Chapter 2

Food Authenticity: Provenancing. A Case Study of Fish

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Additional information is available at the end of the chapter

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Abstract

Authentication of food products is of ongoing interest to consumers in developed countries. Recently, a general interest in the sustainability of food productions, from both societal and environmental perspectives, developed and added a new dimension. Fish and fish products are common targets for food adulteration. The most important issue is fish management, e.g., the environmental impact of overfishing. Analytical means would be helpful for verification. The aim of the present study was to evaluate various marker groups for the distinction of European plaice from the North Sea from European plaice from other geographical origins: volatile organic compounds (VOCs), fatty acids (FA), and isotope ratios. VOCs were analyzed using proton transfer reaction mass spectrometry (PTR-MS); the FA composition was analyzed using gas chromatography with a flame ionization detector, and carbon, hydrogen, nitrogen; and sulfur isotope ratios were analyzed using isotope ratio mass spectrometry. In a principal component analysis, FA profiling appeared the best option to distinguish European plaice from the North Sea from those originating from other seas.

Keywords: European plaice, fatty acid composition, geographical origin, isotope ratio, PCA, *Pleuronectes platessa*, PTR-MS

1. Introduction

In food science and technology, considerable attention to the preservation and quality of foods has been paid throughout history. Food authenticity is a typical food quality issue and is not new either. Some food authenticity infringements were reported over 2000 years ago, and the first related food standards appeared 150 years ago. The classical form of food authenticity violations is the removal or addition of undeclared ingredients or constituents. However,

more recently, a general interest in the sustainability of food productions, from both societal and environmental perspectives, developed and added a new dimension. With advancing technology and increased awareness of social corporate responsibility, new questions have emerged in the food authenticity arena. How are production in terms of raw materials and processing mirrored in unique product characteristics? Do special production (farming management systems, geographical origin, etc.) result in special traits? How to discern and measure these relevant characteristics? And, can these characteristics be used for discriminatory purposes and/or substantiate the produce quality?

Adulteration has been defined as "the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller" by the United States Pharmacopeial Convention (USP). Fish and fish products are common targets for food adulteration [1]. There are different issues in regard to the traceability of fish: (1) the species of the fish, (2) geographical origin, and (3) the production method [2]. During production, fish types can be completely interchanged or mixed with cheaper or less sustainable fish types. According to European law (Regulation 1379/2013), information concerning the commercial designation, the catch area, and the production method shall be available at each stage of marketing of the species concerned [3]. Next to the fact that geographical origin of food products is laid down in legislation, consumers find it important because of economic, safety, and sustainability reasons. Different types of food adulteration can be distinguished: most types influence the product composition by, e.g., substitution or dilution, while other issues involve cultivar and variety of products of plant origin, species of products of animal origin, geographical origin of foods, production system, and processing. Geographical origin has become a more important issue in recent years for economic, safety, and sustainability reasons [4]. Counterfeiting occurs, for example, with the European Union's protected designation of origin, like certain cheeses or olive oils.

Authenticity and traceability research aims to combine different complementary analytical strategies to determine the authenticity of materials and commodities. Various techniques have been studied based on organic constituents, mineral contents or composition, light- or heavy-element isotope ratios, or combinations thereof. If the components have sufficient discriminatory power, the set of their concentrations will form a characteristic pattern or "fingerprint" relating to the geographical origin of the sample. Chemometrics provides the ability to detect these patterns, and is essentially helpful when the number of components necessary to differentiate samples from different geographical origins increases [5]. The three major groups of analytical techniques are: mass spectrometry techniques, spectroscopic techniques, and separation techniques.

Markers that have been successfully used for the verification of the geographical origin of food and feed products are volatile organic compounds (VOCs), the fatty acid (FA) profile, and isotope ratios. These markers can be analyzed using mass spectrometry (like isotope ratio mass spectrometry—IRMS; and proton transfer reaction mass spectrometry—PTR-MS), spectroscopic techniques (like NMR and infrared spectroscopy), and separation techniques (such as liquid chromatography—LC; and gas chromatography—GC) [6].

1.1. VOC profile in the verification of geographical origin

VOCs have shown to satisfactorily predict the country of origin of olive oil, truffles, Grana Padano cheese, as reviewed by Luykx et al. [5] and crude palm oil, butter, cumin cheese, dry cured hams, and coffees, as listed by Pustjens et al. [6]. More recently, VOCs have also shown to successfully verify geographical origin of Chinese cabbage [7], plant-related liquors [8], saffron [9], rosemary [10], teas [11], and capers [12]. For plant products, the difference in VOC profile was ascribed to differences in climate and soil type [13]. This can be translated to fish, since the sea bed and climate will vary for fish from different geographical origins. The VOC profile of fish has been linked to lipid type and source and would therefore be determined by the diet they consumed [14].

1.2. FA profile in the verification of geographical origin

FAs have shown to satisfactorily predict the country of origin of milk and olive oils, as reviewed by Luykx et al. [5] and chocolate and wheat, as listed by Pustjens et al. [6]. More recently, FAs have also shown to successfully verify geographical origin of berries [15], cockles [16], and almonds [17]. FAs in the fish feed are incorporated into its tissues; therefore, the FA profile of the fish tissue reflects the diet over a longer period of time [18]. Especially the content of polyunsaturated fatty acids (PUFAs) seems to be dependent on the geographical origin, i.e., the latitude, and thus broadly related to temperature [19]. They found that at a higher latitude, marine organisms contain more n-3 long-chain PUFAs.

1.3. IR in the verification of geographical origin

All food products have their own isotopic composition, which is determined by the animal's diet (carbon and nitrogen), climate (hydrogen and oxygen), and soil composition (sulfur) [6]. IRMS analysis combined with or without other techniques and/or chemometric models has been successfully applied to determine the geographical origin of dairy and animal products, vegetables, natural flavors, honey, wines, coffee, and fruit, as reviewed by Luykx et al. [5] and cereals, fish and crustaceans, and olive oil and wine, as listed by Pustjens et al. [6]. IRMS analysis of carbon, hydrogen, nitrogen, and oxygen has shown to be able to verify geographical origin of different fish species [20, 21].

1.4. Case study: geographical origin of European plaice

European plaice (*Pleuronectes platessa*) is the principal commercial flatfish in Europe [22]. European plaice live at the bottom of the sea not far from the shore, predominantly in the North Sea, but extend to the Baltic Sea, the Barents Sea, and the sea around Ireland and Iceland [23]. Juvenile European plaice feed on a variety of microbenthic species [24], whereas larger European plaice mainly feed on Polychaeta and mollusks [25]. The habitat of European plaice is dependent on: (a) their life stages: they gradually move from shallow coastal nurseries into deeper water [26, 27] and (b) climate change: European plaice

have shifted toward deeper and more northern areas [28]. This information can be used to find specific groups of markers potentially useful for the verification of their geographical origin.

The aim of this study was to examine how well VOCs, FAs, and isotope ratios are able to distinguish European plaice from the North Sea from European plaice from other provenance.

2. Materials and methods

2.1. Samples

A reference set of 49 European plaice were collected by local fishermen from five different locations in the Northeast Atlantic ocean—FAO area 27: the North Sea, subarea IV a, b, c (n = 31); other research institutes from the Baltic Sea, subarea IIId (n = 3); the Barents Sea, subarea I (n = 5); the sea around Ireland, subarea Va (n = 5); and the sea around Iceland, subarea VIIj2 (n = 5). Locations are labeled as NS, BalS, BarS, SIr, and SIc, respectively, in **Figure 1**. European plaice from the North Sea were both collected in 2013 and 2014; all other samples were collected in 2014. Samples were transported on dry ice to our institute, filleted and stored at −18°C until further analyses. In 2014, a set of market samples were also collected: 11 non-breaded and non-marinated frozen European plaice fillets from Italian (n = 6), German (n = 4), and Dutch (n = 1) supermarkets. According to the package, they originated from the Northeast Atlantic Ocean (FAO zone 27). For some fish, the North Sea, which is part of this FAO catch area, was mentioned specifically.

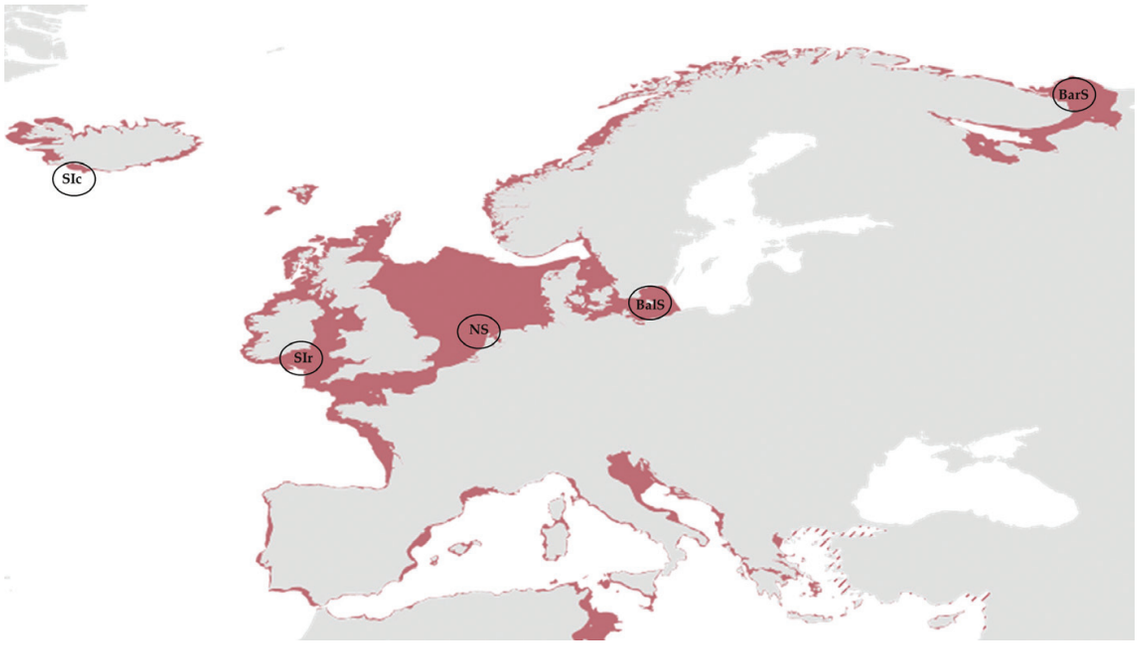

Figure 1. Habitat of European plaice, with the sample locations marked as NS—North Sea, BalS—Baltic Sea, BarS—Barents Sea, SIr—sea around Ireland, and SIc—sea around Iceland. Original figure from FAO Fish Finder (23).

2.2. Chemicals

All chemicals used were of analytical grade purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), or VWR International (Radnor, PA, USA), unless stated otherwise.

2.3. Analytical methods

2.3.1. PTR-MS

The samples were thawed to room temperature in their package and 1 gram of finely sliced material was placed in a 250-mL glass bottle. The headspace above the samples was equilibrated in a waterbath at 25°C for 30 minutes and kept at 25°C for the duration of the measurement. Two independent replicates of each sample were analyzed. The headspace was drawn from the sample flask at a rate of 60 ml/min, which was led through a heated transfer line into a high-sensitivity PTR-MS system (Ionicon GmbH, Innsbruck, Austria). A constant drift voltage of 600 V and a pressure of 2.20 ± 0.02 mbar were maintained in the reaction chamber. Data were collected for the mass range m/z 20–160 using a dwell time of 0.2 s/mass, resulting in a duration of 28 s/cycle. The instrument was operated at a standard E/N (ratio of electric field strength across the drift tube, E, to buffer gas density, N) of 138 Td (1Td = 10^{-17} cm^2 V $molecule^{-1}$). Inlet and drift chamber temperatures were 60°C. Masses are analyzed after H_3O^+ ionization in a quadrupole mass spectrometer and detected as ion count/s, resulting in a fingerprint of the volatiles. Each sample was analyzed for five full mass cycles. The headspace concentrations of the compounds during the second, third, and fourth cycles were calculated as described by Hansel et al. [29]. Blank measurements (empty bottle) were performed for five cycles, with the average of the third, fourth, and fifth mass cycles subtracted from the samples' spectra as background correction. These corrected headspace concentrations (ppbv) were subsequently used for further statistical analysis.

2.3.2. GC flame ionization detection (GC-FID)

The samples were freeze-dried. Fat was extracted from the samples (2 g) using chloroform:methanol (2:1, v/v) as described by Tres et al. [30]. Fatty acids were methyl esterified according to Guardiola et al. [31]. The obtained methyl esters were analyzed in duplicate using an Agilent 7890A GC system, fitted with a flame ionization detector, using a CP7419 50 m × 0.25 mm FAME column (Agilent, Santa Clara, CA, USA). Temperature was kept at 100°C for 1 minute and then increased to 230°C with 5°C/min, after which the temperature was kept at 230°C for 9 min. Helium was used as a carrier gas using a flow of 0.9 ml per min and a split ratio of 1:50. Fatty acids were identified by comparing retention times with those of standard mixtures (Supelco 37 component FAME mix (Supelco, St. Louis, MO, USA)). Results were expressed as normalized peak areas (% of total fatty acids).

2.3.3. IR-MS

The dried defatted samples (1 g) were placed in a paper filter and washed three times with 10 ml of distilled water for removal of sodium sulfate, then three times with 10 ml of acetone to accelerate drying, and then placed into glass containers and dried in vacuum overnight.

For analysis of δ^2H, 250–300 μg of dried sample material was weighed into a silver capsule. δ^2H was determined using a high-temperature pyrolysis system working at 1450°C on the basis of ceramic tubes with glassy carbon reactor filling coupled with a Thermo Finnigan Delta-Plus XL IRMS system (Thermo Fisher Inc.), designed for continuous flow isotope ratio mass spectrometry (CF-IRMS) of hydrogen isotopes in helium carrier gas.

For the other isotopes (for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), 3–4 mg of sample material was weighed into tin capsules. The measurements of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were carried out on an elemental analyzer (Vario EL, Elementar, Hanau, Germany) coupled with an isotope-ratio mass spectrometer, IRMS (IsoPrime, GV Instruments, Manchester, UK). All isotopes were analyzed in duplicate.

The values of the isotope ratios are expressed in δ (‰) and correspond to international standards (V-SMOW for δ^2H, V-PDB for $\delta^{13}C$, air for $\delta^{15}N$, and V-CDT for $\delta^{34}S$) according to the relation:

$$\delta\ (‰) = 1000\ x\ \frac{R_{sample} - R_{standard}}{R_{standard}} \quad (1)$$

where R represents the ratio of the higher mass to the lower mass isotopes measured in the sample (R_{sample}) and in the standard ($R_{standard}$).

2.4. Data analysis

Normality of the distribution of VOC profiles, FA profiles, and isotope ratios within the groups of geographical origin was checked using the Shapiro-Wilk test. Distributions appeared to be nonnormal (Shapiro-Wilk $P < 0.05$). Therefore, Kruskal-Wallis test for group comparisons was performed among the provenance groups using SPSS version 23.0.0.2 (IBM Corp., Armonk, NY, USA). Principal component analysis (PCA) was performed using the Unscrambler (Version X 10.3). Raw data were auto-scaled prior to PCA.

3. Results and discussion

3.1. Distinction of the provenance of European plaice

3.1.1. VOC profiles analyzed by PTR-MS

The reference set of 49 European plaice samples was subjected to PTR-MS analyses. Most abundant masses of protonated VOCs of European plaice collected from the North Sea are m/z 33, 45, and 59. Subsequently, significant differences in mass intensities between European plaice originating from the North Sea versus other provenance were examined (Kruskal-Wallis $P < 0.05$). Results are presented in **Table 1**.

In order to explore the data, PCA was carried out. PCA (**Figure 2A**) revealed grouping of samples, with two-third of the samples from the North Sea showing high positive scores in

the first dimension. Samples from the other seas demonstrated high negative scores in this dimension. Remarkable is the fact that there is more variation in European plaice from the North Sea than in European plaice from the other seas. This might be caused by the fact that the European plaice from the North Sea have been sampled at six different locations within the North Sea, while the other seas have been sampled only on one location. For this reference set, distinction of the North Sea European plaice from other European plaice by their VOCs is a promising approach.

However, the VOC profiles are affected not only by the fish diet [14] but also by the freshness of the fish [32, 33]. This factor may interfere in the analysis. VOCs formed during storage are alcohols, aldehydes, esters, organic acids, and sulfur compounds, which can be either of microbial or nonmicrobial origin [34]. Their non-protonated masses range from m/z 46 for ethanol to m/z 176 for 3-hydroxy-2-butanone. This is a complicating factor and can influence the robustness of the method.

Protonated VOC (m/z)	NS (n = 31)	BalS (n = 3)	BarS (n = 5)	SIr (n = 5)	SIc (n = 5)	P-value NS versus other seas
33	398 ± 623	43 ± 14	60 ± 15	30 ± 17	26 ± 3	0.000
38	3 ± 1	3 ± 0	4 ± 0	4 ± 0	4 ± 0	0.000
39	10 ± 1	10 ± 1	12 ± 1	11 ± 2	10 ± 1	0.063
41	14 ± 7	12 ± 4	11 ± 2	12 ± 3	7 ± 4	0.044
42	39 ± 43	2 ± 1	1 ± 0	0 ± 0	0 ± 0	0.000
43	23 ± 12	13 ± 4	10 ± 1	7 ± 1	16 ± 5	0.001
45	301 ± 240	204 ± 161	69 ± 34	101 ± 29	579 ± 239	0.044
46	7 ± 5	4 ± 4	2 ± 1	2 ± 1	13 ± 6	0.051
47	6 ± 8	14 ± 12	3 ± 1	2 ± 1	55 ± 25	0.793
55	6 ± 3	4 ± 1	6 ± 1	5 ± 1	5 ± 1	0.983
57	10 ± 10	4 ± 1	3 ± 1	25 ± 13	2 ± 1	0.049
59	168 ± 112	79 ± 8	53 ± 17	45 ± 13	66 ± 52	0.000
60	6 ± 4	3 ± 0	2 ± 1	2 ± 0	2 ± 2	0.000
63	1 ± 1	2 ± 2	1 ± 0	1 ± 0	2 ± 0	0.001
69	7 ± 2	3 ± 2	4 ± 1	3 ± 1	4 ± 2	0.000
71	4 ± 2	1 ± 0	0 ± 0	1 ± 0	3 ± 1	0.000
73	7 ± 2	8 ± 1	9 ± 4	6 ± 1	6 ± 3	0.369
87	3 ± 2	1 ± 1	2 ± 0	1 ± 0	1 ± 0	0.000

Table 1. Average protonated VOC composition (ppbv) of European plaice sampled from the North Sea (NS), the Baltic Sea (BalS), the Barents Sea (BarS), the sea around Ireland (SIr), and the sea around Iceland (SIc); mean intensity, standard deviation, and significant differences between European plaice from the North Sea and European plaice caught in other seas (Kruskal-Wallis).

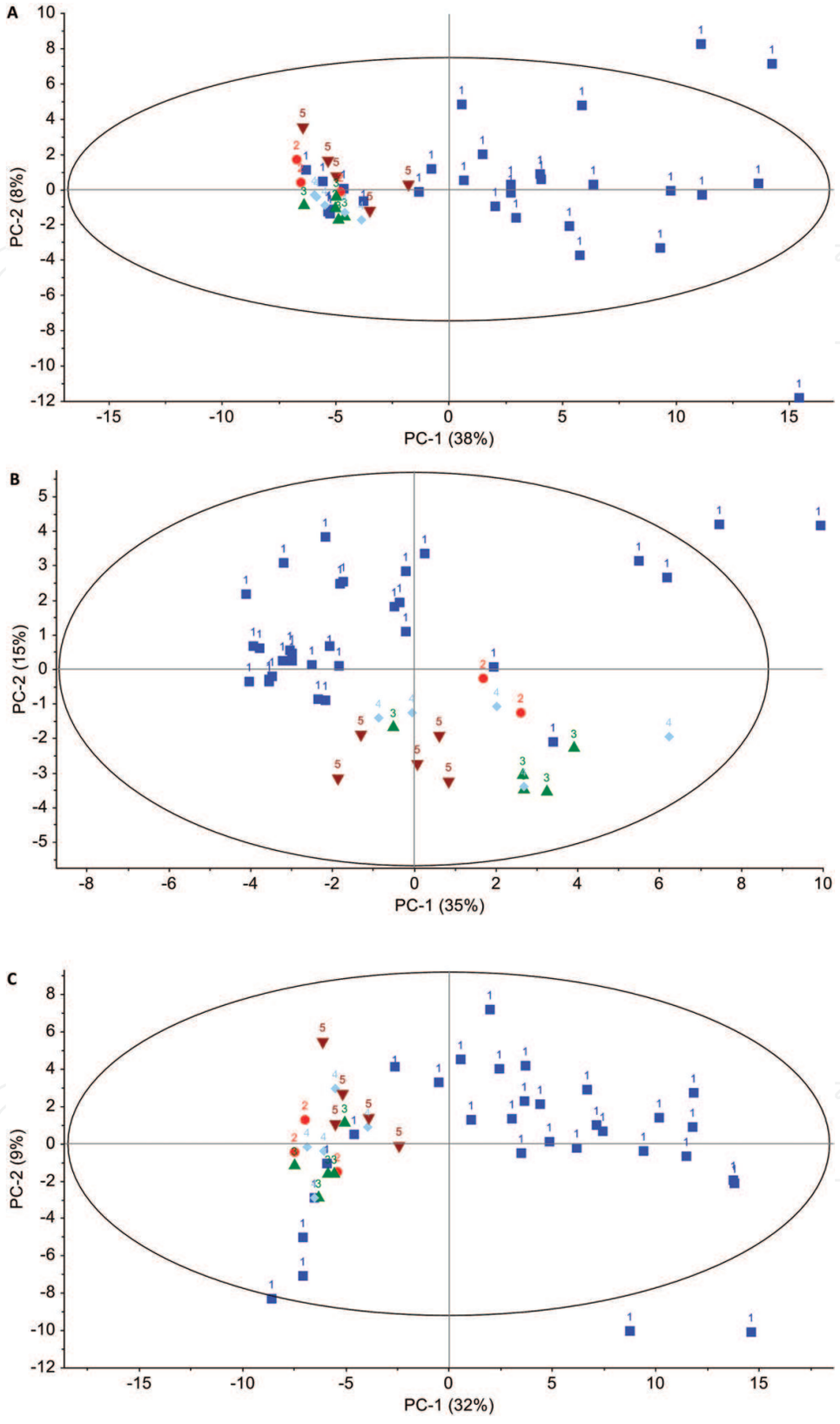

Figure 2. Principal component analysis plot of (A) PTR-MS data, (B) fatty acid profile, and (C) all data combined, on European plaice from the North Sea (1—box), the Baltic Sea (2—dot), the Barents Sea (3—triangle), the sea around Ireland (4—diamond), and the sea around Iceland (5—inverted triangle).

FA	NS (n = 31)	BalS (n = 3)	BarS (n = 5)	SIr (n = 5)	SIc (n = 5)	P-value NS versus other seas
C12:0	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.03	0.01 ± 0.01	0.436
isoC14:0	0.03 ± 0.05	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.02	0.00 ± 0.01	0.146
C14:0	2.02 ± 0.72	2.01 ± 0.28	2.54 ± 0.24	3.10 ± 0.91	2.69 ± 0.83	0.002
C14:1n5	0.15 ± 0.13	0.06 ± 0.01	0.06 ± 0.04	0.04 ± 0.04	0.03 ± 0.02	0.000
isoC15:0	0.06 ± 0.04	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.02	0.01 ± 0.01	0.000
C15:0	0.74 ± 0.16	0.95 ± 0.05	0.70 ± 0.04	0.76 ± 0.11	0.50 ± 0.10	0.332
isoC16:0	0.26 ± 0.19	0.38 ± 0.00	0.35 ± 0.07	0.28 ± 0.09	0.26 ± 0.06	0.004
C15:1n5	0.02 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	0.05 ± 0.03	0.279
C16:0	17.22 ± 2.06	17.57 ± 0.23	15.89 ± 1.83	18.89 ± 2.84	20.02 ± 0.68	0.134
C16:1n9	0.43 ± 0.17	0.65 ± 0.06	0.53 ± 0.06	0.70 ± 0.16	0.42 ± 0.04	0.006
C16:1n7	4.28 ± 1.97	7.64 ± 2.21	16.74 ± 3.10	4.88 ± 1.24	6.45 ± 1.53	0.000
isoC17:0	0.43 ± 0.16	0.36 ± 0.05	0.39 ± 0.08	0.33 ± 0.11	0.28 ± 0.06	0.033
anteisoC17:0	0.40 ± 0.29	0.52 ± 0.05	0.49 ± 0.12	0.34 ± 0.14	0.35 ± 0.13	0.140
C17:1n7	0.31 ± 0.17	0.65 ± 0.03	0.28 ± 0.05	0.34[a] ± 0.15	0.25 ± 0.08	0.371
C18:0	4.53 ± 0.64	4.07 ± 0.04	3.16 ± 0.43	4.15 ± 0.42	3.94 ± 0.73	0.001
Trans C18:1 + C18:T2	0.38 ± 0.33	0.49 ± 0.10	0.40 ± 0.15	0.91 ± 0.40	0.65 ± 0.23	0.001
C18:1n9	6.38 ± 2.41	8.60 ± 1.06	8.81 ± 1.41	8.46 ± 1.62	8.21 ± 1.23	0.000
C18:1n7	3.31 ± 0.93	4.50 ± 0.47	4.17 ± 0.62	3.03 ± 0.66	4.23 ± 0.53	0.018
C18:2n6t	0.09 ± 0.07	0.14 ± 0.01	0.08 ± 0.02	0.09 ± 0.06	0.13 ± 0.06	0.253
c9t12C18:2	0.01 ± 0.03	0.02 ± 0.03	0.01 ± 0.02	0.02 ± 0.03	0.03 ± 0.05	0.189
t9c12C18:2	0.21 ± 0.11	0.14 ± 0.00	0.09 ± 0.02	0.23 ± 0.09	0.10 ± 0.02	0.001
C18:2n6c	0.84 ± 0.95	0.85 ± 0.08	1.21 ± 0.29	1.20 ± 0.57	0.41 ± 0.12	0.095
C18:3n6	0.07 ± 0.05	0.13 ± 0.03	0.16 ± 0.03	0.09 ± 0.02	0.06 ± 0.01	0.019
C18:3n3 + C20:0	0.38 ± 0.18	0.41 ± 0.02	0.57 ± 0.18	0.63 ± 0.19	0.29 ± 0.08	0.057
C20:1n9	1.16 ± 0.52	1.99 ± 0.07	2.45 ± 0.71	3.49 ± 1.36	1.98 ± 0.57	0.000
C21:0	0.07 ± 0.06	0.12 ± 0.02	0.08 ± 0.01	0.04 ± 0.02	0.09 ± 0.03	0.482
C20:2n6	0.35 ± 0.19	0.53 ± 0.01	0.40 ± 0.09	0.69 ± 0.33	0.30 ± 0.11	0.054
C20:3n6	0.14 ± 0.05	0.17 ± 0.04	0.09 ± 0.06	0.13 ± 0.05	0.05 ± 0.01	0.044
C20:3n3 + C20:4n6 + C22:0	6.00 ± 2.20	3.44 ± 0.26	3.28 ± 1.20	4.24 ± 0.75	2.44 ± 0.91	0.000
C22:1n9	0.19 ± 0.26	0.41 ± 0.04	0.41 ± 0.07	0.64 ± 0.17	0.48 ± 0.15	0.000
C20:5n3	17.70 ± 3.27	13.72 ± 1.45	13.19 ± 3.05	15.63 ± 3.33	19.44 ± 2.70	0.075
C22:2n6	0.07 ± 0.03	0.06 ± 0.00	0.10 ± 0.04	0.04 ± 0.05	0.05 ± 0.02	0.991

FA	NS (n = 31)	BalS (n = 3)	BarS (n = 5)	SIr (n = 5)	SIc (n = 5)	P-value NS versus other seas
C24:0	0.15 ± 0.08	0.19 ± 0.05	0.23 ± 0.12	0.15 ± 0.11	0.07 ± 0.05	0.889
C24:1n9	0.23 ± 0.29	0.47 ± 0.07	0.25 ± 0.08	0.52 ± 0.15	0.41 ± 0.12	0.000
C22:6n3	15.78 ± 4.22	13.31 ± 2.72	6.35 ± 1.30	10.60 ± 4.34	11.45 ± 3.24	0.000
SFA*	26.51 ± 1.62	25.77 ± 0.08	23.50 ± 1.83	28.15 ± 2.00	28.05 ± 0.77	0.788
MUFA	16.31 ± 4.99	24.42 ± 3.93	32.07 ± 4.75	20.10 ± 3.57	21.52 ± 2.78	0.000
PUFA	41.52 ± 6.35	32.75 ± 4.23	25.31 ± 4.74	33.14 ± 6.83	34.53 ± 2.89	0.000
OFA	15.66 ± 3.89	17.06 ± 0.38	19.12 ± 1.90	18.60 ± 5.64	15.90 ± 1.24	0.016
Omega 3	39.05 ± 7.85	27.32 ± 4.17	20.01 ± 3.83	26.58 ± 6.68	31.05 ± 2.66	0.000
Omega 6	1.38 ± 1.04	2.05 ± 0.20	2.12 ± 0.36	2.48 ± 0.74	1.14 ± 0.21	0.002
Omega 3/omega 6 ratio	38.09 ± 18.72	13.51 ± 3.33	9.90 ± 3.66	11.75 ± 5.44	28.21 ± 6.26	0.000

*SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, OFA = other fatty acids.

Table 2. Average fatty acid composition of European plaice sampled from the North Sea (NS), the Baltic Sea (BalS), the Barents Sea (BarS), the sea around Ireland (SIr), and the sea around Iceland (SIc); mean concentration, standard deviation, and significant differences between European plaice from the North Sea and European plaice caught in other seas (Kruskal-Wallis).

3.1.2. FA profiles by GC-FID

Subsequently, the reference set of European plaice was subjected to GC-FID analysis. European plaice contains around 0.8% fat [35]. This was extracted and its FA composition was analyzed. The most abundant FAs in European plaice were palmitic acid (C16:0), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) (**Table 2**). Karl et al. [35] found a similar FA profile for European plaice from the North Sea.

When comparing the origins for each FA, 19 out of 35 FAs revealed significant differences between European plaice from the North Sea and European plaice from other seas (Kruskal-Wallis $P < 0.05$; **Table 2**). European plaice from the Barents Sea seems to have a rather distinct FA profile compared to the other seas. When considering groups of FAs instead of individual FAs, contents of MUFA (monounsaturated FA), PUFA (polyunsaturated FA), OFA (other FA), omega-3 FAs, and omega-6 FAs were significantly different in North Sea European plaice from European plaice originating from other seas (Kruskal-Wallis $P < 0.05$). Colombo et al. [19] suggested that fish originating from a higher latitude would contain more PUFA. However, this was not confirmed in our study. European plaice from the sea with the highest latitude, the Barents Sea, showed to have the lowest PUFA content, namely 25%. The sea around Ireland, which has the lowest latitude, showed a rather average PUFA content, namely 33%.

PCA reveals clustering of samples (**Figure 2B**). Generally, the samples from the North Sea and those from the other seas are separated in the second dimension. Therefore, the FA profile seems to be a suitable option to distinguish North Sea European plaice from those

originating from other seas. This was expected since the FA composition of the fish tissue is reflected by their diets and these will vary for the different geographical locations [18].

3.1.3. Isotope ratios by IR-MS

Finally, all reference samples were subjected to IR-MS for analysis of carbon, hydrogen, nitrogen, and sulfur isotope ratios. Results are visualized in a scatter plot (**Figure 3**). Hydrogen isotope ratio (δ^2H) is severely depleted compared to sea water, ranging from −43.7 to −112.5‰ (versus Vienna Standard Mean Ocean Water). This can be explained by the fact that organic tissue is synthesized from metabolized products and their isotopic composition is referenced to local food chains rather than dissolved water isotopes [36]. Sulfur isotope ratio ($\delta^{34}S$) is enriched in European plaice, ranging from +4.6 to +20.1‰. This is typical for marine fish, whereas freshwater fish typically shows depleted $\delta^{34}S$ [37]. Carbon isotope ratio ($\delta^{13}C$) is depleted, ranging from −14.6 to −21.1‰. Nitrogen isotope ratio ($\delta^{15}N$) is enriched, ranging from +8.9 to +13.0‰. Both $\delta^{13}C$ and $\delta^{15}N$ ratios are in accordance with previous research on flatfish from the southern North Sea and claimed to reflect the European plaices' diet [38].

In both scatter plots, grouping of the samples according to their geographical origin is clearly visible. Only samples from the seas around Ireland and Iceland seem to group together. Nitrogen and hydrogen isotope ratios in the North Sea European plaice were significantly different from the ratios in European plaice from other seas (Kruskal-Wallis $P < 0.05$). This was expected based on the results previously described on the carbon, hydrogen, nitrogen, and oxygen isotope ratios of various fish species [20, 21].

3.1.4. All data combined

All VOC, FA, and isotope ratio data acquired in this study were also combined and subjected to PCA plot (**Figure 2C**). Again, groups of samples from the same geographical origin cluster together, with 80% of the European plaice from the North Sea demonstrating high positive

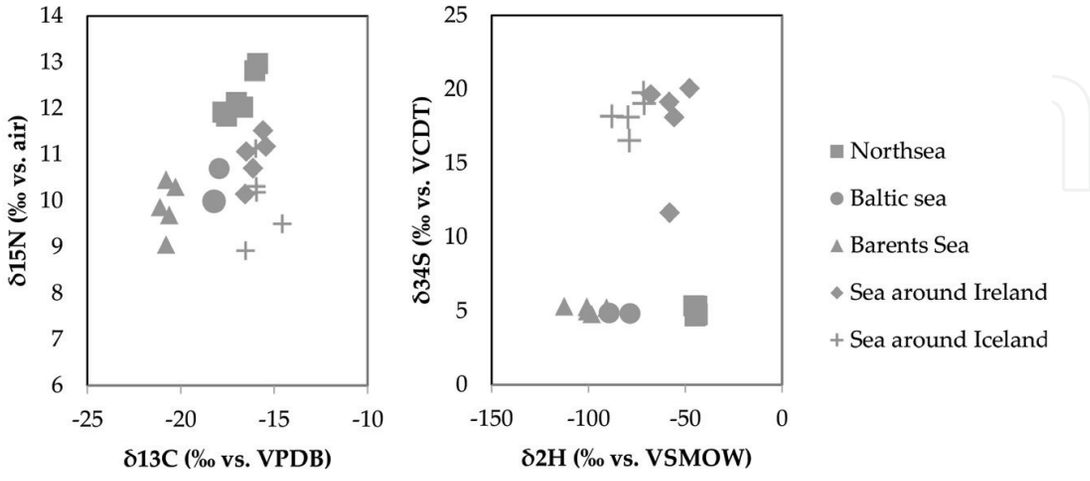

Figure 3. Scatter plot of IRMS data on European plaice: $\delta^{13}C$ versus δ15N (left) and δ^2H versus $\delta^{34}S$ (right) on European plaice from the North Sea (box), the Baltic Sea (dot), the Barents Sea (triangle), the sea around Ireland (diamond), and the sea around Iceland (plus).

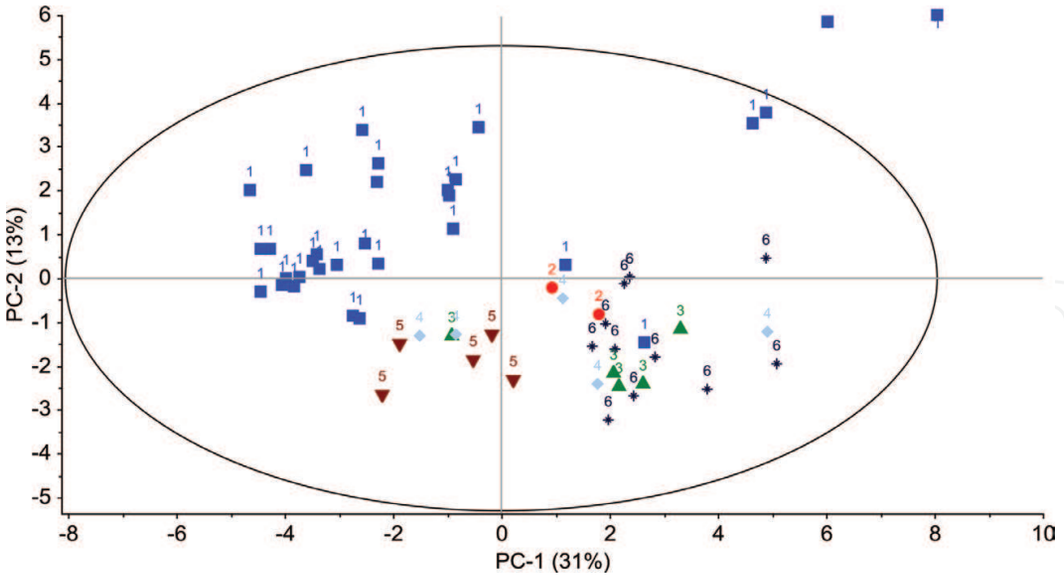

Figure 4. PCA plot of fatty acid profile of European plaice from the North Sea (1—box), the Baltic Sea (2—dot), the Barents Sea (3—triangle), the sea around Ireland (4—diamond), the sea around Iceland (5—inverted triangle), with projection of retail European plaice samples (6—star).

scores in the first dimension and samples from other seas clustering together with the rest from the North Sea and showing negative scores in the first PCA dimension. Distinction of North Sea European plaice from others appears less effective with all data combined in comparison to some of the individual datasets.

3.1.5. Retail European plaice samples

Eventually, a set of 11 non-breaded and non-marinated European plaice fillets from German, Italian, and Dutch supermarkets were analyzed for their FA profiles and compared with the reference set, since FA profiling showed a good distinction and is a robust method. They were projected on the PCA plot of the FA profiles as a sixth class (**Figure 4**). All retail samples cluster in the same region as European plaice samples from the Baltic Sea, the Barents Sea, and the sea around Ireland. According to their label, they originated from the North Sea specifically (n = 4), or more generally from FAO zone 27 (n = 7). In this phase of the research, it cannot be explained why all retail European plaice samples would cluster together away from the samples from the North Sea. This is possibly caused by the fact that samples were collected in a different year and season. However, more research would be needed to evaluate the seasonal effect on the FA composition.

4. Conclusion

This case study showed that the geographical origin of food products is reflected in its product characteristics, which can be measured by several analytical techniques. From the techniques evaluated in this study, it appears that VOC, FA, and isotope ratios all provide

relevant information regarding the provenance of European plaice. VOC analysis by PTR-MS allows rapid analysis, but may be influenced by factors like freshness and packaging. FA and isotope ratio analyses are more robust from that perspective, and both allow automated routine analyses. More samples should be collected to provide insight into differences between seasons and for building chemometric classification models. Nevertheless, the approaches are promising for future provenance verification of European plaice. The results may help to underpin sustainable fishing. Sustainable fishing is an important topic for governmental and possible certification organizations.

In a broader perspective, provenance of food products has become an important topic in food authenticity because of: (a) a quality perspective: the geographical origin of a food product influences its composition and thereby its quality and (b) an environmental perspective: some production locations are more sustainable than others. Consumer awareness on these topics is growing rapidly; they want their food to be of high quality and with a clear geographical identity.

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Conflict of interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

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