A Reference Material Suite for Evaluating Seafood Authenticity and Safety

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Abstract

Seafood is one of the most highly-traded international commodities. Pricing at import is determined by weight, species and provenance, including whether the food is from aquacultured or wild-caught sources. Each of these characteristics can be falsified, resulting in an inflated payout, encouraging unsustainable practices and affecting the US domestic seafood economy. The inclusion of additives such as gels, dyes and antibiotics commonly found in fish feed can also endanger the health of consumers. The US Food and Drug Administration (FDA) and US Customs and Border Protection (CBP) would benefit from seafood authenticity reference materials to aid in identifying cases of import fraud. To address this need, the National Institute of Standards and Technology (NIST) is using shrimp and salmon, two of the three most consumed seafood products in the US, to develop authenticity reference materials. Wild-caught and aquacultured Coho salmon and wild-caught and aquacultured shrimp were procured from reliable sources and processed to include only the edible portions. Each of the four materials was cryomilled and bottled at the NIST Reference Material Production Facility in Charleston, SC. Preliminary analysis reveals differences in metabolomic profiles between materials of differing origin. The anticipated differences in DNA profiles, elemental ratios and total polar/semi-polar constituents will allow regulatory agencies to better identify fraudulent trade items at import and provide a standardization tool for typical analyses conducted at inspection laboratories.

Materials

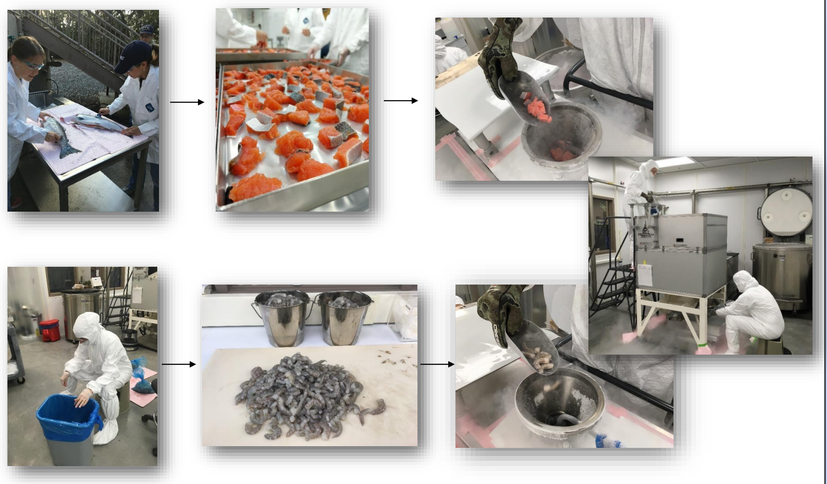
All materials used to generate the seafood suite were obtained from sources verified authentic by the National Oceanic and Atmospheric Administration Marine Forensics Unit:

- Wild-caught Coho salmon (NIST RM 8256)
  - *O. kisutch* hook and line caught ~15 mi off the coast of Alaska
- Aquacultured Coho salmon (NIST RM 8257)
  - *O. kisutch* procured from a land-based aquaculture facility in Washington

- Wild-caught shrimp (NIST RM 8258)
  - *F. aztecus* trawl caught off the coast of South Carolina
- Aqaucultured shrimp (NIST RM 8259)
  - *L. vannamei* procured from an aquaculture facility in Texas

Processing Methods

- Salmon was scaled, fileted, chopped (including skin) and frozen
- Shrimp was headed, peeled, chopped and frozen

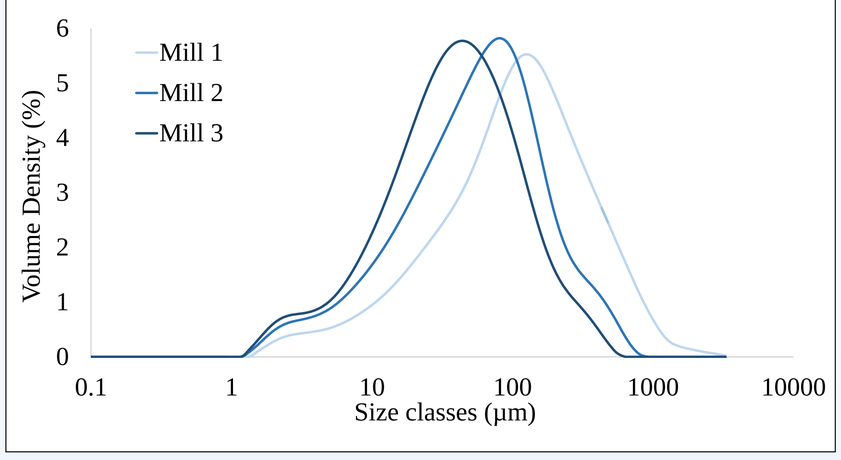


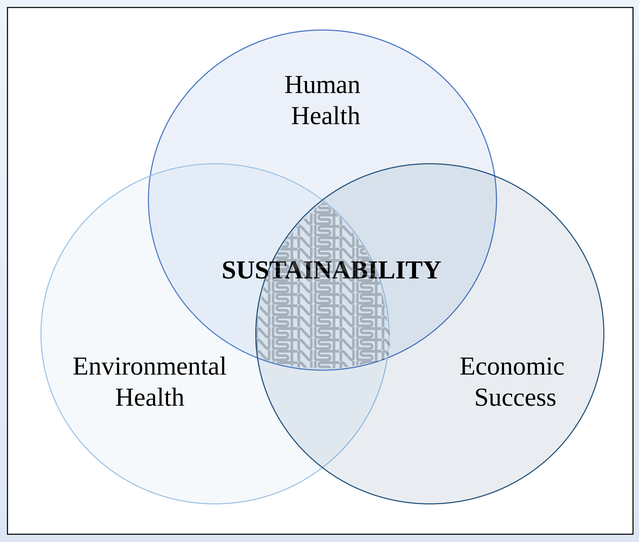
- Materials were cryohomogenized three times using a Palla VM-KT Vibrating cryomill, generating a fine fresh-frozen powder

Processing Results

- Fresh-frozen powder was subsampled during each of the three rounds of cryohomogenization for a preliminary homogeneity assessment using a Malvern Mastersizer 3000 for laser-diffraction particle size analysis
- A small amount of powder was added to ethanol dispersant in the sampling reservoir and 10 measurements were made per subsample to obtain an average particle size for each round of milling
- Three rounds of cryomilling proved sufficient to effectively homogenize the materials according to ISO standard 13320 for particle size distribution (1)

	Mill 1	Mill 2	Mill 3
Avg D_v (50) (µm)	111.00	58	39
1x SD	3.75	0.955	0.238
RSD (%)	3.39	1.65	0.61

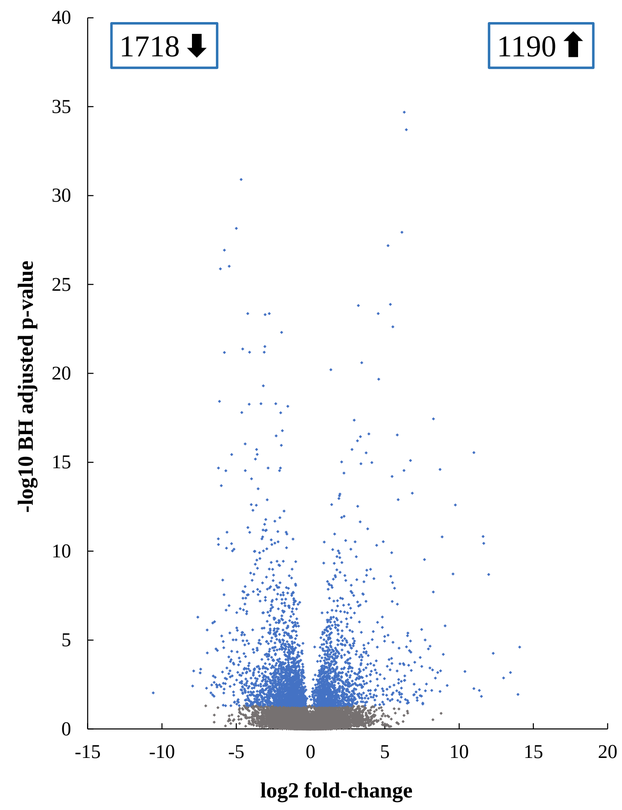




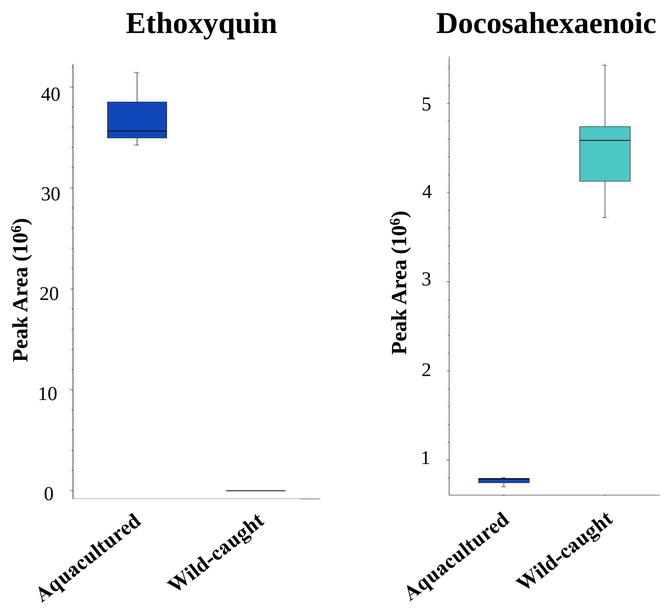
Analytical Methods

- 2 g aliquots of each wild-caught and aquacultured salmon material were extracted using hydroxylamine and acetonitrile, dried with magnesium sulfate and vortexed
- Supernatant was transferred to a clean tube, taken to dryness and reconstituted in ascorbic acid solution in acetonitrile according to AOAC published methods (2)
- Extracts were analyzed by LC-MS/MS using high resolution accurate mass (HRAM) with a Thermo Vanquish UPLC, Agilent XDB-C18 column, and coupled to a Thermo Fusion Lumos to generate full MS (120,000 resolution) and MS/MS (15,000 resolution) with high collisional dissociation (HCD)
- The data were analyzed using Compound Discoverer 2.1 to identify feature differences between the wild-caught and aquacultured materials
- Associated p-values were corrected using a Benjamini-Hochberg (BH) procedure to control the false discovery rate

Analytical Results



- A volcano plot was used to visualize feature differences between replicate aliquots of wild-caught and aquacultured salmon materials
- Statistical differences were evident between the two materials using a log fold Δ of 2 and a BH-adjusted p-value of 0.05
- Preliminary analysis of the two salmon materials suggests they are fit-for-purpose in providing the authenticity community a tool to help verify seafood source at import



- Specific compounds differentially present in the two salmon materials were explored
- Ethoxyquin, a fat stabilizer used in fish feed in the US, was found in the aquacultured, but not the wild-caught salmon
- Docosahexaenoic acid, an omega-3 fatty acid was found at much higher levels in the wild-caught salmon compared to the aquacultured salmon

Future Work and References

- In the future, HRAM MS and MS/MS scans of the wild-caught and aquacultured shrimp materials will be compared to ensure differences can be identified
- All materials will be analyzed by targeted and non-targeted LC-HRMS, ICP-MS and GC-MS using optimal sample preparation methods to identify polar and non-polar compounds useful in differentiating wild-caught and aquacultured materials
- Materials will be assessed by genetic methods for species determination and to evaluate models for use in species and provenance confirmation
- All RMs will be made available to the seafood authenticity community to aid in identification and source of imported goods

1. ISO 13320 (2009). Particle size analysis – Laser diffraction methods
2. Schneider and Andersen (2015). Journal of AOAC International. 98(3), 658-670

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