Determination of the Fatty Acids in Fish Tissue and Feed — Comparison of Different Methods and Statistical Evaluation

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With the aim to reinforce laboratory competence in the field of testing the quality of fish from aquaculture, a study on the precision of fatty acid (FA) analyses in fish meat and fish feed was undertaken. Different methods were performed in laboratories. In situ transesterification method and extraction of lipids from the fish were followed by capillary gas chromatography with flame ionization detection. The reproducibility ($R$) values of the majority of FAs were less than 3% of their absolute values. Differences in calculating ionization detector response factors and/or autoxidation caused by faulty sample-handling could lead to variation in quantification of FAs in fish, especially for FA C22:6n-3. Statistical analysis showed a significant correlation between the two laboratories’ quantifications of FAs in fish and fish feed (Pearson's correlation coefficient; $r = 0.987$, $r = 0.994$, and $r = 0.997$; for fish Z [trout], fish S [rainbow trout], and fish feed, respectively). Overall, adequate accuracy was obtained in this study. The proposed method provides a fast and efficient means of identifying fish and feed for quality control purposes.

**Keywords:** Fish tissues, fatty acids, quantitation, gas chromatography, reproducibility

**Introduction**

Fish diet has a major impact on the chemical composition of fish tissues and especially on the fatty acid (FA) composition of the fish lipids [1–4]. The FA composition of fish can also be influenced by environmental factors such as a temperature, water quality, season, and the size and age of the fish [5–10]. The impact of diet on the FA composition in fish tissue has been examined in numerous farmed and wild fish species [5, 11, 12]. Determination of the FAs in fish and marine oils is predominantly performed by gas chromatographic methods after methylation and detects corresponding methyl esters [13–16]. Separation can be obtained on different columns, and quantification is usually performed using internal standards [17, 18].

Extraction of lipids is a prevailing problem for accurate determination of FA composition and depends on the binding of FAs to the matrix of the sample. There are various extraction methods with different types of solvents or solvent mixtures to isolate lipids from tissues. One of the most widely used methods for the extraction of lipids is that proposed by Soxhlet [19–21]. The method is simple and efficient, and the main disadvantage is its long duration and use of large amounts of solvent, usually petroleum ether. Other methods were proposed by Folch et al. [22], Blight and Dyer [23], and Hara and Radin [24], and there are also more contemporary extraction methods such as pressurized liquid extraction (PLE) and accelerated solvent extraction (ASE) [25, 26]. Some researchers have prepared in situ direct methylation of FAs without lipid extraction and purification steps [27–30].

There are various procedures for the esterification of FAs that can be applied to different classes of lipids [13], and which vary in require expenditure of time and effort. However, absolute accuracy in such studies is not the ultimate priority. The main problem for the analysis of n-3 polyunsaturated fatty acids (PUFAs) is that PUFAs are rather unstable, so that calibration cannot be performed using quantitative standards. The other problem concerns the use of detector response factors. When flame ionization detectors are used, small correction factors can be applied, when high precision is required, to compensate for the fact that the carboxyl carbon atom in each ester is not appreciably ionized during combustion [13]. There are also small effects of this kind due to the absence of hydrogen atoms at double bonds. The degree of correction necessary is greatest for FAs of shorter chain length or with a high degree of unsaturation. Whereas some analysts use empirical response factors (ERFs), other trust in theoretical response factors (TRFs), but many do not use correction factors at all [31–33]. Careful calibration with pure standards is necessary in this instance. When an analysis has been completed, the results can be expressed directly as weight percentages of the FAs. High accuracy of gas chromatography for FA quantitative composition was completely reviewed by Bannon et al. [33], Craske and Bannon [32], and Ackman [31].

Inter-laboratory tests are a useful tool to detect systematic errors associated with a specific method or inadequate application of the method in the laboratory [34]. Unacceptable differences may exist between supposedly identical measurements that are performed in different laboratories. Thus, the important question that has to be answered in the evaluation of analytical methods is how much between-laboratory variability exists [34]. Analysts can very well check between-laboratory variability, but other analysts cannot be verified in other laboratories. This situation suggests that results of analysis of reference standards can vary from laboratory to laboratory [35].

Quality control of food analysis is of great importance because only precise analytical results can allow valid assumptions to be drawn between the quality of different types of food and the risks associated with human activities [36]. In many cases, the analyses used can lack the required quality. Generally, in addition to errors associated with sampling, there are three sources of errors which can occur in the analysis: the pretreatment of the sample (digestion, extraction), the final measurement (calibration error,
overlapping peaks), and insufficient experience (lack of training,
careful work, pure vessels and equipment) [37].

Numerous comparative studies have been conducted with dif-
f erent aims, e.g., to test different methods of analysis [38, 39], to
determine the suitability for application of one or more methods
[40], to establish precision of the methods by determining their
reproducibility limits and make recommendation for their use in
laboratory practice [41, 42], to evaluate and possibly improve an-
alytical methods or laboratory performance, or to certify refer-
ence materials [43–45]. Due to the heterogeneity of FAs in
animal fats, it is also necessary to study the extraction and derivi-
 tization procedure in order to obtain accurate quantitative and
 qualitative results [38].

With the aim to reinforce laboratory competence in the field of
testing the quality of fish from aquaculture, a study on the preci-
sion of two differing analytical methods for quantitative determi-
nation of FAs in fish meat and fish feed was undertaken. The
results of the FA compositions and total lipids were compared in
tROUT (Salmo truta) and rainbow trout (Oncorhynchus mykiss),
and fish feed with different methods in two laboratories. The pre-
cision of laboratories participating in this comparative study was
determined, and reproducibility was compared. The real aim of
this study was to achieve a practical inter-changeability of the test
results.

Materials and Methods

Sample Preparation. Two representative fish samples meat
without bones of trout (Z) (Salmo truta) and rainbow trout (S)
(Oncorhynchus mykiss) and one sample of fish feed were
prepared for analysis in accordance with International
Organization for Standardization (ISO) standard method [46].
The samples were homogenized by using a homogenizer
Grindomix GM 200 (Retch, Germany) at 5000–6000 rpm for
20 s and stored under vacuum in plastic bags at −18 °C until
use. Participants were required to determine the content of
total lipids and FA composition by the most appropriate
methods of their choice. Participants were requested to report
results in duplicate in g/100 g of FA to two significant figures
or two decimal places, whichever was appropriate. Analytical
results were exchanged via correspondence. Laboratories were
 requested to report their uncertainties as standard deviations of
two measurements.

FA Analysis by Capillary Gas Chromatography by
Method 1. Total lipids in fish feed were determined by the ISO
standard method [20]. Lipids were extracted with petroleum ether
(burning point ranged from 40 to 60 °C) after acid hydrolysis of the
sample with hydrochloric acid. The FA composition of the samples was
determined by gas chromatography. The method used was in situ
transesterification [48] as modified by Polak et al. [29]. Briefly,
after adding 3 mL of 0.5 M NaOH in methanol and 0.3 mL of
methylene chloride to homogenized sample, in situ
transesterification was performed by heating sample at 90 °C for
40 min and continued heating after adding 14% BF3 in
methanol. The FAMEs were extracted into hexane after cooling
determined by capillary gas chromatography on GC Agilent
Technologies 6890 with a flame ionization detector and HP-88
capillary column (100 m × 0.25 mm × 0.20 μm, Agilent
Technologies). Separation and detection were performed under
the following temperature conditions: 150 °C, hold for 10 min,
rate of 1.5 °C/min to 180 °C, hold for 40 min, and rate of
3 °C/min to a final temperature of 240 °C. Total analysis
time was 95 min. The injector and detector temperatures
were 250 °C and 280 °C, respectively. The carrier gas was
helium at a flow rate of 2.3 mL/min. Injected volume was
1 μL, and injector split ratio was 1:30. Nitrogen was used as the make-up gas at a flow rate of 45 mL/min; detector gases were hydrogen and synthetic air (21% O2) at a rate of 40 mL/min and 450 mL/min, respectively.
The FAMEs were determined through their retention times in
comparison to the relevant standard mixtures using: 37 Compo-
nents FAME mix (Supelco, Bellefonte, USA); PUFA No. 1, an-
imal source (Supelco, Bellefonte, USA); linoleic acid methyl
ester cis/trans isomer Mix (Supelco, Bellefonte, USA); cis-7-
octadecenoic methyl ester (Supelco, Bellefonte, USA) and
cis-11-octadecenoic methyl ester (Supelco, Bellefonte, USA);
methyl stearidionate (Fluka, Switzerland); and Nu-Chek

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>NIST 3275-2 reported by manufacturer</th>
<th>Quantified by method 1*</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>3.45 ± 0.40</td>
<td>3.43 ± 0.06</td>
<td>99.4</td>
</tr>
<tr>
<td>C16:0</td>
<td>8.01 ± 0.44</td>
<td>7.94 ± 0.21</td>
<td>99.1</td>
</tr>
<tr>
<td>C16:1</td>
<td>5.83 ± 0.45</td>
<td>5.81 ± 0.15</td>
<td>99.7</td>
</tr>
<tr>
<td>C18:0</td>
<td>12.94 ± 0.62</td>
<td>12.85 ± 0.36</td>
<td>99.3</td>
</tr>
<tr>
<td>C18:1</td>
<td>22.10 ± 1.60</td>
<td>21.99 ± 0.36</td>
<td>99.5</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.00 ± 0.42</td>
<td>2.97 ± 0.16</td>
<td>99.1</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.42 ± 0.12</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>C20:5</td>
<td>394 ± 17</td>
<td>424 ± 10</td>
<td>107.6</td>
</tr>
<tr>
<td>C22:5</td>
<td>67.6 ± 2.3</td>
<td>67.1 ± 2.0</td>
<td>99.3</td>
</tr>
<tr>
<td>C22:6</td>
<td>187 ± 8</td>
<td>189 ± 6</td>
<td>101.1</td>
</tr>
</tbody>
</table>

*Extraction of lipids.
Total lipids, % 2.44 ± 0.07 1.75 ± 0.39 6.51 ± 0.08 6.23 ± 0.21 0.69 0.28 ** NS

C22:6
C22:5
n-
C20:4
C22:1
C20:5
C20:3
C18:3
C18:1
C18:0 3.44 ± 0.01 2.75 ± 0.11 3.21 ± 0.10 2.88 ± 0.03 0.69 0.33 NS NS

Results and Discussion

Results showed that the laboratories had very good inter-laboratory control of their methods (Tables 1 and 2). Altogether,

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>BCR-163 reported by manufacturer</th>
<th>Quantified by method 2</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>2.29 ± 0.04</td>
<td>2.3 ± 0.11</td>
<td>104.0</td>
</tr>
<tr>
<td>C16:0</td>
<td>25.96 ± 0.30</td>
<td>25.88 ± 0.22</td>
<td>99.7</td>
</tr>
<tr>
<td>C16:1</td>
<td>2.58 ± 0.16</td>
<td>2.73 ± 0.08</td>
<td>105.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>18.29 ± 0.16</td>
<td>18.11 ± 0.23</td>
<td>99.0</td>
</tr>
<tr>
<td>C18:1</td>
<td>38.34 ± 1.36</td>
<td>38.45 ± 0.45</td>
<td>100.3</td>
</tr>
<tr>
<td>C18:2</td>
<td>7.05 ± 0.17</td>
<td>7.12 ± 0.12</td>
<td>101.0</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.86 ± 0.14</td>
<td>0.88 ± 0.08</td>
<td>102.3</td>
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</table>

*In situ transesterification.

Table 3. Results and uncertainties of the fatty acid composition (% of total fatty acids) and total fat content in fish as reported by laboratories

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Z1</th>
<th>Z2</th>
<th>S1</th>
<th>S2</th>
<th>R</th>
<th>Z</th>
<th>R</th>
<th>S</th>
<th>R</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.17 ± 0.00</td>
<td>1.07 ± 0.02</td>
<td>1.47 ± 0.00</td>
<td>1.62 ± 0.03</td>
<td>0.10</td>
<td>-0.15</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>C15:0</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.14 ± 0.00</td>
<td>0.14 ± 0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>12.69 ± 0.01</td>
<td>11.15 ± 0.47</td>
<td>11.96 ± 0.03</td>
<td>11.20 ± 0.03</td>
<td>1.54</td>
<td>0.76</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1-7</td>
<td>2.05 ± 0.01</td>
<td>1.56 ± 0.03</td>
<td>2.46 ± 0.01</td>
<td>2.30 ± 0.02</td>
<td>0.49</td>
<td>0.16</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17:0</td>
<td>0.17 ± 0.00</td>
<td>0.16 ± 0.00</td>
<td>0.18 ± 0.00</td>
<td>0.21 ± 0.00</td>
<td>0.01</td>
<td>0.03</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>C18:0</td>
<td>3.44 ± 0.00</td>
<td>2.73 ± 0.11</td>
<td>3.21 ± 0.10</td>
<td>2.88 ± 0.03</td>
<td>0.69</td>
<td>0.33</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>C18:1-9</td>
<td>39.04 ± 0.04</td>
<td>37.21 ± 1.30</td>
<td>46.07 ± 0.03</td>
<td>43.27 ± 0.08</td>
<td>1.83</td>
<td>2.80</td>
<td>NS</td>
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<tr>
<td>C18:2-6</td>
<td>15.11 ± 0.01</td>
<td>14.96 ± 0.33</td>
<td>16.78 ± 0.06</td>
<td>18.52 ± 0.17</td>
<td>0.15</td>
<td>–1.74</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>C20:0</td>
<td>0.17 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.22 ± 0.00</td>
<td>0.32 ± 0.00</td>
<td>0.10</td>
<td>–0.10</td>
<td>NS</td>
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</tr>
<tr>
<td>C18:3-6</td>
<td>0.30 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>0.43 ± 0.00</td>
<td>0.42 ± 0.01</td>
<td>0.30</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>C18:3-9</td>
<td>1.41 ± 0.01</td>
<td>2.77 ± 0.02</td>
<td>1.67 ± 0.06</td>
<td>3.05 ± 0.07</td>
<td>–1.36</td>
<td>–1.38</td>
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<tr>
<td>C20:1-9-11</td>
<td>3.87 ± 0.01</td>
<td>2.27 ± 0.11</td>
<td>3.81 ± 0.02</td>
<td>2.57 ± 0.10</td>
<td>2.30</td>
<td>1.24</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>C20:2-6</td>
<td>0.76 ± 0.02</td>
<td>0.80 ± 0.02</td>
<td>0.94 ± 0.00</td>
<td>1.00 ± 0.01</td>
<td>–0.04</td>
<td>–0.06</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20:3-6</td>
<td>0.53 ± 0.01</td>
<td>0.66 ± 0.02</td>
<td>0.46 ± 0.00</td>
<td>0.63 ± 0.01</td>
<td>–0.13</td>
<td>–0.17</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>C20:3-9-3</td>
<td>0.64 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>o</td>
<td>o</td>
<td>–</td>
<td>–</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>C20:4-6</td>
<td>o</td>
<td>1.12 ± 0.02</td>
<td>o</td>
<td>0.84 ± 0.10</td>
<td>o</td>
<td>–</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>C22:1-9</td>
<td>o</td>
<td>0.53 ± 0.03</td>
<td>o</td>
<td>0.35 ± 0.02</td>
<td>o</td>
<td>–</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20:5-3</td>
<td>1.61 ± 0.01</td>
<td>2.69 ± 0.17</td>
<td>0.96 ± 0.00</td>
<td>1.44 ± 0.04</td>
<td>–1.08</td>
<td>–0.48</td>
<td>NS</td>
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<tr>
<td>C22:5-3</td>
<td>1.02 ± 0.02</td>
<td>1.10 ± 0.04</td>
<td>0.29 ± 0.01</td>
<td>0.62 ± 0.14</td>
<td>–0.08</td>
<td>–0.33</td>
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<tr>
<td>C22:6-3</td>
<td>14.75 ± 0.01</td>
<td>17.56 ± 0.04</td>
<td>6.85 ± 0.01</td>
<td>7.01 ± 0.05</td>
<td>–2.81</td>
<td>–0.16</td>
<td>NS</td>
<td>NS</td>
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<td></td>
</tr>
</tbody>
</table>

Total lipids, % 2.44 ± 0.07 1.75 ± 0.11 6.51 ± 0.08 6.23 ± 0.01 0.69 0.28 *** NS

Values are reported as mean ± standard deviation; samples: Z, trout (Salmo trutta); S, rainbow trout (Oncorhynchus mykiss); 1, method 1—extraction of lipids; 2, method 2—in situ transesterification; R, reproducibility; NS, not significant for major constituents R ≤ 3%; R ≥ 3%; NS — not significant for minor constituents R ≥ 0.5%; o, overlapping peaks; for total lipids: NS — not significant R ≤ 0.5%, *** R ≥ 0.5%.
good correspondence between FA composition determined by direct methylation and by Folch extraction prior to the methylation step [28]. Statistical analysis of the results of FA composition and total lipids content in the two fish samples and fish feed with uncertainties are presented in Table 3.

The reproducibility (R) values for the majority of FAs that were analyzed in this study were less than 3% of absolute values [49], i.e., less than the maximum allowable difference. Mean values for the contents of oleic acid (C18:1cis-9) were more varied between the two laboratories in both fish samples, but the difference was not higher than 3% of the absolute values. However, for the mean values of C18:1cis-9 and C18:2n-6, significant differences were observed between a greater number of laboratory groups in Iberian pork fat [51]. Other monounsaturated fatty acids (MUFA) like C16:1n-7 and C20:1 had a very good R values (lower than 0.5%).

As can be observed (Table 3), the R values for the saturated fatty acids (SFAs) (C14:0, C15:0, C17:0, C18:0, and C20:0) were less than 0.5% for the minor constituents in both fish samples. The R value for palmitic acid (C16:0), the most abundant of the SFAs, was less than 3% of the absolute value. R values for the n-6 polyunsaturated fatty acids (PUFAs) (C18:2n-6, C18:3n-6, 20:3n-6, and C20:2n-6) were less than 3% for major constituents and less than 0.5% for minor constituents. R values for the n-3 PUFAs (C18:3n-3, C20:5n-3, and C22:6n-3) were less than 3% for major constituents (C18:3n-3, −1.36, −1.38; C20:5n-3, −1.08, −0.48; C22:6n-3, −2.81, −0.16 in fish Z and in fish S, respectively). Differences in calculating ionization detector response factors in the laboratories could lead to variation in the results, especially for C22:6n-3. However, differences in the contents of C20:5n-3 (EPA) and C22:6n-3 (DHA) might be due to calibration errors of the gas chromatographic methods with the calibration standards and/or possibly in the correction factor used. Empirical correction factor must be adapted for accurate EPA and DHA analyses conducted at temperatures causing thermal losses [52]. Also, a major potential source of loss of PUFAs might be autoxidation caused by faulty sample-handling [13]. Difficulties in determining EPA and DHA have been reported in marine and other oils [50].

It can be concluded that adequate accuracy of the FA composition of fish was achieved in this study. Higher R values for the total lipids content (>5%) and the FA composition in fish Z than in fish S might be a consequence of greater variability of the fish Z samples. This is confirmed by the reproducibility limit of the total fat determination in fish by ISO procedure [19]. An R value higher than 0.5% was obtained in fish Z (0.69%), while it was smaller in fish S (0.28%). According to Horwitz [34], gravimetric determination of the total fat content in meat with a higher fat content resulted in a higher coefficient of variation (%) and a higher standard deviation than did determination of the same in meat with a lower fat content. On the other hand, Smith and Hansen [50] reported that R values worsen for analytes below 0.5% (weight percent) or less. It is not easy to give an objective assessment of the standard of accuracy that should be possible in routine analyses of FAs, but in a collaborative study of the International Union of Pure and Applied Chemistry (IUPAC) methodology for FA analysis, typical coefficients of variation (%) at various concentrations were 15 for 2% level of FAs, 8.5 for 5% level of FAs, 7 for 10% level of FAs, and 3 for 50% level of FAs [13].

Statistical analysis of the results of FA composition and total lipids content in the fish feed with uncertainties are presented in Table 4.

The R values of the main FAs (present in feed in amounts >5%) ranged from −1.14 (C16:0) to 1.23 (C18:2n-6) in the fish feed. The R values of the n-3 PUFAs ranged between −1.06 (C20:5n-3) and 0.97 (C22:6n-3). The difference expressed as R value of the total lipids content was not significant (0.12%). The mean values for the content of oleic acid (C18:1cis-9) also varied between the two laboratories, similar to oleic acid measurements in the fish. R values of the n-3 PUFAs (C18:3n-3, C20:5n-3 and C22:6n-3) were lower than 3% in the fish feed and differences were not significant.

To enable comparison of the two different methods, and taking into account the stricter criteria of the method for analyses of animal and vegetable fats and oils by gas chromatography without a total extraction step, correlation between the FA composition of fish and feed was performed in two laboratories. Pearson’s correlation coefficient for fish Z and fish S were r = 0.987 and r = 0.994, respectively. Corresponding t values were 37.50 and 54.65 (tcalc = 2.09, P = 0.05), indicating a statistically significant correlation between the FA composition of the fish determined in the two laboratories. The obtained correlation coefficient and corresponding t values for FA composition indicated worse precision and probably higher variability of fish Z samples in both laboratories. Higher correlation coefficient and t values were obtained for fish S samples in both laboratories as well as for between-laboratory performances, i.e., R values.

Pearson’s correlation coefficient for the fish feed was r = 0.997. The t value was 78.06 (tcalc = 2.09, P = 0.05), indicating a statistically significant correlation between the FA composition of the fish feed determined in two laboratories.

The FA contents in the two fish species and feed sample analyzed in laboratories were very similar, with the exception of the FA C18:1cis-9 (somewhat higher content measured by method 1) and C22:6n-3 (higher content measured in both fish by method 2). According to our knowledge, there were two collaborative studies organized for the determination of n-3 and n-6 fatty acids in vegetable fats and oils by capillary gas chromatography [53]. It was found rather high reproducibility coefficient of variation, but overall, the results were satisfactory [53].

Results of reproducibility obtained for the most of the FAs in the fish samples showed that the accuracy of the gas chromatography methods used were adequate for the analysis of fish. Similar reproducibility values of the total lipids content and FA composition in fish feed were observed. At the same time, the methods for extracting the total lipids content with either application of an organic solvent or the in situ methylation method resulted in statistically almost equal composition of most of the FAs.

Conclusion

The reproducibility values of the majority of FAs analyzed in this study were less than values recommended by the ISO standard method as a maximum allowable difference. Therefore, adequate accuracy was obtained in this study for quantification of most of the FAs. Reproducibility of most of the FAs in the fish showed that the accuracy of the gas chromatography with flame ionization detection was suitable and adequate for quantification of these compounds in this animal tissue matrix. The FA contents in the two fish species and feed sample analyzed in laboratories were very similar, with the exception of the FA C18:1cis-9 (somewhat higher content measured by method 1) and C22:6n-3 (higher content measured in both fish by method 2). At the same time, the two methods for extracting total lipids (ASE and AOAC) produced statistically equal content of total lipids in the fish and the fish feed. Reproducibility values of the in situ transmethylation method and extraction of lipids with transesterification followed by gas chromatography with flame ionization detector were satisfactory for most of the FAs. Overall, adequate accuracy was obtained in this study. The proposed method provides a fast and efficient means of identifying fish and feed for quality control purposes.

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