td>Mackerel</td>
<td>12 days</td>
<td>Iced</td>
<td>20.1</td>
<td>70.7</td>
</tr>
<tr>
<td></td>
<td>28 h</td>
<td>Room temperature</td>
<td>20.1</td>
<td>76.3</td>
</tr>
<tr>
<td>Bluefish</td>
<td>10 days</td>
<td>Iced</td>
<td>22.0</td>
<td>67.3</td>
</tr>
<tr>
<td></td>
<td>34 h</td>
<td>Room temperature</td>
<td>20.9</td>
<td>63.0</td>
</tr>
<tr>
<td>Monkfish</td>
<td>10 days</td>
<td>Iced</td>
<td>21.2</td>
<td>69.7</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>Room temperature</td>
<td>20.1</td>
<td>70.7</td>
</tr>
</tbody>
</table>

Figure 4—Development pattern of volatile base (TVB-N), trimethylamine (TMA-N), apparent ammonia, and expert sensory analyses during storage of whole mackerel (Scomber scombrus) held on ice at refrigeration temperatures (4 to 5 °C) and at room temperature (21 to 22 °C)

Figure 5—Development pattern of volatile base (TVB-N), trimethylamine (TMA-N), apparent ammonia, and expert sensory analyses during storage of whole bluefish (Pomatomus saltatrix) held on ice at refrigeration temperatures (4 to 5 °C) and at room temperature (21 to 22 °C)
samples reached the limit of sensory acceptability when the apparent ammonia values were lower than 22 mg/100 g. However, as the fish became badly spoiled, the ISE measurements had a tendency to become erratic, a trend that was documented in previous research (Pivarnik and others 1998). Therefore, to eliminate the impact of badly spoiled fish, a final regression was conducted on samples containing <30 mg/100 g apparent ammonia (N = 91) with sensory scores ranging from 11 to 88. This cut-off was statistically determined using residual plot and regression analyses to obtain...
maximum correlation, while retaining a balanced representation of fish over the full quality range. As the electrode values increased above 30, the correlation between sensory scores decreased. Visual examination of residual plots clearly illustrated that the outermost points that broke down the linear regression model were distinctly divided at electrode values above 30. Sensory values of > 50 corresponded to the sensory limit for decomposition. Of 107 total samples originally tested, 16 were eliminated, leaving 34 failed representative fish samples (compared to the original 50 failures). This resulted in an $R^2$ between ISE and expert sensory analysis at 0.78, and predicted apparent ammonia of 19.6 mg/100 g in fish tissue. This value corresponded to the sensory limit of 50, regardless of storage conditions (Figure 9, Table 1).

Table 3 illustrates the correlation and predicted apparent ammonia values for each species evaluated when the sensory value was 50. While the fatty fish (mackerel and bluefish) ap- peared to have a slightly lower correlation when compared to the lean species, all samples tested showed clearly that the measurement of apparent ammonia with the ISE procedure could be used as a predictor of borderline quality and decomposition.

### Conclusions

**Electrode or ISE Values for Apparent Ammonia and Expert Sensory Scores** had a good correlation, regardless of storage conditions. The ISE procedure could be used as a rapid screening method to determine borderline fish quality or initial stages of decomposition. A value of 20 mg/100 g, as determined by the ISE procedure, is proposed as a numerical guideline to reflect borderline quality or unacceptable product, as it correlated with the expert sensory failure over 50. These results were consistent for all species and temperatures tested. The 20 mg/100 g cut-off is meant to serve as a guideline since all species may not follow the same spoilage patterns.

### References


**Table 3—Summarized descriptive statistics for expert sensory panel and ISE determinations for each fish species when ISE$<30$ mg/100 g**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Fish species</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Tilefish</td>
</tr>
<tr>
<td>R²</td>
<td>0.83</td>
</tr>
<tr>
<td>Predicted</td>
<td>19.5</td>
</tr>
<tr>
<td>ISE (Y) when sensory (X) = 50</td>
<td>14</td>
</tr>
</tbody>
</table>

**Figure 9—Correlation of expert sensory scores and apparent ammonia (ISE) determinations for all fish samples stored on ice or at room temperature when the ISE values were <30 mg/100 g of fish tissue (N = 91)**

![Graph showing correlation](image-url)
Ammonia ISE Method for Seafood Quality

Assurance for Seafood Conference; Newport, OR May 16-18. Sponsored by Oregon Sea Grant Program. Abstract ORESU-W-93-001

This study was funded by a grant from the Natl. Oceanic and Atmospheric Admin./Natl. Marine Fisheries Service (NOAA/NMFS) under Grant Nr NA76FD0140. This project has been assigned Contribution Nr 3863 by the U.S. Dept. of Agriculture at the Univ. of Rhode Island, Agricultural Experiment Station. The project directors would like to thank the Rhode Island Dept. of Health for its support of this project. Finally, the project directors acknowledge the work of Santiago Rossi and Anne Carpenter from URI/FSN, and Mike DiLiberti and Marion Parko from NMFS/Sensory Branch, without whom this project would not have been successful.

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