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Shelf life prediction of expired vacuum-packed chilled smoked salmon based on a KNN tissue segmentation method using hyperspectral images.

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Abstract

Ready-to-eat foods that does not receive a heat treatment before being consumed can be at risk of foodborne hazards and spoilage, so it would be of great interest to have a method for monitoring their safety. This work expands on and enhances previous successfully studies with hyperspectral imaging in the SW-NIR range. Specifically, a k-nearest-neighbours model was developed to classify the salmon tissue into white myocommata stripes (fat) and muscle (lean) tissue. Partial Least Squares models developed confirm that a spatial segmentation should be performed before a shelf life model can be calculated. Employing the fat spectra and only the 7 most correlated wavelengths, a support vector machine model was calculated to classify into days 0, 10, 20, 40 and 60 with 87.2 % prediction accuracy. These results make the method developed very promising as a non-destructive method to analyse the shelf life of vacuum-packed chilled smoked salmon fillets.

Keywords: Hyperspectral imaging; Tissue segmentation; SW-NIR; Fish shelf life; Smoked salmon; SVM.

1. Introduction and motivation

There is an increasing demand for ready-to-eat foods that are wholesome and require less handling and preparation (Mhurchu et al., 2013). However, this kind of food does not receive a heat treatment before being consumed, so there could be a risk of foodborne hazards and spoilage. Although chilled smoked salmon can be cooked, it is usually eaten as it is, so it would be of great interest to have a method for monitoring its safety.

Destructive analyses are used in chilled smoked salmon for quality assurance (Jónsdóttir et al., 2008), Listeria innocua growth control (Tomé et al., 2008), detecting frozen-thawed salmon (Fernández-Segovia et al., 2012) and evaluating spoilage (Zaragozá et al., 2014). Although these methods are effective, it would be better to use non-invasive techniques like hyperspectral imaging, which leaves samples intact.

Hyperspectral imaging has been proven as an effective tool for quality analysis of fish, as shown by the large volume of research and potential applications that have been developed (Cheng & Sun, 2014). Specifically for salmon, there are studies that predict microbial numbers (Tito, Rodemann, & Powell, 2012), autolytic changes (Sone, Olsen, Dahl, & Heia, 2011), freshness (Kimiya, Sivertsen, & Heia, 2013), detection of frozen-thawed salmon (Uddin & Okazaki, 2004), determination of drip loss and pH distribution (He et al., 2014). However, these studies have been performed in raw unpacked salmon with the exception of this work.

It is known that different kinds of tissues in fish have different biodegradation rates or contaminants, so this should be taken into account when assessing freshness (Einen & Thomassen, 1998; Sivertsen, et al., 2011; Ivorra et al., 2013; El-Moselhy et al., 2014). This segmentation can be performed using sensors with good spatial resolution like CCD cameras (Marty-Mahé et al., 2004; Stien et al., 2007) or desktop scanners (Kause et al., 2008) based on colour differences. Other sensors like X-rays, computed tomography (CT) scans or magnetic resonance imaging (MRI) have been also used for lipid tissue location and quantification in fish (Kolstad, et al., 2004; Collewet et al., 2013). Although all these sensors could be used, in this study we decided to employ the hyperspectral sensor for tissue segmentation because of the good contrast of the different tissues in the SW-NIR

spectrum and because this meant that there was no need for any additional sensor for the shelf life estimation.

This study expands on and enhances a previous work (Ivorra et al., 2013) that introduces a non-destructive method using hyperspectral imaging SW-NIR spectroscopy with tissue segmentation to analyse the shelf life of vacuum-packed chilled smoked salmon fillets. Specifically, the current study employs a complete different approach for segmenting tissues, where segmentation is performed using the spectra and not the RGB colour as was employed previously. Other important additions were the spectral study for selecting the most relevant wavelengths and the shelf life model employing the non-linear SVM algorithm instead of a PLS-DA model.

2. Materials and methods

2.1 Sample preparation

Con formato: Sangría: Izquierda: 0 cm, Sangría francesa: 1,25 cm

The study was carried out on 30 samples of chilled smoked salmon, which were obtained from a local supermarket. Samples were from three different batches and brands, ten from each, but with the same expiry period in order to establish an initial freshness parameter. Samples were repackaged in aseptic conditions under vacuum.

Image analysis (visual and SW-NIR) was carried out at 0, 10, 20, 40 (expiry date given by the producer) and 60 days (in order to ensure shelf life), although destructive analyses were only carried out at 0, 20, 40 and 60 days.

Images were acquired with the packed samples. After opening the packages aseptically, a representative sample was taken for the subsequent destructive analysis after grinding and homogenizing. During the study the samples were stored at 4 ºC.

2.2. Destructive analyses

The control of biochemical state of samples during storage was carried out based on Total Volatile Basic Nitrogen (TVB-N), determined according to the procedures described by Malle and Tao (Malle & Tao, 1987), and the aerobic plate counts (expressed as cfu g^{-1}) were estimated following the method proposed by the UNE (ISO, 2003, p. 200). Tenfold dilutions in 0.1% peptone water were prepared from each sample obtained from each container on each measurement day and 1-ml aliquots were plated in duplicate. Aerobic counts were determined using Plate Count Agar (Merck, Darmstadt, Germany). Duplicate pour plates were prepared for each dilution and incubated at 28 ºC for 48 h. All destructive analyses were carried out in triplicate after grinding and homogenizing samples.

2.3. Data processing

The method developed is divided into two stages; the objective of the first stage is to develop a classifier of salmon tissues into white myocommata stripes (fat) and muscle (lean) tissue, while the objective of the second stage is to develop a shelf life model taking into account the kind of salmon tissue. Fig.1 is a schematic diagram of the method employed to detect expired salmon, from acquisition to obtaining the result. Each step is explained in detail in the following subsections.

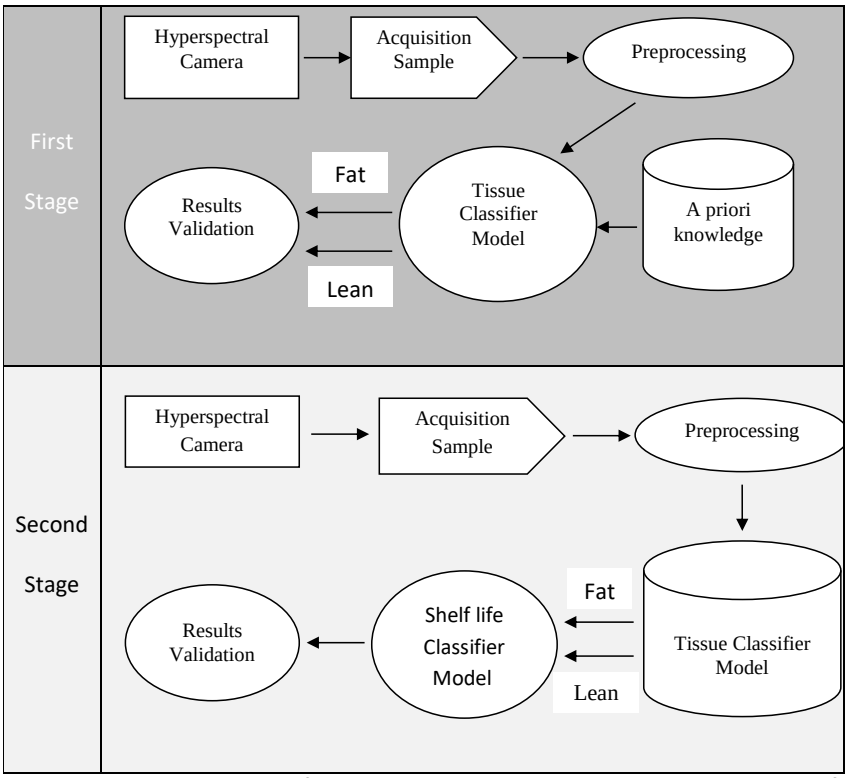

Fig 01 Schematic diagram of the technique employed to detect expired salmon, from acquisition to obtaining the result.

2.3.1 Image acquisition and preprocessing

Images were taken using a Photonfocus CMOS camera MV1-D1312 40 GB 12 (Photonfocus AG, Lachen, Switzerland) and SpecimImSpector V10 1/2″ filter (Specim Spectral Imaging, LTD., Oulu, Finland), which works as a linear hyperspectral camera. The illuminant was an ASD illuminator reflectance lamp (ASD Inc., Boulder, USA), which produces stable illumination across the full working spectral range (Fig. 2), and it was placed at a sufficient distance so as not to warm the samples. The hyperspectral camera was mounted over a conveyor belt in order to be able to scan whole samples line

by line. The conveyor belt was controlled by an Altivar31 adjustable speed drive controller (Schneider Electric Rueil-Malmaison, France).

The position of the illuminant and camera relative to the sample was always constant in order to control the lighting conditions and obtain a constant image size. The image obtained (scanned line) was composed of 256-level grey scale (8 bits). The diffuse reflectance spectrum was recorded using 53 different wavelengths (each wavelength was digitized at 8 bits) distributed equally between 400 and 1000 nm where each wavelength is the mean value of 20 pixels of the CMOS sensor. The scanned line was composed of 1312 points, so that an image was recorded with a resolution of 1312 x 1060 pixels. Original CMOS sensor resolution is 1312 x 1082 but the SpecimImSpector filter projects to 1312 x 1060 (53 x 20) pixels with the current set-up. Samples were placed on a conveyor belt and scanned directly at room temperature (21ºC), without any sample treatment.

A preprocessing step was performed in order to normalize the non-linear light source reflectance. This preprocessing step also removes the effect of the pixel-to-pixel sensitivity variations across the array, as well as the effect of dust or scratches on the CMOS window or camera lens optics, and the dark charge related to the integration time. This was done by applying equation 1, where rW is the reflectance value of a white pattern reflectance acquired under the same conditions as the sample, rD is the dark measure covering the camera's lens and rS is the sample reflectance.

$$R(\lambda) = \frac{(rS - rD)}{(rW - rD)} \qquad (1)$$

Other operations carried out on the spectra for further statistical processing were mean-centering and unit variance normalization. These operations are very common and correct different variable scaling. Furthermore, they are a requisite for building partial least square models.

The camera was operated using our own software developed based on the SDK Photonfocus-GigE_Tools using the C++ programming language. Furthermore, image reflectance calibration and preprocessing were performed using our own code developed on Matlab R2012a (The Mathworks, Natick, Massachusetts, USA).

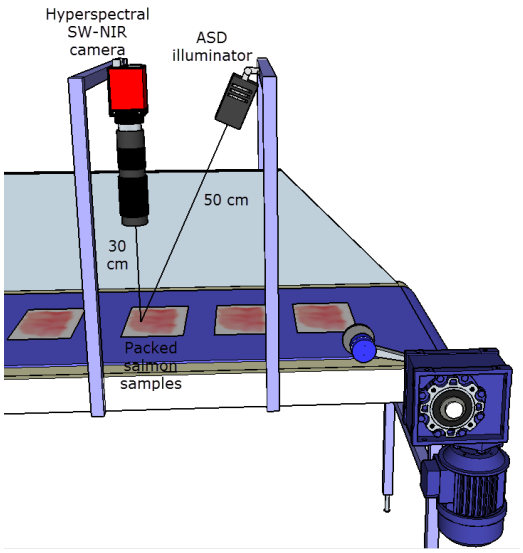

Fig. 02 Spectral acquisition setup.

2.3.2: Tissue Classifier Model

Tissue classification was based on the *k*-nearest-neighbours (KNN) pattern recognition approach. This classification algorithm has been used successfully in numerous food research (Sánchez, Albarracin, Grau, Ricolfe, & Barat, 2008; Y. He, Xu, Khanna, Boushey, & Delp, 2014). Furthermore, Balabin et al, 2010 ranked the KNN method

among the top 3 of "highly accurate methods" in multivariate techniques that includes also the support vector machine and probabilistic neural network methods. KNN predicts the classification label of a given query feature vector based on the K closest training vectors. The majority vote of its K nearest neighbours classify this query object. It means that a sample is classified into the training class that is most common amongst its K nearest neighbours. The distance measure chosen in this case was the Euclidean distance (L2 norm) that usually results in good classification accuracy. The K number of neighbours employed was 17. The choice of K is equal to the square root of the number of samples, as suggested by (Duda & Hart, 1973). In addition, as 17 is an odd number this avoids tied votes.

The database employed for training the KNN model was obtained as follows: two different spectra were acquired for lean and lipid tissues, respectively, for each sample (30) and each acquisition day (5), meaning that a total of 300 (2 tissues x 30 samples x 5 days) spectra were used to train the model. The acquisition line was different for each sample in order to create a more robust model. Moreover, this acquisition line was manually delimited in order to assure the tissue class and create the ground truth of 300 known tissue spectra.

2.3.3: Shelf life Classifier Model

Once the spectra was classified into fat and lean tissue, they were studied together and individually for predicting the shelf life. The method chosen for this study was a partial least squares (PLS) regression model (Bharati & Champagne, 2004). Spectral data usually involve many variables with redundant information thus the use of PLS is very convenient to get an efficient model to reduce the dimension of data and to eliminate

multicollinearity. Specifically, PLS method finds a linear regression model by projecting the predicted variables (acquisition time in this case) and the observable variables (spectra) to a new space that enhances the prediction. In addition, a wavelength selection was performed using a forward interval partial least squares (iPLS) regression model (Norgaard et al., 2000). The basic idea of this method is adding in each iteration the wavelength that provide the lowest cross validation error. This method optimizes the predictive power of PLS regression models and aids their interpretation.

A Support Vector Machine supervised classification model (using nu-support vector regression) (Schölkopf, Smola, Williamson, & Bartlett, 2000) was used to predict the shelf life of chilled smoked salmon. The basic idea of SVM is to find optimal hyperplanes to separate the data into the desired classes. Hyperplanes are chosen so that the margin between them and the nearest data points, called support vectors, between classes are maximized. The model developed is based on a number of support vectors (samples selected from the calibration set) restricted by the nu parameter and non-linear model coefficients which define the non-linear mapping of variables (in this case wavelengths). The SVM is currently wide used in food application (Mizushima & Lu, 2013) due the flexibility and potential to adapt to different classes. Furthermore, the SVM outperformed PLS in some spectral applications (Thissen, Pepers, Anstan, Melssen, & Buydens, 2004) and was ranked between the top three as was mentioned before in section 2.3.2.

2.3.4: Validation of the results

The measure employed to estimate how well the calibration data fitted the models developed was the Root Mean Square Error of Calibration (RMSEC). It is defined as:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \qquad (2)$$

where $\hat{y}_i$ are the values of the predicted variable when all samples are included in the model formation, y_i are the known values and n is the total number of samples.

Other measures employed for evaluating the goodness of the developed models were:

- R^2 is the square of the Pearson correlation coefficient (Nagelkerke, 1991) between the observed and the predicted data values of the dependent variable. A value of 1 it is a perfect fitting.
- Bias is the arithmetic mean value of the prediction errors and should be near zero except when there is a systematic error.

A random cross-validation method (CV) was employed to evaluate the models developed (KNN and PLS models). In this method, subsets of n/4 random samples are used to test the model developed without them, n being the total number of samples. This method was iterated three times to achieve more reliable models. The SVM model was also tested by randomly splitting the samples into ¾ for training and ¼ for testing. It was adjusted so spectra from different days were represented for building and testing.

All statistical procedures were performed on PLS Toolbox 6.3 (Eigenvector Research Inc., Wenatchee, Washington, USA), a toolbox extension within the R2012a computational environment (The Mathworks, Natick, Massachusetts, USA).

3. Results and discussion

3.1 Destructive analysis

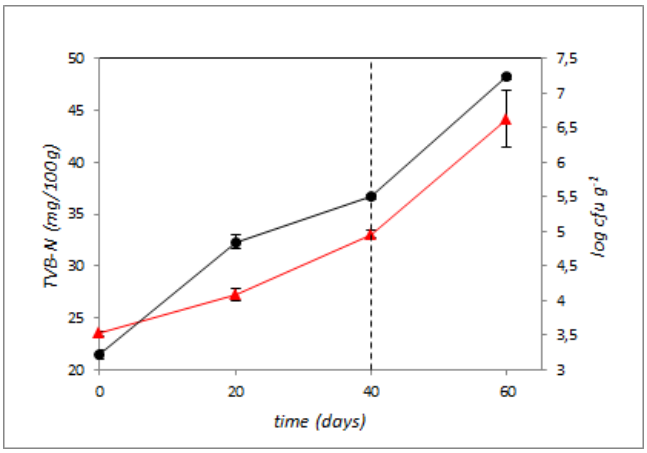

Fig. 03 Evolution of TVB-N (black -o-) and microbiological count (red -Δ-) during product storage. Dashed line indicates expiry date given by the producer.

Figure 3 shows the evolution of TVB-N and microbiological counts and the standard for packaged sliced salmon on days 0, 20, 40 and 60 at 4ºC. TVB-N levels (mg/100 g) increased significantly over time, exceeding the limit value (30 mg/100 g) established for some kinds of fish (Official Journal of the European Union, 2005) at 40 days (expiry date given by the producer). Only some types of fish are legislated for this parameter, although it can give information about the biochemical changes occurring during the storage period of some products such as dry-cured ham (Lorenzo, Fontán, Franco, & Carballo, 2008) and camel meat (Bachir & Zeinou, 2009) without expressing the expiry date of these products. Microbiological counts increased from 3.22 (log cfu g -1) to 7.23 (log cfu g -1), although the values at day 40 (5.5 log cfu g -1) were lower than the legal limit in Spain and those recommended by the EEC (6 log cfu g -1) (Official Journal of Spain, 1991; Official Journal of the European Union, 2001)). The increase was mainly produced by lactic acid bacteria (LAB), which are the dominant flora in vacuum-packed chilled

smoked salmon at the end of the storage period (Leroi, Joffraud, Chevalier, & Cardinal, 1998).

3.2 Tissue classification

Chilled smoked salmon tissues have a different biodegradation process, making it important to study them separately. Therefore, before the shelf life study, salmon tissues were classified into white myocommata stripes (fat) and muscle tissue (lean). In the figure 4 it can be seen that these type of tissues had a differentiate spectrum in the SW-NIR. In one hand, lean spectra had low reflectance values in the visible range except in the red wavelengths and high values from 900 to 1000 nm. On the other hand, fat spectra had a different behavior, showing a high reflectance values in the visible range while in the NIR region it had low values. This fact agrees with the results of others authors that reported high absorbance values at 930 nm on fat fish spectra (ElMasry & Wold, 2008). colour

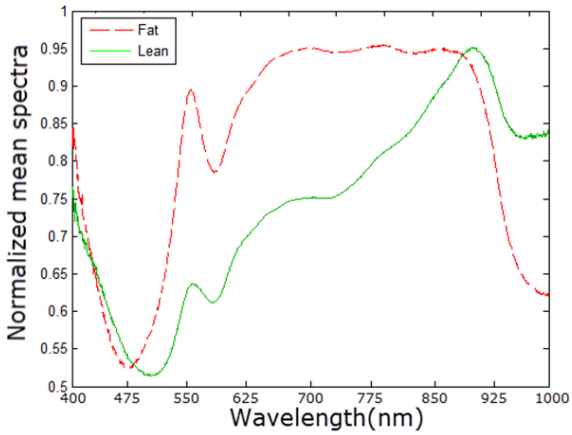

Fig. 04 Normalized spectra of fat (red - -) and lean (black -) tissue in chilled smoked salmon.

A KNN supervised classification model was successfully trained using K=17 and its results can be seen in table 1. The results for lean were the same results than for fat tissue but t with the sensitivity and specificity inverted because it is a binary classification.

Table 1 Statistical results of the Knn model for tissue classification

	Fat	Lean
Sensitivity (Cal)	1.000	1.000
Specificity (Cal)	1.000	1.000
Sensitivity (CV)	0.714	0.750
Specificity (CV)	0.750	0.714
Class. Err (Cal)	0	0
Class. Err (CV):	0.268	0.268

The classification error in CV is due to the variability in samples. This variability is caused by differences between samples from different batches and brands. The random selection of the cross-validation split of samples could cause that some relevant samples (for example a particular brand) were not included in the calibration model causing an under fitting model. Another factor could be that spectra were acquired with packed samples, so the film could make some bright spots appear.

In order to perform an additional qualitative test of the tissue model classifier, image validation was performed. Figure 5b shows the pixels classified as fat by the model compared with an RGB image of the same sample (figure 5a).

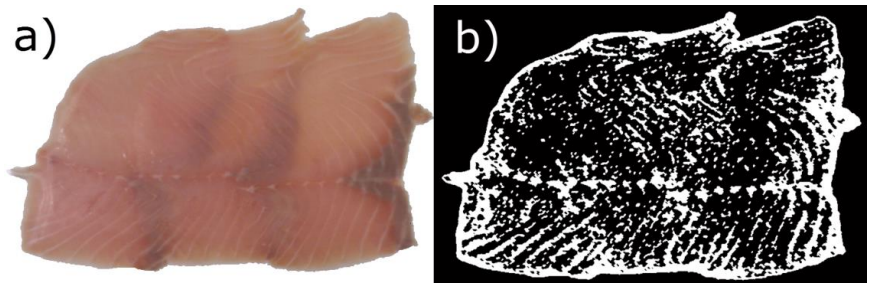

Fig. 05 a) RGB image of chilled smoked salmon b) Result of the fat tissue segmentation performed by the Knn model developed.

3.3 Shelf life prediction

In order to predict shelf life three analyses were performed with Partial Least Squares (PLS) regression between SW-NIR spectra and the time lapse. The models were built using fat tissue, lean tissue and both of these. As can be seen in table 2, a better cross-validation result was obtained using the fat tissue with an error of around 13 days with the same number of latent variables (LV). It was also proven that it is essential to perform tissue segmentation prior to the shelf life study.

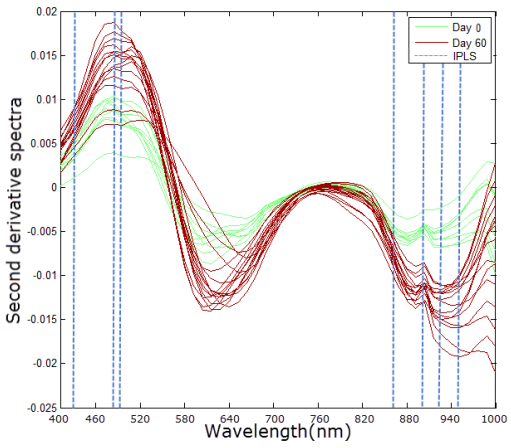

Fig. 06 Second derivative of the spectra acquired at day 0 and day 60. Vertical dashed lines are the wavelengths selected by the iPLS model.

Table 2: Statistical results of vacuum-packed chilled smoked salmon PLS models taking into account the different tissues.

	Fat	Lean	Fat & Lean
Num. LVs	10		
RMSEC (days)	9.13	10.9	15.35
RMSECV(days)	12.72	18.45	17.94
Bias	$2.12183e^{-014}$	$-5.68644e^{-014}$	$-2.53863e^{-014}$
CV Bias	-0.12	-0.02	-0.07
R^2 Cal	0.89	0.86	0.69
R^2 CV	0.80	0.66	0.54

Beyond this point, the spectra employed were only the spectra acquired from fat. In order to increase the resolution, the numbers of independent variables available were reduced, selecting the main spectrum points with the best correlation with storage time. The wavelengths obtained, employing the variable selection algorithm of forward iPLS, were 433, 489, 500, 867,900, 922 and 944 nm. These wavelengths are in the visible and near infrared region of the spectrum, justifying the fact that the complete wavelength range should be used. In addition, as can be seen in figure 6, these regions matched the fact that the difference between the spectra of day 0 and the spectra of last day 60 was maximum. The selected wavelengths indicated zones referring to different functional groups. Wavelengths from the visible zone (433, 489 and 500) have been related to unsaturation into carbon chains, while those from the infrared zone (867, 900, 922 and 944) are into the 3rd overtone of CH, 2nd overtone of OH, and aromatic compounds. If we observe the

functional groups, the results could be attributable to modifications in fat tissue as a result of oxidation and metabolization of polyunsaturated acids by bacteria during storage.

Furthermore, wavelength selection improved the result of correlation between the spectra and storage time, achieving a RMSECV of 7.70 and a R^2CV of 0.89 with a PLS model of 11 latent variables. This improved result is consistent with (Grau et al., 2011) where a reduction of wavelengths helped the PLS-DA classification.

A SVM model was built for classifying spectra into 5 classes corresponding to the image acquisition days. Specifically, there are three classes for spectra acquired before the expiry date (days 0, 10 and 20), one class that is on the expiry date (day 40), and a final class that destructive analyses establish as the limit when it can be consumed safely (day 60). According to the results of the previous PLS, only the spectra acquired from adipose tissue was used with the 7 selected wavelengths. The optimal parameters for the SVM model were nu = 0.14143 and gamma = 0.03162 with 44 support vectors and a radial basis function (RBF). Taking advantage of the known spectra, a RBF kernel was selected, as it is known that it selects solution that are smooth (Scholkopf et al., 1997). The optimal values of nu and gamma were obtained using a search over the grid and cross-validation results. The detailed results obtained can be seen in table 3 and figure 7. The mean classification success in terms of prediction was 87.2%. The SVM model mixed some spectra between classes 10 and 20 because in this kind of processed products during this early period there are few physical-chemical changes (Cardinal et al., 2004) and, consequently, small changes in the spectrum.

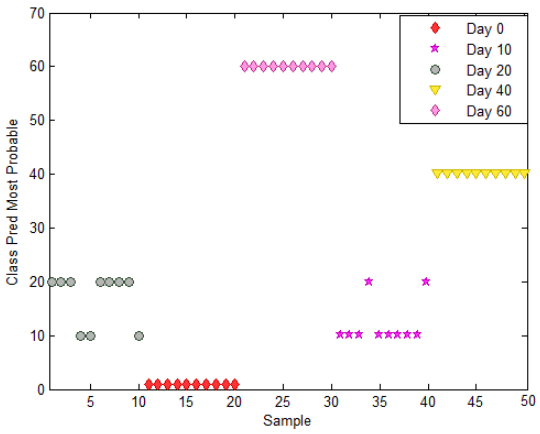

Fig. 07 SVM most probable predicted classes of the spectra used only to validate the model.

The results obtained in this study were better than those obtained in our previous work (Ivorra et al., 2013), where the R^2 was of 0.83 and where the classification consisted of just two classes (expired and non-expired). This could be due to the better tissue segmentation step, where the entire wavelength range was used, and the wavelength selected with iPLS.

Table 3: Statistical results of vacuum-packed chilled smoked salmon SVM model using only adipose tissue.

	0	10	20	40	60
Sensitivity (Cal)	0.778	0.333	0.667	0.933	1.000
Specificity (Cal)	0.987	0.797	0.812	0.928	1.000
Sensitivity (Pred)	1.000	0.467	0.733	0.867	0.933
Specificity (Pred)	1.000	0.922	0.824	0.980	0.980
Class. Err (Cal)	0	0.21	0.15	0.03	0
Class. Err (Pred)	0	0.31	0.22	0.07	0.04

4. Conclusions

This work confirms hyperspectral imaging spectroscopy in the SW-NIR range as a valid technique for non-destructive shelf life prediction in vacuum-packed chilled smoked salmon. Experimental results proved that different salmon tissues should be segmented and processed separately, which is consistent with the findings of previous studies. A KNN supervised model using all the wavelengths in the SW-NIR range was developed to achieve a successful tissue segmentation into fat and lean tissue. Furthermore, the developed model could be a promising tool for estimating the fat content. PLS regression models built with lean, fat and both tissues showed that fat correlated better with the salmon's shelf life ($R^2CV=0.8$). In addition, a reduction to the 7 most correlated wavelengths was performed using an iPLS method. A supervised model based on SVM obtained a mean classification error of 12.7% when predicting the shelf life time of the samples in the following groups: day 0, day 10, day 20, day 40 and day 60. Moreover, the method presented in this study could easily be applied to other food products with different types of tissue.

In terms of future work, we are considering using pattern recognition algorithms in order to also use spatial resolution for the tissue segmentation as in other works using RGB images (Stien et al., 2007; Merkin, Stien, Pittman, & Nortvedt, 2013) and extending the study to estimate the fat content of the salmon.

Acknowledgements

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