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Use of impedance spectroscopy for monitoring freshness of sea bream (*Spaurs aurata*)

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ABSTRACT

In the present study, the use of a rapid portable system based on impedance spectroscopy to assess fish freshness has been tested. The evolution of different physical and chemical parameters (moisture, fat, pH and TVBN) and impedance measurements (modulus and phase at different frequencies) of six different batches of sea bream (*Sparus aurata*) were analysed. Impedance spectroscopy was able to classify raw matter in 6 groups according to differences in composition, and also to classify samples put in storage for a period of time between 0 and 15 days in different groups according to the freshness degree. Finally, a model obtained from Partial Least Squares predicted with high accuracy TVBN values, one of the most

usual parameter to assess shelf life period of fish samples, confirming the potential application of the impedance spectroscopy for monitoring sea bream freshness.

Key words: Sea bream; Fish; Freshness; Impedance spectroscopy; Non-destructive sensor; PLS regression

1. Introduction

Freshness, defined as the degree of deterioration suffered by fish due to the action of endogenous autolytic enzymes and/or the development of a diverse flora of contamination is considered to be one of the most important parameters of fish in most markets (Olafsdóttir et al., 1997). Fish and fishery products are usually highly perishable food products whose freshness and quality rapidly decline post-mortem. As a result of this spoilage, volatile compounds (trimethylamine, ammonia, mercaptans, ...) appear, providing fish odour, softening the muscle, oxidizing lipids and hemoproteins and changing the colour of the meat (Pascual-Anderson, 2000).

Due to the importance of monitoring fish freshness during the commercialization and consumption of fish, a myriad of different enzymatic, physical and physiological analytical methods have been developed to measure modifications in the characteristics of the fish. However, these methods are tedious, destructive, requiring highly skilled operators and time-consuming (Barat et al., 2008). These drawbacks imply that these methods are unsuitable for *in situ* fish quality control.

Accordingly, development of rapid, low-cost and non-destructive methods for monitoring freshness has been one of the most interesting research fields of food industry in last years. Among various alternatives, impedance spectroscopy appears to be an emerging technique with great potential in food quality control. In this sense, impedance has been successfully applied to control fruit ripening (Bauchot, Harker, & Arnold, 2000), determine water and salt levels in different food product (Masot et al., 2010), control ham meat quality (Oliver et al., 2011) or detect additives in water or beverages (Zia et al, 2013).

In fishery products, assessing fish body composition has been the traditional usage of impedance. Duncan et al. (2007) described a method based in bioimpedance to assess body composition in cobia. Composition of other species, such as tuna (Willis & Hobday, 2008), grass carp and tilapia (Zhang, Shen, & Luo, 2010) and catfish (Bosworth & Wolters, 2001) have also been studied. Regarding food process monitoring, Rizo et al., (2012), employed an impedance spectroscopy system for on-line monitoring of the salting-smoking process of salmon. In the same year, Fernandez-Segovia et al. (2012) employed impedance spectroscopy to differentiate between unfrozen and frozen-thawed salmon. There is also research into the application of impedance measurements for evaluation of fish quality (Chevalier, Ossar, & Ghommidh, 2006) and freshness. Zhang, Shen and Luo (2011), employed impedance to estimate freshness of grass carp, finding a good correlation between impedance measurements and total aerobic count, total volatile basic nitrogen and sensory assessment.

However, despite the importance of the described works, studies evaluating the possibility of assessing the freshness of the fish over the days after slaughter by impedance spectroscopy are preliminary and need to be confirmed with deeper research on other species (Zhang, Shen, & Luo, 2011). In the same line, development of portable non-destructive devices based on impedance spectroscopy fish freshness monitoring would be an important advance for industry, governments and consumers.

The objective of this study is to evaluate the feasibility of an easy-to-use system based of Impedance Spectroscopy for rapid evaluation of sea bream freshness.

Materials and methods

2.1. Fish samples

Cultured gilthead sea bream (*Sparus aurata*) with a size of 400-600 grams provided between February and July from two different suppliers were employed. The first group of samples was obtained directly from a fish farm located in the eastern coast of Spain (Mediterranean Sea, Spain). Samples were slaughtered by immersing in ice-cold water (hypothermia) and delivered to the laboratory in insulated polystyrene boxes containing ice within 2 h of harvesting. It was considered that these samples had the highest freshness that could be expected from a fish, and therefore were considered the controlled group (C). The second group of samples was purchased from a local supermarket in Valencia (Spain). Sea breams came from a fish farm set in-shore area in the region of Lagonisi (Greece). In contrast to C, there was no information available about background of fish samples (feed composition, handling or transport conditions) and were labelled as commercial not controlled group (NC). However, a freshness guarantee was provided by the seller.

Upon arrived at the laboratory, fish from two groups, were individually weighed. The weight value of whole fish was 561±28 g and 565±30 g (Mean± SD) for C and NC groups, respectively. Subsequently, sea bream were beheaded gutted, washed and filleted. Fillets were placed individually in sterile bags of polyamide-polyethylene 1920-1960 (Verpackungen GmbH, Sulzberg, Germany), which were heat sealed and stored under refrigeration (4 °C) for a total of 15 days. Samples were analysed at 0, 1, 3, 5, 7, 9, 12, and 15 days of storage. Three different fillets were used at each sampling point and all the analyses were performed in triplicate (n=9).

2.2 Physicochemical determinations

Moisture and lipid content were determined according to the AOAC methods 650.46 and 991.36 (AOAC, 1997), respectively. Total Volatile Basic Nitrogen (TVBN) was assessed by steam distillation according to the method described by Malle and Tao (1987) and expressed as mg N/100 g of muscle. pH measurement was made using a pH meter (Crison Basic 20 +, Crison Instruments SA, Barcelona, Spain) with puncture electrode (Crison 5231) being applied directly to the fish flesh in six different locations on the fillet.

2.3 Electronic system

The system and sensor for measuring impedance in fish samples was developed by the Instituto de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) at the Universitat Politècnica de València (UPV) (Masot et al., 2010). The measurement system applies an electric signal to the sample and measures its response to 50 frequencies from 1Hz to 1MHz.

The sensor employed in this study was a double electrode. This electrode was made of two stainless needles (Ø = 1 mm, L = 15 mm) fixed on a non-conducting support and disposed in parallel with a distance of 10 mm. The impedance measurements were carried out by inserting the sensor into the sample at an angle of 45º to the muscular fibres of the fish. The penetration depth of the electrode into the sample was constant in all analyses. Measurements were performed on samples taken from chilled chamber keeping the samples on an ice-bath. Temperature was taken during the impedance measurements, maintaining the same in 8.0±1.5 ºC.

2.4 Data analysis

An analysis of variance (One-Way ANOVA) was conducted for each evaluated parameter, to test whether there were significant differences between the samples. Physicochemical parameters were considered as dependent variables in these analyses and storage time was the factor in these analyses. The LSD procedure (least significant difference) was used to test for differences between averages at the 5% significance level.

The ability of impedance spectroscopy to classify samples according to groups, batches or storage time from impedance data was studied by a discriminant analysis.

A PLS method was employed to estimate physical-chemical parameters studied in the samples from impedance data (modulus and phase). For the study, samples were divided into two groups for the PLS model development. The first group of samples (training set) was used for the establishment of the regression model with a full cross-validation, and was made up of 66 % of the sample measurements. The second one (external validation set) consisted of 33% of the samples and was used to validate the PLS model. The square of the correlation coefficient (R^2) were used to indicate the model quality.

Statistical treatment of the data was performed using the Statgraphics Centuriun XV (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1 Physicochemical parameters

Moisture, lipid content, TVBN as well as pH values of cultured gilthead sea bream fillets of different batches and origin groups at day 0 of the study are shown in Table 1.

Table 1. Physicochemical parameters in sea bream form the different origin groups (S: supermarket, F: fish farm) (average value and standard deviation, n=3)

Origin group	Batch	Moisture (g/100 g)	Fat (g/100 g)	TVBN (mg N/100 g)	pH
C	1	68,94±3.37^{a}	10,43±1.5b^{ab}	18.96±0.08^{a}	6.02±0.07^{a}
C	2	69,93±0.96^{a}	11,30±1.03^{a}	18.80±0.00^{ab}	6.02±0.05^{a}
C	3	69,84±0.43^{a}	9,58±0.21^{a}	18.89±0.16^{b}	6.08±0.06^{a}
NC	1	72,78±1.34^{b}	5,97±1.15^{c}	16.08±1.04^{c}	6.20±0.05^{b}
NC	2	73,21±2.00^{b}	5,39±0.09^{c}	16.93±1.94^{bc}	6.31±0.05^{c}
NC	3	73,32±0.60^{b}	7,07±0.54^{c}	19.47±1.06^{a}	6.10±0.06^{d}
		***	***	*	***

Values with different letters in the same row are significantly different at p-value * 0.05>p>0.01; *** p<0.001

Data obtained in the physicochemical characterization of samples belonging to groups C and S at the day 0 of the study are consistent with data obtained for farmed sea bream by other authors (Alasalvar et al., 2001; Cakli et al., 2007; Orban, Sinesio,

& Paoletti, 1997). Lipid content of F samples was higher than S samples, with correspondingly lower moisture content. This was probably due to differences in composition in the feed supplied in both farms, since different batches from the same fish farm did not differ among them. There is evidence that proximate composition of muscle and fat deposition is affected by feeding characteristics of the fish, although, in many cases, relations among dietary and quality parameters seem to be rather complicated (Grigorakis, 2007).

Samples from different batches of the same origin group do not differ significantly in moisture, fat and pH. However, statistically significant differences between fish groups were found in TVBN content. Variation in this parameter is related to the fish non-protein nitrogen content which in turns depends on type of fish feeding and environmental factors, among other factors such as season of catching or fish size or in the stage of degradation (Goulas & Kontominas, 2007). For this fish species, a wide range of TVBN contents in fresh fish can be found in the literature (between 15.9 to 26.0 mg N/100 g at day 0 of storage (Kyrana, Lougovois, & Valsamis, 1997). TVBN content in freshly caught fish is typically between 5 and 20 mg/100 g, whereas levels of 30-35 mg/100 g are generally considered as the legal limit for fish stored in ice (EEC, 1995). All samples were below this acceptability limit for consumption at the time of analysis. As it can be observed, TVBN content in F samples was similar for all the batches analysed, mainly due to samples were analysed at the same post-mortem time; however, samples purchased at the supermarket post-mortem time and handling conditions cannot be exactly controlled.

Samples from F supplier showed similar pH near to pH=6.0 in all the analysed batches, indicating the freshness of the sample. pH values of samples from S supplier

were higher than F samples, and also differed among batches ($p < 0.001$). These differences in the pH could be explained by the frequent oscillation in pH values of depending on season, species and other factors (Cakli et al., 2007), but also could be due to a lower initial freshness of the samples. During the storage period values of pH increase as consequence of the production of basic compounds such as ammonia, trimethylamine as well as other biogenic amines by fish spoilage bacteria (Goulas & Kontominas, 2007).

Throughout refrigerated storage, moisture, fat, pH and TVBN content for each batch were determined. There were no significant differences ($p < 0.05$) for the moisture and fat parameters during the period of refrigerated storage (data not shown).

pH values increased progressively during the storage period from pH 6.0 to 6.2 in the fillets of C group and from pH 6.2 to 6.4 in fillets of NC group, maintaining samples from NC group higher values.

Changes in TVBN content, a parameter widely used as an indicator of fish spoilage, along the study for sea bream fillets are shown in Fig. 1. TVBN values remained stable during the first days of study for two groups, being these values slightly higher for F samples. From day 5 of storage, this content increased progressively, being the increase more pronounced for S samples. Also, two batches belonging to NC group, exceeded the limit of acceptance for the TVBN set by the European Union (EEC, 2005) for fresh fish of 35 mg N/100 g fish (horizontal line in Fig. 1). Due to samples of C group maintained the freshness along the 15 days of storage meanwhile samples of NC group lost the acceptance criteria of commercialization and consumption in the first week of storage according to values of TVBN a hypothesis about a different freshness degree of samples belonging to different batches of NC group was

formulated. These differences in the initial freshness of NC samples could be due to a longer-than-declared storage period before the sale of the samples or due to a systematic breakdown of the cold chain in all of the batches during transportation from the farm to the supermarket.

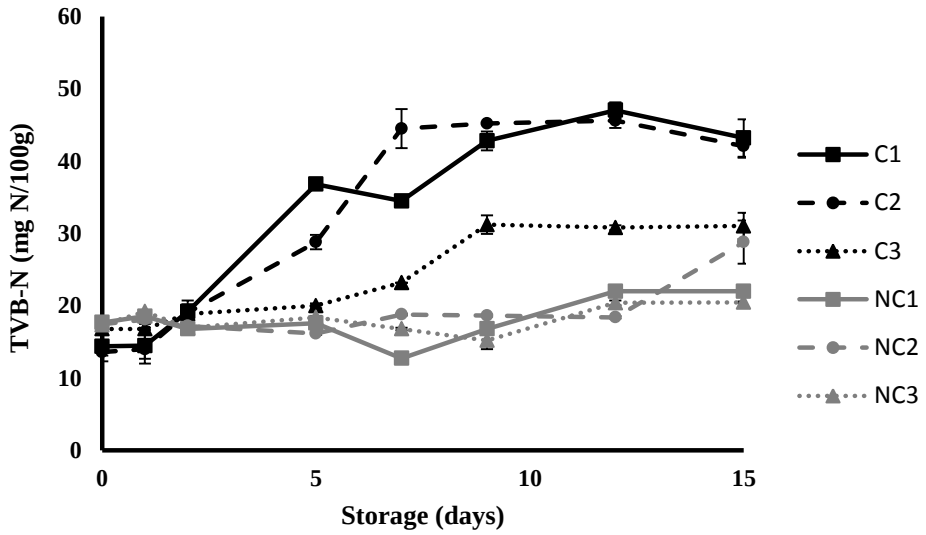

Fig. 1. Evolution of values (Mean± SD; n=3) of total volatile basic nitrogen (TVBN) in samples of sea bream form different origins (C and NC) and batches (batch 1 (●), 2 (■), and 3 (▲)), for 15 days storage at 4 ºC. Upper areas of horizontal line are unacceptable.

3.2 Impedance spectroscopy measurements

Impedance measurements were taken on fresh sea bream from both populations. Modulus and phase impedance spectra of sea bream are shown in Fig. 2.a and 2.b, respectively. Differences between different batches belonging to S and F origins were observed in both, modulus and phase.

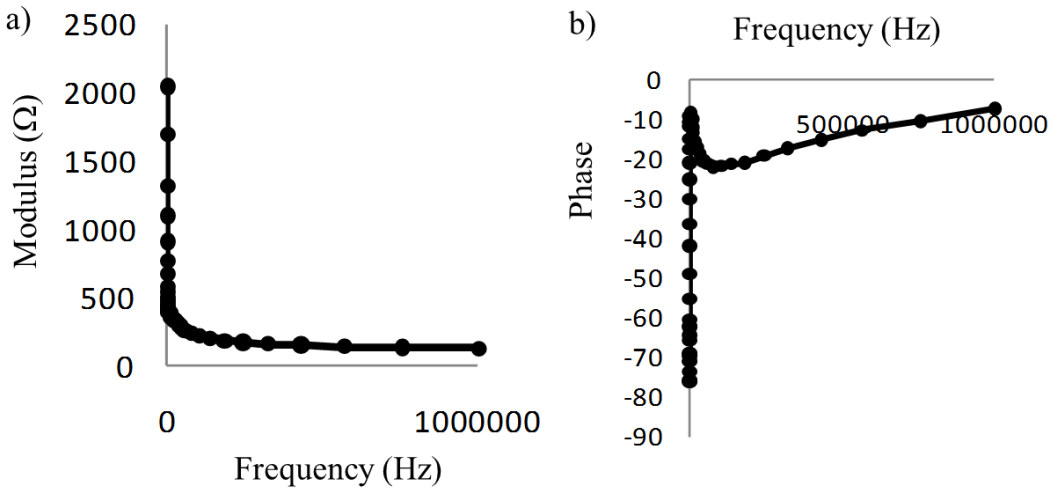

Fig 2. Typical curves representing the module (a) and phase (b) of the impedance of the cultured sea bream analysed.

3.2.1 Raw matter classification

In order to determine the feasibility of the portable impedance equipment to classify fresh samples into the different batches, once known that different batches differed in the composition, a Discriminant Analysis (DA) was carried out using the 50 values of modulus and 50 values of phase at different frequencies. For the analysis, the number of batch was included as a factor and data of modulus and phase of impedance were considered the variables.

Figure 3 shows the LDA plot. The first two functions allowed explain the 97.78% of the variance (F1 88.65% and F2 9.13%). Discriminant function 1 (DF1) determined the separation of samples in two regions concurring with samples C and S. Within each of these two regions the (DF2) allowed the differentiation between each of batches of both group of samples.

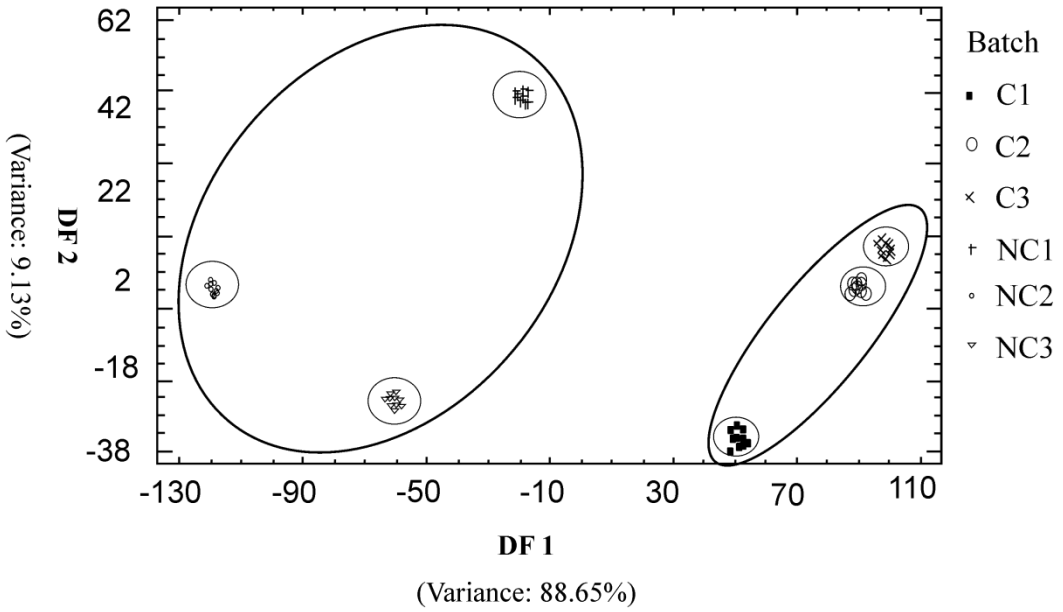

Fig. 3. Projections impedance values of sea bream samples at day 0 of storage in the space defined by the LDA discriminant function (DF) 1 and 2.

These results are consistent with differences found initial composition of the 6 batches analysed summarized in Table 1, where it was observed that samples belonging to C group were more homogeneous among each other than samples from NC group.

3.1.2 Stored samples classification

A LDA was used to assess the feasibility of impedance spectroscopy for monitoring sea bream freshness along 15 days of storage. LDA results for C samples are shown in Fig. 4a. The plot of the first two discriminant functions not only showed an excellent and clear separation of the samples according to the days of storage, but this classification also allowed the grouping of the samples in 4 areas according to storage days (0, 1-3, 4-9, and more than 10 days of storage). These 4 different areas

correspond with the 4 moments of change in a fish muscle during post-mortem storage defined by Caballero et al. (2009). In the hours just after post-mortem (day 0) muscle structure remains intact. From the next day (day 1-3), loss of fibre–fibre adhesions compared to muscle samples obtained at 0 h post- mortem is observed. Finally on day 4 myofibrils are slightly affected, and by 7 day muscle fibres start to detach from the myocommata (day 4-9). From 10 to 14 post-mortem days most of the myocommata has detached from fibres and muscle structure is evidently damaged. This excellent classification of samples according to days of storage and changes produced in the fish muscle confirm the feasibility of impedance to detect changes in the freshness of sea bream fillets. This proven ability of impedance to predict changes in the muscle integrity during storage, which is related with the freshness of the fish, may be explained by the increase in conductivity that occurs when metabolic products are released from the cells as a consequence of muscle microbiological or enzymatic degradation (Marshall & Wiese-Lehigh, 1997).

The same analysis was performed in S samples. However in this case, the plot of the first two discriminant functions (Fig 4b.) only allowed differentiating 3 areas (0, 1-3, and more than 5 days of storage), so that the hypothesis of the lower freshness of S samples built by physicochemical values could be confirmed also by impedimetric analysis.

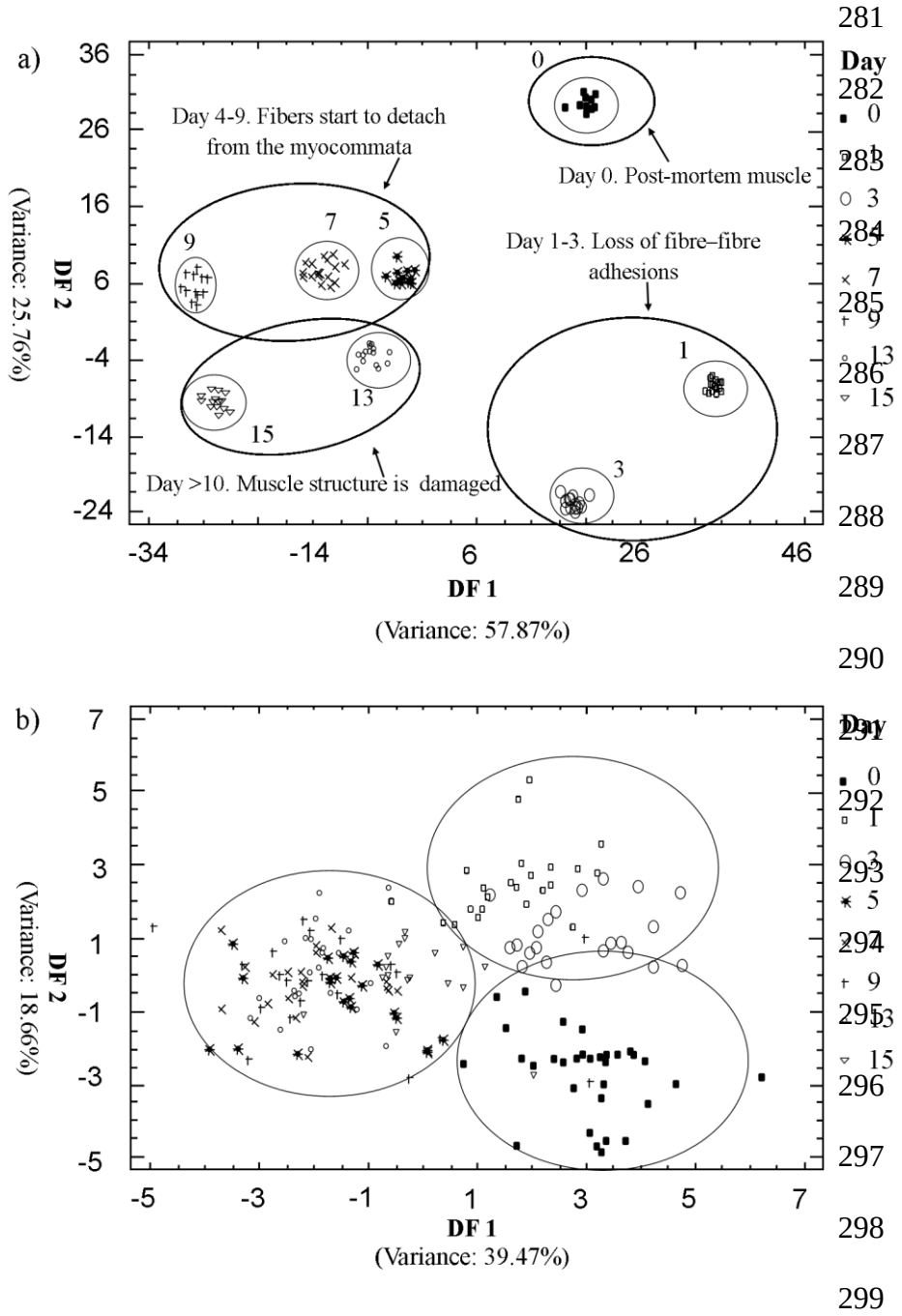

Fig 4. Projections of impedance values of sea bream samples along 15 days of storage in the space defined by the LDA discriminant function (DF) 1 and 2 for group F (a) and S (b).

3.5 Partial least squares regression for establishing relationships between impedance and control parameters

Since the LDA analysis of the impedance data were able to classify samples according to the spoilage degree of the muscles, a PLS was used to predict days of storage and physicochemical values from the impedimetric data. Statistical models were tested for day of storage, moisture, lipid content, pH and TVBN. Table 2 shows the coefficients of determinations (R^2) for both, calibration and validation, of selected models for each of the different measurement parameters. The better correlation between measured and predicted values with the impedance measurements is obtained (Prediction R^2=0.72) is achieved for TVBN values.

Next to TVBN, moisture and lipid content, two of the most responsible components of current passage or obstruction through a sample, are the parameters for which a higher correlation is obtained are. The lesser correlation between these parameters is associated with the uniformity of these values among different samples.

Figure 5 shows the experimental versus the predicted values by the PLS statistical model for the TVBN values and how a preliminary evaluation of the accuracy of the created prediction model can be made by visually inspecting the differences between the measured and the predicted values.

Table 2. PLS Results obtained in the Partial Least Regression (PLS). R^2: Coefficient of determination.

Parameter	Number of PLS factors	Calibration R^2	Prediction R^2
Day of storage	10	0.54	0.33
Moisture	8	0.62	0.53
Lipid Content	8	0.66	0.59
pH	7	0.45	0.38
TVBN	8	0.79	0.72

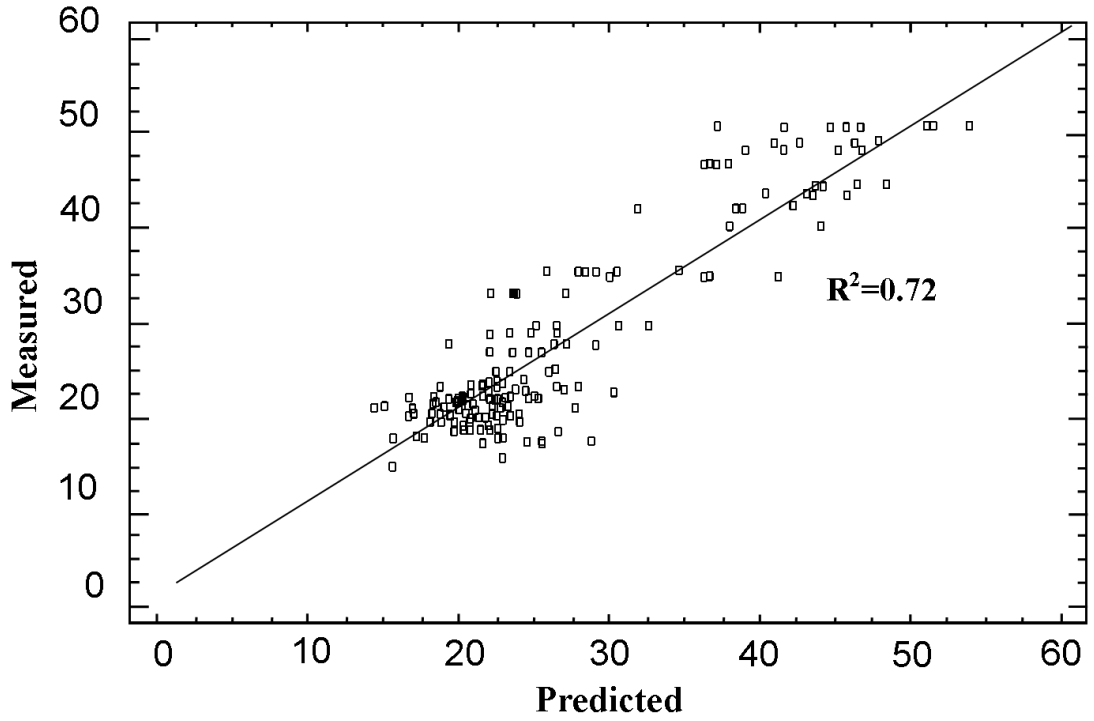

Figure 5. Experimental versus predicted TVBN values by the PLS statistical model.

Keeping in mind that TVBN is one of the most important parameter to establish fish spoilage the potential usefulness of the impedimetric system and sensor developed at the UPV for monitoring sea bream freshness could be confirmed.

4. Conclusions

Six different batches of sea bream fillets with two different levels of information available about background of fish samples (C and NC) have been analysed by means of physicochemical analysis and impedance spectroscopy. Initial differences in composition and freshness between controlled (C) and not controlled commercial (NC) samples were found, showing NC samples a lower shelf life.

These differences in composition and freshness were easily predicted by the impedance system, so that fillets belonging to different batches at day 0 of storage and fillets with different degradation degree were easily classified. Furthermore, PLS statistical analyses allowed the creation of a model to correlate with the impedance modulus and phase data with the content of TVBN. Thus, the feasibility of the developed portable and easy-to-use impedance spectroscopy system for monitoring sea bream fillets freshness has been demonstrated.

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