A Research Note

Cadmium Determination of Frozen Cod: An Interlaboratory Comparison

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ABSTRACT

Results of analyses of cod portions by eight laboratories indicate that a substantial lack of agreement exists among laboratories and methods for cadmium analysis. Consistent results for cod samples of low Cd content (biologically bound) were reported by only three of eight participating laboratories. These laboratories reported a mean Cd content of 0.323 ppm with a coefficient of variation of 38%. Results were better when cod samples were spiked with Cd acetate at the 78 ppm level. Six of the eight laboratories correctly reported that Cd content at the 78 ppm level (mean 71.2 ppm with a coefficient of variation of 13%). Lack of consistency in determining biologically bound cadmium in cod tissue is attributed in part to loss of Cd during dry ashing of tissue. Laboratories utilizing atomic absorption spectrophotometry or neutron activation analysis generally reported more accurate results than the laboratory utilizing argon plasma atomic emission spectrometry.

In recent years, trace metals have attracted attention from the standpoint of nutrition and food safety. Cadmium is one of the trace elements that is not needed for normal metabolism of the human body. Instead, cadmium is known to cause various clinically manifested conditions (9). Since seafoods have the potential of carrying relatively high levels of cadmium (10), it is important to know the reliability of data generated by different laboratories. Currently, there are five methods that are commonly used for cadmium determination. These include colorimetry, emission spectroscopy, neutron activation, atomic absorption spectrophotometry and differential pulse anodic stripping voltammetry. Accuracy and reproducibility of these methods have been discussed by Friberg et al. (5) and Jones et al. (8). Their discussion applies, however, to results obtained under research conditions and may not be an accurate estimate of accuracy and precision obtained by commercial laboratories.

The study reported here was conducted to determine the precision and accuracy of reported cadmium concentrations that can be expected when submitting fish samples for analysis to laboratories typical of those available to the commercial producer.

MATERIALS AND METHODS

Ten blocks of frozen cod portions weighing 36.3 kg each were thawed and comminuted in a silent cutter (Hobart Mfg. Co., Model T21SGA), mixed 15 min in a food mixer (Leland Mfg. Co., Model H200DAH) and packaged in 100-g portions using flat wire polyethylene pouches (11.4 x 22.9 cm) (Albert Mojonnier, Inc.). Sample A was chosen to represent frozen cod portions currently available on the market. Sample B was spiked by adding 100 ml of a cadmium acetate solution containing 5.0 g of Cd(CH3CO2)2·2H2O to 27 kg of comminuted tissue before mixing and packaging as above. All samples were frozen at -29°C, randomized, packed in dry ice (3 pouches of each sample) and shipped by air freight.

The participating laboratories were requested to mix the contents of each pouch thoroughly before removing three replicate samples from each. Mixing of samples by each analyst after thawing was essential to reduce variability in cadmium content due to possible formation of "drip". No recommendations were given for sample preparation or method of analysis. Instead, analysts were expected to use the procedure normally followed in their laboratory and later provide details of the method used.

Eight laboratories collaborated in this study. They included three from industry, two from government and three from universities. In laboratories 1, 3 and 4, the samples were dry-ashed, in laboratories 2, 5, 7 and 8, wet ashing was employed, and in laboratory 6, the material was freeze-dried in preparation for analysis (Table 1). In laboratory 3, argon plasma emission spectrometry was used, in laboratory 6, neutron activation was used, and the remainder of the analysts used atomic absorption spectrophotometry for cadmium determination.

The cadmium concentrations reported by the cooperating analysts were evaluated using analysis of variance (13) and Duncan multiple range test (4) to determine significant differences in replicate samples within and among participating laboratories.

RESULTS AND DISCUSSION

Cadmium concentrations reported by collaborating

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analysts for Sample A are summarized in Table 1. Some laboratories analyzed one sample per pouch instead of the three requested. Since no significant differences were detected at the 5% level within replicate results of any analyst reporting nine determinations, individual cadmium values were used in further statistical analyses. The absence of significant differences among replicate samples is also strong support for the homogeneity of the samples. Data obtained from laboratories 1 and 2 were not included in analyses because values reported as “less than” do not lend themselves to statistical analysis.

Sample A represented edible cod portions, hence the cadmium concentration was anticipated to be between 0.02 and 0.20 ppm (11,12). As shown in Table 1, the cadmium concentrations reported by laboratory 3 were considerably lower and those of laboratory 8 considerably higher than results reported by other participating analysts. The laboratory 8 results were rejected as anomalously high, based on Duncan multiple range test, and the laboratory 3 results were rejected as anomalously low, based on comparison with the results reported by other analysts and the literature values. It should also be noted that laboratory 3 determined cadmium concentrations by argon plasma emission spectrometry, a technique that was found by one of the authors (RDK) to be extremely difficult to use for accurate determination of cadmium at the levels found in seafood samples (Table 2). Consequently, the Duncan multiple range test was recalculated for the data reported by laboratories 4 through 7. This analysis indicates that values received from laboratory 4 were outside the range of cadmium concentrations reported by the majority of analysts and were probably in error.

The mean cadmium concentration in cod portions as determined by laboratories 5 through 7 was 0.323 ± 0.122 ppm. This value was relatively high in comparison to most values reported in the literature, but was not unusual (7,12). We do not know the actual cadmium concentration in Sample A, so a direct determination of the accuracy of the results of labs 5 through 7 cannot be made. However, the overall results indicate that, at most, only three of the eight participating laboratories correctly reported the cadmium content of Sample A.

The coefficient of variability for data generated by laboratories 5 through 7 was relatively high (38%), but compared relatively well with coefficient of variability reported by Capar (2), which ranged between 14 and 21% for samples with similar cadmium content. Coefficient of variability for cadmium values within a single laboratory in the present study are acceptable for most purposes (Table 1).

Cadmium acetate was added to Sample B, resulting in a calculated concentration of about 78 ppm wet weight. Although the amount of cadmium added was high relative to the amount of cadmium present in the unaltered tissue, such a concentration can at times be found in seafoods (10). A summary of the cadmium concentrations reported by the cooperating analysts for Sample B is shown in Table 2. Here, again, individual values were used in initial statistical analysis using the Duncan multiple range test for reasons stated below. There was no significant difference within replicate samples for four laboratories reporting nine determinations, except laboratory 3 and 2 showing significant differences at the 5 and 0.1% level, respectively. The coefficient of variance for each pouch of laboratory 3 was 0.7, 1.5 and 0.4%. These values were relatively close to the coefficient of variance when using all nine cadmium values, thus justifying the use of individual cadmium concentrations in statistical analysis. The above data also indicate good homogeneity of the spiked sample.

The highly significant difference (0.1%) in cadmium content detected among the three pouches by laboratory 2 was a result of the exceptionally low coefficient of variability for each pouch (0.82, 0.13 and 0.07%). Since this level of precision was not attained by other analysts, the mean cadmium content of each pouch was best used in subsequent statistical analysis. It should also be noted that the significant difference among replicate samples in labo-

### Table 1. Cadmium concentrations (ppm) detected in frozen cod portions (Sample A).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Preparation</th>
<th>Analysis</th>
<th>No. of determinations</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Duncan (1%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>550°C</td>
<td>AAb</td>
<td>(3)</td>
<td>&lt;0.100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Wet ashing</td>
<td>AA</td>
<td>(9)</td>
<td>&lt;0.600</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>450°C</td>
<td>Em. Spctr.e</td>
<td>9</td>
<td>0.008</td>
<td>0.002</td>
<td>a</td>
</tr>
<tr>
<td>4</td>
<td>500°C</td>
<td>AA</td>
<td>8</td>
<td>0.050</td>
<td>0.031</td>
<td>a</td>
</tr>
<tr>
<td>5</td>
<td>Wet ashing</td>
<td>AA</td>
<td>3</td>
<td>0.209</td>
<td>0.046</td>
<td>a</td>
</tr>
<tr>
<td>6</td>
<td>Freeze drying</td>
<td>Ntr. Actv.d</td>
<td>3</td>
<td>0.363</td>
<td>0.012</td>
<td>a</td>
</tr>
<tr>
<td>7</td>
<td>Wet ashing</td>
<td>AA</td>
<td>3</td>
<td>0.398</td>
<td>0.165</td>
<td>a</td>
</tr>
<tr>
<td>8</td>
<td>Wet ashing</td>
<td>AA</td>
<td>9</td>
<td>2.067</td>
<td>0.427</td>
<td>b</td>
</tr>
<tr>
<td>Total</td>
<td>Laboratories 3 to 8</td>
<td></td>
<td>35</td>
<td>0.628</td>
<td>0.894</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Laboratories 4 to 7</td>
<td></td>
<td>17</td>
<td>0.195</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Laboratories 5 to 7</td>
<td></td>
<td>9</td>
<td>0.323</td>
<td>0.122</td>
<td></td>
</tr>
</tbody>
</table>

aMean cadmium concentrations not showing the same letter were significantly different at the 1% level (P<0.01).
bAtomic absorption spectrophotometry.
cArgon plasma emission spectrometry.
dNeutron activation.

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TABLE 2. Cadmium concentrations (ppm) detected in frozen cod portions (Sample B).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>No. of determinations</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Duncan (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9</td>
<td>13.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21</td>
<td>a</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>14.07</td>
<td>0.34</td>
<td>a</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>58.84</td>
<td>11.23</td>
<td>b</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>65.44</td>
<td>4.35</td>
<td>bc</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>71.20</td>
<td>0.66</td>
<td>cd</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>73.67</td>
<td>2.52</td>
<td>cde</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>76.22&lt;sup&gt;***&lt;/sup&gt;</td>
<td>2.07</td>
<td>de</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>85.34</td>
<td>4.05</td>
<td>e</td>
</tr>
<tr>
<td>Total</td>
<td>Laboratories 3, and 7 excluded</td>
<td>36</td>
<td>69.31</td>
<td>10.18</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sample preparation and method of analysis are given in Table 1.

<sup>b</sup>Mean cadmium concentrations not showing the same letter were significantly different at the 1% level (P<0.01).

<sup>c</sup>Significant differences at 5% and 0.1%<sup>***</sup> level were detected within replicate values of laboratories reporting nine determinations.

Laboratory 2 does not indicate poor homogeneity of the spiked sample, since the coefficient of variance for Sample B, using mean cadmium values of each pouch as reported by laboratory 2, was only 1.5%.

Based on the Duncan multiple range test (4), cadmium concentrations reported by laboratories 3 and 7 were significantly different from those reported by any other laboratory and were, therefore, excluded from subsequent calculations. Furthermore, values reported by laboratory 3 and 7 were unacceptable since they account for only 18% of the cadmium known to be present in the sample. Cadmium content reported by the remaining laboratories was 69.31 ± 10.18 ppm for 36 observations (Table 2) or 71.78 ± 9.10 ppm for 18 observations, weighing results at three observations per laboratory. These figures represent 89 and 92% recovery with a 15 and 13% coefficient of variation for the two ways of handling the data, respectively. These results are considered to be within the error limits of adding cadmium to the sample.

Determination of cadmium concentrations in Sample B in the present study compares quite well with results of a study using mean cadmium values of each pouch as reported by laboratory 2, was only 1.5%.

It is generally agreed that whenever elemental concentrations in biological samples are determined, a reference sample of similar composition containing a known concentration of the element of interest be included (1). Reference samples for this purpose are generally provided by the analyst. It must be recognized, however, that suitable reference samples are not always readily available and a laboratory performing the analyses may not be able to provide a suitable reference sample to match an unknown sample sent in by a user. In the present study, it is evident that many analysts did not make proper use of a suitable reference sample. If such a sample had been included in the analysis, the problems associated with the ashing of Sample A would have been detected. The observation that the cadmium in Sample B apparently reacted differently than in Sample A points out the need for adequate matching of the reference sample to the composition of unknown samples.

In conclusion, it is apparent that accuracy is lacking for determining the concentration of biologically bound cadmium in seafoods by different laboratories. Only three of eight participating laboratories reported values that were within experimental limits. The difficulty in determining the concentration of biologically bound cadmium has been attributed, in part, to dry ashing. Cadmium determinations of samples spiked with cadmium acetate were acceptable, but values reported by a laboratory chosen at random may be in error, since two of eight laboratories provided incorrect values. Atomic absorption spectrophotometry and
neutron activation procedures appear to be reliable methods for analyzing seafoods for cadmium; however, values reported by a laboratory using argon plasma emission spectrometry were in error.

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References to trade names do not imply endorsement or preference by the Maine Agriculture Experiment Station and the National Fisheries Institute.

REFERENCES


Wilson et al., cont. from p. 121