NIST Special Publication 260-214

Analysis of Seafood Reference Materials: RM 8256, RM 8257, RM 8258 and RM 8259

Wild-Caught Coho Salmon (RM 8256)
Aquacultured Coho Salmon (RM 8257)
Wild-Caught Shrimp (RM 8258)
Aquacultured Shrimp (RM 8259)

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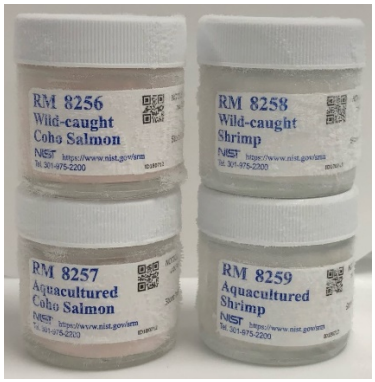

National Institute of Standards and Technology
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Abstract

NIST Reference Material (RM) 8256 Wild-Caught Coho Salmon, RM 8257 Aquacultured Coho Salmon, RM 8258 Wild-Caught Shrimp, and RM 8259 Aquacultured Shrimp were generated for use in authentication and food safety-related determinations, including crude fat, fatty acids, and crude protein. Each unit consists of two jars containing 6 g to 8 g of fresh frozen powder homogenate. RMs of the same type (i.e., fish or shellfish) can be used individually or in tandem for comparative studies. This publication documents the sourcing, measurement results, and statistical analysis employed in characterizing each material.

Key words,

Fatty acids, food safety, total protein, salmon, seafood, shrimp.

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1. Introduction

Food fraud has become an area of increasing concern, costing the global food industry an estimated $10B to $15B USD per year [1]. Food fraud can include the partial or complete substitution of a product or ingredients in a product, providing false claims regarding product origin or source and mislabeling of a product, all of which effect the authenticity of the food. Generally, intentional substitutions are economically motivated. High-priced commodities are typically targeted, such as wine, olive oil, spices, and seafood, and less expensive or less desirable products are substituted for those that are more expensive or rare. In addition to economic implications, these methods of counterfeiting can also pose health risks to consumers, particularly those with allergies or food sensitivities.

Seafood is one of the most highly traded international commodities and pricing at import is determined by weight, species, and provenance, which includes source (i.e., wild-caught or aquacultured). To meet growing demand, seafood can be sourced directly from another country or caught domestically, shipped internationally for processing, and imported back to the US. During these transactions, both species and source could be misrepresented. As such, two of the three most consumed seafood products in the US, shrimp and salmon, were chosen for the development of a food safety and authenticity reference material series. Wild-caught and aquacultured coho salmon and wild-caught and aquacultured shrimp reference materials were generated for use in nutritional, food safety, and authentication determinations.

2. Material Acquisition and Preparation

When procuring raw materials, special care was taken to ensure the seafood products were obtained from authentic sources. The National Oceanic and Atmospheric Administration (NOAA) Northwest Fisheries Science Center Forensic Laboratory provided expertise in identifying verified fisheries and facilities capable of providing authentic seafood products to ensure the appropriate materials were used. Additionally, because the final RMs were intended to represent food items, only those portions of the organisms typically consumed were included in production.

2.1 Salmon Materials

Wild-caught coho salmon (*Oncorhynchus kisutch*) was purchased from a commercial fishing operation based in Alaska, USA. Fish were caught via hook and line 19 km (12 miles) to 24 km (15 miles) offshore between Yakutat and Prince of Wales Island during the 2017 summer fishing season. Fish were processed (headed and gutted) and frozen on the boat to maintain maximum freshness. Frozen fish were shipped to NIST Charleston on blue ice packs and were stored at −40 °C until processing for RM 8256.

Aquacultured coho salmon were purchased from a land-based aquaculture facility in the northwest region of the US and represent immature fish culled from their stocks under normal conditions. Whole, immature fish were shipped to NIST Charleston on wet ice and stored at −40 °C until processing for RM 8257.

Both RM 8256 and RM 8257 were processed in the same manner. Salmon were partially thawed at −4 °C then scaled, rinsed with fresh water and fileted. Edible meat (including the skin) was cut from the carcass, chopped into small chunks, and refrozen at −40 °C overnight. The frozen tissue pieces were stored in liquid nitrogen (LN_2) vapor phase freezers (at or below −150 °C) until the material was cryomilled [2]. The fresh-frozen homogenates were bottled in 6 g to 8 g aliquots and stored at or below −80 °C until analysis.

2.2 Shrimp Materials

A mixture of wild-caught brown shrimp (*Farfantepenaeus aztecus*) and Atlantic white shrimp (*Litopenaeus setiferus*) were trawl-caught by a commercial shrimper off the coast of South Carolina, USA. Headed shrimp were delivered to NIST Charleston on wet ice and stored at −40 °C until processing for RM 8258.

Aquacultured whiteleg shrimp (*Litopenaeus vannamei*) were purchased from a land-based aquaculture company located in the southeastern region of the US. Shrimp were headed prior to freezing individually, shipped to NIST Charleston on wet ice and stored at −40 °C until processing for RM 8259.

Both RM 8258 and RM 8259 were processed in the same manner. Shrimp were thawed, rinsed with fresh water, peeled and chopped, then refrozen at −40 °C. The frozen tissue pieces were stored in LN_2 vapor phase freezers (at or below −150 °C) until cryomilling [2]. The fresh-frozen homogenates were bottled in 6 g to 8 g aliquots and stored at or below −80 °C until analysis.

3. Crude Fat and Fatty Acid Analysis

Ten jars were randomly selected from each production batch for crude fat analysis, and six jars were randomly selected from each production batch for fatty acid analysis. One jar of SRM 1946 Lake Superior Fish Tissue and one jar of SRM 1947 Lake Michigan Fish Tissue were chosen as controls for the crude fat analysis of the salmon materials. One jar of SRM 1946 Lake Superior Fish Tissue was chosen as a control for the crude fat analysis of the shrimp materials. All jars were shipped on dry ice to Eurofins Microbiology Laboratories, Inc. for value assignment. Crude fat measurements were completed according to AOAC Official Method 948.15 [3] and fatty acid measurements were completed according to AOAC Official Method 996.06 [4]. As the reported values were in units of g per 100 g fatty acids as triglycerides, all values were converted to g per 100 g free fatty acids using the conversion factors in Table 1 [4]. These units are well accepted in the food and nutrition community and convey that the source of the free fatty acids, whether they exist as mono-, di- or tri-glycerides for example, is unknown.

Table 1. Conversion factors used to convert fatty acids as triglycerides to free fatty acids

Fatty Acid	Conversion Factor
C 10:0 (Capric acid)	0.9314
C 12:0 (Lauric acid)	0.9405
C 14:0 (Myristic acid)	0.9473
C 14:1 (Myristoleic acid)	0.9470
C 15:0 (Pentadecanoic acid)	0.9502
C 15:1 (Pentadecenoic acid)	1.0002
C 16:0 (Palmitic acid)	0.9529
C 16:1 (Palmitoleic acid)	0.9525
C 17:0 (Margaric acid)	0.9552
C 17:1 (Heptadecenoic acid)	0.9549
C 18:0 (Stearic acid)	0.9573
C 18:1 (Oleic acid)	0.9570
C 18:1n7 (Vaccenic acid)	0.9570
C 18:2n6 (Linoleic acid)	0.9568
C 18:3n3 (alpha-Linolenic Acid)	0.9564
C 18:3n6 (gamma-Linolenic Acid)	0.9564
C 18:4n3 (Stearidonic acid)	0.9561
C 20:0 (Arachidic acid)	0.9609
C 20:1 (Eicosenoic acid)	0.9607
C 20:2n6 (Eicosodienoic acid)	0.9605
C 20:3n3 (Eicosatrienoic acid)	0.9602
C 20:3n6 (homo-gamma-Linolenic acid)	0.9602
C 20:4n6 (Arachidonic Acid)	0.9600
C 20:5n3 (Eicosapentaenoic acid)	0.9999
C 21:0 (Heneicosanoic acid)	0.9626
C 22:0 (Behenic acid)	0.9641
C 22:1n9 (Erucic acid)	0.9639
C 22:2n6 (Docosadienoic acid)	0.9637
C 22:6n3 (Docosahexaenoic acid)	0.9628
C 22-5n3 (Docosapentaenoic acid)	0.9631
C 22-5n6 (Docosapentaenoic acid)	0.9631
C 23:0 (Tricosanoic acid)	0.9655
C 24:0 (Lignoceric acid)	0.9998
C 24:1 (Nervonic acid)	0.9666
C 6:0 (Caproic acid)	0.9016
C 8:0 (Caprylic acid)	0.9192

3.1 Salmon RM Data and Homogeneity

Total crude fat was measured in NIST SRM 1946 Lake Superior Fish Tissue and SRM 1947 Lake Michigan Fish Tissue as quality control materials to verify laboratory performance, with a summary of the results compared to their respective Certificates of Analysis (COAs) provided in Table 2. The measured crude fat in the individual samples of RM 8256 and RM 8257 and their respective data summaries are shown in Table 3. Based on the results from the measurement of the RMs, the laboratory crude fat measurement variability is, conservatively, 3.4 % relative standard deviation (RSD). The SRM results obtained should include their own estimates of uncertainty and can be compared to the certified values using methods previously described [5]. When the RSD associated with the experimental method is applied to the single measurements for SRM 1946 and SRM 1947 using this approach, no significant differences between the measurement results and the reference values were detected, indicating the experimental method is not biased.

Table 2. Crude fat (%) results from SRMs 1946 and 1947

[a] The estimated uncertainty was calculated using methods described previously [5].

	This Study		COA	
	Measurement	Estimated Uncertainty[a]	Mass Fraction (%)	Expanded Uncertainty
SRM 1946	11.08	+/- 0.84	10.17	0.48
SRM 1947	10.76	+/- 0.87	10.4	0.5

Table 3. Results for crude fat (%) in RM 8256 and RM 8257

Reference Material	Jar No.	Crude Fat (%)
RM 8256	Jar 16	3.59
	Jar 176	4.04
	Jar 322	3.75
	Jar 400	3.7
	Jar 554	3.74
	Jar 684	3.84
	Jar 834	3.75
	Jar 916	3.47
	Jar 1075	3.68
	Jar 1168	3.75
	Mean	**3.73**
	StDev	**0.15**
	RSD (%)	**4.0**
RM 8257	Jar 84	9.58
	Jar 172	10.1
	Jar 335	9.97
	Jar 443	9.75
	Jar 606	10.07
	Jar 657	9.68
	Jar 809	9.25
	Jar 968	9.75
	Jar 1019	10.17
	Jar 1149	10.41
	Mean	**9.87**
	StDev	**0.34**
	RSD (%)	**3.4**

The homogeneity plots for RM 8256 and RM 8257 are shown in Figures 1 and 2, respectively. The dotted black line represents the mean of the values and the solid red lines represent one standard deviation from the mean. No trends were observed for total crude fat associated with the bottle number, suggesting that the material is homogenous across the entire material stock within the limits of uncertainty of the measurement method used to assess homogeneity.

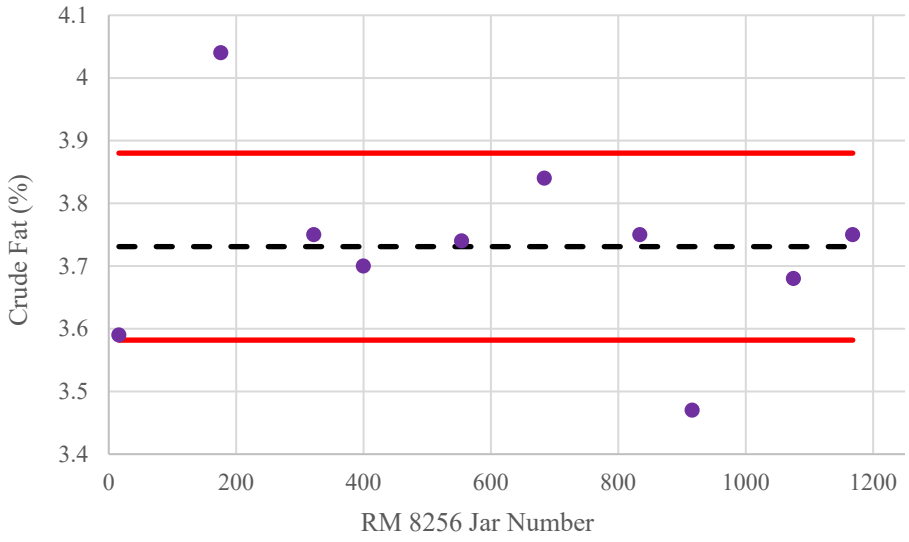

Figure 1. Homogeneity of crude fat in RM 8256

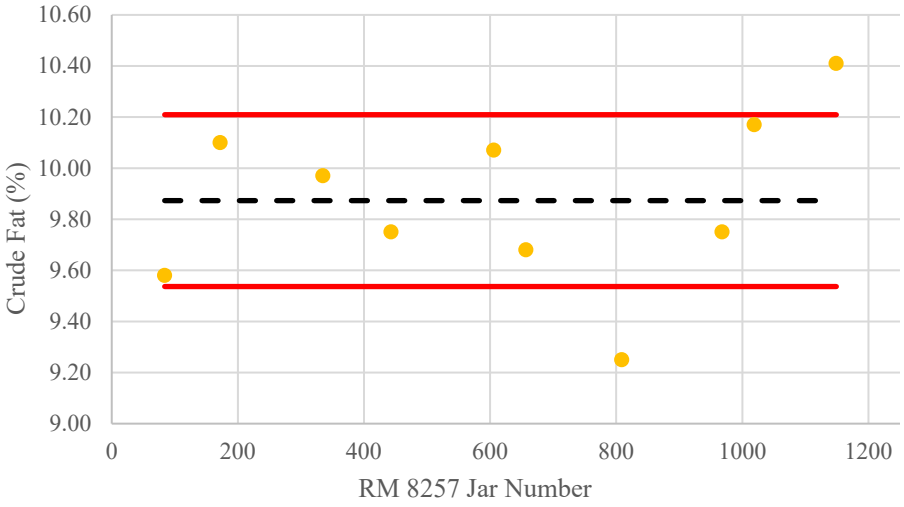

Figure 2. Homogeneity of crude fat in RM 8257

Individual fatty acids were also measured in six samples of each RM 8256 and RM 8257 and the mean, standard deviation and RSD was calculated for each analyte. The results of the fatty acid profile measurements, including summary statistics, are shown in Tables 4 and 5, where “ND” indicates compounds that were not detected. Differences in both composition and abundance of fatty acids can be observed, which is likely attributed to a difference in diet between the wild-caught and aquacultured fish.

Table 4. Results for individual fatty acids in RM 8256[a]

[a] Results are in g / 100 g as free fatty acids

RM 8256									
Jar No.	Jar 87	Jar 324	Jar 530	Jar 671	Jar 836	Jar 1229	Mean	StDev	RSD (%)
C 10:0 (Capric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 12:0 (Lauric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 14:0 (Myristic acid)	0.118	0.126	0.132	0.126	0.131	0.127	0.127	0.005	3.9
C 14:1 (Myristoleic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 15:0 (Pentadecanoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 15:1 (Pentadecenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 16:0 (Palmitic acid)	0.484	0.498	0.508	0.504	0.515	0.500	0.502	0.011	2.1
C 16:1 (Palmitoleic acid)	0.096	0.099	0.101	0.102	0.103	0.100	0.100	0.002	2.5
C 17:0 (Margaric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 17:1 (Heptadecenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:0 (Stearic acid)	0.097	0.099	0.098	0.101	0.101	0.099	0.099	0.002	1.6
C 18:1 (Oleic acid)	0.349	0.345	0.333	0.343	0.347	0.336	0.342	0.006	1.9
C 18:1n7 (Vaccenic acid)	0.044	0.044	0.044	0.045	0.045	0.044	0.044	0.001	1.2
C 18:2n6 (Linoleic acid)	0.055	0.048	0.045	0.047	0.046	0.046	0.048	0.004	7.6
C 18:3n3 (alpha-Linolenic Acid)	0.031	0.033	0.032	0.033	0.033	0.032	0.032	0.001	2.5
C 18:3n6 (gamma-Linolenic Acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:4n3 (Stearidonic acid)	0.050	0.054	0.054	0.054	0.054	0.053	0.053	0.002	3.0
C 20:0 (Arachidic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:1 (Eicosenoic acid)	0.133	0.136	0.134	0.138	0.136	0.135	0.135	0.002	1.3
C 20:2n6 (Eicosodienoic acid)	0.021	0.023	0.023	0.023	0.024	0.024	0.023	0.001	4.8
C 20:3n3 (Eicosatrienoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:3n6 (homo-gamma-Linolenic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:4n6 (Arachidonic Acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:5n3 (Eicosapentaenoic acid)	0.216	0.222	0.222	0.230	0.225	0.219	0.222	0.005	2.2
C 21:0 (Heneicosanoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:0 (Behenic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:1n9 (Erucic acid)	0.022	0.023	0.023	0.023	0.023	0.023	0.023	<0.001	1.8
C 22:2n6 (Docosadienoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:6n3 (Docosahexaenoic acid)	0.645	0.664	0.661	0.697	0.674	0.658	0.667	0.018	2.6
C 22-5n3 (Docosapentaenoic acid)	0.050	0.053	0.052	0.054	0.053	0.052	0.052	0.001	2.6
C 22-5n6 (Docosapentaenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 23:0 (Tricosanoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 24:0 (Lignoceric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 24:1 (Nervonic acid)	0.038	0.039	0.038	0.040	0.039	0.038	0.039	0.001	2.1
C 6:0 (Caproic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 8:0 (Caprylic acid)	ND	ND	ND	ND	ND	ND	–	–	–
Monounsaturated Fat	0.68	0.69	0.67	0.69	0.69	0.68	0.68	0.01	1.2
Omega-3 fatty acids	0.99	1.03	1.02	1.07	1.04	1.01	1.03	0.03	2.7
Omega-6 fatty acids	0.08	0.07	0.07	0.07	0.07	0.07	0.07	<0.01	5.7
Polyunsaturated Fat	1.07	1.10	1.09	1.14	1.11	1.08	1.10	0.02	2.3
Saturated Fat	0.70	0.72	0.74	0.73	0.75	0.73	0.73	0.02	2.4
Total Fat	2.45	2.50	2.50	2.56	2.55	2.48	2.51	0.04	1.7

Table 5. Results for individual fatty acids in RM 8257[a]

[a] Results are in g / 100 g as free fatty acids

RM 8257									
Jar No.	Jar 71	Jar 344	Jar 490	Jar 660	Jar 974	Jar 1105	Mean	StDev	RSD (%)
C 10:0 (Capric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 12:0 (Lauric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 14:0 (Myristic acid)	0.378	0.374	0.386	0.392	0.405	0.405	0.390	0.013	3.4
C 14:1 (Myristoleic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 15:0 (Pentadecanoic acid)	0.032	0.031	0.032	0.033	0.034	0.034	0.033	0.001	3.7
C 15:1 (Pentadecenoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 16:0 (Palmitic acid)	1.498	1.484	1.520	1.518	1.574	1.570	1.527	0.037	2.4
C 16:1 (Palmitoleic acid)	0.583	0.575	0.589	0.586	0.615	0.604	0.592	0.015	2.5
C 17:0 (Margaric acid)	0.033	0.033	0.034	0.033	0.035	0.034	0.034	0.001	2.4
C 17:1 (Heptadecenoic acid)	0.020	0.019	0.020	0.020	0.021	0.021	0.020	0.001	3.7
C 18:0 (Stearic acid)	0.389	0.384	0.391	0.386	0.401	0.399	0.392	0.007	1.8
C 18:1 (Oleic acid)	1.893	1.852	1.896	1.884	1.962	1.929	1.903	0.038	2.0
C 18:1n7 (Vaccenic acid)	0.276	0.272	0.277	0.273	0.285	0.282	0.278	0.005	1.8
C 18:2n6 (Linoleic acid)	0.659	0.653	0.664	0.696	0.720	0.717	0.685	0.030	4.4
C 18:3n3 (alpha-Linolenic Acid)	0.130	0.129	0.131	0.130	0.135	0.134	0.132	0.002	1.8
C 18:3n6 (gamma-Linolenic Acid)	0.020	0.019	0.020	0.019	0.020	0.020	0.020	0.001	2.6
C 18:4n3 (Stearidonic acid)	0.098	0.098	0.096	0.095	0.098	0.098	0.097	0.001	1.4
C 20:0 (Arachidic acid)	0.023	0.021	0.022	0.021	0.023	0.022	0.022	0.001	4.1
C 20:1 (Eicosenoic acid)	0.136	0.134	0.135	0.135	0.140	0.137	0.136	0.002	1.6
C 20:2n6 (Eicosodienoic acid)	0.057	0.056	0.056	0.055	0.057	0.057	0.056	0.001	1.4
C 20:3n3 (Eicosatrienoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:3n6 (homo-gamma-Linolenic acid)	0.023	0.022	0.023	0.022	0.024	0.023	0.023	0.001	3.3
C 20:4n6 (Arachidonic Acid)	0.094	0.093	0.094	0.093	0.097	0.096	0.095	0.002	1.7
C 20:5n3 (Eicosapentaenoic acid)	0.612	0.605	0.617	0.608	0.631	0.622	0.616	0.010	1.6
C 21:0 (Heneicosanoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:0 (Behenic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:1n9 (Erucic acid)	0.020	0.019	0.020	0.019	0.020	0.020	0.020	0.001	2.6
C 22:2n6 (Docosadienoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:6n3 (Docosahexaenoic acid)	0.980	0.960	0.971	0.956	0.990	0.964	0.970	0.013	1.3
C 22-5n3 (Docosapentaenoic acid)	0.259	0.255	0.259	0.255	0.265	0.261	0.259	0.004	1.5
C 22-5n6 (Docosapentaenoic acid)	0.035	0.035	0.035	0.035	0.036	0.035	0.035	<0.001	1.2
C 23:0 (Tricosanoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 24:0 (Lignoceric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 24:1 (Nervonic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 6:0 (Caproic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 8:0 (Caprylic acid)	ND	ND	ND	ND	ND	ND	—	—	—
Monounsaturated Fat	2.93	2.87	2.94	2.92	3.04	2.99	2.95	0.06	2.0
Omega-3 fatty acids	2.08	2.05	2.07	2.04	2.12	2.08	2.07	0.03	1.4
Omega-6 fatty acids	0.89	0.88	0.89	0.92	0.95	0.95	0.91	0.03	3.4
Polyunsaturated Fat	2.97	2.92	2.97	2.96	3.07	3.03	2.99	0.05	1.8
Saturated Fat	2.35	2.33	2.38	2.38	2.47	2.46	2.40	0.06	2.4
Total Fat	8.25	8.12	8.29	8.26	8.59	8.48	8.33	0.17	2.1

3.2 Shrimp RM Data and Homogeneity

Total crude fat (%) was measured in NIST SRM 1946 Lake Superior Fish Tissue to verify laboratory performance, with a summary of the results as compared to the COA provided in Table 6. The measured crude fat (%) in the individual samples of RM 8258 and RM 8259 and their respective data summaries are shown in Table 7. Based on the results from the measurement of the RMs, the laboratory crude fat measurement variability is, conservatively, 9.7 % relative standard deviation (RSD). The SRM results obtained should include their own estimates of uncertainty and can be compared to the certified values using methods previously described [5]. When the RSD associated with the experimental method is applied to the single measurement for SRM 1946 using this approach, no significant difference between the measurement result and the reference value was detected, indicating the experimental method is not biased. Though, the calculated fat content is still within the accepted limits of quantification for the method, the relatively high RSD is likely due to the low fat content of the shrimp materials approaching the lower limit of quantification for the measurement technique, resulting in less measurement precision.

Table 6. Crude fat (%) results from SRM 1946

[a] The estimated uncertainty was calculated using methods described previously [5].

	This Study		COA	
	Measurement	Estimated Uncertainty[a]	Mass Fraction (%)	Expanded Uncertainty
SRM 1946	8.62	+/- 2.03	10.17	0.48

Table 7. Results for crude fat (%) in RM 8258 and RM 8259

Reference Material	Jar No.	Crude Fat (%)
RM 8258	Jar 26	0.67
	Jar 205	0.74
	Jar 337	0.77
	Jar 495	0.64
	Jar 598	0.72
	Jar 737	0.59
	Jar 837	0.81
	Jar 974	0.78
	Jar 1118	0.71
	Jar 1173	0.78
	Mean	**0.72**
	StDev	**0.07**
	RSD (%)	**9.7**
RM 8259	Jar 24	0.68
	Jar 138	0.54
	Jar 360	0.59
	Jar 389	0.59
	Jar 601	0.51
	Jar 747	0.78
	Jar 799	0.51
	Jar 907	0.80
	Jar 1051	0.61
	Jar 1237	0.82
	Mean	**0.64**
	StDev	**0.12**
	RSD (%)	**18.6**

Homogeneity plots for crude fat determinations in RM 8258 (Figure 3) and RM 8259 (Figure 4) indicate reasonable reproducibility across each production batch. The black dashed line represents the mean and red solid lines represent one standard deviation from the mean. The relatively high RSD observed, particularly in RM 8259, is likely the result of low fat content in the shrimp materials. However, the lack of an observable trend in total crude fat across the production batch indicates the homogeneity is fit-for-purpose with regard to crude fat content.

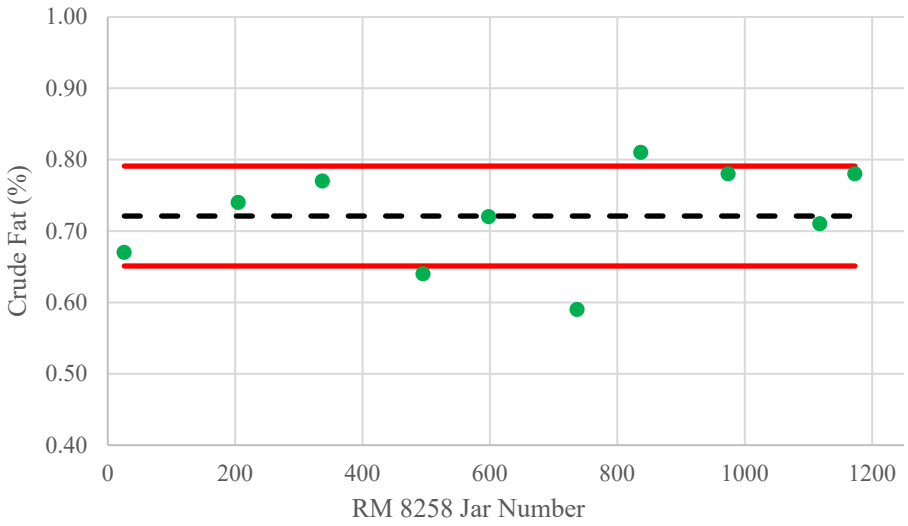

Figure 3. Homogeneity of crude fat in RM 8258

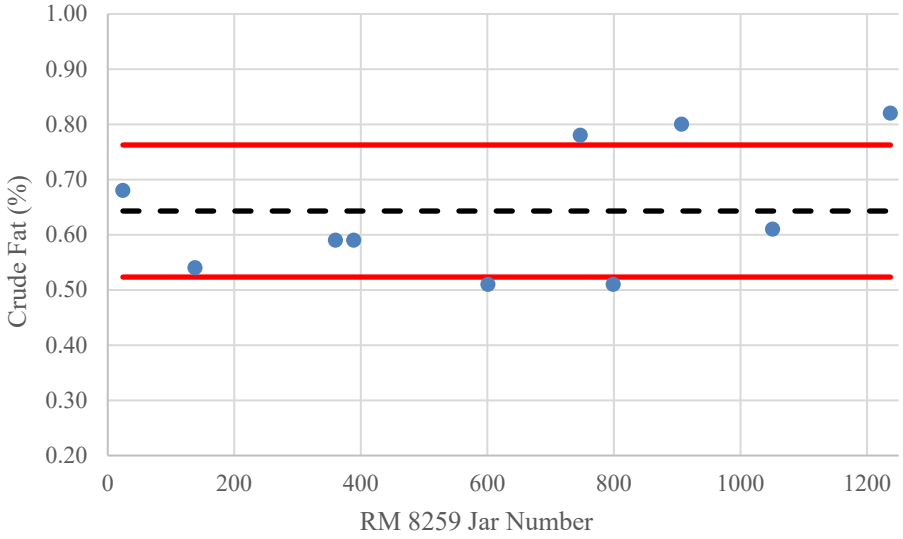

Figure 4. Homogeneity of crude fat in RM 8259

Individual fatty acids were also measured in six samples of each RM 8258 and RM 8259 and the mean, standard deviation and RSD was calculated for each analyte. The results of the fatty acid profile measurements, including summary statistics, are shown in Tables 8 and 9, where “ND” indicates compounds that were not detected.

Table 8. Results for individual fatty acids in RM 8258[a]

[a] Results are in g / 100 g as free fatty acids

RM 8258									
Jar No.	Jar 69	Jar 273	Jar 452	Jar 796	Jar 845	Jar 1061	Mean	StDev	RSD (%)
C 10:0 (Capric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 12:0 (Lauric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 14:0 (Myristic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 14:1 (Myristoleic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 15:0 (Pentadecanoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 15:1 (Pentadecenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 16:0 (Palmitic acid)	0.067	0.068	0.083	0.080	0.073	0.073	0.074	0.006	8.8
C 16:1 (Palmitoleic acid)	0.026	0.027	0.030	0.029	0.030	0.029	0.028	0.002	5.6
C 17:0 (Margaric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 17:1 (Heptadecenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:0 (Stearic acid)	0.058	0.058	0.065	0.061	0.066	0.066	0.063	0.004	5.9
C 18:1 (Oleic acid)	0.033	0.033	0.059	0.047	0.037	0.036	0.041	0.010	25.7
C 18:1n7 (Vaccenic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:2n6 (Linoleic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:3n3 (alpha-Linolenic Acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:3n6 (gamma-Linolenic Acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 18:4n3 (Stearidonic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:0 (Arachidic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:1 (Eicosenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:2n6 (Eicosodienoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:3n3 (Eicosatrienoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:3n6 (homo-gamma-Linolenic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 20:4n6 (Arachidonic Acid)	0.036	0.036	0.040	0.039	0.040	0.040	0.039	0.002	4.9
C 20:5n3 (Eicosapentaenoic acid)	0.079	0.078	0.086	0.083	0.087	0.087	0.083	0.004	4.8
C 21:0 (Heneicosanoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:0 (Behenic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:1n9 (Erucic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:2n6 (Docosadienoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22:6n3 (Docosahexaenoic acid)	0.057	0.058	0.066	0.063	0.065	0.067	0.063	0.005	7.2
C 22-5n3 (Docosapentaenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 22-5n6 (Docosapentaenoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 23:0 (Tricosanoic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 24:0 (Lignoceric acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 24:1 (Nervonic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 6:0 (Caproic acid)	ND	ND	ND	ND	ND	ND	–	–	–
C 8:0 (Caprylic acid)	ND	ND	ND	ND	ND	ND	–	–	–
Monounsaturated Fat	0.058	0.059	0.089	0.075	0.067	0.065	0.069	0.012	16.8
Omega-3 fatty acids	0.136	0.136	0.152	0.146	0.152	0.154	0.146	0.009	5.8
Omega-6 fatty acids	0.036	0.036	0.040	0.039	0.040	0.040	0.039	0.002	4.9
Polyunsaturated Fat	0.172	0.172	0.193	0.185	0.193	0.195	0.185	0.010	5.6
Saturated Fat	0.125	0.126	0.148	0.141	0.139	0.139	0.137	0.009	6.6
Total Fat	0.356	0.357	0.430	0.402	0.399	0.399	0.390	0.029	7.3

Table 9. Results for individual fatty acids in RM 8259[a]

[a] Results are in g / 100 g as free fatty acids

RM 8259									
Jar No.	Jar 169	Jar 266	Jar 561	Jar 652	Jar 1011	Jar 1238	Mean	StDev	RSD (%)
C 10:0 (Capric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 12:0 (Lauric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 14:0 (Myristic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 14:1 (Myristoleic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 15:0 (Pentadecanoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 15:1 (Pentadecenoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 16:0 (Palmitic acid)	0.106	0.116	0.113	0.111	0.105	0.104	0.109	0.005	4.6
C 16:1 (Palmitoleic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 17:0 (Margaric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 17:1 (Heptadecenoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 18:0 (Stearic acid)	0.062	0.070	0.066	0.066	0.060	0.058	0.064	0.004	6.7
C 18:1 (Oleic acid)	0.071	0.081	0.104	0.077	0.073	0.072	0.080	0.013	16.0
C 18:1n7 (Vaccenic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 18:2n6 (Linoleic acid)	0.098	0.109	0.104	0.103	0.095	0.095	0.101	0.006	5.8
C 18:3n3 (alpha-Linolenic Acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 18:3n6 (gamma-Linolenic Acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 18:4n3 (Stearidonic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:0 (Arachidic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:1 (Eicosenoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:2n6 (Eicosodienoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:3n3 (Eicosatrienoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:3n6 (homo-gamma-Linolenic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 20:4n6 (Arachidonic Acid)	0.029	0.031	0.030	0.029	0.027	0.027	0.029	0.002	5.4
C 20:5n3 (Eicosapentaenoic acid)	0.081	0.087	0.086	0.082	0.077	0.076	0.081	0.005	5.5
C 21:0 (Heneicosanoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:0 (Behenic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:1n9 (Erucic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:2n6 (Docosadienoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22:6n3 (Docosahexaenoic acid)	0.051	0.055	0.055	0.052	0.049	0.048	0.052	0.003	5.5
C 22-5n3 (Docosapentaenoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 22-5n6 (Docosapentaenoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 23:0 (Tricosanoic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 24:0 (Lignoceric acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 24:1 (Nervonic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 6:0 (Caproic acid)	ND	ND	ND	ND	ND	ND	—	—	—
C 8:0 (Caprylic acid)	ND	ND	ND	ND	ND	ND	—	—	—
Monounsaturated Fat	0.071	0.081	0.104	0.077	0.073	0.072	0.080	0.013	16.0
Omega-3 fatty acids	0.132	0.142	0.141	0.134	0.126	0.124	0.133	0.007	5.5
Omega-6 fatty acids	0.126	0.140	0.134	0.132	0.122	0.122	0.129	0.007	5.7
Polyunsaturated Fat	0.258	0.282	0.275	0.266	0.248	0.246	0.262	0.015	5.5
Saturated Fat	0.168	0.186	0.179	0.177	0.165	0.162	0.173	0.009	5.4
Total Fat	0.497	0.549	0.559	0.519	0.486	0.480	0.515	0.033	6.5

3.3 Assignment of Non-Certified Values and Uncertainties

For each analyte the estimated value is the mean of the measurements for that analyte, with the standard uncertainty being the standard error of that mean, and the expanded uncertainty (*k*) being a multiple of the standard uncertainty [6]. Value assignments and expanded uncertainties can be found in Tables 10 through13.

Table 10. Non-certified values for fatty acids (as free fatty acids) and crude fat in RM 8256

Analyte	Common Name	Mass Fraction g/100 g [(a)]	*k*
Tetradecanoic acid (C14:0)	Myristic acid	0.127 ± 0.005	2.57
Hexadecanoic acid (C16:0)	Palmitic acid	0.502 ± 0.011	2.57
Hexadecenoic acid (C16:1)	Palmitoleic acid	0.100 ± 0.002	2.57
Octadecanoic acid (C18:0)	Stearic acid	0.099 ± 0.002	2.57
(Z)-9-Octadecenoic acid (C18:1n-9)	Oleic acid	0.342 ± 0.007	2.57
(Z)-11-Octadecenoic acid (C18:1n-7)	Vaccenic acid	0.044 ± 0.001	2.57
(Z,Z)-9,12-Octadecadienoic acid (C18:2n-6)	Linoleic acid	0.048 ± 0.004	2.57
(Z,Z,Z)-9,12,15-Octadecatrienoic acid (C18:3n-3)	alpha-Linolenic acid	0.032 ± 0.001	2.57
(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic acid (C18:4n-3)	Stearidonic acid	0.053 ± 0.002	2.57
Eicosenoic acid (C20:1)	Eicosenoic acid	0.135 ± 0.002	2.57
(Z,Z)-11,14-Eicosadienoic Acid (C20:2n-6)	Eicosodienoic acid	0.023 ± 0.001	2.57
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)	EPA	0.222 ± 0.005	2.57
(Z)-13-Docosenoic acid (C22:1n-9)	Erucic acid	0.0230 ± 0.0004	2.57
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid (C22:5n-3)	DPA	0.052 ± 0.001	2.57
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3)	DHA	0.667 ± 0.019	2.57
(Z)-15-Tetracosenoic acid (C24:1n-9)	Nervonic acid	0.038 ± 0.001	2.57
Monounsaturated Fat		0.683 ± 0.009	2.57
Omega-3 fatty acids		1.026 ± 0.028	2.57
Omega-6 fatty acids		0.071 ± 0.003	2.57
Polyunsaturated Fat		1.097 ± 0.026	2.57
Saturated Fat		0.727 ± 0.017	2.57
Total Fat		2.508 ± 0.043	2.57
		%	*k*
Crude Fat		3.73 ± 0.11	2.26

[(a)] The assigned value is the average of the values measured for the analyte, and the corresponding expanded uncertainty (*k*) listed here is a suitable multiple of the standard uncertainty to achieve 95 % coverage.

Table 11. Non-certified values for fatty acids (as free fatty acids) and crude fat in RM 8257

Analyte	Common Name	Mass Fraction, g/100 g [(a)]			*k*
Tetradecanoic acid (C14:0)	Myristic acid	0.390	±	0.014	2.57
Pentadecanoic acid (C15:0)	Pentadecanoic acid	0.033	±	0.001	2.57
Hexadecanoic acid (C16:0)	Palmitic acid	1.527	±	0.039	2.57
Hexadecenoic acid (C16:1)	Palmitoleic acid	0.592	±	0.016	2.57
Heptadecanoic acid (C17:0)	Margaric acid	0.034	±	0.001	2.57
Heptadecenoic acid (C17:1)	Heptadecenoic acid	0.020	±	0.001	2.57
Octadecanoic acid (C18:0)	Stearic acid	0.392	±	0.007	2.57
(Z)-9-Octadecenoic acid (C18:1n-9)	Oleic acid	1.903	±	0.040	2.57
(Z)-11-Octadecenoic acid (C18:1n-7)	Vaccenic acid	0.277	±	0.006	2.57
(Z,Z)-9,12-Octadecadienoic acid (C18:2n-6)	Linoleic acid	0.685	±	0.032	2.57
(Z,Z,Z)-9,12,15-Octadecatrienoic acid (C18:3n-3)	alpha-Linolenic acid	0.132	±	0.002	2.57
(Z,Z,Z)-6,9,12-Octadecatrienoic acid (C18:3 n-6)	gamma-Linolenic Acid	0.020	±	0.001	2.57
(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic acid (C18:4n-3)	Stearidonic acid	0.097	±	0.002	2.57
Eicosanoic acid (C20:0)	Arachidic acid	0.022	±	0.001	2.57
Eicosenoic acid (C20:1)	Eicosenoic acid	0.136	±	0.003	2.57
(Z,Z)-11,14-Eicosadienoic Acid (C20:2n-6)	Eicosodienoic acid	0.056	±	0.001	2.57
Homo-gamma-Linolenic acid (C 20:3n-6)	homo-gamma-Linolenic acid	0.023	±	0.001	2.57
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid (C20:4 n-6)	Arachidonic Acid	0.095	±	0.002	2.57
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)	EPA	0.616	±	0.010	2.57
(Z)-13-Docosenoic acid (C22:1n-9)	Erucic acid	0.020	±	0.001	2.57
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid (C22:5n-3)	DPA	0.259	±	0.004	2.57
(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid (C22:5n-6)	DPA	0.0348	±	0.0004	2.57
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3)	DHA	0.970	±	0.014	2.57
Monounsaturated Fat		2.948	±	0.064	2.57
Omega-3 fatty acids		2.074	±	0.028	2.57
Omega-6 fatty acids		0.913	±	0.034	2.57
Polyunsaturated Fat		2.987	±	0.056	2.57
Saturated Fat		2.398	±	0.062	2.57
Total Fat		8.333	±	0.180	2.57
		%			*k*
Crude Fat		9.87	±	0.24	2.26

[(a)] The assigned value is the average of the values measured for the analyte, and the corresponding expanded uncertainty listed here (*k*) is a suitable multiple of the standard uncertainty to achieve 95 % coverage.

Table 12. Non-certified values for fatty acids (as free fatty acids) and crude fat in RM 8258

Analyte	Common Name	Mass Fraction, g/100 g[(a)]	*k*
Hexadecanoic acid (C16:0)	Palmitic acid	0.074 ± 0.007	2.57
Hexadecenoic acid (C16:1)	Palmitoleic acid	0.028 ± 0.002	2.57
Octadecanoic acid (C18:0)	Stearic acid	0.063 ± 0.004	2.57
Octadecenoic acid (C18:1)	Oleic acid	0.041 ± 0.011	2.57
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid (C20:4n-6)	Arachidonic acid	0.039 ± 0.002	2.57
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)	EPA	0.083 ± 0.004	2.57
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3)	DHA	0.063 ± 0.005	2.57
Monounsaturated Fat		0.069 ± 0.012	2.57
Omega-3 fatty acids		0.146 ± 0.009	2.57
Omega-6 fatty acids		0.039 ± 0.002	2.57
Polyunsaturated Fat		0.185 ± 0.011	2.57
Saturated Fat		0.137 ± 0.010	2.57
Total Fat		0.390 ± 0.030	2.57
		%	*k*
Crude Fat		0.721 ± 0.050	2.26

[(a)] The assigned value is the average of the values measured for the analyte, and the corresponding expanded uncertainty (*k*) listed here is a suitable multiple of the standard uncertainty to achieve 95 % coverage.

Table 13. Non-certified values for fatty acids (as free fatty acids) and crude fat in RM 8259

Analyte	Common Name	Mass Fraction, g/100 g[(a)]	*k*
Hexadecanoic acid (C16:0)	Palmitic acid	0.109 ± 0.005	2.57
Octadecanoic acid (C18:0)	Stearic acid	0.064 ± 0.005	2.57
Octadecenoic acid (C18:1)	Oleic acid	0.080 ± 0.013	2.57
(Z,Z)-9,12-Octadecadienoic acid (C18:2 n-6)	Linoleic acid	0.101 ± 0.006	2.57
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid (C20:4n-6)	Arachidonic acid	0.029 ± 0.002	2.57
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)	EPA	0.082 ± 0.005	2.57
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3)	DHA	0.052 ± 0.003	2.57
Monounsaturated Fat		0.080 ± 0.013	2.57
Omega-3 fatty acids		0.133 ± 0.008	2.57
Omega-6 fatty acids		0.129 ± 0.008	2.57
Polyunsaturated Fat		0.262 ± 0.015	2.57
Saturated Fat		0.173 ± 0.010	2.57
Total Fat		0.515 ± 0.035	2.57
		%	*k*
Crude Fat		0.643 ± 0.086	2.26

[(a)] The assigned value is the average of the values measured for the analyte, and the corresponding expanded uncertainty (*k*) listed here is a suitable multiple of the standard uncertainty to achieve 95 % coverage.

4. Crude Protein Analysis

Three jars were selected from each production batch for total protein measurement and shipped on dry ice to Eurofins Microbiology Laboratories, Inc. for analysis. Crude protein content in 0.2 g to 0.3 g samples was determined by combustion at ≥ 850 °C in CO_2 with quantitation by thermal conductivity of nitrogen according to AOAC Official Method 968.06 [7]. The nitrogen content determined in the samples was converted to crude protein using a conversion factor of 6.25.

$$\textit{crude protein (\%)} = \textit{nitrogen (\%)} \times \textit{conversion factor}$$

The measured crude protein results for each material and their respective summary statistics are shown in Table 14.

Table 14. Crude protein (%) results from seafood reference materials

Protein (%)						
Jar No.	Jar 1	Jar 2	Jar 3	Mean	StDev	RSD (%)
RM 8256	23.9	23.7	23.7	23.8	0.12	0.5
RM 8257	20.2	20.8	20.8	20.6	0.35	1.7
RM 8258	21.6	21.7	21.6	21.6	0.06	0.3
RM 8259	19.4	19.1	19.4	19.3	0.17	0.9

Non-certified value assignments and uncertainties for crude protein were calculated, where the estimated value is the mean of the measurements for that analyte, with the standard uncertainty being the standard error of that mean, and the expanded uncertainty being a multiple of the standard uncertainty [6]. Value assignments can be found in Table 15.

Table 15. Non-certified values for crude protein (%) in seafood RMs

Reference Material	Total Protein % [(a)]	*k*
RM 8256	23.77 ± 0.29	4.30
RM 8257	20.60 ± 0.86	4.30
RM 8258	21.63 ± 0.14	4.30
RM 8259	19.30 ± 0.43	4.30

[(a)] The assigned value is the average of the values measured for the analyte, and the corresponding expanded uncertainty (*k*) listed here is a suitable multiple of the standard uncertainty to achieve 95 % coverage.

Acknowledgments

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