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# Evaluation of Fourier transform infrared spectroscopy and multispectral imaging as means of estimating the microbiological spoilage of farmed sea bream

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ABSTRACT

The objective of the present study was the evaluation of Fourier transform infrared (FTIR) spectroscopy and multispectral imaging (MSI), in tandem with multivariate data analysis, as means of estimating the microbiological quality of sea bream. Farmed whole ungutted fish were stored aerobically at 0, 4 and 8 °C. At regular time intervals, fish samples (i.e. cut portions) were analysed microbiologically, while FTIR and MSI measurements also were acquired at both the skin and flesh sides of the samples. Partial least squares regression (PLSR) models were calibrated to provide quantitative estimations of the microbiological status of fish based on spectral data, in a temperature-independent manner. The PLSR model based on the FTIR data of fish skin exhibited good performance when externally validated, with the coefficient of determination ($R^2$) and the root mean square error (RMSE) being 0.727 and 0.717, respectively. Hence, FTIR spectroscopy appears to be promising for the rapid and non-invasive monitoring of the microbiological spoilage of whole sea bream. Contrarily, the MSI models' performance was unsatisfactory, delimitating their potential exploitation in whole fish quality assessment. Model optimization results concerning fish flesh indicated that MSI may be propitious in skinned fish products, with its definite competence warranting further investigation.

## 1. Introduction

Fish constitute food of high nutritional value with a long established contribution of their consumption to a balanced healthy diet (Ruxton, 2011; Weichselbaum et al., 2013). Nonetheless, the high perishability of fish has also been well identified, being manifested in the form of various *post mortem* deterioration processes and resulting, essentially, in a short shelf-life of fresh fish even under conditions of storage in ice (Cakli et al., 2006, 2007; Carrascosa et al., 2016; Koutsoumanis, 2001; Koutsoumanis and Nychas, 2000; Parlapani et al., 2013, 2014). Fish freshness has been regarded as one of the most important aspects of raw fish quality definition (Cakli et al., 2006; Hassoun and Karoui, 2017) and is affected and delineated by several factors. Examples of such factors are rigor mortis, autolysis processes and microbiological spoilage, with the latter playing a principal role in the highly perishable character of fish (Cheng et al., 2015b; Hassoun and Karoui, 2017).

Given the importance of microbiological spoilage in fish freshness deterioration, investigation of analytical approaches for its rapid and non-invasive assessment presents significant research interest. Several non-invasive/non-destructive techniques have been evaluated as propitious in fish quality assessment, including spectroscopic techniques and imaging technology approaches (Cheng and Sun, 2015, 2017; Cheng et al., 2015b; Hassoun and Karoui, 2017; Liu et al., 2013; Pérez-Esteve et al., 2014). Fourier transform infrared (FTIR) spectroscopy is a biochemical fingerprinting technique which, in conjunction with multivariate data analysis, has shown significant potential in the detection and quantification of spoilage bacteria in muscle foods, including fresh meat and poultry and processed meat products (Argyri et al., 2013; Cheng and Sun, 2015; Moreirinha et al., 2015; Sahar and Dufour, 2014). Multispectral imaging (MSI), an optical sensing technique which combines spectral and spatial information (Carstensen et al., 2006), has also been proposed as a promising rapid and non-invasive technology for the assessment of the microbiological spoilage of muscle foods (Dissing et al., 2013; Panagou et al., 2014; Tsakanikas et al., 2016). Nevertheless, very limited research data are available in the scientific literature with regard to the use of FTIR spectroscopy in the estimation

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of fish microbiological spoilage (Saraiva et al., 2017), whereas there is no study, to our knowledge, reporting on the corresponding potential of the MSI technology.

Gilthead sea bream (*Sparus aurata*) is one of the main fish species farmed in the Mediterranean countries, and Greece is the largest producer within the European Union (EU) and among the leading producers in the world (FAO, 2018). Moreover, gilthead sea bream is the fourth most important species in terms of economic value of the EU aquaculture production (Eurostat, 2016). Although new processed products (e.g., filleted or frozen products) are being developed, gilthead sea bream marketed in the EU are typically sold fresh, whole and ungutted or minimally processed (i.e. eviscerated). Given the economic significance of sea bream aquaculture in the EU and the scarcity of relevant research data, as mentioned above, the objective of the present study was the evaluation of FTIR spectroscopy and MSI as means of estimating the microbiological spoilage of this fish species.

## 2. Materials and methods

### 2.1. Fish samples, storage conditions and sampling

Farmed whole ungutted gilthead sea bream was studied. Fish from sea-cage farms were provided by Selonda Aquaculture S.A. (Athens, Greece) and were transported to the laboratory in ice within 24 h from harvest. Fish (*ca.* 500 g) were transferred to styrofoam trays (one whole fish per tray), and the latter were wrapped with cling film and stored at different isothermal conditions (0, 4 and 8 °C) in high-precision ( ± 0.5 °C) programmable incubators (MIR-153, Sanyo Electric Co., Osaka, Japan). The incubation temperatures were recorded at 15-min intervals throughout storage using electronic temperature-monitoring devices (COX TRACER®, Cox Technologies Inc., Belmont, NC, USA).

Fish were analysed on the day of arrival to the laboratory (time-zero) and at regular time intervals during storage, depending on the applied storage temperature. Specifically, fish were analysed at 24-h intervals during storage at 0 °C for a total of 276 h, and at 12-h intervals during storage at 4 and 8 °C for a total of 204 and 156 h, respectively. The applied analytical procedures included (i) microbiological analysis and pH measurements; (ii) FTIR spectroscopy measurements; and (iii) MSI acquisition. Two independent experimental replicates (i.e. different times and fishing batches) were conducted and within each replicate, duplicate samples (i.e. different fish) were analysed at each storage temperature and sampling time ($n = 4$).

### 2.2. Microbiological analysis and pH measurements

A 10-g portion from the front dorsal half of each fish was transferred aseptically to a 400-ml sterile stomacher bag (Seward Medical, London, UK) containing 90 ml of sterilized peptone saline diluent (0.1% w/v peptone, 0.85% w/v sodium chloride), and homogenized in a Stomacher apparatus (Lab Blender 400, Seward Medical) for 60 s at room temperature. Appropriate serial decimal dilutions in peptone saline diluent were surface plated on tryptic glucose yeast agar (Plate Count Agar; Biolife, Milan, Italy) and total mesophilic microbial populations (total viable counts, TVC) were determined after incubation of plates at 30 °C for 72 h, according to ISO 4833-2 (2013). The obtained microbiological data were converted to log (colony forming units) per gram of fish (log CFU/g).

Upon completion of the microbiological analyses, the pH values of the fish samples also were measured using a digital pH meter (RL150, Russell pH, Cork, Ireland) with a glass electrode (Metrohm AG, Herisau, Switzerland).

### 2.3. FTIR spectroscopy

FTIR spectral data were collected using a ZnSe 45° HATR (Horizontal Attenuated Total Reflectance) crystal (PIKE Technologies, Madison, WI, USA), and an FTIR-6200 JASCO spectrometer (Jasco Corp., Tokyo, Japan) equipped with a standard sample chamber, a triglycine sulphate (TGS) detector and a Ge/KBr beamsplitter. A small portion from each fish sample was transferred to the crystal plate, covered with a small piece of aluminum foil, and then pressed with a gripper to ensure the best possible contact with the crystal. Two different spectral measurements were acquired for each fish sample, by placing the skin as well as the flesh side of the cut portion in contact with the crystal. The crystal used had a refractive index of 2.4 and a depth of penetration of 2.0 μm at 1000 $cm^{-1}$. Using the Spectra Manager™ Code of Federal Regulations (CFR) software version 2 (Jasco Corp.), spectra were collected over the wavenumber range of 4000 to 400 $cm^{-1}$, by accumulating 100 scans with a resolution of 4 $cm^{-1}$ and a total integration time of 2 min. Prior to the measurements of the tested samples, reference spectra were acquired using the cleaned blank crystal (i.e. no added fish sample). After each measurement, the crystal's surface was cleaned, first with detergent and distilled water and then with analytical grade acetone, and dried using lint-free tissue. The FTIR spectra that were ultimately used in further analyses were in the approximate wavenumber ranges of 3100 to 2700 and 1800 to 900 $cm^{-1}$.

### 2.4. Image acquisition and pre-processing

Images from fish samples (i.e. portions from the dorsal half of fish) were acquired using the VideometerLab system, originally developed by the Technical University of Denmark (Carstensen and Hansen, 2003). This instrument acquires multispectral images in 18 different, non-uniformly distributed wavelengths ranging from UV (405 nm) to short wave NIR (970 nm) (Panagou et al., 2014; Ropodi et al., 2015). Prior to image acquisition, the system was subjected to a light set up procedure known as "autolight" and calibrated radiometrically and geometrically as previously described (Ropodi et al., 2015). Each fish sample was transferred to a Petri dish and the latter was placed inside an Ulbricht sphere, in which the camera is top-mounted, and the corresponding multispectral image of the sample's surface was taken. Similarly to the procedure followed for the FTIR spectral measurements (section 2.3), two different images, corresponding to the skin and flesh side of each fish sample, were acquired.

In order for redundant information (related to the background and not to the sample) to be removed, image segmentation was performed using the VideometerLab system software (version 2.12.39) and previously described procedures (Panagou et al., 2014; Ropodi et al., 2015). In brief, the contrast between the sample material and the other irrelevant objects was maximized, in order to enable a threshold operation. Then, canonical discriminant analysis (Duda et al., 2000) was employed as a supervised transformation building method so as to divide the image into regions of interest. In this way, a segmented image was produced, where the region of interest was used for the extraction of spectral data. After the segmentation process of each image, the mean reflectance spectra (i.e. mean intensity of pixels within the informative area) along with the corresponding standard deviation values were calculated.

### 2.5. Data analysis

The results corresponding to a total of 158 different fish were used for the purpose of data analysis. The data derived from the two abovementioned analytical approaches were evaluated along with the corresponding microbiological data using partial least squares regression (PLSR). In this context, the collected FTIR and MSI data were individually used as input (independent) variables while the TVC as an output (dependent) variable.

Specifically, with regard to the FTIR data related to the skin side of the tested fish samples, the second derivative of the acquired spectra in the ranges of 3100-2700 and 1800-900 $cm^{-1}$ were calculated, using the second derivative Savitzky-Golay numerical algorithm with a second-order polynomial and a 9-point window. A PLSR model was then developed with the number of significant latent variables being determined based on the results of leave-one-out cross-validation. A further feature selection step was performed using Martens Uncertainty Test. Regarding the FTIR data corresponding to the flesh side of the fish samples, Savitzky-Golay smoothing was applied on the acquired spectra in the aforementioned wavenumber ranges, and a PLSR model was then developed as described previously. With reference to MSI data, PLSR was performed using the 18 raw (i.e. no pre-processing step) mean and standard deviation values of the reflectance spectra, both in the case of skin and flesh sides of the fish samples.

In order for the developed models to be able to provide predictions in a temperature-independent manner, model calibration was based on the data (spectral/microbiological) derived from the fish storage experiments at both 0 and 8 °C ($n = 96$). Model validation (prediction) was performed using external data sets obtained during fish storage at the intermediate temperature of 4 °C ($n = 62$). In the case of MSI data, in addition to the aforementioned data partition scheme, an alternative calibration/validation data set structure was also applied in order to evaluate the enhancement potential of the prediction models' efficiency (please refer to section 3.4). In the latter scheme, the validation data set was derived by random sampling of the full data set (~20% of the total sample size using a uniform random generator), consisting of 30 and 29 samples for skin and flesh, respectively. Multivariate data analysis was performed using The Unscrambler® ver. 9.7 software (CAMO Software AS, Oslo, Norway).

## 3. Results and discussion

### 3.1. Microbiological spoilage of sea bream

The mean (± standard deviation) initial level of TVC in sea bream fish (i.e. time-zero of storage experiments) was 4.34 (± 0.50) log CFU/g. In general, gilthead sea bream has been shown to carry an initial bacterial load of 3–4 log CFU/g, depending on the fish culture and handling conditions as well as on the laboratory culture media used for microbial enumeration (Koutsoumanis and Nychas, 2000; Koutsoumanis et al., 1999; López-Caballero et al., 2002; Parlapani et al., 2013, 2014). With temperature being regarded as the most influential parameter affecting microbial responses, the rate of microbial growth was, as expected, strongly dependent on the storage temperature. The TVC of sea bream exceeded the level of 7 log CFU/g, which has been considered as the maximum acceptability limit for fish (ICMSF, 1986), at 180, 84 and 60 h of storage at 0, 4 and 8 °C, respectively (Fig. 1). Similar findings have been reported by other researchers who studied the evolution of spoilage bacteria during aerobic storage of sea bream under various isothermal conditions (Koutsoumanis and Nychas, 2000; Koutsoumanis et al., 1999; Parlapani et al., 2014).

The high vulnerability of fresh fish to microbiological spoilage is largely dictated by specific intrinsic factors including its poikilotherm nature, the high *post mortem* pH of fish flesh and the presence of large amounts of non-protein nitrogen (Gram and Huss, 1996). Bacteria are mainly found on the skin as well as in the gills and the gastrointestinal tract of live fish (Gram and Huss, 1996), and the bacterial concentration in the flesh of intact (i.e. whole and ungutted) freshly harvested fish is expected to be very low (Carrascosa et al., 2016). The microbiota of temperate water fish is primarily constituted of psychrotrophic Gram-negative, rod-shaped bacteria belonging to the genera *Pseudomonas*, *Shewanella*, *Acinetobacter* and *Flavobacterium* (Gram and Huss, 1996). Also, it has been well established, using both traditional and molecular techniques, that bacterial species of the genus *Pseudomonas* constitute the specific spoilage organisms of Mediterranean gilthead sea bream stored under aerobic conditions and at temperatures ranging from 0 to 15 °C (Carrascosa et al., 2016; Koutsoumanis and Nychas, 2000; Koutsoumanis et al., 1999; Parlapani et al., 2013, 2014; Tryfinopoulou et al., 2002). Therefore, the TVC enumerated in this study are expected to be principally comprised of *Pseudomonas* spp., originating mainly from the fish skin.

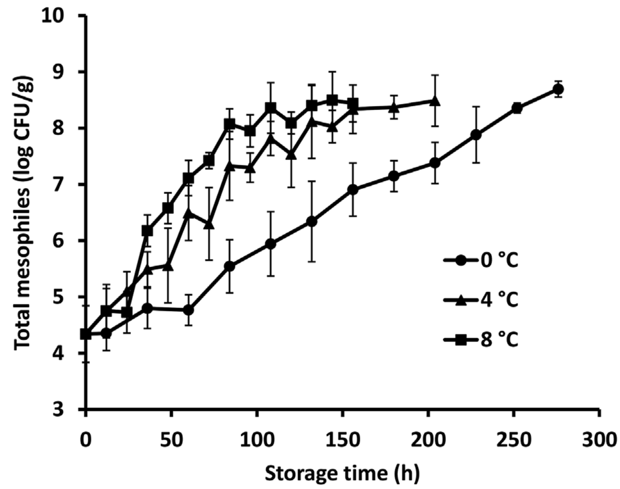


**Fig. 1.** Total mesophilic microbial populations (mean ± standard deviation, $n = 4$) of gilthead sea bream during aerobic storage at different isothermal conditions.

### 3.2. pH data

The mean (± standard deviation) initial pH of sea bream fish was 6.26 (± 0.08), and the pH value measured at the final time point of analyses was 6.45 (± 0.11), 6.30 (± 0.10) and 6.23 (± 0.02) for storage at 0, 4 and 8 °C, respectively. Overall, no considerable differences in the pH values of fish were observed among the different storage temperatures (Fig. 2). The initial pH values of fish measured in this study are in agreement with the pH range reported in the literature for farmed sea bream, namely 6.1 to 6.8 depending on several factors such as season and breeding conditions (Grigorakis et al., 2003; Kyrana et al., 1997; Yazgan et al., 2017). Regarding the pH changes during storage, these appeared to be insignificant under the conditions of this study, where a noteworthy pH increase was observed only after 252 h (*ca.* 11 days) of storage at 0 °C (Fig. 2). The increase of pH during storage of fish has been associated with the production of alkaline bacterial metabolites (Kyrana et al., 1997).

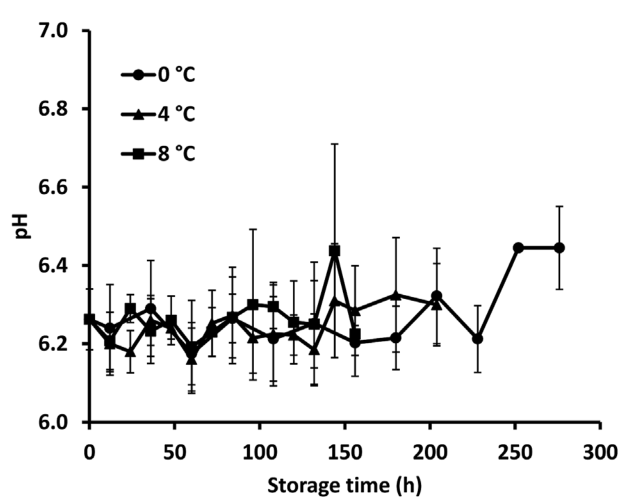


**Fig. 2.** Values of pH (mean ± standard deviation, $n = 4$) of gilthead sea bream during aerobic storage at different isothermal conditions.

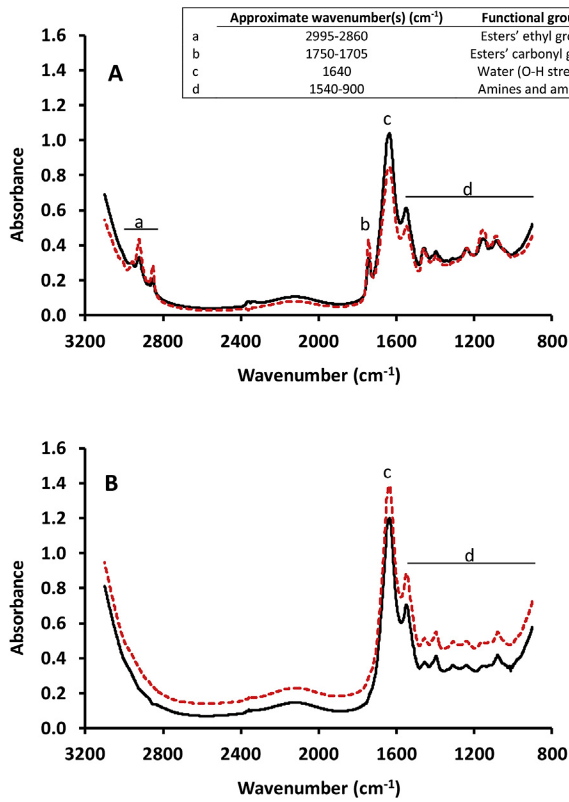


**Fig. 3.** Representative Fourier transform infrared spectra, in the wavenumber range of 3100 to 900 cm$^{-1}$, corresponding to gilthead sea bream skin (A) and flesh (B) of samples with low (fresh fish, black solid line) and high (fish stored at 8 °C for 6 days, red dashed line) microbial populations. Functional groups potentially associated with absorption at the observed peaks (Socrates, 2001) are presented in the embedded table. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 3.3. Estimation of fish spoilage using FTIR spectral data

Examples of typical FTIR spectra in the wavenumber range of 3100 to 900 cm$^{-1}$, corresponding to the skin and flesh of fresh (i.e. time-zero of storage experiments) and spoiled fish samples, are illustrated in Fig. 3; the spoiled fish samples in this figure correspond to fish stored at 8 °C for 144 h, with mean ( ± standard deviation) total microbial population of 8.50 ( ± 0.51) log CFU/g. As mentioned in section 2.3, the FTIR spectra used in further analyses were in the approximate wavenumber ranges of 3100 to 2700 and 1800 to 900 cm$^{-1}$, spectral regions that have been shown to provide useful fingerprints with regard to fish freshness (Armenta et al., 2006; Karoui et al., 2007; Saraiva et al., 2017).

As illustrated in Fig. 3, the acquired spectra of the skin and the flesh of the fish samples appeared to be fairly different, both initially (i.e. in fresh samples) and during the course of fish storage. Starting from the peak observed at around 1640 cm$^{-1}$, which is most likely due to water (O-H stretch) (Saraiva et al., 2017; Socrates, 2001), an absorption decrease and increase was observed during storage in fish skin and flesh, respectively (see peaks labelled as “c” in Fig. 3). Such a differential response may be indicative of a reversed moisture shift in these two sites of the tested cut fish portions. It can also be noted that absorbance peaks at approximately 2920, 2850 and 1740 cm$^{-1}$ were only observed and appeared to increase during storage in skin samples (see peaks labelled as “a” and “b” in Fig. 3A) but not in flesh samples (Fig. 3B). Among the various metabolites that have been studied as fish spoilage index candidates, aldehydes, ketones and most importantly ethyl esters (e.g., ethyl isovalerate, ethyl tiglate) have been most strongly associated with the metabolic activity of *Pseudomonas* spp. (Parlapani et al., 2014, 2017). Indeed, peaks in the spectral regions of 1750-1705 and 2995-2860 cm$^{-1}$ have been associated with the vibration of esters' functional groups i.e. the carbonyl and ethyl group, respectively (Socrates, 2001). Given that *Pseudomonas* spp. are anticipated to be the specific spoilage organisms of sea bream under the conditions of this study, the aforementioned increased absorption peaks observed for fish skin (Fig. 3A) may actually be indicative of the production of certain microbial metabolites such as ethyl esters. With reference to the FTIR spectra acquired for the flesh of the fish samples, absorbance increases with storage time were observed in the region of approximately 1540-900 cm$^{-1}$ (see peaks in the region labelled as “d” in Fig. 3B). Since peaks in this region are commonly due to amines and amides, such absorbance changes may reflect an increase in the concentrations of peptides and free amino acids as a result of autolytic and/or microbiological proteolysis (Saraiva et al., 2017; Sone et al., 2011; Tito et al., 2012).

The separation potential of the transformed FTIR spectra (see section 2.5) in terms of storage time can be visualized in Fig. S1, where the spectra are presented graphically along with the first three PLS components. When the collected FTIR spectral data were evaluated along with the corresponding microbiological data using PLSR, the developed model for fish skin exhibited a good performance, as demonstrated by the performance metrics briefed in Table 1. Specifically, the value of the coefficient of determination ($R^2$) between observed and estimated microbial populations was 0.952, 0.874 and 0.727 for model calibration, cross-validation and prediction, respectively, whereas the corresponding values of the root mean square error (RMSE) were 0.318, 0.519 and 0.717. With particular reference to model prediction (i.e. external validation), approximately 89% (55/62) of the predicted TVC values were within the ± 1 log unit area of the actually observed ones (Fig. 4A). The robustness of the developed PLSR model is also demonstrated by the fact that it is capable of providing reliable estimations of microbial populations based on FTIR spectra of fish skin, regardless of storage temperature. Given that the applied storage conditions can affect not only the dominant spoilage microorganisms but also their metabolic activity (Nychas et al., 2007), it becomes evident that the development of temperature-independent chemometrics approaches is of great importance. On the other hand, the PLSR model based on the FTIR spectra of fish flesh exhibited a poor performance (Table 1; Fig. 4B). Actually, the $R^2$ value was 0.756 and 0.173 for model calibration and prediction, respectively, whereas the corresponding RMSE values were 0.715 and 1.249 (Table 1). Furthermore, only 50% (31/62) of the predicted by the model TVC values were within the ± 1 log unit area of the actually observed ones (Fig. 4B). The most likely explanation for the above model performance results is the higher foreseen microbial contribution to the biochemical changes occurring on fish skin compared to fish flesh. Indeed, skin is one of the fish sites

**Table 1**
Performance metrics of the PLSR models correlating total mesophilic microbial populations in fish samples on the basis of FTIR spectral data, using the data set derived from storage at 0 and 8 °C ($n$ = 96) for model calibration and the data set from storage at 4 °C ($n$ = 62) for model prediction.

| Fish sample [a] | Data set | Slope | Offset | $R^2$ | RMSE |
|---|---|---|---|---|---|
| Skin | Calibration | 0.952 | 0.327 | 0.952 | 0.318 |
| | Cross-validation [b] | 0.883 | 0.808 | 0.874 | 0.519 |
| | Prediction | 0.936 | 0.558 | 0.727 | 0.717 |
| Flesh | Calibration | 0.756 | 1.650 | 0.756 | 0.715 |
| | Cross-validation [b] | 0.657 | 2.313 | 0.457 | 1.076 |
| | Prediction | 0.539 | 2.894 | 0.173 | 1.249 |

$R^2$: coefficient of determination; RMSE: root mean square error.

[a] FTIR spectra were acquired for both the skin and flesh surfaces of the fish samples.

[b] Leave-one-out cross-validation.

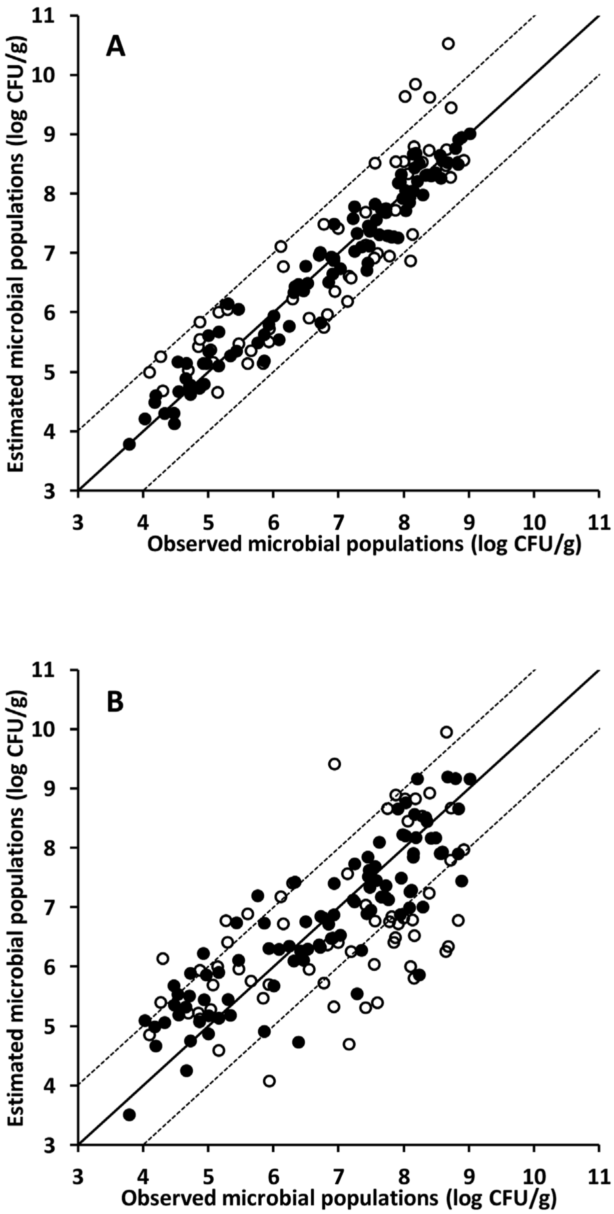


**Fig. 4.** Comparison between the observed and estimated by the PLSR models total mesophilic microbial populations based on the FTIR spectral data of gilthead sea bream skin (A) and flesh (B) for the calibration (solid symbols) and the prediction (open symbols) data sets (solid line: the ideal $y = x$ line; dashed lines: the ± 1 log unit area).

(along with gills and viscera) with the highest bacterial contamination (Gram and Huss, 1996). Although microbial growth can also take place in fish flesh (Carrascosa et al., 2016), its exact role in the biochemical reactions taking place may not be easily discernible from that of autolytic processes which are expected to be extensive in fish muscle (Ghaly et al., 2010; Sone et al., 2011).

Very limited research data are available with regard to the use of FTIR spectroscopy in fish freshness characterization, with most of the published studies being related to authentication and adulteration issues (Hassoun and Karoui, 2017). The available data, specifically referring to the application of FTIR spectroscopy in the evaluation of fish microbiological quality, are even scarcer. To our knowledge, the only study reporting on the potential of FTIR spectroscopy as a means of quantitative monitoring of the microbiological spoilage of fish is that conducted by Saraiva et al. (2017). The latter researchers developed a PLSR model allowing for the estimation of the microbiological quality

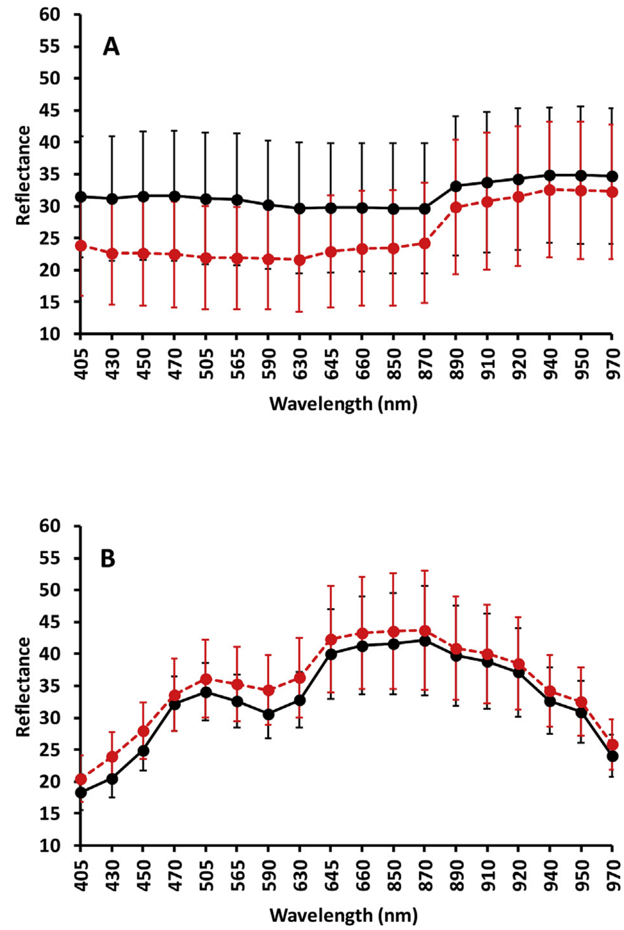


**Fig. 5.** Representative reflectance spectra (mean ± standard deviation) of gilthead sea bream skin (A) and flesh (B) of samples with low (fresh fish, black solid line) and high (fish stored at 8 °C for 6 days, red dashed line) microbial populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of salmon fillets, based on microbiological and infrared spectral data collected during storage at different temperatures and atmospheres (Saraiva et al., 2017). The results of the present study contribute to the enrichment of the currently available information, by demonstrating that FTIR spectroscopy is a promising method also for the estimation of the microbiological spoilage of a distinct fish species/product, namely whole gilthead sea bream, based on the infrared spectra of fish skin.

### *3.4. Estimation of fish spoilage using MSI data*

Typical reflectance spectra (mean intensity of pixels within the informative area along with the corresponding standard deviation values) corresponding to fresh and spoiled fish samples, as previously described (section 3.3) are illustrated in Fig. 5. It is evident that fish skin exhibits a much larger variation in spectral reflectance values for all the wavelengths, compared to the flesh side of the tested samples (Fig. 5). Such finding was to some extent expected, given the more homogeneous and relatively stable colour of sea bream flesh (white) compared to skin (silvery grey), as this is evident even macroscopically. However, in order for this natural one-to-one feature (i.e. wavelength) variation to be taken into account in model development, both the mean and standard deviation values of pixels' intensity were analysed in the context of this study.

Similarly to the FTIR spectra, the MSI data (see section 2.5) and the first three PLS components are illustrated for first-line visualization purposes (i.e. exploratory data analysis) in Fig. S1. Building a microbial

**Table 2**
Performance metrics of the PLSR models correlating total mesophilic microbial populations in fish samples on the basis of multispectral imaging data, using the data set derived from storage at 0 and 8 °C ($n$ = 96) for model calibration and the data set from storage at 4 °C ($n$ = 62) for model prediction.

| Fish sample [a] | Data set | Slope | Offset | $R^2$ | RMSE |
|---|---|---|---|---|---|
| Skin | Calibration | 0.589 | 2.777 | 0.589 | 0.927 |
| | Cross-validation [b] | 0.526 | 3.202 | 0.460 | 1.074 |
| | Prediction | 0.442 | 3.653 | 0.315 | 1.136 |
| Flesh | Calibration | 0.526 | 3.199 | 0.526 | 0.995 |
| | Cross-validation [b] | 0.478 | 3.548 | 0.393 | 1.138 |
| | Prediction | 0.609 | 2.378 | 0.591 | 0.879 |

$R^2$: coefficient of determination; RMSE: root mean square error.
[a] Multispectral images were acquired for both the skin and flesh surfaces of the fish samples.
[b] Leave-one-out cross-validation.

population prediction model using the data partition applied in the case of the FTIR spectral data (i.e. model calibration on the fish storage data at 0 and 8 °C and model prediction on the data at 4 °C), appeared to be challenging in the case of the MSI data. The poor performance of the developed models (Table 2) can be explained by the nature of the acquired spectra, in which most frequency bands lie in the visual region. Excluding a storage temperature from model calibration did not seem to be the best model building strategy in this case; most likely, the calibration data set lacks of critical information in order to allow the PLSR model to "learn" and consequently predict microbial populations at an "unknown" temperature. Having this in mind, a more informative (in terms of storage temperatures) data partition scheme was then applied in calibration and prediction data subsets. In detail, an approximate percentage of 20% of the total sample size (considering all three temperatures) was excluded via a uniform random data generator, and used for model prediction purposes. In this manner it was ensured that the prediction data set consisted of data from all three temperatures and from all the range of measured microbial populations. The exact sizes of the calibration/prediction data sets used in this alternative data partition, as well as the performance metrics of linear regression for observed vs. estimated TVC values (Fig. 6) are presented in Table 3. As demonstrated by comparing the performance metrics (i.e. slope, offset, $R^2$ and RMSE) presented in Tables 2 and 3, the models' performance was enhanced, particularly in the case of fish flesh. Specifically, with regard to model prediction (Fig. 6), the value of $R^2$ between observed and estimated TVC was 0.683 and 0.682 for fish skin and flesh, respectively, whereas the corresponding RMSE values were 0.711 and 0.813 (Table 3). Hence, the resulting MSI models appeared to perform better under this data partition scheme, albeit still not as well as the temperature-independent model based on FTIR spectra of fish skin. The better performance of the model based on MSI data of fish flesh samples compared to that based on skin samples may be attributed to the larger variation on spectral reflectance values of the latter compared to the former (Fig. 5), as discussed previously. Neither the application of various pre-processing treatments on the MSI data nor the utilization of the non-linear regression approach of support vector machines using radial basis function kernel resulted in considerable improvement of model performance (data not shown). A further optimization attempt would be the employment and evaluation of alternative algorithms, such as neural networks (where the system is able to engineer hyperfeatures not obvious for other systems already used), on a considerably bigger, however, data set, an approach which should certainly be addressed in future research.

With reference to the use of imaging technology approaches in fish quality assessment, hyperspectral imaging has been mainly studied and reported in the scientific literature as promising in this area (Cheng and Sun, 2015, 2017; Cheng et al., 2015a; He and Sun, 2015; Kamruzzaman et al., 2015; Liu et al., 2013; Wu and Sun, 2013). On the other hand,

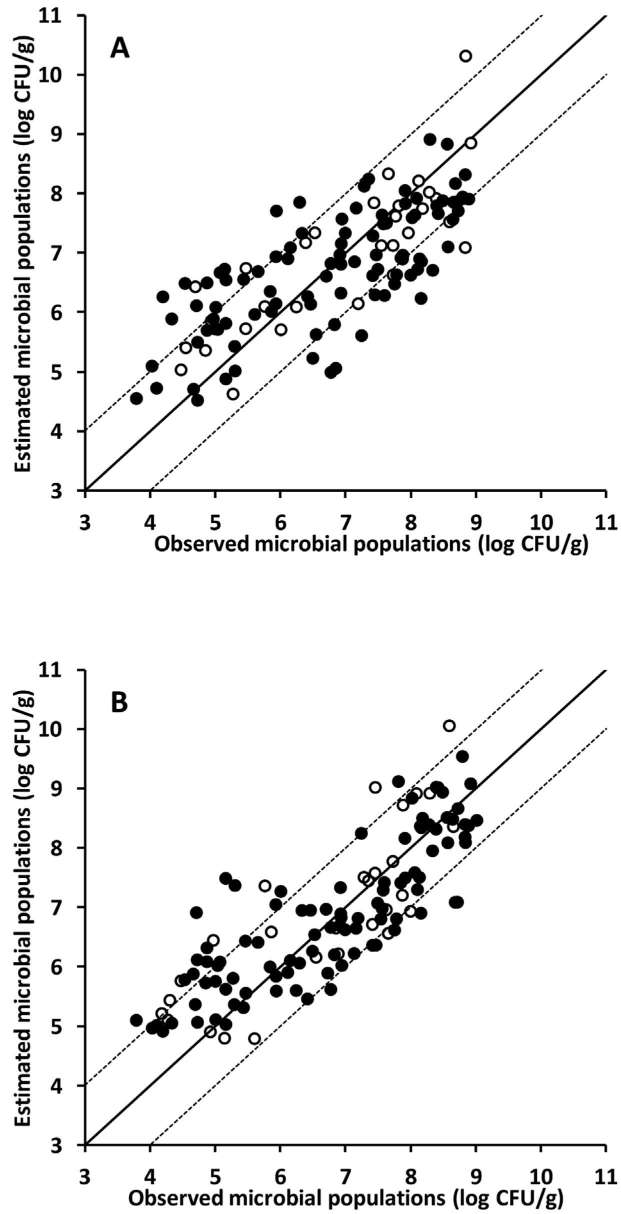


**Fig. 6.** Comparison between the observed and estimated by the PLSR models total mesophilic microbial populations based on the multispectral imaging data of gilthead sea bream skin (A) and flesh (B) for the calibration (solid symbols) and the prediction (open symbols) data sets (solid line: the ideal $y = x$ line; dashed lines: the ± 1 log unit area).

**Table 3**
Performance metrics of the PLSR models correlating total mesophilic microbial populations in fish samples on the basis of multispectral imaging data, with the prediction data set being derived by random sampling of the full data set, using a uniform random generator.

| Fish sample [a] | Data set | Slope | Offset | $R^2$ | RMSE |
|---|---|---|---|---|---|
| Skin | Calibration ($n$ = 128) | 0.584 | 2.810 | 0.575 | 0.713 |
| | Cross-validation [b] | 0.658 | 2.311 | 0.658 | 0.687 |
| | Prediction ($n$ = 30) | 0.706 | 2.096 | 0.683 | 0.711 |
| Flesh | Calibration ($n$ = 129) | 0.682 | 2.171 | 0.682 | 0.666 |
| | Cross-validation [b] | 0.759 | 1.644 | 0.758 | 0.612 |
| | Prediction ($n$ = 29) | 0.813 | 1.523 | 0.682 | 0.813 |

$R^2$: coefficient of determination; RMSE: root mean square error.
[a] Multispectral images were acquired for both the skin and flesh surfaces of the fish samples.
[b] Leave-one-out cross-validation.

and despite its well identified potential in the estimation of the microbiological spoilage of other muscle foods (Dissing et al., 2013; Estelles-Lopez et al., 2017; Panagou et al., 2014; Tsakanikas et al., 2016), MSI has not been evaluated as a means of assessing the microbiological spoilage of fish. The only studies reporting on the utilization of MSI in fish quality assessment refer to the use of this technology for the appraisal of fish chemical spoilage and of the exudative characteristics of frozen-thawed fish (Cheng et al., 2016a, 2016b). To our knowledge, the present study is the first one assessing the potential of MSI as a means of evaluating the microbiological spoilage of whole fish. The unsatisfactory performance of the developed MSI models (i.e. low $R^2$ values) appears to delimitate the potential exploitation of multispectral vision technology in whole fish quality assessment, and may be considered as a limitation of the present study's findings. Even so, it should be noted that the results presented here do not completely disqualify imaging methods for microbiological spoilage assessment but they demonstrate that the studied wavelengths (mainly in the visible spectrum) do not seem to be adequate for such an application. Moreover, the obtained model optimization results indicate that MSI should not be overruled as a potentially propitious sensor-based method for the efficient estimation of fish microbiological quality, especially in the case where fish flesh is considered for sampling purposes (i.e. in skinned fish products such as fish fillets). However, the definite competence of MSI certainly requires further investigation, in terms of both data acquisition and statistical treatment.

## 4. Conclusions

According to the results of this study, FTIR spectroscopy appears to be promising for the rapid and non-invasive assessment of the microbiological quality of whole gilthead sea bream. On the other hand, the MSI models' performance was unsatisfactory, delimitating their potential exploitation in whole fish quality assessment. Model optimization results concerning fish flesh indicated that MSI may be propitious in skinned fish products, with its definite competence, however, warranting further investigation. As also suggested by the collected data, the two tested technologies may find applications in distinct fish products, i.e. FTIR spectroscopy in whole fish whereas MSI in fish fillets, or may be regarded as complementary approaches in the quantitative monitoring of fish microbiological spoilage when mixed samples are considered. In any case, the findings of this study should be substantiated by additional research data, while data fusion strategies potentially enhancing the prediction performance of the developed models should also be explored.

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## Appendix A. Supplementary data

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