TITLE: Rapid Quality Assessment of Mahi-mahi and Tuna

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Abstract
Mahi-mahi and tuna are currently inspected and graded by trained sensory experts by either the Food and Drug Administration (FDA) or the United States Department of Commerce's Seafood Inspection Program. These two species of finfish pose risks as vectors for scombroid poisoning. The major cause of scombroid poisoning is histamine, found in the muscle of various finfish species, including tuna and mahi-mahi. Histamine and other biogenic amine compounds that can act as co-indicators of scombroid poisoning were detected and quantified using a variety of methods. These methods included acetic acid specific Dräger tubes, a novel colorimetric method that detects volatile biogenic amines, and a histamine specific ELISA assay. The quantification results of all three methods correlated with the sensory grading system of mahi-mahi samples. Proximate compositional differences between the mahi-mahi and tuna may have affected the ability of these assays to quantify biogenic amines in tuna as effectively as with mahi-mahi. The colorimetric strips and ELISA were used for a method of standard addition (MSA) procedure to observe the matrix effect of the fish flesh. These results demonstrated how both grade (i.e. level of degradation and presence of spoilage compounds) and compositional differences (including lipid and protein makeup) affected the ability of the colorimetric strips and ELISA to accurately detect and quantify the biogenic amine compounds. The MSA study also demonstrated the effect that the phase of the analyte had on detection, as the compositional nature of the fish might affect the measurement of gas and liquid phase compounds differently.

Methodology

- Graded tuna and mahi samples were received from the FDA. Grade 1 was a high quality sample, grade 7 was a very poor quality sample (fail).
- Acetic Acid Dräger Tubes
    - 50 g samples (7 per grade)
    - 20 minute headspace accumulation
    - 2 minute pull via vacuum pump (Flow rate was approximately 2000 mL/min)
- Colorimetric Strips
    - 50 g samples (5 per grade)
    - Sealed in mason jars with bromophenol blue (BPB) strips
    - 45°C water bath for 60 minutes
    - Colorimeter measurement, focusing on b* portion of L* a* b* reading
- Histamine-Specific ELISA
    - Neogen Veratox® kit
    - 10 g samples (5 per grade)
- Method of Standard Addition (MSA)
    - Biogenic amine standard cocktail of equal parts histamine, tyramine, cadaverine, dimethylamine, trimethylamine

- 0, 10, 20 and 50 ppm spikes run in duplicate for each grade of fish
- Both Colorimetric strips and ELISA were performed for both unspiked and spiked (MSA) samples
- Results were statistically analyzed using one-way ANOVA and Tukey-Kramer post-hoc for means differentiation

Results

Table 1. Mahi-Mahi BPB and ELISA Results

Sample Grade	Average Volatile Biogenic Amines - BPB (ppm)	ELISA Histamine (ppm)
M-1	6.019 a ± 1.9	8.399 a ± 2.9
M-2	10.03 a ± 7.1	0.65 a ± 0.20
M-3	24.47 ab ± 2.7	3.690 a ± 1.5
M-4	5.354 a ± 3.3	1.261 a ± 0.23
M-5	28.93 b ±7.6	1.438 a ± 0.32
M-7	34.20 b ± 0.28	155.3 b ± 20

Means ±SE for quintuplicate determinations. The different superscripts in the same column indicate significant differences between samples within that column.

Table 2. Tuna BPB and ELISA Results

Sample Grade	Average Volatile Biogenic Amines - BPB (ppm)	ELISA Histamine (ppm)
T-1	6.179 ab ± 1.0	0.2362 a ± 0.072
T-2	7.850 b ± 0.77	0.1329 a ± 0.13
T-3	6.562 ab ± 1.7	0.789 a ± 0.27
T-4	1.720 a ± 0.86	0 a ± 0.0
T-5	2.691 ab ± 1.2	81.99 a ± 28
T-6	8.204 bc ± 2.1	197.7 b ± 71
T-7	7.064 bc ± 1.3	36.09 a ± 6.8

Means ±SE for quintuplicate determinations. The different superscripts in the same column indicate significant differences between samples within that column.

Table 3. Calculated Amines in Mahi-mahi using the MSA Procedure

Grade	Volatile Biogenic Amine - BPB (ppm)	Histamine (ppm) - ELISA for Spiked Samples	Histamine (ppm) - ELISA for Unspiked Samples
M-1	35.17	3.389	8.399
M-2	34.67	0.4086	0.65
M-3	81.49	0.7989	3.690
M-4	41.83	0.3830	1.261
M-5	33.53	0.9583	1.438
M-7	99.05	24.21	155.3

Table 4. Calculated Amines in Tuna using the MSA Procedure			
Grade	**Volatile Biogenic Amine - BPB (ppm)**	**Histamine (ppm) - ELISA for Spiked Samples**	**Histamine (ppm) - ELISA for Unspiked Samples**
T-1	25.37	0.0867	0.2362
T-2	256.0	0.0130	0.1329
T-3	58.60	0.0663	0.789
T-4	111.0	0.0753	0
T-5	194.6	5.239	81.99
T-6	44.50	5.090	197.7
T-7	27.10	0.5320	36.09

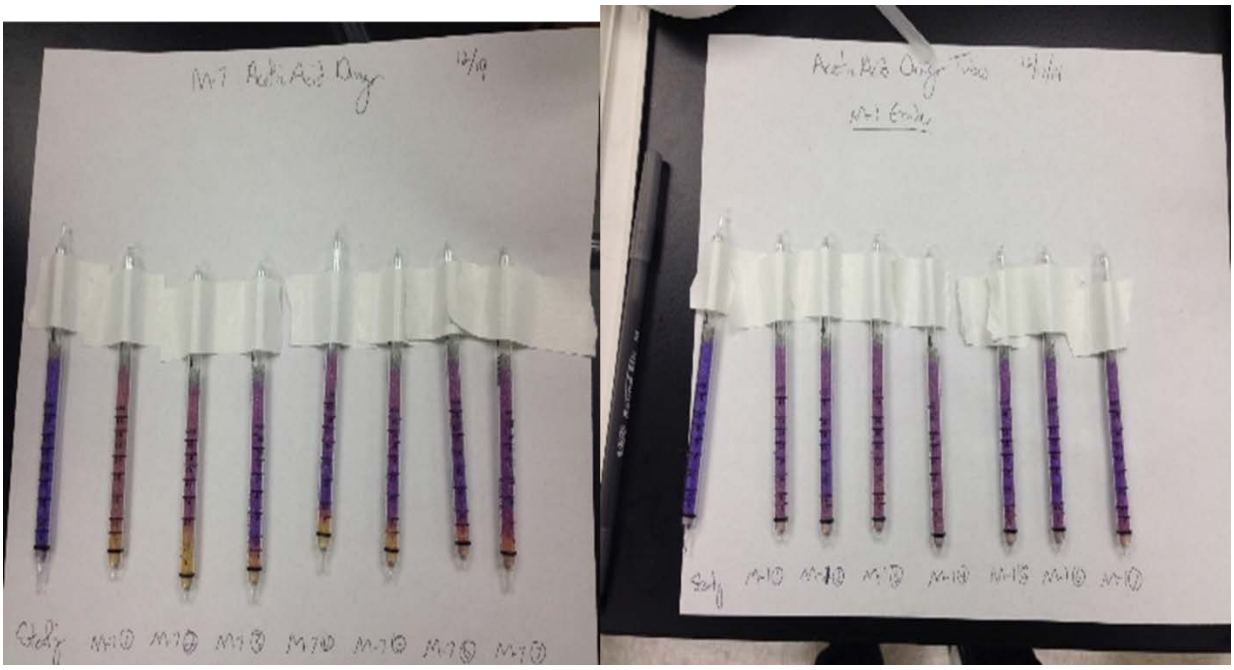

Figure 1. Mahi-mahi Grades 1 (left) and Grade 7 (right) Acetic Acid Dräger Tube Results

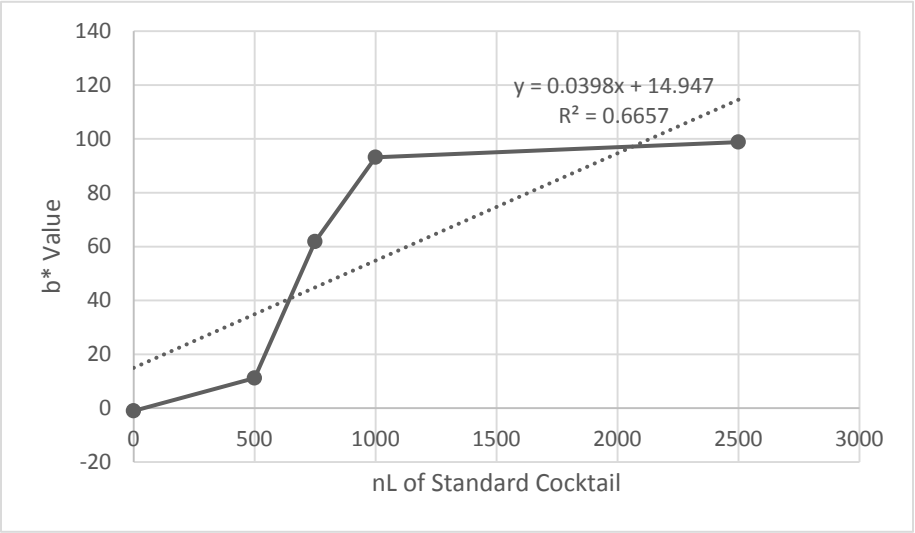

Figure 2. Biogenic Amine Cocktail Standard Curve

Major Takeaways from the Research

- ELISA-MSA results showed an increase in histamine as the quality of both mahi-mahi and tuna decreased (Tables 3 & 4)
- Matrix effect was stronger for volatile compounds than aqueous compounds (Tables 3 & 4)
    - Lipophilic nature of volatile compounds could play a role
    - The analyte being a specific compound (histamine) vs class of compound (biogenic amines) could impact measurement
- Role of lipids
    - Histamine has been found to be more concentrated in red muscle (fattier portion of fish muscle)
    - This could contribute to uneven distribution of histamine throughout the fish
- Further research: determine what proportion of volatile biogenic amines are present in liquid and gas phases at each grade to better reflect the accuracy of the grading in terms of safety

Comparison of three different assays

- For mahi-mahi, all three assays correlated with sample grade
- Grade to assay correlation was not as strong with the tuna samples
- Compositional and physiological differences can explain why this is the case
    - Lipid/protein and enzyme content differences
    - Temperature adaptation differs between the species
- Factors including post-harvest conditions need to be taken into account
- Further research: the BPB method can be further refined and developed to be used for finfish grading

Conclusions

Histamine and volatile biogenic amines were found to act as co-indicators of spoilage in the two types of finfish studied, and therefore can be used as quality indicators. There is a matrix effect that impacts detection for gas phase biogenic amines. There may also be an impact of matrix on aqueous histamine detection. The BPB method correlates biogenic amine concentration well with quality grades for mahi-mahi. Exogenous factors including post-harvest conditions, temperature abuse, and grade sampling (location of filets due to uneven distribution of histamine) are also important to consider when interpreting the results of these studies.

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