Research Note

Development of a Novel Method for Rapid Discrimination between Wild and Farmed Sea Bream

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ABSTRACT

A simple method based on direct sampling analysis, coupled with a time of flight mass spectrometer, was developed to discriminate between wild and farmed sea bream on the basis of the docosahexaenoic and arachidonic fatty acid ratio. Good precision in repeatability and reproducibility (relative standard deviation < 15%) was obtained. The fatty acid ratios of the two types of fish were statistically significant (Student’s t < 0.001). The use of a simple, rapid, and cost-effective tool could aid in the detection of commercial fish fraud, increase the number of controlled samples, and strengthen control along the entire commercial chain.

HIGHLIGHTS

- The sale of farmed fish as wild fish is a common fraudulent practice.
- A rapid method was validated to discriminate between wild and farmed sea bream.
- The DHA/AA ratio between fatty acids allows differentiation of farmed from wild sea bream.

Key words: Direct sample analysis; Farmed fish; Mass spectrometry; Sea bream; Wild fish

The nutritional and quality attributes of fish make it an excellent food for human health. Assessment of seafood origin is key in the perception of product quality by final consumers (14). Fish production can originate from wild herding or aquaculture. Choosing a farmed product means having the certainty of a product available year-round, of standard dimensions, and with stable quality characteristics. Purchasing a wild sea product means the uncertain availability of a product, with prices and characteristics of leaner meats varying by season. As the world wild fish stocks are limited and the market demand has increased in recent decades (3), the fish farming sector enjoys good growth. In fact, more than half of the fish products consumed worldwide now come from farms. The most commonly farmed fish and shellfish are carp, salmon, sea bream, sea bass, croaker, amberjack, eel, trout, tuna, sturgeon, crustaceans, and molluscs.

European legislation provides that consumers be informed about the origin and the characteristics of the products they purchase. As regards food products, legislation requires the provision of additional and clear information to retailers and consumers so that they are better aware of the food purchases they make and the effect of production methods on nutrition and food quality and safety (3). Legislation regulating food product traceability and labeling is given in Regulations (EC) 178/2002 and 1169/2011; Regulation 1379/2013 covers requisites for labeling of seafood products, their origin, and their species name. In addition to the name of the fish species and area of production, the label should include the method of production, that is, whether the fish comes from aquaculture or has been caught from the sea. The best documented cases of food fraud involve fish and seafood products: 72% of illegal activities in the fish sector involve incorrect labeling and lack of traceability. The sale of farmed fish as wild fish is a fraudulent practice. To protect consumers from commercial fraud and to ensure food traceability, reliable analytical methods based on sensitive, rapid, and low-cost techniques need to be developed and applied.

Studies have demonstrated differences in the fatty acids composition of farmed and wild fish products and that the diet is the main reason for these differences (8, 17). Fatty acids are monocarboxylic acids characterized by a long nonbranched hydrocarbon chain. A wide diversity of fatty acids is present in most marine organisms (2). In marine fish, the fatty acids docosahexaenoic acid (22:6n–3; DHA), eicosapentaenoic acid (20:5n–3; EPA), and arachidonic acid (20:4n–6; omega 6 AA) carry out a variety of essential physiological functions (6). Linoleic (18:2n–6; LA) omega 6 and α-linolenic (18:3n–3; ALA) omega 3 acid are...
polysaturated fatty acids, so-called essential because they must be introduced with the diet. Once introduced through food, they are converted enzymatically into other polyunsaturated fatty acids.

The lipid content and fatty acids profile of fish vary between and within species. The fatty acids composition of lipids from fish tissue reflects the fatty acid content of the lipid taken in with the diet. Accordingly, comparing the lipid content and fatty acids composition between wild and farmed fish can be a useful approach to distinguish between them (9–11, 17).

Because consumers do not have the necessary tools or skills to visually determine whether a product actually corresponds to what is indicated on the label, controls to protect them from commercial fraud are desirable (14). Several techniques have been developed in the past decade to detect wild-farmed substitution fraud in seafood (13). Macroscopic examination of fish is of little value due to the lack of specific targets in body integrity and loss of morphological traits at the point of sales. Other types of analysis, such as genomics and proteomics patterns, have limited application owing to the difficulty in selecting reliable markers (14).

The aim of the present study was to develop and validate a rapid method that discriminates between wild and farmed sea bream. For this study, we used direct sampling analysis farmed sea bream by the difference in the composition of fatty acids. The technique allows for the identification and quantification of [FA-H] adduct within a few seconds. The fatty acids are revealed by deprotonated molecule [M-H]− formation, and the reading is rapidly obtained with a linear detector. Statistical analysis was performed to verify the applicability of the method; method performance was evaluated by estimating its specificity, repeatability, and robustness.

MATERIALS AND METHODS

Samples. In total, 60 samples of sea bream (n = 24 farmed and n = 36 wild) were bought at fishmongers in Genova, Italy, and supermarkets in Turin, Italy, between July and January. All fish products were stored in a refrigerator with controlled temperature (4°C) until analysis. All samples weighed within a range of 200 to 1,500 g. Weight and geographical origin were recorded for each sample.

Chemicals and reagents. n-Hexane (89%) was supplied by Sigma (St. Louis, MO). The calibration standard for APCI-TOF-MS was purchased from Agilent Technologies (Santa Clara, CA).

Fatty acids extraction. A volume of 10 mL of n-hexane was added to 5 g of homogenized muscle tissue. The samples were then vortexed for 5 min and centrifuged at 2,000 × g for 1 min. The resulting supernatant from each extraction was used for APCI-TOF-MS analysis.

Equipment. Fatty acids analysis was performed using a Axion2 TOF-MS, coupled with DSA in negative mode. The instrument allows to process six samples during the same analysis. Under room temperature, in the DSA system, charged molecules are generated using high purity nitrogen (99.9%) as the nebulizing gas with APCI. Errors in mass accuracy, peak identification, and data acquisition were calculated using the Axion software (PerkinElmer).

Analytical procedure. DSA was performed by using 10 mL of each extract. The instrumental parameters were as follows: corona current, –6 μA; DSA source temperature, 300°C; flight tube, +10 kV; end plate, 400 V; capillary exit, –95 V; drying gas flow rate, 1 L/min; skimmer, –25 V; and detector, 3,500 V. High-resolution mass spectra were acquired in a scan range of 20 to 2,000 (m/z) at an acquisition rate of 1 spectrum per s. The mass calibration was performed by infusing a standard solution (Agilent APCI) at a rate of 10 μL/min.

Method performance. Based on a data set obtained from a total of 24 samples for each type of fish (n = 48), a specific correlation was created that could discriminate between wild and farmed fish products according to the fatty acids ratio. Method performances were evaluated by determining the specificity, robustness, repeatability, and reproducibility of the method expressed as relative standard deviation (RSD%). Strict repeatability was evaluated on seven samples; three replicates for each sample were performed. Reproducibility was evaluated on seven samples; two aliquots of each sample were collected and analyzed by two operators for the extraction procedure and mass spectrometric analysis; and three replicates for each aliquot were performed. The robustness of the method was evaluated on 12 wild samples by modifying the following parameters: muscle weight (±10%), extraction volume (±10%), and fish storage time before extraction (from 3 to 5 days).

Statistical analysis. Data analysis was performed using STATA 15.1. statistical software (StataCorp LLC, College Station, TX). A nonparametric linear (local) regression model was applied (5) because assumption of normality of distribution was not fulfilled. A nonparametric receiver operating characteristic (ROC)

<table>
<thead>
<tr>
<th>Geographical origin</th>
<th>Farmed sea bream</th>
<th>Wild sea bream</th>
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</thead>
<tbody>
<tr>
<td>Geographical origin</td>
<td>Croatia</td>
<td>Greece</td>
</tr>
<tr>
<td>Wt (g)</td>
<td>500</td>
<td>360–700</td>
</tr>
<tr>
<td>Sample no.</td>
<td>6</td>
<td>6</td>
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curve (15) was estimated to define a DHA/AA value that could effectively discriminate between farmed and wild sea bream. To construct the ROC curve, wild and farmed sea bream were considered as negative and positive samples, respectively.

RESULTS AND DISCUSSION

Table 1 reports the geographical origin and weight of the fish products. The fatty acids adducts [M-H]$^-$ in the mass spectrum were 279.2324, 301.2167, 303.2324, and 327.2324 for [LA-H]$^-$, [EPA-H]$^-$, [AA-H]$^-$, and [DHA-H]$^-$, respectively. All four analytes were correctly identified in the extracted samples (Table 2), with an error in mass identification of less than 5 ppm and a good probability (score) to correctly identify the molecules. To compensate for species variability and discriminate between wild and farmed sea bream, we evaluated and compared the ratio between the four fatty acids. The ratio between the most abundant peak ($m/z$) observed in the mass spectrum [DHA-H]$^-$ and the lowest peak [AA-H]$^-$ proved to be the best discriminator tool. In detail, for the 24 wild sea bream samples the ratio between the two adducts was within a range of 0.9 ± 0.04 and 2.7 ± 0.22, whereas the ratio for the 24 farmed sea bream samples was within a range of 2.9 ± 0.12 and 12 ± 0.43 (Fig. 1).

The nonparametric regression model showed DHA/AA values higher than 3.25 on average for farmed sea bream compared with wild sea bream. This difference was statistically significant ($P < 0.001$). The goodness of adaptation of the model was $R^2 = 0.4545$. For each observed DHA/AA value, the cut-off was determined by construction of the ROC curve using the percentages of sensitivity, specificity, and correct sample classification. On the basis of the last criterion, the most discriminating DHA/AA value was 2.944, a value that correctly identified 97.92% of the samples. Sensitivity, expressed as the ability of the technique to correctly identify the farmed fish, was 95.83%; specificity, expressed as the ability to correctly identify the wild fish, was 100%. For the ROC curve, the estimated area under the curve was 99.65%.

Method performance was evaluated on seven samples by determining repeatability and reproducibility (Table 3). The mean repeatability was 7.2 (RSD%) for the wild fish and 10.8% for the farmed fish. The mean reproducibility was 8.81% for the wild fish and 13.03% for the farmed fish. Size, weight, period, and origin of fish capture did not influence the distinction between the two. Robustness was measured on 12 samples by modifying weight, solvent volume, and storage period to obtain a ratio in the range of its category that would indicate the method as robust.

Under European Union legislation, fish products must be labeled to provide the consumer with information on the geographical origin of the product and its production method. The growth in aquaculture production to meet consumer demand for fish has resulted in a greater number of farmed fish species entering the marketplace. This, in turn, has led to the need for accurate analytical methods to assess the authenticity and traceability of food products. Indeed, the detection of false and misleading labeling has become one of the major challenges for stakeholders in the seafood industry. Robust and verifiable methods that can distinguish farmed from wild product need to be developed to maintain consumer confidence and enforce regulations.

The majority of studies analyzing fatty acids in fish have focused on the nutritional property of fish (7) or on methods to discriminate fresh from frozen sea products.

**TABLE 2. List of fatty acids, their molecular formula, theoretical and experimental $m/z$ adduct, error in mass identification, and score.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular formula</th>
<th>Theoretical $[M-H]^-$</th>
<th>Experimental $[M-H]^-$</th>
<th>Error (ppm)$^a$</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid</td>
<td>C₁₈H₃₂O₂</td>
<td>279.2324</td>
<td>279.2324</td>
<td>1.29</td>
<td>0.95</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>C₂₀H₃₀O₂</td>
<td>301.2167</td>
<td>301.2184</td>
<td>−3.51</td>
<td>0.4</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>C₂₀H₃₂O₂</td>
<td>303.2324</td>
<td>303.2331</td>
<td>−0.46</td>
<td>0.71</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>C₂₂H₃₂O₂</td>
<td>327.2324</td>
<td>327.2324</td>
<td>1.68</td>
<td>0.74</td>
</tr>
</tbody>
</table>

$^a$ Error in mass identification in all analyses was less than 5 ppm.
(12). Previous studies of methods to discriminate between farmed and wild sea bass were performed using total lipid content, fatty acids proportions, and trace mineral composition (1). Typical analytical techniques to identify commercial fraud in fish involve physiochemical, DNA-based, chromatographic, and spectroscopic methods. Although the chemometric approach is useful and exhaustive, the disadvantage is that it needs a huge number of samples and data to process, such as the study of the authentication of sea bass by near-infrared spectroscopy (14).

As reported in a previous study, DSA-APCI-TOF-MS is a good technique for the selective and rapid determination of target compounds in food because of its excellent accuracy and precision in determining m/z values (4, 16). Furthermore, farmed and wild sea bream were differentiated by means of only one fatty acids ratio, thus drastically reducing the analysis process yet maintaining good precision. Also, the ratio approach parameter was robust because it was not influenced by sample weight, extraction volume solvent, fish freshness, or origin of capture.

The use of a simple, rapid, and cost-effective tool can aid in fighting commercial fraud in the seafood sector, increase the number of controlled samples, and strengthen controls along the entire commercial chain.

ACKNOWLEDGMENT

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REFERENCES


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<tr>
<td>--------------------------------------------</td>
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<tr>
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a RSD%, relative standard deviation.