

Analytical, Nutritional and Clinical Methods

Prediction of chemical composition and origin identification of European sea bass (*Dicentrarchus labrax* L.) by near infrared reflectance spectroscopy (NIRS)

G. Xiccato ^{a,*}, Angela Trocino ^a, Francesca Tulli ^b, E. Tibaldi ^b

^a Dipartimento di Scienze Zootecniche, Università degli Studi di Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro (Padova), Italy

^b Dipartimento di Scienze della Produzione Animale, Università degli Studi di Udine, via S. Mauro 2, 33010 Pagnacco (Udine), Italy

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Abstract

Near infrared reflectance spectroscopy (NIRS) analysis was used to predict proximate chemical composition and identify the rearing system of 236 European sea bass caught in four Italian fish farms (extensive ponds, semi-intensive ponds, intensive tanks and intensive sea-cages). Three types of sample preparation (intact fillet portions; whole fresh minced fillet; freeze-dried minced fillet) were compared. NIRS provided good reliability in the prediction of chemical composition of sea bass fillets but weaker results in crude protein prediction. NIRS prediction of chemical composition proved to be more accurate with fresh minced fillets than intact fillet portions. The merely slight improvement of NIRS accuracy with freeze-dried samples did not justify the latter treatment, which was necessary, however, to obtain reliable information on the sea bass rearing system.

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1. Introduction

European sea bass (*Dicentrarchus labrax* L.) is one of the main aquaculture fish products in the European Union and concerns an annual production of around 51,000 tons (year 2001) (FEAP, 2002). The wide competition among producing countries in the Mediterranean area (Greece, Italy, Turkey, etc.) and the consequent lowering of market prices are demanding the differentiation and characterization of sea bass quality that has also occurred for other local foods (Piozzi & Manfredi, 2001; Smart, 2001). The different rearing systems and feeding regimes used for sea bass production may affect flesh quality, especially in terms of fat concentration and quality (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002; Lanari et al., 1999; Poli et al., 2001). Moreover, prices often differ widely according to origin and are highest for wild fish.

Near infrared reflectance spectroscopy (NIRS) analysis may provide wide and quick information on fish quality, and has already been successfully used to predict the chemical composition of various species, such as salmon, trout, cod and halibut (Cozzolino, Murray, & Scaife, 2002; Mathias, Williams, & Sobering, 1987; Nortvedt, Torrisen, & Tuene, 1998; Solberg & Fredriksen, 2001), but less information is available on sea bass. Sample preparation technique is one of the main problems with NIRS analysis of fish or any other fresh product due to the high absorbance of the NIRS signal by water, which disturbs and masks information on the other chemical constituents (Shenk, Workman, & Westerhaus, 1992). The development of methods capable of reducing sample preparation and analysis time and avoiding damage to the commercial product is of fundamental interest in NIRS applications. NIRS fish analysis was initially performed on freeze-dried fillets, then on fresh minced samples; later studies were conducted on whole fillets and intact fish, but with less accurate predictions in this case (Downey, 1996b; Isaksson,

* Corresponding author. Tel.: +39-0498272639; fax: +39-0498272669.
E-mail address: gerolamo.xiccato@unipd.it (G. Xiccato).

Tøgersen, Iversen, & Hildrum, 1995; Rasco, Miller, & King, 1991).

As described by Downey (1996a, 1998), suitable statistical treatment of NIR spectra permitted to identify the origin and characterize a wide range of vegetal foods (from fruit juice to coffee). Although NIRS has been successfully used in several studies on meat to identify species, production system (Fumiere, Sinnaeve, & Dardenne, 2000; McEhinney, Downey, & Fearn, 1999) and processing history (Downey & Beauchêne, 1997; Windham, Barton II, & Lyon, 1995), it has been applied only rarely to fish identification and characterization (Sigernes et al., 1997; Solberg, 1996).

This study aimed to use NIRS analysis to predict the proximate chemical composition and identify the rearing system of European sea bass, comparing three types of sample preparation (intact fillet portions; whole fresh minced fillet; freeze-dried minced fillet) in order to minimize sample damage and analysis time while maintaining suitable prediction accuracy.

2. Material and methods

2.1. The set of samples and chemical analyses

Over a 4-month period from October to January, 236 European sea bass were caught in four Italian fish farms with different rearing systems (extensive ponds, semi-intensive ponds, intensive concrete tanks and intensive sea-cages). The main technical and environmental characteristics of the fish farms are summarized in Table 1.

Two different commercial sizes were selected from each fish farm. From the extensive system, a polycul-

tured fish valley in the Venice lagoon (Veneto), 48 sea bass were caught: 21 fish at 334 ± 70 g live weight and 27 fish at 732 ± 233 g live weight. Another 56 sea bass were taken from a semi-intensive farm located in the Marano lagoon (Friuli-Venezia Giulia): 28 sea bass (live weight: 263 ± 56 g) were caught from concrete tanks (first sampling) and 28 (live weight: 1187 ± 184 g) from large ponds (second sampling). In the intensive farm in the Orbetello lagoon (Toscana), sea bass were taken from concrete tanks: 27 fish at 516 ± 81 g live weight and 35 at 790 ± 168 g live weight. At the fish farm in the Gulf of Trieste (Friuli-Venezia Giulia), the sea bass were taken from sea-cages: the first group was constituted by 35 lighter specimens (374 ± 148 g), the second group by 35 heavier examples (1067 ± 148 g). These differences in number and average weight were imposed by the fish available at the farms.

The fish were slaughtered by immersion in ice slurry, immediately transported to the laboratory in thermally insulated boxes, and submitted to dissection within 4–5 h after catch. The right fillet was immediately separated and used for NIRS and chemical analyses.

The fillets were freeze-dried to determine water concentration and then analysed for proximate composition following AOAC (1990) methods. Half of the samples (118), homogeneously selected according to their origin and weight, were analysed to measure gross energy concentration by adiabatic bomb (Martillotti, Antoniovanni, Rizzi, Santi, & Bittante, 1987).

2.2. NIRS analysis

NIRS analysis was performed by a monochromator spectrometer (InfraAlyser 500, Bran + Luebbe GmbH, Norderstedt, Germany) in the 1100–2500 nm range with

Table 1
Technical and environmental characteristics of the fish farms

	Extensive	Semi-intensive	Intensive	Sea-cages
Location	Venice lagoon North Adriatic Sea Veneto region	Marano lagoon North Adriatic Sea Friuli-Venezia Giulia region	Orbetello lagoon Central Tyrrhenian Sea Toscana region	Gulf of Trieste North Adriatic Sea Friuli-Venezia Giulia region
Rearing system	Large natural lagoon (fish valley)	First sampling: concrete tanks Second sampling: ponds	Concrete tanks	Sea-cages (700 m ³)
Stocking density	10–20 kg/ha (other species: 100–150 kg/ha)	First sampling: 10–15 kg/m ³ Second sampling: 0.4 kg/m ³	25–30 kg/m ³	8–12 kg/m ³
Water temperature	Winter: 2–5 °C Summer: 28–31 °C	Winter: 9–15 °C Summer: 25–30 °C	Winter: 6–8 °C Summer: 26–30 °C	11–20 °C
Dissolved O ₂	Winter: 6–10 mg/l Summer: 2–10 mg/l	6–8 mg/l	4–7 mg/l	6–7 mg/l
Feed characteristics	No artificial feeding	Ether extract: 22% Crude protein: 43%	Ether extract: 22% Crude protein: 44%	Ether extract: 11% Crude protein: 45%

a 2 nm step. Each fillet was analysed according to the following sample preparation sequence: (1) three intact circular portions separated from the anterior (portion a), medium (portion b) and posterior (portion c) epiaxial muscles; (2) whole fresh minced fillet; (3) freeze-dried minced fillet.

Spectra were collected in absorbance using Sesame software (version 3.00 Bran + Luebbe). Each sample was scanned twice and average spectra were exported into Unscrambler software (version 7.0, CAMO ASA, Trondheim, Norway) using the JDX format, and then transformed in second derivative, calculated over a 20-point smoothing segment (CAMO ASA, 1998).

Calibration equations were calculated on transformed spectra by partial least square regression (PLSR) to predict water (%), crude protein (%), lipid (%), and gross energy (MJ/kg) concentration. The number of factors used as independent variables in the prediction equations was fixed at a maximum of 20 (i.e. less than 10% of the number of samples used in the calibration) in order to avoid overfitting (Shenk & Westerhaus, 1994). The optimal number of factors was chosen as a function of the first local minimum in the validation residual variance plot. Full cross-validation was used (Martens, 1999). Outliers were identified and excluded on the base of Malanobis value ($H > 3$) (CAMO ASA, 1998).

Prediction equations were evaluated in terms of coefficient of determination in calibration (R^2c) and cross-validation (R^2cv), standard error of calibration (SEC) and of cross-validation (SECV).

Principal component analysis (PCA) was used to calculate models for each cluster of samples on the basis of the rearing system using full cross-validation. Soft independent modelling class analogy (SIMCA) was used to measure the model-to-model distance and classify samples by the rearing system. Cluster membership in SIMCA was tested at $P < 0.05$ level. Both PCA and SIMCA were performed using Unscrambler software.

3. Results

3.1. NIRS prediction of chemical composition

The proximate composition of the sea bass fillets is shown in Table 2. Both water (62.9–78.2%) and lipid (0.9–16.4%) concentrations varied widely, while crude protein varied in a closer range.

Calibration and validation results for the prediction of chemical composition are reported in Table 3. As regards sample preparation, calibration on intact portions always involved a higher number of factors selected (5–14) than calibration on either fresh and freeze-dried minced fillets (1–3 factors). Among the

Table 2
Chemical composition of sea bass (no. = 236)

	Min	Max	Average	SD
<i>Extensive (no. = 48)</i>				
Water (%)	71.4	78.2	76.1	1.5
Crude protein (%)	18.0	20.3	19.1	0.6
Ether extract (%)	0.9	8.1	2.7	1.5
Gross energy (MJ/kg) ^a	4.89	7.00	5.72	0.65
<i>Semi-intensive (no. = 56)</i>				
Water (%)	64.2	77.0	70.7	3.6
Crude protein (%)	17.1	20.3	19.1	0.7
Ether extract (%)	1.4	16.1	8.5	3.7
Gross energy (MJ/kg) ^a	5.45	10.34	7.86	1.52
<i>Intensive (no. = 62)</i>				
Water (%)	62.9	71.9	67.6	2.3
Crude protein (%)	17.2	20.6	19.3	0.7
Ether extract (%)	5.8	16.4	10.8	2.8
Gross energy (MJ/kg) ^a	7.05	10.64	8.53	0.93
<i>Sea-cages (no. = 70)</i>				
Water (%)	66.5	73.4	70.3	1.7
Crude protein (%)	18.1	20.8	19.3	0.6
Ether extract (%)	4.2	13.6	8.3	2.1
Gross energy (MJ/kg) ^a	6.44	9.50	7.92	0.76
<i>All samples (no. = 236)</i>				
Water (%)	62.9	78.2	70.9	3.8
Crude protein (%)	17.1	20.8	19.2	0.7
Ether extract (%)	0.9	16.4	7.9	3.9
Gross energy (MJ/kg) ^a	4.89	10.64	7.75	1.39

^a no. = half samples.

three intact portions, the highest correlation and the lowest prediction errors were obtained with portion b (medium muscles). This result partially depended however on the higher number of selected factors (12–14) compared to portions a and c (5–9). Predictions calculated on the spectra of fresh minced fillets showed high correlation both in calibration and cross-validation and a great improvement of accuracy compared to intact portions. When freeze-dried fillets were analysed, the coefficient of determination did not increase substantially (apart from crude protein prediction) although the average estimate error was, instead, slightly reduced.

NIRS prediction of water concentration provided good coefficients of determination using portions b and c (R^2cv : 0.77 and 0.69). When the whole fillets were minced (fresh or freeze-dried), R^2cv rose substantially (0.95 and 0.97 in fresh and freeze-dried samples, respectively) with a very low prediction error. The same accuracy was observed for lipid prediction, with R^2cv increasing from around 0.70 in portions b and c to 0.97 in minced fillets and SECV decreasing from 2.39% (on average) to 0.66%. Due to the close relationship between lipid and energy concentrations, the same figure was recorded for energy prediction.

NIRS prediction of crude protein in sea bass fillets was less successful. Coefficients of determination for intact portions were lower than 0.30 and therefore not

Table 3

Coefficients of determination in calibration (R^2c) and cross-validation (R^2cv) and standard errors of calibration (SEC) and cross-validation (SECV) obtained by partial least-square regression to predict the chemical composition of sea bass fillets analysed by NIRS after different sample preparations

	Calibration			Full cross-validation	
	Factors	R^2c	SEC	R^2cv	SECV
<i>Water (%)</i>					
Intact portion a	9	0.81	1.60	0.47	2.76
Intact portion b	14	0.97	0.62	0.77	1.79
Intact portion c	6	0.78	1.73	0.69	2.09
Fresh minced fillet	2	0.95	0.85	0.95	0.87
Freeze-dried fillet	3	0.97	0.66	0.97	0.70
<i>Ether extract (%)</i>					
Intact portion a	9	0.81	1.62	0.48	2.81
Intact portion b	13	0.95	0.78	0.69	2.12
Intact portion c	6	0.76	1.87	0.66	2.25
Fresh minced fillet	2	0.97	0.68	0.97	0.70
Freeze-dried fillet	2	0.98	0.60	0.97	0.62
<i>Crude protein (%)</i>					
Fresh minced fillet	7	0.61	0.39	0.30	0.55
Freeze-dried fillet	12	0.81	0.26	0.68	0.35
<i>Gross energy (MJ/kg)</i>					
Intact portion a	5	0.58	0.87	0.28	1.20
Intact portion b	12	0.98	0.16	0.61	0.86
Intact portion c	6	0.82	0.56	0.67	0.78
Fresh minced fillet	2	0.96	0.26	0.96	0.28
Freeze-dried fillet	1	0.96	0.28	0.96	0.29

reported in tables. Only NIRS analysis of freeze-dried fillets gave fairly good results ($R^2cv = 0.68$; $SECV = 0.35\%$).

3.2. SIMCA analysis of spectral data

SIMCA analysis was performed on spectra taken from fresh and freeze-dried minced fillets.

As a first step, principal component analysis within cluster on the fresh minced spectra selected 3, 3, 4 and 4 principal components for the extensive, semi-intensive, intensive and sea-cage systems, respectively. In SIMCA, the model-to-model distance was higher or equal to three (the minimum distance required for a suitable cluster separation) only when comparing the extensive system with the others (from 2.99 with respect to the sea-cage system to 6.14 with respect to the intensive system in tanks) (Table 4). When classifying the fillets by the four-cluster SIMCA, 65% of fresh minced samples belonging to the extensive system were correctly assigned to their own cluster (Table 5). Only 37% of samples from the intensive system was correctly assigned, while 43% was assigned to two or more clusters and 18% was not assigned at all. The percentage of wrong classification was very low (1–4%).

When SIMCA was performed on spectra obtained from freeze-dried fillets, the optimal number of selected

Table 4

Model-to-model distances in SIMCA for clusters of fresh minced fillets (above the diagonal) and freeze-dried minced fillets (below the diagonal)

	Extensive	Semi-intensive	Intensive	Sea-cages
Extensive	1	3.7	6.1	3.0
Semi-intensive	20.9	1	1.8	1.9
Intensive	30.4	10.2	1	1.9
Sea-cages	29.1	15.5	8.4	1

Table 5

Performance of four-cluster SIMCA analysis of fresh and freeze-dried minced fillets

	Correctly classified (%)	Classified in two or more clusters (%)	Wrongly classified (%)	Not classified (%)
<i>Fresh minced fillet</i>				
Extensive	65	16	4	15
Semi-intensive	58	25	2	15
Intensive	37	43	2	18
Sea-cages	45	38	1	16
<i>Freeze dried-minced fillet</i>				
Extensive	83	0	0	17
Semi-intensive	80	0	2	18
Intensive	74	0	2	24
Sea-cages	83	0	1	16

factors was 3, 2, 3 and 4 for the extensive, semi-intensive, intensive and sea-cage systems, respectively. The model-to-model distance increased greatly in comparison with results on the fresh minced fillets (Table 4). The highest model-to-model distance (>20) was measured once again between the extensive system cluster and the other clusters. The lowest distance was observed between intensive and sea-cages systems. When classifying sea bass by means of SIMCA, more than 80% of freeze-dried samples from the extensive, semi-intensive and sea-cage systems and 74% of fillets from the intensive system were correctly and exclusively assigned to the corresponding cluster (Table 5). No spectra was assigned to two or more clusters, while a certain number (17–24%) was not assigned to any class. Only 1–2% of samples was classified incorrectly.

4. Discussion

Several applications of NIRS analysis in fish flesh have been performed in recent years with special attention on minimal sample preparation. Downey (1996b) attempted to analyse whole skinned farmed salmon by a fibre optic probe in the 400–1100 nm range and obtained satisfactory calibrations for water ($R^2c = 0.69$; $SEP = 1.45\%$) and lipid ($R^2c = 0.70$; $SEP = 2.04\%$). Even better results were achieved for lipid prediction ($R^2c = 0.79$;

SEP = 1.1%) by Solberg (2000) on whole salmon analysed by diode-array. Moreover, NIRS accuracy improved greatly when analysing the whole salmon fillet ($R^2c = 0.88$; SEP = 0.82%). Lee, Cavinato, Mayes, and Rasco (1992) achieved the highest accuracy for NIRS prediction of lipid in the range from 700 to 1050 nm, when whole rainbow trout was analysed by a fibre optic probe in the position midway between the posterior insertion of the dorsal fin and the anterior insertion of the adipose fin ($R^2c = 0.81$; SEP = 2.27%).

Calibrations for the prediction of water and lipid concentrations obtained in our study analysing intact portions of sea bass were quite satisfactory. Better results could have been achieved by correlating the spectra of each portion with its exact composition, as performed by Lee et al. (1992), rather than with the composition of the whole fillet, as performed in our study. In fact, the heterogeneity of fat distribution along the fillet and between the dorsal and ventral sites has been proved (Downey, 1996b; Fjellanger, Obach, & Roselund, 2001). Literature suggests, moreover, that the use of short-wavelength NIR region (700–1100 nm) would permit the improvement of calibration on not homogenous and highly scattering samples, such as whole fish, fillets or intact portions, by providing deeper penetration of light in the sample and a higher instrumental signal to noise ratio (Downey, 1996b; Isaksson et al., 1995; Lee et al., 1992).

Compared to our results on intact fillet portions, Rasco et al. (1991) provided more reliable calibrations for water prediction ($R^2c = 0.82$ and SEP = 1.1%) when analysing cross sections of frozen and thawed trout fillets in the range from 900 to 1800 nm. Crude protein prediction was fairly good ($R^2c = 0.74$; SEP = 5.4%), while lipid prediction was less successful than ours ($R^2c < 0.50$ and SEP = 3.1%).

In our study, prediction accuracy for water, lipid and energy increased remarkably with fresh minced fillet compared to intact portions but remained only fair for protein concentration. Analysing ground salmon fillets, Isaksson et al. (1995) obtained higher prediction accuracy for protein (SECV = 0.37%), while fat and moisture prediction was similar to our results. According to Rasco et al. (1991), NIRS prediction of crude protein in fish tissue may be impaired by the presence of several non-protein nitrogen compounds, such as trimethylamine oxide, urea, taurine, small peptides and amino acids, nucleotides and other purines. Cozzolino et al. (2002), however, successfully predicted total volatile nitrogen ($R^2v = 0.83$; SECV = 0.35%) in fresh minced raw fish (various species).

Using the transmission mode in the short wavelength NIR region (700–1100 nm), Sollid and Solberg (1992) predicted fat concentration in fresh minced salmon fillets with a R^2v of 0.99 and SEP of 0.5%. Similarly, Solberg (1996) obtained good prediction for water

($R^2c > 0.96$; SEP < 0.21%), protein ($R^2c = 0.92$; SEP = 0.22%) and lipid ($R^2c = 0.98$; SEP = 0.33%) in whole minced cod and capelin and in minced salmon fillets. Good NIRS prediction accuracy for chemical composition was confirmed by Solberg and Fredriksen (2001) in fresh minced capelin at different physiological states (pre-spawning, spawning and post-spawning) and by Nortvedt et al. (1998) in minced Atlantic halibut fillets (SECV = 0.27%, 0.52% and 0.42% for lipid, protein and water, respectively).

Our results showed that NIRS prediction of sea bass chemical composition was not substantially improved by the use of freeze-dried fillets rather than fresh minced fillets. The correlation remained substantially similar and the merely slight reduction of the prediction error did not justify the additional cost and time required by freeze drying. The prediction of crude protein improved in terms of error but continued to show a moderate coefficient of determination ($R^2cv = 0.68$). Valdes, Atkinson, Hilton, and Leeson (1989), on the contrary, obtained better calibrations than ours for protein ($R^2v = 0.76$; SEP = 0.18%) but worse results for energy (SEP = 1.7 MJ/kg) using freeze-dried rainbow trout. High prediction accuracy for lipid and protein were also obtained by Mathias et al. (1987) when analysing freeze-dried samples taken from freshwater fish.

The lower prediction performance that we obtained compared to other studies most likely depended on the wide variations of chemical characteristics in our set of sea bass, which included great differences in fish rearing systems and weight. A more specific and local calibration would have probably provided more accurate but less robust prediction equations.

In our study, freeze drying was necessary to obtain satisfactory classification of sea bass by origin. Spectral data of sea bass taken from the extensive system (based on natural feeding regime) were different from those caught in other rearing systems (based on artificial feeding regimes), and probably related to different body conditions (lipid concentration, in particular). Despite a similar fat concentration, the clusters of the three other rearing systems also differed considerably however. Therefore, spectral variations among clusters did not depend exclusively on the feeding regime but more probably on a combination of other factors (water quality, growth pattern, muscular activity, competition, etc.).

Unlike for meat and other foods, very few applications of multivariate analysis are available for fish authentication and classification by NIRS (Downey, 1996b, 1998). Mathias et al. (1987) reported no difference in spectra of freeze-dried muscles of *Oncorhynchus mykiss* and *Salvelinus alpinus*, while Solberg (1996) succeeded in classifying by PCA the minced fresh fillets of three species (*Gadus morhua*, *Mallotus villosus* and *Salmo salar*).

5. Conclusions

NIRS showed good reliability in the prediction of water, lipid and energy but weaker results for crude protein in sea bass fillets characterized by wide variation in size and chemical composition. Comparing different sample preparations, NIRS prediction of chemical composition proved satisfactory using intact fillet portions and improved further when fresh minced fillets were used. The only slight improvement in chemical composition prediction accuracy obtained using freeze-dried samples did not justify the additional costs and time required for sample preparation.

NIRS analysis succeeded in giving reliable information on the sea bass rearing system; for this purpose, the freeze-drying of the samples was required for correct classification by origin.

Our results indicate NIRS as a promising method for sea bass characterization, traceability and authentication.

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