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Assessments and Improvements in Methods for Monitoring Seafood Safety in Response to the Deepwater Horizon Oil Spill

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Supporting Information

ABSTRACT: As a result of the April 2010 Deepwater Horizon oil spill, sensory testing protocols were established for reopening closed seafood harvest areas. In order to improve this method and quantitatively assess petrochemical taint, a new method using solid phase microextraction (SPME) and a 5975T transportable GC/MS was developed. This method can analyze 40 samples per instrument per day and could be an alternative to the human sensory panel. In seafood samples collected from supermarkets in the Washington D.C. area and the Gulf of Mexico, all compounds related to petrochemical taint were below the method detection limit (MDL) (0.14−2.6 ng/g). Additionally, to address consumer concerns regarding the presence of *n*-alkanes and iso-alkanes in seafood, these compounds were investigated in samples purchased in the Washington D.C. area and the Gulf of Mexico. Concentrations in Gulf of Mexico finfish ranged from 0.066 to 1.2 mg/kg, which is within the same background range of iso- and *n*-alkanes measured in seafood samples purchased in the Washington D.C. area (0.0072−1.6 μg/g). These automated methods provide a transportable option to obtain rapid results for compounds indicative of petroleum taint and iso- and *n*-alkanes in case of a future disaster.

KEYWORDS: *SPME, oil spill, alkanes, PAHs*

INTRODUCTION

The Deepwater Horizon oil spill, which began with a drilling rig explosion on April 20, 2010, resulted in approximately 4.9 billion barrels of crude oil being released into the Gulf of Mexico.[1] As a result, the National Oceanic and Atmospheric Administration (NOAA) began closing federal waters for recreational and commercial fishing. Federal waters were reopened once seafood contamination was no longer considered a risk to consumers. The conditions for reopening were that areas must be free of visible oil, and seafood must pass the analytical (testing of polycyclic aromatic hydrocarbons (PAHs)) and sensory testing protocol (free of petroleum taint) developed by the Food and Drug Administration (FDA) and NOAA in consultation with the EPA and Gulf Coast states.[2] Levels of concern (LOCs) were determined for PAHs in crustaceans (shrimp and crab), mollusks (oysters), and finfish.[2] Based on the calculations for PAHs with noncancer risks, LOCs ranged from 123 to 1846 μg/g for crustaceans, 133−2000 μg/g for mollusks, and 32.7−490 μg/g for finfish.[2] LOCs of PAHs that pose a cancer risk ranged from 0.132 to 132 μg/g for crustaceans, 0.143−143 μg/g for mollusks, and 0.035−35 μg/g for finfish.[2]

In order to improve sensory testing by generating quantitative results for reopening seafood harvest areas, a SPME (solid phase microextraction) method for the analysis of compounds indicative of petroleum taint (sensory compounds) was developed that required minimal sample processing and was compatible with on-site analysis. In comparison with other published SPME methods for the analysis of PAHs,[3] the advantage of this method is the use of an internal standard based calibration curve instead of standard addition. Also, a screening method for iso- and *n*-alkanes, which are major components of oil that are of low toxicity, was developed to address consumer concerns. The SPME methods reported here incorporate compounds responsible for petroleum taint and iso- and *n*-alkanes.

Olfactory or sensory testing has been historically used for identifying petroleum taint in seafood.[4−6] Studies have associated petroleum taint with benzene, toluene, and xylene in eels and salmon.[4−6] The sensitivity of the human nose to distinguish taint in seafood has been estimated to be in the low μg/g range.[4,7] One sensory panel was able to distinguish between concentrations of total aromatics at 0.9 μg/g (untainted) and 13 μg/g (tainted) fish.[4] On the basis of this previous work, the compounds chosen as markers of petroleum taint in this study were benzene, toluene, ethylbenzene, xylene, indane, tetralin, mesitylene, and the low molecular weight PAHs naphthalene and 1-methylnaphthalene.

Louisiana crude oil is composed of 28% straight chain alkanes (*n*-alkanes) and branched alkanes (iso-alkanes).[8] After an oil spill, the biodegradation of these compounds in the environment is quite rapid, with greater than 90% of the straight chain alkanes (*n*-alkanes) being degraded in days or months by microorganisms.[9,10] The ratio of lighter (C6−C16) *n*-alkanes to heavier (C16−C35) *n*-alkanes is often used as an indicator of biodegradation,[9,11] because the lighter *n*-alkanes degrade more rapidly. Analysis of weathered oil recovered from the shores of Louisiana after the Deepwater Horizon incident found that after 18 weeks the concentrations of C6−C16 were

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near the detection limit, and the ratio of lighter to heavier *n*-alkanes had dropped 1−2 orders of magnitude from 2.7 to 0.025−0.16.[12] On the basis of the low water solubility of these compounds (0.8−2.9 ng/mL),[13] low toxicity, and rapid biodegradation, their potential impact on seafood is expected to be minimal.

The FDA has not established any LOCs for the straight or branched chain alkanes. Oral reference doses for aliphatic alkanes were calculated by the Total Petroleum Hydrocarbon Working Group to be 5 mg/kg/day for C5 to C8, 0.1 mg/kg/day for C9−C18, and 2 mg/kg/day for C19−C32,[14] which are all at least an order of magnitude higher than the oral reference dose established in seafood for naphthalenes (0.02 mg/kg/day). Because of their low toxicity, potential biogenic origin, and rapid biodegradability, aliphatic alkanes were not included in the LOCs for seafood by the FDA and NOAA. The naphthalene LOCs simply serve as a conservative baseline for data comparison of the less toxic aliphatic alkanes.

n-Alkanes can have biogenic origins in addition to petrogenic origins and are naturally found in all marine plants, animals, and organisms.[15] Because organisms have specific biosynthetic pathways, they tend to have characteristic peaks in certain regions of their *n*-alkane profiles.[15] For example, benthic and planktonic algae have predominant peaks at C15 (pentadecane), C17 (heptadecane), C19 (nonadecane), and C21 (heneicosane).[16,17] Scallops taken from areas contaminated by petroleum showed an even distribution of *n*-alkanes, while scallops taken from an unpolluted location had low concentrations of *n*-alkanes with the exception of C19 and C21.[15] The branched alkanes pristane and phytane, which are derived from chlorophyll, can have both biogenic and petrogenic origins.[18−20] The ratio of pristane to phytane is often used to distinguish between biogenic and petrogenic origins in marine organisms. If the ratio is greater than 1, it is a reliable indicator that the source is of biogenic origin, and values less than 1 generally indicate anthropogenic origin, but with less confidence.[21] Because of the complexity of *n*-alkane sources in seafood, the investigation of individual *n*-alkanes in seafood is necessary to determine if their contributions are from natural and/or petroleum sources. Since concentrations of *n*-alkanes and the branched alkanes pristane and phytane can be used to differentiate between petroleum and natural sources, these compounds were chosen to further investigate the impact of petroleum hydrocarbons on seafood, which will aid in addressing consumer concerns.

The objectives of this research were threefold: first to quantify background concentrations of compounds responsible for petroleum taint and iso- and *n*-alkanes in seafood purchased from local supermarkets in the Washington D.C. area (see Table SI.1 for details on sample locations). Once characterized, these samples will be used to generate a matrix match calibration curve in seafood to quantify potentially contaminated samples. The second goal was to identify the compounds responsible for petroleum taint and develop and validate a rapid method for the analysis of these compounds and iso- and *n*-alkanes in finfish, oysters, and shrimp using SPME-GC/MS (gas chromatography/mass spectrometry). The third objective was to analyze finfish samples from the Gulf of Mexico and determine current concentrations of sensory compounds and iso- and *n*-alkanes in these seafood samples.

MATERIALS AND METHODS

Standards. The analytical standards benzene, toluene, ethylbenzene, *p*-xylene, mesitylene, indane, tetralin, naphthalene, 1-methylnaphthalene, and an *n*-alkane mix ranging from C8−C40 that also contained the iso-alkanes phytane and pristane were obtained from Sigma-Aldrich (St. Louis, MO). For SPME analysis, stock solutions were made in water-miscible solvents, typically methanol or acetone. The following five isotopically labeled standards were obtained from Sigma Aldrich: d_8-toluene, d_{10}-*p*-xylene, d_{12}-mesitylene, d_8-naphthalene, d_{10}-1-methylnaphthalene for the quantification of nine native compounds indicative of petroleum taint. The alkane standards from Sigma Aldrich—d_{22}-decane—and Cambridge Isotopes (Andover, MA)—d_{26}-dodecane, d_{30}-tetradecane, d_{40}-nonadecane, d_{42}-eicosane, d_{50}-tetracosane—were used for the semiquantification of 10 *n*-alkanes and their corresponding isomers (branched alkanes).

Sample Information. Seafood samples for method development (5 finfish, shrimp, oysters) were obtained from local supermarkets in the Washington D.C. area (including farmed and wild-caught) and also during the Roy Martin Young Anglers Tournament (15 finfish) in Dauphin Island, Alabama, in July 2011 to monitor the post-spill recovery process. This event is considered one of the largest fishing tournaments in the world, and samples were collected from several locations in the Gulf of Mexico. The north and south fishing boundaries were the Gulf Coast and 28° latitude line, and the east and west boundaries were the 85° and 91° longitude lines. Details on these samples including fat content and place of origin can be found in the Supporting Information (Table SI.1).

Sample Preparation. All seafood samples were kept frozen at −20 °C and thawed in a refrigerator prior to use. Once thawed, the edible portions of the samples were placed in a Cuisinart Mini-Prep food processor and ground for ∼1 min. Aliquots of the ground seafood (2 g) were placed in 20 mL amber SPME screw top vials (Supelco, Bellefonte, PA). The addition of 4 mL of several different types of solutions to the ground seafood was tested during method development: deionized water, 10% salt (NaCl) water, 10% Triton-X in water, and 10% KOH. In the final method, the vials were vortexed for 20 s, spiked with 225 ng/g of internal standards, and vortexed again for 20 s. When samples were spiked for method validation, the vials containing fish homogenate were allowed to sit for at least 4 h to equilibrate before analysis. Equilibration was not required for direct sample analysis.

SPME Optimization. SPME analysis was optimized for fiber type, incubation time, incubation temperature, extraction time, sample size, and addition of sample modifiers. Optimizations were performed in triplicate, and the results can be found in Figures SI.1 and SI.2. The best fiber for both analyses was found to be the DVB/CAR/PDMS fiber. This dual-layer fiber efficiently traps the volatile components onto the carboxen and the less volatile components onto the copolymer divinyl benzene (DVB)/polydimethyl siloxane (PDMS). In some cases, (e.g., extraction time), the highest response was not chosen due to the need to minimize analysis time. This did not appear to have a significant effect on the detection limits (<0.27 ng/g) of these compounds. Full details of the final optimized method can be found in Table SI.2.

The addition of potassium hydroxide (KOH) has been previously used with SPME for the analysis of polychlorinated biphenyls (PCBs) in milk with varying fat content with quantification by standard addition.[22] The addition of potassium hydroxide to fatty matrix samples followed by heat saponifies triglycerides and breaks them up into alcohols and carboxylates.[22] The addition of KOH was necessary in developing an SPME method that was both accurate and repeatable in measuring sensory compounds and iso- and *n*-alkanes in finfish, oysters, and shrimp that range in fat content from 0.45% to 6.6%. Due to the complex matrix, the addition of the five internal standards d_8-toluene, d_{10}-*p*-xylene, d_{12}-mesitylene, d_8-naphthalene, and d_{10}-1-methylnaphthalene was also required to maintain an internal standard based matrix match (seafood) calibration curve that could accurately quantify over 30 samples and avoid the use of standard addition.

GC/MS Optimization. An Agilent 5975T LTM (low thermal mass)-GC (gas chromatography)/MS (mass spectrometry) with a DB-5 ms UI column (30 m × 0.250 mm i.d. × 0.25 μm film thickness) was interfaced to a Gerstel MPS 2 robotic sampler that has options for SPME, headspace, and liquid injection. The LTM column increases sample throughput by rapidly and efficiently heating (1800 °C/min) and cooling (<1 min) the column, resulting in shorter analytical cycle times. This instrument is fully transportable, requires 120 V power, and is ideal for rapid analyses in the field.

Two separate instrumental methods were developed for the analysis of sensory compounds and iso- and *n*-alkanes due to differences in inlet optimization parameters. Splitless injection was necessary for the *n*-alkanes, because split injection causes discrimination in the inlet for *n*-alkanes with greater than 16 carbons. When split injection was used, calibration curves could not be generated for *n*-alkanes with more than 16 carbons because an increase in concentration did not result in an increase in response. Splitless injection combined with a low initial oven temperature (which was necessary for low molecular weight *n*-alkanes) resulted in large peak tailing for benzene and toluene, most likely due to the use of a thin film (0.25 μm) column, so split injection was necessary for the sensory compounds. Details on the instrumental parameters for each method can be found in the Supporting Information (Tables SI.2 and SI.3).

Both SIM and SCAN data were collected simultaneously during GC/MS analysis with the scan collection ranging from 50 to 450 *m*/*z*. In SIM mode, ions monitored for the sensory compounds were benzene *m*/*z* 78, 52, toluene *m*/*z* 91, 92, ethylbenzene and *p*-xylene *m*/*z* 91, 106, mesitylene *m*/*z* 105, 120, tetralin *m*/*z* 104, 132, indane *m*/*z* 117, 118, naphthalene *m*/*z* 128, 127, and 1-methylnaphthalene *m*/*z* 142, 141. The calibration curve ranged from 5 to 100 ng/g, which is well below the estimated threshold of the human nose (~1 μg/g)[4] and within the range of a more conservative threshold of 50 ng/g. For total iso- and *n*-alkanes, *m*/*z* 57, which is the major ion produced by both the straight and branched chain alkanes, was monitored over the range of C9 to C18. Concentrations in seafood were calculated by using the average relative response factor (RRF) of all individual *n*-alkanes (C9−C18 and pristane and phytane) for all points of the calibration curve (50, 500, 1000, 1500, 2000 ng/g). Due to other interfering compounds present in seafood that produce the *m*/*z* 57 ion (e.g., fatty acids), the total iso- and *n*-alkane values are slightly overestimated.

QA/QC. Percent recoveries for the sensory compounds ranged from 79% to 110% for all seafood samples, and the precision was less than 7% RSD. During sample analysis, a blank sample (consisting of 6 mL of water) and a spiked tilapia sample (275 ppb) were run every six samples to verify the accuracy of the quantification for both the compounds indicative of petroleum taint and the iso- and *n*-alkane methods. For the sensory compounds, the standard was required to fall within 15% of the nominal concentration, and for the semiquantitative iso- and *n*-alkane method, the standard was required to fall within the correct screening range (e.g., 50−500, 500−1000, 1000−1500, 1500−2000 ng/g).

Method detection limits (MDLs) ranged from 0.14 to 0.27 ng/g for the compounds indicative of petroleum taint. Fiber blanks were also run to determine the amount of carryover from the DVB/CAR/PDMS fiber. Calibration curves were run with deionized water followed by five water blanks. This was performed two times throughout the analysis (10 samples), and these blanks ranged from MDL to 2.6 ng/g for the compounds indicative of petroleum taint, which is still lower than the limit of quantification (5 ng/g). Compounds' specific method detection limits were increased to account for the blank carryover values. For iso- and *n*-alkanes, the concentration measured in the fiber blanks was less than 3 ng/g for all compounds. Since the lowest reporting screening range is <50 ng/g, and this is a semiquantitative method, no adjustments were necessary.

Determination of a Matrix Match. Seafood purchased from local supermarkets in the Washington D.C. area were tested for their background concentrations of sensory compounds as well as iso- and *n*-alkanes to determine their suitability for matrix match calibration curves. Background concentrations of sensory compounds and iso- and *n*-alkanes in all seafood types were measured using standard addition. The concentrations of sensory compounds were all below method detection limits in all seafood types. The concentrations of iso- and *n*-alkanes were above the MDL in all seafood samples, and results can be found in Figure 1. Naturally occurring iso- and *n*-alkanes

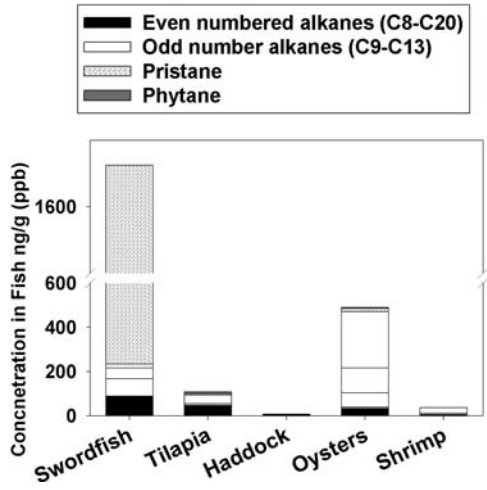

Figure 1. Concentrations of *n*-alkanes ranging from C8 to C20 and the iso-alkanes pristane and phytane in seafood samples (finfish, oysters, and shrimp) collected from local supermarkets in the Washington D.C. area.

made up over 50% of the total iso- and *n*-alkane concentrations in all seafood types and up to 94% and 92% for swordfish and oysters. The concentration of pristane in swordfish is 2 orders of magnitude higher than other seafood types, which is likely due to the swordfish being a top predator compared to the tilapia, haddock, shrimp, and oysters.[19] Swordfish are also oily fish and store their oils in the edible flesh portion and in the cavity around the gut, compared to whitefish (e.g., tilapia, haddock), whose oil is stored in the liver. This results in oily fish being more susceptible to bioaccumulation in edible tissue than whitefish.[23]

Tilapia (farm-raised) was used for matrix match calibration curves for both whitefish and oily fish because of the relatively low concentration of iso- and *n*-alkanes (~100 ng/g) and a fat content of 1.7%, which is between the low and high range (0.45% to 6.6%) of other types of finfish to be analyzed. These fat contents were not measured in this study, but are reference values obtained from the USDA nutrition database.[24]

RESULTS AND DISCUSSION

Validation of Olfactory SPME Method. The optimized SPME method for sensory compounds was validated for accuracy and precision by running a matrix-matched calibration curve using farm-raised tilapia purchased from a local grocery store in the Washington D.C. area. Two calibration curves were tested: one with the addition of 4 mL of 10% KOH solution to 2 g of tilapia, and the second with the addition of 4 mL of deionized water to 2 g of tilapia. The 7-point calibration curves ranged from 5 to 100 ng/g for each analyte. The r^2 of the linear regression curves made up in a 10% KOH solution ranged from 0.991 to 0.998, which was an improvement over the calibration curves made up using 4 mL of water, which had r^2 values ranging from 0.942 to 0.994, and all regressions had *p*-values less than 0.05 for the *x*-variable. Both calibration curves were verified for accuracy at each calibration concentration, by ensuring that each measured concentration was within 15% of the nominal concentration. The matrix-matched calibration curves were then used to analyze seven replicate samples of

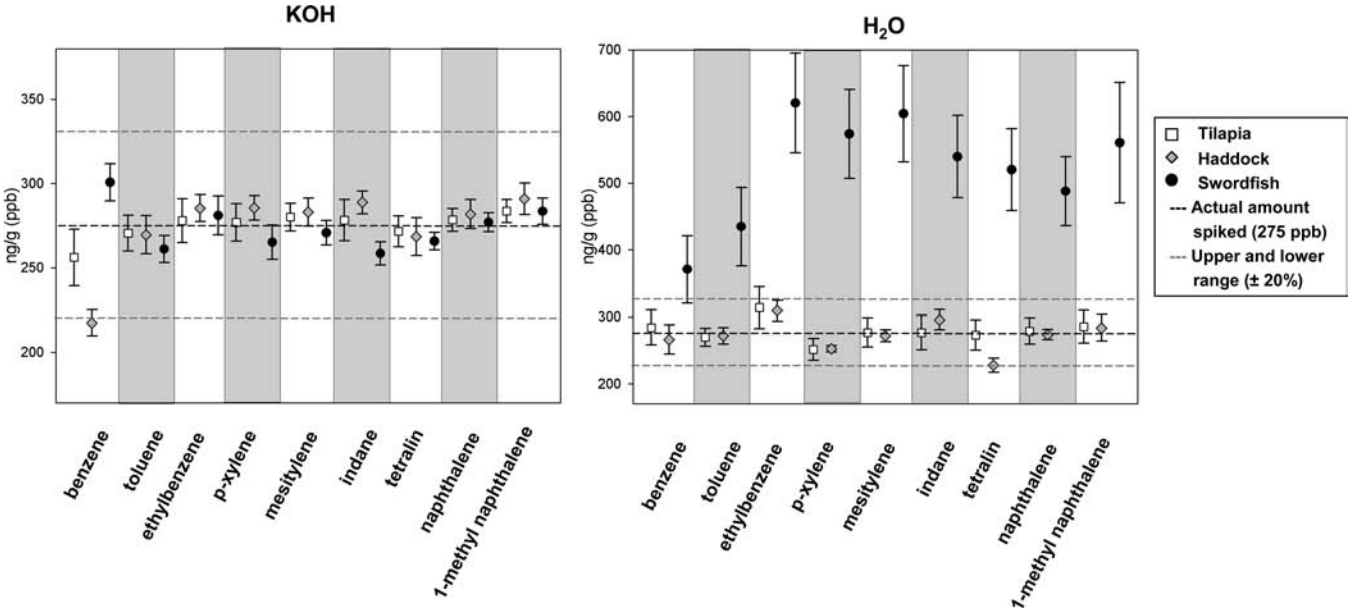

Figure 2. Averages and standard deviations of seven replicate measurements spiked at 275 ng/g for tilapia, haddock, and swordfish in both KOH and water solutions. Dashed lines indicate the spiked concentration ± 20%.

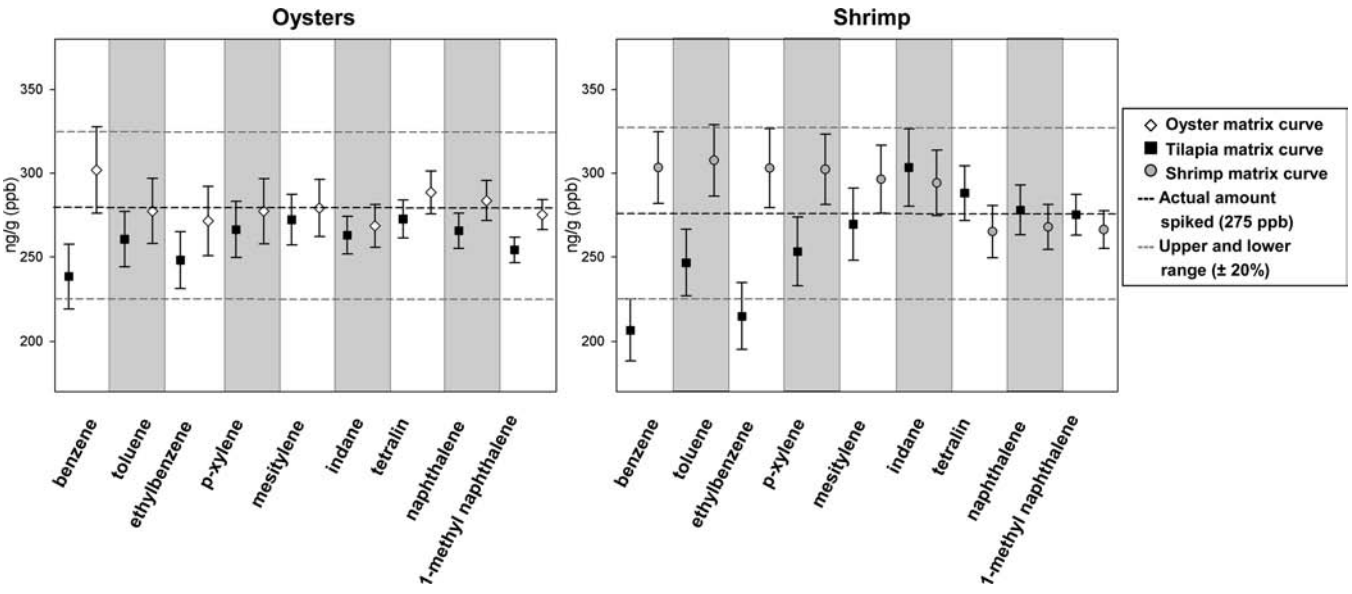

Figure 3. Average and standard deviations of seven replicate samples of oysters and shrimp calculated using a tilapia matrix match calibration curve (for both oysters and shrimp), an individual matrix match calibration curve for oysters, and an individual matrix match calibration curve for shrimp.

each of the following seafoods: tilapia, haddock, and swordfish, which were spiked at a concentration of 275 ng/g of each sensory compound. The accuracy and precision of this data can be found in Figure 2. The addition of KOH was necessary for the accurate analysis of these compounds in swordfish, which has the highest percentage (6.6%) of fat, when using a calibration curve made up in tilapia (1.7% fat) for quantification (Figure 2).

This method, with a 10% KOH solution, was able to accurately and precisely quantify all 28 samples within 20% of the actual value (Figure 2). Spiked shrimp and oyster matrixes were also analyzed using the tilapia matrix match calibration curve for quantification (Figure 3). Individual matrix match calibration curves using oysters and shrimp were also tested for accuracy in quantifying oyster and shrimp samples, respectively (Figure 3). For oysters, the percent difference ranged from 0.86% to 13% with compounds quantified using the tilapia curve and from 0.086% to 9.8% for compounds quantified using the oyster calibration curve. In shrimp, these differences ranged from 0.064% to 24% for the tilapia calibration curve and from 3.2% to 12% for the shrimp calibration curve. For future analyses, the tilapia calibration curve can be used to accurately quantify oyster samples, since these values deviate less than 15%; however, for the shrimp matrix, a shrimp calibration curve is recommended for accurate analysis. Typically SPME analysis in complex matrixes requires standard addition to obtain accurate results; in this case one matrix-matched calibration curve was used for several different types of finfish and oysters. By saponification of the fish tissue (simplifying the matrix), by the use of appropriate internal standards, and due to the

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relatively low affinity of these analytes for the lipid matrix (log K_{OW} < 4), this method remained accurate for at least 30 samples in at least five different seafood tissues with varying fat content using the same calibration curve and SPME fiber.

This method was further used to quantify sensory compounds in 15 fish samples collected during the fishing rodeo in Dauphin Island. Triplicates of each sample were analyzed, and all analyte concentrations were below the 5 ng/g limit of quantification, including naphthalene and 1-methylnaphthalene. For comparison, the LOC calculated by the FDA for naphthalene and 1-methylnaphthalene is 32.7 μg/g in finfish.[2]

***n*-Alkane and iso-Alkane Concentrations in Seafood.** A rapid screening method was developed to estimate total concentrations of iso- and *n*-alkanes in seafood. Due to the many isomers of branched alkanes present in crude oil, individual compounds could not be measured. Analytical standards were obtained for two specific iso-alkanes (pristane and phytane) to aid in differentiating between biogenic and petrogenic origins. Initially a quantitative method using SPME was attempted, but due to the high affinity of iso- and *n*-alkanes to the lipid matrix (log K_{OW} 5−10), an accurate and repeatable method was not possible even with the addition of 10% KOH solution and additional internal standards. Instead, a semiquantitative screening method was developed. The screening concentrations were 0−50, 50−500, 500−1000, 1000−1500, and 1500−2000 ng/g for iso- and *n*-alkanes ranging from C9 to C18, which is the carbon range with the lowest oral reference dose estimated by the Total Petroleum Hydrocarbon Working Group.[14] In order to verify the accuracy of the screening method, individual *n*-alkanes and the iso-alkanes pristane and phytane were measured in tilapia, haddock, swordfish, oyster, and shrimp samples from local markets in the Washington D.C. area, and totals were compared to those calculated using standard addition methods. Figure 4 shows that the standard addition results fall within the correct screening concentrations or are slightly overestimated (oysters) by the iso- and *n*-alkane screening method.

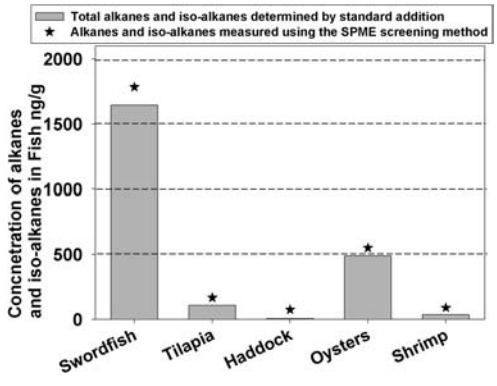

Figure 4. Comparison of total (C9−C18) *n*-alkanes and iso-alkanes pristane and phytane measured by standard addition and using the SPME screening method.

Finfish samples collected throughout the Gulf of Mexico during the Roy Martin Young Anglers Fishing Tournament in July 2011 were analyzed for total iso- and *n*-alkanes using the SPME screening method. Each fish sample was analyzed in triplicate, and the results can be found in Figure 5. The highest concentrations were measured in the 1000 to 1500 ng/g range,

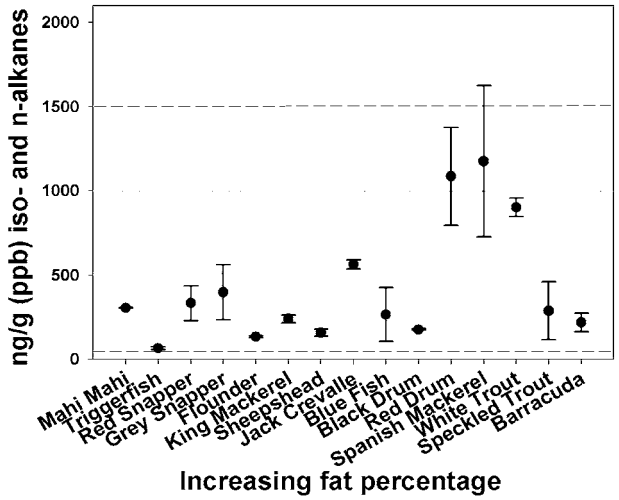

Figure 5. Concentrations of total iso- and *n*-alkanes (C9−C18) measured in 15 different fish samples collected in the Gulf of Mexico during July 2011.

while a majority of the samples had total concentrations of 500 ng/g or less. These samples fall within the same background range as those measured in finfish samples from local Washington D.C. area supermarkets (7.2−1600 ng/g or 0.0072−1.6 μg/g). These values are also greater than an order of magnitude lower than the 32.7 μg/g LOC established for the much more toxic naphthalene in finfish. Additionally, each sample was analyzed individually for pristane and phytane using the alkane screening method. For five of the oily fish, the concentration of pristane was in the 500−1000 ng/g screening range and the concentration of phytane was in the 0−50 ng/g screening range. In these cases, it can be verified that the pristane to phytane ratio would be greater than 1 and indicates biogenic sources. In two oily and three whitefish the pristane concentration was in the range 50−500 ng/g and the phytane concentration was 0−50 ng/g. For the remaining five whitefish, the concentrations of both pristane and phytane were below 50 ng/g. In these two cases, a ratio cannot be estimated.

Implications. The improvements in the method for the analysis of sensory compounds in seafood replace the human sensory panel (qualitative) with a quantitative method. The ability to measure multiple finfish species using an internal standard based calibration curve with little sample preparation is advantageous for high-throughput analyses in the field and can lead to decreased closing times of federal waters in the case of a future oil spill. The semiquantitative SPME iso- and *n*-alkane screening method can be used to rapidly assess iso- and *n*-alkane concentrations in seafood after an oil spill in order to address future consumer concerns of alkane-contaminated seafood if needed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Further details on seafood samples, SPME, and instrumental optimization. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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