Rapid field-deployable method for determination of seafood decomposition by compact mass spectrometry

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Abstract

There is a pressing regulatory need for alternatives to sensory testing to assess seafood decomposition, particularly those which can be performed in non-laboratory field environments. The current work attempts to meet this need with a novel method involving compact mass spectrometry with sensory-driven modeling. An international sampling effort produced samples of 23 different seafood products, which were subjected to controlled decomposition of varying levels on ice. This produced samples of each product for a wide range of decomposition states, and these were scored by a sensory expert on a standard 1-100 scale. Samples were then analyzed by a novel technique involving headspace analysis and compact mass spectrometry. The mass spectrometry data was used to create computer models, guided by the sensory data, to generate a calculated score analogous to a sensory score. Models based on a training set of samples were then used to calculate scores for a test set, and accuracy was assessed by comparing these calculated scores to original sensory data. Calculated scores agreed with sensory findings with respect to overall decomposition state for 96.3% of samples (n=547), with seven false positive (1.3%) and 13 false negative (2.4%) findings. Reproducibility was also assessed via triplicate analysis on separate days for low, middle, and high decomposition states. All sample ranges for these replicates were within 20 points, with 89% (n=66) within 15 points, 73% within 10, and 35% within 5. Given the performance of the method, along with the small spatial footprint and manageable operation requirements of the instrument as well as simplicity of sample preparation, this appears to offer a potentially useful technique for field testing of seafood decomposition.

Keywords: ambient ionization, mass spectrometry, field deployable instrumentation

1. Introduction

As part of its mission to protect consumer safety and ensure sanitary production and transportation conditions, United States Food and Drug Administration (USFDA) field laboratories perform routine sampling and testing of seafood products for decomposition,

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also referred to as spoilage [30]. This testing primarily utilizes sensory analysis, following procedures developed in collaboration with the Canadian Food Inspection Agency (CFIA) and the National Oceanic and Atmospheric Administration (NOAA) [10]. Sensory analysis is an effective, accurate technique for this analysis, however there are several drawbacks associated with its use. First, training of sensory analysts is an intensive process, requiring significant commitment of time and effort [1,17,31]. Furthermore, testing uniformity can be a potential concern [22,24,33].

Techniques currently in official use for confirmation of sensory results are limited to the analysis of histamine [3] or indole [2] content. While histamine analysis can be useful, it is typically only effective for a relatively small class of products [12,32]. Indole content can be an effective indicator of warmer temperature decomposition in shrimp, but is otherwise of limited use [7]. Neither of these tests provide results which are directly comparable to sensory testing, therefore an alternative method is preferable when exploring alternatives to these techniques.

Similar chemistry-based techniques have been explored, including those based on trimethylamine [18], or other biogenic amines [9,27], and larger selections of volatile indicators have also been targeted [4,13-15]. Instruments with sensor arrays known as ‘electronic noses’, of various designs, have also been explored [8,19-20,35]. These have shown adequate results in many cases and may prove useful given a comparison to sensory testing on a wider range of species of regulatory interest. Non-targeted techniques involving mass spectrometry and computer-based modeling have also been employed [16,21], and the current work explores similar techniques.

Initial work involving liquid chromatography-mass spectrometry (LCMS) and computer modeling was performed using six species of salmon [25]. Following the success of that work, and driven by a directive from Congress [29], that work was expanded to 23 products [26]. That method demonstrated strong performance with respect to accuracy and reproducibility via comparison to sensory results but requires shipping of samples to a fully equipped laboratory and sample analysis times typical of LCMS. The current study was performed in tandem, on the same products, but employs a process intended to be more amenable to field deployment.

Due to the time sensitive nature of seafood decomposition analysis [30], there has been interest in performing some testing in the field environment. USFDA operates numerous field sampling stations at border crossings, port facilities, and other settings. These operations are typically run from small office environments which do not have true laboratory facilities or personnel, however, and these limitations must be respected. This study, employing a small form-factor ‘Compact Mass Spectrometer’ (CMS) system, which has been explored for use in field environments [11,28,34], and for other food safety applications [23], with a streamlined, non-extractive sample preparation by headspace sampling attempts to bridge this gap.

Table 1. Products sampled

Common name	Scientific name	Form	Origin
Chum salmon	*Oncorhynchus keta*	Skin-on filet	Alaska
Coho salmon	*Oncorhynchus kisutch*	Skin-on filet	Alaska
Croaker	*Micropogonias furnieri*	Skin-on filet	Guyana
Escolar	*Lepidocybium flavobrunneum*	Skin-off filet	Ecuador
Grouper[a,b]	*Epinephelus fuscoguttatus*	Skin-off filet	Vietnam
Mahi mahi[b]	*Coryphaena hippurus*	Skin-off filet	Ecuador
Pacific Ocean Perch	*Sebastes alutus*	Skin-off filet	Alaska
Peruvian scallop	*Argopecten purpuratus*	Shucked, raw, roe-off	Ecuador
Pink salmon	*Oncorhynchus gorbuscha*	Skin-on filet	Alaska
Pollock	*Gadus chalcogrammus*	Skin-on filet	Alaska
Red snapper	*Lutjanus campechanus*	Skin-on filet	Guyana
Shrimp	*Litopenaeus vannamei*	Raw, headless, shell on	Ecuador
Snapper	*Lutjanus sanguineus*	Skin-off filet	Vietnam
Sockeye salmon	*Oncorhynchus nerka*	Skin-on filet	Alaska
Squid[c]	*Loligo spp.*	Tubes and tentacles	Vietnam
Swordfish[a]	*Xiphias gladius*	Steaks	Vietnam
Weakfish	*Macrodon ancylodon*	Skin-off filet	Guyana
Yellowfin tuna[a]	*Thunnus albacares*	Skin-on filet	Vietnam
Yellowfin tuna	*Thunnus albacares*	Canned in broth	Ecuador

[a]: Carbon monoxide treated and non-treated forms collected
[b]: Aquacultured products
[c]: Mixed spp. in genus

2. Materials and Methods

2.1 Experimental Overview

This work was performed in conjunction with a partner study, using the same sample set, sensory data, and sample handling. Full details can be found in that work [26], but a brief summary is given here. Samples of 23 seafood products (Table 1) were collected by teams led by USFDA certified National Seafood Sensory Experts (NSSE). Samples were collected by these teams in processing facilities in Kodiak, Alaska (USA); Guayaquil, Ecuador; Georgetown, Guyana; and Huy Toa, Vietnam. Products sampled included four species of finfish which were treated with carbon monoxide (CO) (grouper, mahi-mahi, swordfish, and yellowfin tuna), along with their non-treated counterparts. Carbon monoxide treatment is commonly used to preserve/enhance color of seafood products, and these were collected to ensure applicability to these products. Samples were collected in the freshest state possible, and allowed to spoil on ice, then transferred to -20 °C storage in sensory-controlled stages to create seven discreet increments, ranging from the freshest available (1) to very advanced decomposition (7). However, at NSSE discretion, eight such

increments were collected for swordfish (non-CO treated), and nine for canned tuna.

Sensory evaluations were then made for each sample, following typical regulatory procedure [10]. These were performed by a single, highly qualified NSSE in lieu of a panel, as described in agency policy [30]. Each sample then received a numerical sensory score (1-100), with higher scores indicating lower quality. Samples receiving scores greater than 50 are considered decomposed, with lower scores considered non-decomposed.

Five sample portions (approximately 200g) for each quality increment of each product were used in the study. Each of these were tested in duplicate on separate days, and a third replicate for one sample each from the low, middle, and high decomposition ranges was analyzed on a third separate day to establish reproducibility.

Each sample was subjected to the headspace/CMS analysis described below, and sample responses for a panel of known decomposition-indicating volatile organic compounds (VOCs) (Table 2) described in the literature [4,16] were used to create sensory-driven statistical models as described in the companion work.

2.2 Equipment and Reagents

2.2.1 Reagents

Dry ice (Airgas, Woodinville, WA, USA) was used to assist in sample preparation for some products. Pyridine (Certified ACS grade, Fisher Scientific, Hampton, NH, USA) was used as an internal standard. High-purity water was produced by a Milli-Q® purification system with an LC-Pak® polisher (MilliporeSigma, Burlington, MA, USA).

2.2.2 Sample Preparation Equipment

Products were sampled using Miltex® 8 mm biopsy punches (Integra LifeSciences, Princeton, NJ, USA). Sample portions were weighed on a model PC4400 top-loading balance (Mettler-Toledo, Columbus, OH, USA). These were transferred to 20 mL glass headspace vials with 18 mm magnetic screw-top caps (Restek, Bellefonte, PA, USA). Heating steps were carried out with a four-block drybath with six 25 mm slots per block (Thermo Scientific, Waltham, MA, USA).

2.2.3 Instrumental Equipment

Headspace samples were analyzed using an Advion expressIon® S compact mass spectrometer (Advion, Ithaca, NY, USA) with the "volatile atmospheric pressure chemical ionization" (vAPCI) ion source. This source uses a one-meter heated transfer line with an internal diameter of 2.5 mm, and approximately 6 cm of metal tubing exposed on the end. Gaseous samples are introduced to this end and aspirated into the source directly via Venturi pump. A normally-open pushbutton switch was added to the 'digital input 1' port on the instrument, and acts as a manual signal to start the analysis. The instrument was operated using the vendor-provided Mass Express (version 1.0) software package.

Table 2. List of decomposition-indicating compounds with protonated masses.

Compound	M+H
(E)-2-penten-1-ol	87
(E)-2-pentenal	85
1-(2-furanyl)ethanone	111
1,2-dimethylbenzene	107
1-butanol	75
1-penten-3-ol	87
1-propanol	61
2-butanol	75
2-butanone	73
2-cyclopenten-1-one	83
2-ethyl-1-hexanol	131
2-furancarboxaldehyde	97
2-furanmethanol	99
2-methoxy-4-methylphenol	139
2-methoxyphenol	125
2-methyl-1-butanol	89
2-methyl-1-propanol	75
2-methyl-2-cyclopenten-1-one	97
2-methylphenol	109
2-pentanone	87
2-propanol	61
2-propanone	59
3-hexanone	101
3-methylbutanal	87
3-methylcyclopentanone	99
3-pentanone	87
benzaldehyde	107
butanal	73
butyl acetate	117
cyclopentanone	85
ethanol	47
ethyl acetate	89
hexanal	101
methylbenzene	93
pentamethylheptane	171
phenol	101

trimethylamine	60

2.3 Headspace Extraction

Samples of scallops, shrimp, and squid were first homogenized using dry ice as described in the companion work and previous literature [6]. All other products were sampled directly using biopsy punches to remove core-like portions, cutting directly from the interior downward, avoiding the skin if present. These were taken close to the center of each piece, to ensure the best representation. These core pieces (or homogenized sample) (2.0 ± 0.2 g) were transferred to headspace vials with 1.00 mL of high-purity water containing 1 ppm (v/v) pyridine. Vials were then capped and heated at 90 °C for 10 minutes, with staggered starting times to maximize throughput. Samples were removed from heat in groups of four to minimize cooling time prior to analysis.

2.4 Instrumental Analysis

For each sample, the external pushbutton was used to initiate analysis, followed by five seconds of scanning prior to sample introduction to establish background spectra. Each vial was then uncapped and immediately introduced to the mass spectrometer by inserting the tip of the transfer line into the headspace vial, approximately 5 cm, carefully avoiding any solid or liquid material. Each sample was scanned for ten seconds.

The instrument was operated with an atmospheric pressure chemical ionization (APCI) source with the heated transfer line provided by the manufacturer. Nitrogen (100 psi, 10 L/min) was used as the Venturi pump/APCI gas. The transfer tube temperature was 100 °C. The ionization source settings included a capillary temperature of 250 °C, capillary voltage at 180 V, source voltage offset at 30 V, source voltage span at 20 V, source gas temperature at 350 °C, and the APCI corona discharge setting was 5. The instrument was tuned on a weekly basis using the positive ion tuning solution and procedures provided by the manufacturer. Samples were scanned in positive ion mode from m/z 45-200, with a 1000 ms scan time and a scan speed of 155 mz/s, for a total run time of fifteen seconds.

2.5 Data Analysis

The instrument software was set to export each datafile to the “netCDF” format, binned at 0.5 m/z. Complete sets of these datafiles for each product were further processed using the R (3.6.3) programming environment. The first five scans, consisting of air background data, were segregated, and the responses were averaged for each m/z value. Scans 8-13 were used as the sample response and similarly averaged. Scans 6-7, taken during the transition period between background and sample analysis, and scans 14-15, taken during the end of the run, were not used to avoid any potential consistency issues arising from slight differences in sample introduction or removal. Averaged background data for each m/z value was then subtracted from the corresponding sample data, and any resulting differences with values less than zero were replaced with zero.

One third of the samples, rounding up and using a 'set.seed' value to ensure reproducibility, were randomly selected to be the test set. The remaining samples comprised corresponding numerical sensory score. Variables were then restricted to include only m/z values corresponding to the $[M+H]^+$ adduct of known decomposition-indicating VOCs (Table 2). Models were then created using these matrices, applying the Random Forest algorithm [5], in the regression mode, fitting to the sensory data with 2000 trees. These models were then used to predict sensory-like data scores for each sample in the test set.

3. Results and Discussion

3.1 Internal Standard

The initial design of the method included addition of pyridine to the headspace vials for use as an internal standard. Pyridine was chosen for its volatility and low mass. Furthermore, its major ion (m/z 80, corresponding to the protonated molecular ion) does not overlap with that of the decomposition-indicating compounds of interest. However, application of the internal standard in modeling, by using the ratio of the signal from decomposition-indicating compounds to that of pyridine in calculations, gave erroneous results for some products. This appears to be due to suppression of the pyridine signal in highly decomposed samples. To ensure consistency and simplicity, the internal standard was therefore disregarded for all products. Further study may yield a more reliable internal standard procedure; however, the results of the current work seem to indicate this may not be necessary.

the training set. A data matrix was created for each product with the background-subtracted data from each sample in the training set and

3.2 Data Selection

As a means of ensuring accuracy of the computer models, the mass spectral data used in creating them was limited to the m/z values corresponding to the M+H ion of known decomposition-indicating VOCs as described in Section 2.5 above. In some cases, multiple decomposition-indicating compounds generate ions at the same m/z values. However, the identity of the specific compound responsible is not relevant for modeling. Furthermore, non-decomposition-indicating compounds may potentially have interfering m/z values, but the models appear to handle this adequately.

3.3 Method Performance

3.3.1 Accuracy

As described in Section 2.5 above, the model-calculated results for samples in the test sets of all products were compared to the original sensory result for the same samples, as shown in Figure 1. The combined results show quite strong agreement between these techniques.

False positive findings, defined as a calculated score greater than 50 with a sensory score below 50, were observed in 1.3% of samples (seven samples out of 547 total). These were identified in two escolar samples, and one each of non-CO treated grouper, pink salmon, pollack, red snapper, and snapper. The range of calculated scores responsible for false positive findings was 51-54, and all of these arose from samples scoring 48 via sensory testing. As all

of these are very close to the cutoff score, these are very promising results.

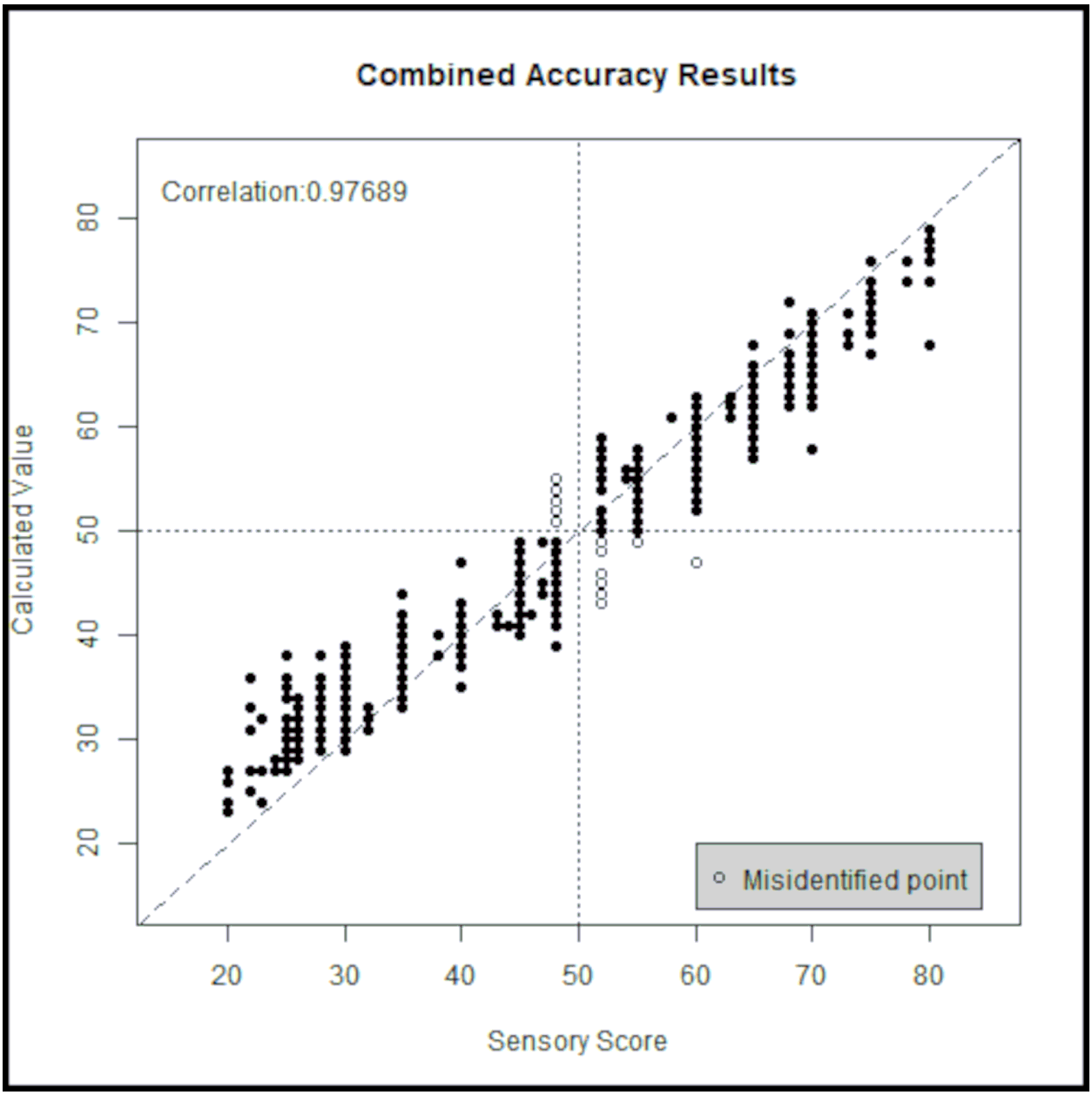

Figure 1: Combined results of test samples for all products, comparing sensory scores to calculated results.

False negative findings, defined as a calculated score below 50 with a sensory score greater than 50, were observed in 2.4% of samples (13 samples out of 547 total). These arose from four samples of Pacific Ocean perch, two each for sockeye salmon and CO-treated yellowfin, and one each for CO-treated grouper, scallops, non-CO treated swordfish, weakfish, and non-CO treated yellowfin. The range of calculated scores responsible for false negative findings was 43-49. The sensory range for these samples was 52-60. Again, these remain very close to the cutoff score. False positive and false negative samples were both classified as 'misidentified points' in Figure 1 and can be found in the upper-left and lower-right quadrants, respectively.

The very high percentage of samples for which the designation of decomposed or non-decomposed was identified consistently with the sensory scores (96.3%, n=547), and the proximity to the cutoff for each of the misidentified samples indicate that the accuracy of this method is very high when compared to sensory findings. So long as care is taken with scores near the cutoff, this technique should provide a reliable indicator of decomposition state.

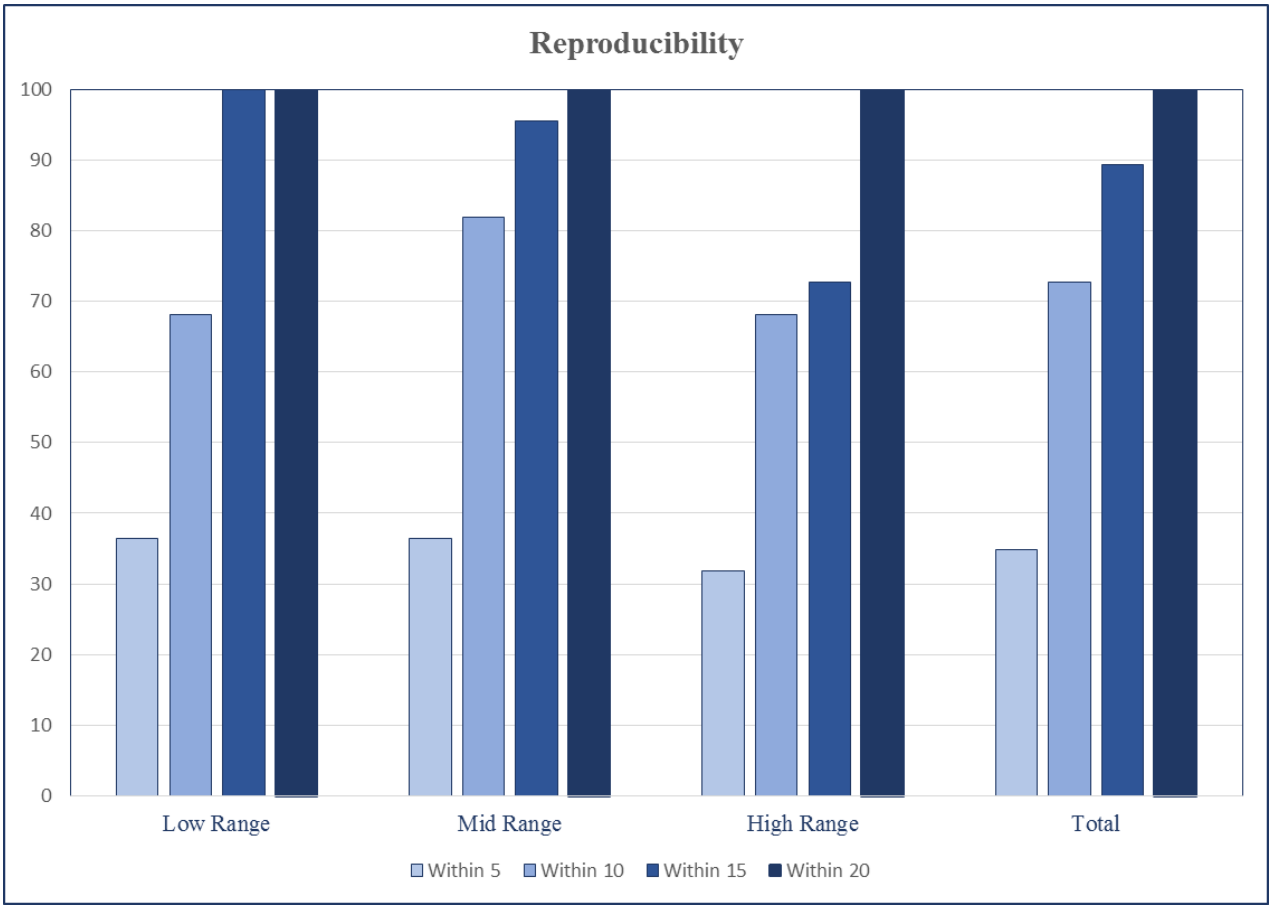

Figure 2: Reproducibility of triplicate measurements, showing the percentage of measurement ranges (n=66) within 5, 10, 15, and 20 points.

3.3.2 Reproducibility

As described in Section 2.1 above, reproducibility of the method was evaluated using triplicate measurements, on different days, of selected samples from low, middle, and high decomposition states for each product. Unfortunately, the third dataset for pink salmon was not completed due to an instrument error, but all other products were completed as planned. For each decomposition state of the remaining products, the range of calculated scores for the triplicate samples was determined. The distribution of these ranges is shown in Figure 2. All of these ranges were within 20 points, with 89% (n=66) within 15 points, 73% within 10, and 35% within five. The results were mostly consistent across the three ranges, although slightly better in the lower and middle ranges as compared to the high range. The overall reproducibility of the method is slightly inferior to that of the similarly obtained LCMS data from the companion work [26], as expected, but still appears adequate for the intended application.

3.4 Field Applicability

A primary goal for the current study is to provide an analytical technique which can be deployed to non-laboratory field settings for regulatory use. For USFDA, this may include, for example, border posts, port offices, or international mail facilities. These settings lack services available to most laboratories, have space limitations, and are typically staffed with non-technical personnel. Furthermore, the goal of field deployment is to provide a rapid screen of samples to reduce the need for full laboratory analysis.

With these goals in mind, the Advion CMS was a promising contender for this type of work. Importantly, the instrument requires only a 110 V power supply, which in many of these settings may be the only available source. The only other service needed is a supply of nitrogen gas. This is readily available from either a small nitrogen generator or tank. The instrument also has a very small footprint (approximately 27 x 52 x 56 cm), which is ideal for small environments. The availability of easy non-chromatographic sample introduction is also key for use by non-laboratory personnel.

In addition to instrumental concerns, the remainder of the method was designed to be performed without laboratory services or personnel. This includes the use of the biopsy punches which proved to be a reliable, reproducible technique for sampling these products. For most samples, a core removed with the punch had a mass very near 1 g. With practice, it may even be possible to eliminate the need for weighing using this technique. The headspace portion of the analysis is also straightforward and should require little in training for even non-laboratory personnel. By staggering the starting times for heating as described, it was possible to analyze a set with over 70 samples in approximately 30 minutes, providing ample throughput for this application.

4. Conclusions

This study explores the expanded use of a previously established technique for the evaluation of decomposition in seafood products. Application of the compact mass spectrometer and simple preparatory techniques allows this work, using sensory-

based computer modeling, to potentially be performed without laboratory services or personnel. Furthermore, the performance of the method appears to be adequate for this type of work, with a correct identification rate greater than 96% and established reproducibility comparable to previous LCMS work [26]. This indicates that it may provide a useful tool to complement existing procedures in these regulatory settings.

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6. Conflicts of Interest

The authors declare no conflict of interest.

7. Disclaimer

The views presented in this article are those of the authors and do not necessarily represent those of the US Food and Drug Administration. No official support or endorsement of this article by the Food and Drug Administration is intended or should be inferred. Mention of brand or firm name does not constitute an endorsement by the US FDA over others of a similar nature not mentioned.

8. Data Availability

Due to the very large quantity of data, this will be made available upon request.

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